Volatile Organic Sulfur Compounds in a Meromictic Alpine Lake

Flüchtige organische Schwefelverbindungen in einem meromiktischen Alpensee

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Keywords: Volatile Organic Sulfur Compounds, Carbon Disulfide, Methanethiol, Dimethyl Sulfide, Dimethyl Disulfide, Meromixis

Summary: The full spectrum of volatile sulfur compounds was detected in the water column of the permanently stratified meromictic Lake Cadagno. Besides hydrogen sulfide it included methanethiol, carbonyl sulfide, dimethyl sulfide, carbon disulfide, and dimethyl disulfide. Their distribution in the water column suggests that these compounds are of biogenic origin. Except for carbon disulfide which is present in all layers of Lake Cadagno, these volatile organic sulfur compounds are restricted to the anoxic part of the lake. For methanethiol, dimethyl sulfide, and carbon disulfide maximum concentrations were observed in the redox transition zone and in the sediment porewater. Carbon disulfide is the most abundant volatile organic sulfur compound with concentrations of up to 60 μmol L^{-1}. The concentrations of the methylated sulfides are in the nmolar range. Although their concentrations varied during the summer months, seasonal trends of the concentrations of volatile organic sulfur compounds did not follow a consistent pattern. The restriction of most sulfur species to the anoxic layers of the lake indicates that their production originates from anaerobic microbial degradation of biomass and not from its release from a specific precursor like dimethylsulfoniumpropionate as in marine environments.

Schlagwörter: Flüchtige organische Schwefelverbindungen, Schwefelkohlenstoff, Methylmercaptan, Dimethylsulfid, Dimethyldisulfid, Meromixis

Zusammenfassung: Im dauernd geschichteten, meromiktischen Cadagnosee konnten in der Wassersäule alle bekannten flüchtigen Schwefelverbindungen nachgewiesen werden: neben Schwefelwasserstoff waren dies Schwefelkohlenstoff, Methylmercaptan, Dimethylsulfid und Dimethyldisulfid. Die Verteilung in der Wassersäule lässt vermuten, dass die flüchtigen organischen Schwefelverbindungen biogenen Ursprungs sind. Mit Ausnahme von Schwefelkohlenstoff, welcher in allen Schichten des Cadagnosees gefunden werden kann, ist das Vorkommen dieser flüchtigen Schwefelverbindungen auf den anoxischen Teil des Sees beschränkt. Maximale Konzentrationen an Methylmercaptan, Dimethylsulfid und Dimethyldisulfid fanden sich in der Redoxübergangszone und im Porenwasser des Sediments. Schwefelkohlenstoff ist die häufigste Verbindung in Konzentrationen bis zu 60 μmol L^{-1}. Die Konzentrationen der methylierten Sulfide lagen im Nanomolar-Bereich. Obschon ihre Konzentrationen während der Sommermonate deutlich schwankten, ergab sich kein jahreszeitliches Muster für die Konzentrationen der flüchtigen organischen Schwefelverbindungen. Das ausschließliche Vorkommen der meisten Schwefelverbindungen im anoxischen Bereich des Sees lässt den Schluss zu, dass deren Bildung im anaeroben mikrobiellen Abbau der Biomasse zu suchen ist und dass sie nicht, wie in marinen Ökosystemen, aus einer spezifischen Vorstufe wie Dimethylsulfoniumpropionat hervorgehen.

1 Introduction

Since a few decades models of the biogeochemical cycle of sulfur include volatile organic sulfur compounds (VOSCs) to balance the exchange of sulfur between the oceans and terrestrial systems by the atmosphere. Furthermore, volatile organic sulfur compounds deriving from biogenic sources received increasing attention because of their importance in the regulation of the global climate [1, 2]. Reduced VOSCs emitted into the atmosphere become oxidized and form sulfate aerosols which are a major source of cloud condensation nuclei. So far, main interests concerned the production, the distribution, and the fluxes of VOSCs in marine environments where dimethyl sulfide (DMS) is the major VOSC species. There DMS mainly derives from dimethylsulfoniumpropionate (DMSP), an osmolyte in marine phytoplankton and in the seagrass *Spartina alterniflora* [3–5]. In contrast, less is known about the occurrence and biogenic production of reduced volatile organic sulfur compounds in freshwater ecosystems [6–9]. In this study Lake Cadagno, a remote meromictic alpine lake, was investigated for the presence of reduced volatile organic sulfur compounds and their vertical and seasonal distribution.

