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Biochemical Changes of the Organism of Apodemus flavicollis (Rodentia: Muridae) Under Conditions of Environmental Anthropogenic Pollution by Heavy Metals in Northern Areas of Ukraine

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Abstract. The present research dedicates the integral assessment of biochemistry indexes of nature populations of rodents under conditions of environment pollution by heavy metals. The raised content in soils of mobile forms Pb, Cd, Cr, Ni and Co was revealed on distance of 500 m to the South-West from Tripillya Thermal Power Plant (Kyiv region, Ukraine). That's considerably (3–5 times) exceeds levels for territory of Kaniv Nature Reserve (Cherkassy region, Ukraine). Territory of National Nature Park "Holosiivsky" (Kyiv, Ukraine) characterized by rather increased content of active form of researched heavy metals especially Pb. Increase of the concentration of diene conjugates (3–7 times) and malonic dialdehyde (2–4 times) in yellow-necked mouse liver (*Apodemus flavicollis*) of under pollution by heavy metals has been discovered. Insignificant increasing of content of Schiff basis in liver cells of rodents in region of impact of Tripillya TPP (in 2 times in spring and in summer, in autumn – in 2.5 times) was detected. Seasonal dynamics of the maintenance of lipid peroxidation has been revealed. The registered changes of biochemical indicators testify about presence ecological-biochemical stress in an organism of the yellow-necked mouse in the district of influence of Tripillya TPP.

Key words: heavy metals, *Apodemus flavicollis*, lipid peroxidation, pollution.

Introduction

Man-caused chemical pollution of ecosystems is an actual problem at the present. Concentration of industrial enterprises in big cities close by small cities and villages makes difficult to monitor its negative impact on environment (AIJUAN *et al.*, 2011; AL-SHAYEB & SEAWARD, 2001; BAKER & CHESNIN, 1975). Intensive production results in releasing of considerable amounts of heavy metals that extend in atmosphere and involve in circulation of elements. Their ranges in many times exceed the natural amount of metals in different levels of biosphere. Lead, mercury, cadmium, copper and zinc widely distributed in biosphere among man-caused metals. They are releasing mainly on burning of mineral fuel. The main products that are forming on burning of coal are ashes, nitric oxides, sulfur anhydride, vanadium pentoxide, carbon oxide and benzapilene. In coal ashes concentrated by heavy metals the maintenance of Cu is 35 mg/kg, Zn – 85 mg/kg, Pb – 20 mg/kg, Cr – 123 mg/kg, Ni – 62 mg/kg, Cd – 4 mg/kg,

Co – 114 mg/kg (KABATA-PENDIAS & PENDIAS, 1984). Anthropogenic emission is so strong that on burning of bituminous coal much more metals are dispersing in atmosphere than human extracts them from deposits (LEYGONIE, 1993). In snow cover nearby big thermoelectric power stations, the regions of pollutions form in radius 10–20 km. Winds contribute to formation of dispersion halo (MAATOUG *et al.*, 2007). Kyiv region is one of the most contaminated in Ukraine. 443 industrial enterprises are exposing as sources of air pollution in Kyiv region. Among areas of region the most pollutants are in Obuchiv district, amount of its industrial discharges arranges from 83000 ton per year. Among them the strongest pollutant is Tripillya TPP – over 21000 ton per year. It forms 84% of all discharges in atmosphere by industrial enterprises in Kyiv region (BESKOROVAYNIY, 2011).

Because of it the clarification of mechanisms and parameters of impact on environment by large plant had placed in densely populated region (e.g. thermoelectric power station) is actual. Small mammals are the most convenient objects for such investigations as long as they are one of some animals that inhabit transformed ecosystems directly next to human. Thanks to short life circle forests rodents have time to reflect the impact of environment on their organism (SALDIVA & BOHM, 2002). It is known that the activation of lipid peroxidation processes of cellular membranes is one of display of toxic heavy metals' impact (BRIGANTI & PICARDO, 2003). Liver is most important organ of detoxication. The present research dedicates the integral assessment of biochemistry indexes of nature populations of rodents under conditions of environment pollution by heavy metals.

Material and Methods

Study area

Researches were conducted on nature population of yellow-necked mouse (*Apodemus flavicollis* Melchior, 1834), which is living on territories with different level of anthropogenic pollution. Environment of this species closely concerned with soil bedding. Therefore, *A. flavicollis* may come as a biomonitor of man-caused pollution of environment. Three areas with different level of anthropogenic loading were chosen for comparative analysis. The territory of Kaniv Nature Reserve (Cherkassy region, Ukraine) that is the nature reserve of highest protection status was chosen as the least disturbed landscape. Territory of National Nature Park "Holosiivsky" (Kyiv, Ukraine) is similar on phytocenosis structure (hornbeam forest) to Kaniv Nature Reserve. It feels direct and indirect impact of human activity starting with disturbing factor and ending with air pollution by discharges. The zone of impact of Tripillya TPP (Obuchiv district, Kyiv region) where are the small hornbeam plantation adjusts very close to the South-West edge of industrial area of thermoelectric power plant (about 500 meters). Industrial area rather works on bituminous coal getting under the torch of dispersion (PHATEEV & PASHCHENKO, 2003).

