MEDICAL UNIVERSITY - PLEVEN
FACULTY OF PUBLIC HEALTH
DEPARTMENT OF HYGIENE, MEDICAL ECOLOGY, OCCUPATIONAL DISEASES AND EMERGENCY MEDICINE

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STUDY OF THE CONTENT OF METALS (LEAD AND CADMIUM) IN FOOD AND HEALTH RISK ASSESSMENT

ABSTRACT

of a dissertation work

for awarding the educational and scientific degree "Doctor"

Doctoral program: Hygiene (including occupational, communal, school, radiation, nutrition, etc.)

Research supervisor: Assoc. Ph.D. Vanya Boycheva (Birdanova), MD

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The dissertation contains 175 standard pages, illustrated with 30 figures, 22 tables and 5 appendices.

The bibliographic list contains 282 titles, of which 105 are in Cyrillic and 177 are in Latin.

In connection with the dissertation work, 3 publications and 3 scientific announcements were made at national and international forums.

The materials used in the dissertation work are published and available on the website of the Medical University of Pleven: http://www.mu-pleven.bg/index.php/bg/

CONTENTS

ABBREVIATIONS USED

BFSA – Bulgarian Food Safety Agency
EC – European Commission
EU - European Union
EFSA – European Food Safety Authority
FA – Food Act
CCA - Codex Alimentarius Commission
MH - Ministry of Health
MAF - Ministry of Agriculture and Food
MPA - Maximum permissible amounts
UN - United Nations Organization
WHO - World Health Organization
FAO - Food and Agriculture Organization
BMDL - Benchmark Dose Lower Confidence Limit
bw – body weight
CCCF - Codex Committee on Contaminants in Foods (Codex Alimentarius)
FAO/WHO - Food and Agriculture Organization of the United Nations/World Health Organization FAO/WHO
CONTAM - Panel on Contaminants in the Food Chain (Expert group/panel on contaminants in the food chain of the European Food Safety Authority)
LB - lower bound
LOD - limit of detection
LOQ - limit of quantitation
MOE - Margin of Exposure
NOAEL - no observed adverse effect level (dose at which no adverse effect is observed)
TWI - tolerable weekly intake
UB - upper bound

I. INTRODUCTION

Heavy metals are chemical environmental pollutants that are found in nature, both naturally and as a result of human activity. The concept of chemical contamination of food is a clear indication of the presence of chemical substances where they should not be or are present in an amount that is in a higher concentration than the amount that is considered safe.

It is accepted that metallic elements that have a relative mass greater than 40 and a relatively high density (over 0.5 g/cm^3) are called heavy metals. Some heavy metals (copper, zinc, iron, etc.) belong to the so-called microelements, because in certain concentrations they are vitally necessary for the functioning of the human body. These substances can have an adverse effect when their amount in the body exceeds the necessary.

The other heavy metals such as lead, cadmium, etc. are not essential for the body, many of them exhibit toxicity, have a significant migration capacity. They are not biologically necessary and have only a detrimental effect on human health. Heavy metals have the ability to remain in the environment for a long time, as well as a tendency to bioaccumulate, which is a prerequisite for their long-term toxic effects. Heavy metals have an adverse effect on human health upon chronic exposure even in small doses.

Food and drinking water intake is the main route of exposure to heavy metals. Dietary exposure represents over 75.8% of the total daily intake of metals [Lopez-Alonso M. et al., 2017; Wang R. et al., 2018].

Ensuring food safety is one of the priority directions of the state policy in the field of food. European legislation has high requirements regarding food quality and safety. The European Commission has established with Regulation (EU) 2023/915 the maximum permissible amounts for lead and cadmium content in certain foods. In 2009, the European Food Safety Authority (EFSA) carried out a human health risk assessment related to the presence of cadmium in food and established a tolerable weekly intake (TWI) of 2.5 μg cadmium/kg body weight (bw)/week. To assess the health risk resulting from dietary exposure to lead, the EFSA-recommended margin of exposure (MOE) method is applied, as there is a lack of scientific data for a threshold for adverse health effects caused by lead intake.

In Bulgaria, as well as in other European countries, the content of lead and cadmium in food is monitored. Every year, a national food control program for the content of contaminants is planned and implemented, which is part of the multi-year national control plan of the Republic of Bulgaria, drawn up according to the requirements of the European Union (EU) legislation.

It is not possible to completely eliminate the presence of lead and cadmium in the environment, therefore it is important to study their concentrations in food, to assess exposure through the intake of foods of plant and animal origin, and to carry out risk assessment for human health related to the detected content of heavy metals.

II. OBJECTIVE, TASKS, HYPOTHESES

1. Objective of the study

To study the content of heavy metals (lead and cadmium) in foods and to assess the health risk associated with the intake of these metals in food.

2. Tasks of the study

1. To investigate the concentrations of heavy metals (lead and cadmium) in foods offered on the market for the period 2013-2020.
2. To assess the dietary exposure.
3. To assess the degree of potential health risk as a result of intake of heavy metals with food.
4. To develop proposals for specific recommendations to reduce the dietary intake of heavy metals.

3. Hypotheses

Based on the presented review of the available literature and the accumulated solid scientific evidence on the adverse effects of heavy metals as contaminants in food, the following hypotheses were formulated:

1. The official control and monitoring of food contaminants in Bulgaria, including heavy metals, creates conditions for ensuring the implementation of harmonized EU legislation in the field of food safety related to lead and cadmium content. As a result, a significant number of foods of animal and plant origin contain lead and cadmium within the legally established maximum permissible amounts.

2. In relation to young people, there are disorders in eating behavior, which are risk factors for the occurrence and development of nutritional imbalances. Prerequisites for nutritional imbalances are created, which support the emergence of the adverse effects caused by the chemical contaminants of food - lead and cadmium, in case of chronic dietary exposure.

3. High chronic dietary exposure to lead increases the risk of damage to human health due to the inherent chronic toxicity of lead.

III. MATERIALS AND METHODS

1. Subject of the study

The subject of the present study is the content of lead and cadmium in food and the assessment of the risk associated with the intake of these heavy metals with the food of the studied group of young people.

The target population group of the study is individuals in the age group of 19 - 29 years. This part of the population is at risk of nutritional imbalances, which may contribute to the onset of adverse effects caused by food-borne chemical hazards. Therefore, I focused on dietary exposure to lead and cadmium, which may increase health risk due to their chronic toxicity.

2. Object of the study and units of observation

To analyze and study the concentrations of lead and cadmium, data from laboratory results for metal content in food were used: 3,508 food samples of plant and animal origin, examined for lead content and 2,979 food samples of plant and animal origin, examined for cadmium content, offered on the Bulgarian market for a seven-year period from 2013 to 2020, inclusive. To assess the dietary exposure to lead and cadmium, 60 healthy individuals in the age group 19-29 years from the city of Pleven were examined.

3. Study setting

STUDY PERIOD - 2013 to 2020 and 2022.

PLACE OF THE STUDY - Department of Hygiene, Medical Ecology, Occupational Diseases, and Disaster Medicine at the Medical University - Pleven and Ambulatory for individual practice for primary medical care "Dr. Pavlinka Lzhovska - Individual practice for primary care" EOOD.

SETTING OF THE STUDY - a combined retrospective cross-sectional epidemiological study was conducted, which was implemented at the following stages:

- First stage - Research and analysis of heavy metal concentrations in food samples for lead and cadmium content, offered on the Bulgarian market for the period 2013 - 2020. The samples were analyzed in the Central Laboratory for Chemical Testing and Control of the Bulgarian Food Safety Agency the foods. Heavy metal content was measured by graphite furnace atomic absorption spectrometry (GF-AAS). The data from the laboratory analyzes were provided with an official letter from Prof. Dr. Georgi Georgiev, MD, PhD, Director of the Center for Risk Assessment in the Food Chain, Ministry of Agriculture, Food and Forestry with a letter with ex. No. C – 357/27.05.2021;
- Second stage - study of the nutrition of the studied population, in the age group 19 - 29 years, for the period June-September 2022 by means of a 24-hour dietary recall method on two non-consecutive days (48-Hour Dietary Recall Method) and by anthropometric indicators;
- Third stage – assessment of dietary exposure and assessment of the degree of potential health risk of the examined persons, as a result of intake of lead and cadmium with food, development of specific recommendations for reducing the dietary intake of heavy metals.

Selection of the study group for the second stage of the study:

FORMATION OF THE SAMPLE. It is a simple, stratified, proportional representative sample. The study included 60 individuals (30 women and 30 men) selected randomly from the patient list of a general practitioner from an outpatient clinic for individual practice for primary care in the city of Pleven.

Criteria for inclusion of study participants:
- residence in the city of Pleven;
- age 19 - 29 years;
- healthy individuals.

Criteria for exclusion of study participants:
- refusal to participate;
- residence outside Pleven and the region;
- persons who take food supplements.

95.6% of the invited persons responded to the survey.

Primary data collection and anthropometric measurements were carried out by the researcher personally. Information was collected through an active interview.

Ethical aspects of the study

The study was approved by the Ethical Commission for Scientific Research at the Medical University - Pleven. The development is supported and partially financed by a scientific project, on the topic: "Assessment of dietary exposure to heavy metals (lead and cadmium) of

young people" - Scientific project of the Medical University of Pleven №14/2022; approved by the the Ethical Commission for Scientific Research with Protocol No. 681 – KENID /03.06.2022.

4. Methods

4.1. Method of comparative analysis

The assessment of food safety in terms of the content of heavy metals - lead and cadmium was carried out using the method of comparative analysis based on the laboratory results of food samples tested for heavy metals - lead and cadmium and the maximum permissible amounts defined in European legislation. The analysis was made on the basis of Commission Regulation (EU) 2023/915 of April 25, 2023 regarding the maximum permissible amounts of certain contaminants in food and the repeal of Regulation (EU) No. 1881/2006 [Regulation (EU) 2023/915, 2023].

4.2. Method for handling left-censored data

Data on the concentration of chemical contaminants are available from laboratory analyzes of food samples. Analytical methods have certain limits of quantification (limit of quantification, LOQ). By definition, results below the LOQ cannot be quantified and are referred to as left-censored results. In practice, in laboratory protocols such results are reported as "not quantified". They show that the analytical method cannot detect the possible amount of contaminant contained in the food sample tested.

The substitution method involves three substitution scenarios:

- lower bound (Lower Bound, LB) scenario or lower bound values, where all left-censored data are replaced with a value of zero;
- middle bound (MB) scenario or mean values, where all left-censored data are replaced by a value equal to half (½) of the LOQ value;
- upper bound (UB) scenario where all left-censored data are replaced by a value equal to the LOQ value.

The guidelines recommend that the lower bound (LB) and upper bound (UB) scenarios be used for chemical contaminants that are likely to be present in food, such as naturally occurring contaminants such as lead and cadmium.

4.3. Method for collecting data on food consumption - 24-Hour Dietary Recall Method.

The method is a structured interview designed to reproduce detailed information about all the foods consumed by the respondent for the previous 24 hours, most often from midnight to midnight of the previous day. [Żukowska, M. Biziuk, 2008]. A personal study of young people's nutrition was conducted using an active interview with two 24-hour dietary intakes recorded on two non-consecutive days. A total of 60 people aged 19 to 29 were included.

4.4. Method of bioelectrical impedance analysis

Body weight in kg was measured while stepping on the scale with the help of a Tanita BCA-TBF-300M professional body analyzer [Gibney et al., 2009].

Anthropometric indixes

Body mass index (BMI) - weight in kg divided by height in m^2 is determined by a professional body analyzer Tanita - BCA–TBF-300M.

4.5. Method for assessing dietary exposure

Dietary exposure to chemical contaminants is calculated using the following formula:

$$\text{dietary exposure} = \frac{\text{consumption (kg) x concentration (mg/kg)}}{\text{body weight (kg)}}$$

Chronic dietary exposure was calculated for each participant using food consumption data from the self-administered dietary survey. The contribution (in %) of each food category to the estimated (total) dietary exposure of the chemical pollutant for the entire study group is also calculated.

4.6. Statistical method

The primary information collected was entered and processed using Microsoft Excel. Statistical processing was performed with the IBM SPSS v.25 computer package. The results are described through graphs, tables and figures. Numerical values are used - percentages, coefficients, average values, standard deviation, minimum, maximum value. The statistical methods applied include basic statistics:

A. For non-numerical indicators

- frequency distributions;
- crosstabs.

B. For the numerical indicators - input and newly calculated

- descriptive statistics with the standard indicators: mean value, standard deviation, median, sum, etc.;
- analysis of the average values of dietary exposure to lead and cadmium for each of the 60 studied participants in a new file;
- analysis of mean values with parametric and non-parametric statistics and testing of statistical hypotheses (t-test, Mann – Whitney U test);
- graphical analysis (histograms, boxplot).

Correlation, regression, and graphical analysis were used to study cause-effect relationships and present the data.

IV. RESULTS

1. Study of the concentrations of heavy metals (lead and cadmium) in foods offered on the market for the period 2013-2020.

The assessment of the content of heavy metals was carried out on the basis of laboratory studies of the content of lead and cadmium. A total of 3,508 food samples were examined for lead content and 2,979 food samples for cadmium content. The study covers the period 2013 - 2020. The studied foods are of plant and animal origin, such as: vegetables, leafy vegetables, root crops, fruits, cereal crops, legumes, pulses, nuts, oilseeds and spices, products based on cereals, fruit and vegetable juices and nectars, coffee, cocoa, tea, milk and milk products, meat and meat products, fish and seafood, etc.

For the purpose of the study and for the assessment of dietary exposure, the laboratory data on the concentration of lead and cadmium in the analyzed foods were grouped/systematized according to the food classification system FoodEx2 developed by EFSA, in the following food categories:

- Cereal crops and products based on cereals;
- Vegetables and vegetable products;
- Rhizomes and tubers with a high starch content and their products, plants for sugar production;
- Legumes, oilseeds, nuts and spices;
- Fruits and fruit products;
- Meat and meat products;
- Fish, seafood, reptiles, amphibians and invertebrates;
- Milk and milk products;
- Sugar and similar, sugar products and water-based sweet desserts;
- Animal and vegetable fats or oils and their primary derivatives;
- Fruit and vegetable juices and nectars (including concentrates);
- Coffee, cocoa, tea and potions/infusions.

I do not have laboratory data on the content of lead and cadmium in the food categories: Eggs and egg products; Alcoholic beverages; Drinking water; Soft drinks; Food for infants and young children; Products for special nutritional use; Compound foods (including frozen products) and Food additives, flavorings, adjuvants, Products for non-standard diets/nutrition and nutritional supplements, Seasonings, sauces and flavorings.

Commission Regulation (EU) 2023/915 of April 25, 2023 sets the maximum permissible amounts (mg/kg) of heavy metals in food [Regulation (EU) 2023/915]. The maximum permissible amounts for contaminants in food are set mainly for agricultural products used as raw materials for food production. Some measured amounts of heavy metals cannot be compared with the maximum permissible content because there is no regulations. For example, semi-processed meat products, meat products, dairy products, sugar and confectionery, eggs, soft drinks and many others.

For any food that has been tested for heavy metal content, it can also be determined whether it contains heavy metals in an amount that is likely to pose a risk to human health, i.e. to carry out an assessment of the health risk, even in cases where the concentrations of a chemical pollutant found in the laboratory analysis are below the maximum permissible amounts. Often, heavy metals for which dietary exposure assessment is performed, as main stage of health risk assessment, are present in the studied foods below the LOQ as traces [Żukowska, M.Biziuk, 2008].

In the study, it is found that many of the food groups showed significant variation in the LOQ of the method used. The LOQ depends on the matrix and method used, and interlaboratory variation must also be accepted. The sensitivity of the method is usually established by the laboratory to examine the samples for compliance with legal requirements in official control, although the technique is capable of under-reporting, which may lead to inaccuracy in the calculation of dietary exposure of the study population.

1.1. The content of lead in the studied foods

Table 6 presents the distribution of the relative share of the samples tested for lead content by food category in relation to the total number of samples tested.

