Survey for microalgae and cyanobacteria in a drinking-water utility supplying the city of Florence, Italy

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ABSTRACT

The occurrence of algae was investigated in surface water of the Arno river and in a drinking-water treatment utility serving the city of Florence. Throughout the 12 months monitoring period, raw water and treated water samples were collected and analysed for the number and species of microalgae and cyanobacteria; settled and filtered water samples were only examined for counts of organisms. Chlorophyll concentrations, temperature, pH, turbidity, flow rate and other indicators of surface water pollution were measured in raw water samples. Microalgae, mainly diatoms (46.1%, with a predominance of the genus *Melosira*) and green algae (35.1%: non flagellate 30.7%, green flagellate 4.4%) were constantly found in river water samples. Cyanobacteria were rarely detected except in summer, mainly of genera *Oscillatoria* and *Anabaena*. In treated water only depigmented and inactive algal cells, mainly of green algae, were detected for which the treatment procedure was found to be less effective. The average algal removal by drinking-water treatment resulted in 97.4% for all samples and in 98.3% when significant algal concentrations were present in raw water. The combination of chlorine dioxide and coagulants resulted in a removal of 90.1%.

Key words | algal removal, cyanobacteria, drinking-water utility, monitoring, planktonic microalgae

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INTRODUCTION

In the last 30 years, the Italian freshwater ecosystem has been affected by anthropic pollution of different origin, such as the use of significant quantities of organic chemical compounds as well as detergents and fertilisers, leading to increased nitrogen and phosphorus concentrations. Algal blooms in surface water, from which drinking water is often produced (as in the case of the Arno river), are a direct consequence of these factors. Algae species commonly found in freshwater which can also give rise to algal blooms are Chlorophyceae (chlorophyta), Bacillariophyceae (diatoms), Dinoflagellated (dinoflagellata), Cyanophyceae (cyanobacteria or blue-green algae) and Euglenophyceae (euglenophyta). The total removal of the algal biomass is a difficult task for any drinking-water facility; moreover, algal metabolites interfere with disinfectants, leading to the formation of by-products that affect water quality. Treatment with high concentrations of chlorine has the disadvantage of dissolving large quantities of algal metabolites and of forming trihalomethanes (THMs), suspected to be carcinogenic (Scully *et al.* 1988; Wardlaw *et al.* 1991; Amirtharajal *et al.* 1993; WHO 1996; Steynberg *et al.* 1996). In fresh water, certain algal species and cyanobacteria may produce toxins such as (i) hepatotoxins (e.g. microcystins, produced by Microcystis, *Oscillatoria* and *Anabaena*); (ii) neurotoxins (e.g. anatoxins, produced by species of *Anabaena*, *Oscillatoria*, *Nostoc*, *Aphanizomenon*, *Cylindrospermum*); and (iii) lipopolysaccharides (produced by various species) (National Rivers Authority 1990). Algal blooms of these toxigenic strains in fresh water may therefore present risks for public health (Falconer *et al.* 1983; Carmichael 1985, 1991; Bourke & Runnegar 1986).

Algal-related problems in drinking-water treatment utilities include a reduced efficiency of coagulation and procedures for the removal of dissolved and particulate organic matter (Steynberg *et al.* 1996), filter clogging, the

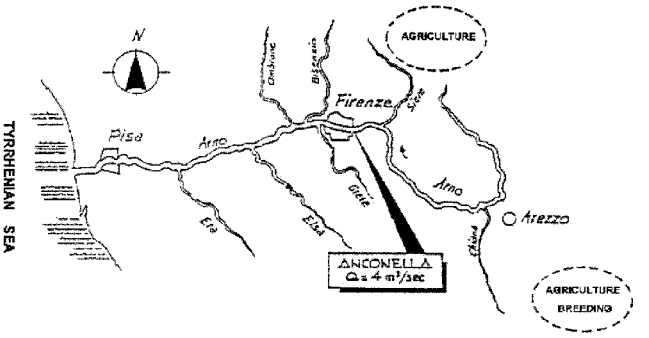
release of organic matter, off-tastes, odour problems and colour alterations (Hu & Chiang 1996).

