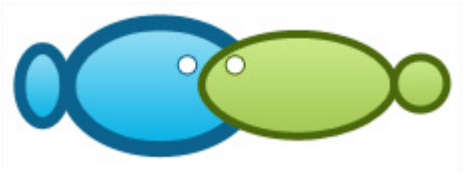

Preliminary study on bacteriological and physicochemical water profile of cyprinid fish ponds

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Abstract. The optimal parameter values related to bacteriological and physicochemical water quality in the on-growing ponds, are important for the fish biomass optimal growth. In fish farming, the fish must be correctly fed and must have the appropriate water environment conditions. This study aims to determine the water quality of cyprinid fish ponds with regards to the value of main bacteriological indicators and of some physicochemical parameters. Water samples were collected from two different cyprinid ponds P1 and P2, Movileni fish farm, Iaşi district, Romania. The average temperature of water in sampling points varied between 20.44 and 22.08°C. The water pH values in all sampling points were alkaline (> 8.50). The average values of nitrate ranged from 1.36 to 4.98 mg L^{-1}, showing highest values in the outlet points of the ponds (site E1 - 4.98 mg L^{-1} and site E2 - 4.90 mg L^{-1}). The maximum concentration of dissolved oxygen (17.92 mg L^{-1}) was observed in inlet source (A) and the lowest concentrations of 5.41 and 3.27 mg L^{-1}, were recorded on evacuation points E1 and E2, respectively. In ecology the physicochemical parameters have a direct influention on the microbial population, indirectly affecting fish development. The average values of total number mesophilic aerobic bacteria were higher in the fish farm intake source (site A – 5.45 log CFU mL^{-1}). The coliform bacteria have been identified in all water samples of the two ponds and their numbers varied between 16 500 and 75 000 CFU mL^{-100}. The water intake source (A) was characterised by a higher number of coliforms bacteria (45 000 CFU mL^{-100}). According to Romanian aquaculture legislation, the water quality of the ponds P1 and P2 of Movileni fish farm, Iaşi district, Romania could be classified as IIIrd quality class, moderate to critical contamination.

Key Words: ponds water quality, mesophilic aerobic bacteria, coliform bacteria, physicochemical parameters.

Introduction. In aquaculture there is a wide range of systems, from small ponds to large-scale commercial systems. Successful pond fish farming depends on the water physical, chemical and biological characteristics and on the species nutrition management. In pond fish farming all these factors are inter-related and require careful and constant monitoring to avoid contamination and/or degradation of the aquaculture environment (Sapkota et al 2008; Gorlach-Lira et al 2013).

The aim of fish farmers is to produce high quality fish with high yields and economic value in order to attain profit maximization. This involves different methods of increasing the yield, for example, by fertilizing the ponds which have low natural food productivity with organic manure in order to naturally stimulate the food production (Ampofo & Clerk 2010). This significant growth in fish consumption has enhanced people's diets around the world through diversified and nutritious food. In 2013, fish accounted for about 17 percent of the global population's intake of animal protein and 6.7 percent of all protein consumed. Moreover, fish provided more than 3.1 billion people with almost 20 percent of their average per capita intake of animal protein (FAO 2016). Fish is the favorite source of animal protein compared to pork, poultry, beef, or mutton. Fish is cheaper and easily accepted all over the world as there is almost no religious bias, which gives an advantage over other animal protein sources (Phillips et al 2004). High

quality water is an important aspect in sustaining the life of fish along with all the other organisms in the aquaculture environment. The main bacteriological indicator for the quality of the water is Enterobacteriaceae which is a non-sporing gram-negative bacteria. Moreover, the fish bacterial flora is shown by the bacteriological quality of the water (Soon et al 2014). Microorganisms have a very important function within the aquatic organisms because they participate in the nutrients conversion and these may affect the disease control of various water quality parameters such as pH, dissolved oxygen and ammonia (Moriarty 1997).

The bacteriological quality of water plays a vital role in the diseases spreading in farmed fish, fact that must be well known by the fish farmers who should understand the importance of mantaining a proper bacteriological water quality of the pond. In aquaculture bacterial pathogens represent an important cause of fish infections and mortalities (Arifin et al 2013). Furthermore, the fish bacterial flora is the main source of occupational diseases affecting the fish handlers and some bacteria from the fish ponds, such as Enterobacteriaceae, are directly related to factors like: suspended matter, dissolved oxygen, organic detritus and nutrient salt. These may show positive or negative correlations in the management of the commercial systems. As a result, the bacterial number in the pond ecosystem is directly linked to the pond management (Behera et al 2012).

