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The Effectiveness of the Use of Aquatic Plants (*Lemna perpusilla, Landoltia punctata* and *Azolla pinnata*) in the Phytoremediation Process of Catfish Aquaculture Wastewater

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Authors' contributions

This work was carried out in collaboration among all authors. Author Amyati designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ZH and DS managed the analyses of the study. Author HH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The purpose of this study was to determine aquatic plants (*Lemna perpusilla, Landoltila punctata* and *Azolla pinnata*) that have the most effective ability as phytoremediation agents for catfish cultivation wastewater.
Study Design: This research was conducted by an experimental method, with a Completely Randomized Design (CRD) consisting of three treatments and four replications.
Place and Duration of Study: This research is located at the Wet Laboratory (Ciparanje) of the Faculty of Fisheries and Marine Sciences, Padjadjaran University. Water quality testing is carried out at the Water Resources Laboratory of the Faculty of Fisheries and Marine Sciences. Research was carried out on 20 July-24 September 2019.

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Methodology: The test wastewater was collected from catfish pond culture and put into twelve aquarium with a volume of 8 litre. Physical and chemical parameters observed include BOD_5, dissolved oxygen, CO_2 acidity, temperature, nitrate, and phosphate.
Results: *Lemna perpusilla, Landoltia punctata* and *Azolla pinnata* were able to improve the quality of catfish culture wastewater such as increasing the concentration of dissolved oxygen (DO) and pH, and reducingBOD_5, CO_2, nitrate and phosphate. *Lemna perpusilla* was able to reduce the concentration of BOD_5, CO_2,carbondioxide, nitrate and phosphate from catfish cultivation wastewater by75%; 77.7%; 23.4%; 44.2%, respectively, during the phytoremediation process, and *Landoltia punctata* was able to reduce the concentrations same parameters by 52%; 68.3%; 17.8%; 18.3% and *Azolla pinnata* by 41%; 84%; 15.3% and 28.4%.
Conclusion: *Lemnaper pusilla*may improve the quality of catfish culture wastewater better than *Landoltia punctata* and *Azolla pinnata. Lemna perpusilla* can be recommended as phytoremediation agents catfish cultivation wastewater to remediate organic matter before being discharged directly into water bodies.

Keywords: Azolla pinnata; catfish; Landoltia punctata; Lemna perpusilla; phytoremediation.

1. INTRODUCTION

Catfish (*Clarias* sp) is one type of fish that is easily maintained, can grow quickly and has high economic value,. This potential encourages community interest in conducting cultivation activities. Aquaculture activities produce solid waste and liquid waste originating from feces, urine and fish food waste. The accumulation of waste can cause a decrease in water quality that affects the physiological processes, behavior, growth, and mortality of fish [1].

The amount of accumulation of aquaculture waste produced by each fish is different. Aquaculture waste in floating cages in the Cirata Reservoir has a water content of 92.5–92.4%, nitrogen 1.11-3.22% and phosphorus 0.43–0.93% [2] while catfish cultivation produces liquid nitrogen waste an average of 1.32%, nitrogen solid waste 6.23%, phosphorus liquid waste 2.64% and phosphorus solid waste 4.46%, and potassium liquid waste0.35 %,potassium solid waste 3.21%. As for the concentration of C-organic liquid waste, an average of 0.63% and an average solid waste of 21.67%, the value of the C / N ratio of the average solid waste is 6.71% [3].

The amount of waste generated from catfish farming can cause a decrease in the quality of aquaculture media. Decrease in the quality of aquaculture water, among others, decreased concentrations of dissolved oxygen (DO), increased turbidity of water and increased waste, especially organic nitrogen and phosphate [4]. A decline in aquaculture water quality can results in disruption to fish survival, therefore applications are needed that can improve the quality of aquaculture wastewater so that the survival of fish is more awake and does not pollute the surrounding environment.

