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The relationship between the content of heavy metals Cd and Cu in some components of the environment, fish as food and human health

Elica Valkova[1*], Vasil Atanasov[1], Tatyana Vlaykova[2], Tanya Tacheva[2], Yanitsa Zhelyazkova[2], Dimo Dimov[2] and Kristian Yakimov[3]

[1]*Trakia University, Agriculture Faculty, Department of Biochemistry, Microbiology and Physics, 6000 Stara Zagora, Bulgaria*
[2]*Trakia University, Medical Faculty, Department of Chemistry and Biochemistry, 6000 Stara Zagora, Bulgaria*
[3]*Trakia University, Agriculture Faculty Faculty, 6000 Stara Zagora, Bulgaria*
[*]*Corresponding author: Elica_Valkova@abv.bg*

Abstract

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The aim of the study was to establish the relationship between the content of Cd and Cu in the air, drinking water, musculature of fish (*Cyprinus carpio* L.) and the blood serum of patients with and without COPD. The amount of PM10 has highest values in 2017 (average annual value 25.2 µg/m^3).

The results regarding the amounts of cadmium in the air of the Stara Zagora region clearly show the absence of pollution. Determination of the amounts of the studied heavy metals in drinking water and the blood serum of the patients was carried out by the method of atomic absorption. The values of Cu, registered during the year-long study into the water of Stara Zagora and Radnevo are much lower than those adopted in Bulgarian legislation norms of 2 mg/l. The highest value of Cd is characterized by the drinking water in Stara Zagora Town from January 2020 (0.0047 mg/l), the value of which almost reaches the norm of 0.005 mg/l, defined in the normative documents. The cadmium concentrations measured during the same period in the drinking water of the Radnevo City are significantly below the accepted norms.

Concentrations of copper in musculature of common carp of from our study from 2015 were significantly lower than the norms in force at that time (Regulation №31 of 2004, laying down maximum levels for certain contaminants in foods). Ovcharitsa Dam (0.60 mg/kg) is characterized by the highest values, far below the norms regulated in the then current Regulation 31 (10 mg/kg). Minimum concentration was measured in the muscles of carp inhabiting the Pastren Dam (0.27 mg/kg). Against the background of extremely low values of the element cadmium in the muscles of the studied specimens of the species *Cyprinus carpio L*. the highest is the concentration measured in the samples from Opan Dam (0.0110 mg/kg), and the lowest in the samples from Pastren Dam (0.006 mg/kg). These concentrations are much lower than the MAC specified in the then active Regulation 31, as well as in the current Regulation №5 and Regulation 1881 (EU).

Keywords: cadmium; copper; blood serum; COPD; musculature; carp; environment
Abbreviations: AAV – Annual average values; COPD – Chronic obstructive pulmonary disease; DNA – Deoxyribonucleic acid; DOAS system – differential optical absorption spectroscopy – automatic sampling and analysis, averaged every hour; EEA – Executive Environment Agency; MAC – Maximum allowable concentration; PM10 – Particulate matter 10; RHI – Regional Health Inspectorate

Introduction

Nowadays, one of the deepening, serious problems is environmental pollution and the resulting consequences for all living organisms (including humans). Environmental factors have a direct impact on metabolic processes and the reproduction of animal organisms and humans (Atanasov et al., 2017). The main vector for the movement of pollutants, poisons, heavy metals and other components of the environment in the food chain is the following: air, water, fish, human. Ingested food (in particular, the musculature of the fish) also constitutes an important factor directly affecting the metabolic reactions. Often change of this factors are accompanied by the appearance and development of disease conditions in humans as a result of serious disorders of biochemical reactions (Tzanova et al., 2017). This type of disease is chronic obstructive pulmonary disease (COPD), which is a growing problem worldwide.

Chronic Obstructive Pulmonary Disease (COPD) is characterized by chronic bronchitis (bronchoconstriction and persistent airflow limitation) and emphysema (destruction of the alveoli walls, resulting in a disturbance of their function in exhalation, presenting with dyspnea) (Singh et al., 2019).

