

## RESEARCH ARTICLE

### Cell-wall images of marine algae by metachromatic method

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#### Abstract

Metachromatic image of cell-walls were investigated for seven algae. Species; red: *Acanthophora nayadiformis*, *Chondria capillaris*, *Phyllophora crispa*, brown: *Petalonia fascia*, *Cystoseira barbata*, *Dictyota dichotoma* and green: *Ulva linza*. The tested methachromatic dyes were azur II, methylene blue and thioflavine. In this work, locations of algal acidic and sulfated polysaccharides in cell-wall of red and brown algae were demonstrated by metachromasy, except in green algae. The metachromatic method clearly showed the cell wall constituents of algae. Cuticle and epidermal cells intensely stained but also the inner cell wall polysaccharide accumulation clearly shown. The most obvious staining and color change was seen by azur II.

**Keywords:** Metachromasy, algae, cell wall components, acidic polysaccharides

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#### Introduction

Numerous papers were published on the constituent of cell-wall of marine algae such as: cellulose as the major component (Kylin 1915; Cronshaw *et al.* 1958; Gretz *et al.* 1980), xylan (Mirande 1913; Naylor and Russel-Wells 1934), mannan (Jones 1950; Frei and Preston 1961), alginic acid (Jones 1930; Frei and Preston 1962; Kreger 1962; Evans and Holligan 1972; Chiowiti *et al.* 1997; Andrade *et al.* 2004), agar (Ji *et al.* 1996; Chiovitti *et al.* 1997; Senni *et al.* 2006), carrageenan (La Claire and Dawes 1976; Chiovitti *et al.* 1997), galactan

(Anderson *et al.* 1965), fucoidan (Senni *et al.* 2006), porphyran, ulvan (Ray and Lahaye 1995), sulphated xylan ( $\beta$ -1,3), (Yamagaki *et al.* 1997), protein (Thompson and Preston 1967), silicon, minerals, calcite and aragonite (Jones 1950). The methods used for the identification of these polysaccharides are X-ray diffraction (Nicolai and Preston 1952; Chronshaw *et al.* 1958), IR / FTIR (Mc Candless *et al.* 1981; Peats 1981; Sakai *et al.* 1993; Haslin *et al.* 2000; Balkan *et al.* 2005), NMR (Usov 1984). Another method, metachromasy was first found by Ehrlich (1887) for histological screening of mast cells. This phenomenon was elucidated by Lison (1935) as the change of the absorption band of the dyes from long to shorter wavelengths. Many substances showed metachromasy such as animal sulfated polysaccharide, heparine (Jorpes 1939; Guven and Ertan 1984; Guven *et al.* 1991), raparin obtained from *Rapana venosa* (Guvener *et al.* 1991) and synthetic anionic surfactants (Guvener *et al.* 1994; Guven and Coban 2013). Metachromatic method was also used for algal polysaccharides in food (Soedjak 1994) and tooth pastes (Guvener *et al.* 1988). Metachromasy of algal acidic and sulfated polysaccharides were investigated for agar by using cresyl blue (Lison 1935), agar and carrageenan and alginic acid (Bank and Bungenberg de Jong 1939; Pal and Shubert 1962; Stone *et al.* 1963). The differentiation of algal polysaccharides: agar, carrageenan and alginic acid was carried out based on the change of lambda max levels of the dye used (Guvener and Guvener 1985a, 1985b).

This method was used for the examination: determination and localization of algal acidic and sulfated polysaccharides in cell (Ando 1955) and cell-walls (Okamura and Oishi 1905; Mc Cully and Margaret 1966; Parker and Dibol 1966; Gordon and Mc Candless 1975; La Clair and Dawes 1976).

In this work, metachromasy phenomenon was used for the identification of acidic and sulfated polysaccharides in algal cell-wall. Images of cell walls were investigated among 7 algae of 3 divisions by using 3 dyes.

## Materials and Methods

The algae samples were collected from the Turkish coasts, as *Acanthophora nayadiformis* from Bozcaada in May 2012 and the other samples from Çanakkale (Yapıldak coast) in May 2013.

