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A study of accumulation and quantity of microelements with high toxic effects on bone and muscle tissue of reptiles in urbanized areas of Absheron

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The purpose of this research is to determine the quantity of some toxic microelements in bone and muscle tissue of background reptile species, compare this quantity with average standard quantity and thereby to study the impact of environmental pollution on reptiles. The accumulation and amount of microelements with high toxic effects have been studied in bone and muscle tissue of following Reptile species - Water snake (*Natrix tessellata* Laurenti, 1768), Mediterranean turtle (*Testudo graeca* Linnaeus, 1758) and Caspian bent-toed gecko (*Cyrtodactylus caspius* Eichwald, 1831) that have been collected from urbanized areas of Absheron peninsula. Studied microelements are copper Cu, nickel Ni, lead Pb, cadmium Cd and zinc Zn. All the five microelements that we have studied are mostly accumulated in the body of Caspian bent-toed gecko. It should be noted that the Caspian bent toed gecko is very small and more functionally active among background reptile species that we studied. Its occurrence in oil, gas wells and in areas exposed to technogenic and antropogenic pollution, indicates its plasticity and tolerance to toxic microelements.

Keywords: *Technogenic, deionized water, spectrometer, microelement, bone and muscle tissue, biodiversity, urbanization*

INTRODUCTION

As it is known urbanization is the transformation of natural and agricultural areas into towns and cities and as a result the number of urban population increases. At the same time the different fields of industry develops in connection with urbanization. All these factors cause the contamination of soil, pollution of water systems and it can result in health threats to urban fauna (Croteau et al., 2008) According to the literature, macro and microelements are not synthesized in the animal body, so they enter the organism from the external environment - inside food, water, air and take part in the synthesis of high-molecular organic and inorganic substances (Grajdankina, 2001; Chernykh et al., 2004; Baimova et al., 2007; Orbelis et, al., 2008; Bikova, 2014).

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In recent years, the Absheron peninsula has been exposed to impact of urbanization. Our rese- arch have been done with reptiles collected from urban areas of Absheron peninsula. The strongly urbanized areas of Absheron peninsula differ from other territories with the development of oil and gas, metallurgic and medicinal industries. Due to anthropogenic and technogenic factors, this area is polluted with variety of pollutants, as well as heavy metals. Heavy metals are known to be naturally occurring compounds, but anthropogenic activities introduce them in large quantities in different environmental compartments. This leads to the environment`s ability to foster life being reduced as human, animal and plant health become threatened. This occurs due to bioaccumulation in the food chains as a result of the nondegradable state of the heavy metals (Masindi et al., 2017).

The long-term exposure to heavy metals in the environment represents a threat to wild populations, affecting communities and putting ecosystem integrity at risk. Therefore this type of exposure represents a threat to a biodiversity (Tovar-Sanhez et al., 2018). It has been studied that aquatic and terrestrial ecosystems in urban environments often exhibit a loss of sensitive species, loss of species numbers (decrease in diversity) and increases in numbers of pollution-tolerant organisms and it can lead a loss of particular species from the area. (Croteau et al., 2008).

Thus, the studying of the accumulation and quantity of toxic microelements in bone and muscle tissue of reptiles in urbanized territories of Absheron peninsula, becomes more relevant. These animals are directly related to the soil and vegetation, and the probability of transferring the microelements from the soil to plants and from plants to the body of reptiles is high. (Kist, 1987; Khlebnikova et al., 2013). The turtles are feeding on plants, the geckoes mainly on insects and water snakes on aquatic invertebrates. So, heavy metals, may enter the trophic chain through primary producers (plants) and invertebrates that live in the soil and in water (Tovar-Sanhez et al., 2018). Some important anthropogenic sources which significantly contribute to the heavy metal contamination in the environment include automobile exhaust which releases lead Pb; smelting which releases copper Cu and zinc Zn; burning of fossil fuels which release nickel Ni (Masindi et al., 2017).