The application of wastewater management technology has been developed with the aim of not polluting the surrounding waters. One of the wastewater management technologies is cultivation with phytoremediation. The advantages of phytoremediation are that it can work on organic and inorganic compounds, the process can be done in situ and ex situ, easy to apply and does not require high costs, not harmful to the environment, can reduce contaminants in large numbers and there is a synergy relationship between the environment and organisms [5], while the weakness of phytoremediation is that the process takes a long time, depending on climatic conditions, can cause the accumulation of heavy metals in plant tissues and biomass and can affect the balance of the food chain in the ecosystem [6]. The use of aquatic plants as a phytoremediation agent in wastewater management has now been widely carried out including research by Satya et al. [7], who used the *Eichhornia crassipes* plant in an effort to remove nutrients contained in wastewater in semi-recirculated fish ponds. *Ipomoea aquatica*, *Salvinia molesta* and *Eichhornia crassipes*are also studied in an effort to remove nutrients from the waste making tempeh [8].

Aquatic plants *Lemna perpusilla* and *Landoltia punctata* belong to the family of Lemnaceae that can function as phytoremediation agents, which have biological filters and ability to improve the quality of aquaculture wastewater. According to Landesman [9] the type of aquatic plant Lemnaceae (*Lemna perpusilla* and *Landoltia punctata*) can multiply the biomass in only 2 days under optimum conditions, so that the lemnaceae has a high enough growth power. The difference between *Lemna perpusilla* and *Landoltia punctata* is from the width and color of the lower leaves. *Landoltia punctata* has a red color on the underside of its leaves with a slimmer leaf size while *Lemna perpusilla* is green on the underside of the leaf and has a more circular leaf size.

Another aquatic plant that has good potential to be a phytoremediation agent is *Azolla pinnata*. *Azolla pinnata* is widely distributed in many areas. According to Arifin [10] *Azolla pinnata* has fast growth, is able to adapt to acidity, infertile soil and pollutants are quite high. Sadheghi *et al.*[11] revealed that *Azolla* sp growth is fast, 2-5 days to be able to double the biomass.Based on the description, *Lemna perpusilla, Landoltia punctata* and *Azolla pinnata* aquatic plants have the ability as phytoremediation agents. The aquatic plants are used in remediating catfish aquaculture wastewater. The purpose of this study was to determine aquatic plants (*Lemna perpusilla, Landoltia punctata* and *Azolla pinnata*) that have the most effective ability as phytoremediation agents for catfish cultivation waste water.

2. METHODOLOGY

The research was carried out on July-September 2019 at the Green House, Ciparanje Area, Padjadjaran University. Eight litre of waste water for each treatment aquaria were collected from the catfish ponds of the Ciparanje area, Padjadjaran University. Water plants consist of 3 types (*Lemna perpusilla, Landoltia punctata* and *Azolla pinnata*), were supplied from Limnology Research Center. The plant weight used for each treatment was 20 g. The study was conducted for 14 days by measuring the concentration of BOD_5, Nitrate, Phosphate and carbondioxide. Chemical analysis was carried out at the Laboratory of Aquatic Resources, Faculty of Fisheries and Marine Sciences, Padjadjaran University carried out in September 2019. The method used in this study was an experimental method with Completely Randomized Design (CRD) consisting of three treatments with four replications each.

2.1 Research Stages

The steps taken during the phytoremediation process were:

1. 12 aquariawere cleaned and given a code:
   LM = *Lemna perpusilla*
   LD = *Landoltia punctata*
   AP = *Azoll apinnata*
2. Aquatic plants (*Lemna perpusilla, Landoltia punctata* and *Azolla pinnata*) were acclimatized for two days.
3. Aquatic plants wereput into an aquarium containing 8 L catfish cultivation wastewater.
4. The measurement of nitrate and phosphate concentration uses The Spectrophotometric Method and BOD_5 concentration uses The Winkler Method.

