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Old Problems in the Face of New Challenges

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Introduction- Humanity is currently facing new challenges in the context of the evolving COVID-19 (SARS-CoV-2) pandemic. The epidemiological situation in connection with COVID-19 causes the greatest tension in society around the world. The situation throughout the world, unfortunately, is getting worse. In this regard, new measures for the prevention of coronovirus infection are actively recommended and are being developed. One of such measures to prevent the spread of the disease, recommended by WHO and Rospotrebnadzor of the Russian Federation, along with the use of masks and gloves, is the use of disinfectants and sanitizers at work places, in transport, educational institutions, and at home. They are liquid (rarely gel) agents that destroy most harmful microorganisms and viruses, as stated by the manufacturer. The composition of most of these products that enter the distribution network includes ethyl or isopropyl alcohol, triclosan, propylene glycol, formic acid, sometimes salicylic acid, all kinds of fragrances and other substances. Moreover, if traditionally, in order to guarantee the effectiveness of an antiseptic, clinical trials are necessarily carried out with the issuance of an opinion on behalf of a certified scientific center, in the case of sanitizers, usually classified as a cosmetic product, manufacturers do not face many difficulties.

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Old Problems in the Face of New Challenges

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I. INTRODUCTION

Humanity is currently facing new challenges in the context of the evolving COVID-19 (SARS-CoV-2) pandemic. The epidemiological situation in connection with COVID-19 causes the greatest tension in society around the world. The situation throughout the world, unfortunately, is getting worse. In this regard, new measures for the prevention of coronovirus infection are actively recommended and are being developed. One of such measures to prevent the spread of the disease, recommended by WHO and Rospotrebnadzor of the Russian Federation, along with the use of masks and gloves, is the use of disinfectants and sanitizers at work places, in transport, educational institutions, and at home. They are liquid (rarely gel) agents that destroy most harmful microorganisms and viruses, as stated by the manufacturer. The composition of most of these products that enter the distribution network includes ethyl or isopropyl alcohol, triclosan, propylene glycol, formic acid, sometimes salicylic acid, all kinds of fragrances and other substances. Moreover, if traditionally, in order to guarantee the effectiveness of an antiseptic, clinical trials are necessarily carried out with the issuance of an opinion on behalf of a certified scientific center, in the case of sanitizers, usually classified as a cosmetic product, manufacturers do not face many difficulties.

The use of disinfectants recommended by WHO will increase (Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19), Geneva: World Health Organization; 2020 (https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf). The use of disinfectants and antiseptics increasingly requires consideration of indirect environmental and health impacts. There is only limited information on the effects of the use of disinfectants and antiseptics (including detergents and sanitizers) on health, which makes it timely and necessary to conduct research on animal and human. (https://doi.org/10.1093/occmed/kqaa036, accessed 10 May 2020; Key Messages and Actions for COVID-19 Prevention and Control in Schools. Geneva; World Health Organization; 2020 (https://www.who.int/docs/defaultsource/coronaviruse/key-messages-and-actions-for-covid-19-prevention-and-controlin-schools-march-2020.pdf; List N: Disinfectants for Use Against SARS-CoV-2, US EPA. 2020 (https://www.epa.gov/pesticide-registration/list-ndisinfectants-use-against-sars-cov-2).

However, the massive use of sanitizers can lead to poorly predictable consequences for animal and human health. Most of the populations of countries that actively applying sanitizers notice signs of dry skin, peeling, sometimes redness and flushing of the skin, shortness of breath, etc. after several days of use. Water-washed sanitizers end up in wastewater. Currently, there are no special methods for wastewater treatment from these agents and their metabolites as well as from specific viruses. Thus, the concentrations of sanitizers in wastewater, and then in natural waters (as a result of insufficiently purified waters entering natural water bodies, including those used for fisheries!) will rapidly increase. This undoubtedly causes concern among ecologists, doctors, specialists of environmental departments, and the population. To a greater extent, such accumulation of sanitizers or their metabolic products in the surrounding aquatic environment can damage the condition of aquatic animals and plants.

