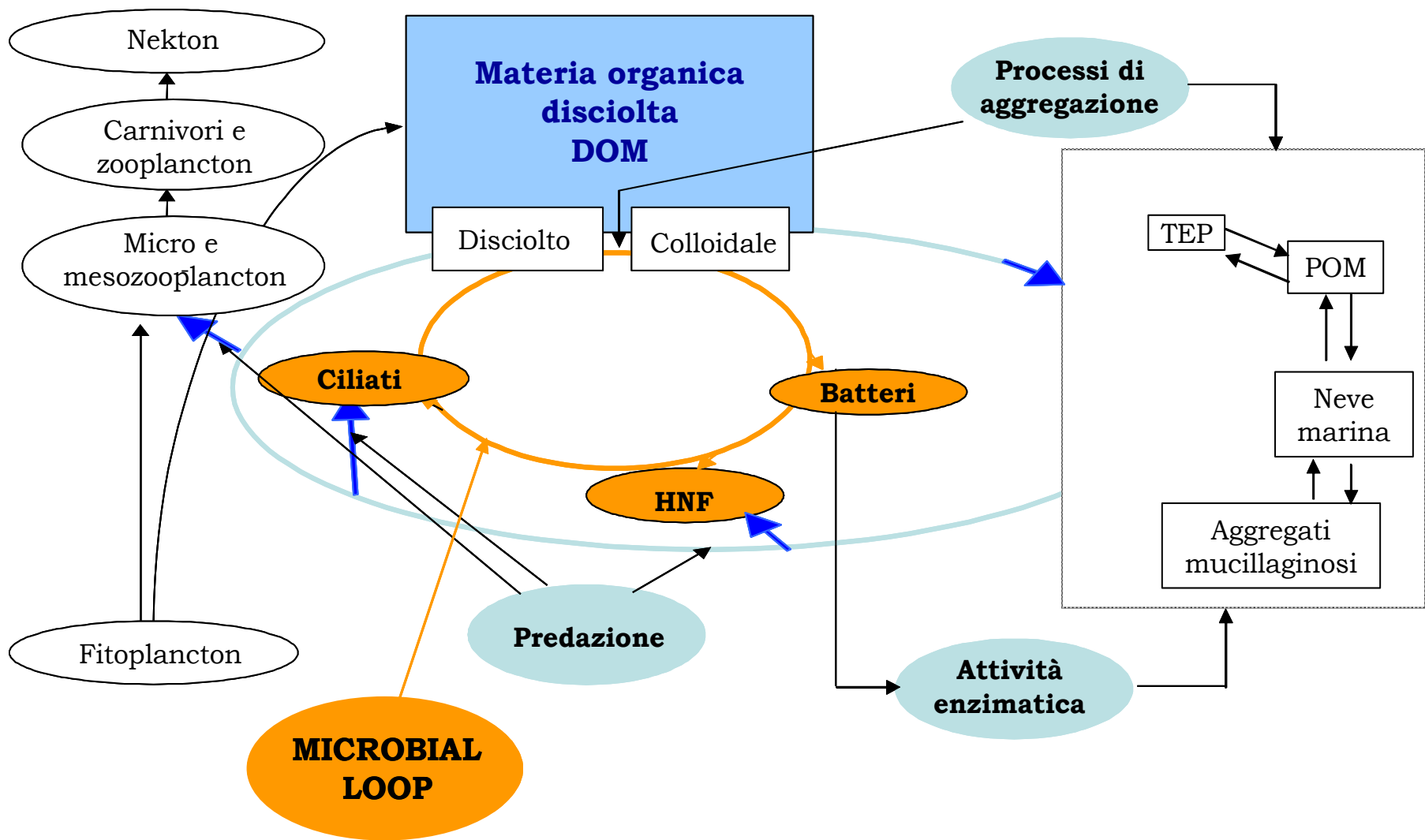
The background image shows a coastal scene. In the foreground, there is a stone pier with a decorative metal railing. The sea is choppy with white foam from the waves. In the distance, a red lighthouse is visible on a small island. The sky is overcast and grey.

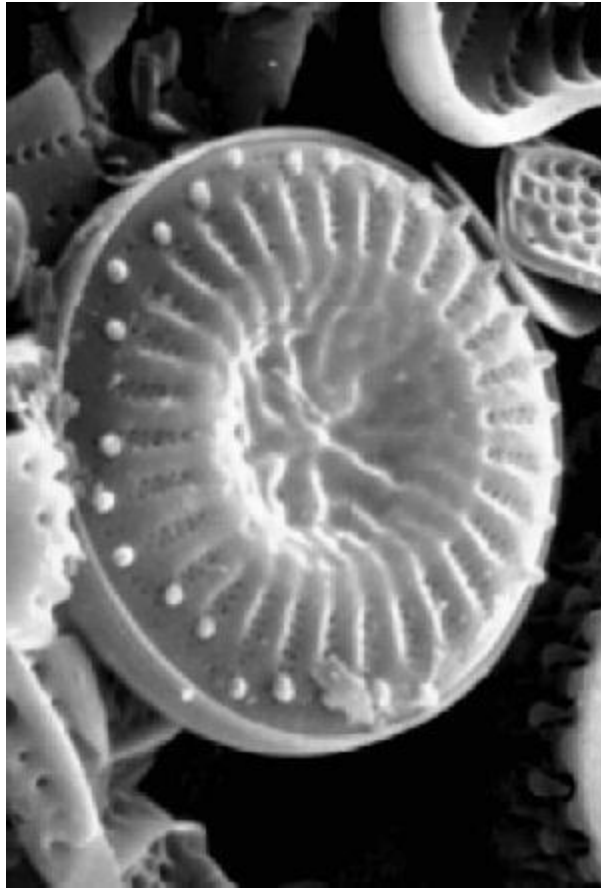
# La sostanza organica marina di natura macromolecolare: ruolo ecologico e metodologie di indagine

CASO STUDIO 1:  
Ricerche svolte nel periodo 2000-2010

# Catena trofica pelagica

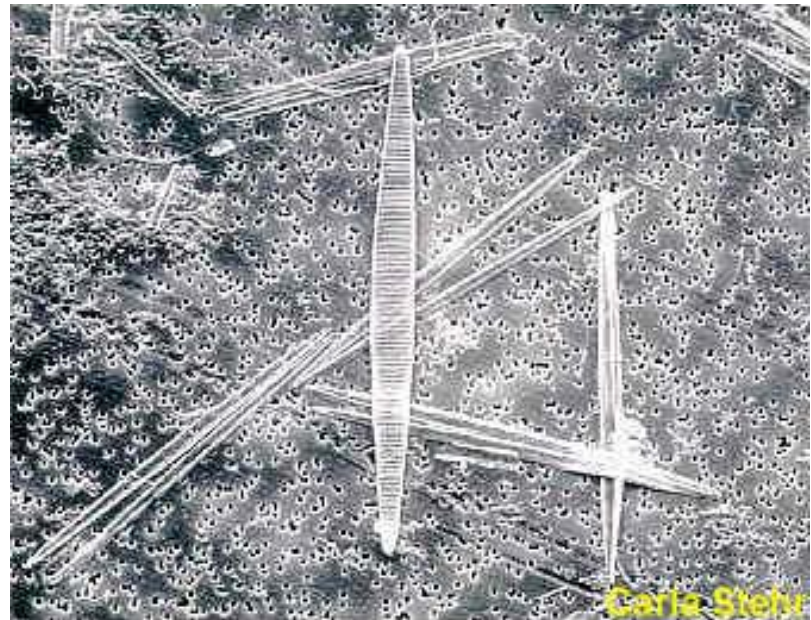
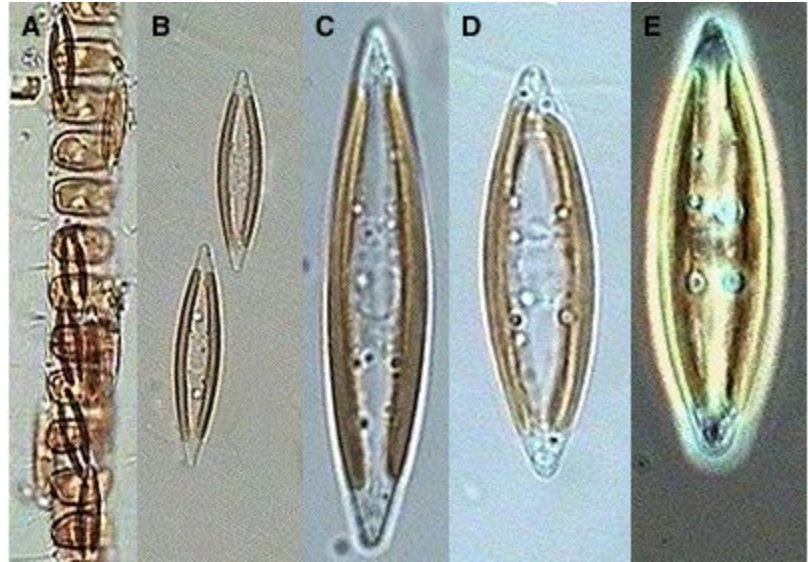


# Diatomee



*Coscinodiscus*

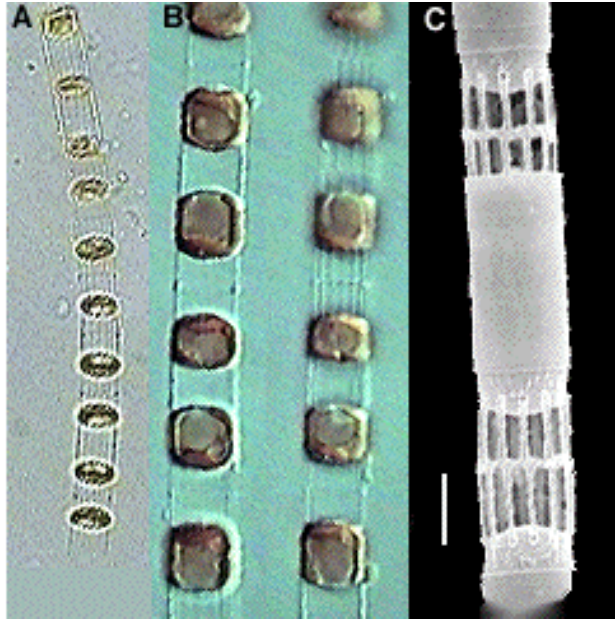
# *Navicula*



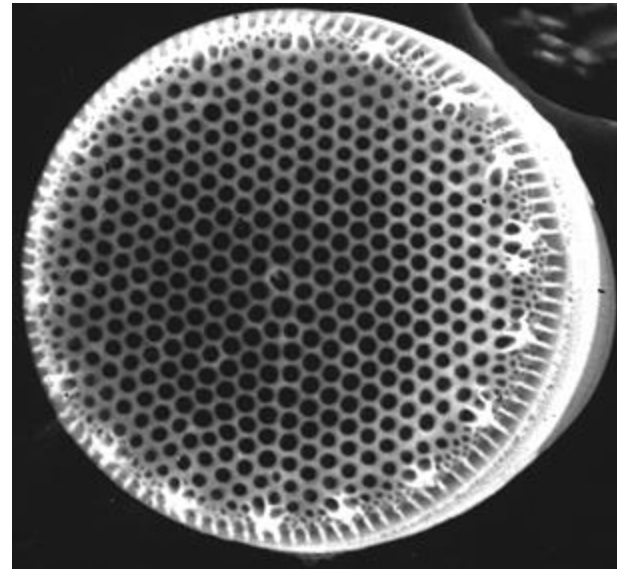
*Pseudo-Nitzschia*

Carla Stehr

*Skeletonema costatum*

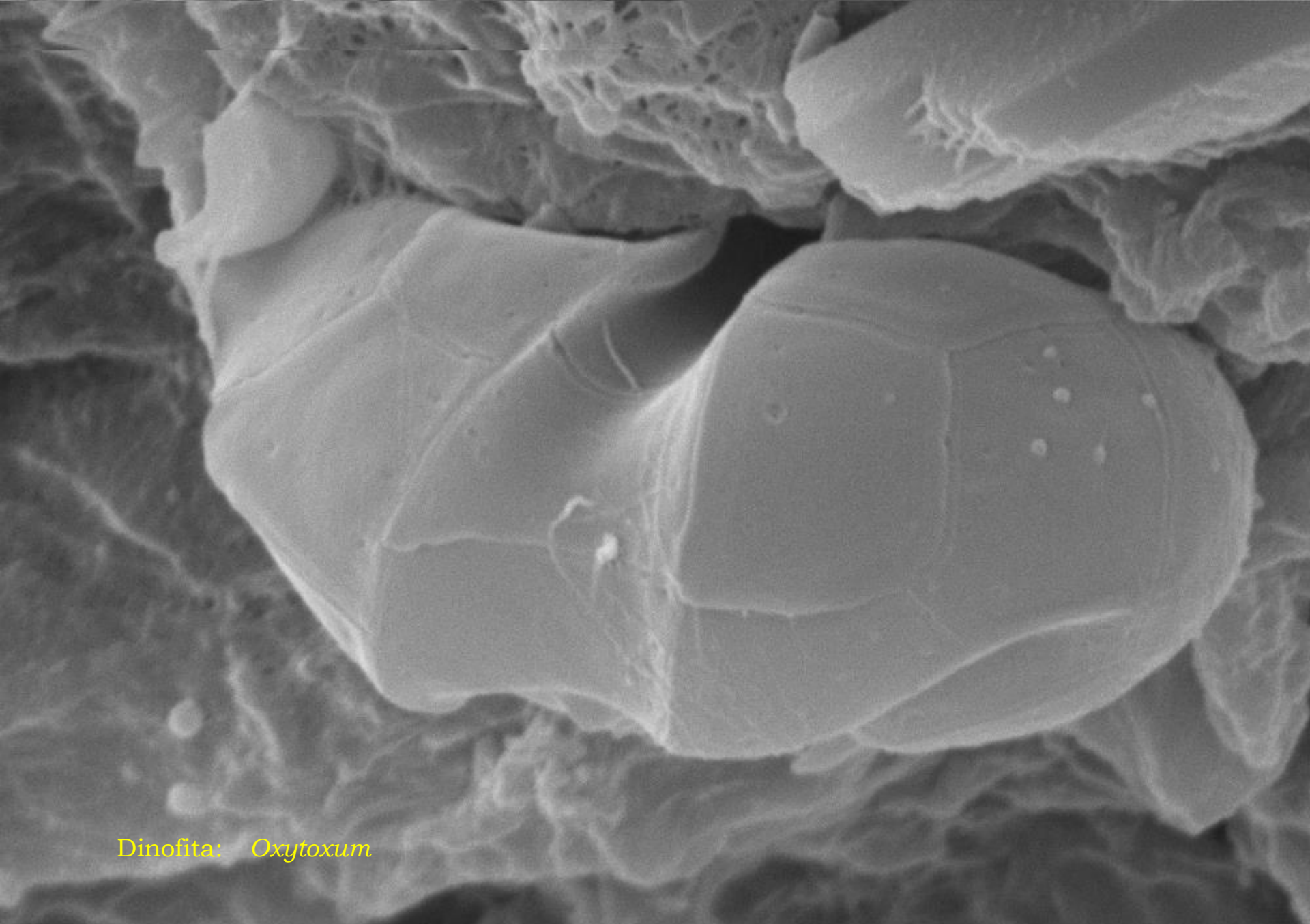


*Thalassiosira*



*Chaetoceros*





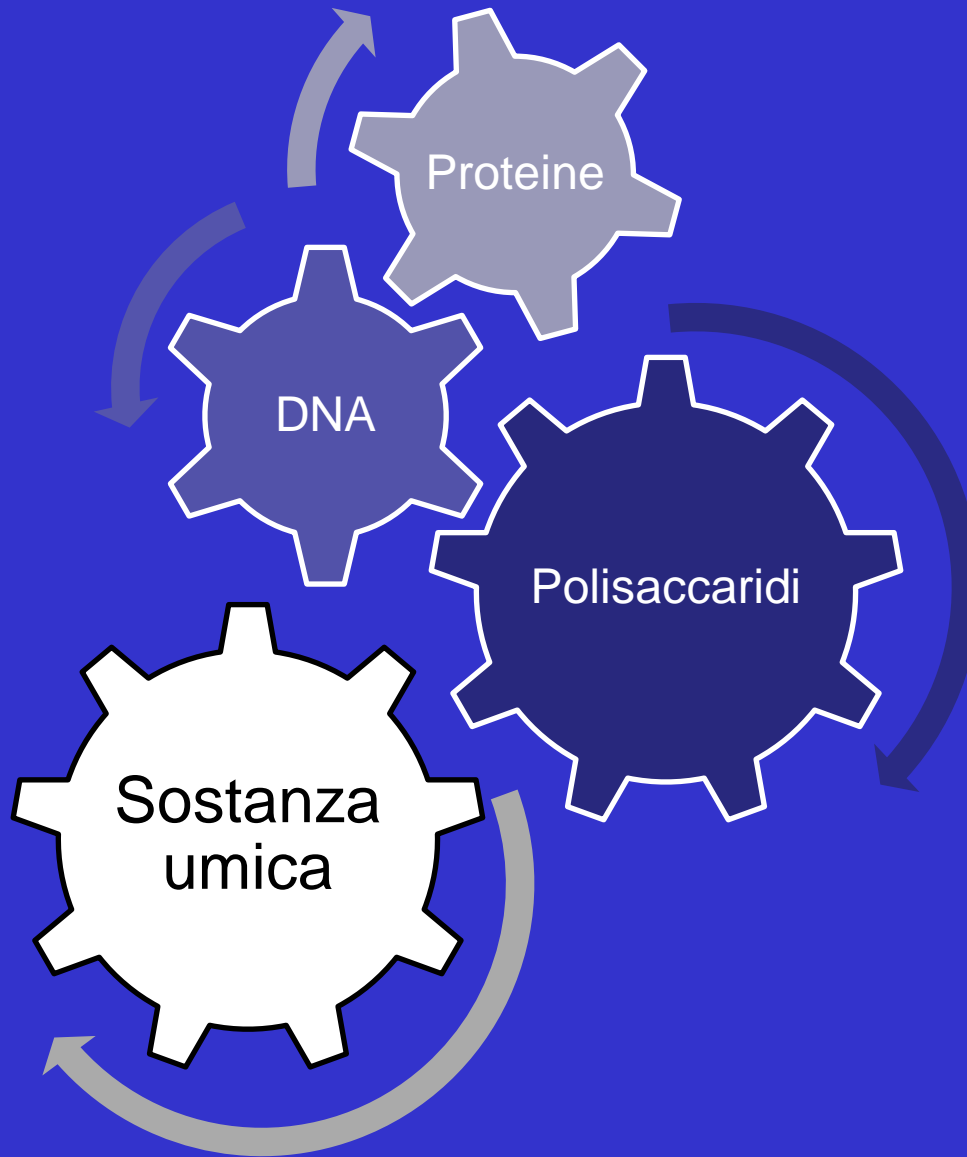
Dinofita: *Oxytoxum*

EHT=13.31 kV WD= 16 mm  
1µm

Mag= 9.98 K X Detector= SE1

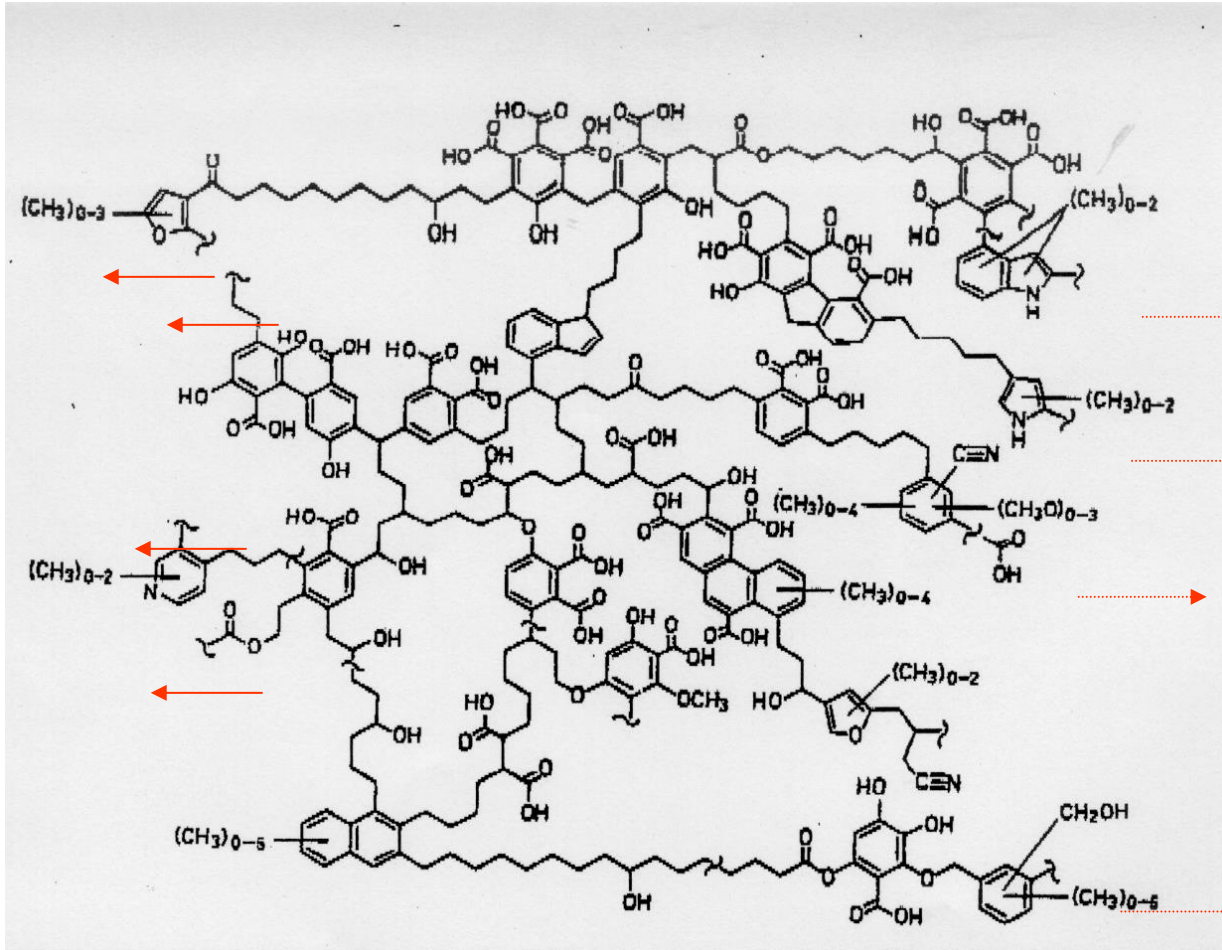
Photo No -1490

# BIOPOLIMERI MARINI



Sostanza umica

- acidi fulvici: basso P.M., pochi gruppi aromatici
- acidi umici: alto P.M., più gruppi aromatici
- umina: alto PM, alto grado di reticolazione



# Obiettivi della ricerca sui biopolimeri marini

1. Isolare e purificare i singoli biopolimeri;
2. determinarne la struttura primaria (composizione e concatenamento);
3. determinare le proprietà delle catene (grado di polimerizzazione  $\leftrightarrow$  PM, dimensione media  $\leftrightarrow$  raggio di girazione);
4. determinare le proprietà aggreganti/gelificanti;
5. studiare la stabilità dei biopolimeri rispetto alla degradazione enzimatica (biodegradazione);
6. individuare la relazione tra proprietà chimiche e funzione biologica (fisiologica, biochimica, ecologica, etc.);
7. individuare i marcatori biologici (biomarkers) relativi alle singole specie produttrici.
8. studiare la variazione stagionale mediante monitoraggio mensile dei composti macromolacolari su scala di bacino.



