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Effect of Mercury Stress on the Growth and Lipid Content of *Euglena* sp. and *Echinodorus palaefolius*

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Abstract. *One way to reduce the adverse effects of the heavy metals mercury in the aquatic environment are using organisms to break down or convert toxic substances into non-toxic forms, either by phytore- mediation or phycoremediation. This research aimed to analyze the growth and lipid content of Euglena sp. after mercury exposure. This research also aimed to analyze the growth of E. palaefolius which is associated with Euglena sp. In this study, the bioremediation ability of Euglena sp. and Echinodorus palaefolius through treatment with mercury concentrations of 5 ppm, 10 ppm, 15 ppm, and 20 ppm, as well as association and non-association treatments. The parameters are the growth of Euglena sp. and the association between Euglena sp. andE. palaefolius measurement and lipid content. The result of the growth of Euglena sp. experienced a significant increase. Lipid content in Euglena sp. was also seen high at 10 ppm mercury concentration. In E. palaefolius, the ability to adsorb heavy metals was also shown by the large diameter of the stems and also the plant growth which has optimal growth in the treatment of 10 ppm mercury stress.*

Keywords: *Euglena sp., Echinodorus palaefolius, growth, mercury, stress*

Citation
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INTRODUCTION

Mercury (Hg) is a toxic metal that can cause adverse effects on the environment and the organisms inside. Mercury can be chemically changed, translocated, and accumulate in the atmosphere for 0.5 to 2 years. In general, Hg exists abundantly in three primary forms, namely elemental, organic, and inorganic. Inorganic mercury (Hg2+) can turn into organic mercury when it is present in an aqueous environment, where there is no oxygen through the methylation process. This makes mercury more dangerous because organic mercury has the highest toxicity among other forms of mercury. Organic mercury that accumulates in the aquatic environment can penetrate into the membranes and accumulate in the cells of aquatic organisms. Accumulation of mercury increase when organisms that

live in these waters are consumed by other organisms (Kumari et al. 2020; Danouche et al. 2021).

One of the methods to lessen the adverse effect of toxicants or pollutants in the environment is bioremediation. Bioremediation is a method that uses organisms to break down or change the form of a toxic substance into a non-toxic form. Phytoremediation is a technique for reducing pollutant toxicity in polluted environments using plants and their interactions with other organisms (Kumari et al., 2020). Meanwhile, phycoremediation is the reduction or biotransformation of harmful pollutants and chemicals using macro or microalgae (Azubuike et al., 2016).

Euglena sp. is a photosynthetic, microscopic, unicellular algae that lack of cell wall. *Euglena* sp. can carry out photosynthesis because it contains chloroplasts and can move with flagella. *Euglena* sp. can be used in various fields including nutrition, energy, health, and the environment. *Euglena* sp. lives in aquatic environments and can live in extreme conditions, such as heavy metal stressful habitats. There are several survival strategies of *Euglena* sp. when it is exposed to heavy metal stress. The mechanisms consist of internal metabolism by glutathione, phytochelatin, cysteine, and the production of several enzymes that function to neutralize ions from heavy metals. Under stress conditions like salinity stress, nitrogen deficiency, and heavy metal stress like Cd & Cr, the lipid content of microalgae increases (Suyono et al., 2015; Khatiwada et al., 2020; Yoshioka et al., 2020; He et al., 2021).

Echinodorus palaefolius is a semi-aquatic plant that comes from the Alismataceae family. *E. palaefolius* is a herbaceous plant, that has hollow stems, broad leaves, and fibrous roots (Nur & Slamet, 2020). This plant is considered capable as a phytoremediator because of its ability to absorb heavy metals. Besides that, *E. palaefolius* is easy to obtain, economical, and not consumed by people. It has a superior aspect compared to other plants in the purpose of phytoremediation by accumulating toxicants in specific organs so it does not interfere with the course of metabolism (Nur & Slamet, 2020). This plant can retain and accumulate mercury in the endodermis, passing through the Casparian band and entering the cell with an apoplast mechanism. The presence of high concentrations of heavy metal pollutants can even cause plants to show symptoms of chlorosis due to heavy metals being able to penetrate vascular tissue (Zhang et al., 2019). This research aimed to analyze the growth and lipid content of *Euglena* sp. and of *E. palaefolius* after mercury exposure.