MATERIALS AND METHODS

The objects of our research are the Mediterranean turtle (*Testudo graeca* Linnaeus, 1758) from the order Turtles (*Testudines*), the water snake (*Natrix tessellata* Laurenti, 1768) from the order Snakes (*Serpentes*) and the Caspian bent-toed gecko ***(****Cyrtodactylus caspius* Eichwald, 1831) from the order Lizards (*Sauria*). All these orders belong to the class of Reptiles. These species are predominantly exposed to the effects of anthropogenic and technogenic factors during the urbanization process in the Absheron Peninsula. The route method was used for the collection of materials. The amount of the following microelements - nickel, copper, lead, cadmium and zinc, which have toxic effects and are needed for the normal functioning of the body, have been studied in the muscle and bone tissue of the selected reptile species. These microelements have toxic effects when their amount is higher than the norm, and in the case of their deficiency, the functional activities of the body are impaired. However, the proper amount of these microelements is actively involved in regulating the physiological and biochemical functions of the body.

For the biochemical analysis 10 specimens were taken from each of the Caspian bent-toed geckoes and water snakes, as well as 7 specimens from the Mediterranean turtles. It should be noted that, taking into consideration the sharp decline in the number of Mediterranean turtles in recent years and their inclusion in International Union for Conservation of Nature’s Red List of Threatened Species, samples of the turtles have been taken from bone and muscle tissue of damaged or dead bodies that were found on the roadside. Furthermore, during the extraction of the material from the Caspian bent-toed gecko, it was sampled from both muscle and bone tissues for the chemical analysis of microelements, because, due to its small quantity it was not well anatomized. Accordingly, the muscle and bone tissue of the water snakes that we investigated were analyzed together. Since both tissues are a major component of the supporting apparatus, it is more advisable to perform this analysis by using both muscle and bone tissue. Bone tissue and somatic muscles (that form a separate tissue group) are important for the body due to their biological and physiological properties. Considering this point, we aimed to study the amount of toxic microelements that have been found in both tissues.

The quantitative analysis of the collected material was carried out at the Institute of Radiation Problems of ANAS in the Laboratory of “Physics and Chemistry of Harmful Impact on the Environment”. The analysis was performed using the AAS-Atomic Absorption Spectroscopy method. To collect research animals we have used the routing method. Atomic Absorption Spectrometer 220 FS was used for determining the accumulation and quantity of toxic microelements in the bone and muscle tissue of the animals we investigated. The advantage of Atomic Absorption Spectrometer is that it is possible to identify several elements in the same solution with high sensitivity, selectivity, and spending little time. The process of analyzing with this device is carried out in 3 stages: (1) Preparation of tubes; (2) Sample preparation; (3) Analysis stage.

Preparation of tubes: Firstly, water is poured into the deionizer (MILLIPORE) and it is removed from ions to obtain deionized water. The tubes are first rinsed with normal water and shampoo and then washed 2-3 times with deionized water. Then the tubes are kept in 10% HNO3 solution for 2 days. After it the acid is removed from them and they are washed 3 times with deionized water. Then the washed tubes are put into a microwave oven (MILESTONE ETHOS PLUS High Performance Microwave Labstation) at 150 0C for 15 minutes for cleaning. After 1 day, the acid is removed from them, they are washed with deionized water 3 times and dried in the drying oven (Oven / Incubator). Pestle and mortar which are used in the homogenizing of samples are cleaned with the above mentioned procedures and dried in a drying oven for 5 minutes.

Sample Preparation: Dried samples are homogenized with the pestle and mortar. The aim here is to increase the touch surface area of the sample and acid in order to reduce the solubility time. The samples are then poured into the microwave's oven of 1g each, then 10 ml 65% HNO3 and 5ml 30% H2O2 are added and kept closed for 1 day. Then the microwave containers are opened, are relieved the pressure, and then are closed again and are placed in a microwave's oven to let all the metal contained transfer to the acid.