Disinfectants are often and successfully used in agriculture and aquaculture. The use of disinfectants in these cases increasingly requires consideration of the indirect effects on the environment and human health. Currently, there is only limited information available on the effects of a number of disinfectants, and therefore such information is needed to assess the potential risks of adverse effects, often delayed!, on animal and human health, taking into account the potential for synergistic effects, which include such multi-component aqueous systems like surface water.

In all cases, US EPA (May 15, 2019) recommends the use of detergents of various natures and compositions before disinfection. Surfaces should always be cleaned with soap and water or detergent to remove organic matter first and then disinfect. There are several groups of disinfectants, the most common are chlorine-based and alcohol-containing products. The most widely used cationic detergents are:

degmicide, cerigel, chlorhesidin, ethonium, dimexil, potassium soap, miramistin, containing active chemical elements, for example, nitrogen atoms in cerigel, etc.

Hypochlorite-based products include liquid (sodium hypochlorite), solid or powder (calcium hypochlorite) formulations. These compounds dissolve in water, creating a dilute aqueous chlorine solution in which undissociated hypochlorous acid (HOCl) is active as an antimicrobial compound. Hypochlorite has a

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broad spectrum of antimicrobial activity and is effective against several common pathogens at various concentrations. For example, hypochlorite is effective against rotavirus at a concentration of 0.05% (500 ppm), but for some highly resistant pathogens such as *Candida auris* and *Candida difficile* (Pereira et al., 2015; Kohler et al., 2018), higher concentrations of 0.5% (5000 ppm) are required in medical settings.

The recommendation to use 0.1% (1000 ppm) hypochlorite solution in the context of COVID-19 (SARS-CoV-2) is a conservative concentration that will inactivate the vast majority of other pathogens that may be present in healthcare settings. However, for operational cases with the possibility of blood spills and body fluids (that is, more than 10 ml), a concentration of 0.5% (5000 ppm) is recommended. Hypochlorite is rapidly inactivated in the presence of organic matter; therefore, regardless of the concentration used, it is important to first clean surfaces thoroughly with soap, water or detergent, washing or wiping. High concentrations of chlorine can lead to metal corrosion and skin or mucosal irritation, in addition to the potential chlorine odor side effects for vulnerable individuals such as people with asthma. Thus, the ratio of benefits and harms from the use of disinfectants of different classes (sanitizers) is actively discussed in the scientific literature and in clinical practice.

Commercial sodium hypochlorite products in various concentration levels are readily available for use in a variety of conditions. In Europe and North America, chlorine concentrations in commercially available products range from 4% to 6%. The concentration may also vary according to national regulations and manufacturers' formulas. In non-health care settings, sodium hypochlorite can be used at the recommended concentration of 0.1% (1000 ppm). Alternatively, the use of 70-90% ethyl alcohol is recommended to disinfect surfaces.

In addition, the present reality necessitates the widespread use by the population of household antiseptics for hand skin - sanitizers. Sanitizers may be identical in composition to professional antiseptics or may differ from them due to additives for the purpose of moisturizing and caring for the skin, flavors, food colors and other components.

Summarizing the available information on the composition of sanitizers, the following components can be distinguished:

- ethyl or isopropyl alcohol
- chlorhexidine
- propylene glycol
- panthenol
- glycerin
- triethanolamine
- quaternary salts: benzalkonium chloride
- flavors and skin care products: vitamins, plant extracts, fragrances, etc.

At the same time, manufacturers of sanitizers usually classify these preparations as cosmetics, which eliminate the need for an examination confirming the effectiveness of these preparations and their composition.

Thus, the currently observed mass (both in terms of coverage of the population and in quantity) use of sanitizers may lead in the future to uncontrolled releases into the natural environment of the components that make up these preparations and their metabolites that can cause biological response effects in natural living organisms, incl. - negative.

