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The relationship between the content of heavy metals Pb and Zn in some components of the environment, fishes as food and human health

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Abstract

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The aim of the study was to establish the relationship between the content of Pb and Zn in the air, water, musculature of fish (*Cyprinus carpio* L.) and the blood serum of patients with and without COPD. The 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 concentrations of Pb in the air do not exceed the requirements of Regulation 12 of 15.07.2010. The levels of lead found in the drinking water of the of Stara Zagora Town in the period June 2019 – July 2020 often approach the limit value determined by Regulation N of 16.03.2001 (0.01 mg/l). The established concentrations of zinc in the drinking water of the cities of Radnevo and Stara Zagora during the reported period are lower than the MAC of 4 mg/l defined in the normative documents.

The analysis of the data on our study of 2015 for the content of Zn in the musculature of common carp does not show an excess of the MAC set by the then current Regulation 31. The highest levels of zinc, which do not even approach the established norms were reached in muscle samples from fish delivered from Ovcharitsa Dam (8.09 mg/kg). With the lowest measured concentrations characterized Pastren Dam (2.69 mg/kg). Musculature samples from all studied water bodies do not exceed the MAC for lead, indicated in the then valid Regulation 31 and now the current Regulation $N_{0}5$ of the Bulgarian legislation and EC Regulation $N_{1}881$ of 2006 with amendment from 2010 for determining the maximum permissible concentrations of some contaminants in foods. Although the values obtained are much lower than the regulated MAC, the highest concentrations are characterized carps from Lake Pastren Dam (0.04 mg/kg), and the lowest those from the Ovcharitsa Dam (0.01 mg/kg).

Keywords: lead; zinc; 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

A significant problem of in the present time of agrarian and industrial impact is the pollution of the environment at all possible levels – air, water, soil, plants, animals and human. The components of the environment and the factors that influence them affect the existence and reproduction of organisms (including hydrobionts and humans) (Valkova et al., 2015; Atanasov et al., 2017). The human diet often includes fish and other aquatic organisms suitable for consumption, which is why fish meat can also have a direct effect on the human health. As a result of the changes in the environment, the state of the human body changes, which leads to changes at the cellular level and metabolic processes (Tzanova et al., 2017). Often it is a prerequisite for the emergence and development of chronic disease conditions such as Chronic Obstructive Pulmonary Disease (COPD).

There are two pathological conditions in COPD – chronic bronchitis and emphysema (https://medlineplus.gov/copd. html) (ISAAC, 1997; Global Initiative for Chronic Obstructive Lung Disease, 2009).

Agricultural and industrial activities cause changes in the components of the environment, such as the concentration of certain heavy metals (Pb and Zn) in the air, water and food entering the body (fish meat), which have an impact on human health. (including COPD). The entry of lead and zinc into the body can happen in different ways – by ingesting the accepted water and food, inhalation and absorption of the skin (Valkova, 2015). In previous studies in South Korea's are established systemic toxic effects of lead and zinc on renal function and diabetes (Braunwald et al., 2001; International Program on Chemical Safety, 2013; Valkova, 2015). It has also been found that an increase in serum levels of these metals leads to the development of diseases such as COPD.

From a biochemical point of view, zinc ions (Zn^{2+}) play a key role in many processes, such as DNA and RNA synthesis, energy synthesis, in many metabolic reactions and in the regulation of the immune system (Valkova, 2015).

The accumulation of zinc in the musculature of fish (common carp) can lead to its entry into the human body in high doses (Valkova et al., 2016). Zinc ions (Zn^{2+}) in high concentrations cause structural changes in the main classes of lipids (Popova, 2004), a significant decrease in the levels of malonic dialdehyde and diene conjugates (Gabrielak et al., 2002). It has been found that zinc produces a significant reduction of the activity of cytochrome P-450 in liver (Arizono et al., 1993; Correia et al., 2011). High levels of zinc can also cause occupational asthma (Malo et al., 1993).