2.2 Research Parameters

Observation of DO, pH, temperature and CO_2 wascarried out every day, while analysis of BOD_5, nitrate, phosphate wasdone every 7 days. The method used are presented in Table 1.

2.3 Analysis of Data

This research used quantitative descriptive analysis. Decreased concentrations of BOD_5, nitrate, phosphate and CO_2 were analyzed using ANOVA with the F test (p = 0.05). Differences between treatments were analyzed using Duncan's Multiple Range Test (DMRT) (p = 0.05). Analysis of the calculation of the reduction rate of BOD_5, phosphate, nitrate and CO_2 based on calculations from [11] the formula used is:

$$P = \left(\sqrt[t]{\frac{Ct}{Co}} - 1 \right) \times 100\ \%$$

Table 1. Physical and chemical parameters of water

	Parameters	Unit	Method	Analysis Tools
Physical Parameters	Temperature	^0C	-	Thermometer
Chemical Parameters	BOD_5	mg/L	Wingkler	Wingkler Bottle
	DO	mg/L	Potentiometric	DO Meter
	Nitrate	mg/L	Spektrofotometric	Spektofotometer
	Phosphate	mg/L	Spektrofotometric	Spektofotometer
	CO_2	mg/L	Titration	Erlenmeyer Glass
	pH	-	Potentiometric	pH Meter

Information:

P= Rate of decline BOD_5, phosphate, nitrate and CO_2 (%)
Ct= Water concentration after phytoremediation (mg/L)
Co= Water concentration before phytoremediation (mg/L)
t= Trial time (days)

3. RESULTS AND DISCUSSION

3.1 Water Quality Parameters

3.1.1 Biochemical Oxygen $Demand_5$ (BOD_5)

The treatment of *Lemna perpusilla, Landoltia punctata* and *Azolla pinnata*, during the phytoremediation process, can reduced the BOD_5 concentration respectively by 75%, 52%, 41% (Fig. 1). The difference in BOD_5 decrease of *Lemnaperpusilla, Landoltia punctata* and *Azolla pinnata* might have influenced by the root characteristics of each aquatic plant. The three aquatic plants have different root characteristics. *Lemna perpusilla* has a longer root network with more amount than *Landoltia punctata* and *Azolla pinnata* that helped *Lemna perpusilla* to be superior in reducing organic matter.The high microbes in the roots will affect the degradation activity of organic matter. The length of *Lemna minor* roots is around 0.5-15 cm [12,13]. Lee *et al*. [14] stated that the root length of *Landoltia punctata* is 0.81-3.16 cm and has the number of root hairs which is 7-12 [15]. *Azolla pinnata* has roots of 1-5 cm long and has 3-6 root hairs [16].

Decreased BOD_5 concentration of catfish aquaculture wastewater by *Lemna perpusilla, Landoltia punctata* and *Azolla pinnata*, during the phytoremediation process was able to increase the concentration of dissolved oxygen which is the result of photosynthesis of aquatic plants and phytoplankton. Oxygen from photosynthesis is used by bacteria to decompose organic matter in water. Organic matter is degraded by microorganisms that grow on the surface of the media and plant roots [17].

3.1.2 Dissolved Oxygen (DO)

Phytoremediation of catfish cultivation waste using *Lemna perpusilla, Landoltia punctata* and *Azolla pinnata* increased the concentration of dissolved oxygen due to the photosynthesis process of aquatic plants that produce oxygen. Dissolved oxygen (DO) is used by aquatic organisms for the process of respiration and decomposition oforganic matter so that an increase in dissolved oxygen supports the decomposition of organic matter by the organism. Phytoremediation of aquatic plants could increase dissolved oxygen because of the photosynthesis process that produces oxygen [18]. (Fig. 2).