In addition, some products manufactured by companies do not have the properties stated in their descriptions. For example, there are cases when products manufactured by pharmaceutical companies did not meet the proclaimed requirements and effects on. So, The U.S. Environmental Protection Agency announced a settlement with Clorox Professional Products Company for selling one of the company's disinfectant bleach products used in hospitals was not effective against the bacterium that causes tuberculosis. Clorox has removed the claim from its product, marketed as "Dispatch Hospital Cleaner Disinfectant with Bleach." "Labels that are false or misleading put people at risk," said Jared Blumenfeld, EPA's Regional Administrator for the Pacific Southwest. "Companies must test and correctly label these disinfectant products to protect the health and safety of hospital patients and staff."(US EPA 2005) https://www.epa.gov/archive/epa/newsroom/2015-news-releases-date.html).

The biological effects of the use of such products, as well as the physiological and biochemical mechanisms of adaptation of aquatic organisms to sanitizers and detergents, have not been studied enough (Slye et al., 2011;Gagné et al., 2012; Gilles , 2012; Messina et al., 2014, etc.). Even less studied are the possible synergistic effects of their combined action in the presence of, for example, heavy metals (HMs) in surface waters.

HMs, such as Cu, Zn, Pb, Cd, Hg, As, etc., which are priority environmental pollutants, have bioavailability for living organisms. Understanding the factors that determine the bioavailability and features of the penetration of elements into living organisms, as well as the mechanisms and ways of excretion from living organisms is one of the important fundamental tasks of aquatic ecotoxicology and environmental safety (Moiseenko, 2009). Thus, the relevance of studying the biological effects of the substances indicated above is beyond doubt.

It seems relevant to study the possible biological effects of exposure in various combinations of sanitizers, detergents and salt solutions to the most toxic heavy metals for aquatic organisms (presumably

Cu, Zn, Pb and Cd) in different microconcentrations, with different exposure times of animals in them, on indicators of the state of oxidative stress. At the same time, it is possible to assess the presence in the experimental solution of precisely labile forms of HMs in water, and not just their total content, since the greatest danger to biota is represented by labile forms characterized by high biochemical activity and the ability to accumulate in natural environments and animal tissues (e.g., Ravero, 2001; Levit et al., 2020).

As test objects in future studies, it seems interesting to us to use mollusks of the family Unionidae (Unio spp.), widely distributed throughout freshwater areas, and for marine areas, the Mediterranean mussel *Mytilus galloprovincialis* Lam., or the White Sea mussel *Mytilus edulis* L., as well as representatives of Crustacea - higher crayfish (eg. *Astacus leptodactylus* Esch. and/or *Procambarus clarkii*). The species of mollusks and crayfish listed above are traditionally used as bioindicator species in biomonitoring of pollution of aquatic ecosystems (Elder, Collins, 1991; Salanki et al., 2003; Depledge, Galloway, 2005; Kuklina et al., 2013), as well as in experimental toxicological experiments (Handy, Depledge, 1999; Curtis et al., 2000; Kuznetsova et al., 2010; Hook et al., 2014, etc.).

There are several reasons for choosing these animals as bioindicators. Summing up the opinions of various authors (Widdows, Donkin, 1992; Gruber et al., 1994; Kramer, Foekema, 2001; Nikinmaa 2014, etc.), we obtain:

1. They are widely distributed and can be easily caught.
2. Most of them live in shallow waters, in coastal waters - places most prone to various types of pollution.
3. Inactive animals (low locomotor) or with a sedentary life.
4. These are animals with a rather long life cycle.
5. Large enough to collect and analyze tissue for contaminants.
6. Many species are quite sensitive to various types of pollution, and at the same time have some resistance, which allows them to accumulate pollutants, which, however, does not lead to death.
7. Many substances show dose-dependent effects on many physiological and biochemical processes in animals.

Studies of the bioavailability of many HM substances hazardous to organisms show that the total concentrations of HMs in water and in sediments do not always correlate with their concentrations in animal tissues (due to differences in ecotoxicity, metal interactions in natural environments, and due to protective physiological and biochemical mechanisms in living organisms).

Thus, the question remains whether mollusks and crustaceans can serve as indicators of pollution of coastal waters by domestic wastewater containing sanitizers, detergents, HMs, and their metabolites. Currently, there are few such studies.

