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QUANTIFICATION OF MUCILAGE-ASSOCIATED SUSPENDED MATTER IN THE GULF OF TRIESTE (ADRIATIC SEA)

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ABSTRACT

Mucilage-associated suspended matter in the Gulf of Trieste has been quantified using different approaches during the 2000 event. Remote sensing, video recording and image analysis were used to assess the quantity of surfaced mucilage. The quantity of water column mucilage was estimated taking quantitative samples of mucilage using a peristaltic pump and using in situ *photography, video recording and image analysis. Samples of surface and water column mucilage were assessed for wet mass immediately after sampling, while for further analyses they were deep-frozen and analysed later. Our results indicate that during culmination of the 2000 event on average less than 2% of sea surface was covered by mucilage in offshore areas. The quantity of particulate matter in offshore surface mucilage was on average 220 mg carbon/L, however, mucilage was limited to less than 0.5 cm thick surface layer. Concentrations were significantly lower for the water column mucilage (47.6±1.7 mg C/L for mucilage clouds, 5.9±4.2 mg C/L for mucilage network). Integrating obtained values for the water column to a depth of 25 m we estimated the average quantity of mucilage-associated particulate matter in mid-June to be about 80 g C/m².*

Key words: mucilage, Adriatic Sea, quantification, video recording, remote sensing, image analysis, particulate matter accumulation

QUANTIFICAZIONE DELLA MATERIA SOSPESA ASSOCIATA ALLE MUCILLAGINI NEL GOLFO DI TRIESTE (MARE ADRIATICO)

SINTESI

Durante il manifestarsi delle mucillagini nell'anno 2000, con diversi approcci gli autori hanno quantificato la materia sospesa associata al fenomeno. A tale scopo sono state utilizzate immagini telerilevate da elicottero successivamente elaborate con programmi grafici (Adobe Photoshop, CorelPhoto-Paint) e con il programma per l'analisi di immagini satellitari ENVI. I campioni per l'analisi quantitativa delle mucillagini nella colonna d'acqua sono stati raccolti con l'aiuto di una pompa peristaltica. La quantità di mucillagini è stata calcolata come massa umida, sostanza secca e carbonio. I risultati dimostrano che durante la comparsa del fenomeno nel giugno 2000, in media meno del 2% della superficie marina è rimasta coperta dalle mucillagini. Nella colonna d'acqua la rete mucosa ha avuto un contenuto medio di 5.9±4.2 mg C/L, mentre le nubi mucose di 47.6±1.7 mg C/L. Gli autori hanno inoltre calcolato che nella colonna d'acqua di 25 metri la quantità di carbonio ha raggiunto, all'apice del fenomeno nel giugno 2000, valori medi di 80 g C/m².

Parole chiave: mucillagini, mare Adriatico, quantificazione, remote sensing, elaborazione di immagini, accumulo di materia sospesa

INTRODUCTION

Recent events (1988, 1989, 1991, 1997, 2000) of massive mucilage accumulation in the northern Adriatic have inflicted acute changes on the ecological dynamics of this marine system and have created serious problems for different human activities in the region. Due to the frequency of occurrence, geographical scale, subsequent ecological and socio-economic impacts as well as their intricacy, mucilage events have become a matter of high environmental concern. All this has prompted extensive research in the last decade and mucilage has become one of the priorities of the national marine research programmes of the Adriatic countries as well as that of major EU funded projects (Hopkins *et al.*, 1999). Numerous scientific papers have been published and up-to date knowledge has been synthesised in special volumes of scientific journals (Vollenweider *et al.*, 1995; Funari *et al.*, 2000). Yet, many aspects of this peculiar phenomenon, which in its extensiveness seems to be unique to the northern Adriatic, have remained unresolved.

