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- 1. Submitted to the journal "Jurnal Penelitian Pendidikan IPA" (13-06-2024)**
- 2. Revision (17-7-2024)**
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## [JPPIPA] Submission Acknowledgement

1 pesan

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Prof. Aris Doyan, M.Si., Ph.D <jppipa@unram.ac.id>

13 Juni 2024 pukul 13.31

Kepada: Maria Ulfah <mariaulfah@upgris.ac.id>

Maria Ulfah:

Thank you for submitting the manuscript, "Bioremediation of Nitrate and Nitrite on the Rehabilitation of Contaminated Jatibarang Landfill and the Application of Calla Lily" to Jurnal Penelitian Pendidikan IPA. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Manuscript URL: <https://jppipa.unram.ac.id/index.php/jppipa/authorDashboard/submission/8095>

Username: mariaulfah520

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Prof. Aris Doyan, M.Si., Ph.D

Jurnal Penelitian Pendidikan IPA (JPPIPA)

**2.Revision (17-7-2024)**



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## [JPPIPA] Editor Decision

1 pesan

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17 Juli 2024 pukul 11.13

Kepada: "Ulfah, Maria" <mariaulfah@upgris.ac.id>, "Sari, Octavia Devi Puspita" <octdevipuspitasari@gmail.com>, "Hayat, Muhammad Syaipul" <m.syaipulhayat@upgris.ac.id>

Ulfah, Maria, Sari, Octavia Devi Puspita, Hayat, Muhammad Syaipul :

We have reached a decision regarding your submission to Jurnal Penelitian Pendidikan IPA, "Bioremediation of Nitrate and Nitrite on the Rehabilitation of Contaminated Jatibarang Landfill and the Application of Calla Lily".

Our decision is: **Revisions Required**

Jurnal Penelitian Pendidikan IPA (JPPIPA)



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### **[JPPIPA] Editor Decision**

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We have reached a decision regarding your submission to Jurnal Penelitian Pendidikan IPA, "Bioremediation of Nitrate and Nitrite on the Rehabilitation of Contaminated Jatibarang Landfill and the Application of Calla Lily".

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**3.Revised version received (26-7-2024)**

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# Bioremediation of Nitrate and Nitrite on the Rehabilitation of Contaminated Jatibarang Landfill and the Application of Calla Lily

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**Abstract:** Currently, waste processing is a problem because it is not yet optimal. The high content of nitrates and nitrites results in environmental pollution which can cause losses because it affects health and biodiversity. One effort to control environmental pollution is phytoremediation using calla lily (*Zantedeschia aethiopica*) to remediate nitrate and nitrite levels that do not comply with quality standards so that pollution can be minimized. This research aims to determine the effectiveness of calla lily as a phytoremediation agent for landfill leachate nitrate and nitrite. This experimental research method uses a Completely Randomized Design (CRD) which consists of three treatment levels, namely variations in calla lily biomass of 0 grams, 200 grams, and 400 grams with 3 repetitions, and measurements of nitrate and nitrite levels are carried out on day 1, day 2, 3, and 7. The results showed that the most optimal percentage reduction in leachate nitrate levels occurred in the P1 on the day 7, namely 45%, while the most optimal percentage reduction in leachate nitrite levels occurred in the P1 on the day 7, it was 53%. The final nitrate content after the research was in accordance with the quality standards of PP RI No.22 of 2021, while the leachate nitrite content after treatment still exceeded the quality standards of PP RI No.22 of 2021. Calla lily has the potential to reduce nitrate and nitrite levels due to landfill leachate pollution.

**Keywords:** Leachate; Nitrate; Nitrite; Phytoremediation; Water Lily; Phytoremediation; Water Lily; Leachate; Nitrate; Nitrite

## Introduction

Currently, development developments have an impact on increasing activity in various sectors which is accompanied by the entry of waste into the environment. Waste processing to date is not optimal, it contains nitrate and nitrite pollutants. As a result, the leachate content in the landfill does not fully comply with the expected quality standards. JPPA Jatibarang Landfill is one of the final disposal sites for rubbish and waste in Semarang, located close to residential areas and the Kreo River. Common pollutants found in leachate can be categorized as organic compounds (BOD, COD), inorganic compounds such as heavy metals, ammonia

commonly found as ammonium iron (Abiriga et al., 2021), (Abiriga et al., 2021) nutrients (nitrogen and phosphorus compound) (Lucero-Sobarzo et al., 2022)(Lucero-sobarzo and Beltr, 2022). The organic and inorganic materials contained in accumulated waste decompose into leachate, which is very concentrated organic material containing heavy metals (Nuruddin 2012 in Zamhar, KN; Dewi, 2015).

The leachate in the Jatibarang Semarang landfill contains nitrates and nitrites. If these substances are in large quantities, it will cause a population explosion large algae. This causes losses because it affects health and biodiversity. In previous research, pollutants in the leachate media of the Jatibarang landfillg Semarang TPA

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had nitrate parameters of 8.79 mg/L and nitrite of 10.6 mg/L (Nindrasari, 2011)(Nindrasari et al., 2011). The nitrate value complies with the quality standard, but the nitrite value exceeds the quality standard based on the [Government Regulation of the Republic of Indonesia Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning the Implementation of Environmental Protection and Management](#).

One effort to control pollution from these pollutants is phytoremediation using calla lily (*Zantedeschia aethiopica*). Phytoremediation is one effort that can be done to reduce pollutant content. This process utilizes plants and microorganisms to minimize and detoxify pollutants because plants have the ability to absorb (Kasman et al., 2019)(Kasman, Riyanti and Kartikawati, 2019). [Phytoremediation is a technique that offers multiple contaminants removal at a natural manner with minimal costs \(Mahajan & Kaushal, 2018\)\(Mahajan, P. and Kaushal, I., 2018\).](#)

Calla lily are included in the aquatic plants which are naturally able to absorb pollutants by breaking them down in the phytoremediation process, so that water lilies get nutrients for their growth. Plants will absorb nutrient elements through their roots as nutrients for growth and other elements that become pollutants. Aquatic plants provide a place for microorganisms to grow and attach to their roots and stems, which play a role in decomposing organic compounds contained in liquid waste. Therefore, the heat released by aquatic plants suppresses damaging pathogenic microorganisms (Dewi, 2017)(Green and Dhoibie, 1996 in Dewi, M.T.K. 2017).

Against this background, the research aims to determine the effectiveness of calla lily in the phytoremediation of nitrate and nitrite leachate from the Jatibarang landfill, Semarang. The expected results from this research are that the levels of nitrate and nitrite contained in the Jatibarang landfill in Semarang are in accordance with quality standards based on Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning the Implementation of Environmental Protection and Management. PP RI No.22 of 2021 states that the quality standard for nitrite is 0.06 mg/L and the quality standard for nitrate is 10 mg/L.

