The Impact of the Ecological Situation on Blood Parameters of Pigeons

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Abstract--- The authors of the research studied the ecological situation in Almaty, Kazakhstan, using rock pigeons as bioindicators. Pigeons were captured in three areas of the city characterized by different ecological conditions. They measured the content of heavy metals (lead and cadmium) in the feathers and internals of the birds. They determined the morpho-functional peculiarities, as well as the hematological and biochemical blood parameters of the species. The study of hematological blood parameters revealed an increase in the number of red cells in the blood of birds from areas having an unfavourable ecological situation. The biochemical blood analysis of birds from areas having an unfavourable ecological situation showed an increase in the glucose and urea levels in blood serum, which can be used as a new biomarker for studies of bioindicators on the example of rock pigeons.

Keywords---- Heavy metal contamination; Bioindicators; Columbalivia; Hematological blood parameters

I. INTRODUCTION

The problem of environmental pollution is one of the most important problems for mankind. Areas with a high anthropogenic load (big megapolises), as well as areas where heavy and light industry enterprises are located are most susceptible to contamination. The mining and chemical industries are important elements of the economy of the Republic of Kazakhstan; however, they have a great adverse effect on the ecological situation in the country [27].

Environmental contamination with heavy metals (copper, iron, manganese, zinc, chromium, mercury, lead and cadmium) is one of the most urgent problems. Lead and cadmium are highly toxic and have acute and chronic effects on human health and the environment. The content of these metals in food, drinking water and the atmosphere must be strictly controlled. Lead belongs to Class 1 hazardous substances and is included in the WHO list of priority pollutants. The primary sources of lead inflow include mining, smelting, production and processing of recycled materials, the use of ethylized fuels and lead paints, as well as the use of lead in plumbing systems [32]. Lead enters living organisms mainly from the atmosphere and soil. It has a highly toxic effect, including on the nervous, excretory, immune, cardiovascular and reproductive systems [21]. At the molecular level, it affects various enzymes, as well as cell membranes, cell receptors, and DNA transcription mechanisms [4].

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Environmental pollution by cadmium is caused by the use of combustible minerals, work with metalcontaining ores, as well as garbage burning [24]. Like lead, cadmium has acute and chronic toxicity, which leads to impaired function of the nervous system, kidneys, liver, and blood formation. In addition, cadmium affects cell proliferation and apoptosis, interacting with the mechanisms for DNA repair and the formation of reactive oxygen species [25].

Lead and cadmium are cumulative toxins that accumulate in the soil, water, plants and other living organisms. In the body of vertebrate animals, lead accumulates primarily in the bones and teeth [4]. Although cadmium mainly accumulates in these animals' liver and kidneys, it can be found in all internals [24].

Due to their bioaccumulation property, the content of heavy metals in animals' bodies often directly affects the content of these metals in the environment. The use of bioindicators best reflects the ecological situation in a particular area as compared with the direct measurement of abiotic environmental pollution [2, 14]. A large number of studies focus on the accumulation of heavy metals in the bodies of various bird species, including such synanthropic species as *Passerdomesticus, Corvussp.*, *Picapica*, etc. [8]. The rock pigeon (*Columbalivia*) is one of the most common species used as a bioindicator of contamination with heavy metals [3, 6, 12, 22, 23]. Rock pigeons are typical synanthropes. Seeds, berries of plants and food leftovers are the primary sources of food for them. Rock pigeons are tied to their nesting sites and have a fairly long lifespan [3, 26].

The aforementioned makes rock pigeons a suitable species for the assessment of the effects of heavy metals on living organisms. Besides food, the primary sources for the accumulation of heavy metals in the bodies of pigeons include atmospheric air, as well as the ingestion of stones and pieces of asphalt contaminated with lead micro-particles [22].

Although there have been numerous studies of bioindicators on the example of rock pigeons, most of them are only aimed at measuring the content of heavy metals in various internals of these birds. The introduction of additional parameters for assessing the state of bioindicator species allows determining the amount of ingested toxic substances, as well as the possible long-term effects on other species, including humans. The authors of the article determined the content of lead and cadmium in the internals and feathers of rock pigeons captured in several districts of Almaty. In addition, they carried out comparative analysis of the hematological and biochemical blood parameters of the birds.

