

## A kinetic study of chlorophyll "a" and Cyanophyta algae biomass grown in different IMTA systems

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**Abstract.** The scope of this research was to perform a 24 hours kinetic analysis of cyanobacterial and total chlorophyll "a" concentration from algae biomass grown in the pond technological water of two different integrated multi trophic aquaculture (IMTA) systems. The research was performed in two different ponds, with an area of 0.45 ha each. The first pond (PCP –polyculture carp pond) was used for rearing in polyculture common carp (*Cyprinus carpio*) with grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*). The second pond was divided by using a net, and stocked as follows: first part with an area of 0.15 ha (CP – with common carp pond) and the second part with an area of 0.30 ha (PP - polyculture pond). Cyanobacterial, total chlorophyll "a" concentration and also turbidity 24 hours kinetic were determined in nine sampling points, as follows: inlet and outlet channels, inlet and outlet area of each pond (PCP, CP and PP) and also the middle area of PCP pond. The maximum concentrations of cyanobacterial chlorophyll "a" were recorded between 22.87-86.80  $\mu\text{g L}^{-1}$  at 14:00 o'clock and the minimum concentration between 13.87-58.70  $\mu\text{g L}^{-1}$  at 02:00 o'clock. The maximum concentration of total chlorophyll "a" was recorded during the dawn (08:00 am), between 145.63-216.57  $\mu\text{g L}^{-1}$ , while the minimum values were encountered during the night (02:00 am), between 71.97-123.03  $\mu\text{g L}^{-1}$ . Significant high cyanobacterial and total chlorophyll "a" concentrations during the day period, compared with night period are recorded only in case of classical cyprinides polyculture pond production system (PCP). The modern IMTA production system (CP-PP) has offered more stability in terms of 24 hours kinetic of cyanobacterial and total chlorophyll "a" concentration.

**Key Words:** common carp, cyanobacterial chlorophyll "a", IMTA, polyculture, total chlorophyll "a".

**Introduction.** Definitions of integrated aquaculture are widespread (Muir 1981; Edwards et al 1988; Liu & Huang 2008; Angel & Freeman 2009; Barrington et al 2009) but in essence related to an aquaculture production system where the output (waste) of one sub system is utilized by another sequential linked sub system resulting in a greater efficiency of the overall system. Therefore, integrated Multi-Trophic Aquaculture (IMTA) refers to farming of different aquaculture species together in a way that the system comprises fed aquaculture, organic extractive and inorganic extractive species in the same production module to reduce organic and inorganic wastes (Neori et al 2004). In an IMTA system, the organic matter comprising uneaten feed and feces from fed aquaculture (for example, fish or shrimp) is consumed by the deposit feeders (for example, sea cucumber). In addition, the inorganic nutrients, mainly nitrogen and phosphorus are absorbed by seaweed (Sumbing et al 2016).

Algae represent an important nutritive base and have a significant effect on the biological productivity of a water body. However, algae are considered to be disastrous when in bloom. The ponds used for carp culture often develop dense algae blooms. Among different algae, blue-green algae (Cyanobacteria/Cyanophyta) are considered to form spectacular water blooms in fish ponds. Nutrient enrichment by the addition of fertilizers, supplementary feeding and other eutrophication processes may cause proliferation of blue-green algae. The factors which are responsible for the preponderance of these algae over other algal groups are their ability to assimilate a variety of biogenic

organic compounds (Smith 1973), being therefore better adaptable to different environmental factors (Sevrin-Reyssac & Pletikoscic 1990) and the plasticity of their photosynthetic apparatus (Krogmann 1973).

Chlorophyll "a" is the primary photosynthetic pigment found in all species of algae, used as an index of algal biomass (Wetzel 2001) or for assessing the intensity of phytoplankton development (Lampert & Sommer 2007). Chlorophyll "a" concentration is not a direct measure of algal biomass, as the concentration of chlorophyll varies depending by species and environmental conditions (Laxson & Kelting 2014).

Increases in chlorophyll "a" are generally associated with increased algal production, as the concentration of chlorophyll "a" is widely considered as the most direct measure of the trophic state of lakes and other aquatic resources. Algal biomass is affected by nutrient availability, water temperature and light, so there can be considerable variation in chlorophyll "a" concentrations throughout the year, depending on which of these three factors is limiting growth at a particular time (Laxson & Kelting 2014).

It is not clear what the precise effects of carp are on nutrient cycling and water clarity in whole lakes as most studies have used relatively small experimental areas or theoretical calculations (Weber & Brown 2009; Morgan & Hicks 2013).

The common carp (*Cyprinus carpio*) is one of the world's most invasive fish (Kulhanek et al 2011). It is often called an "ecological engineer" because of its ability to modify the habitat and biotic communities of the lakes it invades (Matsuzaki et al 2009). Common carps reduce water quality through their feeding activities by physically disturbing sediments and recycling nutrients (Chumchal et al 2005). Carps root in the bottom while searching for food and have been shown to drive rapid declines in macrophytes and increases in water turbidity (Lougheed et al 1998; Zambrano et al 2001; Bajer et al 2009; Matsuzaki et al 2009; Bajer & Sorensen 2015), also these activities can result in an increase in chlorophyll "a" value (Williams et al 2002; Parkos et al 2003). The carp has also been hypothesized to play an important role in nutrient transport from the sediments into the water column due to bioturbation and excretion (Lamarra 1975; Breukelaar et al 1994; Parkos et al 2003; Morgan & Hicks 2013). For all of these reasons, carp removal is often recommended as an important element of lake restoration (Meijer et al 1990).

The main objective of this research was to perform a 24 hours kinetic analysis of cyanobacterial and total chlorophyll "a" concentration from algae biomass grown in the pond technological water of two different integrated multi trophic aquaculture (IMTA) systems.

## Material and Method

**Study sites.** The research was conducted at the "S.C. Piscicola Iasi" fish farm, which is situated in the Larga Jijia village, 24 km north-west from Iasi city, Romania (GPS coordination 47.354731, 27.370649). The total fish farm surface area is 1000 hectares, partitioned in 24 ponds. Both inlet and outlet are made gravitationally from Jijia River, by using monk hydraulic constructions.

**Experimental design.** The research was performed in two different ponds, with an area of 0.45 ha each and an average water depth of 1.5 m (Figure 1).

The first pond (PCP - polyculture carp pond) was used for rearing in polyculture the following fish species: common carp (*Cyprinus carpio* – 2500 specimens) with grass carp (*Ctenopharyngodon idella* – 100 specimens), bighead carp (*Hypophthalmichthys nobilis* – 40 specimens) and silver carp (*Hypophthalmichthys molitrix* - 40 specimens).

The second pond was divided by using a net, and stocked as follows: first part with an area of 0.15 ha (CP – carp pond, with 2000 common carp specimens with an individual average of biomass weight of  $61.2 \pm 11.60$  g and an individual average total length of  $8.5 \pm 0.90$  cm) and the second part with an area of 0.30 ha (PP – polyculture pond, with 500 common carp specimens with an individual average of biomass weight of  $60.0 \pm 10.45$  g and an individual average total length of  $8.3 \pm 0.7$  cm, 40 silver carp

specimens with an individual average of biomass weight of  $2044.0 \pm 289.80$  g and an individual average total length of  $38.7 \pm 1.80$  cm, 40 bighead specimens with an individual average of biomass weight of  $1824.1 \pm 182.59$  g and an individual average total length of  $34.7 \pm 1.20$  cm and 100 grass carp specimens with an individual average of biomass weight of  $199.4 \pm 20.00$  g and an individual average total length of  $17.1 \pm 1.00$  cm) (Figure 2).