The main components of the environment influencing the progression of human health disease include the concentration of certain heavy metals (Cd and Cu) in the air and drinking water, food intake (in particular fish's musculature), smoking and the region in which the settlement is located, especially if it is characterized by increased environmental risk (Ganeshamurthy et al., 2008).

It was found that the concentrations of cadmium and copper in the air, drinking water end food intake are closely related to serum levels of these metals in humans and development of disease (include COPD) (International Program on Chemical Safety, 2013).

Copper ions (Cu^{2+}) play an important role in living cells. In very small amounts, copper is absolutely necessary for the proper functioning of a number of enzymes in the liver, brain and muscles. This trace element enters the structure of cytochrome oxidase – terminal enzyme of the respiratory chain in mitochondria and therefore it is necessary for the processes of energy generation in the cells. It is also an integral part of the antioxidant enzymes (Valkova, 2015; Liu et al., 2018).

Copper-containing enzymes (superoxide dismutase) are important antioxidants, which prevent the production of free radicals or neutralize them (Sevcikova et al., 2011; Ighodaro & Akinloye, 2018).

The toxicity of the Cu element is directly dependent on the physicochemical properties of water (pH, alkalinity, solutes, hardness, etc.) (Di Giulio, 1995; Meyer, 2008). The concentration of free copper and cupric ions (Cu^{2+}) is increased in direct proportion to the acidity of the water. Copper hydroxide predominates in waters with pH 8 or higher values (Tao et al., 2001).

The cellular toxicity of the heavy metal copper can be explained by its involvement in the Fenton reaction (Jan et al., 2015).

The cuprous ion (Cu^{+}) can catalyze the formation of hydroxyl radicals. To induced oxidative damage under the effect of the elements copper may be added and acceleration of lipid peroxidation and DNA damage. Occurring oxidative stress leads to subsequent destruction of lung tissue, anemia, kidney and liver disease (Valkova, 2015; Liguori et al., 2018).

Cu accumulates mainly in the liver of aquatic organisms (fishes) and humans, but in the presence of very large amounts in the body can increase levels in the musculature. Fish is a common source of heavy metals because it is present in the human diet (Valkova, 2015).

Among the heavy metals with high toxicity, the element **cadmium (Cd)** stands out (Valkova et al., 2016). For this metal is characteristic to be normally present in the environment as a component of the soil, the air, sediment and even uncontaminated marine and fresh waters (Zhelyazkov et al., 2018). It occurs mainly in the form of inorganic salts (Bueno et al., 2018).

From a biochemical point of view, an increase in the activity of succinate dehydrogenase and transaminases in the blood plasma is observed with long-term intake of Cd, which is a signal of damage to cell membranes (Korotkov et al., 1996, Adugna et al., 2004).

Studies worldwide found that Cd induced oxidative stress, which leads to increased lipid peroxidation and the production of the end products of this process – the reactive oxygen species (ROS) leading to tissue damage (Kim et al., 2003, Tandon et al., 2003; Valkova et al., 2015; Branca et al., 2020).

One of the main sources of cadmium exposure in humans is tobacco smoke. Because the absorption of Cd by the lungs is much higher than by the gastrointestinal tract, smoking contributes significantly to the overall workload of the body (Jarup, 2003; Jin et al., 2004; Figureoa, 2008; Ming-Ho, 2008).

In non-smokers, the main reason for cadmium exposure is food. Musculature of common carp adopted in nutrition is often a good source of cadmium in humans (Valkova et al., 2016). Chronic exposure to cadmium leads to renal glomerular and tubular damage, disorders of calcium and bone metabolism, malignancies and cardiovascular and respiratory diseases (International Program on Chemical Safety, 2013; Satarug et al., 2017).

The intake of the studied metals copper and cadmium can also be achieved by entering the drinking water and food in the form of ions.

In this reason the aim of the study was to establish the relationship between the content of Cd and Cu in the air, drinking water, musculature of fish (*Cyprinus carpio* L.) and the blood serum of patients with and without COPD.

