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CONCENTRATION AND SIZE DISTRIBUTION OF INORGANIC PARTICLES AND CELLS IN LAKE BOVILLA (ALBANIA)

PËRQENDRIMI DHE SHPËRNDARJA E GRIMCAVE IMORGANIKE DHE QELIZAVE NË LIQENIN E BOVILLËS (SHQIPËRI)*

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Abstract

Flow cytometry allows to analyze size distribution of the suspended particles in the drinking water reservoir of Tirana. The total particle size distribution follows a Poisson pattern with a size of approximately of 0.3 µm being the most frequent. Based on autofluorescence, inorganic particles and phototrophic organisms can be distinguished and with the use of specific dyes heterotrophic and Gram-positive bacteria can be quantified separately.

Keywords: Drinking water reservoir of Tirana (Al), Flow cytometry, suspended particles, autofluorescence,

Përmbledhje

Citometria me rrjedhje ka lejuar analizën sipas madhësisë të përbërjes së grimcave pezull në ujëmbledhësin e ujit të pijshëm të Tiranës. Shpërndarja e përgjithshme e grimcave sipas madhësisë ndjek ecurinë Poisson, ku madhësia rreth 0.3 µm ishte më e pranishmja. Mbështetur tek autofloreshenca, mund të dallohen grimcat inorganike dhe organizmat fototrofë; ndërsa duke përdorur ngjyrues të posaçëm mund të përcaktohet veç e veç sasia e baktereve heterotrofe dhe atyre Gram-pozitive.

Introduction

Lake Bovilla is an artificial reservoir completed in 1998. It supplies the city of Tirana with at present more than 850'000 inhabitants with drinking water. When completely filled the lake has a volume of about 80 x 10^6 m^3, with Terkuza River feeding the lake annually with 50 x 10^6 m^3. The depth was originally about 60m, in recent times due to heavy sedimentation from the catchment only 45 m were measured near the dam.

Figure 7-1. Sampling moments in Bovilla, September 28th, 2008. / Çaste nga marrja e mostrave në Bovillë, në 28 shtator 2008 (Photos: Hoxha).

* *Translations in Albanian language made by Prof. A. Miho, Tirana University*

Based on the chemistry of the water, oxygen, nutrients and chlorophyll concentrations, Lake Bovilla is in an oligotrophic state during most of the year. In contrast, the Secchi disk transparency was unexpectedly low, ranging between 2.2 and 3.2 m. This is due to a high concentration of total suspended solids (TSS) which often exceeded 25 mg/L, the water quality standard for raw drinking water quality.

The phytoplankton concentration varied strongly with depth and season. The main growth period occurred in late spring with a second smaller one in autumn. In spite of the high turbidity the productive layers extended down to 15 m. The maximum of productivity was observed between 5-10 m depth. The most frequent species dominating in the phytoplankton were centric diatoms of *Cyclotella* sp.*,* mainly *C. commensis*, a species typical for reservoirs throughout the region; its number often exceeded 90% of the total number of phytoplankton cells. The microbiological parameters concerning the hygienic quality fulfilled quality class A_1 of EC Directive 75/440.

The present paper will describe the size distribution of the particles suspended in the lake water. By measuring autofluorescence of phototrophic cells and the fluorescence of specific dyes binding to DNA or to cell surfaces, inorganic particles can be discriminated from cells of planktonic algae and bacteria.

Material and methods

Samples were collected at the deepest site of Bovilla Lake on September 28th 2008 (Fig. 7-1), at depths of 1, 3, 5, 10, 15, 20, 30, 40, and 45 meters using a Ruttner water sampler (Hydro-Bios). Depth profiles were obtained immediately on the lake for water temperature, pH, conductivity and nephelometric turbidity, and Secchi disk transparency was measured. Dissolved oxygen (DO) was determined according the standard Winkler procedure. Total phosphate, nitrate, nitrite, ammonium, DO, alkalinity, total suspended solids, chlorophyll *a*, *b*, *c* and phaeophytin were determined using the APHA (1988) standard procedures (for details see Çullaj *et al.,* Nr. 2 *this volume*).

Samples for flow cytometry were taken from 1, 10, and 40 m, a series fixed with p-formaldehyde (3% final concentration) and a second without preservation, and kept at 5^{o}C until analysis.