Many researchers consider massive accumulations of mucilage as an extreme case of particulate matter aggregation. Aggregates are ubiquitous in marine and limnetic environments and are formed mainly from smaller particles in the water column. Coagulation is an important mechanism controlling aggregate size distribution. However, other features are also considered to be important in large aggregate formation such as abundance of TEP (transparent exopolymer particle) (Alldredge *et. al.*, 1993), presence of particular phytoplankton group/species (Kiorboe & Hansen, 1993), spontaneous assembly of dissolved polymers forming stable gels (Chin *et al.*, 1998), and sustained residence of aggregates in surface waters (Riebesel, 1992). Classical coagulation theory underlines three main mechanisms for particle collision, an important step in formation of aggregates: laminar and turbulent shear, Brownian diffusion and differential sedimentation. To increase their size particles have to adhere upon collision. Adhesion rate depends mainly on the stickiness of particles; thus, formation of large aggregates is determined by both quantity and quality of available particles (Alldredge & Jackson, 1995). Resistance to disaggregation and reduced settling velocity are further conditions for accumulation of aggregates in the water column. Particle spectra in the oceans were found to be in general agreement with those expected from coagulation theory (Jackson & Burd, 1998). This theory, however, appears unsatisfactory in the case of the mucilage phenomenon.

Due to the dramatic spatial extent of mucilage, some events affected areas of over 10,000 km^2 and accumulated at the surface in layers that were several cm thick (Stachowitsch *et al.*, 1990; Rinaldi *et al.*, 1995) it was suggested that mucilage-associated particulate matter reached very high values, although there were no field data to support this presumption. Variable forms and changeable vertical and horizontal distribution in the water column and at the surface make quantification of total mucilage-associated matter a very difficult task. The most promising approaches seemed to be *in situ* visual monitoring using underwater cameras (Rinaldi *et al.*, 1995), although classical methodology for assessment of suspended matter was also used (Faganeli *et al.*, 1995). Satellite remote sensing of the event in 1989 followed temporal changes of surface mucilage (Zambianchi *et al.*, 1992) while Berthon *et al.*, (2000) used a bio-optical data set collected by an off-shore oceanographic tower off Venice to study optical properties of the water column in the presence of mucilage. Neither of these studies tried to estimate the quantity of mucilage-associated particulate matter.

In order to provide quantitative estimates of mucilage accumulation in the water column and on the surface we investigated this phenomenon in the Gulf of Trieste. Using different approaches *i.e.* remote sensing, video recording and quantitative sampling we sought to quantify different forms of mucilage aggregates.

MATERIALS AND METHODS

We have made quantification of mucilage-associated suspended matter on the basis of data collected during event in early summer (June-July) of 2000.

Quantification of surfaced mucilage

The quantity of surfaced mucilage was assessed from pictures taken by video camera that was mounted on an aircraft. The eastern part of the Gulf of Trieste was over flown by helicopter and the sea surface surveyed for mucilage on 10, 11 and 14 June 2000. The survey on 14 June followed the path depicted in figure 1 (Plate I). It was carried out between 1 and 2 p.m. The height of this flight was between 400 and 500 m, and the velocity of the aircraft between 110 and 120 km h^{-1}. The snapshots of the video were taken every 15 seconds and we got 234 pictures; among them we selected 154 for further statistical analysis. The remaining pictures were not taken into account because they either partly showed land or displayed the same scenes due to aircraft manoeuvring or path repetition.

To estimate the spatial coverage of mucilage in the studied area, analysis of pictures taken from the aircraft, was carried out using two procedures. In the first, we utilized graphical software (Adobe Photoshop, Corel Photo-Paint) to manually paint mucilage areas white and the mucilageless sea surface black. The second method of picture elaboration was thresholding using the software tool ENVI for image elaboration. We used contour plot to determine the threshold between mucilage and the sea surface without mucilage, applying one contour on which we varied the levels. Before further classification of images we carried out the "decorrelation stretch" to remove the high correlation commonly found in multispectral datasets. The 'isodata' method, *i.e.* unsupervised classification was then applied to destretched images. This method calculates class means and iteratively clusters the remaining pixels using minimum distance technique (Tou, 1974). From all classified images, descriptive statistics and histograms were calculated.

Example of application of these procedures to the same situation at sea is presented in figures 2-4 showing the original video snapshot of mucilage masses at sea (Fig. 2); manual elaboration of the picture, using graphical software (Fig. 3); and corrected 'isodata' (unsupervised classification) picture after thresholding and application of decorrelation stretch (Fig. 4). Results obtained by different methods were rather similar (Tab. 1).