One of the factors that influences the concentration of dissolved oxygen (DO) is temperature. Temperature during phytore mediation research continued to allow organisms to carry out metabolic processes and respiration. The increase in dissolved oxygen in treatment aquatia of *Lemna perpusilla, Landoltia punctata* and *Azolla pinnata* in sequence on the second day was recorded7.10 mg/L; 5.93 mg/L and 6.9 mg/L that have met the SNI [19]which is a minimum of 3 mg/L.

3.1.3 Carbondioxide (CO_2)

Phytoremediation of catfish cultivation waste using *Lemna perpusilla, Landoltia punctate* and *Azolla pinnata* reduced carbon dioxide concentrations. Carbondioxide ($CO_{2)}$ absorption by *Lemn aperpusilla, Landoltia punctata* and *Azolla pinnata* during the phytoremediation process took place in sequence, namely 77.7%; 68.3%; 84% (Fig. 3). Carbondioxide (CO_2) absorption of *Azolla pinnata* was quite high compared to that of *Lemna perpusilla* and *Landoltia punctata*, this was influenced by the biomass of the three plants. The average *Azolla pinnata* biomass at week 1 and 2 was the highest so the CO_2 demand was in line with the high biomass because it was used for photosynthesis. The presence of carbon dioxide in water is needed by plants for photosynthesis. Carbondioxide (CO_2) concentrations are used in the photosynthesis process which triggers plant development and triggers the emergence of new shoots [20].

The temperature during the research ranged from 27.4 to 30^0C which is the optimum temperature for the growth of *Azolla* sp. The appropriate temperature for growth of *Azolla microphylla* ranges from 20-35^0C [21]. The optimum temperature of Lemna is around 6-33^0C [22].The temperature range of *Lemna* sp is lower than *Azolla* sp so that in this study *Azolla* sp grows more optimally.

3.1.4 Acidity

The pH value of catfish culture wastewater before phytoremediation test was 6.26. Wastewater generally has an acidic pH [23].The initial pH value of catfish aquaculture wastewater does not meet the quality standards based on the SNI [19] which is 6.5-8.(Fig. 4)

The average pH during the phytoremediation process was volatile but tends to increase towards neutral pH. Phytoremediation could increase the pH of wastewater to neutral pH[24].An increase in pH indicates the occurrence of the nitrification process. The increase in pH during the research took place meeting the SNI [19]which amounted to 6.5-8.

3.1.5 Temperature

The average temperature during the research showed fluctuating figures. The lowest temperature value was27.4^{0}C and the highest was30^{0}C (Fig. 5).Factors affecting water temperature include the presence of shade (trees, buildings, plants), air temperature, weather and climate [25].

The temperature during the study was relatively high, this is because the research was located in a greenhouse which in principle had been designed so that the optimum room temperature could be maintained. The optimum water temperature for aquatic plants is in the range of 26.69-28.34^{0}C [26]. The water temperature in the test media during the research fulfilled the SNI [19]which was 25-30^{0}C.