Flow cytometric analyses were performed with a portable “Microcyte” flow cytometer (BioDetect AS, Oslo, Norway). Scatter and fluorescence are separately determined in 240 channels corresponding to a log particle size distribution of 0.2 to 15 µm. Calibration was performed with standard kits of monodisperse particles between 1 and 10 µm diameter purchased from BioDetect and Molecular Probes (Eugene, OR, USA). The dyes SYTO 62, wheat germ agglutinin (WGA Alexa 633) were obtained from Molecular Probes. SYTO was used in a final concentration of 50 nmol/L, WGA in a final

concentration of 2 µg/ml. Dyes were added to 1 ml water samples and incubated in the dark for 60 min at room temperature prior to analysis. Each measurement is the average of 50 successive 1 µL-countings by the instrument.

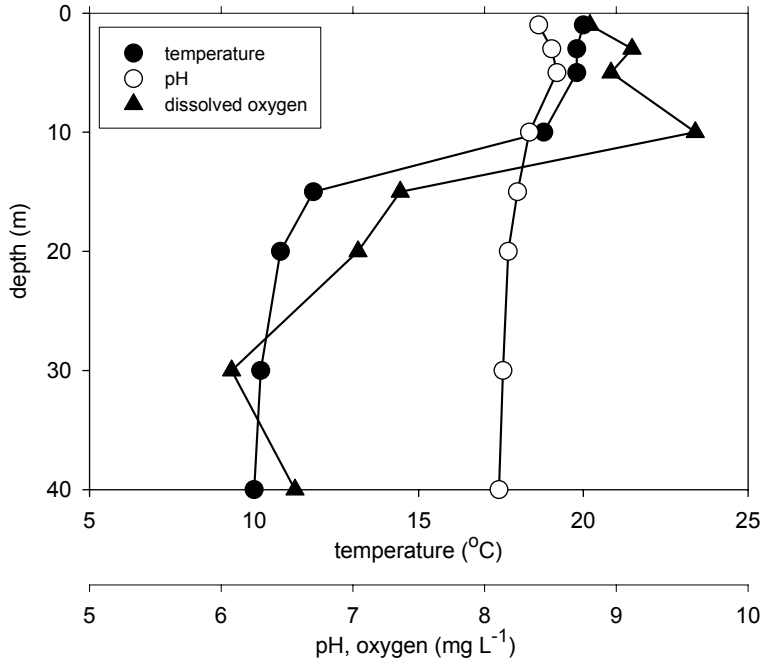


Figure 7-2a. Depth profiles of temperature, pH and oxygen concentration on September 28th, 2008. / Ecuria në thellësi e temperaturës, pH dhe oksigjenit në 28 shtator 2008.

Results and discussion

Figure 7-2 shows the chemistry of the lake at the date of sampling. The temperature in the uppermost 5 m layer is close to 20^{o}C and the thermocline lies between 10 and 15 m depth. The pH is throughout the whole water column above 8.1, with a peak of pH 8.6 at 5 m. The oxygen concentration is saturated between the surface and 10m and drops in the thermocline to 70%

with a minimum at 30 m. Turbidity is with 4.2 NTU units highest at the surface, drops to 2.0 at 15 m and increases again towards the sediment surface. Phosphate concentrations are low throughout the profile, with 3.4 to 4.5 µg/L P, while nitrate is depleted at the surface with 0.03 mg/L N but increases to 0.24 mg/L N in the hypolimnion. The profiles show that the water from 1 m and 10 m is very similar concerning most parameters, but very different from the water at 40 m depth.

