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STUDYING THE EFFECTS OF HEAVY METAL SALTS ON THE BLOOD PROTEINS OF AGRICULTURAL ANIMALS USING ALBUMIN PROTEIN IMITATION LABORATORY METHODS

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Abstract
This article investigates the effects of heavy metal salts (lead, copper, zinc, mercury) on the blood plasma proteins of agricultural animals. The study analyzes how heavy metal salts form complex compounds with blood plasma proteins upon entering the organism.Biochemical and simulation statistical methods were employed to determine the binding properties of heavy metal ions to protein molecules and to elucidate their toxic mechanisms. The research findings explain the decline in quality and safety of livestock products due to heavy metal contamination. This scientific work holds significant importance in the fields of environmental protection, veterinary toxicology, and food safety.

Keywords: Imitation, heavy metals, lead, copper, mercury, zinc, blood proteins, plasma proteins, toxic effects, immunological disorders, blood biochemistry, binding properties, toxicity, mechanism, environmental safety, toxicology.

Introduction

Relevance of the topic. In recent years, the intensification of industrial enterprises and agricultural technologies, along with the resulting technogenic pollution of the environment, has led to the contamination of soil, water, and plants with heavy metals. This has caused a sharp increase in the amount of toxic substances entering animal organisms through the food chain. According to the

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above sources, heavy metal salts such as lead, zinc, copper, and mercury exhibit high toxic properties, and their toxic and allergic effects on living organisms, particularly agricultural animals, are increasing.

After entering the organism, these heavy metal ions react with blood plasma proteins to form complex high-molecular-weight protein compounds. This disrupts the organism's biological stability, impairs metabolic and immunological processes, and leads to decreased productivity and resistance in animals. Consequently, the quality and safety of animal products intended for human consumption deteriorate significantly from a toxicological perspective.

Therefore, studying the toxic effects of heavy metals on the animal organism, particularly the mechanisms of their interactions with blood proteins, holds significant scientific and practical importance from the perspectives of modern veterinary toxicology, food safety, and environmental protection. The relevance of this topic is also reflected in a number of laws and resolutions issued by our government.

Specifically, this includes the Law of the Republic of Uzbekistan No. 754-XII "On Environmental Protection" adopted on December 9, 1992; Law No. 935-XII dated September 3, 1993; Law No. UzR -397 dated December 29, 2015; and the newly revised Law No. UzR -864 "On Veterinary Medicine" dated August 21, 2023. Additionally, normative legal documents such as the Resolution No. UzRM -804 of the Cabinet of Ministers of the Republic of Uzbekistan dated November 30, 2024, regarding the "Green Zone" national project, prioritize environmental safety, improvement of livestock product quality, and protection of animal health. In particular, these documents emphasize the need to prevent risks associated with pollutants, including heavy metals, mitigate their negative effects, and ensure ongoing monitoring.

Decree No. PF-5853 of the President of the Republic of Uzbekistan dated October 23, 2019, "On Approving the Strategy for the Development of Agriculture of the Republic of Uzbekistan for 2020–2030."

This decree is aimed at modernizing agriculture, ensuring environmental safety, and improving the quality of agricultural products through the introduction of advanced technologies. Resolution No. 571 of the Ministry of Agriculture of the Republic of Uzbekistan dated July 9, 2019, "On the Organization of the Activities of the Ministry of Agriculture of the Republic of Uzbekistan." This resolution defines the main tasks, functions, and authorities of the Ministry of Agriculture, including activities related to the control of agricultural product quality and ensuring environmental safety.

Decree No. PD-113 of the President of the Republic of Uzbekistan dated April 5, 2023, "On Measures to Develop the Production and Export of Agricultural Products in 2023." This decree outlines measures aimed at increasing the production of agricultural products, improving their quality, and enhancing export potential. Furthermore, in accordance with Presidential Decree No. PD–114 of the Republic of Uzbekistan dated January 28, 2022, "On Measures to Accelerate Reforms in the Field of Ecology and Environmental Protection," the system of environmental monitoring is being strengthened, including control over the health of agricultural animals and the safety of products derived from them. Therefore, studying the biological effects of heavy metal salts in the animal organism is of not

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only scientific, but also practical significance. Such research contributes to ensuring the environmental safety of livestock products, protecting human health, and enhancing biosafety. [16]

In recent years: the issue of studying the negative effects of heavy metals on the physiological and biochemical systems of humans and animals has become one of the primary challenges in modern toxicology and veterinary science, not only in Uzbekistan but worldwide. Numerous researchers have conducted scientific studies on the harmful impact of heavy metals on the organism and environmental ecology across various topics.

Several scientific studies have been conducted in Uzbekistan in this area. For instance, M.F. Uktamova investigated the toxic effects of lead, cadmium, and mercury salts on the blood and liver tissues of rats. She found that heavy metals negatively affect erythrocyte and hemoglobin levels. O.J. Tojikulova explained the harmful effects of heavy metal ions on mitochondria and ways to reduce these effects with the help of natural antioxidants.

A research team led by S.T. Matkarimov is conducting scientific and practical studies on monitoring, assessment, and ensuring environmental safety in areas contaminated with heavy metals.

