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SIGNIFICANCE OF BIOINDICATORS IN EVALUATING POLLUTION FACTORS ON THE ROMANIAN BLACK SEA COAST: A MINIREVIEW

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Rezumat: *Acest studiu examinează importanța utilizarii bioindicatorilor în monitorizarea nivelurilor de poluare de pe coasta româneasca a Mării Negre. De asemenea, plecând de la caracteristicile ecologice distinctive ale Mării Negre, studiul subliniază și presiunile induse de om, care în prezent sunt agravate de războiul din regiune. De aceea, este important utilizarea anumitor specii acvatice ca bioindicatori (moluste si crustacee), care sunt sensibile la schimbările din mediu și pot servi ca semnale de avertizare ale sănătății unui ecosistem. Totodată, acest studiu promovează implementarea programelor de evaluare bazate pe bioindicatori, care sunt esențiale pentru înțelegerea și reducerea efectelor poluării, și facilitarea utilizării durabile a acestui fragil ecosistem.*
Cuvinte cheie: *bioindicatori, moluste, crustacee, zona costiera romaneasca.*

Abstract: *This review examines the importance of using bioindicators to monitor pollution levels on the Romanian Black Sea coast. Also, based on the distinctive ecological characteristics of the Black Sea, the study highlights the human-induced pressures, which are currently aggravated by the war in the region. It is therefore important to use certain aquatic species as bioindicators (mollusks, crustaceans), which are sensitive to environmental changes and can serve as warning signals of an ecosystem's health. At the same time, this study promotes the implementation of bioindicator-based assessment programs, which are essential for understanding and reducing the effects of pollution and facilitating the sustainable use of this fragile ecosystem.*
Key words: *bioindicators, mollusks, crustaceans, Romanian coastal zone.*

Introduction

This review examines the critical importance of bioindicators in monitoring pollution levels on the Romanian Black Sea coast. Furthermore, the study highlights the distinctive ecological characteristics of the Black Sea, as well as human-induced pressures, which are currently exacerbated by wars in the region. Therefore, the usefulness of aquatic species as bioindicators, which are sensitive to changes in the environment and can serve as warning signals of an ecosystem's health, cannot be neglected. This study aims to promote the implementation of bioindicator-based assessment programs, which are essential for understanding and reducing the effects of pollution and facilitating the sustainable use of this fragile ecosystem.

The Black Sea is known as an inland sea with brackish waters (average salinity value around 15 PSU), communicating with the Sea of Marmara through the Bosphorus strait, and by the Dardanelles with the Aegean Sea, having a major geopolitical position, being positioned at the border of Europe with Asia. Romania's access to the Black Sea stretches over a distance of 225 km, and the aquatic ecosystem that contains over 150 species of fish is an important food source for local communities. Also, this strategic position, in addition to its importance for tourism (bathing tourism) and maritime transport (Midia and Constanta ports), can be considered an important energy resource for the future. As an EU member, Romania has aligned itself with the new European requirements on "blue growth strategies", which must be based in the coming years on renewable energy sources and energy efficiency due to climate change responsible for declining marine biodiversity worldwide [1].

The Black Sea basin at depths of 100-200 meters is known as an anoxic basin, with an area of 508 000 km^2, whose oceanographic characteristics make it distinctively different from other basins. The distinguishing characteristic of the Black Sea basin, in comparison to other basins, lies in its topographical features that facilitate the coexistence of coastal and deep-sea ecosystems inside a single enclosure [2]. Also, the Black Sea current system is represented as a closed, unique circulation of water masses, with different characteristics from one area to another. Thus, the main current runs counterclockwise along the shoreline, parallel to the coastline, for a width of 10-39 nautical miles and covers the entire sea in the continental shelf area in the form of a closed circle [3].

The Black Sea ecosystem is highly vulnerable due to continental pressures from coastal and maritime activities. In addition, the current geopolitical situation, determined by the present Russian-Ukrainian conflict, is currently adding to these problems.

