Actinomycetes, cyanobacteria and algae causing tastes and odours in water of the River Arno used for the water supply of Florence

Eudes Lanciotti, Chiara Santini, Emanuela Lupi and Daniela Burrini

ABSTRACT

The occurrence of actinomycetes, cyanobacteria and microalgae in surface water of the Arno River, used for water supplies in Florence, was investigated in this study on taste and odour compounds (such as geosmin and 2-methylisoborneol (MIB)). Throughout the 12-month monitoring period, raw water samples were collected and analysed for the number and genera of actinomycetes, cyanobacteria and microalgae.

The research has shown that actinomycetes (*Streptomyces*, *Nocardia*, *Micromonospora*) are numerous in autumn and winter and decrease in spring and summer; in contrast, diatoms (*Melosira*, *Navicula*), green algae (*Chlorella*, *Pediastrum*) and cyanobacteria (*Anabaena*, *Oscillatoria*, *Nostoc*) present a completely opposite trend and are numerous in spring and summer and reduced in autumn and winter. The monitoring of the two odour-causing metabolites gives rise to a trend that is more similar in time to that of cyanobacteria than that of actinomycetes. The constant occurrence of geosmin may be a sign of a synergy between microalgae, cyanobacteria and actinomycetes, while MIB could derive from the activity of cyanobacteria or other species of actinomycetes present in a low concentration.

Key words | actinomycetes, algae, cyanobacteria, tastes and odours components, water supply

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INTRODUCTION

Some microorganisms living in surface waters can cause unpleasant tastes and odours that are then found in drinking water used for human consumption. Among the numerous metabolites that give rise to unpleasant tastes and odours, attention has focused on two volatile terpenes, geosmin (1α, 10β-dimethyl-9α-decanol), which confers a musty-muddy off-flavour, and 2-methylisoborneol [MIB (1-*R-exo*)-1,2,7,7-tetramethyl-bicyclo-(2,2,1)-heptan-2-ol], causing a typical musty-muddy and camphor-like taste and odour (Chorus 1993; Nakajima *et al.* 1996; Palmentier *et al.* 1998). These secondary metabolites are produced primarily by actinomycetes but also by cyanobacteria and some kinds of algae (Silvey 1953*a*; Aoyama 1990; Jensen *et al.* 1994; Hu & Chiang 1996). Moreover some genera of microalgae and cyanobacteria can host actinomycetes: *Melosira*, *Navicula*

among the diatoms, *Chlorella*, *Pediastrum*, *Cladophora* among the green algae and *Anabaena*, *Oscillatoria* and *Nostoc* among cyanobacteria. Diatoms and green algae can also produce other types of metabolites that give rise to unpleasant odours. Surface water bodies are the natural habitat of algae and cyanobacteria. Actinomycetes, soil microbes, can enter the water through the wash-in of spores and during the vegetative phase; they can also be hosted inside the above-mentioned algae (Silvey 1953*b*; Cross 1981).

Episodes of unpleasant tastes and odours in water used for drinking water treatment have occurred in the last few decades in Italy (Volterra & Fadda 1994). These phenomena are mainly due to the eutrophication of surface water bodies caused by phosphorus and nitrogen of domestic and agricultural origin.

Both odour compounds and the organisms that produce them can cause problems for drinking water plants. The former because they generally present a very low threshold of perception (10–20 ng l^{-1}) and are extremely resistant to treatment processes, the latter (mainly actinomycetes) because, through the spores, they can colonise the structures of the drinking water plant itself and the distribution network favouring the production of odour-causing compounds in finished water (Chorus *et al.* 1993; Bao *et al.* 1997; Palmentier *et al.* 1998).

These problems are of major importance in Tuscany, especially in the district around Florence, which uses the water from the River Arno as its only source for drinking water (in the drinking water plant, the withdrawal of the raw water is direct).

The river water has repeatedly given rise to unpleasant tastes and odours, which the drinking water plant has been able to remove by using advanced treatment techniques such as adsorption on activated carbon and advanced oxidation with ozone (Lalezary *et al.* 1986; Lalezary-Graig *et al.* 1988).

The present study had the following objectives: (i) monitor actinomycetes, cyanobacteria, microalgae and odorous metabolites (geosmin and MIB); (ii) evaluate the environmental parameters that favour the development of these microorganisms; and (iii) identify the microorganisms responsible for the production of unpleasant tastes and odours and the relations between them.

MATERIALS AND METHODS

Sampling and study site

From May 1996 to April 1997, 24 water samples were analysed every two weeks. Samples of raw water from the Arno river were collected at the intake level of the drinking water plant of the city of Florence.