MATERIALS AND METHODS

The research was done from September to October 2022 in the Biotechnology Laboratory, Faculty of Biology, Universitas Gadjah Mada, and Nogotirto Algae Park, Nogotirto, Gamping, Sleman. The research methods included cultivating *Euglena* sp., administering mercury treatment, measuring the growth of *Euglena* sp., and *E. palaefolius*, as well as lipid content in *Euglena* sp. Cultivation of *Euglena* sp. conducted at the Biotechnology Laboratory, Faculty of Biology, Universitas Gadjah Mada. Cultivation was carried out by growing *Euglena* sp. in CM medium (Cramer & Myers, 1952). The cultivation composition consisted of 100 mL of algae and 400 mL of nutrient medium. The culture was grown for seven days before being treated. Then various mercury treatments were given at Nogotirto Algae Park, Nogotirto, Gamping, Sleman. The treatment consisted of mercury concentrations of 0 ppm (T1), 5 ppm (T2), 10 ppm(T3), 15

ppm (T4), and 20 ppm(T5). The initial density of cultivated algae was 120,000 cells/ml. The association treatments between *Euglena* sp. and five individuals of *E. palaefolius* in one clump was carried out with0 ppm (T6), 5 ppm (T7), 10 ppm (T8), 15 ppm (T9), and 20 ppm (T10). The growth of *Euglena* sp. and *E. palaefolius* was measured on days 3 and 12. The growth of *Euglena* sp. was measured by the cell counting method manually with a haemocytometer modification from the methods of Suyono et al. (2015). The amount obtained was then analyzed with the following formula:

$$\text{Cell/mL} = \frac{\textit{Number of counted cells}}{N} \times 10^4$$

The growth of *E. palaefolius* was measured by measuring stem height, number of stems, and stem diameter. Lipid levels were measured on day 12 using the Bligh & Dyer (1959) method. *Euglena* sp. cells were harvested by centrifugation, added methanol, and chloroform solvents then homogenized. The sample was then added to distilled water, homogenized, and centrifuged. The supernatant was taken, the lipid at the base was taken and incubated until the solvent evaporated, and then the lipid content in the sample was weighed. Quantitative data were analyzed by analysis of variance (ANOVA) at the 95% level to determine the effect of the treatment and continued with the Duncan Multiple Range Test (DMRT) at the 95% level if there was a significant difference. The data analysis was carried out using Ms Excel and SPSS 2.0 software.

RESULTS AND DISCUSSION

Based on Figure 1, growth for all groups has increased significantly, and the treatment gave the density of *Euglena* sp. to grow more. The highest density was found in group T1, namely the control with a density of 597.500 cells/mL on the 12th day of observation. Meanwhile, the lowest density was in T9 and T10 on 3rd day of treatments with a density of 0 cells/mL.