Increased incidence of obstructive pulmonary diseases is associated with lower daily intake of zinc with food (Lin et al., 2010). In hydrobionts and humans has been established rather deficiency than an excess of zinc due to its involvement in a significant number of enzymes and hormones. This is thought to be due, at least in part, to the protective effects of Zn against cadmium, whose metal ion has similar chemistry but high toxicity and accumulates in large doses in smokers (Shenkin, 1995; Southar et al., 1997; Grasseschi et al., 2003; Lin et al., 2013; Sakr et al., 2007).

With respect to cadmium, zinc is a metabolic antagonist. Thanks to this Zn it is possible to reduce the toxic effect of Cd to some extent. Excessive doses of zinc in humans lead to various types of anemia, damage to the lungs, skin, reproductive system and nervous system (Nriagur, 2007; Atanasov et al., 2011).

Lead (Pb) is characterized by multilateral toxicity. This metal has lower chemical mobility and a ubiquitous distribution. It exists in the form of metallic lead, inorganic ions and salts (Harrison, 2001).

Lead not only does not play a significant role in the biochemical aspect, but has a powerful toxic effect on organisms. Over 90% of lead is transported by erythrocytes. In high doses this metal exhibits embryotoxic effect (Avram et al., 1995), causes disturbances in heme formation and erythropoiesis (Iavicoli et al., 2003), neurotoxic action and binding to thiol groups (Dabrowska-Bouta et al., 1996).

High levels of this metal in humans can cause depression, diabetes, dyslexia, epilepsy, fatigue, gout, impaired glycogen metabolism, hallucinations, impotence, infertility, inflammation, renal dysfunction, decreased libido, migraine headaches, multiple sclerosis and tooth decay (Bekyarov, 2009).

The main routes of lead intake in humans are through ingestion of food (including fish) and water, inhalation and dermal adsorption (Valkova et al., 2016). Lead, which is spread through the air transfer, is deposited in soil and water. Due to this fact, lead exposure occurs mainly through the food chain and drinking water, which is the main route of exposure for the adult population. Intake of food, including fish meat, increases the possibility of lead entering in the human body, as these aquatic organisms have the ability to accumulate heavy metals such as lead (Valkova, 2015).

As a result of a number of studies, a significant impairment of lung function has been found in those working with batteries and exhaust gases, who have higher levels of lead in the blood than the control groups of people (Bagci et al., 2004).

Heavy metals lead and zinc can also enter the body through 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 Pb and Zn in the air, drinking water, musculature of fish (*Cyprinus carpio L.*) and the blood serum of patients with and without COPD.

Material and Method

Data were requested from the archives of the Executive Agency for Environment – Sofia regarding the content in the air of the Stara Zagora region of FPM10 and the heavy metal Pb for the period 2014 – 2019.

Upon realization of the objective of the 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 conducted, to track the concentrations studied heavy metals Pb and Zn. For storage, drinking water samples are treated with 5 cm³ to HNO₂.

During the research conducted by us in 2015, control catches were made in the dams Ovcharitsa, Opan and Pastren, located on the territory of Stara Zagora district. Common carp (*Cyprinus carpio L.*) specimens were transported in ice bags. Storage was achieved at -18° C. In order to prepare for mineralization, the samples were thawed at room temperature and washed with deionized water. Medium samples were taken from carp musculature.

In fulfilling the goals and objectives of this study patients with chronic obstructive pulmonary disease (COPD) were tested, who are residents of Stara Zagora and Radnevo and are suitable for inclusion in the study and the control group of patients without this disease. The patients were recruited in the Clinic of Internal Medicine, University Hospital, Trakia University, Stara Zagora, Bulgaria. The conducted work was approved by the Ethics Committee of the Medical Faculty, University of Thrace, Stara Zagora, Bulgaria. Informed consent was obtained from patients and controls prior to the study.

In order to determine the content of tested heavy metals, samples carp musculature and blood serum samples were mineralized by wet burning in a microwave oven Perkin Elmer Multiwave 3000 in a mixture of 1 cm³ k HCl and 6 cm³ k HNO₃.

