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Technical Note: Comparison of storage strategies of sea surface microlayer samples

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Abstract

The sea surface microlayer (SML) is an important biogeochemical system whose physico-chemical analysis often necessitates some degree of sample storage. However, many SML components degrade with time so the development of optimal storage protocols is paramount. Using freshwater and saline SML samples from a river-estuary, we interrogated temporal changes in surfactant activity (SA) and the absorbance and fluorescence of chromophoric dissolved organic matter (CDOM) over four weeks, following selected sample treatment and storage protocols. Some variability in the effectiveness of individual protocols most likely reflects sample provenance. None of the various protocols examined performed any better than dark storage at 4 °C without pre-treatment. We thus recommend this as the optimal strategy, coupled with minimal storage times as far as practicable. Future studies of SML properties should validate their chosen storage protocols independently.

1 Introduction

The sea surface microlayer (SML) is only tens to hundreds of micrometers deep, but it is a physically, chemically and biologically distinct environment. It contains unique microbial communities, is a site for the synthesis and concentration of organic matter components, including transparent exopolymer particles (TEP) implicated in marine snow formation, and surface active substances (SAS) that cause damping of surface turbulence and subsequent suppression of air-sea gas exchange (Ćosović, 2005; Upstill-Goddard et al., 2003; Cunliffe et al., 2011; Wurl et al., 2011; Salter et al., 2011). SAS in seawater are predominantly natural phytoplankton exudates, such as polysaccharides, proteins and lipids, and their degradation products (Gašparović, 2012), with additional contributions in coastal waters from terrestrial humic and fulvic acids. Production of SAS is thus seasonal and leads to strong seasonality of SML properties and air-sea gas exchange (Wurl et al., 2011).

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Notwithstanding the effects of SML sampling methods that can influence sample integrity (Zuev et al., 2001; Cunliffe et al., 2013), the complex physico-chemical nature of the SML and the strong seasonality and reactivity of some of its main components also present a challenge to sample handling and storage. Routine analyses providing valuable SML characterisation include total surfactant activity (SA) (Ćosović and Vojvodić, 1982) and chromophoric dissolved organic matter (CDOM) absorbance (Helms et al., 2008; Frew et al., 2002) and fluorescence (Hudson et al., 2007). However, for logistical reasons even these relatively straightforward measurements are usually only possible in the laboratory. Delays between sampling and analysis are thus inevitable and in the case of open ocean research cruises they may be several weeks. Even where instrumentation is readily available the measurements can be time-consuming, making storage a significant issue for at least some samples. As some degree of SML sample storage is unavoidable the development of storage protocols that minimise temporal degradation and contamination are essential.

There is currently little consensus regarding appropriate maximum storage times or recommended sample treatments for the routine SML analyses outlined above. Although earlier studies addressed sample storage for individual sea water components, reported results are sometimes conflicting and as far as we are aware the simultaneous evaluation of several storage protocols for several sea water analytes has not been adequately undertaken, and this is certainly so for the SML. To address this deficiency we examined the effects of several established storage protocols on the analysis of SA and CDOM absorbance and fluorescence in SML samples.

2 Materials and methods

SML samples were collected from the Tyne estuary (NE UK) on 17 March 2011 (salinities 0 and 17.4), 12 May 2011 (salinity 17.0) and 1 June 2011 (salinity 15.8) using a Garret screen (Garrett, 1965) (mesh 16, wire diameter 0.36 mm, opening 1.25 mm) and transferred to the laboratory in "aged" plastic bottles (i.e. all leachable components

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removed). All sampling and laboratory equipment was acid washed with 10 % HCl and rinsed three times with ultra-pure water (Milli-Q, Millipore System Inc., USA) prior to use. Glass equipment additionally was baked at 450 °C overnight. Samples were processed in the laboratory as detailed below and subsequently stored in 15 ml sterile polypropylene plastic tubes or 20 ml glass bottles for later analysis. The treatments are listed in Table 1.