Tab. 1: Comparison of different methods for assessment of mucilage coverage applied to the situation in figure 2.
Tab. 1: Pokrovnost morske površine s sluzjo na primeru s slike 2 (ocenjena z različnimi metodami).

Method	Mucilage (% of surface)	Mucilageless (% of surface)
Manual separation	6.9	93.1
Thresholding	6.0	94.0
Unsupervised classification	7.0	93.0

On 11 June we also followed changes in the coverage of the sea surface by mucilage over a day (from 8 a.m. to 8 p.m.) using still photography of the same near-shore location. Pictures were elaborated manually using graphical software (Fig. 6).

Surfaced mucilage was sampled manually using 0.5 to 1.5 l polyethylene bottles for quantification of suspended matter and its quantity determined using the same procedures as for water column samples (see below).

Quantification of the water column mucilage

Field surveys, *in situ* video-recording and sampling of the water column mucilage were carried out approx. weekly from 6 June, when mucilage was first observed by divers, to 26 July, 2000, when no mucilage aggregates of any form were seen in the studied area. Field observations were most frequent from 11 to 21 June, when the mucilage event peaked in the studied area. Therefore, quantification of different mucilage forms and layers (surfaced mucilage, water column mucilage network and clouds) was based on the data collected during this period.

Sampling. Concentration of the mucilage-associated suspended matter in the water column was estimated on the basis of quantitative sampling using a peristaltic pump that was operated by SCUBA diver. While sampling, we distinguished two types of water column mucilage (Malej *et al.*, 2000): 1) loose network of mucous material stretching in layers up to 10 m thick, that were considered "quasi homogeneous suspended matter field", and 2) mucilage "clouds", that were spatially well defined and were surrounded by particle-poor water. In both cases, 35 litres of mucilage matter were sampled into large glass containers that were brought to the laboratory within one hour. Quantification of the mucilage network was derived from the premise that sampling by pump provided an average sample of the homogeneous layer. In the case of mucilage clouds our methodology provided data on particulate matter associated with these clouds. Additionally, traditional sampling using 5 L Niskin bottles at standard depths was carried out.

Analyses. Collected samples were analysed in the laboratory using the same procedure for all three types of mucilage (surfaced, mucilage network, mucilage clouds). Mucilage-associated suspended matter, that was collected in large (35 litre) containers, in all cases compacted in the surface layer. Further analysis consisted of determination of the volume of this compacted mucilage layer (cms-concentrated mucilage sample) using measuring cylinders, followed by an estimate of the remaining, presumably mucilage-free, water (mfw). Quantitative subsamples were then taken from both (cms and mfw) for suspended matter determination. The ratio of total sample volume to cms volume was calculated and used in calculation of the integrated water column mucilage-associated particulate matter. For the purposes of this paper conversion factors from volume to dry mass and carbon content of mucilage were taken from our previous study, done during the 1997 event (Flander, 1999; Malej *et al.*, 2000): mean dry mass content of mucilage was 1.6 (±0.9)% of wet mass; and mean carbon content of 10.7 (±3.2)% of dry mass.

Two examples of pictures, taken in the water column by SCUBA divers using an underwater camera, were also analysed applying the same methodology of picture analysis as for the surfaced mucilage. The method applied to water column video snapshots was unsupervised "isodata" classification (see above).

RESULTS AND DISCUSSION

Time-course of the mucilage event in 2000

In contrast to previous mucilage events in the last decade (1991 and 1997), the 2000 phenomenon appeared earlier in the season. While in 1991 and 1997 the water column mucilage accompanied by surface masses were observed in mid-July (Flander Putrle *et al.*, 2000), during the 2000 event visible mucilage has already appeared in the water column in the first days of June with surface accumulations starting from 10 June. The phenomenon reached its peak in mid-June and dissipated by mid-July. Already by 28 June some portion of mucilage has settled to the bottom and after mid-July mucilage was not observed in the water column. Mucilage completely disappeared from the studied area by the end of July. Most results used for calculation of mucilage-associated matter are based on samples collected from June 11 to 21. In this period we carried out measurements using all reported techniques (remote sensing, quantitative sampling of different mucilage forms, viderecording, standard oceanographic techniques).

