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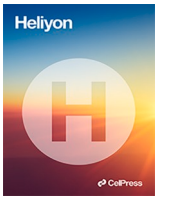

Studying the concentration of xenobiotics in milk and developing the biosensor method for their rapid determination

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ABSTRACT

In this article the content of toxic xenobiotics (heavy metals and pesticides) in cow milk collected from 5 districts of Eastern Kazakhstan was examined and their cumulative properties were determined. The content of organochlorine pesticides (HCCH, DDT) was not detected in the analyzed milk. The content of mercury and arsenic in milk samples does not exceed the maximum allowable concentration (0.005 and 0.05 mg/kg, respectively). The content of cadmium above the maximum allowable concentration (0.03 mg/kg) was found in milk sampled from Shemonaikha and Katon-Karagai districts. The content of lead and zinc above the maximum allowable concentration (0.1 and 5.0 mg/kg, respectively) was found in milk samples taken from all 5 studied districts. The content of copper above the maximum allowable concentration (1.0 mg/kg) was found in milk samples collected from 4 districts under study (Borodulikha, Beskaragai, Shemonaikha and Katon-Karagai). Based on the analysis of information data the need to develop an accelerated method of determining toxic xenobiotics in milk was substantiated. The basic directions of modernization of the biosensor for determination of cadmium and lead salts in milk and dairy products were selected. A new approach to the process of immobilization of the enzyme on the surface of a substrate for cadmium and lead salts determination in milk has been developed. The efficiency of using a polymeric plate with a graphite conducting layer as a basis for the enzyme biosensor was established.

1. Introduction

Globally, the quality and safety of food products is a priority of state policy for the development of the agricultural sector of the economy. The level of contamination of animal origin raw materials with xenobiotics affects the quality and safety of food products. Much attention is paid to pollution of animal raw materials with heavy metals and pesticides. There is growing evidence that confirms the toxic effects on the human body of heavy metals and pesticides, even in small amounts [1,2]. The main sources of intake of these

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xenobiotics into the human body are food and drinking water [3].

Heavy metals have toxic effects if they are not metabolized in the body and accumulate in various body tissues. As a result, they can negatively affect the central nervous system and cause diseases such as memory, attention and intelligence disorders, cardiovascular disease, kidney failure, cancer, and other diseases [4,5]. As noted by the Agency for Toxic Substances and Disease Registry (ATSDR), even at low concentrations, heavy metals have a variety of adverse effects on human health, especially neurotoxic, hepatotoxic, mutagenic and carcinogenic effects [6].

Many of the elements that are classified as heavy metals are essential trace elements. However, when their concentrations exceed the maximum allowable levels in the living organism, they have toxic effects. Such elements as arsenic, cadmium, mercury, lead have a toxic effect even in low concentrations. They are not biodegradable and are stable in the environment [7,8].

Contamination of soil and plants with heavy metals is caused by the operation of mining and processing enterprises, and improper utilization of waste [9,10]. Soil and vegetation belong to the first part of the biogeochemical food chain. The presence of heavy metals in soil, water, and vegetation is harmful to animals and humans because of their bioaccumulation. The heavy metals enter livestock products (meat and milk) through the food chain [11,12].

The contamination of milk with toxic elements is a serious problem. Milk and dairy products are everyday products. The colloidal phase of milk has a high cumulative capacity towards heavy metals. It is noted that in the production of milk products the concentration of elements such as cadmium, copper, lead, arsenic is higher than in the raw milk. It has also established that arsenic is more concentrated in cow milk than in meat [13,14].

Each group of xenobiotics has adverse effects on the ecosystem and human health. But, for industrial-agrarian countries, the pollution of the environment with heavy metals and pesticides causes serious global ecological problems [15,16]. Since the foodstuffs are the main sources of hazardous xenobiotics entering the human body, monitoring their content in food is an important factor in reducing the rate of diseases among the population.

Traditional methods such as chromatography, spectrophotometry and others are used to determine the presence of xenobiotics in raw materials and foodstuffs. These methods are characterized by high sensitivity and accuracy of xenobiotics detection, but they are expensive to handle and require a lot of time for analysis.

In recent years, biosensors for detecting and monitoring environmental pollutants such as pesticides, heavy metals, polycyclic aromatic hydrocarbons, toxins and others have become widespread. They are easy to use, portative, cost-effective and allow real-time monitoring of contamination of objects under study in situ [17,18].

Enzymes, microorganisms, antibodies, nucleic acids, etc. Are used as biological components in biosensor development. Depending on the type of transducer, optical, electrochemical, piezoelectric and other biosensors are available [19,20].