The selected treatments are all in common use. Filtering removes bacteria and thereby reduces bio-degradation but it also removes particulate matter and hence a significant portion of SAS. Consequently it has been recommended to measure SA on unfiltered samples (Ćosović, 2005). Poisoning samples by various means arrests bio-degradation but can lead to cell lysis and the leaching of SAS (Lee and Fisher, 1992; Gardner et al., 1983). Our selected procedures examined the net result of all of these. Poisoning by $AgNO_3$ and $HgCl_2$ were examined. Acidification was not considered because it modifies the sample matrix (Spencer et al., 2007), for example by altering CDOM absorbance (Andersen et al., 2000) and fluorescence intensities (Patel-Sorrentino et al., 2002) and introducing wavelength shifts (Mobed et al., 1996).

Sample filtration (Table 1) used a peristaltic pump. To make sample handling as consistent as possible, unfiltered samples were pumped through empty filter holders. For all protocols, the first sample (t_0) was analysed as soon as possible after treatment (i.e. the same or following day). All samples were kept in the dark and all except treatment 7 which was kept frozen at −20 °C were kept at 4 °C following common practice (Coble et al., 1998; Baker, 2002; Stedmon et al., 2003; Wickland et al., 2007; Fellman et al., 2009; Hood et al., 2009; Lapworth et al., 2009). Subsequent analyses were carried out after one, two, and four weeks. Treatments 1 and 6 were stored both in glass and polypropylene bottles to examine the comparative influences of these materials. All others were stored in polypropylene only.

SA was measured by phase-sensitive AC voltammetry (Metrohm 797 VA Computrace, Metrohm, Switzerland) with a hanging mercury drop (Ćosović and Vojvodić, 1982), a silver/silver chloride reference electrode and a platinum wire auxiliary

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electrode. Calibration used the non-ionic soluble surfactant Triton T-X-100. Samples were brought to salinity 35 prior to measurement by adding surfactant-free 3 mol L^{-1} NaCl solution. For each measurement, a new mercury drop was created and the first few drops discarded. Surfactants accumulated on the drop at $V = -0.6$ V for 15 s with stirring (1000 rpm). Alternating voltage scans of 10 mV at 75 Hz produced a current which was measured. Each response was corrected for the added NaCl solution and expressed as an equivalent T-X-100 concentration.

CDOM absorbance and fluorescence were determined by UV/VIS spectrophotometry (Varian Cary 100 Bio) and UV/VIS spectrofluorometry (Varian Cary Eclipse Fluorescence Spectrophotometer), respectively (Varian Inc, USA). Both used 10 mm path length quartz cuvettes, rinsed three times with ultra-pure water and once with sample before each measurement. Ultra-pure water blanks were measured at the start and end of each run. Absorbance was measured over the wavelength range 800 nm–200 nm in 1 nm steps. Acquired spectra were corrected for drift by subtracting the mean 700 nm to 800 nm absorption (samples are transparent in that range) and the blank spectrum was subtracted. Spectral slopes S (Helms et al., 2008) and the 250 nm to 365 nm absorption ratio (also called $E_2 : E_3$) were used to indicate dissolved organic matter (DOM) composition (or "quality"). $E_2 : E_3$ tracks changes in DOM molecular size and S is an index of average DOM characteristics (chemistry, source, diagenesis). Both are largely independent of CDOM concentration (Helms et al., 2008). For estimating S we used non-linear regression on the wavelength region 350 nm to 400 nm. CDOM fluorescence used excitation wavelengths 250 nm–450 nm in 5 nm steps and emission wavelengths 280 nm–600 nm in 4 nm steps. EEMs were averaged over 0.1 s. Blank scans were subtracted from the acquired EEM matrices, which were then corrected for inner filter and instrument effects (Cory et al., 2010). $HgCl_2$ quenches DOM fluorescence (Fu et al., 2007; Yamashita and Jaffe, 2008), hence we did not measure the fluorescence of $HgCl_2$ poisoned samples. In total, 186 resulting EEMs were modelled with parallel factor analyses (PARAFAC; Stedmon and Bro, 2008). Five different fluorophores were identified (Table 2).