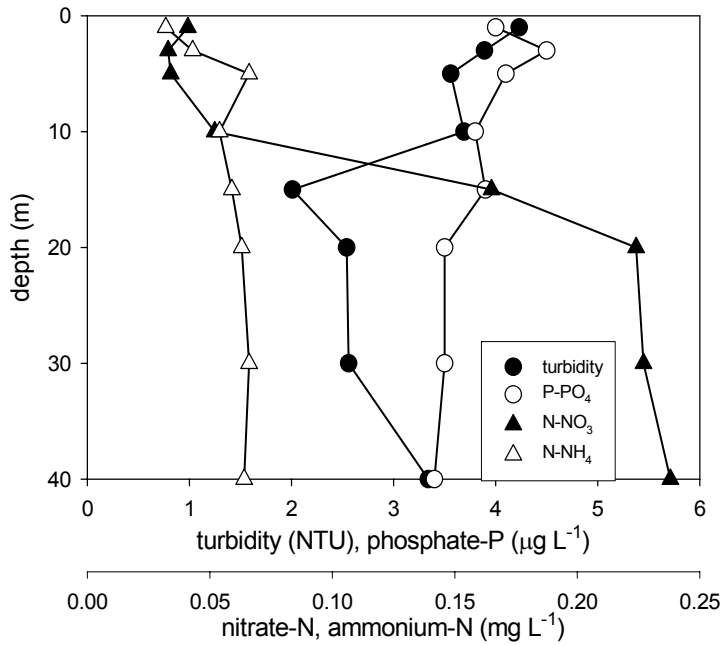


Figure 7-2b. Depth profiles of turbidity and concentrations of phosphate, nitrate and ammonia on September 28th, 2008. / Ecuria në thellësi e turbullisë, fosfateve, nitrateve dhe amoniumit në 28 shtator 2008.

Table 7-1 gives an overview on the total number of particles counted by flow cytometry at each depth and the portion of fluorescent particles of the total in the absence and the presence of dyes. In the counting range of the instrument of 0.2 to 15 µm diameter a total number of 6.4 to 9.0 x 10^5 particles/ml were measured, with the highest value in the surface layer and the number

decreasing with depth. Particle numbers correlate well with the turbidity measured on the lake (Tab. 7-1).

Table 7-1. Total number of particles, percentage of autofluorescent particles and percentage of fluorescent particles after staining with Syto-62 and wheat germ agglutinin (WGA) at different depths. / Numri i përgjithshëm i grimcave, përqindja e grimcave autofluoreshente dhe përqindja e grimcave fluoreshente pas ngjyrimit me Syto-62 dhe aglutininën e embrionit të grurit (WGA) në thellësi të ndryshme.

Depth, m	Total particle number / ml x 10^5 (1)	Auto-fluorescent particles (% of total) (1)	Fluorescent particles stained with Syto-62 (% of total) (2)
-1	9.0	1.3	18.4
-10	7.9	1.3	12.2
-40	6.4	0.6	45.9

Depth, m	Fluorescent particles stained with WGA (% of total) (3)	Turbidity (NTU)	Number of algae/ml* Diatoms - *Chlorophyceae*
-1	16.7	4.23	2980 - 58
-10	17.4	3.69	1676 - 48
-40	16.7	3.34	243 - 2

** counted by microscopy (Koni et al., this volume); (1) mean values of 20 countings; (2) mean values of 10 countings; (3) mean values of 3 countings*

Autofluorescence is the result of naturally occurring fluorescent particles. Activated by the measuring beam of the instrument of 633 nm wavelength red fluorescence is induced from particles containing chlorophylls and phycobilins. In aquatic systems fluorescence signals originate from eukaryotic algae and phototrophic bacteria. At 1 and 10 m depth these organisms amount for 1.3% of the total counts, while in the deep water they are reduced to about half. As phototrophic organisms depend on light the autofluorescent particles in the hypolimnion are sedimented ones from the production layer and probably decaying.

The dye SYTO-62 serves as a general DNA stain, it is incorporated into the DNA helix. As the dye permeates through membranes of most prokaryotic and eukaryotic cells, it stains both living and dead cells, but it may also stain free DNA adsorbed on inorganic particles.

By incubating the samples with SYTO-62 the portion of the fluorescent particles is highly increased with almost half of them stained at 40 m depth. This is interpreted that heterotrophic non-fluorescent bacteria become now

visible and can be distinguished from purely inorganic material. The high proportion at 40 m depth may be an indication for heterotrophic decay processes. However, these must not be free-living organisms but rather it suggests that tiny bacteria adhere to inorganic particles and live on them.