Biosensors are employed for the detection of various toxic elements in milk and dairy products. An amperometric biosensor has been developed for rapid and specific detection of *Salmonella Typhimurium* in milk. The biosensor was tested on samples of skimmed and whole milk, allowing for the rapid detection of *S. Typhimurium* without the enrichment step. For the rapid detection of bacteria and their toxins in milk, a colorimetric biosensor has been developed [21,22]. Various types of biosensors have been developed for the detection of organophosphorus pesticides, urea residues, and heavy metal salts in milk [23–27].

Considering the prospects and relevance of the use of biosensor technology in assessing the quality of raw materials and food, the aim of the work is to select biological material and carrier to develop an express method for determining the content of the most common and dangerous xenobiotics in milk in the intensively developed areas of East Kazakhstan region.

2. Materials and methods

2.1. Samples

Milk samples of cows were collected in August 2022 from 5 districts of East Kazakhstan region - Borodulikha, Ulan, Beskaragai, Shemonaikha, Katon-Karagai. Two to 3 L of milk were taken from each district from 5 to 7 private farms. Milk samples from each district were mixed in a cooling tank. These districts were selected by the direction of location (north, northwest, south, southeast) from the city of Ust-Kamenogorsk as a major industrial center. These five districts are characterized by large pasture lands and oriented to the development of farms, which are the main suppliers of raw milk to dairy processing plants.

2.2. Determination of heavy metals

The concentrations of lead, cadmium, mercury, arsenic, copper, and zinc in milk were analyzed using an inductively coupled plasma mass spectrometry instrument, specifically the Varian 820-ICP MS (Varian Company, Australia). The mass spectrometer sensitivity was calibrated using a diluted calibration solution, Var-TS-MS IV-ICPMS-71 A (Inorganic Ventures Company, USA), containing various elements at a concentration of 10 μg/L. Detector calibration was conducted using three calibration solutions, IV-ICPMS-71 A, containing cadmium, lead, copper, and zinc elements, diluted to concentrations of 10, 50, and 100 μg/L.

The samples of 1–2 g were combusted at the temperature of 400 °C for 4 h and then to 600 °C for 2 h in a muffle furnace. For the digestion of milk samples, a representative 1 g (dry weight) sample is digested with additions of 3 ml nitric acid (HNO_3) and 2 ml of hydrogen fluoride in a microwave for 20 min. After microwave digestion the samples were diluted with 1% HNO_3 in a 10 ml vessel [28].

The quantified concentrations showed discrepancies below 10% compared to certified values. The operating parameters of the Varian ICP 820-MS instrument were as follows: plasma flow of 17.5 L/min, auxiliary flow of 1.7 L/min, sheath gas flow of 0.2 L/min,

nebulizer flow of 1.0 L/min, sampling depth of 6.5 mm, RF power of 1.4 kW, pump rate of 5.0 rpm, and stabilization delay of 10.0 s. All analyses were conducted in triplicate, and the results were expressed as mg/100 g sample.

2.3. Determination of pesticide

The study of pesticide content in milk was conducted by chromatography in a thin layer in the scale range of measurements of the mass concentration of organochlorine pesticides 0.05–5.0 mg/kg [29]. The analysis was performed on a 6890/5973 N gas chromatograph with a mass spectrometric detector (Agilent, USA) equipped with a CombiPAL autosampler (CTC Analytics AG, Switzerland). The sample volume of 1.0 μl was injected with the autosampler into a sample injection unit that was heated to 250 °C in the no-flow splitting mode. To separate, a DB-35MS capillary column (Agilent, USA) with a length of 30 m, an inner diameter of 0.25 mm, and a film thickness of 0.25 μm was used.

The carrier gas (helium "A" grade) was supplied at a constant flow rate of 1.0 ml/min (average linear flow rate 36 cm/s). The column thermostat temperature was programmed from 40 °C (1 min exposure) to 160 °C (3 min exposure) at a heating rate of 20 °C/min, followed by heating to 300 °C (15 min exposure) at a rate of 3 °C/min. The interface, quadrupole, and ion source temperatures of the mass spectrometric detector were 300, 150, and 230 °C, respectively. The voltage at the electron multiplier and other detector fine-tuning parameters were optimized using the autotuning function implemented in the MSD ChemStation software (Agilent, USA).

2.4. Determination of functional cumulation of heavy metals and pesticides

The determination of functional cumulation of heavy metals and pesticides was performed by calculating the cumulation coefficient based on the results of experiments conducted under in vivo conditions [30].

