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# EFFECTS OF CULTURE CONDITIONS ON MYCELIAL GROWTH AND FRUIT BODY OF LINGZHI MUSHROOM *Ganoderma orbiforme* (Fr.) Ryvarden

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## ABSTRACT

Lingzhi mushrooms (*Ganoderma* spp.) are well-known for their numerous health benefits in disease prevention and treatment. These benefits come from their rich content of biologically active compounds. However, there are limited studies on the *Ganoderma orbiforme* species in Vietnam. This study aimed to identify the optimal growth culture conditions of *G. orbiforme* strain Go1, which was collected from Xuan Son National Park, Phu Tho province, Vietnam. The results showed that the mycelial of strain Go1 grew well in a PDA medium at pH 6.0 - 8.0 and at a temperature of 25 - 30°C. Rice, millet, and wheat can be used as substrates for producing the mother spawn of strain Go1. Among these, rice and wheat promoting the fastest mycelial growth. Strain Go1 was able to form fruiting bodies when cultivated on substrates, including rubber sawdust and corn cobs. The substrate composition significantly affected the biological performance of strain Go1. The optimal formula consisted of 89% sawdust, 10% rice bran, and 1% calcium carbonate. Using this formula biological efficiency reached  $8.94 \pm 0.23\%$ .

**Keywords:** *Ganoderma orbiforme*, pH, medium, temperature, cereal grains, substrates.

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## 1. INTRODUCTION

The genus *Ganoderma* was first described in 1881 by Karsten, with *Ganoderma lucidum* P. Karst as the type species. It is a large genus within the family Ganodermataceae, comprising nearly 300 species, primarily distributed in tropical regions [1]. As of June 2023, 493 species of *Ganoderma* are recorded on Index Fungorum. *Ganoderma* species are highly valued, particularly in traditional Chinese medicine [1]. About 430 chemical compounds have been identified within this genus, most of which exhibit strong biological activity. These compounds are distributed across 23 *Ganoderma* species [1, 2].

*Ganoderma orbiforme* (Fr.) Ryvarden, along with species such as *G. lucidum*, *G. applanatum*, *G. cappense* and *G. austral*, is recognized for significant medicinal properties. Compounds like

ganoboninketal C, ergosta-4,6,8,22-tetraen-3-one found in *G. orbiforme* demonstrate inhibition of superoxide anion and elastase, indicating potential in inflammation treatment [3]. Additionally, triterpenoids such as 3,4-seco-27-norlanostane, ganoboninketals E and F, ganoboninones G and H, along with novel lanostanes like C30 (24Z)-3 $\beta$ ,27-dihydroxylanosta-7,9,24-triene, have exhibited antibacterial activity [4]. Furthermore, lanostanoids in the 3 $\beta$ - and 15 $\alpha$ -acetoxy groups have shown anti-tuberculosis activity [5].

Despite its medicinal potential, research on *G. orbiforme* remains limited globally and in Vietnam. Most existing studies focus on this species' diversity, morphology, and phylogeny, with only a few exploring its secondary metabolites. In Vietnam, beyond research into the distribution of *Ganoderma* species, the studies by Phuong *et al.* (2023) [6] and Luyen *et al.* (2023) [7]

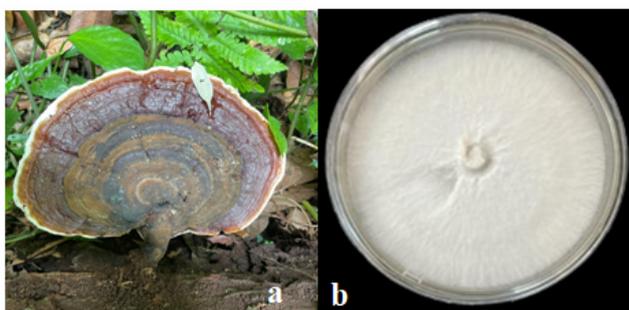
represent some of the first investigations into the medicinal properties and cultivation characteristics of *G. orbiforme*. Phuong *et al.* (2023) identified *G. orbiforme* as one of seven medicinally valuable *Ganoderma* species [6]. Meanwhile, Luyen *et al.* (2023) published findings on suitable cultivation conditions for the *G. orbiforme* FM6 strain, including fructose (15 g/L), NH<sub>4</sub>Cl (2 g/L), and a substrate of sawdust supplemented with barley bran at a 10% ratio [7].

This study aims to assess the growth characteristics of the mycelial and the development of the fruiting body of the *G. orbiforme* strain Go1, collected from Xuan Son National Park, Phu Tho province. The results will contribute valuable information on the growth and cultivation conditions of *G. orbiforme*, aiding mushroom farms and households in optimizing cultivation practices for this species.

## 2. MATERIALS AND METHODS

### 2.1. Fungal strain

The strain Go1 was collected at Xuan Son National Park, Phu Tho province (GPS: 21°08'07"N104°56'59"E). The mushroom samples were collected and processed according to the method of Trinh Tam Kiet (2011) [8]. The mycelial of the mushroom was grown on PDA medium (Potato Dextrose Agar, including 200 g/l potato extract, 20 g/l D-glucose, 20 g/l agar) and stored at 10°C.



**Figure 1. The fruiting body in natural (a) and the mycelial (b) of the strain Go1 on PDA medium**

### 2.2. Methods

#### 2.2.1. DNA extraction and PCR reaction

DNA from the mycelium of strain Go1 was extracted according to the method of

Winnepeninckx *et al.* (1993) [9] with modifications. The ITS (Internal Transcribed Spacer) sequence region was amplified by polymerase chain reaction (PCR) using the primer pair ITS1/ITS4 (TCCGTAGGTGGAACCTGCGG/TCCTCCGCTTATTGATATGC) [10]. The components of the reaction include: Reaction buffer: 5.0 µL, DNA polymerase: 0.5 µL, forward primer ITS1: 0.5 µL, reverse primer ITS4: 0.5 µL, distilled water: 15.5 µL, template DNA: 3.0 µL. After being purified by ExoSAP-IT (Thermo Fisher Scientific), the PCR product was sequenced at 1st Base Company.

#### 2.2.2. DNA sequence analysis

The raw nucleotide sequences were edited using Chromas 2.6.6 software and compared for similarity with sequences on NCBI using the BLASTn tool. The phylogenetic tree based on the nucleotide sequences of the ITS region was constructed using Mega X software using the Maximum likelihood method with a bootstrap coefficient of 1000 replicates with the K2+G+I substitution model [11]. *Tomophagus colossus* (voucher NgoAnh s.n.) and *Tomophagus cattienensis* (voucher Ct199 and Dai 18487) were used as outgroups.

#### 2.2.3. Effect of pH on the mycelial growth of the fungus strain Go1

The mycelial of the strain Go1 was cultured on PDA medium. The medium preparation method was performed according to Bich Thuy *et al.* (2019) [12]. Potatoes were peeled, washed, cut into pieces measuring 1.0 x 1.0 cm; boiled in 500 ml of water for 30 minutes and filtered to get the extract. Dissolve D-glucose and agar into the extract to get the culture medium. Use 1N NaOH and 1N HCl solution to adjust the pH value of the culture medium to 5, 6, 7, 8, and stabilize the pH value with 7.0 g/l HEPES buffer. The culture medium was autoclaved at 121°C for 20 minutes [13]. Divide the medium into sterilized Petri dishes (Ø 9.0 cm). Each Petri dishes was inoculated with a piece of mother spawn (Ø 8.0 mm) at the center of the dish, and incubated at 25°C under dark conditions [14].

#### 2.2.4. Effect of carbon sources on the mycelial growth of the strain Go1

The mycelia of strain Go1 was cultured on PA media (including 200 g/l potato extract, 20 g/l agar) supplemented with different carbon sources, including D-glucose, fructose, sucrose, maltose, and lactose at a concentration of 20 g/l. The pH value of the medium was the optimal pH value selected in section 2.2.3.

#### 2.2.5. Effect of temperature on the mycelial growth of the strain Go1

The optimal environment for the mycelial growth of the strain Go1 will be used as the basic environment to evaluate the impact of temperature. The pH value of the environment is the optimal pH value selected in Section 2.2.3. The mycelial of the strain Go1 was incubated at different temperatures: 20, 25, 30, and 35°C under dark conditions.

#### 2.2.6. Effect of spawn substrates on the mycelial growth of the strain Go1

Use cereal grains, including rice, millet, bobo, and wheat, as substrates for propagating the Go1 strain. The seeds were soaked in water for 10 - 12 hours, cleaned of impurities, removed empty seeds, boiled, cooled, and mixed well with 1.0% CaCO<sub>3</sub> powder. The propagation substrate was sterilized by autoclaving at 121°C for 60 minutes [15]. After sterilization, the substrate was divided into test tubes (250 x 20 mm). Each test tube containing the substrate was inoculated with 1 piece of Go1 grade I mushroom spawn (10 x 10 mm) and cultured in a room at 25 ± 1°C, under dark conditions.

#### 2.2.7. Effect of substrate composition on the fruiting body of the strain Go1

Rubber sawdust and crushed corn cobs were the main materials for cultivating the strain Go1. Sawdust and corn cobs, after being treated with lime water (pH 13 - 14) were incubated for 7 days [16]. The growing substrate was mixed according to each formula: Formula 1: 89% sawdust + 10% rice bran + 1% CaCO<sub>3</sub> powder. Formula 2: 67% sawdust + 22% corn cobs + 10% rice bran + 1% CaCO<sub>3</sub> powder. Formula 3: 45% sawdust + 44% corn

cobs + 10% rice bran + 1% CaCO<sub>3</sub> powder. Formula 4: 22% sawdust + 67% corn cobs + 10% rice bran + 1% CaCO<sub>3</sub> powder. Formula 5: 89% corn cobs + 10% rice bran + 1% CaCO<sub>3</sub> powder. The substrate was adjusted to a humidity of 65% and packed into PE plastic bags (19 x 37 cm), weighing 1.0 kg/bag. The substrate bags were sterilized at 121°C for 180 minutes. After cooling, the substrate bags were inoculated with grain spawn in 1% of the substrates wet weight and incubated at 25 ± 1°C under dark conditions. At the end of the mycelial stage, the strain Go1 was cultured at 25 ± 1°C, air humidity 85 ± 5% to stimulate the formation and development of fruiting bodies [14].

#### 2.2.8. Data collections

The mycelial growth rate (mm/day) was calculated after 8 days of incubation. Mycelial density was observed and evaluated at four levels based on the criteria described by Jo *et al.* (2009) [13]: Compact (C); somewhat compact (SC); somewhat thin (ST); thin (T). Mycelial biomass (grams) was determined based on the method outlined by Gonkhom *et al.* (2022) [17]. In this method, the mycelial was boiled, the agar medium was rinsed with hot water, and then the mycelial was dried to a constant weight (<13% moisture content).

The spawn running period (days) was recorded from inoculation until the mycelial fully covered the substrate. The day of pinhead formation (days) was considered the day of inoculation to the primordia. The mushroom yield (grams/bag) was determined by the fresh mushroom mass harvested per substrate bag. Biological efficiency (BE%) was calculated as the percentage of fresh mushroom yield obtained per 1.0 kg of dry substrate (13% moisture content) [14].

#### 2.2.9. Statistical methods

The optimal growth parameter data was carried out using statistical analysis programs with triplicate. The research results were statistically processed using Excel 2019 and Graphpad Prism 9.1 software. The differences between the formulas were evaluated by one-way ANOVA analysis of variance and multiple group

comparisons according to Tukey’s standard at a significance level of  $P < 0.05$ .