# Funzione *biologica* degli EPS

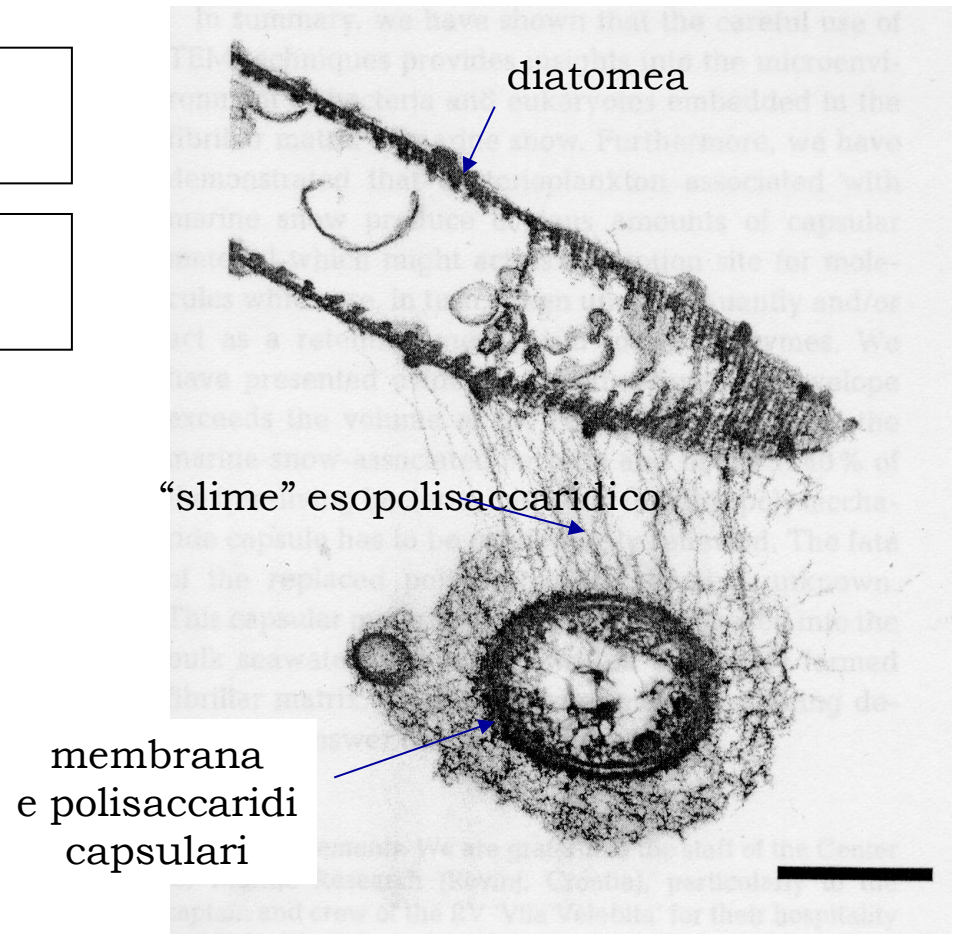
1. sistema tampone contro le variazioni chimiche e biologiche del microambiente (tossine, agenti virali e batterici, inquinamento da metalli pesanti, ecc.);

2. azione sequestrante e di concentrazione dei nutrienti;

3. azione adesiva alle superfici;

4. movimento e mantenimento delle relazioni simbiotiche;

5. protezione contro la predazione e l'azione di enzimi digestivi;



# Funzione *ecologica* degli EPS

1. La maggior parte del carbonio organico nei mari e negli oceani è presente come materia organica in forma disciolta (DOM), in forma colloidale (COC) o come particolato organico (POM).

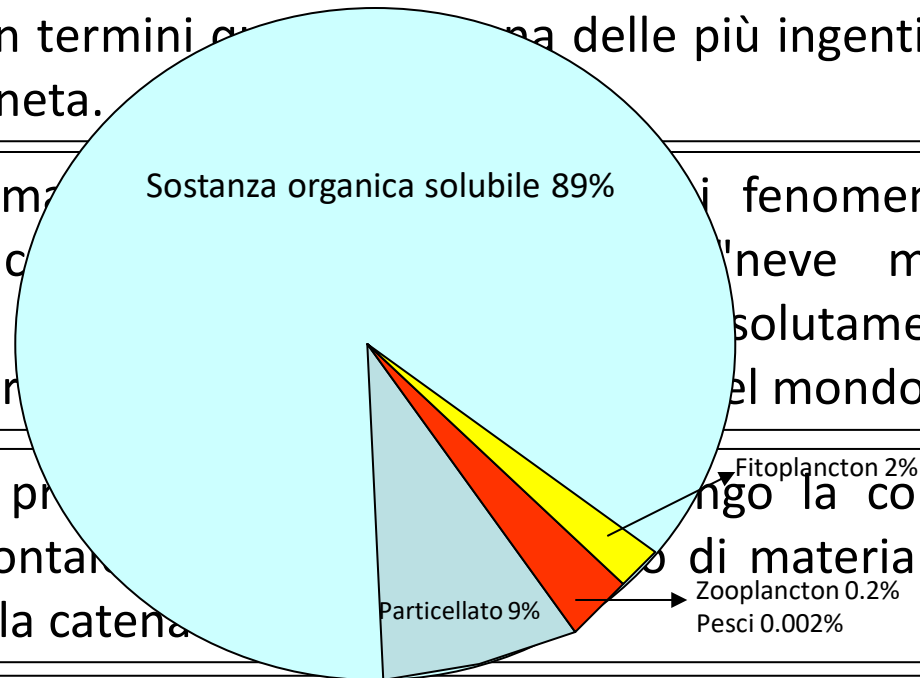
2. Il COC contenuto in particelle sospese di dimensioni comprese tra 1 e 1000 nm costituisce in termini quantitativi una delle più ingenti riserve di carbonio organico del pianeta.

3. Il prodotto marino è un fenomeno aggregativo della materia organica, "neve marina" di origine fitoplanctonica, assolutamente naturale ed indispensabile per la vita nel mondo.

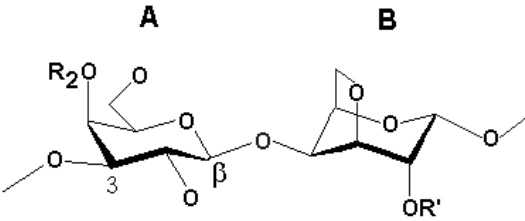
4. Attraverso i processi di diffusione orizzontale e verticale, la disponibilità nella catena alimentare è influenzata dal movimento di materia verso il fondo e la risalita.

5. Nella forma macroscopica, costituiscono la riserva alimentare per molti animali.

6. Fungono da adsorbenti della materia organica disciolta (DOM) e come tali da veicolo del DOM fino ai più alti livelli trofici.



# Struttura primaria



(Carrageenans)

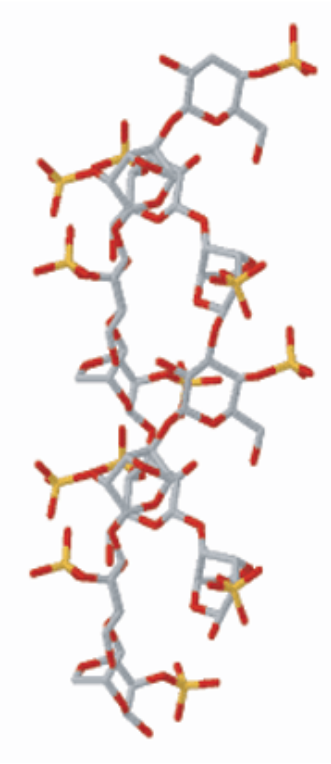
- λ R' = R<sub>2</sub> = SO<sub>3</sub><sup>-</sup>
- κ R' = H R<sub>2</sub> = SO<sub>3</sub><sup>-</sup>
- β R' = R<sub>2</sub> = H

In λ, the A unit is D-Gal (---- 2-sulphate), the B unit is D-Gal-2,6-bisulphate.

Degradazione batterica

# Relazione struttura/ "destino"

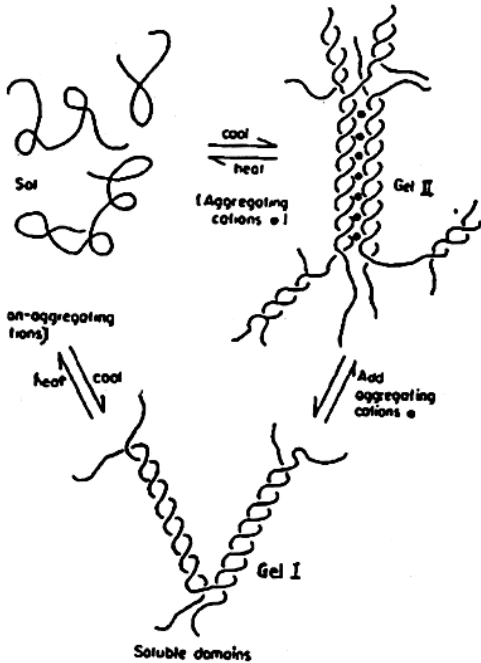
# Struttura secondaria



# Polisaccaridi

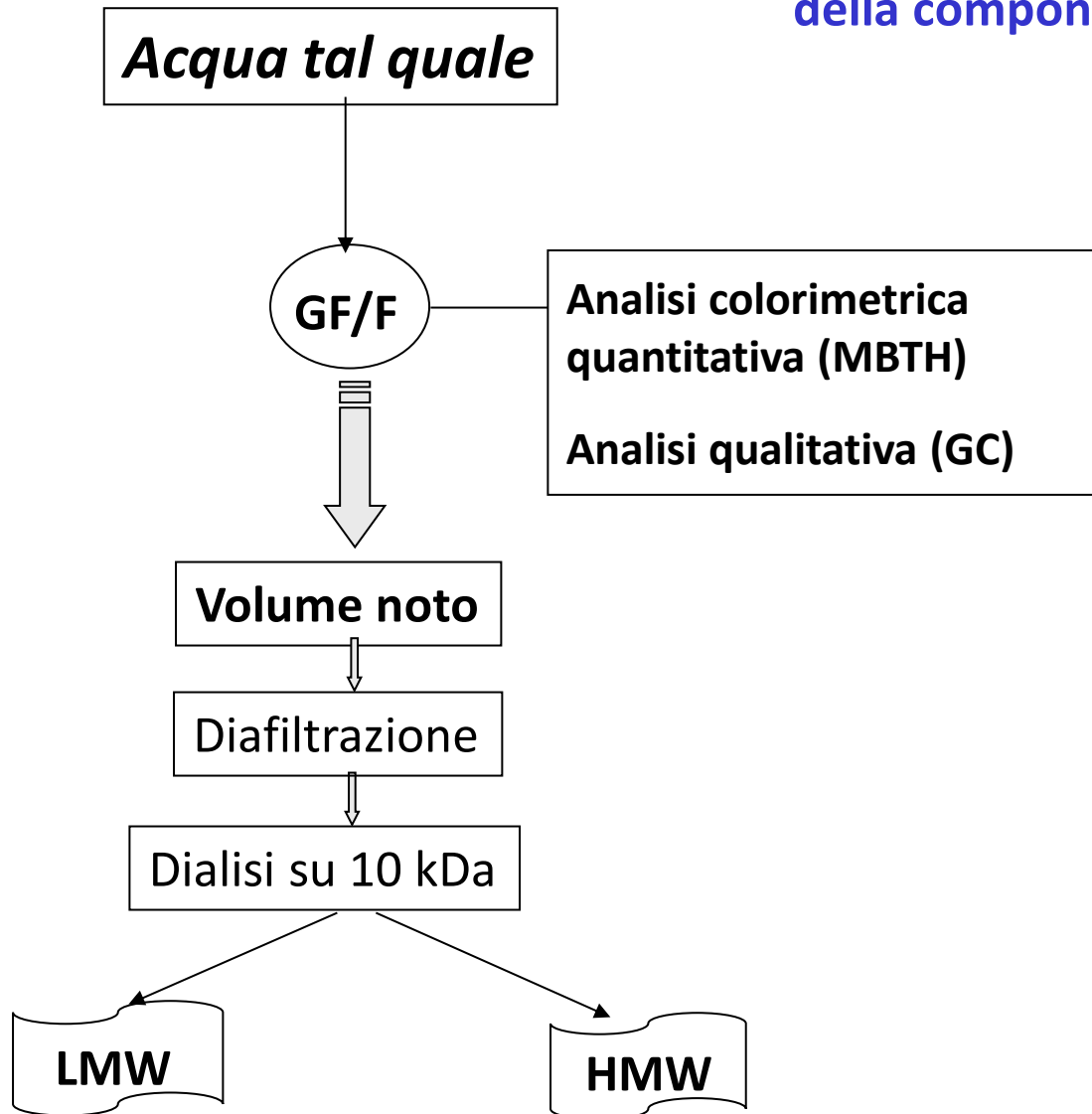
# Polisaccaridi amorfi e GEL

# Aggregazione

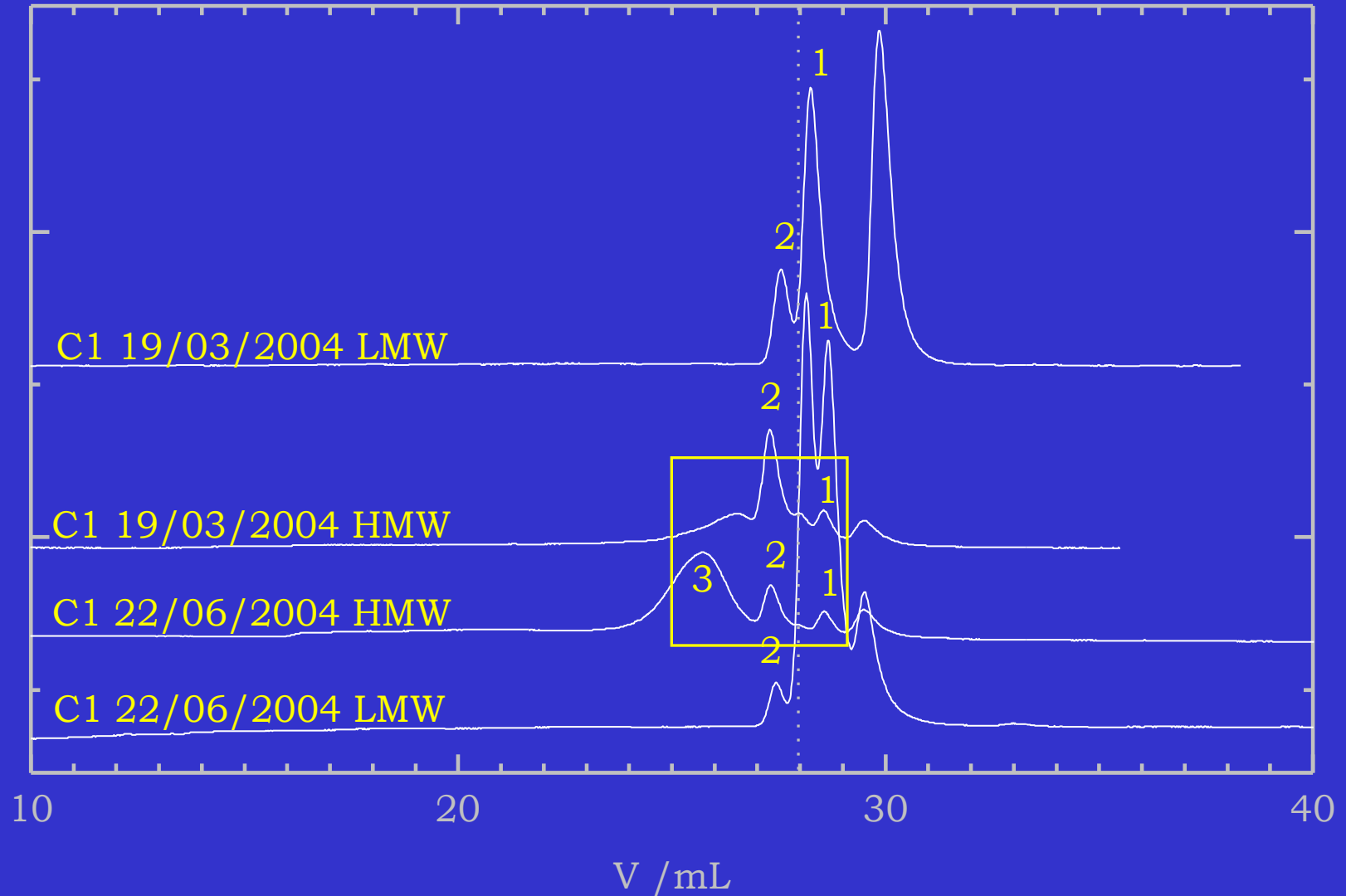


Soluble domains

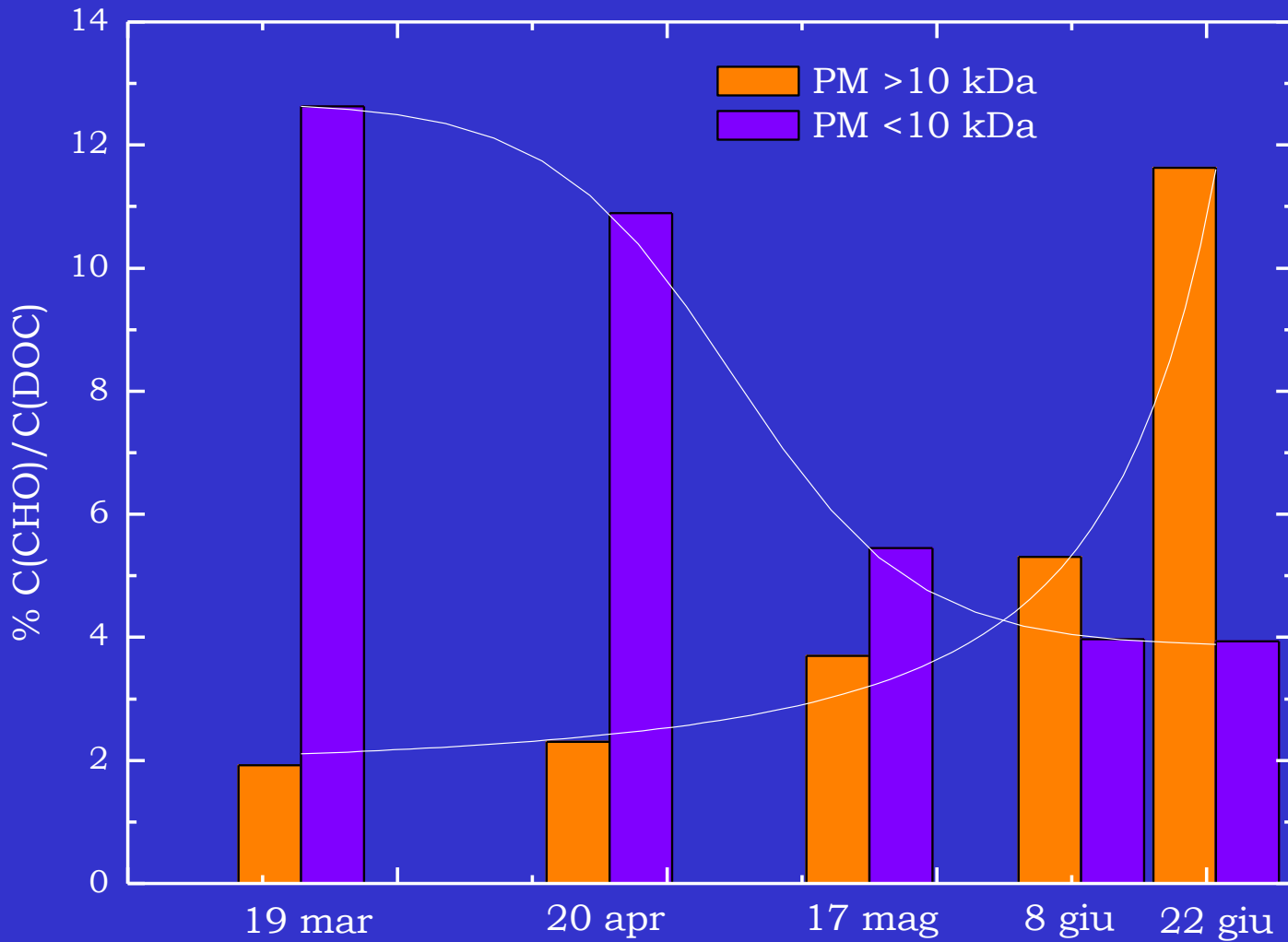
## Partizione ed analisi della componente saccaridica del DOC



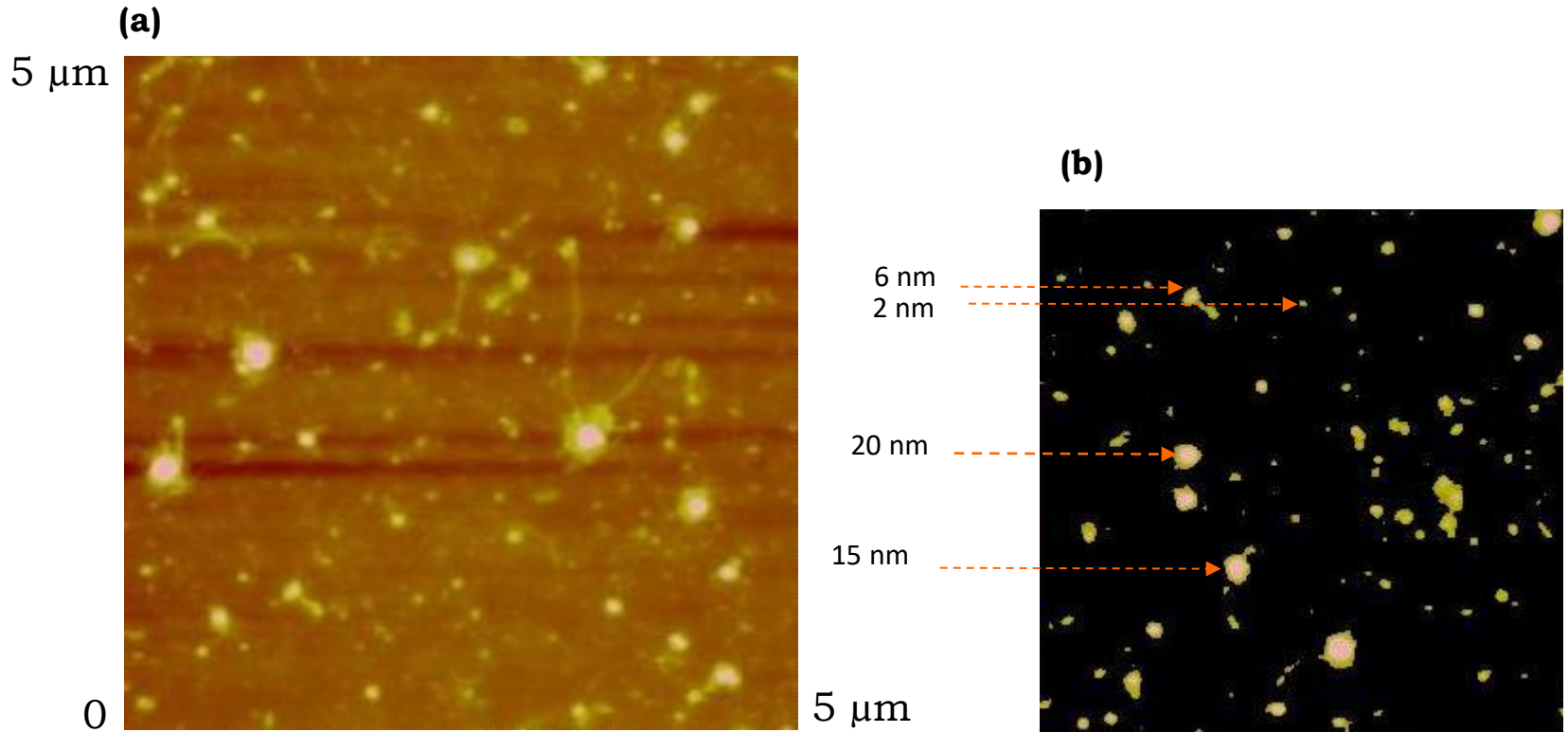
# Distribuzione dei pesi molecolari dei campioni HMW e LMW ottenuti per cromatografia HPSEC.