Among foreign researchers, J. Stamler (USA) studied the binding of heavy metal ions to proteins through S-nitrosylation, while L. Zolla (Italy) investigated the structure of protein-metal complexes using proteomic methods.

Additionally, P. Harris (United Kingdom) conducted research on assessing and monitoring the risks of heavy metals entering the human body through animal products. [9,15]

Research Objective: to study the interactions of heavy metal salts (lead, zinc, copper, cadmium, mercury, etc.) with blood plasma proteins and egg proteins of agricultural animals under imitation laboratory conditions; to determine their binding properties to protein molecules; to analyze the mechanisms of protein-metal complex formation; and to substantiate the diagnostic significance of these toxic processes.

Research Methods: biochemical, mathematical-statistical, and chemical-toxicological methods.

Research Object and Location: the study was conducted at the Pharmacology and Toxicology Laboratory of the Tashkent Branch of Samarkand State University of Veterinary Medicine, Animal Husbandry and Biotechnology, as well as at peasant and farmer farms in the Yangiyo‘l district.

Research Results: according to data from the World Health Organization, there are currently over 6 million chemical compounds, of which 500,000 are in practical use by humans. Among these, 40,000 are harmful to humans, and 12,000 exhibit toxic effects on the organism. [5,8]

Preventive toxicology deals with studying methods to protect living organisms from the harmful effects of chemical substances found in industry, agriculture, services, and food. **Clinical**

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toxicology, on the other hand, involves the diagnosis and development of treatment methods for harmful chemical substances affecting humans and animals.

Heavy metals are chemical elements characterized by their metallic properties and significant atomic weight or density. These metals naturally occur in the Earth's crust. The primary heavy metals include mercury, arsenic, selenium, calcium, magnesium, silver, barium, cadmium, chromium, cobalt, copper, iron, lead, nickel, tin, zinc, and aluminum. Among these, the most hazardous are lead, cadmium, mercury, arsenic, chromium, and aluminum. [1,10]

According to a report by the United Nations Environment Programme (UNEP), seven metals and three metalloids are listed among the most hazardous heavy elements. These include copper, tin, vanadium, chromium, molybdenum, cobalt, nickel, and the metalloids antimony, arsenic, and selenium.

Based on their level of danger to human and animal health, heavy metals are classified into the following categories:

Class 1: cadmium, mercury, selenium, lead, zinc;

Class 2: cobalt, nickel, copper, molybdenum, antimony, chromium;

Class 3: barium, vanadium, tungsten, manganese, strontium.[11]

Among various pollutants, heavy metals and their compounds are distinguished by their widespread distribution and high toxicity. Many of them possess the ability to accumulate in living organisms. These metals are widely used in various industrial processes, and despite treatment efforts, their concentrations in industrial wastewater remain relatively high. They enter the environment through domestic wastewater, emissions, and industrial dust.

Many metals form stable organic compounds, and the high solubility of these complexes facilitates the migration of heavy metals in natural water bodies. Although most heavy metals occur naturally, some originate from anthropogenic sources. Industrial and wastewater containing heavy metals are considered the main sources of hydrosphere contamination. Another pathway for heavy metal distribution is the discharge of wastewaters rich in these elements into aquatic ecosystems.

Heavy metals interact with various components of the environment—such as water, soil, and air—and exert their effects on humans, animals, and other living organisms through the food chain. In humans and animals, heavy metals disrupt numerous biochemical processes and cause toxicity in various bodily systems. For example, they may induce nephrotoxicity, neurotoxicity, hematotoxicity, dermatotoxicity, and have significant effects on the cardiovascular system.

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Below, we examine the pathological effects of certain heavy metals with high toxic potential on the organism.

Lead (Pb) Poisoning

Lead compounds have a strong tendency to bind with organic molecules. Contamination of soil and untreated water sources leads to the presence of lead in wastewater, and this, in turn, results in the accumulation of lead in plants irrigated with such water. Consequently, large and small ruminants are often exposed to lead and its compounds, resulting in toxic effects.

The global annual production of lead is approximately 3 million tons. Each year, around 600,000 tons of lead are deposited onto the Earth's surface from the atmosphere via natural water sources.Out of this amount, approximately 400,000 tons of lead settle onto the Earth's surface. Additionally, about 250,000 tons of lead are released into the environment through exhaust gases emitted by vehicles burning fuel.

Lead exerts toxic effects on the body by disrupting the function of proteins and enzymes. It is absorbed into nerve and muscle cells, where it initially forms lactate and then phosphate compounds, blocking the entry of calcium ions into cells. As a result, lead accumulates in bone tissue by replacing calcium and subsequently affects the nervous system and kidneys. In plants, the average concentration of lead is about 2–3 mg/kg. On average, humans ingest approximately 250 micrograms of lead daily through food and about 20 micrograms through drinking water. Lead enters drinking water through lead-based plumbing joints. The maximum permissible concentration of lead is **0.05 mg/L in water** and **3 µg/m³ in air**.