Quantification of surfaced mucilage

The spatial extent of the surfaced mucilage was assessed when the phenomenon reached its culmination in the studied area, *i.e.* on 14 June. According to our previous observations, which indicated that ascension of mucilage to the surface occurred predominantly during late morning, we made our estimate of spatial coverage of the sea surface on the basis of measurements done between 1 and 2 p.m. We assumed that later during the day the quantity of surfaced mucilage did not increase significantly.

Results of descriptive statistics of 154 snapshots taken from aircraft are given in table 2 and figure 5. Only about 10% (16) images showed mucilage coverage greater than 3% and the situation presented in Table 1 and figure 2 *i.e.* about 7% coverage was rather rare. In a majority of the images (120) coverage by mucilage was less than 2% of sea surface and it was nil in 12 snapshots. Applying an overall mean coverage of 1.6% of sea surface by mucilage (Tab. 2) to the whole Gulf of Trieste (about 600 km^2) gives an estimated mucilage-covered area of about 10 km^2.

Tab. 2: Descriptive statistics of 154 snapshots, taken from aircraft on 14 June 2000.
Tab. 2: Opisna statistika obdelanih videoposnetkov površinske sluzi junija 2000.

Item	% coverage by mucilage
Minimum	0
Maximum	21.0
Mean	1.6
1st quartile (Q1)	0.3
Median	1.0
3rd quartile (Q3)	1.9
Variance	6.2
Standard deviation	2.5

Higher surface coverage was observed along shores during late afternoon hours where the surfaced mucilage accumulated because it was driven by sea breeze towards the shoreline. Figure 6 gives an indication of the variability of surface coverage by mucilage in such a near shore area. Coverage was nil before 9 a.m., increased to about 30% at 11 a.m. and reached over 50% between 01 and 02 p.m. These data indicate that past estimates, if based on near shore data, probably overestimated the quantity of surfaced mucilage. Alternately, previous events were more intense.

Wet, dry and carbon mass of surfaced mucilage. Measurements of mucilage wet mass gave rather different values for surfaced mucilage collected offshore as compared to values of more compacted mucilage that accumulated near shore. Offshore mucilage had an average wet mass of 130±2 g per litre of seawater in contrast to a significantly higher mean wet mass of that collected near shore (870±18 g per litre sea water). Applying conversion factors (dry mass to wet mass) of 1.6% and 10.7% carbon content, estimated average dry mass was 2.1 g DW/L and 0.22 g C/L for offshore surface mucilage. Respective values for mucilage that accumulated near shore were 13.9 g DW/L and 1.5 g C/L. However, these extremely high particulate mass values were limited only to top layers thinner than 0.5 cm offshore and 5 cm near shore.

Surface mucilage assessed by this technique represented only a fraction of the water column mucilage that ascended to surface. Therefore we tried to quantify particulate mass of the water column aggregations.

Quantification of the water column mucilage

Particulate matter. Average concentration of particulate matter in the water column during the mucilage event estimated on the basis of Niskin samples was 3.8±1.8 mg DW/L. Values were higher below 20 m depth (5.6±2.1 mg DW/L) than in the upper 15 m (3.0±0.9 mg DW/L). Using the conversion factor of 3% carbon to dry mass, average estimated particulate carbon was 0.1 mg C/L.

Mucilage network. Quantification of particulate matter associated with the mucilage network on the basis of sampling by pump indicated a mean mucilage wet mass of 3.5±2.6 g WW per litre seawater. Using the same conversion factors as for surface mucilage (1.6% wet to dry mass, 10.7% carbon content) we estimated that this mucilage layer on average contained 55.6±40 mg DW/L and 5.9±4.2 mg C/L. Over the time from June 10 to June 20, the wet mass of mucilage network increased from 1.2 g WW/L (2.0 mg C/L) to 7.1 g WW/L (12.1 mg C/L) indicating that "older" mucilage had a greater particulate mass per unit volume. In contrast, sampling this layer during the event using Niskin bottles gave significantly lower DW of 5.2±3.9 mg DW/L (0.2±0.1 mg C/L).

