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INFLUENCE OF SUBLETAL CONCENTRATIONS OF COBALT (II) IONS ON HEMATOLOGICAL INDICATORS IN THE ORGANISM OF FRESHWATER FISH

The study examined changes in selected biochemical indicators (glucose, lactate, pyruvate levels, and lactate dehydrogenase activity) in the blood of crucian carp (*Carassius gibelio* Bloch) and pike (*Esox lucius* L.) exposed to 0.1 and 0.25 mg/dm³ of $Co^{2+}$ ions in water. The results demonstrated that changes in the hematological parameters of fish are species-specific and depend on the concentration of metal ions in the aquatic environment.

Despite exposure to sublethal concentrations of cobalt ions, the metal content in the blood of both fish species remained unchanged; however, in crucian carp, it was an order of magnitude lower than in pike. An increase in glucose concentration was observed in both species at both cobalt concentrations. Lactate levels in crucian carp increased under 0.1 mg/dm³ exposure, whereas in pike, they rose at 0.25 mg/dm³. Pyruvate concentration in crucian carp increased at 0.25 mg/dm³, whereas in pike, it decreased proportionally to the cobalt concentration in water.

A positive correlation was observed between the lactate/pyruvate ratio and lactate dehydrogenase activity in the blood of both species at elevated cobalt concentrations. These findings suggest that in pike, at higher cobalt concentrations (0.25 mg/dm³), glycolytic processes play an increased role in the organism's energy supply.

*Key words: pike, crucian carp, blood, cobalt.*

Надійшла 29.11.2024.

UDC 547.458:664.16 doi: 10.25128/2078-2357.24.3–4.3

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# STUDY OF SUCRALOSE CONTENT IN CARBONATED DRINKS OF DIFFERENT MANUFACTURES

It was established that spectroscopic and chromatographic methods of analyzing were used to determine sucralose. Given that the spectroscopic method of analysis requires specific preparation of samples is insufficiently accurate and sensitive, and the chromatographic method is expensive, the electro-analytical method of determination was chosen for the research. This is justified by the structure of the molecule (it contains functional groups that can be oxidized electrochemically). Electrochemical experiments were carried out using a potentiostat-galvanostat on a three-electrode setup with a glassy carbon electrode as a working electrode, a platinum electrode as an auxiliary electrode and a silver chloride electrode for comparison. The study utilized non-carbonated beverages Continente (Portugal) and Xixo (Hungary), as well as carbonated beverages Sumol Zero (Portugal) and Fanta Shokata (Ukraine).The manufacturer indicated the presence of sucralose in all of them. Based on the conducted research and corresponding calculations, it was established that the beverages are safe for consumption.

Natural processes of photo-, photoelectro-, and electrodegradation in soil and wastewater reduce the problems of sucralose accumulation in the environment. Therefore, to prevent diffusion to the anode space with the release of gaseous chlorine, membrane electrolysis is applied (the membrane is made of polyvinylpyridine), which separates the cathode and anode spaces and does not allow chloride ions to reach the anode. In this case, water electrolysis or electrooxidation of hydroxyl ions occurs at the anode with the formation of gaseous oxygen.

Electroanalytical determination of sucralose was also conducted using the method of cyclic voltammetry. The fact that the electrochemical determination of sucralose took place can be judged by a gradual but sharp increase in the current value at certain potential values. In this case, the intensity of this increase depends on the concentration of the sweetener.

A solution with a neutral pH level was used as the background electrolyte. Materials based on carbon (graphite, carbon nanotubes) were used as the working electrode.

We proposed a new method for determining sucralose, associated with the dependence of the peak current value during its electrochemical oxidation on the concentration. At the same time, a linear relationship between the peak current value and the sweetener concentration is maintained.

