203.3

i

i

85 HE

RW) 1993

NATIONAL INSTITUTE FOR WATER RESEARCH

INSTITUTE OF WATER POLLUTION CONTROL (SOUTHERN AFRICAN BRANCH)

AND

WATER RESEARCH COMMISSION

HEALTH ASPECTS OF EUTROPHICATION

ΒY

W.E. SCOTT, R.A. VAN STEENDEREN AND D.I. WELCH NATIONAL INSTITUTE FOR WATER RESEARCH

Paper to be presented at Symposium on The Impact of Phosphate on South African Waters CSIR Conference Centre, Pretoria 22 November 1985

LEE CALLA ED DE SERVICIO E SUIDERSUITE PUNCTA - CO-SERVICIO PERFECTO - COLARIA A MANA - LEMA CO-SERVICIO PERFECTO A ELOS (2000)

This paper may <u>NOT</u> be published without the permission of the National Institute for Water Research, the Institute of Water Pollution Control and the Water Research Commission. 203.3-85HE-2022

S. J. L. 141/142 6420 isin 2022 203.3 85HE LO:

.

4 10 B

HEALTH ASPECTS OF EUTROPHICATION

W.E. SCOTT, R.A. VAN STEENDEREN* and D.I. WELCH**

M.Sc. (UOFS), Sci. Nat.; *D.Sc. (University of Pretoria), M.I.W.P.C. and **Ph.D. (Aberdeen), C. Biol., M.I. Biol. National Institute for Water Research, CSIR, P.O. Box 395, Pretoria, 0001

1001. , 85

ABSTRACT

Phosphate a key nutrient in eutrophication may be indirectly responsible for various health problems in water supplies. Health problems may be connected with the development of large amounts of plant biomass in the form of algal or cyanobacterial blooms. The two features of algae-rich waters which are most likely to have health implications are, firstly, production of various cyanobacterial toxins and, secondly, organohalide formation after treatment by chlorination. Antibacterial substances produced by certain algae may on the other hand be responsible for an improvement in water quality.

EKSERP

Fosfaat, 'n sleutelvoedingstof in die eutrofikasieproses, kan indirek vir 'n aantal gesondheidsprobleme in watervoorrade verantwoordelik wees. Gesondheidsprobleme kan verbind word aan die ontwikkeling van groot hoeveelhede plantmateriaal in die vorm van alg- of sianobakteriële opbloeie. Die twee kenmerke van algbevattende water wat waarskynlik die grootste gesondheidsimplikasies het, is eerstens, die vorming van verskeie toksiese stowwe van sianobakteriële oorsprong en, tweedens, die vorming van organohalogeenverbindings na waterbehandeling deur chlorering. Sekere alge kan antibakteriële stowwe produseer wat 'n heilsame invloed op watergehalte kan hê.

INTRODUCTION

The development of large amounts of algae, especially cyanobacteria (or blue-green algae), in lakes or impoundments as a result of excessive phosphorus loading or eutrophication is well known (Toerien, 1977; OECD, 1982). It is not always realized that algae may be involved in a number of ways in the health aspects of eutrophied waters. The potential pathogenic effects of some algae are perhaps not as well known as the diseases caused by bacteria and/or fungi, since properly documented incidents have been few in number and in many cases the evidence of algal responsibility is circumstantial or not recognized. The literature on animal and human diseases caused by algae is scattered in many diverse journals and reports, but several helpful reviews have appeared over the years (Schwimmer and Schwimmer, 1964; Schwimmer and Schwimmer, 1968; Gorham and Carmichael, 1979; Carmichael, 1981; Stein and Bordon, 1984 and Carmichael, Jones, Mahmood and Theiss, 1985).