Material and Methods

Data are required from the archives of the Executive Environment Agency – Sofia regarding the content in the air of the Stara Zagora Region of FPM10 and the heavy metal Cd for the period 2014 – 2019.

For the purpose of this study sampling, archiving and storage of samples of drinking water from the towns of Stara Zagora and Radnevo for 12 months (June 2019 – May 2020) were carried out, to monitor the concentrations of the investigated heavy metals Cd and Cu. For storage purposes, drinking water samples were treated with 5 cm^3 to HNO_3.

In the survey conducted by us in 2015 control catches were made in the dams Ovcharitsa, Opan and Pastren, located on the territory of Stara Zagora Region. The caught carp specimens (*Cyprinus carpio* L.) were transported in ice bags. Storage was performed at – 18°C. Prior to mineralization, the samples were thawed at room temperature and washed with deionized water. Medium samples were taken from carp muscles.

For the purposes of this study, patients with chronic obstructive pulmonary disease (COPD) were tested, who are residents of the cities of Stara Zagora and Radnevo and are suitable for inclusion in the study and the control group of patients without the presence of this disease. The patients were recruited in the Clinic of Internal Medicine, University Hospital, Trakia University, Stara Zagora, Bulgaria. The work was approved by the Ethic committee of Medical Faculty, Trakia University, Stara Zagora, Bulgaria. Informed consents were obtained from patients and controls before the study.

To determine the content of the tested heavy metals, samples carp muscles and blood serum samples were prepared by wet burning in a microwave oven Perkin Elmer Multiwave 3000 in a mixture of 1 cm^3 conc. HCl and 6 cm^3 conc. HNO_3.

The content of the heavy elements Cd and Cu in the resulting acid solutions and water samples was determined by atomic absorption spectrometer (AAS) "A Analyst 800" Perkin Elmer on a cuvette and flame system by using acetylene-oxygen combustion

Statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc.).

Results and Discussion

Human, as a last unit of the nutritional chain is often exposed to the toxicants of different nature such as pesticides and their metabolites, chlorine compounds, heavy metals and others. The bioaccumulation of heavy metals in the animal and human body is a long-term process and has an impact on health after a few years.

The content of heavy metals in the air and ingested food and water is an essential factor that triggers the development of diseases of various kinds, including diseases of the respiratory system, such as COPD. In this regard, tracking levels of heavy metals in these sources is essential due to the impact on the occurrence and development of this disease.

Content of PM10 in the air of the Stara Zagora region in the period 2014 – 2019

The presence of high concentrations of fine dust particles in the inhaled air significantly complicates breathing due to their adhesion to the alveoli of the lungs. Heavy metals such as cadmium, lead, nickel, etc., present in the air contained in a large percentage precisely in fine powder (Thomas, 2013). Due to this fact, the study of the content of PM10 in the air of the Stara Zagora region is necessary, especially since the region is risky in terms of environmental pollution.

With a view to take into account this important component of the air was requested and provided by the EEA – Sofia data on the average daily values for PM10 (PM10) from DOAS – Razhena Village for the period from 01.01.2014 to 31.12. 2019. The received information is processed and presented in Figure 1.

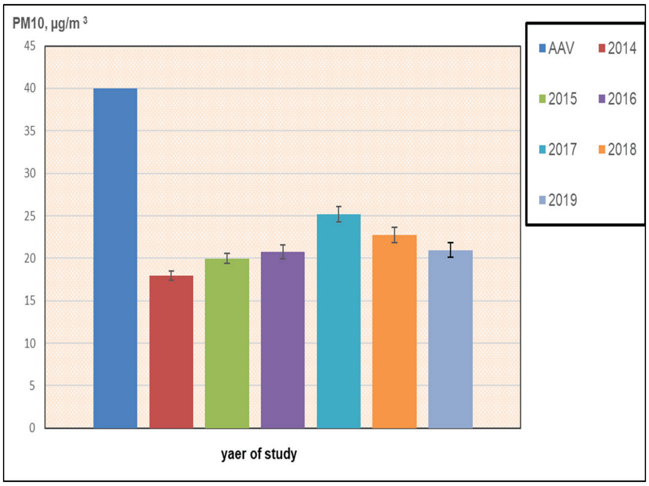

Fig. 1. Content of PM10 in the air of the Stara Zagora region in the period 2014 – 2019