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All measurements were in triplicate. Statistical tests evaluated any significant differences. For examing glass vs. plastic, we fitted a linear model with generalised least squares and varying variances. An analysis of variance then determined whether any of the factors were zero, which indicates that a variable exerts no influence. Rejections were at the 5 % level. For comparing compatibility with no change, each variable (SA, CDOM S, CDOM $E_2 : E_3$) and each treatment were tested separately using an analysis of variance. When the statistics did not pass the quality checks, results were discarded. Except for the statistical tests which operated on the original values, results were normalised to those for no treatment at t_0 to facilitate direct comparisons of changes during storage. Errors are expressed as the standard deviation of the triplicate analyses.

3 Results and discussion

We found that in all instances except CDOM $E_2 : E_3$ for samples treated with $HgCl_2$, storage in either glass or polypropylene did not significantly affect our analytical results (SA $p = 0.45$, CDOM S $p = 0.55$, CDOM $E_2 : E_3$ for silver filter $p = 0.74$).

The selected treatments produced varying SA responses (Fig. 1). Formalin (treatment 2) produced an initial increase of 10 % at t_0 , which may reflect additional DOM leached from dying cells. If so it is evidently essentially complete immediately following the addition. The filtered frozen sample (treatment 7) initially showed lower SA, likely due to particulate matter removal, whereas the unfrozen filtered sample (treatment 5) did not. This could be explained by clogging, causing a change in the effective filter pore size. Poisoning apparently partly compensated surfactant removal by filtering by introducing leached material (treatments 4 and 6). The untreated sample showed a maximal change of −10 %. Only the untreated sample ($p = 0.52$), frozen ($p = 0.17$) and poisoning with $HgCl_2$ ($p = 0.06$) are compatible with no change over time.

CDOM responses are shown in Figs. 2 and 3. Formalin introduced significant absorption even in blank water samples, which precludes its use in CDOM storage protocols. $HgCl_2$ significantly changed the absorption at small wavelengths: the 250 nm to 365 nm

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absorption ratio changed 10-fold (off scale in Fig. 3). Silver filtration and freezing also led to large changes. $AgNO_3$ gave the best performance with changes up to 15 % in $E_2 : E_3$ and 20 % in S, with the untreated sample showing changes up to 20 %.

Table 3 shows the results of the statistical test against no change over time (disregarding initial change compared to the untreated sample) for all variables and treatments. Overall none of the sample storage protocols examined performed any better than "no treatment".

Perhaps unsurprisingly, changes during storage depend on the initial SAS concentration and composition. Figure 4 shows SA vs. time for the four samples examined: high SA freshwater and three lower SA estuarine waters. Only the estuarine sample from 01/06 showed any clear downward trend in SA; none of the others showed any significant temporal change ($p = 0.12$, $p = 0.63$, and $p = 0.53$, respectively). Initial changes due to the treatments (e.g. SAS leaching due to poisoning) also showed significant scatter for the different sampling locations and times indicating that treatment effects cannot be easily predicted (data not shown).

Different SAS components degrade differently. Figure 5 shows relative changes in fluorescence components in the untreated sample. Tryptophan-like substances (component 5) and reduced humic-like groups (component 4) apparently degrade more rapidly than humic-like substances (components 1–3).