The parameters which play a vital role in the identification and establishing the indicators for the most adequate habitual environment for the good development of cyprinidae species are the physicochemical and bacteriological parameters (Ntengwe & Edema 2008). Therefore, this study aims to determine temperature T (°C), pH, dissolved oxygen DO (mg L^{-1}) and nitrates $N\text{-}NO_3$ (mg L^{-1}), total number of mesophilic aerobic bacteria (log CFU mL^{-1}), the total number of coliform bacteria (CFU mL^{-100}) of water samples from two cyprinid ponds, P1 and P2, from Movileni fish farm, Iaşi district, Romania.

Material and Method

Details of study area. The researches were conducted in the Movileni Fish Farm, Iaşi district, Romania. The water source of the farm is represented by Jijia river. The water inlet and outlet from the ponds are made gravitationally, by using „monk" type hydraulic constructions. The summer of 2016 (August) in which the measurements were performed has been an extremely dry season, following a very dry season in 2015.

Experimental design. The experiment used two ponds with an area of 0.45 ha each and an average water depth of 1.5 m. The experimental design and sampling areas are presented in Figure 1.

The first pond (P1) has been divided by using a net as follows: first part with an area of 0.15 ha dedicated to carp monoculture (P1C) and the second part with an area of 0.30 ha dedicated to cyprinid polyculture (P1P). The second pond (P2) was used for rearing polyculture common carp (*Cyprinus carpio*) with grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypothalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*). The fishponds were stocked at the beginning of summer with juveniles of cyprinids which were kept there until the end of autumn. Our experimental research has lasted for 48 hours in August, 2016 (9 and 10 August, 2016).

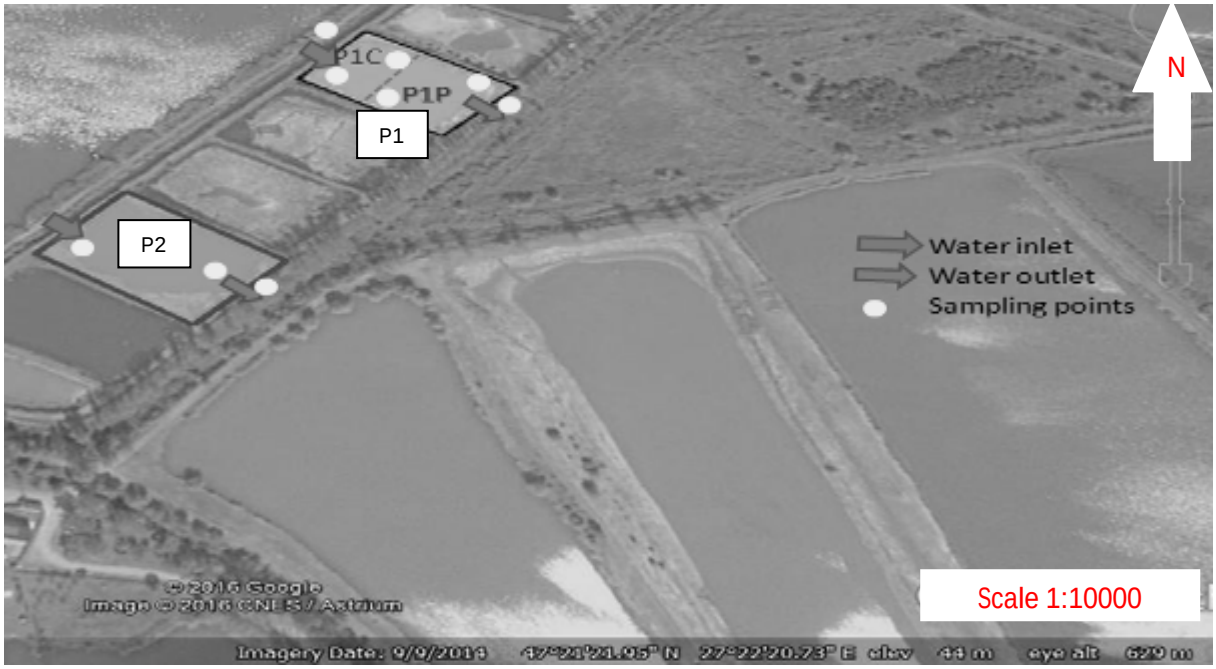


Figure 1. Details of study area, Movileni Fish Farm, Iaşi district, Romania.

Sampling of water. The water samples were collected in plastic bottles of 0.5 L and 1 L capacity from each of the two ponds, P1 pond with four sampling points coded - I.1.1., O.1.1., I.1.2, O.1.2. and P2 pond with two sampling points coded - I.2.1., O.2.1. Also, there were collected samples on intake water source, coded A and the ponds outlet coded E1 and E2. For the estimation of different parameters all water samples were collected in August, every two hours from the nine sampling points of the ponds. The samples were pre-treated on the field to fix them and immediately brought to the laboratory for physicochemical and bacteriological analysis of various parameters following the standard methods (APHA 1989).

