



Infrared spectroscopy and biochemical parameters of rat tissues under heavy metal poisoning conditions

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Abstract. The increasing level of anthropogenic environmental pollution and effective means to reduce the negative impact of xenobiotics on animal and human health is an urgent problem today. Considering this, the purpose of the study is to examine the effect of heavy metals on accumulation processes under poisoning conditions, and biochemical parameters in the body of rats. Analogue groups were formed of rats of the same age, gender, and body weight to conduct the study. Rats were poisoned with solutions of copper sulfate, zinc sulfate, cadmium sulfate, and lead nitrate for 14 days. Using the method of infrared spectroscopy, substantial differences in the spatial structure of protein components in intact and poisoned animals were established. The difference between the spectral characteristics of the examined tissues is clearly demonstrated by the statistical indicators of skewness and kurtosis. It was determined that poisoning of rats with copper, zinc, cadmium, and lead ions affects the course of glycolysis reactions and the tricarboxylic acid cycle, which leads to a likely increase in serum concentrations of lactate and pyruvate, oxaloacetate and α -ketoglutarate and a decrease in Malate content compared to intact rats. It was established that under the conditions of poisoning, there is also a substantial increase ($P < 0.05$) in the content of the examined heavy metals in the blood, liver, and kidneys. In animals poisoned with heavy

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metals, a decrease in the pool of free amino acids in the kidneys is observed. In particular, the content of aspartic acid, valine, glycine, tyrosine, and cystine (more than 1.5 times) in the kidneys of such rats decreases; alanine, leucine, serine, taurine, threonine, phenylalanine (more than 2.0 times), lysine – 3.4-4.9 times. Therewith, an increase in the level of isoleucine and methionine by 1.3-1.5 times, ornithine – by 1.8-2.1 times, and glutamic acid – by 4.4-5.3 times in rats of the experimental group compared to intact ones was identified. The results of the study can be helpful in the professional activities of doctors of veterinary medicine, toxicologists, biologists, and environmentalists and used to control the quality of livestock products, conduct toxicological studies, and analyse environmental objects

Keywords: copper; zinc; cadmium; lead; blood; internal organs

Introduction

A large number of various chemical compounds, including heavy metals, that enter the environment require research to establish their impact on the biota and find ways and means to reduce the negative impact on animal and human health (Cao *et al.*, 2018; Engwa *et al.*, 2019; Djordjevic *et al.*, 2019). Balali-Mood *et al.* (2021) investigated the impact of anthropogenic environmental pollution with heavy metals, toxic to all living things, which necessitates a thorough study and establishing the biochemical effect on the body individually and under complex impact conditions. Wallace & Buha (2020) determined the complex effects of heavy metals and pesticides on the body and their probable involvement in carcinogenesis. Still, their work lacks consideration of biochemical parameters and the use of infrared spectroscopy. The body has the ability to accumulate heavy metals at the cellular, tissue, and organ levels of the organisation of living matter, which is primarily due to the specific features of metal accumulation in various tissues and organs, and the action of protective mechanisms that limit their migration (Kumar & Sharma, 2019; Ohta & Ohba, 2020; Schaefer *et al.*, 2020).

Schaefer *et al.* (2020), showed that the constant increase in anthropogenic load on environmental objects in the form of chemical,

physical, and biological compounds has quite serious consequences, but they focused on toxicological aspects without investigating biochemical parameters and did not use the method of infrared spectroscopy. Targeted impact on one of the objects of the environment through a chain reaction of relationships in the biosystem can cause changes in the state of the other, which in turn is a real threat to the gene pool of all living beings and can lead to an increase in mutagenic pressure on the population of both animals and humans. Therewith, total pollution of atmospheric air, soil, drinking water, and food products with a wide range of xenobiotics can cause genetically determined pathologies manifested by congenital malformations, cytogenetic disorders in germ and somatic cells (Kumar & Sharma, 2019; Li & Saleem, 2022).

Ortiz *et al.* (2022) examined an environmental biota that is constantly in contact with a large a wide variety of chemical compounds. The authors note that not only substances that are part of food products and are contained in water and enter the air are essential for the body (proteins, lipids, carbohydrates, vitamins, macro - and microelements), but also those that do not represent nutritional value or, even in certain doses, are able to show toxic effects on the mammalian body being components of both

natural (essential oils, dyes, alkaloids, tannins) and artificial origin (preservatives, flavouring and aromatic additives, pesticides, and medicinal and hormonal preparations that were added during animal feeding), without investigating the effect on metabolic processes. Ohta & Ohba (2020) indicated that a large group of substances not involved in metabolism are called xenobiotics. Solving numerous problems due to the negative impact of xenobiotics on a living organism is extremely relevant and one of the priority tasks for humanity; however, the existing studies did not investigate the effect of heavy metals on metabolic processes and did not use the infrared spectroscopy method, as noted in the materials of this paper.

It is important to establish and examine specific molecular targets that ensure the selectivity of xenobiotics biotransformation and detoxification to understand their biological effect and the limits of the body's tolerance to them. As Stefanac *et al.* (2021) note, detoxification for organic compounds is associated with their chemical transformation, including partial oxidation of molecules, and in the case of metal ions, the neutralisation process consists in their deposition as part of specific functional groups and molecules. Aemere *et al.* (2020) report that this process occurs due to the specific features of the binding constants of metal ions to certain ligands.

Thus, the scientific literature does not fully cover the issue of animals poisoned with heavy metals, namely their effect on metabolism in tissues depending on the type of poisoning and the specific features of their effect on the content of other heavy metals, using the method of infrared spectroscopy and simultaneous monitoring of biochemical parameters.

Objective – to investigate how heavy metals affect the accumulation of substances under toxic conditions, and biochemical parameters in the body of rats.

