The ecotoxicological aspects of using the marine benthic invertebrates skeleton in the production of active calcium substances

A A Oripova, E A Ovsuk, O I Sergienko, N B Uliyanov and U Oripov

1 ITMO University, 9, Lomonosova st., St. Petersburg, 191002, Russia

E-mail: a.a.oripova@niuitmo.ru

Abstract. Marine benthic invertebrates are susceptible to the accumulation of heavy metals and other pollutants from the environment, leading to the impossibility of their use as raw materials in the food production and pharmaceutical industry. In this paper, the accumulation of heavy metals in the shells and needles of Strongylocentrotus sea urchins was studied to assess the safety of the use of this raw material in the production of active calcium substances for food and medical purposes with the use of voltammetry analysis and capillary electrophoresis. High levels of strontium accumulation in the studied samples were revealed, indicating the unfavorable situation of commercial waters with respect to the presence of mobile forms of strontium.

1. Introduction

Marine invertebrates represent approximately 60% of the total diversity of marine animals and are rich sources of biologically active compounds. The biotechnological potential of marine benthic invertebrates is increasingly attracting scientific and economic interest worldwide. Biological features, chemical composition, mechanical characteristics of benthic invertebrates determine a great interest in them in different areas of industry: they are the sources of raw materials for food production, the sources of biologically active substances and medicines, animal feed, fertilizers, etc. Great interest is shown in them in chemistry, microbiology and pharmacy.

The Arctic region of Russia, including the Barents Sea, is one of the main sources of fish wealth (including marine benthic invertebrates), surpassing other marine regions in terms of fish stocks. The most common genus of sea urchins Strongylocentrotus in the Arctic region, belonging to the class of echinoderms (Echinodermata), is an object of active fishing. Sea urchins are mainly caught in order to extract gonads from them. The gonads (caviar) of a sea urchin are the sources of raw materials for food production, and extraction of valuable biologically active substances for pharmaceuticals, chemistry and microbiology.

Due to the fact that sea urchins are the sources of biologically active substances, it is necessary to have information about the pollution in their populations habitats. This article is devoted to the study of heavy metals accumulation in the shells and needles of the Strongylocentrotus sea urchins to assess the safety of their use as raw materials in the production of active calcium substances.
2. Materials and methods

2.1. Study area and research materials

The Arctic ecosystems with all their richness and diversity are the most fragile and vulnerable due to climatic and geographical features of the region. The problem of rational use of marine resources has arisen for a long time and includes the great economic pressure on the main commercial species of hydrobionts. Rational nature management implies complexity in the processing of natural resources, which is not sufficiently applied in the field of marine benthic invertebrates.

In the extraction process of gonads from the sea urchins about 70% of their body weight goes to waste consisting mainly of the shells and needles, which are known for their high content of unique biologically active substances of various classes, such as proteins, quinoid pigments, and calcium [1, 2].

The main component that forms the skeleton of a sea urchin is calcium carbonate: in the *S. franciscanus* species, 91.08% of their skeleton consists of calcium carbonate [3], *S. droebachiensis* – 90.77%, *S. intermedium* and *S. nudus* – 73.4% [4]. The shells of sea urchins also contain magnesium carbonate - in sea urchins of the *Strongylocentrotus* genus it varies between 2-14% [6].

The carbonate skeleton of sea urchins is easily neutralized with decomposition by solutions of organic acids. The calcium salts of organic acids obtained by neutralizing the carbonate skeleton can be widely used in the food and pharmaceutical industries. Calcium isolated from the considered raw materials can serve as an additive to feed in animal husbandry, as well as the basis for biologically active substances (BA) and functional food products.

Aquatic benthic invertebrates are susceptible to the accumulation of heavy metals and other pollutants from the environment, which can lead to the impossibility of their use as raw materials for the above described purposes and to a decrease in their commodity characteristics. Therefore, the measurement of heavy metals in the shells and needles of sea urchins is an important component in assessing the safety of their use as raw materials for the isolation of biologically active calcium.

This article is devoted to the study of heavy metals accumulation in the shells and needles of the *Strongylocentrotus* sea urchins to assess the safety of their use of as raw materials in the production of active calcium substances.