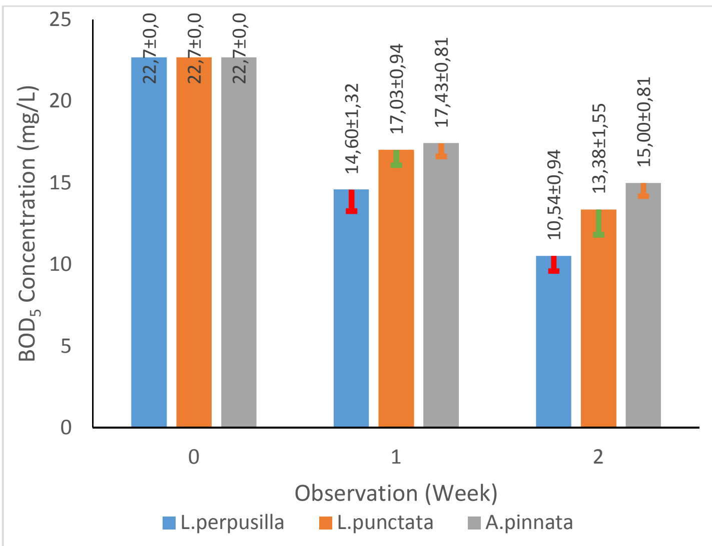

Fig. 1. The BOD_5 values during the observation

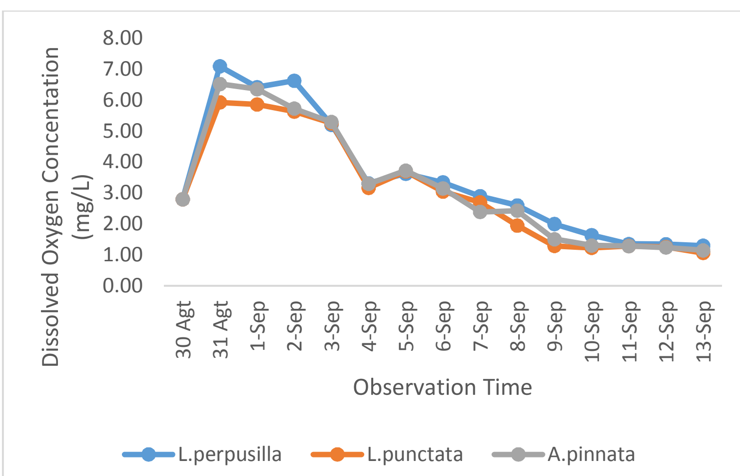

Fig. 2. The daily dissolved oxygen value

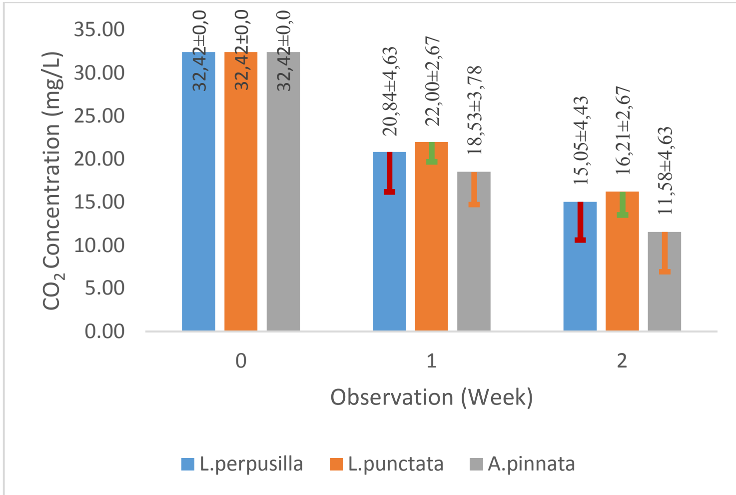

Fig. 3. The CO_2 values during the observation

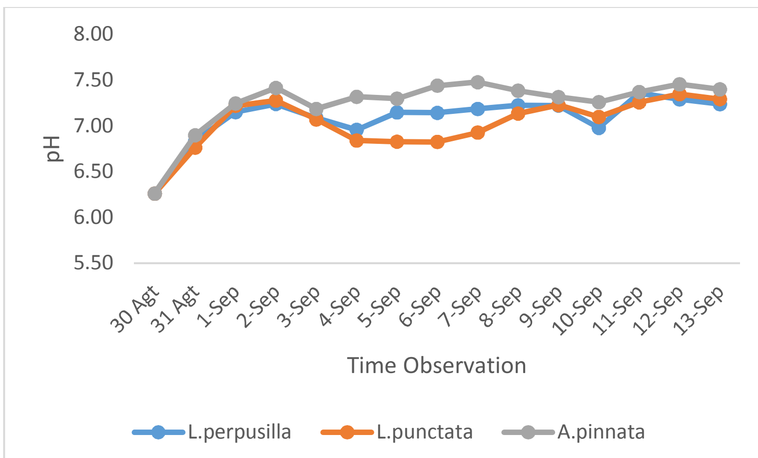

Fig. 4. The daily pH value

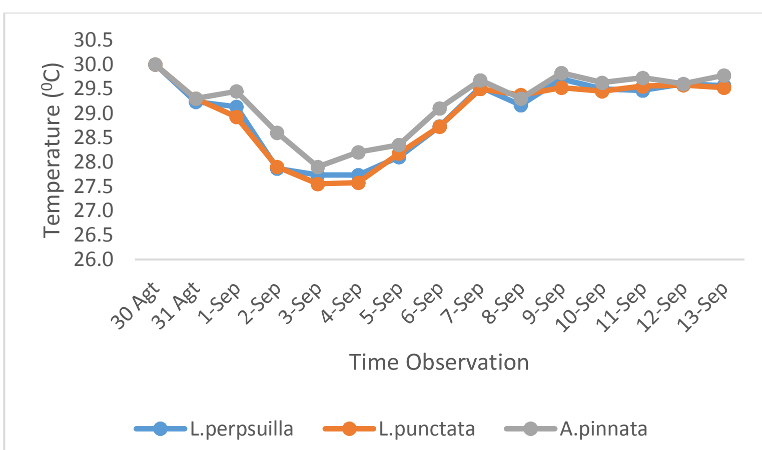

Fig. 5. The daily temperature value

3.2 Nitrate Concentration

Nitrate is a form of nitrogen in the waters that functions as nutrients for plants for the growth process. Decrease in nitrate concentration of wastewater occurs because it is absorbed by aquatic plants so that aquatic plants experience an increase in biomass. Plants absorb nitrates for cell growth through their roots [27]. Concentration of nitrate in water are influenced by several parameters such as dissolved oxygen and organic matter content [28] Dissolved oxygen concentration is low, the activity of microorganisms in decomposting of organic matter and nitrification process will be affected. Increasing the concentration of dissolved oxygen (DO) in the first week supports the activity of microorganisms in decompossed organic matter and the process of nitrification.

Nitrate is a source of nitrogen that can be utilized directly by aquatic plants, because nitrates are very soluble in water. Nitrates are formed from ammonia which is oxidized to nitrite with the help of the bacterium Nitrosomonas, then nitrite