Two groups (experimental and control) of snails (6 snails in each) weighing 13–14 g and 1.5 months old were prepared for the study.

Lethal dose of the studied xenobiotics (LD_{50}) was determined experimentally on the "control" group of snails. The lethal dose for each xenobiotic was determined by the maximum amount of the toxic element that resulted in the death of 50% of snails within 1 day.

For a period of one month, a group of snails from the experimental group were placed daily, at the same time each day, in Petri dishes containing 20 ml of a solution. The solution contained a fraction of the lethal dose of the investigated xenobiotics, which is known to cause the death of 50% of the experimental subjects.

During the study, the daily administered dose of lead, cadmium, mercury, arsenic, and organochlorine pesticides was 0.1 LD_{50}, since these elements and pesticides exhibit toxic effects even in low concentrations. The daily dose of zinc and copper was 0.2 LD50 as less toxic elements.

The cumulation coefficient (C_c) of the studied xenobiotics was calculated according to the following equation (1):

$$C_C = n_{50} \times d / LD_{50} \tag{1}$$

where: n_{50} – total average lethal dose at n-fold exposure;

D – daily dose (multiple of LD_{50})

LD_{50} – average lethal dose at a single injection.

For a comparative assessment of the cumulative properties of the xenobiotics under study we applied the classification proposed by Kagan [31] (Table 1).

2.5. Determination of the activity of catalase

The activity of catalase was determined by gas-metric method using the Warburg method [32]. The activity of catalase can be judged from the volume of oxygen released as a result of hydrogen peroxide decomposition. In a round bottom flask containing 15 ml of substrate (distilled water or milk) 0.001 g of crystalline catalase at 37 °C was added and 5 ml of 3% hydrogen peroxide was added under continuous stirring. The flask was hermetically sealed with a silicone tube and connected to a volumeter. As a result, the volume of released gas was measured, the value of which was adjusted to normal conditions according to Clapeyron's unified gas law (equation (2)).

$$\frac{P_1 V_1}{T_1} = \frac{P_2 V_2}{T_2}, \text{ or } \frac{PV}{T} = const, \tag{2}$$

Table 1
Classification of cumulative effects of xenobiotics.

Cumulation rate	C_c value
Supercumulation	<1
Expressed cumulation	1–3
Moderate cumulation	3–5
Low cumulation	5

where:

P_1 – initial pressure, kPa.

P_2 – final pressure, kPa.

V_1 – initial volume, ml.

V_2 – final volume, ml.

T_1 – temperature of the first gas, K.

T_2 – final temperature, K.

The effect of heavy metal ions was studied by the same methodology, with the addition of 0.015 mg of lead salt per 15 ml of substrate and 0.0045 mg of cadmium salt per 15 ml of substrate.

Water and milk without heavy metal salts were used as a control sample. As a test sample was used.

- water containing lead salts (0.015 mg per 15 ml of water) and cadmium (0.0045 mg per 15 ml of water);
- milk with lead salts (0.015 mg per 15 ml of water) and cadmium (0.0045 mg per 15 ml of water).

In the experimental samples of water and milk were added lead and cadmium salts concentration, exceeding the maximum allowable standard by 10 times.

2.6. Statistical analysis

Statistical analysis of the results was performed using Statistica 12.0 (STATISTICA, 2014; StatSoft Inc., Tulsa, OK, USA). The differences between samples were evaluated using one-way ANOVA. A p-value <0.05 was considered statistically significant.

3. Results and discussion

3.1. Determination of heavy metals and pesticides in milk sampled from eastern part of Kazakhstan

The results of the study of the content of heavy metals and organochlorine pesticides in the milk samples collected from the 5 study areas are presented in Table 2. Milk samples from all five areas in the eastern part of Kazakhstan have exceeded the maximum allowable concentration (MAC) for lead, with Shemonaikha having the highest concentration at 190.0 μg/kg and Beskaragai having the lowest at 110.0 μg/kg. The MAC for lead is 100 μg/kg according to the regulatory norm of Kazakhstan.

The milk samples from Borodulikha, Shemonaikha, and Katon-Karagai also exceeded the MAC for cadmium, which is 30 μg/kg. Shemonaikha had the highest concentration at 56.0 μg/kg, while Ulan had the lowest at 13.0 μg/kg. For copper and zinc, all five areas exceeded the MAC of 1000 μg/kg and 5000 μg/kg respectively. Beskaragai had the highest concentration of copper at 1850 μg/kg while Ulan had the lowest at 560 μg/kg. Shemonaikha had the highest concentration of zinc at 6900 μg/kg while Ulan had the lowest at 5200 μg/kg.

