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Phytolithes (SiO_2 Microparticles) of some multicellular brown algae

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ABSTRACT

In this study we present the data of investigation of the biomineral (SiO_2) particles of the brown algae: Fucus evanescence (C.Agardh 1820), Turbinaria ornate (Turner 1807), Sargassum miyabei (Yendo, 1907), Dictyota dichotoma (Hudson) J.V.Lamouroux 1809. 2 wide-spread morphotypes of mineral particles were found using optical microscopy: irregular smooth (15-47,34%) and irregular ruminate (27-64%).

Keywords: phytoliths; algae; biomineral; biosilica

INTRODUCTION

Silicon microparticles (phytoliths) were found in many groups of plants [1-3] and in mushrooms [4]. In particular, earlier we found similar silicon formations in multicellular seaweed [3].

Results of findings of phytoliths in the talloms of some brown multicellular seaweed are presented in this work: Fucus evanescence (C.Agardh 1820), Turbinaria ornate (Turner 1807), Sargassum miyabei (Yendo, 1907), Dictyota dichotoma (Hudson) of J.V.Lamouroux 1809.

MATERIALS AND METHODS

Sample collection

Fucus evanescence was collected in the coastal zone of Aniva Gulf (Sakhalin Island) to the southern Okhotsk Sea. *Sargassum miyabei* and *Turbinaria ornate* were collected in the coastal zone of the South Chinese Sea (the Socialist Republic of Vietnam). *Dictyota dichotoma* was collected in the coastal zone of Petra Velikogo Gulf, Japan Sea.

Samples of mature algae (5 samples of each species, aged 2–3 years) were collected at a depth of 3–6 m, placed into plastic containers, and refrigerated at −5 °C. Preparations of phytoliths were made over 2 days.

Sample preparation

Phytoliths were excised using the modified Piperno technique [2]. The thallomes (approximately 30–50 g per sample) were burned in covered, ceramic-enameled crucibles in a muffle furnace at 450 °C for 4 h. The ashes were

then transferred into glass centrifugal test tubes and washed thoroughly (up to 10 mL of rinse solution were added for 10 minutes, with periodic stirring of the test tube) with a solution of 10% hydrochloric acid and concentrated nitric acid (JSC "Neva-Reactiv"; Saint-Peterburg, Russian Federation). The tubes were then rinsed twice with 10 mL of distilled water. After the last wash and subsequent centrifugation for 10 minutes at 1000 g (OPn-8, Dastan, Kyrgystan), the water was decanted, leaving 0.5 mL in the test tube. A further 200 µl of solution were removed from the test tube bottom with a pipette and subjected to microscopy. The remaining solution was used for X-ray diffraction analysis.

Optical microscopy

The processed samples were individually placed on a microscope slide (JSC BioVitrum, Saint-Peterburg, Russian Federation) and covered with a cover slip (JSC BioVitrum, Saint-Peterburg, Russian Federation). They were examined within one hour on an AxioScope A1 light microscope (Zeiss; Oberkochen, Germany) using an AxioCam 3 digital video camera (Zeiss; Oberkochen, Germany) at 100x to 630x magnification.

The length and width of the visible image of each particle were measured using the Axio Vision 4.2 program (Zeiss; Oberkochen, Germany), a component of the optical microscope software. The thinnest site was measured as the width of shapeless formations. As measurements were taken from two-dimensional images, the actual dimensions of the studied objects may differ.

Scanning electronic microscopy

Substantial analysis was performed using a JEOL JXA 8100 scanning electron microscope with energy-dispersive spectrometer by Oxford Instruments. The sample deposition for the electron microscopy was made with platinum.

Phytolith analysis

The definitions of morphotypes as well as the descriptions of phytoliths and other unidentified mineral particles were according to the International Code for Phytolith Nomenclature 1.0 [1].

RESULTS AND DISCUSSION

Using light microscopy we observed morphological features of phytoliths (see Tab. 1).