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2 Materials and Methods

Study site

Lake Cadagno is a small alpine lake (surface 26 ha, depth 21 m) situated at 1 921 m above sea level in the Piora valley, in the southern part of the Central Alps in Switzerland. The geology of the Piora valley determines the chemistry of the water. The bedrock of the valley partly consists of a karstic system and contains gypsum and dolomite. Subaquatic springs constantly supply the hypolimnion of Lake Cadagno with salt rich water (calcium, magnesium, sulfate, and hydrogen carbonate are the major ionic species) leading to permanent density stratification of the lake. This leads to three chemically and biologically different lake compartments, the oxic mixolimnion which is low in nutrients (N, P), the anoxic monimolimnion which is high in nutrients, and a narrow redox transition zone in between with steep chemical gradients. Water close to the sediments contains about 2 mmol L^{-1} Ca^{2+}, 1.4 mmol L^{-1} Mg^{2+}, 2.6 mmol L^{-1} SO_4^{2-} and 2.8 mmol L^{-1} [CO_3^{2-} + HCO_3^-] (DelDon, personal communication). The sediment and the monimolimnion (below 14 m) are constantly anoxic and the bacterial population is rich in sulfate reducing bacteria resulting in hydrogen sulfide concentrations higher than 1 mmol L^{-1} [10–12]. In the redox transition zone between the oxic mixolimnion and the anoxic monimolimnion steep gradients of oxygen, hydrogen sulfide, redox potential, and conductivity are formed. During summer a dense community of phototrophic sulfur bacteria (mainly *Chromatium okenii* and *Amoebobacter purpureus*) and various non-phototrophic bacteria becomes established in this layer [13, 14]. These microbial populations show a high productivity in photo- and chemoautotrophic CO_2 assimilation and act as nutrient filter between the mixo- and the monimolimnion. The element sulfur shows two spatially distinct cycles. In a short one photo- and chemolithotrophic sulfide oxidation is directly coupled to sulfate reduction within the bacterial layer while in a larger cycle sulfide formed in the sediment and the monimolimnion diffuses up to the bacterial layer in the redox cline where it becomes oxidized again by the metabolism of the sulfide oxidizers.

Sampling

Water samples were taken from a platform at the site of the maximum depth of 21 m. Samples of 2 liters were collected from different depths using a PVC-tube and a pump. After allowing the water to overflow for a minute, the bottles were carefully sealed with butyl rubber septa to exclude air. The samples were stored at 4 °C and analyzed within 4 hours.

Sediment fractions were taken from 40 cm long gravity cores (16.8 cm diameter) obtained from 21 m depth. To eliminate oxidation during sample preparation the cores were extruded and sliced to 5 cm layers under nitrogen. For the determination of the volatile sulfur compounds sediment samples were diluted with 510 mL nitrogen saturated sterile water (to a final volume of 800 mL) and sealed with butyl rubber septa. Volatile compounds were extracted from sediment and water samples by a purge and trap technique.

Chemicals

All chemicals were obtained from Fluka (Buchs, Switzerland), except when otherwise declared. The lignosulfonate solution (trade name: "Attisol 10 B") was obtained from Cellulose Attisholz AG (Luterbach, Switzerland).

Chemical determinations

Hydrogen sulfide was determined photometrically according to Gilboa-Garber [15], oxygen (mg L^{-1}) and turbidity (formazin turbidity units) were measured with a multisensor unit (HPT from Züllig AG, Rheineck, Switzerland).

For bacteriochlorophyll-a analysis 100 mL of water were filtered through a glass microfibre filter (Whatman GF/F; Ø 2.5 cm) and the pigments extracted with 3 mL acetone/methanol (7 : 2; v/v) for 2 hours in the dark. After centrifugation (3000 rpm, 10 minutes) the absorption was measured at 770 nm and an extinction coefficient of 75 $mmol^{-1}$ L cm^{-1} was used for the calculation of the concentration of bacteriochlorophyll-a.

Volatile sulfur compounds were purged from the water and sediment samples, preconcentrated by cryoadsorption (on dry ice cooled Tenax TA, 60...80 mesh; Chrompack, Switzerland) and analyzed by gas chromatography (DANI 86 HT-gas chromatograph; J&W GS-Q (30 m × 0.53 mm I.D., 20 µm film) megabore column; flame photometric detector (FPD); flowrate: 4.2 mL min^{-1} helium; temperature program: isothermal at 50 °C for 3 minutes, heating rate of 17.5 °C min^{-1} up to 225 °C, isothermal at 225 °C for 7 minutes; detector temperature: 250 °C; injector temperature 250 °C). The volatile organic sulfur compounds were identified by comparing the retention times with those of standards. To quantitate methanethiol (MT), DMS, carbon disulfide (CS_2), and dimethyl disulfide (DMDS) separate calibration curves were obtained for every sulfur compound. Increasing amounts of reference compounds were added to 2 L distilled oxygen free water which was treated similarly as the lake samples. The detection limits were 0.04 nmol L^{-1} for MT, 0.1 nmol L^{-1} for DMS, 0.02 nmol L^{-1} for CS_2 and 0.02 nmol L^{-1} for DMDS, respectively, with standard deviations (in % from mean) ranging between 4.2 and 12.3 % for the 4 compounds. A Hewlett Packard G1800A GCD-System equipped with a Chrompack Pora PLOT-Q column (25 m × 0.32 mm, 4 µm film) was used to identify MT, DMS, and CS_2 in the natural water samples by their specific mass spectra. All measurements were performed with a flowrate of 0.8 mL min^{-1} of dried oxygen free helium. Temperatures for the injector and the electron ionization detector were set at 250 °C.