## Method

The research was located at ~~TPA~~ Jatibarang ~~Landfill~~ Semarang in 2023. The research used a Completely Randomized Design (CRD) with 3 treatments and 3 replications. Treatment consists of: P0 (10 liters of leachate + 0 grams of calla lily (control)), P1

(10 liters of leachate + 200 grams of calla lily), P2 (10 liters of leachate + 400 grams of calla lily).

### Leachate Water Sampling

Taking leachate water samples at the research station, totaling 90 liters of leachate water. Put 10 liters of leachate into the research unit basin with a capacity of 15 liters.

### Calla Lily Sample Selection

Selecting research samples as a phytoremediation agent for nitrate and nitrite leachate using calla lily, because it has hypertolerant and hyperaccumulator properties. The calla lily plants used are mature plants. This research uses a variation of biomass consisting of 0 grams (P0 or control), 200 grams (P1), and 400 grams (P2). The more biomass a calla lily has, the denser the root fibers become and spread out in different directions for optimal absorption of pollutants.

### Calla Lily Acclimatization

Before the research, acclimatize the calla lily for one week. The acclimatization process at the research location involves placing calla lily in a 15 liter capacity basin containing 10 liters of distilled water. This acclimatization activity aims to adapt calla lily with new environmental conditions. The acclimatization process lasts for one week assuming the calla lily are considered to have adapted to the new environment. After one week of acclimatization, then move the calla lily into a 15 liter capacity basin containing 10 liters of leachate according to the research treatment code.

### Primary Data Collection

Collect primary data/nitrate and nitrite content of leachate on the first, third and seventh days of the research by taking 2.5 liter samples of leachate water from each research unit, then testing the nitrate and nitrite content. The leachate's nitrate and nitrite content was tested at the UPTD Environmental Laboratory, Environmental Service, Semarang, Indonesia.

### Secondary Data Retrieval

Collect secondary data by measuring environmental conditions on days 1, 3 and 7. Measuring environmental conditions including temperature, light intensity and pH.

## Result and Discussion

### Results

The measurement of nitrate levels before treatment was 8.79 mg/L, where this figure is in accordance with established quality standards, but

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decreased after giving calla lily phytoremediation treatment. The effectiveness of calla lily in reducing nitrate levels can be seen in tables 1, 2 and 3.

The measurement of nitrite levels before treatment was 0.8 mg/L, which exceeds the established quality standards. However, nitrite levels decreased after giving calla lily phytoremediation treatment. The

effectiveness of calla lily in reducing nitrite levels can be seen in tables 4, 5 and 6.

**Table 1.** Nitrate Levels on day 1

Treatment	Initial average	Repetition			Amount	Average	% Decrease
		1	2	3			
P <sub>0</sub>	8,79 mg/L	5,982	5,85	7,987	19,819	6,606	25
P <sub>1</sub>		9,99	7,398	5,73	23,118	7,706	12
P <sub>2</sub>		5,821	5,97	5,538	17,329	5,776	34

\*) Quality standards of PP RI No.22 of 2021

**Table 2.** Nitrate Levels on day 3

Treatment	Initial average	Repetition			Amount	Average	% Decrease
		1	2	3			
P <sub>0</sub>	8,79 mg/L	7,334	7,171	6,824	21,329	7,109	19
P <sub>1</sub>		5,459	8,302	5,323	19,084	6,361	28
P <sub>2</sub>		5,29	5,407	5,306	16,003	5,334	39

\*) Quality standards of PP RI No.22 of 2021

**Table 3.** Nitrate Levels on day 7

Treatment	Initial average	Repetition			Amount	Average	% Decrease
		1	2	3			
P <sub>0</sub>	8,79 mg/L	7,41	7,73	8,057	23,197	7,732	12
P <sub>1</sub>		4,666	4,911	4,888	14,465	4,821	45
P <sub>2</sub>		5,023	5,184	4,637	14,844	4,948	44

\*) Quality standards of PP RI No.22 of 2021

**Table 4.** Nitrite Levels on day 1

Treatment	Initial average	Repetition			Amount	Average	% Decrease
		1	2	3			
P <sub>0</sub>	0,8 mg/L	0,498	0,486	0,691	1,675	0,558	30
P <sub>1</sub>		0,883	0,634	0,474	1,991	0,663	17
P <sub>2</sub>		0,483	0,497	0,456	1,436	0,478	40

\*) Quality standards of PP RI No.22 of 2021

**Table 5.** Nitrite Levels on day 3

Treatment	Initial average	Repetition			Amount	Average	% Decrease
		1	2	3			
P <sub>0</sub>	0,8 mg/L	0,628	0,612	0,579	1,819	0,606	24
P <sub>1</sub>		0,448	0,721	0,435	1,604	0,534	33
P <sub>2</sub>		0,432	0,443	0,433	1,308	0,436	46

\*) Quality standards of PP RI No.22 of 2021

**Table 6.** Nitrite Levels on day 7

Treatment	Initial average	Repetition			Amount	Average	% Decrease
		1	2	3			
P <sub>0</sub>	0,8 mg/L	0,63	0,66	0,688	1,978	0,659	18
P <sub>1</sub>		0,364	0,387	0,385	1,136	0,378	53

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P<sub>2</sub> 0,398 0,414 0,361 1,173 0,391 51  
 \*) Quality standards of PP RI No.22 of 2021

Based on table 1, it can be seen that calla lily are effective in reducing nitrate levels in leachate. This can be seen from the nitrate levels in the P treatment<sub>2</sub> with a decrease percentage of 34%. Based on the data in Table 1, a graph can be made of the average effectiveness of calla lily phytoremediation on nitrate levels on day 1.

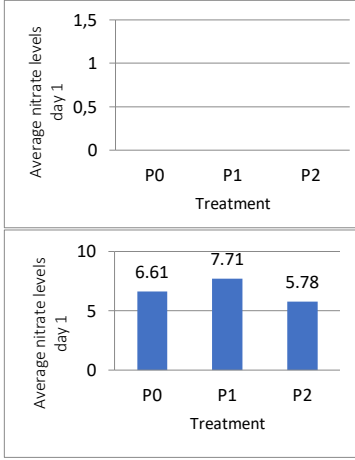


Figure 1. Average day 1 nitrate levels

Based on table 2, it can be seen that calla lily are effective in reducing nitrate levels in leachate. This can be seen from the nitrate levels in the P<sub>2</sub> with a decrease percentage of 39%. Based on the data in table 2, a graph can be made of the average effectiveness of calla lily phytoremediation on nitrate levels on the day 3.

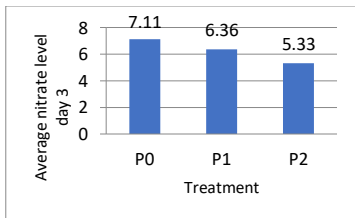


Figure 2. Average day 3 nitrate levels

Based on table 3, it can be seen that calla lily are effective in reducing nitrate levels in leachate. This can be seen from the nitrate levels in the P treatment<sub>1</sub> with a decrease percentage of 45%. Based on the data in table 3, a graph can be made of the average effectiveness of

water lily phytoremediation on nitrate levels on the day 7.