In this study, the tested algae were:

- |              |                                                                                                                                                          |
|--------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Rhodophyta:  | <i>Acanthophora nayadiformis</i> (Delile) Papenfuss,<br><i>Chondria capillaris</i> (Hudson) M.J. Wynne,<br><i>Phyllophora crispa</i> (Hudson) P.S. Dixon |
| Ochrophyta:  | <i>Petalonia fascia</i> (O.F. Müller) Kuntze,<br><i>Cystoseira barbata</i> (Stackhouse) C. Agardh,<br><i>Dictyota dichotoma</i> (Hudson) J.V. Lamouroux  |
| Chlorophyta: | <i>Ulva linza</i> Linnaeus                                                                                                                               |

Algaebase ([www.algaebase.org](http://www.algaebase.org)) was used for current names of algae (Guiry and Guiry 2014). Collected algae samples were carried to the laboratory in seawater. Each sample was manually cross-sectioned and slides were prepared for staining.

Dyes: Azure II (Sigma), Methylene blue (Merck) and Thioflavine (Gurr). Dyes solutions 0.01 mg dye was solved in 10 ml distilled water. Cellulose was obtained in our laboratory from *Ulva intestinalis* (Kiran *et al.* 1980).

Staining: A drop of the solution was placed via glass rod on the section. The sample was let stand two minutes for penetration. The optical images were taken using Olympus BX51 microscope.

## Results and Discussion

The location of algal polysaccharide in cell-wall of algae examined is shown in Figure 1.

Cross sections of the algae show that they are formed of an outer cuticle (cu) and a similar outer wall (owl) with epidermal cells beneath (ep), cortex (cor) and a core medulla (med) (Figure 1d). With an electron microscope the algal layers of the outer wall (owl) and epidermal cells (ep) where the most intense staining appears, can be examined in detail, while with a light microscope they cannot be clearly distinguished. However this is not the case for cortex and medulla cells. With a light microscope the cortex and medulla cells are conspicuously apparent. In some samples the intercellular matrix (im) appears clearly (Figure 1e). The inner wall layer (iwl) is clearly seen in all samples. However this part cannot be clearly distinguished from the middle lamella due to the little cellulose it contains.

Depending on the type of stain, agar within the algae was stained with different intensities. In the *A. nayadiformis* taxon methylene blue and azure II only stained the outer layers while thioflavin stained all layers (Figure 1a-c). In this situation in the *A. nayadiformis* taxon not only outer wall layer but inner wall layer had similar densities in the agar and thioflavin may be used for this aim.

In *C. capillaris* methylene blue and azure II stained all layers other than the cortex while thioflavin again stained all layers effectively. In addition, in the intercellular matrix a small amount of staining occurred (Figure 1d-e). In *Phyllophora crispera* the most effective staining was by azure II and thioflavin (Figure 1g-i) and while methylene blue stained the outer wall layer intensely it did not sufficiently stain the cortex and medulla.

As with *Rhodophyta* members, *Ochrophyta* members showed similar results to staining. In *P. fascia* methylene blue stained all layers very well, while

thioflavine was most effective for the outer wall layer (owl) and azure II again stained the outer layers best (Figure 1j-l). In *C. barbara* thioflavin stained all layers while methylene blue and azure II mostly stained the outer wall layer (owl) (Figure 1m-o).

*D. dichotoma* appeared to be best stained by thioflavin while methylene blue and azure II partly stained the inner wall layer (iwl) in addition to the outer wall layer (Figure 1p-s).

On all sections and with all stains the cuticle part was stained more intensely (Figure 1a-s). In general cortex cells are smaller than medulla cells. Epidermal cells are much smaller than the cells forming the cortex and medulla. While the thickness of the cuticle could not be fully distinguished, the thickness changed according to the type and environment (Figure 1e-l). Some are consisted of a very thin layer (Figure 1f-i-p).

Using *U. linza* surficial staining with the three types of stain showed that the cell-walls were also completely stained (Figure 2a-c). The cellulose obtained from the *Ulva intestinalis* was stained by the same dyes (Figure 2d-f).