According to Regulation №12/15.07.2010 of the Bulgarian legislation, determining the norms for sulfur dioxide, nitrogen dioxide, fine dust particles, lead, benzene, carbon monoxide and ozone in the atmospheric air, the average annual norm (AAN) for protection of human health for one calendar year for PM10 is 40 μg/m^3. According to the data presented in Figure 1 with the highest values, this indicator is characterized in 2017 (average annual value 25.2 μ/m^3). All reported average annual values of PM10 are below the SGN, defined in the legislation, and are almost twice lower than the regulated concentration.

These data, as well as those obtained from the EEA – Sofia show that despite the relatively low average annual levels has not yet achieved qualitative and sustainable reduction of air pollution from this pollutant in the area controlled by RIEW – Stara Zagora. This to some extent determines the increase of some heavy metals such as Cd, Pb, Ni and others in the atmospheric air of the region during this period.

In a special report of the European Court of Auditors from 2018 in 2016 Bulgaria is one of the countries in Europe where there is a deviation from the norms of the EU (European Union) regarding the number of exceedances of PM10 concentrations.

(https://www.eca.europa.eu/Lists/ECADocuments/SR18_23/SR_AIR_QUALITY_BG). pdf).

The statistical analysis of the data for content of PM10 in the air of the region of Stara Zagora in the period 2014 – 2019 is an indication of good reliability of the obtained results ($p < 0.05$).

Content of Cd in the air of the Stara Zagora region in the period 2014 – 2019

The high content of heavy metals in the inhaled air is a good reason for their accumulation in the walls of the alveoli and their entry into the blood. This often leads to diseases of the lung tissue such as COPD and others.

Therefore, data were requested and provided by the EEA – Sofia on the average annual values of the more toxic heavy metal cadmium from DOAS – Razhena for the period from 01.01.2014 to 31.12.2019, due to lack of such for the element copper in the air during this period. The data on cadmium concentrations in the air of Stara Zagora in the period are presented in Figure 2.

The MAC regulated in Regulation № 11 of 14 May 2007 on standards for arsenic, cadmium, nickel and polycyclic aromatic hydrocarbons in ambient air of 5 ng/m^3 was not reached during the study period. But as mentioned above in a special report of the European Court of Auditors from 2018 in 2016 is stated that Bulgaria is one of the countries in Europe where there is a deviation from the norms of the EU (European Union) on the number of exceedances of concentrations of heavy metals and fine dust particles.

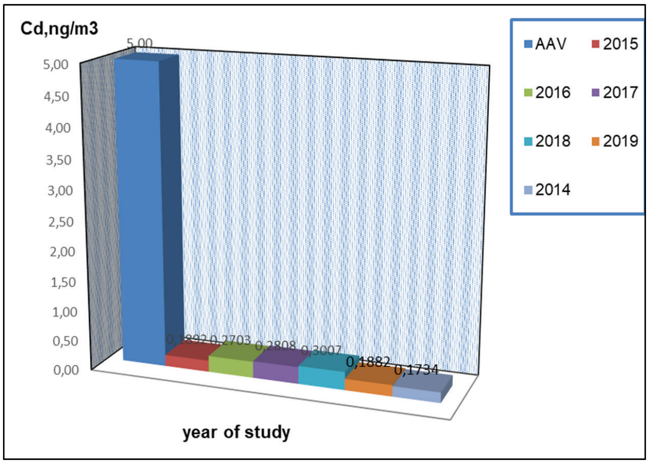

Fig. 2. Content of Cd in the air of the Stara Zagora region in the period 2014 – 2019

(https://www.eca.europa.eu/Lists/ECADocuments/SR18_23/SR_AIR_QUALITY_BG.pdf).

The data on the content of Cd in the air of the Stara Zagora region in the period 2014 – 2019 are characterized by good statistical reliability ($p < 0.05$).