Algae are known to produce organic extracellular products as a normal part of their growth (Fogg, 1971). The concentration of dissolved organic compounds in water will increase upon the death and lysis of The nature of many of these compounds is still unknown and algal cells. although conventional water treatment practices such as flocculation, sedimentation and filtration will remove algal cells, numerous dissolved organic compounds will remain in the water. Chlorination of organic compounds of direct or indirect algal origin during water treatment can lead to the formation of potentially carcinogenic and/or mutagenic The higher the level of organic precursors, the more organohalides. intensive chlorination will be required. A considerable volume of literature on water chlorination has appeared as a result of the publication of the proceedings of a series of four biannual conferences (Jolley, 1978; Jolley, Gorchev and Hamilton, 1978; Jolley, Brungs and Cumming, 1980 and Jolley, Brungs, Cotruvo, Cumming, Mattice and Jacobs, 1983) but only a comparative limited amount of information on the role of algae or algal extracellular products as precursors or organohalides has been published (Hoehn, Randall, Goode and Shaffer 1978; Morris and Baum, 1978; Briley, Williams, Longley and Sorber, 1980; Pilkington and van Vuuren, 1981; Bernhardt, 1982 and Wachter and Andelman 1984).

In this paper we discuss algal associated health aspects which are linked with eutrophied water sources. We also present some additional information on the formation of total organohalogen precursors in laboratory algal cultures. The paper is concluded with a consideration of possible beneficial effects of algae in eutrophic waters.

HARMFUL OR TOXIC SUBSTANCES ASSOCIATED WITH ALGAL BLOOMS

Gastrointestinal disorders

According to Schwimmer and Schwimmer (1968) the earliest report of an algae-caused disease dates from 1842 when Farre identified filaments of the cyanobacterium Oscillatoria in the stool of a thirty-five-year-old woman suffering from dyspepsia. Farre felt that the Oscillatoria originated from the drinking water supply in London.

Several reports from Canada during the 1950's describe at least fourteen cases of human algal illnesses after involuntary and/or accidental consumption of lake water containing cyanobacteria (Schwimmer and Schwimmer, 1968). In all cases headache, nausea, vomiting and diarrhea symptoms developed. The illnesses were mostly contracted by children who had swum in various lakes. The cyanobacteria Microcystis, Anabaena and Aphanizomenon were identified in stool and vomitus samples from various patients. An unknown number of children from a rowing team from a Johannesburg high school became ill after drinking water from the It is known that eutrophic Roodeplaat Dam in the summer of 1978/79. Roodeplaat Dam contains various species of Microcystis and Anabaena during the summer months (Scott, Barlow and Hauman, 1981). Attempts to obtain more information about the incident were unsuccessful since most of the children recovered after 2 or 3 days and the school involved did not want to or was unable to supply additional information.

Zillberg (1966) supplied circumstantial evidence that blooms of Anabaena flos-aquae were responsible for gastroenteritis in European infants in Salisbury (now Harare), Zimbabwe. The blooms occurred in Lake McIlwaine which supplied water to the (then) European suburbs. The non-European townships received water from a different source and here the incidence of gastroenteritis was significantly lower. Carmichael et al. (1985) reported on nine incidents from 1975 to 1981 in three different states of the USA where municipal or recreational water supplies were involved in occurrences of blooms of cyanobacteria (mostly Anabaena spp.) which resulted in human illnesses. In some cases alternative water supplies had to be employed until the blooms disappeared. Direct evidence of a diarrhea-producing toxin present in extracts of Microcystis has been given by Aziz (1974). Allergenic reactions manifested as skin and/or eye irritations after swimming in water containing cyanobacterial blooms have been documented by Stein and Borden (1984) and Carmichael et al. (1985).

Liver toxins produced by Microcystis

Reports on cyanobacterial toxicity in South Africa are usually associated with Microcystis aeruginosa (also described as Microcystis toxica). Most of these reports occurred in eutrophic impoundments and farm dams in the Transvaal and Orange Free State (Steyn, 1945; Stephens, 1949; Louw 1950; Toerien, Scott and Pitout 1976; Amann and Eloff, Eloff, 1981; Scott et al. 1981 and Scott, 1985). M. aeruginosa 1980; is the most common algal bloom former in this country and is of considerable importance in local eutrophication problems. Two morphological forms of M. aeruginosa can be distinguished in field material. Irregularly shaped colonies with a net-like appearance containing individual cells with a diameter of 4-6 μ m are known as M. aeruginosa forma aeruginosa, while compact spherical or lens-shaped colonies containing individual cells with a diameter of 2.5 - 5.5 µm are know as M. aeruginosa forma flos-aquae (Komárek 1958). Toxicity as measured with a mouse test is usually present in the forma aeruginosa and not in the forma flos-aquae (Scott et al., 1981).