The presence of microalgae in water used for the production of drinking water requires high chlorine dosages for pre-chlorination in order to neutralise surface loads of algal particles and to facilitate their agglomeration during the floc formation. High chlorine dosages are likewise required for post-chlorination to control bacterial regrowth in distribution networks.

For an improvement in water quality, the sources of anthropic environmental pollution need to be limited so as to reduce algal blooms in surface water, and water treatment processes need to be optimised to reduce risks to public health.

As not much research has been carried out in Italy on the evaluation of planktonic microalgae and cyanobacteria contents in drinking water and on the removal of algae and algal toxins in treatment facilities, the authors considered the study of this problem to be of great importance.

The aims of this study were as follows: (i) to monitor microalgae contents in Arno river water used for the production of drinking water for the city of Florence, and (ii) to investigate the efficiency of algal removal by the treatment procedure used in the above-mentioned facility.



Parameter	Min	Max	Mean
Turbidity (FTU)	2.2	100.0	20.4
Flow rate (m^3 s)	3.7	189.9	30.1
Temperature (°C)	6.5	30.0	17.3
pH	7.7	8.3	8.0
TOC (mg l^{-1})	1.8	5.2	3.7
NH_3 (mg l^{-1})	0.0	0.8	0.2
NO_3^- (mg l^{-1})	0.2	12.1	6.2
O_2^* (mg l^{-1})	7.8	10.5	8.9
O_2^* saturation (%)	70.0	139.0	93.8

* Oxygen dissolved

Figure 1 | Map of Arno river basin and main physico-chemical parameters of raw water collected from December 1993 to November 1994 at the intake of the Anconella drinking-water plant.

MATERIALS AND METHODS

Sampling area

The Arno river basin

Figure 1 shows the Arno basin and a number of raw water parameters. The Arno River is 241 km long with a basin of 8,228 km^2. It flows through Tuscany and is therefore affected by the pollution typical of this region: agriculture, animal breeding, and urban effluent. Near Florence, the river chiefly shows signs of pollution caused by domestic wastewater, as there is little industry in the basin upstream. The river shows wide seasonal flow variation. There have been long periods of drought with increasing frequency in recent years. Rainfall in the basin is irregular and influenced by the configuration of depressions and Apennine ridges that direct moisture bearing winds from the sea.

The drinking-water utility

The city of Florence is served by two utilities using water from the Arno river for a production of 300,000 m^3 d^{-1}. About 80% of the drinking water is produced by the main utility (Anconella), which has a maximum capacity of 4,000 m^3 s^{-1} using a treatment procedure consisting of the following phases:

pre-oxidation with chlorine dioxide and treatment with powdered activated carbon (PAC);
coagulation/flocculation with aluminium polychloride and sedimentation;

sand filtration;
ozonation;
post-chlorination with chlorine dioxide.

Water sampling

During the period December 1993–November 1994 the following samples were collected every 2 weeks: 24 raw water samples, and 24 treated water samples. Microalgae and cyanobacteria were counted and their species determined in all 48 samples. Settled and filtered water samples (24 of each type) which were examined only for their algal units.

Chlorophyll-a, b and c were determined in raw water samples.

Physico-chemical parameters, such as turbidity, temperature, pH, TOC, NH_3, $NO_{3\_}$ and dissolved oxygen were also determined. The river flow rate was recorded for correlation with the presence of algae.

Microalgae evaluation

After having made a comparison between concentration techniques proposed by various authors (*Standard Methods for the Examination of Water and Wastewater* 1992) (centrifugation, filtration and sedimentation) the authors chose the latter method because it is less damaging to cells which is very important for observing the morphological aspects of algae.

Cylindrical settling chambers were used with a thin glass bottom that permits the viewing of organisms settled directly in the inverted microscope without further manipulation.

Every chamber has a grid subdivided into squares of 1 mm^2.