*Key words: sucralose, electrochemical determination, cyclic voltammograms, membrane electrolysis, carbonated drinks, background electrolyte.*

Sucralose is almost entirely excreted from the body, does not penetrate the brain, does not raise blood sugar levels, is resistant to oral bacteria, and its use as a sugar substitute is increasing. At the same time, the widespread use of sucralose, its relatively high stability, minimal biodegradation in sewage systems and treatment facilities, prompted researchers to consider the possibility of using this compound as a reliable molecular marker for identifying sources of pollution in natural water bodies. The use of sucralose and its impact on life processes are described in many scientific works [1–5]. These studies have drawn attention to chlorinated sweeteners that are metabolically active and can disrupt metabolism by negatively affecting thyroid hormone metabolism. It has also been established that there are certain metabolites of sucralose whose safety profiles are currently unknown [3, 4, 5]. Additionally, tests using several different methodologies have shown the presence of mutagenic properties in sucralose hydrolysis products, as well as the possibility of forming potentially toxic compounds—dioxins and tetrachlorodibenzofurans—under high temperatures. This factor is more pronounced when sucralose and its derivatives are used as components in anti-corrosion agents.

Therefore, there is a pressing need to determine the concentration of sucralose in various biological fluids, in carbonated beverages, and in the environment. In a number of works [6–10], methodologies used for determining sucralose are described. Specifically, the analytical profile of sucralose was studied using spectroscopic and chromatographic methods of analysis. However, the first method requires specific sample preparation, is insufficiently accurate and sensitive. The second method, although characterized by a high level of sensitivity, uses rather expensive equipment. Moreover, both methods are quite slow and labor-intensive.

The polarimetric method of analysis is widely used in pharmaceutical and food industries for analyzing substances, intermediates, determining the content of sugary substances or medicinal products, as well as for identifying specific substances and their origins. For identifying the composition of oils, the polarimetric method is used together with refractometry. The approaches analyzed in the scientists' works [6–10] were successfully utilized. Therefore, we decided to use the electroanalytical method of determination as an alternative, considering that sucralose is electrochemically active since it contains functional groups capable of electrochemical oxidation. To justify the use of this approach, we considered the following advantages: modernity, low cost, speed, selectivity, accuracy, and sensitivity.

**The purpose** of the article is to conduct the determination of sucralose in carbonated beverages and the environment.

**Research task** is to select the most modern, inexpensive, sensitive, and selective methods for determining sucralose.

## Materials and methods of research

Electrochemical determination of sucralose was conducted using a potentiostat-galvanostat with curve registration, connected to an electrochemical cell. Electroanalytical determination of sucralose was performed using the method of cyclic voltammetry. A potential is applied to the anode, the magnitude of which cyclically changes within certain limits at a constant rate. In this process, an electric current passes through the cell containing a solution with sucralose, and the dependence of the current on the potential is graphically depicted. Here, the potential is plotted on the x-axis, and the current on the y-axis. Since the potential changes cyclically within certain limits, the graph of the dependence between the current and potential is called a cyclic voltammogram. The fact that the electrochemical

determination of sucralose took place can be judged by a gradual but sharp increase in the current value at certain potential values. In this case, the intensity of this increase depends on the concentration of the sweetener.

**Research findings and their discussion**

Sucralose (100 g of a colorless fine crystalline powder) was purchased from the sports nutrition store "Protein in Kyiv" (Kyiv, Ukraine) and used without further purification. Sucralose solutions with concentrations of $10^{-5}$, $10^{-4}$, $10^{-3}$, and $10^{-2}$ mol/L were prepared in distilled water using serial dilution. The study utilized non-carbonated beverages Continente (Portugal) and Xixo (Hungary), as well as carbonated beverages Sumol Zero (Portugal) and Fanta Shokata (Ukraine). In all of them, the manufacturer indicated the presence of sucralose. For the study, 25 ml of each beverage was taken without dilution. Electrochemical experiments were conducted using a PI-50 potentiostat-galvanostat on a three-electrode setup with a glassy carbon electrode as the working electrode, a platinum auxiliary electrode, and a silver-silver chloride (3M) reference electrode. Signal registration was carried out using a Keithley multimeter with data output to Microsoft Excel. At this stage, the potential window was chosen from -2.0 to +2.0 V with a potential sweep rate of 0.1 V/s. Figure 1 shows the cyclic voltammograms of sucralose solutions of known concentrations from $10^{-5}$ to $10^{-2}$ mol/L in concentration decreasing order:

