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# **Non-corresponding contaminants in marine surface sediments as a factor of ARGs spread in the Sea of Azov**

Author links open overlay panelMarina Sazykina a, Timofey Barabashin a b, Elizaveta Konstantinova a, Ameer Abood Karim Al-Rammahi c, Liliya Pavlenko b, Lyudmila Khmelevtsova a, Shorena Karchava a, Maria Klimova a, Irina Mkhitaryan b, Margarita Khammami a, Ivan Sazykin a

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## **Abstract**

The present study aims to analyze the level and total toxicity of the most common pollutants in surface sediments and assess their impact on the occurrence of [antibiotic resistance](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/antimicrobial-resistance) genes (ARGs) in the Sea of Azov. Biotesting using the whole-cell bacterial lux-biosensors showed high integral toxicity of surface sediments and the presence of genotoxicants and substances that cause [oxidative stress](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/oxidative-stress) and protein damage. Using cluster analysis, it was shown that the distribution of pollutants in the Sea of Azov depends on the type of surface sediments. The relative abundance and distribution of 14 ARGs in surface sediments were shown. Principle component analyses results suggest that non-corresponding contaminants do not exert direct influence on the ARGs abundance in the surface sediments of the Sea of Azov. Thus, the need to investigate the significance of non-corresponding pollutants in the selection and distribution of ARGs in the [aquatic environment](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/aquatic-environment) remains a pressing problem.

## **Introduction**

One of the most serious modern global threats to the public health is the spread of antimicrobial resistance in the environment (WHO, 2014; Zhuang et al., 2021). A special case is the development of antibiotic resistance in bacteria (ARB), especially multidrug resistance, due to the spread of antibiotic resistance genes (ARGs). ARGs can be transferred both between bacteria of the same species (vertical gene transfer) and as a result of horizontal gene transfer (HGT) via mobile genetic elements (MGE), including plasmids, transposons and integrons (van Hoek et al., 2011; von Wintersdorff et al., 2016; Zhu et al., 2017; Zhang et al., 2018; Abe et al., 2020). This phenomenon can lead to the spread of ARGs from non-pathogenic microorganisms to pathogens, and, as a consequence, cause difficulties in the treatment of human diseases (Nappier et al., 2020). The development of antibiotic resistance is due to the active use of antibiotics and some other chemicals in medicine, industry, agriculture and other areas of anthropogenic activity (Adegoke et al., 2018; Zainab et al., 2020; Bombaywala et al., 2021a). To date, it has been shown that ARB and ARGs are widespread in the different compartments of terrestrial ecosystems, including soils (Cycoń et al., 2019; Tyrrell et al., 2019; Maurya et al., 2021; Sazykin et al., 2021; Wang et al., 2021a), groundwater (Zainab et al., 2020), and the atmosphere (Li et al., 2018a). With runoffs, sewage discharges and leaching, both antibiotics and their metabolites, as well as ARGs and bacteria carrying them, enter aquatic ecosystems and are found in surface water (Gao et al., 2018; Adegoke et al., 2018; Nnadozie and Odume, 2019; Chen et al., 2020; Nappier et al., 2020), biofilms (Guo et al., 2018; Kaeseberg et al., 2018; Abe et al., 2020) and sediments (Su et al., 2014; Zhu et al., 2017; Yang et al., 2018; Mootapally et al., 2019; Sazykin et al., 2019; Altuğ et al., 2020; Zhang et al., 2020a), the latter of which become a major pool of the environmental resistome.

The development of antibiotic resistance, including the distribution and variability of ARGs in natural environments, is also influenced by the presence of other non-corresponding pollutants, such as potentially toxic elements (PTEs) (Su et al., 2014; Poole, 2017; Yang et al., 2018; Chen et al., 2020; Ohore et al., 2020), polycyclic aromatic hydrocarbons (PAHs) (Maurya et al., 2021; Sazykin et al., 2021), polychlorinated biphenyls (PCBs) (Gorovtsov et al., 2018), organochlorine pesticides (OCPs) (Dealtry et al., 2014), etc. Even subtoxic levels of PTEs in the environment can change the composition of the microbial community and contribute to multiple resistance in bacteria not only to metal stress, but also decrease antibiotic susceptibility (Poole, 2017; Guo et al., 2018; Zhang et al., 2018; Bombaywala et al., 2021b; Zhuang et al., 2021). In addition, PTEs can form complexes with antibiotics, holding them back, which increase bacterial tolerance to antibiotics. The co-selection as well as cross-selection mechanism in the bacterial community may be involved in the conjugative transfer of ARGs induced by PTEs (Ohore et al., 2020). PCBs and OCPs pollution can cause co-selection, co-resistance and cross-resistance of ARB in the same way as it was shown for PTEs (Gao et al., 2018; Gorovtsov et al., 2018). It was shown that PAHs, a major component of petroleum, can accelerate the propagation of ARGs in seawater through the HGT (Wang et al., 2017).