The chemical structure of five toxins isolated from laboratory cultures and field material collected in the Transvaal has been determined (Botes, 1985). The toxins are potent liver toxins and as a group have been given the name cyanoginosins. Cyanoginosins are cyclic heptapeptides and all contain as a constant feature the following five amino acids: D-alanine, D-glutamic acid, erythro- β -methylaspartic acid, N-methyldehydro alanine and a novel β -amino acid known as Adda (3-amino-9-methoxy-10-phenyl-2-6-8-trimethyl-4-6-dienoic acid).

Human liver intoxication suspected of being caused by cyanobacterial toxins has been reported in Australia. In 1979 a water-borne outbreak of hepatoenteritis occurred among 139 children and 10 adults on Palm Island, Queensland (Bourke and Hawes, 1983). The disease was connected with a water supply containing a dense water bloom. The water was not the time of the incident and the identity of the sampled at cyanobacterium responsible for the bloom is uncertain. Shortly after the incident Anabaena flos-aquae was present in the phytoplankton. Strong evidence of liver injury has been obtained from the population of Armidale, New South Wales during a period of a bloom of toxic M. aeruginosa in the water-supply reservoir, Malpas Dam. At a time when a toxic bloom was present a significant elevation of the level of y-glutamyltranspeptidase could be demonstrated in plasma specimens of the population receiving their drinking water from the reservoir (Falconer, Beresford and Runnegar, 1983). No such elevated enzyme activity could be demonstrated in an adjacent population receiving water from a different source or at a time when the toxic bloom was absent.

An investigation into the effects of two cyanoginosin toxins on rats showed that pathological lesions caused by cyanoginosin LA was similar to that caused by cyanoginosin LR (K. Jaskiewicz, P.G. Thiel and D.P. Botes, 1985, unpublished results). Toxin LR differs structurally from LA by the replacement of an L-alanine residue by L-arginine. These purified toxins when applied by intraperitoneal infection to test animals (rats or vervet monkeys) proved to be up to five times more toxic than application by mouth. Freeze-dried algal material dosed to monkeys by intragastric intubation also appears to be more toxic than purified toxin. The reason for these differences are not yet understood (P.G. Thiel, personal communication).

Other non-liver toxins and diseases associated with algal blooms

An outbreak of pyrogenic reactions in patients at a haemodialysis centre in Washington D.C. was traced to endotoxins present in tap water used to prepare the dialysis fluid. The endotoxin was thought to originate from an increase in algae in the local water source (Hindman, Favero, Carson, Petersen, Schonberger and Solano 1975). The algae (cyanobacteria?) in the water source were not identified. Subsequent studies in Pennsylvania have proven that a number of cyanobacteria are capable of producing endotoxins and that the presence of endotoxins in drinking water was correlated to the presence of cyanobacteria in water sources (Sykora and Keleti, 1981). Mutagenic properties of reservoir water in Missouri could be matched with a bloom of cyanobacteria (Collins, Gowans, Garro, Estervig and Swanson, 1981). Extracellular products of cyanobacteria are capable of supporting the growth of the bacterium Legionella pneumophila which causes Legionnaires disease (Tison, Pope, Cherry and Fliermans, 1980). Subsequently it has been demonstrated that L. pneumophila occur naturally in a wide range of aquatic habitats (Fliermans, Cherry, Orrison, Smith, Tison and Pope 1981). Cyanobacteria have also been implicated in Haff's disease. The disease is expressed as acute muscular pain accompanied by decomposition of muscular tissue with liberation of myoglobin which results in a brownish-black urine. Haff's disease can be fatal and is cause by eating fish (e.g. Berlin, 1948). Soviet researchers believe that the development of *M. aeruginosa* in water, sources at the time of appearance of toxic fish is responsible for the disease (Birger, Malyarevskaya and Arsan, 1974).

WATER CHLORINATION AND ORGANOHALOGEN FORMATION AS A RESULT OF EUTROPHICATION

One of several research needs identified by the Organisation for Economic Co-operation and Development (OECD, 1982) is to establish, the extent to which eutrophied waters contribute, with chlorination treatment, to organochloride formation in drinking water, due to the high level or organic precursors and the more intensive chlorination they receive at various stages of treatment and transportation.