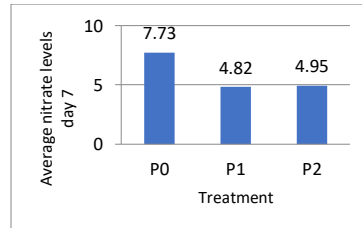


Figure 3. Average day 7 nitrate levels

Based on table 4, it can be seen that calla lily are effective in reducing nitrite levels in leachate. This can be seen from the nitrite levels in the P<sub>2</sub> with a reduction percentage of 40%. Based on the data in table 4, a graph can be made of the average effectiveness of calla lily phytoremediation on nitrite levels on day 1.

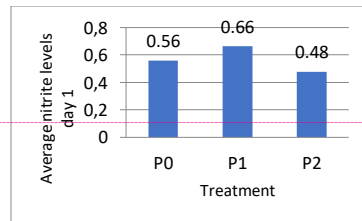


Figure 4. Average day 1 nitrite levels

Based on table 5, it can be seen that calla lily are effective in reducing nitrite levels in leachate. This can be seen from the nitrite levels between treatments and the percentage reduction in nitrite levels which appeared to decrease on the 3rd day. Based on the data in table 5, a graph can be made of the average effectiveness of calla lily phytoremediation on nitrite levels on day 3.

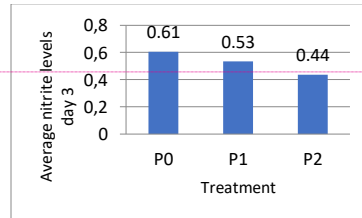


Figure 5. Average day 3 nitrite levels

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Based on table 6, it can be seen that calla lily are effective in reducing nitrite levels in leachate. This can be seen from the nitrite levels in the P treatment1 with a decrease percentage of 53%. Based on the data in table 6, a graph can be made of the average effectiveness of calla lily phytoremediation on nitrite levels on day 7.

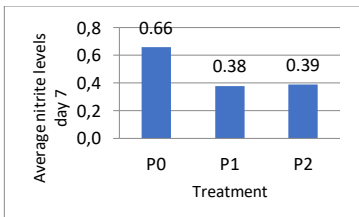


Figure 6. Average day 7 nitrite levels

Discussion

Nitrate is a type of inorganic nitrogen found in water and a key nutrient for plant and algae growth. Nitrate can also be defined as the main form of nitrogen found in natural aquatic environments (Setyorini & Maria, 2019)(Setyorini and Maria, 2019). Nitrite (NO<sub>2</sub>) is an oxidized form of nitrogen and is commonly found in wastewater treatment plants (Putri et al., 2019)(Putri et al., 2019). According to Juliasih And al., (2017)Juliasih et al. (2017), nitrite levels are one of the parameters that are an indicator of pollution. Excessive nitrite content in waters causes the balance of the ecosystem to be disturbed and if it enters the human body, it also has the potential to cause chronic health problems.

Based on the results of research that has been carried out, the treatment that has the highest effectiveness in reducing nitrate is P1 on day 7 treatment, as well as the treatment that had the lowest effectiveness in reducing nitrate levels, namely P1 on day 1 treatment and P0 on day 7 of treatment. Meanwhile, the treatment that has the highest effectiveness in reducing nitrite is P1 on day 7 treatment, and the treatment that had the lowest effectiveness in reducing nitrite levels was P1 on day 1 of treatment. The decrease in nitrate and nitrite levels that occurred in this study was caused by the phytoremediation process carried out by calla lily through the process rhizofiltration. Therefore, calla lily plants have the ability to adapt to survive in an environment that is stressed by nitrite and have the power to absorb nitrite in the waste leachate media so that the nitrite levels in the leachate media decrease and the nitrite levels in the calla lily plants increase. Apart from that, the nutrients needed by microorganisms have been successfully absorbed by the plant roots so that the

plants can reach their optimal point. This condition causes a balance between the growth and death of microorganisms which is often referred to as stationary phase (Kasman et al., 2019)(Kasman, Riyanti and Kartikawati, 2019).

A study conducted by (Belmont & Metcalfe, 2003)Belmont and Metcalfe (2003), stated that calla lily is used in units wetlands to degrade nitrogen. Aquatic plants need nitrogen to grow. Therefore, the presence of nitrates and nitrites is used by plants as nutrients, the ability of the roots of calla lily is effective in absorbing dissolved ions and anions in the water layer. This is confirmed by research according to Moloantoa et al. (2022)Moloantoa et al. (2022), that nitrate functions as a nutrient for aquatic plants because it is easily used as a source of N for protein synthesis and the rapid development of chlorophyll which is used for photosynthesis. There is a photosynthesis process that produces oxygen and some of it is released through the roots, so the microorganisms in the root area will get oxygen.

Compounds in water can be absorbed by the root tips because of the attractive forces between water molecules in plants so that compounds absorbed by the roots will enter the stem through the xylem and then be passed on to the leaves. Organic pollutants or nitrites in plants can then be detoxified through enzyme degradation. These organic compounds are then stored in vacuoles for further metabolism (Fitriana & Kuntjoro, 2021)(Fitriana and Kuntjoro, 2020).

Reducing nitrate really requires the availability of sufficient dissolved oxygen which will be released by plant roots in the rhizosphere zone for microorganisms to decompose organic substances in leachate. The absorption of nitrate by plants causes the nitrate concentration to decrease in the leachate. ~~There is an increase in the uptake of nitrate content in calla lily because all the substances contained in the leachate can be absorbed by the roots of calla lily which are able to absorb a liquid without prior selection (Eptein, 1972).~~ Nitrate that has been absorbed by plants is then converted into protein with the help of carbon dioxide (CO<sub>2</sub>) found in free air and sunlight.

Nitrate is the end result of nitrification which will be transported by plants or will diffuse to the roots where it will be converted into N<sub>2</sub> and N<sub>2</sub>O in the denitrification (Fandya & Soewondo, 2011)(Fandya, 2011). The decreasing concentration of nitrate is also due to the denitrification (Saputra et al., 2016)(Saputra et al., 2016). The decrease occurred because the growth of nitrifying bacteria was slower than heterotrophic bacteria (Effendi, 2003)(Hefni Effendi, 2003). High nitrogen levels in waters are the main cause of very rapid growth of algae which causes eutrophication. Nitrification, which is the process of oxidizing ammonia

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into nitrites and nitrates, takes place under aerobic conditions. Ammonia (NH<sub>3</sub>) give nitrite (NO<sub>2</sub>) will be converted first through the nitrification into the form of nitrate (NO<sub>3</sub>) can then be absorbed by calla lily. Calla lily can directly absorb nitrate (NO<sub>3</sub>) whose function is to meet nutritional needs in the growth of calla lily.