At the same time, it is known that Biological Early Warning Systems (BEWs) have long been actively used to monitor water quality, in which living organisms are successfully used as biosensors of natural water pollution. Developed in the 1980s–1990s, automated systems for non-invasive registration of the heart rate in crustaceans and mussels at the Marine Biology Laboratory in Plymouth made it possible to assess the degree of influence of certain heavy metals on the cardiac activity of animals (Depledge and Andersen, 1990; Depledge et al., 1995, US EPA, 2005, etc.).

Heart rate variability (HRV) is one of the fundamental physiological properties of living organisms, and can serve as a basis for early diagnosis of the deterioration of the physiological state (PS) of an organism. Among aquatic invertebrates, the most analogies in the general structure, functioning, and systems of regulation of cardiac activity, in comparison with mammals, are known for mollusks. The main parameters of the heart rate of mollusks, calculated using clinical cardiology algorithms developed for humans, intersect with similar values for human rhythmograms (Bychkov et al., 1997). However, both in the world and in Russia, studies of the cardiac activity of crayfish are quite rare, especially when using automated systems for non-invasive heart rate monitoring (Kholodkevich et al., 2009; Kholodkevich et al., 2021).

In early studies by foreign scientists, it was shown that crayfish can change the rhythm of heart activity in the presence of HMs (Spicer, Weber, 1991; Styrishave et al., 1995), as well as in the presence of chemicals used in the treatment/disinfection of water in aquaculture (Kozak et al., 2009), for example, during its chlorination or chloramination (Kuklina et al., 2014). These works can be the basis for research on the effects of sanitizers on the functional indicators of crustaceans and mollusks.

The effect of chlorine-containing substances on the cardiac activity of crayfish has not been sufficiently studied, despite the fact that organochlorine compounds, being the strongest toxicants, can enter water bodies with wastewater, posing a danger to the flora and fauna of these water bodies. Active chlorine and its compounds are widely used in industry, in water treatment processes at waterworks, in various disinfections, including in aquaculture to combat parasitic infections. Thus, 10 mg/L of biocide as chloramines-T is considered as a commonly used in industry and aquaculture, at the same time in experiments on crayfish *Astacus leptodactylus* (Esch., 1823) the clear exposure effect was shown only after 1 day exposure to 50 mg/L of chloramines-T (Kuklina et al., 2014). According to heart rate changes, the 1-h exposure did not adversely affect crayfish at either concentration, as well as during daily exposure to 10 mg/L. As assessed by the heart rate, the 24-h exposure to 50 mg/L of chloramine-T was toxic for crayfish and led to substantial loss of energy (Kuklina et al., 2014).

It is known that the biocenosis reacts to a change in the quality of the habitat by changing the intensity of metabolism. The efficiency of aerobic energy exchange in hydrobionts, which can be estimated from the rate of oxygen consumption, can serve as an indicator of the quality of the aquatic environment (see Kolupaev, 1992; Martin et al., 2007). The advantage of using this particular functional indicator, the change of which, as a rule, is associated with the organism's attempt to avoid or compensate for adverse effects, lies in the possibility of detecting the initial effects of pollutants on a living organism and early signs of deterioration in animal health.

The biological effects of the use of sanitizers and detergents, as well as the physiological and biochemical mechanisms of adaptation of aquatic organisms to them, have not been sufficiently studied. Studies on the effects of detergents on living organisms are also rare. It is noted that synthetic detergents (SDs) and surfactants, which are part of them, have a negative impact on the PS of living organisms, water quality for biota, and the self-cleaning capacity of water bodies (Ostroumov, 2001). Pollution of water by them is further complicated by the fact that the products of chemical and biological decomposition in some cases are more toxic than the original substances (Ostroumov, 2001, 2006, etc.). The criterion for changes in the toxicity of SDs in long-term experiments of Ryabuhina et al. (Ryabuhina et al., 2007) was the dynamics of the survival of Ceriodaphnia in water samples compared with the control. In the experiments, an increase in the toxicity of solutions with a SDS concentration of 25 mg/l was revealed on the 15th day of the experiment (Ryabukhina et al., 2007).