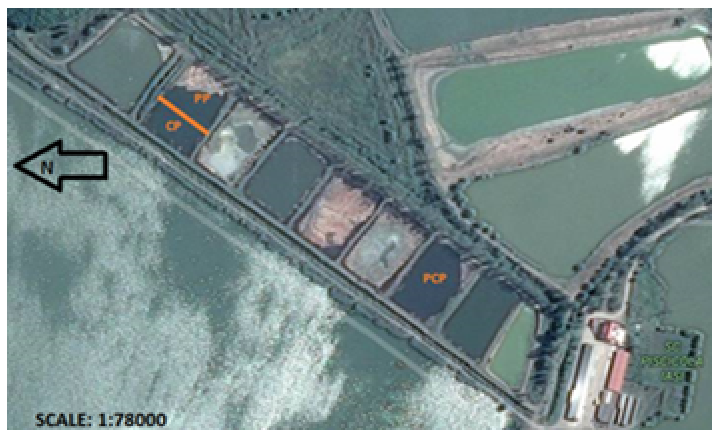


Figure 1. Top view of the experimental fish ponds situated at SC Piscicola Iasi farm (CP - carp pond; PP - polyculture pond; PCP - polyculture carp pond).

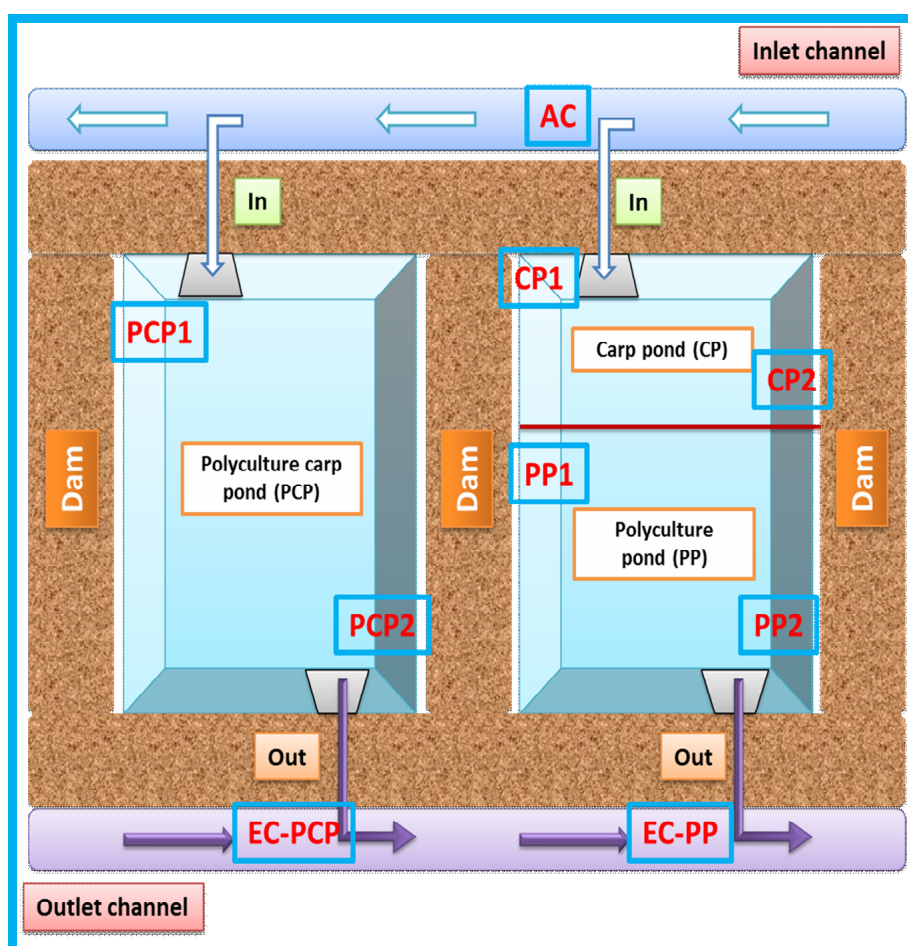


Figure 2. Experimental design and sampling areas. Sampling areas: AC – inlet channel; EC-PCP – outlet channel from polyculture carp pond; EC-PP – outlet channel from polyculture pond; PCP1 – polyculture carp pond 1; PCP2 – polyculture carp pond 2; CP1 – carp pond 1; CP2 – carp pond 2; PP1 – polyculture pond 1; PP2 – polyculture pond 2.

The administered feed had a crude protein content of 28% and was represented by a mix of cereals (wheat lees, dry maize dregs, sunflower groats) in equal amounts and flour protein. Feed was manually administered twice a day, only in PCP and CP, for five days in a week, that makes a total of 59 days of feeding during the entire production cycle. A 1.5% body weight daily feeding ratio was used.

This experimental design was used for a growth research that had lasted for 83 days, from 15 June to 5 September 2016. However, this paper aims to present a 24 hours kinetics of chlorophyll "a" cyanobacterial algae biomass grown, between 24–25 August 2016. It was considered as being very important to study both day and night kinetics of chlorophyll "a" cyanobacterial algae biomass grown, in certain weather conditions. This kinetics study was part of a longer monitoring period, over the 83 days production cycle. Between 24–25 August 2016 the weather was sunny, with an average temperature of 22.78°C during the day and 20.8°C during the night. The light intensity varied from 108 000 lux for direct sunlight at noon, to less than 12 lux at afternoon and the photoperiodicity was 13 day: 11 night hours.

No fertilizers were added in the ponds both at the beginning and during the experimental period.

Regarding the water circulation system, it must be mentioned that both PCP and CP-PP are part of a group of nine ponds (Figure 1), therefore having the same inlet and outlet channels. The outlet of the ponds is made gravitationally. The recirculating flow is 5 L s<sup>-1</sup> ha<sup>-1</sup> for both ponds

Considering the interactions between chlorophyll "a" and cyanobacterial algae biomass grown and organic matter, nutrients concentration, oxygen, temperature and pH, the following parameters were determined: dissolved oxygen - DO (mg L<sup>-1</sup>), temperature (°C), pH, phosphorus (P<sub>2</sub>O<sub>5</sub> - mg L<sup>-1</sup>), nitrate nitrogen (N-NO<sub>3</sub> - mg L<sup>-1</sup>), nitrite nitrogen (N-NO<sub>2</sub> - mg L<sup>-1</sup>), ammonium nitrogen (N-NH<sub>4</sub> - mg L<sup>-1</sup>), water transparency (cm), turbidity (FTU), total and cyanobacterial chlorophyll "a" (µg L<sup>-1</sup>). These parameters were analyzed in nine sampling stations, as presented in Figure 2. The water samples were collected and the chlorophyll "a" and cyanobacterial algae biomass grown were determined once every six hours, as follows: August 24, 2016 at 08:00 am, 14:00 pm and 20:00 pm, respectively in August 25, 2016 at 02:00 am and 08:00 am.

**Water quality assessment.** For each of the sampling area, the oxygen, temperature and pH of the water were monitored using the portable equipment HQ40d for pH, DO and the Multi-Parameter (HACH). Also, water samples were collected in order to determine the concentration of phosphorus (P<sub>2</sub>O<sub>5</sub> - mg L<sup>-1</sup>), nitrate nitrogen (N-NO<sub>3</sub> - mg L<sup>-1</sup>), nitrite nitrogen (N-NO<sub>2</sub> - mg L<sup>-1</sup>), ammonium nitrogen (N-NH<sub>4</sub> - mg L<sup>-1</sup>) and chemical oxygen demand (COD - mg L<sup>-1</sup>) with Merck kits, by using the Spectroquant Nova 400 photometer, in the Research Laboratory of Aquaculture, Environmental Science and Cadastre Department from "Dunarea de Jos" University of Galati, Romania. For determination of biological oxygen demand (BOD<sub>5</sub>) was used Velp IP54 analyzer consisting in a stirring unit, BOD sensors, supports for absorbing carbon dioxide, special bottles for BOD and magnetic stirrers. The analyzer was used together with a cooling incubator Velp FTC 90I, small capacity, equipped with an auto-tuning temperature regulator. All the water samples were taken from a 20 cm depth.

The transparency of the water was evaluated by using the Secchi disc in each of the sampling area.

