

## RESEARCH ARTICLE

# Identifying prospective water plants for reducing nutrients and total coliform in sub-surface constructed wetlands with limited oxygen

Ridwan Muhamad Rifai<sup>1</sup>, Anie Yulistyorini<sup>1\*</sup>, Jenvia Rista Pratiwi<sup>1</sup>, Muhammad Aris Ichwanto<sup>2</sup>

<sup>1</sup> Study Programme of Environmental Engineering, Faculty of Engineering, The State University of Malang, Malang 65145, Indonesia

<sup>2</sup> Study Programme of Building Engineering Education, Faculty of Engineering, The State University of Malang, Malang 65145, Indonesia

**Abstract:** Extensive aeration is required for constructed wetlands (CWs) since dissolved oxygen is essential in nutrients and pathogen removal. On the other hand, plants typically used in CWs can release oxygen into the system, lowering the need for external aeration. Hence, this study tried to uncover the oxygenation rate of three species of plants in the CWs system with limited oxygen and their capability to treat wastewater. *Canna sp.*, *Heliconia sp.*, and *Typha sp.* were used and compared to uptake nutrients and reduce coliform numbers in domestic wastewater using sub-surface CWs in low levels of initial oxygen. In the meantime, oxygen release from the plant root was monitored in real-time using an IoT-based module. On the detention time of 24 hours, CWs planted with *Typha sp.* were able to reduce 61%  $\text{NH}_4^+$  and 71%  $\text{PO}_4^{3-}$ . The system was also able to remove coliform with a magnitude of 1.39 log units. In addition, *Typha sp.* was observed to generate a higher oxygenation rate to the CWs system compared to the other two plants at 0.175 %/h, on average. These observations suggested that *Typha sp.* had the best prospect to be used in a sub-surface CWs system with the least external aeration needed.

**Keywords:** Absence of oxygen, coliforms, nutrients, sub-surface constructed wetlands, water plants

**Correspondence to:** Anie Yulistyorini, Departement of Civil Engineering and Planning, Faculty of Engineering, Universitas Negeri Malang, Malang 65145, Indonesia; E-mail: [anie.yulistyorini.ft@um.ac.id](mailto:anie.yulistyorini.ft@um.ac.id)

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## 1 Introduction

Water pollution is a severe concern in developing countries such as Indonesia. Climate change, anthropogenic consequences, and population growth have affected water ecosystems (Bienfang et al., 2011). Globally, 10% of the world's population faces the risk of clean water contamination (Yesilay et al., 2023). Environmental quality deterioration and water-borne diseases remain the main issues in Indonesia since approximately 5% and 14% of black water in urban and rural areas, respectively, along with 51-53% of grey water, is discharged directly to the environment (Sururi et al., 2023; Widayarani et al., 2022).

Excreta from animals and humans consist of pathogenic bacteria that pollute the aquatic environment, but this contaminated water is still used for household needs such as bathing or laundry (Sururi et al., 2023). Untreated wastewater also causes eutrophication due to the presence of suspended nutrients in wastewater that lead to the reduction of dissolved oxygen in water bodies (Sengupta et al., 2015). Therefore, it is critically important to provide sanitation facilities for wastewater treatment and to prevent the water pollution of clean water resources.

Sustainable wastewater technology, which has high effi-

ciency in pollutant removal but less cost in operation and maintenance, is in demand in Indonesia. Constructed wetlands (CWs) are engineered nature-based technology systems designed to optimise the biodegradation process found in a natural environment and are considered sustainable technology for wastewater treatment (Dotro et al., 2021). CWs are also considered a low-cost and practical design for decentralised wastewater treatment and are suitable for implementation in developing countries (Kadlec et al., 2008). Although CWs need less energy than other wastewater treatment technologies, CWs' application for wastewater treatment requires more extensive land and is challenging to implement in urban areas.

Recently, new designs of CWs with extensive aeration have been developed to overcome the limitations of wastewater treatment, especially on land requirements. Numerous aeration strategies, such as continuous and intermittent aeration, have been applied to enhance dissolved oxygen (DO) in the sub-surface constructed wetlands (John et al., 2020). Increasing DO could enhance the removal of nitrogen and biodegradation of organic matter in CWs. A previous study showed that enhancing DO in CWs improved the removal of COD by up to 94%, TN for 92%, TP for 52%,  $\text{NH}_4^+ - \text{N}$  for 98%, and up to 100% for  $\text{NO}_3^- - \text{N}$  (John et al., 2020;

Wu et al., 2014). However, the additional energy and equipment needed to enhance aeration in CWs is considered quite expensive in Indonesia.