Literature Review

Heavy metals can often interact with biological systems, losing one or more electrons and forming metal cations that are related to the nucleophilic centres of vital macromolecules. Several acute and chronic toxic effects of heavy metals affect various organs in the body. Gastrointestinal and kidney dysfunction, nervous and immune system disorders, skin damage, vascular damage, birth defects, and cancer are examples of complications of heavy metal toxicity. In particular, Kumar & Sharma (2019) indicate that simultaneous exposure to two or more metals can have a carcinogenic effect. However, they did not determine their effect on biochemical parameters and did not examine tissues using infrared spectroscopy, which is presented in this study. The fact that heavy metals are carcinogenic to mammals is an important aspect of chronic exposure. Although the exact mechanism is not fully understood, abnormal changes in the genome and gene expression are considered the main process. Carcinogenic metals such as arsenic, cadmium, and chromium can disrupt the synthesis and renewal of DNA. Kim *et al.* (2019), covered the toxic mechanism of action of heavy metals on the body, which functions in a similar way, usually through the formation of reactive oxygen species, inactivation of enzymes, and inhibition of antioxidant protection, yet infrared spectroscopy was not used.

Li *et al.* (2022) showed that some heavy metals cause toxicity according to a specific scheme and selectively bind to specific macromolecules. The involvement of metalloproteins (metal + carrier protein) by tissues is conducted by their fixing on specific membrane receptors, in combination with which they enter cells, where they are destroyed by lysosomal enzymes, and the metal is restored again and can be reused in metabolism. As is often the case, the implementation of biological functions depends on the interaction between ligand-binding residues

and metal ions, and the molecular mechanism involves the binding of metal ions to specific residues in proteins. Some of these amino acid residues are stored in the protein family, and they can create key stable interactions or play an important functional role. Each family has its own preserved residues, which are involved in protein recognition and metal ion binding. It can combine with cadmium with high affinity and play a role in detoxifying heavy metals and maintaining a stable state of major metal ions in cells. Among them are metallothioneins, which, due to their unique metal binding characteristics, can provide resistance to cadmium and contribute to the stable state of zinc.

Gazwi *et al.* (2020), investigated the formation of reactive oxygen species and release of cytochrome C, which may play a role in the hepatotoxic effects of heavy metal exposure. An analysis of male *Wistar* rats exposed to heavy metals showed intensive development of oxidative stress and activation of caspase-9, the initiator of apoptosis signalling.

Entering cells, heavy metals bind mainly to functional groups of proteins and others (for example, the formation of metallothioneins or compounds involved in biotransformation (Kim *et al.*, 2019; Aemere *et al.*, 2020; Samuel *et al.*, 2021), which may be related to detoxification mechanisms. Therewith, this is the cause of metabolic disorders and an explanation of the high toxicity of heavy metals (Saha & Paul, 2019; Proshad *et al.*, 2020). However, Kim *et al.* (2020) and Luo *et al.* (2020) note that the binding strength of heavy metal ions to the functional groups of various biopolymers may vary, which is a manifestation of the different toxicity of these toxicants.

The interaction of metals entering the cell can cause antagonism, synergy, and affect to a certain extent the content of individual metals in cells and, accordingly, in tissues. This is best demonstrated by researchers on the example of

plants, when the cadmium content in the soil is too high, there is an obstacle to the supply of iron to the plant, which causes growth retardation, chlorosis, and other symptoms similar to iron deficiency (Asad *et al.*, 2019; Kashif Irshad *et al.*, 2020). The described symptoms are caused by direct or indirect interaction between cadmium and iron. The toxic effects of cadmium can cause phosphorus deficiency or reduce manganese intake and prevent the intake and transport of several essential nutrients (such as calcium, magnesium, phosphorus, and potassium) and water by plants. In addition, Kashif Irshad *et al.* (2020) note that cadmium is able to enter the cell through carriers of basic elements. Due to the lack of specificity of these vectors, it is usually assumed (Engwa *et al.*, 2019) that it is absorbed by transporters of basic elements (such as zinc, iron, and calcium). The following research expands and deepens knowledge about the effects of heavy metals on the animal body.

Materials and Methods

The research was conducted during 2020-2022 based on the National University of Life and Environmental Sciences of Ukraine. Experimental studies were accompanied by the use of three-month old rats (males) with a similar body weight of 200 ± 15 g, kept in a vivarium under standard conditions. From animal analogues, five groups were formed: the first – control (intact), rats of the second, third, fourth, and fifth groups were orally administered solutions of copper sulfate, zinc sulfate, cadmium sulfate, and lead nitrate, respectively, in established toxicological doses. Intoxication of animals was conducted for fourteen days, then they were decapitated under ether anaesthesia, followed by the collection of blood samples and pieces of organs (liver, kidneys) for further research.

All work was conducted in accordance with the provisions of the European Convention for the protection of vertebrate animals used

for experimental and other scientific purposes (1986). The content of copper, zinc, cadmium and lead in blood, liver, and kidney samples selected for research was determined by atomic absorption spectrometric method using the absorption mode on AAS SpectrAA-55b, Varian Company (USA). Standard solutions of these metals were used as controls.

Infrared spectroscopy was used to examine the internal organs (liver, kidneys) of rats, which was performed on the infrared (IR)-Fourier spectrophotometer “Nicolet 380”, manufactured by Thermo electron corporation (USA). The obtained spectra were recorded in steps of 4 cm^{-1} using a device for light reflection from the sample, the working wavelength range was $650\text{--}4000\text{ cm}^{-1}$. The obtained absorption spectra were processed using specially developed programs – LabSolution IR, IR Tutor V1.1 Software Kit, MetRe-C (Smith B.C., 2016; Vinet & Anil, 2020).

