



EFFECTIVENESS OF NITRATE AND PHOSPATE UPTAKE BY PLANT'S OF *Lemna perpusilla* AND *Landoltia punctata*

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KeyWords

Phytoremediation, Lemnaceae plant's, Nitrate, Phospate, Growth.

ABSTRACT

The aim of this research was to determine the rate of nutrient absorption of $N-NO_3$ and $P-PO_4$ and growth from the two plants studied. The research was held in July - August 2018. The research was carried out and divided into two, namely *ex situ* and *in situ*. Sampling, water quality testing, and observation of lemnaceae plants were carried out *in situ* at LIPI Limnologi, Cibinong, Bogor. Furthermore, the analysis of nitrate, phosphate, TSS, and VSS was carried out *ex situ* in the Testing Laboratory of the Limnology Research Center, Indonesian Institute of Sciences, Cibinong, Bogor, West Java. The method used was an experimental method. The results showed that *L. perpusilla* is more effective at absorbing nitrate and phosphate by 0,79 mg / L / day and 0,37 mg / L / day compared to *L. punctata* plants absorbing nitrate and phosphate by 0,71 mg / L / day and 0,35 mg / L / day.

INTRODUCTION

Activities in the field of fisheries, specifically aquaculture in the process not only involves fish as a subject, but natural or artificial involve other organisms that are mutually influence. According to Islami et al. (2017), Factors influencing water quality in aquaculture activities include: water temperature, dissolved oxygen (DO), acidity (pH), alkalinity, ammonia, nitrite, nitrate, carbon dioxide, and other dissolved organic matter. Feed is one of the factors that affect water quality. Remaining metabolism and non-inedible feed residues either dissolved or deposited in aquaculture ponds can affect the chemical and physical parameters of water quality in water in aquaculture ponds. The accumulation of food waste and excretion can produce toxic compounds. The high concentration of toxic metabolites caused fish to get stress, decreased appetite, susceptibility to disease, impaired growth, and increased mortality (Sugama 2002).

Phytoremediation is defined as washing pollutants mediated by plants, including trees, grasses and aquatic plants. Washing can mean the destruction, inactivation or immobilization of pollutants into harmless forms (Chaney *et al.* 1995) . In general, plants will absorb nutrients that dissolve in water and from the soil through their roots. All plants have the ability to absorb nutrients accumulated by plants into energy to grow (Wolverton and Mcknown 1975).

Plant Lemnaceae (*Lemna perpusilla* and *Landoltia punctata*) are aquatic plants that can theoretically absorb water and the elements contained in it so that it can be used as a media for testing nutrient elimination capacity. The choice of *L. perpusilla* and *L. punctata* as plants in this research because these two aquatic plants have root systems and leaves that are able to absorb nutrients well. Leaf diameter of *L. perpusilla* and *L. punctata* plants ranges from 1-5 mm (Cheng *et. al* 2002). Difference between *L. perpusilla* and *L. punctata* is from the color of the lower part of the leaf and the width of the leaf. Color on the bottom of the leaves of plants *L. punctata* is red and the width of the leaves is slimmer, whereas in *L. perpusilla* is green and more circular (Crishmadha and Mardiaty 2011). The purpose of this study was to determine the rate of absorption of nutrients $N-NO_3$ and $P-PO_4$ and the growth of the two plants studied.

METHODS

The experiment was conducted in July to August 2018. Research was conducted at the Testing Laboratory of the Limnologi Research Center, Indonesian Institute of Sciences, Cibinong, Bogor, West Java.

The research method used is the experimental method with 2 treatments 10 repetitions. Plant samples *L. perpusilla* were taken from catfish ponds and *L. punctata* plants taken from carp ponds as much as 25 g for one study container.

The research preparation phase begins by preparing 20 aquariums with a volume of 20 L as a media storage container. Tools and materials are prepared. Then, liquid fertilizer is used as the experimental medium , taken with a volume of 200 ml then diluted with water to 40L. The diluted fertilizer is divided into 20 experimental aquarium containers and each container is filled with 2 L, from which the container is added and diluted again with water to 20 L. *L. perpusilla* and *L. punctata* taken as much as 25 g and acclimatized before the research is carried out. The final stage of preparation, water samples of research media were analyzed first before the research was conducted. The parameters to be analyzed are pH, temperature, DO, conductivity, TDS, turbidity, TSS, VSS, nitrate and phosphate.