For treated water the settling chamber used had a base diameter of 10 cm and a height of 8 cm for a maximum capacity of 628 ml. The sample volume was 500 ml and the height of the water column was 6.37 cm.

A smaller chamber measuring 2 cm in diameter and 4 cm in height for a maximum capacity of 50 ml was utilised for raw water. The volumes analysed were 5 or 10 ml depending on the algal density; the height of the water column was either 0.4 or 0.8 cm. The water samples were collected in one-litre glass bottles and stored in a dark and cool place.

Raw water samples were analysed as follows: the bottle was shaken for suspending cells and live samples of 5 and 10 ml were poured into the settling chambers. After having added Lugol's solution to the remaining sample in the bottle, thus obtaining a transparent, brownish colour, the third settling chamber was filled with 10 ml of the preserved sample.

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

For settled water, preserved and live samples of 250 ml were prepared.

For filtered and treated water, fresh and preserved samples of 500 ml were prepared. The sedimentation time was 8 or 24 h, depending on the sample type and volume. For treated water 24 h were sufficient to obtain a complete sedimentation.

In the presence of floating algae the surface tension was higher than the force of gravity; a longer sedimentation time is thus of no advantage.

The samples were preserved according to *Standard Methods* (1992).

Live samples were generally used, as they are useful for taxonomy analyses, but in the case of abundance of motile organisms or species that tended to float, preserved samples were examined.

Microalgae counts

The algal specimens were observed directly in the settling chambers with an inverted Nikon Diaphot-TMD microscope equipped with 10×, 20×, 40× and 60× objectives.

For counts of microalgae and the determination of their genus 20× and 40× objectives were generally used, and a good quality objective of 600× magnification was used to resolve smaller details.

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, and 300 squares in the chamber containing the other sample volumes, 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: $N/\text{ml} = N_i A/an_i V$, 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; and V = the sample volume in ml.

For genera determination the taxonomic keys of the Photographic Atlas of Palmer (1977), Bourrelly (1972) and Streble & Krauter (1984) were used.

The single specimens were measured using an ocular micrometer.

Chlorophyll determination

To calculate the amount of chlorophyll in raw water, the sample was filtered 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 densities of the extracts were determined using a Perkin-Elmer (model 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):

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

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 extract (10 ml), l the length of the optical path (1 cm) and V_g the volume of filtered water (litres).

RESULTS AND DISCUSSION

Algal monitoring of raw water

Algal counts in raw water showed a predominance of Bacillariophyceae (diatoms) followed by Chlorophyceae, Cyanophyceae, Euglenophyceae and Dinoflagellated (Figure 2). Both diatoms and green algae were found in all samples; their number was reduced in winter but extremely high in spring–summer. Other groups of microalgae were sporadic and only found in summer. Therefore, the overall quantity of microalgae detected in autumn and winter (October 1993–February 1994, October–November 1994) was low, whereas from March to September 1994 it increased sharply with a predominance of diatoms. Presumably this pattern is correlated to the highly variable flow of the Arno river, and hence closely related to changes in turbidity and flow rate as shown in Figure 3. The increase in turbidity is mainly due to an increased quantity of suspended debris and a higher flow rate of the river leading to algal stress and cell destruction.

The only species that may survive are Bacillariophyceae thanks to their thick outer layer. This is also confirmed in the literature (Reynolds & Glaister 1993). Moreover, between June and September 1994, longer exposure to solar radiation, higher water temperatures (up to 29°C), lower flow rates and therefore an increase in nutrients favoured the growth of diatoms and green algae, as well as other classes of algae.

The authors did not find a high diversification of genera in any algal class. The following specimens were predominant: *Fragilaria* and *Melosira* among diatoms, *Chlorella* among green algae and *Oscillatoria* among cyanobacteria. The highest diversification was found among green algae, and during the summer (*Chlorella*, *Tetrahedron*, *Scenedesmus*, *Ankistrodesmus*, *Pediastrum*, *Crucigenia*).