DMSP was measured as DMS after cold hydrolysis. Water samples free from DMS and other volatile sulfur compounds were treated with 5 mol NaOH per litre (final concentration) for 20 hours at 4 °C and analyzed for DMS. For DMSP quantification in biomass 10 L of water from different depths were filtered (Whatman GF/C; Ø 2.5 cm), the filters digested in Hungate tubes with 3 mL of 1N NaOH at 4 °C for 24 hours [3] and the headspace gas was analyzed for DMS.

Sediment slurries (45 mL) from Lake Cadagno were prepared with nitrogen saturated distilled water and amended with potential precursors of volatile organic sulfur compounds and anaerobically incubated in 100 mL vials. Details are given in Table 1. VOSCs formed were analyzed in 100...500 µL headspace samples with a DANI 86 HT-gas chromatograph. For calibration 100 mL vials were filled with 45 mL nitrogen saturated distilled water mixed with different amounts of standards to final concentrations of 0...40 µmol L^{-1} and headspace gas analyzed after incubation at 30 °C overnight.

3 Results

Figures 1 and 2 (left) show profiles of some general physical and chemical parameters which characterize the summer situation of the meromictic Lake Cadagno. The oxygen profile showed a maximum at 6 m depth followed by a steep decrease down to total depletion at about 12 m. Due to temperature dependent density stratification during summer the oxic part of the lake is well stabilized. Just below the oxycline, dense populations of anaerobic phototrophic bacteria, mainly *Amoebobacter purpureus* and *Chromatium okenii*, and non-phototrophic bacteria developed in early summer causing a strong turbidity in the redox transition layer. The biomass in the layer was dominated by phototrophic bacteria, in summer mainly by the large *Chromatium okenii* cells [13, 14, 16]. Hydrogen sulfide was found throughout the anoxic monimolimnion where the dominant bacterial activity was dissimilatory sulfate reduction [10–12, 16, 17]. Hydrogen sulfide, the major reduced sulfur compound, reached occasionly maximum concentrations of more than 1 mmol L^{-1} in the sediment near

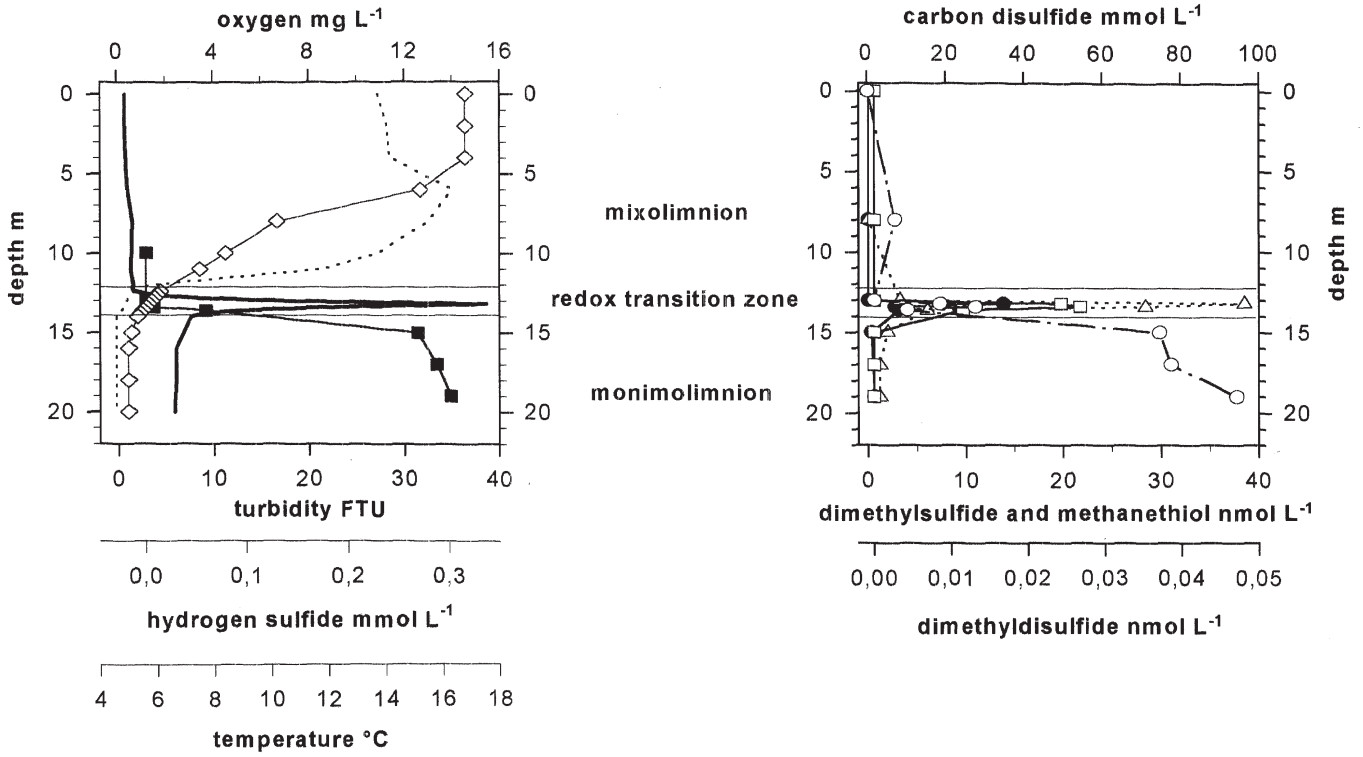

Fig. 1: Typical summer depth profiles in Lake Cadagno (31 July 1996).