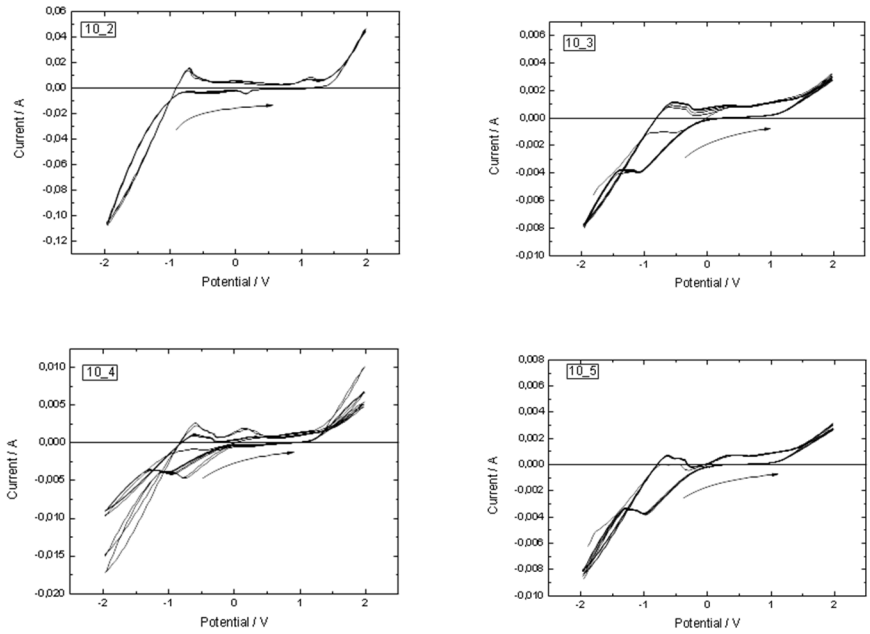


Fig. 1. Cyclic voltammograms of sucralose solutions with decreasing molar concentration.

As can be seen from the patterns of current increase depending on the potential, the electrooxidation of sucralose exhibits a sufficiently pronounced voltammetric profile. The first peak, which height depends on the concentration, is observed at a potential of -0.7 V and corresponds to the electrooxidation of the non-chlorinated hydroxymethyl group. In this process, an equal number of protons and electrons are released. The second peak, which potential approaches +1 V, corresponds to the oxidation of sucralose at the ring hydroxyl groups. The cathodic and anodic peaks at low and high potentials, respectively, describe the onset of the water electrolysis process. Regarding the oxidation of sucralose, the process is quasi-reversible at low concentrations of sucralose and irreversible at high concentrations, indicating the dependence of the kinetics of the oxidation process on pH. The electroanalytical process proceeds in a diffusion or kinetic mode depending on the concentration. There is a dependence between the peak current value and the concentration:

$$I=kC \quad (1)$$

Here, I – is the current, C - is the concentration, and k is the polarographic coefficient, which for this process is found by the formula:

$$k=\frac{I_2}{c_2}=\frac{0,018}{0,01}=1,8 \quad (2)$$

The results of the voltammetric study of non-carbonated beverages Continente (Portugal) and Xixo (Hungary) are shown in Figure 2.