The next research stage is, 600 ml of liquid fertilizer media treated with phytoremediation is carried out to analyze the parameters of TSS, VSS, Nitrate and Phosphate at the beginning of the research and repeat every 7 days for 21 days. Then, initial spread of plant biomass weighing 25 g is carried out. *L. perpusilla* and *L. the punctata* is harvested every week after the phytoremediation process and is put back as much as 25 g into the media container. Measurement of water quality parameters was carried out for 28 days. After the liquid fertilizer media water was given phytoremediation treatment for one month, *L. perpusilla* and *L. punctata* is harvested in total.

Analysis of Rate of Decrease Nitrate and Phosphate

Determining the rate of decrease in phosphate and nitrate concentrations can be used formula as follows:

$$\mu = \left(\sqrt[t]{\frac{C_t}{C_o}} - 1 \right) \times 100\%$$

Description:

μ = rate of decrease in phosphate / nitrate (%)
 C_t = Final concentration (g)
 C_o = Initial concentration (g)
 t = Trial Time duration (days)

Analysis of Rate Absorption Nitrate and Phosphate

Chrishmadha and Mardiaty (2011), to determine the rate of absorption of phosphate and nitrate concentrations can be used formula as follows:

$$P = \frac{C_t - C_o}{A \cdot t}$$

Description:

P = Absorption Rate (g / m² / day)
 C_t = Initial Concentration (g)
 C_o = Initial Concentration (g)
 A = Media Container Area (m²)
 T = Trial time duration (days)

RESULT AND DISCUSSION

Nitrate

Nitrate is produced from the oxidation process of nitrogen compounds in the waters (Effendie 2003). According to Hendrawati (2007), the use of nitrogen is mostly by plants in the form of nitrates. Based on the results of the research that has been carried out there is a decrease in nitrate concentration which can be seen in Figure 1.

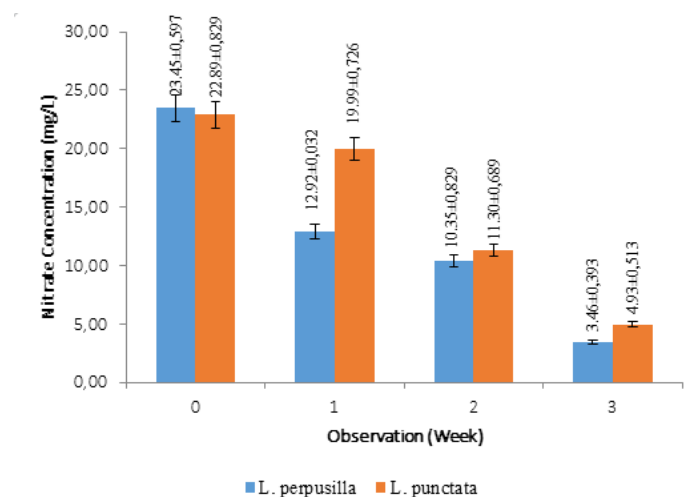


Figure 1. Nitrate concentration during research

Based on figure 1. The concentration of nitrate in liquid fertilizer media at the beginning of the study ranged from 22.89 ± 0.829-23.45 ± 0.597 mg / L. Decreased nitrate concentration began to decline at first week. According to Effendi (2003), aquatic plants can reduce nitrate concentration but absorption is not as fast as ammonia concentration.

The value of nitrate concentration decreased for the second week and the third week has a value that is not too far away. This is because, at the 2nd and 3rd week of ammonium which is in the growing medium, it has undergone a nitrification process (Effendie 2003), so that to meet the need to grow *L. punctata* absorbs nitrates for cell growth through its roots.

Effendi (2003), plant age also affects the ability to reduce NO_3 compounds. At the age of the plant is still young to reach a certain size, its ability to reduce the burden of nitrite, nitrate, ammonia and phosphate is higher, but after reaching the size (certain wet weight), its ability decreases. This can be understood because during the growth period, a living creature usually needs nutrients in large quantities, but after reaching the adult size, the need is reduced. The rate of absorption and elimination of t-days is presented in Table 1.