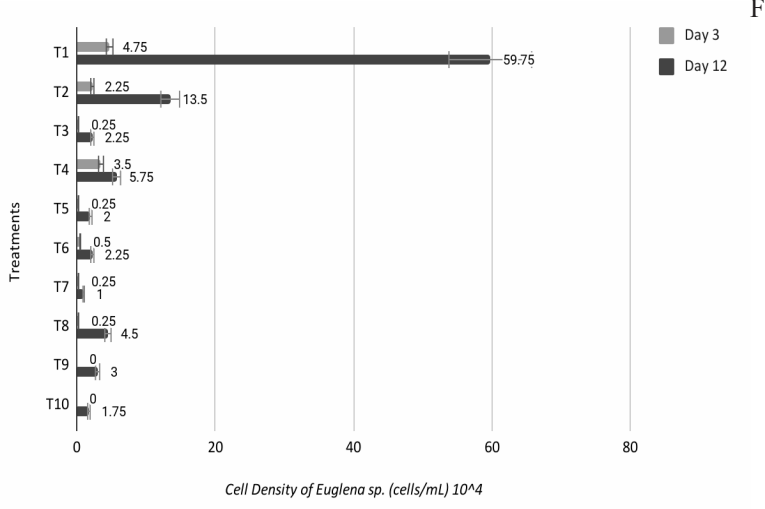

Figure 1. T1= Treatment of 0 ppm Hg in *Euglena* sp., T2= Treatment of 5 ppm Hg in *Euglena* sp., T3= Treatment of 10 ppm Hg in *Euglena* sp., T4= Treatment of 15 ppm Hg in *Euglena* sp., T5= Treatment of 20 ppm Hg in *Euglena* sp., T6= Treatment of 0 ppm Hg in association between *Euglena* sp. and *E. palaefolius*., T7= Treatment of 5 ppm Hg in association between *Euglena* sp. and *E. palaefolius*., T8= Treatment of 10 ppm Hg in association between *Euglena* sp. and *E. palaefolius*., T9= Treatment of 15ppm Hg in association between *Euglena* sp. and *E. palaefolius*., T10= Treatment of 20 ppm Hg in association between *Euglena* sp. and *E. palaefolius*

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The measurement result showed that the control treatment has the highest number of cells compared to other treatments both in the control of *Euglena* sp. and in the control association *Euglena* sp. with *E. palaefolius*. In the control treatments, *Euglena* sp. can grow well because there is an optimal condition for cell division (Suyono et al. 2015).

Figure 2 shows that the diameter of the *E. palaefolius* stems still increases despite exposure to mercury stress. The highest in- crease in stem diameter was obtained in treat- ment T8 (10 ppm) by 0.1 cm, and the low- est increase in diameter was found at T9 (15 ppm) by 0.03 cm. Meanwhile, in T7 (5 ppm) treatment, the diameter stem is decreasing from 0.25 cm to 0.20 cm.

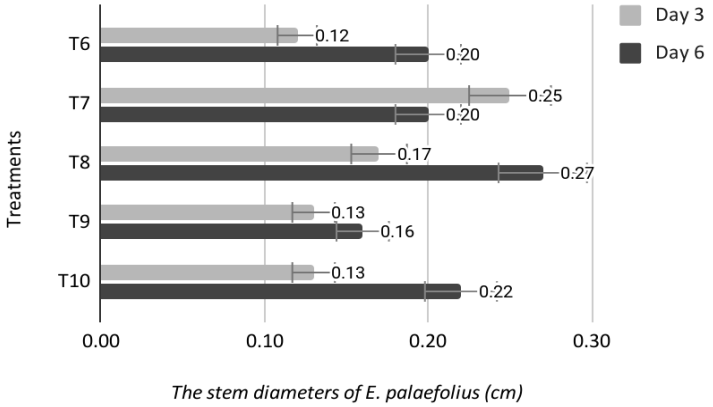

Figure 2. T6= Treatment of 0 ppm Hg in association between *Euglena* sp. and *E. palaefolius*., T7= Treatment of 5 ppm Hg in association between *Euglena* sp. and *E. palaefolius*., T8= Treatment of 10 ppm Hg in association between *Euglena* sp. and *E. palaefolius*., T9= Treatment of 15ppm Hg in association between *Euglena* sp. and *E. palaefolius*

Under conditions of stress on heavy metals, aquatic plants such as *E. palaefolius* will experience changes in morphological and anatomical structures, one of which can be seen from the size of the stem diameter. Changes in stem diameter can occur due to the role of the stem as a distributor of water and nutrients to other organs. When the water being transported contains mercury, the rods will act as a mechanism to reduce the levels of mercury present (Claro et al., 2009). This mechanism affects the anatomical structure of plant stems. Anatomical differences can be seen in the metaxylem diameter and cortical thickness. The cortical cells of stressed aquatic plants will show an increase. Further more, there was a significant increase in the aerenchyma of aquatic plants under heavy metal stress and metaxylem. Plants do this to accumulate heavy metals, so they do not go further and become toxic to plants (Batool et al., 2014). The trunk of plants has a peripheral parenchyma cell layer that acts as an assimilator because it contains chloroplasts (Claro et al., 2009).

Based on the study's results, it was known that there was an increase in stem diameter in all treatments. The largest increase in diameter occurred in the T8 treatment (10 ppm). In comparison, the minor increase/decrease in diameter occurred in the T9 treatment (15 ppm). The results obtained are par-

tially in accordance with the existing theory, the more mercury stress given, the diameter increase. The trend of stem diameter growth shows a decrease with increasing mer- cury concentration.

