Sanitary implications associated with the use of eutrophic freshwater

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Summary. - This review presents the problem of eutrophication of lakes whose waters are used also for potable use. The indirect negative impact of algal blooms as well as the direct consequences of the overgrowth of toxic Cyanophyta are considered. Problems for water treatment plants processing eutrophic raw water are exposed. Basic treatment will not easily remove algae or their by-products and increased use of chlorine will give rise to high levels of chlorinated by-products such as THM. Possible alternatives and improvements are suggested for the treatment of poor quality raw water to obtain high quality drinking water.

Key words: freshwater, eutrophication, Cyanophyta, biotoxins.

Riassunto (Implicazioni sanitarie connesse con l'uso di acque dolci eutrofiche). - Nella presente memoria si dibatte il problema della eutrofizzazione dei bacini lacustri la cui acqua è utilizzata anche per scopi potabili. Vengono considerati gli effetti negativi diretti ed indiretti che alge della classe delle Cianofizze hanno nei confronti di organismi superiori, incluso l'uomo. I trattamenti di potabilizzazione tradizionali non sono in grado di abbattere la carica algale e di eliminare le biotossine eventualmente prodotte e il conseguente maggiore impiego di cloro determina la formazione di alti livelli di prodotti intermedi, quali i trialometa. Vengono esposti i problemi che si incontrano nel processo di potabilizzazione di acque eutrofiche. Vengono infine proposti possibili alternative e miglioramenti per il trattamento di acque grezze di scarsa qualità per ottenere acqua potabile di buona qualità.

Parole chiave: acqua dolce, eutrofizzazione, Cianobatteri, biotossine.

Introduction

Initially, the word "eutrophication" was intended to mean the enrichment of a water basin with phosphorous and nitrogen. In a broader sense, "eutrophication" refers to the biological reaction to the increase of nutrients, with an excessive growth of macrophytes and of macro- and micro-algae. This can be observed through the visible loss of transparency of the waters and by the appearance of anomalous water colour. Often such episodes, are accompanied by the presence of foam and scum, as well as by altered organoleptic characteristics caused by substances actively secreted by or released from algae [1].

Though there are two recognized macronutrients (phosphorous and nitrogen), at present the trophic classification of water reservoirs is based only on phosphorous and on two potential productivity indexes: the phytoplankton biomass (indirectly estimated according to the chlorophyll "a" content) and turbidity (Table 1).

The importance of a balance between inorganic nitrogen and phosphorous as orthophosphate, however, is underlined by some researchers who have found nitrogen to be a limiting factor in fresh waters when N/P=5, and phosphorous a limiting factor when N/P=10 [3].

In particular seasons and hydrological conditions, "blooms" of algae may develop in eutrophic freshwater reservoirs and lakes. According to Vollenweider algal "blooms" mean an algal population of such density as to render it visible to the human eye. This means abundant quantities which, depending on the algal species, generally consist in over 1,000,000 cells/l. Typical characteristics of these blooms are the chlorophyll "a" content (>10 g/l) and a tendency to be largely of one species [4, 5].

Even though algal blooms can be formed by green algae and diatoms, the most undesirable blooms both from the ecological point of view and for human uses such as drinking water or recreation, are of cyanobacteria or blue-green algae [6].

This paper is a review dealing with the problems and possible human health implications associated with the use of eutrophic waters.

When an eutrophic water is used for drinking purposes, the following problems can occur: 1) increase of organic substances in drinking water; 2) formation of high levels of by-products after chlorination; 3) development of
Table 1. - Classification of trophic levels in lakes according to OECD criteria [2]

<table>
<thead>
<tr>
<th>Level</th>
<th>Phosphorous total (µg/l)</th>
<th>Chlorophyll &quot;a&quot; (µg/l)</th>
<th>Transparency (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>average</td>
<td>maximum</td>
</tr>
<tr>
<td>Ultraoligotrophy</td>
<td>&lt;4</td>
<td>&lt;1.0</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>Oligotrophy</td>
<td>&lt;10</td>
<td>&lt;2.5</td>
<td>&lt;8.0</td>
</tr>
<tr>
<td>Mesotrophy</td>
<td>10-35</td>
<td>2.5-8</td>
<td>8-25</td>
</tr>
<tr>
<td>Eutrophy</td>
<td>35-100</td>
<td>8.25</td>
<td>25-75</td>
</tr>
<tr>
<td>Hypereutrophy</td>
<td>&gt;100</td>
<td>&gt;25</td>
<td>&gt;75</td>
</tr>
</tbody>
</table>