Typisches Sommertiefenprofil im Cadagnosee (31. Juli 1996).

left: - - - oxygen, — turbidity, ■ hydrogen sulfide, ◇ temperature
right: ● methanethiol, △ dimethylsulfide, □ dimethyldisulfide, ○ carbon disulfide

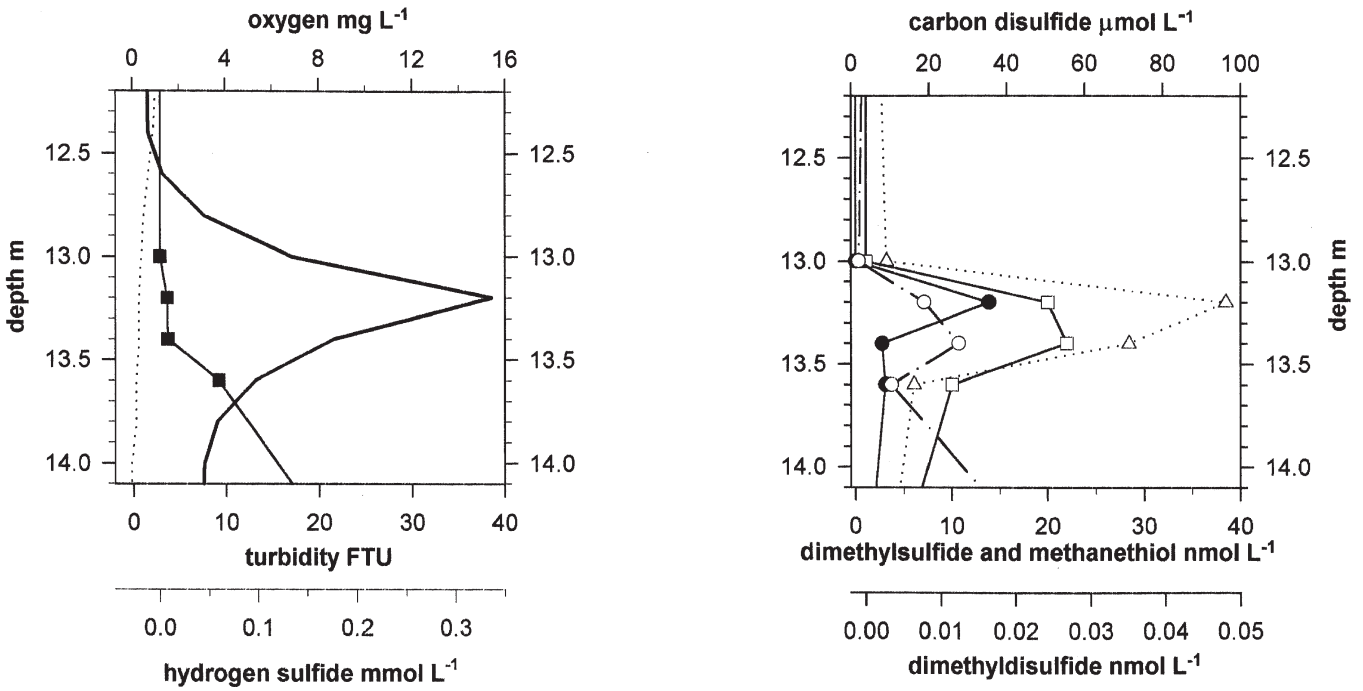

Fig. 2: Typical summer depth profiles in the redox transition zone of Lake Cadagno (31 July 1996).

Typisches Sommertiefenprofil in der Redoxübergangszone im Cadagnosee (31. Juli 1996).

left: - - - oxygen, — turbidity, ■ hydrogen sulfide
right: ● methanethiol, △ dimethylsulfide, □ dimethyldisulfide, ○ carbon disulfide

water layer. Although hydrogen sulfide is the main exogenous electron donor for the phototrophic bacteria near the chemocline, its concentration was often low or even below the detection limit at the depth of their highest population density.

Figures 1 and 2 (right) show the profiles of volatile organic sulfur compounds taken at the same time. CS_2 was the only volatile sulfur compound found throughout the whole water column, in the anoxic as well as in the oxic part. However, maximum concentrations were also observed in the redox transition layer and close to the sediment water interface.

Methylsulfides were restricted to the anoxic layers of the lake. Maximum concentrations for MT and DMS (23 nmol L^{-1} and 50 nmol L^{-1}, respectively) were found in the layer of highest bacterial density, they decreased towards the sediment. DMDS was generally restricted to the redox transition layer, however, in a few cases traces were found in the mixolimnion as well.