The algal profile of raw water differed from season to season: in autumn, non flagellate green algae were the

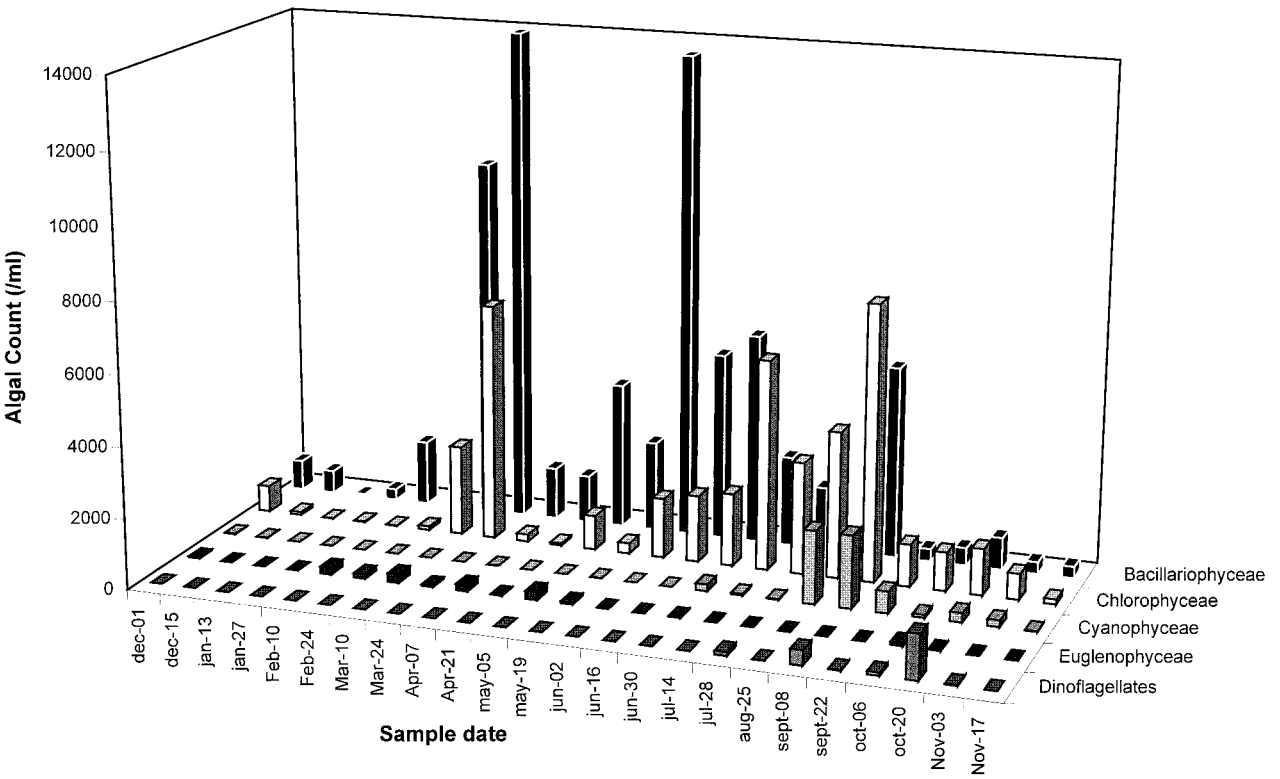


Figure 2 | Distribution of classes of microalgae in raw water of the Arno river from December 1993 to November 1994.

most numerous (21.3%), followed by centric diatoms (genus *Melosira*, 18.2%); in winter, diatoms were predominant (pennate 46.1% and centric genus *Melosira* 34.6%), followed by non flagellate green algae (15.4%). In spring the most numerous specimens were diatoms (pennate 15.2% and centric *Melosira* 61.9%) followed by green algae (20.0%); while in summer, as in autumn, non flagellate green algae (41.0%) were more abundant than pennate (1.7%) and centric (genus *Melosira*, 34.7%) diatoms.