II. RESEARCH METHODS

Research place and time

The authors of the aricle examined rock pigeons (*Columbalivia*) living in three zones of Almaty that differ in ecological situation (Figure 1) from July to September 2018.



Figure 1: Almaty Map with Observation Zones (Google maps)

Zone 1. The eastern part of the city, the submontane area, the well-ventilated Kok-Tobe zone, the Zhaskanat microdistrict (hereinafter, Kok-Tobe) is marked by the most favourable ecological situation (control);

Zone 2. The south-western part of the city, the Tastak microdistrict;

Zone 3. The north-western part, the area of Almaty-1 Railway Station is marked by the most unfavourable ecological situation.

Capture and sampling of animals

A total of 90 pigeons between one and three years old – 30 birds in each study zone – were captured in the evening while they were sleeping in attics and roofs to eliminate injury and emotional stress. The birds were transported to the place of study in cages covered with dark cloth. When the birds got adapted to the standard living conditions and were prepared for research within 2-3 days, they were used for further experiments. The authors of the article carried out visual examination, which included a description of parameters, in particular, the birds' appearance, overall condition, body mass and length. The body mass was measured on an electronic scale with an accuracy of up to 0.0001 g. The length of the birds' bodies was measured by a ruler with an accuracy of up to 1 mm.

For the acquisition of blood samples, the birds were anesthetized by intraperitoneal administration of sodium thiopental solution (1.5 mg / kg). Blood samples were taken from the brachial or axillary artery. An incision, 2-3 cm long, was made on the skin from the elbow to the shoulder. After exposing and legating an artery, a polyethylene cannula, 1-2 mm in diameter, was inserted through the puncture. Sometimes the researchers had to additionally pierce the incision with a 0.1% procaine hydrochloride solution, because due to the strong reactivity and increased vascular tonus of pigeons, the lumen of the artery was so severely narrowed that it was difficult to push the cannula towards the shoulder, although the birds underwent anesthesia and slept. After obtaining blood samples, the researchers collected feathers and samples from the internals (kidneys, liver and spleen).

The specimen of the biological samples of pigeons' internals (kidney, liver, spleen) carefully collected from their bodies and previously cleared of blood clots were placed in a polyethylene package and stored in a refrigerator at a temperature of -10^{0} . Each sample weighed 500 mg (0.5 g). The acquisition of feather samples was carried out by symmetrically cutting off most of the overlying secondary flight feathers from the right and left wings – no less than 1000 mg (1 g). The resulting biomaterials of internals and feathers were further used to determine the content of heavy metals.

The work was carried out at the base of the Institute of Human and Animal Physiology of the Ministry of Education and Science of the Republic of Kazakhstan. For experimental purposes, the birds underwent anesthesia in accordance with the European Charter Treaty on the Humane Treatment of Laboratory Animals and the Rules for conducting medical and biological experiments, preclinical (non-clinical) and clinical research, as well as the Requirements for preclinical and clinical bases (Order No. 141 of the Minister of Health of the Republic of Kazakhstan of April 2, 2018).

Determination of heavy metals

The content of heavy metals in the feathers and internals (liver, spleen, kidneys) was determined by the atomic absorption method using a Hitachi atomic absorption spectrophotometer (AAS, Hitachi, Ltd., Japan) as described in [30]. To analyze the biomaterials, the researchers prepared the samples in a muffle furnace by the dry ashing method, gradually increasing the temperature to 450°C and extracting the ash at this temperature within 8-9 hours. The resulting ash was dissolved through heating in a 1M solution of nitric acid. The sensitivity of the technique was $0.1 \,\mu\text{g}$ / ml and $0.005 \,\mu\text{g}$ / ml for lead and cadmium, respectively.

Analysis of hematological and biochemical blood parameters

The Sysmex KX - 21 hematological analyzer (XN; Sysmex, Kobe, Japan) was used to determine the hemoglobin content and hematological parameters in the obtained samples. To determine the biochemical blood parameters (total protein, urea, glucose), the authors of the article used the BS200 Mindray biochemical analyzer (Mindray, Shenzhen, China) [5, 10, 13, 17]

Statistical analysis

The data is presented in the form of mean and standard deviations (SD). The authors used Version 6 STATISTICA software (Dell) to process the statistical research results. To ensure statistical reliability, they carried out one-way analysis of variance. The research results were viewed as reliable when p < 0.05 (unless stated otherwise).