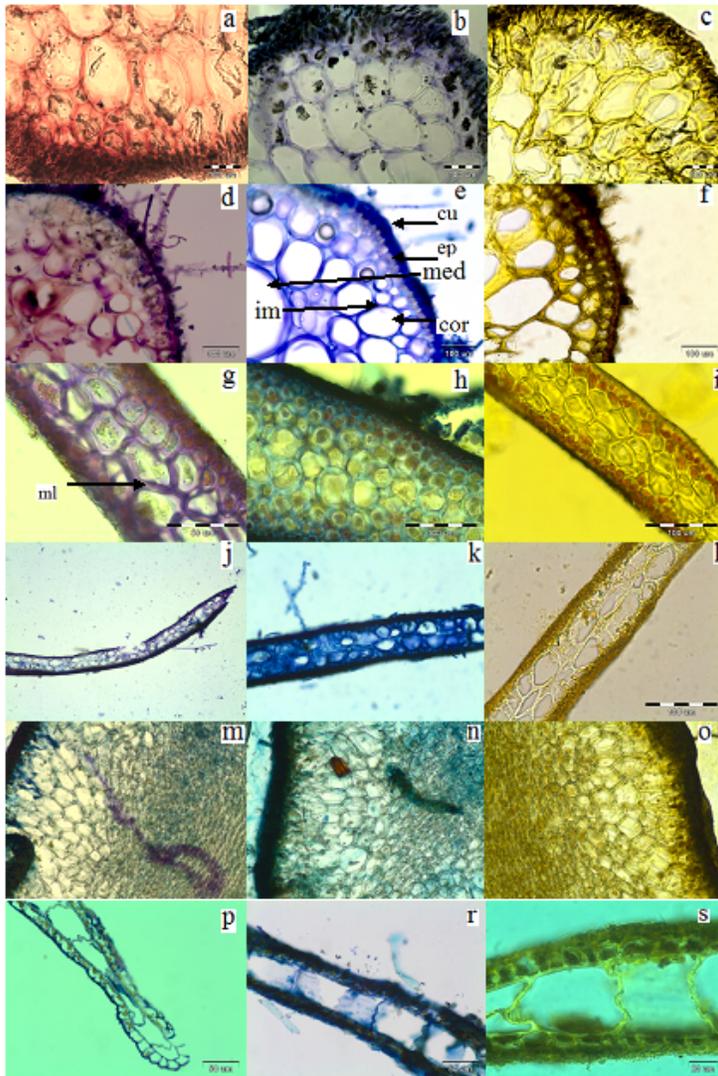
*Rhodophyta*, *Ochrophyta* and *Chlorophyta* species showed some color changes as a result of staining. Staining of red algae with azure II produced red tones (Figure 1a-d-g) while brown algae samples appeared with blue tones (Figure 1j-m-p). Staining of green algae produced a very distinctive purple color (Figure 2a-d). Staining with methylene blue generally produced blue tones with only *A. nayadiformis* observed to have a purple color (Figure 1b). Thioflavin staining generally produced a yellow color, sometimes brown. The brown color appeared most in the cuticle and epidermal cells (Figure 1 c-f-i-o).

The three dyes stained the cuticle, epidermal cells, cortex and medulla cell-walls by different amounts depending on the taxon. This indicates that depending on the type of algae these parts house different amounts of polysaccharides.