Methods

The results of control catches of *A. flavicollis* on chosen areas were used for material. The control catches were conducted according to generally accepted methods in spring, in summer and in autumn 2012 (CRISTALD & MASCARZON, 1990; ZADYRA & LUKASHEV, 2012). General amount of individuals were analyzed is 89 specimens. Among them 36 specimens were from Kaniv Nature Reserve, 23 specimens – from National Nature Park "Holosiivsky", 30 specimens – from Tripillya TPP. The collection of material was conducted on registered spots by square (3025 m^2) which are chosen for assessment of density of individuals' distribution.

The heavy metals content in upper soil stratum of 5 cm and in liver were determined by flame atomic-absorption spectrophotometer C115-M1 (SELMI-CHROM, Ukraine) with heavy hydrogen corrector of the background and computer complex CAS-120. The content of acid-soluble metals and their exchange fraction in soil have been analyzed by extraction by

acetate-ammonium buffer (pH 4.8) according to standard methods (ALLEN, 1989). The content of metals in recoveries was estimated in mg per kg of masses of air dried recoveries (HANNA, 1964).

The portion of products of lipid peroxidation was estimated on maintenance of primary (diene conjugates – DC), second (malonic dialdehyde – MDD) and terminal (Schiff bases – SB) metabolites. During the lipid peroxidation on stage of formation of free radicals in molecules of polyunsaturated higher fatty acids the system of conjured binary connections is forming. As a result conjugated dienes are forming. It is accompanying by appearance of new maximum in spectrum in absorption on wave of 223 nm (BRIGANTI & PICARDO. 2003).

Since conjugated dienes differ by intensive absorption in UV specters spectrophotometric methods was used for identification of DC. Identification of contents of MDD was conducted by spectrophotometric methods (SHIMADZU, UV mini-1240 model, Japan). MDD was detected in reaction with 2-thiobarbituric acid (TBA) (ORECHOVICH, 1977). Calculation of concentration of TBA-active compounds was conducted taking into account the value of coefficient of molecular extinction of malonic dialdehyde on 552 nm. Content of DC and MDD were calculated in nmol per mg of sample. Content of Schiff basic was calculated in conventional units per 1 ml of sample that was detected by spectrofluoremetric method (the length of excitation wave is 360 nm; the length of emission wave is 420 nm; SHIMADZU, RF-1501 model, Japan) (BRIGANTI & PICARDO, 2003).

The samples for definition of products of lipid peroxidation were obtained by preparation of 10% liver homogenate counting on 1 gm of liver tissue per 10 ml of 0.9% solution of sodium chloride (physiological solution).

Because of misfit of normal distribution of some sampling variative ranks of investigated indexes the average size of products of lipid peroxidation and content of metals were presented as median (*Me*). Standard declination of median (SD_{Me}) was used as index of variation. Man-Whitney's U-Index was used for comparative characteristic of sampling parameters. For all statistical analysis the STATISTICA 6.0 statistical package was used (StatSoft, 2001).

Results and Discussion

Soil is a specific component of biosphere as long as it not only accumulates contaminants but appears as natural buffer that control the transfer processes of chemical elements and compounds from lands to atmosphere, hydrosphere and living organisms (PHATEEV & PASHCHENKO, 2003). The lifetime of contaminants' being in soil is too much longer than in other components of biosphere. Therefore, contamination of soil particularly by heavy metals is practically perpetual. Compounds of heavy metals that are falling on ground surface together with atmospheric precipitates are accumulating in soil thickness especially in upper humus levels and slowly are removing under leaching, erosion, deflation and extraction by plants. The period of semi-extraction for Pb from soil is a few thousand years, for Cd is to 1.1 thousand years, for Zn – to 0.5 thousand years (ALLOWAY, 1990). It may suppose that under permanent source of air contamination gradual increase of heavy metals' content in upper level of soil will occur. It may serve as index of contamination of researched territory (GROMOW & EMELINA, 1994). Examination of content of heavy metals (Pb, Cd, Cr, Ni, Co) in soil samples displays the significant differences of selected regions under exchange fraction (Table 1).