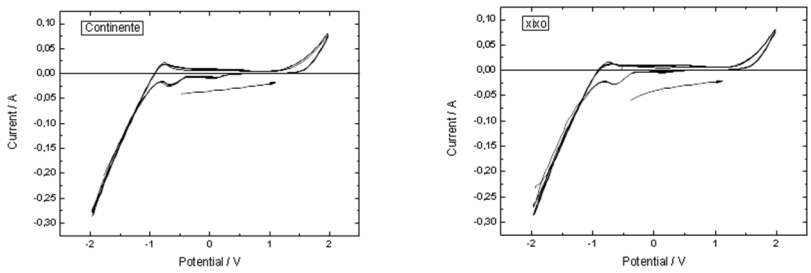


Fig. 2. Cyclic voltage-current curves of non-carbonated drinks.

The voltammetric peak that describes the electrooxidation of sucralose is present on both curves, confirming the presence of sucralose as declared by the beverage manufacturer. The appearance of additional reduction peaks may indicate both the quasi-reversibility of the sucralose electrooxidation process and the reduction of other components of the beverage (especially in the sample of the Portuguese-made beverage). Given that the peak current value in the Continente beverage sample is 0.018 A, the sucralose concentration in it will be:

$$C=\frac{I}{k}=\frac{0,01926}{1,8}=0,0107 \text{ M}$$

As for the Hungarian-made Xixo beverage, the peak current value was 0.01728 A. Accordingly:

$$C=\frac{I}{k}=\frac{0,01728}{1,8}=0,0096 \text{ M}$$

Thus, the concentration of sucralose in non-alcoholic non-carbonated beverages is approximately 0.01 mol/L.

Calculating the amount of sucralose in a 200 ml package bottle of the Portuguese-made beverage:

$$m=CvM=0,0107*0,2*397,5=0,85_{\Gamma}$$

The volume of the Hungarian-made beverage package bottle is 1/3 L. Therefore:

$$m=CvM=0,0\frac{096*1}{3}*397,5=1,27_{\Gamma}$$

The maximum permissible daily intake of sucralose is 880 mg/kg of body weight. Therefore, the amount of sucralose in both beverages is within acceptable limits.

For a person weighing 80 kg, this corresponds to 70 g of sucralose, i.e. 0.177 mol. Accordingly, such an amount of sucralose would be contained in (taking into account the concentration of sucralose in the beverages):

$$v=\frac{m}{CM}=\frac{0,177}{0,0096}=18,43$$ l of the Hungarian-made beverage

та

$$v=\frac{m}{CM}=\frac{0,177}{0,0107}=16,54$$ l of the Portuguese-made beverage

Therefore, both beverages are safe for consumption.

Electrochemical studies of carbonated beverages containing sucralose from Portugal (left) and Ukraine (right) are presented in Figure 3:

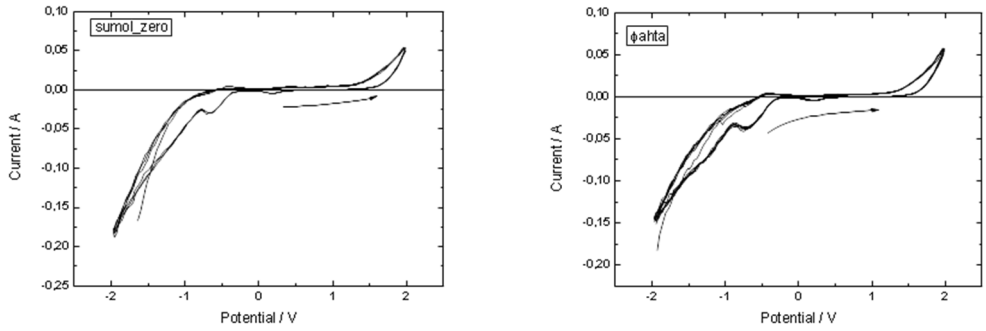


Fig. 3. Cyclic voltage current curves of carbonated drinks.

In this case, the sucralose oxidation peaks appear slightly at a potential of -0.5 V. This is because carbonated beverages contain orthophosphoric acid as an acidity regulator, and since the electrooxidation of sucralose occurs with the release of protons, according to Le Chatelier's principle, the equilibrium of the redox process will shift towards reducing their concentration, i.e., the reduction process, which is reflected by the cathodic peaks on both curves. Moreover, in carbonated beverages, the partial pressure of carbon dioxide is quite high, which also lowers the pH value. Therefore, to determine sucralose in carbonated beverages, the medium needs to be neutralized.