The release of pollutants into the environment due to anthropogenic activities is complex (Klenkin et al., 2008). The microbiota experiences the total toxic effect of pollutants of various chemical nature. One of the most promising approaches for assessing total toxicity of ecosystem compartments is biotesting using whole-cell bacterial luminescent sensors (Shemshedinova et al., 2020; Moraskie et al., 2021). Whole-cell bacterial lux-biosensors can effectively and quickly detect various toxic substances: DNA-tropic compounds; substances causing oxidative stress, damaging proteins and membranes; heavy metals; polychlorinated biphenyls, etc. They possess higher sensitivity to toxicants. Detection limits range from milligrams per liter to micrograms per liter (Elad and Belkin, 2017; Woutersen et al., 2011). These strains are constructed by coupling the luxCDABE genes to a promoter that is involved in a specific stress response. In the presence of compounds that induce a specific type of stress, luminescent response of biosensors is recorded. It is directly correlated with the total amount and potency of these compounds. Microbial whole-cell biosensors have been shown to be effective for assessing environmental pollution by PTEs, PAHs and petroleum products (PPs), sulfonates, nitrates, phenol, etc. (Tsybulskii and Sazykina, 2010; Sazykina et al., 2012; Rampley et al., 2020; Li et al., 2021a).

Internal seas, bays and estuaries are the end body of global geochemical cycles, and surface sediments that form in such environments are a depositing medium for various pollutants that come both as a result of economic activities in the coastal zone and in the catchments of the rivers they drain (Su et al., 2014; Guo et al., 2018; Chen et al., 2020; Zhang et al., 2020a). Thus, sediments of mediterranean ecosystems are of significant interest in terms of identifying modern and historical pollution at the level of the entire basin (Birch, 2017). The Sea of Azov is a shallow shelf sea in Eastern Europe bounded by Russia and Ukraine. Previous studies have found the occurrence of OCPs (Klenkin et al., 2008), PTEs (Kurilov et al., 2009; Korablina et al., 2018; Bufetova, 2020), PPs (Pavlenko et al., 2018; Korablina et al., 2021; Tikhonova et al., 2021) and radionuclides (Matishov et al., 2002, Matishov et al., 2020; Mkhitaryan and Korablina, 2020) in marine surface sediments in the Sea of Azov, but the prevalence of ARGs in the Sea of Azov remains unexplored. The specific objectives of the present study are to 1) identify and quantify the ARGs in surface sediments of the Sea of Azov; 2) evaluate the levels, spatial patterns and total toxicity of PTEs, PPs, PCBs, OCPs and technogenic cesium (137Cs) in surface sediments of the Sea of Azov; and 3) reveal the relationship between abundance and distribution of ARGs and non-corresponding contaminants in the marine environment.

## **Section snippets**

## **Study area and sample collection**

The Sea of Azov belongs to the Mediterranean basin of the Atlantic Ocean. Its area is about 38,000 km2, the volume is 290 km3 (Rosneft, 2019). The Sea of Azov is the shallowest sea in the world with an average depth of 7 m. The surface is relatively flat, sloping gently from the coast to the center, composed mainly of modern terrigenous sediments (Matishov et al., 2019). The largest rivers flowing into the sea are the Don and Kuban Rivers.

The basin of the Sea of Azov is directly and indirectly

## **Sediment characterization**

The studied samples of surface sediments of the Sea of Azov were subdivided into 6 types according to the composition and texture (Fig. 1). In the eastern part of Taganrog Bay, surface sediments were composed of loose sand with an admixture of shell rock. In shallow areas (depth up to 10 m) in the northeastern, eastern and southwestern parts of the Sea of Azov, surface sediments were represented by shell rock or shell crumb with an admixture of sand or silt. In the deepest Central part of the

## **Conclusions**

As a result of anthropogenic activities, pollutants of various nature enter the Sea of Azov, in particular PTEs, PPs, PCBs, OCPs, and radiocaesium. In general, their level in marine surface sediments corresponds to or slightly exceeds the target values. The spatial distribution of pollutants in the sediments of the Sea of Azov is uneven. Clustering of the studied sediments by the levels of pollutants showed that the composition of surface sediments is the main factor in the redistribution of

## **CRediT authorship contribution statement**

**Marina Sazykina:** Conceptualization, Resources, Writing – original draft, Supervision, Funding acquisition. **Timofey Barabashin:** Investigation, Writing – original draft. **Elizaveta Konstantinova:** Formal analysis, Visualization, Writing – original draft. **Ameer Abood Karim Al-Rammahi:** Investigation. **Liliya Pavlenko:** Validation, Investigation. **Lyudmila Khmelevtsova:** Validation, Investigation. **Shorena Karchava:** Investigation. **Maria Klimova:** Investigation. **Irina Mkhitaryan:** Investigation. **Margarita**

## **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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