Wheat germ agglutinin (WGA) coupled to a fluorescent marker is another possibility to selectively stain prokaryotic cells. In contrast to SYTO-62 WGA acts like an antibody and binds to *N*-acetylglucosamine and *N*-acetylgalactosamine residues of the outer cell wall. Gram-positive bacteria, including their spores, become stained with fluorescently labeled WGA, whereas Gram-negative are not (Sizemore *et al.,* 1990; Fife *et al.*, 2000; Holm & Jespersen, 2003). Furthermore vegetative cells of fungi are stained as well, because of their chitin content in the cell walls (Cohen, 2001). The amount of particles stained with WGA in the three Buvilla samples is hardly variable with depth; Gram-positive bacteria seem to be in a rather constant ratio to the total counts.

The size range of the instrument is divided into 240 separate channels; it is thus possible to measure the size distribution of the suspended particles over the range from 0.2 to 15 µm. As illustrated in figures 7-3, the number of scattering particles (filled circles) peaks at approx. 0.3 µm and declines asymmetrically towards larger sizes. The shape is similar for the three depths investigated. Phototrophic organisms detected as autofluorescence (open circles) have a completely different size distribution. A large part of the particles belongs to size class > 1 µm, especially in the 1m and 10 m sample. The 40 m sample has only a much reduced number of fluorescent particles, as the live cells become decomposed during sedimentation.

Particle size distribution has been studied in Lake Brienz (Switzerland), which can be compared with Bovilla Reservoir. Both have influents with a high load of inorganic particles. Lake Brienz has for most of the year a TSS concentration of between 3.5 and 7.7 mg/L, a particle concentration (0.1 to 2.0 µm) of 1.7 to 6.6 x 10^7 ml^{-1} and a turbidity of 1 to 6.5 FTU units (Chanudet & Filella, 2007), similar to the results obtained from Bovilla. The size distribution of particles in Lake Brienz based on TEM measurements resulted in a shape similar to Bovilla, with a peak at about 0.3 µm in frequency (Chanudet & Filella, 2008).

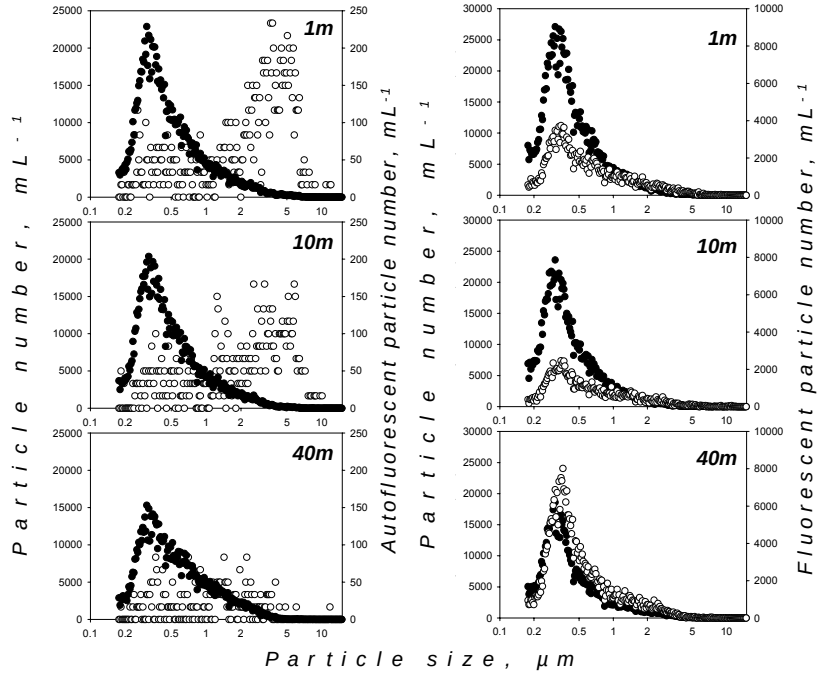
In contrast to the low number of autofluorescent cells 10 to 50 % of the particles became fluorescent after SYTO-staining. The shape of the size distribution of the fluorescent signals follows the same pattern of the one of the scatter signals (Fig. 7-4). However, the decline to the larger sizes is slightly slower which results in a higher portion of stained cells in the larger size classes (*see* also Tab. 7-2).