Physicochemical parameters. The water physicochemical parameters such as temperature T (°C), pH, dissolved oxygen (mg L^{-1}) and nitrates N-NO_3 (mg L^{-1}) were determined. The instruments used for water anayses were: the HQ40d Portable pH, Dissolved Oxygen, Multi-Parameter (HACH), respectively Spectroquant photometer, Nova 400 for nitrate, with Merck kits.

Microbiological analysis. The total number of coliform bacteria and total mesophilic aerobic bacteria as main bacteriological indicators for the bacteriological quality of water used for the cyprinid farm were determined. The number of total mesophilic aerobic bacteria were carried out in pour plate using plate count agar followed by incubation at 37°C for 48 h, method provided by the Romanian Standard STAS 3001-91. Coliforms at 37°C were determined through the most probable number (MPN), with three sets of three tubes according to STAS ISO 4831-92.

The water physicochemical parameters were determined at the Research Laboratory of Aquaculture, Environmental Science and Cadastre Department from "Dunarea de Jos" University of Galati.

Statistical analysis. The one-way analysis of variance (ANOVA) has been performed on the data of bacterial and physicochemical variables using Statistica (version 5) software. Mean values and standard errors were generated for each physicochemical parameter and microbial group. The significance of differences was defined at $p < 0.05$.

Results. The results of the physicochemical parameters of the water samples from the nine sampling points on the two cyprinid ponds, P1 and P2 are presented in Figures 2-5.

The minimum and maximum temperatures of water were observed on all the sites from each pond (Figure 2). For P1 pond the minimum value was 19.00°C and the maximum value was 24.10°C. For P2 pond, the minimum value was 18.90°C and the maximum value was 24.30°C. The average temperature of water in sampling points varied between 20.44°C and 22.08°C. The differences of the temperature values were insignificant ($p > 0.05$) in the analyzed cyprinid ponds P1 and P2.

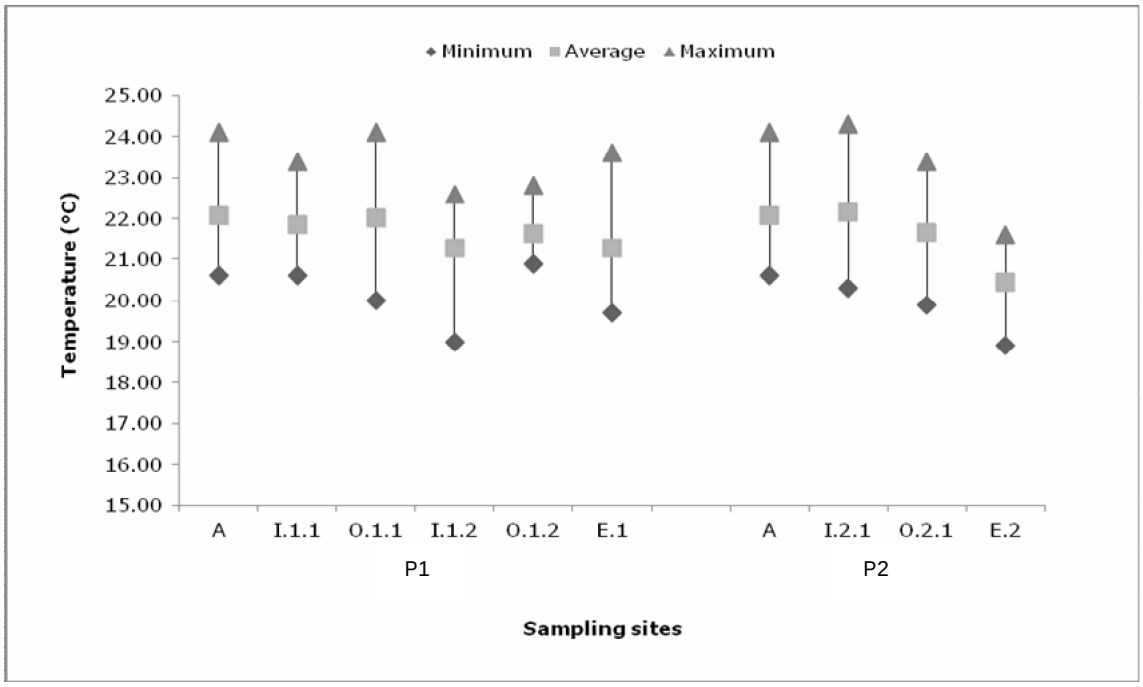


Figure 2. The water temperature variation in the cyprind ponds, P1 and P2.