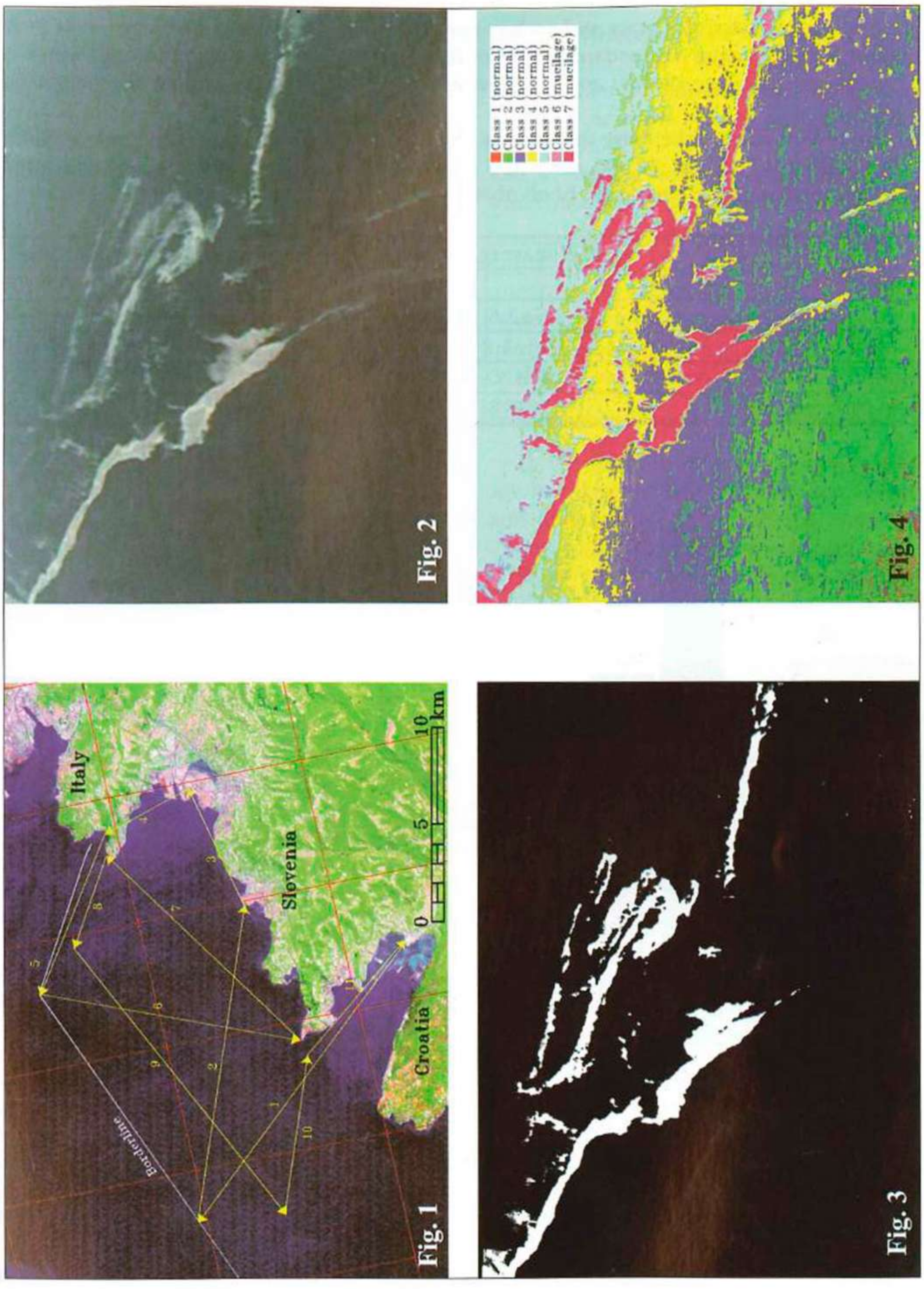

PLATE I / TABLA I:

Fig. 1: The helicopter flight path during video recording of the southeastern part of the Gulf of Trieste.
Sl. 1: Pot helikopterja med snemanjem površinske sluzi v jugovzhodnem delu Tržaškega zaliva.
Fig. 2: Original video snapshot of surface mucilage taken from aircraft on 14 June 2000.
Sl. 2: Originalen videoposnetek površinske sluzi 14. junija 2000.
Fig. 3: Manually elaborated video snapshot from figure 2 using graphical software.
Sl. 3: Grafično obdelan videoposnetek s slike 2.
Fig. 4: Corrected unsupervised classification ('isodata' method) - decorrelation stretched situation on Fig. 2.
Sl. 4: Prikaz površine morja z videoposnetka na sliki 2 po opravljenem klasifikacijskem postopku.