III. RESULTS

Determination of heavy metals in internals

When analyzing the content of lead and cadmium in different internals of pigeons (feathers, spleen, and liver), the authors of the article recorded significant differences in the accumulation degree of these metals (Table 1). There were differences both across the internals and across the zones where pigeons were captured. The concentration of lead and cadmium increased in the internals of the pigeons as the unfavourability of the ecological situation increased from the lowest (in the Kok-Tobe zone) to the highest (the Almaty-1 zone). Lead was effectively accumulated in all the studied internals of the birds. The authors of the article observed similar dynamics for cadmium (Table 1). Interestingly, the accumulation degree of cadmium was lower in the kidneys than in the liver.

Table 1: Content of Heavy Metals in Internals of Rock pigeons from Different Areas of Almaty (mg/kg \pm

	St		Pb		Cd		Pb		С	Р	C	Р		С
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							0.		0	0		0		0
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	Та		0.1		0.0		0.		0	0	0	0		0
stak		22 *		19*		089*		.019*		.076±*	.017±*	.083±	.007±*	
microdist	rict		±0.		±0.		± 0		±	0	0	*		0
		001		004		.001		0.001		.047	.001	0.001	.001	
	Al		1.3		0.0		1.		0	1	0	1		0
maty 1		80 *		58 *		146 *		.031 *		.194 *	.028 *	.228 *	.010 *	
			±0.		±0.		± 0		±	±	±	±		±
		101		002		.094		0.002		0.073	0.002	0.110	0.002	
Note * reliable when p< 0.05														

SD wet weight)

Determination of morphological peculiarities of rock pigeons

The authors of the article carried out visual examination of the captured birds, which included a description of parameters, in particular, the birds' appearance, overall condition, body mass and length. Table 2 presents the results of the examination.

Table 2: Mass, Body Length and Feather Color of Pigeons Captured in Different zones of Almaty

Parameters	s	Study zones of Almaty					
	-	Kok-Tobe	Tastak microdistrict	Almaty-1			
Mass (g)		339±16	319±18	279±17			
Body leng	th (mm)	209 ± 3	197±3	193±3*			
Feather	Brown	not applicable	3	not applicable			
color (%)	Red	6	not applicable	not applicable			
	Gray	93	65	39			
	Black	1	32	61			
Note* relia	able when p< 0.05	·					

The average body mass and height of birds differed across the zones. The highest and lowest values of body mass and height were observed in Kok-Tobe and the area of Almaty-1 Railway Station, respectively. The body mass of the birds tended to decrease as the tensions of the ecological situation increased. With respect to the length of the birds' bodies, the authors of the article observed similar trends.

The pigeons used in the research had four morphs: gray, black, red and brown feather colors. Pigeons with a gray morph prevailed (93%) in the most ecologically favourable zone (Kok-Tobe). As the city's ecological situation deteriorated, the number of pigeons with black feathers increased (Table 2). This data complies with literature data describing that the number of pigeons with dark feathers increases in parallel to the increase in the urbanization degree [1].

Besides, there were differences in the overall condition and appearance of pigeons, depending on the ecological situation in the regions where the birds were captured. The pigeons captured in the Kok-Tobe microdistrict were relatively calm, with clean radiant feathers and a moderate appetite. The pigeons captured in the north-western part of Almaty-1 Railway Station were disturbed, drank a lot of water and ate frequently. Their feathers were faded and uneven.

Hematological parameters of studied birds

To assess the impact of the accumulation of lead, cadmium and other heavy metals in the bodies of the studied birds, the authors of the research studied their hematological and biochemical blood parameters. The analysis of the pigeons' blood parameters in the conditions of the megalopolis of Almaty revealed a tendency of changes in the birds' blood parameters, depending on their habitat (Table 3).

Parameters	Kok-Tobe	Tastak microdistrict	Almaty-1
Red blood cells, million/mm ³	3.46±0.20	3.72 ±0.23*	3.96 ±0.25*
Hemoglobin, g/L	13.50±0.50	13.90±0.40	14.20±0.50
White blood cells, thousand/ mm ³	18.91 ±0.20	19.45 ±0.30	32.56±0.50*
Glucose, mmol/L	6.80±0.25	7.80±0.35*	8.20±0.40*
Total protei, g/L	52.6±2.5	44.9±3.0	37.8±2.0*
Urea, mmol/L	1.83±0.10	3.78±0.23*	4.09±0.20*
Note * reliable when $p < 0.05$		·	

Table 3: Blood Parameters of Pigeons Captured in Different Zones of Almaty

The number of red blood cells and the concentration of hemoglobin in the blood of the studied pigeons were in the range of values given in the literature [16, 29]. The significant increase in the number of red blood cells is due to the increase in the pollution of the birds' habitat. Hemoglobin levels were marked by a similar tendency. It is likely that the observed reaction is compensatory and indicates the adaptive capabilities of synanthropic pigeons in a megalopolis.

