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Correlation and regression analysis of unionised ammonia ions, NH_3 and pH in ornamental fish aquatic environment

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ABSTRACT

Assessing ammonia in water is very important for fish farmers as it is one of the pollutants that may affect the water quality. In water, ammonia can be found either in the form of unionised ammonia (NH_3) or ionised ammonia (NH_4^+), which contribute to a total ammonia nitrogen (TAN). In assessing the ammonia toxicity, NH_3 provides a more accurate measure of toxicity and needs to be monitored consistently, rather than relying solely on TAN. However, the method of measuring NH_3 in water can be complex and challenging. Therefore, the ability to determine the correlation between NH_3 and other parameter which is easier to be measured (such as pH) is crucial to enable the estimation of NH_3. This study was conducted to determine the correlation between NH_3 and pH based on the data collected from Redgold Aquatics ornamental fishponds located in Sik, Kedah. The pH was assessed by a commercial measuring device, while the NH_3 was calculated using an appropriate mathematical equation that requires the data of pH, TAN, and temperature. The correlation and regression analysis were conducted via the IBM SPSS tool and Microsoft Excel. The result shows that NH_3 and pH are highly correlated with regression equation of NH_3 = 0.01pH – 0.07. In addition, factors that may affect the total ammonia value have been discussed thoroughly in this work. The analysis of these water quality parameters may assist farmers in estimating the toxic ammonia, NH_3 from the known water pH.

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1. INTRODUCTION

Since the 1980s, aquaculture production has rapidly developed due to the high demand for fish and food production [1-2]. The ornamental fish industry is one of the aquaculture producers that has contributed to the progress of the aquaculture industry in Malaysia [3-4]. Malaysia is currently the world's eighth largest ornamental fish producer, contributing 9% of ornamental fish to worldwide commerce, according to reports from the Department of Fisheries (DOF) [3]. In 2016, the DOF recorded 259 ornamental fish exporters in Malaysia. The ornamental fish industry has become a significant source of income for local farmers [4]. The management of water that is supplied to the fishpond plays a major role in ornamental fish farming. Excellent water management will almost certainly enhance fish quality and boost fish production for future commerce [5]. Water quality is an essential component of every aquaculture operation. It is very important for fish health, and any decrease in water quality causes stress and illness in fish [1], [3], [6].

Water quality provides significant information in determining the factor of success or failure of an aquaculture operation [1], [2], [6-7]. Various physicochemical characteristics, such as pH, ammonia, temperature, dissolved oxygen, hardness, and others, influence the quality of water resources [1], [6], [8–10]. The pH of water is one of the parameters that may be affected by the effluents discharged from various pollution sources in the environment. In general, fish can tolerate a pH range of 6–9 in freshwater ecosystems [1], [8-9]. However, aquatic organisms cannot thrive in environments with pH levels that are too high or too low [11].

Ammonia nitrogen is regarded as one of the most significant contaminants in the freshwater ecosystems. It can be present in natural aquatic systems via direct means, such as animal excretion of nitrogenous waste, and also indirect means, such as the breakdown of natural nitrogenous organic matter in contaminated sediments [1], [9-10], [12–14]. In general, concentrations less than 0.02 ppm are considered safe. However, unionised ammonia, NH_3 levels may be lowered and transformed into harmless nitrates by biological processes [11]. Total ammonia nitrogen (TAN) in the aqueous solution is composed of two major forms: ionised or ammonium ion (NH_4^+) and unionised ammonia, NH_3, the relative concentrations of which are pH-dependent and temperature-dependent [13–15]. Even though the concentration of ammonia is only measured in parts per million (ppm), it is exceedingly dangerous and should be investigated thoroughly as the toxicity of ammonia nitrogen in water is mostly a result of dissolved ammonia [12], [14], [16]. Furthermore, as NH_3 has high lipid solubility, it may penetrate cell membranes and cause more damage to complex animals [10], [16]. Meanwhile, NH_4^+ has a minimal impact on cultured substances. Based on an article by the California State Water Resources Control Board, ammonia toxicity is highly dependent on pH and temperature. The toxicity of ammonia will increase as pH increases because NH_4^+ will be converted to NH_3, and it will affect the water quality and further impact the aquatic flora and fauna [15-16].