For untreated samples stored at 4 °C, Hunter and Liss (1981) found small SA losses from samples dark-stored at 6 °C for up to a week, but an increase of 20 % after 34 days, in agreement with our findings. Mitchell et al. (2000) found no change in the CDOM absorbance of samples refrigerated for less than 24 h, while Hudson et al. (2009) reported fluorophore-specific declines in fluorescence intensity dependant upon sample provenance. For frozen samples, two studies of a range of freshwaters found that after freeze/thaw absorption coefficients and fluorescence intensities showed both increases and decreases (Spencer et al., 2007; Hudson et al., 2009), although overall CDOM loss was observed and protein, humic and fulvic-like fluorophore intensities all declined (Hudson et al., 2009). Both studies concluded that there were no simple

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relationships between initial sample characteristics and changes during freeze/thaw, which also corresponds with our findings, and that correcting their data for this effect was therefore not possible. On the other hand Yamashita et al. (2010) found CDOM absorbance to be unaffected by freezing although fluorescence data were compromised and Gao et al. (2010) found that changes after freezing were less than 15 %. All these findings strongly support our own results, which show that changes in sample characteristics during storage can vary greatly, dependent not only on the selected sample treatment and storage time, but also on the initial sample composition. Consequences for the subsequent analysis of SML samples stored according to various protocols may therefore be difficult to predict with any great confidence.

4 Conclusions

A storage experiment using SML samples of varying salinities and seven different storage protocols showed that measured surfactant activity and CDOM absorption and fluorescence all depended on sample provenance as well as initial sample treatment and subsequent storage times. Moreover, all analyses showed significant scatter between triplicates and none of the several protocols examined performed any better than that of no treatment. This highlights the difficulty of devising adequate storage protocols for SML samples. Given the potential problem of organic material leaching from dying cells on poisoning, the likely removal of significant particulate organic matter on filtration and the health and safety issues associated with sample poisoning (e.g. $HgCl_2$) we must conclude that where the storage of samples for SAS and CDOM analysis is necessitated by circumstances, such samples should remain untreated and be stored at 4 °C in the dark for as short a time as possible. For storage of 7 days we found this protocol to result in an error of less than 12 % in SA compared to samples analysed immediately following collection. For some studies, for example those considering large nearshore-offshore gradients in SA and CDOM properties, such an error may be acceptable, whereas for others, for example those examining short-term changes in SA

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and CDOM at a single site, they are likely to prove more problematic. We strongly recommend that all future studies of SML physico-chemical properties carry out routine evaluations of their selected protocols for sample storage.

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Table 1. Treatments used for the SML samples. All samples were stored in the dark and, with the exception of treatment 7, at 4 °C.

No.	Treatment	Sampling location
1	No treatment	Estuarine & riverine
2	Fixed with 1 % Formalin (final concentration) (Wurl et al., 2009)	Estuarine
3	Poisoned with 6 µmol L^{-1} $AgNO_3$ (Kim et al., 2008). The salt was baked at 200 °C to remove remnants of surfactants before making up the poison	Estuarine
4	Filtered with silver filter	Estuarine & riverine
5	Filtered with 0.2 µm surfactant free cellulose acetate (SFCA) filter	Estuarine & riverine
6	Filtered with 0.2 µm surfactant free cellulose acetate (SFCA) filter and poisoned with $HgCl_2$	Estuarine & riverine
7	Filtered with 0.2 µm surfactant free cellulose acetate (SFCA) filter and frozen at −20 °C	Estuarine & riverine

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Table 2. Characteristics of identified PARAFAC components. Secondary excitation maxima are shown in parentheses. Description contains previously assigned characteristics and names of similar components as of Murphy et al. (2008) ([†]), Fellman et al. (2010) (*) and Cory and McKnight (2005) ([#]).

Comp.	Max. Ex λ (nm)	Max. Em λ (nm)	Description
1	265	533	Humic-like terrestrial DOM[†]
2	< 250 (305)	425	Humic-like, low molecular weight[†]*
3	< 250 (365)	479	Humic-like, high molecular weight*
4	265	429	Reduced, humic-like group[#]
5	280	342	Tryptophan-like, amino acids free or bound[†]*

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Table 3. Statistical evaluation of no effect of storage time (p value). Tests were run separately for each treatment and variable. Unmeasured data are shown as n/a; statistics that did not pass the quality control as "–".