Microwave sample containers (that were kept in the acid) should be washed twice with deionized water and dried in a 40^{o}C drying oven for 10 minutes. Samples which are removed from microwave should be diluted to the required concentration for analysis with the AAS device. Deionizing water is used for dilution. The containers used for the dilution are also cleaned according to the above cleaning rules. Then we stick the sample name, number, weight and dilution percentage (DF-dilution factor) on tubes.

Samples are emptied from microwave ovens into centrifugal tubes of 50 ml, the walls of the tubes are washed with deionized water and added to the samples. The volume is then completed to 50 ml with deionized water.

Analysis stage: The next step is to prepare standards for each metal to be determined. The standard solutions used to form a calibration chart when performing element analysis in AAS are prepared by diluting the stock standard of that element. The dilution is made with 0.5% HNO3 solution. The samples are then poured into the tubes after being diluted 10 times, 0.1 ml of each metal stock standard, 2ml HNO3, 1ml of H_2O_2 are added and then deionized water is added in order to complete solution to 100ml. The prepared solutions are put into a spectrometer for determining the amount of microelements.

RESULTS AND DISCUSSION

The issue of microelement accumulation in various tissues of animals found in urbanized areas or areas contaminated by technogenic factors, was not properly studied in Azerbaijan. The Absheron Peninsula is an area of our republic that has been strongly urbanized and has been exposed to technogenic pollution in recent years. This is due to the intensive development of the peninsula's oil and gas industry, the existence of chemical industry, factories and plants, as well as, a strong tendency of living in urban environments among population. All the factors mentioned above, including anthropogenic factors, have a great impact on wild fauna. Wildlife fauna, including reptile species, that are our research objects, feed on plants, invertebrates (Caspian bent-toed geckoes and Mediterranean turtles), and partially primary vertebrates (water snakes). Thus, the microelements that exist in the body of these creatures, pass through the food chain to the body of the reptiles we investigate and accumulate in their different tissues. Since, the reptiles are generally involved in food chain of biocenosis, studying these micronutrients also has a practical importance (Orbelis et al., 2008; Maksimyuk et al., 2015).

As shown in the table, copper is more commonly found in the bone and muscle tissue of the Caspian bent-toed gecko (6.025 mg/kg), approximately half of this amount has been observed in Mediterranean turtle (4.364 mg/kg) and least amount was found in the water snake (1.322 mg/kg).Copper is released into environment during industrial activities such as cement production, also most domestic water pipes are copper -based, resulting in frequent contact between water and copper sources in urban areas (Croteau et al., 2008).

Copper is an essential micronutrient that performs vital physiological functions in both human and animal organisms. Copper exists inside many vitamins, hormones, enzymes, respiratory pigments and takes part in metabolism and tissue respiration, stimulates normal blood composition and blood-forming function of bone marrow, regulates the amount of blood cholesterol, intensifies the formation of erythrocytes and leukocytes, strengthens the bone tissue. Low dosage leads to reduction of carbohydrates in blood and affects the metabolism of minerals; the deficiency of this microelement causes the reduction of the growth rate, depigmentation of hair, decrease in hemoglobin levels in blood, damages to cardiac muscles, disruption in the structure of the connective tissue and destruction of blood vessels. In animals, the reduction of absorption and usage of iron occurs which is accompanied by diarrhea and weakness, causing anemia (Diorditsa, 2006; Orbelis et al., 2008; Dobryanskaya et al., 2014;) Excess amount of copper in the body causes functional disorders of respiration, reduction of other microelements - zinc, molybdenum and manganese and long-term excess amount of copper leads to intoxication and poisoning of the body (Chernykh et al., 2004; Dobryanskaya et al., 2014; Sheybak, 2014).