Table 1. Absorption and Elimination of Nitrate per day during research

| | Nitrate Absorption rate/day (%) | Nitrate Elimination/day (mg/L/day) |
|----------------------|------------------------------------|---------------------------------------|
| <i>L. perpusilla</i> | -8,71 | -0,79 |
| <i>L. punctata</i> | -7,05 | -0,71 |

Based on the results of calculations from table 1. Nitrate concentration data obtained a value of the specific absorption rate of nitrate per day. The *L. perpusilla* plant is more effective in absorbing nitrate with a percentage of absorption of 8.71% per day with a nitrate elimination rate of 0.79 mg / L per day, whereas in *L. punctata* plants it is 7.05% per day with a nitrate elimination rate of 0.71 mg / L per day. The minus value on the numbers indicates that plants carry out absorption and elimination of nutrients. Given the *L. perpusilla* plant has a higher growth rate compared to *L. punctata*, nutrient absorption capacity is certainly different. This is evidenced by the absorption of nutrients absorbed by *L. perpusilla* higher when compared to *L. punctata*. The leaf size of the two plants can be used as a reference, where *L. perpusilla* has a broad leaf shape than the *L. punctata* plant (Ozengin & Elmachi 2007), so *L. perpusilla* is able to absorb nutrients superior to the growth process.

Percentage of nitrate absorption by *L. perpusilla* for 21 days at 8.71 % per day with the final result of nitrate concentration so low at the final test that is equal to 3.46 ± 0.393 mg / L. Phytoremediation research of *L. perpusilla* plant was also conducted by Chrismadha and Mardiaty (2011), suggesting that *L. perpusilla* has a N- NO_3 absorption capacity of 3.9 g / m^2 days in media taken from Saguling Reservoir water with nitrate absorption capacity of 74.05%.

Percentage of nitrate absorption by *L. punctata* for 21 days amounting to 7.5 % per day with the final result of nitrate concentration so low in the final test that is equal to 4.93 ± 0.513 mg / L. Cheng *et al* (2012), stated that *L. punctata* plants had nitrate absorbing capacity of 2.03 g / m^2 days in media taken from pig slaughter wastewater with N- NO_3 absorption rate of 54.25 %.

The final nitrate concentration from the research of the two test plants was 3.46 ± 0.393 mg / L and 4.93 ± 0.513 mg / L. This value is below the quality standard of nitrate concentration in water which is 10 mg / L, based on Government Regulation Number 82 of 2001. This indicates that the two plants are effective in improving the quality of the waters (phytoremediation) of nitrogen, especially nitrates in the waters.

Phosphate

Phosphorus (P) is said to be one of the keys to life, because its function is very central in the life process. The function of P in plants is to store and transfer energy in the form of ADP and ATP. Energy is obtained from photosynthesis and metabolism of carbohydrates stored in phosphate mixtures for use in growth and production processes. The process will not take place in the absence of P elements. The amount of phosphorus in plants is smaller than Nitrogen and Potassium (Effendie 2003). Based on the results of the research that has been carried out there is a decrease in nitrate concentration which can be seen in Figure 2.

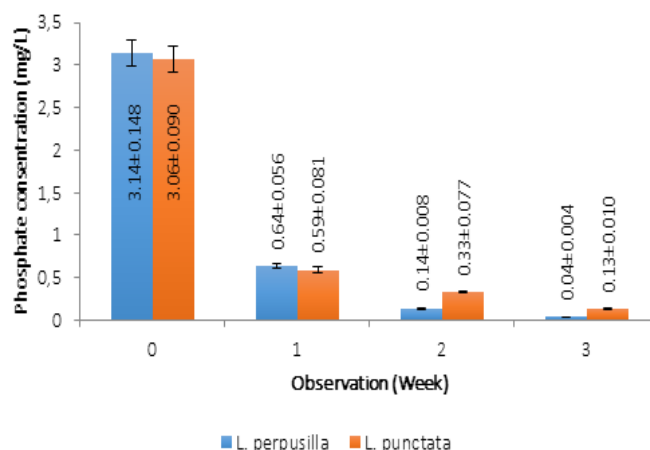


Figure 2. Phosphate concentration during research

Figure 2. The use values based phosphate concentration of media research at the beginning of the week for *L. perpusilla* of $0.148 \pm 14 \text{ mg / L}$ and for *L. punctata* plants amounted to $3.06 \pm 0.090 \text{ mg / L}$. Compared with the nitrate concentration data in Figure 1 in the initial week, the value of phosphate concentration is smaller. This is because the plant needs to be an element of P is not as much an element of N and K (Effendie 2003).