Thus, assessing and monitoring these parameters is essential to identify the magnitude and source of any pollution load that may harm fish [1], [15]. However, typical methods of measuring ammonia by manual sampling through on-site sample collection and testing has many limitations, such as sample degradation, complex sample preparation and tedious setup. Furthermore, the use of measurement devices to test ammonia is rather costly, which can be a financial constraint for the farmer. There is also work on developing an automated system for general ammonia measurement [27]. The work however requires a complex process in designing and developing the system itself, hence increasing the challenges for actual implementation. Thus, the correlation study between ammonia toxic ammonia, NH_3 and pH in the freshwater system would significantly help in reducing the complexity and resolving the issue of manual process, as ammonia can only be predicted from the pH value. By using this correlation relationship, the effect of NH_3 can be quickly assessed based solely on pH measurement.

Therefore, the primary objective of this work is to determine the correlation between ammonia, specifically the unionised ammonia, NH_3 and pH in the aquatic environment to observe the dependency of

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one variable on others. Correlation analysis assesses the degree of similarity between independent and dependent variables [17]. This type of analysis aims to determine the nature of the connection between the variables and, as a result, offers a system for prediction or forecasting. The correlation analysis of these characteristics may have specific impacts, particularly for small-scale fish farmers, as it may help them manage their fishponds more effectively. Next, the regression between pH and NH_3 will also be analysed to obtain the mathematical equation where both variables can be related. Statistical analysis technique is to be used for both correlation and regression analysis. The significance of this work lies in the ability to predict the ammonia, specifically NH_3 based on the obtained correlation and regression model, thus helping the fish farmers to consistently predict their water quality condition based on the use of low-cost measurement devices.

2. METHODOLOGY

2.1 Unionised ammonia (NH_3) measurement

Total ammonia nitrogen (TAN) test kit

The API ammonia test kit was chosen to measure the TAN concentration in the water because it is suitable for both saltwater and freshwater solutions. The test kit is based on the salicylate method and can measure ammonia levels ranging from 0 to 8 ppm. The value of TAN was determined by comparing the colour of the tested solution with the colour chart provided with the test kit. Fig. 1 shows the API ammonia colour chart used as a reference. Upon mixing the sample and reagent, the transition of solution colour will be referred to this chart.

Fig. 1. API ammonia test kit with its colorimetric colour chart

Colorimeter mobile apps

As the colour chart has a limited range of values in between the scale to determine the level of TAN, the colorimeter mobile application [Lab Tools] was used as complimentary method for measuring various ranges of TAN values that the chart cannot measure. The application was run by scanning the colour of the solution using the mobile phone camera and the application would present the value. By using the colorimetric mobile application, a wide range of TAN measurements can be performed, resulting in better data collection.

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The calibration of the colorimetric application was performed by capturing the colour chart of the test kit so that the mobile application could define the colour, as presented in Fig. 2. During the calibration, the environment light was standardised, and all the colour samples were captured from the same position. However, the colour might differ slightly from the actual colour chart due to the lighting conditions. Five colour samples with absorbance ranging from 0 to 2 were selected for calibration purposes. Then, as the application includes colour samples in its database, an application of API colour chart was developed, which would include three new tabs of ‘Database’, ‘Inputs’, and ‘Train’. In the ‘Database’ tab, the saved colour samples were listed. Then, after selecting the the desired colour sample, the value of TAN for each colour was manually entered based on values from the colour chart. These TAN values served as the reference values of the Y-axis of the calibration graph.