Table 6. Relative share (in %) of samples tested for lead content by food category

Main food categories	Number of samples (n)	% of total number of samples (n)
Animal and vegetable fats and oils or oils and their primary derivatives	10	0,3
Coffee, cocoa, tea and decoctions/infusions	27	1
Fish and other seafood	272	8
Fruits and fruit products	234	7
Fruit and vegetable juices and nectars (including concentrates)	29	1
Cereal crops and cereal-based products	401	11
Legumes, oilseeds, nuts and spices	276	8
Meat and meat products	665	19
Milk and milk products	425	12
Rhizomes and tubers with high starch content and their products, plants for sugar production	303	8,7
Sugar, confectionery and water-based sweet desserts	78	2
Vegetables and vegetable products	788	22
Total number of samples (n)	3508	100

Data analysis shows **lead** concentrations (min – max value) expressed in mg/kg wet weight from 0.000011 mg/kg to 15.44 mg/kg wet weight (Table 7).

Lead content in a minimum value of 0.000011 mg/kg and within the limits of the legally determined maximum permissible amounts for the relevant food product [Regulation (EU) 2023/915] has been established in the following food categories:

- Animal and vegetable fats and oils or oils and their primary derivatives;
- Fish and other seafood;
- Fruit and vegetable juices and nectars (including concentrates);
- Rhizomes and tubers with a high starch content and their products, plants for sugar production;
- Cereal crops and products based on cereals;
- Legumes, oilseeds, nuts and spices.
- Milk and milk products.

For coffee and tea, there are no legally established maximum permissible quantities.

Measured values of lead above the maximum permissible amounts have been found in food products in the following food categories:

- Meat and meat products;
- Fruits and fruit products;
- Vegetables and vegetable products.

Table 7. Lead content in the studied food categories (min – max value) in mg/kg and established maximum permissible amounts (MPA)

Main food categories	Minimum value	Maximum value	MPA
Animal and vegetable fats and oils or oils and their primary derivatives	0,021	0,03	0,10
Coffee*, cocoa, tea* and decoctions/infusions	0,001	2, 68	-
Fish and other seafood	0,0015	0,3	0,30
Fruits and fruit products	0,000066	0,49	0,10-0,20
Fruit and vegetable* juices and nectars	0,026	0,026	0,03-0,05
Cereal crops and cereal-based products	0,000011	0,1	0,20
Legumes, oilseeds*, nuts* and spices	0,000011	0,18	0,20
Meat and meat products* (including offal)	0,0018	15,44	0,10 0,10-0,20
Milk and milk products*	0,001	0,19	0,020
Rhizomes and tubers with high starch content and their products, plants for sugar production	0,00002	0,1	0,10
Sugar, confectionery and water-based sweet desserts	0,0005	0,1	0,10
Vegetables and vegetable products (including mushrooms)	0	2,1	0,30

*No maximum permissible amount is regulated, according to Regulation 2023/915

Reported excess concentrations of lead above the maximum permissible amount were found in single food products from 2 food categories of animal origin and 2 food categories of plant origin (Fig. 5):

- Meat and meat products with a maximum measured value in:

- meat from a wild pig (boar) – 15.44 mg Pb /kg at a norm of 0.10 mg Pb /kg, which is 154 times above the MPA. These levels are significantly higher than the maximum permissible amount of lead in meat from cattle, sheep, pigs and poultry [Regulation (EU) 2023/915].

- mammalian kidney - 0.42 mg Pb /kg at a norm of 0.15 mg Pb /kg; 3 times above the MPA;

- poultry liver - 0.42 mg Pb/kg at a norm of 0.10 mg Pb/kg; 4 times above the MPA;

- 0.30 mg Pb/kg at a norm of 0.15 mg Pb/kg; 3 times above the MPA.

• Milk and milk products:
- milk - 0.19 mg Pb/kg at a norm of 0.020 mg Pb/kg; 9.5 times above MPA;
• honey - 1.06 mg/kg at a norm of 0.10 mg/kg - 10.6 times above the MPA.
• Vegetables and vegetable products:
- spinach - 2.1 mg Pb /kg at a norm of 0.30 mg Pb /kg - 7 times above the MPA;
- peppers - 0.9 mg Pb/kg at a norm of 0.050 mg Pb/kg; 18 times above the MPA;
- lettuce - 0.96 mg Pb /kg at a norm of 0.30 mg Pb /kg; 3 times above the MPA.
• Fruits and fruit products:
- apples - 0.50 mg Pb /kg at a norm of 0.10 mg Pb /kg - 5 times above the MPA.

From the category Coffee, cocoa, tea and decoctions/infusions, lead was found in herbal tea - 2.68 mg Pb /kg, but there are no regulated MPAs.

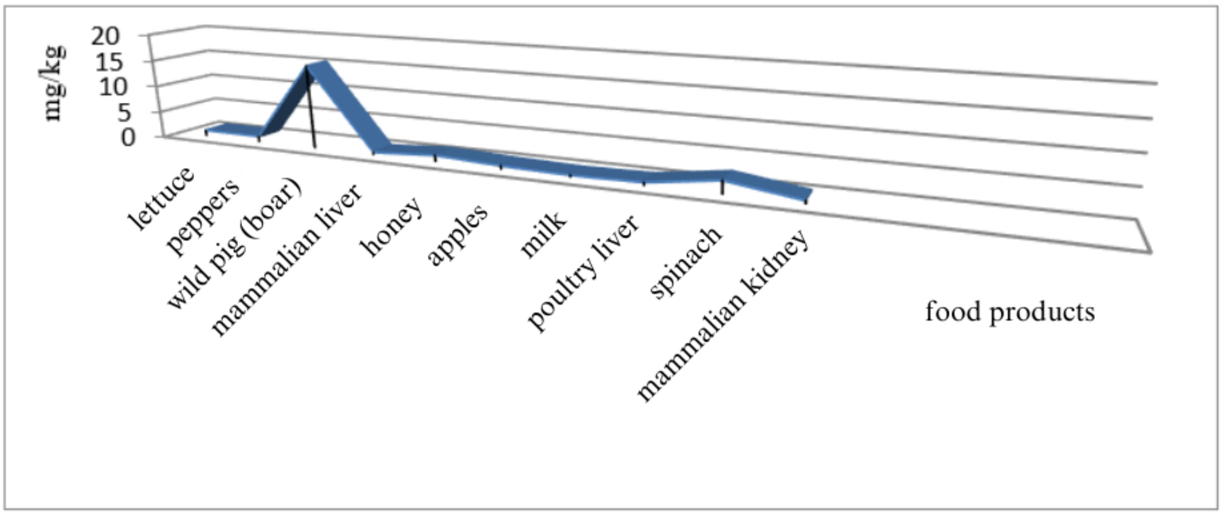

Figure 5. Food products with reported excess lead concentrations

1.2. Cadmium content in the studied foods (in mg/kg)

Table 8 presents the distribution of the relative share of samples tested for cadmium content by food category compared to the total number of samples tested.

The results show **cadmium** concentrations in the studied food samples (min – max value), expressed in mg/kg wet weight, from 0.00001 mg/kg to 0.946 mg/kg (Table 9).

Cadmium content in a minimum value of 0.00001 mg/kg and within the limits of the legally defined maximum permissible amounts - Regulation (EU) 2023/915 was found in only three categories of food, respectively:

• Cereal crops and products based on cereals;
• High starch rhizomes and tubers and their products, sugar producing plants.

Cadmium values above the maximum permissible amounts [Regulation (EU) 2023/915] were measured in the following food categories:

• Meat and meat products (including offal);
• Fish and other seafood;
• Fruits and fruit products;
• Vegetables and vegetable products.

In the food category - Meat and meat products (including offal), the maximum measured concentration of cadmium of 0.946 mg/kg of all those examined was found.

Table 8. Distribution of the relative share of samples tested for cadmium content by category (in %)

Main food categories	Number of samples (n)	% of the total number of samples
Coffee, cocoa, tea and infusions	24	1
Fish and other seafood	227	7,6
Fruits and fruit products	226	7,6
Fruit and vegetable juices and nectars (including concentrates)	8	0,3
Cereal crops and cereal-based products	363	12,2
Legumes, oilseeds, nuts and spices	253	8,5
Meat and meat products (including offal)	525	18
Milk and milk products	119	4,0
Rhizomes and tubers with high starch content and their products, plants for sugar production	320	11
Sugar, confectionery and water-based sweet desserts	75	2.5
Vegetables and vegetable products (including mushrooms)	810	27.3
Total number of samples	2979	100

According to Regulation (EU) No. 2023/915, maximum permissible amounts for cadmium are not regulated in: Coffee, cocoa tea/infusions (they are regulated in cocoa and in fresh herbs); Fruit and vegetable juices (regulated MPAs for cadmium in fruit juices for infants); Milk and milk products; nuts; Sugar, confectionery and water-based sweet desserts.

Concentrations of cadmium above the maximum permissible amounts were reported in single food products from 2 categories of food of animal origin and 2 categories of food of plant origin (Fig. 6):

- Meat and meat products:

- horse meat, with the highest measured concentration of cadmium 0.946 mg Cd /kg at the norm - 0.20 mg/kg;

- meat from wild pig (boar) 0.25 mg Cd /kg, at the norm - 0.050 mg/kg;

- Fish and other seafood:

- fish meat (salmon) 0.433 mg Cd /kg, at the norm - 0.050 mg Cd /kg;

- Fruits and fruit products:

- apples 0.44 mg Cd /kg with a norm of 0.020 mg Cd /kg;

- Vegetables and vegetable products:

- Oyster mushroom (*Pleurotus ostreatus)* 0.53 mg Cd /kg, at the norm 0.15 mg Cd /kg;
- spinach - 0.45 mg Cd /kg at a norm of 0.20 mg Cd /kg;
- lettuce 0.2 mg Cd/kg at a norm of 0.10 mg Cd/kg;
- herbs 0.45 mg Cd /kg at a norm of 0.20 mg Cd /kg in herbs;
- peppers 0.18 mg Cd /kg with a norm of 0.020 mg Cd /kg.

Table 9. Content of cadmium in the studied food categories (min – max value) in mg/kg and established maximum permissible amounts (MPA)

Main food categories	Minimum value	Maximum value	MPA
Coffee*, cocoa, tea* and infusions*	0,038	0, 57	0,60(cocoa)
Fish and other seafood	0,0004	0,433	0,050-0,25
Fruits and fruit products	0,0023	0,44	0,020-0,20
Fruit* and vegetable* juices and nectars (including concentrates)	0	0,06	-
Cereal crops and cereal-based products	0,00001	0,086	0,040-0,20
Legumes, nuts*, oilseeds	0,000024	0,633	0,10-1,20
Meat and meat products* (including offal)	0,001	0,946	0,050-0,20
Milk* and milk products*	0,00046	0,137	-
Rhizomes and tubers with high starch content and their products, plants for sugar production	0,00001	0,08	0,10
Sugar, confectionery and water-based sweet desserts *	0,0002	0,026	-
Vegetables and vegetable products (including mushrooms)	0,00001	0,53	0,020-0,15-0,50

*No maximum permissible amount is regulated, according to Regulation (EU) 2023/915

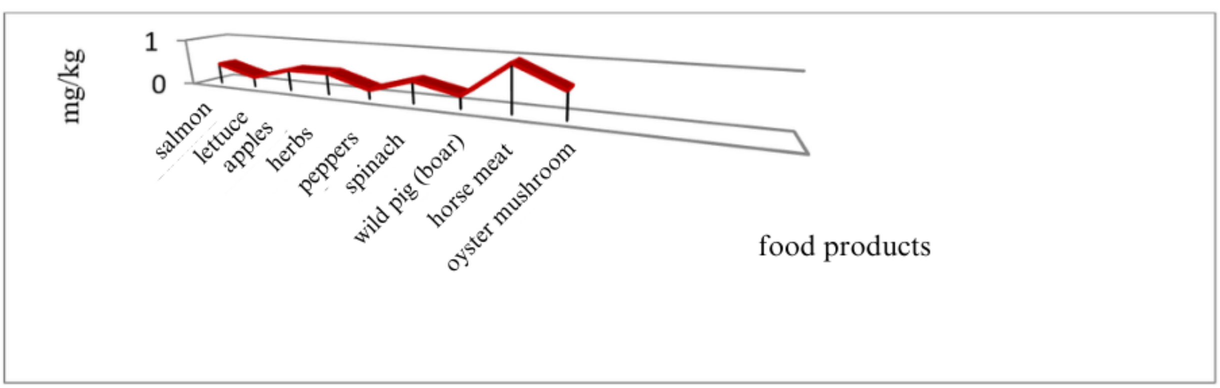

Figure 6. Food products with reported excessive cadmium concentrations

1.3. Left censored laboratory results

In my study, I found that many of the food groups showed significant variation in the LOQ of the analytical method used. The LOQ depends on the matrix and method used, and interlaboratory differences must also be accepted. The sensitivity of the method is usually established by the laboratory to examine the samples for compliance with legal requirements in the official control, although the technique is capable of reporting lower results. This leads to an increase in the number of results below the LOQ, which may lead to inaccuracy in the calculation of dietary exposure of the study population.

In this study, much of the analytical data for lead content and for cadmium content is "left-censored", i.e. the data were below the limit of quantification (LOQ) (Fig. 7). In this case, it is used the substitution method as described in the Materials and Methods section of the dissertation, with the application of the upper limit (LB) and lower limit (UB) scenarios in the assessment of exposure to lead and cadmium.

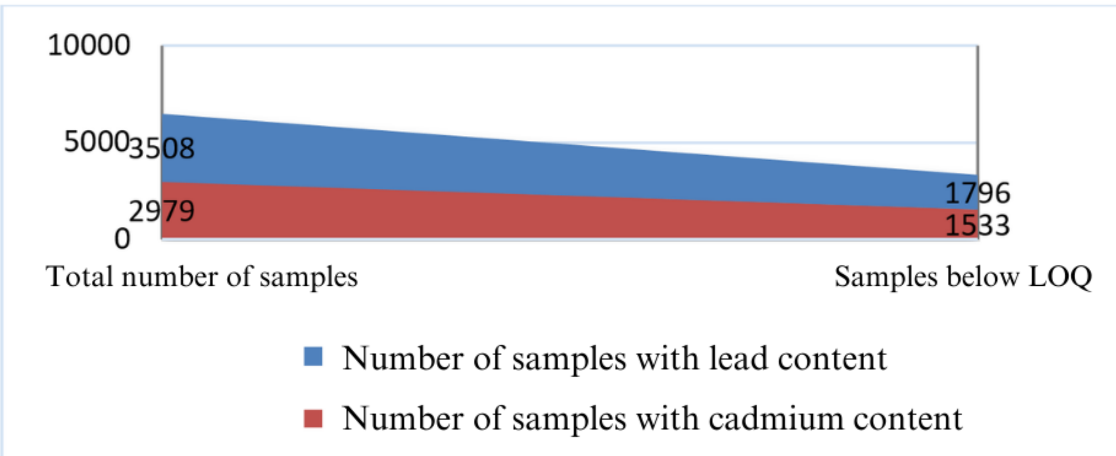

Figure 7. Number of food samples tested for lead and cadmium content, of which number of samples with a result below the LOQ

In the tested food samples for lead content, the relative proportion of samples with a result below the LOQ was 51% of the total number of tested food samples (Fig. 8). Of the 3,508 food samples tested for the presence of lead, 1,796 samples (51%) were below the limit of quantification (LOQ).

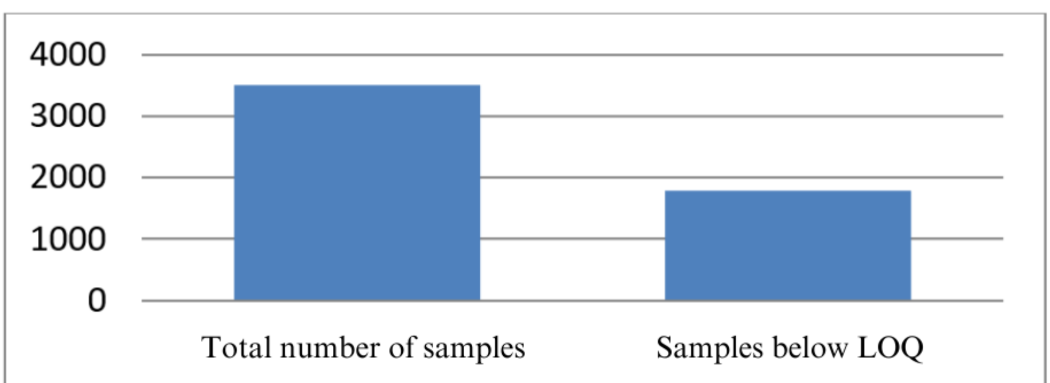

Figure 8. Relative proportion of food samples tested for lead content with a result below the limit of quantification

With the highest relative proportion of food samples tested for lead content with a result below the LOQ are the following food categories of plant origin:

- Cereal crops and cereal-based products - 90%;
- Rhizomes and tubers with high starch content and their products, plants for sugar production - 89%;
- Legumes, oilseeds, nuts and spices - legumes - nuts - 88%;
- Fruits and fruit products - 85%;
- Fruit and vegetable juices and nectars (including - 97 %.
- Vegetables and vegetable products - 72%.