Table 1 Morphometry features of seaweed phytoliths

Type	Quota, %	Area, µm^2	Width, µm	Length, µm	Perimeter, µm
Fucus evanescence					
gypsum crystals	93	1703,63±1449,94	49,2±21,79	75,89±22,6	230,43±87,93
smooth elongated rectangular	7	13542,46±8946,45	131,07±48,41	237,42±122,24	582,86±260,69
irregular smooth	15	1189,33±920,81	35,03±15,14	49,56±16,71	137,19±50,32
irregular ruminate	27	1266,7±1013,6	38,08±17,35	48,63±21,66	144,22±64,98
long smooth	8	15360,54±7496,32	16,42±5,08	800,89±199,53	1654,2±433,91
Turbinaria ornate					
irregular smooth	38,67	85,78±61,75	9,56±3,77	12,15±4,58	36,31±13,21
irregular ruminate	54	133,52±118,04	11,6±4,72	14,87±6,11	44,65±17,86
round	2	152,09±29,89	13,65±1,56	13,82±1,35	46,79±4,69
«yellow» (thick)	5,33	296,93±231,65	17,82±7,29	22,04±9,57	66,23±28,03
Sargassum miyabei					
irregular smooth	33,33	84,66±40,57	9,64±2,5	12,63±4,28	37,52±10,41
irregular ruminate	64	123,67±93,72	11,34±4,44	14,9±5,15	44,33±15,28
«yellow» (thick)	2,67	111,30±31,67	10,11±2,55	14,71±0,87	43,31±5,82
Dictyota dichotoma					
irregular smooth	47,34	64,97±40,69	8,26±2,62	10,75±3,22	32±9,37
irregular ruminate	33,33	79,87±55,1	8,84±3,05	12,26±4,36	35,99±11,74
oval	10	56,2±26,99	7,28±2,01	10,03±2,19	29,76±6,59
round	2	63,37±18,74	8,78±1,54	8,95±1,67	30,24±4,47
«yellow» (thick)	7,33	118,8±91,66	11,3±5,99	15,93±8,31	45,95±21,94

We observed dozens of sponge spicules and Diatomic spp in all samples (Fig. 1).

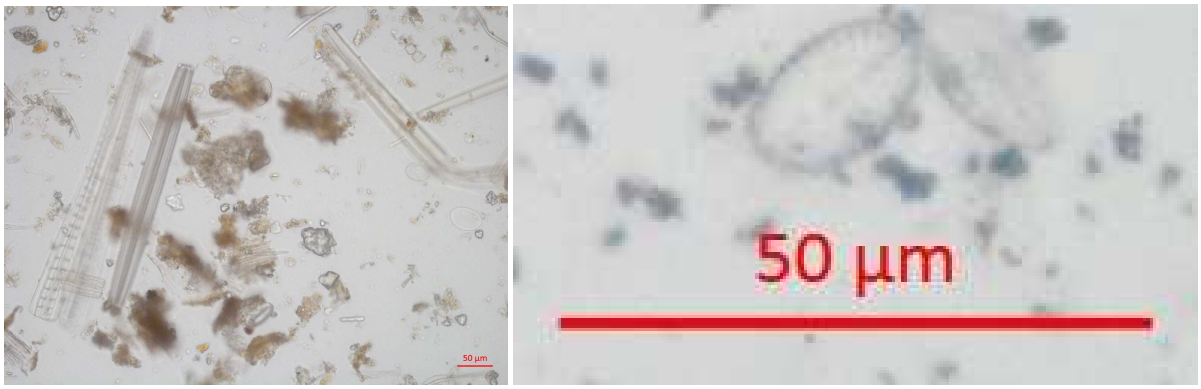
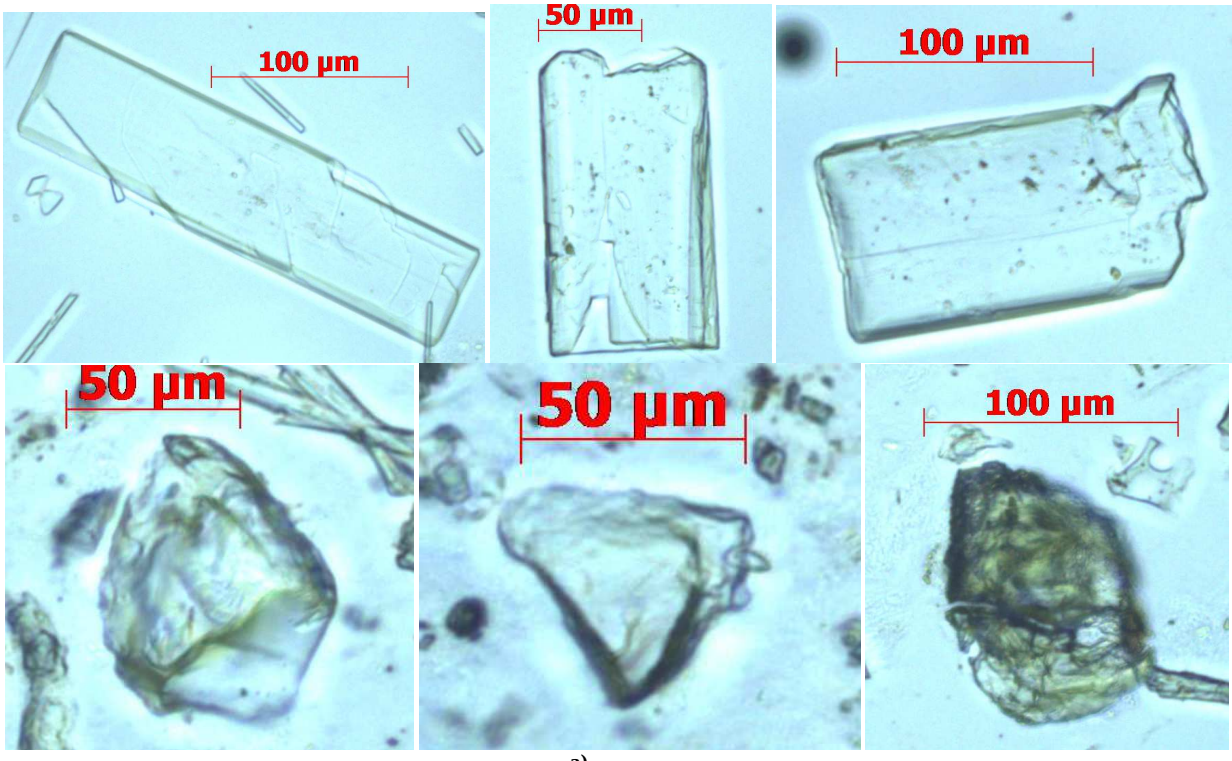