Tab. 3: ***Comparison of wet mass, dry mass and carbon concentrations the during mucilage event in 2000: particulate matter (Niskin samples, taken at standard depths of 0.5, 5, 10, 15, 20, m above bottom); water column mucilage: mucilage network, mucilage clouds; and surfaced mucilage: a) offshore samples, b) concentrated mucilage along shore.***
Tab. 3: Primerjava mokre mase, suhe mase in ogljika v sluzi l. 2000: lebdeča snov (vzorčenje z Niskinovo posodo na standardnih globinah 0,5, 5, 10, 15, 20 m in nad dnom); sluz v vodnem stolpcu: sluzasta mreža in sluzasti oblaki; površinska sluz: a) na odprtem morju, b) ob obali.

	Wet mass (g/L)	Dry mass (mg/L)	Carbon (mg/L)
particulate matter (Niskin)		3.8±1.8	0.1±0.09
mucilage network	3.5±2.6	55.6 ±40	5.9±4.2
mucilage clouds	27.8±1.8	445±16	47.6±1.7
a) 'offshore' surfaced mucilage	120±20	2,100	220
b) 'along shore' surf. mucilage	870±18	13,900	1,500

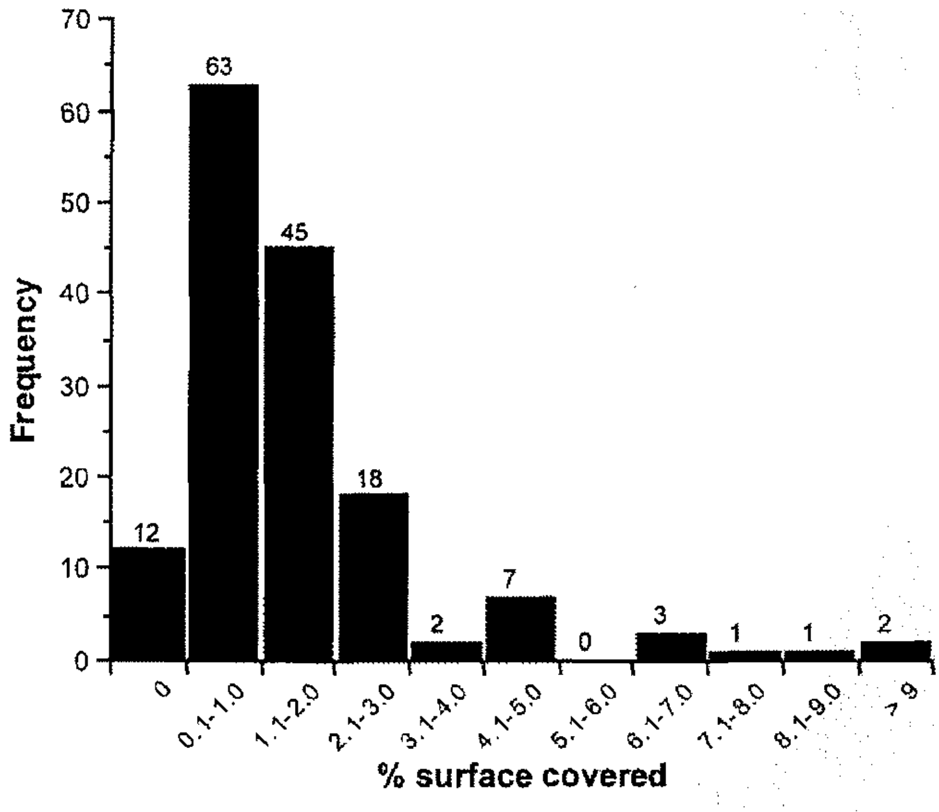

Fig. 5: Frequency distribution of results on coverage of sea surface by mucilage obtained by analysis of 154 video snapshots taken on June 14, 2000.
Sl. 5: Frekvenčna razporeditev rezultatov obdelave 154 videoposnetkov, ki kažejo pokrovnost s sluzjo 14. junija 2000.