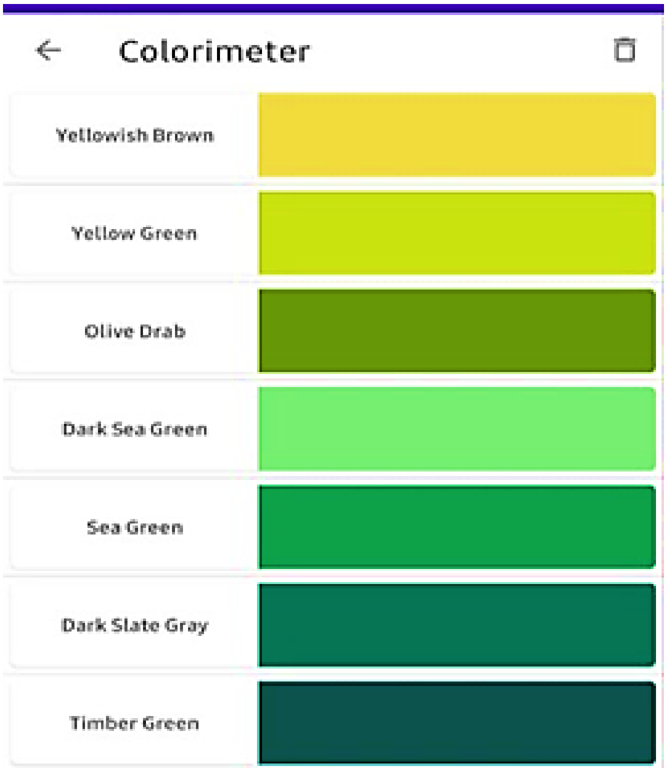

Fig. 2. List of colours on colorimeter mobile application for TAN measurements

The reference values were displayed in the Inputs tab, as shown in Fig 3. Finally, after setting the value of each colour, the model needed to be trained. Subsequently, during TAN measurement, the solutions were scanned by the camera, and the TAN value was displayed on the screen.

The application would automatically train the model and produce a graph of TAN values against colour values, as shown in Fig. 3.

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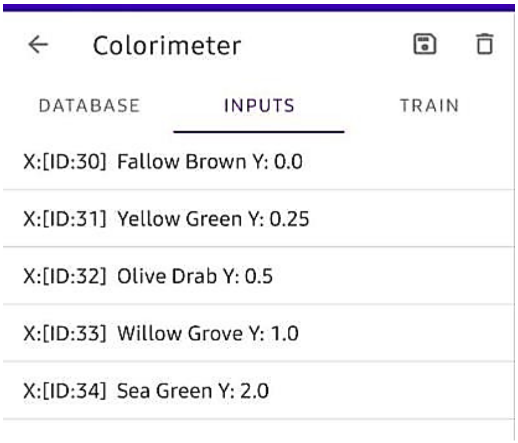

Fig. 3. TAN value for each colour on colorimeter mobile application

Calculation of NH_3 from TAN

The API ammonia test kit detected the TAN and it presented two chemical components: Unionised ammonia, NH_3 and ionised ammonia NH_4^+. Thus, a separate calculation was needed to determine the value of NH_3.

If the *pH* and temperature values from the sample are available, the Emerson method equation allows for the determination of the NH_3 percentage from the total ammonia measured in freshwater [15]. The calculation process started by firstly calculating the ionisation constant of ammonium ion, *pKa*. The following equation is used to calculate the sample's pKa value as shown in Eq. (1)

$$pKa = 0.09018 + 2727.92/T \quad (1)$$

where T represents the temperature in K.

Next, the following equation is used to compute the fraction of NH_3 as compared to total ammonia, TAN, denoted by *f* in Eq. (2)

$$f = 1/(10^\wedge(pK_a - pH) + 1) \quad (2)$$

By using the fraction, the NH_3, can be obtained by multiplying *f* and TAN obtained from the measured ammonia as given by Eq. (3)

$$un\text{-}ionised\ ammonia,\ NH_3 = total\ ammonia,\ \mathrm{TAN} \times f \quad (3)$$

where TAN is the measurement of total ammonia obtained through colorimetric method.

2.2 pH and temperature measurement

pH and temperature measurement were measured using a commercial thermometer (Hanna Instruments), as shown in Fig. 4. Both parameter are required for the calculation of NH_3 during colorimetric test activities.

The device is a waterproof *pH* tester with a 0.1 *pH* resolution and an accuracy of ±0.1 *pH*. *pH* Calibration was achieved based on two-point calibration techniques using buffer solutions of *pH* 4.01 and 10.01.

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Fig. 4. A commercial thermometer by Hanna Instruments

2.3 Data collection

pH and NH_3 data were collected at the Redgold Aquatics Ornamental Fishpond in Sik, Kedah, Malaysia. The data were collected from 12.00 p.m. to 3.00 p.m. Fig. 5 shows the data collection activities performed on-site. It shows how the colorimetric test was performed to obtain the colour transition after mixing with the reagent.

Fig. 5 On site ammonia measuring process using API ammonia test kit

In this work, 45 fishponds were selected as the sampling ponds for data collection purposes. The pH and temperature values were collected using the commercial device while the TAN was collected using the test kit. The calculation of NH_3 required temperature values; thus, the commercial thermometer was used to determine the water temperature. Apart from that, the data collection was also divided into two categories: ponds with the presence of fish and aquatic plant density. The purpose of dividing into these categories is to observe which factor influences the value of parameter to be monitored.