Enumeration and identification of actinomycetes, cyanobacteria and microalgae were carried out, together with measurement of chlorophyll and the odour-causing metabolites geosmin and MIB. Turbidity, temperature and pH were measured and the flow rate of the Arno river was

Table 1 | Comparison of 24 water samples for the number of colonies (CFU) that developed on BAIA (bacto actinomycete isolation agar) and SCA (starch casein agar)

CFU l^{-1}	BAIA (N=24)	SCA (N=24)
Max	17,800	4,000
Min	120	200
Mean	4,372	1,242
Grade of growth (%)*	19/24	4/24
	(79.2)	(16.7)

* Number of samples grown on BAIA or SCA (in one sample the number of CFU l^{-1} is the same).

recorded. The drainage basin of the Arno river covers 8,228 km^2. The river flows through most of Tuscany and is influenced by the typical anthropic activities of this region: agriculture, animal breeding and urban effluent discharges. The stream-like character of the river, with alternating periods of drought and flooding, variations in flow, turbidity, temperature and the washing of the soil could promote the development of microorganisms responsible for the unpleasant odours. Besides these variables, we should also consider the alkaline pH (ranging from 7.2 to 8.5), which favours the growth of actinomycetes (Cross 1981).

Analysis of actinomycetes

Selective media and colony selection

For the determination of actinomycetes the most suitable volume was 5 ml of raw water, filtered on to sterile membranes of cellulose acetate with a pore size of 0.45 μm (Sartorius). To find the most suitable medium for the growth of actinomycetes, we used two selective media: bacto actinomycete isolation agar (BAIA) and starch-casein agar (SCA) supplemented with antibacterial compounds (cycloheximide and novobiocine) (Goodfellow *et al.* 1988). The medium which gave the best results was BAIA (Table 1) with a higher yield (79.2%) compared with SCA. The filter membranes placed on BAIA and SCA were

incubated for 7 days at 30°C, the time necessary for the growth of the actinomycete colonies but also for out-competing saprophytic microflora (fungi, bacteria). Unlike fungi and bacteria, actinomycetes can penetrate the pores of the filter membrane and develop on the surface and beneath it. They can therefore grow directly on the culture medium. Hence, the membrane was transferred after the first 7 days of incubation on to a new plate of the same medium. Both plates were then incubated for another 7 days at 30°C. The observation and count of colonies was carried out on both the 7th and the 14th day. The incubation was then extended to the 21st day to allow colonies with a slower growth to develop hyphae big enough to reach the sporulation phase ('mature' colonies). The transfer of the membrane on the 7th day of growth made it possible to isolate and count the actinomycetes more easily by comparing the number of colonies present on the first plates with those on the plates with the filtering membrane. Lastly, to confirm the colonies of actinomycetes of uncertain identification, the mycelium of the substratum was observed on the optical microscope by turning the Petri plates upside down.

Isolation and identification

After counting the actinomycetes, we proceeded to isolate the colonies on the same type of media on which they had been developed, BAIA or SCA–in this phase devoid of antibiotics–and also on yeast malt agar (ISP$_2$), a medium favourable to the development of spores. For each isolated colony, we described the macroscopic, morphological features (appearance, form and margins of the colonies, presence and colour of the mycelium in the substratum and/or in the air, presence of coloured pigments in the medium) (Bergey's Manual 1994) and the presence of odorous metabolites. The colonies isolated were observed under the optical microscope (with a direct light and in phase contrast) directly from the upturned plate, using enlargements of $100 \times$ and $200 \times$. To obtain a clearer image of the colonies, they were grown directly on the cover-slide as follows: one part of the isolated colony was ground in a glass microhomogeniser by adding a drop of sterile water; the homogenised part of the colony was then put on a cover-slide formerly fixed on solid ISP$_2$ medium

plate (according to the International Streptomyces Project, DIFCO) at an angle of about 45°; the plate was then incubated at 30°C. After 7 days, the cover-slide on which the isolated colony was developed was observed under an optical microscope at $200 \times$, $400 \times$ and $1,000 \times$. The morphological features of the colony and mycelium made it possible to define the genus of the isolated colonies. Colonial morphologies of actinomycetes were singled out, based on some peculiar features of the colonies: form (round and sometimes slightly irregular), section (convex, flat, umbonated), margins (eroded and sometimes whole) or aspect (powdery, at times opaque). Actinomycetes can be distinguished from other micro-organisms present in the water by the presence of the substratum and aerial hyphae, the type of colouring of both myceli and the production of coloured pigments that can spread in the soil.

Analysis of algae and cyanobacteria

Evaluation and counts

A comparison was made among concentration techniques proposed by various authors (*Standard Methods* 1992) (centrifugation, filtration and sedimentation). Sedimentation was chosen because it is less damaging to cells, an important factor for observing the morphology (Burrini *et al.* 2000). After collecting water samples in one-litre glass bottles and storing them in a dark, cool place, cylindrical settling chambers with a thin glass bottom were used. This technique permitted the viewing of organisms, settled directly in the inverted microscope without further manipulation; the chamber had a grid subdivided into squares of 1 mm^2. The settling chamber for raw water had a base diameter of 2 cm and a height of 4 cm for a maximum capacity of 50 ml. Depending on the algal density, 10 or 20 ml volumes were analysed; the height of the water column was either 0.4 or 0.8 cm. The following procedure was employed to analyse raw water samples: the bottle was shaken to suspend cells and live samples of 10 and 20 ml were poured into the settling chambers. The third chamber was filled with 10 ml of the preserved sample, following the addition of Lugol's solution to the remaining sample in the bottle. Sedimentation time

was 4 or 8 h, depending on the sample volume (Furet & Benson-Evans 1982).