3. RESULTS AND DISCUSSION

3.1. ITS region nucleotide sequencing

Table 1. GenBank BLAST search results of ITS sequences of *G. orbiforme* species from this study against the GenBank database

| Specie              | Voucher/GB accession no | Per. identity (%) | Query cover % (QC) | Locality | Reference |
|---------------------|-------------------------|-------------------|--------------------|----------|-----------|
| <i>G. orbiforme</i> | MK345446                | 99.53             | 92.0               | Laos     | Genbank   |
|                     | PP660631                | 99.20             | 89.0               | China    | Genbank   |
|                     | MK345448                | 99.67             | 87.0               | Thailand | Genbank   |
|                     | MK313109                | 99.87             | 86.0               | China    | Genbank   |
|                     | PP660618                | 99.65             | 91.0               | China    | Genbank   |
|                     | MG279186                | 97.73             | 90.0               | China    | Genbank   |
|                     | PP660625                | 97.72             | 89.0               | China    | Genbank   |

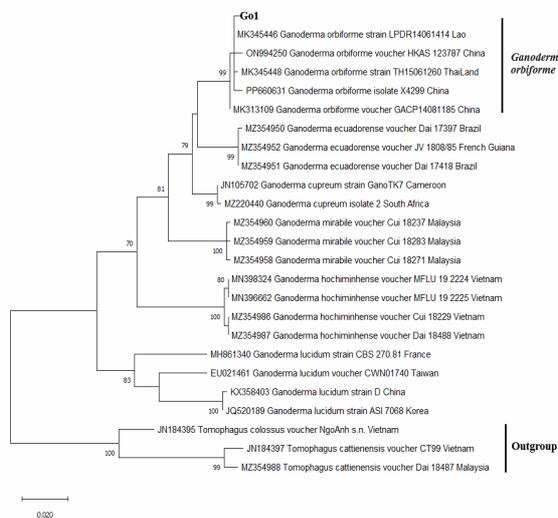


Figure 2. Maximum likelihood phylogenetic tree of the strain Go1 inferred from the internal transcribed spacer (ITS). Bootstrap frequencies are greater than 50%.

The ITS region nucleotide sequence of strain Go1 is 583 base pairs (bp) in length and exhibits a high similarity to *G. orbiforme*, ranging from 97.72% to 99.87% (Table 1). The ITS dataset includes the sequence of strain Go1 along with 18

sequences retrieved from GenBank, using *Tomophagus colossus* Ngo Anh and *Tomophagus cattienensis* CT99 as outgroups. The molecular phylogenetic tree was constructed using the Maximum Likelihood method in MEGA X software. The results indicated that the nucleotide sequences of the ITS region within the genus *Ganoderma* clustered into a single group, distinct from the outgroups. Specifically, the ITS region nucleotide sequence of strain Go1 and that of *G. orbiforme* formed a branch with a high bootstrap support value of 99% (Figure 2).

3.2. Effect of pH

The mycelial of the *G. orbiforme* strain Go1 grows well at pH values ranging from 6.0 to 8.0. Within this pH range, the mycelial growth rate varied from  $3.99 \pm 0.039$  to  $4.05 \pm 0.037$  mm/day, while mycelial biomass reached  $0.1051 \pm 0.0054$  to  $0.1095 \pm 0.0073$  g after 8 days of culture. The mycelial density remained thick throughout this range (Table 2, Figure 3). The differences in these results were not statistically significant ( $P > 0.05$ ).

Table 2. Effect of pH on mycelial growth of strain Go1

| pH value | Mycelial growth rate (mm/day) | Mycelial biomass (g)  | Mycelial density |
|----------|-------------------------------|-----------------------|------------------|
| pH 5.0   | $2.91^b \pm 0.063$            | $0.0643^b \pm 0.0045$ | C                |
| pH 6.0   | $3.99^a \pm 0.039$            | $0.1051^a \pm 0.0054$ | C                |
| pH 7.0   | $4.02^a \pm 0.069$            | $0.1061^a \pm 0.0015$ | C                |
| pH 8.0   | $4.05^a \pm 0.037$            | $0.1095^a \pm 0.0073$ | C                |

Notes. C: Compact. Values are the means  $\pm$  SD of mycelial growth rate (mm/day), and mycelial biomass (g). Values in the same letter differ significantly according to Tukey’s multiple range test ( $P < 0.05$ ).



**Figure 3. The mycelial of the strain Go1 after 8 days of incubation with different pH conditions**

Mushrooms are known to adapt and grow within an environmental pH range of 2.0 to 9.0 [18]. Lee *et al.* (2008) [19] reported that the optimal pH for mycelial growth in *Ganoderma* species falls between 5.5 and 6.0, and some strains of *G. lucidum* can grow across a broader pH range of 5.0 to 9.0. Similarly, Magday *et al.* (2014) [20] found that mycelial growth and biomass of *G. lucidum* were consistent at pH values of 6.0 and 6.5. In the Bich Thuy *et al.* (2019) study, a pH range of 4.0 to 12.0 was found suitable for a *G. lucidum* strain [16]. Luangharn *et al.* (2019) [21] also reported that *G. tropicum* and *G. resinaceum* species grew best at pH 7.0 to 8.0. Wannasawang *et al.* (2023) [14] observed

that pH 4.0 to 8.0 was ideal for the growth of *G. sichuanense* mycelial, while pH 4.0 to 6.0 was optimal for *G. orbiforme* species collected in Thailand. These studies demonstrate that *Ganoderma* species can grow across a wide range of pH levels, with optimal growth conditions varying depending on the specific strain and species. Notably, the *G. orbiforme* isolate obtained in Vietnam exhibits a pH tolerance range similar to that of the *G. orbiforme* strain from Thailand.

**3.3. Effect of carbon sources**

The carbon sources were optimized using different sugars, with D-glucose identified as the best carbon source for the mycelial growth of the Go1 strain. The mycelial growth rate on D-glucose was  $4.06 \pm 0.044$  mm/day, with a biomass yield of  $0.1098 \pm 0.0007$  g after 8 days of cultivation, and the mycelial density was high (Figure 4). In contrast, the mycelial grew the slowest on a lactose-supplemented medium, with a growth rate of just  $1.41 \pm 0.037$  mm/day and a biomass yield of  $0.0520 \pm 0.0099$  g.

**Table 3. Effect of carbon sources on mycelial growth of the strain Go1**

| Carbon sources (20 g/L) | Mycelial growth rate (mm/day) | Mycelial biomass (g)  | Mycelial density |
|-------------------------|-------------------------------|-----------------------|------------------|
| D-Glucose               | $4.06^a \pm 0.044$            | $0.1098^a \pm 0.0007$ | C                |
| Fructose                | $3.07^c \pm 0.078$            | $0.0873^b \pm 0.0081$ | SC               |
| Sucrose                 | $2.89^c \pm 0.061$            | $0.0932^b \pm 0.0022$ | SC               |
| Maltose                 | $3.67^b \pm 0.101$            | $0.0897^b \pm 0.0014$ | ST               |
| Lactose                 | $1.41^d \pm 0.037$            | $0.0520^c \pm 0.0099$ | C                |

Notes. C: Compact. SC: Somewhat Compact. ST: Somewhat thin. Values are the means  $\pm$  SD of mycelial growth rate (mm/day), and mycelial biomass (g). Values in the same letter differ significantly according to Tukey’s multiple range test ( $P < 0.05$ )



**Figure 4. The mycelial of the strain Go1 after 8 days of incubation with different carbon sources**

Glucose, along with its structural or stereoisomers (fructose, galactose) and epimers (mannose), is generally the most effective carbon source for the growth and development of mycelial in most basidiomycetes. Among these, glucose is the most suitable because it is rapidly absorbed and metabolized by the mycelial, providing the energy

needed for cellular activities [22 - 24]. Chandra and Purkayastha (1977) [25] also reported that most tropical fungi preferentially utilize glucose over other carbon sources. Indeed, glucose has been shown to be the optimal carbon source for many fungi, including *Ganoderma lucidum*, *G. applanatum*, *G. sinense*, *Amauroderma subresinosum*, *Tremella fuciformis*, and *Agaricus blazei* [7, 26]. However, Luyen *et al.* (2023) noted that fructose was the optimal carbon source for the mycelial growth of the *G. orbiforme* FM6 strain collected in Pu Mat, Nghe An, Vietnam. The effect of carbon source on mycelial growth can vary depending on the fungal strain and culture conditions [7].

3.4. Effect of temperature

Table 4. Effect of temperature on mycelial growth of the strain Go1

| Temperature (°C) | Mycelial growth rate (mm/day) | Mycelial biomass (g)         | Mycelial density |
|------------------|-------------------------------|------------------------------|------------------|
| 20               | 0.31 <sup>c</sup> ± 0.041     | 0.0102 <sup>c</sup> ± 0.0013 | T                |
| 25               | 4.06 <sup>a</sup> ± 0.047     | 0.1091 <sup>b</sup> ± 0.0017 | C                |
| 30               | 4.17 <sup>a</sup> ± 0.039     | 0.1226 <sup>a</sup> ± 0.0095 | C                |
| 35               | 1.07 <sup>b</sup> ± 0.045     | 0.0222 <sup>c</sup> ± 0.0019 | C                |

Notes. C: Compact. T: Thin. Values are the means ± SD of mycelial growth rate (mm/day), and mycelial biomass (g). Values in the same letter differ significantly according to Tukey's multiple range test (P < 0.05)

The mycelial growth of the strain Go1 exhibited significant differences (P < 0.05) across a temperature range of 20 - 35°C. The optimal temperature for mycelial growth was found to be between 25°C and 30°C. At these temperatures, the mycelial growth rate was similar, reaching 4.06 ± 0.047 mm/day at 25°C and 4.17 ± 0.039 mm/day at 30°C. However, the mycelium grown at 30°C had a higher density than at 25°C, which was reflected in the biomass yield 0.1226 ± 0.0095 g at 30°C compared to 0.1091 ± 0.0017 g at 25°C. Conversely, at 20°C, the mycelium of strain Go1 exhibited minimal growth, with a growth rate of just 0.31 ± 0.041 mm/day, a biomass yield of 0.0102 ± 0.0013 g after 8 days of cultivation, and a thin mycelial density (Figure 5).

Temperature is a crucial environmental factor that influences mushrooms' growth, development, and productivity [27]. It affects the assimilation of nitrogen and carbon and the respiratory activity and biosynthetic processes in mushrooms [15]. Additionally, temperature directly impacts the activity of decomposing enzymes such as laccase, xylanase, cellulase, amylase, and protease. Deviations from the optimal temperature can disrupt mushrooms' physiological and biochemical processes.

For the *G. orbiforme* strain Go1, the suitable temperature ranges for mycelial growth is from 25°C to 30°C, with the optimum at 30°C. These results are consistent with the findings reported by Wannasawang *et al.* (2023) [14], who noted that

the optimal temperature range for the growth and development of *Ganoderma* species, including *G. orbiforme*, is between 25°C and 30°C. Specifically, *G. orbiforme* exhibited the best growth at 25°C and 30°C, with mycelial biomass reaching 0.0707 ± 0.0246 g and 0.0512 ± 0.0006 g, respectively. In contrast, low temperatures, such as 20°C, inhibited the growth rate of several *Ganoderma* species, including *G. sinense* [15], *G. sichuanense*, *G. lucidum* [23], and *Amauroderma subresinosum* [26]. Subedi *et al.* (2021) [23] also observed that the mycelial growth rate of *Ganoderma lucidum* peaked at 30°C but decreased when the temperature increased to 35 - 40°C.

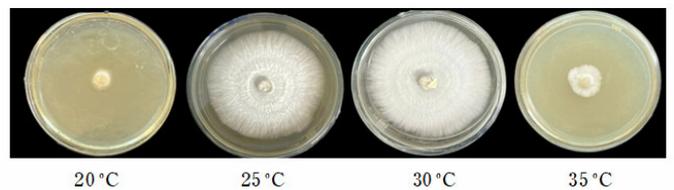


Figure 5. The mycelia of the strain Go1 after 8 days of incubation at different temperatures

3.5. Effect of spawn substrates

The mycelium of the strain Go1 can grow on all four types of cereal grains tested: Rice, millet, sorghum, and wheat. The fastest mycelial growth was observed on wheat and rice grain substrates, with growth rates of 8.165 ± 0.143 mm/day and 7.93 ± 0.084 mm/day, respectively. In contrast, the slowest growth was recorded on sorghum grain, with a rate of 4.54 ± 0.138 mm/day.