# Concentrazioni percentuali di HMW-DCHO e LMW-DCHO nel DOC in funzione del tempo di campionamento.

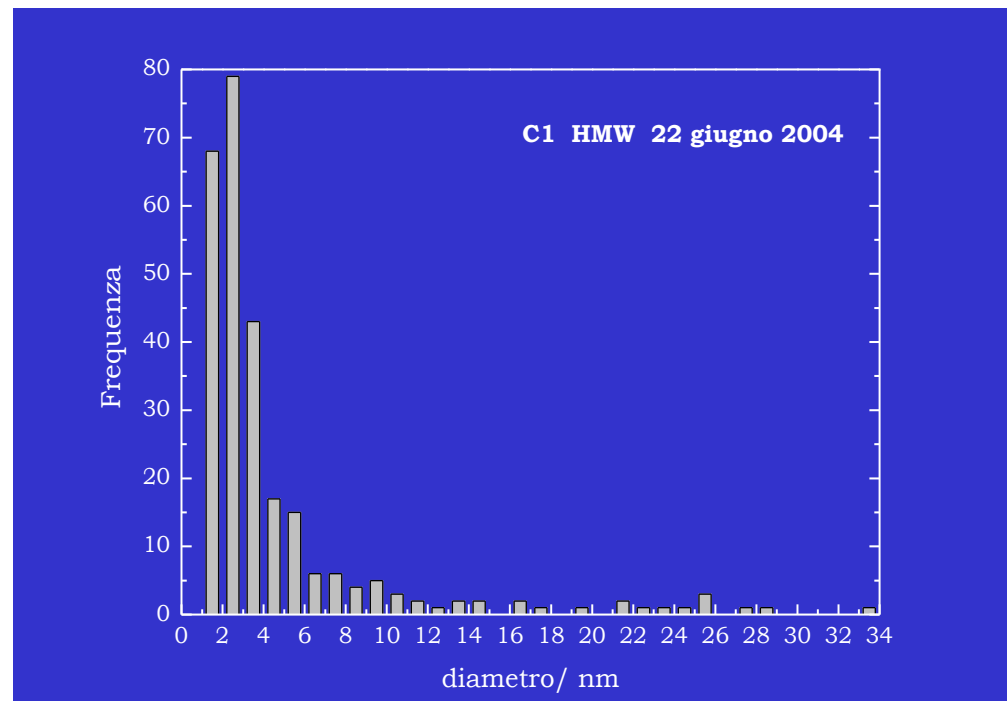
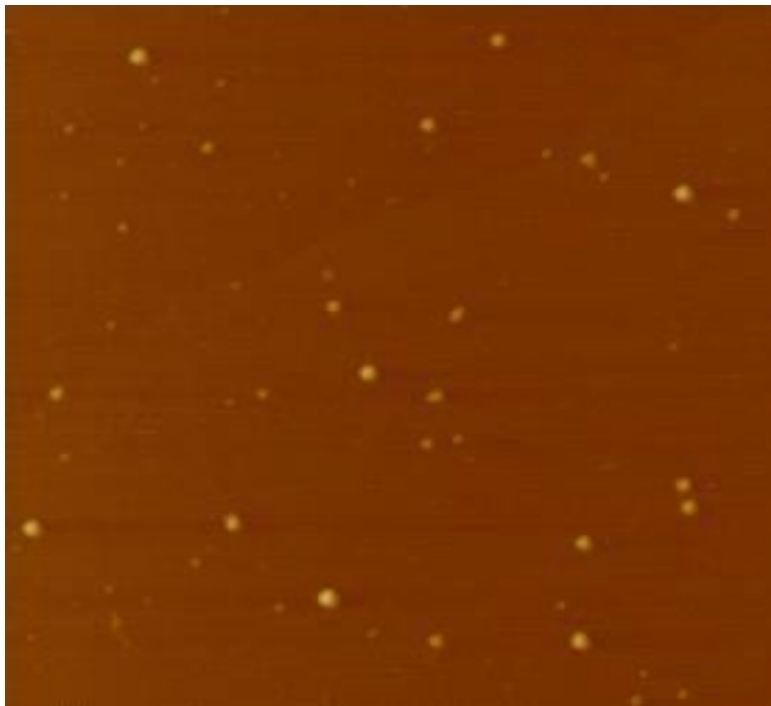


# AFM



**(a)** Immagine AFM di una soluzione del campione HMW-DOC del 22 giugno 2004 in DMSO.

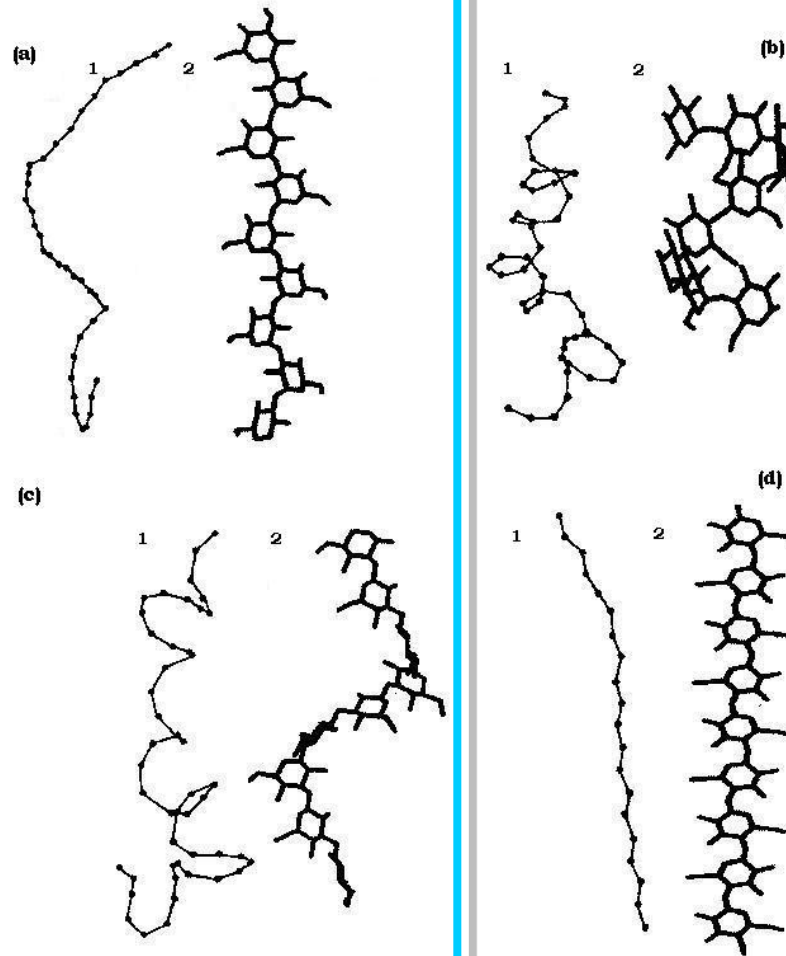
**(b)** Ingrandimento di un'area e rielaborazione dell'immagine per la valutazione delle dimensioni del particellato.



**Distribuzione dei diametri delle particelle per il campione HMW-DOC del 22 giugno 2004.**

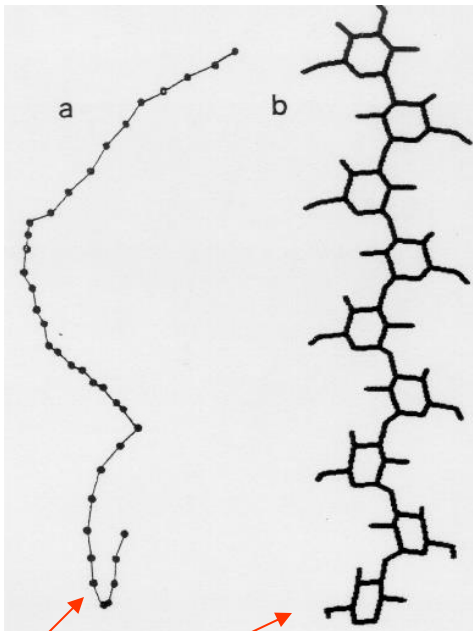
**Immagine AFM di una soluzione del campione LMW-DOC del 22 giugno 2004 in DMSO.**



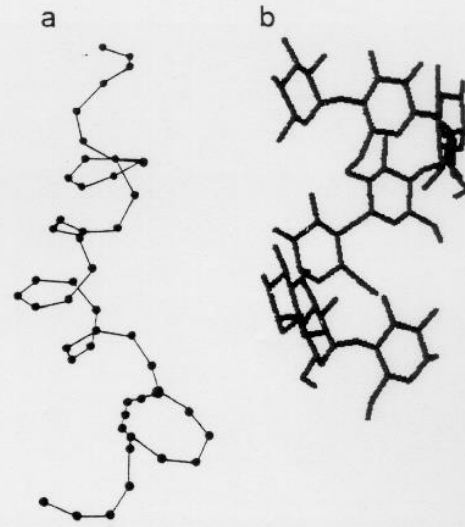


**Conformazioni di catene polisaccaridiche del glucosio (glucani) con diversa concatenazione e configurazione del carbonio anomero (1) in soluzione ed (2) allo stato solido. (a)  $\alpha$ -(D)-(1,3)-glucano (b)  $\alpha$ -(D)-(1,4)-glucano (c)  $\beta$ -(D)-(1,3)-glucano (d)  $\beta$ -(D)-(1,4)-glucano (Urbani et al., 2001).**

poli[ $\beta$ -(1-4)-glc]

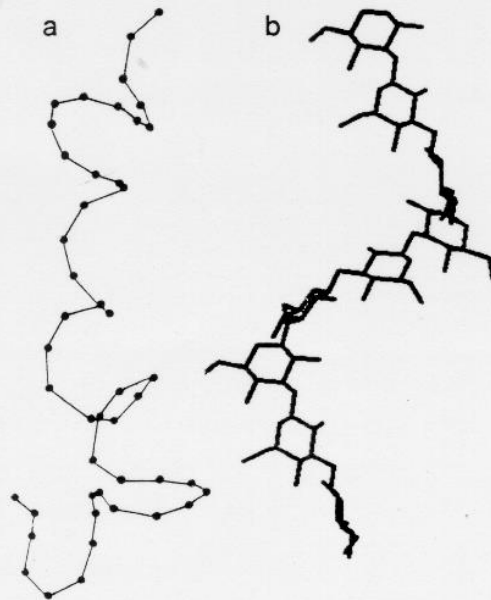


poli[ $\alpha$ -(1-4)-glc]

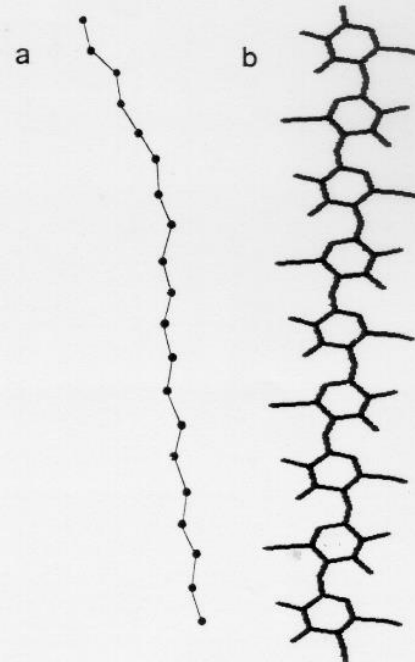


a: *soluzione*  
b: *stato solido*

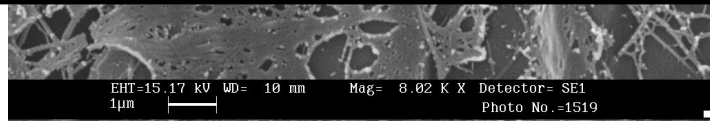
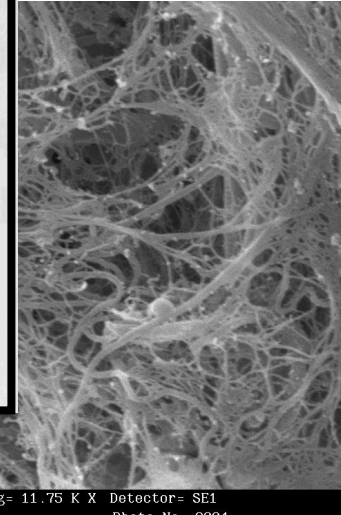
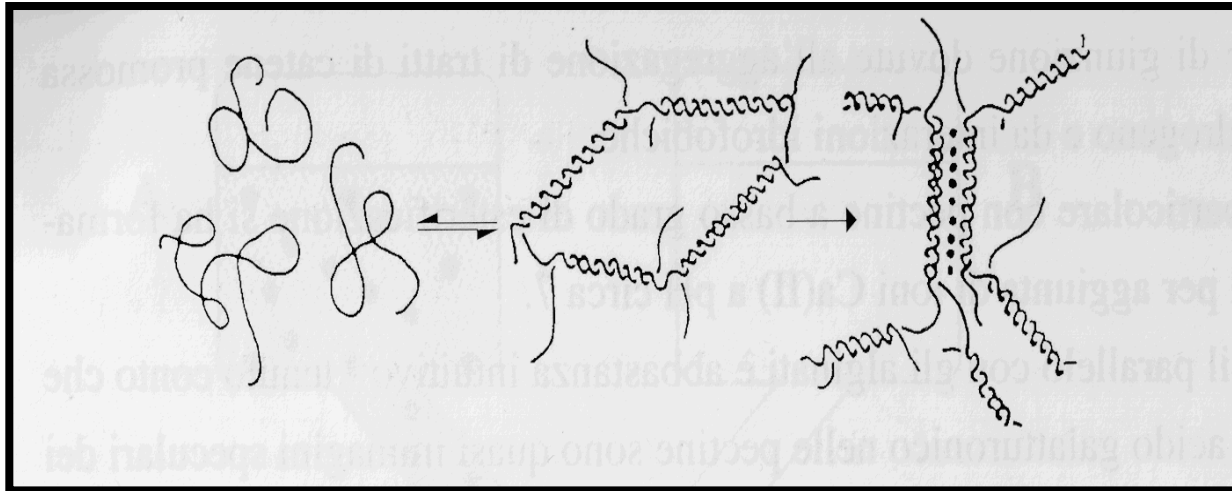
poli[ $\beta$ (1-3)-glc]



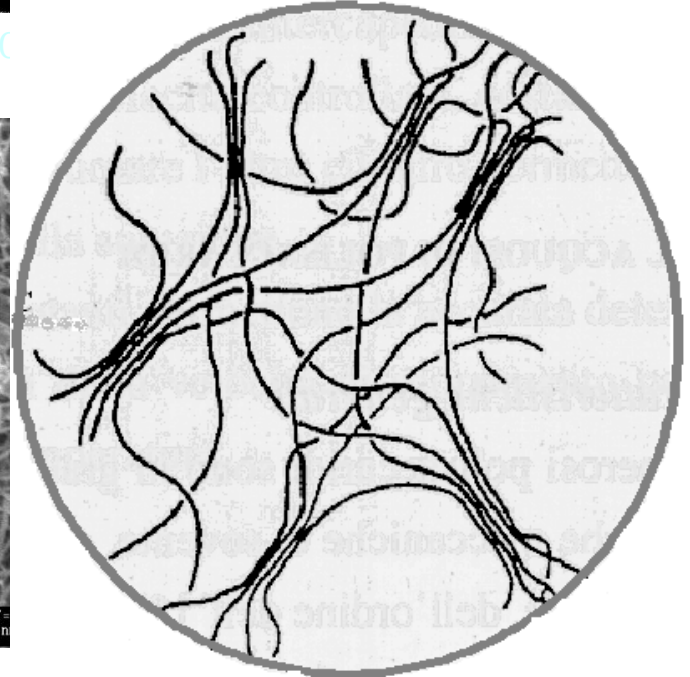
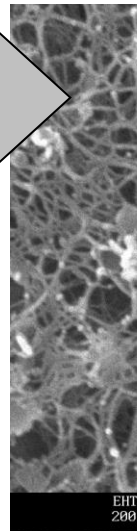
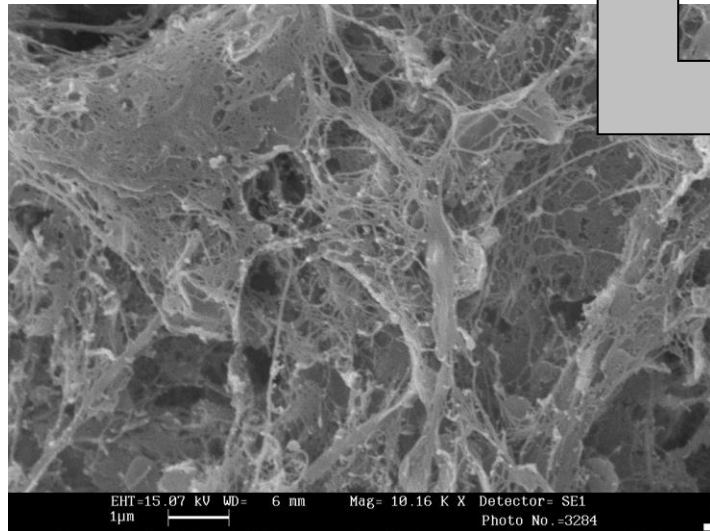
poli[ $\alpha$ -(1-3)-glc]



# Microscopia elettronica a scansione



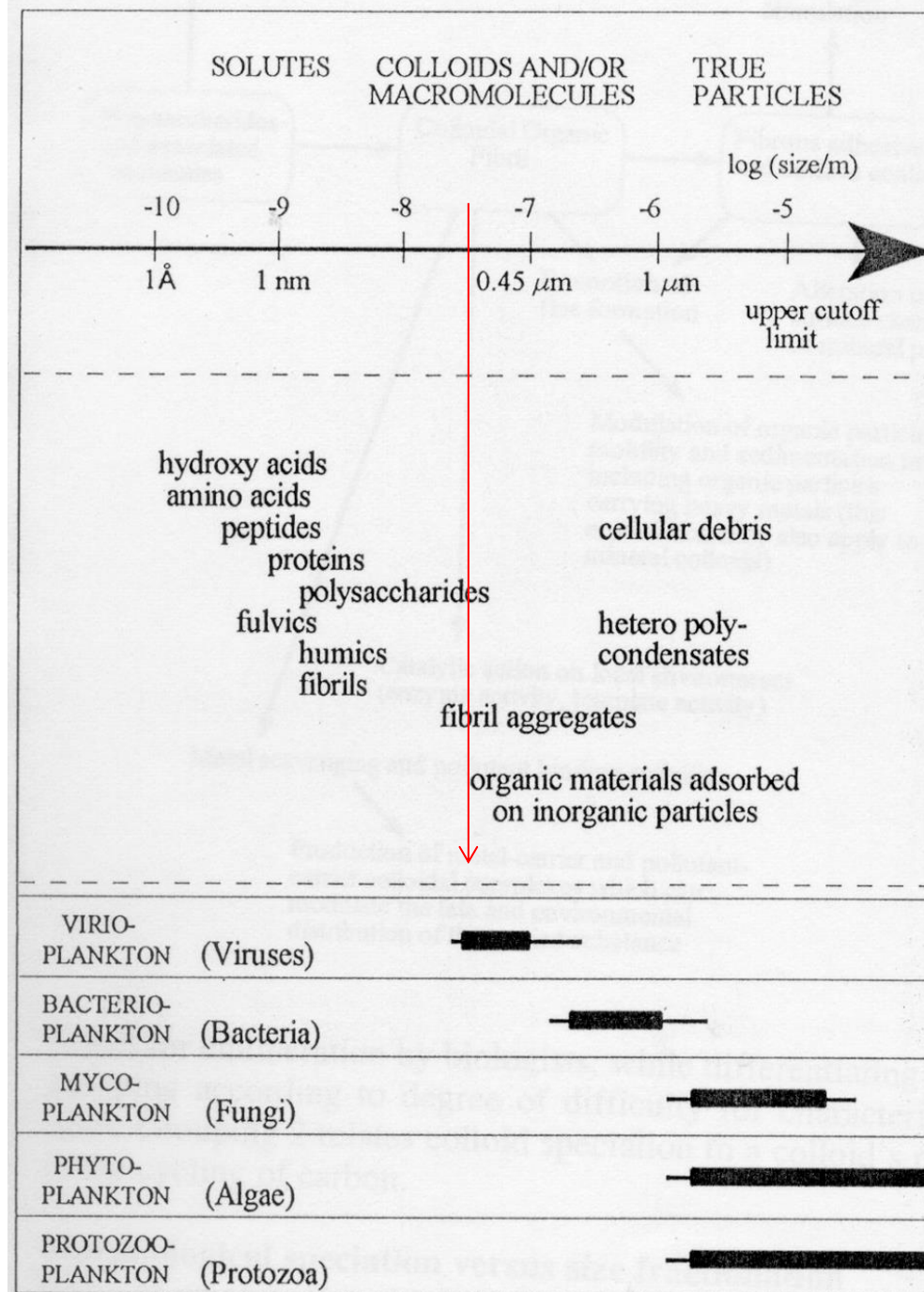
Campioni di mucillagine nativa (Urbani e Sist, 2003)



Soluzioni concentrate di polisaccaridi purificati da mucillagine (Urbani e Sist, 2003)

# Sostanza organica macromolecolare nel mare e classi dimensionali

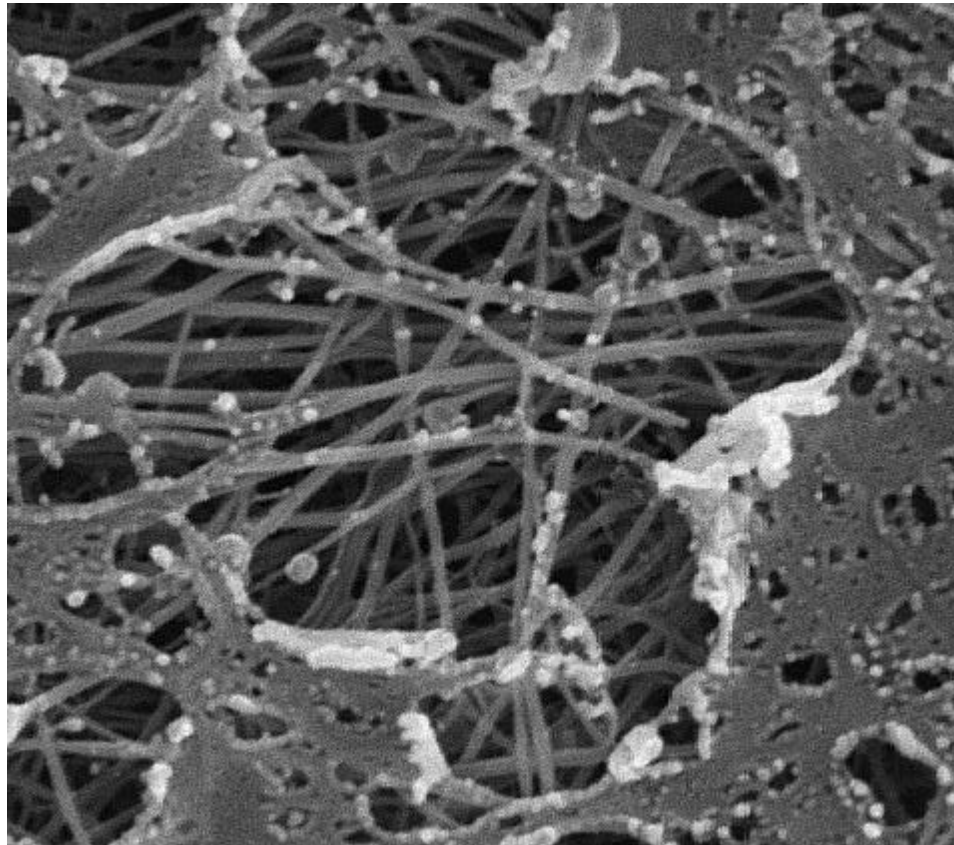
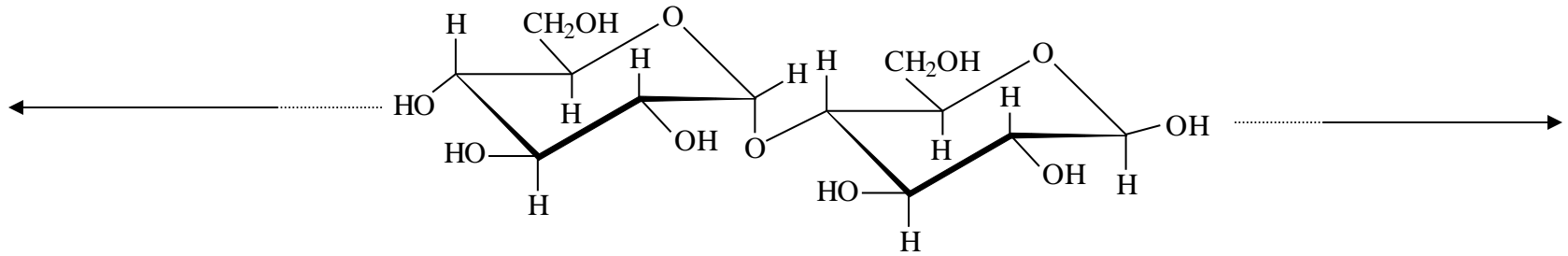
- Polisaccaridi ed oligosaccaridi
- Sostanza umica
- Proteine e peptidi
- Acidi nucleici