Metabolites of the tricarboxylic acid cycle (malate, oxaloacetate, α -ketoglutarate) and glycolysis (lactate, pyruvate) in blood serum were determined by enzymatic methods (Vlizlo *et al.*, 2012). At the beginning of the biochemical analysis, blood serum was obtained by centrifugation on an MPW-352 centrifuge (Poland) with a rotation speed of 3000 rpm, for 15 min. Specific reagents of lactate dehydrogenase (LDH, EC 1.1.1.27), malate dehydrogenase (MDG, EC 1.1.1.37) from Reanal (Hungary) and oxidised and reduced forms of pyridine nucleotides from Serva (USA) were used for the analytical study of metabolites of intermediate carbohydrate metabolism (glycolysis, tricarboxylic acid cycle). The concentration of metabolites was determined spectrophotometrically on Specord M40 UV-VIS, manufactured by Carl Zeiss (Germany) at $\lambda = 340\text{ nm}$. Determination of the content of free amino acids in the kidneys was conducted by ion exchange chromatography on an amino acid analyser model AAA t-339m of Mikrotechna (Czech Republic).

Experimental data were processed using generally accepted methods of variation statistics. Statistical data calculations were performed using Microsoft Excel 2007.

Results and Discussion

The cumulative properties of heavy metals in the body of rats were examined. Figure 1 (A) shows the copper content in the blood, liver, and kidneys of rats. It was established that during their experimental poisoning, the content of this metal increased: in the blood by 1.4 times, in the liver by 1.6 times, and in the kidneys by 1.5 times compared to animals of the control group.

For poisoning experimental rats with zinc (Fig. 1 (B)), the content of the test metal increased as follows: in the blood by 1.5 times, in the liver – by 2.5 times, and in the kidneys – by 1.6 times, compared with the control group of animals. Poisoning of rats with cadmium ions led to an increase in its content: in the blood – by 27 times, in the liver – by 243, and in the kidneys – by 23 times compared to animals of the control group (Fig. 1 (B)).

In addition, the mechanisms of interaction of the examined heavy metals with each other in the rat body were examined. The results of experimental studies showed that in the blood of rats poisoned with copper sulfate, cadmium increased by 7.5 times and zinc by 1.8 times, compared with intact animals. In the liver of animals poisoned with copper ions, the content of cadmium increased by 6.9 times, zinc – by 1.8 times, and lead – by 1.4 times compared to intact animals. In the kidneys, cadmium content increased by 2.2 times, zinc and lead, respectively, by 1.5 times, compared with the intact group of rats (Table 1).

Poisoning of rats with zinc sulfate resulted in an effect on the content of heavy metals in all types of biological material studied. Thus, in the blood and liver of poisoned rats, the level of cadmium increased by 5.8 and 6.5 times,

respectively, the copper content remained unchanged, and lead increased only in the liver by 1.3 times (Table 2). Therewith, the content of

cadmium in the kidneys increased by 2.4 times, copper – by 1.2 and lead – by 1.7 times compared to intact animals.

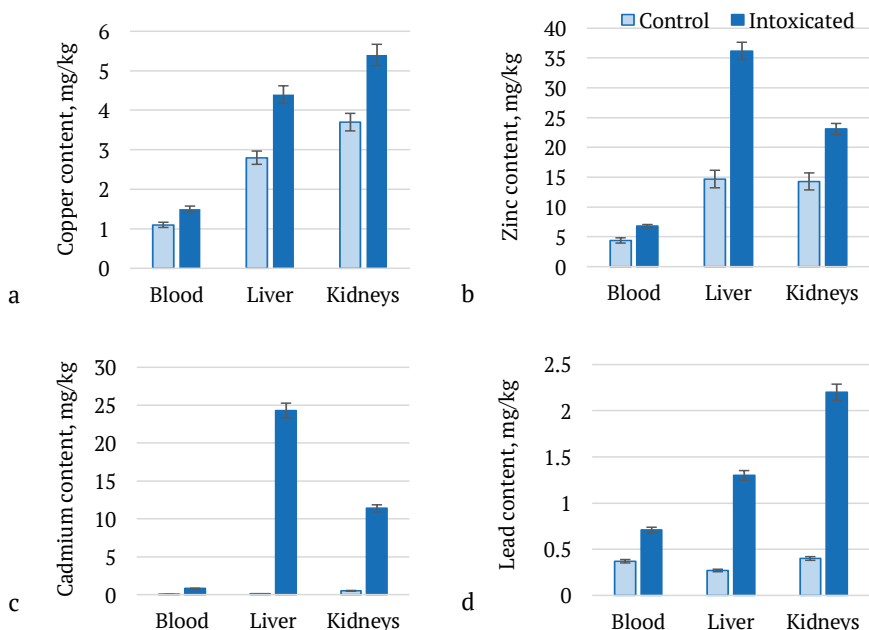


Figure 1. Content of heavy metals (a – copper, b – zinc, c – cadmium, d –lead) in the blood, liver, and kidneys of toxic rats

Notes: ($M \pm m$, $n = 8$), $*P < 0.05$, compared to the control

Table 1. Content of cadmium, zinc, and lead in the blood and internal organs of rats poisoned with copper sulfate, mg/kg ($M \pm m$, $n = 8$)

Metal	Intact rats	Poisoned rats
<i>Blood</i>		
Cadmium	0.04 ± 0.002	0.30 ± 0.02*
Zinc	2.39 ± 0.71	4.23 ± 1.12*
Lead	0.38 ± 0.02	0.43 ± 0.03
<i>Liver</i>		
Cadmium	0.14 ± 0.01	0.96 ± 0.04*
Zinc	18.74 ± 2.34	34.25 ± 3.17*
Lead	0.29 ± 0.02	0.40 ± 0.02*
<i>Kidneys</i>		
Cadmium	0.52 ± 0.01	1.15 ± 0.05*
Zinc	12.73 ± 1.82	19.24 ± 2.71*
Lead	0.43 ± 0.02	0.64 ± 0.03*

Note: $*P < 0.05$, compared to intact rats

Table 2. Content of cadmium, copper, and lead in the blood and internal organs of rats with zinc sulfate poisoning, mg/kg ($M \pm m$, $n = 8$)