COS was occasionally detected in the monimolimnion and in the sediment. In cases of high hydrogen sulfide concentrations COS became difficult to quantify.

DMSP, the major source of DMS in seawater, was not detected in Lake Cadagno. Neither water samples from any layer of the lake nor phytoplankton or phototrophic bacteria (enriched by filtering 10 L of water from different depth of the euphotic zone) released traces of DMS or other volatile sulfur compounds after cold hydrolysis with NaOH.

All volatile organic sulfur compounds detected in the anoxic monimolimnion were found in the sediment as well. The average concentrations measured for MT, DMS and CS_2 were 20.78 nmol L^{-1}, 52.55 nmol L^{-1}, and 303.73 µmol L^{-1}, respectively, which amounts for a 2.1, 7.3, and 5.1 fold increase in the porewater compared to the average concentrations found in the monimolimnion. As shown in Figure 3 a slight increase for DMS and CS_2 with increasing depth was observed. In contrast MT showed a maximum in the 5...10 cm fraction and decreased with increasing depth. Hydrogen sulfide was the most abundant volatile sulfur species in the sediment at concentrations of 1...2.5 mmol L^{-1} [11].

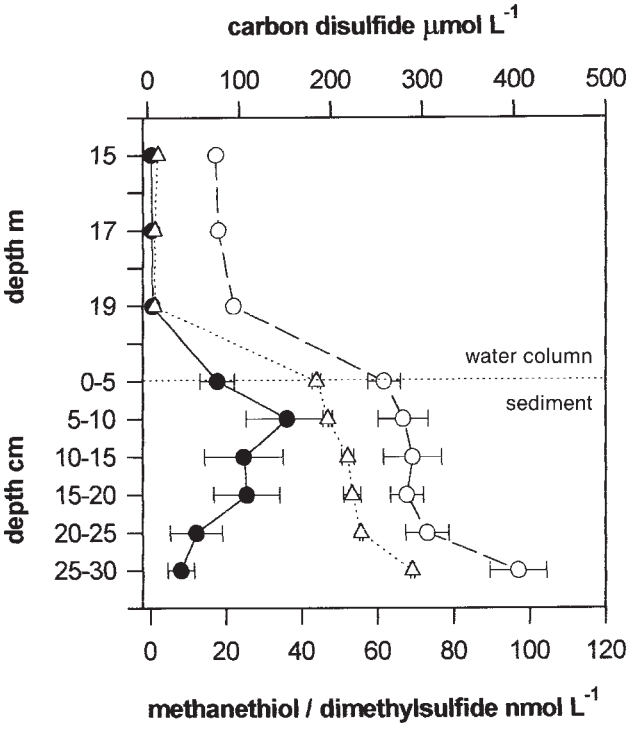

Fig. 3: Volatile organic sulfur compounds in the sediment porewater (6 July 1997); means of 3 samples ± 1 standard deviation. The concentrations in the sediment were calculated from the differences between fresh and dry weight.

Flüchtige organische Schwefelverbindungen im Porenwasser des Sedimentes (6. Juli 1997); Mittel von 3 Proben ± 1 Standardabweichung. Die Konzentrationen im Sediment wurden aus der Differenz zwischen Frisch- und Trockengewicht berechnet.

● methanethiol, △ dimethylsulfide, ○ carbon disulfide

Changes in lake chemistry including VOSCs were monitored during early summer to late fall, the time the lake is accessible for sampling. Most volatile organic sulfur compounds yielded their maximum values in the layer with the high density of phototrophic bacteria. The vertical extension of the bacterial plate ranged from 0.8 to 1.2 m and started downwards at the depth of nearly complete depletion of oxygen.

Depending on its size 4 to 5 samples in 20 cm-intervals were analyzed and the average concentrations of the different VOSCs calculated. Figure 4 shows the seasonal variations of VOSCs in the bacterial zone. MT and CS_2 followed similar patterns during summer 1996. The concentrations of both compounds increased from July to September yielding maxima in September, then decreased towards the end of the summer. Interestingly on August 23th and October 10th no MT was detected in the bacterial layer. Average DMDS concentrations hardly varied until beginning of September, but increased significantly at the end of the summer. Its maximum concentration was recorded in October, coinciding with the depletion of MT and the time when fall circulation introduced oxygen into the chemocline. Average DMS concentrations were in the range of 3...20 nmol L^{-1} and peaked in September, similar to MT and CS_2. Compared to the volatile organic sulfur compounds the bacteriochlorophyll-a concentrations in the same water volume as a parameter for the biomass of purple sulfur bacteria varied only by a factor of 2 during summer with maxima on July 18th and on September 13th. Although the maxima of all VOSCs coincided with the maximum in September no correlation between bacteriochlorophyll-a and any of the volatile sulfur compounds was found.