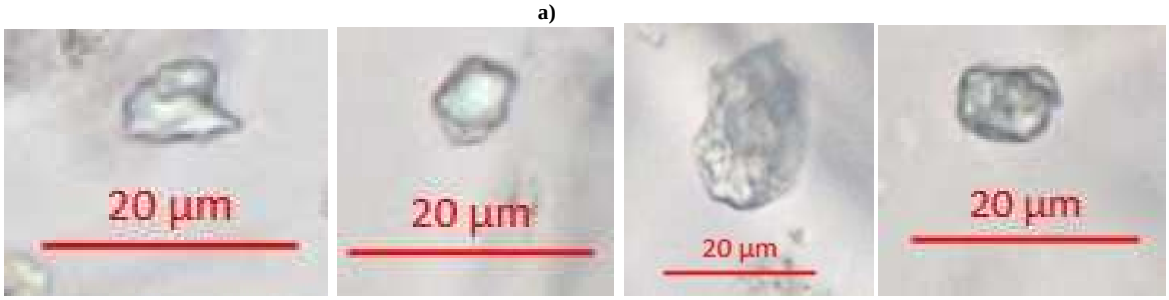
Fig. 1. Diatoms spp. in samples a) Turbinaria ornate, b) Dictyota dichotoma

We excluded these artifacts from morphological research in this study.

As we can see the majority of phytoliths in the studied seaweed have no specific form and belong to 2 morphotypes: irregular smooth and irregular ruminate (Fig. 2 a-d).

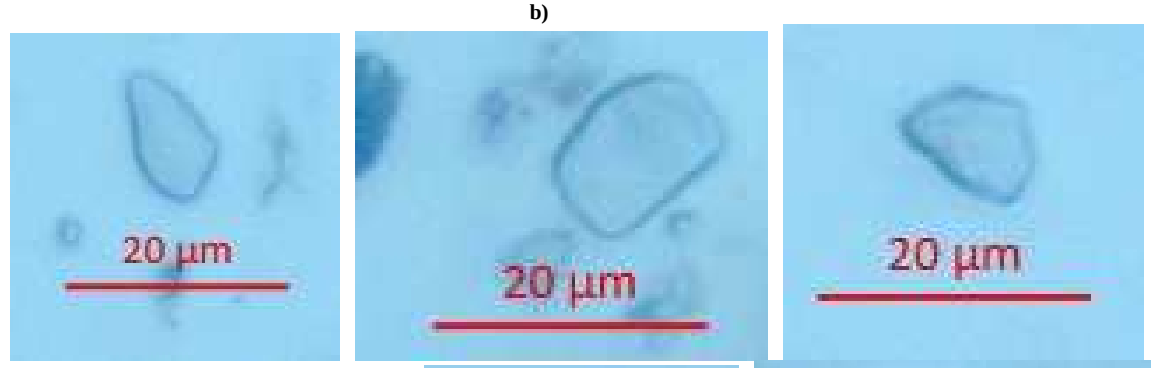


a)