The examination of white blood cells in the pigeons' peripheral blood revealed an increase in the total number of white blood cells, depending on the increase in the unfavourability of the ecological situation. In the ecologically favourable zone of Kok-Tobe, the total number of white blood cells was in the range of 18890-18930 in 1 mm³. Interestingly, this value is a little lower than that noted in a number of studies that determined the number of wild and domestic pigeons' white blood cells [28, 29]. Oppositely, the birds captured in the area of Almaty-1 Railway Station showed significant leukocytosis.

Biochemical blood parameters of studied birds

To determine the overall health condition of the birds, the authors of the article carried out biochemical studies of peripheral blood samples. The glucose levels of the birds in the control group was in the range of regular values. Nevertheless, as the tension of the ecological situation increased, the researchers observed an increase in the blood glucose levels up to a maximum of 8.60 mmol / L, i.e. by 12.4% higher than that of pigeons from Kok-Tobe (Table 3). The opposite effect was observed in the total protein levels in blood serum. The pigeons captured in the zone of Almaty-1 Railway station showed the most pronounced decrease in the total protein levels (Table 3). Although the total protein levels of this group of pigeons was within the standard range for birds, it was statistically much lower than that of pigeons from the Kok-Tobe zone. The analysis results are consistent with the results of previous studies [9].

An important indicator of the renal function implies the determination of nitrogen metabolism and watersalt metabolism. For mammals, the primary indicator of the renal function presupposes the determination of the urea levels in blood serum. For birds, however, uric acid is the final product of nitrogen metabolism. While a number of authors believe that urea analysis has no diagnostic value for birds, others observed an increase in urea in the blood of birds during shocks and stress, as well as dehydration of the body [15, 18]. The authors of the article analyzed the presence of urea in pigeons' blood plasma and found that the urea concentration in the blood of pigeons captured in the Kok-Tobe microdistrict was relatively low. As the tension of the ecological situation increased, the urea concentration more than doubled.

IV. DISCUSSIONS

Pigeons living in different parts of Almaty differ significantly both in the content of heavy metals in their internals and in their morphological peculiarities, as well as the hematological and biochemical blood parameters, which reflects the overall anthropogenic load on the study areas. Similar results were previously obtained in other studies where rock pigeons were used as bioindicators [3, 6, 12, 23]. Pigeons captured in the Kok-Tobe microdistrict located in the submontane zone of Almaty showed the lowest content of heavy metals in their internals and feathers. Nevertheless, this indicator was higher for pigeons captured in the Tastak microdistrict. The pigeons caught in the area of Almaty-1 Railway Station had the highest content of heavy metals. The area of Almaty-1 Railway Station is marked by an increased anthropogenic load, including heavy traffic and urban density. Besides, it is located in a closed depression, an area with atmospheric static conditions. These factors apparently affect the accumulation of particles containing heavy metals in the atmosphere and soil and, later, in living organisms.

In most studies, the accumulation of heavy metals in birds' bodies is determined by their content in feathers and (or) internals. The collection of feathers is the least invasive, so they serve as a convenient source of material [20]. Depending on the method of processing feathers (whether the material is washed before the analytical analysis or not), it is possible to assess both the endogenous and exogenous accumulation of heavy metals. The exogenous accumulation occurs due to the sedimentation of particles containing heavy metals onto feathers [14]. For more comprehensive assessment of the ecological situation in the study areas, the authors of the article combined the use of feathers and internals. They analyzed the content of lead and cadmium in pigeons' liver and kidneys, as well as the spleen, which turned out to effectively accumulate both lead and cadmium. It is known that lead effectively binds to red blood cells, significantly destabilizing their membranes [4]. Lead accumulates in the spleen, where red blood cells are destroyed. Cadmium, in its turn, accumulates in many types of tissues, including the spleen. Increased cadmium levels in the kidneys are apparently associated with the transfer of cadmium ions together with metallothionein proteins from the liver to the kidneys, as well as other similar mechanisms [31]. Nevertheless, there is a limited amount of data on the molecular mechanisms of the accumulation of heavy metals in the bodies of birds, in particular, rock pigeons.