Cyanobacterial and total chlorophyll "a" concentration were determined by using the AlgaeTorch (bbe Moldaenke GmbH, Schwentinental, Germany) – a fluorescence measurement device specifically developed to perform field measurements of total chlorophyll "a" and cyanobacterial chlorophyll "a" (Pobel et al 2011). This instrument is based on the same principle as the bbe FluoroProbe (Beutler et al 2002) and provides rapid measurement of the concentrations of cyanobacterial and total chlorophyll "a" in the water. The bbe AlgaeTorch uses seven LEDs for fluorescence excitation. These LEDs emit light at three selected wavelengths (470 nm, 525 nm, 610 nm; two LEDs each). An additional LED is used for turbidity estimations based on the reflected light of any particles in the water (Kalaji et al 2016).

In this study, the AlgaeTorch instrument was immersed to a depth of 20 cm in each of the nine sampling areas, once at six hours. In each sampling areas were performed three replicate measurements. Each of the replicate measurements were done in the same position, perpendicular with the water surface.

**Statistical analysis.** The obtained results were statistically analyzed using descriptive statistics and One Way ANOVA test (Tukey – Duncan, significance level  $p \leq 0.05$ ). Programs used were Microsoft Excell 2010 and IBM SPSS Statistics 20.0. The results were presented as minimum, maximum and mean  $\pm$  standard deviation.

**Results and Discussions.** The 24 hours kinetics of water DO, oxygen saturation, temperature and pH, in each of the sampling points of both IMTA experimental ponds (PCP and CP-PP), are presented in Figures 3 to 6.

The maximum concentration of DO were generally recorded on 24.08.2016 at 20:00 pm (AC – 10.72 mg L<sup>-1</sup>; EC-PP – 8.03 mg L<sup>-1</sup>; CP1 – 9.76 mg L<sup>-1</sup>; CP2 – 9.26 mg L<sup>-1</sup>; PP1 – 8.95 mg L<sup>-1</sup>; PP2 – 10.30 mg L<sup>-1</sup>; PCP1 – 9.54 mg L<sup>-1</sup>; PCP2 – 10.24 mg L<sup>-1</sup>; EC-PCP – 8.30 mg L<sup>-1</sup>), while the minimum values were generally recorded at dawn (24-25.08.2016 at 08:00), as follows: AC – 5.43 mg L<sup>-1</sup>; EC-PP – 3.34 mg L<sup>-1</sup>; CP1 – 4.98 mg L<sup>-1</sup>; CP2 – 4.38 mg L<sup>-1</sup>; PP1 – 4.17 mg L<sup>-1</sup>; PP2 – 5.42 mg L<sup>-1</sup>; PCP1 – 4.98 mg L<sup>-1</sup>; PCP2 – 4.28 mg L<sup>-1</sup>; EC-PCP – 3.69 mg L<sup>-1</sup> (Figure 3). The DO 24 hours kinetics shows a clear upward tendency during the day (from 08:00 till 20:00), followed by a strong downward tendency during the night (from 20:00 till 08:00) in each of the sampling points of both IMTA experimental ponds (PCP and CP-PP) (Figure 3). The low DO values during the night are the result of no algal oxigenation. Also, cyanophyta algae biomass take the oxygen during the night, due to the lack of light for photosynthesis.

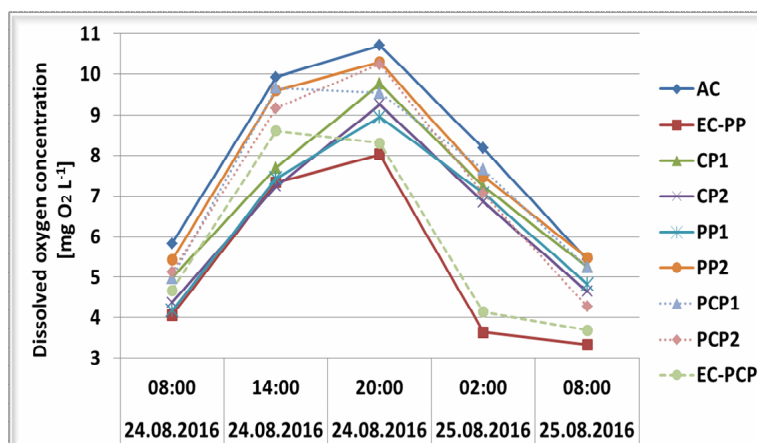


Figure 3. Water dissolved oxygen (DO) 24 hours kinetics, in PCP and CP-PP.

It must be pointed out that the lowest values of DO are recorded in case of the outlet channel (EC-PP and EC-PCP), while the highest DO is registered in case of the inlet channel (AC). Also, significant statistical results ( $p < 0.05$ ) are registered between EC-PP and EC-PCP, most probably due to the position of the sampling point along the outlet channel (EC). Therefore, EC serves as an outlet channel for a battery of nine ponds, in which PCP is the third pond and PP is pond number eight (Figure 1), fact that generates an organic matter accumulation at the end of the channel due water hydraulics and therefore, lower DO concentration.

The high DO concentration in PP2 is due to the fact that no food was administrated in this pond section. No significant differences were recorded between CP2 and PP1 as the sampling points were situated in the center part of CP-PP experimental pond, while in case of CP1, PCP1 and PCP2, the feeding process induced lower DO values, compared with PP2, where no food was administrated.

The maximum percentages of oxygen saturation were generally recorded on 24.08.2016 at 20:00 pm (AC – 154.30%; EC-PP – 106.36%; CP1 – 123.57%; CP2 – 128.42%; PP1 – 113.31%; PP2 – 147.24%; PCP1 – 114.76%; PCP2 – 133.30%; EC-PCP

– 103.10%), while the minimum values were generally recorded at dawn (24 - 25.08.2016 at 08:00), as follows: AC – 72.00%; EC-PP – 45.14%; CP1 – 71.23%; CP2 – 55.30%; PP1 – 59.43%; PP2 – 76.17%; PCP1 – 63.13%; PCP2 – 53.20%; EC-PCP – 41.30% (Figure 4). The oxygen saturation 24 hours kinetics shows a clear upward tendency during the day (from 08:00 till 20:00), followed by a strong downward tendency during the night (from 20:00 till 08:00) in each of the sampling points of both IMTA experimental ponds (PCP and CP-PP) (Figure 4). The oxygen saturation 24 hours kinetics is relatively similar with the DO 24 hours kinetics, described above.

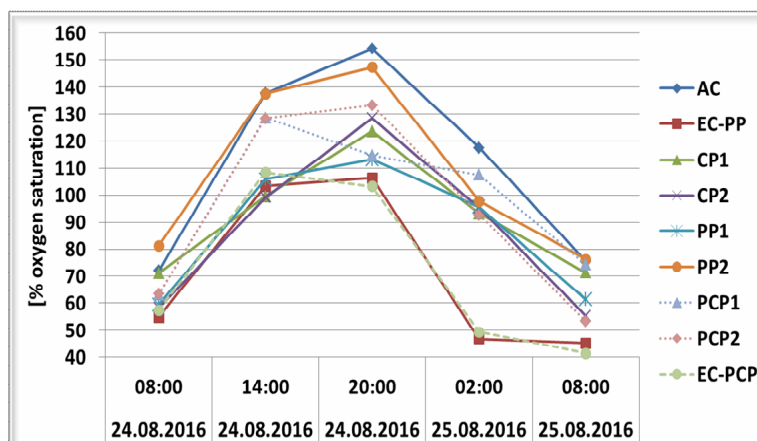


Figure 4. Water oxygen saturation 24 hours kinetics, in PCP and CP-PP.