Based on Figure 3, it is known that the treatment data that always shows a significant growth in height is the *E. palaefolius* plant grown in the control treat- ment (T6), which is able to show a height of 40 cm on day 12th. In the treatment T7, the plant actually showed a significant decrease, so that at the last harvest, the plant height was 21 cm. Plants in the T8, T9, and T10 treatments showed a positive or increasing trend, even though the growth rate was relatively slow.

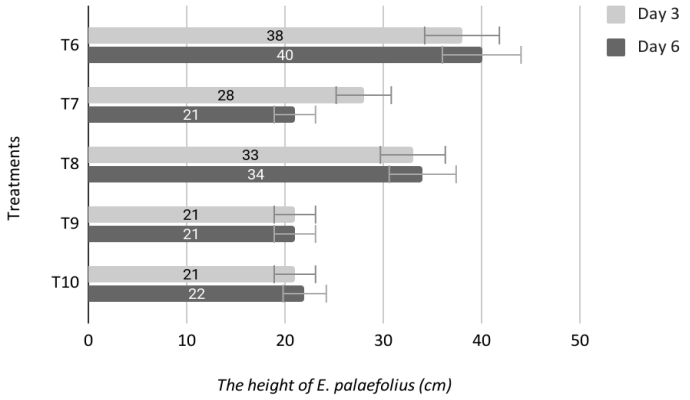

Figure 3. T6= Treatment of 0 ppm Hg in association between *Euglena* sp. and *E. palaefolius*., T7= Treatment of 5 ppm Hg in association between *Euglena* sp. and *E. palaefolius*., T8= Treatment of 10 ppm Hg in association between *Euglena* sp. and *E. palaefolius*., T9= Treatment of 15ppm Hg in association between *Euglena* sp. and *E. palaefolius*

The presence of mercury as a pollutant affects the plant's height. Heavy metals can cause inhibition of cell division and elon- ga- tion, absorption of water and nutrients, and enzymatic activity causing inhibition of growth rate. Hg accumulation in plants can in- hibit root and shoot growth (Hamim et al., 2019). *E. palaefolius* can still maintain itself to stay alive even though it is grown in mercury stress, so it can be said that this plant can carry out phytoremediation. Even so, the level of concentration of mercury exposure also needs to be considered. If the plants are in conditions where the mercury concentration exceeds the plant's tolerance ability, this will cause the plants to die. *E. palaefolius* can accumulate heavy metals in specific organs, such as roots, which will be translocated to the air tissue and enter the xylem, so it does not inhibit plant metabolism (Nur & Slamet, 2020). If the pollutant load is very high, it causes metabolic disturbances and can cause the death of these plants one by one . Giving pollutant concen- trations that are too high for a long time can cause chlorosis in plants, inhibiting chloro- phyll synthesis. Heavy metals as pollutants can also inhibit the action of enzymes that catalyze chlorophyll synthesis (Nur & Slamet, 2020). If plant metabolism is disrupted for a long term, it will inhibit the growth of the plant and will fatally cause death in the plant.

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The lipid content of *Euglena* sp. living at various mercury stresses in the FWS-CW system can be seen in Figure 4. Treatment T0 until T5 are 0.067 mg/ml, 0.033 mg/ml, 0.111 mg/ml, 0.047 mg/ml and 0.029 mg/ml. The highest lipid levels were in the 10 ppm treatment, and the lowest was in the 20 ppm mercury concentration treatment. The trend graph for lipid levels in *Euglena* sp. tended to decrease with increasing concentration of mercury solution. Based on Duncan's test, it was known that the differences between treatments were not significantly different. This shows that differences in mercury concentrations did not cause a significant decrease in lipid content in *Euglena* sp.