The value of phosphate concentration at first week in *L. perpusilla* and *L. punctata* plants decreased from the previous value of $0.64 \pm 0.056 \text{ mg / L}$ and $0.59 \pm 0.081 \text{ mg / L}$. The decrease in phosphate concentration is in line with increasing plant biomass (Chaney *et. Al.* 1995). Decrease in phosphate concentration occurred at week 2 of 0.14 ± 0.008 and $0.33 \pm 0.077 \text{ mg / L}$, and at week 3 of 0.04 ± 0.004 and 0.13 ± 0.001 . The reduction in phosphate in the second and third weeks was not as drastic as the first week, because plants had adapted it to culture media.

The decrease in phosphate concentration in the two plants was not significantly different at first week, because the initial phosphate concentration values were not much different. The difference in absorption of both plants was seen in the 2nd week where *L. perpusilla* was able to reduce phosphate concentration more than *L. punctata*. This is because *L. perpusilla* plants have the ability to multiply twice as much as ordinary plants (Landesman 2005). The width of the leaves from *L. perpusilla* is larger than *L. punctata*, so that more nutrients are needed (Ozengin and Elmachi 2007). Absorption and Elimination of phosphate per day are presented in Table 2.

Table 2. Absorption and Elimination of Phosphate per day during research

| | Phosphate Absorption rate/day (%) | Phosphate Elimination/day (mg/L/day) |
|----------------------|--------------------------------------|---|
| <i>L. perpusilla</i> | -18,76 | -0,369 |
| <i>L. punctata</i> | -13,96 | -0,349 |

Calculation results from the data in table 2. Phosphate concentration obtained values from the specific absorption rate of phosphate per day. The *L. perpusilla* plant is superior in absorbing phosphate with the absorption rate of 18.76% per day with a phosphate elimination rate of 0.369 mg / L per day, whereas in *L. punctata* plants it is 13.96% per day with a nitrate elimination rate of 0.349 mg / L per day. This is due to the width of the leaves of the plant *L. perpusilla* wider than the *L. punctata* plant (Les and Crawford 1999), the large leaf width indicates the amount of chlorophyll content, so relatively more phosphorus is needed to store and transfer energy in the form ATP and ADP (Effendie 2003).

Percentage of phosphate absorption by *L. perpusilla* for 21 days at 18.76% per day with the result that the phosphate concentration was $0.04 \pm 0.004 \text{ mg / L}$. Chrismadha and Mardiaty (2011), suggest that *L. perpusilla* has P-PO₄ absorption capacity of $6.7 \text{ g / m}^2 \text{ / day}$ in media taken from Saguling Reservoir water with a P-PO₄ absorption rate of 73.36%.

Percentage of phosphate absorption by *L. punctata* for 21 days equal to 13.96% per day with the final result of phosphate concentration which is so low in the final test which is equal to 0.13 ± 0.01 mg / L. Cheng *et al* (2012), stated that *L. punctata* plants had P-PO₄ absorption capacity of 0.18 g / m² days in media taken from pig slaughter wastewater with a rate of absorption of P-PO₄ of 34.22%.

The final phosphate concentration value of *ris et* both plants was 0.04 ± 0.004 mg / L and 0.13 ± 0.01 mg / L. This value is already below the quality standard of phosphate constellation in water, which is 0.2 mg / L, based on Government Regulation Number 82 of 2001. This indicates that the two plants are effective in improving water quality (phytoremediation), especially phosphate in the waters.

Water quality

pH

The optimum pH value that supports the nitrification process is pH 8-9, at pH <6 the reaction will stop. The nitrification process is carried out by a group of nitrifying bacteria and the process occurs in aerobic conditions (Effendi 2003). The pH value during the research is presented in Figure 3.

