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Studies on structure and organization of calcium carbonate deposits in algae

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The structure and organization of calcium carbonate deposits is studied in species of *Halimeda*, *Udotea*, *Neomeris* (Chlorophyta) and *Padina* (Phaeophyta). It was found that in *Halimeda* aragonite deposition takes place outside the cell wall and in the intercellular spaces, while in *Udotea* aragonitic needles get arranged in layers parallel to the axis of filament within a sheath. In the case of *Neomeris*, crystallization takes place around the walls of sporangia. In *Padina*, aragonite crystals are randomly oriented on the surface of cells.

Almost all algal phyla have some genera which have the ability to accumulate various inorganic substances within or around the cell. However, the predominant mineral deposits of algae are either 'calcite' or 'aragonite'[1]. The way of lime deposition, and the type and amount of calcification vary from group to group and even from genus to genus[2]. A study of the structure and organization of calcium deposits is very much essential in the taxonomy of calcareous algae, which is possible by using SEM. Various studies have proved that this tool offers a great potential. Aragonitic deposition in the members of nemaliales and *Padina* is studied by Levy and Strauss[3]. McConnell and Colinvaux[4] studied the mineral components of Udoteacean forms.

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THE calcareous algae of India have been studied in the literature for their mineral deposits[5], but their structures and organization remain uninvestigated. In the present work an attempt is made to study the surface morphological features of some calcareous algae. The position, mode and orientation of aragonitic needles are studied in detail.

To study the structure and organization of calcium carbonate, species of *Helimed*, *Udotea* (Chlorophyta – Udoteaceae), *Neomeris* (Chlorophyta – Dasycladiaceae), *Padina cavonica* (Phaeophyta – Dictyotaceae) collected from Agatti, Lakshadweep Atoll (8°–12°13'N, 71–74°E) were dehydrated slowly through acetone grades. They were first coated with carbon and gold to avoid excessive charging, as suggested by Borowitzka *et al.*[2] The observations were carried out with camera CAMEBAX model 571 Probe Microanalyser.

Halimeda tuna, *H. opuntia* and *H. simulans* showed that needles of aragonite completely fill up the intercellular spaces of segments. In *H. tuna* the crystals were 10 μm long and 0.3–0.6 μm in width (Figure 1 *a*), while in *H. simulans* and *H. opuntia*, mature crystals were of uniform size, about 0.08–0.3 μm wide and 4–4.5 μm long needles tapering slightly near the ends (Figure 1 *c*). The orientation of the crystals in all the three species appeared to be random. Deposition of these crystals was observed outside the cell wall but within the intercellular spaces (Figure 1 *b*).

In the case of *Udotea indica* and *U. flabellum* aragonite the needles were outside the cell wall but within the sheath. These needles were 0.07 × 0.4 μm in size and occurred in bundles (Figure 1 *d*). *Neomeris annulata* and *N. van-Bosseae* showed typical aragonite needles. Mature needles are 0.3–0.6 μm wide and 5–6 μm long in size (Figure 1 *e*, *f*). In both the species the orientation of the crystals was observed to be random. In younger parts of the thallus the crystals were found around the walls of sporangia (Figure 1 *g*). But in older parts of the thallus the intercellular spaces become filled with crystals.

Deposits in *Padina pavonica* showed aragonitic $CaCO_3$ outside the cell wall (Figure 1 *h*). Orientation of crystals was random (Figure 1 *i*). Needles were about 2–4 μm long and 0.3–0.4 μm wide. In the oldest part of thallus crystals lose their needle shape (Figure 1 *j*). These intercellular spaces are completely isolated from the external seawater by the outer layer of closely appressed utricles. The aragonite needles completely fill the intercellular spaces of the segment. Wilbur *et al.*[6] reported that in *H. monile* the needle-like crystals get formed in the fibrous material of the filament wall when the segments are 36–38 h old. Crystal formation seems to coincide with the development of chloroplast formation and with the fusion of the outer layer of filaments, which isolates the intercellular spaces from the outside[7,8]. According to Borowitzka[2], the process of calcification in the genus *Halimeda* may be a purely physical mechanism resulting from a combination of anatomical and physiological properties of this alga[2].