Content of heavy metals Cu and Cd in the drinking water of the cities of Radnevo and Stara Zagora in the period June 2019 – May 2020

The content of heavy metals in drinking water is essential for the state of the immune system and the development of chronic lung diseases such as COPD. Therefore, it was necessary to monitor the levels of some heavy metals (Cd and Cu), both in the air and in the water taken by the patients.

The quantities of copper, established in period of one year in the drinking water of cities Radnevo and Stara Zagora are presented in Figure 3.

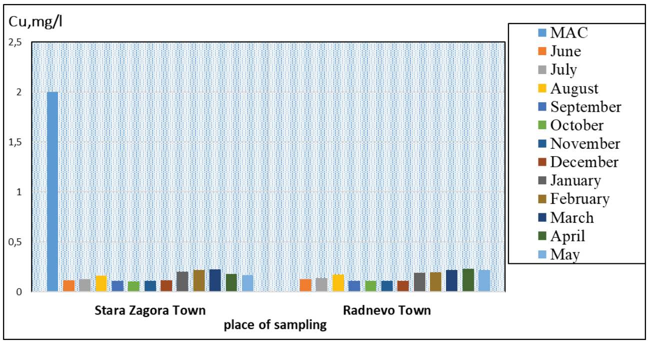

Fig. 3. Content of Cu in the drinking water of the cities of Radnevo and Stara Zagora in the period June 2019 – May 2020

According to Regulation №9 of 16.03.2001 from the Bulgarian legislation the normative requirement for Cu in drinking water is 2 mg/l. The values of this element registered in the water of the two studied cities are much lower than the accepted norms.

In the Report on the activity of RHI – Stara Zagora for 2017 (https://www.rzi-starazagora.org/images/otcheti/Godishen_otchet_RZI-St.Zagora_2017.pdf) it is stated that deviations of the studied metals Cu and Cd on an annual scale are observed in only 10% of the samples drinking water taken, as they do not exceed the MPC by more than 5%. In response to our letter from the RHI – Stara Zagora regarding the values of the studied heavy metals (including Cu), it was stated that there are no deviations from the accepted norms for drinking water in the legislative system.

The levels of cadmium measured in a period of one year in the drinking water of cities Radnevo and Stara Zagora are presented in Figure 4.

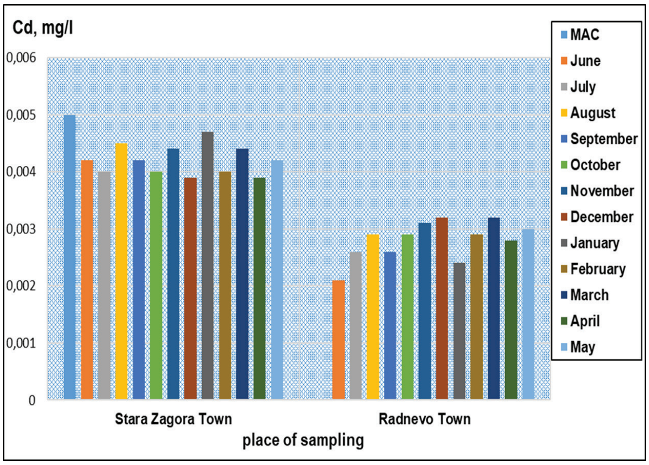

Fig. 4. Content of Cd in drinking water of cities and Radnevo Stara Zagora in June 2019 – May 2020

As is apparent from Figure 4, the levels of cadmium in the drinking water of the studied cities are significantly higher than the reported amounts of copper in the samples. The concentrations of Cd, registered in the reported period in the water used for domestic and drinking needs in the town of Stara Zagora often approach the normative requirements, determined by Regulation №9 of 16.03.2001. The highest value of this metal is characterized by drinking water taken in January 2020 (0.0047 mg/l). With higher values differ and water sampled in August and November 2019 and March 2020 in both studied cities.

