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EFFECTS OF SYNTHETIC ZEOLITE "A" AND POLYCARBOXYLATES ON QUALITY AND QUANTITY OF DIATOM MUCOUS EXUDATES

M. Monti, C. Welker and S. Fonda Umani

Laboratory of Marine Biology, Strada Costiera 336, 34010 Trieste, Italy

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ABSTRACT

With the objective to understand whether the introduction on the market of phosphorus-free detergents, namely of detergents based on Zeolite A and Poly Carboxylic Acid (PCA) as builder system, may have played a role in the development of the mucilage phenomenon in the Northern Adriatic Sea (Italy), a experimental study has been undertaken in laboratory.

Using diatom *Cylindrotheca closterium* (Ehr.) Reimann and Lewin as inoculum, which is considered as the most important microalgal organism responsible for mucilage production, several tests have been carried out, adding the Zeolite A and PCA system, at various concentrations, fractions and ratios, to aged filtered seawater.

Under controlled conditions the mucous aggregates formation has been followed and photographed, collecting and then measuring their volumes and dry weights, and at last calculating density, porosity and excess of density.

Statistical analysis applied to all collected data has revealed an homogenous response of this microalgal species and comparable parameters for the mucous aggregates both in the blank and in the tests with Zeolite A and PCA.

Our experimental study has not revealed any evident causal relationship between the presence of Zeolite A - PCA system and the formation of mucous aggregates.

KEY WORDS: mucilage, phosphorous-free detergents, synthetic Zeolite A, Polycarboxylates, *Cylindrotheca closterium*.

INTRODUCTION

In the summers of 1988 and 1989, and to a lesser extent in 1991, large amounts of mucous aggregates (mucilage) appeared in the Northern Adriatic Sea (Fig. 1). This phenomenon, which had already been described (Herndl and Peduzzi, 1988; Degobbis, 1989; Fanuko et al., 1989; Marchetti et al., 1989; Fanuko and Turk, 1990; Honsell and Cabrini, 1990; Marchetti, 1990; Rinaldi et al., 1990; Stachowitsch et al., 1990; Revelante and Gilmartin, 1991; Volterra and Bruno, 1991; Cabrini et al., 1992) covered several thousand square kilometers and it would seem to be of a similar nature to phenomena recorded in the same sea in the last centuries (Fonda Umani et al., 1989).

Mucous aggregates are complex formations which may have different origins (Silver et al., 1978; Alldredge, 1979; Prezelin and Alldredge, 1983; Beers et al., 1986; Alldredge and Silver, 1988; Lochte, 1991; Monti et al., 1992). Moreover these aggregates form a microhabitat which greately favours various communities of bacteria, microalgae and heterotrophic flagellates, whose concentrations vary according to the time of year and the area concerned (Caron et al., 1982; Alldredge and Youngbluth, 1985; Alldredge et al., 1986; Herndl, 1988; Casaretto et al., 1992; Herndl et al., 1992; Volterra et al., 1992). These macroaggregates must not to be confused with marine snow, a collective term for identifing a variety of fragile, amorphous, macroscopic particles ranging from 0.5 mm to many centimeters. Such kind of formations were noticed regularly each year in all the world seas, included the Northern Adriatic Sea (Herndl and Peduzzi, 1988; Monti and Welker, 1993; Welker, 1993), where the highest concentration appearing after the spring phytoplankton blooms. Macroaggegates more similar to "mucilage" phenomenon, as well as the massive foam banks due to *Phaeocystis* in the North Sea (Lancelot et al., 1994), were observed in the North Sea (Smayda, 1970) during 1947 and 1948, due to *Coscinodiscus concinnus*, and in the Northern Adriatic Sea (Fonda Umani et al., 1989) since 1729.

The research carried out in the Northern Adriatic Sea in the framework of Alpe Adria project, during the last appearances of this phenomenon in the summers 1988, 1989, 1991, pintpointed that they are mainly due to exudates produced by diatoms as *Cylindrotheca closterium, Skeletonema costatum* and some *Chaetoceros* species (Degobbis et al., 1995).

The most commonly accepted hypothesis is that the Po river, the most important river flowing into the Northern Adriatic Sea after a 700 km course through a fertile, industrialized and heavily populated (ca. 15×10^6

inhabitants) area, with its particular ten year pattern of discharges (water and nutrients) might have influenced the plankton communities, selecting species and/or modifying relationships in the food webs, which resulted in a significant production and accumulation of exudates (Degobbis et al., 1995).

Multivariate techniques (Cluster Analysis and Principal Components Analysis) applied to phytoplankton data collected in June, July, August and September 1990, 1991, 1992 and 1993 in the Gulf of Trieste (Northern Adriatic Sea) highlighted a clear temporal pattern from 1990 to 1993. In the summer 1991 phytoplankton communities showed a dramatic increase in the dinoflagellate/diatom ratio. In particular, during June and July 1991 only few diatom specimens (*Cylindrotheca closterium, Proboscia alata, Nitzschia longissima* and *Nitzschia* spp.) were collected, this confirming the diatom scarcity in the phytoplankton communities before and during the appearance in July 1991 of large mucous aggregates (mucilage) in the Gulf of Trieste. The differences observed between the structure of the phytoplankton communities inside the aggregates and in the ambient waters suggested two hypotheses: 1) the aggregates were not produced in the Gulf, but carried into the Gulf by the eastern ascending current, 2) the aggregates produced by a few species with a high exudates production, even if scarce in the ambient water, acted as a selective environment for the same species which can quickly reach a high reproductivity rate within the aggregates (Cataletto et al., in press).