Live samples were generally used, as they are useful for taxonomy analyses. However, in the case of abundance of motile organisms or species that tended to float, preserved samples were examined. An inverted Nikon Diaphot-TMD microscope, equipped with $10 \times$, $20 \times$, $40 \times$, $60 \times$ objectives, was used for directly observing algae and cyanobacteria in the settling chambers. An ocular micrometer was employed for measuring the single specimens. For genera determination the taxonomic keys of the photographic atlas of Palmer (1977), Bourrelly (1972) and Strable & Krauter (1984) were used. For counts of microalgae and the determination of their genus, we generally used $20 \times$ and $40 \times$ objectives; a good quality objective of $600 \times$ magnification was employed to resolve greater detail. After observing that the distribution of organisms was uniform at the bottom of the settling chamber, five non-adjacent squares were chosen in the chamber containing raw water, according to the random number table. Algal units or single cells contained in the square were counted according to Standard Methods for organisms lying on the boundary line. The number of algae per ml of sample was calculated with the formula: N $\text{ml}^{-1} = N_i A / a n_i V$, where N_i = the total number of algae counted in the areas considered; A = the total area of the settling chamber; a = the area of the square; n_i = the number of squares observed; V = the sample volume in ml.

Chlorophyll determination

Besides the microscopic algae analysis, the determination of chlorophyll a, b and c was performed, much more easily and rapidly than by using an optical microscope and representing an additional means of evaluation in the presence of algae. The chlorophyll concentration in raw water was calculated by filtering the sample through glass-fibre filters (Whatman GF/C) under low vacuum, until the filter was sufficiently coloured. The volume was recorded and the membrane homogenised with a glass grinder adding 10 ml of 90% acetone solution saturated with $MgCO_3$. When using cold acetone without grinding, the extraction efficacy was too low, as in the case of green algae. The sample was stored overnight in the refrigerator and the day after the extract was filtered on nylon membrane (*Standard Methods* 1992). The optical density of the extracts was determined using a Perkin-Elmer (mod. Lambda 1 UV/VIS) spectrophotometer at 663 nm for CHL-a, 645 nm for CHL-b and 630 nm for CHL-c. The chlorophyll concentration in extracts was calculated using equations derived by Rodier (1984):

CHL-a $(\mu\text{g l}^{-1}) = (11.64\ E_1 - 2.16E_2 + 0.10E_3)v/(lV_g)$;
CHL-b $(\mu\text{g l}^{-1}) = (20.97E_2 - 3.94E_1 - 3.66E_3)v/(lV_g)$;
CHL-c $(\mu\text{g l}^{-1}) = (54.22E_3 - 14.8E_2 - 5.53E_1)v/(lV_g)$;

where E_1 is the absorbance at 663 nm, E_2 the absorbance at 645 nm, E_3 the absorbance at 630 nm, v the volume of extracts (10 ml), l the length of the optical path (1 cm) and V_g the volume of filtered water (litres).

Analysis of odour-causing metabolites

To determine the quantity of the volatile compounds geosmin and MIB (Figure 1), responsible for unpleasant odours and tastes, a microextraction procedure combined with gas-chromatography-ion-trap-mass spectrometry (GC-ITD-MS) was employed. This methodology is highly sensitive and selective for trace compounds such as geosmin and MIB. One litre of raw water was added to 100 g of NaCl and 10 µl of methanol containing 25 µg ml^{-1} of internal standards (1-chlorooctane and 1-chlorodecane). We then carried out the extraction of odour-causing metabolites using hexane (two parts of 3 ml). The extracts, reduced to 0.1 ml, were analysed using mass spectrometry GC-ITD (Bao *et al.* 1997).

The gas-chromatograph employed was a Varian 3.400 with the following technical features:

> Injector temperature: 200°C
> Column: HP5-MS 30 m × 0.25 µm (Hewlett Packard)
> Transport gas: helium with a pressure of 7 psi
> column head pressure
> Temperature programme: 1 min at 40°C, then by an increase of 4°C min^{-1} from 40°C to 130°C, from 130°C to 200°C (by an increase of 7°C min^{-1}), from 200°C to 290°C (by an increase of 10°C min^{-1}), lastly, for 7 min, at 290°C.