Various inorganic and organic sulfur compounds (potassium thiocyanate, sodium thiosulfate, sodium tetrathionate, *l*-cysteine · HCl, cystine, *dl*-methionine and homocysteine, Table 1) were added to sediment slurries to obtain more information on possible natural precursors of VOSCs, and incubated in the dark under anaerobic conditions. The addition of *dl*-methionine strongly stimulated both the formation of MT and DMS. In all other samples including the control only small amounts of DMS accumulated. Interestingly, the sediment slurry with sodium tetrathionate resulted in a release of methanethiol and carbon disulfide. Although tetrathionate might chemically disproportionate to thiosulfate and elemental sulfur, no stimulation of formation of volatile sulfur compounds by thiosulfate was found. Incubation of the sediment slurry with methoxylated aromatic compounds, syringic acid and 3,4,5-trimethoxybenzoic acid, and the polymer lignin (anaerobically at 30 °C) led also to significant formation of DMS and minor amounts of MT.

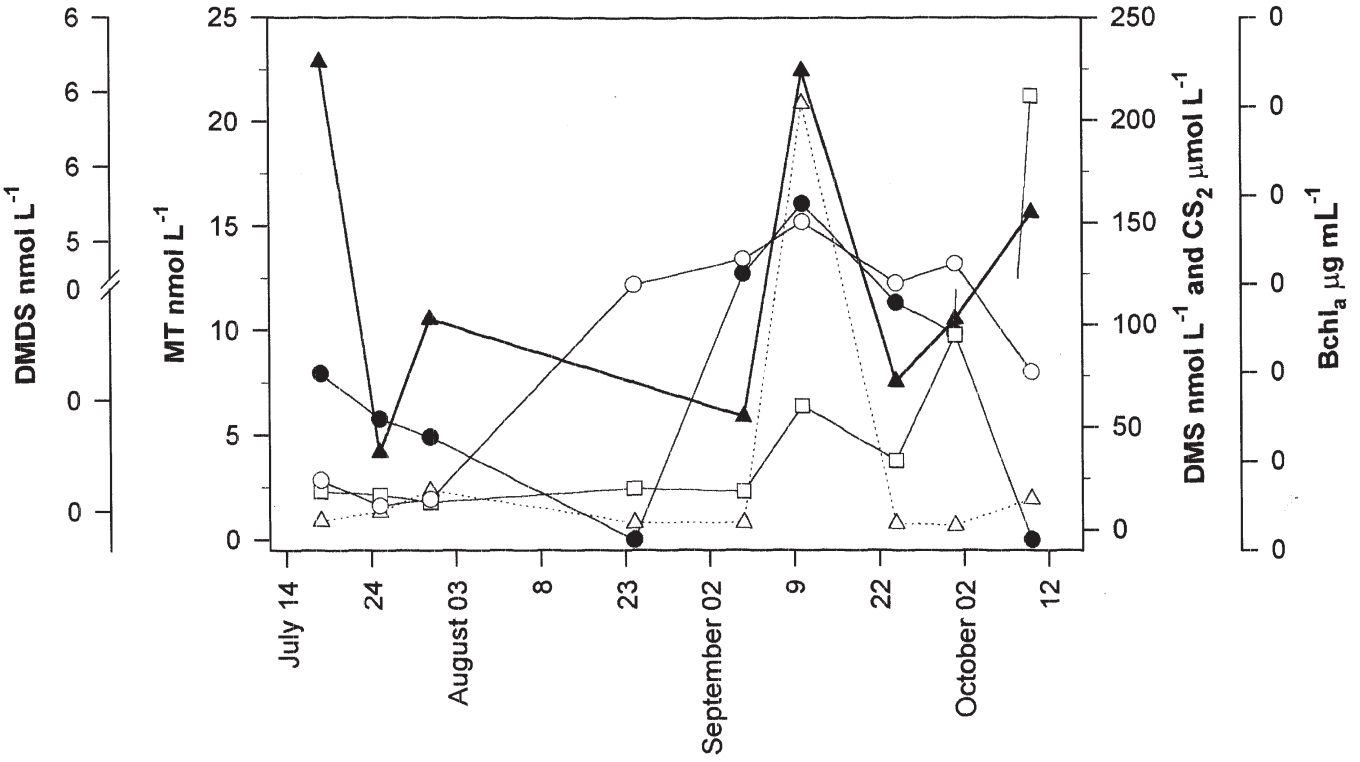

Fig. 4: Average concentrations of volatile organic sulfur compounds and bacteriochlorophyll-a in the layer of the phototrophic bacteria (summer 1996). Depending on the extension of the layer 4 to 5 samples (in 20 cm-intervals) were analysed and used for the calculation of the average concentrations.

Gemittelte Konzentrationen der flüchtigen organischen Schwefelverbindungen und von Bakteriochlorophyll in der Schicht der phototrophen Bakterien (Sommer 1996). Je nach Ausdehnung der Schicht wurden 4 bis 5 Proben (in 20 cm vertikalem Abstand) analysiert und für die Berechnung der mittleren Konzentrationen verwendet.

● MT: methanethiol, △ DMS: dimethylsulfide, □ DMDS: dimethyldisulfide, ○ CS_2: carbon disulfide, ▲ $BChl_a$: bacteriochlorophyll-a

Table 1: Effect of addition of various sulfur sources to sediment slurries (45 mL) on the release of volatile sulfur species. All samples including the control contained H_2S from the sediment (1...2 mmol L^{-1}) and its endogeneous H_2S regenerating system.