Marine pollution and habitat degradation have a significant impact on the sustainable use of oceans and seas and can affect human health through both direct contact with polluted waters and ingestion of contaminated marine nutrient sources. Thus, in the Black Sea basin, there has been a long-standing concern about pollution prevention. Therefore, physical and biochemical measurements, for nutrients, dissolved oxygen, hydrogen sulphide were analysed to describe the possible routes and mechanisms of transport of coastally trapped pollutants into the offshore, deep-basin waters through the serpentine current system, and the redox mechanisms controlling the sub oxic zone located between the oxic and anoxic layers of the Pontic Basin [4].

The main concern for the well-being, efficiency, and ecological diversity of the marine environment in Romania's coastal region is due to human activities in this area. Literature data has shown that about 80% of pollutant emissions are attributed to spills as well as residues released as a result of various economic, industrial, agricultural, tourism and urban activities [5]. The Danube River and its branching rivers are another significant contributor to pollution in the Black Sea. The counterclockwise flow of the currents in the western Black Sea basin plays a significant role in the dispersion of pollutants substances to the coastal regions of Romania.

In protecting the marine ecosystem, researchers place particular emphasis on the use of monitoring studies, which use aquatic organisms as bioindicators of pollution. The significance of these studies lies in their ability to evaluate the well-being of aquatic ecosystems. Organisms serve as highly responsive indicators of environmental conditions, as they provide signals at various levels, including population, individual, and molecular, in reaction to environmental changes [6]. In the Black Sea, there are several studies on the organisms used as bioindicators (*Mytilus galloprovincialis* (Lamarck, 1819), and *Rapana venosa* (Valenciennes, 1846)) for the identification of heavy metals, PAHs, and PCBs, based on their responses to the stress induced by the presence of contaminants in the environment [7-10]. However, the existing literature data lacks comprehensive research studies of the utilization of *Euxinia maeotica* (Sowinsky, 1894), a species indigenous to the Ponto-Caspian Basin, as a bio tester. This species exhibits a preference for consuming organic plant or animal debris, thereby displaying detritivorous feeding behavior. Moreover, specimens of this species serve as a readily available and abundant food source for benthic fish from the environment [11].

Our paper aims to highlight the importance of using bio-testers in monitoring programs to detect and understand the pollutants that could threaten this fragile ecosystem in the Romanian coastal area.

LEGAL FRAMEWORK

Romania, as a UN and EU member state, has aligned its environmental policies with international legislation to "conserve the oceans and ensure their sustainable use". In this regard, Romania joined the other 192 UN member states at the Development Summit in September 2015, adopting the 2030 Agenda for Sustainable Development, a global development agenda for action with a universal character, which promotes balance between the three dimensions of sustainable development – economic, social and environmental. Among the objectives of the program are those relating to the protection of marine and coastal ecosystems, the prevention and reduction of marine polarization and the impact of ocean acidification. An important role in improving the health of the oceans and achieving these objectives will be played by the development of scientific research, particularly in marine technology and environmental engineering [12].

To ensure the protection of the marine environment, Romania has adhered to and signed various international treaties, conventions, and agreements. Thus, maritime transport must be carried out by the measures laid down in the MARPOL 73/78 Convention [13].

On the Romanian coast, the measures adopted by Law 17/1990, which was amended and supplemented by Law 36/2002, on the "Legal regime of inland maritime waters, the territorial sea, the contiguous zone and the exclusive economic zone of Romania" must be respected [14]. The EU Marine Strategy Framework Directive [15] has been transposed in Romania by Law 6/2011 and sets out the strategy for the marine environment to "ensure and maintain the good environmental status of marine waters" [16].