. A more recent hypothesis (Pettine et al., 1992) suggested that the detergent ingredients, Zeolite A - Polycarboxylic Acid (PCA) as builder system, may have played a role in exacerbating the mucilage phenomenon in these years, after the introduction of phosphorous free detergents. This hypothesis is based on the assumption that both Zeolite A and PCA, once discarged into the environment and reaching the sea, become colloidal material. The former would take part in the aggregation process and the latter would enhance coagulation and flocculation processes like other natural polimer such as, for example, humic and fulvic acids. The same authors calculate the amount of Zeolite A reaching the Northern Adriatic Sea through the Po river and indicated 100 µg/l as the average concentration of Zeolite A present at the plume of the river. For PCA a 1:5 fraction respect to Zeolite A is estimated considering the actual ratio of the two ingredients in standard detergents.

Because of the absence of any experimental evidence for this hypothesis we set up a laboratory study with the specific aim to measure experimentally whether Zeolite A and PCA, at concentrations close to those indicated by Pettine et al. (1992), can have a role, if any, in the formation of mucilage. In particular, the quality and quantity of mucous aggregates produced by the diatom *Cylindrotheca closterium* in presence and absence of these detergent ingredients were measured and compared for different test conditions.

The choice of the species used as inoculum in our tests is dictated by the fact that this diatom is the most abundant organism found incorporated in the macroaggregates collected in the Gulf of Trieste (Monti et al., 1993) and is believed to be one of the responsibles for the mucilage formation (Monti et al., 1992).

Furthermore, previous experimentation carried out in laboratory has shown that this species is able to reproduce inside the mucous aggregates (Monti and Welker, unpublished data) and produces high amounts of mucous aggregates under controlled conditions, when subjected to stress such as nutrient starvation (Monti et al., 1994; Welker and Monti, 1994).

MATERIAL AND METHODS

Experimental conditions

The experiments were performed under controlled conditions at 20°C (± 0.5 constant temperature) and under a 16:8 h light/dark cycle (under cool white fluorescent light, scalar irradiance = 0.2×10^{16} quanta $sec^{-1}cm^{-2}$) that resemble the natural conditions under which a large numbers of mucous aggregates were observed (Monti et al., 1993). The seawater was collected 8 miles offshore in the Gulf of Trieste at intermediate depth (10 m) in February and November 1993. The water was filtered with a 0.45 µm filter and stored in darkness at 20°C for five months. We used nutrient depleted aged seawater because during previous tests (Welker and Monti, in press; Monti et al., in press) we observed the highest release of exudates by the species *C. closterium* when stress conditions such as nutrient starvation were applied (Fig. 2).

Four experiments have been carried out. In each experiment 7 transparent plastic tanks (20 l each) have been used, 2 for the blank and 5 for the tests.

10 liter of aged and filtered seawater was added to each tank. Under the experimental conditions described all tanks were first inoculated by *C. closterium* and then only the test tanks were spiked by the detergent ingredients under study.

No stirring was used during the experimentation because, as already observed, these are the most favourable conditions for macroflocs to be performed (Monti et al., 1995).

Fig.1 - Macroaggregates appeared in the Gulf of Trieste in 1991.

Fig.2 - Light microscope micrograph of *C. closterium* (Ehr.) Reimann and Lewin.

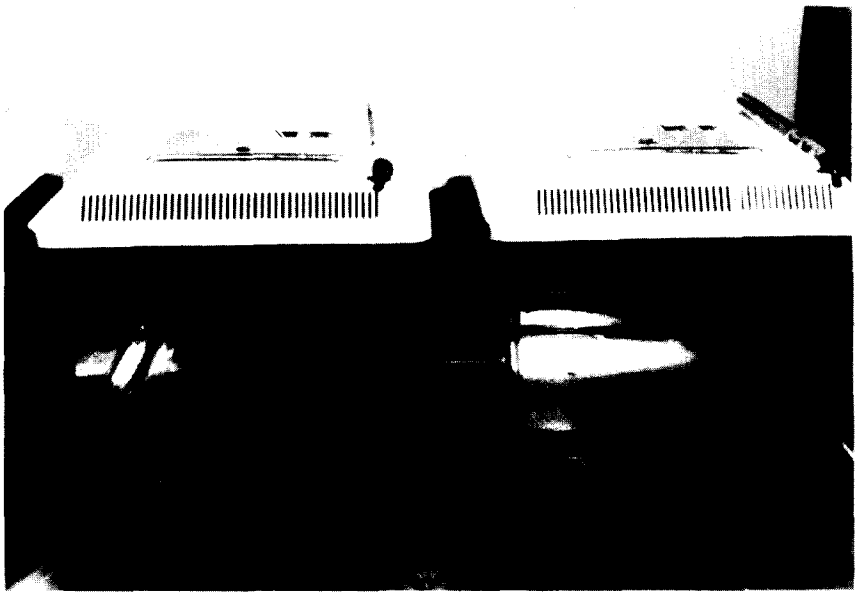

Fig.3 - Plastic tanks with mucous aggregates on the bottom.