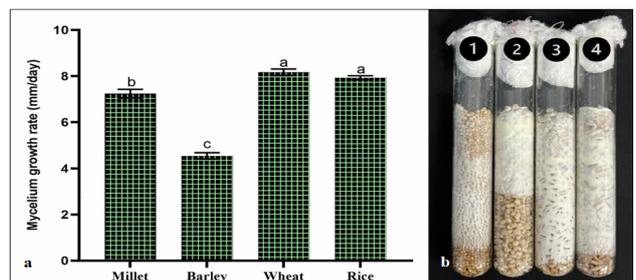


Figure 6. The mycelial growth rate (a), and the mycelial of the strain Go1 after 16 days of incubation on grain substrates

Notes: Values are the means ± SD of mycelial growth rate (mm/day). According to Tukey's multiple range test, values in the same letter differ significantly (P < 0.05). 1: Millet. 2: Barley. 3: Wheat. 4: Rice.

Various types of cereal grains, sawdust, and straw can be used as substrates for mushroom spawn production, with cereals being the most commonly used raw materials [14]. Sorghum grains, in particular, are widely utilized as substrates for producing spawn across different mushroom types [28]. For instance, rye is used for *Agaricus* spp. spawn production, while wheat and sorghum grains are suitable for *G. lucidum* spawn production [14]. Luangharn *et al.* (2019) [21] observed robust growth of *G. australe* mycelial on substrates like barley, corn cobs, millet, and sorghum; recommending these cereals for promoting mycelial growth in spawn production. Lee *et al.* (2014) [29] concluded that cereals are effective substrates for mycelial growth because they provide a sustained nutrient supply over time. In this study, rice, millet, and wheat were found to

be suitable for propagating the *G. orbiforme* strain Go1.

**3.6. Effect of substrate composition**

In the five experimental substrate formulas tested, the mycelial growth rate of the *G. orbiforme* strain Go1 showed no significant statistical difference ( $P > 0.05$ ), with an average growth rate ranging from  $3.34 \pm 0.041$  to  $3.66 \pm 0.057$  mm/day. This corresponds to a mycelial growth period of  $38.67 \pm 1.66$  to  $42.00 \pm 1.51$  days, with the mycelial consistently exhibiting a dense texture (Table 5, figure 7). However, the time required for pinhead formation differed significantly ( $P < 0.05$ ) across the various substrates. The primordia formation time was the shortest recorded on the substrate composed of 89% sawdust, 10% rice bran, and 1% light  $\text{CaCO}_3$  powder, averaging  $47.33 \pm 0.88$  days.

**Table 5. Effect of substrate composition on the growth of mycelial and fruiting bodies of the strain Go1**

| Form-ula | Mycelial growth rate (mm/day) | Spawn running period (days) | Mycelial density | Primordia formation (days) | Yield (g/bag)         | Biological efficiency (BE%) |
|----------|-------------------------------|-----------------------------|------------------|----------------------------|-----------------------|-----------------------------|
| CT1      | $3.66^a \pm 0.057$            | $38.67^a \pm 1.66$          | C                | $47.33^c \pm 0.88$         | $37.23^a \pm 0.95$    | $8.94^a \pm 0.23$           |
| CT2      | $3.54^a \pm 0.184$            | $40.00^a \pm 1.33$          | C                | $48.33^c \pm 1.46$         | $34.70^b \pm 0.50$    | $8.33^b \pm 0.12$           |
| CT3      | $3.56^a \pm 0.192$            | $39.67^a \pm 2.00$          | C                | $50.33^{ab} \pm 1.33$      | $32.73^c \pm 0.78$    | $7.86^c \pm 0.19$           |
| CT4      | $3.61^a \pm 0.452$            | $39.33^a \pm 1.45$          | C                | $49.67^b \pm 0.57$         | $33.23^{bc} \pm 0.95$ | $7.97^c \pm 0.23$           |
| CT5      | $3.34^a \pm 0.041$            | $42.00^a \pm 1.51$          | C                | $52.00^a \pm 0.67$         | $30.90^d \pm 0.44$    | $7.42^c \pm 0.11$           |

*Notes.* CT1: 89% sawdust + 10% rice bran + 1%  $\text{CaCO}_3$  powder. CT2: 67% sawdust + 22% corn cobs + 10% rice bran + 1%  $\text{CaCO}_3$  powder. CT3: 45% sawdust + 44% corn cobs + 10% rice bran + 1%  $\text{CaCO}_3$  powder. CT4: 22% sawdust + 67% corn cobs + 10% rice bran + 1%  $\text{CaCO}_3$  powder. Values are the means  $\pm$  SD of mycelial growth rate (mm/day), mycelial growth time (day), fruiting body formation time (day), yeild (g/bag), and biological efficiency (%). Values in the same letter differ significantly according to Tukey’s multiple range test ( $P < 0.05$ )

The mass of the fruiting bodies also varied significantly across different substrates. The largest fruiting body mass was obtained on the substrate with 89% sawdust, 10% rice bran, and 1%  $\text{CaCO}_3$  powder, averaging  $37.23 \pm 0.95$  g per bag, which corresponds to a biological efficiency of  $8.94 \pm 0.23\%$ . Conversely, the lowest fruiting body mass was observed on the substrate containing 89% corn cob, 10% rice bran, and 1%  $\text{CaCO}_3$  powder, with an average of  $30.90 \pm 0.44$  g per bag and a biological efficiency of  $7.42 \pm 0.11\%$ .

The yield and biological efficiency of strain Go1 were found to be higher compared to

previously reported *G. orbiforme* strains from Thailand and the FM6 strain collected from Pu Mat, Nghe An. Wannasawang *et al.* (2023) [14] reported that when *G. orbiforme* was cultivated on a rubber sawdust substrate, the mycelium fully colonized the substrate within 22 days, with fruiting body formation taking between 41 and 58 days. The resulting mushroom yield was  $16.21 \pm 3.51$  g per bag, corresponding to 20.26 g/kg of substrate. Similarly, Luyen *et al.* (2023) [7] found that the biological efficiency of the *G. orbiforme* strain FM6 grown on sawdust supplemented with 10% barley bran was 8.84%.

#### 4. CONCLUSION

The *Ganoderma* strain Go1 exhibits a high degree of homology in the ITS region nucleotide sequence with *G. orbiforme*. Molecular phylogenetic analysis confirms that the ITS data for strain Go1 clusters with the sequences of *G. orbiforme*, forming a branch with a high support level of 99%.

The mycelial of *Ganoderma* strain Go1 grows well on PDA medium, which contains 200 g/L potato, 20 g/L D-glucose, and 20 g/L agar, with an optimal pH range of 6.0 - 8.0 and incubation temperature of 30°C. Various cereal grains can be used to propagate the Go1 strain, with wheat and rice grains being the most effective in promoting mycelial growth. The Go1 strain can form fruiting bodies on substrates such as rubber sawdust and crushed corn cobs. The composition of the substrate significantly affects the biological efficiency of the Go1 strain. The optimal substrate formula, consisting of 89% sawdust, 10% rice bran, and 1% CaCO<sub>3</sub> powder, yielded a biological efficiency of 8.94 ± 0.23%. These findings provide a crucial basis for further research to exploit and develop promising *G. orbiforme* strains for mushroom production in Vietnam.

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# DETERMINE THE OPTIMAL DOSE OF IRRIGATION WATER AND NPK FERTILIZER FOR TOMATO PLANTS IN SON LA PROVINCE BASED ON SOIL SENSOR DATA

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## ABSTRACT

In the context of precision agriculture, optimizing irrigation and fertilizer application is critical to improving crop yield while minimizing resource use. This study aimed to determine the optimal doses of irrigation water and NPK fertilizer for tomato cultivation in greenhouse conditions in Son La province, Vietnam, using data collected from IoT-based soil sensors. The experiment was conducted on 15 plots with five different fertilization and irrigation treatments (D1 to D5), where soil relative humidity and available N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O levels were monitored in real time throughout the tomato growth stages. Crop indicators including plant height, stem diameter, number of leaves, flowers, fruits, and fruit diameter were measured, and yield was recorded over three harvests. Statistical analyses revealed that increasing fertilization improved growth and yield parameters significantly up to D4. The D5 treatment showed slight improvements, particularly in potassium levels, but with diminishing returns. Regression models demonstrated strong correlations ( $R^2 > 0.82$ ) between fertilizer dose and nutrient increase in soil, and a moderate correlation ( $R^2 = 0.6997$ ) between irrigation and soil moisture. The findings suggest that D4 provides an optimal balance between resource use and crop performance. These results support the development of a sensor-based recommendation system for precise irrigation and fertilization management in tomato production under greenhouse conditions.

**Keywords:** *Internet of Things, soil sensors, tomato plant.*

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## 1. INTRODUCTION

In the context of climate change, resource scarcity and increasing requirements for food safety [1 - 3], precision agriculture, smart agriculture or high - tech agriculture is becoming an inevitable trend in the world and in Vietnam [4]. One of the technological pillars for the development of modern agriculture is the Internet of Things (IoT) [5]. IoT has realized the concept of smart agriculture - the most important innovation field in modern agriculture and is playing an increasingly important role in modernizing agricultural production methods [6]. Specifically, IoT facilitates the intelligent connection and processing of

information through automated irrigation systems and precision monitoring technology. By integrating with blockchain technology, IoT not only contributes to improving operational efficiency, but also helps reduce production costs and losses, while significantly improving the quality of agricultural products [7 - 9].

IoT in agriculture is a system of internet-connected devices to collect, transmit and analyze data in real time, thereby making more accurate farming decisions. The main components of the agricultural IoT system include: Sensors (measuring soil moisture, conductivity, pH, nutrient content, air temperature, solar radiation,

wind speed,...), data transceivers (relaying data from the sensor to the server or cloud platform), data control and analysis software and remote control equipment [10].

Traditionally, irrigation and fertilization regimes have been established primarily based on general crop requirements, seasonal patterns, and pedological characteristics, often without adequate consideration of real-time soil moisture and nutrient status. In contrast, precision agriculture emphasizes the critical role of site-specific soil properties and nutrient indicators as key input variables influencing the effectiveness of crop production and land management strategies [11].

Recent advances in soil sensor technology have significantly improved the accuracy and applicability of real-time soil monitoring. These sensors collect continuous data on essential soil-agricultural parameters and transmit them to centralized systems, allowing for spatiotemporal tracking of soil conditions. This capability enables the formulation of precise, localized recommendations for irrigation and fertilization, thereby enhancing input efficiency and resource use optimization.

However, the full potential of smart Internet of Things (IoT)-based systems in agriculture can only be realized when the input data are standardized, comprehensive, and accurately reflect field variability. This is especially crucial in developing decision-support systems for irrigation and nutrient management. Typically, essential input data include:

- Crop characteristics: water demand, growth stage, level of nutrient consumption according to season.
- Soil characteristics: soil type, motorized composition, structure, etc.
- Fertilizer characteristics: fertilizer form (slow/rapid dissolution), pure nutrient content, absorption time.

Training data and declared data represent critical components for the effective operation of Internet of Things (IoT)-based agricultural systems [12]. Only when the dataset is

comprehensive, accurate, and continuously updated can the IoT system generate crop- and site-specific recommendations, thereby optimizing water and fertilizer use, reducing production costs, minimizing environmental impact, and enhancing overall agricultural efficiency.