The maximum temperature values were generally recorded during the day, on 24.08.2016 at 14:00 (AC – 24.10°C; EC-PP – 23.60°C; CP1 – 23.40°C; CP2 – 22.90°C; PP1 – 22.60°C; PP2 – 22.80°C; PCP1 – 24.30°C; PCP2 – 23.40°C; EC-PCP – 21.60°C), while the minimum values were generally recorded at dawn (25.08.2016 at 08:00), as follows: AC – 20.60°C; EC-PP – 19.20°C; CP1 – 20.60°C; CP2 – 20.00°C; PP1 – 19.60°C; PP2 – 20.90°C; PCP1 – 20.30°C; PCP2 – 19.90°C; EC-PCP – 18.90°C (Figure 5). The temperature 24 hours kinetics shows a clear downward tendency starting from 24.08.2016 (14:00) till 25.08.2016 (08:00). Several studies were made in order to establish how cyanobacteria respond to temperature (Canale & Vogel 1974; Reynolds & Walsby 1975; Konopka & Brock 1978; Tilman et al 1986). Those studies had demonstrated that cyanobacterial dominance generally occurs at higher (> 20°C) water temperatures, whereas optimal temperature for other algal groups tend to be lower than 20°C (Robarts & Zohary 1987).

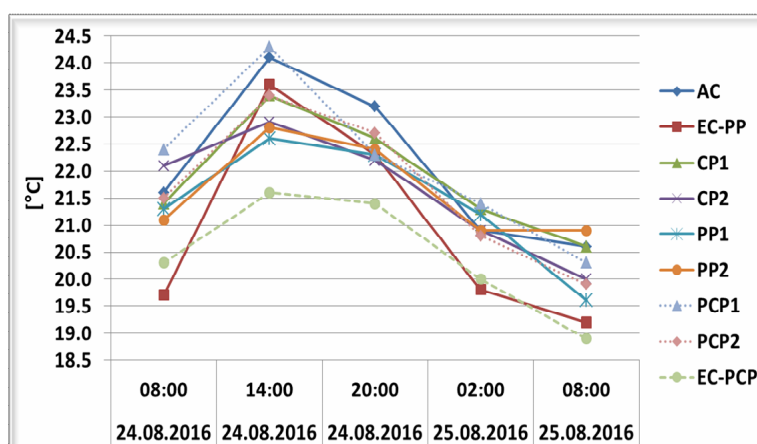


Figure 5. Water temperature 24 hours kinetics, in PCP and CP-PP.

The maximum values of pH were generally recorded on 24.08.2016, between 14:00 and 20:00 (AC – 9.76; EC-PP – 9.51; CP1 – 9.72; CP2 – 9.50; PP1 – 9.56; PP2 – 9.56; PCP1 – 9.52; PCP2 – 9.36; EC-PCP – 9.26), while the minimum values were generally recorded



at dawn (24-25.08.2016 at 08:00), as follows: AC – 8.57; EC-PP – 8.49; CP1 – 8.65; CP2 – 8.69; PP1 – 8.67; PP2 – 8.68; PCP1 – 8.67; PCP2 – 8.70; EC-PCP – 8.28 (Figure 6). The pH 24 hours kinetics shows a clear upward tendency till the midday (from 08:00 till 14:00), followed by a strong downward tendency during the night (from 20:00 till 08:00) in each of the sampling points of both IMTA experimental ponds (PCP and CP-PP) (Figure 6). The highest pH values are encountered in case of CP1 from CP pond compared with PCP and PP ponds, most probably due to the high value of the ratio between, the amount of administrated food and the rearing surface of CP pond section. Also, other studies (Hinnners et al 2015) have mentioned that the growth of cyanobacteria is significantly limited by the relatively high pH values.

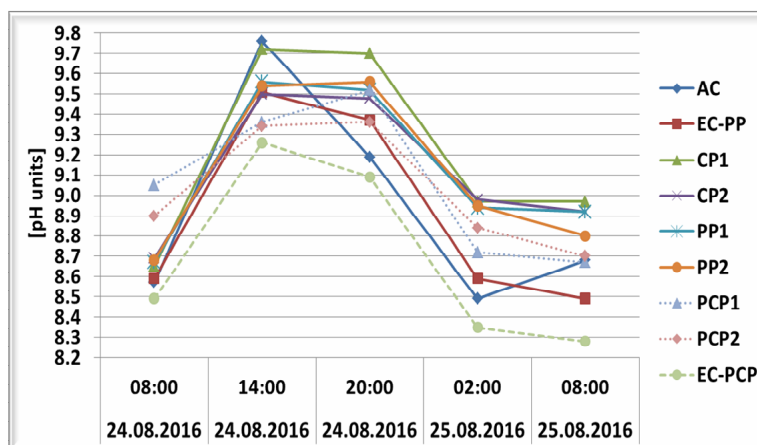


Figure 6. Water pH 24 hours kinetics, in PCP and CP-PP.

The 24 hours kinetics of phosphorus ( $P_2O_5$  -  $mg L^{-1}$ ), nitrate nitrogen ( $N-NO_3$  -  $mg L^{-1}$ ), nitrite nitrogen ( $N-NO_2$  -  $mg L^{-1}$ ) and ammonium nitrogen ( $N-NH_4$  -  $mg L^{-1}$ ), in each of the sampling points of both IMTA experimental ponds (PCP and CP-PP), are presented in Figures 7 to 10.

The maximum concentration values of phosphorus ( $P_2O_5$ ) were recorded at dawn, 24.08.2016 at 08:00, AC – 2.98  $mg L^{-1}$ ; EC-PP – 4.83  $mg L^{-1}$ ; CP1 – 3.53  $mg L^{-1}$ ; CP2 – 3.91  $mg L^{-1}$ ; PP1 – 4.02  $mg L^{-1}$ ; PP2 – 3.15  $mg L^{-1}$ ; PCP1 – 4.07  $mg L^{-1}$ ; PCP2 – 4.17  $mg L^{-1}$ ; EC-PCP – 4.32  $mg L^{-1}$ , while the minimum values were generally recorded during the night (25.08.2016 at 02:00 am), as follows: AC – 1.43  $mg L^{-1}$ ; EC-PP – 3.35  $mg L^{-1}$ ; CP1 – 2.02  $mg L^{-1}$ ; CP2 – 2.17  $mg L^{-1}$ ; PP1 – 2.26  $mg L^{-1}$ ; PP2 – 1.72  $mg L^{-1}$ ; PCP1 – 2.98  $mg L^{-1}$ ; PCP2 – 3.02  $mg L^{-1}$ ; EC-PCP – 2.86  $mg L^{-1}$  (Figure 7). The phosphorus ( $P_2O_5$ ) 24 hours kinetics shows a clear downward tendency during the day (from 08:00 till 20:00), followed by upward tendency at the end of the night (from 02:00 till 08:00), in each of the sampling points of both IMTA experimental ponds (PCP and CP-PP) (Figure 7).

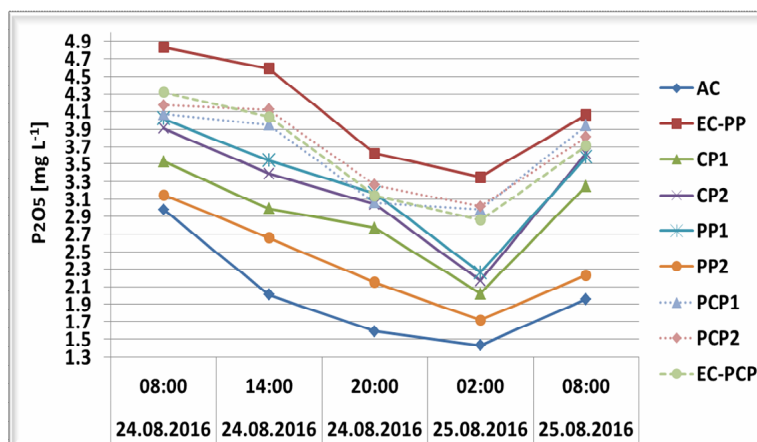


Figure 7. Water phosphorus ( $P_2O_5$  -  $mg L^{-1}$ ) concentration during 24 hours kinetics, in PCP and CP-PP.

It must be pointed out that the lowest concentration of phosphorus ( $P_2O_5$ ) was recorded in case of inlet channel, followed by PP2 (the pond section where no food was administrated), while the outlet channel (EC-PCP and EC-PP) and PCP pond (PCP1 and PCP2) registered the highest phosphorus ( $P_2O_5$ ) concentration, most probably due to the amount of food administrated (in PCP was administrated the highest quantity of fish food).