bacterial biofilms in the pipes with possible triggering of corrosion or crust phenomena; 4) release of reddish waters due to the release of iron and manganese in the pipes, caused by biological corrosive phenomena; 5) accumulation of sediments in the less used parts of the network, particularly in distribution pipes characterized by intermittent flows; 6) production of hydrogen sulphide from sediments and from bacterial tubercles in the biological film; 7) presence in the pipes of protozoa nematodes, insect larvae etc.; 8) release of ammonia, phosphates and silicates from the sediments originated by microbiological activity; 9) unpleasant organoleptic characteristics [7] due to the presence of some compounds such as geosmin, mucidone, methoxyprazine and methylisoborneol, produced by actinomycetes, cyanobacteria, and by certain species of *Pseudomonas* that can establish themselves in the network [8].

### Taste and odour problems

The relationship between algal Cyanophyta and taste and odour of the waters was demonstrated as early as 1883. In addition to the most commonly encountered substances such as geosmin [9] and methylisoborneol [10] (Table 2) there is evidence that many blue-green algae produce several metabolites which can also give odours and tastes to the water [11]. Among these, there are betacyclolactone with its typical tobacco odour, and a wide range of hydrocarbons, fatty acids, aromatic compounds, ketones, terpenoids, amines and sulphides which can even give particular tastes and odours to fish and other water organisms [12].

Cyanophyte species such as *Anacystis cyanae*, *Anacystis nidulans*, *Chlorogloea fritschii*, *Chroococcus turgidus*, *Lyngbya aestuarii*, *Lyngbya lagerhaimii*, *Microcoleus chthonoplastes*, *Microcystis aeruginosa*, *Microcystis weisenbergii*, *Nostoc muscorum*, *Oscillatoria williamssii*, *Phormidium luridum*, *Plecostema terebrans*, *Spirulina platensis* and *Trichodesmium erythraeum* produce aliphatic hydrocarbons and particularly n-heptadecane [13]. This is also the main component produced by *Coccolithus elabens*, *Agmenellum quadruplicatum*, and *Anacystis montana* [14].

Among these metabolites, it is very rare to find hydrocarbons with a smaller chain than 14 carbon atoms; however, n-decane, n-octadecane, n-tridecane have also been isolated from *Cyanidium caldarium* [12].

Several cyanobacteria synthesize C16 and C18 fatty acids and their esters cause taste, together with 2-amino propane, trimethylamine, 1-amino propane and ethanolamine. Methyl-mercaptan and dimethyl-disulphide, found in old cultures of *Oscillatoria chalybea* and of *Microcystis flos-aquae*, could be the result of bacterial degradation of algal cells [15].

Table 2. - Cyanophytes producing geosmin and methylisoborneol

<table>
<thead>
<tr>
<th>Species producing geosmin</th>
<th>Species producing methylisoborneol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symploc a muscorum</td>
<td>Oscillatoria tenuis</td>
</tr>
<tr>
<td>Oscillatoria tenuis</td>
<td>Oscillatoria curviceps</td>
</tr>
<tr>
<td>Oscillatoria prolifica</td>
<td>Lyngbya cryptovaginata</td>
</tr>
<tr>
<td>Oscillatoria cortiana</td>
<td></td>
</tr>
<tr>
<td>Oscillatoria variabilis</td>
<td></td>
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<tr>
<td>Oscillatoria agardii</td>
<td></td>
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<tr>
<td>Oscillatoria splendidia</td>
<td></td>
</tr>
<tr>
<td>Lyngbya aestuarii</td>
<td></td>
</tr>
<tr>
<td>Anaibaena cirinata</td>
<td></td>
</tr>
<tr>
<td>Schizothrix muelleri</td>
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</tbody>
</table>
Toxins produced by cyanobacteria

Cyanobacteria may produce two types of toxins, cyto toxins and biotoxins, according to the way of detection. Cyto toxins are detected by bioassays with cultured mammalian cell lines. No evidence exists on animal deaths due to this kind of toxins [16]. Biotoxins are compounds that most commonly produce intoxications in animals and man.