Nitrite ions can act as a source of nitrogen for plants. The roots of water lilies are effective in absorbing dissolved ions and anions in the water layer. The release of oxygen resulting from photosynthesis in the upper and bottom layers of water which allows the nitrification process to occur or the change of nitrite to nitrate also influences the reduction of nitrite. ~~[This is in accordance with Barnes and P.J (1980), that one of the factors controlling the nitrification process in the water treatment process is dissolved oxygen, where the nitrification process occurs under aerobic conditions, so the presence of oxygen is very important in this process. The decrease in nitrite is also due to the presence of bacteria Nitrobacter which converts nitrite into nitrate which is the core part of the nitrification process (Fandya & Soewondo, 2011)(Fandya, 2011).~~

The decrease in nitrite is also influenced by the symbiosis between calla lily and waste microorganisms. The effect of phytoremediation using calla lily reduces nitrite levels by utilizing the cooperative relationship between calla lily and microorganisms which can convert contaminants or pollutants into less toxic substances. Organic materials experience degradation through an aerobic oxidation process which in turn produces more stable compounds. The use of calla lily acts as a provider of oxygen for aerobic microorganisms. This oxygen comes from photosynthesis carried out by water lilies with the help of sunlight. The oxygen diffuses into the water through the water surface. Aerobic organisms then use oxygen as electron acceptor to oxidize organic compounds in leachate into simple, stable compounds. This compound is a nutrient for plants to carry out photosynthesis. In this case, there is a reciprocal relationship between the calla lily and the aerobic organisms found in the leachate.

The ability of plants to remove nitrates and nitrites depends on the number of plants and the time of remediation. The faster the remediation time, the more plants are needed, if the remediation time used is long enough, the fewer plants will be ~~(Nursagita & Titah, 2021)(Nursagita, Yulyana Suci and Sulistyaning, Harmin, 2021).~~ From this, it is in line with the results of this research that on days 1 and 3 the calla lily plants were more effective in reducing nitrate, namely those in the P2 with a plant weight of 400 grams which is heavier compared to the treatment of P1. Meanwhile, on day 7, the calla lily plants were more effective in reducing nitrate, namely the P1 weighing 200 grams. If it is on nitrite, namely in the P2 with a plant weight of 400 grams

which is heavier compared to the treatment of P1. Meanwhile, on day 7, the calla lily plants were more effective in reducing nitrite, namely the P1 weighing 200 grams.

Plant density and biomass influence the effectiveness of reducing leachate nitrate and nitrite levels. Plants at low density, the evaporation process takes place more perfectly, thus creating better natural growth characteristics. Plants that are too close together tend to have slower evaporation and growth. This affects the process of nutrient absorption and photosynthesis ~~(Ngirfani & Puspitarini, 2020)(Ngirfani, 2020).~~ This is confirmed by research conducted by ~~Herlambang & Hendriyanto (2015)Herlambang and Hendriyanto (2015),~~ showing that variations in plant weight or number of plants have an impact on reducing nitrite levels. The heavier or more numerous the plants are, the greater the decrease in nitrite levels. However, the greater the number of plants, the more plant competition will arise, that is, plant growth can be disrupted and over time they will wilt and even die.

The amount of water lily plant biomass in the P2 less effective in reducing nitrate and nitrite levels on day 7 due to an increase in organic matter in the leachate due to the influence of wilting water lilies. Some of the biomass of aquatic plants has died, causing an increase in aquatic organic matter. Plants do not directly absorb organic material, but aquatic plants provide conditions that allow the decomposition process of organic material to occur by microorganisms. Therefore, during the treatment there was a decomposition process that was still ongoing. So that aquatic plants will be less able to contribute significantly in reducing organic matter ~~(Astuti & Indriatmoko, 2018)(Astuti and Indriatmoko, 2018).~~ In addition, on day 1 and day 3 of the study, reducing nitrate and nitrite levels was less effective compared to day 7 because on day 1 and day 3 there was a time span of 2 days which could only be used by water lily plants to adapt to leachate so that the calla lily plant tissue cannot accumulate nitrates and nitrites optimally. Calla lily which continuously absorb nitrates and nitrites in waste leachate will over time experience a decrease in their ability to absorb nitrates and nitrites. This is because calla lily plants have a limit or capacity to absorb nitrates and nitrites.

Physical and chemical environmental factors influence the survival of calla lily in the phytoremediation process, including temperature, light intensity and pH. pH and temperature measurements aim to determine the acidity and temperature conditions of an environment. Temperature is related to metabolic and photosynthesis processes, so an increase in temperature will indirectly affect the absorption rate. According to ~~Ikawati et al. (2013)Ikawati et al., (2013)~~ the higher the environmental temperature of the plant,

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the higher the level of absorption by the plant. This is confirmed by research by (Hidayat, 2011)(Hidayat, 2011), which revealed that the higher the environmental temperature of the plant, the higher the level of absorption by the plant, where the environmental temperature will cause the photosynthesis process to increase, so that the absorption of nutrients by the plant will also increase. The denser the plants, the less light can enter the waste. This will affect the temperature of the leachate. The denser the plants, the lower the temperature of the water so that the photosynthesis process is reduced. (Latifa & Pantiwati, 2021)(Latifa and Pantiwati, 2021).

Light intensity measurements at the research location were carried out every day (during the research) at 08.00-09.00 WIB because during this time the plants were actively carrying out photosynthesis, which would later influence the nitrite absorption process by the calla lily. High light intensity causes the temperature of the leachate to increase, thereby accelerating the absorption of more metal ions (Monita et al., 2013)(Monita et al., 2013). Increasing temperature can increase respiration which can indirectly increase energy production needed in the active transport process (MD, 2011)(Agustinus, 2011). Increasing temperature can increase respiration which can indirectly increase energy production needed in the active transport process (Agustinus, 2011).

On days 1 and 3 there was an increase in pH. The measurement results prove that phytoremediation using calla lily can increase the pH. This shows that there is a tendency for each treatment to reach a normal pH. The increase in pH in the phytoremediation treatment was due to the mineralization process of organic acids in the leachate. The results of the mineralization of organic acids from leachate waste will be nutrients that will be absorbed by plant roots, causing an increase in pH. The increase in pH is caused by the processes of photosynthesis, denitrification, breakdown of organic nitrogen and sulfate reduction (Fitriyah, 2011)(Fitriyah, 2011). However, on days 3 and 7 there was a decrease in pH. The decrease in pH is caused by the biodegradation process of organic materials. Dead plant parts of water lilies will be broken down by microorganisms in the water, so that in the process of decomposing organic matter it will affect the pH value (Sari et al., 2017)(Sari et al., 2017). The activity of degrading microorganisms allows a decrease in pH because organic compounds have been broken down into organic acids (Purnamawati et al., 2015)(Purnamawati et al., 2015). The cause of the decrease in pH can also be caused by the rotting of calla lily plant parts by the work of acid-producing microbes (Hapsari et al., 2016)(Hapsari et al., 2016).

## Conclusion

Calla lily (*Zantedeschia aethiopica*) is effective as a phytoremediation agent in reducing nitrate levels in leachate wastewater in P1 day 7 (calla lily biomass 200 grams) with a reduction percentage of 45% and in leachate waste nitrite levels at P1 day 7 (calla lily biomass 200 grams) with a reduction percentage of 53.53%.