Treatment	SA	CDOM S	CDOM $E_2 : E_3$
1	0.52	0.12	0.05
2	0.004	n/a	n/a
3	0.004	–	0.14
4	0.002	0.14	0.21
5	0.10	–	–
6	0.06	0.70	0.58
7	0.17	0.36	0.10

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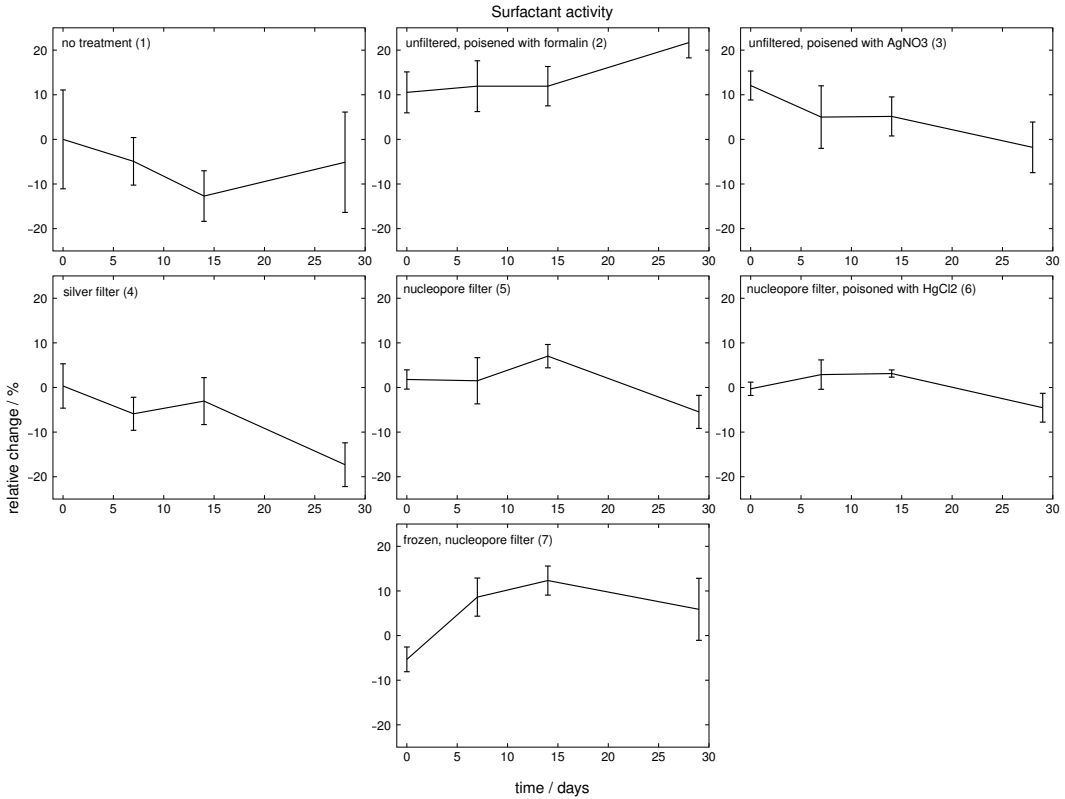

Fig. 1. Relative change of SA vs. storage time.