# Suddivisione dei biopolimeri marini in base alle loro caratteristiche macromolecolari

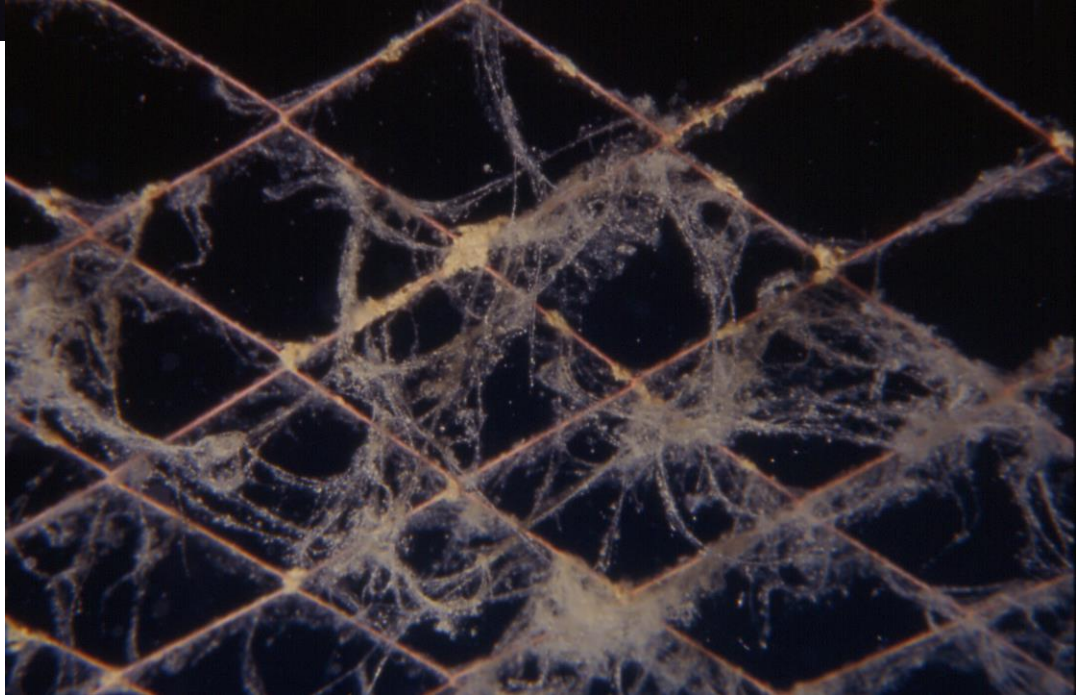
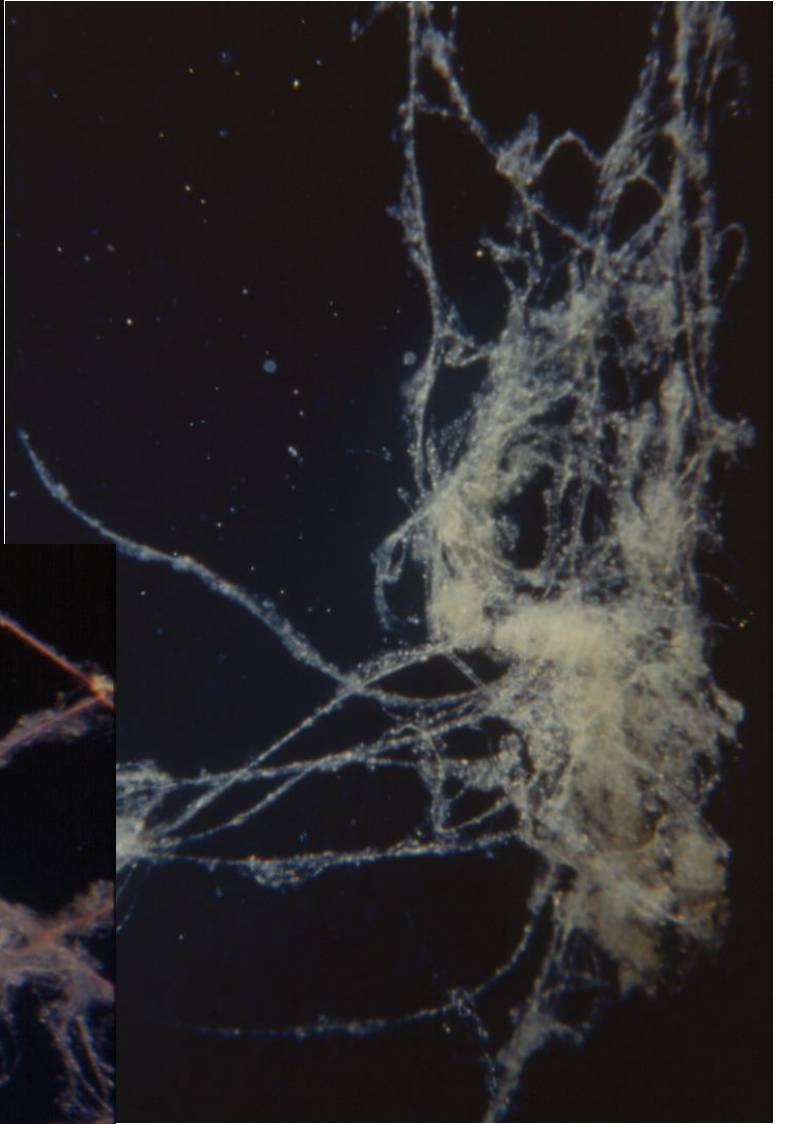
	Natura Origine	Massa molec. (dalton)	Dimensioni (nm)	Struttura supramolecolare	Tempo di vita medio	% della sostanza organica
<b>Biopolimeri RIGIDI</b>	<ul style="list-style-type: none"> <li>- mucopolisaccaridi</li> <li>- peptidoglicani</li> <li>- cellulose</li> <li>- composti pectici (parete cellulare microbica e prodotti extracellulari)</li> <li>- Acidi nucleici</li> </ul>	$10^4 - 10^6$	100 – 1000	<ul style="list-style-type: none"> <li>- struttura fibrillare basata su doppie o triple eliche</li> <li>- struttura a rete tridimensionale (gel)</li> </ul>	<ul style="list-style-type: none"> <li>- sulla superficie: mesi</li> <li>- in profondità: 100 anni-secoli</li> <li>ore - giorni</li> </ul>	10-30
<b>Biopolimeri FLESSIBILI</b>	materiale refrattario (prodotti di ricombinazione di amminoacidi, zuccheri rilasciati dal plancton)	$500 - 10^5$	----	<ul style="list-style-type: none"> <li>- struttura flessibile, disordinata, più o meno estesa</li> </ul>	10 – 7000 anni	fino a 90
	<ul style="list-style-type: none"> <li>- polisaccaridi di riserva (endocellulari)</li> <li>- composti proteici (endocellulari)</li> </ul>			<ul style="list-style-type: none"> <li>- struttura flessibile</li> <li>- struttura globulare</li> </ul>	ore - giorni	
<b>Sostanze umiche</b>	(di derivazione terrestre-fluviale)	500 - 5000	0.5 - 2	<ul style="list-style-type: none"> <li>- struttura globulare</li> </ul>	ca. 500 anni nel suolo	<ul style="list-style-type: none"> <li>- 40-80 laghi</li> <li>- tracce in mare aperto</li> </ul>

# Catene saccaridiche

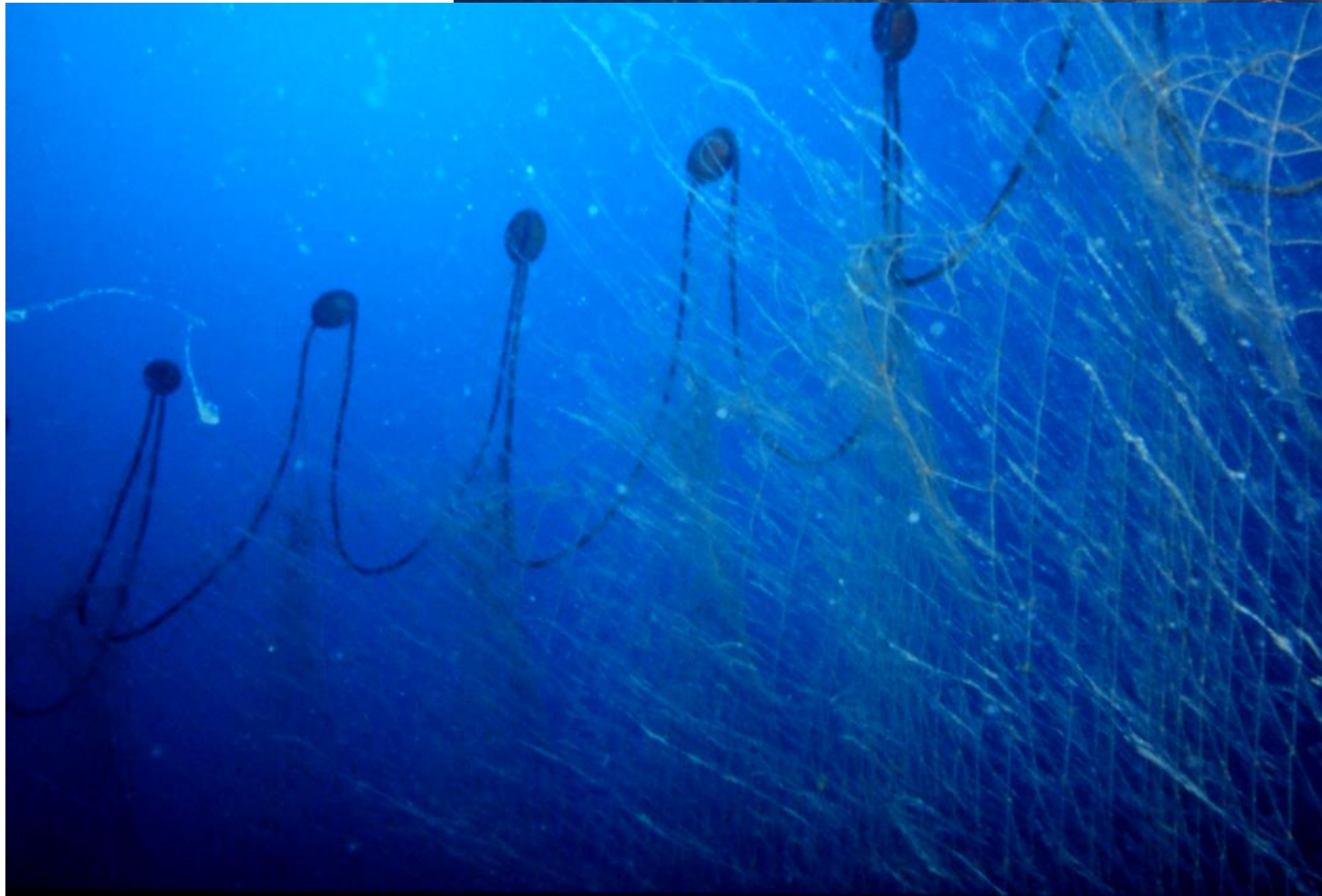


**Neve marina**









# Ragnatele

Golfo di Trieste - mucillagini in sospensione



**Nastri**



An underwater photograph showing a diver in silhouette on the left, holding a white object. To the right, a large, billowing cloud of white sediment, known as a 'nubi', is suspended in the water. The water is a deep blue-green color, and the scene is dimly lit, typical of an underwater environment.

**Nubi**

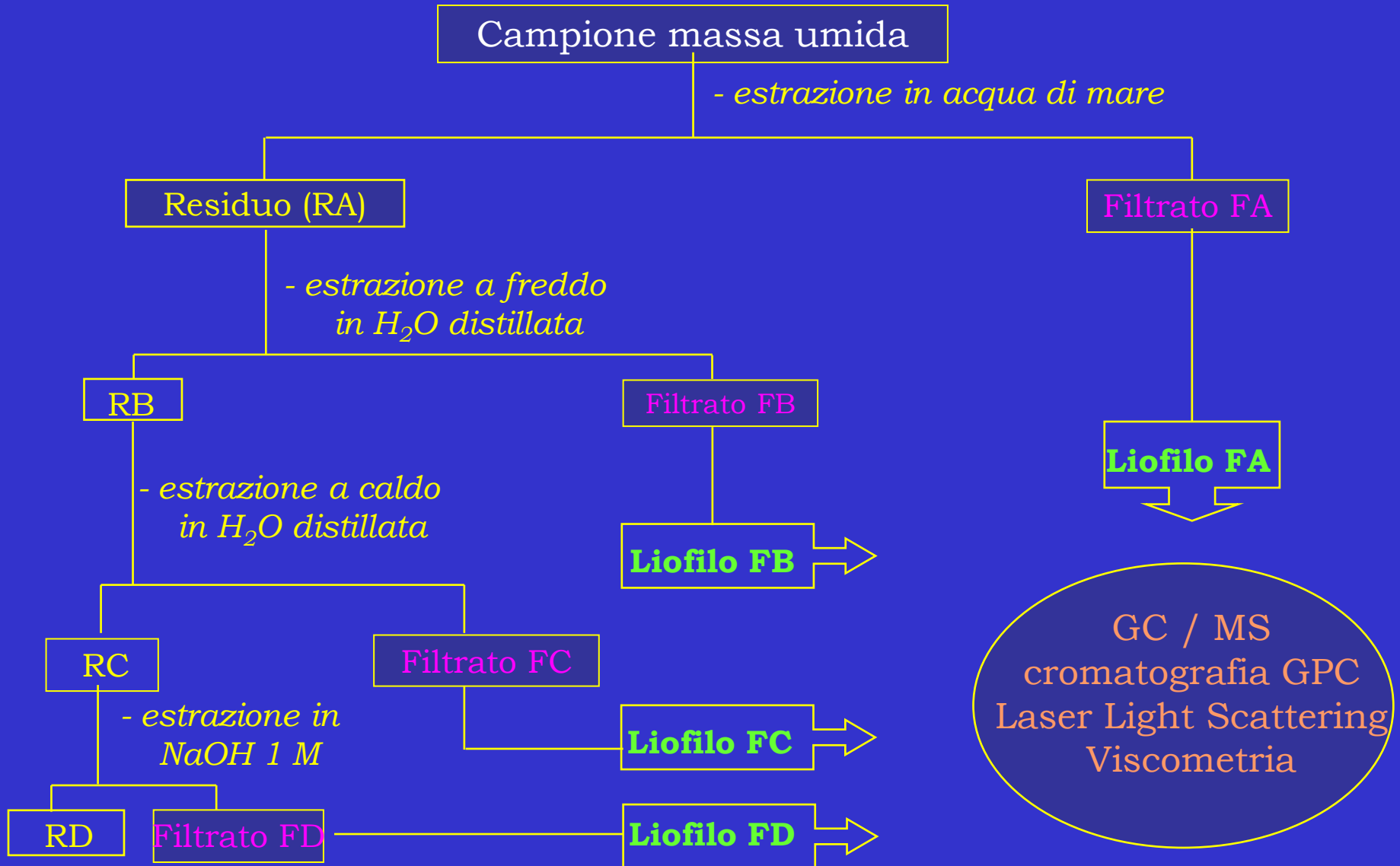
Golfo di Trieste - mucillagini in sospensione



# Strati cremosi superficiali



# Isolamento e purificazione dei polisaccaridi da mucillagini

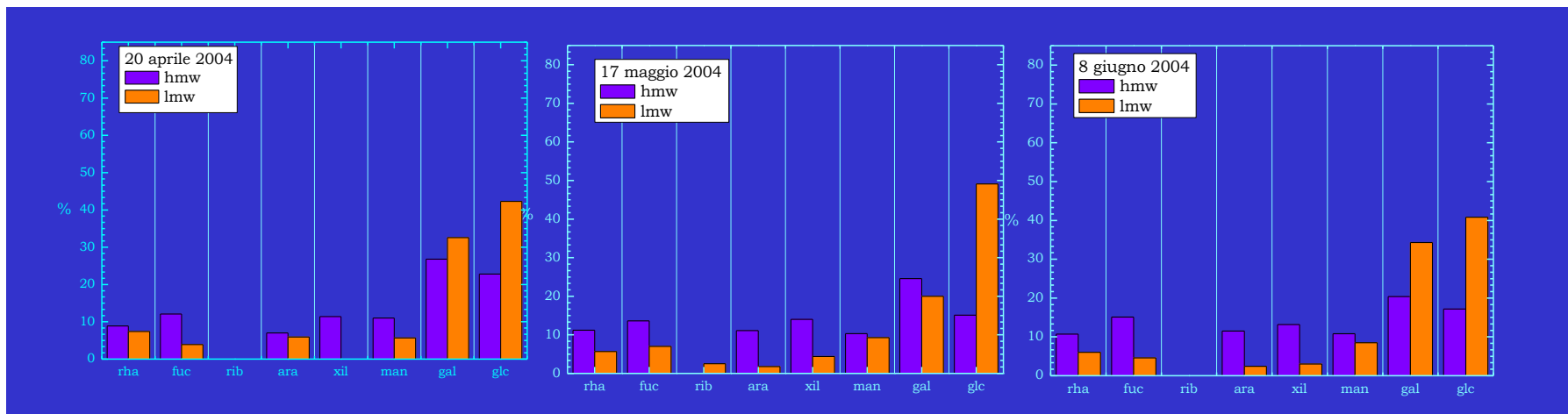


# Determinazione della struttura primaria (composizione e concatenamento)

Analisi gascromatografica degli idrolizzati acidi dei polisaccaridi per determinare la composizione relativa dei vari monosaccaridi;

Analisi di metilazione per determinare i carboni implicati nel concatenamento

Analisi NMR per la definizione della chiralità nel legame anomeroico





## Composizione monosaccaridica (GC-FID)

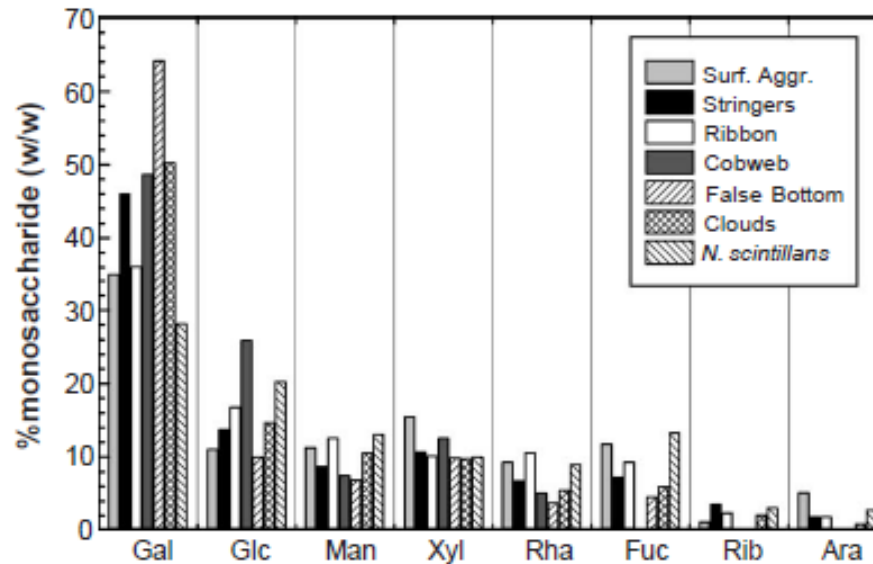


Fig. 8. Average monosaccharide composition (% w/w) of the sugar fraction of different aggregate typologies. Abbreviations: Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Fuc, fucose; Rib, ribose; Ara, arabinose.

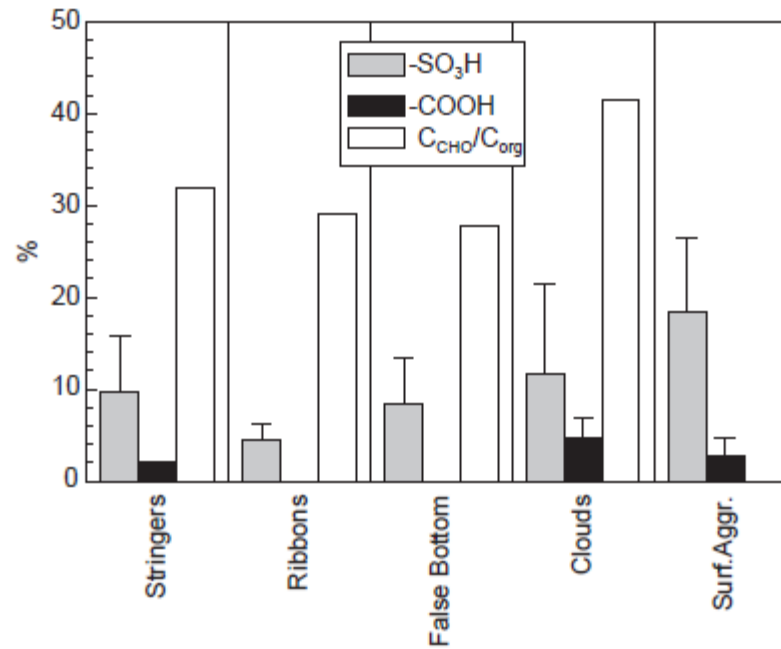
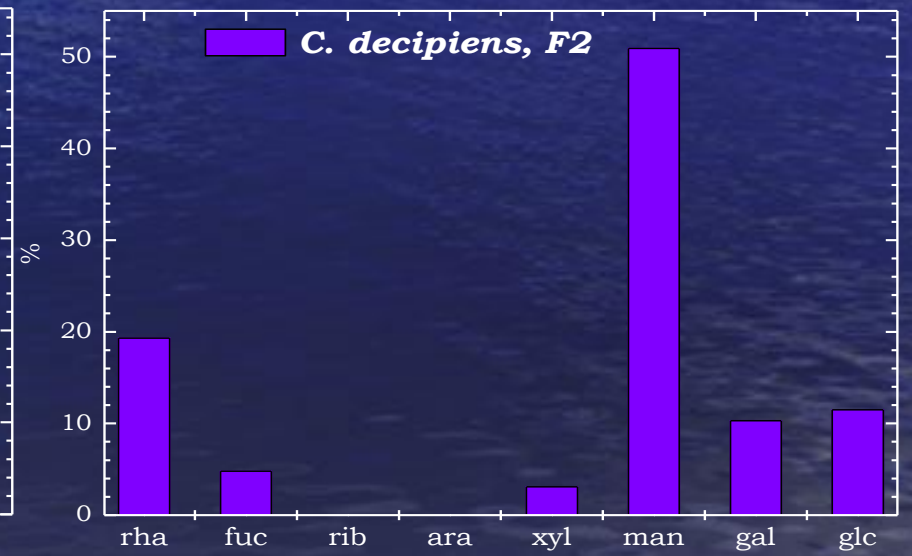
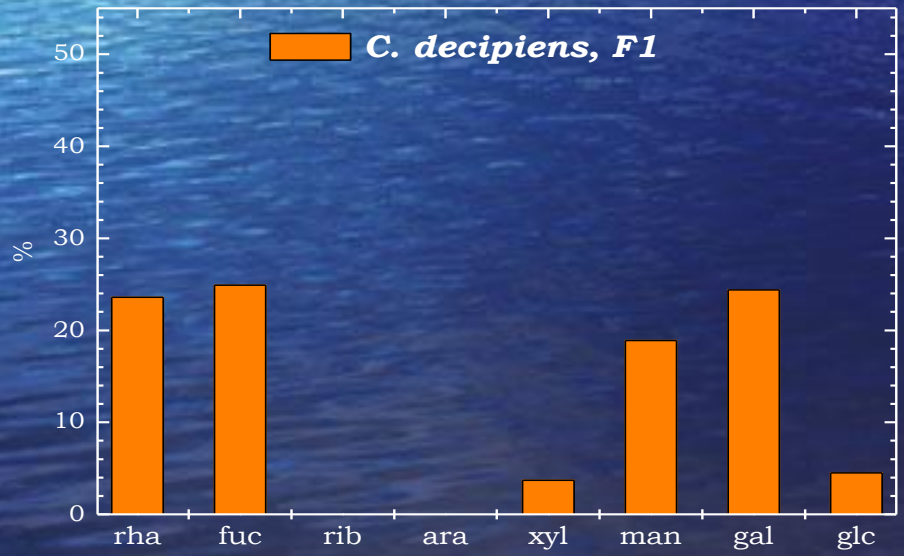
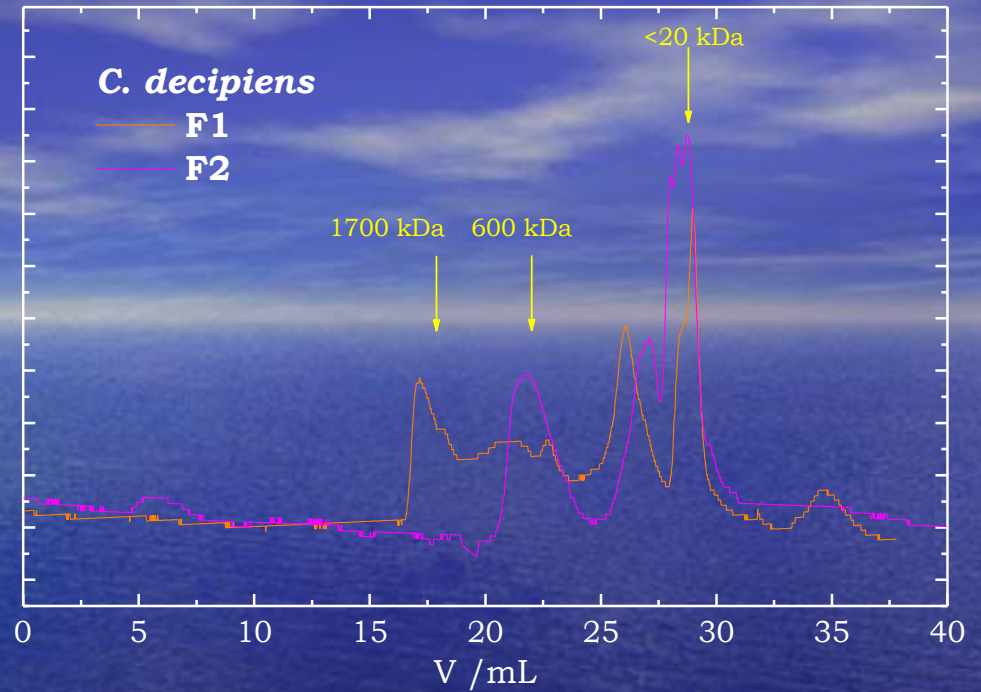


Fig. 7. The  $C_{CHO}/C_{org}$  ratio (% w/w), the sulphate ( $-SO_3H$ ) and uronic acid ( $-COOH$ ) contents (% mol/mol of monosaccharide residues) for different mucilage typologies.