The concentrations of mercury and arsenic in all milk samples were below their respective MACs of 5 μg/kg and 50 μg/kg. Arsenic content varied across the regions, with Borodulikha (9.0 μg/kg) exhibiting the highest concentration, followed by Shemonaikha (6.0 μg/kg), Katon-Karagai (2.0 μg/kg), and Ulan (not detected). Beskaragai did not detect any arsenic in the milk samples. Mercury was not detected in the milk samples from Borodulikha and Katon-Karagai. However, Ulan and Beskaragai had low levels of 2.0 μg/kg and 2.1 μg/kg, respectively. Shemonaikha showed the lowest mercury concentration at 1.4 μg/kg.

The results showed that the concentrations of cadmium (Cd), lead (Pb), and mercury (Hg) were significantly higher than those reported by Parsaei (2019) in a study of raw bovine milk samples from Iran. Parsaei reported the highest concentrations of Cd, Pb, and Hg as 4.05 μg/kg, 12.36 μg/kg, and 5.76 μg/kg, respectively [34]. In comparison to a study by Sidawi (2021) that examined milk samples from Georgia, the concentration range of Pb in the present study falls within the range reported by Sidawi, indicating a similar level of lead contamination [35]. Chirinos-Peinado (2022) reported mean concentrations of Pb and Cd in milk as 15 μg/kg and 505 μg/kg, respectively. In comparison to their findings, the concentration of Pb in the present study is similar while the concentration of Cd is lower [36]. Madani-Tonekaboni (2019) detected mean concentrations of lead and cadmium in raw milks purchased from

Table 2
Content of xenobiotics in milk samples, μg/kg.

District	Borodulikha	Ulan	Beskaragai	Shemonaikha	Katon-Karagai	MAC [33]
Cadmium	$22{,}0 \pm 0{,}33^{c}$	$13{,}0 \pm 0{,}23^{a}$	$18{,}0 \pm 0{,}27^{b}$	$56{,}0 \pm 0{,}85^{e}$	$33{,}0 \pm 0{,}47^{d}$	30
Lead	$130{,}0 \pm 1{,}65^{b}$	$170{,}0 \pm 1{,}80^{d}$	$110{,}0 \pm 1{,}72^{a}$	$190{,}0 \pm 1{,}57^{e}$	$160{,}0 \pm 2{,}71^{c}$	100
Mercury	n/d	2.0 ± 0.02^{b}	$2{,}1 \pm 0.03^{b}$	$1{,}4 \pm 0.02^{a}$	n/d	5
Arsenic	9.0 ± 0.12^{c}	n/d	n/d	6.0 ± 0.11^{b}	2.0 ± 0.03^{a}	50
Zinc	6100 ± 68^{c}	5200 ± 76^{a}	5500 ± 62^{b}	6900 ± 93^{d}	5700 ± 84^{b}	5000
Copper	1410 ± 15^{c}	560 ± 8^{a}	1850 ± 27^{e}	1700 ± 19^{d}	1310 ± 24^{b}	1000
HCCH	n/d	n/d	n/d	n/d	n/d	50
DDT	n/d	n/d	n/d	n/d	n/d	50

$^{a–e}$ means within the same row with different lowercase letters differing significantly among different milk samples ($P < 0.01$).
n/d – not detected; MAC - maximum allowable concentration; HCCH - Hexachlorocyclohexane; DDT – Dichlorodiphenyltrichloroethane.

supermarkets in the east of Iran as 38.15 μg/kg and 4.67 μg/kg, respectively. The concentrations in the present study are notably lower for both lead and cadmium [37]. Monteverde (2022) analyzed milk samples from Italian dairy farms and reported a concentration of 20 μg/kg for Pb. This concentration is similar to the range observed in the present study [38].

Chuanyou Su (2021) investigated milk from industrial regions of China and found levels of Cd and Pb as 0.11 μg/kg and 2.68 μg/kg, respectively. The concentrations of both Cd and Pb in the present study are higher than those reported by Chuanyou Su [39]. Malhat et al. reported that the highest concentration of Zn in cow milk sampled from Egypt was 10,750 μg/kg, for lead 4404 μg/kg which are much higher than the data of present study [40]. Overall, these comparisons suggest that there is variation in heavy metal contamination levels in milk across different regions and countries.