20 µm
20 µm
50 µm

b)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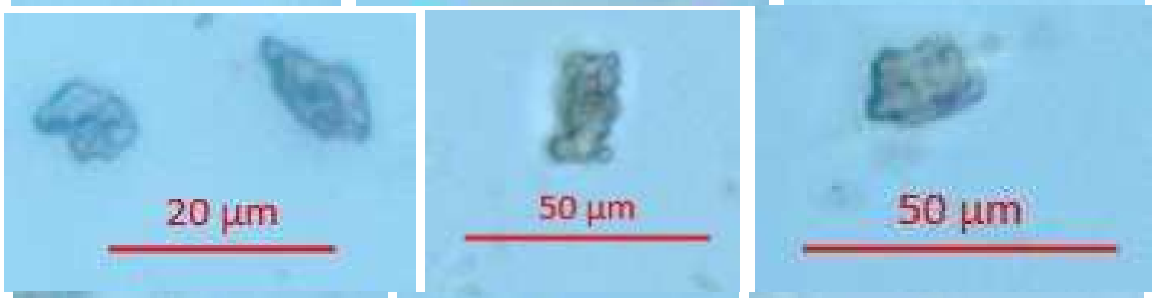
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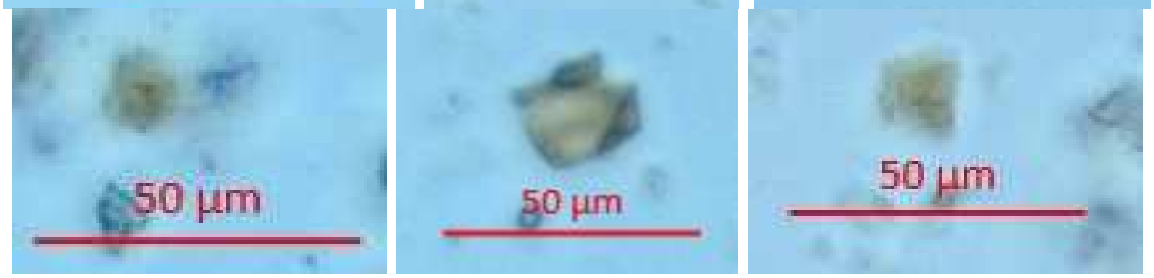
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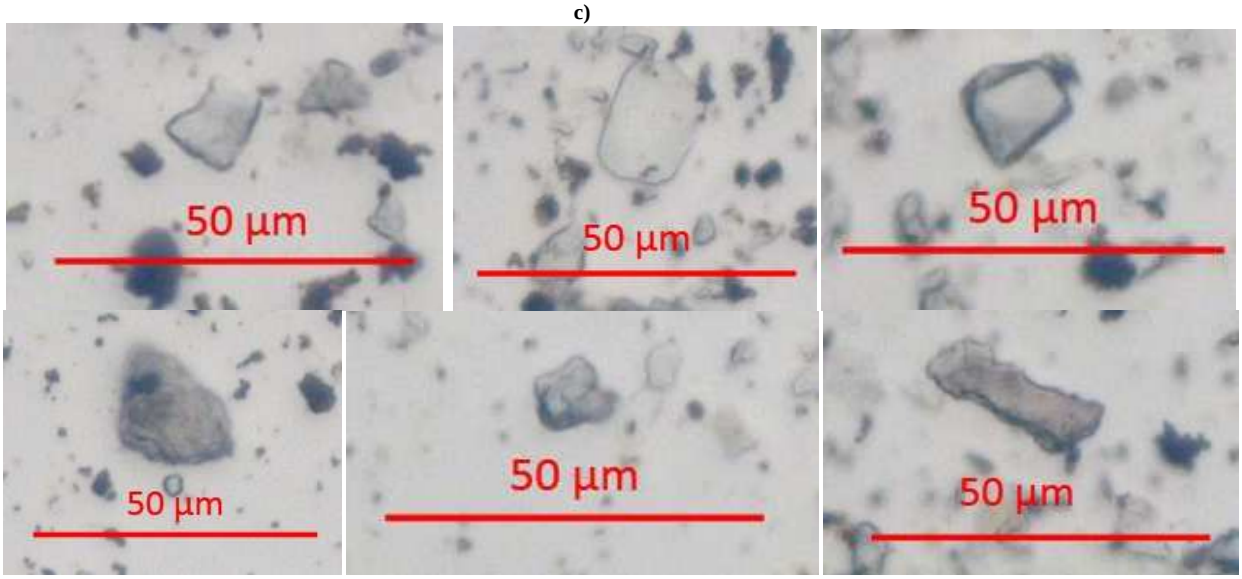
c)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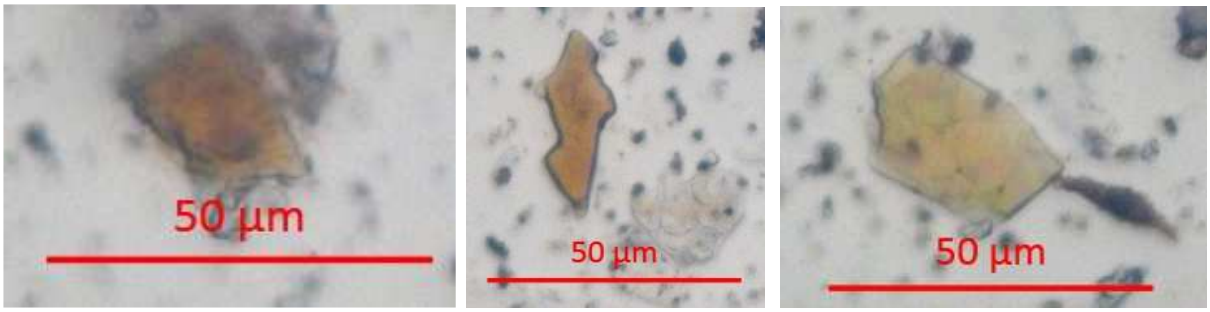