According to the report on the activities of RHI – Stara Zagora for 2017 (https://www.rzi-starazagora.org/images/otcheti/Godishen_otchet_RZI-St.Zagora_2017.pdf) and the required data on the content of these metals in the drinking water of the cities of Radnevo and Stara Zagora for the period June 2019 – May 2020. There are no deviations from the adopted regulatory requirements of Ordinance №9, regulating the MAC for Cd in drinking water of 0.005 mg/l. But in our research in the period June 2019 – May 2020 approaching the standards for cadmium is observed at least five times a year, suggesting some risk of excess the regulatory requirements and the risk of passing larger amounts of cadmium from drinking water in the body of patients studied.

Analysis of the data content of Cd and Cu in the drinking water of cities Radnevo and Stara Zagora during the period June 2019 – May 2020 indicates reliability of the results ($p < 0.05$).

Content of heavy metals Cu and Cd in the musculature of carp (Cyprinus carpio* L.*) in study from 2015

The trace element copper above certain levels is toxic to both fish and humans as a consumer. As can be seen from Figure 5 the content of copper in the samples from 2015 is much lower than the MAC regulated in the then current Regulation 31 on the content of this metal in the musculature of fish intended for consumption. Whit the highest values far below the specified in the then active Regulation 31 norms characterized Ovcharitsa Dam (0.603 mg/kg). Minimum concentration was measured in the musculature of common carp inhabiting the Pastren Dam (0.272 mg/kg).

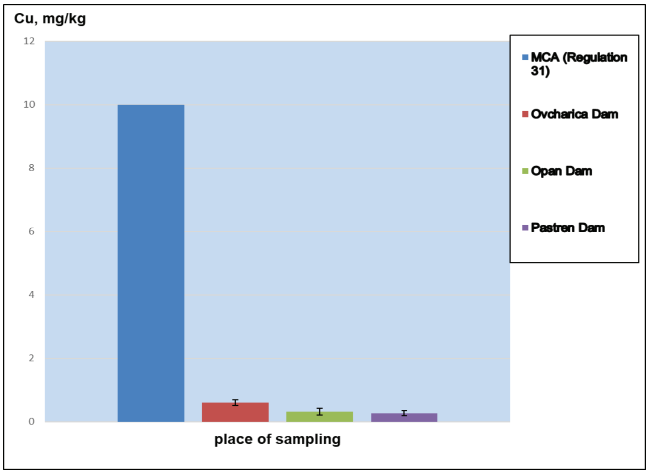

Fig. 5. Content of heavy metal Cu in the musculature of carp (*Cyprinus carpio* L.) in study from 2015 (Valkova, 2015)

The data from our 2015 study unequivocally show that the element copper accumulates mainly in the liver of hydrobionts (De Boeck et al., 2010), and that this organ, being a “universal biochemical laboratory”, retains some heavy metals and prevents their accumulation. in the musculature of the carp we studied (Khoshnood et al., 2010).

The metal **cadmium (Cd)** is not characterized by the implementation of important functions in the metabolism. Even in small doses, this heavy element has a strong toxic effect on all living organisms, including hydrobionts. The toxic effect of Cd is expressed in the replacement of Zn in important enzyme proteins. In this aspect, the study of the amounts of Cd in the muscles of the studied hydrobionts is extremely necessary from a biochemical point of view.

The levels of cadmium in all tested muscle samples (Figure 6) do not exceed the MAC, regulated in the then current Regulation 31 from 2004 and the now active Regulation №5 of the Bulgarian legislation, as well as in EC Regulation №1881 of 2006 as amended in 2010 to determine the maximum permitted concentrations of certain contaminants in foods. Against the background of extremely low values of cadmium in the musculature of carp from the study conducted in 2011, the highest concentration was measured in carp from Opan Dam (0.011 mg/kg) and the lowest in samples from Pastren Dam (0.006 mg/kg).