One µl of the extract was injected into the gas-chromatograph. The detection apparatus was a
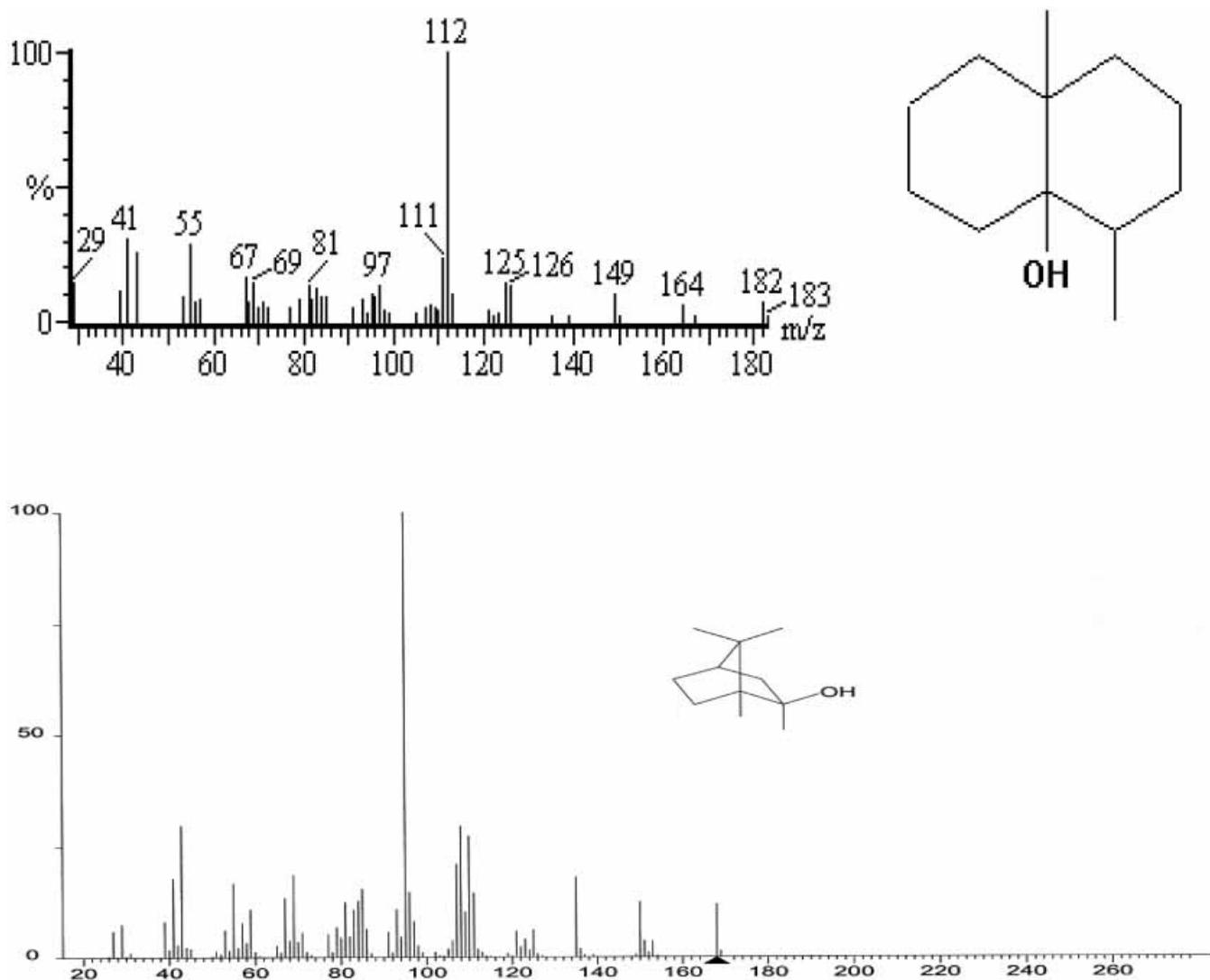
Figure 1 | Formulas of structure and spectra of Geosmin (above) and MIB (below).

Saturn-Varian mass spectrometer with electronic ionisation at 70 eV and mass scanning/range of 40–400 m/z.

RESULTS AND DISCUSSION

Seasonal variation of actinomycetes, cyanobacteria and algae

During the monitoring period (spring 1996–spring 1997) the presence of actinomycetes, algae and cyanobacteria in

the River Arno water followed a seasonal but alternating course (Table 2 and Figure 2). Chlorophyll *a* (typical of all algae and cyanobacteria) reflects the trend of algae in general; chlorophyll *c* values exceed *b* when there is a high quantity of diatoms. The algal classes and cyanobacteria most frequently identified from 1996 to 1997 are shown in Figure 3.