On the other hand, plants are known for releasing oxygen, including in the CWs system. The oxygen provided by the plant was observed to reduce 300.37 mg COD/m<sup>2</sup>.d and provide 0.43–1.12% of BOD (Zhang et al., 2014). Several ornamental plants have been used in CWs due to their performance in reducing pollutants and increasing wastewater quality. *Canna* and *Heliconia* were observed to reduce COD at 0.283 and 0.271 mg/d, respectively (Konnerup et al., 2009). In another study, *Heliconia* showed average removal efficiency for COD, NH<sub>4</sub><sup>+</sup>, and PO<sub>4</sub><sup>3-</sup> as much as 86.8%, 96.4%, and 57.0%, respectively (Almeida-Naranjo et al., 2020). Meanwhile, another ornamental plant, *Canna*, was observed to provide pollutant efficiency removal of 71.34%, 69.34%, and 69.67% for COD, NO<sub>2</sub><sup>-</sup>, and PO<sub>4</sub><sup>3-</sup>, respectively (Zorai et al., 2023).

Unfortunately, limited research has been conducted to observe the released oxygen from common plants used in CWs. Moreover, suppose the oxygen is measured in real-time with the support of Internet of Things (IoT) technology. Therefore, this study aims to observe the real-time oxygen release rate of several common ornamental plants used in CWs and identify which provides the best reduction rate of organic pollutants. The focus was set on pathogen bacteria and nutrient wastewater removal using sub-surface constructed wetlands (SSFCWs) in the absence of the addition of aeration. Eventually, the most prospective plant that can be used in low-aeration CWs will be identified, yet it will provide the most exceptional pollutant removal rate.

## 2 Materials and Methods

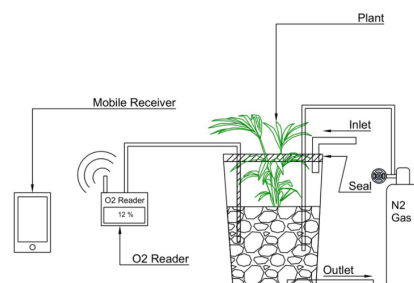
### 2.1 IoT-based O<sub>2</sub> Sensor Assembly

Oxygen partial pressure was measured using an online monitoring system based on an IoT system. The system was arranged on a System on A Chip (SoC) MCU-based IC ESP32 Devkit with Wi-Fi connectivity. An electrochemical-based O<sub>2</sub> sensor (DFRobot SENO322) was embedded in the SoC and coded to generate output as O<sub>2</sub> partial pressure. Real-time reading of O<sub>2</sub> was sent to a spreadsheet through the internet and can be read through a web-based application.

### 2.2 CWs Module Design

The CWs module design was developed according to a previous study (Zhang et al., 2014). Four CWs were constructed using a 45.5 L container filled with expanded clay aggregate as the plant media. Ornamental plants, i.e., *Typha* sp., *Heliconia* sp., and *Canna* sp., were obtained domestically and used for treatment in three containers. Meanwhile, another container was used as a control without any additional plants. The IoT-based O<sub>2</sub> sensors were installed in each container with a depth of around 10 cm beneath the clay surface where the plant root is located. During the operation, containers were supplied with nitrogen gas to deplete the oxygen until

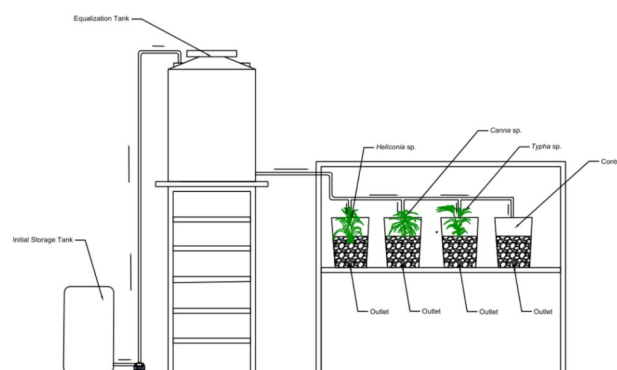
it reached a value of 1.62±1.30%. Containers were sealed using parafilm and petroleum jelly to prevent external oxygen from penetrating the system. CWs module can be seen in Figure 1.