Bachmann et al (1996) reported strong positive correlations between chlorophyll "a" and both total phosphorus and total nitrogen, and strong negative correlations between Secchi transparency and chlorophyll "a", total phosphorus, and total nitrogen in a study of 65 lakes in Florida. Also, in our study, Secchi water transparency registered the following values: AC - 24 cm; EC-PP - 16 cm; CP1 - 19 cm; CP2 - 18 cm; PP1 - 18 cm; PP2 - 23 cm; PCP1 - 21 cm; PCP2 - 19.5 cm; EC-PCP - 12 cm.

Also Costa et al (2014) mentioned that in synthesis, high nutrient concentrations, mainly phosphorus, cause low water quality in these fishponds by increasing cyanobacteria, chlorophyll "a", turbidity, and thermotolerant coliforms, and by depleting dissolved oxygen.

The maximum concentration of nitrate nitrogen ( $N-NO_3$ ) were recorded on 24.08.2016, at 14:00, 20:00 and on 25.08.2016 at 08:00, as follows: AC -  $4.20 \text{ mg L}^{-1}$ ; EC-PP -  $10.80 \text{ mg L}^{-1}$ ; CP1 -  $6.20 \text{ mg L}^{-1}$ ; CP2 -  $7.00 \text{ mg L}^{-1}$ ; PP1 -  $7.50 \text{ mg L}^{-1}$ ; PP2 -  $3.20 \text{ mg L}^{-1}$ ; PCP1 -  $5.40 \text{ mg L}^{-1}$ ; PCP2 -  $5.80 \text{ mg L}^{-1}$ ; EC-PCP -  $12.80 \text{ mg L}^{-1}$ , while the minimum values were generally recorded during the night (25.08.2016 at 02:00; 24.08.2016 at 20:00 in PCP1 and PCP2), as follows: AC -  $2.70 \text{ mg L}^{-1}$ ; EC-PP -  $7.10 \text{ mg L}^{-1}$ ; CP1 -  $3.90 \text{ mg L}^{-1}$ ; CP2 -  $4.80 \text{ mg L}^{-1}$ ; PP1 -  $4.50 \text{ mg L}^{-1}$ ; PP2 -  $1.90 \text{ mg L}^{-1}$ ; PCP1 -  $3.60 \text{ mg L}^{-1}$ ; PCP2 -  $3.20 \text{ mg L}^{-1}$ ; EC-PCP -  $8.30 \text{ mg L}^{-1}$  (Figure 8). It can be observed that the nitrate nitrogen concentration in the outlet channel registered the highest values among the sampling points, fact recorded at noon (20:00), as follows: EC-PP -  $10.80 \text{ mg L}^{-1}$  and EC-PCP -  $12.80 \text{ mg L}^{-1}$ . The nitrate nitrogen ( $N-NO_3$ ) 24 hours kinetics shows a clear upward tendency during the day (from 08:00 till 20:00), followed by downward upward tendency during the night (02:00), in each of the sampling points of both IMTA experimental ponds (PCP and CP-PP) (Figure 8).

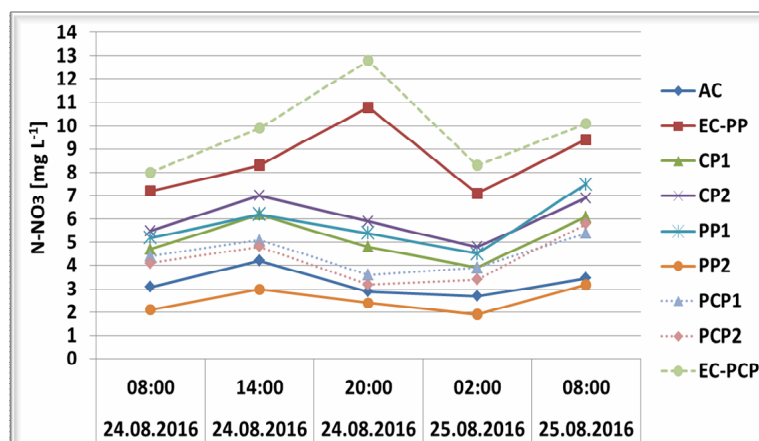


Figure 8. Water nitrate nitrogen ( $N-NO_3 - \text{mg L}^{-1}$ ) concentration during 24 hours kinetics, in PCP and CP-PP.

In case of the outlet channel (EC-PCP and EC-PP) were encountered the highest nitrate nitrogen concentrations, while at PP2 (the pond section where no food was administrated) were registered the lowest nitrate nitrogen concentrations. Also, among the experimental ponds, CP registered the highest nitrate nitrogen concentrations, most probably due to the ratio between the amount of administrated food and the rearing surface of CP pond section, as it was mentioned before.

Regarding nitrite nitrogen ( $N-NO_2$ ) concentration, the highest values are generally recorded at dawn, 24.08.2016 at 08:00 and 14:00, AC -  $0.18 \text{ mg L}^{-1}$ ; EC-PP -  $0.26 \text{ mg L}^{-1}$ ; CP1 -  $0.12 \text{ mg L}^{-1}$ ; CP2 -  $0.13 \text{ mg L}^{-1}$ ; PP1 -  $0.14 \text{ mg L}^{-1}$ ; PP2 -  $0.24 \text{ mg L}^{-1}$ ; PCP1 -



0.21 mg L<sup>-1</sup>; PCP2 – 0.19 mg L<sup>-1</sup>; EC-PCP – 0.23 mg L<sup>-1</sup>, while the minimum values were recorded during the night (25.08.2016 at 02:00), as follows: AC – 0.14 mg L<sup>-1</sup>; EC-PP – 0.23 mg L<sup>-1</sup>; CP1 – 0.07 mg L<sup>-1</sup>; CP2 – 0.10 mg L<sup>-1</sup>; PP1 – 0.09 mg L<sup>-1</sup>; PP2 – 0.17 mg L<sup>-1</sup>; PCP1 – 0.16 mg L<sup>-1</sup>; PCP2 – 0.16 mg L<sup>-1</sup>; EC-PCP – 0.20 mg L<sup>-1</sup> (Figure 9). The nitrite nitrogen (N-NO<sub>2</sub>) 24 hours kinetics shows a clear downward tendency during the day (from 08:00 till 20:00), followed by upward tendency in the night (from 20:00 till 02:00), in each of the sampling points of both IMTA experimental ponds (PCP and CP-PP) (Figure 9). It must be highlighted that the lowest nitrification process does occur in case of CP pond section, due to high stocking density and therefore, high organic matter concentration. The presence of high organic matter concentration accelerates the growth of heterotrophic bacteria, therefore limiting the activity of autotrophic bacterial communities, that generates the oxidation of ammonia to nitrites and then, to nitrates.

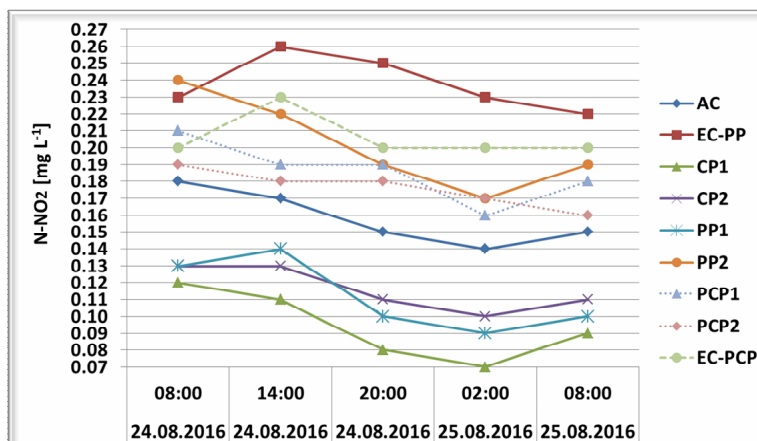


Figure 9. Water nitrite nitrogen (N-NO<sub>2</sub> – mg L<sup>-1</sup>) concentration during 24 hours kinetics, in PCP and CP-PP.