The pH values didn't show fluctuations over the study period. In this research, the average of pH on the sampling sites was between 8.61 and 9.23. The maximum pH value, 9.79, was recorded in the intake water source of the two types of ponds. The water pH values in all sampling points were alkaline (Figure 3).

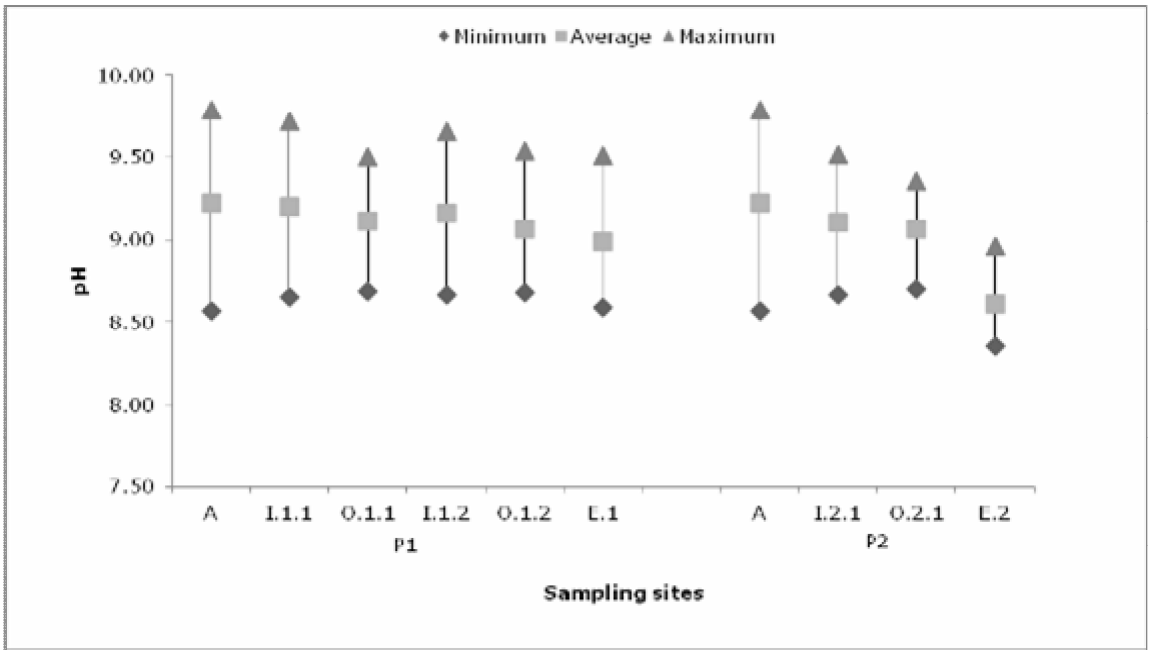


Figure 3. The water pH variation in the cyprinid ponds, P1 and P2.

DO from the analyzed fish ponds did not present significant differences within the two ponds P1 and P2 ($p > 0.05$, $p = 0.843$). The values of DO slightly varied among the analyzed sites. These average values were between 8.14 and 9.82 mg L^{-1}. The maximum concentration of DO (17.92 mg L^{-1}) was observed in the water intake source (A). The lowest concentrations (5.41 and 3.27 mg L^{-1}) were recorded on the outlet points (E1 and E2) (Figure 4).