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Place acknowledgments, including information on grants received, before the references, in a separate section, and not as a footnote on the title page

## Author Contributions

Conceptualization, M. U., O. D. P. S., M. S., H.; methodology; M U.; validation, M. S. H; formal analysis, O. D. P. S.; investigation, M. S., H.; resources, O. D. P. S.; data curation, M. U.; writing – original draft preparation, M. S. H; writing – review and editing; M. U; visualization: M. S. H. All authors have read and agreed to the published version of the manuscript.

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, X.X. and Y.Y.; methodology, X.X.; software, X.X.; validation, X.X., Y.Y. and Z.Z.; formal analysis, X.X.; investigation, X.X.; resources, X.X.; data curation, X.X.; writing – original draft preparation, X.X.; writing – review and editing, X.X.; visualization, X.X.; supervision, X.X.; project administration, X.X.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript.” Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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## Conflicts of Interest

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The authors declare no conflict of interest. Declare conflicts of interest or state "The authors declare no conflict of interest." Authors must identify and declare any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. Any role of the funders in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results must be declared in this section. If there is no role, please state "The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results"

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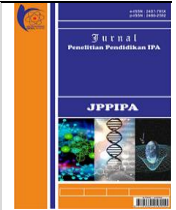
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# Bioremediation of Nitrate and Nitrite on the Rehabilitation of Contaminated Jatibarang Landfill and the Application of Calla Lily

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**Abstract:** Currently, waste processing is a problem because it is not yet optimal. The high content of nitrates and nitrites results in environmental pollution which can cause losses because it affects health and biodiversity. One effort to control environmental pollution is phytoremediation using calla lily (*Zantedeschia aethiopica*) to remediate nitrate and nitrite levels that do not comply with quality standards so that pollution can be minimized. This research aims to determine the effectiveness of calla lily as a phytoremediation agent for landfill leachate nitrate and nitrite. This experimental research method uses a Completely Randomized Design (CRD) which consists of three treatment levels, namely variations in calla lily biomass of 0 grams, 200 grams, and 400 grams with 3 repetitions, and measurements of nitrate and nitrite levels are carried out on day 1, day 2, 3, and 7. The results showed that the most optimal percentage reduction in leachate nitrate levels occurred in the P1 on the day 7, namely 45%, while the most optimal percentage reduction in leachate nitrite levels occurred in the P1 on the day 7, it was 53%. The final nitrate content after the research was in accordance with the quality standards of PP RI No.22 of 2021, while the leachate nitrite content after treatment still exceeded the quality standards of PP RI No.22 of 2021. Calla lily has the potential to reduce nitrate and nitrite levels due to landfill leachate pollution.

**Keywords:** Leachate; Nitrate; Nitrite; Phytoremediation; Water Lily.

## Introduction

Currently, development developments have an impact on increasing activity in various sectors which is accompanied by the entry of waste into the environment. Waste processing to date is not optimal, it contains nitrate and nitrite pollutants. As a result, the leachate content in the landfill does not fully comply with the expected quality standards. Jatibarang Landfill is one of the final disposal sites for rubbish and waste in Semarang, located close to residential areas and the Kreo River. Common pollutants found in leachate can be categorized as organic compounds (BOD, COD), inorganic compounds such as heavy metals, ammonia commonly found as ammonium iron (Abiriga et al.,

2021) nutrients (nitrogen and phosphorus compound) (Lucero-Sobarzo et al., 2022).

The leachate in the Jatibarang landfill contains nitrates and nitrites. If these substances are in large quantities, it will cause a population explosion large algae. This causes losses because it affects health and biodiversity. In previous research, pollutants in the leachate media of the Jatibarang landfill had nitrate parameters of 8.79 mg/L and nitrite of 10.6 mg/L (Nindrasari, 2011). The nitrate value complies with the quality standard, but the nitrite value exceeds the quality standard based on the Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning the Implementation of Environmental Protection and Management.

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One effort to control pollution from these pollutants is phytoremediation using calla lily (*Zantedeschia aethiopica*). Phytoremediation is one effort that can be done to reduce pollutant content. This process utilizes plants and microorganisms to minimize and detoxify pollutants because plants have the ability to absorb (Kasman et al., 2019). Phytoremediation is a technique that offers multiple contaminants removal at a natural manner with minimal costs (Mahajan & Kaushal, 2018).

Calla lily are included in the aquatic plants which are naturally able to absorb pollutants by breaking them down in the phytoremediation process, so that water lilies get nutrients for their growth. Plants will absorb nutrient elements through their roots as nutrients for growth and other elements that become pollutants. Aquatic plants provide a place for microorganisms to grow and attach to their roots and stems, which play a role in decomposing organic compounds contained in liquid waste. Therefore, the heat released by aquatic plants suppresses damaging pathogenic microorganisms (Dewi, 2017).

Against this background, the research aims to determine the effectiveness of calla lily in the phytoremediation of nitrate and nitrite leachate from the Jatibarang landfill, Semarang. The expected results from this research are that the levels of nitrate and nitrite contained in the Jatibarang landfill in Semarang are in accordance with quality standards based on Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning the Implementation of Environmental Protection and Management. PP RI No.22 of 2021 states that the quality standard for nitrite is 0.06 mg/L and the quality standard for nitrate is 10 mg/L.

## Method

The research was located at Jatibarang Landfill in 2023. The research used a Completely Randomized Design (CRD) with 3 treatments and 3 replications. Treatment consists of: P0 (10 liters of leachate + 0 grams of calla lily (control)), P1 (10 liters of leachate + 200 grams of calla lily), P2 (10 liters of leachate + 400 grams of calla lily).

### *Leachate Water Sampling*

Taking leachate water samples at the research station, totaling 90 liters of leachate water. Put 10 liters of leachate into the research unit basin with a capacity of 15 liters.

### *Calla Lily Sample Selection*

Selecting research samples as a phytoremediation agent for nitrate and nitrite leachate using calla lily, because it has hypertolerant and hyperaccumulator

properties. The calla lily plants used are mature plants. This research uses a variation of biomass consisting of 0 grams (P0 or control), 200 grams (P1), and 400 grams (P2). The more biomass a calla lily has, the denser the root fibers become and spread out in different directions for optimal absorption of pollutants.

### *Calla Lily Acclimatization*

Before the research, acclimatize the calla lily for one week. The acclimatization process at the research location involves placing calla lily in a 15 liter capacity basin containing 10 liters of distilled water. This acclimatization activity aims to adapt calla lily with new environmental conditions. The acclimatization process lasts for one week assuming the calla lily are considered to have adapted to the new environment. After one week of acclimatization, then move the calla lily into a 15 liter capacity basin containing 10 liters of leachate according to the research treatment code.

### *Primary Data Collection*

Collect primary data/nitrate and nitrite content of leachate on the first, third and seventh days of the research by taking 2.5 liter samples of leachate water from each research unit, then testing the nitrate and nitrite content. The leachate's nitrate and nitrite content was tested at the UPTD Environmental Laboratory, Environmental Service, Semarang, Indonesia.