Staining with WGA results in a broad size distribution of fluorescent particles in the range between 0.3 and 5 µm; organism stained with WGA seem to be uniformly distributed over a broad size range. A peak at 0.3 µm is only seen in the surface water.

In table 7-2 the data of the 240 channels of the instrument are transformed into four defined size classes. The portion of autofluorescent particles in the two classes smaller than 1.0 µm is less than 1%; these signals possibly are indicators of small algae and phototrophic bacteria, mainly cyanobacteria. However, autofluorescent particles account for more than half in the class greater than 5.0 µm, both in the 1m and 10m sample. These signals originate from larger eukaryotic phototrophic cells.

Figura 7-3: *Scatter and autofluorescence*

Figura 7-4: *Scatter and fluorescence after staining with SYTO-62*



Figures 7-3, 7-4 & 7-5. *Size distribution of particles from Bovilla (for details see text). /* Shpërndarja e madhësisë së grimcave në Bovillë (*shih* tekstin për hollësitë).

Figura 7-5: *Scatter and fluorescence after staining with WGA*

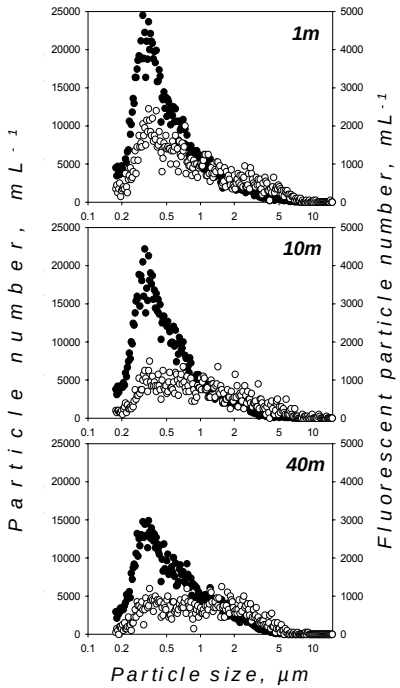


Table 7-2. Size distribution of particles in percentage of the total number for autofluorescent particles and fluorescent particles, after staining with Syto-62 and wheat germ agglutinin (WGA) at different depths. / Shpërndarja e madhësisë së grimcave në përqindje të numrit të përgjithshëm për grimcat autofluoreshente, dhe për grimcat fluoreshente pas ngjyrimit me Syto-62 dhe aglutininë nga embrioni i grurit (WGA) në thellësi të ndryshme.

% autofluorescent particles				
Depth, m	***Particle size, µm***			
	< 0.5 µm	**0.5 – 1.0 µm**	**1.0 – 5.0 µm**	**>5.0 µm**
1 m	0.3	0.5	5.3	53.9
10 m	0.3	0.7	4.6	53.1
40 m	0.3	0.5	1.8	12.3
% fluorescent particles stained with Syto-62				
1 m	14.2	21.7	41.4	97.5
10 m	8.3	17.2	35.4	97.0
40 m	40.3	63.5	65.3	80.5
% fluorescent particles stained with WGA				
1 m	9.8	17.0	38.0	95.4
10 m	7.8	19.4	41.5	95.5
40 m	8.6	17.9	34.3	85.4

The portion of SYTO-62 stained particles of the total, indicative for live cells, increases with size, almost fully covering the largest size class in all depths as seen with autofluorescence. In the 40m sample the proportion of stained particles is clearly higher than in the two epilimnion samples, indicating again the dominance of heterotrophic processes. The high percentage of stained particles agrees with the finding, that depending on environmental conditions 20 up to 70% of the microorganisms are associated with particles (Characklis *et al.*, 2005). However, it must be remembered that several nucleic acid stains may bind unspecific to charged particles.

As the number of stained cells with WGA is relatively constant over the 240 measuring channels (Fig. 7-5), its proportion to the total number of cells increases with particle size in an almost linear way. Again the largest size class is almost completely stained similar to the results with SYTO-62.

When the number of autofluorescent cells is compared with the microscopic counting of the algae, there is about a 4 to 10 fold difference, with clearly higher numbers with flow cytometry. Autofluorescence may also origin from small phototrophic bacteria attached to inorganic particles, which would have been overlooked and not counted by microscopy.

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