Test metal	Intact rats	Poisoned rats
<i>Blood</i>		
Cadmium	0.04 ± 0.002	0.23 ± 0.018*
Copper	1.13 ± 0.053	1.32 ± 0.064
Lead	0.38 ± 0.022	0.41 ± 0.020
<i>Liver</i>		
Cadmium	0.14 ± 0.008	0.91 ± 0.049*
Copper	2.83 ± 0.082	3.29 ± 0.093
Lead	0.29 ± 0.018	0.38 ± 0.022*
<i>Kidneys</i>		
Cadmium	0.52 ± 0.01	1.24 ± 0.06*
Copper	3.73 ± 0.09	4.64 ± 0.10*
Lead	0.44 ± 0.02	0.73 ± 0.04*

Note: * $P < 0.05$, compared to intact rats

For cadmium poisoning of rats, a substantial increase in the content of heavy metals in various biological materials was also noted compared to their quantitative values in intact animals (Table 3). Thus, the content increased: in the blood, copper and zinc, respectively, by

1.6 times, lead – by 1.4 times; in the liver, copper increased by 1.5 times, zinc by 1.8, and lead by 1.2 times. Therewith, the content of copper in the kidneys increased by 5.2 times, zinc by 1.8 times, and cadmium by 1.7 times compared to intact rats.

Table 3. Content of zinc, copper, lead in the blood and internal organs of rats with cadmium sulfate poisoning, mg/kg ($M \pm m$, $n = 8$)

Test metal	Intact rats	Poisoned rats
<i>Blood</i>		
Zinc	2.39 ± 0.71	3.88 ± 0.93*
Copper	1.13 ± 0.05	1.80 ± 0.09*
Lead	0.38 ± 0.02	0.52 ± 0.04*
<i>Liver</i>		
Zinc	18.74 ± 2.34	32.83 ± 3.67*
Copper	2.83 ± 0.08	4.25 ± 0.09*
Lead	0.29 ± 0.02	0.35 ± 0.02
<i>Kidneys</i>		
Zinc	12.73 ± 1.82	23.25 ± 1.99*
Copper	3.73 ± 0.09	19.52 ± 1.79*
Lead	0.44 ± 0.02	0.75 ± 0.04*

Note: * $P < 0.05$, compared to intact rats

Under the conditions of lead poisoning of rats, substantial changes in the content of heavy metals were noted both in the blood and in internal organs (liver, kidneys). Thus, the

blood content of zinc increased by 1.5 times, copper by 1.4, and cadmium – by 12.3 times, compared to intact rats. In the liver, there was an increase in the content of zinc – 1.6 times,

copper – 1.3 times, and cadmium –7.5 times compared to the control group. In the kidneys of rats, an increase in the content of all heavy

metals examined was also noted: zinc by 1.7 times, copper by 4.9 times, and cadmium by 2.6 times compared to intact rats (Table 4).

Table 4. Content of zinc, copper, cadmium in the blood and internal organs of rats with lead nitrate poisoning, mg/kg ($M \pm m$, n = 8)

Test metal	Intact rats	Poisoned rats
	<i>Blood</i>	
Zinc	2.39 ± 0.71	3.46 ± 0.83*
Copper	1.13 ± 0.05	1.53 ± 0.03*
Cadmium	0.04 ± 0.002	0.49 ± 0.03*
	<i>Liver</i>	
Zinc	18.74 ± 2.34	29.19 ± 3.22*
Copper	2.83 ± 0.08	3.78 ± 0.91*
Cadmium	0.14 ± 0.008	1.05 ± 0.05*
	<i>Kidneys</i>	
Zinc	12.73 ± 1.82	21.07 ± 1.95*
Copper	3.73 ± 0.09	18.25 ± 2.82*
Cadmium	0.52 ± 0.01	1.33 ± 0.06*

Note: * $P < 0.05$, compared to intact rats

The results of the examination of infrared spectra of internal organs under conditions of cadmium poisoning of rats provide a general spectrum. It is the sum of the spectra in which the absorption bands corresponding to various functional groups of organic substances and water molecules are calculated. The number of characteristic absorption bands in accordance with

the groups of atoms, and the intensity and maximum positions on the obtained infrared spectra, contribute to determining the component composition of substances that are part of the macromolecules of the examined organs, and the structure of compounds and their interaction and transformation under pathological conditions. The absorption spectra are shown in Fig. 2-5.

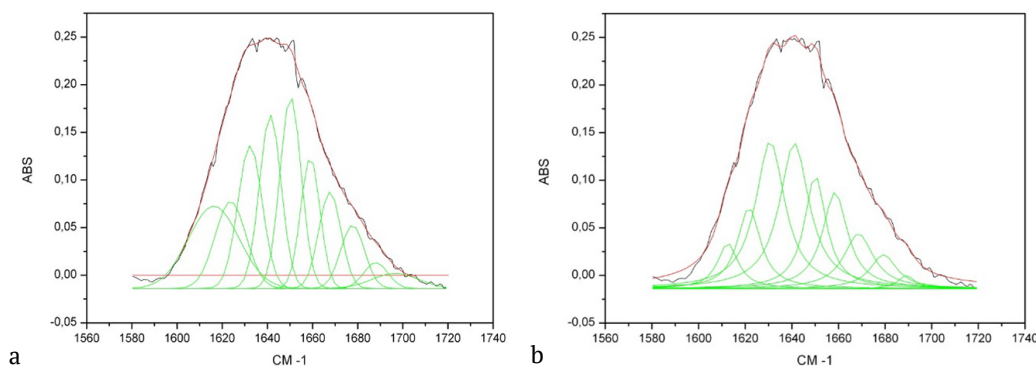


Figure 2. Infrared spectra of examined liver samples from intact rats with Gaussian (a) and Lorentz (b) distributions

Notes: in Figs. 2-5, ABS – degree of absorption of infrared waves; cm-1 - reverse centimetres

