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Acute and Chronic Toxicity of Arsenic to Some Aquatic Organisms

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Arsenic is a naturally occurring metal in aquatic ecosystems, but its levels are increasing due to pollution. The background levels of most unpolluted freshwaters are below 1 μg/L of arsenic. In ocean water the average residues are slightly higher (2 – 3 μg/L) (Moore and Ramamoorthy 1984; Blanck et al. 1989). Rivers crossing industrial areas contain residues of 1 - 20 μg/L, but values between 10 μg/L and 33 μg/L were detected in the streams receiving runoff from agricultural areas treated with herbicides containing arsenic compounds (Moore and Ramamoorthy 1984). The highest levels of arsenic in waters were reported in the vicinity of mining areas where over 5000 μg/L of residues were reported for a small lake receiving mine waters (Wagemann et al. 1978; Wong et al. 1999). The aquatic environment is important in the global cycling of arsenic as arsenic concentrations in aquatic biota are higher than in terrestrial organisms (Phillips 1990). In natural waters, arsenate is dominant, but it may also be present as organic or inorganic complexes. Conversion of all these forms is possible due to chemical and biological activity (Blanck et al. 1989; Jain and Ali 2000).

Phillips (1990) compared water quality objectives, standards and criteria for arsenic prescribed by different regulations and found the permissible concentrations were between 8 and 500 μg/L. The existing knowledge of the behaviour and effects of arsenic in aquatic environment is inadequate, because it is mainly based on laboratory data. The USEPA (1999) published recommended water quality criteria for total arsenic in the water; the Criterion Maximum Concentration (CMC) is 340 μg/L and the Criterion Continuous Concentration (CCC) is 150 μg/L for freshwater ecosystems. CMC is "an estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed briefly without resulting in an unacceptable effect"; in the case of CCC the exposure is indefinitely. A limit of 10 μg/L was set for drinking water by the USEPA (2001) and the WHO (2001).

Arsenic is classified as a metalloid with metallic and non-metallic properties. It occurs in different chemical forms. The most frequently used arsenic compound is arsenic oxide, As_2O_3, which is used for agricultural chemicals and synthesis of various inorganic and organic compounds. Arsenic is present in feed additives, pesticides, pharmaceuticals, diverse industrial products such as paints, glass, alloys, electronic components, and wood preservatives (Phillips 1990).

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Increased arsenic values in the air have been reported in the vicinity of metal smelters and active volcanoes (Mance 1987). An important source of arsenic atmospheric pollution is the combustion of fossil fuels in power plants (Förstner and Wittmann 1981). In the Šalek Valley, a polluted area in Slovenia, the main source of air pollution is the Šoštanj Thermal Power Plant (ŠTPP) (Stropnik et al. 1994). Svetina et al. (1996) studied trace elements in the water of the forested ecosystem at a reference point and two sampling points in the Šalek Valley. The results provided evidence of the influence of the Šoštanj Thermal Power Plant (ŠTPP) on concentrations of arsenic and some other metals in precipitation, leachate, and spring water. The finding of increased concentrations of arsenic in the aquatic ecosystem led us to investigate its toxicity to aquatic organisms.

The purpose of our research was to evaluate the acute and chronic toxicity of arsenic to aquatic organisms from different trophic levels and to compare the sensitivity of selected organisms. A review of the literature showed that acute toxicity to daphnids and fish is known (Moore and Ramamoorthy 1984; Mance 1987), but little information exists about its toxicity to algae or chronic effects. Thus, we studied the acute effects on luminescent bacteria, a mixed bacterial community, daphnids, and fish as well as chronic effects on luminescent bacteria, green algae, and daphnids. The concentrations which caused acute and chronic toxicity were compared to concentrations measured in the water samples near the Šoštanj Power Plant area.

MATERIALS AND METHODS

The standard stock solution of arsenic was prepared by dissolving As_2O_3 in distilled water according to the ISO standard (1982). In the experiments with fish and in the chronic tests with daphnids the concentrations of arsenic in the samples were determined spectrophotometrically (ISO 1982) at the start and end of test. Chemical analyses of arsenic showed that the measured concentrations did not drop below 90% of nominal concentrations. In experiments two replicates were used for each tested concentration and the control unless otherwise stated. At least two trials for each test species were conducted.