As well as the food category:

- Animal and vegetable fats and oils or oils and their primary derivatives - 70%.

Significantly lower proportion of tested food samples for lead content with measured values below the LOQ was found in the food categories of animal origin:

- Meat and meat products (including offal) - 5%;
- Milk and milk products - 7%;
- Fish and other seafood - 12% (Fig. 9)

In the plant-based food categories, the relative share of samples tested for lead content with a result below the limit of quantification ranges from 90% to 85%, in animal-based foods this relative share is between 5% and 12%. For the category Sugar, confectionery and water-based sweet desserts, this share is 19%. With the lowest percentage of samples with a result below the LOQ are the food categories Meat and meat products, Milk and milk products and Fish and other seafood - in the range of 5% to 12%. The category Meat and meat products (including offal) had the fewest samples tested for lead content below the LOQ.

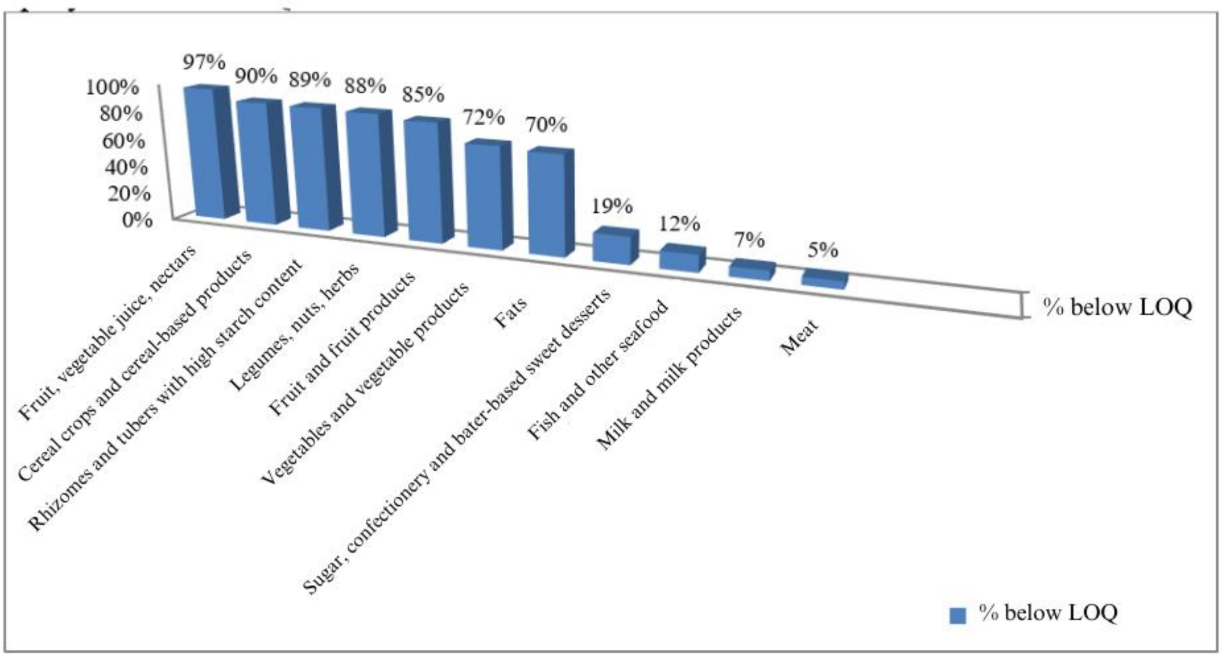

Figure 9. Distribution of the relative proportion of food samples tested for lead content below the limit of quantification (LOQ) by food category

In the present study, the relative proportion of food samples tested for cadmium content with a result below the LOQ was 52 % (Fig. 10)

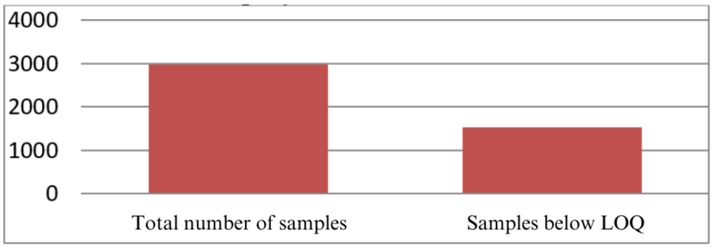

Figure 10. Relative share of food samples tested for cadmium content with a result below the limit of quantification

With the highest relative proportion of samples tested for cadmium content, with a result below the LOQ, are the following food categories of plant origin:

• Fruits and fruit products - 95%;

• Fruit and vegetable juices and nectars (including concentrates) - 88%;

• Cereal crops and cereal-based products - 76%;

• Legumes, oilseeds, nuts and spices -73%;

• Roots and tubers with high starch content and their products, plants for sugar production - 71%;

• Vegetables and vegetable products (including mushrooms) - 63%.

A significantly lower proportion of samples tested for cadmium content with measured values below the LOQ was found in the following food categories:

• Meat and meat products (including offal) - 6%;

• Fish and other seafood - 14%;

• Coffee, cocoa, tea and infusions - 17%.

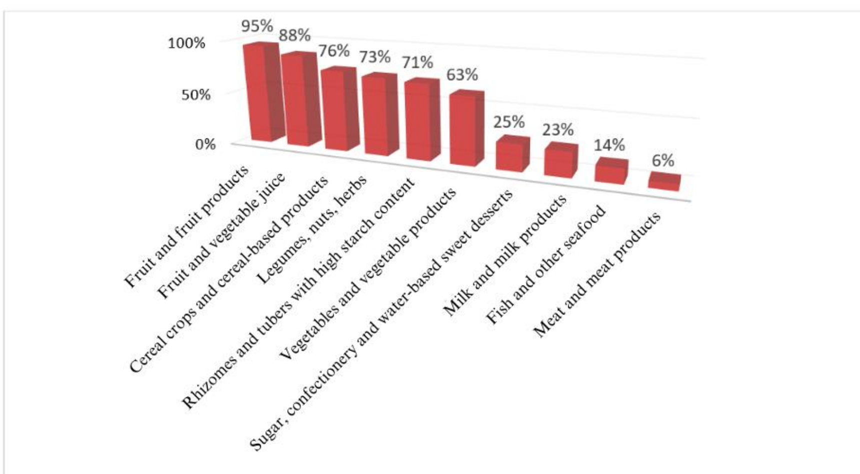

Figure 11. Distribution of the relative proportion of food samples tested for cadmium content with a result below the limit of quantification (LOQ) by food category

For plant food categories, samples scoring below the limit of quantification ranged from 63% to 95%. In the food categories Sugar, confectionery and water-based sweet desserts and

Milk and milk products are 25% and 23%, respectively, and the lowest percentage of samples with a result below the LOQ are in the food categories Fish, seafood and Meat and meat products (Fig. 11).

In order for left-censored data to be taken into account when calculating concentrations (of lead and cadmium) for the food samples studied, I used the substitution method described in Materials and methods, following the EFSA report on the treatment of left-censored data in the assessment of food exposure to chemical substances [European Food Safety Authority; Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010; 8(3):. [96 pp.]. doi:10.2903/j.efsa.2010.1557. Available online: www.efsa.europa.eu] and the recommendations given at a colloquium organized by WHO GEMS/Food-EURO in 1995 on "Reliable assessment of low levels of food contamination" [GEMS/FoodEURO, 1995]

For the calculation of dietary exposures, laboratory results for concentrations below the LOQ were included in two scenarios:

1) lower limit scenario (LB scenario) for concentration;
2) upper limit scenario (UB scenario) for concentration.

Regarding lead, I calculated the lower limit values (LB scenario) for the concentrations of lead in the food samples studied. The obtained results are shown in Table 10.

Table 10. Lower limit values for lead concentrations (LB scenario) for the studied samples of food categories (in mg/kg)

Main food categories under Foodex	LB scenario		
	Mean ±SD*	Me**	P95***
Animal and vegetable fats and oils or oils and their primary derivatives	0,007±0,012	0	0,026
Coffee, cocoa, tea and decoctions/infusions	0,165±0,528	0	0,615
Fish and other seafood	0,031±0,044	0,016	0,124
Fruits and fruit products	0,009±0,045	0	0,040
Fruit and vegetable juices and nectars (including concentrates)	0,001±0,005	0	0,000
Cereal crops and cereal-based products	0,010±0,061	0	0,061
Legumes, oilseeds, nuts and spices	0,008±0,027	0	0,080
Meat and meat products (including offal)	0,113±0,872	0,035	0,215
Milk and milk products	0,011±0,017	0,007	0,017
Rhizomes and tubers with high starch content and their products, plants for sugar production	0,004±0,015	0	0,040
Sugar, confectionery and water-based sweet desserts	0,03±0,12	0,01	0,07
Vegetables and vegetable products	0,024± 0,11	0	0,1

* Mean – lower limit value for average lead concentration
**Me - lower limit value for median lead concentration
***P95 - lower limit value for the 95th percentile of lead concentration

I also calculated the upper limit values (UB scenario) for lead concentrations in the studied food samples. The obtained results are shown in Table 11.

Table 11. Upper limit values for lead concentrations (UB scenario) for the studied samples of food categories (in mg/kg)

Main food categories under Foodex	**UB scenario**		
	Mean ±SD*	**Me****	**P95*****
Animal and vegetable fats and oils or oils and their primary derivatives	0,038±0,029	0,026	0,070
Coffee, cocoa, tea and decoctions/infusions	0,238±0,527	0,044	0,675
Fish and other seafood	0,031±0,044	0,016	0,124
Fruits and fruit products	0,042±0,054	0,031	0,120
Fruit and vegetable juices and nectars (including concentrates)	0,019±0,012	0,022	0,041
Cereal crops and cereal-based products	0,087±0,235	0,044	0,230
Legumes, oilseeds, nuts and spices	0,057±0,043	0,044	0,120
Meat and meat products (including offal)	0,114±0,872	0,035	0,215
Milk and milk products	0,011±0,017	0,008	0,021
Rhizomes and tubers with high starch content and their products, plants for sugar production	0,052±0,077	0,022	0,120
Sugar, confectionery and water-based sweet desserts	0,03±0,12	0,01	0,07
Vegetables and vegetable products	0,11± 0,40	0,04	0,266

* Mean – lower limit value for average lead concentration
**Me - lower limit value for median lead concentration
***P95 - lower limit value for the 95th percentile of the lead concentration

It is evident from the tables that the range between the lower and upper limit values (LB minimum - UB maximum) for the average lead concentration in the studied sample food categories is as follows:

- Animal and vegetable fats and oils or oils and their primary derivatives from 0.007 mg/kg to 0.038 mg/kg;
- Coffee, cocoa, tea and decoctions/infusions from 0.165 mg/kg to 0.238 mg/kg;
- Fish and other seafood from 0.031 mg/kg to 0.031 mg/kg;
- Fruits and fruit products from 0.009 mg/kg to 0.042 mg/kg;
- Fruit and vegetable juices and nectars (including concentrates) from 0.001 mg/kg to 0.019 mg/kg;

- Cereal crops and cereal-based products from 0.0010 mg/kg to 0.087 mg/kg;
- Legumes, oilseeds, nuts and spices - legumes - nuts - spices from 0.008 mg/kg to 0.057 mg/kg;
- Meat and meat products (including offal) from 0.113 mg/kg to 0.114 mg/kg;
- Milk and milk products from 0.011 mg/kg to 0.011 mg/kg;
- Rhizomes and tubers with high starch content and their products, plants for sugar production from 0.004 mg/kg to 0.052 mg/kg;
- Sugar, confectionery and water-based sweet desserts from 0.03 mg/kg to 0.03 mg/kg;

Vegetables and vegetable products from 0.024 mg/kg to 0.11 mg/kg; The range of P95 upper limit values for lead concentration (highest concentrations) varies between 0.021 mg/kg to 0.675 mg/kg.

Regarding cadmium, the approach was the same with the calculation of the lower limit values (LB scenario) for the cadmium concentrations and the upper limit values (UB scenario) for the cadmium concentrations in the studied food samples. The results obtained are shown in Table 12 and Table 13. The range of upper limit values for P95 of lead concentration (highest concentrations) varies between 0.021 mg/kg to 0.675 mg/kg.

Table 12. Lower limit values for cadmium concentrations (LB scenario) for the studied food categories (in mg/kg)

Main food categories under Foodex	LB scenario		
	Mean ±SD*	**Me****	**P95*****
Coffee, cocoa, tea and infusions	0,165±0,156	0,115	0,492
Fish and other seafood	0,010±0,031	0,004	0,032
Fruits and fruit products	0,004±0,033	0	0,002
Fruit and vegetable juices and nectars (including)	0,0075±0,02	0	0,04
Cereal crops and cereal-based products	0,003±0,009	0	0,02
Legumes, oilseeds, nuts and spices	0,016±0,010	0	0,064
Meat and meat products (including offal)	0,118±0,18	0,043	0,53
Milk and milk products	0,006±0,014	0,003	0,016
Rhizomes and tubers with high starch content and their products, plants for sugar production	0,006±0,015	0	0,04
Sugar, confectionery and water-based sweet desserts	0,002±0,004	0,001	0,005
Vegetables and vegetable products (including mushrooms)	0,02±0,05	0	0,1

* Mean – lower limit value for average cadmium concentration

**Me - lower limit value for the median concentration of cadmium

***P95 - lower limit value for the 95th percentile of the cadmium concentration

Table 13. Upper limit values of cadmium concentrations (UB scenario) for the studied food categories (in mg/kg)

Main food categories under Foodex	UB scenario		
	Mean ±SD	**Me**	**P95**
Coffee, cocoa, tea and infusions	0,306±0,286	0,220	0,800
Fish and other seafood	0,017±0,043	0,004	0,140
Fruits and fruit products	0,016±0,03	0,015	0,038
Fruit and vegetable juices and nectars (including)	0,002±0,005	0	0,02
Cereal crops and cereal-based products	0,05±0,17	0,007	0,085
Legumes, oilseeds, nuts and spices	0,30±0,164	0,02	0,09
Meat and meat products (including offal)	0,12±0,18	0,043	0,53
Milk and milk products	0,006±0,015	0,0025	0,016
Rhizomes and tubers with high starch content and their products, plants for sugar production	0,03±0,011	0,016	0,046
Sugar, confectionery and water-based sweet desserts	0,002±0,004	0,001	0,005
Vegetables and vegetable products (including mushrooms)	0,07±0,020	0,02	0,5

* Mean – lower limit value for average cadmium concentration

**Me - lower limit value for the median concentration of cadmium

***P95 - lower limit value for the 95th percentile of the cadmium concentration

As can be seen from the tables, the range between the lower and upper limit values (LB minimum - UB maximum) for the average concentration of cadmium in the studied sample food categories is as follows:

- Coffee, cocoa, tea and infusions from 0.165 mg/kg to 0.306 mg/kg;
- Fish and other seafood from 0.010 mg/kg to 0.017 mg/kg;
- Fruits and fruit products from 0.004 mg/kg to 0.016 mg/kg;
- Fruit and vegetable juices and nectars (inclusive) from 0.0075 mg/kg to 0.002 mg/kg;
- Cereals and cereal-based products from 0.003 mg/kg to 0.05 mg/kg;
- Legumes, oilseeds, nuts and spices - legumes - nuts - spices from 0.016 mg/kg to 0.30 mg/kg;
- Meat and meat products (including offal) 0.118 mg/kg to 0.12 mg/kg;
- Milk and milk products from 0.006 mg/kg to 0.006 mg/kg;
- Rhizomes and tubers with high starch content and their products, plants for sugar production from 0.006 mg/kg to 0.03 mg/kg;
- Sugar, confectionery and water-based sweet desserts from 0.002 mg/kg to 0.002 mg/kg;

- Vegetables and vegetable products (including mushrooms) from 0.02 mg/kg to 0.07 mg/kg.

The range of upper limit values for P95 of cadmium concentration (highest concentrations) varies between 0.016 mg/kg to 0.800 mg/kg.