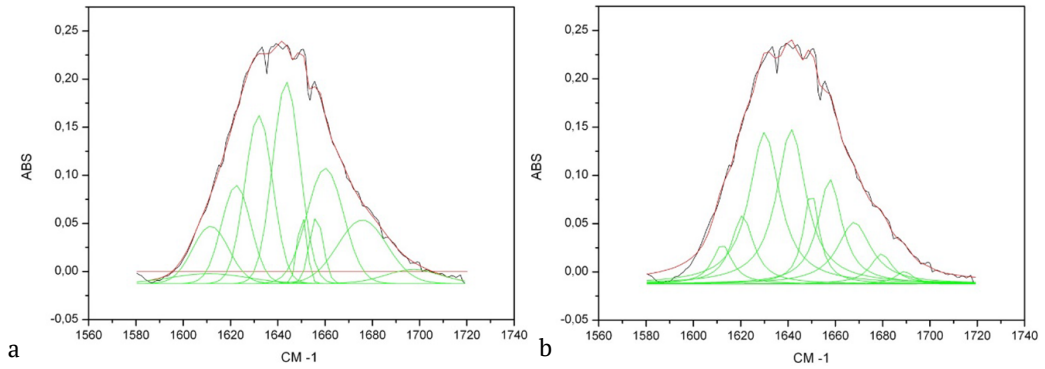


Figure 3. Infrared spectra of the examined kidney samples in intact rats with Gaussian (a) and Lorentz (b) distributions

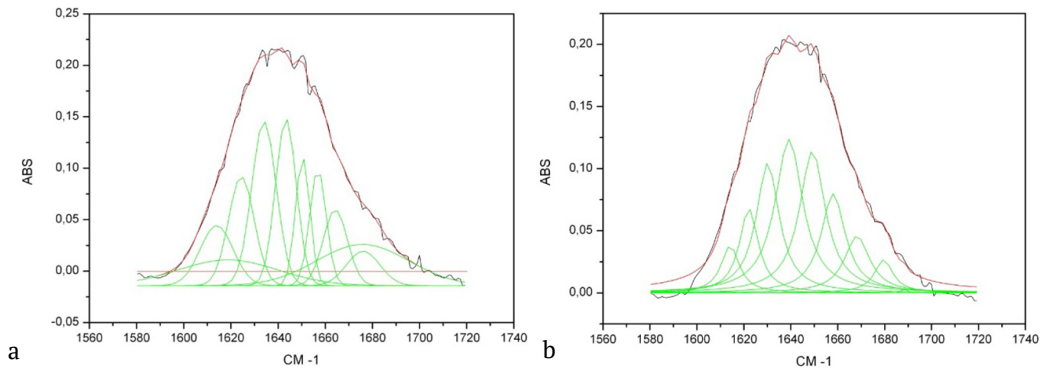


Figure 4. Infrared spectra of examined liver samples from cadmium-poisoned rats with Gaussian (a) and Lorentz (b) distributions

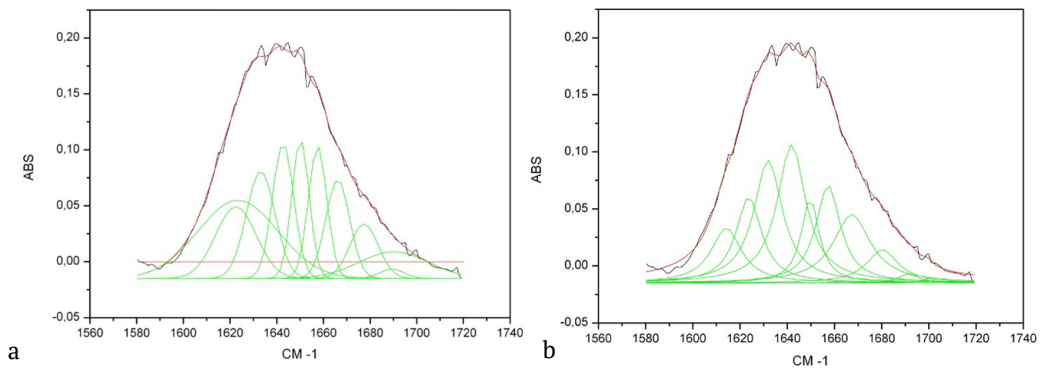


Figure 5. Infrared spectra of examined kidney samples from cadmium-poisoned rats with Gaussian (a) and Lorentz (b) distributions

Figs. 2-5 show the obtained original infrared absorption spectra of components of liver and kidney samples in intact and cadmium-poisoned animals with a Gaussian (a) and Lorentz (b) distribution. As a result of the analysis of the obtained infrared spectra, it was established that the spectrograms are represented by curves that have absorption bands with intensities of different degrees. In addition, in investigating the obtained frequency range, it was found that the maximum absorption bands at the following wavelengths: 1616 cm^{-1} , 1632 cm^{-1} , 1650 cm^{-1} , 1659 cm^{-1} , 1667 cm^{-1} , 1677 cm^{-1} , 1688 cm^{-1} , 1696 cm^{-1} . The analysis of the recorded wavelengths showed that the most intense are bands with a frequency of 1650 cm^{-1} and 1659 cm^{-1} .

Results of detailed analysis of the maximum intensity absorption bands at frequencies of 1650 cm^{-1} and 1659 cm^{-1} allow stating that these bands are characteristic of α -spiral configurations of the polypeptide chain, which indicates a predominance in the spatial structure of the liver and kidney parenchyma α -helical conformation of proteins. The data obtained are consistent with the previously published results (Brian C. Smith, 2016).

As a result of further analysis of the infrared spectra, absorption bands were detected within the wavelength range from 1640 cm^{-1} up to 1620 cm^{-1} , which is typical for β -folded protein conformations. Moreover, the maximum absorption bands with a frequency of 1622 cm^{-1} , 1627 cm^{-1} , and 1633 cm^{-1} correspond to the examined liver samples of intact animals. Notably, in the mentioned frequency range (1640-1620 cm^{-1}), there is the absorption of antiparallel types β -structures (Socrates, 2004).