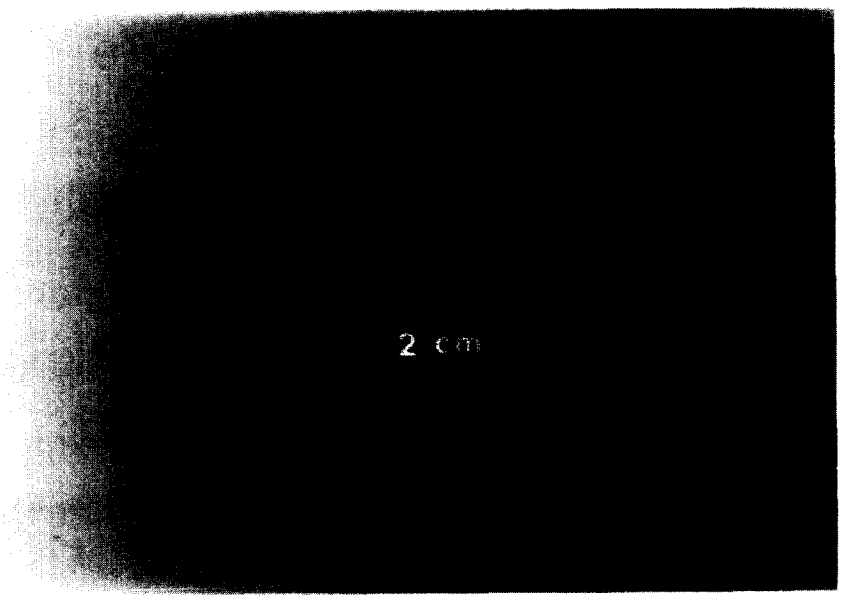

Fig.4 - Mucous aggregates formed (in tank n.° 7) under controlled conditions

The experiments were conducted over a time period of 15 months.

Microalgal inoculum

C. closterium was isolated from mucous aggregates collected at intermediate sea depth at the above station on July 24, 1991. *C. closterium* was mantained in batch culture at 20°C and under a 16:8 light/dark cycle (under cool white flourescent light, scalar irradiance = $0.2x10^{16}$ quanta $sec^{-1}cm^{-2}$) in enriched seawater (f/2 medium according to Guillard, 1975). The species before being inoculated was transferred to 16°C and lower light intensity (under cool white flourescent light, scalar irradiance = $0.1x10^{16}$ quanta $sec^{-1}cm^{-2}$) because, as previous tested, these stress conditions can enhance exudate production (Monti et al., 1995). In each tank the same predetermined concentration of *C. closterium*, in its exponential growth phase, was inoculated (1.000.000 cell/dm^3) on the first day of the experiments. The inoculum of cells in exponential growth phase in depleted aged seawater seems to accelerate the reaching of the stationary phase in comparison to the normal batch cultures. The formation of visible flocks is probably due to a sudden release of the components accumulated during the exponential growth phase (Welker and Monti, 1994).

Zeolite A and Poly Carboxylic Acid (PCA)

Detergent-grade Zeolite A ($Na_2O \cdot Al_2O_3 \cdot 2SiO_2 \cdot 4H_2O$) was equilibratedunder pH and temperature control in seawater for a week. A Ca/Na/Mg Zeolite was thus obtained whose molar composition by analysis resulted to be:

Ca/Na/Mg Zeolite	Na_2O	CaO	SrO	MgO	Al_2O_3	SiO_2
mol fraction (sc)	0.46	0.15	0.11	0.26	1.0	1.97

This produced the standard sample of Zeolite A with a water content of 19.8 % and an average particle size (μm) between 3.5 and 4.5 used in our experimentation. From the standard sample a fraction (ca. 5%) of a fine particle sample with an average particle size of 1.25 μm but with the same composition was also obtained.

As to PCA a detergent-grade product constituted by a copolymer of acrylic and maleic acid with an average molecular weight of 70.000 was used as standard sample. Also in this case a short chain sample of PCA, with an average molecular weight of 10.000, was taken into account.

Suspension of the Zeolite A and Polycarboxylate (PCA) system

Fresh suspensions were prepared adding to 1 liter deionized water under ultrasonic vibration, first 10 mg of PCA and then the right amount of Ca/Na/Mg Zeolite in order to have a 1:3 and 1:5 ratio of the two ingredients. 33.3 or 20 ml of this suspension was added to the 10 liter seawater contained in the test tanks. The resulting concentrations in the tanks are thus: ratio 1:3, PCA 33 μg/l and Zeolite 100 μg/l; ratio 1:5, PCA 20 μg/l and Zeolite 100 μg/l. These concentrations correspond to those hypothesized by Pettine et al. (1992) to be present at the river Po mouth (the worst case scenario).

Four experiments were carried out. In the 1° experiment standard PCA and standard Zeolite A were added in a single step at the 1:3 ratio, while the ratio was 1:5 in the 2° experiment. In the 3° experiment standard PCA and standard Zeolite A at 1:5 ratio were added in 6 steps additions during the first 6 days of the experiment. In the 4° experiment short chain PCA and fine particle Zeolite A were dosed in a single step.

An overview of the dosage is summarized below:

	Experimentation			
	1°	**2°**	**3°**	**4°**
Ca/Na/Mg Zeolite, standard, μg/l	100	100	100	-
Ca/Na/Mg Zeolite, fine, μg/l	-	-	-	100
PCA, standard, μg/l	33	20	20	-
PCA, short chains, μg/l	-	-	-	20
Single step addition	x	x	-	x
Stepwise addition	-	-	x	-

The Zeolite A and PCA were provided by the producing industries.