The most common bloom-forming cyanobacteria producing biotoxins, are Microcystis, Anabaena, Anphanizomenon oscillatoria, Nodularia and Nostoc. Other species are shown to produce also these toxins, i.e. Coelosphaerium, Cyclotropspermopsis, Fischerella, Gloeothrixia, Gomphosphaeria, Hapalosiphon, Microcoleus, Schizothrix, Scytonema, Spirulina, Symploca, Tolypothrix and Trichodesmium.

Two different kinds of biotoxins are produced by cyanobacteria: neurotoxins and hepatotoxins. These may be distinguished by the effects that lethal doses cause in intraperitoneally (i.p.) injected mice. Hepatotoxic blooms are almost twice as common as neurotoxic ones. Statistical associations were found between hepatotoxicity and incidence of Microcystis aeruginosa, M. viridis, M. wesenergii, Aphanizomenon spheroides and Anabaena flos-aquae. Neurotoxicity was statistically associated with Anabaena lemmermannii, Anabaena flos-aquae, Gomphosphaeria naegeliana, Anphanizomenon flos-aquae.

Effects of algal biotoxins on animals

Aphatoxins produced by Aphanizomenon flos-aquae are fast-acting neurotoxins that inhibit nerve conduction by blocking sodium channels without affecting permeability to potassium, the transmembrane resting potential, or membrane resistance [17].

The pre-death symptoms, induced in mice treated with extracts of A. flos-aquae, include: spastic irregularities, open mouth, loss of coordination and violent tremors. Injected i.p., it acts within 1 and 2 min, with a latency period up to 5 min, and has a minimum lethal dose ranging from 0.05 to 0.1 mg of algal lyophilisate/kg body weight in mice [18].

The toxic extract of the alga injected i.p. in the fish Fundulus heteroclitus induces a darker pigmentation, extending from the head to the tail with loss of balance and consequent death by respiratory block. This phenomenon of progressive darkening is similar to that described for saxitoxin [19]. The lethal dose for F. heteroclitus is 29 μg/kg body weight [20].

Anatoxin-a was the first toxin from a freshwater cyanobacteria to be chemically defined. It is a secondary amine whose chemical name is, 2-acetyl-9azabicyclo(4-2-1)non-2-ene with a molecular weight of 166 Da [21]. The lethal dose of anatoxin-a administered orally to calves is 6-8 times greater than that derived from intraperitoneal injection (i.p.) in mice while for rats it is 25 times greater than that for mice. For birds, as for mammals, the sensitivity to the toxin varies with the lethal dose by the oral or intra-peritoneal routes for pheasants 2-4 times greater than that for ducks [22].

Signs of poisoning with anatoxin-a in field reports for wild and domestic animals include staggering, muscle twitching, gasping, convulsions, and opisthotonos (head and neck extended over the back in birds). Death by respiratory arrest occurs within minutes to a few hours depending on species, dosage and prior food consumption. The LD₅₀ after i.p. administration to the mouse of the purified toxin is about 200 μg/kg body weight, with survival time of 4-7 min. Animals need to ingest only a few millilitres to a few litres of the toxic surface bloom to receive a lethal dose [23-25].

Microcystins are cyclic heptapeptides (M.W. = 909-1067 Da) of which more than 24 different variants have been described to date [26]. The main target organ of microcystins is the liver. Investigations on its distribution in the body have shown that it is preferentially transported into hepatocytes [27-30].

Microcystins at 50-100 nM concentration cause rapid blebbing of hepatocytes, decreases in GSH content, activation of phosphorylase A and increases in cytoplasmic levels of calcium but with no change in cyclic AMP levels. The results suggest that the disruption of cytoskeletal structures is accompanied by disturbances in cellular calcium homeostasis and by decreased protection against oxidative damage to the cells [31]. Hepatotoxins induce changes in the actin microfilaments, forming part of the cells’ cytoskeleton leading to a dense aggregation of the microfilaments near the centre of the cell [32]. As a result of this loss of cellular support the cells round up leading to destruction of the sinusoids of the liver, lethal intrahepatic haemorrhage and/or hepatic insufficiency.

Toxins similar to microcystins have been isolated from several different Cyanobacteria. These toxins which have similar toxicological properties and share some common structural features constitute a peculiar link between taxonomically very distinct species. They are often produced in great amounts (up to 1% of the dry weight, in certain Microcystis strains).

