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STRUCTURE AND MINERAL ELEMENT COMPOSITION OF THE LYOPHILISED FRESHWATER BRYOZOAN *HYALLINELA PUNCTATA*

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Abstract

Structure of the lyophilised colonies of the freshwater bryozoan *Hyalinella punctata* (Hancock, 1850) and their mineral element composition were studied by X ray diffraction, scanning electron microscopy (SEM) and inductively coupled plasma (ICP) spectrometry for the first time ever. The X ray measurements showed amorphous structure of the bryozoan, while SEM indicated porous connected structure without regular structural motifs. Considerable presence of phosphorus (7949.69 µg/ml), sulphur (6204.7 µg/ml) and calcium (6139.58 µg/ml), as well as low heavy metal content was found by ICP. The obtained results jointly indicate the potential of the investigated animal species as a base for bioscaffolds and nanocomposites.

Introduction.

Freshwater bryozoans are sessile invertebrates that grow on submerged substrates. They live in lotic and lentic water and feed on suspended organic particles, which they capture with a whorl of ciliated tentacles. *Hyalinella punctata* (Hancock, 1850) is not a very frequent bryozoan species, but it has been noticed in more European countries including Serbia [1]. Most of previous studies have focused on bryozoans of marine origin, particularly for the reason of their bioactive natural products [2], while freshwater bryozoans have been practically neglected till date. Our structural and elemental study of the lyophilised bryozoan *H. punctata* is aimed to determine its usefulness in term of new natural material for making scaffolds for cell growth and/or the base for construction of the new bionanomaterials. Although there is an increasing number of studies on using invertebrates such are marine sponges, for making bioscaffolds for cell growth and tissue regeneration [3,4], freshwater bryozoans have not been previously studied from this aspect.

Materials and Methods

The colonies of the freshwater bryozoan *Hyalinella punctata* (Hancock, 1850) were collected in Belgrade (the river Danube, Serbia) in November 2011. Voucher specimen has been deposited in the Zoology Collection of the Department of Biology and Ecology of the University of Novi Sad, Serbia (BRY 003). The lyophilised material was used for the experiments.

Crystal structure of powdered bryozoan sample was analysed by X-ray diffraction (XRD) using filtered CuK α radiation (Siemens D5000) preceded by the angular correction with high quality Si standard. The microstructural observation of as-received bryozoan samples was performed by scanning electron microscopy (SEM), on the samples of whole tissue, using a JEOL 6300F microscope at 3 kV accelerating voltage.

Due to mineral element analysis, the lyophilised material (0.2 g) was microwave digested with nitric acid and hydrogen peroxide for 1 h. The elements were determined by an inductively coupled plasma optical emission spectrometer with axial and radial viewing plasma configuration (ICP-OES, SpectroGenesis EOP II, Spectro Analytical Instruments GmbH, Kleve, Germany).

Results and discussion

X-ray diffraction pattern (Fig. 1A) reveals significant broadening of profile diagram indicating the amorphous nature of the bryozoan sample.

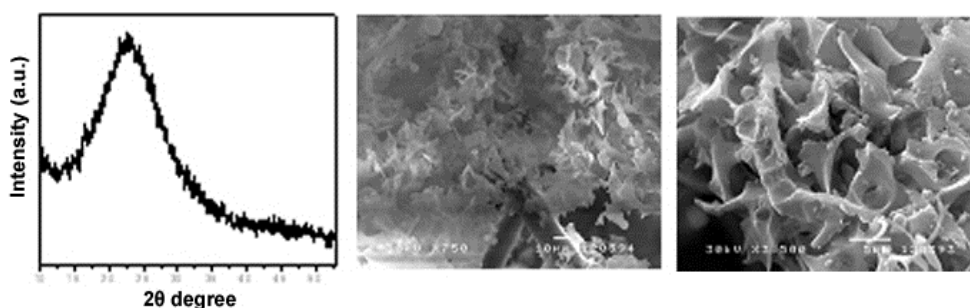


Figure 1. A) X ray diffractogram and B) SEM images of *H. punctata*.

SEM imaging of the bryozoan samples (Fig. 1B) depicts highly porous connected structure with no regular structural motifs, suitable for using as biomimetic materials.

The element composition (in $\mu\text{g/g}$ dry weight) determined by ICP is as follows: P 7949.69, S 6204.7, Ca 6139.58, Fe 3918.96, Al 3128.33, Mg 2036.46, K 1854.69, Na 695.83, Si 335.31, Mn 166.15, Zn 80.89, Ba 24.71, Cu 23.48, B 15.75, Li 14.39, Sr 11.49, Pb 7.12, Cr 5.95, Ni 3.91, Mo 3.42, As 3.28, Se 1.94, Cd 1.61, Co 1.19 and Hg n.d. Considerable presence of phosphorus, sulphur and calcium, as well as non-toxic content of heavy metals [5] is obvious. The bryozoans are calcifying animals, having the structure based on calcium carbonate; indeed,

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mineralisation of bryozoan skeleton makes these organisms proven bioindicators [6]. On the other hand, high content of sulphur may originate from its biologically active secondary metabolites containing sulfur, such as alkaloids, which have been found in considerable amounts in some other bryozoans [7]. From mineral element point of view the bryozoan based material seems to be applicative in humans, but it should be seasonally analysed.

In conclusion, the porous connected structure of the *H. punctata* together with its mineral content indicate its potential as a base for bioscaffolds and nanocomposite materials.

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