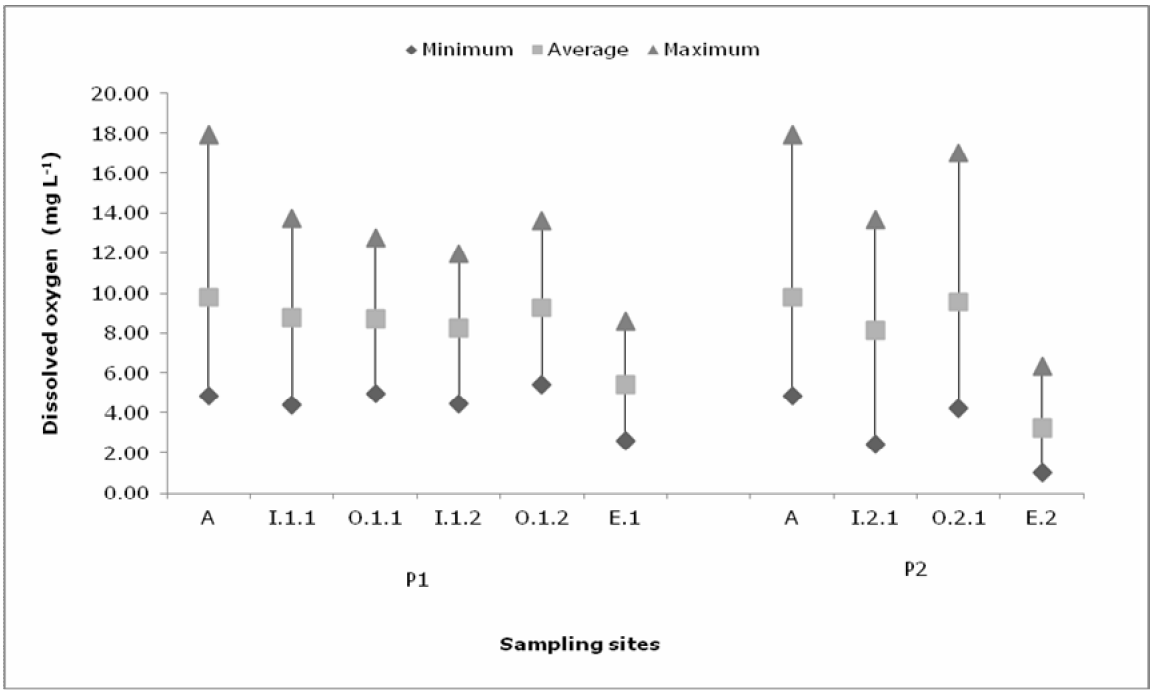


Figure 4. The variation of water dissolved oxygen in the cyprinid ponds, P1 and P2.

The average values of nitrate ranged from 1.36 to 4.98 mg L^{-1}, showing highest values in evacuation sites (Figure 5).

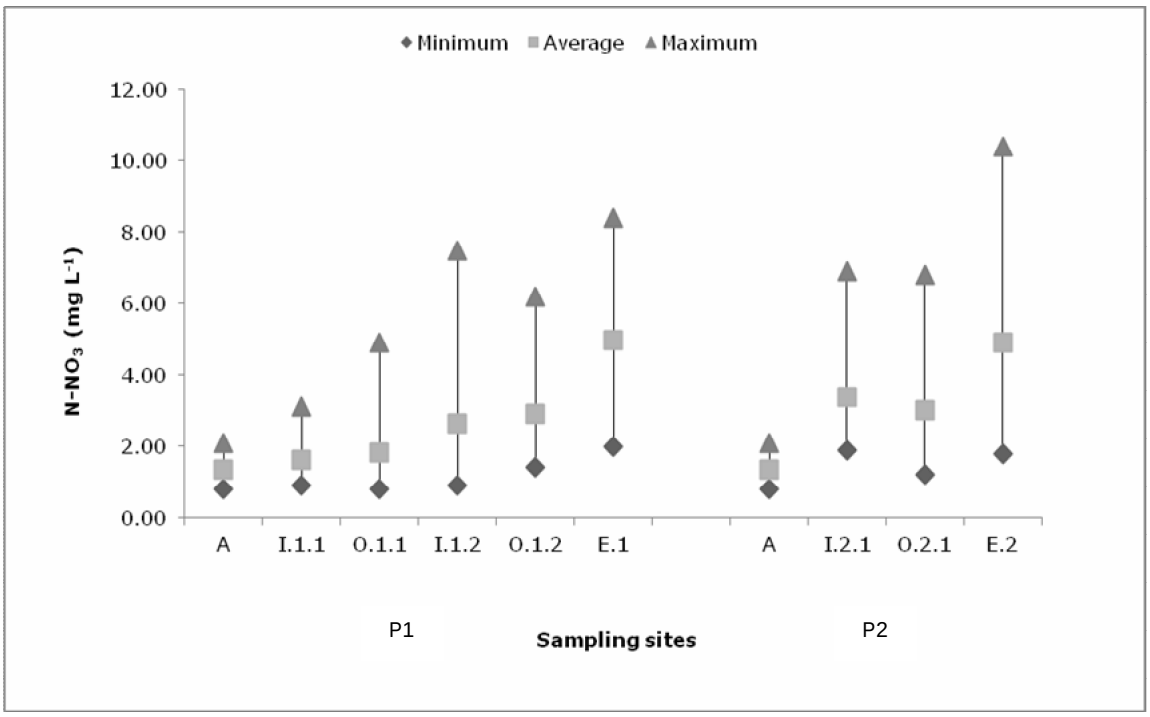


Figure 5. Variation of $N\text{-}NO_3$ in the water of the cyprinid ponds, P1 and P2.

The total number of mesophillic aerobic bacteria ranged from 4.14 to 4.66 log CFU mL^{-1} in the water samples. The average value of the total number of mesophilic aerobic bacteria were higher in the farm water intake source (site A – 5.45 log CFU mL^{-1}) than in the inlet and outlet studied fish ponds P1 and P2 (Figure 6). The coliform bacteria were found in all water samples of P1 and P2 ponds and their numbers varied between 16500 and 75000 CFU mL^{-100} respectively, in August (Figure 7).

