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Drinking water quality assessment in the Danube Delta Biosphere Reserve

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SUMMARY. This research is aimed mainly at the assessment of water quality of several drinking water treatment plants and groundwater wells, from the Danube Delta Biosphere Reserve and the habits of the local population in terms of water consumption and use. Thus, the study highlights the presence and distribution of prokaryotic and eukaryotic assemblages, specifically the algal communities which emerged as a dominant group in the investigated drinking water sources. The spatial variation of the pH, EC, turbidity, dissolved oxygen (DO), temperature, algae species and biomass was measured in five drinking water treatment plants (DWTP) and three groundwater wells from villages located in the Danube Delta Biosphere Reserve. No PCR product was present for either genes coding anatoxin, microcystin and saxitoxin. The bbe-Moldaenke technique identified the presence of sligthly more algal groups, than the light microscopic technique; both methods show clear results: the water treated in the drinking water treatment plants has significant algal biomass. The water quality indicated by the algal communities shows oligo-β-mesosaprobic conditions, despite the high number of taxa and individuals from Sf. Gheorghe and C. A. Rosseti samples, they reflect oligotrophic conditions.

Keywords: algae, cyanobacteria, cyanotoxins, drinking water.

Introduction

Due to the practical experience mentioned into international decree for many (particularly physico-chemical) parameters, the concentrations present in drinking water sources would rarely result in any breach of limit values (Commission Directive, 2015).

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On the other hand, microorganisms present in drinking water sources and the substances produced by them have been reported more frequently in similar studies performed in other countries. These findings could affect the health of people and their offspring (Cabral, 2010; Semenza *et al.*, 2012; Sweileh *et al.*, 2016).

Prokaryotic and eukaryotic microorganisms are naturally present in many water resources and can enter, grow and colonize water treatment and transport systems (including reservoirs, pipes or plumbing). Studies worldwide have reported the presence of protozoa, algae, invertebrate and fungi which can constitute a public health concern. The potential pathogenicity of some genera like *Acanthamoeba*, *Candida* and *Aspergillus*, can become a health concern by harboring a variety of pathogenic bacteria or by the presence of toxic compounds produced by cyanobacteria (Belila *et al.*, 2016; Fosso-Kankeu *et al.* 2008; Ibelings *et al.* 2014; Říhová Ambrožová *et al.*, 2009). Microorganisms in drinking water systems have the tendency to attach to any surface they come in contact with, resulting in so called "biofilms" (Douterelo *et al.*, 2014; Fish *et al.*, 2015). Despite of rigorous standards for regulatory purposes and systematic promulgation (modifications or completions of these), in the Romanian legislation no information can be found, about preventive measures that include control of the presence of algae and algal toxins or the presence of biofilms in the drinking water distribution systems (Decision 974, 2004; Law no. 458, 2012).

An overview of the drinking water quality in Europe by the reporting period 2011-2013, under the Drinking Water Directive 98/83/EC (Council Directive, 1998), showed that the development of the drinking water quality is based on the spatial scale of the Water Supply Zone. It could include a distinction between large and small water supply zones.

Small water supply zones serve less than 1,000 m³/day and less than 5,000 people. In case of these water supplies, no obligation to report the drinking water quality every three years is needed (Overview of the drinking water quality in Romania, 2016). The water supply zone in the Danube Delta Biosphere Reserve could be included into the category of small water supply zone, due to the low number of inhabitants. Information provided by the national database on drinking water quality in the Danube Delta Biosphere Reserve is insufficient. The references on contamination with eukaryotic microorganisms are mentioned only in case of wells. This study is bound to bring more information and clarification about the quality of the drinking water in the Danube Delta and it shows the importance of conducting more similar studies.

Materials and methods

Drinking water sources

Inhabitants of the Danube Delta Biosphere Reserve have access to fresh water directly from the Danube River, through the drinking water treatment plants (DWTP) and drinking water distribution systems (DWDS) or groundwater wells.

The distribution network of the drinking water does not include a polyvinyl chloride (PVC) piping network in all locations from the the Danube Delta Biosphere Reserve. The routine maintenance of drinking water standards includes the periodical pumping of drinking water through the piping network in case of Sf. Gheorghe, Sulina, Chilia, Partizani, Pardina and Maliuc. There are no DWTPs and DWDSs in case of C.A. Rossetti, Letea and Vulturul.