The summary analysis of the studied concentrations of lead and cadmium in the examined foods offered on the Bulgarian market demonstrates that:

- The values of lead and cadmium in a significant number of the examined food samples are within the limits of the legally established maximum permissible amounts and this confirmed the first hypothesis;
- Excessive concentrations of both metals were found in single samples from the food categories "Meat and meat products (including offal), "Vegetables and vegetable products" (including mushrooms) and "Fruits and fruit products" - meat from mammals, apples, spinach, lettuce and peppers.
- The highest content of lead was found in boar meat samples (15.44 mg/kg) - 154 times above the MPA; peppers (0.9 mg/kg) – 18 times above the MPA; honey (1.06 mg/kg) – 10.6 times above the MPA; milk (0.19 mg/kg) – 9.5 times above the MPA; spinach (2.1 mg/kg) - 7 times above the MPA; apples (0.50 mg/kg) - 5 times above the MPA; liver (0.30 mg/kg) and kidney of mammals (0.42 mg/kg) - 3 times above the MPA; liver from poultry (0.42 mg/kg) - 4 times above the MPA; lettuce (0.96 mg/kg) - 3 times above the maximum limit.
- The highest content for cadmium was reported for apple samples (0.44 mg/kg) - 22 times above the MPA; fish meat (0.43 mg/kg) and peppers (0.18 mg mg/kg) - 9 times above the MPA; horse meat (0.946 mg/kg) and wild boar meat (0.25 mg/kg) - 5 times above the maximum permissible limit; mushrooms (0.53 mg/kg) – 3.5 times above the MPA; spinach (0.45 mg/kg), lettuce (0.2 mg/kg) and herbs (0.45 mg/kg) - 2 times above the MPA.
- The data are comparable with contemporary literature data in Europe;
- Over half of the laboratory results of the tested food samples were reported as "not detectable", i.e. with values below the limit of quantification of the method. These left-censored results for lead account for 51% of all tested food samples and for cadmium - 52%;
- The summarized data show that no significant contamination with lead and cadmium was found in the investigated foods offered on the Bulgarian market for the period 2013-2020.

2. Evaluation of the food exposure of the examined individuals

2.1. Results for the amount of food consumed by the individuals

The average consumption (g/day) of the study participants is important for calculating their dietary exposure and the average consumption is presented in Table 14:

Table 14. The average consumption (g/day) of the studied population by food category

Food category	Average consumption (g/day)
Cereal crops and cereal-based products	265.9
Vegetables and vegetable products	214.9
Rhizomes and tubers with high starch content and their products, plants for sugar production	97.6
Legumes, oilseeds, nuts and spices	12.0 8.1
Fruits and fruit products	169.5
Meat and meat products	190
Fish, seafood, reptiles, amphibians and invertebrates	28.4
Milk and milk products	155.6
Sugar, confectionery and water-based sweet desserts	5.23
Animal and vegetable fats or oils and their primary derivatives	8.9
Fruit and vegetable juices and nectars (including concentrates)	73.5
Coffee, cocoa, tea and potions/infusions	3

The number of products consumed by the studied population is 1,866.

2.2. Results of the anthropometric data of the studied individuals

The study included 60 healthy volunteers in the age group 19-29 years from the city of Pleven, with an average age of 23.8±1.9 years (Me - 24 years). No statistical differences in age were found in both sexes. The distribution of respondents by gender was: 50% (n=30) female and 50% (n=30) male.

The descriptive characteristics by age and anthropometric indicators are presented in Table 15.

Table 15. Descriptive characteristics of the studied sample (n=60)

Metrics	Minimum	Maximum	Mean value± SD	Mediana
Age	20	29	23,8±1,9	24,0
Height (cm)	155	193	172, 7 ± 9,4	172,5
Weight (kg)	47	115	68, 7±14,9	66,5
BMI (kg/m2)	16,9	32,2	22,8±3,2	22,9

Table 16 presents the anthropometric status of the examined individuals, determined on the basis of the body mass index (BMI), according to the criteria of WHO, 1995. The relative share of respondents with a normal body mass (BMI - from 18.5 to 24.9) is the highest - nearly ¾ of the studied participants (70%) are of normal weight. The prevalence of overweight and obesity prevails in 5% of the individuals examined. Respondents who are overweight are 18.3%, and those who are underweight are 6.7% - only at the expense of women.

Statistically significant gender differences were found in the distribution of anthropometric status ($\chi^2 = 9.169$; $p < 0.027$).

Table 16. Body mass index of the study participants by gender

Body weight categories (by BMI)	Men (n=30) n	%	Women (n=30) n	%	Total (n=60) n	%
Underweight < 18,5	0		4	13,3	4	6,7
Normal weight 18,5 – 24,99	19	63,3	23	76,7	42	70
Overweight ≥ 25,0	9	30	2	6,7	11	18,3
Obesity ≥ 30,0	2	6,7	1	3,3	3	5

I linked the data on food consumption with the data on gender, age and body weight (and height). The summarized results of the anthropometric status of the studied individuals show:

- Statistically significant gender differences are found in the anthropometric status of women and men.
- 70% of the participants are of normal body weight.
- Underweight was found only in women.
- Men are 3 times more overweight.
- The relative proportion of obesity was twice as high in the male as compared to the female group.
-

2.3. Dietary lead exposure of the examined individuals

The dietary lead exposure of the examined individuals under the LB and UB scenarios is listed in Table 17.

Table 17. Dietary exposure to lead of the examined individuals at Scenario LB and Scenario UB (in µg/kg bw/day)

Dietary exposure	N*	Mean value ± SD	Median	95th percentile (P95)
Dietary exposure LB	1258	0,024 ± 0,014	0,02	0,050
Dietary exposure UB		0,052 ± 0,028	0,021	0,095

N* number of food products

2.4. Dietary exposure to cadmium of the examined individuals

Dietary exposure to cadmium of the examined individuals under scenarios LB and UB is listed in Table 18.

Table 18. Dietary exposure to cadmium in LB and UB for 7 days

Dietary exposure	N*	Mean value ± SD	Median	95th percentile (P95)
Dietary exposure LB	1220	**0,21** ± 0,0165	**0,2**	**0,41**
Dietary exposure UB		**0,38 ± 0,0279**	**0,32**	**0,73**

N* number of food products

2.5. Results of estimated chronic dietary exposure to lead, distribution by gender and mean value

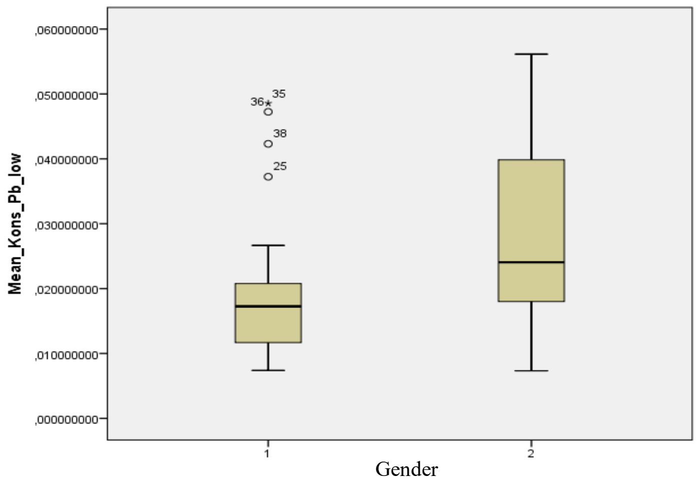

Figure 12. Mean values of dietary exposure to lead in LB in the subjects, distribution by gender (1 male, 2 female)

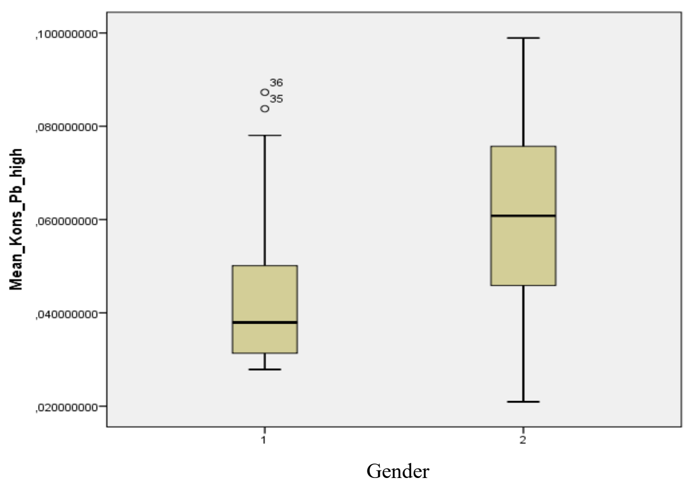

Figure 13. Mean values of dietary lead exposure at UB in both sexes (1 male, 2 female)

2.6. Results of the estimated chronic dietary exposure to cadmium - distribution by gender and mean value (Fig. 14)

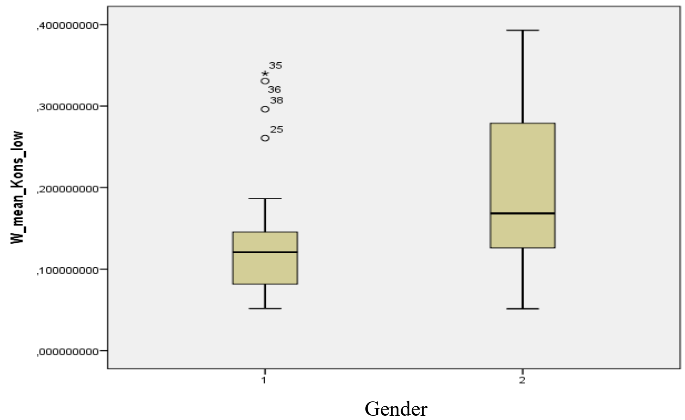

Figure 14. Dietary exposure to cadmium mean values by gender (1 male, 2 female) LB

The results show that dietary exposure to cadmium in women is higher compared to that in men (Fig. 15).

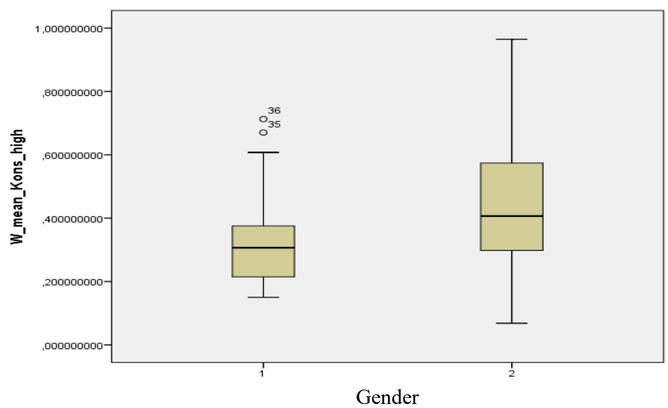

Figure 15. Dietary exposure to cadmium mean values by gender (1 male, 2 female) UB

2.7. Food categories contributing to chronic dietary lead exposure

The category Meat and meat products contributed the most to the total dietary exposure (59%) of lead, followed by the categories: Vegetables and vegetable products (15.2%), Cereal crops and cereal-based products (8%), Milk and milk products products (4.3%) and Fruit and fruit products (4.3%).

Wild pig (boar) meat, which was found to be 154.4 times higher than the reference value, did not contribute to dietary lead exposure because respondents did not report consuming game meat during the study period.

2.8. Food categories contributing to chronic dietary exposure to cadmium

The category Meat and meat products has the largest contribution to the total dietary exposure (62%) of cadmium, followed by the categories: Vegetables and vegetable products (17%), Cereals and cereal-based products (4%), Milk and milk products products (3.1%).

Although the content of Cd in the studied meat and meat products is low, their significant contribution to the dietary intake of Cd can be explained by their high consumption (190 g/day).

It is noteworthy that from the group "Legumes, oilseeds, nuts and spices" a high contribution to the dietary intake of Cd is made by nuts (5.4%), which is due to the established high average content of Cd (0.17 mg/kg) in them. Although this product is consumed in small amounts by the study population, it may significantly contribute to cadmium intake at the individual level.

Horse meat - no contribution to dietary cadmium exposure because respondents did not report consuming horse meat during the study period.

The data presented give rise to the following conclusions:

- The average value of the food exposure to lead in the studied group, calculated on the basis of the two accepted scenarios for the lower bound and upper bound of lead concentration in food, is within the limits of 0.024 µg/kg body weight. bw/day to 0.052 µg/kg bw/day; the median chronic dietary exposure to lead was between 0.02 µg/kg bw/day and 0.021 µg/kg bw/day and the 95th percentile of dietary exposure for high consumers was within 0.050 µg/kg bw/day and 0.095 µg/kg bw/day. These values are lower than the established by the European Food Safety Authority BMDL0.1 of 1.5 µg lead/kg bw/day for effects on systolic blood pressure and BMDL10 of 0.63 µg lead/kg pp./day in connection with the occurrence of chronic kidney diseases. Therefore, our second working hypothesis is not confirmed;
- The dietary exposure to cadmium in the studied group, calculated on the basis of the two accepted scenarios for the lower bound and upper bound of the concentration of cadmium in food, with an average value ranging from 0.21 µg/kg body weight. bw/week up to 0.38 µg/kg bw/week; the median chronic exposure was in the range of 0.2-0.32 µg/kg bw/week and the 95th percentile of dietary exposure for high consumers was in the range of 0.41 µg µg/kg bw/week week up to 0.73 µg/kg bw/week does not exceed the toxicological reference value. These values do not exceed the toxicological reference value established by the European Food Safety Authority of 2.5 µg/kg bw/week as a tolerable weekly intake. Therefore, our second working hypothesis is not confirmed;
- Data from the present study indicate that dietary exposure to lead and cadmium in women is higher compared to that in men. Based on this, they could be assessed as a higher risk group for chronic exposure to lead and cadmium than men.
- The food category Meat and meat products has the largest relative contribution to the total dietary exposure to lead (59%) and cadmium (62%) in the studied individuals, followed by the categories: Vegetables and vegetable products (15% for lead and 17% for cadmium) and Fruit and fruit products (2% for lead and 4% for cadmium). The significant contribution of meat and meat products to the chronic dietary exposure of lead and cadmium in the studied individuals is due to their large average daily consumption in the studied group.

3. Assessment of the degree of potential health risk as a result of the intake of the metals lead and cadmium with food

In order to assess whether dietary exposure to cadmium may pose a health risk, the estimated chronic dietary exposure levels are compared to the EFSA established Tolerable Weekly Intake (TWI) of 2.5 µg/kg bw/week, which is the amount that can be taken weekly with negligible or virtually no risk of adverse health effects in humans. [EFSA, Panel on Contaminants in the Food Chain (CONTAM), 2011]

Since any level of exposure to lead, which has been identified as a probable human carcinogen, can lead to cancer, the EFSA experts concluded that no Tolerable Daily Intake (TDI) for lead in food can be determined. In such cases, the margin of exposure (MOE) approach, which is calculated as the ratio of the BMDL to the estimated dietary exposure, can be used to assess the potential health risk. The lower limit with 295% confidence of the reference/benchmark dose is called the BMDL (lower limit of a benchmark dose). The benchmark dose (BMD) is the dose derived from animal toxicity studies at which a percentage (eg, 1%, 5%, or 10%) increase in the adverse effect is observed. EFSA experts have assessed and determined that the Margin of Exposure (MOE) is a range within which lead is likely to cause a small but measurable potential adverse effect. The BMDL is the lower limit of the MOE. The greater the ratio between the BMDL and the estimated dietary exposure, the lower the risk of an adverse health effect.

3.1. Assessment of the degree of potential health risk as a result of lead intake with food

In 2010, EFSA's Panel (Working Group) on Contaminants in the Food Chain (CONTAM Panel) assessed the health risks of lead exposure and identified cardiovascular effects and nephrotoxicity as critical health effects in adults [EFSA, 2010].

To characterize the risk of dietary exposure to lead, EFSA's CONTAM Panel recommends calculating the Margin of Exposure (MOE). The MOE is calculated by dividing the BMDL by the estimated dietary exposure. The MOE gives an indication of the level of concern for human health safety related to the presence of a toxic substance in food and is not a toxicological reference value, such as the TWI, determined for dietary intake of cadmium. The calculated value of the MOE only indicates (guideline) how to prioritize the order of taking the necessary measures to reduce the health risk.

In view of this, the CONTAM Panel determined for each of the identified critical health effects a BMDL as a reference dose for lead intake as follows:

- for effects on systolic blood pressure - BMDL0.1 of 1.50 µg/kg bw/day;

- for effects on kidney function - BMDL10 of 0.63 µg/kg bw/day [EFSA, 2012].