Numerous studies in the literature have indicated that temperature, season of the year, pH at which chlorination occurs, organic content of the source water, the point of chlorination, the chlorine dose and chlorine contact time all influence the final amount of organohalogens formed in finished waters. A survey of the literature has revealed no specific information on the role that M. aeruginosa may play as a possible source of organohalogen precursors. For this reason total organohalogen precursors were estimated after chlorination of a unialgal NIWR laboratory culture of M. aeruginosa, strain WR 133. Cyanobacterial cultures were grown at 30°C in five litres modified Volk and Phinney's culture medium (Scott et al. 1981) under a light:dark regime of 16:8 at three levels of added phosphate: 600 μ g P/ ℓ , 60 μ g P/ ℓ and no added P. Growth of WR133 was monitored as chlorophyll a and dissolved organic carbon (DOC) and total organohalogen formation potential (TOHp) present in the cyanobacterial-free culture medium was monitored at selected Chlorination of the medium was at pH 9 for 24 hours intervals. utilizing commercial bleach (NaOC1). Some preliminary results are presented in Figure 1.

In all three cultures a steady increase in DOC was observed as cultures aged. The two cultures with added phosphate also showed a steady increase of TOHp with time. The culture with no added P (Figure 1C) died on the 8th day as the chlorophyll value dropped to zero and at the same time TOHp value increased to a value of $6700 \ \mu g \ CHCl_3/\ell$. After 14 days all three cultures showed TOHp concentrations in excess of 1 000 $\mu g \ CHCL_3/\ell$. The cultures were not bacteria-free and were regularly examined for contamination under phase contrast microscopy. It was noticed that the culture with 600 $\mu g \ P/\ell$ (Figure 1A) had a heavy infection of bacteria on the 7th day. Contamination may have been introduced during subsampling. The other two cultures did not appear to suffer heavily from bacterial contamination.

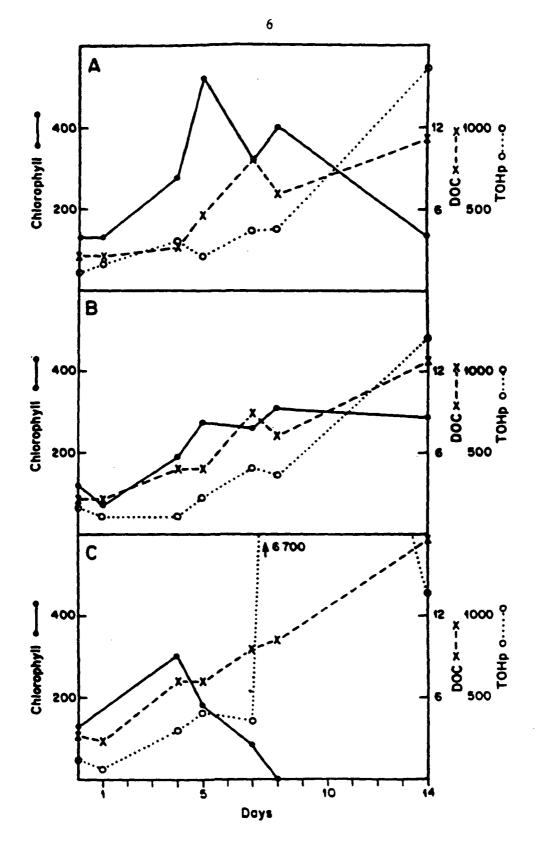


FIGURE 1

ć

Changes in chlorophyll $(\mu g/\ell)$ and dissolved organic carbon (mg/ℓ) and total organohalogen formation potential (as μg CHCl₃/ ℓ) in cultures of *M. aeruginosa* with different amounts of phosphate added. A:600 μg P/ ℓ ; B:60 μg P/ ℓ , C:no phosphate added.

The values of TOHp in excess of $1\ 000\ \mu g\ CHCO_3/\ell$ recorded here are higher than values reported in the literature for other algae or cyanobacteria (Table 1). From the data in Table 1 it is interesting to note that the cyanobacteria (Anabaena and Pseudanabaena) generally showed higher TOHp values than green algae.

POSSIBLE BENEFICIAL EFFECTS OF ALGAE IN EUTROPHIC WATERS

Thus far this paper has only considered the harmful aspects of algal blooms. We wish to conclude this paper by pointing out that there are also a number of potential beneficial effects associated with algae. Many of these beneficial aspects still require added research and should receive attention especially in situations where eutrophic conditions are difficult to control.