The amounts of heavy metals in the mineralized acid solutions and aqueous samples were determined with a Perkin Elmer atomic absorption spectrometer (AAS) "A Analyst 800" on a cuvette and a flame system using acetylene-oxygen combustion.

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

Results and Discussion

The way of life of humans and their place at the end of natural food chains determines the possibility of direct exposure to various pollutants such as heavy metals, pesticides, drugs and others (Thompson & Darwish, 2019). Since the accumulation of heavy metals, pesticides and other toxicants in the plant, animal and human organism is a long process; we followed the levels of zinc and lead from 2015 to 2020. The complex impact of high levels of heavy metals in the inhaled air, water and accepted food (fish meat), complemented by smoking and the location of the settlement in a region at increased risk for the environment is a good reason for the emergence and development of chronic diseases such as COPD. This determines the need to track the values of heavy metals in these sources because of their importance for the emergence and development of the disease studied.

Content of Pb in the air of the Stara Zagora Region in the period 2014 – 2019

The levels of heavy metals in the air are a factor that largely determines the condition of the alveoli of the lungs in humans, as well as the condition of most organs and systems of the body as a whole. The presence of high concentrations of these elements in the inhaled air favors depositions into the alveoli and into tissues and organs such as the liver, kidneys, gonads and others. This was a good reason to request data from the EEA – Sofia on the average annual values of the metal with high toxicity lead from DOAS – Razhena for the period from 01.01.2014 to 31.12.2019, due to lack of such for the element zinc in air during this period. The provided information is processed and presented in Figure 1.



Fig. 1. Content of Pb in the air of the Stara Zagora Region in the period 2014 – 2019

Figure 1 clearly shows that the AAV for lead in air referred to in Regulation № 12 of 15.07.2010 for the levels of sulfur dioxide, nitrogen dioxide, particulate matter, lead, benzene, carbon monoxide and ozone in ambient air is much higher values than those provided by the EEA – Sofia for that period. All reported concentrations are extremely low compared to the requirements of Regulation №12 of 15.07.2010, but here it is a question of the lack of exceeding the average annual values of lead. Unfortunately, however, in the special report of the European Court of Auditors from 2018. It is stated that 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 concentrations of heavy metals and fine dust particles.

(https://www.eca.europa.eu/Lists/ECADocuments/ SR18_23/SR_AIR_QUALITY_BG.pdf; https://op.europa.eu/webpub/eca/special-reports/air-quality-23-2018/bg/ www.nsi.bg).

This means that the average annual values are normal, but the number of exceedances of these indicators is greater than the required purity of air on Bulgarian territory in the mentioned period.

The statistical analysis of the data for Pb content in the air of Stara Zagora region in the period 2014 - 2019 shows good reliability of the obtained results (p <0.05).

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

Water is a major component of the environment, but also an environment in which to carry out all life processes. The components of water largely determine the acidity of the medium and, accordingly, the direction of the biochemical reactions (Zhelyazkov et al., 2018). The levels of heavy metals in drinking water are crucial for the condition of the human body (Modupe et al., 2018). The entry of large amounts of metal ions leads to the formation of superoxide radicals and a significant reduction in immune protection, which is often the cause of chronic diseases such as COPD. This necessitated the study of the levels of some heavy metals (Pb and Zn), both in the air and in the drinking water adopted by patients.

Zinc (Zn) plays an essential role in the metabolism of the human body, participating in many transport and regulatory proteins (insulin). In excessive doses in drinking water however this metal can cause very negative consequences affecting the nervous and endocrine system, as well as the immune system.

During the study we found that the concentrations of zinc in the drinking water of the cities of Radnevo and Stara Zagora during the reporting period (Figure. 2) are significantly lower than the MAC of 4 mg/l. The highest value of Zn was reported in the samples of drinking water from Stara Zagora in February 2020, as this value is almost 4 times lower than the one regulated in Regulation №9 of 16.03.2001 concentration (1.35 mg/l). Similar levels of zinc were reported in the water from Stara Zagora, subjected to analysis in April 2020. The concentration of this metal in the drinking water of the town of Radnevo is even lower, as the highest registered value is in April 2020 (1.23 mg/l).