Differences of soil in researched regions under content of acid-soluble fraction of heavy metals were founded statistical insignificant and were corresponded to value of regional clarke typical for forest-steppe zone of Ukraine (PHATEEV & PASHCHENKO, 2003). Excess of normative indexes of boundary permissible concentrations for arable soil have not founded. However, the analysis of exchange fraction of heavy metals has showed what

Table 1. Content of exchange fraction of heavy metals in samples of upper soil level in researched territories

Content of metals, mg/kg / Territory of investigation	Pb		Cd		Cr		Ni		Co	
	Me	SD_{Me}	Me	SD_{Me}	Me	SD_{Me}	Me	SD_{Me}	Me	SD_{Me}
Kaniv Nature Reserve (Cherkassy region, Ukraine)	< 0.19	–	< 0.002	–	0.07	0.03	0.08	0.07	0.05	0.04
National Nature Park "Holosiivsky" (Kyiv, Ukraine)	0.27	0.09	< 0.003	–	0.12	0.02	0.10	0.06	0.11	0.06
Region of impact of Tripillya TPP (Kyiv region, Ukraine)	0.34	0.05	0.03	0.00	0.17	0.14	0.30	0.05	0.25	0.01

its content in Kaniv Nature Reserve is the least. Soil at National Nature Park "Holosiivsky" characterized by increased content of Pb. In region of impact of Tripillya TPP the content of active forms of all researched heavy metals in soil are greatly (in 3–5 ones, $p<0.05$) exceeding the levels are typical for nature reserve territory. Thus it may affirm that the consistent increase in soil the part of biological available fraction of researched heavy metals was observed: Kaniv Nature Reserve < National Nature Park "Holosiivsky" < region of impact of Tripillya TPP. Increase of content of exchange fraction of heavy metals in soils at last two regions probably was conditioned by processes of atmospheric contaminants transportation and falling out (the presence of big city nearby with National Nature Park "Holosiivsky" and powerful Tripillya TPP). It will be showed that in every case the exceeding of normative indexes of boundary permissible concentrations of heavy metals for arable soils was not determined. It may understand by existing environmental standards as a satisfactory ecological situation in all researched territories.

The same results of pollution of soils by heavy metals at territories were adjacent to Tripillya TPP were obtained by other researchers (KRASOVSKIY *et al.*, 2005). Estimation is showing that thanks to irregular dispersion of smoke fumes discharges of station 26.3–36.0 ton per km^2 of man-caused dust are falling out in South-East direction annually. Because of it soils are enriching by compounds of Cd, Pb, Cr. Thus it may be contend that region around Tripillya TPP under content of heavy metals (Pb, Cd, Cr, Ni, Co) is a man-caused contaminated territory. Territory of National Nature Park "Holosiivsky" characterized by rather increased content of moveable form of researched heavy metals especially Pb.

In National Nature Park "Holosiivsky" significant increase of density of yellow-necked mouse's population during different seasons of year: in spring – 5 individuals per hectare, in summer – 6 individuals per hectare, in autumn – 8 individuals per hectare. In Kaniv Nature Reserve and in region of impact of Tripillya TPP the highest density was observed in summer (17 individuals per hectare and 16 individuals per hectare accordingly). The value of density was minimal in spring and in autumn: 5 individuals per hectare 8 individuals per hectare accordingly in Kaniv Nature Reserve; 6 individuals per hectare and 7 individuals per hectare accordingly on territory next to Tripillya TPP. Thus maximum density on most contaminated territory next to powerful TPP was observed in spring while minimum density is observed in autumn.

Analyzing the content of heavy metals in yellow-necked mouse's liver on researched territories the insignificant increase of content of Cu, Zn, Cr, Mn in region of impact of Tripillya TPP was detected. Thus the content of Cu and Cr in liver on 17% greater on contaminated

territory than on reserved territory, the content of Zn and Mn – on 22%. So the heavy metals are accumulating in yellow-necked mouse's liver from population in region of impact of Tripillya TPP. It probably may cause physiological disturbance in organism.

Biochemical changes in organism are one of most accurate indexes of direct impact of heavy metals (CHRISTINA & CHANG, 2005). The products of lipid peroxidation in homogenate of yellow-necked mouse's liver in natural populations were analyzed for confirmation of presence of biochemical indexes' changes. Analysis of content of products of lipid peroxidation in animals' organism from researched territories showed considerable differences ($p<0.05$) (Table 2).