With the objective of developing a precise recommendation system for irrigation and macronutrient fertilization in greenhouse tomato (*Solanum lycopersicum*) cultivation in Son La province, a series of studies has been initiated. These include regional assessments, fertilization trials, and the integration of IoT technology and soil sensors for real-time soil condition monitoring. Our previous research demonstrated that the sensor-based IoT system could estimate available nitrogen, phosphorus, and potassium contents with an average error rate below 9% [13].

The present study reports the findings of a fertilization experiment coupled with the collection of sensor-based data, including soil relative humidity and available macronutrient (N, P, K) levels. The primary aim is to determine the optimal rates of irrigation and NPK fertilization, thereby contributing to the development of a robust input dataset for a decision-support system tailored to tomato cultivation, based on soil moisture dynamics and available nutrient status.

## **2. MATERIALS AND METHODS**

### **2.1. Study areas and sites**

The study was conducted in Moc Son ward, Son La province, a region characterized by typical features of a mountainous urban area in the Northwestern highlands of Vietnam. The terrain is marked by rugged high mountains interspersed with broad valleys, with an average elevation of approximately 1,080 meters above sea level. The area includes extensive and relatively flat plateaus suitable for agricultural cultivation. Short-term vegetable monoculture in this region is primarily practiced on yellow-red soils, which have a moderately deep soil profile, medium to heavy texture, moderate to high organic matter and nutrient content, slight acidity, and low levels of exchangeable bases and readily available nutrients.

The study site was located within the Rau An Toan Tu Nhien Cooperative. The crop selected for the study was tomato (*Solanum lycopersicum*), cultivar Chia Tai CTV 40. Prior to the initiation of the experiment, the soil was mechanically tilled and basal fertilization was applied at a rate of 40,000 kg of farmyard manure, 60 kg of nitrogen (N), 60 kg of phosphorus ( $P_2O_5$ ), and 25 kg of potassium ( $K_2O$ ) per hectare.

## 2.2. Experimental design

At the study site, designed with 15 experimental plots (size 3 m x 5 m). At each plot, 01 soil sensor (7 in 1 RS485 Modbus RTU), with the specifications shown in Hoang *et al.* (2025) [13], is connected to other hardware of the IoT system by wires to collect data.

Based on the results of previous studies on tomato plants and soil characteristics in the study area [14 - 16], we determined that the recommended amount of NPK fertilizer for tomato cultivation per hectare is 200 kg N, 200 kg  $P_2O_5$ , and 200 kg  $K_2O$ . Therefore, in the experimental plots, soluble inorganic fertilizer (KAISTOM, NPK 17-17-17) was applied 12 times throughout the tomato growing period, according to the following dosage schedule:

D1: 3.4 kg N/ hectare + 3.4 kg  $P_2O_5$ /hectare + 3.4 kg  $K_2O$ /hectare+ 0.4 L/m<sup>2</sup> water in plot 01, 06 and 11.

D2: 6.8 kg N/ hectare + 6.8 kg  $P_2O_5$ /hectare + 6.8 kg  $K_2O$ /hectare + 0.8 L/m<sup>2</sup> water in plot 02, 07 and 12.

D3: 10.2 kg N/ hectare + 10.2 kg  $P_2O_5$ /hectare + 10.2 kg  $K_2O$ /hectare + 1.2 L/m<sup>2</sup> water in plot 03, 08 and 13.

D4: 13.6 kg N/ hectare + 13.6 kg  $P_2O_5$ /hectare + 13.6 kg  $K_2O$ /hectare + 1.6 L/m<sup>2</sup> water in plot 04, 09 and 14.

D5: 17.0 kg N/ hectare + 17.0 kg  $P_2O_5$ /hectare + 17.0 kg  $K_2O$ /hectare + 2.0 L/m<sup>2</sup> water in plot 05, 10 and 15.

## 2.3. Measuring crop indicators

In the experimental plots, the growth, development, and yield indicators of tomato plants

were monitored and recorded at different growth stages following fertilizer application. The growth stages and corresponding monitoring indicators include:

- Seedling stage: Plant height, stem diameter, and number of true leaves.

- Vegetative stage: Plant height, stem diameter, and number of flowers.

- Flowering and fruiting stage: Plant height, number of flowers per plant, number of fruits per plant, and fruit diameter.

- Harvesting stage: Fruit diameter and yield (kg/plant).

## 2.4. Online sensing and monitoring data collection

The online data collection and monitoring system includes components: 7-in-1 RS485 Modbus RTU soil sensor, 12V DC power supply, Raspberry Pi Compute Module 4 industrial computer and 4-core RS485 connection fire conductor system. Data of soil properties to be collected including relative humidity (Rh), available N content, available  $P_2O_5$  and available  $K_2O$  are stored and extracted according to each growth stage of the tomato plants mentioned above.

## 2.5. Data processing

*2.5.1. Determine the requirements of the plant for moisture and N, P, K content in the soil*

Data on soil properties were collected in accordance with the growth and development stages of tomato plants. The indicators presented by the average value are collected and aggregated in stages.

The requirements of the plant for moisture and nutrient content N, P, K in the soil are the indicators in the fertilizer application test to achieve the most optimal crop indicators.

*2.5.2. Develop a correlation formula between the dose of irrigation and fertilizer and the increase in monitoring indicators in the soil*

Following each fertilization event, sensor data were collected at hourly intervals up to the

subsequent fertilization in order to assess the effects of different fertilizer application rates on key monitoring indicators. Specifically, for soil relative humidity, data acquisition commenced immediately after fertilizer application and continued on an hourly basis until the implementation of routine daily irrigation. Throughout the data collection period, standard daily irrigation practices were consistently maintained. The recorded data were aggregated, and average values were utilized for further analysis.

The Pearson correlation algorithm was implemented to determine the correlation formula between the dose of irrigation and fertilizer and the increase in monitoring indicators in the soil in the form  $y = ax + b$ , which will be the basis for calculating the dose of irrigation water and fertilizer for the recommendation system.

*2.5.3. Statistical analysis*

**Table 1. Comparison of difference in average value of crop monitoring indicators under different fertilization doses**

| Treatment                          | Seedling stage (n=3) |                         |                        | Vegetative stage (n=3) |                    |                         |
|------------------------------------|----------------------|-------------------------|------------------------|------------------------|--------------------|-------------------------|
|                                    | Plant Height (cm)    | Stem Diameter (mm)      | Number of leaves       | Plant Height (cm)      | Stem Diameter (mm) | Number of flowers/plant |
| D1                                 | 29.7a                | 3.7a                    | 8.3a                   | 80.0a                  | 10.7a              | 3.3a                    |
| D2                                 | 32.0ab               | 4.3ab                   | 9.0ab                  | 88.3ab                 | 11.3a              | 3.7ab                   |
| D3                                 | 32.7ab               | 5.3b                    | 9.3b                   | 96.3bc                 | 13.3b              | 4.0bc                   |
| D4                                 | 35.3bc               | 7.7c                    | 9.7bc                  | 101.7c                 | 15.3c              | 4.7cd                   |
| D5                                 | 36.7c                | 8.3c                    | 10.7c                  | 105.0c                 | 15.7c              | 5.0d                    |
| Flowering and fruiting stage (n=3) |                      |                         |                        |                        |                    |                         |
| Treatment                          | Plant Height (cm)    | Number of flowers/plant | Number of fruits/plant | Fruit diameter (cm)    | -                  | -                       |
| D1                                 | 101.0a               | 5.3a                    | 12a                    | 3.3a                   | -                  | -                       |
| D2                                 | 106.0ab              | 5.3a                    | 12a                    | 4.0ab                  | -                  | -                       |
| D3                                 | 110.0bc              | 6.3a                    | 13ab                   | 3.7a                   | -                  | -                       |
| D4                                 | 111.3c               | 5.7a                    | 16b                    | 4.3bc                  | -                  | -                       |
| D5                                 | 113.3c               | 6.0a                    | 15b                    | 4.7c                   | -                  | -                       |
| Harvesting stage (n=3)             |                      |                         |                        |                        |                    |                         |

A one-way analysis of variance (ANOVA), used to examine the differences in soil available nitrogen, phosphorus, and potassium contents, soil relative humidity, and crop performance indicators among treatments, and the Pearson correlation analyses described above were performed using IBM SPSS Statistics version 22.0.

**3. RESULTS AND DISCUSSIONS**

**3.1. Indicators of crop monitoring under different fertilization doses**

Table 1 presents the results of monitoring the growth and productivity indicators of tomato plants under the influence of five different fertilizing doses (D1 to D5), through the development stages including seedling, vegetative, flowering - fruiting and harvesting. The data were statistically processed with LSD letter grouping at a significance level of 0.05, in order to identify significant differences among the treatments.

| Treatment | 1st harvested fruit diameter (cm) | 1st harvested yield (kg/plant) | 2nd harvested fruit diameter (cm) | 2nd harvested yield (kg/plant) | 3rd harvested fruit diameter (cm) | 3rd harvested yield (kg/plant) |
|-----------|-----------------------------------|--------------------------------|-----------------------------------|--------------------------------|-----------------------------------|--------------------------------|
| D1        | 3.7a                              | 0.3a                           | 3.7a                              | 0.52a                          | 3.5a                              | 0.4a                           |
| D2        | 4.3b                              | 0.4b                           | 4.2b                              | 0.66b                          | 4.1b                              | 0.5b                           |
| D3        | 4.9c                              | 0.5c                           | 4.8c                              | 0.86c                          | 4.6c                              | 0.8c                           |
| D4        | 5.4d                              | 0.8d                           | 5.5d                              | 1.13d                          | 5.2d                              | 1.1d                           |
| D5        | 5.5d                              | 0.8d                           | 5.5d                              | 1.19d                          | 5.3d                              | 1.1d                           |

*Note: Different alphabet characters indicate statistically significant differences,  $p < 0.05$ .*

The results of monitoring the growth indicators and yield of tomato plants in the four stages of development (seedling, vegetative, flowering – fruiting, and harvesting) under the fertilizing treatments from D1 to D5 show a marked increase in most agronomic characteristics as fertilizer levels increase. In the seedling stage, the plant height increased from 29.7 cm in D1 to 36.7 cm in D5, while the stem diameter increased from 3.7 mm to 8.3 mm, and the number of true leaves also increased from 8.3 to 10.7 leaves/plant. Similarly, at the vegetative stage, the plant reaches a height of 80.0 cm (D1) to 105.0 cm (D5), and a stem diameter of 10.7 mm to 15.7 mm. The number of flowers gradually increased from 3.3 in D1 to 5.0 flowers/plant in D5, showing that vegetative and vegetative growth of plants are improved by fertilizing.

At the flowering - fruiting stage, the improvement continued to be evident with the plant height increasing from 101.0 cm (D1) to 113.3 cm (D5), the number of flowers/plant

ranged from 5.3 to 6.3 but there was no clear statistical difference. However, the number of fruits on the plant increased significantly, from 12 fruits (D1 and D2) to 15 - 16 fruits in D4 and D5. The fruit diameter also increased from 3.3 cm (D1) to 4.7 cm (D5) respectively, reflecting the positive effect of fertilizer on fruit size. By the harvesting stage, the yield indicators show a very clear difference: the 3rd harvested yield of D1 is only about 1.2 kg/plant, while D5 reaches a total of about 3.1 kg/plant, more than 2.5 times. At the same time, the fruit diameter in D5 remained at the highest level in all three acquisitions, indicating that fertilizing not only improved in quantity but also increased the weight of the fruit.

### 3.2. Requirements on relative humidity and content of available N, P, K in the soil of tomato plants

Table 2 provides information on soil moisture content and available N, P, K content in the soil corresponding to each fertilizer application in the four main growth stages of tomato plants.