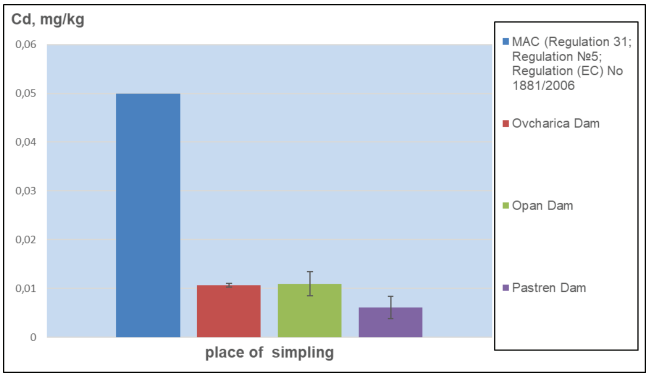

Fig. 6. Content of heavy metal Cd in the musculature of carp (*Cyprinus carpio* L.) in study from 2015 (Valkova, 2015)

As a result of the conducted statistical analysis, a high degree of reliability (p = 0.0001) of the results regarding the amounts of copper and Cd in the musculature of common carp, studied in the 2015 study, was established.

Content of heavy metals Cu and Cd in the blood serum of patients with established COPD and those who do not have such a disease in the period June 2019 – May 2020

The entry of heavy metals into the human body is carried out mainly through inhaled air, water and food. Over time observed accumulation of metals in certain tissues and organs. Upon entry into the body, these elements increase their levels in the blood, reaching all the cells.

Upon the occurrence and development of chronic inflammatory conditions such as COPD, most of the trace elements in the ionized state are mobilized to increase the energy state, the immune system and the rate of detoxification, which is accompanied by a decrease in their levels in the blood (blood serum). The development of COPD is accompanied by a gradual deterioration of respiration and the need for mechanical ventilation, which causes a decrease in serum levels of elements such as Cu, Zn, Fe, Co, Ni Mo et al. (El-Attar et al., 2009; Sylvester et al., 2012).

In order to establish the relationship between COPD and the concentrations of heavy metals in the blood, it was necessary to conduct a study of the content of certain metals in the blood serum of patients with established COPD and a control group of patients who do not have this disease.

The results of this study are presented in Figures 7 and 8.

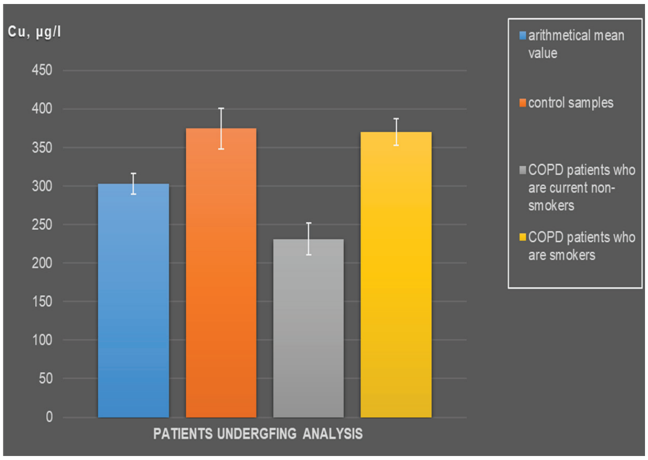

Fig. 7. Content of Cu in blood serum of patients with established COPD and those who do not have such a disease in the period June 2019 – May 2020

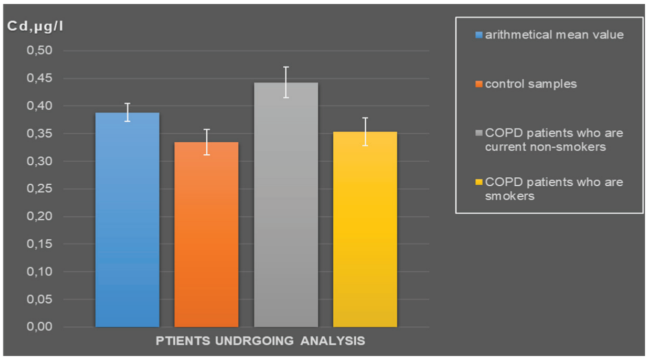

Fig. 8. Content of Cd in the blood serum of patients with established COPD and those who do not have such a disease in the period June 2019 – May 2020