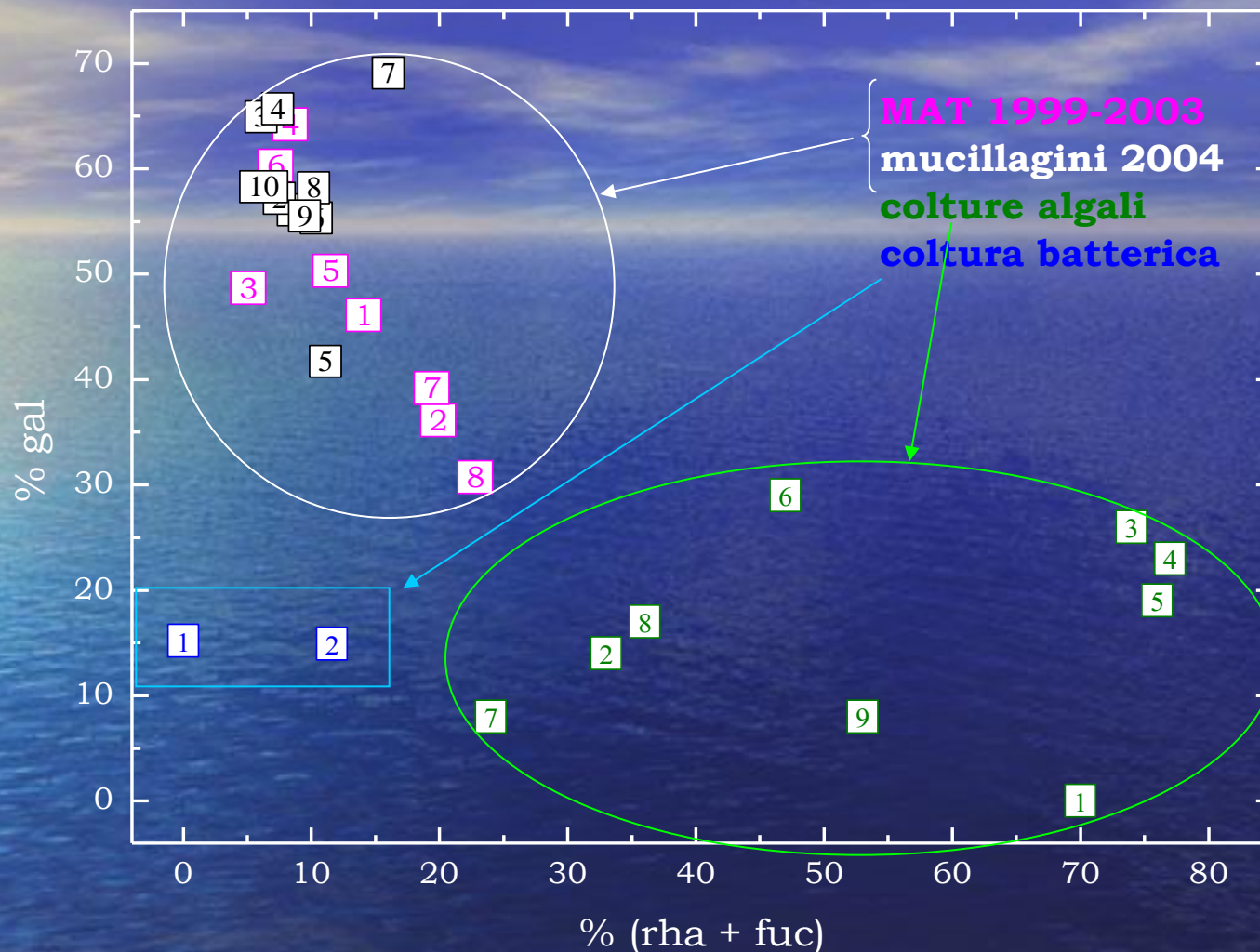
**Catene polielettrolitiche !**

**Frazionamento  
dell'esopolisaccaride  
disciolto  
da coltura axenica**



## Rapporti molari dei monosaccaridi componenti F1 ed F2

	<b>Rha</b>	<b>Fuc</b>	<b>Xil</b>	<b>Man</b>	<b>Gal</b>	<b>Glc</b>
<i>Chaetoceros decipiens</i> F1	7	7	1	4	5	1
<i>Chaetoceros decipiens</i> F2	7	2	1	14	3	3
<i>Chaetoceros decipiens</i> (Myklestad, 1995)	7	7	0.5	1	3	0.5



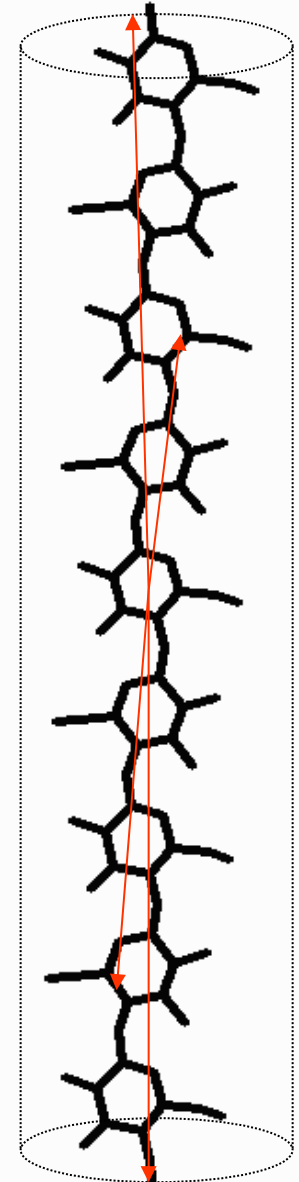
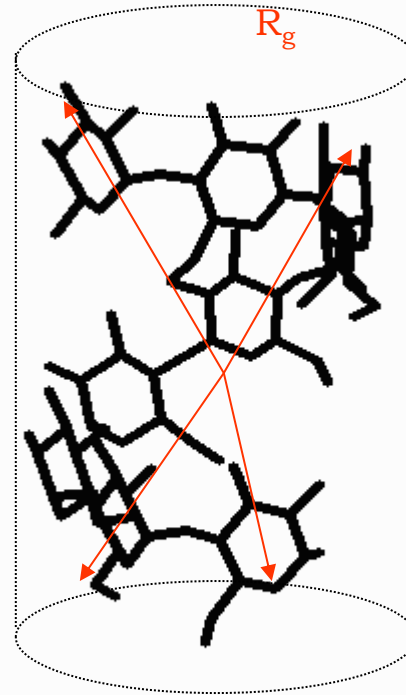
Correlazione tra le percentuali di **galattosio** e la somma (**fuc+rha**) in campioni mucillaginosi adriatici ed in esopolisaccaridi prodotti da colture axeniche algali e batteriche (dati da letteratura).

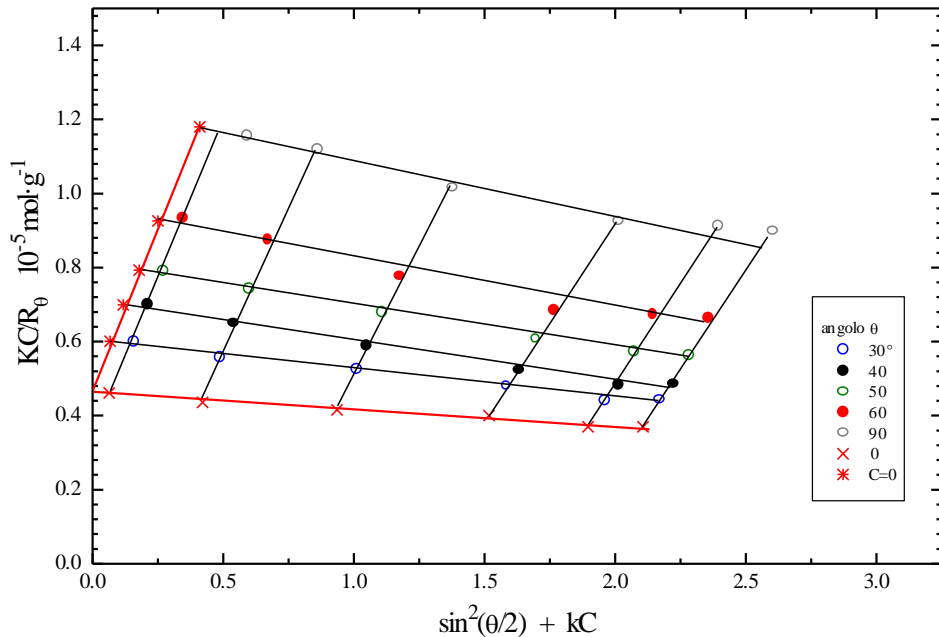
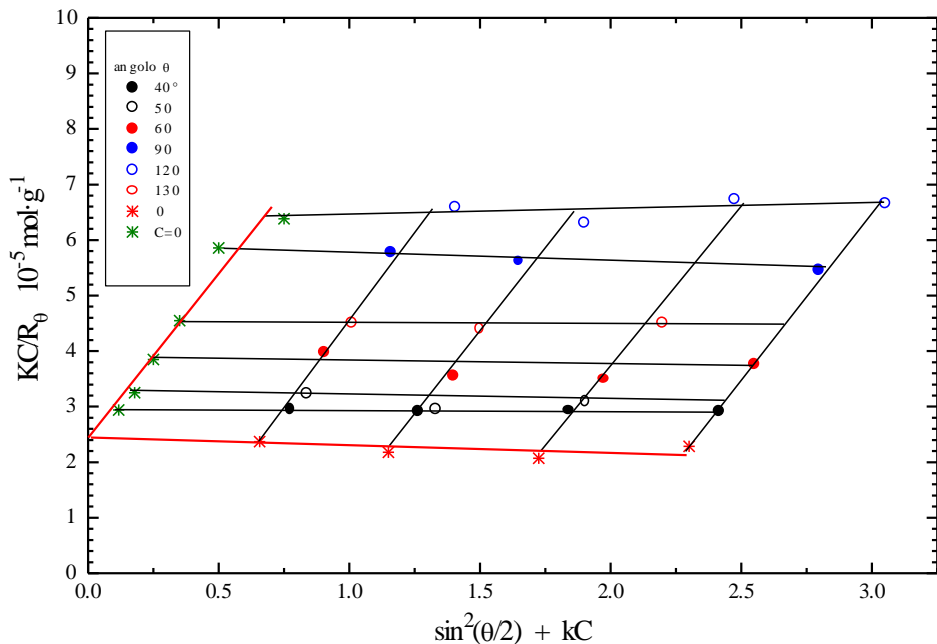
## Analisi di metilazione

<b>Derivato</b>	<b>Legame</b>	<b>%</b>
<b>2,3,4-Me<sub>3</sub> Rha</b>	<b>t-Rha</b>	<b>21.4</b>
<b>2,3,4-Me<sub>3</sub> Fuc</b>	<b>t-Fuc</b>	<b>10.7</b>
<b>2,3,4,6-Me<sub>4</sub> Man</b>	<b>t-Man<sub>p</sub></b>	<b>3.2</b>
<b>3,4-Me<sub>2</sub> Rha</b>	<b>2-Rha</b>	<b>8.3</b>
<b>2,3,4,6-Me<sub>4</sub> Glc</b>	<b>t-Glc<sub>p</sub></b>	<b>4.0</b>
<b>2,3,4,6-Me<sub>4</sub> Gal</b>	<b>t-Gal<sub>p</sub></b>	<b>3.2</b>
<b>3,4-Me<sub>2</sub> Fuc</b>	<b>2-Fuc</b>	<b>2.7</b>
<b>3,4,6-Me<sub>3</sub> Man</b>	<b>2-Man<sub>p</sub></b>	<b>2.3</b>
<b>2,3,4-Me<sub>3</sub> Man</b>	<b>6-Man<sub>p</sub></b>	<b>3.3</b>
<b>3,5-Me<sub>2</sub> Xyl</b>	<b>2-Xyl<sub>f</sub></b>	<b>1.0</b>
<b>Fuc</b>	<b>2,3,4-Fuc</b>	<b>5.4</b>
<b>3,4,6-Me<sub>3</sub> Gal</b>	<b>2-Gal<sub>p</sub></b>	<b>13.4</b>
<b>2,3,6- Me<sub>3</sub> Gal</b>	<b>4-Gal<sub>p</sub></b>	<b>10.5</b>
<b>2,3,6-Me<sub>3</sub> Glc</b>	<b>4-Glc<sub>p</sub></b>	<b>2.4</b>
<b>3-Me Xyl</b>	<b>2,4-Xyl<sub>p</sub></b>	<b>1.8</b>
<b>3,6-Me<sub>2</sub> Man</b>	<b>2,4-Man<sub>p</sub></b>	<b>1.9</b>
<b>4,6-Me<sub>2</sub> Man</b>	<b>2,3-Man<sub>p</sub></b>	<b>1.0</b>

# Determinazione delle proprietà delle catene (PM, raggio di girazione)

- Esperimenti di Diffusione della luce ( $R_g$ , PM, coefficiente del viriale) - **Laser Light Scattering**
- Esperimenti di Cromatografia di Permeazione su Gel (PM) - **GPC**
- Esperimenti di **Viscometria** (volume e raggio idrodinamico, conformazione)





sample	$M_{NaCl} = I$	$M_w$ /dalton	$A_2 \cdot 10^6$ / $cm^3 \cdot mol \cdot g^{-1}$	$R_G$ /nm
TH14B13 (2000)	0.10	264,000	-0.41	77
	0.30	145,000	-0.81	83
	0.50	218,000	-1.3	99
	0.70	234,000	-4.8	94
Rovinj, A7 (2001)	0.30	222,000	-0.43	155
	0.70	217,000	-0.20	131
			-0.45	
			-0.56	

	$R_G$ /nm
Pullulan	24
Welan	68
Alginate	73
TH14B13 (2000)	77
A7 (2001)	155



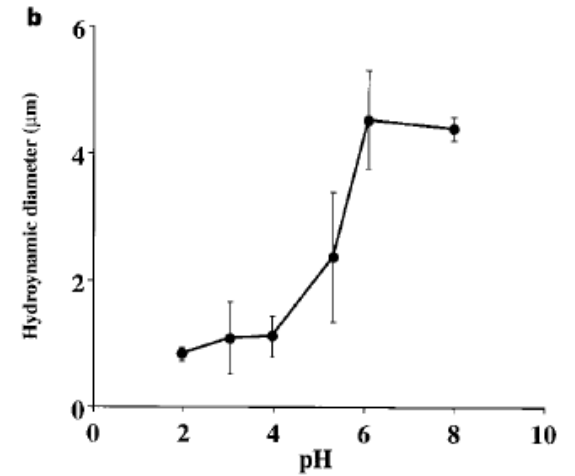
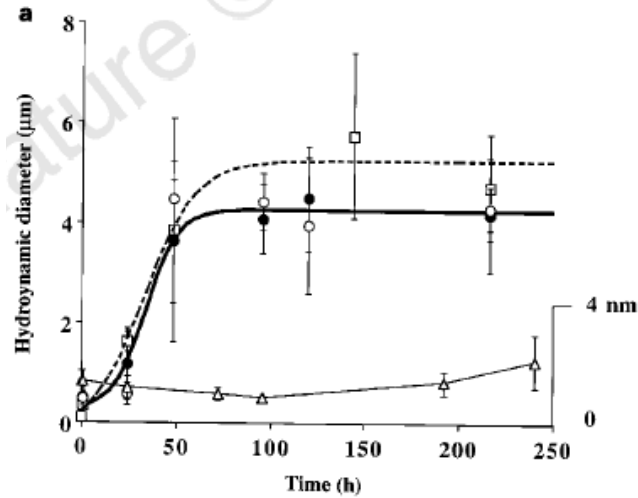
# DLS

«Dynamic Laser Light Scattering»

## Spontaneous assembly of marine dissolved organic matter into polymer gels

Wei-Chun Chin, Mónica V. Orellana & Pedro Verdugo

Department of Bioengineering, University of Washington, Box 357962, Seattle, Washington 98195, USA



Urbani & Sist, 2004

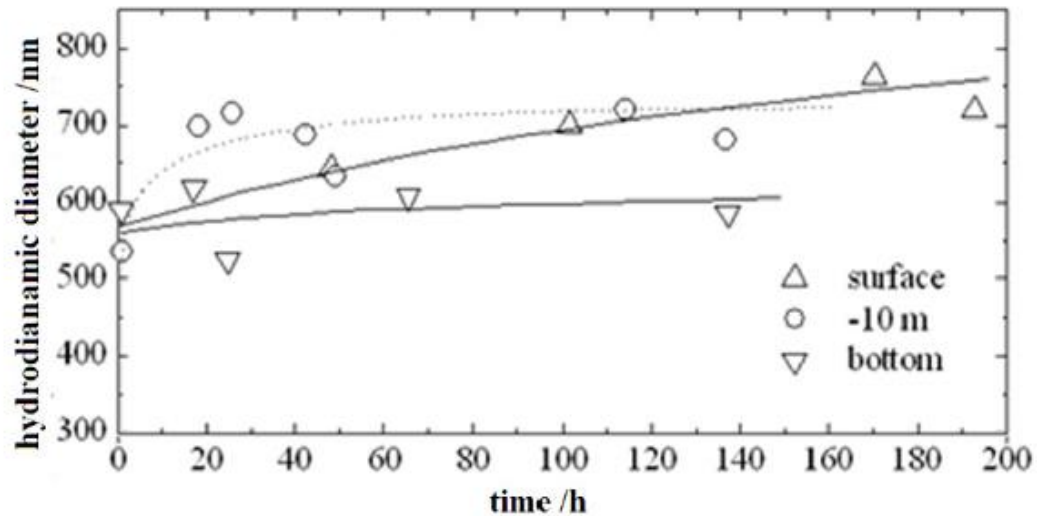
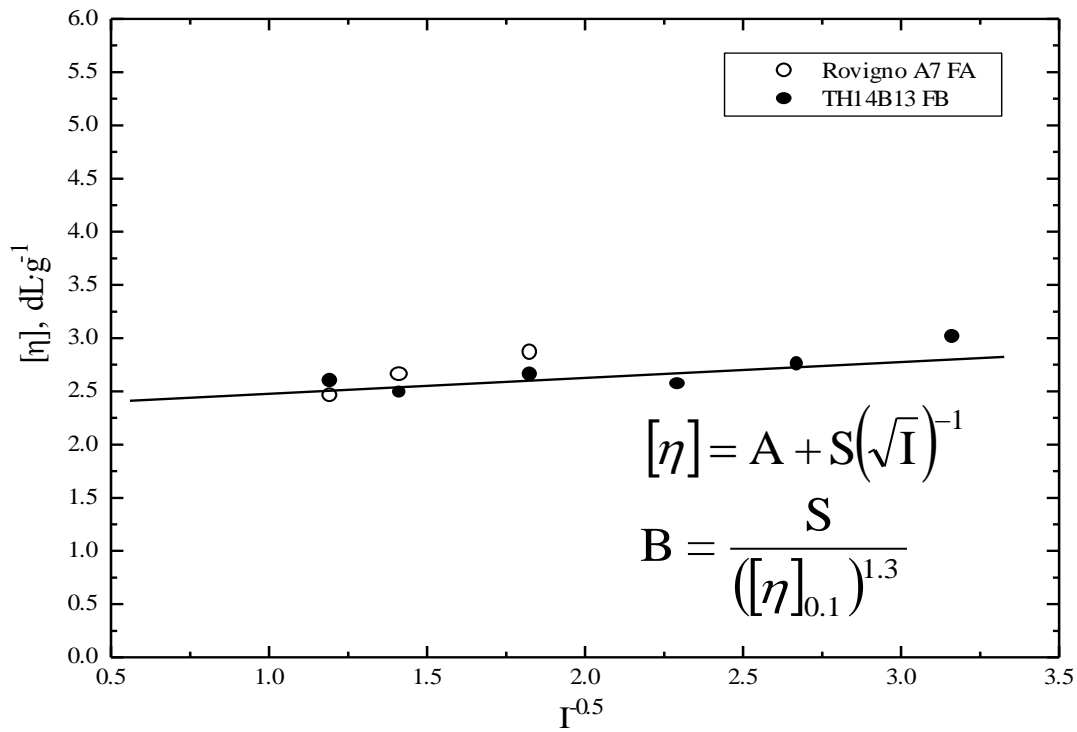


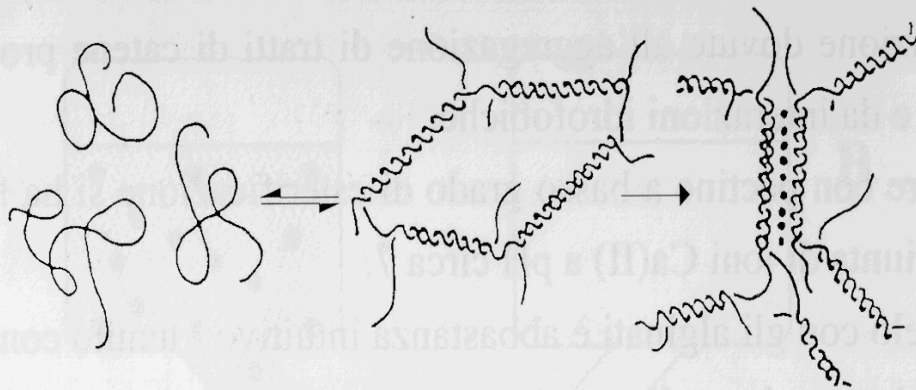
Figure 7. Hydrodynamic diameter in dependence on time for Adriatic seawater samples.