Deficiencies of calcium, iron, copper, and magnesium in the body can increase lead absorption into the bloodstream.[14]

Bluish-gray spots may appear on the gums, and the concentration of lead in urine increases. When the body is exposed to lead and its compounds, the majority is excreted through the kidneys and intestines. However, significant amounts accumulate in the liver, bones, and nervous tissue.Once absorbed into the bloodstream, approximately 90% of lead binds with enzymes, amino acids, and sulfhydryl and other functional groups in proteins, leading to disruptions in the functioning of the central nervous system.

Cadmium (Cd) Poisoning

One of the natural sources of cadmium emission into the atmosphere is volcanic activity. However, cadmium contamination of the biosphere primarily occurs due to anthropogenic factors. These include zinc mining and metallurgy, the electronics and semiconductor industries, pigment production, electrical engineering, and the manufacture of superphosphate fertilizers.

Cadmium is primarily used for coating steel products to protect their surfaces from corrosion, as a stabilizer in polyvinyl chloride (PVC), as an additive pigment in plastics and glass, and as electrodes in nickel-cadmium batteries. Approximately 15,000 tons of cadmium are produced worldwide annually. Cadmium, in the form of dust, smoke, fumes, and vapor, is highly toxic to humans and animals.Cadmium poisoning primarily affects the heart muscles and respiratory organs, particularly causing oncological and malignant tumors in the lungs. Additionally, paralysis of the central nervous system is observed. Cadmium acts as an antagonist to zinc, cobalt, and

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selenium. Its presence disrupts iron and calcium metabolism, with accumulation mainly occurring in the liver and kidneys. Due to the high toxicity of cadmium compounds, their medical use has been discontinued in recent times.Because cadmium-containing paints are toxic, many countries have banned the use of cadmium in the manufacture of children's toys. Exposure to as little as 0.03 grams of cadmium sulfate can cause poisoning in humans. [3, 6]

Cadmium that enters the organism binds with proteins. It interacts with sulfhydryl groups of enzymes in the blood, disrupting their normal functions. In cases of cadmium compound poisoning in humans, symptoms such as bloody diarrhea are observed, along with the accumulation of lipids in the liver and heart, and inflammation of the kidneys. Cadmium absorbed by the body is excreted very slowly through the stomach and urinary tract. Additionally, lead compounds also exhibit strong toxic effects on warm-blooded organisms. [3, 7]

During the study, blood samples were collected from animals, and blood plasma was separated. To observe the reactions between protein substances in the blood plasma and heavy metal salts, mixtures with various metal compounds were prepared under laboratory conditions. The experiment utilized 1% lead acetate (Pb(CH3COO)2), 1% ferric chloride (FeCl3), 1% copper sulfate (CuSO4), 1% zinc acetate (Zn(CH3COO)2), and 40% concentrated magnesium sulfate (MgSO4) solutions. The formation of complex compounds—precipitates—resulting from the interaction of these metal salts with blood plasma proteins and the mechanisms of their formation were thoroughly investigated. Additionally, when heavy metal salts were applied to both simple and complex egg proteins for imitation purposes, various colored precipitates appeared in the solutions.

Colored Precipitates Resulting from the Interaction of Heavy Metals with Blood Plasma Proteins

Table 1

№	1% ($Pb(CH_3COO)_2$)	1% ($FeCl_3$)	1% ($CuSO_4$)	1% ($Zn(CH_3COO)_2$)	40 % ($MgSO_{4(kons)}$)
1. Protein sample	Yellowish-black precipitate	Reddish-yellow precipitate	Blue precipitate	White precipitate	White precipitate

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As demonstrated in the above experiment, metal ions such as Pb^{2+}, Fe^{3+}, and Cu^{2+} exhibit high toxic effects on animal blood plasma proteins, whereas less toxic ions such as Zn^{2+} and Mg^{2+} serve as examples of lower toxicity metal ions.

On colored precipitates in imitation egg albumin exposed to heavy metals

Table 2

№	1% ($Pb(CH_3COO)_2$)	1% ($FeCl_3$)	1% ($CuSO_4$)	1% ($Zn(CH_3COO)_2$)	40 % ($MgSO_{4(kons)}$)
1. Protein sample	Whitish-black precipitate	Dark orange precipitate	Light blue precipitate	White precipitate	White precipitate

It was established that all metal ions, namely Pb^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, and Mg^{2+}, exhibit toxicity when interacting with imitation egg albumin proteins. The results demonstrated that heavy metal ions bind to protein molecules in blood plasma, forming specific complexes. This process leads to disruptions in immune and metabolic processes and induces toxic effects in the organisms of animals. In particular, lead, iron, and copper compounds cause severe poisoning and significant functional impairments in the organism.

Conclusion

The results of the study showed that lead and other heavy metals bind to protein molecules in the bodies of animals, forming harmful toxic complexes. This condition negatively affects the health of animals by weakening their immune systems and disrupting metabolic processes. Therefore, contamination with heavy metal salts poses a serious risk to agricultural animals and is likely to adversely affect the quality of their food products. The findings emphasize the necessity of developing measures to reduce the impact of heavy metals, as ecotoxicants, within the field of ecological toxicology—a sub-discipline of toxicology.

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