It can be seen from Figure 7 that the highest values are characterized by the concentrations of copper recorded in the control samples, which were taken from patients in whom no presence of COPD was detected. The lowest levels are observed in patients in whom the presence and development of this disease is confirmed, but they belong to the group of current non-smokers. The total average of copper is lower as the value obtained in the control group of patients as well as in that of the samples taken from smokers. According to studies conducted in this direction in patients suffering from chronic diseases such as COPD, the concentrations of metals such as Cu, Zn, Ni, Mn, etc., which belong to the so-called essential elements are significantly reduced in the blood due to their mobilization in protein synthesis (enzymes, transport proteins, hormones, etc.), necessary for the immune defense and energy state of the organism (Karadag et al., 2004, Berger et al., 2004). This explains the result of our study, in which the copper measured in the control serum samples, was almost five points higher than that found in COPD patients who were smokers and more than 100 points higher than the values, registered with current non-smokers.

The levels of copper are affected not only by smoking, but also by the working environment, inhaled air, water and food intake, and more. It has been found that the addition of honey in normal doses stimulates the recovery of abnormal elastin fibers in the emphysematous lungs in COPD, which favors disease control (Janssen et al., 2019).

The data illustrated in Figure 8 show some differences in the levels of cadmium in the blood serum of the studied groups of patients in the period 2019-2020. The quantities of Cd recorded in the samples from the control group of patients were 0.020 points lower than those found in COPD patients who belonged to smokers and 0.110 µg/l lower than the values found in current non-smokers. The analysis shows that in healthy organisms with COPD, the levels of this element are lower than those measured in those suffering from this disease, which is understandable.

Cadmium concentrations in the blood are also influenced by other factors such as food and other ingested substances.

In general, a greater risk of intake of cadmium exist in smokers who are exposed daily to the fumes generated in the working environment, but also to another type of gas and dust (Branca et al., 2020).

Statistical processing of data on the content of Cd and Cu in the blood serum of patients with established COPD and those who do not have such a disease in the period June 2019 – May 2020 shows the presence of reliability of the obtained results ($p < 0.05$).

Conclusions

All reported average annual values of PM10 in the atmospheric air of Stara Zagora region are below the SGN defined in the legislation. The data received from the EEA – Sofia, as well as those indicated in a report of the RIEW – Stara Zagora in the period 2013 – 2014 and a special report of the European Court of Auditors from 2018 certify that, despite the relatively low average annual levels of PM10, a qualitative and sustainable reduction of air pollution by this indicator has not yet been achieved. The results regarding the quantities of cadmium (Cd) in the air of the Stara Zagora Region in the period 2014-2019 show the absence of pollution on an average annual basis. The values of the elements Cu, registered in the drinking water of the cities Stara Zagora and Radnevo are much lower than accepted norms. The highest value of Cd is characterized in drinking water in Stara Zagora Town, taken in January 2020 (0.0047 mg/l). The content of copper in the samples from 2015 is much lower than the MAC regulated in the then current Regulation 31 on the content of this metal in the musclature of fish intended for consumption. Whit the highest values far below the specified in the then active Regulation 31 norms characterized Ovcharitsa Dam (0.6028 mg/kg). Minimum concentration was measured in the musclature of common carp inhabiting the Pastren Dam (0.2716 mg/kg). Against the background of extremely low values of cadmium in the musculature of common carp from the study conducted in 2015, the highest concentration was measured in carp from Opan Dam (0.0110 mg/kg) and the lowest in samples from Pastren Dam (0.0061 mg/kg). The highest values of Cu in the blood serum of the studied groups of patients were found in the control samples. The amounts of Cd recorded in the serum samples from the control group of patients were 0.020 µg/l lower than those found in COPD patients who belonged to smokers and 0.110 µg/l lower than those found in current non-smokers.

The results of our study suggest that there is a relationship between the content of heavy metals Cd and Cu in some components of the environment (air, drinking water, food intake (musculature of the carp species studied)) and socially significant diseases such as COPD.

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