Fig. 2. Content of Zn in the drinking water of the cities of Radnevo and Stara Zagora during the period June 2019 – May 2020

According to the commentary above and according to the activity report of RHI – Stara Zagora Town 2017 (https:// www.rzi-starazagora.org/images/otcheti/Godishen_otchet_ RZI-St.Zagora_2017.pdf), and the required by RHI – Stara Zagora Town 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 accepted regulatory requirements of Regulation №9, regulating the MAC for Zn in drinking water of 4 mg/l. In the study conducted by us for the period June 2019 – May 2020 not observed approaching the standards of zinc, which implies the lack of risk of exceeding the regulatory requirements and the danger of the passage of larger amounts of zinc from drinking water in the body of the studied patients.

In Figure 3 presents the registered data on the concentrations of lead in the drinking water of the cities of Radnevo and Stara Zagora during the studied period of one calendar year.

Lead is an element that not only does not perform specific biological functions in cells, but also contributes to the formation of large amounts of free radicals, leading to the oxidation of vital molecules such as proteins and lipids. Regulation №9 of March 16, 2001 of the Bulgarian legislation determines the normative requirement for Pb in drinking water to be 0.01 mg/l. The levels of lead found in the drinking



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

water of the city of Stara Zagora in the period June 2019 – July 2020 often approach the limit defined by normative documents, in July 2019 and January 2020 the concentration of this element is aligned with the MAC (0.01 mg/l).

The quantities of lead registered in the drinking water of the town of Radnevo in the mentioned period are lower, but in March 2020 the value slightly approaches the limit – 0.007 mg/l. Studies conducted by us were compared with data of RHI, Stara Zagora received in previous years and during the survey period. The report on the activity of RHI Stara Zagora from 2017 (https://www.rzi-starazagora.org/ images/otcheti/Godishen otchet RZI-St.Zagora 2017.pdf) contains data that deviations in some heavy metals (including Pb and Zn) on an annual basis are found in only 10% of the samples taken for drinking water, as they do not exceed the MAC by more than 5%. As a result of our consultation with the RHI - Stara Zagora regarding the values of the studied heavy metals (including Pb), it was stated that there are no deviations from the accepted regulatory requirements for drinking water.

Analysis of the data content of Pb and Zn in the drinking water of cities Radnevo and Stara Zagora in the period from June 2019. – May 2020, performed in 2020, shows the presence of reliability of the obtained results (p < 0.05).

Content of heavy metals Zn and Pb in the musculature of carp (Cyprinus carpio L.) in study from 2015

Being on top of the food chain, the man consumed primarily musculature of hydrobionts and far less caviar and liver (and their products such as fish oil) in certain types of fish. The musculature of hydrobionts is important, both from a physiological-biochemical and from a dietary point of view. The content of Zn and Pb was regulated in the Regulation N_{231} in force at the time of the study, but in the currently active normative documents (Regulation N_{25} of the Bulgarian legislation and Regulation (EU) 1881) there is a restriction only for the element lead.

For the element Zn (Figure 4) the MAC, regulated in the Ordinance 31 of the Bulgarian legislation on the content of this metal in the musculature of fish, in force during the study (2015), has not been reached.



Fig. 4. Content of heavy metal Zn in the musculature of carp (*Cyprinus carpio* L.) in study from 2015 (Valkova, 2015)

The analysis of the data from all studied water bodies in 2015 does not show exceeding of the MAC, determined by the then valid Regulation 31. The highest levels of Zn, which do not even approach the established norms, were reached in the musculature samples from fish delivered from Ovcharitsa Dam (8.09 mg/kg). With the lowest measured concentrations is characterized Pastren Dam (2.69 mg/kg). The results correspond to the worldwide researches on the content of zinc in the musculature of fish (Yousafrai et al., 2012). The results of these studies are clear evidence that musclature is characterized by low levels of such metals due to their concentration in organs such as the liver, ovaries, testes, gills and kidneys.