Freeze-dried bacteria *Vibrio fischeri* NRRL-B-11177 were obtained from the manufacturer (Dr. Lange GmbH, Düsseldorf, Germany). The luminescence was measured on a luminometer (Lumistox) at $15\pm0.2^{\circ}C$ after 30 min for acute toxicity assessment and after 24 hr to measure chronic effects of arsenic (Lange 1989). The acute and chronic toxicity results for each concentration were calculated as percentage inhibition relative to the control sample.

The microorganisms of activated sludge from the aeration tank of the municipal laboratory wastewater treatment plant were used in the toxicity test as the mixed bacterial community. A low concentration of microorganisms was desired so that concentration in the final test mixtures was 150 mg/L of suspended solids (ISO 1986). Oxygen consumption was measured with an oxygen electrode (WTW Oximeter, OXI 96) following biochemical degradation of meat extract, peptone, and urea every 30 min during 3 hr. The oxygen consumption rate was calculated for each

arsenic concentration. The percentages of inhibition were calculated and plotted against corresponding concentrations of arsenic on semilogarithmic paper.

The results obtained in the bacterial tests were expressed as effective concentrations that reduced the luminescence or oxygen consumption by 20%, 50%, and 80%. The EC20, EC50, and EC80 values were determined using linear regression analysis. The EC20 was taken as the toxicity threshold.

The green alga *Scenedesmus subspicatus* Chodat 1926 (CCAP 276/20) was obtained from the Culture Collection of Algae and Protozoa, Cumbria, United Kingdom. The stock culture of algae was maintained in a nutrient solution according to Jaworski (Thompson et al. 1988) at a constant room temperature of $21\pm1^{\circ}C$ and under continuous fluorescent illumination (4000 lux) provided by four Osram L18 W/10 cool-white fluorescent lights. Flasks were agitated at 150 rpm for 15 min alternating with 15 min resting on an orbital shaker (Tehtnica Železniki, type EV 403) to encourage gas exchange.

In the toxicity test, algal growth was determined by measuring the cell density (EEC 1992). Test flasks were constantly shaken at the same frequency as stock flasks and were illuminated with four Osram L 40W/20S cool-white fluorescent lights giving an illumination of 7000 lux. Algal density was determined by counting cells in a Bürker counting chamber at 0, 24, 48 and 72 hr. The numbers of algal cells were plotted against days of exposure and growth curves were constructed. The inhibition of algal growth was determined by a comparison of areas under the growth curves and with a comparison of growth rates. The percentages of inhibition of algal growth and specific growth rates were plotted on semilogarithmic paper; the E_bC_{10}, E_bC_{50}, E_bC_{90} for biomass and the E_rC_{10}, E_rC_{50}, E_rC_{90} for growth rate were estimated with linear regression analysis.

Daphnia magna Straus 1820 were obtained from the Institut für Wasser, Boden und Lufthygiene des Umweltbundesamtes, Berlin. They were cultured at $21\pm1^{\circ}C$ in 3-L aquariums covered with glass plates containing 2.5 L of modified M4 medium (Kühn et al. 1984) illuminated with fluorescent bulbs (approx. 1800 lux) for 12 hr per day. They were fed a diet of the algae *Scenedesmus subspicatus* Chodat 1926 corresponding to 0.13 mg C/daphnia per day. One day before the start of testing reproductive daphnids were isolated to obtain neonates about 24 hr old for the test. Each test solution was prepared by adding appropriate amounts of a stock solution to reconstituted water. Acute toxicity to daphnids was evaluated by counting the immobile daphnids after a 24 hr and 48 hr exposure period. Daphnids not able to swim within 15 s after gentle agitation of the test container were considered to be immobile (EEC 1992).

A chronic toxicity test with *Daphnia magna* was performed in a semi-static exposure system according to the OECD Guideline (1993). Individual daphnids were each placed in a 100 mL beaker containing 50 mL solution; ten replicates were used for each concentration and a control. Modified M4 medium was used as dilution water. The room temperature was maintained at $21\pm1^{\circ}C$ and a photoperiod of 16 hr light: 8 hr dark was provided. On Mondays, Wednesdays, and Fridays the

surviving daphnids were transferred to freshly prepared test solutions and fed a diet of *Scenedesmus subspicatus* at a ratio of 0.15 mg C/day per daphnid. Young daphnids were counted daily and then removed. On renewal days the pH, temperature, and dissolved oxygen concentration were measured. The endpoints of chronic toxicity test were mortality of daphnids, appearance of the first offspring, and total young per female after 21 d.