Seawater analyses

Every 3-4 days samples of seawater were collected for the following analyses:
- counting of phytoplankton cells which were performed under an inverted microscope Leitz Fluovert at a magnification of 320 X according to Uthermöl method (1958);
- particulate organic Carbon (POC) and nitrogen (PON) which were performed by a Perkin Elmer 2400 CHNS/O Elemental Analyzer after treatment of sample with HCl 1N and drying at 55°C. Concentration of POC and PON were measured by filtration of 100 ml on a precombusted (3 h, 450°C) 25 mm Whatmann GF/F glass fiber filter (0.7 μm).

The Cellular Carbon Content (CCC) was derived from POC divided by the cells number.

Mucus analyses

Upon appearance (7-15 days) the mucus produced in each tank (Fig. 3) was gently collected with a plastic microtube (0.3 mm I.D.) then placed in a glass Petri, measured, photographed (Fig. 4) and analyzed as follows:
- quantitative analyses which were performed by staining the mucus with Rose bengal, followed by vigorous stirring in a vortex mixer, and filtering through a 0.45 μm HA millipore filter. The filters were dried at 70°C for about 1 h, placed on glass slides, cleared with several drops of immersion oil, and examined at a magnification of 1000 X. Depending on the abundance of material on the filters, in 10 to 45 randomly selected fields the cells were counted;
- dry weight determination obtained by filtering onto preweighed filters, dried as described above, and prior to mounting the filter on a microscopy slide, reweighed to the nearest 0.1 mg. Using the dry weight and the volume, the density, porosity and excess of density were calculated, according to Alldredge and Gotschalk (1988);
- POC and PON analyses on mucous aggregates which were performed as described above.

Multivariate analyses

At last multivariate analyses were performed using Lagonegro and Feoli (1985) software. Data obtained from each experiment were organized in a matrix (variables/samples), then normalized and processed by using Cluster analysis (Sum Square Agglomeration) based on euclidean distance matrix between samples. Data related to mucus were organized in one matrix (5 variables/28 samples) then standardized and treated as the other ones. Also on mucous data Principal Component Analysis has been performed. The scattergram of principal components, including both variables and samples, has been obtained with BIPLOT method.

RESULTS

All the water analysis results are collected in Table 1 and the mucus characteristics in Table 2.

Seawater analysis

Algal concentration

In the 1° experiment, maximum algal concentration appeared on the seventh day with the only exception of one of the tests (n. 7) that reached the maximum at 11th day and one of the blanks that constantly decreased (Tab. 1).

In the 2° experiment, maximum was reached on the 11th day with the only exception of one of the tests (n. 1) that reached the maximum on the last day (18th day).

In the 3° experiment, maximum was reached on the last day of the experiment (14th day) with the only exception of one of the tests (n. 3) that reached its maximum on the 4th day and then decreased constantly.

In the 4° experiment, in four tanks the maximum appeared on the last day (17th day) and in the other three on the 14th day.

1° experiment

days		blanks		tests				
		1	2	3	4	5	6	7
3	algal conc. cell/dm^3	158057	63429	81296	125071	125965	173313	93480
	POC µg/dm^3	2548.16	1170.36	1004.96	1292.36	1317.56	730.16	815.76
	CCC µg/cell	0.0161	0.0185	0.0124	0.0103	0.0105	0.0042	0.0087
7	algal conc. cell/dm^3	110777	159019	305531	184033	208154	238529	270890
	POC µg/dm^3	529.76	441.96	584.56	591.56	336.16	687.36	401.96
	CCC µg/cell	0.0048	0.0028	0.0019	0.0032	0.0016	0.0029	0.0015
11	algal conc. cell/dm^3	42882	37521	89681	89681	21441	166167	348414
	POC µg/dm^3	363.56	475.16	301.36	340.56	290.96	789.36	452.16
	CCC µg/cell	0.0085	0.0127	0.0043	0.0049	0.0136	0.0048	0.0013
15	algal conc. cell/dm^3	11842	12812	31412	35614	33982	37150	36052
	POC µg/dm^3	377.96	385.96	326.16	644.16	239.76	376.76	483.96
	CCC µg/cell	0.0319	0.0301	0.0104	0.0181	0.0071	0.0101	0.0134

2° experiment

days		blanks		tests				
		1	2	3	4	5	6	7
4	algal conc. cell/dm^3	148520	190246	180410	164318	223945	210406	239346
	POC µg/dm^3	1167.90	1162.10	616.90	626.10	728.90	656.30	400.90
	CCC µg/cell	0.0079	0.0061	0.0034	0.0038	0.0033	0.0031	0.0017
7	algal conc. cell/dm^3	34842	37521	155445	29461	209049	75042	104523
	POC µg/dm^3	349.70	275.90	349.70	369.30	397.90	493.50	556.90
	CCC µg/cell	0.0100	0.0074	0.0022	0.0125	0.0019	0.0066	0.0053
11	algal conc. cell/dm^3	58089	205474	658412	971963	734348	785270	535127
	POC µg/dm^3	484.30	280.30	476.10	517.70	433.50	488.90	249.10
	CCC µg/cell	0.0063	0.0014	0.0007	0.0005	0.0006	0.0006	0.0005
15	algal conc. cell/dm^3	141152	182247	337693	416309	480631	374321	436643
	POC µg/dm^3	289.70	297.70	425.70	501.90	522.70	626.50	512.30
	CCC µg/cell	0.0021	0.0016	0.0013	0.0012	0.0011	0.0017	0.0012
18	algal conc. cell/dm^3	223342	180606	276944	482418	513686	513686	497605
	POC µg/dm^3	398.10	345.70	506.50	727.70	406.50	451.70	293.30
	CCC µg/cell	0.0018	0.0021	0.0018	0.0015	0.0008	0.0009	0.0008