The average estimated chronic exposure to lead is:

- 0.024 µg/kg bw/day in scenario LB;
- 0.052 µg/kg bw/day in scenario UB.

The 95th percentile (P95) of chronic dietary exposure for lead calculated for our study population is as follows:

- 0.050 µg/kg bw/day in scenario LB
- 0.095 µg/kg bw/day in scenario UB.

Table 19 gives the estimated dietary lead exposures of the study population and BMDL0.1 for cardiovascular effects and BMDL10 for nephrotoxicity.

Table 19. Estimated chronic dietary exposure to lead for the study population (in µg/kg bw/day)

Dietary exposure to lead	Average value ± SD	Median	95th percentile	$BMDL_{0,1}$	$BMDL_{10}$
LB scenario	0,024 ± 0,014	0,02	0,05	1,5	0,63
UB scenario	0,052 ± 0,028	0,021	0,095	1,5	0,63

To characterize the risk of dietary lead exposure of the study population, I applied the MOE method using the BMDLs set by EFSA and the calculated chronic dietary lead exposures under scenario LB and scenario UB, according to the following formula:

MOE = BMDL (µg/kg bw/day) / dietary exposure (µg/kg bw/day)

The CONTAM panel determines:

- the calculated MOE value is equal to or is > 10, as the absence of a significant risk to the health of the consumer;
- the calculated MOE value is < 10 and > 1, suggests a low level of concern for human health, i.e. there is a low risk of adverse effects such as cardiovascular effects and nephrotoxicity [EFSA, Panel on Contaminants in the Food Chain (CONTAM) , Scientific Opinion on Lead in Food, 2010].

In the study of the estimated exposure margin (MOE) for mean chronic exposure to lead using the LB and UB scenarios for effects on systolic blood pressure were as follows:

MOE = BMDL0.1 (µg/kg bw/day) / exposure under scenario LB or scenario UB (µg/kg bw/day)

MOE scenario LB = 1.5 /0.024 = 62.5

MOE scenario UB = 1.5 /0.052= 28.8

The estimated MOE values for the 95th percentile of chronic lead exposure for the LB and UB scenarios for MOEs for effects on systolic blood pressure are as follows:

MOE scenario LB = 1.5 /0.050= 30

MOE scenario UB = 1.5 /0.095= 15.8

In the study of the calculated exposure margin (MOE) for effects on renal function were as follows:

MOE = BMDL10 (µg/kg bw/day) / exposure under scenario LB or scenario UB (µg/kg bw/day)

MOE scenario LB = 0.63 /0.024= 26

MOE scenario UB = 0.63 /0.052= 12

The estimated MOE values for the 95th percentile of chronic lead exposure for the LB and UB scenarios for effects on renal function are as follows:

MOE scenario LB = 0.63 /0.050 = 12.6

MOE scenario UB = 0.63 /0.095= 6.6

The estimated margin of exposure (MOE) of respondents' chronic exposure to lead suggests a low level of concern for their health, i.e. there is a low risk of the harmful effects caused by lead.

Table 20 shows the estimated margin of exposure (MOE) for the mean chronic exposure and the 95th percentile of the chronic exposure to lead for the study group, respectively, for the two scenarios (LB and UB) for the concentration of lead in the study foods. Estimated MOEs exceed 10, except for the 95th percentile of exposure under scenario UB for the critical effect nephrotoxicity.

Table 20. MOE values for chronic exposures (mean and P95) calculated for scenarios LB and UB

Adverse health effect	MOE			
	Average exposure		P95	
	LB	UB	LB	UB
Cardiovascular effects	62,5	28,8	30	15,8
Nephrotoxicity	26	12	12,6	7

3.2. Assessment of the degree of potential health risk resulting from dietary intake of cadmium.

In 2011, the European Food Safety Authority's Panel on Contaminants in the Food Chain (CONTAM Panel) confirmed that the current Tolerable Weekly Intake (TWI) of 2.5 μg/kg bw/week for cadmium is still considered appropriate and no changes are necessary to protect consumers. Tolerable weekly intake is the estimated amount of a chemical contaminant in food that can be ingested daily over a lifetime without significant risk to health [http://www.efsa.europa.eu/en/efsajournal/pub/1975.htm].

To characterize the cadmium intake risk of the study population, I compared the estimated weekly cadmium exposures under scenario LB and scenario UB to those determined by the CONTAM TWI Panel for cadmium.

Table 21 shows the dietary exposure to cadmium of the study population and the toxicological reference dose for cadmium intake - tolerable weekly intake (TWI).

Table 21. Dietary exposures to cadmium - (Mean value, Median, 95 percentile in scenarios LB and UB and TWI (in μg/kg bw/week)

Dietary exposure to cadmium	Average exposure ± SD	Median	P 95	TWI
LB scenario	0,21 ± 0,017	0,2	0,41	2,5
UB scenario	0,38 ± 0,028	0,32	0,73	2,5

With the calculated P95 values of cadmium exposure from 0.41 μg/kg/body weight/week in the LB scenario to 0.73 μg/kg/body weight/week in the UB scenario, there is no risk of adverse health effects for the subject population.

As a summary of these data, the following more important conclusions can be drawn:

- The health risk associated with dietary exposure to lead for the study group was assessed using the margin of exposure (MOE) approach. The calculated MOE values for the mean dietary exposure, as well as the median and P 95 of the dietary exposure, are higher than 10. Therefore, it can be concluded that there is no significant risk to the health of the study group associated with dietary lead intake. Therefore, our third working hypothesis is not confirmed;
- The results of the present study show that the estimated dietary exposure to cadmium does not exceed the established tolerable weekly intake of 2.5 μg/kg bw/week. Therefore, adverse health effects are not expected for the study population.

4. Proposals for specific recommendations to reduce dietary intake of lead and cadmium

Based on the obtained results, the following specific recommendations can be made in order to reduce the dietary intake of heavy metals (lead and cadmium):

1. Since lead can have health effects at any level of exposure and its presence in food cannot be completely avoided, effective measures should be taken to ensure the control of its content in food. Thanks to advances in analytical capacity, equipment is available to lower the limit of detection for lead and cadmium in analyzed foods.

2. Food producers and processors should understand the danger of food contamination with heavy metals and have as their main goal the reduction of their content in the products they produce to realistically achievable low levels. Their professional associations should promote reliable laboratory testing of food and support the access of food producers and traders to laboratories that have the necessary analytical capacity.

3. Despite the fact that lead and cadmium are present in foods in relatively low concentrations, according to modern knowledge they represent a serious threat to the health of the population, especially when chronic exposure is taken into account. Therefore, it is necessary to constantly monitor their content in the offered foods, as well as their nutritional intake (exposure) depending on the way of eating by the different age groups of the population.

4. Encouragement of organic farming.

5. Informing consumers about the national recommendations for a healthy and varied diet and their relation to the dietary exposure to heavy metals (lead and cadmium).

V. DISCUSSION

Dietary exposure is one of the ways of exposure to heavy metals, and their intake with food is different and depends on the eating pattern. Chronic exposure to heavy metals through the alimentary route can cause adverse health effects due to their bioaccumulation.

In the European Union (EU), Regulations establish maximum permissible amounts (MPA) (maximum levels) for the content of chemical pollutants that are reasonably achievable through the application of good agricultural, fishing and manufacturing practices. Regulatory frameworks (EU Regulations and Directives or national regulations) set the permissible amounts of chemical contaminants that can be present in different foods, in order to enforce and maintain food standards. Maximum permissible amounts for contaminant content are set only for individual foods/raw materials. Nevertheless, a risk assessment can be carried out for any food to determine whether the food contains contaminants in an amount that is dangerous or harmful to human health.

International food safety organizations such as the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Contaminants and Food Additives, JECFA), carry out in-depth assessments of the possible adverse effects on human health of various pollutants. In their opinions, based on the available toxicological data, these organizations establish appropriate toxicological reference values (health-based guidance values, HBGV) for the intake/exposure of chemical contaminants in food (ie for dietary exposure to contaminants), with the aim of protection against acute or chronic risk to human health.

The concepts must be distinguished:

- maximum permissible amounts of contaminants in food; and
- established toxicological reference values for intake of pollutants with food as tolerable weekly intake - TWI and tolerable daily intake - TDI).

Maximum Permissible Amounts (MPA) of contaminants in food are not safety levels. Consumption of foods containing contaminants that exceed the permitted MRLs does not always result in intake of the contaminant above toxicological reference values.

Risk assessment is the scientific part of the risk analysis process and establishes risk as a function of two components: hazard and exposure. Risk assessment assesses the likelihood of occurrence and severity of adverse health effects (damages) resulting from exposure to a hazard.

The study of the assessment of the risk of heavy metals in food is a laborious task, due to the complexity of collecting and processing the primary data characterizing the levels of contamination of food with lead and cadmium, the specific eating pattern of the studied population and the methodological approaches for assessing exposure to lead and cadmium ingested with food.

In this regard, the aim of our study was to analyze and calculate the levels of the heavy metals lead and cadmium in the food products offered on the Bulgarian market for the period 2009-2020 and to carry out a risk assessment of the population I studied, related to the intake of these metals with food. In order to achieve this goal, the tasks were formulated - research and analysis of the data from the examined food samples for lead and cadmium and the left-censored data, based on validated standardized methods; assessment of dietary exposure to lead and cadmium in a young population and health risk assessment, as well as development of recommendations to reduce dietary intake of metals.

Our study is retrospective and is based on a sufficiently long research period, a representative number of food samples offered in the Bulgarian commercial network and a representative number of respondents from the studied population to assess their food exposure.

In the present study, 3508 samples for lead content and 2979 samples for cadmium content were analyzed and processed. For the purposes of the study, I categorized the analyzed food samples into main food categories according to the hierarchical food classification system developed by EFSA of - FoodEx2 [EFSA, FoodEx 2011], which is based on 20 main food categories, further divided into subgroups at 4 separate food products levels.

The 3,508 foods tested for lead content (Fig. 16) were grouped into 12 main food categories according to the FoodEx system.

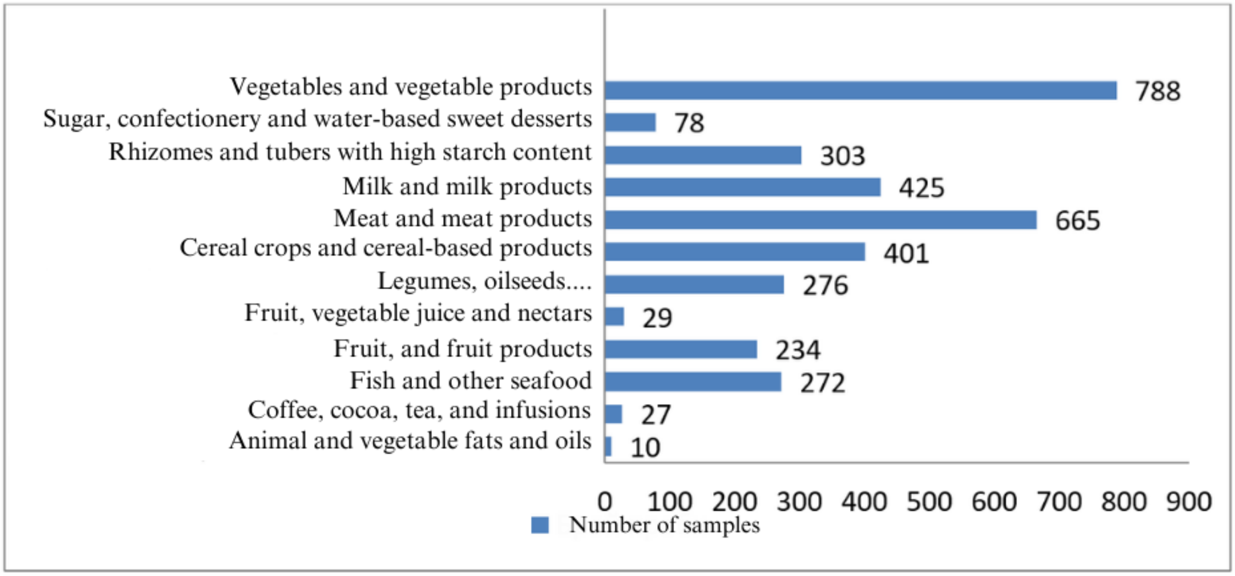

Figure 16. Number of samples tested for lead content by major food categories

In the study, 2,979 food samples tested for cadmium (Fig. 17) were systematized into 11 main food categories.

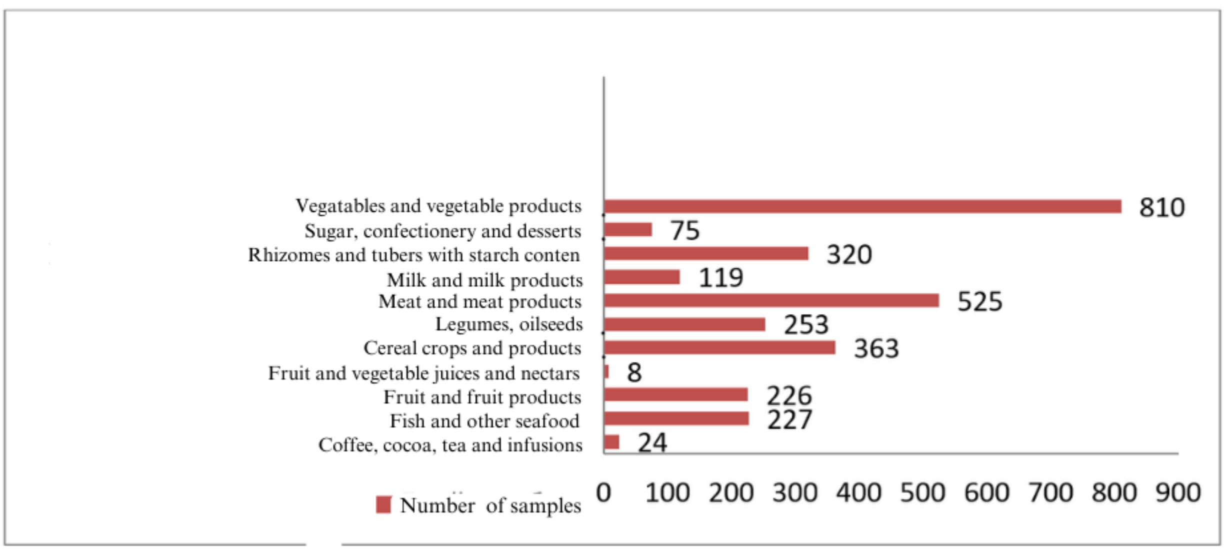

Figure 17. Number of samples tested for cadmium content by main food categories

The results of the study show that no significant contamination with lead and cadmium was found in the studied foods offered on the market in Bulgaria for the study period from 2013 to 2020 inclusive. The presence of lead and cadmium in samples of food products from all studied food categories was found within the limits of the maximum permissible amounts. Excessive concentrations of lead and cadmium were found in food products in 5 of the 12 food categories: Meat and meat products (including offal), Fish and other seafood, Milk and milk products, Fruits and fruit products and Vegetables and vegetable products. As I noted earlier, the maximum permissible amounts (MPA) for the content of lead and cadmium are set only for individual foods and they are mainly for raw materials.

Analyzing lead concentrations by food category highlighted the following:

The lead content is within the limits of the maximum permissible values for the respective foods in 8 of the 12 food categories: Animal and vegetable fats and oils or oils and their primary derivatives; Rhizomes and tubers with high starch content and their products, plants for the production of sugar; Fish and other seafood; Fruit and vegetable juices and nectars; Cereal crops and cereal-based products; Legumes, oilseeds, nuts and spices; Sugar, confectionery and water-based sweet desserts. For foods in the category Coffee, cocoa, tea and infusions, there are no regulated MPA.

Excessive lead content was found in single samples in 4 of the 12 food categories: Meat and meat products (including offal), Milk and milk products, Fruits and fruit products, Vegetables and vegetable products. In 3 of these food categories (without Milk and milk products), the cadmium content is also above the maximum permissible amounts. The highest lead content was found in wild boar (boar) meat samples, which are probably also related to the shooting of these animals with lead cartridges. The very high results for lead content in wild boar meat skew the distribution of lead concentration results in this food category. Values above the MPA have also been found in offal from mammals and birds, milk, in some leafy and fruit vegetables and pome fruits. Of the plant samples, the highest lead content was found in spinach. Values above the MPA in single samples have been found in offal of mammals and birds, milk, in some leafy and fruit vegetables, and honey.