During the period from late spring to summer, from 28 May 1996 to 17 September 1996, the number of colonies of actinomycetes was fairly low (maximum value 2,800 CFU l^{-1}) compared with that of algae (maximum

Table 2 | Statistical analysis of the correlation coefficient test (by Spearman) between the number of three types of microorganism and the concentration of geosmin and MIB (n.e.=not effected)

Water sampling (date)	Cyanobacteria (units ml^{-1})	Algae (units ml^{-1})	Actinomycetes (CFU l^{-1})	MIB (ng l^{-1})	Geosmin (ng l^{-1})
28 May	0	1,377	1,000	0.6	3.6
11 June	5	939	600	1.2	2.8
26 June	4	900	1,600	1.7	3.5
12 July	102	3,396	400	1.9	9.2
23 July	24	2,592	1,000	2.7	4.8
6 Aug	15	293	2,800	3.1	7.3
20 Aug	12	1,259	2,800	2.2	2.9
3 Sep	63	2,565	400	3.0	4.2
17 Sep	20	2,067	120	1.8	2.6
1 Oct	0	188	3,800	0	0.2
15 Oct	1	123	7,200	0.1	1.0
29 Oct	1	300	10,200	0	1.1
12 Nov	0	261	7,800	0.7	2.2
26 Nov	0	31	6,600	0	1.1
13 Dec	0	83	2,400	0	1.0
23 Dec	0	104	5,200	n.e.	n.e.
14 Jan	0	42	5,200	0	1.0
28 Jan	0	85	9,000	n.e.	n.e.
11 Feb	0	198	4,200	0	1.5
4 Mar	0	302	3,600	0	2.0
11 Mar	0	177	3,000	0	2.7
25 Mar	0	522	17,800	0.4	3.1
8 Apr	0	835	2,400	0	2.9
22 Apr	0	814	5,800	1.6	8.9

Table 2 | Continued

	r (Spearman)	P
algae vs. MIB	0.72	< 0.0002
algae vs. geosmin	0.76	< 0.00005
cyanobacteria vs. MIB	0.80	< 0.00005
cyanobacteria vs. geosmin	0.49	< 0.02
actinomycetes vs. MIB	− 0.52	< 0.01
actinomycetes vs. geosmin	− 0.46	< 0.03
algae vs. cyanobacteria	0.68	< 0.0002
algae vs. actinomycetes	− 0.68	< 0.0003

value 3,400 algal unit ml^{-1}). The abundance of algae and cyanobacteria was mainly due to the climatic conditions (higher temperatures, average 22.9°C, a higher exposure to the sun), which were more favourable to cyanobacteria and algae than to actinomycetes. Statistical analysis of annual monitoring (Table 2) seems to confirm these associations: $r = 0.68$ for algae vs. cyanobacteria, $r = -0.68$ for algae vs. actinomycetes.

This period was characterised by an increased variety of algal genera, mostly diatoms (*Melosira*, *Navicula* and *Synedra*) and green algae (*Chlorella*, *Pediastrum* and *Clamydomonas*), and of cyanobacteria (*Anabaena*, *Oscillatoria*, *Phormidium*, *Microcystis* and *Nostoc*). Among them were the genera able to host actinomycetes and their spores and/or produce volatile odour-causing metabolites. These genera are able to coexist with or become colonised by actinomycetes which cause the destruction of host cells leading to the liberation of secondary metabolites which give rise to unpleasant tastes and odours (Silvey 1953a, b; Cross 1981).

From the end of September to October 1996, with different climatic conditions, there was a rapid change with an increase in the quantity of actinomycetes and a decrease in algae, including the genera *Melosira* and *Navicula* that give rise to volatile odour-causing metabolites other than geosmin and MIB, or are able to

host actinomycetes inside them, with an almost complete disappearance of cyanobacteria. This behaviour was closely connected to turbidity (expressed as FTU), flow rate (m^3 s^{-1}) and temperature (°C), affecting the presence of actinomycetes, algae and cyanobacteria. During this period there was an increase of flow rate and turbidity in the river following abundant rainfall; the consequent washing of the soil brought detritus, mud, plants and fouling vegetable remains that could be colonised by autochthonous actinomycetes. In the case of algae and cyanobacteria, the increase of turbidity and flow rate are stress factors as they prevent the penetration of light. Otherwise, changes of temperature (ranging on an average from 22.9°C to 16.8°C) are favourable to actinomycetes and unfavourable to algae and cyanobacteria (Figure 2).