The maximum concentrations of ammonium nitrogen (N-NH<sub>4</sub>) were recorded at dawn, 24-25.08.2016 at 08:00, AC – 0.33 mg L<sup>-1</sup>; CP1 – 0.57 mg L<sup>-1</sup>; CP2 – 0.55 mg L<sup>-1</sup>; PP1 – 0.52 mg L<sup>-1</sup>; PP2 – 0.38 mg L<sup>-1</sup>; PCP1 – 0.60 mg L<sup>-1</sup>; PCP2 – 0.56 mg L<sup>-1</sup>, while the minimum values were generally recorded at the midday (14:00), as follows: AC – 0.09 mg L<sup>-1</sup>; CP1 – 0.28 mg L<sup>-1</sup>; CP2 – 0.31 mg L<sup>-1</sup>; PP1 – 0.35 mg L<sup>-1</sup>; PP2 – 0.13 mg L<sup>-1</sup> (Figure 10). In case of PCP experimental pond (PCP1 and PCP2) and also, for the outlet channel (EC-PP and EC-PCP), the minimum ammonium nitrogen (N-NH<sub>4</sub>) concentration was registered on 24.08.2016 at dawn (PCP1 – 0.44 mg L<sup>-1</sup>; PCP2 – 0.43 mg L<sup>-1</sup>; EC-PCP – 0.62 mg L<sup>-1</sup>, EC-PP – 0.58 mg L<sup>-1</sup>) while the maximum one, at night 25.08.2016 (PCP1 – 0.52 mg L<sup>-1</sup>; PCP2 – 0.46 mg L<sup>-1</sup>; EC-PCP – 1.10 mg L<sup>-1</sup>, EC-PP – 0.86 mg L<sup>-1</sup>).

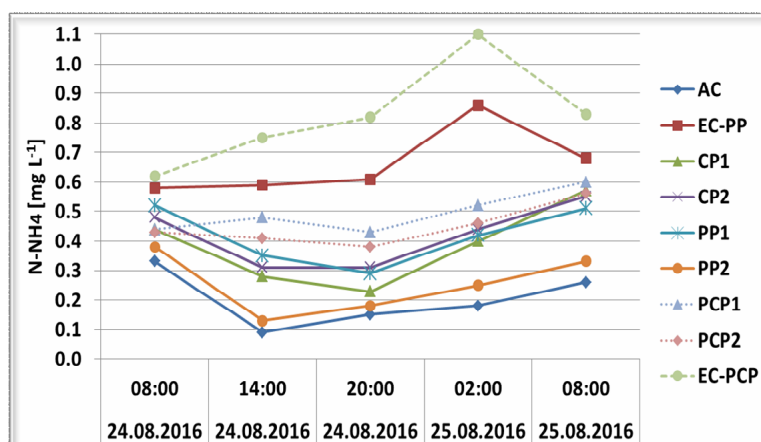


Figure 10. Water ammonium nitrogen (N-NH<sub>4</sub> – mg L<sup>-1</sup>) concentration during 24 hours kinetics, in PCP and CP-PP.

The ammonium nitrogen concentration 24 hours kinetics shows a clear upward tendency during the night in case of all sampling points, except the outlet channel. Also, by analyzing the 24 hours kinetic of all three nitrogen compounds ( $\text{N-NH}_4$ ;  $\text{N-NO}_2$ ;  $\text{N-NO}_3$ ) it can be concluded that the highest intensity of nitrification process was recorded at midday (14:00) in case of inlet channel and CP-PP sampling points, focusing on PP1 (pond section where no food was administrated), where the best nitrification results occur (Figures 8 to 10).

In order to evaluate the organic matter from the experimental systems (CP-PP and PCP) and to correlate it with the cyanobacterial and total chlorophyll "a" concentration from algae biomass grown, a 24 hours kinetic was identified for chemical oxygen demand (COD) and biological oxygen demand - 5 days ( $\text{BOD}_5$ ), presented in Figures 11 and 12.

The maximum concentration of COD were recorded during the dawn (CP1 –  $171.1 \text{ mg L}^{-1}$ ; CP2 –  $165.3 \text{ mg L}^{-1}$ ; PP1 –  $158.4 \text{ mg L}^{-1}$ ; PP2 –  $125.4 \text{ mg L}^{-1}$ ; PCP1 –  $165.7 \text{ mg L}^{-1}$ ; PCP2 –  $157.3 \text{ mg L}^{-1}$ ), while the minimum values were encountered during the night (25.08.2016 at 02:00), as follows: EC-PP –  $141.6 \text{ mg L}^{-1}$ ; CP1 –  $163.9 \text{ mg L}^{-1}$ ; CP2 –  $149.4 \text{ mg L}^{-1}$ ; PP1 –  $144.7 \text{ mg L}^{-1}$ ; PP2 –  $115.3 \text{ mg L}^{-1}$ ; PCP1 –  $155.5 \text{ mg L}^{-1}$ ; PCP2 –  $142.6 \text{ mg L}^{-1}$ ; EC-PCP –  $114.0 \text{ mg L}^{-1}$  (Figure 11). It can be observed that the COD concentration in the inlet channel registered a opposite 24 hours kinetic, compare with the other sampling points (CP-PP, PCP and outlet channel). Also, the highest COD concentration was found in case of CP1 (pond section where the high value of the ratio between the amount of administrated food and the rearing surface), while the lowest were observed in AC and PP2 (pond section where no feed was administrated). Also, significant difference regarding COD concentration were found between the two sampling points from the outlet channel (EC-PP and EC-PCP). The highest value registered for EC-PP is probably due to the position of the sampling point, at the end of the outlet channel (Figure 11).

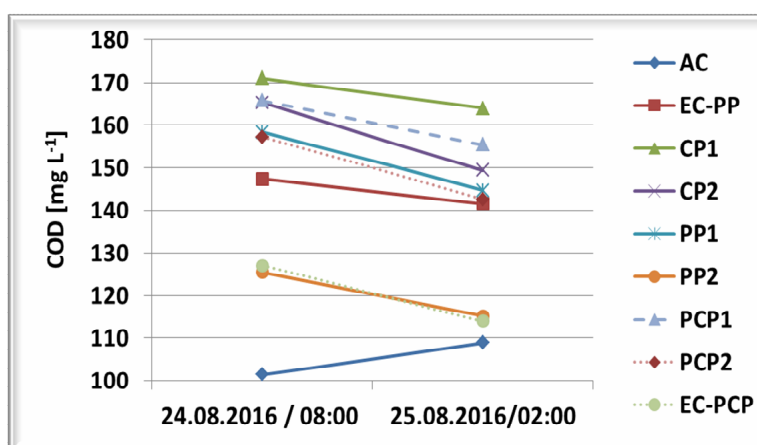


Figure 11. Water COD concentration during 24 hours kinetics, in PCP and CP-PP.

Regarding  $\text{BOD}_5$ , the maximum concentration were recorded during the dawn (AC –  $71.1 \text{ mg L}^{-1}$ ; CP1 –  $98.2 \text{ mg L}^{-1}$ ; CP2 –  $99.7 \text{ mg L}^{-1}$ ; PP1 –  $100.0 \text{ mg L}^{-1}$ ; PP2 –  $87.7 \text{ mg L}^{-1}$ ; PCP1 –  $93.4 \text{ mg L}^{-1}$ ; PCP2 –  $88.8 \text{ mg L}^{-1}$ ), while the minimum values were encountered during the night (25.08.2016 at 02:00), as follows: AC –  $47.7 \text{ mg L}^{-1}$ ; EC-PP –  $66.5 \text{ mg L}^{-1}$ ; CP1 –  $78.4 \text{ mg L}^{-1}$ ; CP2 –  $83.5 \text{ mg L}^{-1}$ ; PP1 –  $86.2 \text{ mg L}^{-1}$ ; PP2 –  $71.2 \text{ mg L}^{-1}$ ; PCP1 –  $77.7 \text{ mg L}^{-1}$ ; PCP2 –  $73.1 \text{ mg L}^{-1}$ ; EC-PCP –  $70.9 \text{ mg L}^{-1}$  (Figure 12). Also, the highest  $\text{BOD}_5$  concentration was found in case of CP2 and PP1 sampling points, while the lowest were observed in AC, EC-PP and PP2 (pond section where no feed was administrated). Also, significant statistical differences ( $p < 0.05$ ) regarding  $\text{BOD}_5$  concentration were found between the two sampling points from the outlet channel (EC-PP and EC-PCP).