3° experiment

days		blanks		tests				
		1	2	3	4	5	6	7
1	algal conc. cell/dm^3	11614	41095	35402	11614	19654	10720	14294
	POC µg/dm^3	352.96	308.76	508.56	255.36	416.76	326.36	215.96
	CCC µg/cell	0.0304	0.0075	0.0144	0.0220	0.0212	0.0304	0.0151
2	algal conc. cell/dm^3	18761	21441	75043	33055	32161	21441	27894
	POC µg/dm^3	331.76	313.16	206.56	263.36	220.36	199.56	328.36
	CCC µg/cell	0.0177	0.0146	0.0028	0.0080	0.0069	0.0093	0.0119
3	algal conc. cell/dm^3	33948	233169	121498	78616	241209	41988	29481
	POC µg/dm^3	367.16	321.76	322.96	356.56	279.96	230.16	263.76
	CCC µg/cell	0.0108	0.0014	0.0027	0.0045	0.0012	0.0055	0.0089
4	algal conc. cell/dm^3	19654	85763	175993	108097	96484	33055	50922
	POC µg/dm^3	312.56	430.96	243.96	306.96	310.16	446.16	235.96
	CCC µg/cell	0.0159	0.0050	0.0014	0.0029	0.0032	0.0135	0.0046
5	algal conc. cell/dm^3	82190	114351	30374	28588	92910	51815	52709
	POC µg/dm^3	517.96	624.76	344.96	446.36	329.96	368.16	411.16
	CCC µg/cell	0.0063	0.0055	0.0114	0.0157	0.0036	0.0071	0.0078
7	algal conc. cell/dm^3	207261	43755	8040	98270	441323	275157	12507
	POC µg/dm^3	446.16	586.76	388.36	562.16	338.36	325.76	327.96
	CCC µg/cell	0.0022	0.0130	0.0483	0.0057	0.0008	0.0012	0.0262
8	algal conc. cell/dm^3	196541	64322	56262	124178	95590	479738	33055
	POC µg/dm^3	387.96	470.16	382.16	397.16	481.76	437.76	261.56
	CCC µg/cell	0.0020	0.0073	0.0068	0.0032	0.0050	0.0009	0.0079
11	algal conc. cell/dm^3	209941	74149	50028	262650	362707	468124	198327
	POC µg/dm^3	392.96	351.36	678.76	414.96	512.16	582.76	215.96
	CCC µg/cell	0.0019	0.0047	0.0136	0.0016	0.0014	0.0012	0.0011
14	algal conc. cell/dm^3	426136	261756	53602	527086	1060427	591409	904981
	POC µg/dm^3	672.96	584.16	428.56	896.76	617.56	604.96	639.56
	CCC µg/cell	0.0016	0.0022	0.0080	0.0017	0.0006	0.0010	0.0007

4° experiment

days		blanks		tests				
		1	2	3	4	5	6	7
3	algal conc. cell/dm^3	2680	7160	4467	10720	1580	18761	41988
	POC µg/dm^3	323.90	490.90	307.50	372.50	398.90	322.50	519.30
	CCC µg/cell	0.1209	0.0686	0.0688	0.0347	0.2525	0.0172	0.0124
6	algal conc. cell/dm^3	5360	22334	8040	33055	2680	12507	16974
	POC µg/dm^3	549.50	415.30	357.30	566.70	566.70	506.70	647.70
	CCC µg/cell	0.1025	0.0188	0.0444	0.0171	0.2115	0.0405	0.0382
10	algal conc. cell/dm^3	26580	232275	29481	38415	8934	33055	216195
	POC µg/dm^3	379.30	503.10	397.90	439.50	440.70	334.30	578.30
	CCC µg/cell	0.0133	0.0022	0.0135	0.0114	0.0493	0.0101	0.0027
13	algal conc. cell/dm^3	34841	67896	40202	25908	53802	24121	268223
	POC µg/dm^3	360.30	320.90	252.90	297.90	301.30	340.70	377.50
	CCC µg/cell	0.0103	0.0047	0.0063	0.0115	0.0056	0.0141	0.0014
14	algal conc. cell/dm^3	275157	470804	219786	226915	197434	244783	334119
	POC µg/dm^3	358.50	382.70	416.10	356.30	331.90	291.70	355.10
	CCC µg/cell	0.0013	0.0008	0.0019	0.0016	0.0017	0.0012	0.0011
17	algal conc. cell/dm^3	629824	32161	252823	62536	485096	90230	427029
	POC µg/dm^3	298.50	331.90	297.30	434.70	252.30	275.50	345.90
	CCC µg/cell	0.0005	0.0103	0.0012	0.0070	0.0005	0.0031	0.0008

Tab. 1. Algal concentration, POC and CCC values in blanks and tests in all the experiments.