In the foods analyzed for lead, as in the foods studied for cadmium, the samples with excessive concentrations of lead in foods of plant origin predominated and the highest concentration was also measured in the category of foods of animal origin.

My study confirmed the data from the EFSA report on the content of lead in the food of the European population [EFSA 2012]. In the case of foods of plant origin, the samples exceeding the maximum permissible amounts indicated by EFSA are - wheat grain, wheat flour and rice. In vegetables, average lead concentrations ranged from 2 μg/kg to 961 μg/kg, with a maximum concentration of 155,000 μg/kg in seaweed. A lead content of 840 μg/kg was measured in potatoes. In legumes, oilseeds, nuts and spices, average lead concentrations ranged from 6 μg/kg in chickpeas to 90 μg/kg. In fruits, the increased results are for apples, strawberries and plums. Lead content values in confectionery vary between 10 μg/kg and 406 μg/kg in chocolate. The maximum lead concentration of 2140 μg/kg was recorded in sunflower oil. In foods of animal origin, EFSA reports average concentrations between 3 μg/kg in poultry meat to 1,143 μg/kg in wild boar meat and also as in my study the maximum lead concentration of 232,000 μg/kg was recorded in wild boar meat. In other foods of animal origin - between 3 μg/kg to 244 μg/kg in an edible sea snail with a maximum concentration of 4,060 μg/kg.

In our dataset of analytical results for lead, there is a high proportion of samples with results below the LOQ (so-called left-censored data). In total, samples with results below the LOQ were 51% of all foods tested for lead, and for cadmium they were 51.5%. This average score varies between food categories and with the highest relative proportion of scores below the LOQ are 5 of the 12 food categories and these are plant-based categories. For most categories, results below the LOQ ranged from 72% to 90%, with the exception of food of animal origin categories, where they ranged from 5 to 13%.

The relative share of left-censored data for lead in the plant food categories is higher than the relative share of left-censored data in these categories for cadmium.

Figure 18 and Figure 19 present the relative share of food product samples with lead content below the LOQ, for food products of plant and animal origin, respectively. As can be seen, the relative share of left-censored data for lead in the samples of animal origin is significantly lower than that in the samples of plant origin.

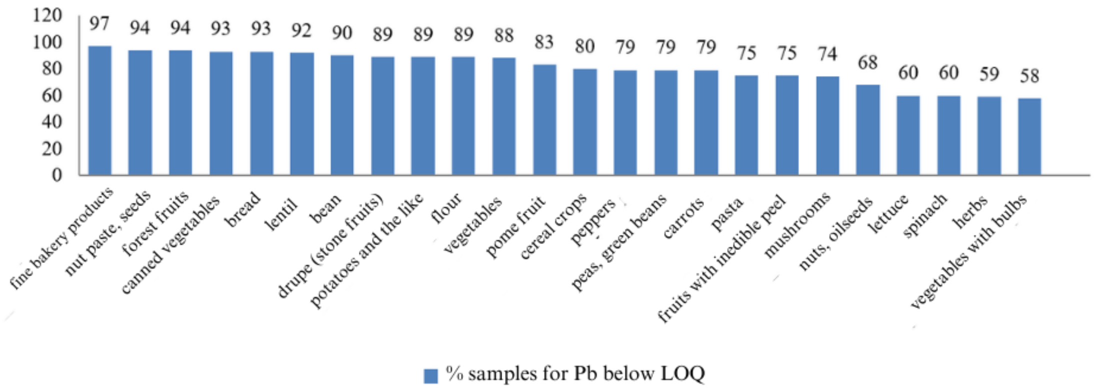

Figure 18. Food products of plant origin with results below quantification (LOQ) (in %)

In all samples citrus fruits, fruit, leafy and leguminous vegetables, the measured lead content was below the LOQ. In contrast to plant foods, the animal food categories had a low relative proportion of samples with results below the LOQ, and for the mammalian kidney and offal samples, the percentage of left-censored data was negligible (Fig. 19).

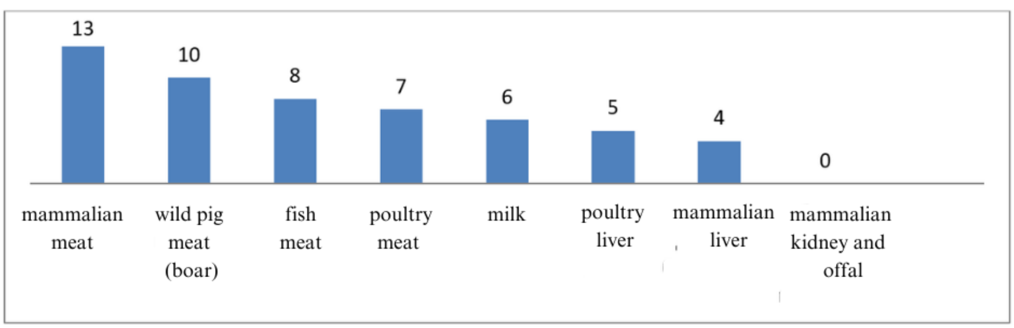

Figure 19. Results below the limit of quantification (LOQ) of food samples of animal origin tested for lead (in %)

The analysis of the data on the content of cadmium in food shows the following:

Cadmium content within the limits of the maximum permissible amounts is established in the food categories: Rhizomes and tubers with a high starch content and products thereof, plants for the production of sugar, Legumes, oilseeds, nuts and spices and Cereal crops and products based on cereals and in the food categories: Coffee, cocoa, tea and infusions (regulated MPA in cocoa); Milk and milk products; Fruit and vegetable juices and nectars; Sugar, confectionery and water-based sweet desserts, cadmium content is not regulated.

Of the 11 food categories examined, excessive concentrations of cadmium were found in single samples of 4 food categories: Meat and meat products (including offal), Fish and other seafood; Fruits and fruit products and in Vegetables and vegetable products. In Fish and other seafood, non-standard single samples and at the same time low lead content values were found. Only 3 food categories: Meat and meat products (including offal), Fruits and fruit products and Vegetables and vegetable products reported the highest cadmium and lead content. Among the investigated products of animal origin, the highest content of cadmium was found in horse meat, and among the samples of plant origin, the highest content of cadmium was found in apples. Values above the MPA in individual samples were found in horse meat, wild boar and fish, in some leafy, bulbous, fruit vegetables and mushrooms.

The analysis performed showed a greater number of samples with excessive concentrations of cadmium in the food categories of plant origin compared to those of animal origin.

The results of the study confirmed the data of EFSA for examined foods in the EU, where the samples exceeding the maximum permissible amounts of cadmium in foods of animal origin were: beef, sheep and goat meat - 0.0090 mg/kg; horse meat: 0.1715 mg/kg; beef, sheep, pig, poultry, horse liver: 0.1160 mg/kg; kidneys of cattle, sheep, pigs, poultry, horses: 0.2009 mg/kg; game liver and kidney - 0.1760 mg/kg; fish 0.0234 mg/kg; seafood 0.2152 mg/kg. For foods of plant origin, the EFSA report indicates the highest values of cadmium content in: mushrooms - 0.2087 mg/kg, celery and spinach [EFSA 2009].

In the samples tested for cadmium content, samples with results below the LOQ were 51.4% of all foods tested for cadmium. Samples with results below the LOQ range widely, with the highest relative proportion being 6 food categories of plant origin out of a total of 11 food categories, where results below the LOQ ranged from 55.6% to 100% (Figure 17) , and in the categories of foods of animal origin, they have the smallest relative share in the range from 1% to 45% (Fig. 21). Among the samples with results below the LOQ for lead, the highest relative proportions were also the food categories of plant origin. With the largest relative share of samples with results below the LOQ is the food category Fruits and fruit products from 90% to 97%, a lower relative share of samples with results below the LOQ from 25% to 45% are for samples of leafy and bulbous vegetables, mushrooms and cereal crops. The relative proportion of samples with results below the LOQ in food products of plant origin is shown in Figure 20.

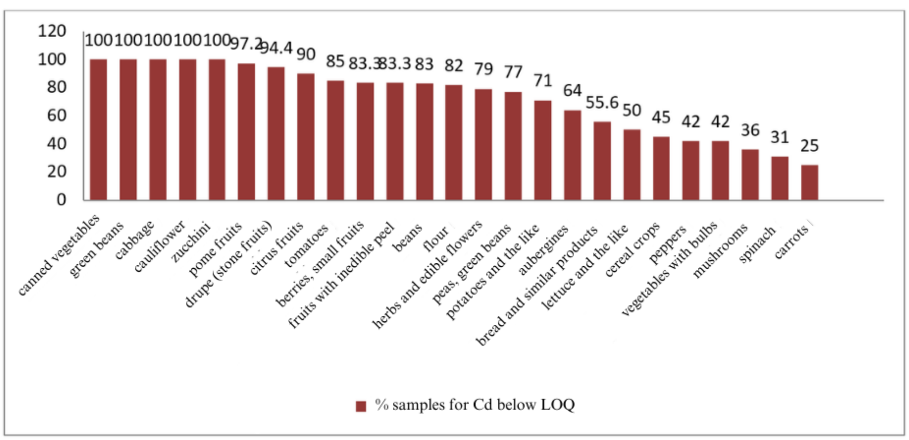

Figure 20. Results below the limit of quantification (LOQ) of plant food samples tested for cadmium

All samples of zucchini, processed or canned vegetables, legumes and leafy greens were below the limit of quantification (LOQ).

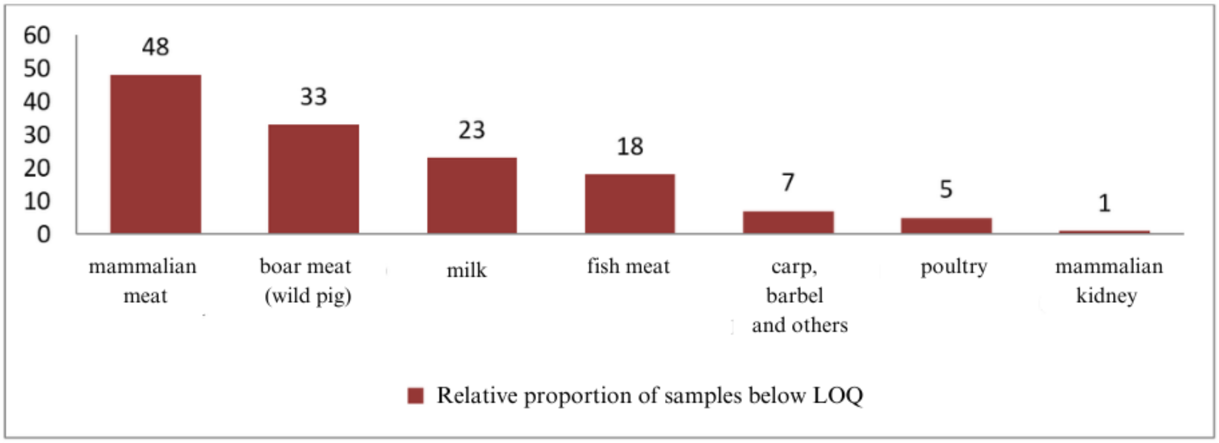

Figure 21. Food samples of animal origin tested for cadmium content with results below the limit of quantification (LOQ) (in %)

The relative proportion of samples with results below the LOQ for food products of plant origin is much higher than for food products of animal origin. Samples with results below the LOQ were almost of equal relative value for both metals, with a slight preponderance of lead content in plant foods. For fats, 2/3 of the samples for lead have results below the LOQ, while for Fish and other seafood and Milk and milk products they are respectively 7-12% - in these two categories for the content of cadmium there are no samples with results below the LOQ. In meat, the relative share for both metals is within 5-6%. There are no cadmium samples for fat in my study. The summary information of measured content of lead and cadmium below the LOQ in the food categories studied is shown in Figure 22.

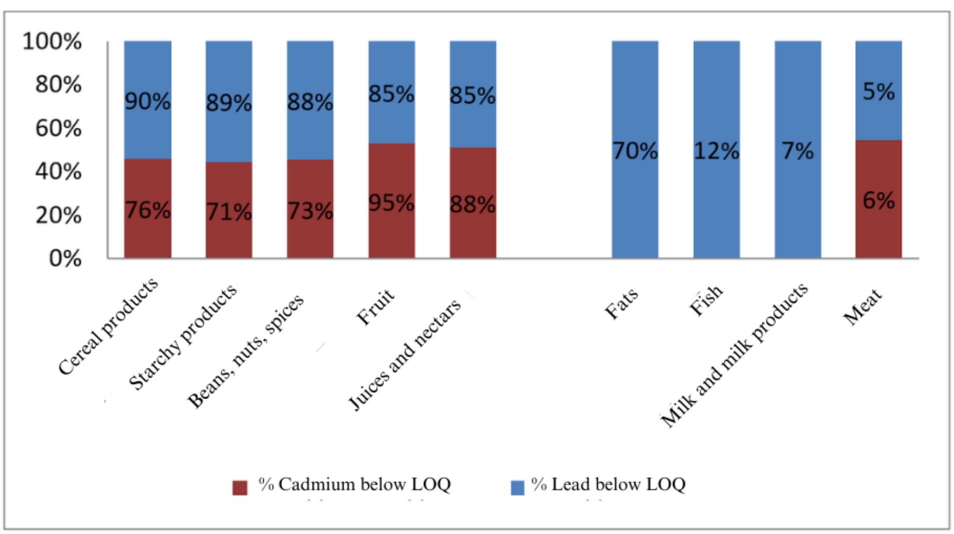

Figure 22. Samples tested for lead and cadmium content with results below the limit of quantification (LOQ) by food category (in %)

Over half of the laboratory results of the tested food samples for the content of lead and cadmium were reported as "not detected", i.e. with values below the limit of quantification (LOQ) of the method. The resulting distribution of values is left-censored, and I treated the left-censored data using the substitution method, which allowed us to use them in the exposure estimates. I combined these qualitative results according to the methodology described in Materials and methods with quantitative (numerical) data, since the reliable assessment of the analytical results of the measurements of lead and cadmium in food is a prerequisite for a realistic assessment of dietary exposure to these pollutants in food. I applied the approach of lower (LB) and upper (UB) limits on lead and cadmium concentrations. Working with left-censored data was a serious challenge. The two accepted scenarios for lower bound and upper bound of lead and cadmium concentration by food categories, determined according to the specified methodology are illustrated in Figure 23 and Figure 24.

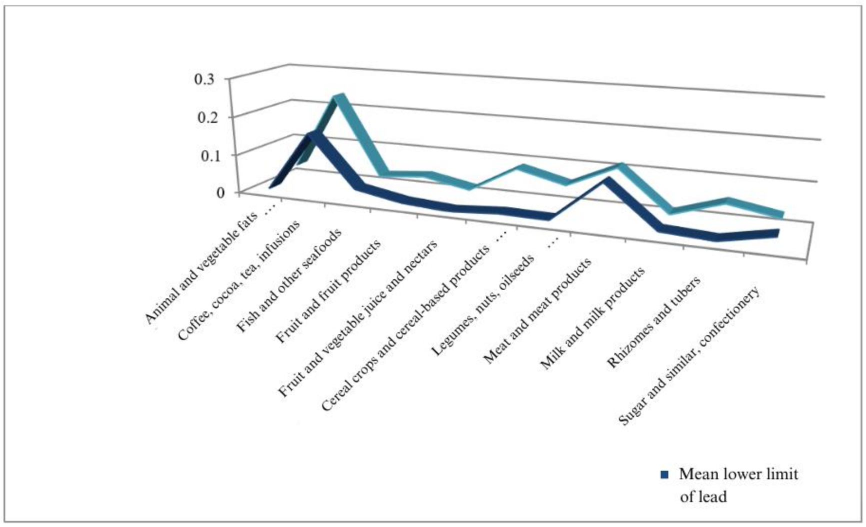

Figure 23. Lead content by food category -Lower (LB) and upper UB) limit of average lead concentration () (in mg/kg wet weight)

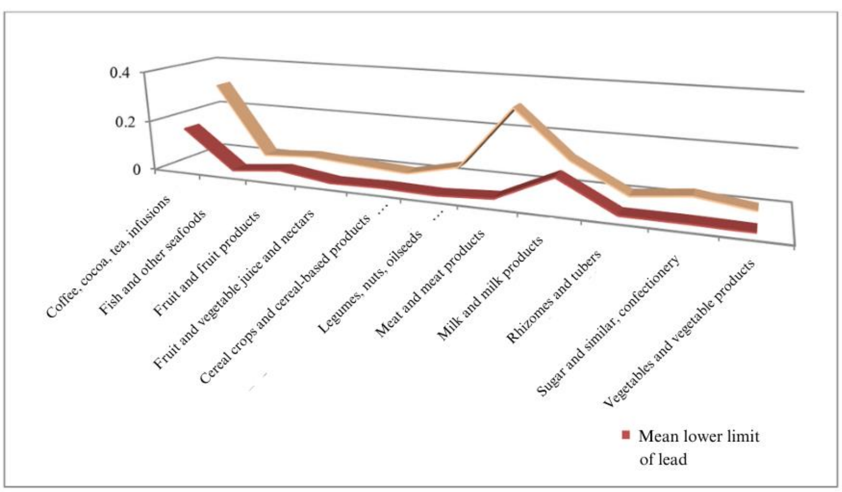

Figure 24. Cadmium content by food category - lower (LB) and upper (UB) limit of the average concentration of cadmium (in mg/kg wet weight)

For lead and cadmium, a significant number of the lower limit and upper limit values for concentration are within the limits of the legally established maximum permissible amounts.