POLLUTION SOURCES IN THE BLACK SEA

Pollutants can be classified according to their chemical composition and physical state, as well as their properties such as solubility, biodegradability, and reactivity. In addition, pollutants can be classified by the medium of action, such as air, aquatic, and/or terrestrial, or by source, such as fuel combustion, industrial or residential. Pollutant categories are predominantly subjective, although the most commonly used classifications are those based on their biological, chemical, and physical characteristics. At the same time, the classification of pollutants based on their functions is related to the existing biological and physiological understanding [17].

Over the last fifty years, the Black Sea ecosystem has been under high anthropogenic pressure due to accelerated economic development in the surrounding areas. The main anthropogenic pressures identified on the Romanian Black Sea coast are caused by the emergence of ports and shipping activities, the presence of oil and petroleum products processing industries (refining and petrochemicals), shipyards, sewage treatment plants discharging insufficiently treated effluents into the sea [5]. Also, tourism and fishing activities cannot be neglected, as well as the influence of the Danube, whose waters are laden with various pollutants and are affecting the vulnerability of the marine ecosystem. Because it is a semi-enclosed sea, the Black Sea accumulates pollutants over time making it a focal point for various types of pollution such as eutrophication, plastic pollution, and chemical contamination [18]. According to the results of the joint Black Sea surveys presented by the EU/UNDP funded EMBLAS-PLUS project (2019), 124 chemicals hazardous to the marine ecosystem and human health have been identified, including persistent organic pollutants, metals, pesticides, biocides, pharmaceuticals, flame retardants, industrial pollutants and personal care products. Shockingly, plastic waste (in bottles, bags, and packaging) found in the Black Sea constitutes for about 83% of marine litter. The main sources of this waste are large rivers (in Ukraine the study included the Danube and the Dniester) [19]. In addition, armed operations related to the present Russian-Ukrainian conflict, as well as those related to training, can also be considered major environmental pollutant sources. Military operations impose numerous stresses on the natural environment and yet, their impact on the overall degradation of the ecosystem has not been adequately acknowledged. There exist multiple rationales for this phenomenon. One reason is that the military is not commonly regarded as an "industry", although it exhibits many characteristics of one. Another issue to consider is the existence of a double standard inside governments, when they exhibit a reluctance to establish the same degree of transparency and accountability for their armed forces as they do for other governmental or civil society entities [20]. The pollutants from military activities are the metal-laden particles of Pb, Cu, Cd, Sb, Cr, Ni, Zn, and Al from weapons residues, which end up in the aquatic environment [21]. Military training operations involve the frequent use of live-fire weapons, creating contamination of inorganic and organic nature, often resulting in reliable site-specific degradation [22]. In addition to this, there is the use of energetic materials such as explosives, pyrotechnics, and fuels, which are used during armed conflicts. In terms of organic chemical contamination, military activities are responsible for the entry of the following pollutants into the aquatic environment: PAH, 2, 4-dinitrotoluene (DNT), 2, 4, 6-trinitrotoluene (TNT), 1, 3, 5-trinitro-1, 3, 5-triazine cyclohexane (RDX), recognized to be resistant to biological degradation or any kind of treatment, thus becoming a source of permanent contamination and potentially harmful to human health and the environment. Nor can the per- and polyfluoroalkyl substances (PFAS) that are present in fire training exercises in aqueous film-forming foams to extinguish hydrocarbon, fuel-type fires be neglected [23], [24]. The intensification of maritime operations in the Northwest Pontic Basin has led to increased oil spills and releases of new pollutants into the Black Sea, in addition to known land-based and shipping emissions. These pollutants pose a significant danger to marine organisms and human welfare, impacting on water quality and seafood safety. Bioindicators are therefore needed to monitor the impact of these pollutants on the ecosystem. They can be used as early warning systems, identifying primary signs of pollution before they progress into more significant ecological disasters.