Freisetzung flüchtiger Schwefelverbindungen nach Zugabe ausgewählter Schwefelquellen zu Sedimentaufschlämmungen (45 mL). Alle Proben einschließlich der Kontrolle enthielten H_2S aus dem Sediment (1...2 mmol L^{-1}) sowie dessen natürliches H_2S regenerierendes System.

compound added		volatile organic sulfur compound released CS_2	COS	MSH	DMS	incubation time
control		–	–	–	6.67 µmol	12 d
potassium thiocyanate	2 mmol L^{-1}	–	–	–	6.68 µmol	12 d
sodium thiosulfate	2 mmol L^{-1}	–	–	–	7.89 µmol	12 d
l-cysteine	5 mmol L^{-1}	–	–	–	6.70 µmol	12 d
homocysteine	2 mmol L^{-1}	–	–	–	6.67 µmol	12 d
sodium tetrathionate	2 mmol L^{-1}	5.7 µmol	–	93 µmol	–	12 d
dl-methionine	2 mmol L^{-1}	–	–	1.5 mmol	30 µmol	6 d
syringic acid	2 mmol L^{-1}	–	–	200 nmol	150 µmol	4 d
3,4,5-trimethoxybenzoate	2 mmol L^{-1}	–	–	230 nmol	70 µmol	6 d

4 Discussion

Six volatile sulfur compounds have been detected in the stratified Lake Cadagno, confirming the analyses from freshwater algal cultures and other anoxic freshwater environments [8]. Dissimilatory sulfate reduction produced H_2S as the major volatile sulfur compound in the monimolimnic water column and in the sediment in concentrations several magnitudes higher than that of organic sulfur species. Typically its concentration increases with depth reaching values of more than 1 mmol L^{-1} close to the sediment water interface [11]. Organic volatile sulfur species show two maxima, one in the sediment, the other in the zone of maximum concentration of phototrophic bacteria, where hydrogen sulfide concentration rap-

idly decreases towards the oxic mixolimnion. The depletion of hydrogen sulfide in the upper part of this zone is due to its oxidation in the metabolism of the phototrophic purple sulfur bacteria present, *Chromatium okenii* and *Amoebobacter purpureus,* and chemolithotrophic sulfide oxidizers not identified so far [10]. CO_2 incorporation studies give evidence of a significant activity of a non-photosynthetic lithotrophic CO_2 fixation [18]. CS_2 is the only volatile organic sulfur compound present in the entire water column and in the sediment porewater. Furthermore its concentration exceeds the ones of methylated sulfur compounds by a factor of 10^3. In contrast to reduced volatile sulfur compounds carbon disulfide is relatively stable under oxic conditions and is likely the only sulfur compound which may be released from the surface of Lake Cadagno into the atmosphere.

CS_2 has been found in other freshwater environments but only in much lower concentrations [8, 19]. In the sediment of meromictic antarctic lakes maximum concentrations of CS_2 reach about 2 μmol L^{-1} [9]. It is also produced in anaerobic mud of the sea floor, anaerobic sediments and by *Spartina alterniflora* [20]. Its quantities evolved are often in the same order as those of H_2S [21]. Although CS_2 is found in many biological systems the mechanism of formation is still unknown. When sediment samples of Lake Cadagno were fed with organic and inorganic sulfur compounds only tetrathionate resulted in a significant increase of CS_2 formation. In soils and rice paddies cysteine, cystine, and thiosulfate were also found to stimulate the formation of CS_2 [22].

Both CS_2 and COS may be formed abiotically from metal sulfides such as FeS_2 but only under oxidizing conditions. Although FeS_2 is present in the anoxic sediment in Lake Cadagno [11], the chemical formation of CS_2 seems thus unlikely.

The drop of CS_2 concentrations at the mixolimnion boundary indicates a sink caused by bacterial breakdown under aerobic conditions. Various *Thiobacillus* species and *Thiothrix ramosa* have been described to use carbon disulfide as sole energy source for growth [23]. However, we were not able to enrich bacteria from the water column growing on CS_2 as sole energy substrate.

Carbonyl sulfide was only occasionally detected in the monimolimnion and in the sediment porewater. The metabolic origin of COS is as well unknown although biogenic production of this compound from cysteine, cystine, thiocyanate, isothiocyanates, lanthionine, and djenkolic acid (*S,S'*-methylenebis[cysteine]) in soils and rice paddies has been described [24]. Sediment slurries from Lake Cadagno were not stimulated to produce COS by addition of these sulfur compounds. Carbonyl sulfide consumption in natural environments may be due to the hydrolysis of COS to CO_2 and H_2S, whereby the latter could then be used by the different sulfide oxidizing bacteria [22].