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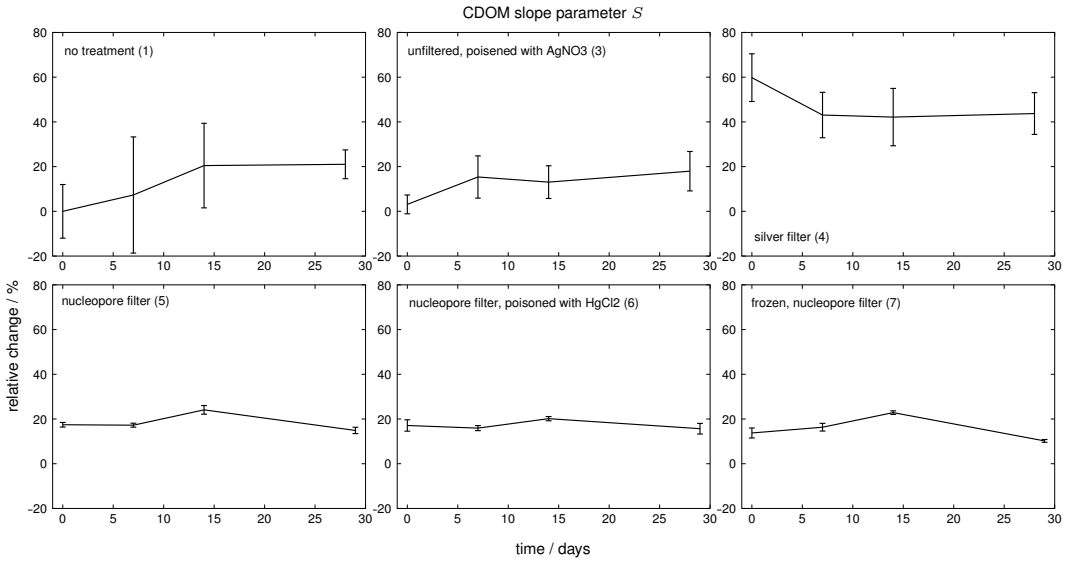

Fig. 2. Relative change of CDOM slope parameter *S* vs. storage time.

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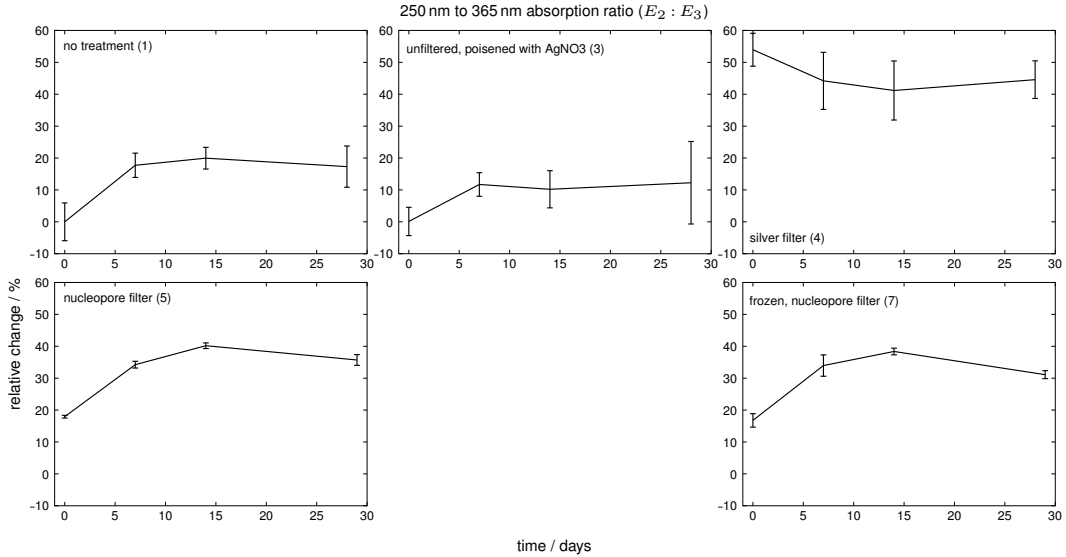

Fig. 3. Relative change of 250 nm to 365 nm absorption ratio ($E_2 : E_3$) vs. storage time. Note that $HgCl_2$ is off scale and thus not shown.