BIOINDICATORS

Monitoring systems play a crucial role in promptly and accurately determining the characteristics of pollutants and their corresponding effects on the environment. By using biodiversity as a means of addressing the presence and impact of pollutants, as well as changes in essential processes or the capacity of species to accumulate them, it could represent the most effective indicators that exist in the natural environment. Bioindicators are influenced by several environmental factors, such as light transmission, water temperature, and the presence of suspended particles [25]. Moreover, with the use of bioindicators, it becomes possible to observe the environmental conditions of a certain region or to assess the level of pollution. Currently, more and more emphasis is placed on the development of indicators for water management, which include both human and environmental aspects, which could be very advantageous in obtaining information about water quality. Indicator organisms refer to biological species that possess the ability to define distinctive traits or attributes in their respective environments. The specified phenomena are obtained by directly observing the biotester's environment, with a specific emphasis on the aspects of development, feeding, and reproduction. It is crucial to emphasize that the different viable species in a population may not consistently display identical reactions to contaminants, nor do they respond uniformly to every contaminant [17].

Bioindicators are living organisms, such as plants, plankton, or animals, that provide insights into the health of their environment. Because these organisms are sensitive to changes (chemical, physical, and biological) in their environment, they can be used to monitor environmental conditions as they provide vital information on the existence and consequences of pollutants [26]. Researchers use modifications of bioindicators to measure the levels of pollutants such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs), which may not be detected by traditional monitoring tools.

According to Parmar et al., (2016) study [26], the benefits linked to the utilization of bioindicators (Figure 1) are outlined below:

- The determination of biological effects is possible.
- To observe the combined and divergent impacts of different contaminants on the environment.
- The early detection and detrimental impacts of pollutants on plants and humans can be monitored.

- The prevalence of these entities allows for their easy quantification.
- This alternative is economically affordable, by comparing with other specialized measuring systems.

In conformity with the recommendations of the Water Framework Directive (2000) and the Marine Strategy Framework Directive (2008), which propose the establishment and implementation of biological quality issues and the formulation of early warning methodologies for environmental health assessment and monitoring, research in the Black Sea coastal areas has been aligned with European environmental policies [15], [27].

As information on the assessment of pollutants in biota is quite limited in the Black Sea region, the activities carried out by Romanian scientists together with partners from Consortium in the framework of the CBC Project "Assessment of the Vulnerability of the Black Sea Marine Ecosystem to Human Pressures" (ANEMONE) (2018-2023) aimed to provide a comprehensive study on chemical contamination of aquatic organisms.