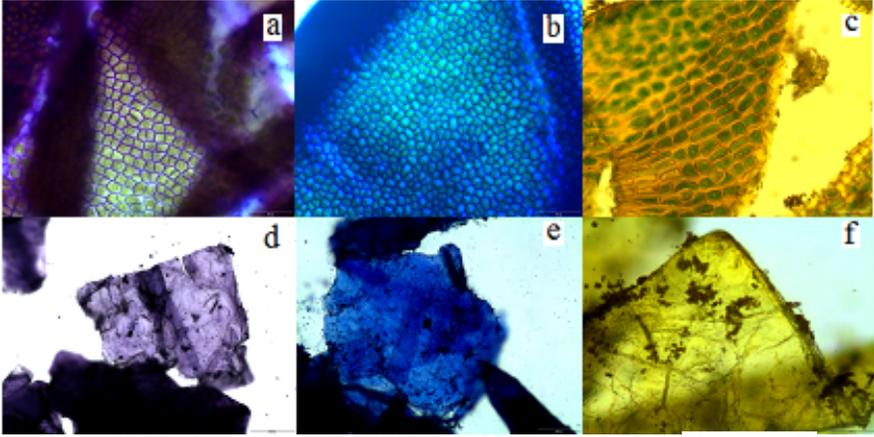
In *Rhodophyta* and *Ochrophyta* members these layers contain a small amount of cellulose in addition to agar, carrageenan and alginate (La Claire and Dawes 1976). These acidic polysaccharides build up in every layer with different rates in accordance with the type of algae and the environment they inhabit (McHugh 2003; Barnes 2005). In general while alginates are found in the cell-walls, in brown algae they are found in the intercellular matrix of the medulla (Barnes 2005). This data was imaged in this study using different stains.

It is thought that the intense staining in the cuticle and outer wall layers is due to the small size of the cells. In general in the medulla the cells are large, so no intense staining is observed. However when the volume is considered, it is noticed that they contain at least as much polysaccharide as the outer wall layer.

As shown in Figure 1 a, c, e, g, i and s the middle lamella (ml) appears to contain a significant amount of polysaccharide.



**Figure 1.** *Acanthophora nayadiformis* (a.Azur II, b.Methylene blue, c.Thioflavine), *Chondria capillaris* (d.Azur II, e.Methylene blue, f.Thioflavine), *Phyllophora crispa* (g.Azur II, h.Methylene blue, i.Thioflavine), *Petalonia fascia* (j.Azur II k.Methylene blue, l.Thioflavine), *Cystoseira barbata* (m.Azur II n.Methylene blue, o.Thioflavine), *Dictyota dichotoma* (p.Azur II, r.Methylene blue, s.Thioflavine). cu: cuticle, ep: epidermal cells, cor: cortex, im: intercellular matrix, med: medulla, ml: middle lamella



**Figure 2.** *Ulva linza*: (a. Azur II, b. Methylene blue, c. Thioflavine), *U. intestinalis* cellulose: (d. Azur II, e. Methylene blue, f. Thioflavine)

This method was previously used by Ando (1955) and it was suggested that basing metachromatic reaction musilaginous cells of *Laminaria religiosa* contained fucoidine while *Undaria pinnatifida* contained alginic acid in their cells. In another study, *Gelidium pacificum* agar of histochemical intracellular matrix and outer layer of the wall of parenchyma cells in medullary and cortical regions showed Metachromasy with toluidine blue and alcian blue (Akatsuka and Iwamoto 1979). La Claire and Dawes (1976) investigated the localization of carrageenan in the inner and outer wall of *Euclima nudum* by metachromatic dye toluidine blue and alcian blue.

In this work the first time seven algae at all 3 different divisions were tested and the localization of acidic and sulfated polysaccharides was clearly visualized in cell-walls of red and brown algae but algae contained cellulose did not show metachromasy through three dyes as in our earlier report (Kıran *et al.* 1980).

## Metakromatik Metotla Deniz Alglerinin Hücre-duvarı resimlerinin İncelenmesi

### Özet

Yedi algin hücre duvarlarının metakromatik resimleri araştırılmıştır. Bu algler: Kırmızı: *Acanthophora nayadiformis*, *Chondria capillaris*, *Phyllophora crispa*, Kahverengi: *Petalonia fascia*, *Cystoseira barbata*, *Dictyota dichotoma* ve Yeşil: *Ulva linza*'dır. Metakromatik boyalar: Azur II, metilen mavisi ve tiyoflavindir. Bu çalışmada, Metakromazi yöntemiyle, yeşil alg dışında, kırmızı ve kahverengi alglerin hücre zarındaki alg polisakaritleri gösterilmiştir. Metakromatik metot hücre zarı içeriğini başarılı bir şekilde göstermiştir. Kuticle ve epidermal hücreler derinlemesine boyanırken

iç duvardaki polisakkarit birikmesi ortaya çıkmıştır. En belirgin boyanma ve renk değişimi Azur II ile görülmüştür.

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