These findings highlight significant regional variations in the levels of heavy metals present in the milk samples, indicating potential environmental contamination. The presence of high concentrations of cadmium, lead, copper, and zinc in certain regions raises concerns about the potential health risks associated with the consumption of contaminated milk. It is essential to address these issues through proper monitoring, control measures, and regulatory interventions to ensure the safety and quality of milk products in the affected areas.

Based on the studies, organochlorine pesticides were not detected in the studied milk. Apparently, the treatment of crops used for animal feed meets the requirements for their use.

Of all the elements tested, the content of mercury and arsenic in the milk samples does not exceed the level of maximum allowable concentration.

The content of cadmium above the maximum allowable concentration was found in milk sampled from Shemonaikha and Katon-Karagai districts. The content of lead and zinc above the maximum allowable concentration was found in milk samples taken from all 5 studied districts.

The content of copper exceeding the maximum allowable concentration was found in milk samples taken from 4 districts under study. These are Borodulikha, Beskaragai, Shemonaikha and Katon-Karagai.

In milk samples taken from 5 districts of East Kazakhstan region, the content of the 4 most common elements was established: lead, cadmium, zinc and copper. In milk samples taken from Shemonaikha district, all 6 elements under investigation were detected. Among the 6 elements tested, the content of mercury and arsenic in milk does not exceed the maximum allowable concentration. Probably, it is connected with mining of polymetals in the area.

From the data obtained a map with a high content of hazardous xenobiotics in milk of East Kazakhstan area is made (Fig. 1).

It is established that the excess of the level of maximum allowable concentration of lead, cadmium, zinc and copper in milk is observed in Shemonaikha district, which is located in the northern direction from the city of Ust-Kamenogorsk. In this connection, this area can be attributed to 1 zone with high content of hazardous xenobiotics in milk. It should be noted that large industrial enterprises

Fig. 1. Location of sampling areas with hazardous xenobiotics in milk in eastern Kazakhstan.

such as "Kazzinc" LLP, "Ust-Kamenogorsk Titanium–Magnesium Plant" JSC and others are located in the northern part of Ust-Kamenogorsk city.

In the southeastern direction from Ust-Kamenogorsk city there is Katon-Karagai district. In milk received from this area, the content of mercury in milk samples was not detected. However, it was found that the level of lead, cadmium, zinc and copper in milk exceeded MPC, but to a lesser extent than in Shemonaikha district. In this regard, this area can be referred to zone 2 with a high content of hazardous xenobiotics in milk.

Borodulikha and Beskaragai districts are located in the northwest direction from Ust-Kamenogorsk city. In milk obtained from these areas, there is an excess of MPC in milk for lead, zinc and copper, in connection with which this area can be attributed to the 3 zone with a high content of hazardous xenobiotics in milk. At the same time, Borodulikha and Beskaragai districts are located in the northern direction from Semey city, where such enterprises as silicate, cement factory are operating. Moreover, there is a cobalt-nickel ore deposit in Beskaragai district. Perhaps, in connection with this fact, in milk samples of Beskaragai district the content of mercury 0.0021 mg/kg was found.

Ulansky district is located to the south of Ust-Kamenogorsk city. In the milk obtained from this area there is a slight excess of MPC of lead and zinc. This area can be attributed to zone 4 with a high content of hazardous xenobiotics in milk.

Exceeding of MPC levels in milk for heavy metals in areas located in the northern, south-eastern and north-western direction from Ust-Kamenogorsk city is probably due to the direction of the wind rose.

The main settlements are located in the northern, north-western, south-eastern and southern directions from Ust-Kamenogorsk city.

Thus, on the basis of studies of the content of xenobiotics in milk samples from 5 districts of the East Kazakhstan region, it was established that the most common and dangerous xenobiotics are lead, cadmium, zinc and copper.

To conduct comprehensive monitoring of food safety in intensively developed areas of East Kazakhstan region at the first stage of the study, we carried out analytical and information work based on laws and regulations of the Republic of Kazakhstan and East Kazakhstan region, as well as statistical data of ministries and departments of the Republic of Kazakhstan. There are 956 active industrial enterprises in East Kazakhstan Region, of which manufacturing industry is more than 71%, and mining industry - more than 23%. According to the analysis of official statistics, East-Kazakhstan region has 22,088 sources of emissions of pollutants, which is 8.3% of the total number of sources in the Republic of Kazakhstan [41].

Based on the previous analysis of [42,43], it is necessary to constantly monitor the content of toxic xenobiotics in milk, as raw materials of animal origin, namely the content of cadmium, lead, mercury, arsenic, zinc, copper and organochlorine pesticides.