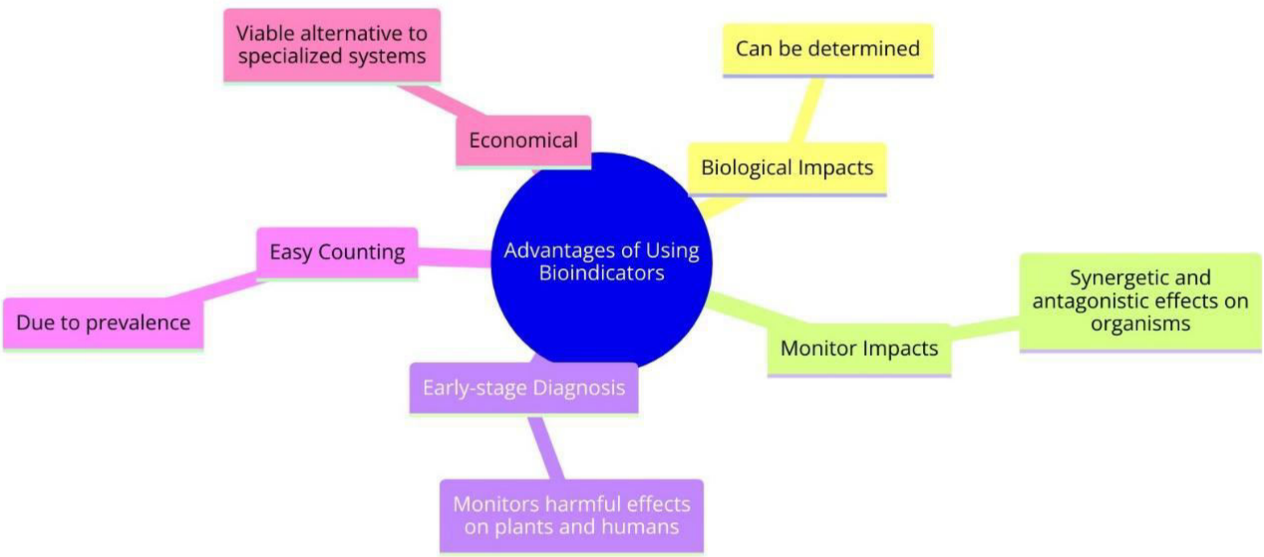

Fig. 1. Advantages of using Bioindicators in environment assessing

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Literature data showed that Mediterranean mussels (*M. galloprovincialis*) have gained prominence as bioindicators for marine pollution in the Black Sea environment, providing to the research valuable insights into the presence of microplastics and other contaminants (heavy metals, PAH, PCBs, pesticides, dioxines etc) [29]. Also, Savuca et al., (2022) in their review study raises the alarm about the large amounts of plastic materials (Polyethylene terephthalate (PET), Polyethylene (PE), Polypropylene (PP), Styrene acrylonitrile copolymer (SAC)) was identified in water, sediments and marine organisms (mussels) in the Pontic Basin, with negative impacts on the marine environment and human health [30]. Given their rapid stress response, these organisms are highly suitable for conducting molecular, physiological, and ecological investigations, as well as for biomonitoring applications in the water column [31-33].

The marine predatory snail *Rapana venosa* (Valenciennes, 1848) has the potential to impact both natural and farmed populations of oysters, mussels, and other molluscs. *Rapana venosa* is well recognised as a highly undesirable invasive species, posing a significant hazard to both cultured and natural populations of oysters and mussels. The introduction of this gastropod into the Black Sea basin occurred during World War II through the ballast water of both commercial ships and military vessels, subsequently leading to a notable decrease in the population of mussels and oysters within the basin. This marine organism exhibits a notable ecological aptitude, as evidenced by its elevated fertility, accelerated development rate, and ability to withstand low salinity, extreme temperatures, water pollution, and oxygen deprivation [34], [35].

In contrast, there are few research studies in the literature on the use of the biotester *Euxinia maeotica* (Sowinsky, 1894) as a pollution indicator. This is a crustacean species native to the Ponto-Caspian basin, which feeds preferentially on organic plant or animal debris (detritivorous), and specimens of this species are a rich and easy food source for benthic fishes [11]. The advantages of using this species come from the fact that it is one of the most common species on the Romanian coast, occupying the shallow sandy habitat of the northern Romanian coast, and can be collected very easily, being present on the shore between 0 - 1.5 m. It also tolerates the low water temperatures characteristic of the cold season, the species being present at 2-3 ºC, as well as low salinities, supporting salinity variations from 0-20 ‰. Few studies in literature have shown how these amphipods are influenced in their body metabolism when exposed to pollutants such as heavy metals and hydrocarbons and it could be a novel research study to use *Euxinia maeotica* (Sowinsky, 1894) as a bioindicator [36, 37].

Conclusions

By establishing environmental policies and measures for the management of aquatic ecosystems, the EU has set itself the important goal of preventing marine pollution for the coming decades. The aquatic environment of the Romanian Black Sea coastal area is under continuous threat from pollution due to current geopolitical conditions, military activities as well as economic, industrial, agricultural, tourist, and urban activities taking place in the area.

The significance of bioindicators in environmental monitoring is of utmost importance within the current military conflict. Ongoing research and the establishment of bioindicator-based assessment programs will be essential in safeguarding the key marine corridor along the Romanian Black Sea coast as the crisis progresses. By comprehending and addressing the consequences of pollution, it is possible to safeguard the well-being of the Black Sea and guarantee the long-term viability of its marine biodiversity for subsequent generations.

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