2.4 Data analysis

The collected data were subjected to correlation and regression analysis. The IBM Statistical Package for the Social Sciences (SPSS) software and Microsoft Excel were used as the analysis tools. Regression

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analysis is significant to develop an estimated regression equation. In this study, the estimated regression equation was used to predict the value of ammonia based on the pH values. The analysis parameters evaluated include the R and R^2, the analysis of variance (ANOVA) test, and the unstandardised coefficients [18]. Correlation analysis was carried out as the correlation coefficient quantifies the linear relationship between the two variables. The correlation describes the link between two variables in which changes in the value of one variable affect changes in the value of the other variable [19-20]. The pattern of the graph of NH_3 against pH was evaluated to define the direction and strength of the relationship between the desired parameters. Meanwhile, for correlation analysis, the Pearson product correlation of pH and NH_3 was assessed [17], [21].

Apart from the correlation and regression analysis, the data were also observed from the perspective of other potential contributing factors, the presence of fish and the density of aquatic plants. These factors were thought to have significant effect on the value of TAN detected in the water.

3. RESULTS AND DISCUSSION

3.1 Colorimeter apps calibration

The calibration of the colorimetric application was done in the Train tab. The application automatically trained the model, producing a linear calibration graph of TAN values against colour values, as shown in Fig. 6. The X-axis defines the colour value based on the input type Hue, while the Y-axis defines the TAN value.

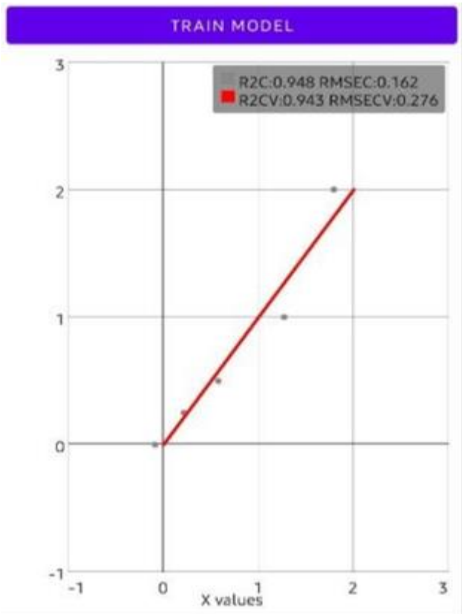

Fig. 6. Calibration graph of the trained model

Fig. 6 shows the trained model of the colorimetric application used for identifying the concentration of TAN value based on colour changes. The right corner of the calibration graph shows the values of R^2 and root mean squared error (RMSE) for calibration (C) and validation (CV). Both R^2 and RMSE were used to assess the regression quality of the trained model. The results indicate an R2C of 0.948 and an R2CV of 0.943. The R^2 values for calibration and validation are close to 1, indicating that the calibration graph

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regression is acceptable. It implies that the values of colour variation may explain 94.8% of the variation in TAN concentration.In other words, the model is good enough to determine the value of a response variable based on the value of a predictor variable. Furthermore, the calibration root mean square error (RMSEC = 0.162) and validation root mean square error (RMSECV = 0.276) are low. It shows that the model fits the dataset, implying that the observed data points are near the predicted values of the model.

3.2 In-situ data collection

The overall pH value of the fishponds is between 6.27 and 9.34 with a mean value of 7.59378, while the overall NH_3 is between 0.00001 and 0.04128 ppm with a mean value of 0.0077396 ppm. This indicates that the fishponds have normal and safe pH values at noon. Similarly, the NH_3 value evaluates the safe conditions for fish to live.

3.3 Data analysis

Fundamentally, correlation analysis is carried out to measure the relationship between two variables. In this study, the analysis was performed to define the strength and direction of the linear relationship between pH and NH_3 and also to prove the hypothesis that there is a significant relationship between pH and NH_3. The graph of NH_3 against pH is shown in Fig. 7. The graph shows a strong, linear positive slope with an R value of 0.799, which is near to 1. The value shows good strength for the relationship between the two designated variables.