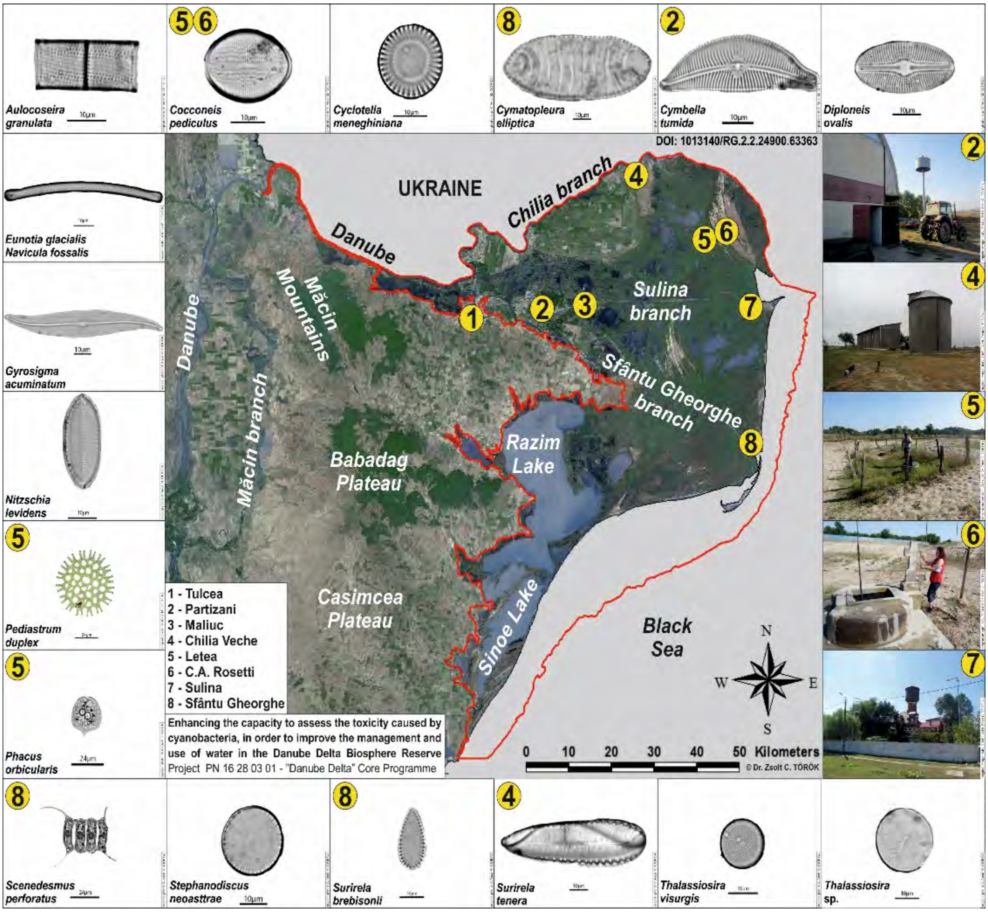

Figure 1. The sampling sites map (1. Tulcea, 2. Partizani, 3. Maliuc, 4. Chilia Veche, 5. Letea, 6. C. A. Rosetti, 7. Sulina, 8. Sfântu Gheorghe) sorrounded by photos of the algal taxa founded in the eight sites and photos from five sites

Sampling design

In situ measurements of water temperature, turbidity, dissolved oxygen concentration (DO) and electrical conductivity (EC) have been performed using HANNA HI 9829 Multiparameter, odors in water were also analyzed. Basic parameters examined in the present study were recommended by the Romanian legislation (Decision 974, 2004).

Ten liters of drinking water, were filtered *in situ* from every sampling site, using a 40 μm phytoplankton mesh size net. The resulting samples were used to determine the taxonomic composition and to measure the algae biomass, using bbe- Moldaenke technique with fluorescence spectrum excitation at 470nm LED (Light Emitting Diode) for green algae, 525nm LED for diatoms, 570nm LED for cryptophyceae and 610nm LED for cyanobacteria. Samples from DWTP (Sulina, Sf. Gheorghe and Chilia) and from groundwater wells (Letea and C.A. Rosetti) were stored in laboratory, in dark conditions for a period of seven days in opaque plastic containers (5L) and in transparent plastic containers (5L).