1° experiment

mucus characteristics	blanks		tests				
	1	2	3	4	5	6	7
density g/cm^3	0.1901	0.1851	0.1797	0.2477	0.2231	0.1047	0.0900
porosity	0.845	0.849	0.854	0.799	0.819	0.915	0.926
ex. of density g/cm^3	0.031230	0.030400	0.029530	0.040890	0.036840	0.017210	0.014850
cell density cells/mg	506203	445517	699549	98213	177779	264474	555751
POC µg/cm^3	3821.75	1466.71	1098.34	657.74	2880.70	1358.56	1284.23
PON µg/cm^3	0.00	128.17	119.03	36.86	0.00	0.00	0.00
CCC µg/cell	0.007549	0.003292	0.001570	0.006697	0.016203	0.005136	0.002310

2° experiment

mucus characteristics	blanks		tests				
	1	2	3	4	5	6	7
density g/cm^3	0.0036	0.1053	0.0032	0.0943	0.0135	0.0075	0.1254
porosity	0.997	0.915	0.997	0.923	0.989	0.994	0.898
ex. of density g/cm^3	0.000595	0.017400	0.000533	0.015600	0.022400	0.001250	0.020800
cell density cells/mg	14014940	309193	16236689	227094	3495230	15210013	462265
POC µg/cm^3	1354.90	2249.31	1590.19	3800.70	1466.99	5218.54	2034.81
PON µg/cm^3	0.00	274.44	15.66	0.00	0.00	0.00	0.00
CCC µg/cell	0.000097	0.007275	0.000098	0.016736	0.000419	0.000343	0.004402

3° esperiment

mucus characteristics	blanks		tests				
	1	2	3	4	5	6	7
density g/cm^3	0.0424	0.0186	0.0207	0.0124	0.0094	0.0123	0.0108
porosity	0.986	0.985	0.983	0.990	0.992	0.990	0.991
ex. of density g/cm^3	0.007001	0.003070	0.003412	0.002040	0.000155	0.002024	0.001789
cell density cells/mg	2972208	4502708	2130479	2088038	2472877	1185995	1185149
POC µg/cm^3	2519.50	1166.10	853.91	921.48	3976.53	1042.47	538.47
PON µg/cm^3	32.47	0.00	0.00	0.00	450.22	0.00	0.00
CCC µg/cell	0.000847	0.000259	0.000401	0.000441	0.001606	0.000879	0.000454

4° experiment

mucus characteristics	blanks		tests				
	1	2	3	4	5	6	7
density g/cm^3	0.0081	0.0236	0.0083	0.0108	0.0174	0.0087	0.0085
porosity	0.993	0.981	0.993	0.991	0.986	0.993	0.993
ex. of density g/cm^3	0.001320	0.003850	0.001350	0.000160	0.002840	0.001430	0.001390
cell density cells/mg	6647339	1534057	3718681	2832100	1619768	3594147	1829656
POC µg/cm^3	2402.70	2097.51	3259.22	2180.03	2974.90	1776.80	1300.80
PON µg/cm^3	223.60	236.80	393.30	474.60	380.10	389.20	303.30
CCC µg/cell	0.000361	0.001367	0.000876	0.000769	0.001836	0.000494	0.000711

Tab. 2. Mucus characteristics in all the experiments.

Particulate Organic Carbon (POC)

In the 1° and 2° experiments the same decreasing trend was observed, even if in the 1st experiment the values were twofold those of the 2° one (Tab. 1). Also in the 4° experiment a similar trend was observed, with even lower values than in the 1° and 2° ones. Viceversa in the 3° experiment the maximum values were reached at the end of the experiment, even if a peak was observed on the 5th and 7th days. The values had the same order of magnitude as the 2° experiment.

Cellular Carbon Content (CCC)

We observed that the Cellular Carbon Content changed during the experiment and among the experiments (Tab. 1). Particularly, in the 1° experiment, the values, ranging between 0.0013 µg/cell and 0.0319 µg/cell, showed a minimum on the 7th day in all the tanks and the maximum on the last day, with the only exception of the tank n. 5.

In the 2° experiment the values, ranging between 0.0005 µg/cell to 0.0125 µg/cell, showed the maximum at the 7th day with the exception of tanks n. 3 and n. 5 which constantly decreased .

In the 3° experiment four tanks reached the maximum on 5th -7th days while the other three showed an almost constant decrease. The values ranged between 0.0006 µg/cell and 0.048 µg/cell.

In the 4° experiment we observed a constant decrease in all the tanks and the values ranged between 0.0005 µg/cell and 0.25 µg/cell.

Mucus production

The same number (ranging from 3 to 5) of aggregates of the same dimension (ranging between 0.3-0.6 cm), appeared simultaneously both in the blanks and in the tests in each experiments, generally around the 7th day in the 1°, 2° and 4° experiments and on the 15th day in the 3° experiment with the fractioned addition. The volume of the aggregates observed ranged from $4x10^{-4}$ to $35x10^{-4}$ cm^3.

Mucus characteristics

Cell density

In the 1° experiment the cell density values within the aggregates did not reach $1x10^4$ cells/mg dry weight, in the 2° experiment the values were extremely variable, while in the 3° and 4° experiments they were more homogeneous and generally ranged between $1x10^6$-$7x10^6$ cells/mg dry weight (Tab. 2).

Density, Porosity and Excess of Density

The density and porosity values were very variable among the blanks and the tests and among the experiments (Tab. 2). The excess of density values were always higher than the seawater density. Only in the 4° experiment the excess of density values were lower both in the tests and in the blanks, for this reason the mucous aggregates were lighter than in the previous experiments.

Particulate Organic Carbon (POC) and Particulate Organic Nitrogen (PON)

Particulate Organic Carbon (POC) measured in the aggregates showed a high and constant variability among the experiments and tanks, ranging between 538 µg/cm^3 and 5219 µg/cm^3.

Particulate Organic Nitrogen (PON) was present in very low concentrations in the first three experiments while in the last one it showed slightly higher and more stable values (Tab. 2).