In the following period (October 1996–January 1997) high concentrations of actinomycetes were recorded in the presence of high values of FTU (maximum values of 10,200 and 9,000 CFU l^{-1}) with a marked decline in the number of algae (from thousands of units per ml in the former period to hundreds or even less) and the disappearance of cyanobacteria (sensitive to an increase of flow rate and turbidity as well as the decrease of sunshine). In this period, the number of algal genera declined with a predominance of diatoms (*Melosira* and *Navicula*) and there
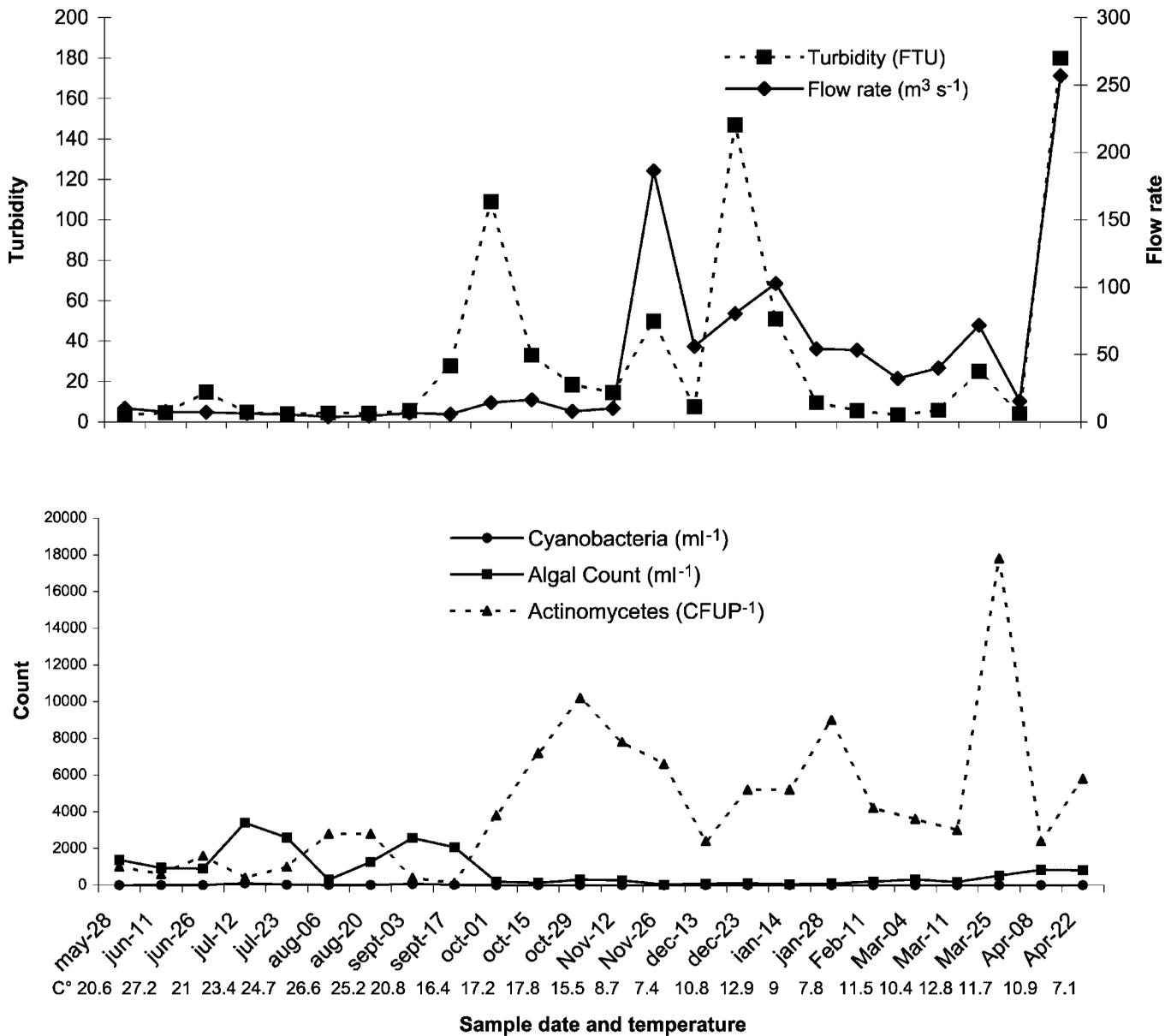
Figure 2 | Seasonal variation of actinomycetes, algae and cyanobacteria able to produce odour-causing metabolites and to host actinomycetes depending on turbidity (FTU), flow rate ($m^3 s^{-1}$) and temperature (°C).

was a marked decrease in the number of green algal species (*Clamydomonas*).

From February to April 1997, we observed a gradual increase in the number of algae, among which *Melosira*, *Navicula* and *Chlorella* are able to host actinomycetes, while actinomycetes varied in number, with values that never went below 2,000 CFU l^{-1}. All this leads us to

assume that, in similar climatic conditions, actinomycetes and algae have a cyclic course, given that the type and number of algae and actinomycetes found in April 1997 were equal to those found in May 1996.

In our monitoring the pH was always above 7, with values ranging from 7.1 to 8.3 in the period from May to September 1996 and 7.9 to 8.5 from the end of October to
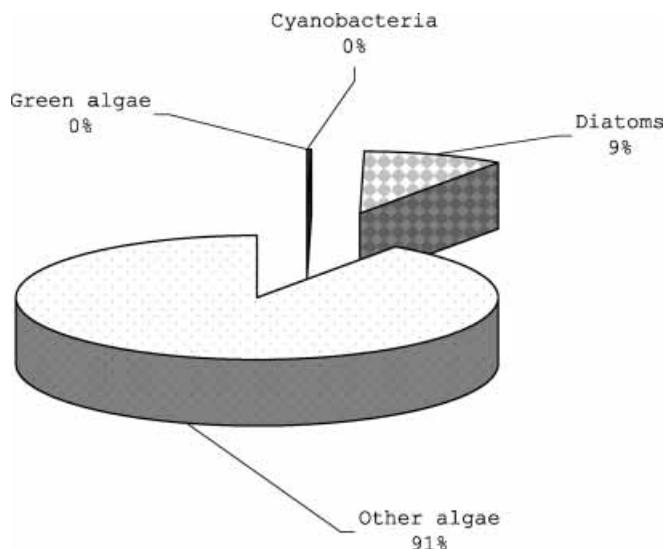
Figure 3 | The algal classes and cyanobacteria most frequently identified.