The negative impact of lead even at low doses determines the need for analysis of the musculature of the surveyed species. The established tendencies regarding the accumulation of heavy metals in the muscles of the studied hydrobionts are preserved in this element as well (Figure 5).

Musculature samples from all studied water bodies do not exceed the MAC for lead, regulated both in the then current Regulation 31 from 2004 on the maximum permissible



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

amounts of contaminants in food, and in the currently active Regulation No5 of Bulgarian legislation and Regulation (EU) No1881 of 2006 with an amendment of 2010 to determine the maximum permissible concentrations of certain contaminants in food. Although the values obtained are much lower than the regulated MAC, with the highest concentrations are characterized the carp from dam Pastren (0,044 mg / kg), and with the lowest these from dam Ovcharitsa (0.009 mg/kg). The statistical data from the analysis of the carp musculature samples from studied water bodies regarding the content of lead and zinc in the studied period of 2015 are characterized by a maximum degree of reliability (p < 0.001).

Content of heavy metals Pb and Zn in the blood serum of patients with established COPD and those who do not have such a disease, in samples taken for analysis in the period June 2019 – May 2020

The intake of heavy metals in the human body can take place in several ways – through the intake of air, in which there are vapors or dust particles containing heavy metals, and the same through the intake water, food or medicines. Passing into the blood, these ions reach all parts of the body, accumulating in vital organs such as the liver, spleen, kidneys and more.

Essential elements such as Cu, Zn, Ni, Co, Mn and others that enter the body are largely absorbed due to their participation in the structure of many enzyme systems and proteins with protective, transport and regulatory function. High levels of micronutrients also lead to intoxication.

Disease states such as COPD are characterized by the mobilization of a large part of the trace elements such as Zn, Cu, Ni, Fe and others in order for use from the body for the

synthesis of a number of enzymatic, hormonal and transport proteins needed to increase immune protection and energy levels in cells. It has been found that serum levels of these elements decrease precisely in case of respiratory distress and the need for mechanical ventilation (El-Attar et al., 2009).

It is clear that the occurrence and development of COPD is directly related to the levels of certain heavy metals in the blood, which determines the need to study their levels in the blood serum of patients with established COPD (smokers and non-smokers) and a control group of patients in which this disease is absent.

The data recorded in the analysis of serum samples regarding the content of heavy metals lead and zinc are presented in Figures 6 and 7.







Fig. 7. Serum Pb content in patients with established COPD and those who do not have such a disease, in samples taken for analysis in the period June 2019 – May 2020

Among the metals essential for metabolism, of course, is the element zinc (Zn). This metal is required for the catalytic activity of approximately 100 enzymes (Solomons, 1998; Prasad, 1995) and plays a role in immune function (Heyneman, 1996; Simmer & Thompson, 1985), protein synthesis, wound healing, DNA synthesis and cell division. Zinc also supports normal growth and development of the fetus during pregnancy, and during childhood and adolescence, it is also necessary for the proper sense of taste and smell (US Department of Agriculture, Agricultural Research Service, 2011). Daily intake of zinc is necessary to maintain a stable condition, as the body does not have a specialized system for storing zinc.

High levels of zinc cause oxidative stress by formation of reactive oxygen species that cause damage to cells via its involvement in oxidative processes.

As mentioned, the concentrations of zinc as an essential element on the one hand and heavy metal on the other are essential for the development of COPD. Therefore, it was necessary to analyze this metal in the blood serum of the studied patients (Figure 6).

As can be seen from the graph (Figure 6), the amounts of zinc measured in the serum of the control group of patients are the highest (2010.44 μ g/l), which is completely normal due to the absence of diseases such as COPD. Zinc as an essential trace element is practically used by the body, which is why the serum of smokers with COPD is characterized by the lowest values (1432.9 μ g/l).