The qualitative assessment was made using light microscopic technique and the identifications were made to the species level (Krammer and Lange-Bertalot, 1986, 1988, 1991; Ettl, 1983). All samples were examined, before and after incubation for seven days. The quantitative assessment was determined by calculating the relative abundance, expressed as percentages. For phytoplankton roughly 400 individuals were counted in one drop of water, from every sample, at 40x magnification. In case of low number of individuals / sample, the entire surface of the slide was counted.

The taxonomic composition of the algal communities were analyzed from the following drinking water samples:

- "*in situ*" filtered samples from DWTPs in Sf. Gheorghe, Maliuc, Chilia, Partizani and from groundwater wells in Letea (1) and (2), C.A. Rosseti.
- before and after seven days of incubation from Sf. Gheorghe, Chilia, Sulina, Letea (1) and C.A. Rosseti.

DNA extraction and PCR amplification of cyanotoxin genes

The water samples were filtered through 0.2 μm-pore-size mixed cellulose ester membrane filters (Fioroni, France), followed by DNA extraction using ZR Soil Microbe DNA kit (ZymoResearch, USA), according to manufacturer's instruction. The optimal reaction component concentrations for the cyanotoxin gene amplification assays were as follows: 1 μL DNA, 5 μL MyTaq Red Mix 5X Buffer Solution, 0.3 μL of each 20 μM forward and reverse primers, 0.3 μL MyTaq Red 5u/μL and RNase/ DNase-free water to a final volume of 25 μL. Cycling protocols for each primer pair was used as recommended in the cited literature (Table 1).

Table 1.

Primer sets used in this study

Primer	Toxin	Sequence (F/R)	T_a°C	Product (bp)	Reference
***ana*C-gen**	Anatoxin	TCTGGTATTCAGTCCCCTCTAT/ CCCAATAGCCTGTCATCAA	58	366	Rantala-Ylinen *et al.*, 2011
***mcy*A-Cd1**	Microcistin	AAAATTAAAAGCCGTATCAAA/ AAAAGTGTTTTATTAGCGGCTCAT	59	297	Hisbergues *et al.*, 2003
***sxt*A**	Saxitoxin	GATGACGGAGTATTTGAAGC/ CTGCATCTTCTGGACGGTAA	60	125	Al-Tebrineh *et al.*, 2010

Results and discussion

Water supply and water use customs

The first water supply service in the Danube Delta was founded in 1920 in the town of Sulina, subsequently this service was extended to other locations from the Danube Delta Biosphere Reserve. Nonetheless, residents have continued to collect water directly from the Danube River for everyday household demands (drinking, cooking, and washing). The water supply service has achieved substantial and significant improvements after 1990, but these improvements are unable to provide the daily water requirements at a proper quantity and quality.

The available quantity of fresh water provided by the water supply services is not continuous during the summer time. The reported cubic meters per capita per days during summer period being as follows: 700-900 m^3/day at Sf. Gheorghe; 600 m^3/day at Sulina and Chilia. However, it is reported a less hours/day continuous water supply for the winter period, around 200 m^3/day.

The physico-chemical characteristics of water quality

The quality of the water is controlled and monitored at the DWTPs by the drinking water suppliers, private companies and by the Public Health Authority.

The research performed by the Danube Delta National Institute for Research and Development in 2016, deals with the assessment of the quality of water supplied to consumers, specifically from the point of view of chemical and biological contamination with algae and cyanobacteria toxins (Report of the project No PN 16 28 03 01, 2016). Cyanobacteria and its toxins are not included in national standards, therefore environmental protection agencies do not monitor their impact on the water quality. Concerns about monitoring and mitigating the negative effects of cyanobacteria and cyanotoxins on the environment, have been raised not only in the academic and scientific research field, but also at the European Enviroment Protection level (Falconer *et al.*, 2005, Nicholson *et al.*, 2001, Říhová Ambrožová *et al.*, 2009).