The most abundant methylsulfide in marine ecosystems and in few lakes is DMS [5, 7], while in most anoxic freshwater samples methanethiol is the dominant species [8, 19]. In Lake Cadagno both compounds, MT and DMS are lacking in the mixolimnion but show a maximum in the anoxic layer with the phototrophic bacteria and a second one in the sediment porewater, which is for methanethiol a 2-fold, and for DMS a 7-fold increase compared to the water above the sediment. A similar distribution for methylated sulfides has been reported in Lake 226 S and Lake 302 S of the Canadian Shield [7], in the Schleinsee in Germany [6], and in meromictic lakes in Antarctica [9].

The most important precursor of DMS in marine environments, DMSP, has not been found neither in water and algal samples from the mixolimnion, nor in purple sulfur bacteria. Although dissolved DMSP has never been found in freshwater samples so far it is of interest that two related freshwater strains of purple sulfur bacteria (*Chromatium minus*, DSM 178, and *Chromatium minutissimum* DSM 1376) do contain DMSP [25].

Our data suggest that the formation of MT and DMS is due to bacterial activity of sulfur-containing biomass under anoxic conditions. The increase of methylsulfides in the zone of the phototrophic bacterial community may be furthermore stimulated by zooplankton grazing. $^{12}C/^{13}C$ ratios suggest that up to 50 % of the biomass digested by zooplankton originates from the bacterial population in the chemocline [18]. Indeed high numbers of Cyclops and Copepod species are frequently found in anoxic water samples obtained from this depth. The high concentrations of methylsulfides in the sediments are probably due to both the biodegradation of organic material deriving from sedimenting biomass and the methylation of the free hydrogen sulfide present. Methionine is likely a source of methanethiol in the sediment rich in organic matter and in the monimolimnion of Lake Cadagno, as MT is an intermediate in the degradation of methionine in anoxic sediments [26]. This and the subsequent methylation of methanethiol to traces of DMS has been confirmed during incubation experiments with sediment slurries. Besides methionine tetrathionate seems to be another source of methanethiol (Table 1). Methylation of hydrogen sulfide is a further mechanism leading to the formation of methanethiol and dimethyl sulfide. Sulfide-dependent thiol methyltransferases are widespread in natural environments [27]. The formation of MT from methoxylated aromatic substrates has been observed before [28]. In hydrogen sulfide containing sediment slurries amended with methoxylated aromatic monomers or the biopolymer lignin methanethiol formation was significantly stimulated.

The depletion of methylated sulfur compounds in the upper part of the bacterial layer may be due to anoxigenic phototrophs and colorless sulfur bacteria acting as sinks for methylated compounds like in marine ecosystems. *Thiocapsa roseopersicina* and *Thiocystis* sp. use DMS as electron donor in phototrophic growth, while in *Rhodobacter sulfidophilus* DMS is the only electron donor in chemoautotrophic growth [29–31]. Recently the complete oxidation of DMS to sulfate by *Thiocapsa roseopersicina* M11 under oxic/light conditions was reported [32]. Such reactions could not be demonstrated by *Chromatium okenii.* Neither DMS nor MT added to water samples taken from the bacterial layer were oxidized in incubation experiments in the light.

DMDS occurs almost exclusively in the bacterial layer below the redox transition layer. It probably originates from the oxidation and dimerization of methanethiol. At the oxycline this is a spontaneous chemical reaction, in the absence of molecular oxygen it could also be catalyzed biologically. Sulfur oxidizers at the oxycline may be a potential sink of DMDS as a strain of *Thiobacillus thioparus* was shown to grow chemolithoautotrophically on DMDS [33].

As indicated by the steep concentration gradients for most volatile sulfur compounds on both sides of the bacterial layer, this zone must play an important role in the metabolism of the VOSCs. Whereas the mean bacteriochlorophyll-a concentrations as a measure for the abundance of purple sulfur bacteria did not vary greatly during the summer (0.45...0.85 μg mL^{-1}) the volatile organic sulfur compounds showed much greater changes in their concentrations.

The average concentrations in the bacterial layer for MT, DMS, DMDS, and CS_2 were in the range of 0...16 nmol L^{-1}, 2...208 nmol L^{-1}, 0.02...6.0 nmol L^{-1}, and 12...150 µmol L^{-1}, respectively. Although a general trend to higher concentrations for all VOSCs was found in September their temporal variations during the summer did not follow a consistent pattern. Except for dimethyl disulfide the concentrations of all the other VOSCs decreased in fall. The maximum concentrations of DMDS on October 10th coincided with the drop of MT below detection limit due to chemical oxidation of the MT as at this time of the year the turnover of the mixolimnion reached the zone of the bacterial layer.

Although the maximum concentrations of VOSCs were found in the bacterial layer, no significant relationship existed between their concentrations and the ones of bacteriochlorophyll-a and protein. Similarly no strong correlation between DMS and chlorophyll-a was found in marine water. The lack of correlation between bacteriochlorophyll-a and VOSCs in the bacterial layer of Lake Cadagno may suggest that heterotrophic bacteria rather than phototrophic ones dominate the production of these compounds. Furthermore VOSCs diffuse rapidly and their turnover is much faster as compared to the growth rates of the bacterial population.

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