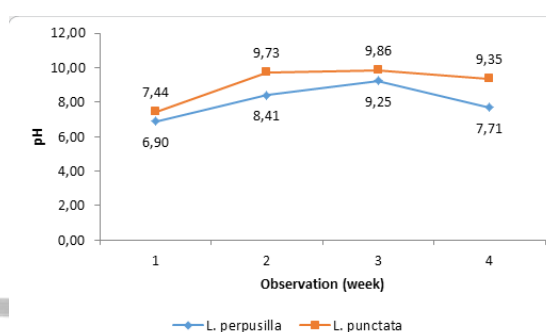


Figure 3. pH value during research

Figure 3, the increase in pH value occurs in the second week. The pH of the plant *L. perpusilla* was 9.25 and in *L. punctata* plants amounted to 9.86. The decrease in pH value took place in the third week for *L. perpusilla* plants at 7.71 and *L. punctata* of 9.35. Cardova (2008), the process of decomposition of organic matter will produce carbon dioxide which if it reacts with water and in which there is a mineral it will make pH become high. *L. perpusilla* and *L. punctata* plants absorb CO₂ content in photosynthesis through roots and leaves in the waters and convert it into simple sugars (glucose), so the pH value in this research is not too high. The reaction that converts carbon dioxide to glucose is called a dark reaction in the Calvin cycle.

Willoughby 1978, basically the presence of carbon dioxide in water in the form of carbon dioxide gas (CO₂), bicarbonate ions (HCO₃⁻), carbonate ions (CO₃²⁻), and carbonic acid (H₂CO₃). The proportion of the four carbons is related to the pH value, shown in Figure 4.

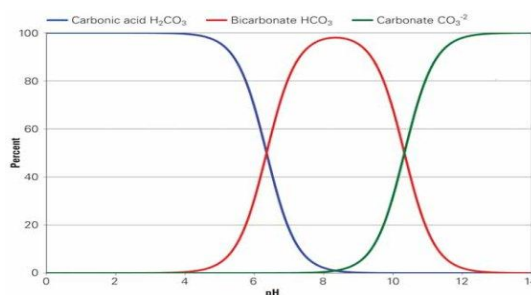


Figure 4. The relationship between the percentage of CO₂ and pH
(Source: Effendie 2003)

Figure 4 shows that when the pH range is at value 8, the percentage of carbon dioxide is close to 0%. The pH range of research from the first week to the end ranges from 6.9 -9.86. This indicates that the percentage of carbon dioxide in the water is absorbed well by plants in the process of photosynthesis.

The pH value will also affect the concentration of dissolved oxygen in it, if the pH value is high then dissolved oxygen will increase, the opposite occurs in an atmosphere of low pH (high acidity) (Kordi and Tancung 2010).

Dissolved Oxygen

Oxygen reduction occurs in the third and fourth week. Dissolved oxygen concentrations that drop significantly in a waters show the decomposition of organic substances and produce foul-smelling gases and endanger organisms (Simanjuntak 2007). These organic substances can come from dead plants. Dissolved oxygen concentration is shown in Figure 5.

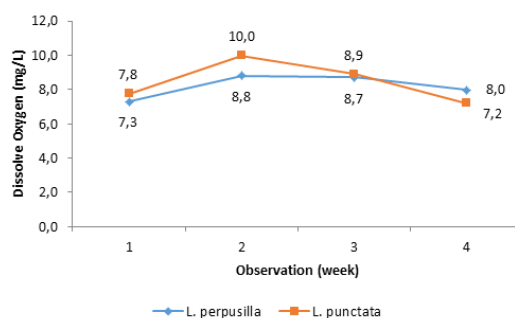


Figure 5. Oxygen dissolved concentration during the research

Figure 5, dissolved oxygen concentration in the first week ranged from 7.3-7.8 mg / L. The concentration increased in the second week. Water plants effectively increase the concentration of oxygen in water through photosynthesis. Carbon dioxide in photosynthesis is absorbed and oxygen is released into the water. The oxygen concentration of *L. punctata* plant has a higher value in the second week because leaf cover in the media does not match the *L. perpusilla* plant, so it does not receive oxygen from the diffusion with the atmosphere. According to Effendi (2003) the source of dissolved oxygen can come from the diffusion of oxygen contained in the atmosphere and photosynthetic activity by aquatic plants. Solar energy is absorbed by chlorophyll and used to decompose water molecules, form oxygen gas and reduce NADP molecules to NADPH.

Temperature

The value of temperature has decreased in the second and third weeks. The temperature of the two plants is different, where the temperature of the water medium in *L. perpusilla* plants is higher because leaf cover in these plants is more dense. The measured temperature range is still good for the growth of the two plants, where the lemnaeae plant has a tolerance of life at a temperature range of 6-33 °C (Leng *et al.* 1994). Air temperature is presented in Figure 6.