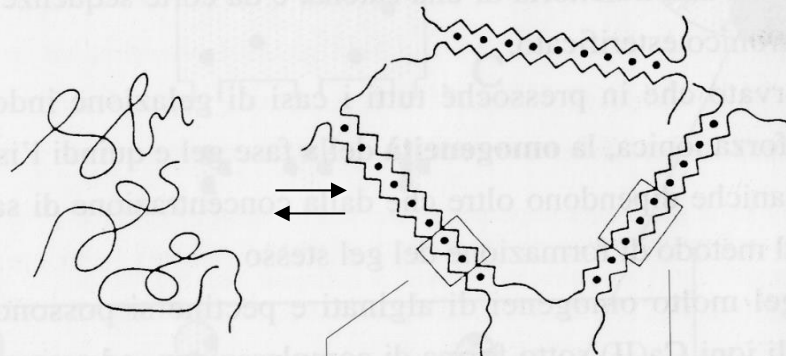
<b>Polymer</b>	<b>B</b>
<i>Polyphosphate</i>	0.44
<i>Poliacrylate</i>	0.23
<i>Dextran sulphate</i>	0.23
<i>Carboxymethyl-amylose</i>	0.20
<i>Carboxymethyl-cellulose</i>	0.065
<i>Hyaluronic Acid</i>	0.065
<i>Mannuronic-rich alginate</i>	0.040
<b>TH14B13 (2000)</b>	<b>0.036</b>
<i>Guluronic-rich alginate</i>	0.031
<i>DNA</i>	0.0055
<i>Xanthan</i>	0.0053

# Determinazione delle proprietà aggreganti/gelificanti

Processo di aggregazione 1



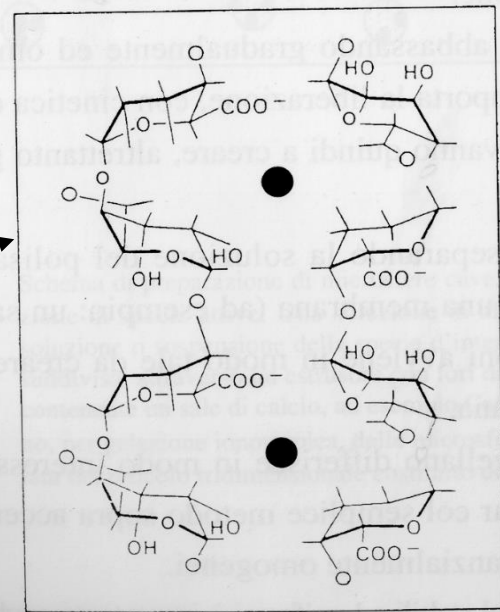
Processo di aggregazione 2



K-carragenano

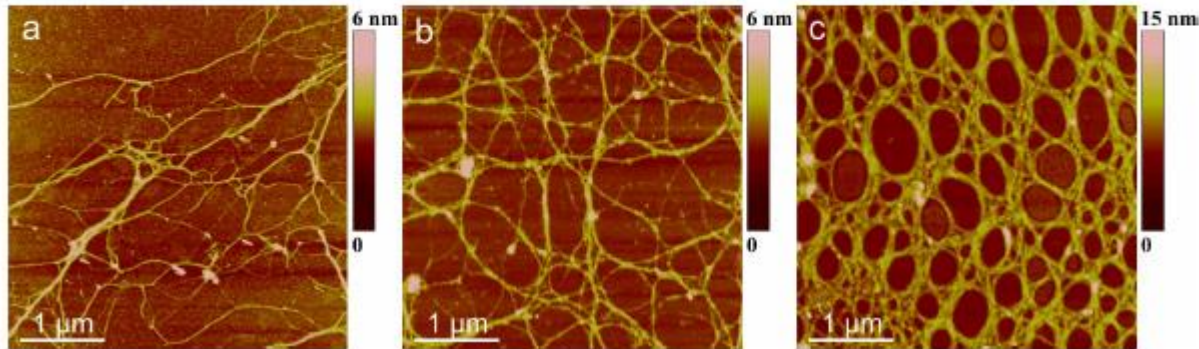
alginato

GEL



**Figure 4.** Evolution of polymer networks in the macroscopic gel phase from (a) to (c): early stage of gel phase formation (a) to condensed gel network of older macroaggregate (c). AFM images are acquired in contact mode and presented as height data, scan size  $4\ \mu\text{m} \times 4\ \mu\text{m}$ .

**AFM**



OPEN ACCESS

*Marine Drugs*

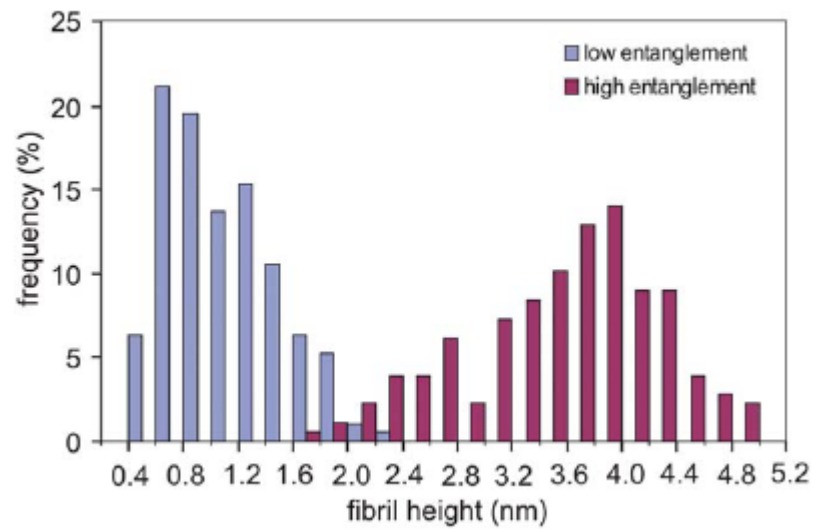
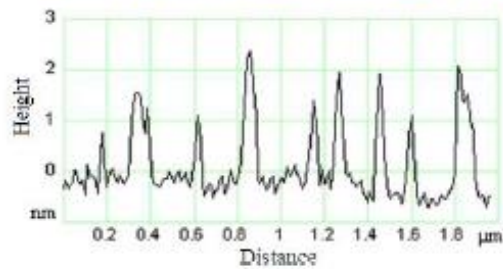
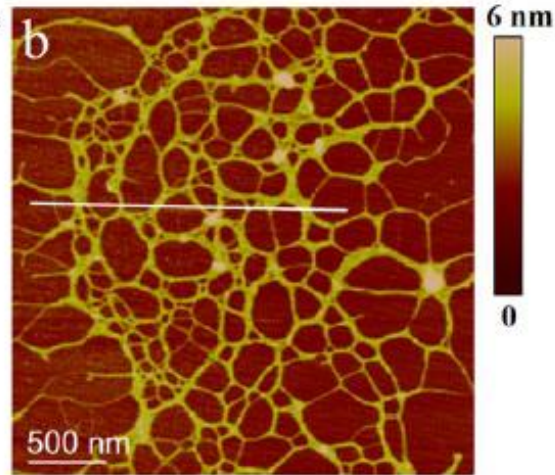
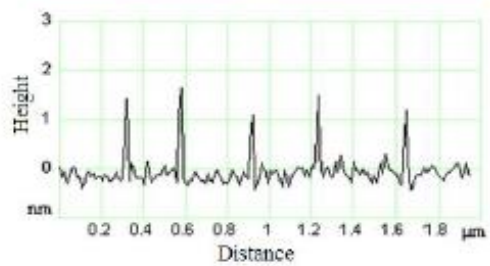
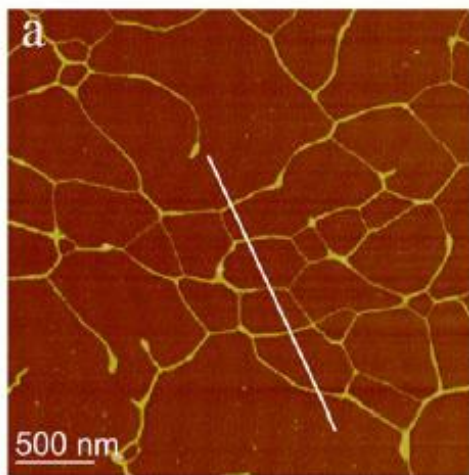
ISSN 1660-3397

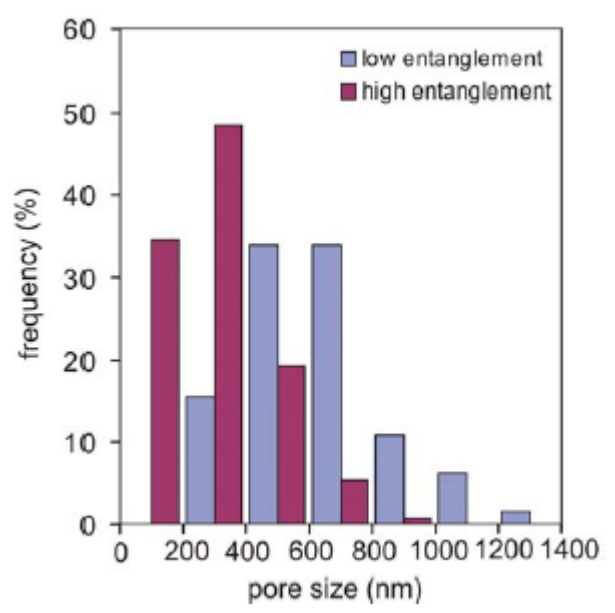
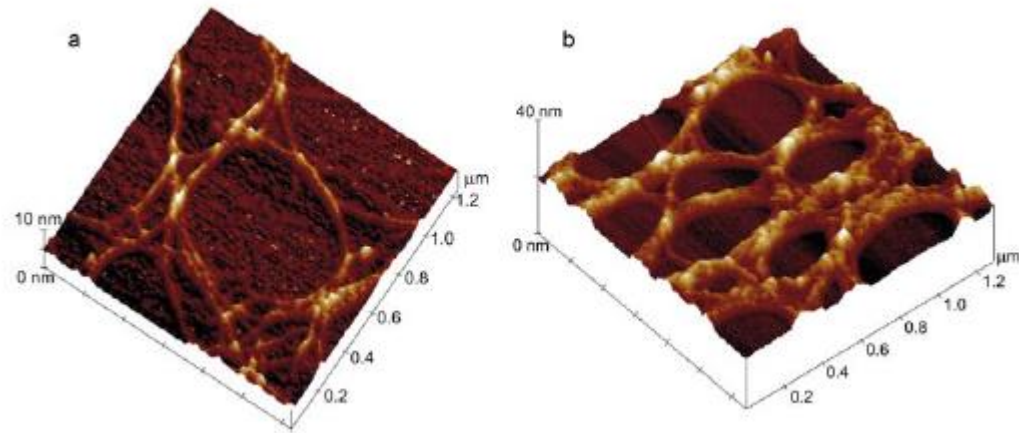
[www.mdpi.com/journal/marinedrugs](http://www.mdpi.com/journal/marinedrugs)

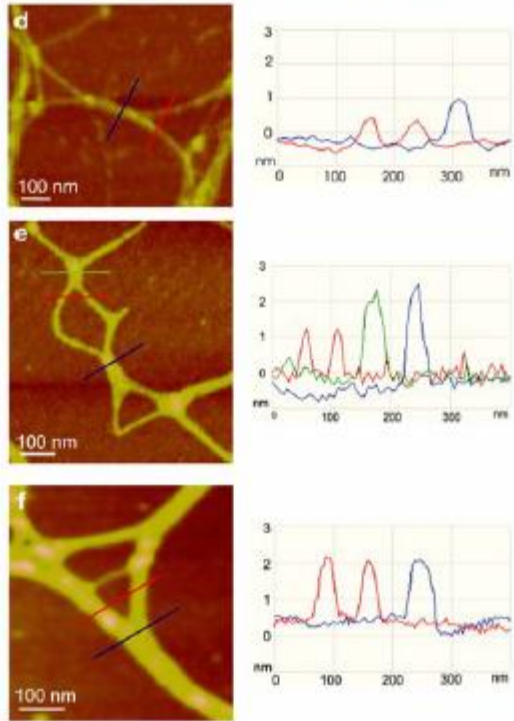
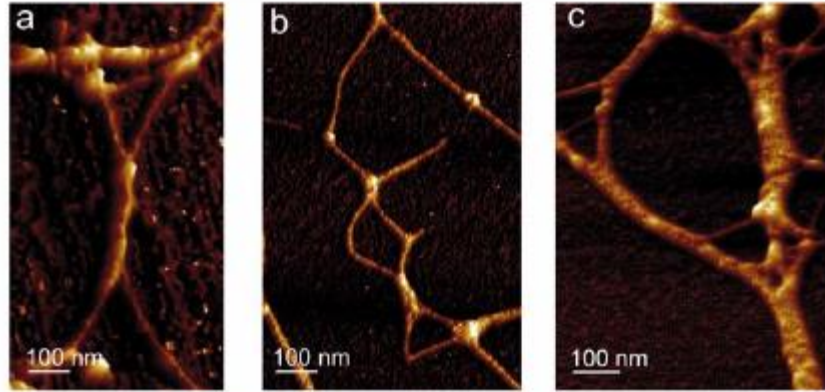
Article

## Polymer Networks Produced by Marine Diatoms in the Northern Adriatic Sea

Vesna Svetličić <sup>1,\*</sup>, Vera Žutić <sup>1</sup>, Tea Mišić Radić <sup>1</sup>, Galja Pletikapić <sup>1</sup>,  
Amela Hozić Zimmermann <sup>1</sup> and Ranieri Urbani <sup>2</sup>

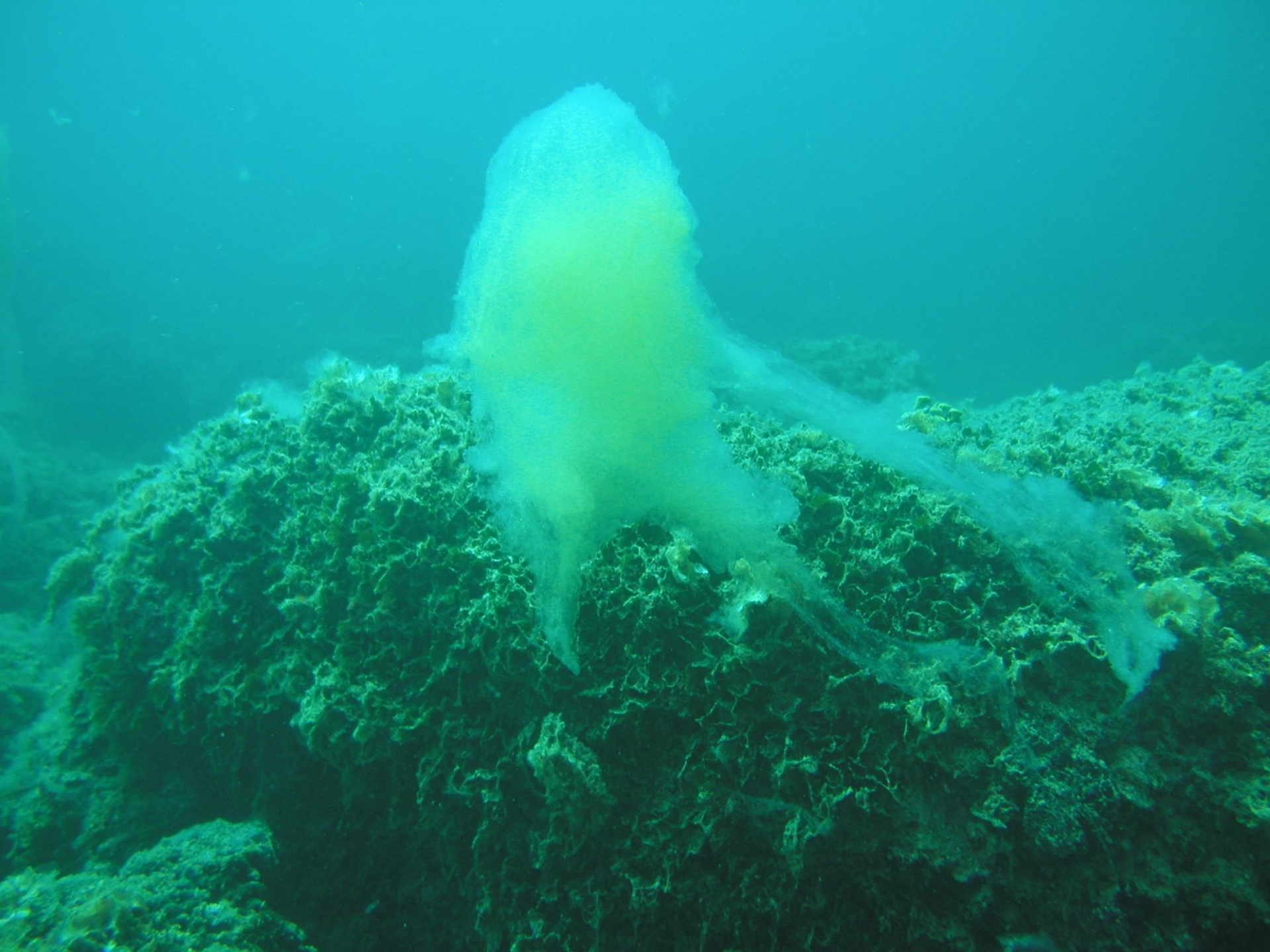






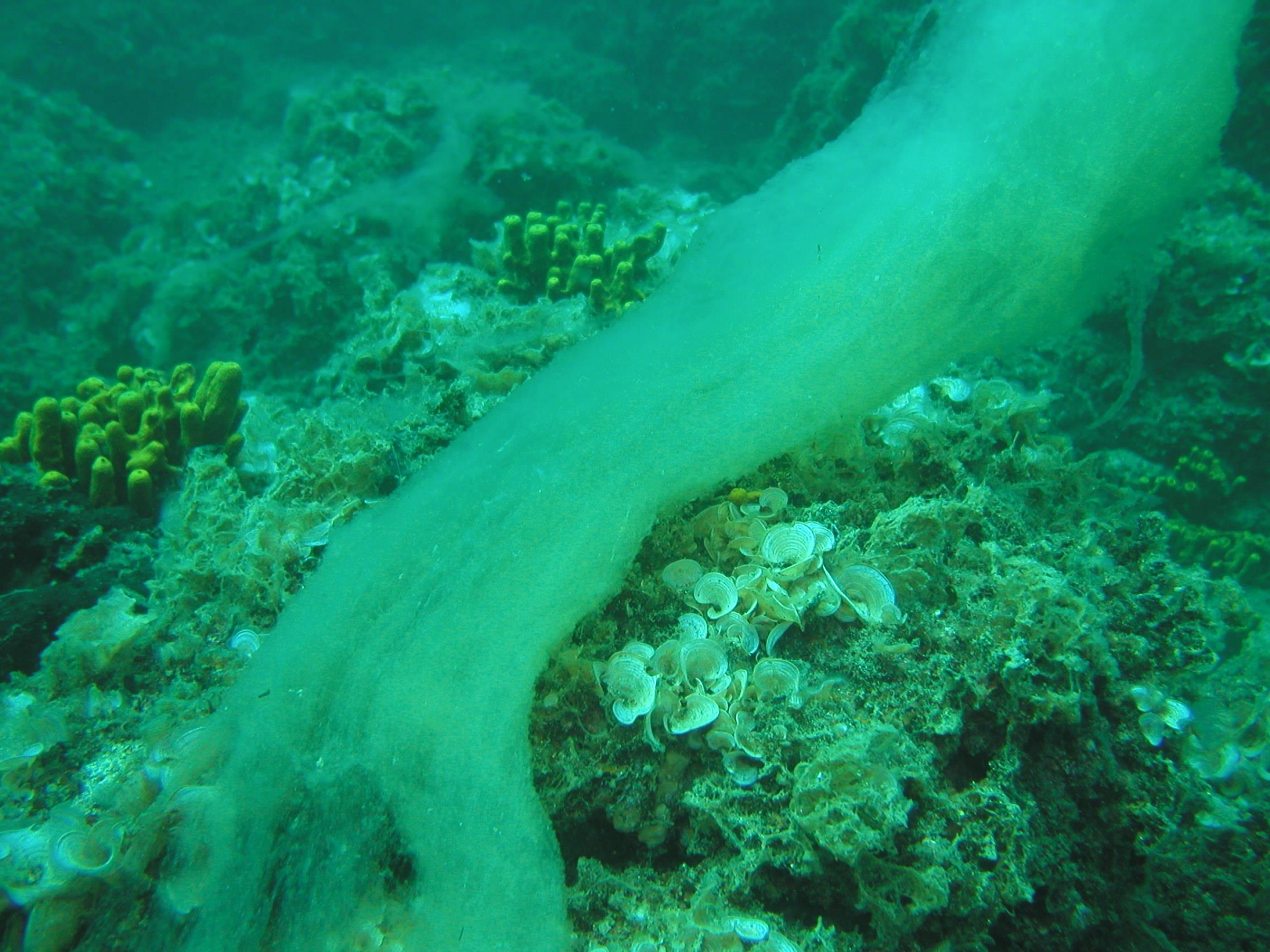
# Microscopia SEM di mucillagini adriatiche