In the present evaluation of the water quality from the DWTPs and groundwater wells, took place in august 2016. All the analyzed water samples were odorless. *In situ* recorded parameters are presented in Figure 2.

The recorded pH values were in the range of standard values. In case of DWTPs the EC values were lower than in the case of drinking water from well sources and lower than standard value (Decision 974, 2004).

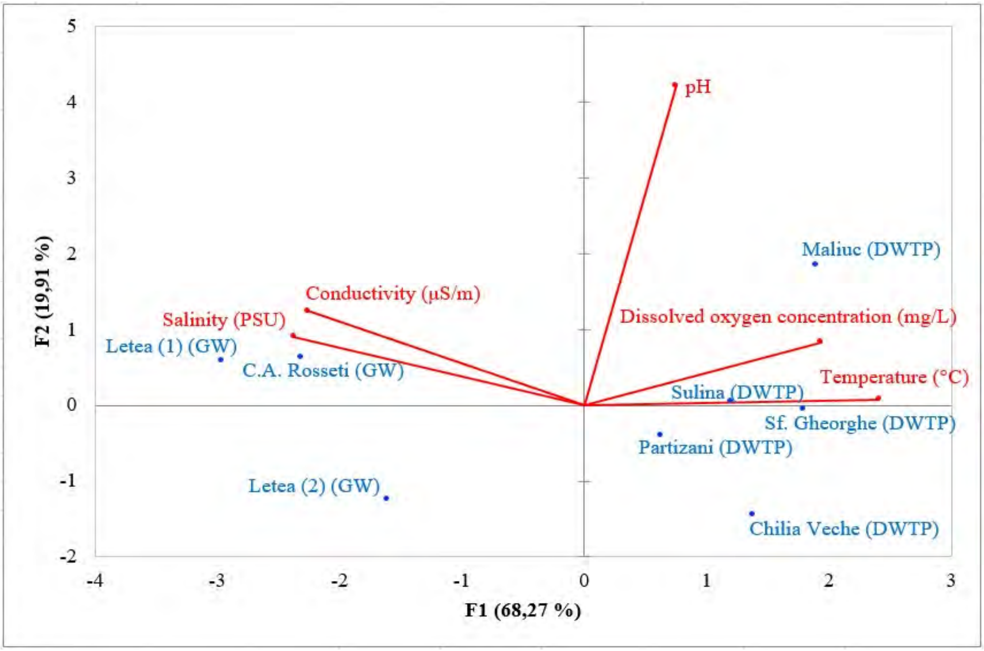

Figure 2. Principal Component Analysis (PCA) biplot (axes F1 and F2: 89.97 %) for the drinking water samples lakes and their aggregation based on physical and chemical parameters (abbreviations: DWTP-drinking water treatment plants, GW-groundwater well, PSU- Practical Salinity Unit)

Molecular detection of cyanotoxins

The presence of targeted cyanotoxin genes in the investigated environmental samples was verified by electrophoresis on a 1.0% agarose gel. No PCR product was present for either genes coding anatoxin, microcystin and saxitoxin. The concentration of cyanobacterial cells in drinking water samples is not always related to concentrations of cyanotoxins or genetic determinants (Szlag *et al.*, 2015). Possible factors causing the lack of cyanotoxin genes could be that some strains of potential toxin-producing species did not have the toxin gene, or that concentrations of cyanotoxin genes are lower in running- than stagnant water.

Algae and cyanobacteria in the drinking water supply

The evaluation of the response to the different treatment steps (decantation, filtration, chlorination), in case of algae and cyanobacteria was assessed at the entrance in the network distribution system. The influence of water treatment on the planktonic community structure was unnoticeable in case of Sf. Gheorghe, Chilia and Partizani (Fig. 3). The results showed higher values of the recorded algal biomass than at the sources from where water is extracted (The Danube River) before it enters the water plant.