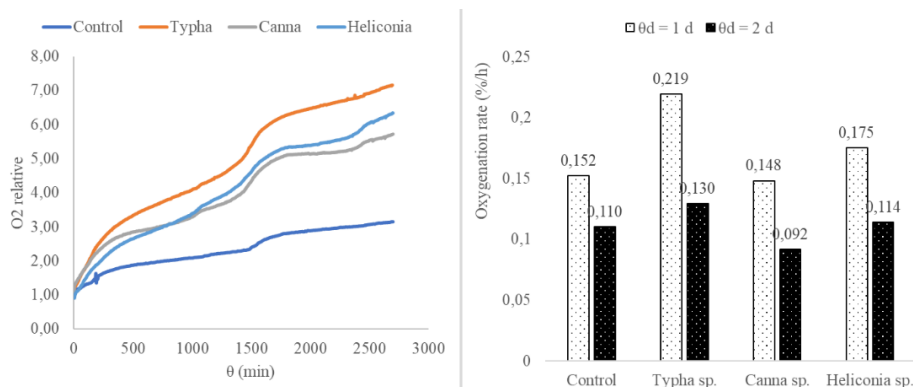
**Figure 1.** Illustration of CWs module and IoT system used in the experiment

### 2.3 Wastewater Quality Analysis

Domestic wastewater consisting of black and greywater was supplied to each container with a flow rate of 1.25 and 0.625 m<sup>3</sup>/s, respectively, for detention time ( $\Theta_d$ ) of 1 and 2 days. Before treatment, wastewater was collected in a storage tank and pumped to a vessel to equalise the flow so the desired flow rate was attained. The scheme of CW installation can be seen in Figure 2. Raw (system inlet) and treated (system outlet) wastewater samples for each  $\Theta_d$  were analysed for pH, dissolved oxygen (DO), ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N), orthophosphate (PO<sub>4</sub><sup>3-</sup>-N), and total coliform using the standard method of wastewater examination (American Public Health Association, 2017). On the other hand, while the treatment was operating, real-time oxygen partial pressure increase rates were filed and monitored. The wastewater and operation of the CWs were analysed for four consecutive weeks.



**Figure 2.** Scheme of CWs used in the experiment



**Figure 3.** Comparison of the oxygenation rate of *Typha sp.*, *Canna sp.*, and *Heliconia sp.*

## 2.4 Performance Analysis

The value of water quality parameters between the inlet and outlet were compared and analysed to determine the nutrient removal efficiency of each plant. Also, the partial pressure increase of each plant was analysed to determine the best-performing CW system. All data were then calculated using multivariate analysis using the Multi-Variate Statistical Package (MVSP ver. 3.1) to generate the most prospective plants for CWs regarding nutrient removal and oxygen production.

## 3 Results and Discussions

### 3.1 Oxygenation Rate and Nutrients Uptake by Plants

An oxygenation rate difference was observed among plants along with detention time, as seen in Figure 3. The average oxygenation rate was higher on the first than on the second day. According to Dalton's law of partial pressure, this might result from oxygen intrusion into the system, where the oxygen with higher partial pressure in the surrounding environment moves into the system. In addition, referring to Henry's law, the oxygen then penetrated the system at a relatively higher rate since there was no oxygen initially (Hua et al., 2017). For both detention time  $\Theta_d = 1$  d and 2 d, *Typha sp.* showed the highest oxygen release rate into the CWs system with average values of 0.219 and 0.130 %/h, respectively. By neglecting the oxygenation rate caused by atmospheric oxygen penetration into the system, the pure oxygenation rate provided by plants was corrected to 0.067 and 0.020 %/h for detention time  $\Theta_d = 1$  d and 2 d, respectively.