Based on the data obtained, the next stage of our study involved calculating the dietary exposure and assessing the health risk of the study population related to the intake of these pollutants. I applied a deterministic approach with the calculation of dietary exposures at the individual level for each participant using data on the individual consumption of food items included in the daily (weekly) diet of the study population. I used a Food Diary designed to provide detailed information on all foods consumed by the respondent for one weekday and one weekend day. The methodological approach makes it possible to determine the intake of pollutants in the entire population based on the distribution of estimated individual dietary exposures, namely the mean, median and P95 of the exposure for the study group as a whole. As a result, it is possible to rank food products according to the level of their contribution to the total dietary exposure and to identify the main groups of products that are significant sources of lead and cadmium.

The determination and evaluation of the dietary intake of lead and cadmium was carried out among young people from the city of Pleven aged 19-29. However, it is known that food consumption in different periods of the year can differ, therefore the nutrition assessment was made for the period June-September 2022.

The dietary exposure of the respondents depends not only on the concentrations of lead and cadmium in the studied foods, but also on the eating pattern.

For the assessment of the chronic dietary exposure to the heavy metals lead and cadmium, the following were taken into account:

- the concentrations of lead and cadmium in foods, according to the provided laboratory results;
- the average amount of food consumed per day;

Thus, the calculated intake of the contaminant with food (i.e. the accepted dose of lead and cadmium) is compared with the corresponding toxicological reference value for cadmium - TWI, and for lead the margin of exposure (MOE) is calculated to characterize the potential risk to health of people.

The amount of heavy metals ingested with food depends to a large extent on the frequency of consumption of the food categories that contain them, as well as on the size of the consumed portion.

Some of the food products that contain excessive concentrations of lead and cadmium were not consumed or were rarely consumed by the individuals in my study - such as game and horse meat. Other food products with high consumption found in the study may contribute significantly to total dietary exposure to lead and cadmium, even if cadmium and lead are present in them at low concentration levels. Therefore, in my study, I analyzed food intake data for all food categories, and looked for high consumers who might have higher intakes of lead and cadmium due to their dietary patterns.

EFSA's short database of European food consumption [EFSA, 2008] provides the dietary patterns of the adult population in Europe and includes 16 countries. Table 22 shows the average food consumption (g/day) for Belgium and Bulgaria, presented in the report [Scientific opinion, Cadmium in food, 2009].

Table 22. Average consumption (g/day) of the studied population, in Belgium and Bulgaria according to the EFSA database for 2008.

Food categories	Amount of food consumed (gr.) Belgium	Amount of food consumed (gr.) Bulgaria	Amount of food consumed (gr.) studied population
Cereal crops and cereal-based products	245	257	265.9
Sugar and sugar products	31	40	5.23
Vegetables, nuts and pulses	230	210	
Vegetables and vegetable products	-	-	214.9
Legumes, oilseeds, nuts and spices - legumes - nuts - spices	-	-	 12.0 8.1 2.0
Starchy roots or potatoes	95	83	97,6
Fruits and fruit products	113	70	169,5
Fruit and vegetable juices, drinks and bottled water	945	207	-
Fruit and vegetable juices and nectars (including concentrates)	-	-	73,5
Coffee, tea, cocoa (presented as liquid)	432	120	3 (изразено като продукт)
Meat and meat products, offal	123	114	190
Fish and seafood	25	40	28.4
Milk and milk products	203	169	155.6

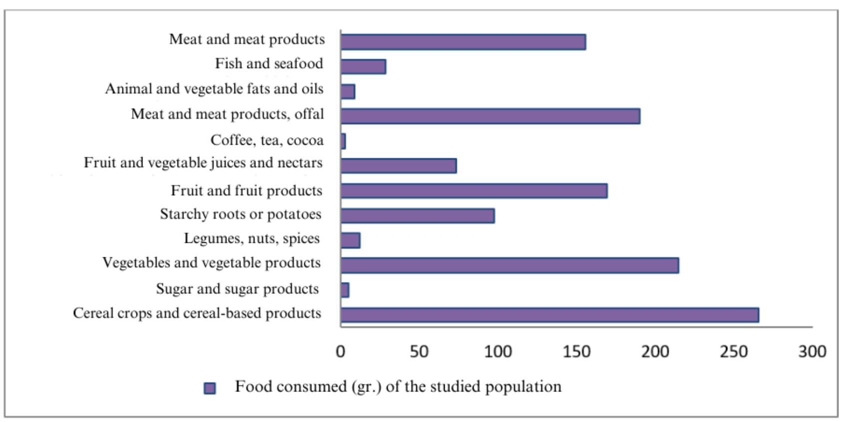

Figure 25. Food categories consumed by the studied population

A total of 1860 analytical results were processed to calculate chronic dietary exposure to lead and cadmium. For the calculation of dietary exposure to lead, 1258 food products were processed, and for the assessment of dietary exposure to cadmium - 1220 food products.

As I indicated, the exposure to cadmium and lead with food includes the objective assessment of the main variables: the content of cadmium and lead in food, the amount of food consumed by the respondents, but also the body weight of the subjects. To estimate the actual levels of heavy metal dietary intake and further risk assessment, I used a methodological approach described in Materials and Methods. Evaluating the method used, it should be noted that its indisputable advantage is the possibility of calculating individual exposure values and determining the risk categories of foods with a contribution to dietary exposure. At the same time, a limitation in the use of this approach is the complexity of the assessment of individual dietary exposure. In my research I followed all these conditions. In this regard, the assessment of the probable levels of intake of lead and cadmium with food was carried out by taking into account the combinations of the statistical parameters of the concentrations of heavy metals in food and the consumption of food products. This approach allows ranking heavy metals and food products according to their contribution to the total value of dietary exposure. The proposed approach is related to the calculation of dietary exposure based on average concentrations based on the lower (LB) and upper (UB) limit scenarios for concentration of lead and cadmium in food (mg/kg) and the average consumption of food products.

Daily dietary lead exposure

The mean dietary exposure to lead in my study ranged from 0.024 µg/kg bw/day (LB) to 0.052 µg/kg bw/day (UB), the median being between 0.02 (LB) and 0.021 µg/kg bw/day (UB), and P 95 was between 0.050 (LB) and 0.095 µg/kg bw/day (UB). According to EFSA, the dietary lead exposure of the European population varies on average between 0.40 and 0.59 µg/kg body weight per day; with a median of 0.50 µg/kg bw per day and P95 ranging between 0.65 and 0.99 µg/kg bw per day. Therefore, the mean, median, and P95 of the dietary lead exposure of my study population are much lower than those found for the European population. The maximum upper P 95 limit of dietary lead exposure in adults in the European population study was estimated at 1.16 µg/kg bw/day [EFSA Scientific report, 2012] - therefore the maximum estimated chronic exposure for lead 0.095 µg/kg bw/day (UB) of the study population is much lower than the established lead intake of the European population.

Chronic exposure results show a gender difference. The potential daily dietary exposure to lead in women is within wider ranges than the dietary exposure in men. Average chronic exposure for men is from 0.011 µg/kg bw/day to 0.02 µg/kg bw/day, and for women about 0.018 µg/kg bw/day to 0.049 µg/kg bw/day.

Daily dietary cadmium exposure

The mean dietary exposure to cadmium in my study ranged from 0.21 µg/kg bw/week (LB) to 0.38 µg/kg bw/week (UB), the median being between 0.2 (LB) and 0.32 µg/kg bw/week (UB), the 95th percentile was between 0.41 (LB) and 0.73 µg/kg bw/week (UB). In the dietary cadmium exposure study of the European population, the mean cadmium exposure ranged between 1.50 and 2.23 µg/kg bw per week, which is within the TWI of 2.5 µg/kg bw/week determined by EFSA. These data, compared with the results of their study, show that the dietary exposure to cadmium of the respondents is at significantly lower levels than the dietary exposure to cadmium of the European population [EFSA Journal, 2012] - the average value of the weekly dietary exposure to cadmium of the study population is between 7 and 6 times lower than that of the European population. The maximum estimated chronic exposure for cadmium was between 0.41(LB) and 0.733 µg/kg bw/week (UB) was between 6 and 7 times lower than the established cadmium intake in a dietary cadmium exposure study in the European population for P 95 of chronic exposure, where it varies from 2.47 to 4.81 µg/kg bw/week [EFSA Scientific report, 2012].

The results of chronic weekly exposure to cadmium also showed a gender difference. Female weekly dietary exposure to cadmium was also within wider mean ranges than male dietary exposure (which was also found for daily dietary exposure to lead, which may correspond to greater differences in body status of the studied females and males.

Contribution of the main food groups to the dietary intake (exposure) of lead and cadmium:

Before calculating the dietary exposure of the considered heavy metals, the individual food products analyzed were grouped into main food categories, according to the FoodEx 2 classification system of the European Food Safety Authority, to show their contribution to the total exposure to lead and cadmium [EFSA , FoodEx, 2011]. The average daily exposure for each category was calculated in order to calculate the contribution (in %) of each of them to the total exposure to lead and cadmium.

The total mean daily dietary exposure to lead (µg/kg bw/day) was obtained by summing the calculated mean daily exposures for each food category. The total average weekly dietary exposure to cadmium was obtained by summing the average daily exposures from all foods, then multiplying by seven. These dietary exposures were calculated based on the average body weight of 70 kg of the participants in the present study. In order to determine the contribution of individual food groups to the intake of lead and cadmium, dietary exposures calculated under the lower (LB) limit scenario for the concentration of the considered heavy metals were used, in order to reduce the effect of left-censored data from laboratory results.Also, the food consumption data of each participant in the present study were used to calculate mean consumption values (g/day) of the food categories.

The food categories with the main contribution to dietary lead exposure in the present study were: Meat and meat products (including offal) had the largest relative contribution to total dietary lead exposure (59%), followed by the categories: Vegetables and vegetable products (15.2%); Cereal crops and cereal-based products" (3.6%). The lowest level of lead intake was with Animal and vegetable fats or oils and their primary derivatives and Fruit and vegetable juices and nectars (including concentrates) (0.19%) (Fig. 26). These results differ from the conducted study of dietary lead exposure in the European population [EFSA Scientific report, 2012], which highlighted Cereal crops and cereal-based products (16.1%) as the main factor for lead exposure through the food.

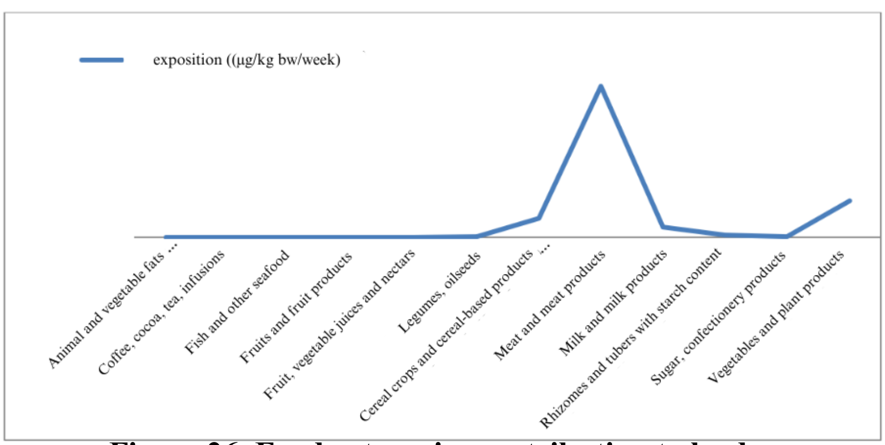

Figure 26. Food categories contributing to lead exposure

The lowest level of cadmium intake with food (% of) is from the category Fish and seafood (1.2%), and the highest (respectively 61.5%) from Meat and meat products (Fig. 27). The highest level of dietary lead intake is also from the category Meat and meat products. A study of dietary exposure to cadmium in the European population [EFSA Scientific report, 2012] highlights the main food categories – Cereal crops and cereal-based products (26.9%); vegetables and vegetable products (16.0 %) and Roots and tubers with a high starch content and their products, sugar-producing plants (13.2 %) as the main factor for dietary cadmium exposure, which does not confirm the results of this survey for the category Meat and meat products (including offal).

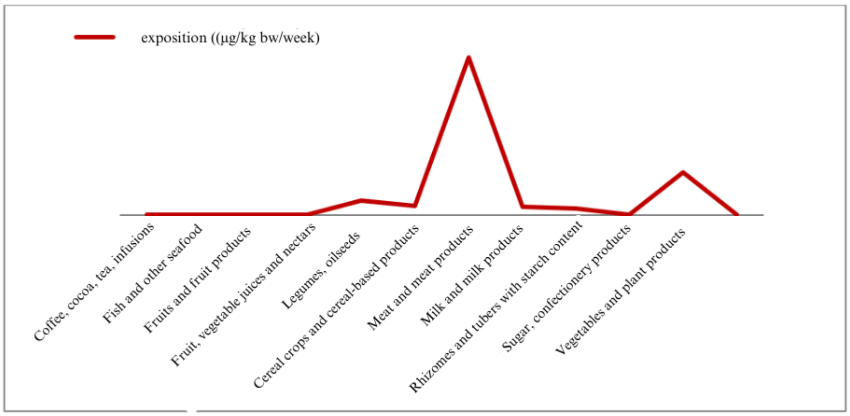

Figure 27. Food categories contributing to cadmium exposure

The characterization of the risk is the fourth stage of the risk assessment, in which the aggregated data from the previous three stages - hazard identification, hazard characterization and exposure assessment serve for a consolidated assessment of the undesirable results that may occur [Bogoeva I., etc. . 2017]. The risk characterization provides an assessment of the possible risk, its nature and the significance of the risk to health. [Dorne J.L.C.M., et al., 2013].

The characterization of the risk of occurrence of the adverse health effects of lead and cadmium taken in food is a quantitative assessment of the probability of occurrence of damage to the health of the studied population, related to the identification of hazards and the assessment of dietary exposure.

To assess the potential health risk, as a result of dietary lead exposure, I calculated an margin of exposure (MOE), which is a ratio between the BMDL (lower 95% confidence limit of the benchmark dose, in μg/kg bw/day) and the EFSA-determined and estimated chronic dietary exposures (in μg/kg bw/day) of the study population for the LB and UB scenarios to assess the level of health concern of the study group of young people.

The average value of dietary exposure to lead in the studied group, calculated on the basis of the two scenarios adopted for the lower bound and upper bound of the concentration of lead in food, the median of the chronic dietary exposure to lead and the 95 percentile (P95) of dietary exposure for high consumers are lower than the European Food Safety Authority's BMDL0.1 values of 1.5 μg lead/kg bw/day for effects on systolic blood pressure and BMDL10 of 0.63 μg lead/kg bw/day in relation to the occurrence of chronic kidney diseases.

Mean values of estimated chronic exposure to lead were between 26 and 12 times lower than the BMDL10 value and 28 times lower than the BMDL0.1 value, respectively. In the studied group, the median dietary exposure was lower than the estimated average value of the dietary exposure to lead, which was also found for the estimated average value of the dietary exposure to cadmium.

At the 95th percentile (P95) of dietary exposure for high consumers in our study population, the maximum estimated chronic exposure for scenario UB is about 6.6 times lower than the BMDL10 value for effects on renal function and about 16 times lower than of BMDL0.1 for effects on systolic blood pressure [EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on Lead in Food, 2010]. At the 95th percentile of dietary exposure for high consumers in our study population, the maximum estimated chronic dietary exposure to lead for the LB scenario was 12 times lower than the BMDL10 value and 30 times lower than the BMDL0.1 value.