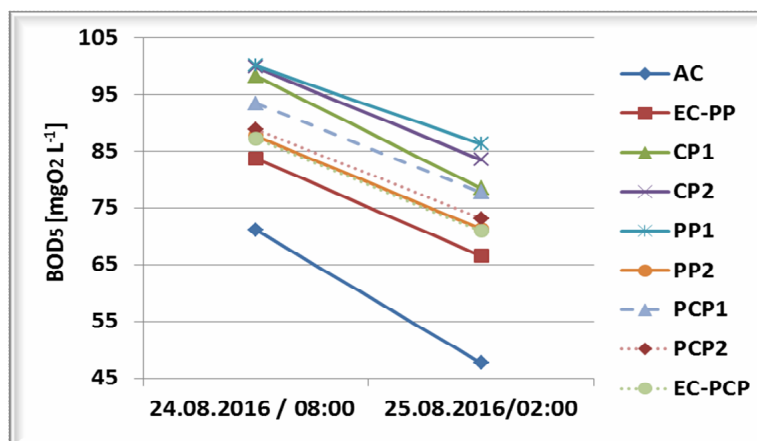


Figure 12. Water BOD<sub>5</sub> concentration during 24 hours kinetics, in PCP and CP-PP.

Therefore, it can be concluded that the highest concentration of organic matter is found in case of CP pond section, while the lowest one, in PP pond section. Also, the results indicates a low organic matter concentration in case of EC-PP, compared with EC-PCP. Those conclusions can be justified by the feeding regime applied for each of the pond section taken into discussion. The findings are confirmed also by the results registered in case of water transparency and mentioned above.

Cyanobacterial, total chlorophyll "a" concentration and also turbidity 24 hours kinetic were determined for each of nine the sampling points (Figures 13 to 15). As was stated by Kalaji et al (2016), the turbidity estimations are used to indicate the reliability of the chlorophyll "a" measurements. At high turbidity levels, the chlorophyll "a" measurements should be interpreted with care, since artefacts such as shading may occur, resulting in measurement errors (Kalaji et al 2016). The turbidity estimations of the AlgaeTorch are not accurate enough for the determination of turbidity as a water quality parameter (Kalaji et al 2016).

Therefore, the maximum concentration of cyanobacterial chlorophyll "a" were generally recorded during the midday (AC – 32.20 µg L<sup>-1</sup>; CP1 – 31.83 µg L<sup>-1</sup>; CP2 – 27.47 µg L<sup>-1</sup>; PP1 – 29.23 µg L<sup>-1</sup>; PP2 – 22.87 µg L<sup>-1</sup>; PCP1 – 37.77 µg L<sup>-1</sup>; PCP2 – 44.73 µg L<sup>-1</sup>; EC-PP – 71.80 µg L<sup>-1</sup>; EC-PCP – 86.80 µg L<sup>-1</sup>), while the minimum values were encountered during the night (25.08.2016 at 02:00), as follows: AC – 19.63 µg L<sup>-1</sup>; CP1 – 15.17 µg L<sup>-1</sup>; CP2 – 16.96 µg L<sup>-1</sup>; PP1 – 18.87 µg L<sup>-1</sup>; PP2 – 13.87 µg L<sup>-1</sup>; PCP1 – 30.07 µg L<sup>-1</sup>; PCP2 – 34.90 µg L<sup>-1</sup>; EC-PP – 33.57 µg L<sup>-1</sup>; EC-PCP – 58.70 µg L<sup>-1</sup> (Figure 13).

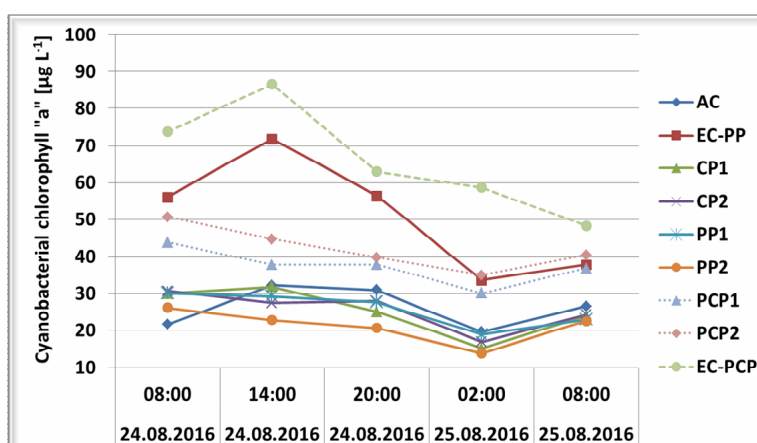


Figure 13. Cyanobacterial chlorophyll „a” concentration during 24 hours kinetics, in PCP and CP-PP.

However, it can be stated that the highest values of cyanobacterial chlorophyll "a" were recorded in case of the outlet channel (EC-PP and EC-PCP). Also, high cyanobacterial chlorophyll "a" values are recorded for PCP, compared with CP-PP experimental pond. Significant high values are also recorded in case of EC-PCP, compared with EC-PP. This fact confirms that there is a significant positive correlation between cyanobacterial

chlorophyll "a" kinetic and nitrogen and phosphorus kinetic. Also, a good positive correlation can be found by taking into consideration the BOD<sub>5</sub> and COD kinetic. Generally, it can be actually observed that cyanobacterial chlorophyll "a" values have a accentuated downward tendency in case of the sampling points situated on the outlet channel and a small downward tendency in case of PCP sampling points, while a relatively constant evolution during the day can be pointed out in case of CP-PP experimental pond sampling areas.

The maximum concentration of total chlorophyll "a" were recorded during the dawn, 24.08.2016 at 08:00 (AC – 186.30  $\mu\text{g L}^{-1}$ ; CP1 – 152.40  $\mu\text{g L}^{-1}$ ; CP2 – 161.30  $\mu\text{g L}^{-1}$ ; PP1 – 156.07  $\mu\text{g L}^{-1}$ ; PP2 – 145.63  $\mu\text{g L}^{-1}$ ; PCP1 – 164.50  $\mu\text{g L}^{-1}$ ; PCP2 – 182.93  $\mu\text{g L}^{-1}$ ; EC-PP – 191.60  $\mu\text{g L}^{-1}$ ; EC-PCP – 216.57  $\mu\text{g L}^{-1}$ ), while the minimum values were encountered during the night (25.08.2016 at 02:00), as follows: AC – 88.90  $\mu\text{g L}^{-1}$ ; CP1 – 108.4  $\mu\text{g L}^{-1}$ ; CP2 – 72.83  $\mu\text{g L}^{-1}$ ; PP1 – 73.23  $\mu\text{g L}^{-1}$ ; PP2 – 71.97  $\mu\text{g L}^{-1}$ ; PCP1 – 123.03  $\mu\text{g L}^{-1}$ ; PCP2 – 119.03  $\mu\text{g L}^{-1}$ ; EC-PP – 101.10  $\mu\text{g L}^{-1}$ ; EC-PCP – 116.10  $\mu\text{g L}^{-1}$  (Figure 14). Significant statistical differences ( $p < 0.05$ ) regarding total chlorophyll "a" values were reported in the first day dawn (24.08.2016 at 08:00), compared with the second day dawn (25.08.2016 at 08:00).