**Table 2. Results of the synthesis of relative humidity and available N, P, K content in the soil collected by the sensor under different fertilization doses**

| Treatment            | Humidity (%) | Soil avail. N (mg/kg) | Soil avail. P <sub>2</sub> O <sub>5</sub> (mg/kg) | Soil avail. K <sub>2</sub> O (mg/kg) | Humidity (%)           | Soil avail. N (mg/kg) | Soil avail. P <sub>2</sub> O <sub>5</sub> (mg/kg) | Soil avail. K <sub>2</sub> O (mg/kg) |
|----------------------|--------------|-----------------------|---|--------------------------------------|------------------------|-----------------------|---|--------------------------------------|
| Seedling stage (n=6) |              |                       |   |                                      | Vegetative stage (n=9) |                       |   |                                      |
| D1                   | 22.0ab       | 56bc                  | 78c   | 178d                                 | 22.2c                  | 53c                   | 76c   | 178d                                 |
| D2                   | 19.7b        | 47c                   | 91c   | 195d                                 | 22.3c                  | 53c                   | 83c   | 191d                                 |
| D3                   | 26.3a        | 89a                   | 124b  | 256c                                 | 24.8b                  | 82b                   | 120b  | 278c                                 |

|                                  |        |       |      |      |                         |      |      |      |
|----------------------------------|--------|-------|------|------|-------------------------|------|------|------|
| D4                               | 21.2ab | 117a  | 164a | 311b | 25.2ab                  | 123a | 169a | 338b |
| D5                               | 21.5ab | 102ab | 175a | 378a | 25.5a                   | 122a | 194a | 428a |
| Flowering – Fruiting stage (n=9) |        |       |      |      | Harvesting stage (n=12) |      |      |      |
| D1                               | 22.3a  | 56c   | 94c  | 209d | 22.3a                   | 58c  | 79c  | 178e |
| D2                               | 23.0a  | 71c   | 94c  | 235d | 22.7a                   | 64c  | 95c  | 218d |
| D3                               | 24.5a  | 93b   | 136b | 315c | 22.9a                   | 85b  | 130b | 292c |
| D4                               | 23.7a  | 129a  | 193a | 420a | 22.9a                   | 117a | 175a | 375b |
| D5                               | 23.2a  | 122a  | 189a | 405b | 22.8a                   | 143a | 217a | 474a |

*Note: Different alphabet characters indicate statistically significant differences,  $p < 0.05$ .*

Data to monitor soil moisture requirements and nutrient content of N, P, K show that tomato plants have increasing nutrient requirements with each stage of growth and according to fertilizing experiments. In the seedling stage, soil moisture ranges from 19.7% to 26.3%, with the highest value in D3, then slightly reduced in D4 and D5 (21.2 - 21.5%). Soil available N content increased drastically from 47 mg/kg (NT 2) to 117 mg/kg (NT 4), while available phosphorus ( $P_2O_5$ ) content ranged from 78 mg/kg to 175 mg/kg and available potassium ( $K_2O$ ) content from 178 mg/kg to 378 mg/kg, which was highest in NT 5. In the vegetative stage, the humidity remains stable in the range of 22.2 - 25.5%, and the content of N, P, and K continues to increase with the trend of fertilizing. Potassium content increased particularly sharply, from 178 mg/kg in D1 to 428 mg/kg in D5.

At the stage of flowering - fruiting and harvesting, soil moisture is no longer significantly different between experiments, fluctuating narrowly around 22 - 24.5%, which indicates that the plant is less sensitive to moisture at the final stage. However, N, P, K content still plays an important role, especially potassium. At D5, available  $K_2O$  reaches 474 mg/kg at harvesting stage, which is significantly higher than D4 (375 mg/kg), while N and P are only slightly different. This is consistent with the results observed in table 1, when the majority of growth indicators between D4 and D5 do not have statistical differences, but D5 tends to be higher in some parameters such as number of fruits or fruit

diameter. This suggests that the slight difference between D4 and D5 in crop characteristics can be explained by the difference in soil available  $K_2O$  content, rather than by N or  $P_2O_5$  contents.

The increase in growth indicators (plant height, stem diameter, number of true leaves) and yield (number of fruits, weight of fruits, and diameter of fruits) corresponding to the increase in fertilizer application from D1 to D5 shows that fertilizer has a significant positive impact on the development of tomato plants.

However, the degree of improvement gradually decreased at D4 and D5: most of the indicators did not differ statistically, although D5 had a slightly higher average value. This indicates that D4 has nearly reached the optimal threshold, increasing the amount of fecal matter in D5 no longer yields a statistically corresponding effect.

While the N and  $P_2O_5$  contents were not much different between D4 and D5,  $K_2O$  increased markedly at D5 (increasing from 420 mg/kg to 474 mg/kg during the harvesting stage). This is consistent with the record of some higher crop indicators (fruit diameter and number of fruits) in D5, although not statistically different from D4. Therefore, potassium can be a factor that plays a role in maintaining or slightly improving yield at the highest level of fertilization.

The results show that D4 is the optimal solution in terms of growth, productivity and efficiency of fertilizer use. D5 has some higher indicators, but not statistically significant, while the requirement for potassium content increases sharply, which may cause fertilizer waste or the

risk of excess nutrients in the soil if applied long-term.

Therefore, the tomato plant's requirements for relative humidity and soil N, P, K content are:

- Seedling stage: 20 - 22% Rh + 100 - 120 mg/kg N + 150 - 200 mg/kg  $P_2O_5$  + 300 - 400 mg/kg  $K_2O$ .
- Vegetative stage: 22-25% Rh + 100 - 150 mg/kg N + 150 - 200 mg/kg  $P_2O_5$  + 350 - 400 mg/kg  $K_2O$ .

- Flowering - Fruiting stage: 22 - 25% Rh + 100 - 120 mg/kg N + 150 - 200 mg/kg  $P_2O_5$  + 400 - 450 mg/kg  $K_2O$ .

- Harvesting stage: 22 - 25% Rh + 100 - 120 mg/kg N + 150 - 200 mg/kg  $P_2O_5$  + 400 - 500 mg/kg  $K_2O$ .

3.3. The formula correlates the dose of irrigation water and fertilizer with the increase in moisture and N, P, K content in the soil

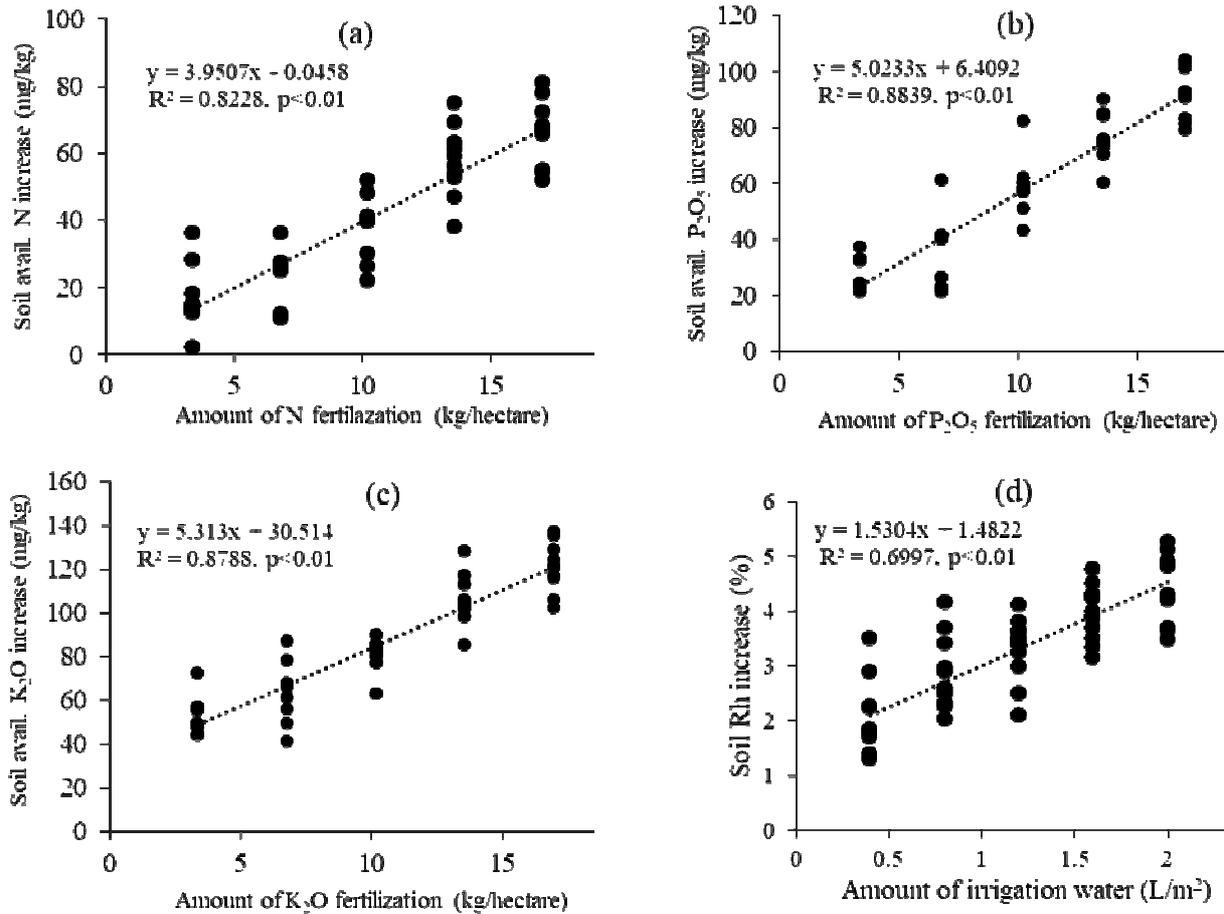


Figure 1. Correlation between the amount of irrigation water and the amount of NPK fertilizer with the increase in humidity and the content of N-P-K collected by the sensor (n=60)

- (a) Correlation between the amount of fertilizer and the increase in N in the soil.
- (b) Correlation of fertilizer intake with  $P_2O_5$  increase in soil.
- (c) Correlation of fertilizer intake with  $K_2O$  increase in soil.
- (d) Correlation between the amount of irrigation water and the increase in the relative humidity in the soil.

The assessment of the relationship between the amount of fertilizer, irrigation water and the fluctuation of physicochemical indicators of the soil is an important foundation in the study of

sustainable agriculture. In this study, sensor data from 60 soil samples (n = 60) were used to construct bivariate correlation models, thereby determining the correlation between NPK

fertilizers with the corresponding increase in soil N, P<sub>2</sub>O and K<sub>2</sub>O contents and the amount of irrigation water with the increase in relative humidity in the soil. The graphs in figure 1 show a linear upward trend between the amount of irrigation water and fertilizer with moisture and N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O content measured in the soil by the sensor. The relationships are shown through highly statistically significant correlation graphs and regression formulas ( $p < 0.01$ ), reflecting the practicality and reliability of the results.

Between the amount of fertilizer with N increase in soil:  $y = 3.9507x - 0.0458$ ,  $R^2 = 0.8228$ : Each kg/hectare of nitrogen fertilizer (N) applied to the soil increases the N content in the soil by about 3.95 mg/kg. The base coefficient (-0.0458) is close to zero, indicating a linear correlation starting near the origin, reflecting the fact that digestible protein is a nutrient that is easily lost and difficult to accumulate in the soil.  $R^2 = 0.8228$  shows that the model explains 82.28% of the variation in N content in soil from the amount of N fertilizer, which is a high correlation, reflecting that the conversion efficiency of N fertilizer into measurable mineral form in soil is reliable. However, there are still 17.72% unexplained errors that may result from evaporation, leaching or adsorption by soil glue.

Between the amount of fertilizer with an increase in P<sub>2</sub>O<sub>5</sub> in the soil:  $y = 5.0233x + 6.4092$ ,  $R^2 = 0.8839$ : Per kg/hectare P<sub>2</sub>O<sub>5</sub> applied, the content of P<sub>2</sub>O<sub>5</sub> in the soil increases by about 5.02 mg/kg. A constant value of 6.4092 indicates that there is a baseline level of phosphorus available in the soil or an effect from residues from previous crops.  $R^2 = 0.8839$  is very high, which proves that the model has good reliability. A slope greater than N indicates that the phosphate fertilizer has more retention and less loss due to leaching, thanks to its strong adsorption with soil glue or interaction with iron and aluminum.