Floating mats of cyanobacteria consisting mainly of Spirulina sp., Phormidium tenue, Chroococcus turgidus and Nostoc commune are prepared as edible food for humans in Mexico (Ortega 1972). Natural blooms of Spirulina also form part of the diet of the Kanembou tribe north of lake Chad. Several commercial undertakings successfully market Spirulina originating mostly from Mexican (Durand-Chastel, 1980) and Taiwanese lagoons as health foods. A flourishing Chorella industry has developed in Japan and Taiwan in the form of tablets, extracts and other health food items.

Commercial exploitation of algal blooms as a protein source is still in its infancy and costly, thus restricting its usefulness, where applicable, to the health food market. Of more practical importance is the production of antibacterial and/or growth-inhibiting substances. Chrost (1975) has demonstrated that the development of actual blooms of algae produced substances which inhibited bacterial growth. Gräf and Baier (1981) found that two freshwater algae Hydrodictyon reticulatum and Aphanothece nidulans (a cyanobacterium) had strong antibacterial effects against a range of pathogenic organisms. A large number of marine algae have been screened for antibiotic or other medicinal effects (Stein and Borden, 1984) but information in this field must still be explored in freshwater eutrophic envirofnments.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. A van Rossum for expert technical assistance. NIWR research on health aspects of drinking-water and toxic cyanobacteria is partly funded by the Department of Health and Welfare.

TABLE 1 Published values of TOHp's involving algal or cyanobacterial sources

Type of material	Organohalogens (µg/ℓ)	Reference
Reservoir water containing 100 µg/ℓ chlorophyll/ℓ	500 ¹	Hoehn <u>et al</u> , (1978)
'Soluble' chlorophyll preparation (1.7 mg Chl/ℓ)	250 ²	Morris and Baum (1978)
Anabaena culture	600 ¹	Briley <u>et al</u> . (1980)
Roodeplaat Dam Water	200 ¹	Pilkington and van Vuuren (1981)
Rietvlei Dam Water	1501	
Hartbeespoort Dam Water	160 ¹	
<u>Pseudanabaena</u> culture Green algae cultures Diatom culture	$400^{3}_{300^{3}}_{200^{3}}$	Bernhardt (1982)
Anabaena culture	450 ³	Wachter and
Green algal cultures Chlorophyll preparation (5mg/l)	100 ³ 300 ³	Andelman (1984)

Total Trihalomethane potential Chloroform production Total organohalogen (expressed as µg CHCl₃)

BRARY

--

TERMATIONAL REFERENCE CENTRE CH COMMUNITY WATER SUPPLY AND -ALE ANDA (IRC)

REFERENCES

AMANN, M.J. and J.N. ELOFF 1980. Preliminary study of the toxins of different *Microcystis* strains. S. Afr. J. Sci., vol. 76, pp. 419-420.

AZIZ, K.M.S. 1974. Diarrhea toxin obtained from a waterbloom-producing species, *Microcystis aeruginosa* Kützing. <u>Science</u>, vol. <u>183</u>, pp. 1206-1207.

BERLIN, R. 1948. Haff disease in Sweden, Acta med. scand., vol. 129, pp. 560-571.

BERNHARDT, H. 1982. Beeinträchtigung der Chlorung als Wasseraufbereitungsverfahren durch algenbürtige Substanzen. In: H. Müller, F. Jüttner and U. de Haar (Eds). <u>Schadstoffe im Wasser</u>, Bd. 3, Algenbürtige Schadstoffe, Deutsche Forschungsgemeinschaft, H. Boldt Verl., Boppard, pp. 152-159.

BIRGER, T.I., MALYAREVSKAYA, A.Y. and O.M. ARSAN. 1974. Etiology of the Haff (Yuksov-Sartlan) Disease. <u>Hydrobiol. J.</u>, vol. 9, no. 2, pp. 71-80.

BOTES, D.P. 1985. Cyanoginosins - Isolation and structure. In: Steyn P.S. and R. Vleggaar (Eds). <u>Mycotoxins and Phycotoxins</u>, Elsevier Science Publishers, Amsterdam, in press.