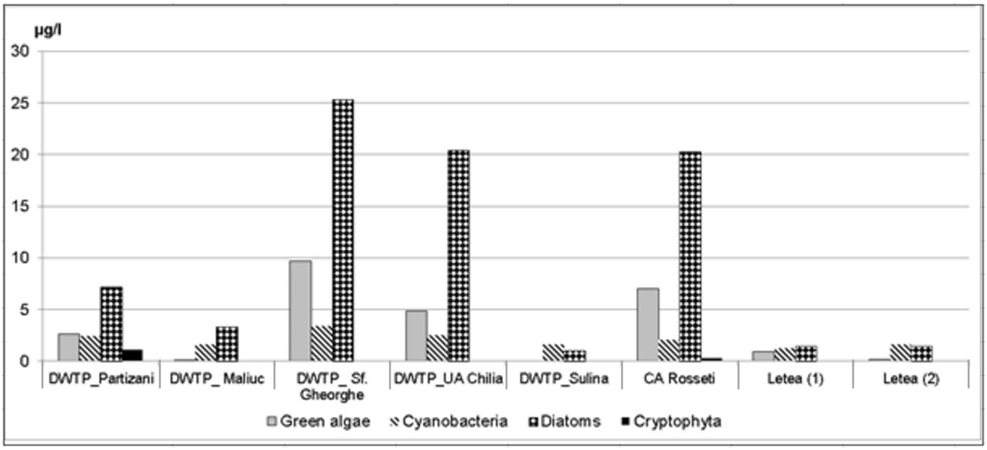

Figure 3. Algal biomass distribution in drinking water sources in the Danube Delta

The highest number of taxa were found in the "*in situ*" filtered samples from DWTPs from Sf. Gheorghe (35 taxa), Partizani and Chilia (21 and 20 taxa, respectively), Sulina (12 taxa) and only 8 taxa were found in samples from Maliuc. In comparison the samples from the groundwater wells were similar regarding the qualitative assessment: 27 taxa in C.A. Rosseti, 24 taxa in Letea (1) and only 10 taxa in Letea (2). Consequently three taxa were common in the analyzed samples: *Cyclotella meneghiniana*, *Stephanodiscus neoastraea* and *Thalassiosira weissflogii*.

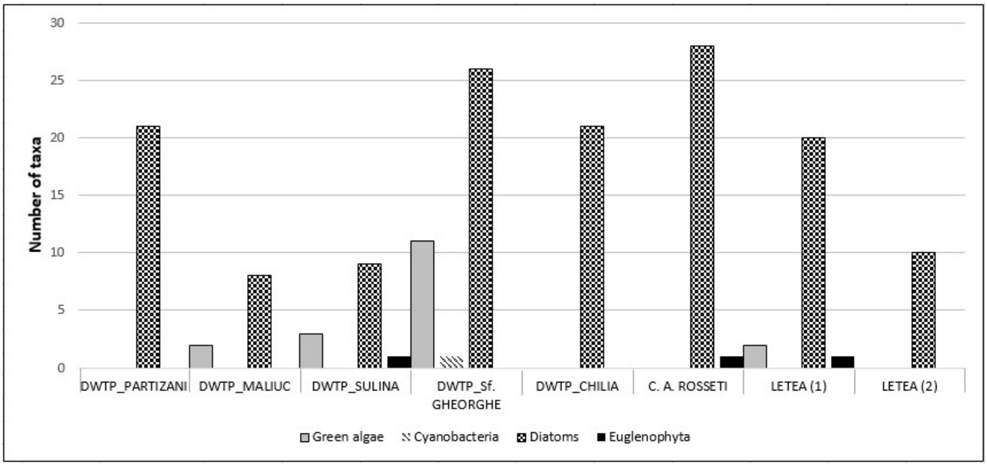

Figure 4. The taxonomic composition of the algal groups identified "*in situ*" the drinking water samples

Figure 3 and 4 show the similarity between the two methods of identifying the algal composition in water samples. Although bbe- Moldaenke technique identified the presence of sligthly more algal groups, both methods show clear results: the water treated in the DWTPs has significant algal biomass. In samples from the ground water Letea (1) and Letea (2) the algal biomass and taxonomic composition was the lowest. In Sulina green algae and other algal communities were found performing the light microscopy method. After analyzing the results, the water samples from DWTP Sf. Gheorghe registered the highest algal biomass (with the highest number of taxa), the dominating taxa being *Cyclotella meneghiniana*. The saprobic level indicated by this taxon is α-mesosaprobic, classifying the water quality in class III-IV (Van Dam *et al.*, 1994), which means the quality of the water is altered (in one drop of water 184 cells were found).