The number of microalgae counted was moderate only in winter. Maximum values of algal blooms of a centric diatom (*Melosira*) were reached in spring (12,000 algal units ml^{-1}) and during the summer months (5,000 algal units ml^{-1}). These algal blooms may cause taste and odour changes in drinking water.

The chlorophyll content of raw water samples confirmed that CHL-a gives the best indication of the river phytoplankton community (Figure 4); moreover, the peak values recorded were mainly related to Chlorophyceae and Cyanophyceae which increase from the end of summer to the beginning of autumn.

Algae removal in the drinking-water utility

Algal removal was found to be highly efficient in the drinking-water utility: mean values of 97.4% were obtained for all samples and of 98.3% when algal concentrations in raw water were significant (10^4 algal units ml^{-1}). It was found that the combined use of chlorine dioxide and flocculant leads to mean values of a 90.1% algal removal. Moreover, sand filtration further reduced the microalgae content, thus enhancing treatment efficiency (97.7%).

Coagulation/flocculation is currently regarded as essential for the removal of algae. Since these organisms

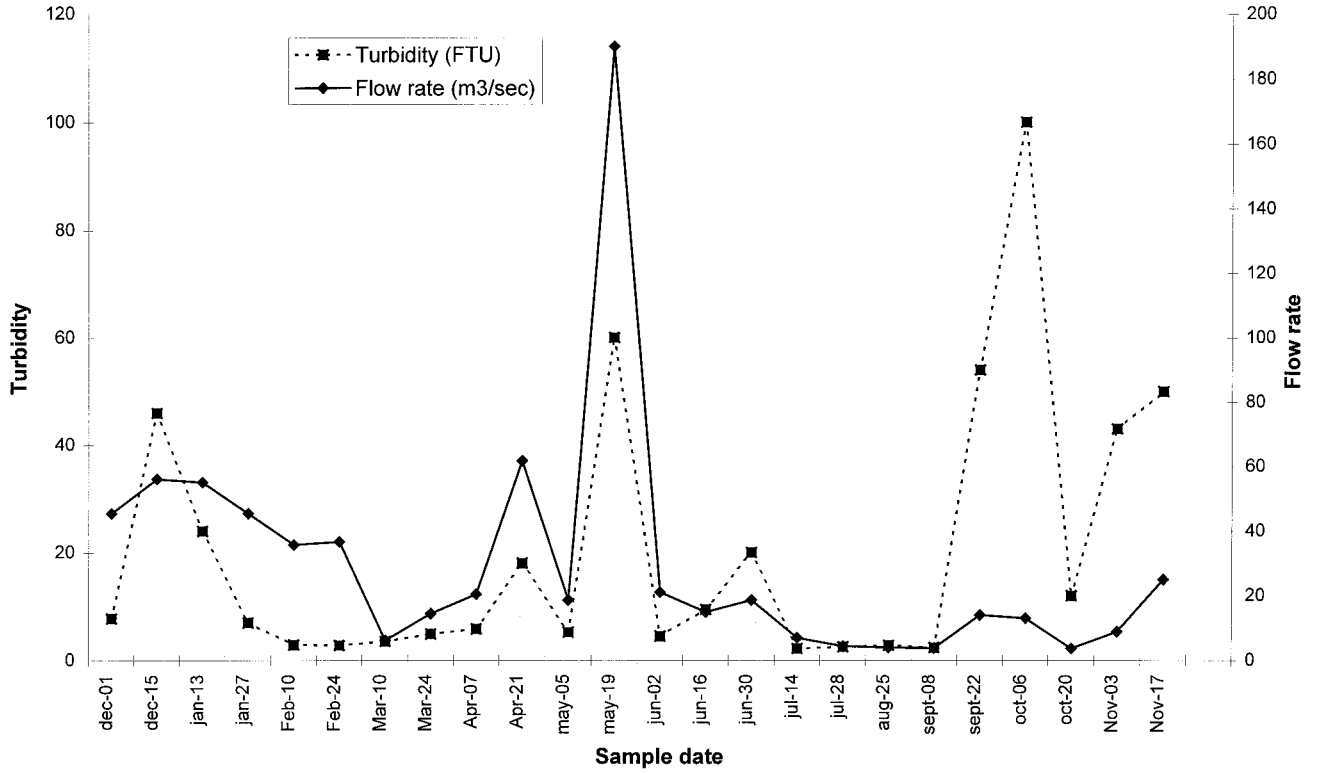


Figure 3 | Turbidity and flow rate of the Arno river from December 1993 to November 1994.