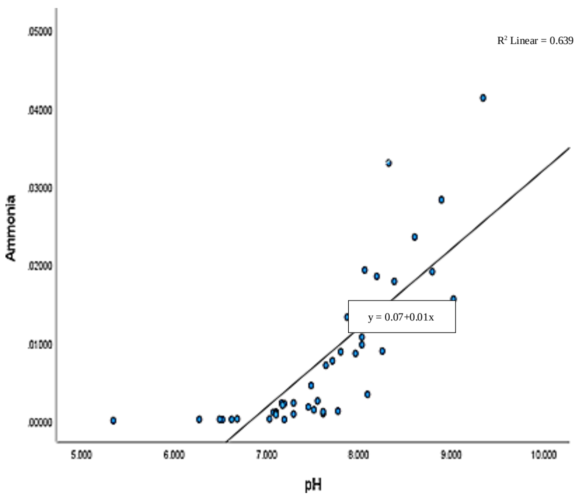

Fig. 7. Graph of NH_3 against pH

Based on the scatter plot, the mathematical formula between NH_3 and pH in freshwater can be interpreted as in Eq. (4).

$$NH_3 = 0.01(pH) - 0.07 \tag{4}$$

Next, the Pearson correlation was applied to describe the relationship between NH_3 and pH, as shown in Table 1. The Pearson product correlation of pH and NH_3 was also found to have a high positive correlation (R = 0.799, p <0.001), hence supporting the hypothesis. The result depicts that an increase in pH would lead to an increase of NH_3 in the fishponds.

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Table 1. Correlation Coefficients of NH_3 and pH

Correlations			
		NH_3	pH
Pearson Correlation	NH_3	1.000	.799
	pH	.799	1.000
Sig. (1-tailed)	NH_3	.	<.001
	pH	.000	.
N	NH_3	45	45
	pH	45	45

Regression analysis is based on the assumption of normality of variance, linearity of relationship among the variables, and homogeneity. In this study, the regression technique was used to assess the strength of the relationship between the independent variable (pH) and the dependent variable, NH_3. The analysis assists in predicting and estimating the value of the dependent variable from the independent variable. In this study, bivariate regression was applied as the study only involves one dependent variable and one independent variable.

The study hypothesises that there is a significant impact of the pH of water on the level of NH_3. The dependent variable pH was regressed on the predicting variable NH_3 to test the hypothesis. Table 2 presents the model summary from the regression analysis. The regression between pH and NH_3 shows an R-value of 0.799, indicating that there is a strong, positive linear relationship between pH and NH_3. The R^2 value of 0.639 shows that the model defines a 63.9% change in NH_3 which can be accounted for by the pH value. The ANOVA test, which was inbuilt in the regression analysis, was also analysed to verify the hypothesis. Table 3 presents the ANOVA results for both NH_3 and pH. From the results, pH significantly predicted NH_3 (F (1,43) = 76.003, p <0.001), indicates that the pH value can play an important role in determining the NH_3 value (b = 0.01, p <0.001). Finally, Table 4 shows the coefficient of regression analysis between NH_3 and pH. It gives the coefficient of regression analysis, β which signifies the amount by which change in pH must be multiplied to give the corresponding average change in NH_3, or the amount NH_3 changes for a unit increase in pH.

Table 2. Model summary of regression analysis for NH_3 and pH

Model Summary[b]				
Model	R	R^2	Adjusted R^2	Std. Error ofthe Estimate
1	.799[a]	.639	.630	.00593660
a. Predictors: (Constant), pH				
b. Dependent Variable: Unionised Ammonia (NH_3)				

Table 3. ANOVA test for NH_3 and pH

ANOVA[a]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.003	1	.003	76.003	<.001[b]
	Residual	.002	43	.000		
	Total	.004	44			
a. Dependent Variable: Unionised Ammonia (NH_3)						
b. Predictors: (Constant), pH						

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Table 4. Coefficients of Regression Analysis between NH_3 and pH

Coefficients[a]					
Model	Unstandardised Coefficients		Standardised Coefficients	t	Sig.
	B	Std. Error	Beta, β		
1 (Constant)	-.069	.009		-7.801	<.001
pH	.010	.001	.799	8.718	<.001
a. Dependent Variable: Unionised Ammonia (NH_3)					

3.4 Potential factors affecting NH_3

The presence of fish

The presence of fish may affect the value of NH_3 in the fishpond. This is because ammonia is the product of excretion from fish and other living things in the water. Thus, the presence of fish in the fishpond is related to the amount of ammonia present.