As the mentioned in the table information shows, the amount of copper in bone and muscle tissue of Caspian bent-toed gecko is higher than in other objects of research. This is due to its activity and intensive metabolism. The amount of the copper in Caspian bent-toed gecko's bone and muscle tissue (somatic muscle) is higher than the standard average quantity (Kist, 1987). The reason for this is the technogenic contamination of the peninsula and on the other hand, we can say that the gecko is highly adaptive to this microelement. Copper's toxicity average is over 250 mg (Dobryanskaya et al., 2014).

Table 1. The accumulation of microelements with high toxicity, in the bone and muscle tissue of Mediterranean turtle, water snake and Caspian bent-toed gecko (mg / kg).

Species	Microelements				
	Cu	Ni	Pb	Cd	Zn
Mediterranean turtle	4.364	1.122	0.547	0.055	212.4
Water snake	1.322	1.392	0.564	0.059	274.7
Caspian bent-toed gecko	6.025	2.499	5.060	0.103	554.2

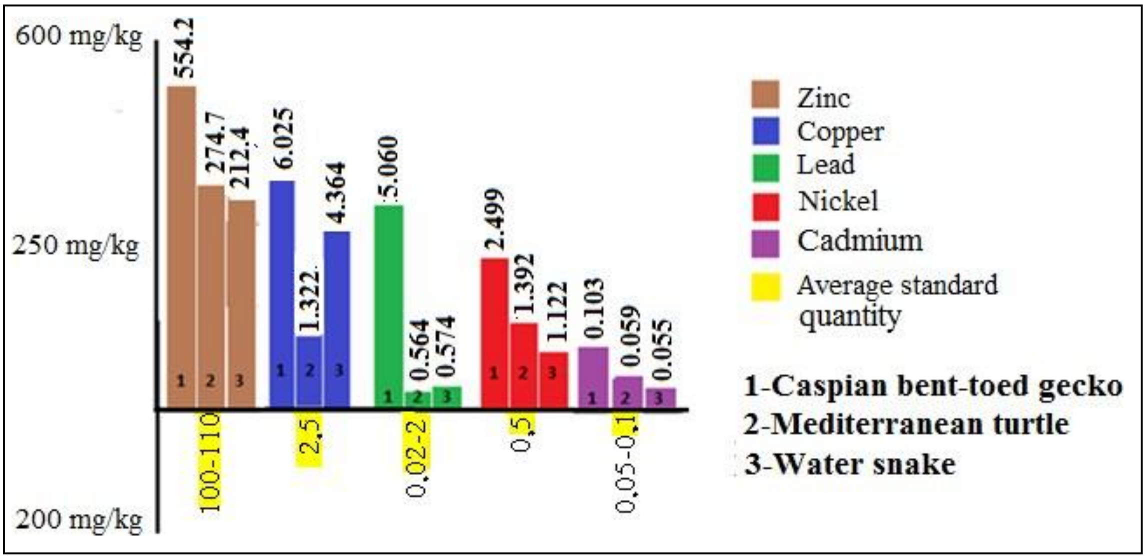

Figure l. The amount of microelements with high toxicity in comparison with the average standard quantity (mg/kg) in the bone and muscle tissue of the Mediterranean turtle, water snake and Caspian bent-toed gecko.

One of the microelements required for the normal development of living organisms is nickel. It is known that the nickel is involved in the fermentation processes in animals and plants. The amount of nickel in the bone and muscle tissues of the reptiles of our research is as follows: 2.499 mg/kg in the Caspian bent-toed gecko, 1.392 mg/kg in the water snake, and 1.122 mg/kg in the Mediterranean turtle. Excess amount of nickel leads to eye diseases (Kornick et al., 2008). This microelement has high allergic properties, so, in 2008 the American Contact Dermatitis Society recognized nickel as "Allergen of the Year" (Diorditsa, 2006). In human and animal organisms, nickel is accumulated in the pancreas and parathyroid gland, accelerates the oxidation of the ascorbic acid and speeds up the passage of sulfhydryl group into the disulfide. As it seems, nickel is one of the most important microelements in the body, which its deficiency and excess amount in the body leads to impairment of physiological processes (Kornick et al., 2008).