Juvenile rainbow trout *Oncorhynchus mykiss* Walbaum 1990, about 6 cm in length, were obtained from the fish farm Povodje. Zebrafish *Brachydanio rerio* Hamilton Buchanan were obtained from a commercial supplier. They were acclimated to the test temperature at least seven days prior the beginning of an experiment. During acclimatization the fish were fed with commercial fish food and the tanks were illuminated with fluorescent bulbs for 12 hr per day. Unpolluted brook water with a total hardness of 250 mg $CaCO_3$/L, alkalinity 234 mg $CaCO_3$/L, and pH 8.4 was used as holding and dilution water. The toxicity tests with rainbow trout and zebrafish were conducted in a static exposure system at $12\pm0.5^{\circ}C$ and $21\pm1^{\circ}C$, respectively (EEC 1992). Zebrafish were exposed in 3-L aquariums containing 2.5 L of test solution and rainbow trout in 40-L tanks containing 35 L of sample. Test solutions were slightly aerated. The endpoint of acute toxicity test was survival of fish during the 96 hr of exposure; every 24 hr dead fish were counted and removed.

The USEPA (1994) statistical computer programs were used for toxicity data analyses. The percentages of immobile daphnids and fish mortality were calculated. Probit analysis was used to calculate the EC10, EC50 (with 95% confidence limits) and EC90 for daphnids as well as the LC10, LC50 (with 95% confidence limits) and LC90 for fish. The daphnid reproduction data were analysed by the linear interpolation method to calculate the IC10 and IC25 and by the one-tailed Dunnett's test providing the NOEC and LOEC values.

RESULTS AND DISCUSSION

Results of toxicity tests with bacteria are summarised in Table 1. The acute toxicity to *Vibrio fischeri* increased slowly with increasing concentrations; 50% reduction of luminescence (30min EC50) occurred at 72.4 mg/L.

Table 1. Arsenic toxicity to luminescent bacteria and mixed bacterial community.

Luminescent bacteria		Mixed bacterial community
Acute toxicity	Chronic toxicity	
30min EC20 = 13.4 mg/L	24hr EC20 = 3.7 mg/L	120min EC20 = 28.8 mg/L
30min EC50 = 72.4 mg/L	24hr EC50 = 20.4 mg/L	120min EC50 = 41.7 mg/L
30min EC80 = 395 mg/L	24hr EC80 = 115 mg/L	120min EC80 = 58.9 mg/L

A threshold concentration, the concentration at which significant inhibitory effects on activated sludge were first observed, is reported to be at 0.1 mg/L of arsenic (Koopman and Bitton 1986). Kahru et al. (1996) studied toxicity of some pesticides to luminescent bacteria using the Biotox method and found a 5min EC50 at 7.6 mg/L and an EC20 at 1.9 mg/L for As_2O_3. It should be mentioned that arsenic

toxicity depends highly on experimental conditions, the reason for the highly variable toxicity data.

Arsenic was toxic to algae; the EC50 values for algal biomass and the specific growth rate were 34.7 mg/L and 60.3 mg/L, respectively (Table 2).

Table 2. Toxicity of arsenic to algae.

	Cell growth (biomass)	Specific growth rate
72hr EC10 (mg/L)	9.4	34.7
72hr EC50 (mg/L)	34.7	60.3
72hr EC90 (mg/L)	128.8	104.7

The biomass determination was a more sensitive endpoint than the growth rate. This compared favorably to practical experience on differences in the EC50 values derived from biomass and growth rates from two ring tests (Nyholm 1985). Ring tests are designed for different purposes; in this case they were used to compare the results obtained from biomass derived EC50 estimates with EC50 estimates derived from growth rates. In his conclusion Nyholm (1985) proposed that EC50 be determined from the growth rate is less dependent on parameters in the test system used.

A review of the literature revealed little information on toxicity of arsenic to algae. Highly variable data are reported for arsenic compounds due to different experimental conditions. "No effect" concentrations have a wide range, from 0.16 to 1000 mg/L of arsenic, depending on the chemical form, test conditions, test species, and test endpoints (Moore and Ramamoorthy 1984). Peterson et al. (1996) found that 0.3 mg/L of arsenic inhibited the growth of green algae by 25%. Arsenate toxicity to algae is highly variable due to nutrient conditions in water. Arsenate is chemically similar to phosphate and phosphate levels in water play an important role in the toxicity of arsenate. When phosphorus is plentiful in water, the toxicity of arsenate is low; conversely, arsenate is very toxic at low levels of phosphorus (Blanck et al. 1989).