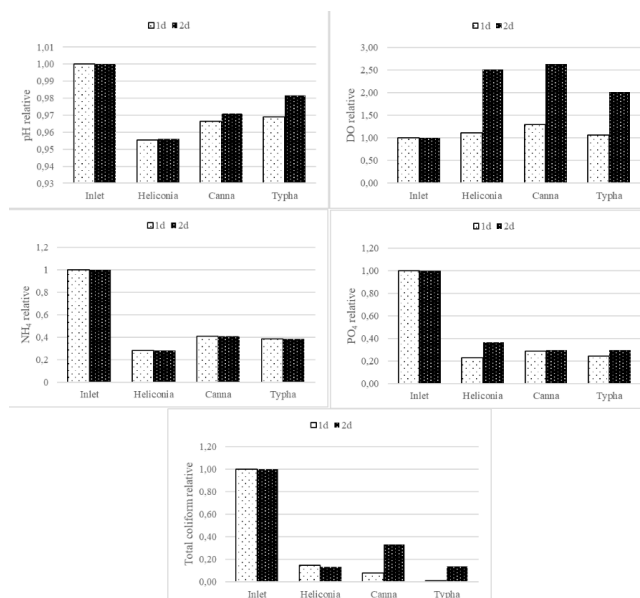
The oxygenation rate generated from *Typha sp.*, *Canna sp.*, and *Heliconia sp.* plant roots links directly to the average DO concentration measured in each system, which were 2.56 mg/L, 3.1 mg/L, and 2.89 mg/L, respectively. These DO levels could stimulate phosphorus removal of 73%, 69.5%, and 69%, respectively, for three plants. The DO level of 2.74–5.66 mg/L improved the phosphorus removal from 23–81% (Chand et al., 2021). (Biswas and Patidar, 2022) reported

that *Typha angustifolia* in CWs plays an essential role in total phosphorous removal because it has a high capacity for nutrient uptake.

Figure 4 compares the inlet and outlet of CWs for respective plants. At  $\alpha = 0.05$ , no significant pollution reduction difference between  $\Theta_d = 2$  d compared to  $\Theta_d = 1$  d for parameter  $\text{NH}_4^+ - \text{N}$  ( $p\text{-val} = 0.361$ ) and  $\text{PO}_4^{3-} - \text{P}$  ( $p\text{-val} = 0.137$ ). This pattern was also observed for coliform reduction ( $p\text{-val} = 0.126$ ). These observations indicated that  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ , and coliform removal occurred mainly on the first day when oxygen concentration was lower than the second. The additional oxygen concentration in regards to prolonging the detention time appeared not to provide a significantly higher removal rate of each pollutant. At this point, we can assume that there was a correlation between lower oxygen concentration in the system, the urge of plants to produce oxygen and uptake nutrients, and the removal rate of nutrients and coliform. Nonetheless, the dissolved oxygen was significantly higher on the  $\Theta_d = 2$  d compared to  $\Theta_d = 1$  d ( $p\text{-val} < 0.050$ ), showing plants' capability to release oxygen over time, indicating the pollutant removal process was still occurring.

### 3.2 Coliform Removal by CWs System

The total coliform number reduction ability was observed in each CW respective to the plant used for treatment. On average, the CWs system was able to reduce total coliforms from their initial numbers by as much as 85.65%, 86.43%, and 95.93% for systems planted using *Heliconia sp.*, *Canna sp.*, and *Typha sp.*, respectively. These numbers equal 0.84, 0.87, and 1.39 log reduction units. *Typha sp.*'s performance was superior to the others in reducing coliform numbers. *Typha sp.* can reduce total coliforms from such wastewater. A coliform reduction of 45% (5.80 log units) was reported from a system using *Typha latifolia* in zeolite substrate, treating a mixture of industrial and domestic wastewater for a maximum detention time of three days (Hamad et al., 2020). Another report showed that *Typha sp.* planted in gravels and boulders reduced 86.17% (0.86 log units) from domestic wastewater with a maximum detention time of 216 days (Chand et al.,



**Figure 4.** Various pollution removal and dissolved oxygen increase among plants with detention time difference.

2021).