Between the amount of fertilizer with an increase in available K<sub>2</sub>O content in the soil:  $y = 5.313x + 30.514$ ,  $R^2 = 0.8788$ : Per kg/hectare K<sub>2</sub>O application will increase 5.313 mg/kg K<sub>2</sub>O in the soil, with a fairly high initial background level

(30.514 mg/kg). The high correlation coefficient ( $R^2 = 0.8788$ ) shows a reliable relationship between the amount of fertilizer K<sub>2</sub>O and the increase of K<sub>2</sub>O in the soil. K is the average mobile element, so that a part can be washed away, but the large angular coefficient indicates that the potassium application efficiency is high. However, a relatively large base (30.514) indicates that the soil was initially capable of providing good natural K, possibly from primary minerals such as mica or feldspar.

Between the amount of irrigation water and the increase in relative humidity in the soil:  $y = 1.5304x + 1.4822$ ,  $R^2 = 0.6997$ : Each liter of irrigation water/m<sup>2</sup> will increase the soil humidity by about 1.53%. The constant component (1.4822) reflects the natural background moisture.  $R^2 = 0.6997$  is average - decent, showing relatively wide data dispersion, and the model explains only about 70% of the variation. This is appropriate because soil moisture is affected by many factors including soil structure, soil texture, rate of water infiltration, and evaporation.

#### **4. CONCLUSION**

Fertilization at different levels has a marked effect on tomato growth and yield. D4 is the most effective level of fertilization in this research condition, helping to maximize agronomic indicators without causing significant excess nutrients in the soil. Increasing the stool to D5 level does not make a significant statistical difference, except for some small improvements in yield, mainly due to higher potassium.

Regression models have shown that the amount of fertilizer has a marked impact on the increase in nutrient content in the soil with a high  $R^2$  coefficient (above 0.82), confirming the efficiency of fertilizer in improving the quality of agricultural land. Specifically, the slope of the regression lines shows that each unit of fertilizer can significantly increase the content of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O in the soil, with the degree of variation of this content being explained mainly by the amount of fertilizer. The correlation between the amount of irrigation water and the relative humidity of the soil with a lower  $R^2$  coefficient (0.6997), shows that the

influence of irrigation water on soil moisture depends not only on the amount of water but also on other factors such as the motorized composition of the soil, structure and water retention.

Thus, with the high reliability required by tomato plants in terms of relative humidity and N, P, K content in the soil, the relative humidity data and N, P, K content in the soil measured in real time by the sensor and the linear regression formula of these indicators can become the input data of the system recommending watering and macronutrient fertilizing (N, P, K) for tomato plants grown in greenhouses in Son La province.

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# POSITIVE INFLUENCE OF VERMICOMPOST RATES AND HB101 PLANT VITALIZER ON GROWTH AND YIELD OF BUSH - TYPE COMMON BEAN

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## ABSTRACT

The combined use of organic fertilizers and foliar applications is increasingly recognized as a strategy to enhance early crop growth and yield. The field experiment was conducted to determine the effects of vermicompost rates and spraying frequency of HB101 plant vitalizer on the growth and yield of bush-type common bean. The two - factor experiment was designed using a randomized complete block design (RCBD). Vermicompost was applied at three levels of 4, 8 and 12 tons ha<sup>-1</sup>. The treatments of HB101 spraying were: H0 (water spraying – as control), H1 (spraying at 5-day intervals), H2 (spraying at 10-day intervals). The results showed that increasing vermicompost rates led to increased plant growth and yield traits. Application of 12 tons ha<sup>-1</sup> of vermicompost produced the highest values of LAI, SPAD, dry weight, pod number, pod weight, pod weight per plant, pod yield of bean. Spraying HB101 positively affected the growth indicators, physiological traits and yield parameters, which were statistically higher than those of the control treatment. Combination of 12 tons ha<sup>-1</sup> of vermicompost with spraying HB101 at 5-day or 10-day intervals resulted in better plant performance, with comparable pod yield being 5.12 and 4.61 tons ha<sup>-1</sup>.

**Keywords:** *Bush-type common bean, vermicompost, plant vitalizer, pod yield.*

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## 1. INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a widely cultivated species globally and in Vietnam. Beans are rich in protein, minerals, fiber, energy, and vitamins, making them a viable meat substitute in underdeveloped and developing countries [1]. The bush-type common bean is a new variety with high adaptability to Vietnam climate and a yield potential comparable to that of climbing bean varieties. However, until now, research on cultivation techniques for this bean kind is still limited.

Organic fertilizers - particularly vermicompost - have been proven effective in enhancing crop yields and minimizing adverse environmental impacts. Vermicompost contains a balanced amount of macronutrients and micronutrients,

which are easily absorbed by plants. They also contain a diverse microbial system, plant hormones and growth-regulating compounds, that improve soil quality, stimulate plant growth and enhance crop yields [2, 3]. However, vermicompost, as other organic fertilizers, typically act slowly due to the slow mineralization, making it necessary to combine with foliar fertilizers at the early stages of plant growth. Foliar fertilization enables nutrients to be absorbed directly through the leaf surface, resulting in higher nutrient-use efficiency, particularly under nutrient-poor soil conditions [4]. Dang *et al.* (2025) [5] concluded that combining organic fertilization with foliar application positively impacts the soil quality and plant growth, decreases the physiological disorders in durian, contributing to improving durian

production. HB101 is a natural organic solution extracted from cedars, cypress, pines, and plantain plants, acting as both a nutritional source and a plant vitalizer for crops. Some previous studies concluded HB101 solution being effective in promoting plant growth and yield, such as potato [6], red cabbage [7].

However, the effectiveness of combining basal organic fertilizers with foliar plant vitalizers varies depending on the crops, cultivars, and the ecological conditions of the research area. This study was conducted to determine the optimal amount of vermicompost combined with HB101 foliar application for bush-type common bean under the cultivation conditions in Gia Lam, Ha Noi city.

**2. MATERIALS AND RESEARCH METHODS**

The two-factor field experiment was conducted on VA.007 bush-type common bean variety in spring-summer season of 2022 in Gia Lam, Ha Noi city. The experiment was arranged using a randomized complete block design (RCBD) with three replications, with an experimental plot of 7 m<sup>2</sup>. The first factor was vermicompost with three rates: T1 - 4 tons ha<sup>-1</sup>, T2 - 8 tons ha<sup>-1</sup>, and T3 - 12 tons ha<sup>-1</sup>. The second factor was the frequency of HB101 spraying, with three treatments: H0 - water spraying (control), H1 - spraying HB101 at 5-day intervals, and H2 - spraying HB101 at 10-day intervals. The used vermicompost contained nutrients of 2.5% N, 1.8% P<sub>2</sub>O<sub>5</sub>, and 1.4% K<sub>2</sub>O. The nutritional components of HB101 plant vitalizer are as follows: 0.1% total protein, pH 4.0, 0.4% total lipids, 41 mg L<sup>-1</sup> Na, 33 mg L<sup>-1</sup> Ca, 1.8 mg L<sup>-1</sup> Fe, 3.3 mg L<sup>-1</sup> Mg, and 7.4 mg L<sup>-1</sup> Si.

Seeds were sown at a spacing of 50 cm x 30

cm, with 3 - 4 seeds per hole, and then 2 plants per hole were left at 9 days after sowing (DAS). Vermicompost was applied 10 days before sowing. HB101 solution was diluted at a ratio of 3 drops per L of water, and applied during the experimental period with a rate of 1 L of solution for 1 m<sup>2</sup> of land.

Growth indicators (plant height, aboveground fresh weight (AFW)) and physiological parameters (leaf area index (LAI), SPAD, aboveground dry weight (ADW)) were measured at flowering stage (37 DAS) and at harvesting stage (50 DAS). SPAD index was measured using a chlorophyll meter (SPAD-502, Osaka, Japan). LAI was measured using the formula: LAI = P2/P1 \*100\* plant number m<sup>-2</sup>, where P1 is the fresh weight of 1 dm<sup>2</sup> of leaves, P2 is the total fresh weight of leaves on the plant. The aboveground part of plant samples were oven - dried at 80°C until the constant weight for determining ADW. The yield components, including pod number, pod weight, and pod weight per plant, were collected. The actual pod yield was determined as the total pod weight of all plants from each experimental plot.

The data were statistically analyzed using Statistix 8.0 software with analysis of variance (ANOVA) to determine the influence of treatments and their interaction. The least significant difference (LSD) was used to compare the mean values between treatments.

**3. RESULTS AND DISCUSSION**

**3.1. Effects of vermicompost rates and HB101 spraying frequencies on the growth of bush-type common beans**

**Table 1. Effects of vermicompost rates and HB101 spraying frequencies on growth indicators of bush-type common beans**

| Vermicompost rates | HB101 spraying frequencies | Plant height (cm)   |                     | AFW (g plant <sup>-1</sup> ) |                     |
|--------------------|----------------------------|---------------------|---------------------|------------------------------|---------------------|
|                    |                            | Flowering stage     | Harvesting stage    | Flowering stage              | Harvesting stage    |
| V1                 | H0                         | 41.49 <sup>b</sup>  | 46.67 <sup>b</sup>  | 33.91 <sup>c</sup>           | 53.51 <sup>d</sup>  |
|                    | H1                         | 44.61 <sup>ab</sup> | 48.45 <sup>ab</sup> | 44.11 <sup>ab</sup>          | 68.39 <sup>bc</sup> |
|                    | H2                         | 44.96 <sup>ab</sup> | 49.51 <sup>ab</sup> | 41.12 <sup>b</sup>           | 63.34 <sup>c</sup>  |
| V2                 | H0                         | 44.85 <sup>ab</sup> | 48.41 <sup>ab</sup> | 40.03 <sup>bc</sup>          | 63.40 <sup>c</sup>  |
|                    | H1                         | 46.63 <sup>a</sup>  | 50.53 <sup>ab</sup> | 43.36 <sup>ab</sup>          | 70.19 <sup>bc</sup> |

|                            |    |                    |                     |                     |                     |
|----------------------------|----|--------------------|---------------------|---------------------|---------------------|
|                            | H2 | 46.11 <sup>a</sup> | 51.30 <sup>a</sup>  | 45.12 <sup>ab</sup> | 68.81 <sup>bc</sup> |
| V3                         | H0 | 44.97 <sup>a</sup> | 49.61 <sup>ab</sup> | 46.40 <sup>ab</sup> | 68.83 <sup>bc</sup> |
|                            | H1 | 46.90 <sup>a</sup> | 52.13 <sup>a</sup>  | 49.21 <sup>a</sup>  | 79.36 <sup>a</sup>  |
|                            | H2 | 46.83 <sup>a</sup> | 50.83 <sup>a</sup>  | 46.50 <sup>ab</sup> | 74.76 <sup>ab</sup> |
| Vermicompost rates         | V1 | 43.69 <sup>b</sup> | 48.21 <sup>b</sup>  | 39.71 <sup>b</sup>  | 61.75 <sup>c</sup>  |
|                            | V2 | 45.86 <sup>a</sup> | 50.08 <sup>ab</sup> | 42.84 <sup>b</sup>  | 67.47 <sup>b</sup>  |
|                            | V3 | 46.23 <sup>a</sup> | 50.86 <sup>a</sup>  | 47.37 <sup>a</sup>  | 74.32 <sup>a</sup>  |
| HB101 spraying frequencies | H0 | 43.77 <sup>b</sup> | 48.23 <sup>a</sup>  | 40.11 <sup>b</sup>  | 61.91 <sup>b</sup>  |
|                            | H1 | 46.05 <sup>a</sup> | 50.37 <sup>a</sup>  | 45.56 <sup>a</sup>  | 72.65 <sup>a</sup>  |
|                            | H2 | 45.97 <sup>a</sup> | 50.54 <sup>a</sup>  | 44.25 <sup>a</sup>  | 68.97 <sup>a</sup>  |

Note: Values with different letters in the same column of individual or interactive factors indicate significant differences in LSD test at 5% significance level.