BOURKE, A.T.C. and HAWES R.B. 1983. Freshwater cyanobacteria (bluegreen algae) and human health. Med. J. Aust., vol. 1, pp. 491-492.

BRILEY, K.F., WILLIAMS, R.F., LONGLEY, K.E. and C.A. SORBER. 1980. Trihalomethane production from algal precursors. In: Jolley (1980) pp. 117-129.

CARMICHAEL W.W. (Ed.) 1981. The Water Environment Algal Toxins and Health. New York: Plenum Press. 491 p.

CARMICHAEL, W.W., JONES, C.L.A., MAHMOOD, N.A. and W.C. THEISS. 1985. Algal toxins and water-based diseases. <u>CRC Critical Reviews in</u> Environmental Control, vol. 15, pp. 275-313.

CHR6ST, R.J. 1975. Inhibitors produced by algae as an ecological factor affecting bacteria in water. Acta microbiol. polon. B., vol. 7, pp. 167-176.

COLLINS, M.D., GOWANS, C.S., GARRO, F., ESTERVIG, D. and T. SWANSON. 1981. Temporal association between an algal bloom and mutagenicity in a water reservoir. In: Carmichael (1981) pp. 271-284.

DURAND-CHASTEL, H. 1980. Production and use of Spirulina in Mexico. In: G. Shelef and C.J. Soeder (Eds). <u>Algae Biomass</u>. Elsevier/North-Holland Biomedical Press. pp. 51-64. ELOFF, J.N. 1981. Autecological studies on *Microcystis*. In: Carmichael (1981) pp. 71-96.

FALCONER, I.R., BERESFORD, A.M. and M.T.C. RUNNEGAR. 1983. Evidence of liver damage by toxin from a bloom of the blue-green alga, *Microcystis* aeruginosa. Med. J. Aust., vol. 1, pp. 511-514.

FLIERMANS, C.B., CHERRY, W.B., ORRISON, L.H., SMITH, S.J., TISON, D.L. and D.H. POPE. 1981. Ecological distribution of Legionella pneumophila. Appl. Environm. Microbiol., vol. 41, pp. 9-16.

FOGG, G.E. 1971. Extracellular products of algae in freshwater. Arch. Hydrobiol. Beih. Ergebr. Limnol., vol. 5, pp. 1-25.

GORHAM, P.R. and W.W. CARMICHAEL. 1979. Phycotoxins from blue-green algae. Pure Appl. Chem., vol. 52, pp. 165-174.

GRäF, W. and W. BAIER 1981. Hygienische und mikrobiologische Beeinflussung natürlicher Gewässerbiotope durch Algen und Wasserpflanzenaufwuchs 1. Zbl. Bakt. Hyg., I. Abt. Orig. B, vol. 174, pp. 421-442.

HINDMAN, S.H., FAVERO, M.S., CARSON, L.A., PETERSEN, N.J., SCHONBERGER, L.B. and J.T. SOLANO 1975. Pyrogenic reactions during haemodialysis caused by extramural endotoxin. Lancet, vol. 2, 732-734.

HOEHN, R.C., RANDALL C.W., GOODE, R.P. and P.T.B. SHAFFER 1978. Chlorination and water treatment for minimizing trihalomethanes in drinking-water. In: Jolley *et al.* (1978) pp. 519-535.

JOLLEY, R.L. (Ed.) 1978 <u>Water Chlorination Environmental Impact and</u> <u>Health Effects. Volume 1.</u> Ann Arbor: Ann Arbor, Science Publishers Inc. 439 p.

JOLLEY, R.L., BRUNGS, W.A., COTRUVO, J.A., CUMMING, R.B., MATTICE, J.S. and V.A. JACOBS (eds) 1983. <u>Water Chlorination Environmental Impact</u> and Health Effects. Volume 4. Ann Arbor: Ann Arbor Science Publishers Inc. 1493 p. (2 books).

JOLLEY, R.L., BRUNGS, W.A. and R.B. CUMMING (Eds) 1980. <u>Water</u> <u>Chlorination Environmental Impact and Health Effects</u>. <u>Volume 3</u>. Ann Arbor: Ann Arbor Science Publishers Inc. 1171 p.