There are only a few Russian experimental studies (Gostyukhina et al., 2007; Trusevich et al., 2014; 2017; Kuznetsova, Kholodkevich, 2015) that show the effect of anionic and cationic detergents (TDTMA) and sodium dodecyl sulfate (SDS) at different concentrations on the activity of valve movement and on the heart rate of the Black Sea mussels (*Mytilus galloprovincialis* Lam.). With an increase in the concentration of the active detergent to 1.7 mg/l, the behavior of the mussel is marked by long periods of the presence of the mollusk with closed valves, i.e. lack of filtration. Under these conditions, mussels switch to anaerobic metabolism, in the case of prolonged exposure, this leads to oxygen starvation - hypoxia. The transition of the mollusk to the closed state is a sign of the negative effect of detergent solutions on the functional state of the mussel (Trusevich et al., 2010; 2017; Gaisky et al., 2014; Kuznetsova and Kholodkevich, 2015). However, the same protective reaction prevents the entry of toxic substances into the body cavity of mollusks. In the case of small (smaller) concentrations (0.3-0.5 mg/L) of SDS, mollusks "taste" the water, which manifests itself later in a change in the circadian rhythm of cardiac activity. This indicates the need to take into account the negative effects of low concentrations of detergents, expressed in a significant change in circadian activity, with the loss of the predominance of the active state of mussels at night, which was stressed earlier (Kuznetsova and Kholodkevich, 2015). A higher locomotor (valve opening) during the night, leads to avoidance of vulnerability of mussels to diurnal predators). For the same species of mollusks, changes in biochemical markers of oxidative stress were shown (Messina et al., 2014) under the action of SDS detergent.

In the studies of oxidative stress in hydrobionts in the presence of water pollutants great attention is occupied by the study of detoxification and tissue protection systems, among which the enzymatic antioxidant system (AOS) plays a leading role (Soldatov et al., 2014; Chuiko, 2014). In the presence of the cationic detergent tetradecyl trimethyl ammonium bromide (TDTMA) at a concentration of 0.8 mg/l (a value close to the concentrations of the detergent in the surrounding aquatic environment) for 8 days, the mussels showed a change in AOS indicators, indicating the development of a state of oxidative stress. Significant changes were found in the peripheral tissues of mussels (gills and leg), which were in direct contact with TDTMA. An increased level of TBA-AP was noted by 46 and 11, respectively. Against this background, a significant increase in the activity of SOD, which neutralizes O2-, was noted; in the gills, SOD increased 6 times ($p<0.05$). At the same time, an increase in CAT activity by 1.7 and 3.2 times, respectively, was noted in the gills and leg. The tissue specificity of the AOS response to this detergent was shown, since The AOS system of the hepatopancreas showed the least sensitivity to the action of the detergent, and the gills, on the contrary, showed the maximum sensitivity to such exposure.

In terms of the scale of pollution and the impact on biological objects, HMs compounds occupy a special place among pollutants, and their distribution in the environment is the most serious threat to its environmental safety, which is aggravated over the years. An important feature of metals is that their potential toxicity and bioavailability are largely determined by their form. The forms of elements in natural environments are influenced by the compositional and granulometric composition of the medium, the content and absorbing capacity of mineral and organic sorbents, pH, Eh, the composition of the aqueous phase, and many other factors (Dash et al., 2021). A large amount of scientific literature has been accumulated concerning the distribution and accumulation of HMs in various ecosystems, the ecotoxicological effects of metals on living organisms (Förstner, 1981; Handy and Depledge, 1999; Kapustka et al., 2004; DeForest et al., 2007; Strode, Balode, 2013; Hook et al., 2014; Moiseenko, 2019; Egorov, 2019), while free HM forms are the most toxic (Linnik and Nabivanets, 1986; Depledge and Rainbow, 1990).