Lead and cadmium are toxic metals that cause harmful effects on human organs. Lead has negative effects on all systems of the human body and can cause permanent brain injury, hearing loss, and cognitive impairment. Laws have been tightened to reduce lead intoxication, but lead is still widely used in industry, resulting in emissions that contaminate the environment [44–47]. Cadmium exhibits toxicological effects on the human body, including nephrotoxicity, carcinogenicity, teratogenicity, endocrine and reproductive toxicity [48,49]. Cadmium exposure can lead to renal and hepatic dysfunction, pulmonary edema, osteomalacia, adrenal and hematopoietic damage. Cadmium is a proven carcinogen for humans. It has a long biological half-life and can accumulate in the soft tissues of the human body [50].

Zinc and copper are essential micronutrients. However, they have toxic effects when their concentrations exceed the maximum allowable levels in the living organism. For example, many researchers attribute neurodegenerative disorders, including Alzheimer's and Parkinson's diseases, to the toxic effects of copper in its excessive accumulation in the human body. The occurrence of Wilson's and Menke's diseases, hepatic diseases, necrosis, and hyperemia of blood vessels are also considered to be examples of toxic effects of copper [51,52]. Zinc is an essential and vital element for the body. At the same time, at high doses, zinc reduces the absorption of phosphorus and causes anemia and digestive disorders. Increased intracellular zinc content leads to the Alzheimer's disease and Kuffor-Rakeb syndrome [53,54].

The xenobiotics studied in this work have different toxicological effects on the human body. An in vivo experiment was carried out to quantify the ability of a xenobiotic to accumulate in the body and have an adverse effect. The bioaccumulation factor for each xenobiotic was subsequently calculated.

The procedure for determining the lethal dose (LD_{50}) involves administering various doses of metal to snails and observing their response, including death. Methods such as oral administration or contact with contaminated substrates are often used to assess toxic effects.

As a result of experimental studies, the lethal dose (LD_{50}) for each xenobiotic was determined for the "control" group of snails.

The death rate of 50% of the experimental objects in the group of snails "experimental" was determined on the following days: for zinc - on the 26th day, for copper - on the 22nd, lead - on the 20th, cadmium - on the 21st, organochlorine pesticides (HCCH, DDT) - on

Table 3
Bioaccumulation factor of the analyzed xenobiotics.

Name	Cumulation coefficient	Level of cumulation
- HCCH	1,7	IB – high risk
- DDT	1,9	IB – high risk
Lead	2,0	IB – high risk
Cadmium	2,1	IB – high risk
Copper	4,4	II – moderate risk
Zinc	5,2	III – little risk

the 17th and 19th, correspondingly.

The cumulation coefficient (bioaccumulation factor) is an important indicator in assessing the ecological risk and toxicity of substances, particularly for those with accumulative properties, such as certain heavy metals and pesticides. The bioaccumulation factor is defined as the ratio of the cumulative dose of a toxin, typically causing a specific effect (often lethal) in 50% of the test animals when administered repeatedly and in fractions, to the dose that induces the same effect with a single exposure. This factor is inversely proportional to the intensity of bioaccumulation: the smaller the factor, the greater the bioaccumulation.

The results of the bioaccumulation factor calculation are presented in Table 3. Based on the obtained values of the bioaccumulation factor, the degree of bioaccumulation was determined for each investigated xenobiotic. The xenobiotics were categorized according to their hazard level: highly hazardous (Ib), moderately hazardous (II), and low hazardous (III). Organochlorine pesticides (such as HCH and DDT) exhibit a high degree of bioaccumulation and are classified as highly hazardous substances. Lead and cadmium also show a high degree of bioaccumulation and are classified as highly hazardous substances. Zinc demonstrates a moderate degree of bioaccumulation and is classified as a moderately hazardous substance. Copper exhibits a low degree of bioaccumulation and is classified as a low hazardous substance. These results allow for the assessment of the accumulation level of the investigated xenobiotics in snail organisms. A low value of the cumulation coefficient indicates a potential for adverse effects on organisms, while a high degree of bioaccumulation suggests a lower potential for toxicity of these substances.

3.2. Development of a biosensor method for the determination of heavy metals

To determine lead and cadmium in milk and milk products, as highly dangerous and the most common xenobiotics in the territory of the East Kazakhstan region, we propose a biosensor method. The biosensor method is characterized by the simplicity of the device, efficiency, specificity, and low cost. At the same time, it is characterized by high analytical capabilities and is widely used in practice [55,56].