### 3.3 Weighing of Best-Performing Plants

As described previously, since oxygen plays the leading role in determining the  $\text{NH}_4^+$  removal via aerobic nitrification in anoxic systems such as CWs, the dissolved oxygen concentration directly defines the system's capability to lower the  $\text{NH}_4^+$  concentration in wastewater (Vymazal, 2007). In addition, the higher the dissolved oxygen concentration is, the more  $\text{PO}_4^{3-}$  removal occurs due to using oxygen as an electron acceptor by phosphorous-accumulating bacteria (Liu et al., 2016). This feature has also been observed in coliform reduction since high dissolved oxygen causes oxidative stress on coliforms, eventually inhibiting their growth (Baez et al., 2013). Therefore, despite no significant difference in pollutant removal rate between  $\Theta_d = 1$  d and  $\Theta_d = 2$  d, the dissolved oxygen concentration for  $\Theta_d = 2$  was significantly higher than  $\Theta_d = 1$  d. Hence, according to the data set on the second day, the best-performing plant was considered. Table 1 shows the weighing of the performance of each plant concerning the most optimum nutrient and coliform removal, pH stabilisation, and oxygen supply.

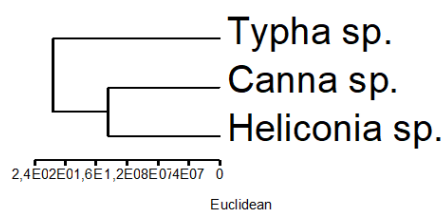
A multivariate analysis was conducted to confirm the weighing by analysing the difference between pollutant con-

centration and oxygen constituents between treated and untreated wastewater among plants. The Euclidian distance-based analysis showed that *Typha sp.* had a distinctive pollutant concentration and oxygen constituents on  $\Theta_d = 2$  d. This confirmed that *Typha sp.* was the most distinctive species among the three and can be deduced as the best-performing plant for reducing organic pollutants by releasing oxygen the most.

## 4 Discussions and Conclusions

Regarding nutrient removal, previous research showed that total nitrogen removal was higher in the constructed wetland bed planted with *Canna* than in *Heliconia* because of the higher growth rate of *Canna* (Konnerup et al., 2009). However, both plants can be used to enhance the aesthetic appearance of the wetlands as wastewater treatment. In vertical flow constructed wetlands, *Typha latifolia* gives the best efficiency in pollutant removal, especially of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  with percentages of 97.93% and 76.97%, respectively, while for *Canna indica* were 96.57% and 69.34%, respectively. The removal of  $\text{PO}_4^{3-}$  was higher in the wetland with *Canna indica* than in *Typha latifolia*, with an efficiency of 69.67% (Zorai et al., 2023).

In our study, three types of plants, *Typha sp.*, *Canna sp.*, and *Heliconia sp.*, revealed no significant difference in pollutant removal with  $\Theta_d$  of 1 d and 2 d. On the other hand, *Heliconia sp.* showed higher effectiveness in removing  $\text{NH}_4^+$  compared to the other plants but lower removal on  $\text{PO}_4^{3-}$ . *Canna sp.* and *Typha sp.* demonstrated similar abilities in removing  $\text{PO}_4^{3-}$  from the wastewater, i.e. 71% removal or 35.99 mg/L. On the other hand, *Heliconia sp.* removed 69% (35.23 mg/L)  $\text{NH}_4^+$  from the influent, while *Canna sp.* and *Typha sp.* removed  $\text{NH}_4^+$  for 61% (28.84 mg/L) and 66% (30.09 mg/L), respectively. Nitrogen uptake by water plants in CWs varied among the types of plants, with



**Figure 5.** Best-performing plant species in line with multivariate analysis

**Table 1.** Overall weight of plant species performance

Plant Species	pH	DO	$\text{NH}_4^+$	$\text{PO}_4^{3-}$	Coliform	$\text{O}_2$ Rate	Overall Weight
<i>Canna sp.</i>	++	+++	+	+++	+	+	+
<i>Heliconia sp.</i>	+	++	+++	++	+++	++	++
<i>Typha sp.</i>	+++	+	++	+++	++	+++	+++



a magnitude of 0.5–40% of the total nitrogen (Saeed and Sun, 2012). The roots stems, and leaves of the plant absorbed inorganic nitrogen ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ) (Preiner et al., 2020) and organic nitrogen (amino acids, nucleotides) (Moreau et al., 2019) from water columns and converted into their constituent substances (Zhang et al., 2023). Nitrification and denitrification by microorganisms also remove nitrogen from wastewater, ranging from 55–95% in CWs (Zhang et al., 2023).