Meanwhile in association with *E. palaefolius*, the highest lipid levels were found in the 5 ppm mercury treatment (T7) and the lowest in the 20 ppm mercury treatment (T10) (Figure 5). The lipid content from T6 until T10 was 0.153 mg/ml, 0.178 mg/ml, 0.029 mg/ml, 0.02 mg/ml, and 0.007 mg/ml. The graphic trend of lipid content in *Euglena* sp. decreased with increasing concentration of mercury solution. This shows the 5 ppm in

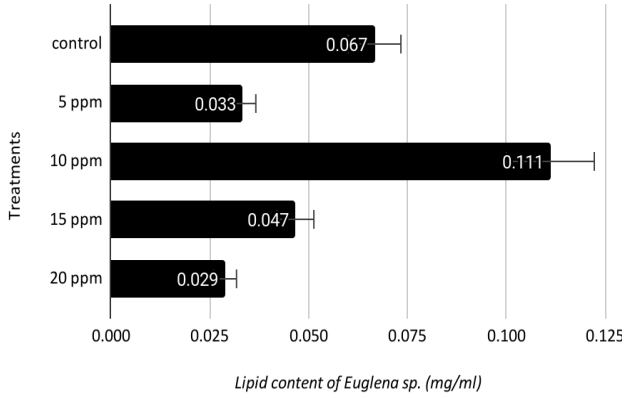

Figure 4. Lipid content of *Euglena* sp. in the variation of mercury stress

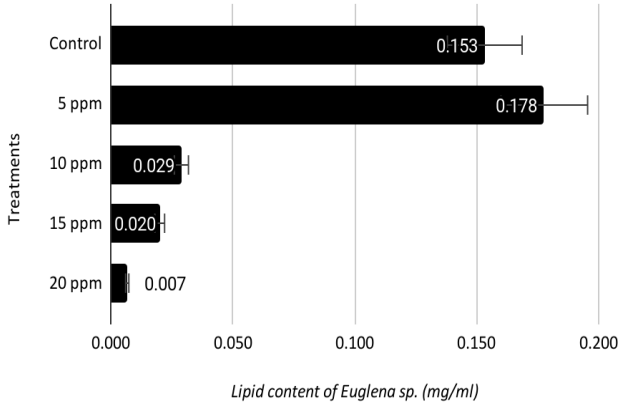

Figure 5. Lipid content of *Euglena* sp. that growth associated with *E. palaefolius* in the variation of mercury stress

the association treatment gave more optimum lipid content than others.

Lipids in microalgae generally increase with increasing stress. Lipid levels can increase after salinity stress, nitrogen deficiency, and heavy metals such as Cd, and Cr. This is because microalgae will produce lipids in large quantities to prevent water from escaping from the microalgae cells. In addition, lipids are also used as cell protectors, because they are constituents of cell membranes, so when stressed, they are produced in large quantities so that the cells remain viable. However, an increased stress concentration that is too high can also cause the lipid levels in microalgae to decrease. This can be because heavy metal stress can cause the formation of ROS, which causes oxidative degradation of lipids so that the lipid struc- ture is damaged. In the results of research on *Euglena* sp. living alone lipid levels lower as the stress concentration increases. This could be due to the higher stress concentration, and the lower number of *Euglena* sp. cells (Rocchetta et al., 2012; Suyono et al., 2015; Chen et al., 2017; Tossavainen et al., 2019; He et al., 2021).

CONCLUSION

Euglena sp. and *E.palaefolius* are known to have the potential as bioremediation agents against mercury pollutants. However, mercu- ry levels that exceed the tolerance limits of both, can stop the growth rate of *Euglena* sp. and *E. palaefolius*, and can be lethal. The 5 ppm mercury in the association treatment gave more optimum lipid content than others. The overall best plant height was in the treat- ment of 10 ppm mercury.

AUTHOR CONTRIBUTION

The contribution of each author in this research is W.E.W. measures the density of *Euglena* sp., D.H.T. measures the lipid content, M.N.R.A. measured the height of the plants; H.N.A. measures the diameter of the stem, D.U.S. analyzed plant height data, T.E. analyzes data of density on *Euglena* sp., K.Q.M. analyzed stem diameter data; R.A. analyzed the lipid content data, D.K. discussed the relationship between the four data and E.A.S. research design and manuscript writing and review.

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CONFLICT OF INTEREST

There is no conflict of interest in this team of the research.

REFERENCES

Azubuike, C. C., Chikere, C. B. & Okpokwasili, G. C. (2016). Bioremediation Techniques-classification Based on Site of Application: Principles, Advantages, Limitations and Prospects. *World J Microbiol Biotechnol,* 32(11), 180. DOI: 10.1007/s11274-016-2137-x.