d)

Fig. 2. Morphotypes of two wide-spread forms: «irregular smooth», «irregular ruminate» : a) Fucus evanescence, b) Turbinaria ornate, c) Sargassum miyabei, d) Dictyota dichotoma

All this phytoliths are consist of SiO_2 (fig. 3).

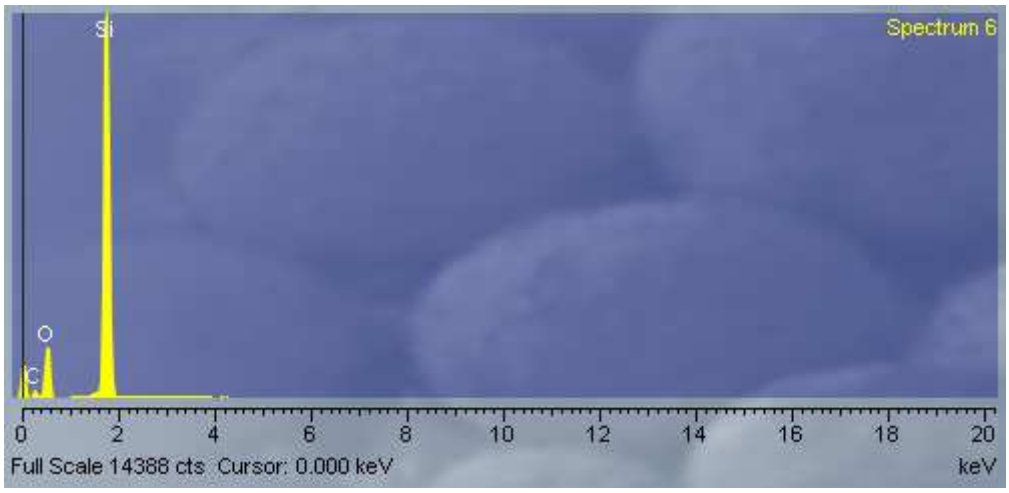

Fig. 3. Spectrum of wide-spread phytoliths

These two main types of phytoliths in different types of seaweed differ only by the size and share. We saw similar results in other groups of plants as well [3, 5].

A conclusion can be made about a certain general function of the morphotypes belonging to various taxonomic groups.

Acknowledgements
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