tend to float, they cannot be completely removed by sedimentation. Moreover, a correct and careful treatment procedure is required, usually involving pre-oxidation with chlorine dioxide that neutralises algal surface loads and favours flocculation (Bernhardt & Clasen 1991; Vlaski *et al.* 1996*a*,*b*).

After the subsequent ozonisation and post-chlorination phases, the removal was no higher than that achieved after filtration (98%, ranging from 96–99%). This value dropped to 90–93% when algal removal in the preceding phases was less efficient. In fact, ozone and chlorine dioxide do not physically eliminate algal cells but favour the oxidation process, thus inactivating any algae that survive the preceding treatment phases and their metabolites.

Although algal removal reached high levels at the end of the above-mentioned treatment phases, the quality of the water produced and then passed into the distribution network nevertheless depended on the raw water quality. In fact, even with a 99% removal of algae, the water distributed was not always free of algal cells. However, the surviving cells were depigmented and inactive. The overall treatment process was less efficient for algal removal in the presence of small microalgae or filamentous algae having a diameter less than 5–10 μm.

To improve the organoleptic water quality, which may be affected by algal metabolites, the use of ozone and PAC was found to be essential.

Algal class distribution

Figure 5 shows the algal classes most frequently identified during the monitoring period in raw water. In this period, the algal units (a.u.) were abundant, motile and pigmented. The dominant microalgae were: centric diatoms, mainly *Melosira* (46.1%), non flagellate green algae (30.7%), pennate diatoms, with a predominance of genera