In this study, out of 45 fishponds, only seven ponds were identified without the presence of fish. Hypothetically, a pond without fish should have a lower ammonia level compared to fishponds with fish. This hypothesis has been considered because the primary source of nearly all ammonia in fishponds is the protein in feed. When feed protein is completely broken down, ammonia is produced within the fish and excreted through the gills into pond water, resulting in a higher level of ammonia.

Random selection revealed some ponds with fish exhibiting low levels of NH_3, while the highest value of NH_3 was found in a pond without fish. The condition could be influenced by other factors. One of the factors is the nitrogen cycle [13]. The nitrogen cycle is a series of processes in which nitrogen and its compounds are interconverted in the environment and living organisms,including nitrogen fixation and decomposition [22]. In the nitrogen cycle, ammonia from fish excretion and uneaten fish food is converted to nitrites by nitrogen-fixing microorganisms. Nitrites are then reduced by *Nitrobacter*, which converts them into nitrates that can be consumed by aquatic plants and algae as food [10]. This process may establish and stabilise thelevel of ammonia in the fishponds.

The density of aquatic plants

The collected data were also categorised according to the density of aquatic plants in the fishponds. The density of the plants was classified by defining the percentage of the plant density with an increment of 25%. The main aquatic plant at the ornamental fishpond is known as water hyacinth (*Eichhornia crassipes*). It is a floating aquatic plant that provides a habitat for fish and is also involved in water purification. Its capacity to absorb nutrients makes it a potential biological alternative for secondary and tertiary treatment of contaminated water. It has been discovered that water hyacinth stabilises *pH* and temperature in ponds, preventing stratification within the water column [10].

In general, all aquatic plants can remove nitrogen directly through absorption and indirectly by enhancing theactivity of microorganisms through changes in the redox environment inside the aqueous environment. The plants are involved in the denitrification process, which can lower the ammonia level in the water [23-26].

Based on the observation, one of the fishponds with 0% aquatic plant cover recorded a high NH_3 value of 0.04128 ppm. The lowest NH_3 value of 0.00001 ppm was recorded in a fishpond with 100% aquatic plant density.

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In addition, most fishponds with a greater amount of aquatic plants showed lower ammonia levels. These data provide evidence to prove that plants have effects on ammonia levels

4. CONCLUSION

From this work, it can be concluded that the main objective of this work in analysing the relationship between NH_3 and *pH* has been successfully achieved. It can be seen that the correlation analysis is a highly useful approach, and the identification of correlations between multiple physicochemical parameters represents a significant advance in surface water quality management. The correlation study shows that *pH* and NH_3 have a high positive linear relationship with an R-value of 0.799, which is close to 1. The regression analysis shows that 63.9% of the variation in NH_3 levels can be affected by the *pH* of water. Besides, based on the data collected, Redgold Aquatic ornamental fishponds have good water quality in terms of *pH* and ammonia levels. The average *pH* of the fishponds is within the ideal *pH* range, and the overall data show low NH_3 levels. In addition, the study reveals a significant role of aquatic plants in reducing ammonia levels.

This research has positive impacts on education and the community. The outcome of the study may serve as a reference for additional research linked to aquaculture. This study can be used to develop a table that estimates the value of ammonia based on *pH* and temperature measurements at a specific fish farm. For future work, the study can be further extended by considering temperature and *pH* as the independent variables to estimate ammonia. Besides, a study on factors influencing ammonia readings in the aqueous solution is recommended.

5. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper. All affiliations and funding sources supporting this study are transparently acknowledged, and no competing financial or non-financial interests exist that could have influenced the outcome of this research.

6. AUTHORS' CONTRIBUTIONS

Fatin Izyani Mohamad Robi: Contributed to technical writing of the manuscript and proofreading process. **Mohamad Naim Ahmad:** Contributed to the design of the study and data collection. **Mohd Suhaimi Bin Sulaiman:** Assisted with the data analysis and provided significant input in interpreting the results. Nur **Syafiqah Nadiah Mohd Zain:** Contributed to the literature review and statistical analysis. **Khairul Anuar Ishak:** Supported the project by providing technical expertise and guidance on the analytical tools. **Mohamad Faizal Abd Rahman:** Conceptualized the research, led the manuscript drafting, and coordinated the entire research team. Conceptualisation, methodology, formal analysis, investigation and writing-original draft, supervision, writing-review and editing, and validation.

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