Furthermore, the distinct reduction peak observed at -0.8 V corresponds to the irreversible reaction of gradual sucralose dechlorination with the formation of chloride ions and deoxygalactosucrose:

$$SucrCl_3 + H^+ + 2e^- \rightarrow SucrHCl_2 + Cl^- \quad (1)$$

$$SucrCl_2 + H^+ + 2\ e^- \rightarrow SucrH_2Cl + Cl^- \quad (2)$$

$$SucrH_2Cl + H^+ + 2e^- \rightarrow SucrH_3 + Cl^- \quad (3)$$

As deoxygalactosucrose, unlike sucralose, is metabolized by a larger number of organisms, dechlorination can serve to purify wastewater from sucralose.

Since, as mentioned above, electrooxidation of sucralose is ineffective in acidic solution, and hydrolysis occurs in alkaline medium, a neutral pH solution maintained by a phosphate buffer should be used as the background electrolyte. Reducing the potential window (the difference between the minimum and maximum sweep potentials) allows for better manifestation of peak current values. It is proposed to use a carbon-based material (graphite, carbon nanotubes) as the working electrode. To increase sensitivity and selectivity, a substance with an active amino group or with a basic organic nitrogen atom can be used as a modifier of the electrode, which, interacting specifically with the chlorine atom in the sucralose molecule, will form a quaternary salt. Therefore, considering the above, the strategy of determination undergoes certain changes.

Determination Strategy:

First Stage: Preparation of sucralose solutions of known concentrations. Conduct cyclic voltammetric studies of these solutions with the addition of background electrolyte and buffer solution, based on which a calibration graph of the dependence of the peak current value on sucralose concentration is constructed.

Second Stage: Similar actions are performed with solutions of common natural carbohydrates without and with the addition of sucralose. This is necessary to determine the possibility and degree of selectivity in measuring the concentration of sucralose in beverages and other media.

Third Stage: Preparation of beverage samples that, according to the manufacturers, contain sucralose. The pH is neutralized using a buffer solution.

Fourth Stage: Conduct voltammetric analysis of beverage samples with sucralose. In this case, the influence of the presence of other substances (mainly ascorbic acid, hydroquinone derivatives, other carbohydrates, as well as sugar substitutes like tagatose and steviol) on the accuracy of the analysis is determined, as well as the possibility of simultaneous determination of sucralose and sugar concentrations. The use of electrode modifiers allows increasing the selectivity (specificity) of the method, reducing the cost of sucralose determination, shortening the analysis time, and increasing the accuracy of concentration determination. Based on the conducted studies, it was established that carbonated beverages contain a safe amount of sucralose and, are therefore they are safe for consumption.

Regarding the problem of sucralose accumulation in the environment, it is addressed by natural processes of photo-, photoelectro-, and electrodegradation in soil and wastewater. Since anodic processes based on the Fenton reaction are not suitable for halogenated compounds due to the formation of toxic gaseous chlorine, cathodic degradation in a strongly acidic medium is applied. It has already been used for chlorarenes and trichloroacetic acid, so it is suitable for sucralose as well. In the main cathodic reaction, sucralose is reduced to deoxyhexoses and chloride ions. To prevent the diffusion of the latter to the anode space with the release of gaseous chlorine, membrane electrolysis is applied. A membrane made of polyvinylpyridine, which separates the cathode and anode spaces, does not allow chloride ions to reach the anode. In this case, water electrolysis or electrooxidation of hydroxyl ions occurs at the anode with the formation of gaseous oxygen. The scheme of sucralose degradation in a membrane electrolyzer is shown in Figure 4.