At the same time, one of the topical problems is the disclosure of patterns of behavior of HMs in the bottom sediments of water bodies and the assessment of potential environmental risks of HM accumulation by bottom sediments, which are components of surface waters. The effect of HM ions on the sorption of various organic toxicants by bottom sediments is considered in literature. The effect of Cd^{2+} and Cu^{2+} ions on the sorption of atracine, one of the most common herbicides, by bottom sediments was studied in (Du Laing, 2009; Gadd, 2004). It is shown that Cd exhibits a synergistic (enhancing) effect on the sorption of atracine, while copper has an antagonistic effect. The processes of sorption of HMs and other hazardous substances by natural sorbents are interrelated and little studied; therefore, understanding the patterns of the mutual influence of these toxicants in sorption processes seems necessary and very relevant. Competitive sorption of heavy metals by bottom sediments is practically not studied. The effect of organic pollutants on the transformation of heavy metal compounds has not been studied either. Biochemists have been studying the mechanisms of the toxic effect of HM ions on living organisms for many years. It has been established that HM ions can accumulate in living organisms, interfere with the metabolic cycle, and suppress the synthesis of proteins, including enzymes (Kováčová, Šturdík, 2002; Moiseenko, 2009, 2019; Gadd, 2004). However, it is equally important to study the effect of biota and its metabolites on the behavior of HMs in the environment. Although monitoring of the level of contamination of sediments of water bodies is still carried out by the total (gross) content of toxic elements, however, it should be noted that only labile hydrated ions or unstable complexes most easily penetrate cell membranes and, therefore, are considered biologically active, therefore, bioavailability is determining factor of HM toxicological impact on aquatic organisms. Labile forms of heavy metals such as Cu, Cd, Zn, Pb are priority environmental pollutants. For benthic organisms, the most accessible are dissolved forms of metals present in the pore (silt) waters of bottom sediments. Therefore, the factors affecting the distribution of metals in the “bottom sediments – pore solution” system are simultaneously the factors controlling their bioavailability (Levit et al., 2014).

When evaluating the biological effects of HM environmental pollution, it is customary to determine the bioaccumulation coefficients of heavy metals (BCF) in animal tissues (Mendosa-Carraza, 2016). Tissue specificity in the accumulation of heavy metals (mainly Cu, Zn, Pb, and Cd) and metal specificity of the effects of such accumulation by mussel’s tissues were shown (e.g., Brown et al., 1998; Brown et al., 2004; Levit et al., 2017; Zarykhta et al., 2019). However, in most ecotoxicological studies, the gross values of HM concentrations in experimental solutions are taken into account, without taking into account the concentration of labile forms of these metals and possible HM transformations in natural waters of various compositions.

A lot of works are devoted to the biological effects of HM action on the physiological and biochemical indicators of the state of aquatic organisms (Gundacker, 2010; Fokina, Nefedova, Nemova, 2010; Moiseenko, 2019). Most of these studies were carried out on bivalves, both marine and freshwater species (Curtis et al., 2000; Chuiko et al., 2014; Kholodkevich et al., 2019). Curtis et al. (2000) evaluated the responses of the mussel’s cardiac system and changes in locomotor behavior (valve movements) to exposure to various concentrations of copper ions in water. The responses of these two functional systems to copper differed significantly and were not always dose-dependent. In the literature, we also find evidence of species specificity in the sensitivity of aquatic animals to HMs and in their accumulation (Levit et al., 2017).

In general, the ability of macrobenthic invertebrates (mollusks and crustaceans) to accumulate heavy metals depends on the form of the metal and the characteristics of the organism; therefore, bioaccumulation should be considered in combination with data on metal concentrations in the abiotic components of the ecosystem (Kudryavtseva et al., 2021). Using stripping voltammetry (IVA), it was found that the amount of IVA-labile forms of heavy metals, such as Cu, Cd, Zn, Pb, depends, among other things, on the pH of the experimental solution, which can be affected by the components of sanitizers and detergents.

A batch of different test species each for a different trophic level is highly recommended in order to study the toxicity of a substance or synergistic effects of its mixture on benthic invertebrates (HELCOM 2014).

It should be noted that a comprehensive study of natural objects using various methodological approaches and algorithms for their implementation will make it possible to predict the state of ecosystems under anthropogenic impacts in the face of new challenges associated with the emergence and spread of a new coronovirus pandemic.

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