Table 2. Content of products of lipid peroxidation in liver homogenate of natural populations of animals from researched territories

Territory of investigation	Season 2012	Diene conjugates, nmol/mg		TBA-active compounds, nmol/mg		Schiff bases, conventional units	
		Me	SD_{Me}	Me	SD_{Me}	Me	SD_{Me}
Kaniv Nature Reserve	spring	0.10	0.08	0.13	0.01	0.0017	0.0003
	summer	0.26	0.05	0.21	0.06	0.0019	0.0003
	autumn	0.25	0.04	0.21	0.03	0.0017	0.0002
National Nature Park "Holosiivsky"	spring	0.27	0.12	0.16	0.03	0.0017	0.0002
	summer	0.54*	0.03	0.25	0.03	0.0028	0.0002
	autumn	0.49*	0.05	0.27	0.05	0.0026*	0.0003
Region of impact of Tripillya TPP	spring	0.72*	0.07	0.42*	0.02	0.0030*	0.0004
	summer	0.73*	0.04	0.81*	0.16	0.0035*	0.0001
	autumn	0.72*	0.07	0.43*	0.09	0.0042*	0.0004

* – statistically significant differences ($p<0.05$)

As a result of investigation of diene conjugates' content in liver homogenate the increasing of these products in yellow-necked mouse on contaminated territory during the different seasons 2012 was determined. In spring it was in 7 times greater in region of impact of Tripillya TPP as against the nature reserve territory, in summer and in autumn it was greater in 3 times accordingly.

As deserve to point out in National Nature Park "Holosiivsky" the maximum concentration of DC was detected in summer while the content of primary metabolites had descended. As it is well known diene conjugates concern to toxic metabolites that has damage impact on enzymes, ferments and nucleic acids (KAPPUS, 1985). Therefore it may assume that content of DC depends on intensity of metabolism in organism. In our investigations the increasing of quantity of toxic product in individuals of yellow-necked mouse was detected during the year on territory next to Tripillya TPP.

The study of content of MDD had showed the increasing of its concentration near territory of Tripillya Thermal Power Plant during 2012 in comparison with reserved territory: increasing on 3 times had been recorded in spring, in summer – on 4 times, in autumn – on 2 times. Such increasing of MDD possibly closely concerned with entry of heavy metals with food. It's possible leads to affection of liver cells' membranes.

Important role in synthesis of prostaglandins, progesterone and other steroids belongs to MDD (BRIGANTI & PICARDO, 2003). The negative role of MDD is lying in lacing of lipids' molecules and deterioration of penetration of membranes. As a result the membrane becomes less labile. The processes concerned with change

of membrane surface (e.g. phagocytosis, pinocytosis, cell migration) were broken (ORECHOVICH, 1977).

Formation of Schiff bases is final product of lipid peroxidation as a result of interaction of secondary products of lipid peroxidation with proteins and phospholipids that contains amides (KAPPUS, 1985). The analysis of content of Schiff basis insignificant but statistically considerable exceeding of its concentration in liver of mouse was detected for region of impact of Tripillya TPP. The clear gradual growth of its content could to note during the vegetative season from 0.0030 conventional units in spring to 0.0042 conventional units in autumn. On reserved territories the content of Schiff basis in liver of animals was practically constant during the vegetative season. Such difference in dynamics of Schiff basis on territories with different level contamination of top-soil it is possible to explain by compliance of accumulation that product to more deep changes of oxide homeostasis (CHRISTINA & CHANG, 2005). Considerable increasing of that index mostly is the consequence of serious tissue affection.

It is well known that the entrance of toxic substances to organism of warm-blooded animals stimulates the generation of active forms of oxygen (BRIGANTI & PICARDO, 2003). Under disturbance or overcharge of molecular mechanisms of inactivation of these radicals intensification of processes of free-radical oxidation and accumulation of products of lipid peroxidation are possible.

Prohibition of these processes is carried out due to endogenous antioxidants – vitamin A, E and others (CRISTALD & MASCARZON, 1990). Accumulation of products of lipid peroxidation under conditions of toxic pollution is concerned with exhaustion of resources of endogenous protectors (DIXON & PAIVA, 1995). Disturbance of cell membranes' structure and ferment system of organism's metabolism that is appearance of intoxication characteristic is a consequence of these.

Conclusions

The results of this study show that content of heavy metals in soil and liver in population of yellow-necked mouse is the highest in region of impact of Tripillya TPP (most contaminated among researched territories) as against Kaniv Nature Reserve what is background territory. Considerable accumulation of products of lipid peroxidation in liver cells of rodents from Tripillya TPP area as against natural reserve territory in spring (diene conjugates are in 7 times; MDD is in 3 times), in summer and in autumn (diene conjugates are in 3 times; MDD is in 4 times and in 2 times) was stated. Insignificant increasing of content of Schiff basis in liver cells of rodents in region of impact of Tripillya TPP (in 2 times in spring and in summer, in autumn – in 2.5 times) was detected.

Thus exceeding of levels of maximum permissible concentrations for soil of researched territories was not detected but biochemical features of disturbance in organism of yellow-necked mouse from the natural populations were observed. Conformity of chemical composition of soils to the maximum permissible concentrations was not conforms to condition of prosperity of animal organism existence of which was concerned with paedosphere. Therefore it may conclude about absolute content of heavy metals in soil is not a marker of ecological conditions of environment. Recorded changes of biochemical parameters are sensible indexes of presence of ecological-biochemical stress for an organism under condition of pollution.

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