JOLLEY, R.L., GORCHEV, H. and D.H. HAMILTON, Jr (Eds) 1978. <u>Water</u> Chlorination Environmental Impact and Health Effects. <u>Volume 2</u>. Ann Arbor: Ann Arbor Science Publishers Inc. 909 p.

KOMÁREK, J. 1958. Die taxonomische Revision der planktischen Blaualgen der Tschechoslowakei. In: J. Komárek and H. Ettl (Eds) <u>Algologische</u> Studien, Czechoslovak Academy of Sciences, Prague, pp. 10-206.

LOUW, P.G.J. 1950. The active constituent of the poisonous algae *Microcystis toxica* Stephens. <u>S. Afr. Ind. Chem.</u>, vol. 4, pp. 62-66.

MORRIS, J.C. and B. BAUM. 1978. Precursors and mechanisms of haloform formation in the chlorination of water supplies. In: Jolley *et al.* (1978) pp. 29-48.

OECD, 1978. Eutrophication of Waters. Paris: Organisation for Economic Co-operation and Development, 154 p.

ORTEGA, M.M. 1972. Study of the edible algae of the valley of Mexico. Bot. Mar., vol. 15, 162-166.

PILKINGTON, N.H. and L.R.J. VAN VUUREN 1981. Formation of trihalomethanes during chlorination of algae laden surface waters. Paper presented at the Australian Water and Wastewater Association, Perth, April 1981, 7 p. (CSIR Reprint RW 812).

SCHWIMMER, D. and SCHWIMMER, M. 1964. Algae and medicine. In: D.F. Jackson (Ed.) Algae and Man, Plenum Press, New York pp. 368-412.

SCHWIMMER, M. and SCHWIMMER, D. 1968. Medical aspects of phycology. In: D.F. Jackson (Ed.) <u>Algae, Man, and the Environment</u>, Syracuse University Press, Syracuse, New York, pp. 279-358.

SCOTT, W.E. 1985. Examination of toxic and non-toxic *Microcystis* aeruginosa in the field and in laboratory culture. In: P.S. Steyn and R. Vleggaar, <u>Mycotoxins and Phycotoxins</u>, Elsevier Science Publishers, Amsterdam (in press).

SCOTT, W.E., BARLOW, D.J. and J.H. HAUMAN. 1981. Studies on the ecology, growth and physiology of toxic *Microcystis aeruginosa* in South Africa. In: W.W. Carmichael (1981) pp. 49-70.

STEIN, J.R. and C.A. BORDEN, 1984. Causative and beneficial algae in human disease conditions: a review. Phycologia, vol. 23, pp. 485-501.

STEPHENS, E.L. 1949. *Microcystis toxica* sp. nov.: a poisonous alga from the Transvaal and the Orange Free State. <u>Trans R. Soc. S. Afr.</u>, vol. 32, pp. 105-112 with one plate.

STEYN D.G. 1945. <u>Poisoning of animals by algae (scum and waterbloom)</u> in dams and pans. Pretoria: Union of South Africa, Department of Agriculture and Forestry, Government Printer, 9 p.

SYKORA, J.L. and G. KELETI. 1981. Cyanobacteria and endotoxins in drinking-water supplies. In: W.W. Carmichael (1981) pp. 285-301.

TISON, D.L., POPE, D.H., CHERRY, W.B. and C.B. FLIERMANS. 1980. Growth of *Legionella pneumophila* in association with blue-green algae (Cyanobacteria). <u>Appl. Environm. Microbiol</u>., vol. 39, pp. 456-459.

TOERIEN, D.F. 1977. <u>A review of eutrophication and guidelines for its</u> <u>control in South Africa</u>. Pretoria: CSIR Special Report WAT 48. 110 p. TOERIEN, D.F., SCOTT, W.E. and M.J. PITOUT 1976. *Microcystis* toxins: isolation, identification, implications. <u>Water S.A.</u>, vol. 2, pp. 160-162.

WACHTER, J.K. and J.B. ANDELMAN. 1984. Organohalide formation on chlorination of algal extracellular products. <u>Environ. Sci. Technol.</u>, vol. 18, pp. 811-817.

ZILLBERG, B. 1966. Gastro-enteritis in Salisbury European children - a five year study. <u>Centr. Afr. J. Med.</u> vol. 12, pp. 164-168.