After seven days of incubating the water in transparent containers (TC) or in opaque containers (OC), the number of taxa dropped (Fig. 5). In each case the only identified taxa were diatoms, with only the siliceous frustule with no organic matter. These results could indicate that stored water bottles or cans, in dark conditions, are preferable for long-term use of the drinking water.

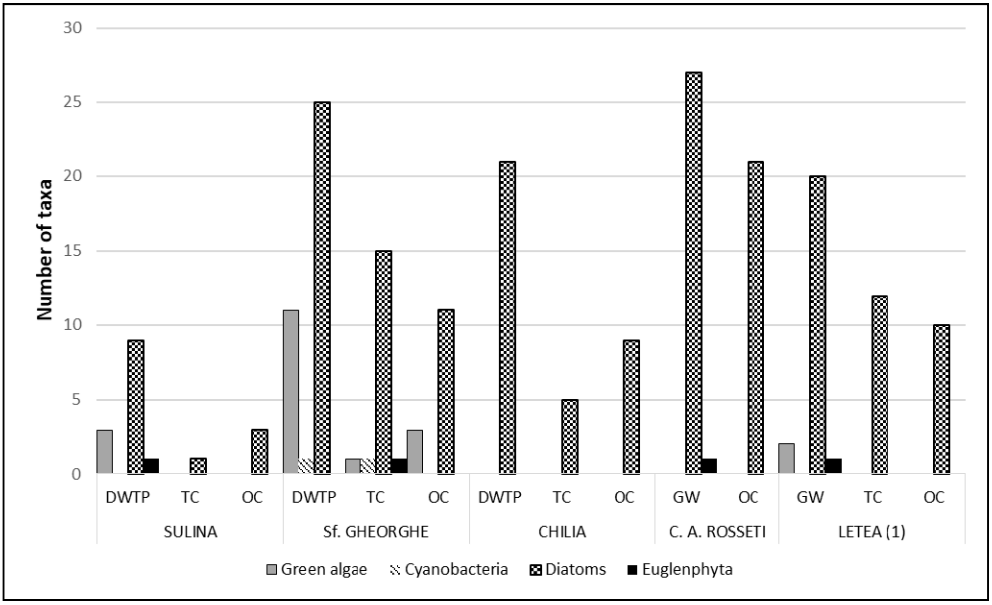

Figure 5. The taxonomic composition of the algal groups identified in the "*in situ*" drinking water samples compared to the incubated samples

The relative abundance was calculated for the following samples: DWTPs Partizani, Sf. Gheorghe and Chilia; groundwater wells C.A. Rosseti *in situ* and after incubation in an opaque can. In the samples from DWTP Sf. Gheorghe the class Mediophyceae (Centrales) was the only one present, with three taxa: *Cyclotella meneghiniana, Stephanodiscus neoastraea and Thalassiosira weissflogii*. These three taxa also ocupied more than 80% of the taxa identified in Partizani and Chilia. In the water samples from the ground water in C.A. Rosseti the diversity was higher. *Eunotia glacialis* being the dominant taxa (more than 50%), followed by *Diploneis elliptica* and *Achnanthes minutissima*. In Fig. 6 the correspondence analysis shows two distinct groups: DWTPs and groundwater. Because of the similarity between *Stephanodiscus neoastraea and Thalassiosira weissflogii*, in the correspondence analysis these species have been counted togheter (in order to avoid mislead identification).