The results of acute and chronic toxicity tests with daphnids indicate great sensitivity of daphnids to arsenic in comparison to other organisms. In our study the EC50 values were 2.7 mg/L after 24 hr and 2.5 mg/L after 48 hr (Table 3). A review of the literature shows that arsenic (III) is not highly toxic to daphnids in short duration exposures; the 48hr EC50 (LC50) values ranged from 1.5 to 9.1 mg/L of arsenic (Mance 1987; Bartell et al. 1992). The EC50 values obtained in our study match these data. The reported 96hr EC50 values for fed and unfed *Daphnia magna* were 4.3 mg/L and 1.5 mg/L, respectively (Richardson and Gangolli 1992).

Table 3. Acute and chronic arsenic toxicity to *Daphnia magna*.

Acute toxicity		**Chronic toxicity**
24hr EC10 = 2.0 mg/L	48hr EC10 = 1.9 mg/L	21d IC10 = 1.3 mg/L
24hr EC50 = 2.7 mg/L	48hr EC50 = 2.5 mg/L	21d IC25 = 1.9 mg/L
(2.6-2.9)	(2.4-2.7)	21d NOEC = 1.85 mg/L
24hr EC90 = 3.8 mg/L	48hr EC90 = 3.4 mg/L	21d LOEC = 3.70 mg/L

Elnabarawy et al. (1986) compared the sensitivity of *Daphnia magna*, *Daphnia pulex*, and *Ceriodaphnia reticulata* to some organic and inorganic chemicals. In the case of arsenic (III) *Ceriodaphnia reticulata* was the most sensitive species; the 48hr EC50 was 1.27 mg/L. The reported 48hr EC50 values were 2.37 mg/L for *D. pulex* and 4.50 mg/L for *D. magna.*

Daphnid survival and reproduction indicated a similar sensitivity to arsenic toxicity (Figure 1). After 21d a 20% mortality was observed in the treatment containing 1.85 mg/L of arsenic, although production of offspring was not significantly lower than in the control sample. In our case the NOEC (1.85 mg/L) and the IC25 (1.9 mg/L) were very similar (Table 3). Biesenger and Christensen (1972) reported results indicating higher toxicity of arsenic to daphnids after three weeks of exposure. Their LC50 and the EC50 values were at 2.85 mg/L and 1.40 mg/L of arsenic, respectively. Reproduction decreased by 16% at a concentration of 0.52 mg/L. Lima et al. (1984) found that 1.32 mg/L of arsenic was the 28d LC50 for *Daphnia magna.* No reproduction occurred with that treatment. There was no adverse effect at 0.633 mg/L. Enserink et al. (1991) studied the toxicity of single metals as well as combined effects of mixtures to *Daphnia magna* and rainbow trout. The reported 21d LC50 was 5.8 mg/L in a semi-static exposure system. The lowest concentration causing a significant decrease of daphnid survival and intrinsic rate of natural increase (i.e. a growth potential of a population assuming no limiting factors) compared to the control was 10 mg/L of arsenic. The lowest concentration causing a significant decrease of the length of daphnids was 3.4 mg/L and this result is similar to our 21d LOEC observed at 3.7 mg/L of arsenic.

The results of acute toxicity tests with zebrafish and rainbow trout are given in Table 4. The 96hr LC50 values were 28.1 mg/L for zebrafish and 15.3 mg/L for rainbow trout. As we expected, arsenic was more toxic to rainbow trout than to zebrafish. Bartell et al. (1992) observed the median lethal concentration for rainbow trout at 13.0 mg/L, which is similar to our results. Dixon and Sprague (1981) reported the 6d LC50 was 13.3 mg/L for rainbow trout and found that pre-exposure to arsenic at a concentration of 3 mg/L affected neither growth nor caused any histological damage.

A review of the literature showed that arsenic toxicity to fish is quite well known. Arsenic is not among the very toxic substances: toxicity started at concentrations higher than 1 mg/L (Dojlido and Best 1993). In general, the LC50 values of sodium arsenite and arsenic trioxide for different fish species are between 0.05 and 59 mg/L depending on age, species, and test conditions (Moore and Ramamoorthy 1984; Crompton 1997).