Therefore, the results obtained show that the proteins of liver samples in intact animals are represented by polypeptide chains β -folded structure of the antiparallel type, which is consistent with the literature data (Smith, 2011).

When analysing the infrared spectra, the maximum absorption band at a wavelength of 1660 cm^{-1} was also established, which is caused by amorphous structures and their vibrations (Smith, 2021).

Furthermore, the presence of absorption bands in the frequency range from 1680 cm^{-1} to 1670 cm^{-1} was established. These bands are caused by fluctuations in which the bond length of carbonyl groups changes. Such fluctuations are caused by certain sites in the structures of protein molecules. The obtained spectra also show maximum absorption bands with wavelengths of 1690 and 1696 cm^{-1} . In the studied samples of liver and kidney parenchyma, absorbances were established, which indicate the presence of reversible turns in certain sections of polypeptide chains at wavelengths in the range of 1670-1688 cm^{-1} (Socrates, 2004).

The comparison of the spectrograms of intact and poisoned rats presented in Figs. 2-5 indicated that in the absorption spectra of the liver samples of animals poisoned with cadmium, there is a notable increase in the intensity of the absorption bands at the maximum frequencies of 1624 cm^{-1} and 1650 cm^{-1} . Therewith, the stability of the intensity of the absorption band at 1633 cm^{-1} was determined, which is caused by the fluctuations of flat, elongated, and twisted β -folded structures (Smith, 2016).

Using the laws of normal Gauss and Lorentz distributions, complex curves with numerical bands were decomposed and the sum of individual curves was obtained for deeper qualitative and quantitative analysis of spectrograms in experimental animals. With such a perfect analysis, substantial differences in the examined spectrograms are clearly established. The difference between the infrared spectra of the examined liver and kidney tissue samples taken from intact and cadmium-poisoned animals was demonstrated using such well-known statistical indicators as skewness and kurtosis

(Brown, 2022). In rats poisoned with heavy metals, there is a substantial increase in the content of lactate and pyruvate in the blood serum (Table 5).

Table 5. Content of glycolysis substrates and tricarboxylic acid cycle intermediates in rat blood serum under heavy metal poisoning conditions, $\mu\text{mol/mL}$ ($M \pm m$, $n = 8$)

Parameter	Intact rats	Poisoned rats			
		copper	zinc	cadmium	lead
<i>Glycolysis substrates</i>					
Lactate	3.602 ± 0.709	$6.122 \pm 0.918^*$	$6.843 \pm 0.955^*$	$9.722 \pm 1.056^*$	$9.016 \pm 1.012^*$
Pyruvate	0.113 ± 0.039	$0.192 \pm 0.071^*$	$0.225 \pm 0.067^*$	$0.853 \pm 0.087^*$	$0.632 \pm 0.077^*$
<i>Intermediates of the tricarboxylic acid cycle</i>					
Malat	0.033 ± 0.001	$0.027 \pm 0.002^*$	$0.026 \pm 0.002^*$	0.034 ± 0.001	$0.027 \pm 0.002^*$
Oxaloacetate	0.083 ± 0.002	$0.106 \pm 0.007^*$	$0.104 \pm 0.007^*$	$0.111 \pm 0.008^*$	$0.109 \pm 0.007^*$
α -Ketoglutarate	0.056 ± 0.001	$0.076 \pm 0.003^*$	$0.075 \pm 0.002^*$	$0.082 \pm 0.001^*$	$0.079 \pm 0.002^*$

Note: * $P < 0.05$, compared to intact rats

An increase in the lactate concentration in the blood serum of rats poisoned with copper ions – 1.7 times, zinc – 1.9 times, cadmium – 2.7 times, and lead – 2.5 times compared to animals of the control group was found. The content of pyruvate in the blood serum changed towards increase as follows: for poisoning with copper ions – by 1.7 times, zinc – by 2 times, cadmium – by 7.6 times, and lead by 5.6 times compared to the control.

The examined indicators of tricarboxylic acid cycle intermediates in the blood of rats under heavy metal poisoning are presented in Table 5.

It was established that when rats were poisoned with copper ions, the concentration of oxaloacetate and α -Ketoglutarate in the blood serum increased by 28 and 36%, respectively, compared to the control group of animals. Therewith, copper ion intoxication caused a 19% decrease in malate content compared to the intact group of rats.

When rats were poisoned with zinc ions, serum concentrations of oxaloacetate (by 25%) and alpha-ketoglutarate (by 34%) increased. Meanwhile, there was a 21% decrease in malate content compared to intact animals.

Rat poisoning with cadmium ions affected the serum intermediates of the tricarboxylic acid cycle as follows: the concentration of oxaloacetate and α -Ketoglutarate increased by 34 and 46%, respectively, and the malate content remained unchanged compared to the control.

As a result of lead ion poisoning, an increase in serum oxaloacetate (by 31%) and alpha-ketoglutarate (by 41%) and a decrease in malate (by 18%) were observed compared to intact rats.

The importance of amino acids in protein biosynthesis and highly active biological compounds has become the main prerequisite for numerous examinations of their content in body fluids and tissues both during experimental studies and during the occurrence of pathologies. In intermediate metabolism, amino acids and their derivatives play a binding role in the integration of major metabolic pathways (Kokaman, 2022).

In addition, as noted in the literature (Mo Zhou *et al.*, 2020), the pool of free amino acids is represented by metabolically and functionally interrelated compounds, the content of which is a regulatory factor in many key stages of metabolism in the mammalian body.

In this regard, the examination of the molecular mechanisms of the formation of the fund of free amino acids *in vivo* is an important part of solving numerous problems of modern biochemistry and clinical veterinary medicine, related to the purposeful regulation of metabolic processes in living organisms under the influence of biologically active natural compounds and various xenobiotics (Oluranti *et al.*, 2021).