Based on (Lasari et al., 2015) research, *Canna sp.* and *Heliconia sp.* effectively remove pollutants such as ammoniacal nitrogen, chemical oxygen demand, and total nitrogen from domestic wastewater. In addition, the effectiveness of *Canna sp.* and *Heliconia sp.* was also found in domestic wastewater treatment in constructed wetland technology. *Canna sp.* and *Heliconia sp.* can also effectively remove substances such as ammonium nitrogen, COD, and total nitrogen from domestic wastewater. However, oxygen depletion in water can affect the removal efficiency of pollutants and coliforms. High concentrations of BOD and COD can lead to oxygen depletion, affecting the removal process of pollutants in waters Almeida-Naranjo et al., 2020).

*Typha sp.*, particularly *Typha angustifolia* and *Typha latifolia*, is used in treating domestic wastewater due to its effectiveness in removing BOD and COD of domestic wastewater and convert total nitrogen into nitrate, thus helping in wastewater treatment (Kadaverugu et al., 2016). Research has shown that *Typha angustifolia* is efficient in removing heavy metal ions, with a removal efficiency of 77.85% in five days, making it a promising method for wastewater treatment (Cristescu et al., 2018). In the treatment of domestic wastewater, *Typha latifolia* has been found to play a significant role in the removal of biological oxygen demand (BOD) and chemical oxygen demand (COD) from the wastewater, as well as in the conversion of total nitrogen into nitrates, thereby contributing to the treatment of wastewater intended for reuse in agriculture (Kadaverugu et al., 2016). Additionally, using *Typha latifolia* in constructed wetlands has been proposed as an eco-friendly technology for removing heavy metals and enteric bacteria from wastewater (Hamad et al., 2020). Therefore, *Typha sp.* shows promise in treating domestic wastewater due to its ability to remove pollutants and heavy metal ions, making it an efficient and cost-effective method for wastewater treatment.

This experiment demonstrated a higher coliform removal number which was probably related to a more suitable climate for *Typha sp.* in removing coliform and using expanded clay aggregate as substrate. Several previous studies have studied the mechanism of *Typha sp.* in reducing coliform numbers. According to the result of this experiment, plant roots release oxygen to the rhizosphere. This condition makes the environment unfavourable for coliform (Kipasika et al., 2016). In addition, oxygen may facilitate predation activities on these bacteria (Kipasika et al., 2016; Shingare et al., 2017). The root of *Typha sp.* itself has been investigated

to possess such an antibacterial property, limiting coliform growth (Shingare et al., 2017). Plant root has also been known for being colonised by some indigenous endophytes. The growth of endophytes may reduce the resources available for coliform due to competition and lead to coliform population decline (Shehzadi et al., 2014).

On the other hand, coliform, which belongs mainly to Gram-negative bacteria, has a negative charge in its outer membrane (Li et al., 2015). In contrast, clay will be positively charged when mixed with water due to increased cation exchange capacity (Mueller, 2015). Expanded clay aggregate has a larger surface area. As the reactive surface area of clay is large enough to adsorb bacteria, the number of coliforms will eventually decrease (Ihekweeme et al., 2021). The combination of adsorption by expanded clay aggregate and an unfavourable environment produced by macrophytes lead to the decrease of coliforms. This state will be more prominent, along with the addition of detention time, since the unfavourable conditions will take longer for coliform to grow (Vymazal et al., 2005).

According to this study, *Typha sp.* was observed to be the most prospective plant for CWs with limited oxygen in treating domestic wastewater. *Typha sp.* was able to generate oxygen at a higher rate into the CWs system compared to the other two plants. This feature helps the system degrade nutrients, especially  $\text{PO}_4^{3-}$ , in the wastewater supplied to the CWs. In addition, some properties belonging to *Typha sp.* make it more advantageous to reduce the coliform numbers than several other plants. Nonetheless, a more complex CWs system is needed to observe plant performance accurately. For instance, to test the plant on a pilot scale involving more individuals and wastewater.

## Author Contributions

R.M.R. carried out the experiment and performed the analysis as well as computation. A.Y. conceived the idea and supervised the project. R.M.R., A.Y., and J.R.P. wrote the manuscript. M.A.I. support in providing IoT system and equipment. All authors discussed the results prior the manuscript finalisation.

## Conflict of Interest

The authors certify that they have no known financial or personal interests that could be interpreted as influencing or compromising the integrity of the work presented in this paper.

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