The accumulation of heavy metals can cause poisoning for birds. Well-defined signs of birds' poisoning include disorders of the nervous, digestive and reproductive systems [11]. The studied birds differed in their overall condition and behaviour, but no signs of acute poisoning were observed. Interestingly, the analysis of hematological parameters did not reveal signs of anemia and a decrease in the number of red blood cells typical of the late stages of lead poisoning. This research outcome is consistent with the observations published by Elezaj and co-authors [9]. The authors of the article recorded a tendency to an increase in the number of red blood cells and hemoglobin as the ecological situation deteriorates, which is probably the body's reaction to hypoxia that characterizes urbanized areas. Nevertheless, there is nearly no research on the oxygen levels in the blood of synanthropic bird species. Besides, the analysis revealed leukocytosis in the blood of birds living in the Almaty-1 area having unfavourable ecological conditions. According to a number of authors, animals' leukocytosis is associated with stress; however, this factor is hardly reliable due to the differences in the response of hematological parameters for different animal species, depending on different types of stress [7]. Besides, there is insufficient data on the level of white blood cells of nondisturbed animals. The comparison of the level of cortisol or other stress-related factors for animals from different zones could be an alternative method for stress assessment. Nevertheless, the cause of leukocytosis in the blood of birds living in the area of Almaty-1 Railway Station could be hypodynamia due to the lack of active flights, as well as parasitic and other infections.

The study of biochemical blood parameters revealed a number of differences in the indicators for pigeons living in areas with favourable (Kok-Tobe) and unfavourable (the Tastak microdistrict and the zone of Almaty-1 Railway Station). As the ecological situation deteriorated, the authors of the article noted a significant increase in the glucose and urea levels in the blood serum, as well as a decrease in the levels of total blood protein. Hyperglycemia of the birds studied was apparently caused by stress, which is noted for many vertebrates, including birds [19]; however, it could also be caused by metabolic disorders. A decrease in the levels of total blood protein may indicate a nutritional deficiency (a statistically significant decrease is observed for pigeons in the area of Almaty-1 Railway Station), as well as a lack of the liver function due to the accumulation of heavy metals. In addition, the authors determined the urea levels in the serum of the studied birds. Although urea is not the primary product of nitrogen metabolism for birds, the authors recorded an increase in the urea levels in the blood of birds during shocks and stresses, as well as dehydration of the body [15, 18]. It is necessary to point out that the accumulation of heavy metals in the liver and kidneys might affect their functioning.

The use of hematological and biochemical blood parameters as an additional method for assessing the state of bioindicator species can be used for the preliminary assessment of the effects of pollutants, on humans. The lack of data on the initial levels of hematological and biochemical parameters in the blood of bioindicator species can be viewed as a disadvantage of this method. Nevertheless, the comparison with the indicators of the reference group makes it possible to eliminate this disadvantage.

V. CONCLUSIONS

1. The authors of the article determined the dependence of the increase in the content of heavy metals in pigeons' bodies on their habitat. The eastern zone (Kok-Tobe) located in the submontane area was characterized by the lowest content of heavy metals in the bodies of pigeons. Compared with the control group, a consistent statistically significant increase in the content of heavy metals was observed in the Tastak microdistrict and the area of Almaty-1 Railway Station. Starting from the south-west zone (Tastak) to the northwest (Almaty-1), there were zones marked by increasing accumulation of toxicants in the body of pigeons. The number of toxicants significantly differed from the indicators of the control group of birds. Lead effectively accumulated in all the studied internals of birds, while cadmium was found in all the studied internals, except for the liver.

2. The morphological (body mass, height, and color of feathers) and hematological parameters of pigeons are very sensitive to the effects of ecological factors, which is caused by the increase in the content of heavy metals. These parameters can be used as biomarkers of the ecological situation in the studied urban areas.

3. The determination of the biochemical parameters of blood, such as the levels of glucose, total protein and urea, may be an additional factor in the assessment of the ecological situation of the study areas. This panel can be extended for more comprehensive assessment of the physiological condition of bioindicator species.

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