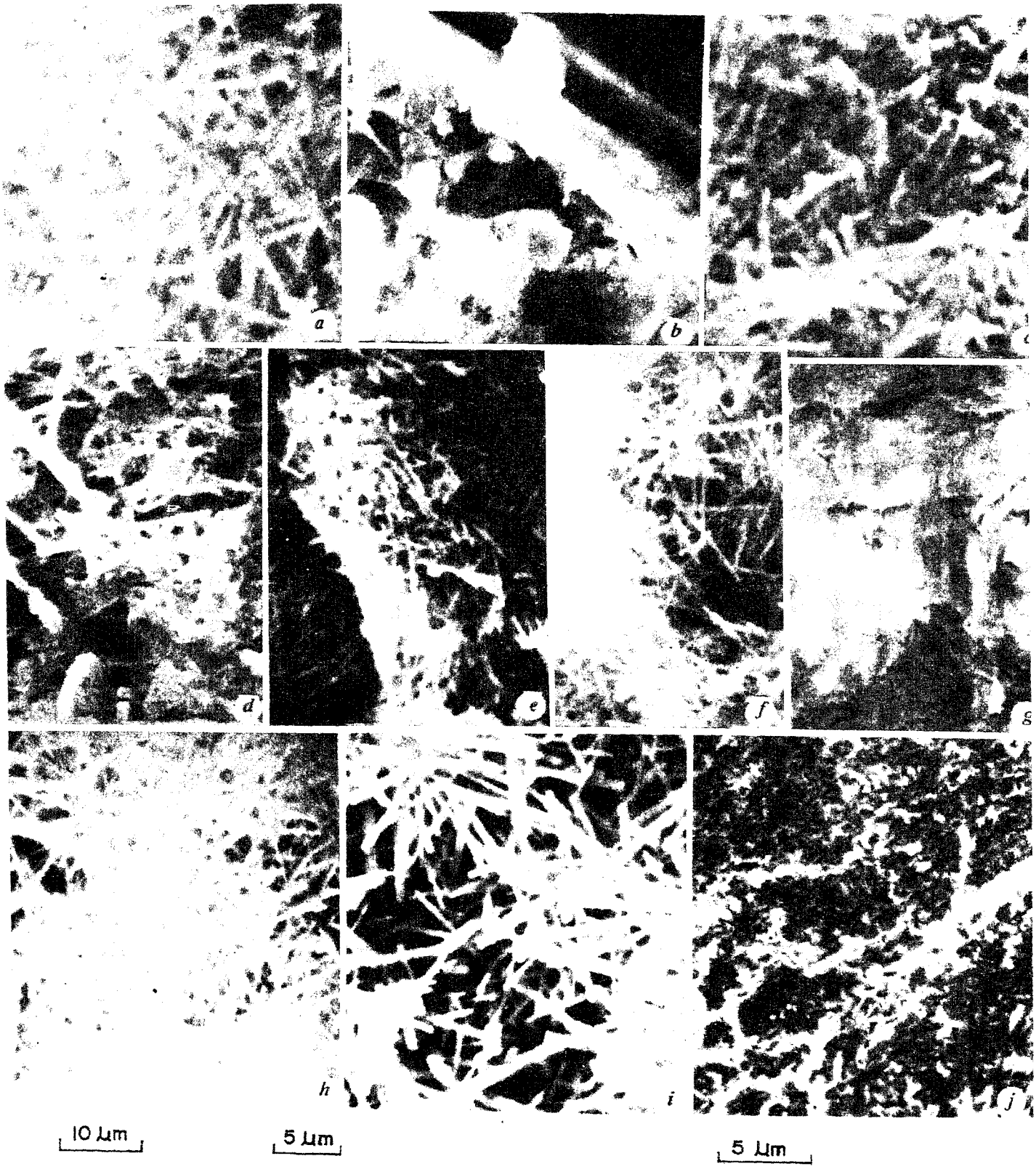

Figure 1. Surface morphologies of *Halimeda*, *Udotea*, *Neomeris* and *Padina* spp.: *a*, aragonitic needles of *H. tuna*; *b*, *H. opuntia*, a transverse section showing the calcification in the intercellular spaces; *c*, aragonite needles of *H. simulans*; *d* and *e*, bundles of aragonitic needles of *U. indica* and *U. flabellum*; *f*, aragonitic needles of *N. annulata*; *g*, calcium deposition around the walls of sporangia; *h*, aragonitic needles of *N. van-Bosseae*; *i*, aragonitic needles of *P. pavonica*; *j*, aragonite crystals losing their needle-shaped appearance at maturity in *P. pavonica*.