Due to elevated concentrations in the vicinity of the Šoštanj Thermal Power Plant in the Šalek Valley the toxicity of arsenic was investigated. Arsenic induced toxic effects in all tested organisms. The most sensitive species was *Daphnia magna*; the 48hr EC50 was determined to be 2.5 mg/L. Daphnids were followed by rainbow trout *Oncorhynchus mykiss* with the 96hr LC50 at 15.3 mg/L. In chronic exposures daphnids were more sensitive than bacteria or algae; the "low effect level" (IC25) was at 1.9 mg/L of arsenic. The laboratory-derived data were compared with

measured concentrations of arsenic in the field published by Svetina et al. (1996). They reported an average concentration of 3.6±2.5 mg/L of arsenic in one year measured in precipitation near the Šoštanj Thermal Power Plant, which supports the statement that the atmospheric deposition is an important source of pollution. The

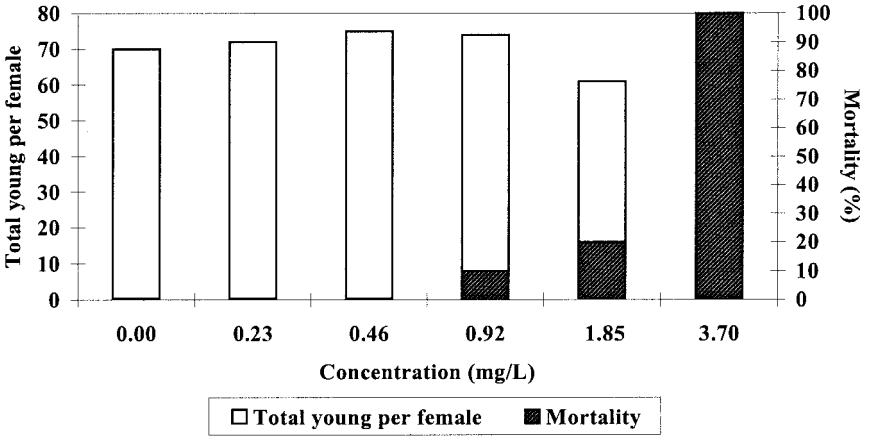

Figure 1. Daphnid mortality and offspring production in samples with different concentrations of arsenic after 21d.

Table 4. Acute arsenic toxicity in rainbow trout and zebrafish.

	All values are in mg/L		
	LC10	LC50 (confidence limit)	LC90
Zebrafish 24hr	27.3	34.9 (31.9-39.9)	44.8
48hr	24.5	32.7 (29.5-36.9)	43.6
72hr	22.2	28.5 (25.6-31.2)	36.7
96hr	21.9	28.1 (25.2-30.7)	36.0
Rainbow trout			
24hr	-	-	-
48hr	15.5	23.2 (19.8-36.3)	34.7
72hr	13.4	17.7 (15.2-20.1)	23.4
96hr	12.1	15.3 (12.9-17.3)	19.4

measured arsenic concentrations in the soil water (1.0±0.7 mg/L) and spring water (1.5±0.9 mg/L) were within the range of concentrations that caused chronic effects on daphnids in our laboratory tests.

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REFERENCES

Bartell SM, Gardner GH, O'Neill RV (1992) Ecological risk estimation. Lewis Publishers, Boca Raton

Biesenger KE, Christensen GM (1972) Effects of various metals on survival, growth, reproduction and metabolism of *Daphnia magna*. J Fish Res Bd Canada 23:1691-1700

Blanck, H, Holmgren K, Landner L, Norin H, Notini M, Rosemarin A, Sundelin B (1989) Advanced hazard assessment of arsenic in the Swedish environment. In: Landner L (ed) Chemicals in the aquatic environment, advanced hazard assessment, Springer Verlag, Berlin, p 256

Crompton TR (1997) Toxicants in the aqueous ecosystem. John Wiley and Sons, Chicester

Dixon DG, Sprague JB (1981) Acclimation-induced changes in toxicity of arsenic and cyanide to rainbow trout, *Salmo gairdneri*. J Fish Biol 18:579-589

Dojlido JR, Best GA (1993) Chemistry of water and water pollution control. Ellis Horwood, New York

EEC (1992) EEC/383A/Part C Methods for the determination of ecotoxicity. Official Journal of the European Communities, Brussels

Elnabarawy MT, Welter AN, Robideau RR (1986) Relative sensitivity of three daphnid species to selected organic and inorganic chemicals. Environ Toxicol Chem 5:393-398