Discussion. The quality of aquaculture water is generally influenced by various physicochemical and bacteriological parameters. The water quality directly influences the productivity and the health of the fish. Theoretically, aquaculture in eutrophic environments is predisposed to pH changes and leads to the possibility of increasing the toxicity of the non-ionized ammonia and nitrite. High temperatures can catalyse those processes turning them into evident and frequent processes. The interactions of these environmental factors can influence fish production by decreasing the rate of food conversion, reducing the weight gain and releasing a higher quantity of soluble nutrients into the environment (Sapkota et al 2008; Gorlach-Lira et al 2013).

There is a very close similarity between the atmospheric temperature and water temperatures due to the depth of ponds. The high temperature and bright sunshine accelerates the process of organic matter decay resulting into the liberation of large quantities of CO_2 and nutrients. The pH value is influenced by the organic and inorganic compunds present in water. Any alteration in water pH is accompanied by the change in other physicochemical parameters. pH maintenance is one of the most important attributes of any aquatic system since all biochemical activities depend on surrounding water pH. Maximum values during summer may be due to increased photosynthesis of the algal blooms resulting into the precipitation of calcium and magnesium carbonates from bicarbonates causing higher alkalinity.

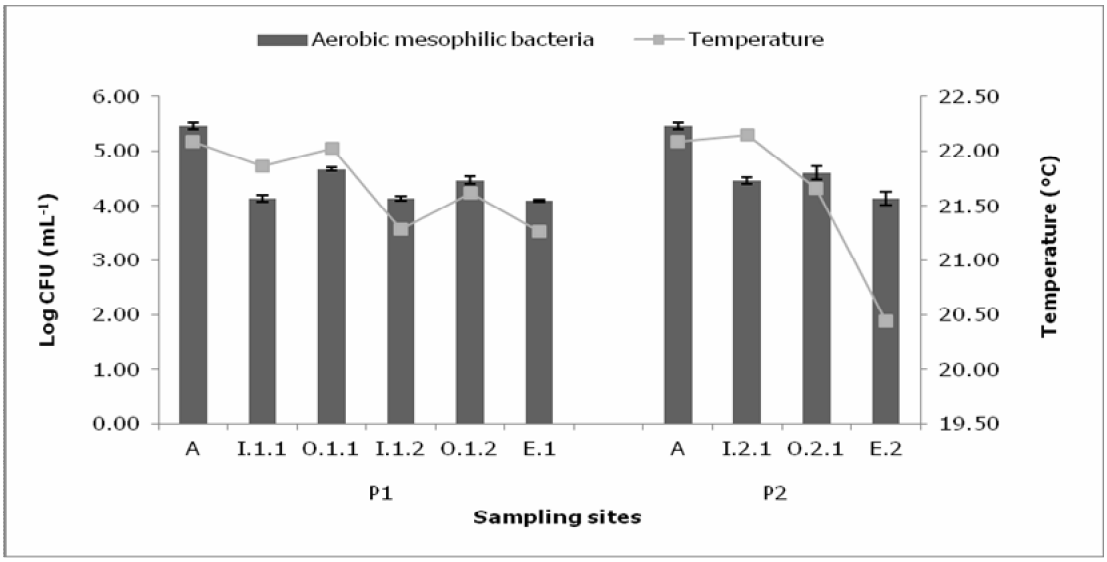
Dissolved oxygen is a very important parameter of water quality and an index of physicalchemical and biological processes which take place in water. DO is of great importance to all living organisms. It may be present in water due to direct diffusion from air and photosynthetic activity of autotrophic organisms. DO concentration of is one of the most important parameter to indicate water purity.

Nitrate concentration depends on the activity of nitrifying bacteria which in turn get influenced by the presence of DO. This may be caused by the higher phytoplanktonic production, decaying macrophytes and concentration of nutrients due to the evaporation of water with subsequent increase in nitrate value. These facts have also been stressed by Agarwal & Rajwar (2010) in their observations. The decrease in nitrate content was probably due to its utilization as nutrient by the algal community as it has been evidenced by the massive growth of algae.

Water physicochemical parameters, such as pH, nutrients and presence of toxic compounds may influence the density of bacterial populations. The importance of the microbial population in the water intake source used for fish farming can be resumed by its influence on the amount and diversity of bacteria in the fish during harvesting.

The levels of total mesophilic aerobic bacteria serve as support in the evaluation of the water in respect to the decomposing activity. Bacterial number in the analysed water from P1 and P2 ponds were in the range of 4 log CFU mL^{-1}. These results were lower than the values recorded by Ntengwe & Edema (2008) in the order of 6 log CFU mL^{-1} from fish culture pond water, but higher than those observed by Al-Harbi & Uddin (2003) in the tilapia pond culture (3 and 4 log CFU mL^{-1}). The differences between the number of total mesophilic aerobic bacteria were insignificant ($p > 0.05$, $p = 0.282$) in the analyzed ponds P1 and P2.