Lead is released to the atmosphere from gasoline, as well as, oil combustion, cement and metallurgic industries cause lead accumulation in the soil and atmosphere. Lead is one of the most important microelements for the body (Diorditsa, 2006; Baimova et al., 2007). Its biological value is not sufficiently studied yet, but experiments on laboratory animals show that it participates in the metabolism of bone tissue, stimulates growth, takes part in iron metabolism, affects the hemoglobin concentration and the activity of some enzymes. Lead levels in human body vary from 2 to 200 mg.

Among the reptiles of our research, the accumulation of lead is more in bone and muscle tissue of the Caspian bent-toed gecko 5.060 mg/kg. It should be noted that, the lead is mainly accumulated in bone tissue, liver, kidney and brain (Diorditsa, 2006; Duskayev et al., 2014). In water snake and in Mediterranean turtle small amounts of lead have been observed, respectively 0.564 and 0.547 mg/kg (Figure 1). Different accumulation of lead in the studied animals' bone and muscle tissue depends on their nutrition. Lead is absorbed directly into the body by food and air (used during breathing), while excess amount is excreted by the feces (Duskayev et al., 2014).

Cadmium is one of the microelements with high toxicity. Atmospheric air deposition and tire wear on asphalt are major urban sources of cadmium (Croteau et al., 2008). Its deficiency and excessive levels can lead to a number of complications in the body. In all living organisms cadmium is found to weigh up to 0.5 mg per kg. Despite the fact that it is present in all living things, its physiological significance has not been studied in details, yet. However, scientists have found out that cadmium is involved in the metabolism of some micronutrients, such as zinc, iron, silver and calcium, takes part in sugar metabolism and in activation of certain enzymes in the body. Cadmium is mainly concentrated in the kidneys (30-60% of the total), liver (20-25%), pancreas, long bones, spleen and other tissues and organs. Sublethal effects of cadmium exposure on herpetofauna have also been demonstrated (Croteau et al., 2008).

It should be noted that although cadmium is considered to be one of the most dangerous toxic elements, its application in medicine has yielded positive results. Thus, patients suffering from heart disorders are provided with nickel-cadmium batteries in their chest cavity in order to supply them with mechanical energy. However, excessive accumulation of cadmium in the body may lead to certain diseases. One more interesting fact: In the 50s of the last century in Japan the local population became infected with a disease called "the Italian disease". The disease was a result of the heavy pollution of the environment. It was revealed that, people in the area where the disease was spread were fed on rice and seafood with high cadmium content, or more precisely, these individuals daily consumed about 600 μg of cadmium. The daily safe dose for a person is 1 μg per kg body weight.

The amount of cadmium in bone and muscle tissues of the reptiles that we investigated is as follows: 0.103 mg/kg in the Caspian bent-toed gecko and approximately equal in the water snake and the Mediterranean turtle (respectively 0.059 mg/kg and 0.055 mg/kg). As it seems, the amount of cadmium in muscle and bone tissue of all three reptiles is much lower than the standard average quantity (Kist, 1987).