Batool, R., Hameed, M., Ashraf, M., Fatima, S., Nawaz, T. & Ahmad, M. S. A. (2014). Structural and Functional Response to Moioioietal Toxicity in Aquatic *Cyperus alopecuroides* Rottb. *Limnologica*, 48, 46-56. DOI: 10.1016/j.limno.2014.06.002.

Bligh, E. G. & Dyer, W. J. (1959). A Rapid Method of Total Lipid Extraction and Purification. *Can J Biochem Physiol*. 37(8), 911-7. DOI: 10.1139/o59-099.

Chen, B.,Wan, C., Mehmood, M. A., Chang, J. S., Bai, F. & Zhao, X. (2017). Manipulating Environmental Stresses and Stress Tolerance of Microalgae for Enhanced Production of Lipid and Value-Added products-A review. *Bioresour.Technol*. 244, 1198–1206. DOI: 10.1016/j.biortech.2017.05.170.

Danouche, M., El Ghachtouli, N. & El Arroussi, H. (2021). Phycoremediation Mechanisms of Heavy Metals Using Living Green Microalgae: Physicochemical and Molecular Approaches for Enhancing Selectivity and Removal Capacity. *Hyelon*, 7(7), e07609. DOI: 10.1016/j.heliyon.2021.e07609.

Claro, K. C., Oliveira, P. S. & Rico-Gray, V. (2009). *Tropical Biology and Conservation Management-Volume V: Ecology.* EOLSS Publications.

Hamim, H., Mutyandini, A., Sulistyaningsih, Y. C., Putra, H. F., Saprudin, D. & Setyaningsih, L. (2019). Effect of mercury on Growth, Anatomy and Physiology of Four Non-edible Oil-producing Species. *Asian Journal of Plant Sciences*, 18(4), pp.164-174. DOI: 10.3923/ajps.2019.164.174.

He, J., Liu, C. C., Du, M., Zhou, X., Hu, Z.,

Lei, A. & Wang, J. (2021). Metabolic Responses of a Model Green Microalga *Euglena gracilis* to Different Environmental Stresses. *Frontiers in Bioengineering and Biotechnology*, 9, 1–12. DOI: 10.3389/fbioe.2021.662655.

Kumari, S., Amit, Jamwal, R., Mishra, N. & Singh, D. K. (2020). Recent Developments in Environmental Mercury Bioremediation and its Toxicity: A Review. *Environmental Nanotechnology, Monitoring & Management*. 13, 100283. DOI: 10.1016/j.enmm.2020.100283.

Nur, F. & Slamet, I. (2020). The phytoremediation of *Echinodorus palaefolius* (Water Jasmine) in Reducing BOD and COD of Liquid Waste-Batik Industry" X" in Pekalongan. *GSC Biological and Pharmaceutical Sciences,* 12(3), 215-222. DOI:10.30574/gscbps.2020.12.3.0303.

Rocchetta, I., Mazzuca, M., Conforti, V., Balzaretti, V. & Molina, M. D. C. R. D. (2012). Chromium Induced Stress Conditions in Heterotrophic and Auxotrophic strains of *Euglena gracilis. Ecotoxicol. Environ*. Saf. 84, 147–154. DOI: 10.1016/j.ecoenv.2012.07.020

Suyono, E. A., Haryadi, W., Zusron, M., Nuhamunada, M., Rahayu, S. & Nugroho, A. (2015). The Effect of Salinity on Growth, Dry Weight and Lipid Content of the Mixed Microalgae Culture Isolated from Glagah as Biodiesel Substrate. *Journal of Life Sciences*, 9, 229-233. DOI:10.17265/1934-7391/2015.05.006

Tossavainen, M., Ilyass, U., Ollilainen, V., Valkonen, K. & Romantschuk, M. (2019). Influence Oflong Term Nitrogen Limitation on Lipid, Protein and Pigment Production of *Euglena gracilis* in Photoheterotrophic Cultures. *PeerJ*. 7:6624. DOI: 10.7717/peerj.6624.

Zhang, P., Li, Qinfeng, Z. & Chaolan., (2019). Root Adaptation in *Echinodorus Osiris* Rataj Plant Under Cadmium Stress. *International Journal of Phytoremediation*, 1–6. DOI: 10.1080/15226514.2019.1686605.