### *Secondary Data Retrieval*

Collect secondary data by measuring environmental conditions on days 1, 3 and 7. Measuring environmental conditions including temperature, light intensity and pH.

## Result and Discussion

The measurement of nitrate levels before treatment was 8.79 mg/L, where this figure is in accordance with established quality standards, but decreased after giving calla lily phytoremediation treatment. The effectiveness of calla lily in reducing nitrate levels can be seen in tables 1, 2 and 3.

The measurement of nitrite levels before treatment was 0.8 mg/L, which exceeds the established quality standards. However, nitrite levels decreased after giving calla lily phytoremediation treatment. The effectiveness of calla lily in reducing nitrite levels can be seen in tables 4, 5 and 6.



**Table 1.** Nitrate Levels on day 1

Treatment	Initial average	Repetition			Amount	Average	% Decrease
		1	2	3			
P <sub>0</sub>		5.982	5.85	7.987	19.819	6.606	25
P <sub>1</sub>	8.79 mg/L	9.99	7.398	5.73	23.118	7.706	12
P <sub>2</sub>		5.821	5.97	5.538	17.329	5.776	34

\*) Quality standards of PP RI No.22 of 2021

**Table 2.** Nitrate Levels on day 3

Treatment	Initial average	Repetition			Amount	Average	% Decrease
		1	2	3			
P <sub>0</sub>		7.334	7.171	6.824	21.329	7.109	19
P <sub>1</sub>	8.79 mg/L	5.459	8.302	5.323	19.084	6.361	28
P <sub>2</sub>		5.29	5.407	5.306	16.003	5.334	39

\*) Quality standards of PP RI No.22 of 2021

**Table 3.** Nitrate Levels on day 7

Treatment	Initial average	Repetition			Amount	Average	% Decrease
		1	2	3			
P <sub>0</sub>		7.41	7.73	8.057	23.197	7.732	12
P <sub>1</sub>	8.79 mg/L	4.666	4.911	4.888	14.465	4.821	45
P <sub>2</sub>		5.023	5.184	4.637	14.844	4.948	44

\*) Quality standards of PP RI No.22 of 2021

**Table 4.** Nitrite Levels on day 1

Treatment	Initial average	Repetition			Amount	Average	% Decrease
		1	2	3			
P <sub>0</sub>		0.498	0.486	0.691	1.675	0.558	30
P <sub>1</sub>	0.8 mg/L	0.883	0.634	0.474	1.991	0.663	17
P <sub>2</sub>		0.483	0.497	0.456	1.436	0.478	40

\*) Quality standards of PP RI No.22 of 2021

**Table 5.** Nitrite Levels on day 3

Treatment	Initial average	Repetition			Amount	Average	% Decrease
		1	2	3			
P <sub>0</sub>		0.628	0.612	0.579	1.819	0.606	24
P <sub>1</sub>	0.8 mg/L	0.448	0.721	0.435	1.604	0.534	33
P <sub>2</sub>		0.432	0.443	0.433	1.308	0.436	46

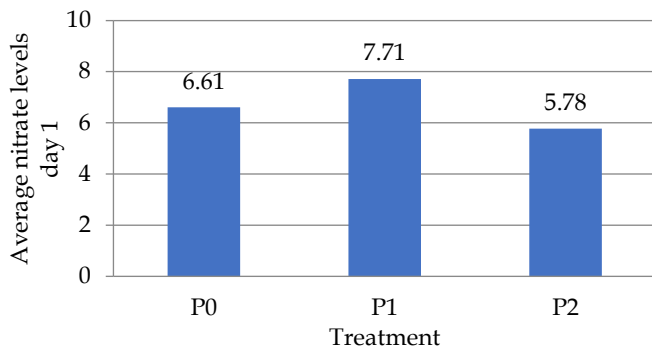
\*) Quality standards of PP RI No.22 of 2021

**Table 6.** Nitrite Levels on day 7

Treatment	Initial average	Repetition			Amount	Average	% Decrease
		1	2	3			
P <sub>0</sub>		0.63	0.66	0.688	1.978	0.659	18
P <sub>1</sub>	0.8 mg/L	0.364	0.387	0.385	1.136	0.378	53
P <sub>2</sub>		0.398	0.414	0.361	1.173	0.391	51

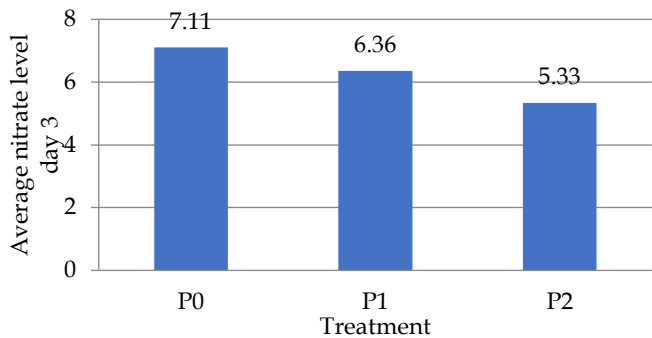
\*) Quality standards of PP RI No.22 of 2021

Based on table 1, it can be seen that calla lily are effective in reducing nitrate levels in leachate. This can be seen from the nitrate levels in the P treatment2 with a decrease percentage of 34%. Based on the data in Table 1, a graph can be made of the average effectiveness of calla lily phytoremediation on nitrate levels on day 1.



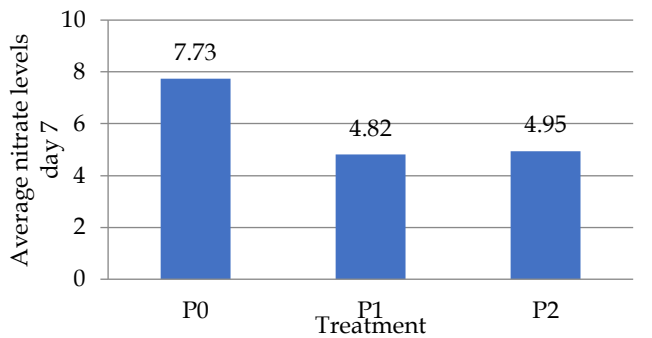
**Figure 1.** Average day 1 nitrate levels

Based on Table 2, it can be seen that calla lily are effective in reducing nitrate levels in leachate. This can be seen from the nitrate levels in the P2 with a decrease percentage of 39%. Based on the data in table 2, a graph can be made of the average effectiveness of calla lily phytoremediation on nitrate levels on the day 3.