The neutral or alkaline pH, the high value of temperature and the DO of sampling water observed in August, are favourable for the growth of aerobic mesophillic bacteria. Such conditions were observed in P1 and P2 ponds which are the subject of this work, where all the samples indicated pH values over 8.0, the water temperature ranged from 22 to 24°C and the values of dissoved oxygen were between 8.14 and 9.59 mg L^{-1}, during the study (Figure 6 a-c).



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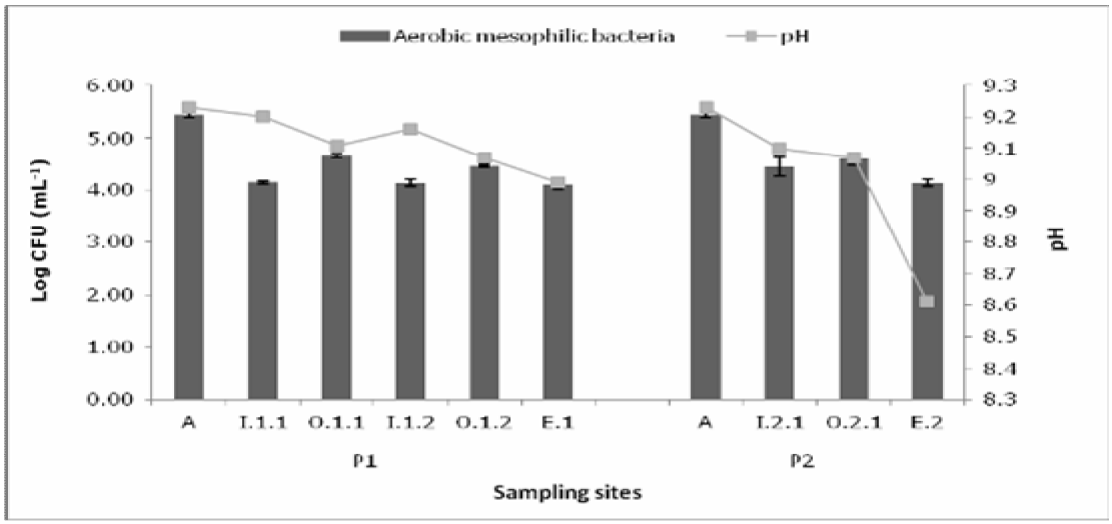

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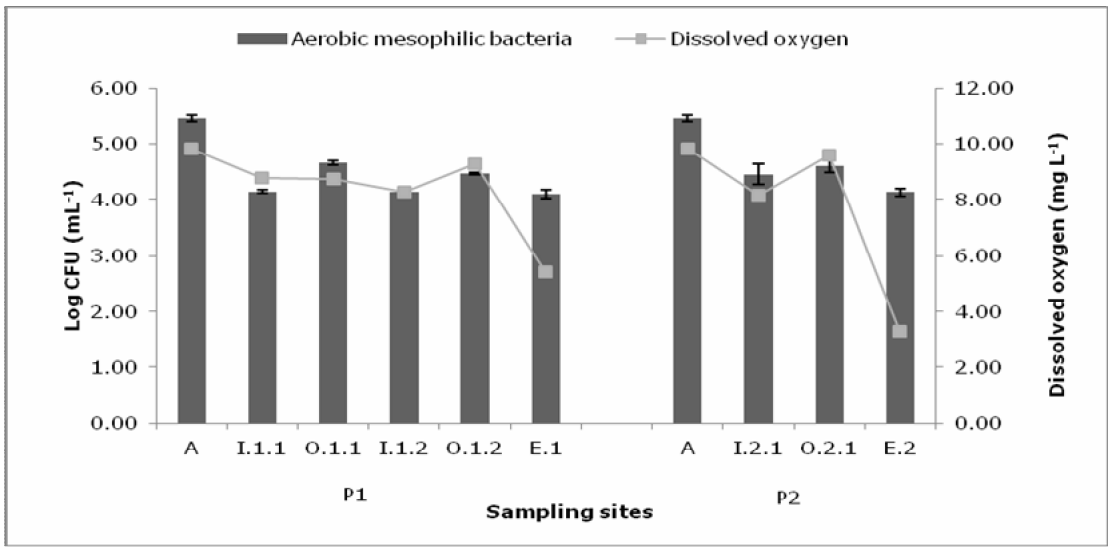

c

Figure 6. Correlations between the total number of mesophillic aerobic bacteria and the physicochemical parameters: temperature (a), pH (b), dissolved oxygen (c).