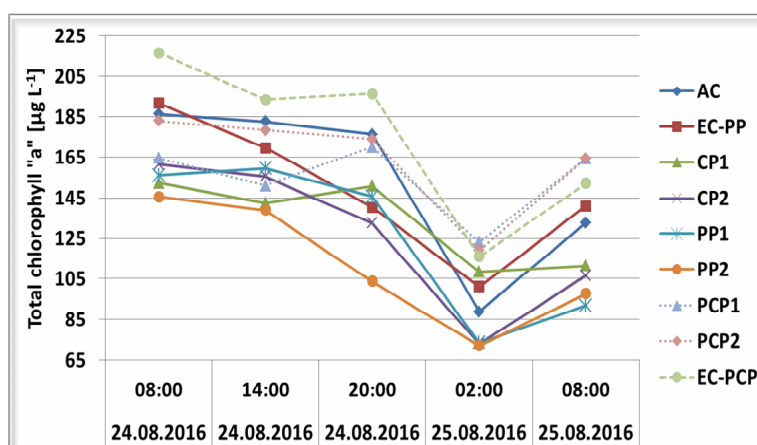


Figure 14. Total chlorophyll „a” concentration during 24 hours kinetics, in PCP and CP-PP.

Also, PP2 pond section, where no fish food was administrated, registered the lowest concentration of both cyanobacterial and total chlorophyll "a" (Figures 13 and 14), compared with PCP, where the highest concentrations were found, among pond sampling points.

In terms of turbidity, the following average values were recorded for each of the nine sampling points: AC – 20.85 FTU; CP1 – 18.01 FTU; CP2 – 12.62 FTU; PP1 – 12.29 FTU; PP2 – 10.87 FTU; PCP1 – 18.40 FTU; PCP2 – 20.83 FTU; EC-PP – 27.96 FTU; EC-PCP – 18.30 FTU (Figure 15).

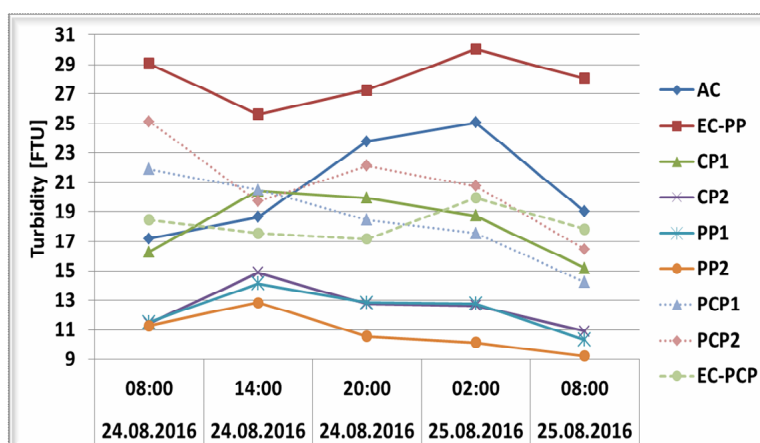


Figure 15. Turbidity during 24 hours kinetics, in PCP and CP-PP.

By analysing the turbidity values, it can be stated that the most reliable results in terms of both cyanobacterial and total chlorophyll "a" are in case of CP2, PP1 and PP2. Also, on the opposite part, less reliable cyanobacterial and total chlorophyll "a" results are encountered in case of EC-PP.

Rohilla (2008) tested three experimental treatments, with a control, as follows: control – no fish, pond A – *Labeo rohita*, *Catla catla*; pond B – *L. rohita*, *C. catla*, *C. idella*, and pond C – *L. rohita*, *C. catla*, *H. molitrix*, *C. idella*. He reported the higher chlorophyll "a" values throughout the duration of the study in case of ponds B and C, but pond A experienced levels that were below the control pond. The amount of algal biomass increased significantly from October to November in all of the ponds containing fish but not for the control pond. Rohilla (2008) also hypothesized that through direct consumption, fish would reduce the biomass of algae, but this increase in chlorophyll "a" was unexpected. Opuszyński (1979) and Voros et al (1997) stated that introduction of silver carp suppressed herbivorous zooplankton populations which consequently reduced grazing pressure on algae.

Dulić et al (2009), conducted a study from April to September in three earthen fish ponds (0.09 ha each), stocked with 400 carp yearling per pond, with average weight of 100 grams, in order to evaluate the seasonal dynamics of primary and secondary production in carp ponds. They obtained a chlorophyll "a" variation between 300-900  $\mu\text{g L}^{-1}$  during August.

Increases in chlorophyll "a" concentrations in the ponds after stocking grass carp in comparison to control pond were indicated by Demir & Kırkağaç (2005). In their study, grass carp was stocked at a rate of 2 fish/100  $\text{m}^2$  with an individual average biomass weight of  $135.7 \pm 17.2$  g and the chlorophyll "a" concentration was higher inside the cage than the outside due to clogging the net cage by algae. In August, they registered a chlorophyll "a" value of  $7.65 \pm 0.09$   $\mu\text{g L}^{-1}$  inside the cage and  $4.44 \pm 0.21$   $\mu\text{g L}^{-1}$  outside the cage.

Costa et al (2014) stated that chlorophyll "a" ranged between 8.7-344.0  $\mu\text{g L}^{-1}$ , with a mean of 104.4  $\mu\text{g L}^{-1}$ , in a study that took place on 30 fish ponds, in southeastern Brazil.

It was also stated that non-consumed fish ration remains in the system and leads to algal blooms, especially cyanobacteria, high chlorophyll "a" concentrations (Mercante et al 2004), high levels of turbidity and oxygen depletion (Simões et al 2008).

Saeed & Batran (2014) reported that chlorophyll "a" ranged between 43.07-170.98  $\mu\text{g L}^{-1}$  for *Oreochromis niloticus* monoculture ponds and 77.63-144.50  $\mu\text{g L}^{-1}$  for tilapia and catfish polyculture ponds.

Ghețu & Costin (2011) made in Iasi city, Romania, a chlorophyll "a" monitoring study and reported values of 41.05  $\mu\text{g L}^{-1}$  at Larga Jijia sampling station, 42.74  $\mu\text{g L}^{-1}$  at Vladeni Pond sampling station, 24.66  $\mu\text{g L}^{-1}$  at Halcenii Lake sampling station and 24.66  $\mu\text{g L}^{-1}$  at Miletin River sampling station.

Ferber et al (2004) made a study in order to identify the relation between cyanobacteria and atmospheric nitrogen. Therefore, they reported relatively low chlorophyll "a" values in May (5–9  $\mu\text{g L}^{-1}$ ), while blooms occurred in mid-June, from mid-July to mid-August, and in mid-September, yielding chlorophyll "a" concentrations to 60, 88–92 and 69  $\mu\text{g L}^{-1}$ .

Kohl & Nicklisch (1988) concluded that because of the structure of the light-harvesting pigment–protein complex, the specific chlorophyll "a" content is low in cyanophyta algal biomass.

Recent research (O'Neil et al 2012) suggests that eutrophication and climate change are two processes that may promote the proliferation and expansion of harmful cyanophyta algal blooms.

The temporal aspect of a cyanophyta algal blooms in a particular ecosystem depends on the extent to which different environmental factors influence bloom dynamics (Havens 2008). Cyanophyta algae dominance often occurs when water temperature rises above 20°C when there is depletion of dissolved inorganic N and free  $\text{CO}_2$  from the water.

**Conclusions.** This study confirms the direct relation between both cyanobacterial and total chlorophyll "a" and nitrogen and phosphorus 24 hours kinetic tendencies. Also, significant high cyanobacterial and total chlorophyll "a" concentrations during the day period, compared with night period are recorded only in case of classical cyprinides polyculture pond production system (PCP). The modern IMTA production system (CP-PP) has offered more stability in terms of 24 hours kinetic of cyanobacterial and total chlorophyll "a" concentration.

Therefore, the modern IMTA production system seems to be a good alternative for the classical cyprinides polyculture in terms of preventing eutrophication, although future studies must be made in order to see the long term evolution of both cyanobacterial and total chlorophyll "a" concentration in the tested experimental ponds production systems (PCP and CP-PP).

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