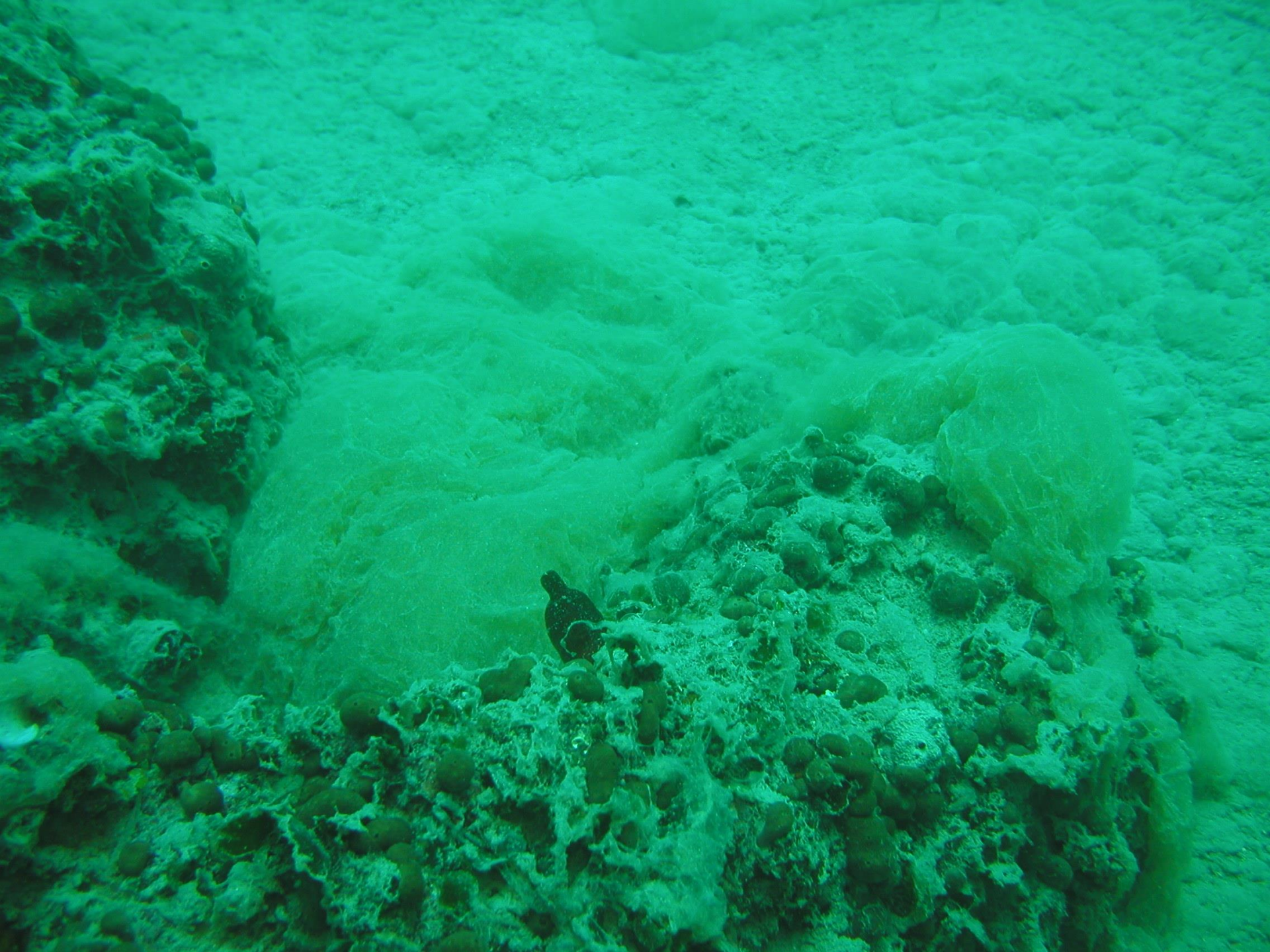


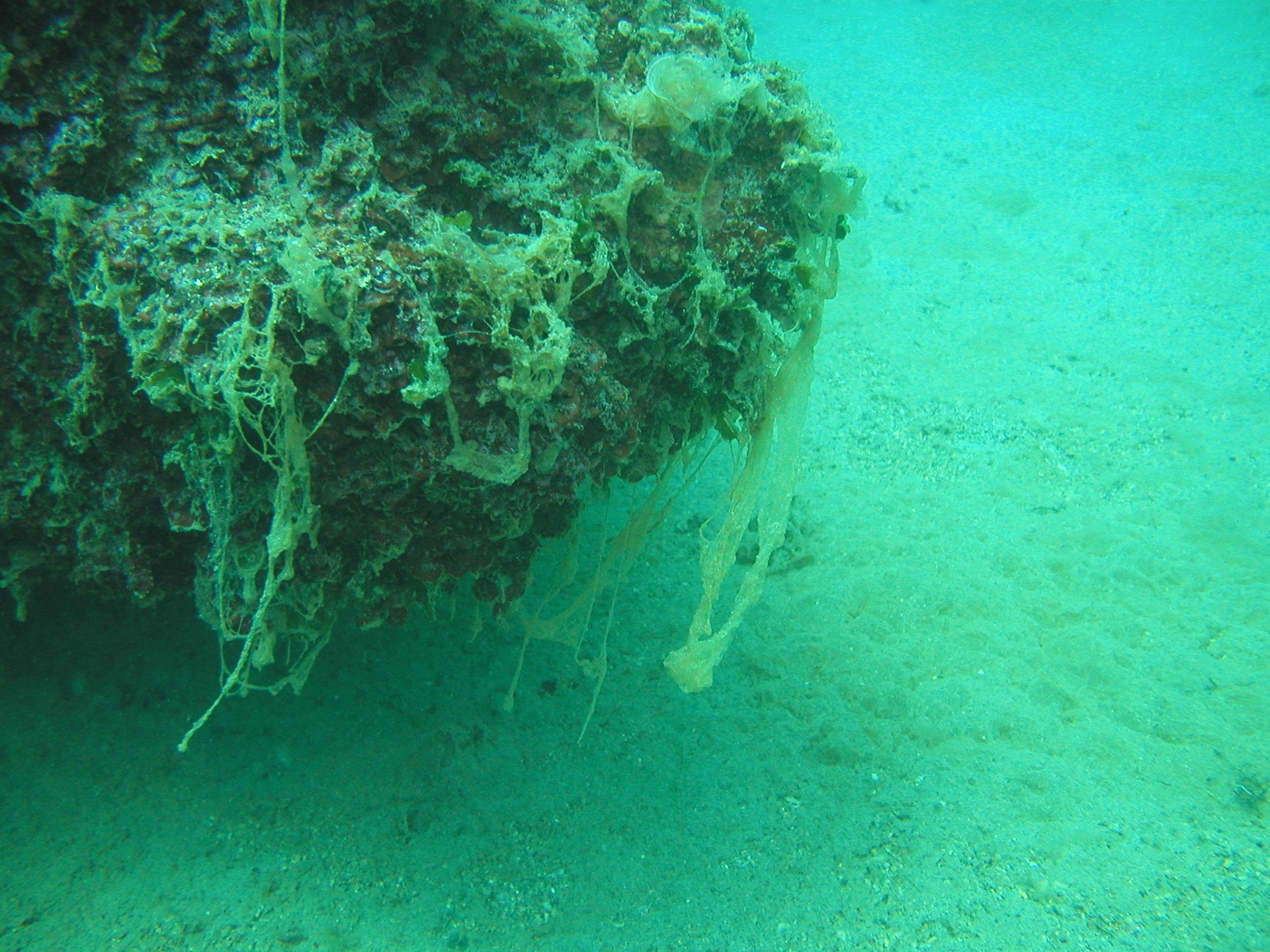














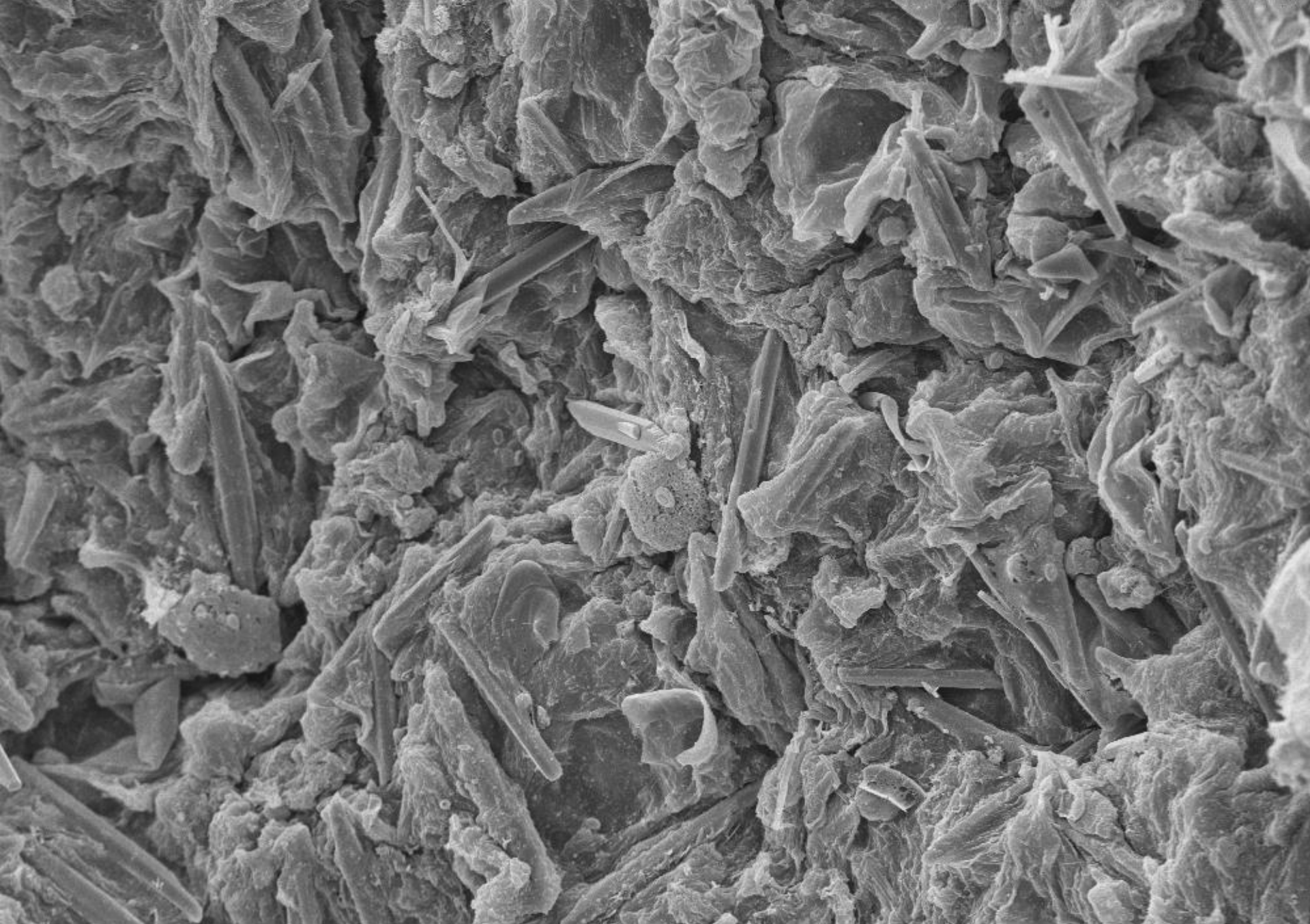










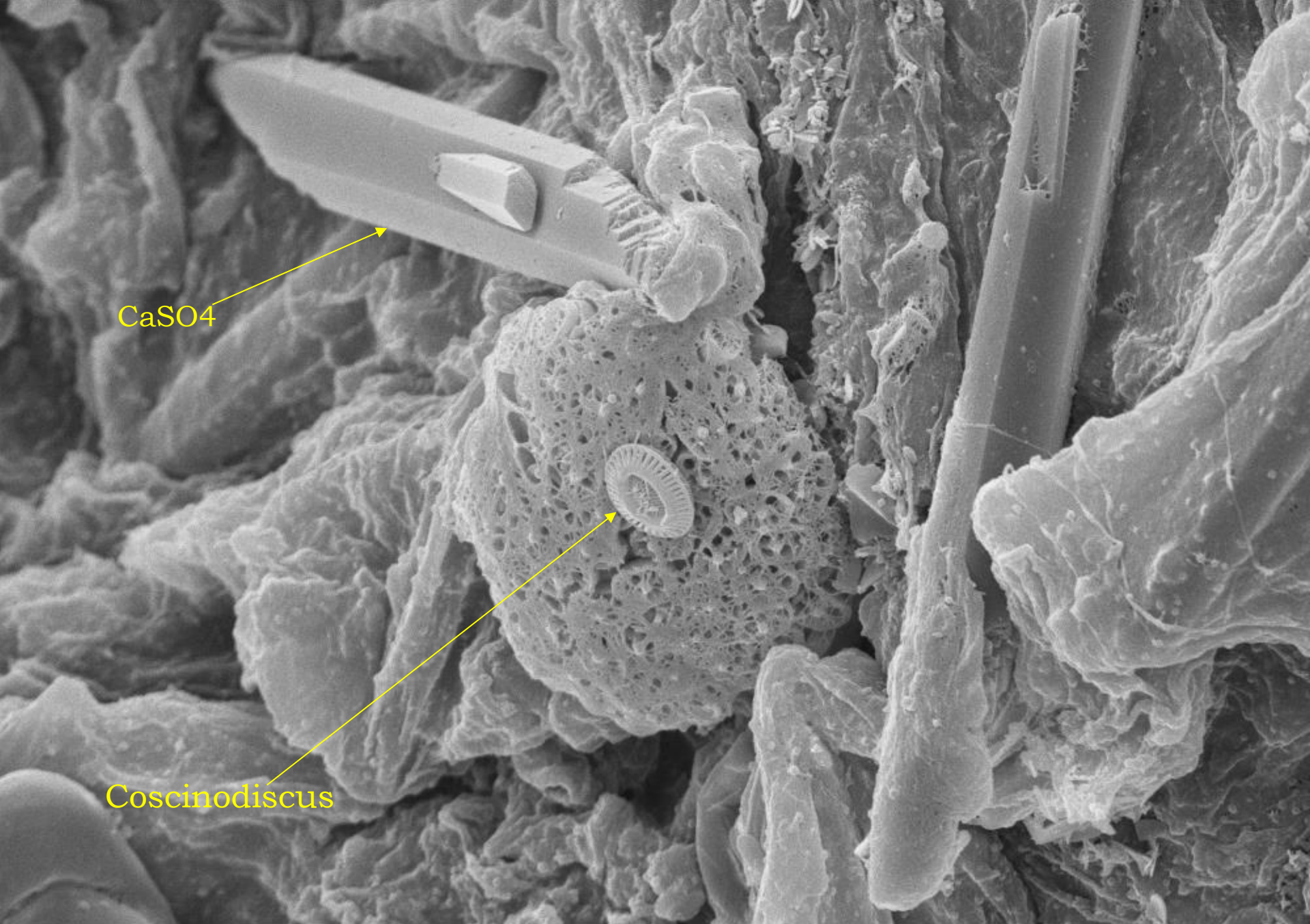


EHT=13.31 kV WD= 16 mm Mag= 600 X Detector= SE1

20µm



Photo No =1482



CaSO4

Coscinodiscus

EHT=13.31 kV WD= 16 mm Mag= 2.70 K X Detector= SE1

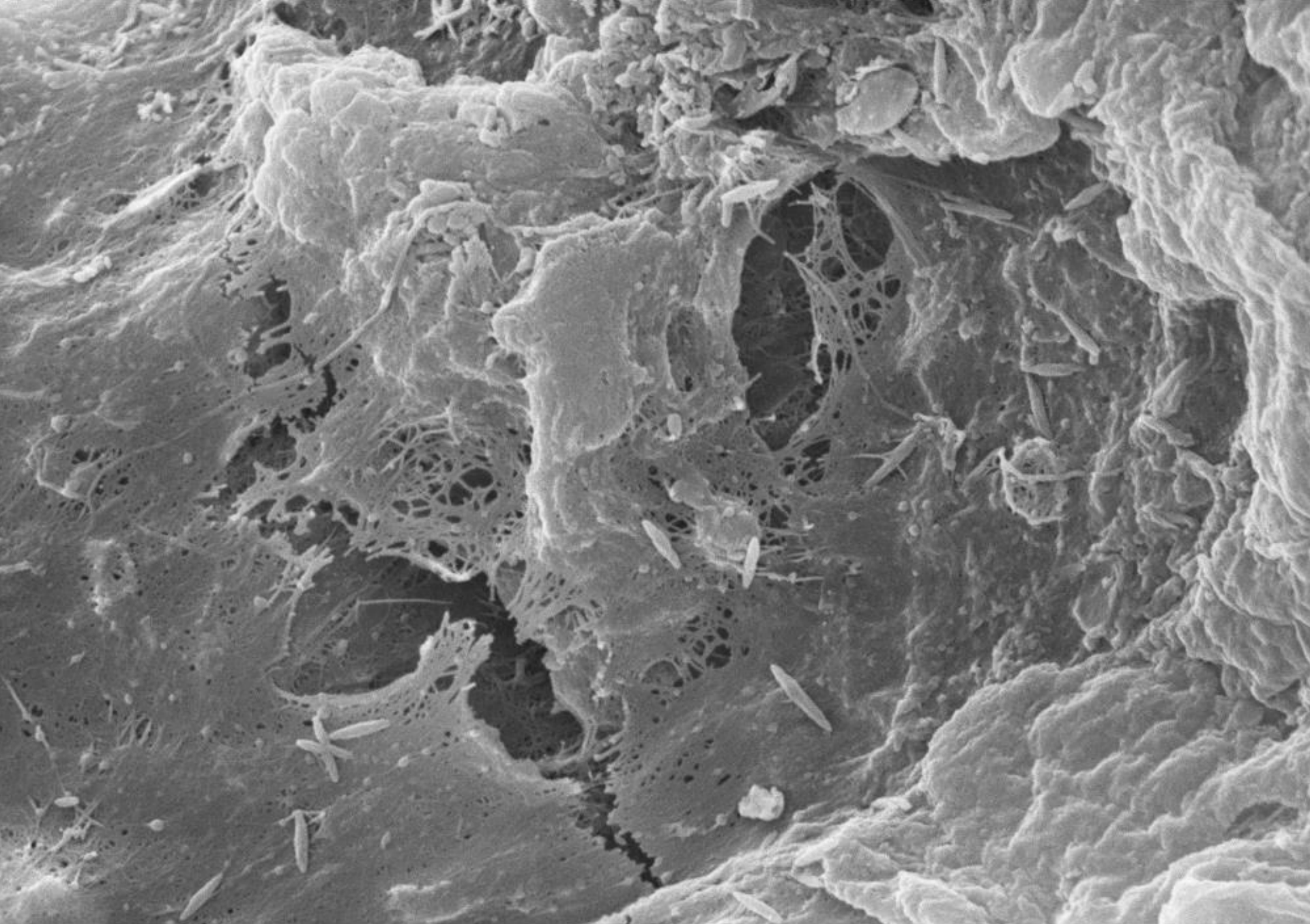
2µm

Photo No =1484



*Diatomea centrica*

EHT=15.17 kV WD= 9 mm Mag= 11.04 K X Detector= SE1  
1µm  Photo No =1499



EHT=13.31 kV WD= 16 mm

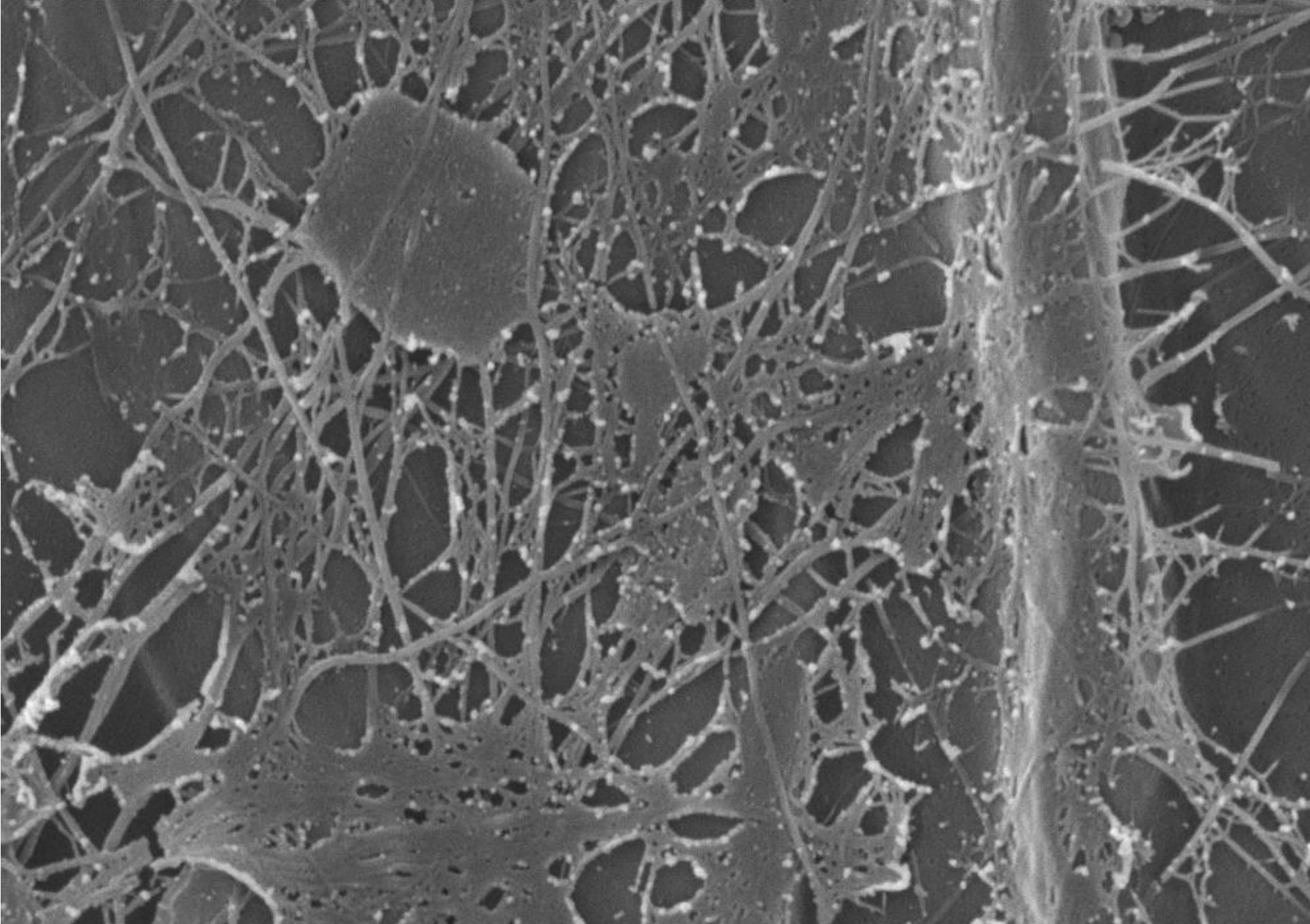
Mag= 4.62 K X Detector= SE1

63

1 $\mu$ m



Photo No =1493



EHT=15.17 kV WD= 10 mm

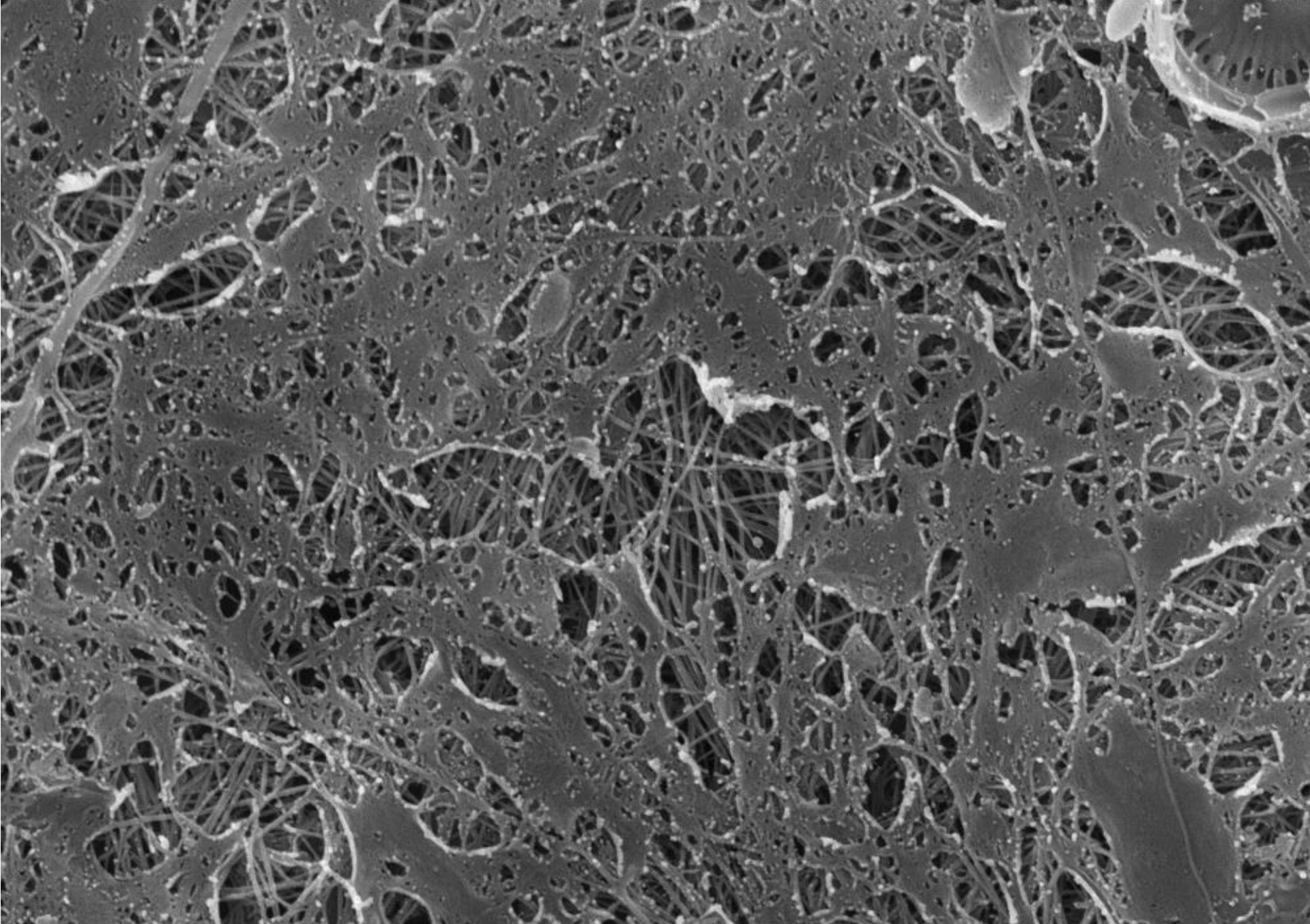
Mag= 8.02 K X Detector= SE1

1 $\mu$ m



Photo No =1519





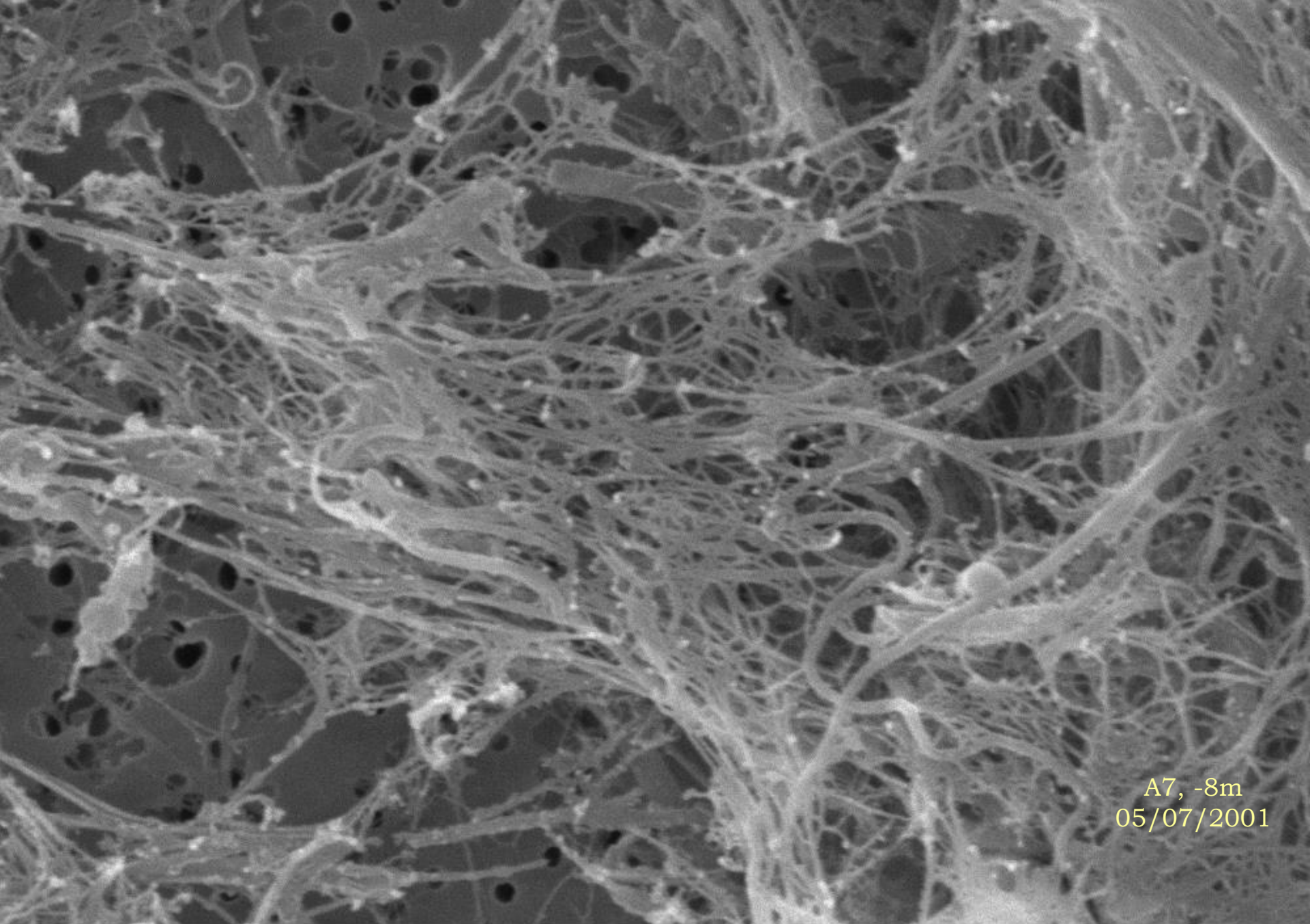
EHT=16.94 kV WD= 15 mm

Mag= 5.03 K X Detector= SE1

1 $\mu$ m



Photo No. =1561



A7, -8m  
05/07/2001

EHT=15.35 kV WD= 11 mm

Mag= 11.75 K X

Detector= SE1

1 $\mu$ m



Photo No =3004

# Microscopia SEM di mucillagini bentoniche tirreniche

# Aggregati algali bentonici tirrenici ed adriatici

*J. Phycol.* 52, 550–563 (2016)  
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DOI: 10.1111/jpy.12415

## ORGANIC AGGREGATES FORMED BY BENTHOPLEUSTOPHYTE BROWN ALGA *ACINETOSPORA CRINITA* (ACINETOSPORACEAE, ECTOCARPALES)<sup>1</sup>

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Istituto Superiore per la Protezione e la Ricerca Ambientale, Chioggia 30015, Italy

*Carla Rita Ferrari*

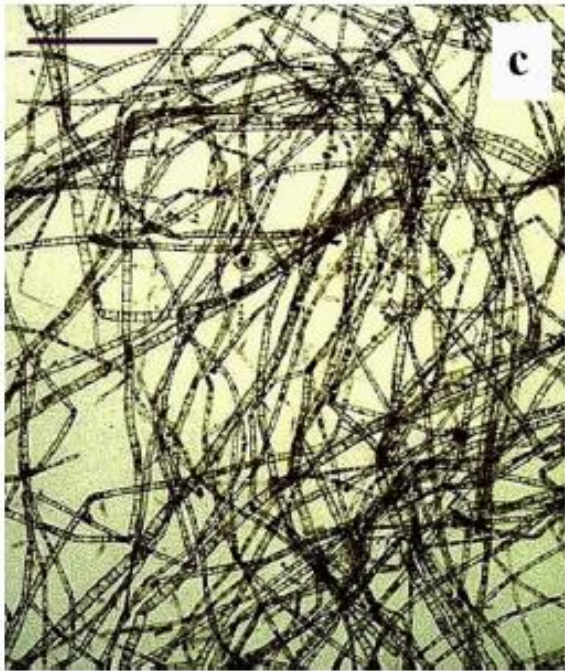
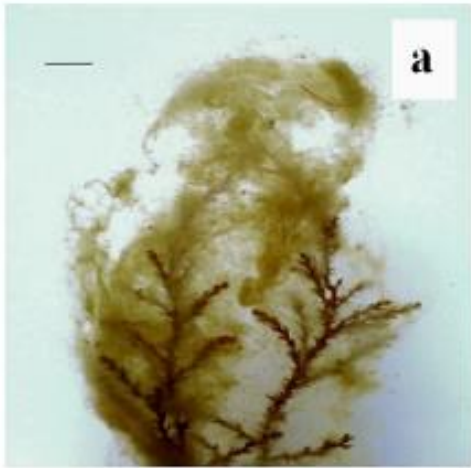
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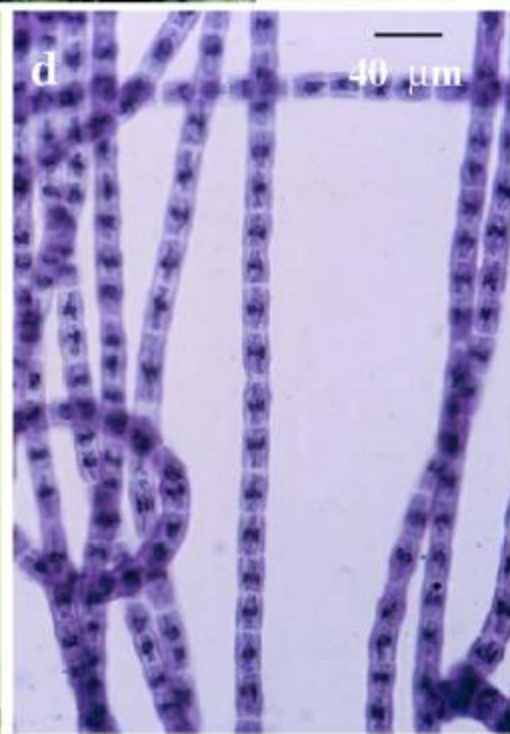
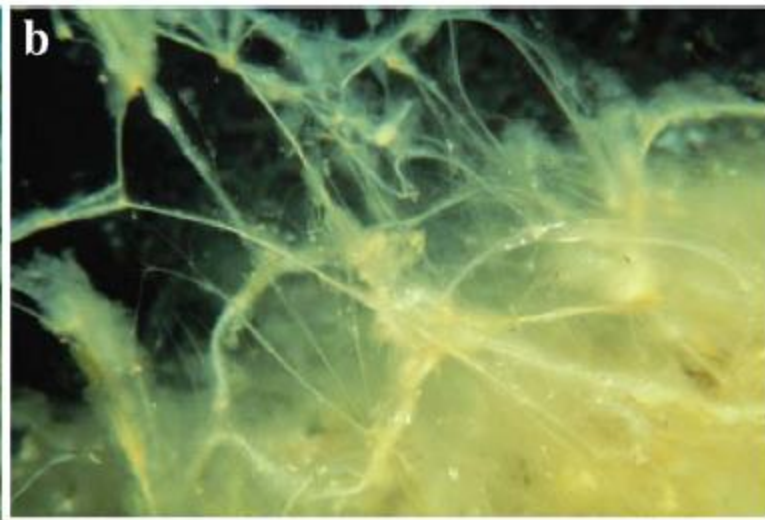
*Mirjana Najdek*

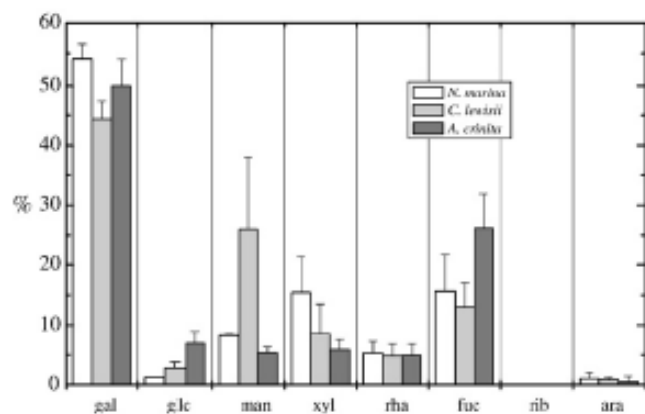
Institut "Ruder Bošković", Center for Marine Research, Rovinj 52210, Croatia

*Paola Sist and Ranieri Urbani*<sup>2</sup>

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**Fig 6.** Relative monosaccharide composition (% w/w) of purified polysaccharides from aggregates produced by different macroalgae.