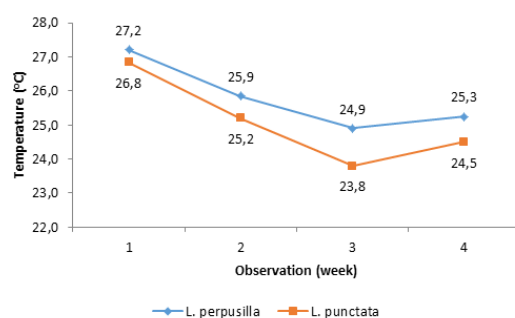


Figure 6. Temperature value during research.

Turbidity

The value of turbidity continues to increase from the first week to the last. The biditas tour value is shown in Figure 7 .

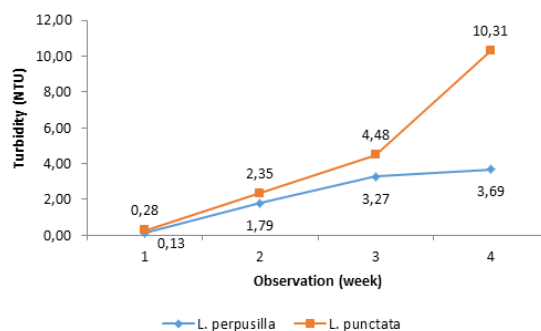


Figure 7. Turbidity value during research

The third week of nutrient research decreased (Figures 1 and 2), causing many plants to die in the fourth week. Decomposition of dead plant detritus can cause disruption of brightness from the conditions of the research media. Turbidity of *L. punctata* plants increased significantly in the fourth week because these plants could not resist the environmental conditions that were minimal in nutrients or close to zero.

TSS and VSS

According to PP RI Number 82 In 2001, the value of the residual suspended for class 2 waters are below 50 mg / L . The concentrations of TSS and VSS throughout the research are presented in Figures 8 and 9 .

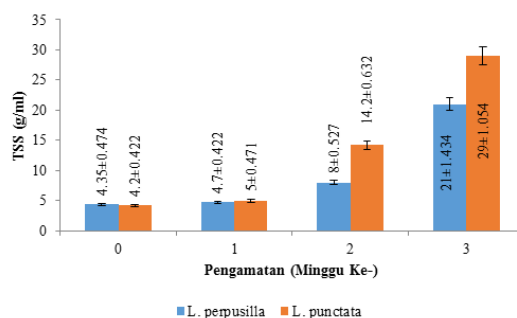


Figure 8. TSS value during research

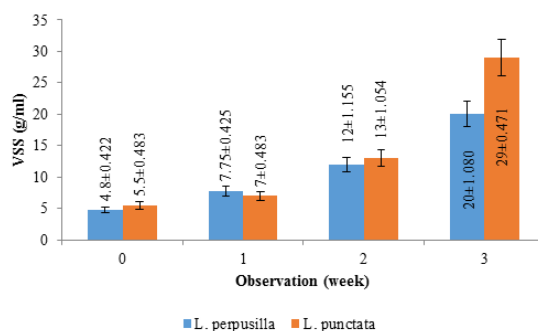


Figure 9. VSS values during research

Energy is the ability to do an effort, the biggest source of energy is from the sun. *L. perpusilla* and *L. punctata* require energy obtained by changing the form of energy that already exists in the environment in the form of nutrients. Both plants interact with their environment. This interaction causes the transfer of energy from the environment absorbed through the roots of both plants. The process of transferring energy is known as energy flow in the ecosystem.

Detritus is the result of decomposition of waste or dead plants and animals. The availability of limited and reduced nutrients during retention time causes many plants to die until the end of the research period. The number of dead plants increases the content of detritus or organic matter in the waters. In line with the results of the research conducted, the concentration of TSS and VSS increased every week. Increasing organic matter is directly proportional to the increasing concentration of TSS and VSS in research media (Effendie 2003).

CONCLUSIONS

The conclusion of this research is *L. perpusilla* were able to absorb nitrate by 0.79 mg / L / day and phosphate by 0.37 mg / L / day compared to *L. punctata* which was able to absorb nitrate of 0.71 mg / L / day and phosphate of 0.35 mg / L / day.

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