The conducted research involved the samples of shells and needles from two species of the sea urchins genus *Strongylocentrotus* with 50-57 mm in diameter caught in the waters of the Barents Sea in winter and summer 2017: a green sea urchin *S. Droebachiensis* (10 specimens) and a lilac sea urchin *S. Purpuratus* (10 specimens).

2.2. Methods and equipment

The study obtained data about heavy metals accumulation in research materials (samples of shells and needles) by carrying out two different methods of analysis: voltammetry [7], and capillary electrophoresis [8].

Studies to quantitatively determine the copper, lead, and cadmium content in the samples by a voltammetry method of analysis were carried out on an ABC-1.1 polarograph. This analyzer is designed to measure the mass concentration of various elements such as copper, lead, cadmium, zinc, mercury, nickel, bismuth, arsenic, iodine, etc., in drinking, natural, and waste waters, food, metals, and other materials according to certified or standardized measurement procedures.

Sample preparation for the quantitative analysis of the copper, lead, and cadmium content on the ABC-1.1 analyzer was carried out using the MC-6 microwave sample preparation system, in which the samples under study were subjected to wet mineralization. The MC-6 microwave sample preparation system is used to decompose samples of food products, food raw materials, soils, environmental materials, biological fluids, etc., when analyzing the chemical composition of samples by various
methods. The principle of the system is to use microwave energy for rapid heating of samples with the addition of oxidizing agents. The prepared sample was then analyzed by the standard addition method and processed according to the standardized measurement procedure (08-01-MVI).

In the conducted studies to determine the content of strontium, calcium and magnesium in the samples of shells and needles using capillary electrophoresis methods, the Kapel-103RT capillary electrophoresis system was used. This system is intended for the qualitative and quantitative determination of the composition of samples in aqueous and aqueous–organic solutions. The capillary electrophoresis method is implemented in capillaries and is based on differences in the electrophoretic mobilities of charged particles in both aqueous and non-aqueous buffer electrolytes.

Sample preparation for the quantitative analysis of the content of strontium, calcium and magnesium was carried out using a microwave sample preparation system MC-6 (subjected to wet mineralization). Then, prepared samples were analyzed by recording electropherograms according to a standardized, approved methodology (PND F 14.1:2:4.167-2000).

3. Results and Discussion
This research covers the sample analysis of shells and needles taken from a green sea urchin *S. Droebachiensis* and a lilac sea urchin *S. Purpuratus*. The obtained data on heavy metals accumulation in the studied samples of shells and needles is shown in Table 1.

| Species      | Sample   | Zn     | Cd      | Pb      | Cu      | Sr       |
|--------------|----------|--------|---------|---------|---------|----------|
| *S. Purpuratus* | needles  | 13.26±1.46 | 0.03±0.002 | 0.23±0.01 | 1.54±0.14 | 54000.00±4850.00 |
|              | shells   | 14.17±1.59 | 0.07±0.005 | 1.83±0.12 | 1.03±0.10 | 26153.85±1875.54 |
| *S. Droebachiensis* | needles  | 17.39±1.87 | 0.03±0.001 | 0.25±0.01 | 0.30±0.02 | 69565.22±5467.65 |
|              | shells   | 18.68±1.95 | 0.10±0.009 | 3.17±0.22 | 0.33±0.02 | 30825.22±2650.23 |

Table 1. Heavy metal accumulation in the shells and needles of studied species, mg/kg.

According to the data obtained, the quantitative content of cadmium and lead in the studied samples corresponds to the standards of the permissible level of toxic elements, presented by Technical Regulation of the Customs Union (TR CU 021/2011).

Analysis of shells and needles samples of the studied sea urchin species revealed the presence of high concentrations of strontium, magnesium and calcium (Table 2).

| Species      | Sample   | Sr      | Ca      | Mg      |
|--------------|----------|---------|---------|---------|
| *S. Purpuratus* | needles  | 54000.00±4850.00 | 760.00±68.45 | 7700.00±742.45 |
|              | shells   | 26153.85±1875.54 | 923.08±87.59 | 9230.77±894.73 |
| *S. Droebachiensis* | needles  | 69565.22±5467.65 | 857.25±74.14 | 7826.09±612.56 |
|              | shells   | 30825.22±2650.23 | 5095.65±479.83 | 10260.87±985.47 |

Table 2. Accumulation of strontium, calcium and magnesium in the shells and needles of the studied sea urchin species, mg/kg.