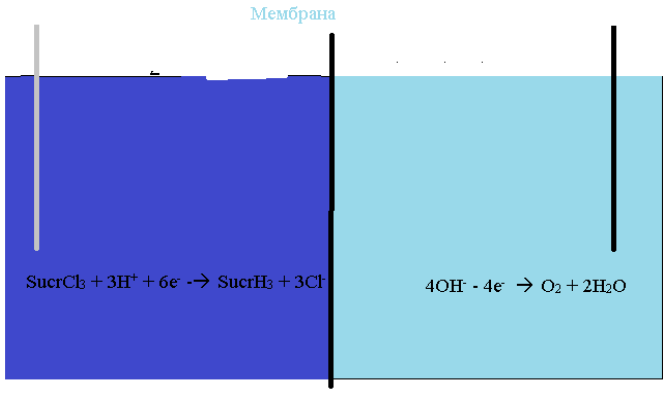


Fig. 4. Scheme of membrane electrolysis during sucralose degradation.

Electroanalytical determination of sucralose is performed using the method of cyclic voltammetry. A potential, the magnitude of which cyclically changes within certain limits at a constant rate, is applied to the anode. In this process, an electric current passes through the cell containing a solution with sucralose, and the dependence of the current on the potential is graphically depicted. The potential is plotted on the x-axis, and the current on the y-axis. Since the potential changes cyclically within certain limits, the graph is called a cyclic voltammogram.

The fact that the electrochemical determination of sucralose took place can be judged by a gradual but sharp increase in the current value at certain potential values. In this case, the intensity of this increase depends on the concentration of the sweetener.

A solution with a neutral pH level is used as the background electrolyte. Materials based on carbon (graphite, carbon nanotubes) are used as the working electrode.

Determination Stages

First Stage: Preparation of sucralose solutions of known concentrations. Conduct cyclic voltammetric studies of these solutions with the addition of background electrolyte, based on which a calibration graph of the dependence of the peak current value on sucralose concentration is constructed.

Second Stage: Preparation of beverage samples that, according to the manufacturers, contain sucralose.

Third Stage: Conduct voltammetric analysis of beverage samples with sucralose. This ensures the possibility of selective, rapid, and sensitive determination of sucralose in carbonated and non-carbonated beverages, which also contain common carbohydrates and other sugar substitutes, as well as in natural environments.

This allows us to compare the concentrations of sucralose with the maximum permissible levels for the human body and the environment. From this, we can judge the admissibility of consuming a particular beverage (pharmaceutical product, chewing gum, pastry).

**Conclusions**

Sucralose is used as a taste sweetener in the food industry and as an excipient (taste corrector) in the pharmaceutical industry during drug production. The wide range of sucralose use in human nutrition makes the process of its electrochemical determination in various environments, as well as its removal from wastewater and natural waters, relevant. We proposed a new method for determining sucralose, associated with the dependence of the peak current value during its electrochemical oxidation on the concentration. At the same time, a linear relationship between the peak current value and the concentration of the sweetener is maintained. Based on the conducted research, it was shown that the concentration of sucralose in non-alcoholic non-carbonated beverages is within the maximum permissible levels, according to the approved procedure by the National Commission of Ukraine with Codex Alimentarius and the Committee on Hygienic Regulation of the Ministry of Health of Ukraine.

This method, after refinement, can be applied for determining sucralose both in samples of biological fluids and in the environment. Our further research will focus on verifying the concentration of the working solution during the synthesis of anti-corrosion composites.