April 1997. The neutral and alkaline pH supports the development of actinomycetes, as shown in the literature (Cross 1981).

Actinomycete colonies

In the present research 11 types of colonial morphologies of actinomycetes were singled out, based on some peculiar features of the colonies. Among the 11 colonial morphologies found in the Arno river, the most frequently isolated were six species: four belonging to the *Streptomyces* genus, one to *Nocardia* and one to *Micromonospora*.

Comparison of occurrence of actinomycetes, algae, cyanobacteria and taste and odour compounds

The concentrations of geosmin and MIB, of which actinomycetes and some cyanobacteria genera are considered to be the major producers, have been determined in the present study. From May to September 1996, high concentrations of the two odour-causing metabolites analysed, were recorded (Figure 4). Geosmin, in particular, reached

its highest concentration ($9.2 \, \text{ng} \, \text{l}^{-1}$) in July (sampling of 12 July 1996) and maintained concentrations above the typical odour threshold ($4 \, \text{ng} \, \text{l}^{-1}$) in August and September. MIB also showed an increase in the period from July to September 1996 (maximum concentration $3.2 \, \text{ng} \, \text{l}^{-1}$) but did not reach the odour threshold ($8 \, \text{ng} \, \text{l}^{-1}$).

Besides high concentrations of geosmin and MIB during this spring-summer period, there was also an increase of some cyanobacteria genera (*Anabaena*, *Oscillatoria*, *Phormidium*, *Nostoc* and *Microcystis*), diatoms (*Melosira*, *Navicula* and *Synedra*) and green algae, while the number of actinomycetes was fairly low. Statistical analysis (Table 2) seems to confirm the positive association between increased production of geosmin and MIB and increase in cyanobacteria and algae number ($r = 0.72$ for algae vs. MIB and $r = 0.76$ for algae vs. geosmin; $r = 0.8$ for cyanobacteria vs. MIB and $r = 0.49$ for cyanobacteria vs. geosmin), but it also shows a negative association ($r = -0.52$ for actinomycetes vs. MIB and $r = -0.46$ for actinomycetes vs. geosmin). All this shows that the production of geosmin and MIB was, in all probability, mainly caused by cyanobacteria and to a lesser degree by actinomycetes freed in water or associated with some algal genera (such as diatoms and green algae).

In the following period—autumn 1996 to winter 1997—we observed that, from the beginning of September, there was a considerable decrease in geosmin concentration and, above all, of MIB which was much lower than the threshold odour value ($8 \, \text{ng} \, \text{l}^{-1}$). In this period the number of actinomycetes increased, while that of algae was minimal and the only group consisted of diatoms, especially the *Melosira* genus, an ideal substratum for the growth of actinomycetes.