The quantitative content of magnesium in the studied samples is confirmed the levels of magnesium carbonate presence in the shell and needles of the studied species [6]. At the same time, the levels of strontium accumulation in the studied samples significantly exceed the content of calcium and magnesium, while the quantitative content of calcium is the lowest one (Figure 1).
The potential reason for high levels of strontium in the samples of shells and needles of the studied sea urchin species is the increased ability of strontium to replace calcium cations in the living organisms: strontium accumulates in the body after ingestion and cannot be excreted. According to available studies, the accumulation of strontium in the aquatic organisms is closely related to the concentration of calcium, and the strontium accumulation coefficient is inversely proportional to the concentration of calcium in the water bodies [9, 10].

![Normalized distribution diagram of strontium, magnesium and calcium in the studied species of the sea urchins.](image)

**Figure 1.** Normalized distribution diagram of strontium, magnesium and calcium in the studied species of the sea urchins.

4. Conclusion
The revealed low levels of calcium compared with the concentration of strontium in the studied samples of shells and needles of *S. Droebachiensis* and *S. Purpuratus* sea urchins indicate an unfavorable situation in the habitat of these species in relation to the presence of mobile forms of strontium.

The results of the study indicate the need for strict regular monitoring of strontium content in the Arctic waters, particularly in the Barents Sea. The obtained dependences in the accumulation and distribution of heavy metals within the studied objects (*S. Droebachiensis* and *S. Purpuratus*) can serve as a scientific basis for more effective safety control and ensuring high quality calcium salts production in the food and pharmaceutical industries when using marine benthic invertebrates, especially sea urchins, as raw materials.

References
[1] Amarowicz R, Synowiecki J, and Shahidi F 1994 Sephadex LH-20 separation of pigments from shells of red sea urchin (Strongylocentrotus franciscanus) *Food Chem.* 51 227–229
[2] Hou Y et al. 2016 Marine shells: Potential opportunities for extraction of functional and health-promoting materials *Crit. Rev. Environ. Sci. Technol.* 4(11–12)1047–1116
[3] Amarowicz R, Synowiecki J, and Shahidi F 2012 Chemical composition of shells from red (Strongylocentrotus franciscanus) and green (Strongylocentrotus droebachiensis) sea urchin *Food Chem.* 3 82–86
[4] Goodwin T W and Srisukh S 1950 A study of the pigments of the sea-urchins, Echinus esculentus L. and Paracentrotus Lividus Lamarck *Biochemistry Journal* 47 69–76
[5] Dincer T and Cakli S 2007 Chemical composition and biometrical measurements of the Turkish
sea urchin (Paracentrotus lividus, Lamarck 1816) Critical Review in Food Science and Nutrition 47 21–26

[6] Magdans U, Hermann G 2004 Single crystal structure analysis of sea urchin spine calcites European Journal of Mineralogy 16(2) 261–268

[7] Yuanyuan Lu et al. 2018 A review of the identification and detection of heavy metal ions in the environment by voltammetry Talanta 178 324–338

[8] Zemann A, Rohregger I and Zitturi R 2018 Determination of small ions with capillary electrophoresis and contactless conductivity detection Capillary Electrophoresis. Methods In Molecular Biology 384 ed Schmitt-Kopplin Ph (Humana Press) 3–19

[9] Selda O T and Nursah A 2012 Relationship of heavy metals in water, sediment and tissue with total length, weight and seasons of Cyprinus carpio L., 1758 from Isikli Lake (Turkey) Pakistan J. Zool. 44(5) 1405–1416

[10] Zimmerman C E 2005 Relationship of otolith strontium-to-calcium ratios and salinity: Experimental validation for juvenile salmonids. Canadian Journal of Fisheries and Aquatic Sciences 62 88–97