Mucilage clouds. Wet mass values were the highest for mucilage clouds with an average of 27.8±0.8 g WW/L. Dry mass in mucilage clouds was 445±16 mg DW/L and carbon concentration 47.6±1.7 mg C/L. On one occasion (20 June; excluded from calculations of average mass) we measured extremely high wet mass (170 g WW/L) in the mucilage cloud which was similar to values of surfaced mucilage. Converting the last value to carbon indicated particulate carbon concentration in the mucilage cloud of over 290 mg C/L.

Summary of quantification of particulate mass associated with mucilage is given in table 4.

Clearly, there are rather big changes in volume-specific wet mass, dry matter and carbon for different mucilage forms that ranged over two orders of magnitude for different mucilage forms. Despite a very high volume-specific value of surfaced mucilage, its limited spatial extent (average < 2% sea surface) and concentration in very thin layer (< 0.5 cm offshore) makes its contribution to the total particulate matter unimportant.

Compared to our values, Müller-Niklas *et al.* (1994) reported significantly higher dry mass and carbon values for water column mucilage, presumably mucilage clouds, during the 1991 event: average dry weight was 1,293 mg DW/L and carbon 192.85±71.6 mg C/L. Values that they reported for water column mucilage are more comparable to our estimates for offshore surfaced mucilage.

To assess the particulate matter in the whole water layer with mucilage clouds including particle-poor surrounding water, additional information is needed: estimate of cloud size, shape and abundance. As a first approximation we defined the typical cloud as a cylinder with conical endings with an average volume of 0.25 m^2 (about 250 L). Cloud abundance was assessed by counting them in the diver's observation field, which was defined as a rotary ellipsoid that has a volume of about 1000 m^3. The number of clouds in this volume varied from nil to 8 with an average of 2. Taking into account quantitative data on carbon mass, we may estimate that the water layer with mucilage clouds had an average concentration of about 0.4 mg C/L. Due to large variability in number, size and mass of particulate matter associated with mucilage clouds this value should be considered as very approximate.

To get an overall picture of the quantity of mucilage-associated particulate matter we integrated obtained values to a 25 m deep water column using as an example the situation observed in the period from 11 to 20 June 2000. For our calculation the following depths of different layers were used: upper 4 m deep layer containing marine snow (Niskin sample), followed by a 10 m deep layer of mucilage network, 6 m deep layer comprising mucilage clouds, that usually developed below the mucilage network layer, and 5 m deep bottom layer with marine snow. Calculation of the mucilage network layer was done taking into account concentration factor of 3 x 10^{-2} (= cms/cms+mwf, see methods section). Total integrated mucilage-associated particulate carbon was estimated to be 82 g C/m^2.

Quantification of the water column mucilage using video recording and image analysis. Applying the same procedure for quantification as for surfaced mucilage, two contrasting water column situations were analysed: the marine snow layer and the mucilage network (Figs. 7, 8, Tab. 4). Results indicated that this method could be a useful supplement to the quantification methodology described above.

Tab. 4: Videorecording and image analysis of water column mucilage. Unsupervised 'isodata' classification was applied to snapshots of the water column with only marine snow present and with mucilage network (Figs. 7, 8).
Tab. 4: Analiza videoposnetkov sluzi v vodnem stolpcu: situacija z morskim snegom in sluzasto mrežo (Sl. 7, 8).

Situation	% Mucilage	% Mucilageless
marine snow (upper water column)	1.1	98.9
mucilage network (description: see methods sec.)	18.3	81.7

In the water layer considered as 'quasi homogeneous mucilage network' elaboration of underwater images thus indicated mucilage coverage less than 20%. Unfortunately, this method was unsuitable for quantification of mucilage clouds. Using average carbon concentration determined for the mucilage network and applying 20% coverage to the whole water column we obtained an estimate of mucilage-associated particulate matter an order of magnitude lower than when using the integration method of different layers (see above).

CONCLUSION

Presented estimates, based on different methodologies, indicate a very high variability in the quantity of mucilage-associated particulate matter. Contrasting values were obtained for particular mucilage forms (mucilage network, clouds, surfaced mucilage) that varied over two orders of magnitude. An additional problem is the calculation of an integrated water column value since it requires information on the vertical distribution of different mucilage forms. Therefore, only a combination of different methods used in parallel could give more reliable quantification of total mucilage-associated particulate matter.