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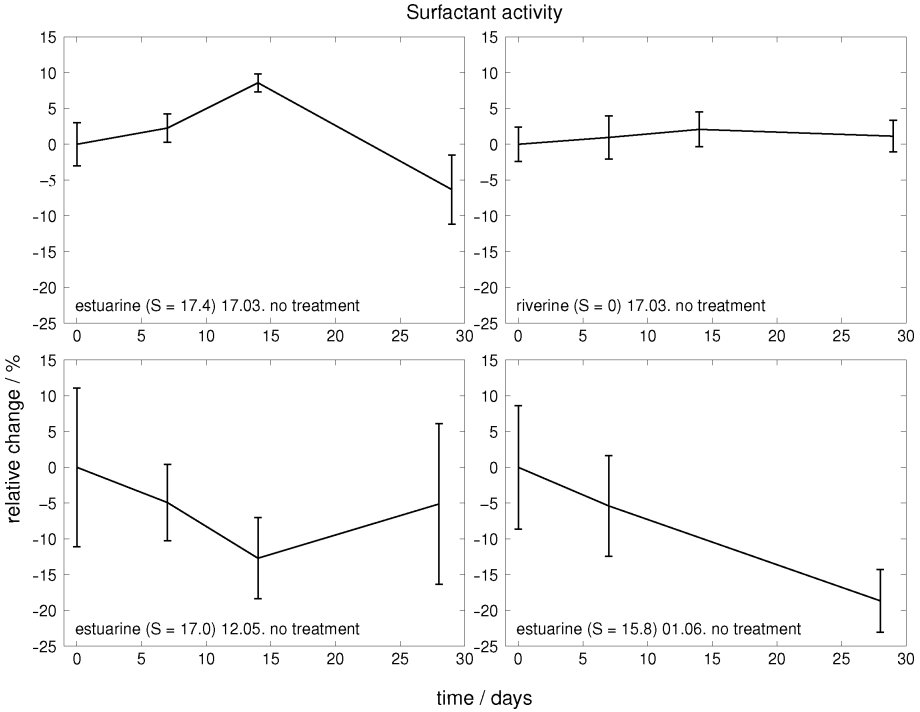

Fig. 4. SA vs. storage time for untreated samples of different origin.

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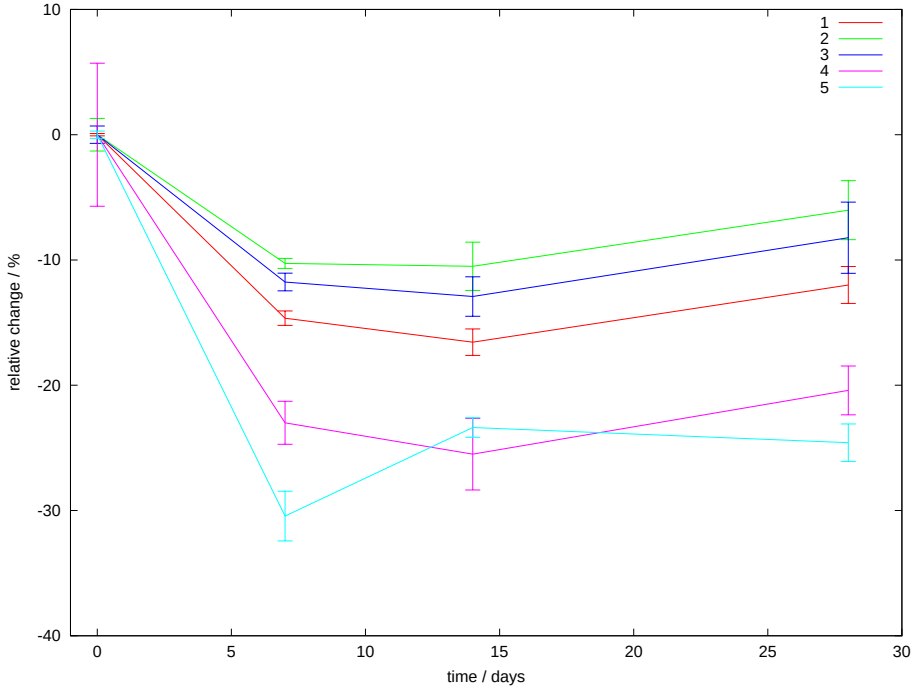

Fig. 5. Relative change of the five different fluorescence components vs. storage time for the untreated sample.

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