**Figure 2.** Average day 3 nitrate levels

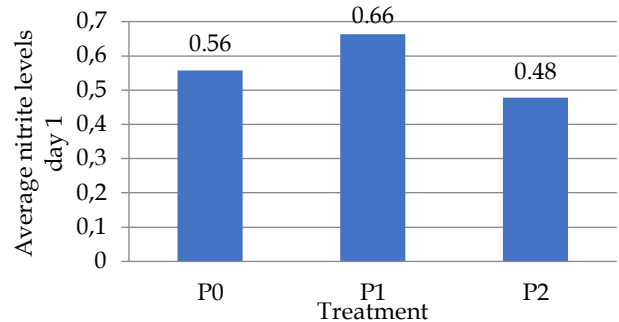
Based on Table 3, it can be seen that calla lily are effective in reducing nitrate levels in leachate. This can be seen from the nitrate levels in the P treatment1 with a decrease percentage of 45%. Based on the data in table 3, a graph can be made of the average effectiveness of water lily phytoremediation on nitrate levels on the day 7.



**Figure 3.** Average day 7 nitrate levels

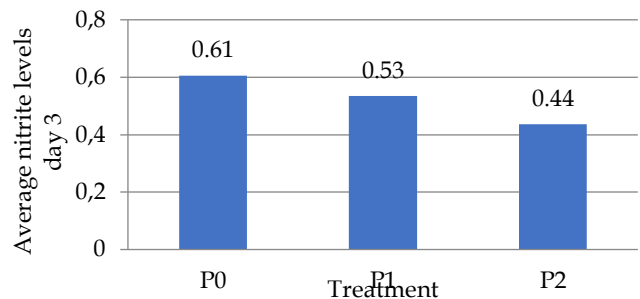
Based on Table 4, it can be seen that calla lily are effective in reducing nitrite levels in leachate. This can

be seen from the nitrite levels in the P2 with a reduction percentage of 40%. Based on the data in table 4, a graph can be made of the average effectiveness of calla lily phytoremediation on nitrite levels on day 1



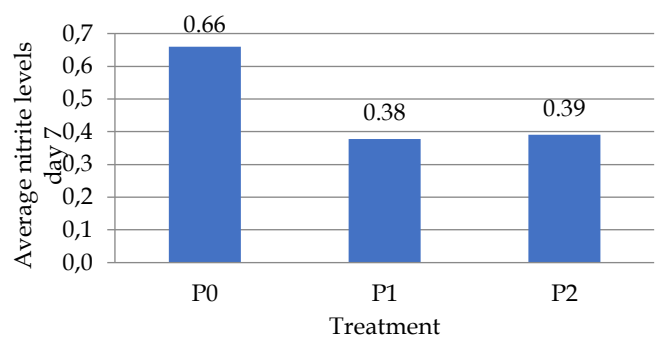
**Figure 4.** Average day 1 nitrite levels

Based on Table 5, it can be seen that calla lily are effective in reducing nitrite levels in leachate. This can be seen from the nitrite levels between treatments and the percentage reduction in nitrite levels which appeared to decrease on the 3rd day. Based on the data in table 5, a graph can be made of the average effectiveness of calla lily phytoremediation on nitrite levels on day 3.



**Figure 5.** Average day 3 nitrite levels

Based on Table 6, it can be seen that calla lily are effective in reducing nitrite levels in leachate. This can be seen from the nitrite levels in the P treatment1 with a decrease percentage of 53%. Based on the data in table 6, a graph can be made of the average effectiveness of calla lily phytoremediation on nitrite levels on day 7.



**Figure 6.** Average day 7 nitrite levels

Nitrate is a type of inorganic nitrogen found in water and a key nutrient for plant and algae growth. Nitrate can also be defined as the main form of nitrogen found in natural aquatic environments (Setyorini & Maria, 2019). Nitrite ( $\text{NO}_2$ ) is an oxidized form of nitrogen and is commonly found in wastewater treatment plants (Putri et al., 2019). According to Juliasih et al. (2017), nitrite levels are one of the parameters that are an indicator of pollution. Excessive nitrite content in waters causes the balance of the ecosystem to be disturbed and if it enters the human body, it also has the potential to cause chronic health problems.

Based on the results of research that has been carried out, the treatment that has the highest effectiveness in reducing nitrate is P1 on day 7 treatment, as well as the treatment that had the lowest effectiveness in reducing nitrate levels, namely P1 on day 1 treatment and P0 on day 7 of treatment. Meanwhile, the treatment that has the highest effectiveness in reducing nitrite is P1 on day 7 treatment, and the treatment that had the lowest effectiveness in reducing nitrite levels was P1 on day 1 of treatment. The decrease in nitrate and nitrite levels that occurred in this study was caused by the phytoremediation process carried out by calla lily through the process rhizofiltration. Therefore, calla lily plants have the ability to adapt to survive in an environment that is stressed by nitrite and have the power to absorb nitrite in the waste leachate media so that the nitrite levels in the leachate media decrease and the nitrite levels in the calla lily plants increase. Apart from that, the nutrients needed by microorganisms have been successfully absorbed by the plant roots so that the plants can reach their optimal point. This condition causes a balance between the growth and death of microorganisms which is often referred to as stationary phase (Kasman et al., 2019).

A study conducted by (Belmont & Metcalfe, 2003), stated that calla lily is used in units wetlands to degrade nitrogen. Aquatic plants need nitrogen to grow. Therefore, the presence of nitrates and nitrites is used by plants as nutrients, the ability of the roots of calla lily is effective in absorbing dissolved ions and anions in the water layer. This is confirmed by research according to Moloantoa et al. (2022) that nitrate functions as a nutrient for aquatic plants because it is easily used as a source of N for protein synthesis and the rapid development of chlorophyll which is used for photosynthesis. There is a photosynthesis process that produces oxygen and some of it is released through the roots, so the microorganisms in the root area will get oxygen.

Compounds in water can be absorbed by the root tips because of the attractive forces between water molecules in plants so that compounds absorbed by the roots will enter the stem through the xylem and then be

passed on to the leaves. Organic pollutants or nitrites in plants can then be detoxified through enzyme degradation. These organic compounds are then stored in vacuoles for further metabolism (Fitriana & Kuntjoro, 2021).

Reducing nitrate really requires the availability of sufficient dissolved oxygen which will be released by plant roots in the rhizosphere zone for microorganisms to decompose organic substances in leachate. The absorption of nitrate by plants causes the nitrate concentration to decrease in the leachate. Nitrate that has been absorbed by plants is then converted into protein with the help of carbon dioxide ( $\text{CO}_2$ ) found in free air and sunlight.

Nitrate is the end result of nitrification which will be transported by plants or will diffuse to the roots where it will be converted into  $\text{N}_2$  and  $\text{N}_2\text{O}$  in the denitrification (Fandya & Soewondo, 2011). The decreasing concentration of nitrate is also due to the denitrification (Saputra et al., 2016). The decrease occurred because the growth of nitrifying bacteria was slower than heterotrophic bacteria (Effendi, 2003). High nitrogen levels in waters are the main cause of very rapid growth of algae which causes eutrophication. Nitrification, which is the process of oxidizing ammonia into nitrites and nitrates, takes place under aerobic conditions. Ammonia ( $\text{NH}_3$ ) give nitrite ( $\text{NO}_2$ ) will be converted first through the nitrification into the form of nitrate ( $\text{NO}_3$ ) can then be absorbed by calla lily. Calla lily can directly absorb nitrate ( $\text{NO}_3$ ) whose function is to meet nutritional needs in the growth of calla lily.