Acute effects of algal toxins on man

First recorded evidence of an outbreak of human disease associated with blooms of algae was registered in 1931. More than 5,000 inhabitants of Sewickley, Pennsylvania (USA) [26] were affected by diarrhoea but a bacterial agent could not be identified. They were served by water coming from a basin with an abundant bloom of Cyanophytes. A modest incubation period, lasting 2-12 hours, was followed by a two day acute
hepatic stage and a lethargic stage of 1-2 days. This was due to the electrolyte imbalance caused by gastrointestinal symptoms, which could last up to 5 days. After the critical stage was over, serum transaminases indicating effects on the liver were elevated for a long period [33, 34].

Measures to treat eutrophic waters for human consumption

Mechanical or physical treatment of the water to remove algal cells without damage is often preferable to chemical treatment in which the cells we damaged and the contents released since the cell components will cause significant problems for treatment.

The operation of the treatment plant will be highly dependent on the particular circumstances and will depend on the species of algae present and their density. Apart from potential toxicity from blue-green algae, algae in general can have a significant deleterious effect on finished drinking water. Algae breaking through filters can contribute to turbidity and decreased efficiency of disinfection and may even give rise to algal growth in storage tanks. This is a particular problem with species such as Oscillatoria which can grow in low light or can use available assimilable organic carbon to grow in the absence of light. In general, apart from the trichomonad species, the Cyanophytes are more difficult to remove from raw water.

Chemical treatment

Chlorination

Chlorine concentrations of 1 mg/l and above cause the destruction of chlorophyll “a” [35] causing cell death. Low concentrations, on the other hand, cause the temporary suspension of photosynthesis. It would be better to avoid breaking the cells because with the release of endocellular substance there can be a release of polysaccharides which can interfere with flocculation [36].

All algae produce exometabolites which are potential precursors of trihalomethanes (THM). THM precursors consist of: 1) chlorophyll and other natural coloured compounds; 2) macromolecules in the cell biomass; 3) extra or exo-cellular products (ECP) [37].

Humic and fulvic acids are major precursors for THM formation in natural waters but it has also long been established that algal products are also important in this respect. Indeed in eutrophic waters with intense algal activity these are probably the dominant precursors. Table 3 summarizes comparative production of chlorofrom, usually the dominant THM, between algal products and commercially available fulvic and humic acids [38].

Within the same class, different species give different yields of THM (Table 4). Among Cyanophyta, for instance, Oscillatoria tenuis produces more precursors of THM than Anabaena flos-aquae, while for the production of organic carbon, exactly the opposite occurs [37].

Alternative oxidants

Eutrophic waters with heavy algal blooms may benefit from disinfection by oxidants other than chlorine. In some countries such as France, ozone may be preferred but chlorine dioxide is another alternative to chlorine. Algae are removed at ozone doses of 0.5 to 2.0 mg/l while 2.2 mg/l ozone can ever kill small invertebrates [39]. However very long contact times of up to 30 minutes may be needed to remove algae completely. Generally ozone is more effective against prokaryotes such as Cyanophyta since their chlorophylls are not protected in chloroplasts [40]. Appropriately applied ozone can help to remove 65-75% of Oscillatoria biomass [41].

An additional advantage of ozone is that it destroys algal products causing tastes and odours which are a source of consumer complaints [42]. Oxidation of organics can be further stimulated by the use of UV with ozone or peroxides [43].

| Table 3. - Percentage of chloroform (guiding THM component) with reference to TOC (total organic carbon). Maximum and minimum values are reported |
|-----------------------------------------|-----------|-----------|
|                                       | CHCl₃/C (TOC) (%) |
| min                                   | max       |
| Extra-cellular products                | 0.04      | 5.0       |
| Cellular biomass                       | 0.3       | 4.0       |
| Fulvic acids                           | 0.3       | 0.9       |
| Humic acids                            | 0.5       | 1.6       |