The results show the statistical differences of plant height and AFW of beans under vermicompost rates as well as HB101 spraying frequencies (Table 1). Generally, V2 and V3 produced taller plants than V1 treatment. Specifically, V3 showed a significantly higher plant height than V1 at both growth stages. AFW followed a similar trend, being highest in V3 (47.37 and 74.32 g plant<sup>-1</sup> at flowering and harvest stages, respectively). HB101 spraying also presented a positive effect on bean plants, when at flowering stage, H1 and H2 significantly increased plant height compared with H0. Likewise, AFW was statistically enhanced by HB101 application as compared to H0 treatment at all measured times.

Combined application of vermicompost with HB101 spraying significantly influenced plant height and AFW of beans at flowering and harvesting stages (Table 1). Generally, increasing vermicompost rates promoted plant height, especially in the interaction with HB101 spraying. Regarding AFW, at flowering stage, higher

vermicompost rates interacted with HB101 increased this indicator, which was highest under V3H1 (49.21 g plant<sup>-1</sup>). At harvesting stage, AFW increased progressively with vermicompost rates, where V3H1 recorded the highest value (79.36 g plant<sup>-1</sup>), being comparable with V3H2 (74.76 g plant<sup>-1</sup>) and statistically higher than all other treatments

### 3.2. Effects of vermicompost rates and HB101 spraying frequencies on physiological traits of bush-type common bean

Across both measurement stages, vermicompost positively influenced LAI (Figure 1). At flowering stage, LAI was the highest under V3 treatment, which was comparable with V2 treatment. At harvesting stage, the statistical higher LAI was recorded under V3 treatment. Regarding HB101 spraying, at flowering stage, H1 treatment yielded the highest LAI, but in harvesting stage, H1 and H2 gave comparable LAI and statistically higher than that of H0 treatment.

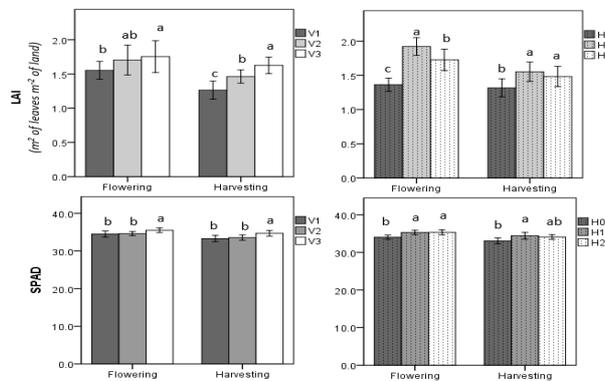


Figure 1. Single effects of vermicompost rates and HB101 spraying frequencies on SPAD readings and leaf area index (LAI) of bush-type common beans

Notes: Columns with different letters within each treatment are significantly different at the 5% level

Similar trends were observed for SPAD, where V3 resulted in statistically better value in both two stages. HB101 application significantly affected SPAD. At flowering stage, HB101 application statistically outperformed watering only (H0

treatment). At harvesting stage, the maximum SPAD was reached in plants treated with H1 treatment, which was at par with H2 treatment and statistically higher than H0 treatment.

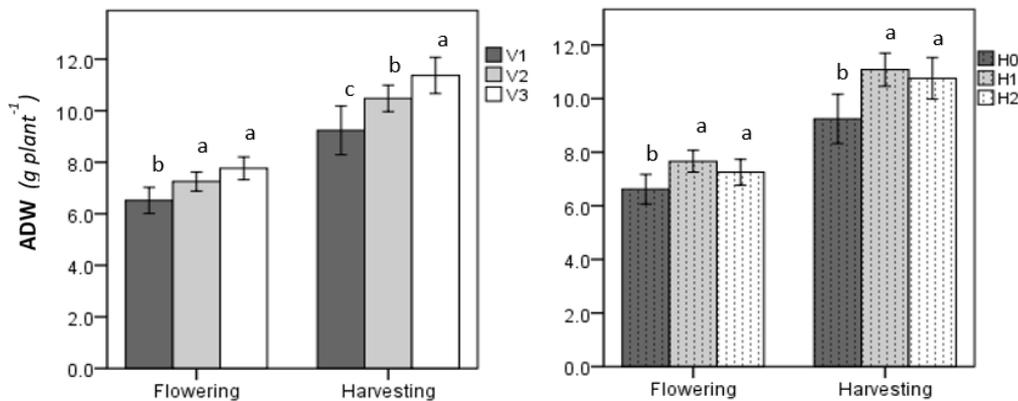
**Table 2. Interactive effects of vermicompost rates and HB101 spraying frequencies on SPAD readings and leaf area index (LAI) of bush-type common beans**

| Vermicompost rates | HB101 spraying frequencies | SPAD                 |                       | LAI (m <sup>2</sup> of leaves m <sup>-2</sup> of land) |                      |
|--------------------|----------------------------|----------------------|-----------------------|--|----------------------|
|                    |                            | Flowering stage      | Harvesting stage      | Flowering stage  | Harvesting stage     |
| V1                 | H0                         | 33.47 <sup>c</sup>   | 32.50 <sup>d</sup>    | 1.34 <sup>d</sup>                                      | 1.10 <sup>e</sup>    |
|                    | H1                         | 35.18 <sup>ab</sup>  | 34.36 <sup>abc</sup>  | 1.72 <sup>bc</sup>                                     | 1.44 <sup>cd</sup>   |
|                    | H2                         | 34.83 <sup>abc</sup> | 33.06 <sup>bcd</sup>  | 1.60 <sup>cd</sup>                                     | 1.26 <sup>de</sup>   |
| V2                 | H0                         | 33.76 <sup>c</sup>   | 32.59 <sup>cd</sup>   | 1.37 <sup>d</sup>                                      | 1.40 <sup>cd</sup>   |
|                    | H1                         | 34.59 <sup>bc</sup>  | 33.73 <sup>abcd</sup> | 1.99 <sup>ab</sup>                                     | 1.52 <sup>abc</sup>  |
|                    | H2                         | 35.52 <sup>ab</sup>  | 34.36 <sup>abc</sup>  | 1.76 <sup>bc</sup>                                     | 1.47 <sup>abcd</sup> |
| V3                 | H0                         | 34.83 <sup>ab</sup>  | 34.06 <sup>abcd</sup> | 1.39 <sup>d</sup>                                      | 1.46 <sup>bcd</sup>  |
|                    | H1                         | 36.12 <sup>a</sup>   | 35.18 <sup>a</sup>    | 2.06 <sup>a</sup>                                      | 1.70 <sup>ab</sup>   |
|                    | H2                         | 35.59 <sup>ab</sup>  | 34.82 <sup>ab</sup>   | 1.82 <sup>abc</sup>                                    | 1.72 <sup>a</sup>    |

Note: Values with different letters in the same column of individual or interactive factors indicate significant differences in the LSD test at the 5% significance level.

The results from table 2 show that, under the same vermicompost rate, spraying HB101 produced higher SPAD compared to the control. Furthermore, higher vermicompost rates combined with HB101 effectively increased chlorophyll content in bean leaves. At both stages, T3H1 treatment resulted in the maximum SPAD, with 36.12 at flowering stage and 35.18 at harvesting stage.

Regarding LAI, at both two phenological stages, increasing vermicompost amount (V2 and V3 treatments) applied along with spraying HB101 (H1 and H2 treatment) increased LAI, even though there were no differences between these treatments. The highest LAI was obtained under V3H1 treatment with 2.06 m<sup>2</sup> of leaves m<sup>-2</sup> of land at flowering stage, and under V3H2 treatment with 1.72 m<sup>2</sup> of leaves m<sup>-2</sup> of land at harvesting stage.



**Figure 2. Single effects of vermicompost rates and HB101 spraying frequencies on aboveground dry weight of bush-type common beans**

Notes: Columns with different letters within each treatment are significantly different at the 5% level.

Vermicompost rates and HB101 spraying frequencies statistically influenced the dry accumulation of beans (Figure 2). The data clearly show a positive trend in the effect of higher vermicompost rates on ADW. At flowering stage, plants treated with V3 showed the highest ADW, being comparable with that under V2 treatment and statistically higher than V1 treatment. At harvesting

stage, ADW under V3 treatment statistically outperformed V1 and V2 treatments. On the other hand, the frequency of HB101 spraying also showed a significant impact on ADW. The data indicate that spraying HB101 (H1 and H2 treatments) had a crucial role in maximizing ADW, which was statistically higher than that in plants treated by water only (H0 treatment).

**Table 3. Interactive effect of vermicompost rates and HB101 spraying frequency on aboveground dry weight of bush - type common beans**

| Vermicompost rates | HB101 spraying frequencies | ADW (g plant <sup>-1</sup> ) |                      |
|--------------------|----------------------------|------------------------------|----------------------|
|                    |                            | Flowering stage              | Harvesting stage     |
| V1                 | H0                         | 5.86 <sup>e</sup>            | 7.63 <sup>e</sup>    |
|                    | H1                         | 7.15 <sup>bcd</sup>          | 10.23 <sup>cd</sup>  |
|                    | H2                         | 6.55 <sup>de</sup>           | 9.85 <sup>cd</sup>   |
| V2                 | H0                         | 6.77 <sup>cde</sup>          | 9.75 <sup>d</sup>    |
|                    | H1                         | 7.67 <sup>abc</sup>          | 11.15 <sup>abc</sup> |
|                    | H2                         | 7.32 <sup>abcd</sup>         | 10.52 <sup>bcd</sup> |
| V3                 | H0                         | 7.23 <sup>abcd</sup>         | 10.35 <sup>cd</sup>  |
|                    | H1                         | 8.17 <sup>a</sup>            | 11.85 <sup>ab</sup>  |
|                    | H2                         | 7.89 <sup>ab</sup>           | 11.90 <sup>a</sup>   |

*Note: Values with different letters in the same column indicate significant differences in the LSD test at 5% significance level.*

The interaction between vermicompost rates and HB101 spraying significantly influenced ADW at both growing stages (Table 3). At flowering stage, V3 treatment consistently resulted in higher ADW across all HB101 treatments, which were comparable with V2H1 and V2H2 treatments, therein V3H1 gave the maximum ADW (8.17 g plant<sup>-1</sup>). At harvesting stage, higher ADW values were reached under V3H2, V3H1 and V2H1 (11.90, 11.85 and 11.15 g plant<sup>-1</sup>, respectively). Generally, higher vermicompost rates interacted with HB101 showed the synergistic effect for improving plant growth. Additionally, at the same vermicompost rates, H2 treatment improved ADW, but further increasing the frequency to H1 did not provide additional benefits.

### 3.3. Effects of vermicompost rates and HB101 spraying frequencies on yield components and pod yield of bush-type common bean

There were significant differences in yield components and pod yield, based on varying vermicompost rates as well as HB101 spraying (Table 4). Increasing vermicompost rate corresponded to better measured parameters, with V3 statistically outperforming V1 and V2 in terms of pod number, pod weight per plant and pod yield, with pod yield under V3 treatment being 4.67 tons ha<sup>-1</sup>. On the other hand, HB101 application had no difference in pod weight. The effect of HB101 spraying was particularly notable in terms of pod number, where H1 treatment gave a statistically higher pod number compared to H0

and H2 treatments. In addition, H1 treatment achieved the highest pod weight per plant (40.80 g plant<sup>-1</sup>) and pod yield (4.24 tons ha<sup>-1</sup>), which were comparable to H2 treatment.