According to him, the layer of appressed utricles separating the intercellular spaces from the external seawater medium increase the length of the diffusion path of ions from seawater to the intercellular spaces, where the calcification takes place.

As far as *Neomeris annulata* and *N. van-Bosseae* are concerned, there is a layer of appressed outer cells separating the intercellular spaces from the external medium, which indicates the absence of a definite outer cell layer. In *U. indica*, filaments are interwoven closely and so help in reducing water movement, creating a long diffusion path required for the production of pH changes and hence stimulating $CaCO_3$ precipitation. The aragonitic needles are arranged in bundles, with *n* layers parallel to the axis of the filament which are enclosed within a sheath. This sheath may be playing a direct role in calcification by providing an organic matrix for the nucleation of crystals[7]. It is also known to play a role in orienting aragonitic needles. Vacuolar inclusions of calcium oxalate must be supplementing this process[1].

It can be concluded that in the genus *Halimeda*, aragonite deposition takes place outside the cell wall and in the intercellular spaces, while in *Udotea* aragonitic needles get arranged in layers parallel to the axis of the filament within a sheath. In case of *Neomeris*, crystallization takes place around the walls of sporangia. In *Padina* aragonite crystals are randomly oriented on the surface of only the young thalli.

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Acute toxicity of ammonium sulphate to the air-breathing organ of the live fish *Heteropneustes (Saccobranchus) fossilis* (Bloch)

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Acute toxicity induced by 2000 ppm (96 h LC_{50} value) of the inorganic fertilizer ammonium sulphate on the inner epithelial lining of the air sac (accessory respiratory organ) of *Heteropneustes fossilis* has been described using histopathological techniques. The goblet cells show cyclic increased (due to hyperplasia and hyperactivity) followed by decreased (due to exhaustion and degeneration) mucogenic activity. Cyclic haemorrhage takes place due to rupture of the tips of the secondary lamellae, which also regenerate several times. This causes hyperplasia of the haphazardly arranged epithelial cells, leading to decreased (secondary) lamellar density. Uncontrolled hyperplasia also causes increased distance of respiratory blood–air barrier, which along with decreased lamellar density results in impaired aerial respiration, leading to asphyxiation and ultimate death of the fish.

AMMONIUM sulphate, a common agricultural fertilizer, is extensively used to increase plankton production[1,2]. Excessive application of this inorganic fertilizer results in acute ammonia toxicity. Amongst the organs affected, the gills exhibit quick but severe damage[3–5], probably due to contact stress. On the other hand, the data associated with ammonia-induced toxicity in air-breathing organs (which in many species are modified gills structures[6,7]) are not available. In *Heteropneustes fossilis*, the accessory respiratory organ (ARO) (air sac or branchial diverticulum) is a pair of sac-like backward extensions of the suprabranchial chamber – embedded deeply in the body myotomes, one on each side of the body – through which the fish respire aerially[6,8]. Hence, this organ does not come under direct contact of the external medium. An attempt is made here to analyse the toxicity of ammonium sulphate (which liberates ionized ammonia also) on the respiratory epithelial lining of the air sac of *H. fossilis* in an attempt to compare (with the available data on gills) the mode of action of this ambient toxicant on these different organs as the mechanism of respiration in them is also different.

H. fossilis (length 16 ± 2 cm) collected locally were stored in the laboratory for 20 d in tap water in plastic aquaria. They were regularly fed with minced goat liver. Feeding was discontinued one day before the start of the experiment. Ten groups of 10 fish each, irrespective of their sex, were exposed to 50 l of 2000 ppm of ammonium sulphate solution (96 h LC_{50} value determined by trimmed Spearman–Karber method[9]) prepared in tap water (dissolved O_2 6 mg/l, water temperature 22 ± 2°C, pH 7.8, hardness 23.2 mg/l). In controls, addition of ammonium sulphate was escaped. After every 24 h, both