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Fig. 6: Coverage of sea surface by mucilage in near shore area followed from 8 a.m. to 8 p.m. on June 11, 2000. Data were elaborated using the same methodology as for airborne images.
Sl. 6: Pokrovnost morja s sluzjo v priobalnem morju, ki smo jo spremljali 11. junija 2000 od jutra do večera. Slike so bile statistično obdelane z enako metodologijo kot videoposnetki s helikopterja.

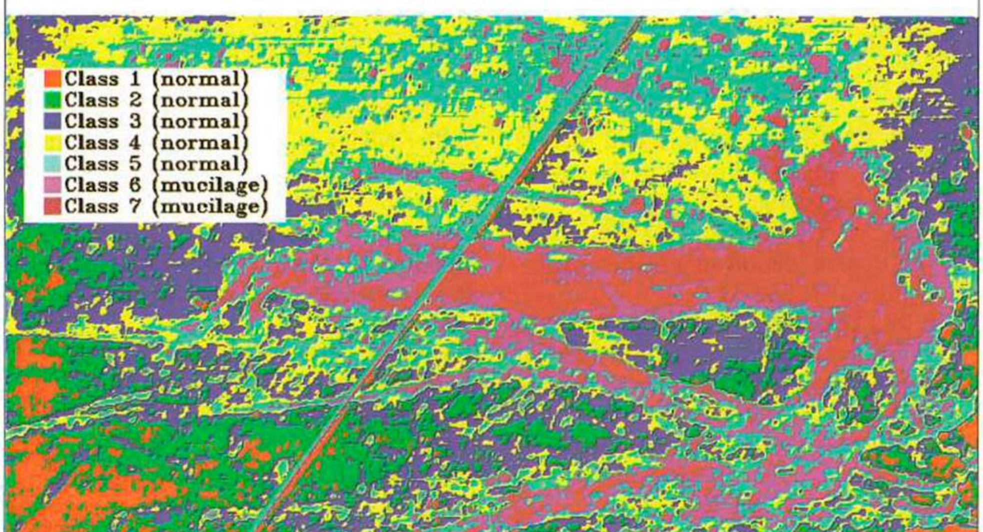

PLATE II / TABLA II:

Fig. 7: Video snapshot of the water column mucilage network.
Sl. 7: Videoposnetek podvodne sluzaste mreže. Posnetek je bil obdelan z enako metodologijo kot helikopterski posnetki.
Fig. 8: 'Isodata' unsupervised classification applied to figure 7. Classification is the same as for figure 4.
Sl. 8: Obdelan videoposnetek s slike 7; klasifikacija je enaka kot na sliki 4.

OVREDNOTENJE KOLIČINE SLUZI V TRŽAŠKEM ZALIVU (JADRANSKO MORJE)

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POVZETEK

Avtorji so z uporabo različnih pristopov ocenjevali količino lebdeče snovi, vezane v sluzastih agregatih Tržaškega zaliva. Leta 2000 so količino površinske sluzi ocenili s pomočjo snemanja iz helikopterja. Slike so analizirali s pomočjo grafične programske opreme (Adobe Photoshop, CorelPhoto-Paint) in programa za analizo satelitskih posnetkov ENVI. Kvantitativne vzorce sluzi v vodnem stolpcu so jemali s pomočjo peristaltične črpalke. Količina sluzi je bila ocenjena kot mokra masa, suha snov in ogljik. Rezultati so pokazali, da je bilo med pojavom sluzi junija 2000 v povprečju manj kot 2% morske površine prekrite s sluzjo. V vodnem stolpcu je sluzasta mreža povprečno vsebovala 5,9±4.2 mg C/L, sluzasti oblaki pa 47,6±1,7 mg C/L. Ocenjena je bila tudi integrirana vrednost ogljika v vodnem stolpcu 25 metrov, ki je junija 2000 med viškom pojava znašala okoli 80 g C/m².

Ključne besede: sluz, Jadransko morje, kvantifikacija, daljinsko zaznavanje, video zapis, akumulacija lebdeče snovi

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