Zinc is the most common microelement in the body after iron. Sources of zinc in urban environments include tire wear, brake lining wear and corrosion from galvanized steel barriers (Croteau et al., 2008) It is a component of more than 2,700 enzymes, and has a catalytic function in up to 70 enzymes (Khlebnikova et al., 2013; Maksimyuk et al., 2015; Khabarov et al., 2012). Due to its antioxidant properties it takes part in DNA reparation. The bio-physiological significance of zinc microelement has been studied extensively in medicine, agricultural animals and some species of wild fauna (Klug, 2010; Shtikova, 2015; Rebezov et al., 2015). Despite the biological importance of a zinc as a microelement, zinc pollution can negatively impact reptiles (Croteau et al., 2008). Its deficiency and excess amount causes certain disorders in the body, primarily the disruption of normal growth and development. In this regard, interesting information is given in the works of N.N.Maximyuk and M.B.Rebezov (Maksimyuk et al., 2015) about accumulation of heavy metals in wild boar meat. The authors suggest that, it is not advisable to use boar meat as food, because heavy metals including zinc accumulate in the internal organs and bones of this animal. Studies show that the zinc microelement is sufficiently deposited in bone tissue of hunting birds and mammals, so it is clear, that it will accumulate also in muscle tissue, which is directly related to bone tissue (Sheybak, 2014; Sheybak, 2015). The accumulation of eco-toxicants, including zinc microelement in wild fauna species, which live in areas where urbanization and technogenic pollution are widespread, contributes to serious disruption of the ecosystem (Klug, 2010; Rebezov et. al., 2015).

Cu, Ni and Zn are essential, serve as micronutrients and are used for redox processes, to stabilize molecules through electrostatic interactions; as components of various enzymes and regulation of osmotic pressure. Too low concentrations of heavy metals lead to a decrease in metabolic activity and too high concentrations lead to toxicity (Rathoure et al., 2017).

As a result of chemical analysis, it was determined that the copper microelement is in the second place after zinc among studied microelements in reptile species. Thus, in bone and muscle tissue of the Caspian bent-toed gecko the amount of zinc was 554.2 mg/kg, while the amount of copper was 6.025 mg/kg; in water snake the amount of zinc and copper was respectively 274.7 mg/kg and 4.364 mg/kg, in Mediterranean turtle the amount of zinc and copper were respectively 212.4 mg/kg and 1.322mg/kg.

Zinc microelement is found in the tissues of all plant and animal organisms. In the body of an adult human the quantity of zinc is about 2 g and this number is twice less than the amount of iron. It is known that, 20% total amount of zinc is accumulated in the bone, 6% in the blood plasma, 2.8% in the erythrocytes, 3% in the liver, and 65% in the muscle tissue (Sheybak, 2014). The amount of zinc in various food products is as follows: 20-40 mg/kg in beef, pork and mutton, 15-30 mg/kg in fish meat and 60-100 mg/kg in oysters. In chicken eggs the amount of zinc is 15-20mg, in carrot, beet and potato is 10 mg/kg, in bone and muscle tissue is about 100-110 mg/kg (Yanovich, 2014; Shtikova, 2015).

CONCLUSION

As mentioned in discussions amount of microelements, that we studied in bone and muscle tissue of reptiles differs depending on species and microelements. Some of them are below, while others are above the standard average and it is related to disturbance of the ecological balance. First of all, these microelements are excessive in external environment and on the other hand, the amount of some microelements exceeds the norm in muscle and bone tissue of reptiles. So, it helps us to come to conclusion that some of these organisms have a high ability to accumulate microelements and adapt to them. All the five microelements that we have studied are mostly accumulated in the body of Caspian bent-toed gecko. It should be noted that the Caspian bent toed gecko is very small and more functionally active among background reptile species that we studied. Its occurrence in oil, gas wells and in technogenic areas indicates its plasticity and resistance to toxic microelements. Therefore, high levels of microelements detected in the Caspian bent-toed gecko do not cause lethal effects. Toxic microelements in bone and muscle tissue of studied reptiles are higher than the standard average and one of the reasons why they do not have a lethal effect is that, these micronutrients are accumulated in functionally active bone and muscle tissue. The toxic effects of microelements are directly related to which organ of body it is accumulated in and this issue has not been studied in detail, yet (Chornix et. al., 2004).