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ДОСЛІДЖЕННЯ ВМІСТУ СУКРАЛОЗИ У ГАЗОВАНИХ НАПОЯХ РІЗНИХ ВИРОБНИКІВ

Встановлено, що для визначення сукралози використовували спектроскопічний та хроматографічний методи аналізу. Враховуючи те, спектроскопічний метод аналізу вимагає специфічної підготовки проб, є недостатньо точний та чутливий, а хроматографічний – дорогий, то для проведення дослідження обрано електроаналітичний метод визначення. Це обгрунтовано будовою молекули сукралози (містить функціональні групи, здатні окиснюватися електрохімічно). Електрохімічні експерименти проводилися з допомогою потенціостату-гальваностату на трьохелектродній установці зі скловугільним електродом в якості робочого електроду, платиновим в якості допоміжного електроду із хлорсрібним електродом для порівняння. У дослідженні було використано негазовані напої Continente (Португалія) та Хіхо (Угорщина), а також газовані напої Sumol Zero (Португалія) та Fanta Shokata (Україна). У всіх із них виробник заявив присутність сукралози. На основі проведених досліджень та відповідних обрахунків встановлено, що напої є безпечними для вживання.

Природні процеси фото-, фотоелектро- та електродеградації у ґрунтових та стічних водах зменшують проблеми накопичення сукралози у навколишньому середовищі. Тому для уникнення дифузії до анодного простору з виділенням газоподібного хлору, застосовується мембранний електроліз (мембрану виготовляють з полівінілпіридину), що розділяє катодний та анодний простір, не пропускає хлорид-йон до аноду. При цьому на аноді відбувається електроліз води або електроокиснення гідроксил-йону з утворенням газоподібного кисню.

Електроаналітичне визначення сукралози проводили також методом циклічної вольтамперометрії. Про те, що електрохімічне визначення сукралози відбулося, можна судити за поступовим, але різким зростанням значення струму у певних значеннях потенціалу. При цьому інтенсивність цього зростання залежатиме від концентрації цукрозамінника.

В якості фонового електроліту використано розчин із нейтральним рівнем pH. В якості робочого електроду використовується матеріал на основі карбону (графіт, карбонові нанотрубки).

Нами запропоновано новий метод визначення сукралози, пов'язаний із залежністю пікового значення струму при її електрохімічному окисненні від концентрації. При цьому зберігається лінійна залежність між піковим значенням струму та концентрацією цукрозамінника.

*Ключові слова: сукралоза, електрохімічне визначення, циклічні вольтамперограми, мембранний електроліз, газовані напої, фоновий електроліт.*

Надійшла 31.10.2024.

UDC 502/504:57(477.81) 577.47: 504.054 doi: 10.25128/2078-2357.24.3–4.4

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# VULNERABILITY OF FRESHWATER MUSSEL *UNIO TUMIDUS* TO WATERBORNE MIXTURE OF PSYCHOACTIVE SUBSTANCES AND MICROPLASTIC

This study investigates the effects of psychoactive substances and microplastics (MP) on the aquatic environment using swollen river mussels (*Unio tumidus*) as bioindicators. Mussels were exposed to microplastic, caffeine, chlorpromazine and their mixture for 14 days and biochemical biomarkers of stress and toxicity were analysed. All exposures caused the signs of toxicity, indicated as the loss of lysosomal membrane stability, inactivation of choline esterase, and decrease of the Zn/Cu ratio. All exposures, particularly MP, increased the glutathione level, indicating the involving of low weight cellular thiols in the stress response. Exposure to MP induced superoxide dismutase, and mixture decreased phenol oxidase activity, confirming the negative cumulative effect of the combine exposure.

*Key words: pharmaceuticals, antioxidants, copper, zinc, cumulative effect.*

The modern life of humankind cannot be imagined without plural pharmaceutical and personal care products [1]. Particularly, nowadays, the psychoactive substances increasingly applicate due to the prevalence of stress, anxiety, and symptoms of post-traumatic stress disorder among the victims of military activities [14]. Correspondingly, they input into the surface waters, and these so-called 'micro pollutants' expected to create the mixtures with cumulative effects on the aquatic organisms [1, 3, 6]. The psychotropic pharmaceutical chlorpromazine (Cpz) was selected for this study as one of most popular drug not only in the schizophrenia curing, but also prospective pharmaceutical due to its antibacterial effect, antitumor activity, inhibition of the replication in different viruses including SARS-CoV-2 [6, 22]. Caffeine (Caff), a central nervous system stimulant, utilized both as medicine