Figure 28 presents the estimated chronic dietary exposure to lead under the LB and UB scenarios compared to the BMDL10 value for nephrotoxic effects.

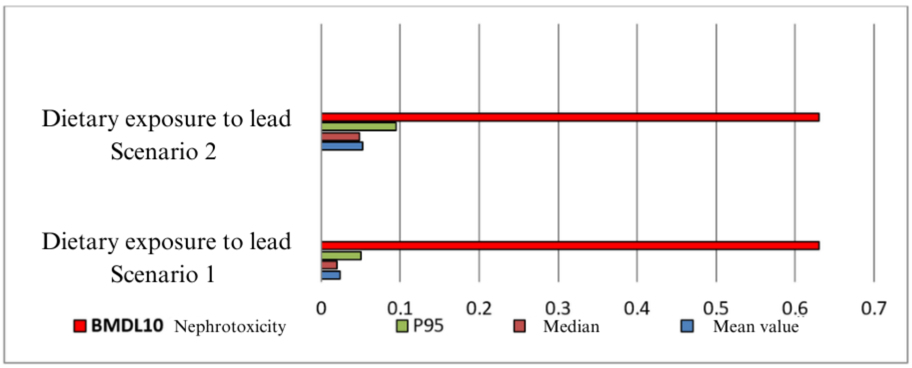

Figure 28. Dietary lead exposure for LB and UB scenarios and BMDL10 value for lead

Figure 29 presents the estimated chronic dietary lead exposure for the LB and UB scenarios compared to the BMDL0.1 value for cardiovascular disorders.

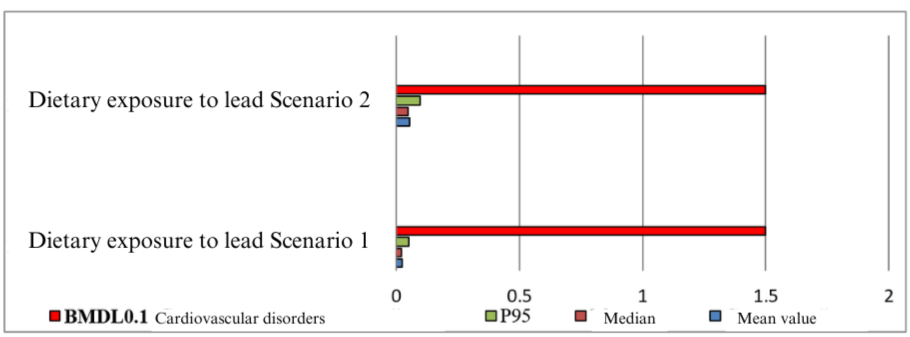

Figure 29. Dietary exposure for scenarios LB and UB and the BMDL0.1 value for lead

For genotoxic carcinogens and some carcinogenic substances, there is no established safe intake such as TWI and TDI. Therefore, for substances such as lead, a margin of exposure (MOE) is calculated [EFSA, 2009a]. The MOE is the ratio of the BMDL to the estimated chronic dietary exposure to lead. I calculated the margin of exposure (MOE) based on the obtained values of dietary lead intake.

In the present study in the study population:

- The MOE value for effects on systolic blood pressure for lead exposure is 62.7 in the LB scenario, which is a number greater than 10;
- The MOE value for effects on systolic blood pressure for lead exposure is 28.8 in the UB scenario, which is a number greater than 10.

Therefore, there is no serious risk to the health of the studied individuals for effects on systolic blood pressure.

In the present study in the study population:

- The MOE value for effects on renal function for lead exposure is 26 in the LB scenario, which is a number greater than 10;
- The MOE value for effects on renal function for lead exposure is 12 in the UB scenario, which is a number greater than 10.

Therefore, there is no serious risk to the health of the studied individuals for effects on kidney function. The potential health hazard to subjects for effects on kidney function is much less likely than the potential health hazard to respondents for effects on systolic blood pressure.

The calculated MOE values, according to the approach of the European Food Safety Authority, namely the ratio between the BMDL and the calculated chronic dietary exposure to lead, are equal to or > 10 for the two defined by EFSA - BMDL10 and BMDL0.1 and show that at the calculated dietary lead exposures do not pose a health risk to the respondents

Determining the probability of an adverse health effect occurring in the study population as a result of dietary exposure to cadmium is the final step in risk characterization. To assess the extent of the potential health risk arising from this intake, I compared the EFSA-defined tolerable weekly intake (TWI) for cadmium of 2.5 5 μg/kg bw/week and the estimated chronic dietary exposure to cadmium for the study population, for to draw a conclusion about the risk to their health. Attribution of exposure to TWI is expressed as a percentage of TWI and indicates, for a given exposure, whether the TWI is exceeded and whether there is, and to what extent, a risk to human health [Dorne J.L.C.M., et al., 2013].

In order to assess the degree of potential risk to the health of the studied individuals, I compared dietary exposure to cadmium in the LB and UB scenarios of the subjects with the tolerable weekly intake for cadmium.

Dietary exposure to cadmium in the study group, calculated on the basis of the two scenarios adopted for the lower bound and upper bound of cadmium concentration in food, as mean value, median of chronic exposure and the 95th percentile of dietary exposure for high consumers do not exceed the European Food Safety Authority's toxicological reference value of 2.5 μg cadmium/kg bw/week, called tolerable weekly intake.

In the current study:

Average dietary exposure values for cadmium are:

- 0.21 μg/kg body weight/week in scenario LB;
- 0.38 μg/kg body weight/week in scenario UB.

The mean values of the chronic dietary exposures we calculated were between 6.7 and 11 times lower than the tolerable weekly intake of 2.5 μg/kg bw/week, respectively. In the study, the median chronic dietary exposure was lower than the estimated mean chronic dietary exposure for cadmium.

The median chronic dietary exposure is:

- 0.2 µg/kg body weight/week in scenario LB;
- 0.32 µg/kg body weight/week in scenario UB.

These are between 12.5 and 8 times lower than the tolerable weekly intake, respectively.

The chronic dietary exposure to cadmium at the 95th percentile for high consumers is:

- 0.41 µg/kg body weight/week in scenario LB;
- 0.73 µg/kg body weight/week in scenario UB.

This represents from 5 times to 3 times lower than the tolerable weekly intake, respectively.

Figure 30 presents the estimated chronic dietary exposure to cadmium under the LB and UB scenarios compared to the tolerable weekly intake (TWI).

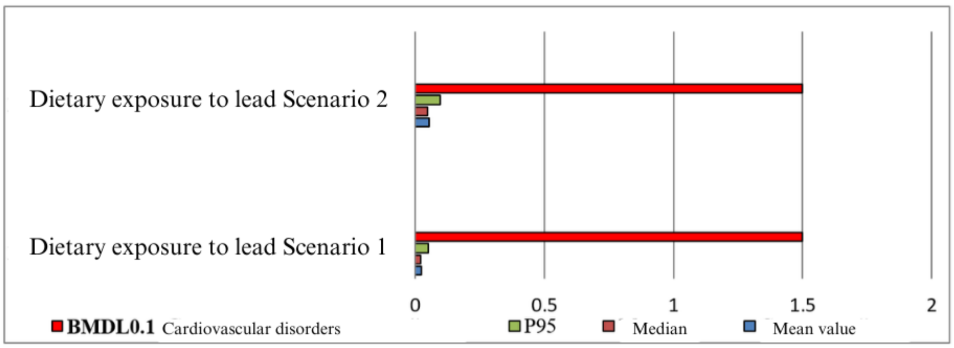

Figure 30. Chronic dietary exposure to cadmium under scenarios LB and UB compared to tolerable weekly intake (TWI)

In the current study, the study population:

- the average value of calculated chronic dietary exposure for cadmium is between 8.4% and 15% of the TWI;
- the median chronic exposure is lower than the estimated mean dietary exposure for cadmium and is between 7.8% and 12.9% of the TWI;
- dietary exposure for cadmium at the 95th percentile for high consumers is between 16.5% and 15% of the TWI.

With the calculated values of dietary exposure to cadmium in scenarios LB and UB, no adverse health effects can be expected in the studied group. The proposed recommendations for reducing the dietary intake of lead and cadmium are related to awareness and education of consumers for more frequent consumption of healthy and safe foods, promotion of organic farming, support of producers by their professional associations, regarding their responsibilities for preventive control to minimize the risks to acceptable levels.

VI. CONCLUSIONS

1. No significant contamination with lead and cadmium was found in the examined foods offered on the Bulgarian market for the period 2013-2020, since in a significant number of the examined food samples, the values of lead and cadmium are within the legally established maximum permissible amounts. Therefore, our third working hypothesis is not confirmed;
2. Excessive concentrations of both metals were found in single samples from the food categories "Meat and meat products (including offal), "Vegetables and vegetable products" (including mushrooms) and "Fruits and fruit products".
3. The highest content of lead was found in boar meat samples (15.44 mg/kg) - 154 times above the MPA; peppers (0.9 mg/kg) – 18 times above the MPA; honey (1.06 mg/kg) – 10.6 times above the MPA; milk (0.19 mg/kg) – 9.5 times above the MPA; spinach (2.1 mg/kg) - 7 times above the MPA; apples (0.50 mg/kg) - 5 times above the MPA; mammalian liver (0.30 mg/kg) and kidney (0.42 mg/kg) - 3 times above the MPA; poultry liver (0.42 mg/kg) - 4 times above the MPA; lettuce (0.96 mg/kg) - 3 times above the MPA.
4. The highest cadmium content was reported for apple samples (0.44 mg/kg) - 22 times above the MPA; fish meat (0.43 mg/kg) and peppers (0.18 mg mg/kg) - 9 times above the MPA; horse meat (0.946 mg/kg) and wild boar meat (0.25 mg/kg) - 5 times above the MPA; mushrooms (0.53 mg/kg) – 3.5 times above the MPA; spinach (0.45 mg/kg), lettuce (0.2 mg/kg) and herbs (0.45 mg/kg) - 2 times above the MPA.
5. Over half of the laboratory results of the tested food samples for the content of lead (51%) and cadmium (52%) were reported as "not detected", i.e. with values below the limit of quantification of the method.
6. The average value of the food exposure to lead in the studied group, calculated on the basis of the two accepted scenarios for the lower bound and upper bound of lead concentration in food, is within the limits of 0.024 μg/kg t .bw/day to 0.052 μg/kg bw/day; the median chronic dietary exposure to lead was between 0.02 μg/kg bw/day and 0.021 μg/kg bw/day and the 95th percentile of dietary exposure for high consumers was within 0.050 μg/kg bw/day and 0.095 μg/kg bw/day. These values are lower than those established by the European Food Safety Authority BMDL01 of 1.5 μg lead/kg bw/ day in relation to the effect on systolic blood pressure and a BMDL10 of 0.63 μg lead/kg bw/day in relation to the occurrence of chronic kidney disease.
7. Dietary exposure to cadmium in the studied group, calculated on the basis of the two accepted scenarios for the lower bound and upper bound of the concentration of cadmium in food, with an average value ranging from 0.21 μg/kg t .bw/week up to 0.38 μg/kg bw/week; the median chronic exposure was in the range of 0.2-0.32 μg/kg bw/week and the 95th percentile of dietary exposure for high consumers was in the range of 0.41 μg/kg bw/week up to 0.73 μg/kg bw/week. These values do not exceed the toxicological reference value established by the European Food Safety Authority of 2.5 μg/kg bw/week as an acceptable weekly intake.
8. Chronic dietary exposure to lead and cadmium in women of the studied age group 19-29 years is higher compared to that found in men. Based on this, they could be assessed as a higher risk group for chronic exposure to lead and cadmium than men.
9. The food category "Meat and meat products" has the largest relative contribution to the total dietary exposure of lead (59%) and cadmium (62%) in the studied

individuals, followed by the categories: "Vegetables and vegetable products" - (15%) for lead and 17% for cadmium) and "Fruits and fruit products" (2% for lead and 4% for cadmium) and Cereal crops and cereal-based products (8% for lead and 4% for cadmium). The significant contribution of meat and meat products to the chronic dietary exposure to lead and cadmium is due to their large average daily consumption in the studied group.

10. The health risk associated with dietary exposure to lead for the study group was assessed using the margin of exposure (MOE) approach. The estimated MOE values for the mean dietary exposure, as well as the median and 95th percentile of dietary exposure, are greater than 10. Therefore, the health risk associated with dietary lead intake is low for the study group
11. Chronic dietary exposure to cadmium does not exceed the established tolerable weekly intake of 2.5 μg cadmium/kg bw/week, therefore no adverse health effects are expected for the study population.

VII. RECOMMENDATIONS

Recommendations to the Ministry of Agriculture

1. The competent authorities should take effective measures to permanently control the content of lead and cadmium in foods offered on the Bulgarian market.
2. The competent authorities should carry out an assessment of the risk of exposure to lead and cadmium in food for different age groups of the population.
3. Professional associations of food producers should support producers' access to laboratories that have the competence to carry out analysis of the low detection limits of lead and cadmium in food.

Recommendations to the Ministry of Health

1. The intake of diverse and safe foods is important for the level of exposure to heavy metals, and in connection with this, activities can be planned to increase the level of awareness of the population over 19 years of age regarding the national recommendations for a healthy diet and the relationship of healthy eating with dietary exposure to heavy metals (lead and cadmium).
2. The Directorates of "Public Health" at the Regional Health Inspectorates, within the framework of their health education activities, can direct resources to increase knowledge and build children's and students' behavior towards healthy eating.

VIII. CONTRIBUTIONS

Contributions of original character

1. A complex systematic study of the content of heavy metals (lead and cadmium) in foods offered on the Bulgarian market was conducted, as well as an assessment of the health risk of the dietary intake of lead and cadmium in a young population, by applying modern methods of analysis, European norms and criteria for health risk assessment.
2. Chronic dietary exposure to lead and cadmium in a young Bulgarian population has been established.
3. The priority categories of foods, which have a significant contribution to the dietary exposure of lead and cadmium in the young Bulgarian population, have been determined.
4. No significant risk to the health of the study group related to the intake of lead with food has been identified, no adverse health effects are expected for the study population related to the intake of cadmium with food.
5. The conducted complex study on the content of heavy metals in foods offered on the Bulgarian market can be a scientific basis for developing strategies for food safety management.

Contributions of a confirmatory and applied nature

1. Values of lead and cadmium were found in a significant number of food products within the limits of the legally established amounts, comparable to the results of the European databases.
2. The food categories and the specific food products with a high content of lead and cadmium have been identified.
3. The important role of a healthy diet in the prevention of chemical hazards related to food is confirmed.
4. Recommendations for reducing the dietary intake of lead and cadmium are formulated, which reflect contemporary policies, national and international health promotion strategies.
5. The obtained results can be used to optimize the monitoring guidelines regarding official food control.

PUBLICATIONS AND SCIENTIFIC ANNOUNCEMENTS RELATED TO THE DISSERTATION

Scientific publications:

1. Average daily consumption of foods and of foods accumulating lead and cadmium in young people, Vanya Birdanova, Irena Stoilova, **Ivelina Ruseva**, Zdravka Radionova, Journal of IMAB 2023 Jan-Mar;29(1), ISSN: 1312-773X
2. Study of the content of heavy metals in foods in Bulgaria, **Ivelina R. Drambozova**, J Biomed Clin Res Volume 14 Number 2, 2021
3. Some aspects of heavy metal contamination of food, **Drambozova I.**, Birdanova V. "Preventive Medicine" magazine is referenced in "Bulgarian Medical Literature" database IX, 2021, 2(20)

Scientific announcements:

1. Study of the levels of metals in Bulgarian food products for period 2013-2019, **Ivelina Drambozova**, Vanya Birdanova, 14th Internat ional co ngress on nutrition: "A place where science meets practice" Belgrade 8-10th November 2021

Participation in forums with international participation in our country:

2. Some aspects of contamination with heavy metals in foods, **I. Drambozova**, V. Birdanova, 11th South-East European Conference of Chemotherapy, Infections and Cancer and 31st Annual Assembly of International Medical Association Bulgaria, 28–31 October 2021 Medical University – Plovdiv, Bulgaria
3. Attitudes towards healthy eating of Bulgarian users of social networks, Birdanova V., Gafurova, Georgiev, Vitkova, **Drambozova I.**, XI National Congress on Nutrition with International Participation, May 26-29, 2022.