In their study, León-Espinosa de los Monteros et al. (2000) also found lower levels of zinc in samples from patients with COPD compared with controls without the disease. In these patients, Zn was introduced into the synthesis of proteins with protective, immune and transport functions necessary to deal with the oxidative stress caused by the disease and tobacco smoke.

Lead is known as an element with high toxicity that has no direct involvement in biochemical processes, but rather large quantities it contributes to the development of oxidative stress and peroxidation of lipids and proteins of the cells. Although the respiratory system is one of the main routes for the introduction of lead, a limited number of studies on the effect of lead on the lungs exist and most of them describe the influence of Pb on hematopoietic, cardiovascular, reproductive and nervous systems, as well as kidney diseases (Bellinger, 2011; Landrigan, 1990). This determines its importance for the occurrence and development of COPD, which is clearly seen at higher doses of this element in the blood and the body as a whole. Analysis of the content of this element in the blood serum of the patients studied is presented in Figure 7.

The data presented in Figure 7 show some variations in lead levels recorded in the study groups of patients. The concentrations of this heavy metal measured in the samples from patients with COPD and were current non-smokers were 0.01 µg/l higher than the total arithmetic mean, by 0.016 µg/l higher than in the control group of patients and by 0.014 μ g/l of the established concentration in smokers. The study shows that the amounts of lead measured in patients who have not developed COPD are the lowest, which is completely understandable. The graph clearly shows that the Pb levels found in the samples from current non-smokers are higher than those obtained in smokers. This result can be explained by the fact that the concentrations of lead in the blood are directly dependent not only on the presence of chronic diseases such as COPD, smoking and air and water intake. This indicator is influenced by a set of factors, which include the intake of food and other substances, as well as work in an environment that allows the intake of heavy metals (in this case lead). Much of the patients studied who are current non-smokers are ex-smokers, which also affects the results obtained.

Lead intake in humans is by ingestion of food and water, inhalation and through the skin. Lead in the air is deposited in soils and waters. Thus the exposure of lead in humans is through direct involvement in the food chain and drinking water, which is the main route of administration of the population of non-smokers as a whole.

Impaired lung function was found among workers in factories for batteries and sources, in which higher levels of lead in the blood are recorded in comparison with control samples (Bagci et al., 2004). In the case studied in our study, there is no data on the working environment of non-smokers and, accordingly, of smokers subjected to analysis. However, the registered data for lead show that the levels of this metal are higher in non-smokers, i.e. these quantities were influenced by factors such as food intake, work environment and others.

Statistical processing of data on the content of Pb and Zn in the blood serum of patients with established COPD and those who do not have such a disease, in samples taken for analysis in the period June 2019. – May 2020, performed in 2020 shows the presence of reliability of the obtained results (p < 0.05).

Conclusions

In an average year plan, the quantities of lead (Pb) in the air of Stara Zagora region between 2014-2019 show no contamination. According to the report of the European Court of Auditors, Bulgaria belongs to 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. The concentrations of the element Zn registered in the drinking water of the cities of Stara Zagora and Radnevo are much lower than the accepted norms. The highest levels of Zn, which do not even approach the established norms, were reached in the muscle samples from fish delivered from Ovcharitsa Dam (8.09 mg/ kg). With the lowest measured concentrations characterized Pastren Dam (2.69 mg/kg). Carp musculature samples from all studied water bodies do not exceed the MAC for lead, regulated both in the then current Regulation 31 from 2004 on the maximum permissible amounts of contaminants in food, and in the currently active Regulation №5 of Bulgarian legislation and Regulation (EU) №1881 of 2006 with an amendment of 2010 to determine the maximum permissible concentrations of certain contaminants in food. Although the obtained values are much lower than the regulated MAC, the carps from the Pastren Dam are characterized with the highest concentrations (0.044 mg/kg), and the lowest those from the Ovcharitsa Dam (0.009 mg/kg). The values of Zn in the blood serum are highest in the control samples 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 smokers.

In our study, it was found that there is a link between the content of Pb and Zn in the air, drinking water, musculature of fishes (*Cyprinus carpio L.*) and socially significant diseases such as COPD.

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