**Table 4**

Polysaccharide chain properties from light scattering and viscometry

Aggregates	[NaCl]/M	$[\eta]/\text{dL g}^{-1}$	$M_w$	$R_G/\text{nm}$	$A_2/\text{cm}^3 \text{ mol g}^{-2}$
<i>C. lewisii</i>	0.4	4.4	974,000	151	$-7.0 \cdot 10^{-8}$
(C1)	0.6		>6,000,000		$-3.0 \cdot 10^{-5}$
<i>C. lewisii</i> ,	0.5	2.52	742,000	101	$4.8 \cdot 10^{-6}$
<i>A. crinita</i>					
(CA1)	0.6	1.54	855,000	117	$-5.2 \cdot 10^{-8}$

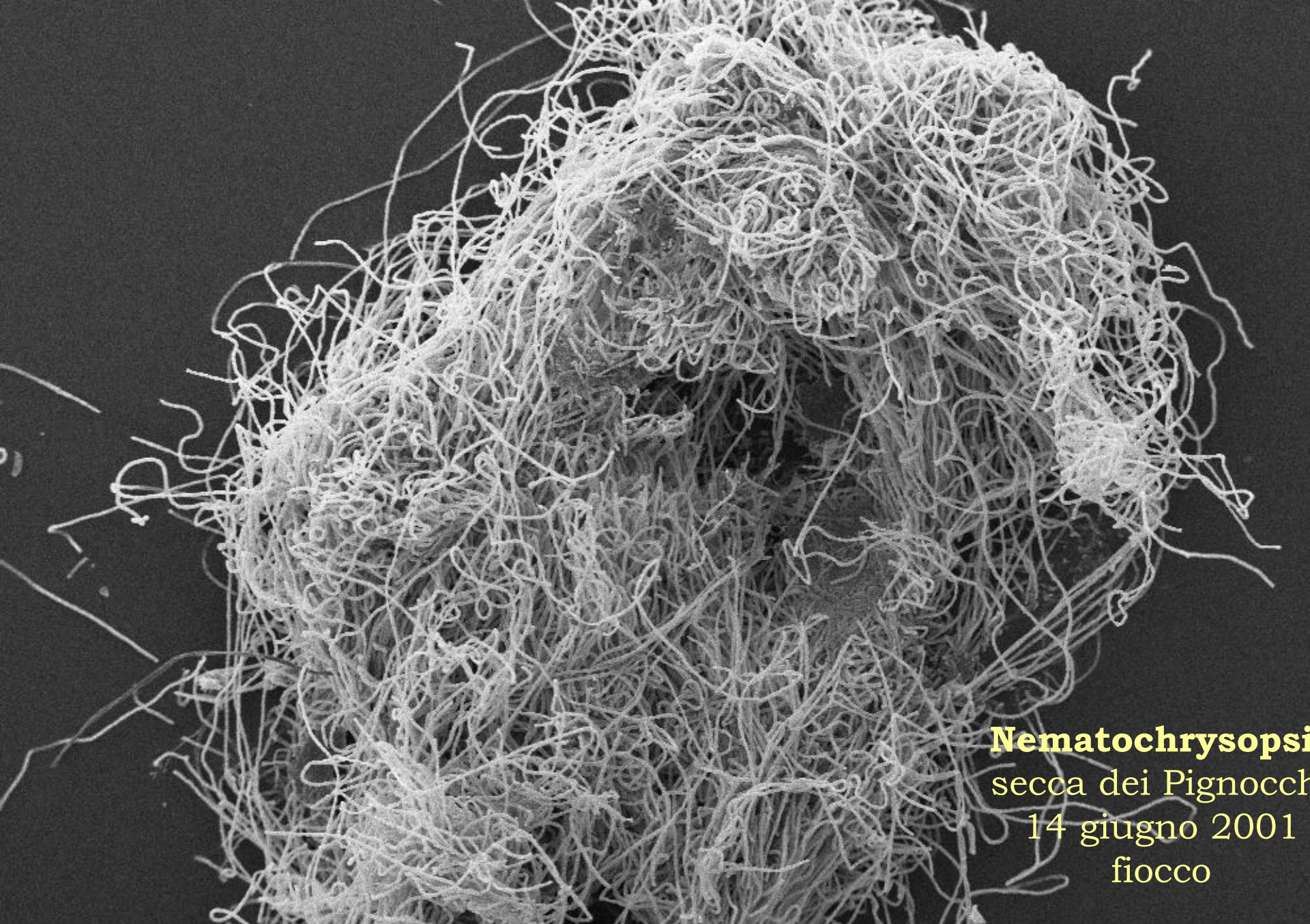













**Nematochrysoptera**  
secca dei Pignocchi  
14 giugno 2001  
fiocco

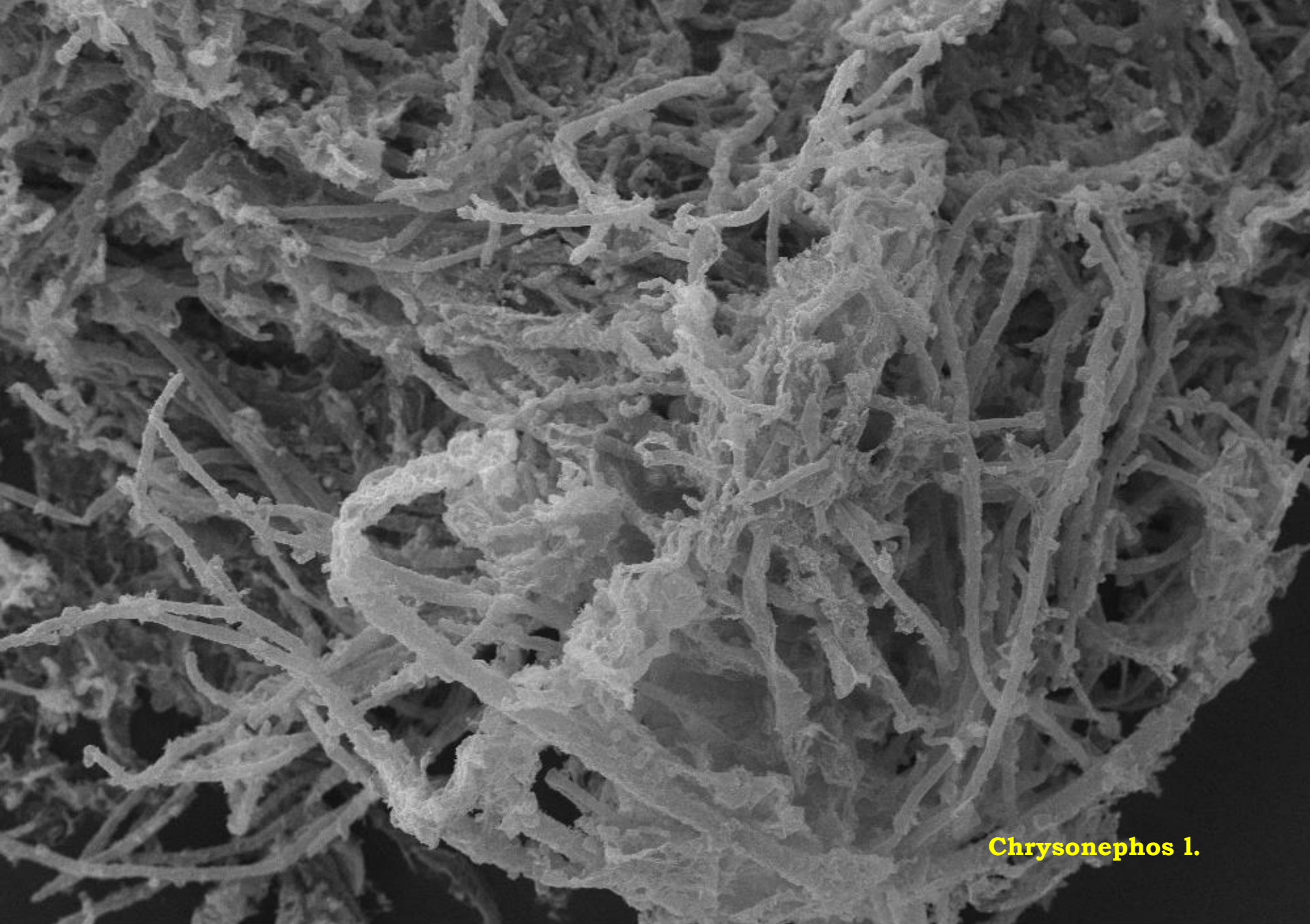
EHT=15.35 kV WD= 11 mm Mag= 35 X Detector= SE1

100µm 

Photo No =3022

Chrysonephos l.  
secca dei Pignocchi  
14 giugno 2001  
fiocco

EHT=14.05 kV WD= 9 mm Mag= 270 X Detector= SE1  
20µm  Photo No.=4360



**Chrysonephos 1.**

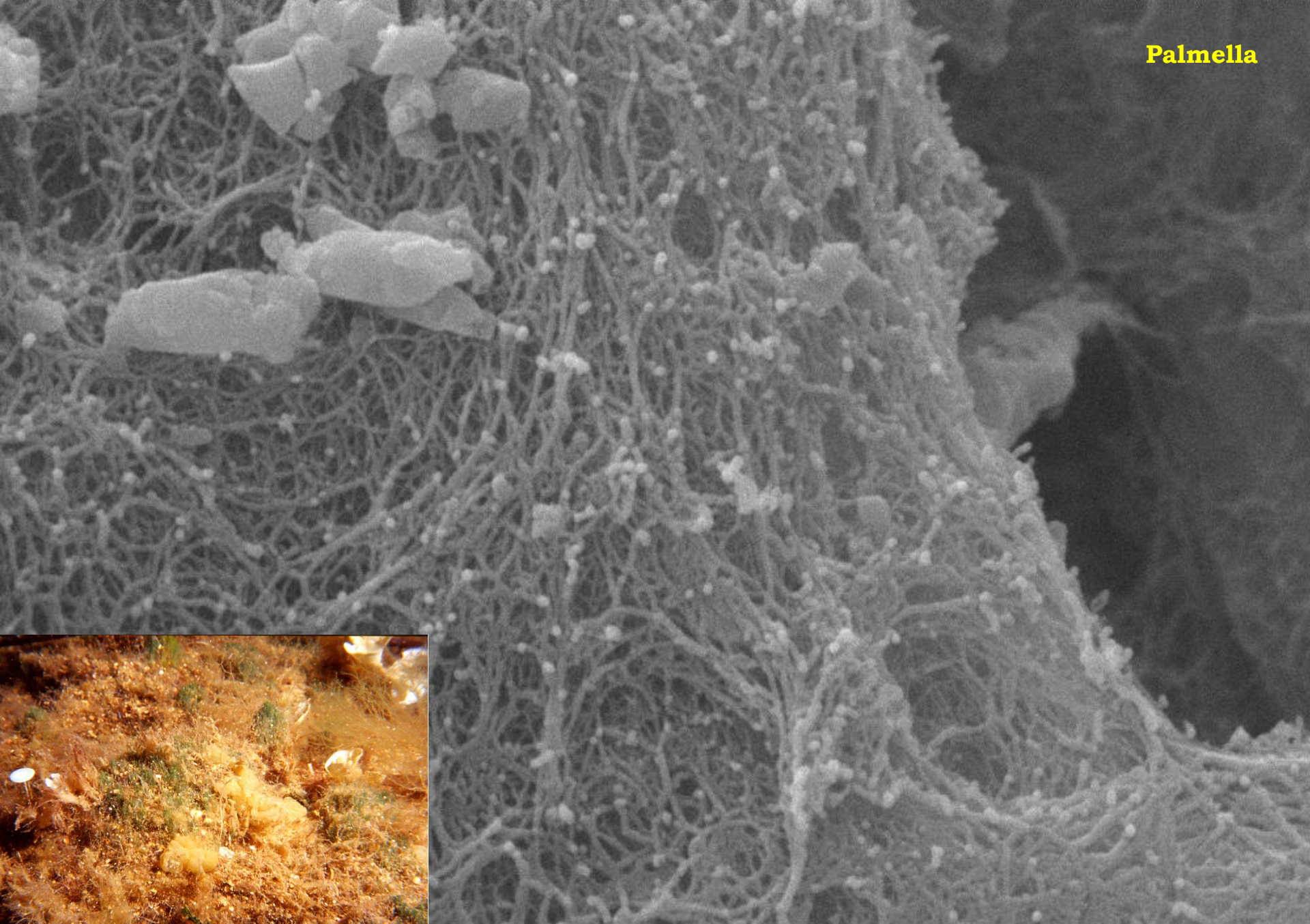
EHT=16.19 kV WD= 9 mm Mag= 124 X Detector= SE1


100µm



Photo No =4365

**Palmella**



EHT=14.05 kV WD= 9 mm Mag= 15.02 K X Detector= SE1  
1µm  Photo No =4361

# Risultati della ricerca

---

La caratterizzazione della frazione polisaccaridica nelle diverse condizioni studiate (colture, colonna d'acqua) permette di trarre delle conclusioni comuni:

- il materiale fresco è principalmente prodotto dalla **componente fitoplanctonica** ed è a più alto peso molecolare.
- I polisaccaridi hanno **struttura polielettrolitica**
- La struttura aggregata **geliforme** è determinata principalmente dai polisaccaridi.
- La composizione di questa frazione semi-labile presenta elevati valori di fucosio che tendono a calare con il procedere della degradazione ad opera della comunità batterica. Sulla base di questi risultati si sono individuati il **gal** e la somma **(fuc+rha)** come bioindicatori dello stato di degradazione della materia organica aggregata.

-



# Risultati della ricerca

- La capacità aggregante dei biopolimeri saccaridici è influenzata dalla forza ionica. I macroaggregati si formano al aloclino con l'augmentare della forza ionica