Today, the number of pathological conditions continues to grow, in the genesis of which the violation of amino acid metabolism is given a leading place (Li & Saleem, 2022).

The ability of the kidneys to concentrate various compounds, the presence of active transport processes and systems of metabolic activation of xenobiotics allow them to be classified as target organs that affect the level of their toxic effects in the body (Engwa *et al.*, 2019).

The results of investigating the effect of heavy metals on the pool of free amino acids in rat kidneys are shown in Table. 6. Notably, among the examined amino acids, a decrease in their total amount was established in rats of all experimental groups: in rats poisoned with copper sulfate and zinc sulfate – by 1.3 times, and cadmium sulfate and lead nitrate – by 1.4 times compared to intact animals. The content of aspartic acid, valine, glycine, tyrosine, and cystine (more than 1.5 times) decreased in poisoned rats; alanine, leucine, serine, taurine, threonine, phenylalanine (more than 2 times), lysine – on average by 3.4-4.9 times compared to the control. Therewith, in rats of the experimental groups, an increase in the level of isoleucine and methionine in the kidneys was observed by an average of 1.3-1.5 times, ornithine – by an average of 1.8-2.1 times, glutamic acid – by an average of 4.4-5.3 times compared to the control.

Table 6. Free amino acid content in rat kidneys before and after heavy metal poisoning, µg/g (M ± m, n = 8)

Amino acid	Animal group				
	Intact	Copper sulfate poisoned	Zinc sulfate poisoned	Cadmium sulfate poisoned	Lead nitrate poisoned
Alanine	723.4 ± 31.14	446.4 ± 20.43*	451.2 ± 21.52*	328.3 ± 18.74*	339.3 ± 19.12*
Arginine	304.6 ± 17.21	231.2 ± 16.32*	247.6 ± 18.41*	212.2 ± 14.27*	217.4 ± 15.18*
Aspartic acid	682.7 ± 27.18	415.3 ± 24.41*	438.4 ± 27.15*	397.6 ± 20.61*	403.8 ± 22.74*
Valine	297.8 ± 22.34	164.3 ± 14.23*	161.7 ± 13.94*	149.4 ± 11.28*	158.9 ± 12.11*
Histidine	214.3 ± 19.57	169.4 ± 15.47*	171.5 ± 16.23*	151.5 ± 10.78*	156.7 ± 11.13*
Glycine	483.2 ± 27.12	339.2 ± 24.38*	344.8 ± 26.82*	318.7 ± 21.32*	321.5 ± 22.17*
Glutamic acid	182.7 ± 25.29	823.7 ± 41.25*	798.2 ± 39.18*	984.1 ± 46.27*	972.3 ± 44.16*
Isoleucine	252.4 ± 23.16	332.4 ± 26.19*	327.9 ± 27.45*	381.4 ± 30.32*	379.8 ± 29.27*
Leucine	475.8 ± 21.17	287.3 ± 19.33*	301.7 ± 20.17*	215.6 ± 16.41*	218.4 ± 17.33*
Lysine	954.5 ± 68.23	243.6 ± 18.47*	281.4 ± 19.53*	195.8 ± 15.84*	197.6 ± 16.21*
Methionine	169.8 ± 17.64	228.3 ± 17.92*	219.7 ± 18.41*	261.3 ± 20.72*	259.7 ± 19.89*
Ornithine	174.6 ± 15.31	319.8 ± 19.23*	317.1 ± 18.42*	364.2 ± 22.81*	356.4 ± 20.45*
Proline	269.3 ± 21.54	238.5 ± 20.34	241.6 ± 20.89	212.5 ± 18.92	214.8 ± 19.17
Serine	489.2 ± 27.12	234.7 ± 19.21*	245.3 ± 20.95*	218.4 ± 17.23*	221.6 ± 18.14*
Taurine	341.4 ± 19.23	182.2 ± 14.36*	194.8 ± 15.21*	145.2 ± 10.03*	148.5 ± 10.72*

Table 6, Continued

Tyrosine	294.7 ± 19.15	193.5 ± 19.32*	191.6 ± 18.93*	176.4 ± 18.27*	179.7 ± 19.44*
Threonine	357.6 ± 32.16	168.3 ± 15.64*	197.2 ± 19.42*	162.8 ± 13.26*	164.6 ± 14.31*
Tryptophan	201.2 ± 21.14	227.1 ± 16.89	221.4 ± 17.23	245.3 ± 18.14	243.8 ± 17.42
Phenylalanine	598.4 ± 25.12	342.3 ± 15.43*	338.1 ± 16.25*	210.1 ± 11.47*	211.4 ± 11.53*
Cystine	187.3 ± 19.23	102.7 ± 6.02*	99.8 ± 5.19*	95.4 ± 4.23*	98.3 ± 4.71*
Sum of amino acids	7654.9	5690.2	5791.0	5426.2	5464.5

Note: * $P < 0.05$, compared to the control group

Increased methionine content, as noted in the literature (Kokaman, 2022), may indicate a violation of the transfer of sulfhydryl groups and methylation of macromolecules. Since isoleucine is utilised in the kidneys to ensure the processes of transamination and synthesis of adenosine triphosphoric acid, the increase in its content when heavy metals are introduced indicates that the affected kidneys are not able to effectively implement this process. Transamination disorders are indicated by an increase in glutamic acid (Li & Saleem, 2022). It is assumed that as a result of heavy metal poisoning, the ability to break down excess amino acids in the urea cycle is clearly impaired, as evidenced by an increase in the concentration of ornithine in the blood serum. In turn, the literature indicates (Samuel *et al.*, 2021) that a decrease in the concentration of aspartic acid can lead to a violation of the formation of oxaloacetate and the normal course of transformations in the Krebs cycle.

The data obtained, consistent with the research (Salama *et al.*, 2016), is associated with kidney damage in rats with lead poisoning, which also indicates the involvement of gamma-Glutamylcysteine in the activation of the expression of the nuclear antigen of proliferating cells, enhancing the regenerative ability of this internal organ, reducing inflammation, and regulating the apoptosis.