As shown from our research, reptiles those are found in urbanized areas of Absheron peninsula are exposed to an impact of heavy metals that generate a great threat for their health and survival. Some of them (Caspian bent-toed gecko) can adapt and survive in urban conditions, while others such as Mediterranean turtle are at risk of extinction. The reptiles are an important ring of the trophic chain and changes in their numbers are directly affect the other animals and it cause the disturbance of the ecosystem. Therefore, we should find the solution ways for protection of these animals from contamination with heavy metals and at the same time for conservation of biodiversity. In our future studies we are planning to determine the negative effects of heavy metals on reptile species and work on the issue of how to preserve them from toxicological effects of heavy metals.

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Abşeronun urbanlaşmış ərazilərindəki sürünənlərin sümük və əzələ toxumasında yüksək toksiki təsirli mikroelementlərin toplanması və miqdarının öyrənilməsi

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Tədqiqat işinin məqsədi sürünənlərin sümük və əzələ toxumasında toplanan toksiki mikroelementlərin miqdarının təyin edilməsi, bu miqdarın standart orta miqdarla müqayisəsi və bu yolla da ətraf mühitin çirklənməsinin reptililərə təsirinin öyrənilməsidir. Abşeron yarımadasının urbanlaşmış ərazilərindən toplanmış reptili növlərinin – Suilanı (*Natrix tessellata* Laurenti, 1768), Aralıq dənizi tısbağası (*Testudo graeca* Linnaeus, 1758) və Xəzər nazikbarmaq gekkonunun (*Cyrtodactylus caspius* Eichwald, 1831) sümük və əzələ toxumasında yüksək toksiki təsirə malik olan mikroelementlərin toplanması və miqdarı öyrənilmişdir. Öyrənilmiş mikroelementlər aşağıdakılardır: mis *Cu,* nikel *Ni,* qurğuşun *Pb,* kadmium *Cd* və sink *Zn.* Məlum olmuşdur ki, öyrənilən 5 mikroelementin hər biri ən çox Xəzər nazikbarmaq gekkonunun bədənində toplanmışdır. Qeyd olunmalıdır ki, Xəzər nazikbarmaq gekkonu öyrənilən növlər arasında ən kiçik və funksional cəhətdən ən aktiv növdür. Bu növün neft və qaz quyularının ətrafında, antropogen və texnogen çirklənməyə məruz qalmış ərazilərdə daha çox yayılması onun plastikliyini və toksiki mikrolelementlərə qarşı tolerantlığını göstərir.

Açar sözlər: *Texnogen, deionizə edilmiş su, spektrometr,mikroelement, sümük və əzələ toxuması, biomüxtəliflik, urbanlaşma*

Изучение накопления и количества высокотоксичных микроэлементов в костной и мышечной тканях рептилий на урбанизированных территориях Апшерона

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Цель этого исследования заключалась в изучении влияния загрязнения окружающей среды на устойчивость рептилий, путем определения количества токсичных микроэлементов, накопленных в их костной и мышечной тканях и сравнения этого количества со стандартными средними. Накопление и количество высокотоксичных микроэлементов определялось в костной и мышечной тканях водяной змеи (*Natrix tessellata* Laurenti, 1768), Средиземноморской черепахи (*Testudo graeca* Linnaeus,1758) и Каспийского тонкопалого геккона (*Cyrtodactylus caspius* Eichwald, 1831), выловленных на урбанизированных территориях Апшеронского полуострова. Изученными микроэлементами являлись: медь (Cu), никель (Ni), свинец (Pb), кадмий (Cd) и цинк (Zn). Показано, что наибольшая концентрация каждого из 5 изученных микроэлементов была отмечена в теле Каспийского тонкопалого геккона. Следует отметить, что Каспийский тонкопалый геккон - самый маленький и наиболее функционально активный среди изученных видов. Широкое распространение этого вида вокруг нефтяных и газовых скважин, в районах, подверженных антропогенному и техногенному загрязнению, указывает на его пластичность и устойчивость к токсичным микроэлементам.

Ключевые слова: *Техногенный, деионизированная вода, спектрометр, микроэлемент, костная и мышечная ткани, биоразнообразие, урбанизация*