Biosensors detect the signal produced by the interaction between the analyte and biological components, which is proportional to the heavy metal concentration. The biosensor schematic consists of a transducer (B) containing a biocatalyst (A) that interacts with heavy metal salts (S) in the product (Fig. 2). As a result of the chemical reaction at the transducer contacts (B), an electrical potential (P) is generated. The electrical potential is output from the transducer as an electrical signal and amplified by an operational amplifier (C). The amplified signal is processed by an Arduino Nano microcontroller (D) using a loaded program and displayed on a screen (E) as a digital image.

In order to develop an express method of determining cadmium and lead in milk, the biological material for the biosensor is selected. In this study, the enzyme catalase of the oxidoreductase class, which catalyzes redox reactions, was used for the experiments. The development of enzyme-based biosensors is promising because of their high selectivity, sensitivity, and low cost [57–59].

During the experimental studies, it was found that the enzyme catalase is the most sensitive to cadmium and lead salts. As a result, we studied the activity of catalase enzyme in aqueous medium and in milk at certain concentrations of lead and cadmium salts and revealed the effectiveness of using catalase enzyme as a biomarker of milk contamination.

Based on the mathematical analysis, the degree of transformation of the substrate was calculated. The results are presented in Fig. 3.

It was found that in control samples (water and milk) the degree of transformation of the substrate differs slightly and was 69–72%. Therefore, we can conclude that this technique can be used in a milk medium. In the experimental samples with cadmium and lead salts concentration exceeding the maximum permissible norm by 10 times, the decrease of the enzyme activity was observed in comparison with the control sample. As can be seen from the graph in Fig. 3, lead has a greater degree of inhibitory activity against the enzyme catalase. In the presence of cadmium ions, the degree of transformation of the substrate was 66–68%, in the presence of lead ions - 57–60%. The data obtained indicate that catalase can be used as an indicator for the detection of heavy metal salts, especially lead.

A polymer substrate was used as the base of the biosensor, on which three types of conductive layers were deposited by sputtering: silver, platinum, and graphite. The preliminary conductivity tests revealed no significant differences between the three types of substrates. However, considering the economic efficiency, it is advisable to use a polymeric substrate with graphite conducting layers as a carrier for enzyme immobilization in further studies.

In this study, the electrode potential was determined using.

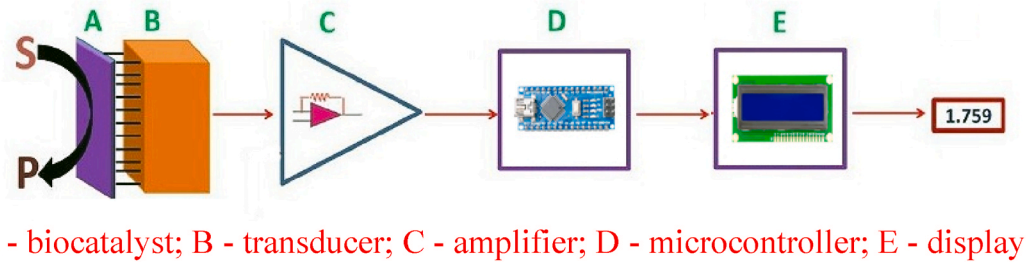


Fig. 2. Biosensor block diagram.

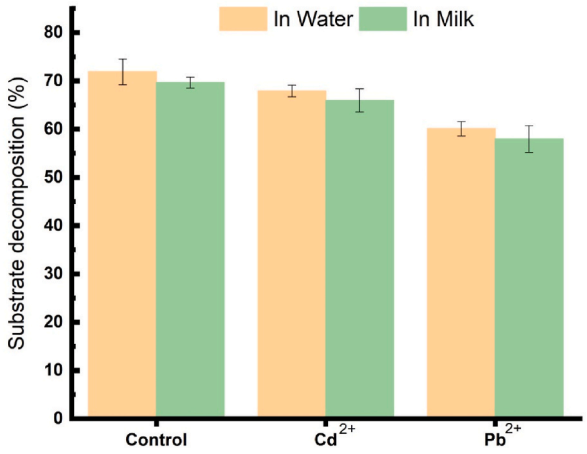


Fig. 3. Study of catalase enzyme activity.

- platinum electrode with the enzyme immobilized on it (A);
- polymer plate with a graphite conductive layer and the enzyme immobilized on it (B). The pure substrate was tested;
- polymer plate with a graphite conductive layer and enzyme immobilized on it (C). The substrate was tested with the addition of cadmium salt;
- polymer plate with a graphite conductive layer and an enzyme immobilized on it (D). The substrate was tested with the addition of lead salt.