Enserink EL, Maas-Diepeveen JL, van Leeuwen CJ (1991) Combined effects of metals; an ecotoxi-cological evaluation. Wat Res 25:679-687

Förstner U, Wittmann GTW (1981) Metal pollution in the aquatic environment. Springer – Verlag, Berlin

ISO (1982) ISO 6595; Water quality - Determination of total arsenic - Silver diethyldithiocarbamate spectrophotometric method. Geneva

ISO (1986) ISO 8192; Water quality - Test for inhibition of oxygen consumption by activated sludge. Geneva

Jain CK, Ali I (2000) Arsenic: Occurrence, toxicity and speciation techniques. Wat Res 34: 4304-4312.

Kahru A, Tomson K, Pall T, Külm I (1996) Study of toxicity of pesticides using luminescent bacteria *Photobacterium phosphoreum*. Wat Sci Tech 33:147-154

Koopman B, Bitton G (1986) Toxicant screening in wastewater systems. In: Dutka BJ, Bitton G (eds) Toxicity testing using microorganisms, vol 2.CRC Press, Boca Raton, p101

Kühn R, Pattard M, Pernack KD, Winter A (1984) Results of the harmful effects of water pollutants to *Daphnia magna* in the 21 d reproduction test. Wat Res 23:501-510

Lange B (1989) Lumistox Operation Manual. dr. Bruno Lange GmbH, Berlin

Lima AR, Curtis C, Hammermeister DE, Markee TE, Northcott CE, Brooke LT (1984) Acute and chronic toxicities of arsenic (III) to fathead minnows, flagfish, daphnids and an amphipod. Arch Environ Contam Toxicol 13:595-601

Mance G (1987) Pollution threat of heavy metals in aquatic environments. Elsevier Applied Science, London

Moore JM, Ramamoorthy S (1984) Heavy metals in natural waters. Springer Verlag, New York

Nyholm N (1985) Response variable in algal growth inhibition tests - biomass or growth rate? Wat Res 19:273-279
OECD (1993) OECD Guideline 202 Part II, Daphnia, reproduction test (draft version). Organisation for Economic Cooperation and Development, Paris
Peterson HG, Nyholm N, Nelson M, Powell R, Huang PM, Scroggins R (1996) Development of aquatic plant bioassays for rapid screening and interpretive risk assessments of metal mining liquid waste waters. Wat Sci Tech 33:155-161
Phillips DJH (1990) Arsenic in aquatic organisms: a review, emphasizing chemical speciation. Aquat Toxicol 16:151-186
Richardson ML, Gangolli S (eds) (1992) The dictionary of substances and their effects. Royal Society of Chemistry, Bugbrooke
Stropnik B, Byrne AR, Smodiš B, Jaćimović R (1994) Air pollution monitoring in the Šalek Valley, Part 2: Elemental composition of size-fractionated lignite coal-combustion particles during Šoštanj TPP operation. Acta Chim Slovenica 41:65-82
Svetina M, Smodiš B, Jeran Z, Jacimovic R (1996) Trace elements in the water cycle of the Šalek valley, Slovenia, using INAA. J Radioanal Nucl Chem 204:45-55
Thompson AS, Rhodes JC, Pettman I (1988) Culture collection of algae and protozoa, catalogue of strains. Culture Collection of Algae and Protozoa Freshwater Biological Association, Cumbria
USEPA (1994) USEPA toxicity data analysis software. Environmental Monitoring Systems Laboratory, Cincinnati
USEPA (1999) National Recommended Water Quality Criteria – Correction. EPA 822-Z-99-001. Website: http://www.epa.gov/ost/pc/revcom.pdf
USEPA (2001) Drinking Water Standard for Arsenic. EPA 815-F-00-015. Website: http://www.epa.gov/safewater/ars/ars_rule_factsheet.html
Wagemann R, Snow NB, Rosenberg DM, Lutz A (1978) Arsenic in sediments, water and aquatic biota from lakes in the vicinity of Yellowknife, Northwest Territories, Canada. Arch Environ Contam Toxicol 7:169-191
Wong HKT, Gauthier A, Nriagu JO (1999) Dispersion and toxicity of metals from abandoned gold mine tailings at Goldenville, Nova Scotia, Canada. Sci Total Environ 228:35-47
WHO (2001) Arsenic in Drinking Water. No.210, Geneva
Website: http://www.who.int/inf-fs/en/fact210.html