Cellular Carbon Content (CCC)

Cellular Carbon Content (CCC) showed a high and constant variability among the experiments and tanks ranging between $0.0097x10^{-2}$ µg/cell and $1.62x10^{-2}$ µg/cell (Tab. 2).

DATA ELABORATION

Cluster Analysis applied to the matrix cellular density/samples pintpointed that in the 1° and 2° experiments the blanks appeared clearly separated from the tests (Fig. 5). For the matrix POC/samples the blanks appeared distinguished from the tests in the 2° and 3° experiments. For the matrix CCC/samples only in the 1st experiment the blanks appear different from the tests.

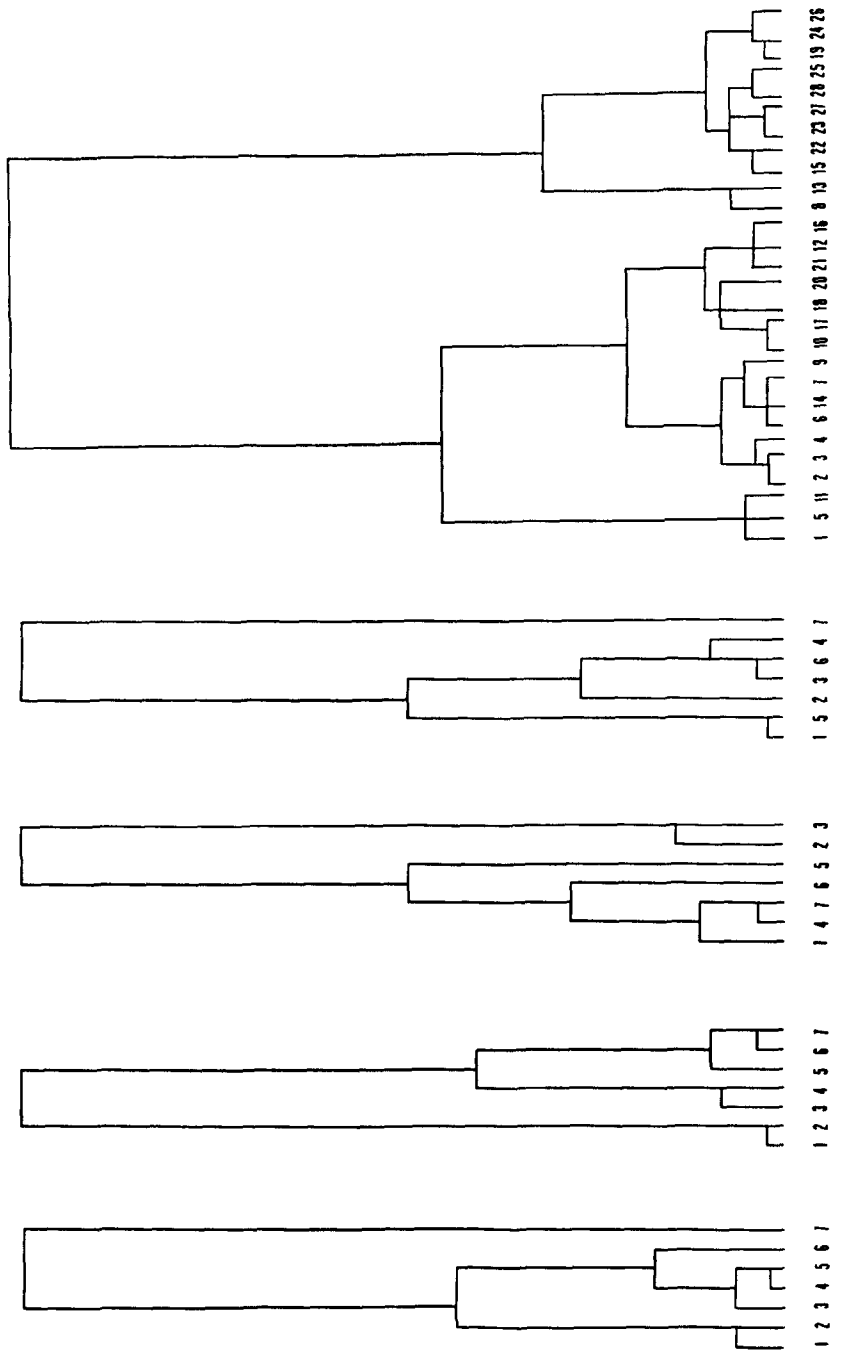

Fig.5 - Dendrogram obtained by processing algal concentration matrix for each experiment with Cluster Analysis.

Fig.6 - Dendrogram obtained by processing mucus characteristics matrix with Cluster Analysis.

Summing up: as regards the cellular growth and characteristics the multivariate analyses confirmed as above described, namely that the blanks were well differentiated particularly in the 1° experiment and to a lesser extent in the 2° and 3°;

As regard the matrix of mucous aggregates characteristics the Cluster Analysis did not show significant differences between the blanks and the tests in any of the experiments (Fig. 6). The Principal Components Analysis confirmed the absence of differences between the blanks and the tests and also among the various experiments (Fig. 7).

The only significant correlation observed was between POC and CCC, less significant negative correlation existed between cell density and excess of density and between cell density and CCC.