In addition, the results presented are the same as those of Selorm Fiat Kenston *et al.* (2018)

concerning the effects of a mixture of heavy metals that reduce kidney function, increase liver damage, and disrupt electrolyte balance in rats.

Identical data were also obtained in relation to glutamate metabolism during the poisoning of rats with even one heavy metal (Li *et al.*, 2020).

The established patterns are consistent with the previous results (Flora *et al.*, 2022), which, in particular, indicate the use of chelation therapy as one of the promising methods in the treatment of heavy metal poisoning.

Thus, the poisoning of rats by the studied heavy metals is reflected in the infrared absorption spectra and characterises α spiral configurations of polypeptide chains and antiparallel types β of structures in liver and kidney tissues. Moreover, in blood serum, an increase in the concentration of lactate, pyruvate, oxaloacetate, and α -Ketoglutarate and a decrease in malate and the total content of amino acids in rat kidney tissues are observed.

Conclusions

When rats are poisoned, the copper content in the blood increases by 1.4 times, in the liver – 1.6 times, and in the kidneys – 1.5 times. Meanwhile, the zinc content in the blood of rats increases by 1.5 times, in the liver – by 2.5 times, in the kidneys – by 1.6 times; cadmium in the blood of rats increases by 27 times, in the liver – by 243 times, in the kidneys – by 23 times; lead in the blood of rats increases by 1.9 times, in

the liver – by 4.8 times, in the kidneys – by 5.5 times compared to the group of intact animals.

The obtained and analysed infrared absorption spectra showed a substantial difference in the spatial organisation of protein components in liver and kidney tissues in intact and cadmium-poisoned rats, which consists in the shift of the maximum bands, their appearance or disappearance, and increase or decrease in the integral intensity. The presence of all forms of secondary structures in the spatial structure of liver and kidney tissue proteins was established, in particular, α -spirals, β -folded structures and amorphous regions. In the case of cadmium ion poisoning, the denaturation of protein molecules and the transformation of their structure into disordered segments were recorded.

The difference between the infrared spectra of liver and kidney tissues in intact and cadmium-poisoned rats was identified and demonstrated using statistical indicators such as skewness and kurtosis.

It was established that poisoning of rats with copper sulfate, zinc sulfate, cadmium sulfate, and lead nitrate ions causes a likely increase in the concentration of lactate and pyruvate in the blood serum and leads to an increase in the content of oxaloacetate and α -Ketoglutarate and a decrease in the concentration of

malate compared to intact rats. This indicates the sensitivity of glycolysis reactions and the tricarboxylic acid cycle to the intake of toxic doses of heavy metals.

Heavy metal poisoning in rats is accompanied by a decrease in the total pool of free amino acids in the kidneys. Thus, the quantitative changes in the pool of free amino acids in the kidneys of rats with the experimental introduction of toxic doses of heavy metals into the body can be an additional criterion for assessing the state of exchange of amino acids, nitrogenous compounds, proteins and nucleotides, internal reserves of the organ, the degree of disintegration of metabolism under the influence of xenobiotics and the adaptive capabilities of the body in general. The obtained research results can be used for clinical studies under the conditions of heavy metal poisoning of animals.

In the future, it is planned to expand the range of research on the effect of heavy metals on intermediate protein metabolism in the body of poisoned rats.

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None.

Conflict of Interest

None.

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Інфрачервона спектроскопія та біохімічні показники тканин щурів за умов отруєння важкими металами

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Анотація. Підвищення рівня антропогенного забруднення навколишнього середовища та пошук дієвих засобів щодо зменшення негативного впливу ксенобіотиків на здоров'я тварин і людей є актуальною проблемою сьогодення. Враховуючи це, метою роботи було вивчення впливу важких металів на процеси кумулювання за умов отруєння та біохімічні показники в організмі щурів. Для проведення дослідження сформовані групи-аналогі з щурів одного віку, статі та за масою тіла. Впродовж 14 діб здійснювали інтоксикацію щурів розчинами сульфату міді, сульфату цинку, сульфату кадмію та нітрату свинцю. Використовуючи метод інфрачервоної спектроскопії, встановлено значні відмінності в просторовій структурі білкових компонентів у інтактних та токсикованих тварин. Різницю між спектральними характеристиками досліджуваних тканин продемонстровано наочно за статистичними показниками скупесу і куртозису. Встановлено, що отруєння щурів іонами міді, цинку, кадмію та свинцю впливає на хід реакцій гліколізу і циклу трикарбонових кислот, що призводить до вірогідного збільшення в сироватці крові концентрації лактату й пірувату, оксалоацетату і α -кетоглутарату та зменшення вмісту малату порівняно з інтактними щурами. З'ясовано, що за умов отруєння також відзначається вірогідне збільшення ($P < 0,05$) вмісту досліджуваних важких металів у крові, печінці та нирках. У токсикованих важкими металами тварин відмічається зменшення в нирках пулу вільних амінокислот. Зокрема, у нирках таких щурів знижується вміст: аспарагінової кислоти, валіну, гліцину, тирозину, цистину (більш ніж у 1,5 рази); аланіну, лейцину, серину, таурину, треоніну, фенілаланіну (більш ніж у 2,0 рази), лізину – у 3,4–4,9 рази. Разом із тим, встановлено зростання рівня ізoleyцину та метіоніну в 1,3–1,5 рази, орнітину – у 1,8–2,1 рази, глутамінової кислоти – у 4,4–5,3 рази в щурів дослідної групи порівняно з інтактними. Результати дослідження можуть бути корисними у професійній діяльності лікарів ветеринарної медицини, токсикологів, біологів, екологів та використовуватися для контролю за якістю тваринницької продукції, проведення токсикологічних досліджень і аналізу об'єктів довкілля

Ключові слова: мідь; цинк; кадмій; свинець; кров; внутрішні органи