On the one hand, the production of geosmin and MIB during the spring-summer season seems to be sustained mainly by cyanobacteria, on the other, in autumn and winter, the origin of odour-causing metabolites (mainly geosmin) seems to be principally caused by actinomycetes, as the only algae present belong to the *Melosira* genus which can host actinomycetes but is incapable of producing geosmin and MIB. Lastly, during the period from February to April 1997, we observed a progressive increase in the geosmin concentration and partly in MIB,
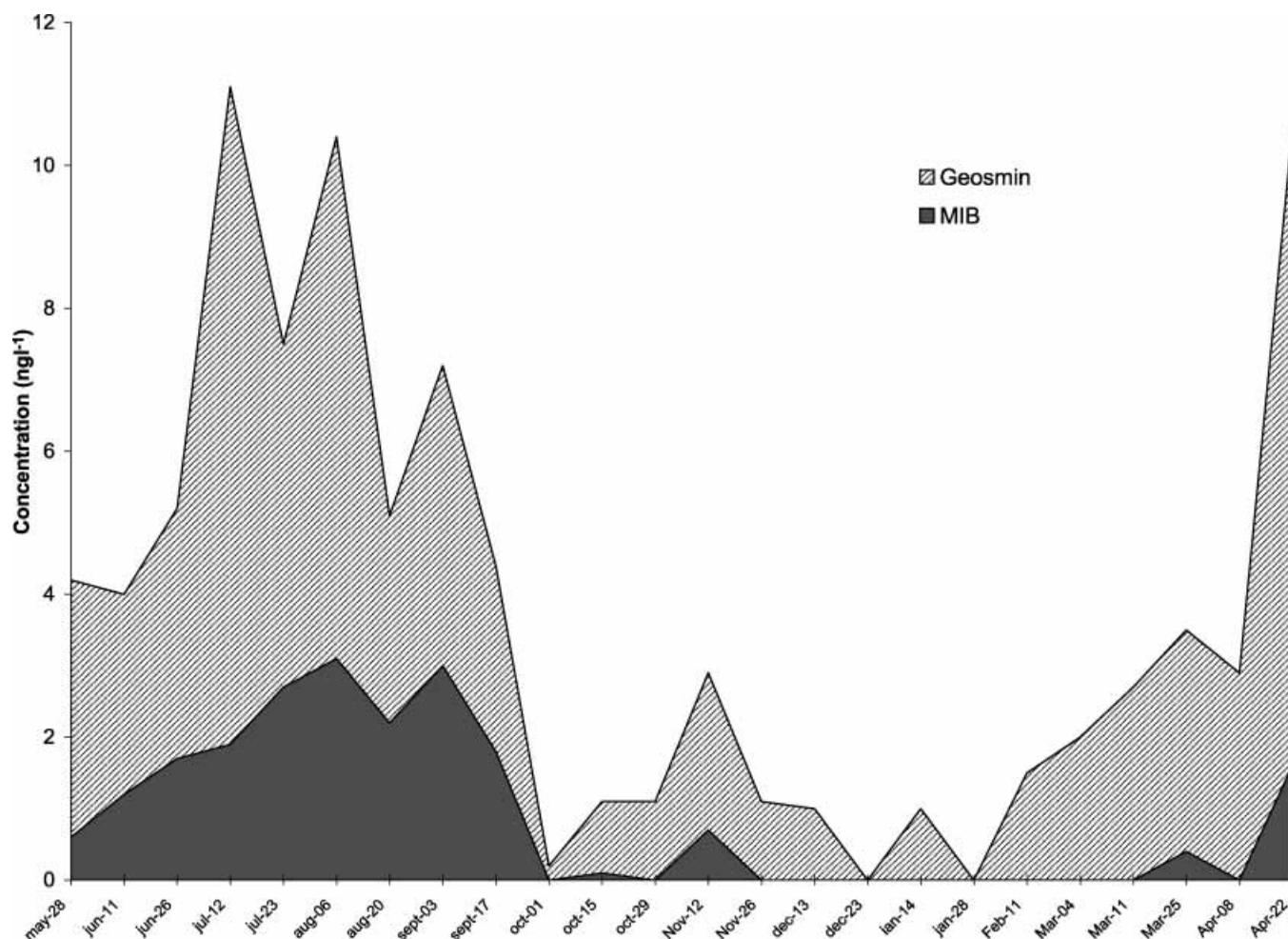
Figure 4 | Temporal trend of geosmin and MIB in the Arno river water.

correlated to the increase of algae and to a varying number of actinomycetes.

CONCLUSIONS

- Monitoring of the Arno river was carried out for the first time to evaluate the occurrence of actinomycetes related to algae and cyanobacteria directly involved in the production of the odour-causing metabolites, geosmin and MIB, or those able to host actinomycetes present in the water.

- Actinomycetes are frequent in autumn and winter ($4,000–18,000$ CFU l^{-1}) and infrequent in spring-summer $100–2,800$ CFU l^{-1}), while algae/cyanobacteria have a diametrically opposite course and are numerous in spring-summer (maximum value $3,400$ units ml^{-1}) and present in reduced numbers in autumn-winter (minimum value <500 units ml^{-1}).

- The most frequent actinomycetes isolates belong to the *Streptomyces*, *Nocardia* and *Micromonospora* genera, while among the algal groups, the most common isolates were diatoms (*Melosira*, *Navicula* and *Synedra*) and green algae (*Chlorella*). Among

the cyanobacteria we found *Anabaena*, *Phormidium Oscillatoria* and *Microcystis*. Among the microorganisms identified, the main producers of odour-causing metabolites were cyanobacteria and, to a lesser degree, actinomycetes either free in water or associated with other genera of diatoms and green algae.

- Above all the *Melosira* and *Navicula* genera seem to be constantly present in varying numbers during the different seasons.

- The seasonal monitoring performed on the odour-causing metabolites, geosmin and MIB, reveals a trend, which, in the course of time, proved to be more similar to that of cyanobacteria than to that of actinomycetes. Indeed, it could be observed that the production of geosmin and MIB was mainly due to cyanobacteria in spring and summer while in autumn and winter, the presence of geosmin seemed to be mainly caused by actinomycetes alone or associated with algae such as the *Melosira* and *Navicula* genera which were always present.

- The probable synergy between the presence and alternation of microalgae/cyanobacteria and actinomycetes can explain the constant occurrence of geosmin, while MIB could be mainly due to the metabolic activity of cyanobacteria or the actinomycetes present in low concentrations.

- Research is still being carried out by laboratory tests to evaluate the capacity of strains of actinomycetes isolates to produce MIB and geosmin.

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