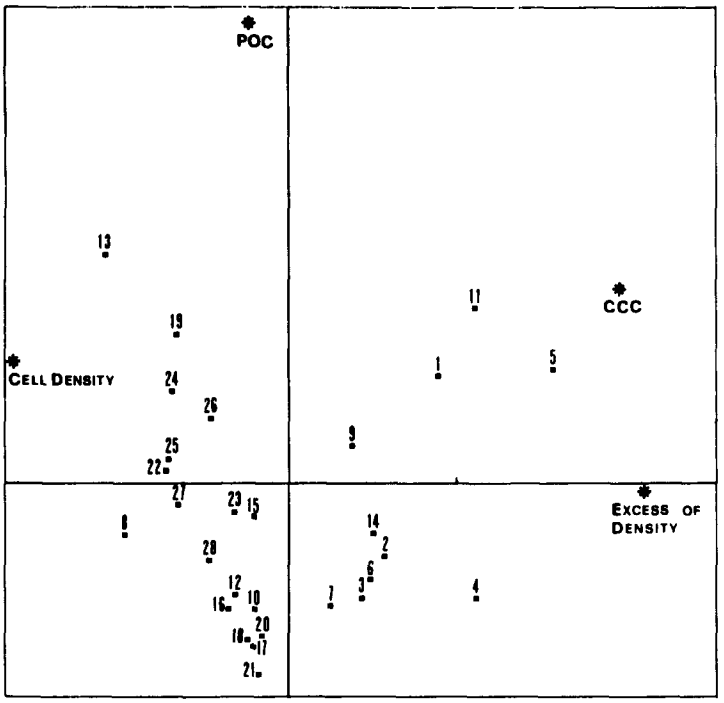

Fig.7 - Scattergram obtained by processing mucus characteristics matrix with Principal Component Analysis.

DISCUSSION AND CONCLUSION

On analysing the results it appeared that in the 1° experiment the microalgal cells reached the maximum 4 days before those of the 2° experiment. In the 3° experiment slower growth appeared as in the 4th. The only significant differences observed, between blanks and tests, were in the 1° and 2° experiments in which the cell density values in the blanks were constantly lower than in the tests.

Viceversa both POC and CCC data suggest that cellular densities in the blanks are lower, than in the tests. This fact may be due to the effect of the addition of aluminium silicate in the tests which could have enhanced the cellular division rate with the result of a higher number of cells with a lower CCC.

The mucous aggregates produced both in the blanks and in the experimental test tanks, appeared extremely variable for all the characteristics considered. The only evident results were that the CCC and the excess of density were generally higher in the 1° experiment than in the other ones.

The mucus produced in the 1° experiment, both in the blanks and tests, showed a lower cellular density with a higher CCC and, consequently, higher excess of density than in all the other experiments. This behaviour can be associated only with a particular physiological phase of the diatom *C. closterium*. In fact, even if we tried to follow the same protocol, both the nutritional situation and the growth phase can present differences within few

hours. Not only, but also the water's nutritional characteristics can change, even using aged seawater mantained in the same conditions, and consequently the results of the experiments can be affected in various ways.

We operated in small unstirred batch cultures because, as we have mentioned above, in previous years we observed the highest rate of aggregation just in these conditions. Of course, calm conditions of water can determine a high sedimentation rate of the Zeolite A and PCA system, but, as observed also by Pettine et al. (1992), aggregation can be possible only in stable water column conditions. In this experiment we did not consider the influences of shearing forces even if some Authors (Herndl et al. 1992) mention that the size of the aggregates may be seen as a function of shear-forces, but in any case can be considered barely pertinent in this respect.

Furthermore it must be kept in mind that our aged seawater was previously filtered throught 0.45 μm mesh and, consequently, it must have been free of particles larger than 0.45. This means that the only particles present in the tanks were derived from the addition of Zeolite A and PCA system. The ratio and quantity used in our experiments were the same as, or even higher than those given as hypothesis by Pettine et al. (1992), for the Northern Adriatic Sea.

The hypothesis of Pettine et al. (1992), in addition, does not take into account a series of facts (Kurzendörfer et al., 1994), namely:

a. most of sewage waters in the Po river catchment area are treated by STP (Sewage Treatment Plant), so that only a small fraction of Zeolite A (ca. 10%) and even less of PCA reaches surface waters and then possibly the Northern Adriatic Sea.

b. Zeolite A is a metastable product. In the environment it decomposes back to mineral constituents and contributes to give amorphous insoluble Ca-Al silicate phosphates, indistinguishable from other minerals common to natural waters and soil (Kuhm and Lortz, 1994)

c. PCA in natural waters becomes insoluble as Ca salts and is incorporated into sediments.

d. Po river conservative modelling calculations, based on the existing STP and river in-flow removal, predict a content for the Zeolite A - PCA system at the river mouth of 20 - 50 μg/l, contributing thus to the average suspended solids (SS) present at the river mouth (40 mg/l) by a maximum of 0.12 % (Cavalli and Clerici, 1994). Because of Zeolite A decomposition, the contribution to SS should be mainly under the form of amorphous Ca - Al silicate phosphates.

All these considerations support the thesis that the concentration of Zeolite A and PCA in the Northern Adriatic Sea should be well lower than that suggested by Pettine et al. (1992), and also used in our laboratory experimentation. We have consequentely worked adopting the worst case scenario.

Pettine et al. (1992) at last have made the hypothesis that the effect of the Zeolite A and PCA system on the aggregation and flocculation phenomenon consists in the production of lighter aggregates due to the lower density of those particles and their capability to produce flocks with greater porosity and, consequently, less density. In this respect another important result of our experiments may be considered the evidence that the amount of mucous aggregates is no different in blanks and tests and that no differences occurin density and porosity, expressed as excess of density, in the aggregates produced in blanks or tests.

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