Servais et al (2005) cited by Hacioglu & Dulger (2009) mentioned that freshwaters polluted by faecal discharges from human and animals may transport a variety of human pathogenic microorganisms. Because the detection of all waterborne faecal pathogens is very difficult, various indicators of faecal contamination are usually used to detect faecal pollution in natural waters. Coliform bacteria are used as microbiologic indicators for water quality (Sood et al 2008). Water intake (A) was characterised by a higher number of coliforms bacteria (45 000 CFU mL^{-100}). The differences of coliform bacteria values between the analyzed ponds P1 and P2, were statistically significant ($p < 0.05$, $p = 0.00017$). According to the Order no. 161 issued on 16 February 2006, the water from the two ponds could be classified as IIIrd quality class, moderate to critical contamination (Order 161, 2006).

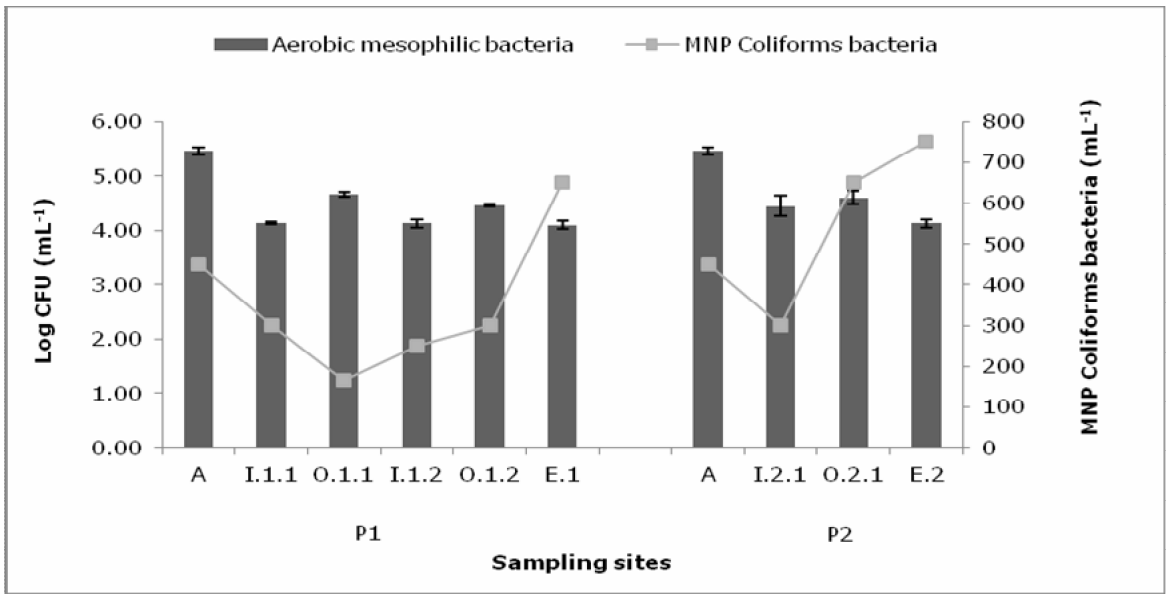

Figure 7. Correlations between the total number of mesophillic aerobic bacteria and the MNP of coliform bacteria.

Agarwal & Rajwar (2010) noted that the growth of bacterial population during summer months is the outcome at the influx of washed organic matter in the water intake source from the surrounding areas. It is natural that the incoming nutrient load finds its way first to the surface, thereby encouraging bacterial proliferation. Santos et al (2012) cited by Gorlach-Lira et al (2013) also observed the presence of coliforms in the water of all six studied fish farms in Brazil. As shown in the present and other works, aquaculture environment is characterised by fluctuations of physicochemical parameters and bacteriological indicators caused by different nutrient loads, trophic changes, climatic characteristics, and therefore special attention should be paid to the frequency of limnological and sanitary aspects of the water.

Conclusions. The results obtained in this study demonstrate that according to Romanian regulations on water quality for aquaculture, the water from the cyprinid ponds P1 and P2, belongs to quality class III. The presence of coliforms bacteria and mesophilic aerobic bacteria in high concentrations of the water samples indicates the potential risks of contamination of the fish. For two systems of cyprind culture, there is a need to apply monitoring programmes regarding not only the physicochemical parameters, but also the analysis of bacteriological indicators that are rarely included in the monitoring of the fish farm water quality and that there is a need for the river which ensures the intake water to be closely monitored by the authorities because the low quality of the surface water could impact the fish farming operations. The bacterial dynamics in such ecosystems should be under constant control and frequent monitoring by using bacteriological indicators and the identification of all bacteria.

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*** STAS ISO 4831-92 - Microbiology. General guidance for determining the number of coliform bacteria. The most probable number technique. [in Romanian]

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