| Table 4. - Percentage production of chloroform, with reference to TOC (total organic carbon) produced by various algae |
|---------------------------------------------------------------|--------|--------|
| Algae                                      | CHCl₃/C (TOC) (%) | TOC (mg/l) |
| Chlorella pyrenoidosa                        | 0.3-2.6 | (2.8-18.9) |
| Scenedesmus quadricauda                     | 0.2-0.5 | (20.7-75.8) |
| Anabaena flos-aquae                         | 0.2-0.7 | (10.9-30.5) |
| Oscillatoria tenuis                         | 0.9-3.9 | (2.1-23.5) |
As with other oxidants, Cyanophyta are more sensitive than other phytoplankton algae to the effects of hydrogen peroxide: Oscillatoria and Microcystis are killed with 1.7 mg/l, while Raphidiopsis requires 3.4 mg/l, Ankistrodesmus 6.8-10.2 mg/l and Anabaena 5-9.8 mg/l. Green algae such as Pandorina are insensitive to treatment with hydrogen peroxide even at very high dose, since pigments are well protected within the chloroplasts [43]. Potassium permanganate is a good oxidizing agent at different pHs, but is more active at an acid pH. Besides its oxidative action it stimulates filtering efficiency because its reduction product (MnO2) acts as an additional filter when it deposits on the filter bed [44]. Potassium permanganate is effective against algae in general and can be used to destroy unidentified biomasses. It has the advantage of being exceptionally soluble, does not alter the pH, does not increase odours and is compatible with other treatments such as chlorination, treatment with lime, and other coagulants.

**Clari-flocculation**

Clari-flocculation was first used to remove suspended solids contributing to turbidity and reduced efficiency of disinfection. Most of these suspended solids were inorganic but bacteria were also removed in the process [45]. Temperature, pH, dose and type of coagulating agent all have a major influence on the removal efficiency and the optimum conditions will vary considerably [46]. The most commonly used coagulating agents are aluminium and iron salts although organic polyelectrolytes are increasingly employed.

Cyanophyta interfere with clari-flocculation because they contain intracellular gas vacuoles which make them buoyant and colonial forms are surrounded by a mucilaginous gariage. They also produce other polysaccharides and mucopolysaccharides which can sequester the metal ions of the coagulating agent and prevent proper formation of the floc blanket. Algae are also associated with pH fluctuations at different times of the day and pH control becomes more difficult. Waters commonly affected by Cyanophyto blooms therefore require very careful design and operation of the clari-flocculation stage of treatment.

Only one third of the mucopolysaccharides produced or released from lysed Cyanophyta can be removed in this section of the works; all the rest passes through the distribution network where it may become easily assimilating organic carbon, which can stimulate a renewed growth of bacteria in the form of aftergrowth in the system.

**Filtration**

Slow sand filters remove suspended solids and bacteria but they also remove organic substances in solution in the highly biologically active surface layers [47]. Rapid gravity filtration has no biological activity and unless it is operated very efficiently algae can penetrate the filters into supply [48, 49]. Filters may vary considerably in both design and the filter media used [50, 51]. The efficiency of filters in removing algal cells and products will vary considerably but heavy Cyanophyto blooms can cause significant operational problems.

**Activated carbon**

Two forms of activated carbon are used as adsorbents in water treatment. These are powdered (PAC) which is dosed intermittently and then removed in flocculation and granular (GAC) which is used as a filter medium, at the last stage in the treatment process, which may also develop biological activity and which can be regenerated [52]. The toxins produced by some cyanophytes are not removed by ordinary filtration, although there may be some removal by the biological region of sand filtering. However, they are removed to some extent by PAC and to a much greater extent by GAC.

**Preventive measures**

In view of the considerable difficulties caused by Cyanophyta blooms it is obviously advisable, where possible, to design and construct reservoirs which do not encourage the growth of algal blooms. For example it would be appropriate to establish a reservoir with a very short retention time which would not give the algae time to build up in numbers or to build in desratification techniques which disrupt the growth-optimal light. However such a design will depend on the particular circumstances and even more important is to prevent the discharge of excessive nutrients to reservoirs from sewage effluent or farming. This will require careful planning and catchment management.

**Conclusions**

Cyanophyta blooms in water bodies used for potable water supply can give rise to major problems in terms of water treatment and water supply. Many of these problems such as interference with water treatment processes are well known but others such as problems arising from toxins produced by Cyanophyta are still under study.

It is essential that the potential for Cyanophyta blooms be given careful consideration in the design and operation of drinking water reservoirs and treatment works. However the best approach is to prevent the discharge of excessive nutrients to such waters.

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