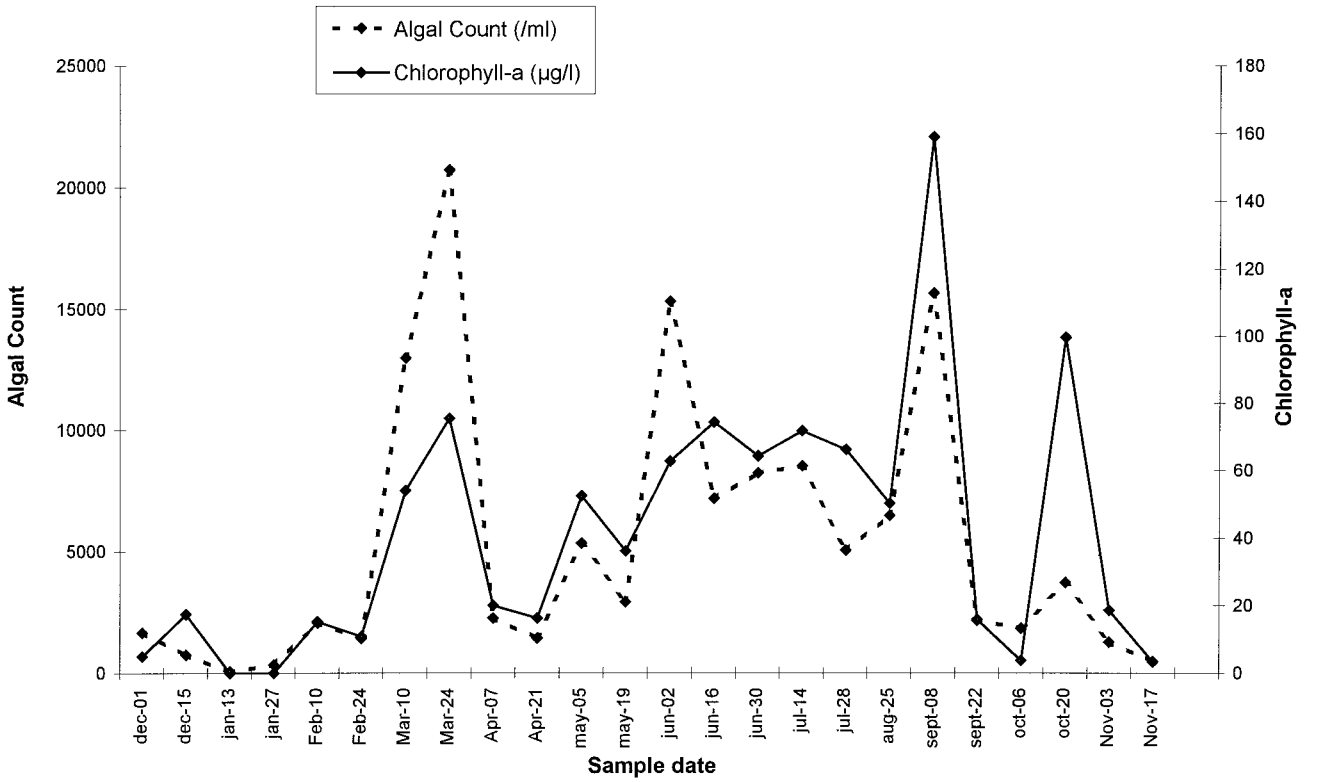


Figure 4 | Algal count and chlorophyll-a concentrations in raw water of the Arno river from December 1993 to November 1994.

Navicula and *Fragilaria* (15.0%), green flagellates (4.4%) and filamentous cyanobacteria (3.8%).

The profile of organisms in treated water (Figure 5) was quite different: the residual algae were non-motile and depigmented, there was a predominance of non flagellate green algae (76.0%) followed by pennate diatoms (8.7%), centric diatoms (8.2%) (mostly *Melosira*), green flagellates (6.1%) and filamentous cyanobacteria (1.0%). The fact that microalgae persisted in the finished water and that a particular algal profile emerged is presumably related to aspects of algal morphology and physiology, such as spherical or fusiform shapes, their small size, motility, cell wall composition and, in some cases, the external mucilaginous layer. The latter features enable these organisms to survive all treatment phases. In spring, summer and autumn, green algae (mainly *Chlorella*) which have these requisites, were predominant in treated water. In winter a predominance of pennate diatoms (63.9%), mainly Fragilaria, can be observed. Others, e.g. Dinoflagellates, were retained because of their exceptional size.

Cyanobacteria monitoring in raw water

Cyanobacteria were substantially absent from raw water during most of the monitoring period, except in summer when they increased to as much as 25% of the algal community. In August their numerical density reached 2,000 algal units ml^{-1} for about 2 weeks, with a predominance of genera *Oscillatoria* and *Anabaena*.

The presence of cyanobacteria is significant for public health and aquatic wildlife since these organisms may produce neurotoxins (e.g. anatoxins) and hepatotoxins (e.g. microcystins) (Carmichael 1992; Fawell *et al.* 1993).

Identification of the species did not enable us to determine whether they were toxic strains. In addition,