A platinum electrode with the enzyme immobilized on it was used as a control sample.

The electrode potential of the experimental samples was measured on a digital dual-channel memory oscilloscope ASK-2034 in constant current mode.

Using the Nernst equation [60], we calculated the value of the electrode potential for the redox reaction of hydrogen peroxide decomposition (equation (3)).

$$E = E^0 - \frac{RT}{nF} \ln \frac{a(Red)}{a(Ox)} \tag{3}$$

where:

R – the universal gas constant, equal to 8.31 J/(mol*K).

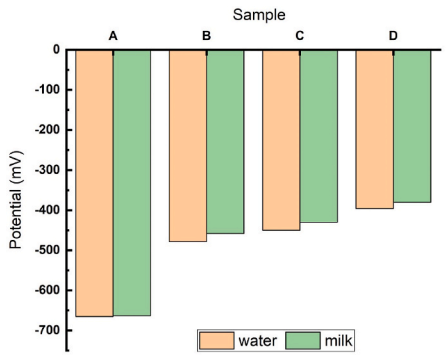
T – absolute temperature.

F – Faraday number equal to 36,500 Cl/mol.

n – number of moles of electrons involved in the process.

The calculated value of the electrode potential under these conditions was 667.2 mV.

When creating enzymatic biosensors, special attention is paid to the process of immobilization of biological material on the



A – control ("ideal conditions") B – test substrate
C – test substrate with $CdSO_4$; D – test substrate with $Pb(NO_3)_2$

Fig. 4. The value of the electrode potential of the control and experimental samples.

substrate surface. The sensitivity of the biosensor and the quality of its measurements will depend on the method of immobilization [61,62]. In this work, we used the layer-by-layer method, in which oppositely charged polyelectrolytes form multilayers on the substrate surface.

Two variants of multilayers were used for enzyme immobilization: in the first one, the combination "chitosan-sodium alginate" and in the second one, "chitosan: sodium carboxymethyl cellulose (CMC). The substrate was immersed in catalase enzyme solution with a concentration of 0.05 mg/ml, then after complete drying, the substrate was weighed and the mass of immobilized catalase was determined. After that, the substrate was immersed in chitosan and sodium alginate/CMC solution in 5-fold repetitions, alternately, until a dense multilayer membrane was formed. As the number of multilayers increased, a very low electrode potential was observed. This is probably due to the fact that with increasing membrane thickness the diffusion of substrate and product molecules to the carrier surface was reduced.

The results of repeated measurements on a substrate with a chitosan- CMC multilayer combination had a large scatter of data, which led to the conclusion that this combination is inexpedient for enzyme immobilization. For immobilization of the enzyme, the multilayer combination "chitosan-alginate sodium" was used. The result of measurements on a substrate with a multilayer of sodium chitosan-alginate combination is shown in Fig. 4.

4. Conclusion

The analysis of the concentration of heavy metals in milk revealed excess of MAC of lead and zinc in milk samples taken from all 5 studied areas of eastern Kazakhstan. The content of cadmium is higher than MAC in milk from Shemonaikha and Katon-Karagai. Also, the concentration of copper is higher than MAC in all districts except Ulan district. The content of mercury and lead is below MAC in milk sampled from all districts. The research found that organochlorine pesticides, lead, and cadmium exhibit a high degree of bioaccumulation and are classified as highly hazardous substances. Zinc demonstrates a moderate degree of bioaccumulation, while copper exhibits a low degree of bioaccumulation, indicating a lower potential for toxicity. Biosensor method for rapid detection of hazardous heavy metals is proposed. Application of the layer-by-layer method for immobilization of the enzyme on the surface of a substrate with a multilayer combination of "chitosan-sodium alginate" increases the sensitivity of the biosensor and the quality of its measurements. The conducted research confirms the efficiency of using a polymeric plate with a graphite conducting layer as a basis for the biosensor.

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Author contribution statement

Yerlan Zharykbasov and Nadir Ibragimov - Conceived and designed the experiments. Klara Zharykbasova, Zhaynagul Kakimova, Gulnara Tulkebayeva - Performed the experiments. Aitbek Kakimov, Klara Zharykbasova, Zhanibek Yessimbekov - Analyzed and interpreted the data. Sandugash Toleubekova, Gulmira Mirasheva, Alibek Muratbayev - Contributed reagents, materials, analysis tools or data. Klara Zharykbasova, Aitbek Kakimov, Zhanibek Yessimbekov - Wrote the paper.

Data availability statement

Data included in article/supplementary material/referenced in article.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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