is oxidized to nitrate with the help of the bacterium Nitro bacter [29]. Factors that cause *Lemna perpusilla* to absorb nitrate higher than *Azolla pinnata* and *Landoltia punctata* that mught be *Lemna perpusilla* has more and longer root hairs so that the absorption range of nitrate waswider and optimal. Decreased nitrate concentrations by *L. perpsilla, Landoltia punctata* and *Azollapinnata* during the phytoremediation process took place sequentially at 23.4%; 17.8%; 15.3% (Fig. 6).

3.3 Removal of Phosphate Concentration

Phytoremediation using *Lemna perpusilla, Landoltia punctata* and *Azolla pinnata*can reduce the concentration of test media phosphate. Decrease of phosphate by *Lemna perpusilla, L. puncatata* and *Azolla pinnata* respectively was 44.2%; 18.3%; 28.4% (Fig. 7). *Lemna perpusilla* reduced phosphate higher than *L. puncatata* and *Azolla pinnata* This was influenced by differences in the size of the leaves and roots which are the parts that absorb nutrients. *Lemna perpusilla* has a greater leaf width than *Landoltia punctata*and *Azolla pinnata*. *Lemna perpusilla* has a leaf diameter of 6-8 mm [30] while *Landoltia punctata* has a leaf diameter of 1-5 mm [31]meanwhile *Azolla pinnata* has a small leaf shape about 1–2 mm in diameter with overlapping leaf positions [32]. The broader leaf identifies the high chlorophyll so that more phosphorus is needed to store and transfer energy in the form of ATP and ADP [28].