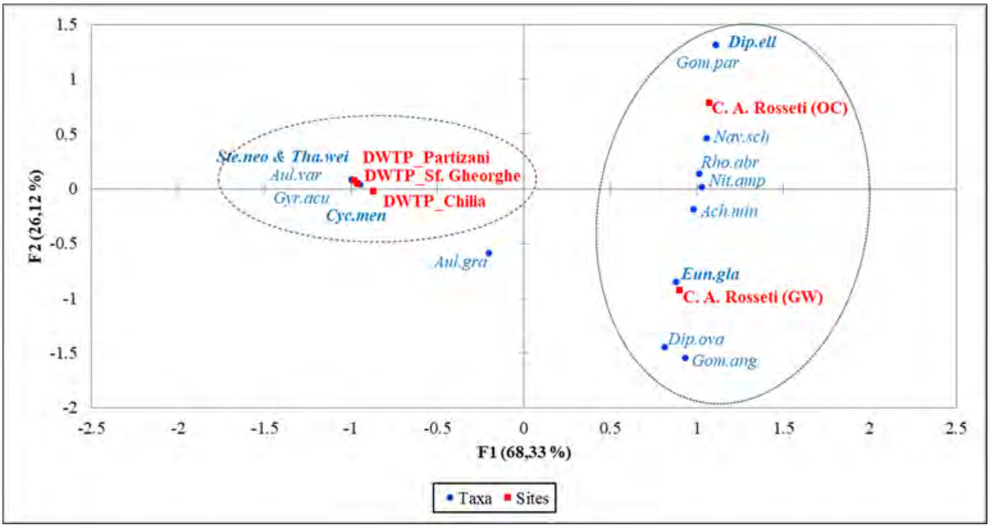

Figure 6. The correspondence analysis (axes F1 and F2: 94,45 %) showing the aggregation of sampling sites with the number of individuals from each algal taxa (abbreviations: *DWTP-drinking water treatment plants, GW-groundwater well, OC – opaque container; Ste.neo.- Stephanodiscus neoastraea, Tha.wei. - Thalasiosira wiesurgis, Aul.var. - Aulacoseira varians, Gyr.acu.- Gyrosigma acuminatum, Cyc.men. - Cyclotella meneghiniana, Aul.gra. - Aulacoseira granulata, Dip.ell. - Diploneis elliptica, Gom.par.- Gomphonema parvulum, Nav.sch.- Navicula schoenfeldii, Rho.abr.- Rhoicospahenia abreviatta, Nit.amp.- Nitzschia amphibia, Ach.min.- Achnanthes minutissima, Eun.gla.- Eunotia glacialis, Dip.ova.- Diploneis ovalis, Gom.ang.- Gomphonema angustum*.)

The water stored for household purposes can contain cyanobacteria, especially in rural areas without sewage systems or DWTP and DWDS, if water is collected from sources that presented a cyanobacterial blooming period. This phenomenon can be worsened if water is carried home and kept in transparent plastic containers. The presence of these microorganisms may affect the water quality, especially due to their ability to use carbon dioxide, light and minerals which allow them to grow and develop under these circumstances (Fosso-Kankeu *et al.*, 2008).

Overall the water quality indicated by the algal communities shows oligo-β-mesosaprobic conditions, but at every site the conditions change, because of the dominating bioindicator taxon. Even though the samples from Sf. Gheorghe and C. A. Rosseti had a higher number of taxa and individuals, they reflect oligotrophic conditions. The water quality could be at a lower level than expected, but as observed at the microscope samples, no high abundance of cyanobacteria was identified.

In the present study, after the incubation period (Table 2) a significant decrease of the total amount of chl-a and a slight increase in cyanobacteria was recorded in case of Sulina and C.A. Rosseti.

Table 2.

Concentration of chl-a and cyanobacteria biomass in DWTPs (abbreviations: CYAN - cyanobacteria, OC - opaque container; TC - transparent container; GW - groundwater wells, DWTP - drinking water treatment plants)

	DWTP/GW		Incubation OC		Incubation TC	
	chl-a (μg/l)	CYAN (μg/l)	chl-a (μg/l)	CYAN (μg/l)	chl-a (μg/l)	CYAN (μg/l)
Sulina	2.64	1.65	3.06	1.90	3.46	2.10
Sf. Gheorghe	38.52	3.44	21	2.92	14.82	2.68
Chilia	27.90	2.57	3.32	1.78	3.80	1.87
Letea (1)	3.72	1.30	3.46	1.51	3.45	1.42
C.A. Rosseti	29.62	2.09	10.19	2.17	7.43	1.84

Conclusions

Results revealed an undesirable amount of algal taxa in the drinking water samples, even after the treatment carried out in the DWTPs. In some cases the algal biomass was significantly reduced in the drinking water sampled from the wells, rather than from the Drinking Water Treatment Plants. The results obtained in this study should raise concern with the authorities and the legislation should contain information and toleration limits about the amount of cyanobacteria toxins and algae found in the drinking water.

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