Nitrite ions can act as a source of nitrogen for plants. The roots of water lilies are effective in absorbing dissolved ions and anions in the water layer. The release of oxygen resulting from photosynthesis in the upper and bottom layers of water which allows the nitrification process to occur or the change of nitrite to nitrate also influences the reduction of nitrite. The decrease in nitrite is due to the presence of bacteria *Nitrobacter* which converts nitrite into nitrate which is the core part of the nitrification process (Fandya & Soewondo, 2011).

The decrease in nitrite is also influenced by the symbiosis between calla lily and waste microorganisms. The effect of phytoremediation using calla lily reduces nitrite levels by utilizing the cooperative relationship between calla lily and microorganisms which can convert contaminants or pollutants into less toxic substances. Organic materials experience degradation through an aerobic oxidation process which in turn produces more stable compounds. The use of calla lily acts as a provider of oxygen for aerobic microorganisms. This oxygen comes from photosynthesis carried out by water lilies with the help of sunlight. The oxygen diffuses into the water through the water surface. Aerobic organisms then use oxygen as electron acceptor

to oxidize organic compounds in leachate into simple, stable compounds. This compound is a nutrient for plants to carry out photosynthesis. In this case, there is a reciprocal relationship between the calla lily and the aerobic organisms found in the leachate.

The ability of plants to remove nitrates and nitrites depends on the number of plants and the time of remediation. The faster the remediation time, the more plants are needed, if the remediation time used is long enough, the fewer plants will be (Nursagita & Titah, 2021). From this, it is in line with the results of this research that on days 1 and 3 the calla lily plants were more effective in reducing nitrate, namely those in the P2 with a plant weight of 400 grams which is heavier compared to the treatment of P1. Meanwhile, on day 7, the calla lily plants were more effective in reducing nitrate, namely the P1 weighing 200 grams. If it is on nitrite, namely in the P2 with a plant weight of 400 grams which is heavier compared to the treatment of P1. Meanwhile, on day 7, the calla lily plants were more effective in reducing nitrite, namely the P1 weighing 200 grams.

Plant density and biomass influence the effectiveness of reducing leachate nitrate and nitrite levels. Plants at low density, the evaporation process takes place more perfectly, thus creating better natural growth characteristics. Plants that are too close together tend to have slower evaporation and growth. This affects the process of nutrient absorption and photosynthesis (Ngirfani & Puspitarini, 2020). This is confirmed by research conducted by Herlambang & Hendriyanto (2015), showing that variations in plant weight or number of plants have an impact on reducing nitrite levels. The heavier or more numerous the plants are, the greater the decrease in nitrite levels. However, the greater the number of plants, the more plant competition will arise, that is, plant growth can be disrupted and over time they will wilt and even die.

The amount of water lily plant biomass in the P2 less effective in reducing nitrate and nitrite levels on day 7 due to an increase in organic matter in the leachate due to the influence of wilting water lilies. Some of the biomass of aquatic plants has died, causing an increase in aquatic organic matter. Plants do not directly absorb organic material, but aquatic plants provide conditions that allow the decomposition process of organic material to occur by microorganisms. Therefore, during the treatment there was a decomposition process that was still ongoing. So that aquatic plants will be less able to contribute significantly in reducing organic matter (Astuti & Indriatmoko, 2018). In addition, on day 1 and day 3 of the study, reducing nitrate and nitrite levels was less effective compared to day 7 because on day 1 and day 3 there was a time span of 2 days which could only be used by water lily plants to adapt to leachate so that

the calla lily plant tissue cannot accumulate nitrates and nitrites optimally. Calla lily which continuously absorb nitrates and nitrites in waste leachate will over time experience a decrease in their ability to absorb nitrates and nitrites. This is because calla lily plants have a limit or capacity to absorb nitrates and nitrites.

Physical and chemical environmental factors influence the survival of calla lily in the phytoremediation process, including temperature, light intensity and pH. pH and temperature measurements aim to determine the acidity and temperature conditions of an environment. Temperature is related to metabolic and photosynthesis processes, so an increase in temperature will indirectly affect the absorption rate. According to Ikawati et al. (2013) the higher the environmental temperature of the plant, the higher the level of absorption by the plant. This is confirmed by research by (Hidayat, 2011), which revealed that the higher the environmental temperature of the plant, the higher the level of absorption by the plant, where the environmental temperature will cause the photosynthesis process to increase, so that the absorption of nutrients by the plant will also increase. The denser the plants, the less light can enter the waste. This will affect the temperature of the leachate. The denser the plants, the lower the temperature of the water so that the photosynthesis process is reduced (Latifa & Pantiwati, 2021).

Light intensity measurements at the research location were carried out every day (during the research) at 08.00-09.00 WIB because during this time the plants were actively carrying out photosynthesis, which would later influence the nitrite absorption process by the calla lily. High light intensity causes the temperature of the leachate to increase, thereby accelerating the absorption of more metal ions (Monita et al., 2013). Increasing temperature can increase respiration which can indirectly increase energy production needed in the active transport process (MD, 2011).

On days 1 and 3 there was an increase in pH. The measurement results prove that phytoremediation using calla lily can increase the pH. This shows that there is a tendency for each treatment to reach a normal pH. The increase in pH in the phytoremediation treatment was due to the mineralization process of organic acids in the leachate. The results of the mineralization of organic acids from leachate waste will be nutrients that will be absorbed by plant roots, causing an increase in pH. The increase in pH is caused by the processes of photosynthesis, denitrification, breakdown of organic nitrogen and sulfate reduction (Fitriyah, 2011). However, on days 3 and 7 there was a decrease in pH. The decrease in pH is caused by the biodegradation process of organic materials. Dead plant parts of water lilies will be broken down by microorganisms in the

water, so that in the process of decomposing organic matter it will affect the pH value (Sari et al., 2017). The activity of degrading microorganisms allows a decrease in pH because organic compounds have been broken down into organic acids (Purnamawati et al., 2015). The cause of the decrease in pH can also be caused by the rotting of calla lily plant parts by the work of acid-producing microbes (Hapsari et al., 2016).

## Conclusion

Calla lily (*Zantedeschia aethiopica*) is effective as a phytoremediation agent in reducing nitrate levels in leachate wastewater in P1 day 7 (calla lily biomass 200 grams) with a reduction percentage of 45% and in leachate waste nitrite levels at P1 day 7 (calla lily biomass 200 grams) with a reduction percentage of 53%.

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## Author Contributions

Conceptualization, M. U., O. D. P. S., M. S., H.; methodology; M. U.; validation, M. S. H.; formal analysis, O. D. P. S.; investigation, M. S., H.; resources, O. D. P. S.; data curation, M. U.; writing – original draft preparation, M. S. H.; writing – review and editing: M. U.; visualization: M. S. H. All authors have read and agreed to the published version of the manuscript.

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## Conflicts of Interest

The authors declare no conflict of interest.

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