The absorption of phosphate in the second week wasnot as high as the first week, this wasinfluenced by the decrease in water plant biomass. Decreased biomass of test water plants causes a decrease in phosphate absorption. Phosphate uptake is in line with the increase in plant biomass [33].

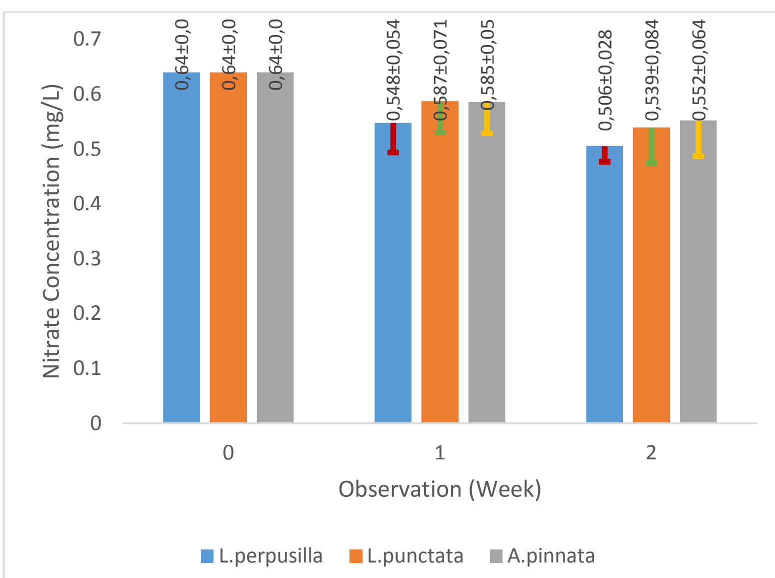

Fig. 6. Average nitrate concentration during research

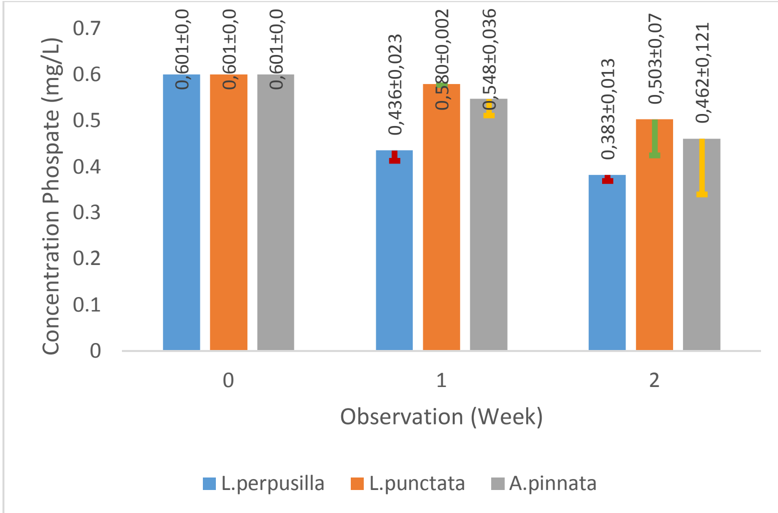

Fig. 7. Average phosphate concentration during research

4. CONCLUSION

Based on the overall results of the study it can be concluded:

- *Lemna perpusilla* can improve the quality of catfish culture wastewater better than *Landoltia punctata* and *Azolla pinnata.*
- *Lemna perpusilla* is able to reduce the concentration of BOD_5, CO_2, nitrate and phosphate from catfish cultivation waste during the phytoremediation process, which took place 75%; 77.7%; 23.4%; 44.2% and *Landoltia punctata* is able to reduce BOD_5, CO_2, nitrate and phosphate concentrations respectivelyby 52%; 68.3%; 17.8%; 18.3%, while *Azolla pinnata* is able to reduce BOD_5, CO_2, nitrate and phosphate concentrations respectively by 41%; 84%; 15.3% and 28.4%.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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