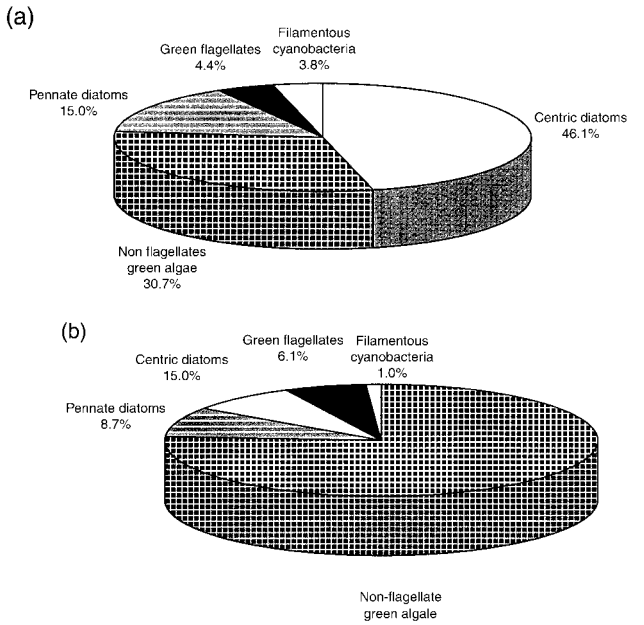


Figure 5 | The algal classes most frequently identified from December 1993 to November 1994 in: a. raw water (24 samples); b. treated water (24 samples). (a.u.=algal units)

physical and chemical parameters such as temperature, pH, solar radiation, nitrogen, phosphorus, growth phase and probably other parameters may influence toxin production (Falconer 1993). In theory, the water samples may or may not have contained algal toxins in the monitoring period.

The lack of evidence of toxicity in fish and birds living near the river suggests that toxins were not present in the river water or were present at concentrations inferior to those that could affect wildlife. However, cyanobacteria can produce toxins that can affect humans but not fish (Chorus & Bartram 1999). Direct laboratory experience and data in the literature show that oxidation with ozone and adsorption on PAC, preferably combined, are able to eliminate algal toxins, including cyanotoxins (Falconer 1993; Mouchet & Bonnélye 1998). Both treatments are employed in the drinking-water utility of Florence, which is therefore equipped for the removal of algal toxins.

CONCLUSIONS

The monitoring revealed the constant presence of microalgae throughout the 12 months study period. Raw water showed a predominance of diatoms (46.1%) and green algae (35.1%; non flagellate 30.7%, green flagellates 4.4%) which affect the quality of treated water as a number of depigmented and inactivated algal cells persist after treatment.

The average algal removal by the drinking-water utility of Florence achieved values of 97.4% for all samples and of 98.3% when algal concentrations were significant in raw water.

Treatments were less efficient for green algae (82.1%: non flagellate 76.0%, flagellate 6.1%) which were predominant in treated water. Even if they do not give rise to any risk to public health, they can increase organic matter in the network, thus leading to the formation of biofilm, and to bacterial regrowth.

Cyanobacteria were not common in raw water, reaching significant levels only in summer (2,000 algal units ml^{-1} in August). The predominant genera were *Oscillatoria*, *Anabaena* and *Phormidium*.

Concerning drinking-water production, the presence of algae in the river should be dealt with separately from that of other microorganisms like bacteria.

The main concepts are as follows:

- it is important to reduce algal growth by limiting organic matter in rivers which serve as a drinking-water source, and reduce nutrients such as phosphorus and nitrogen contained in effluents of wastewater treatment facilities. This is indicated in the EEC Directive 91/271 regarding urban wastewater discharged in areas of environmental importance;
- the total elimination of algal cells in drinking water is not always possible;
- inactivated and oxidised algae may enter the distribution network and therefore water quality has to be maintained by controlling the pipe network and paying attention to end points where the efficiency of post-disinfection is reduced, and by carrying out controls for bacteria and other micro-organisms;

- optimisation of pre-oxidation phases and of coagulation/sedimentation phases is essential as the filtration process for small sized green algae and a number of diatoms is inefficient;
- water treatment processes need to be adjusted continuously to deal with specific algal-related problems;
- certain algal species, such as *Melosira*, may affect the taste and odour of raw water, with repercussions on the final product;
- Cyanophyceae belonging to genera which include toxic strains were identified, but neither aquatic wildlife (fish and birds), nor humans seem to be affected;
- drinking-water utilities in which powdered activated carbon and ozone treatment are employed efficiently remove algal toxins and improve the taste and odour of treated water. This is the case of the utility of the city of Florence which is equipped with these treatment processes.

In conclusion, algae and algae-related problems must be monitored continuously to guarantee the safety of drinking-water production.

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