**Table 4. Effects of vermicompost rates and HB101 spraying frequencies on yield components and pod yield of bush - type common bean**

| Vermicompost rates         | HB101 spraying frequencies | Pod number (Pod plant <sup>-1</sup> ) | Pod weight (g pod <sup>-1</sup> ) | Pod weight per plant (g plant <sup>-1</sup> ) | Pod yield (tons ha <sup>-1</sup> ) |
|----------------------------|----------------------------|---------------------------------------|-----------------------------------|---|------------------------------------|
| V1                         | H0                         | 6.23 <sup>e</sup>                     | 4.24 <sup>b</sup>                 | 26.48 <sup>e</sup>                            | 2.86 <sup>e</sup>                  |
|                            | H1                         | 7.87 <sup>cd</sup>                    | 4.35 <sup>b</sup>                 | 34.43 <sup>cd</sup>                           | 3.37 <sup>de</sup>                 |
|                            | H2                         | 7.20 <sup>de</sup>                    | 4.42 <sup>b</sup>                 | 31.88 <sup>de</sup>                           | 3.45 <sup>de</sup>                 |
| V2                         | H0                         | 7.83 <sup>cd</sup>                    | 4.60 <sup>ab</sup>                | 35.85 <sup>cd</sup>                           | 3.51 <sup>d</sup>                  |
|                            | H1                         | 9.08 <sup>ab</sup>                    | 4.50 <sup>ab</sup>                | 40.78 <sup>abc</sup>                          | 4.24 <sup>bc</sup>                 |
|                            | H2                         | 8.37 <sup>bc</sup>                    | 4.38 <sup>b</sup>                 | 36.66 <sup>bcd</sup>                          | 3.98 <sup>cd</sup>                 |
| V3                         | H0                         | 8.70 <sup>bc</sup>                    | 4.61 <sup>ab</sup>                | 40.03 <sup>bc</sup>                           | 4.27 <sup>bc</sup>                 |
|                            | H1                         | 9.80 <sup>a</sup>                     | 4.80 <sup>ab</sup>                | 47.19 <sup>a</sup>                            | 5.12 <sup>a</sup>                  |
|                            | H2                         | 8.57 <sup>bc</sup>                    | 5.06 <sup>a</sup>                 | 43.26 <sup>ab</sup>                           | 4.61 <sup>ab</sup>                 |
| Vermicompost rates         | V1                         | 7.10 <sup>c</sup>                     | 4.34 <sup>b</sup>                 | 30.93 <sup>c</sup>                            | 3.22 <sup>c</sup>                  |
|                            | V2                         | 8.43 <sup>b</sup>                     | 4.49 <sup>ab</sup>                | 37.76 <sup>b</sup>                            | 3.91 <sup>b</sup>                  |
|                            | V3                         | 9.02 <sup>a</sup>                     | 4.82 <sup>a</sup>                 | 43.49 <sup>a</sup>                            | 4.67 <sup>a</sup>                  |
| HB101 spraying frequencies | H0                         | 7.59 <sup>b</sup>                     | 4.48 <sup>a</sup>                 | 34.12 <sup>b</sup>                            | 3.54 <sup>b</sup>                  |
|                            | H1                         | 8.92 <sup>a</sup>                     | 4.55 <sup>a</sup>                 | 40.80 <sup>a</sup>                            | 4.24 <sup>a</sup>                  |
|                            | H2                         | 8.04 <sup>b</sup>                     | 4.62 <sup>a</sup>                 | 37.26 <sup>ab</sup>                           | 4.01 <sup>a</sup>                  |

*Note: Values with different letters in the same column of individual or interactive factors indicate significant differences in the LSD test at the 5% significance level.*

Vermicompost rates interacted with HB101 spraying had a significant effect on yield components and yield of bean plants (Table 4). At the lowest vermicompost rate (V1), all these indicators remained at lower level regardless of HB101 spraying frequencies, which suggests that limited nutrient input constrained plant response to foliar spraying. At the highest vermicompost rate (V3), pod yield attributes were further markedly enhanced by HB101 spraying, particularly under combining with H1 treatment. V3H1 treatment produced the greatest pod number (9.80 pods plant<sup>-1</sup>), pod weight (4.80 g), pod weight per plant (47.19 g), and the highest

pod yield (5.12 tons ha<sup>-1</sup>). Additionally, V3H2 gave the comparable yield parameters (43.26 g plant<sup>-1</sup> and 4.61 tons ha<sup>-1</sup>). These results clearly indicate that the interaction between vermicompost rates and HB101 spraying is synergistic, leading to better pod yield of bush – type common bean.

### 3.4. Discussions

Vermicompost has emerged as a sustainable soil amendment, reducing the dependency on chemical fertilizers as well as promoting long-term soil fertility. Vermicompost sources are rich in readily available nutrients, beneficial microorganisms, and humic substances, thereby improving soil structure, aeration, and water-

holding capacity, resulting in fostering optimal root development [8]. In addition, vermicompost stimulates plant growth through the release of bioactive compounds, including phytohormones and enzymatic metabolites [3, 8]. In our experiment, application of vermicompost at higher rates (V2 and V3 treatments) led to significant increases in vegetative growth parameters (plant height, AFW, LAI) as well as yield components (pod number, pod weight, pod weight per plant) and pod yield in common bean. These results are consistent with the previous studies. Salwa et al. (2019) [7] reported that higher vermicompost rate promoted plant growth and yield of red cabbage. Regarding Gutiérrez-Miceli *et al.* (2007) [2], adding more vermicompost significantly increased plant height and fruit yield of tomatoes. According to Belmeskine *et al.* (2020) [8], earthworms interact intensively with microorganisms to speed up the decomposition of organic matter, leading to higher plant height, leaf fresh weight, the root length and number of nodules of snap bean (*Phaseolus vulgaris* L.) under vermicompost application. Additionally, vermicompost increased chlorophyll content of leaves, leading to better photosynthesis efficiency with improvement of biological yield of snap beans [8]. In the study of Sangsoda *et al.* (2025) [9], vermicompost showed the highest plant height and dry weight of hemp compared to compost treatment.

Nowadays, the utilization of plant-derived biostimulants offers an environmentally sustainable and effective strategy for advancing modern agricultural practices. According to Godlewska *et al.* (2021) [10], Ertani *et al.* (2016) [11], Mbuyisa *et al.* (2024) [12], the plant extracts generate a high content of bioactive compounds, that enhance plant

growth, quality, photosynthetic activity while improving tolerance to both abiotic and biotic stresses. In our trials, HB101 spraying increased pod yield of common bean with improvement in growth parameters such as AFW, LAI, SPAD and ADW, especially in the interaction with higher vermicompost rates. It suggests that regular application of the HB101 solution positively influences plant growth and development. Our findings are consistent with the previous studies. Ertani *et al.* (2016) [11] recorded better dry weight, contents of protein, glucose, fructose in roots and leaves, as well as chlorophyll content and phenolic acids in leaves of maize treated by vegetal extracts derived from red grape, blueberry fruits and hawthorn leaves, especially at higher spraying rate. These authors confirmed the presence of plant-promoting substances, such as IAA and IPA hormones and different phenolic compounds in the applied extracts. Similarly, Mbuyisa *et al.* (2024) [12] found the macro- and micronutrients, phenolics, flavonoids and antioxidant activity in plant extracts derived from *Ascophyllum nodosum*, aloe vera leaf and moringa leaf, leading to increased plant height, leaf area, leaf chlorophyll index and total tuber yield of potato. HB101 is a natural plant growth enhancer extracted from cedars, cypresses, pines and plantains, which helps to promote plant cell proliferation, activating a mitochondrial enzyme of the plant, and increases the microbial populations in the soil. Salwa *et al.* (2019) [7] recorded better plant growth and yield of red cabbage as a result of improved soil fertility under higher rate of HB101 spraying. These results suggest that HB101 contributes to improved physiological status and reproductive performance, likely through its active ingredients that stimulate growth processes.

**Table 5. Pearson correlation matrix of physiological parameters and yield under the effect of vermicompost rates and HB101 spray frequencies**

|        | LAI-H | SPAD-F | SPAD-H | ADW-F  | ADW-H  | PWP    | PY     |
|--------|-------|--------|--------|--------|--------|--------|--------|
| LAI-F  | 0.43* | 0.42*  | 0.52** | 0.55** | 0.69** | 0.49** | 0.50** |
| LAI-H  |       | 0.66** | 0.70** | 0.73** | 0.68** | 0.68** | 0.72** |
| SPAD-F |       |        | 0.59** | 0.59** | 0.54** | 0.41*  | 0.55** |

|        |  |  |  |        |        |        |        |
|--------|--|--|--|--------|--------|--------|--------|
| SPAD-H |  |  |  | 0.58** | 0.51** | 0.50** | 0.52** |
| ADW -F |  |  |  |        | 0.70** | 0.67** | 0.74** |
| ADW-H  |  |  |  |        |        | 0.74** | 0.71** |
| PWP    |  |  |  |        |        |        | 0.89** |

Note: ns: non-significant; \*:  $p$ -value < 0.05; \*\*:  $p$ -value < 0.01; -F: at flowering stage, -H: at harvesting stage, PWP: Pod weight per plant, PY – pod yield.

On the other hand, vermicompost is a kind of organic fertilizer, its nutrient release (especially N) may be slower, particularly at the early growth stages. In this situation, the inclusion of a plant vitalizer supplying available nutrients or growth-regulating compounds led to plant growth improvement. Our findings indicate that higher vermicompost rates interacted with HB101 spraying enhanced bean growth, thereby promoting LAI, SPAD, and ADW, all of which were significantly and positively associated with final yield (Table 5). For example, between LAI (at flowering stage) and ADW (at harvesting stage):  $r = 0.69$  ( $p < 0.01$ ); between LAI and ADW at harvesting stage:  $r = 0.68$  ( $p < 0.01$ ). Importantly, yield components and pod yield exhibited a higher correlation with ADW. Therein, PWP was significantly positively correlated with ADW at harvesting ( $r = 0.74$ ,  $p < 0.01$ ). ADW was also closely linked with pod yield, with  $r = 0.74$  ( $p < 0.01$ ) at flowering stage and  $r = 0.71$  ( $p < 0.01$ ) at harvesting stage, suggesting that assimilate partitioning into economic yield components is the key determinant of productivity.

Dang *et al.* (2025) [5] found that combining organic manure with foliar fertilizer improved soil fertility, increased leaf nutrient contents, increased fruit yield, TSS content and decreased physiological disorder in durian fruits as compared to organic or foliar fertilization alone. Similarly, Kouam *et al.* (2024) [13] reported utilization of botanical extract along with soil-applied fertilizers provided essential nutrients as well as amino acids, bioactive compounds and phytohormones, leading to better plant height, leaf area, fresh and dry biomass, and fruit yield and quality of strawberry. In terms of HB101, Salwa *et al.* (2019) [7]

confirmed that combining higher vermicompost rates and HB101 showed promoted plant growth and yield traits of red cabbage. Our study demonstrates that higher vermicompost application rates, particularly in combination with HB101 spraying at 5- or 10- intervals, enhanced pod yield of bush-type common beans. This approach offers a promising strategy for sustainable yield improvement through the synergistic effects of organic fertilization and foliar biostimulant application.

#### 4. CONCLUSION

Applying vermicompost at higher rates improved plant growth, physiological indicators of common beans. Application of 12 tons ha<sup>-1</sup> of vermicompost statistically increased pod number, pod weight per plant and pod yield. Additionally, spraying HB101 enhanced fresh and dry weight, LAI and SPAD as well as pod yield traits. Application of vermicompost at 12 tons ha<sup>-1</sup> combined with HB101 spraying at either 5- or 10-day intervals positively influenced plant growth and yield of common bean, resulting in comparable pod yield of 5.12 and 4.61 tons ha<sup>-1</sup>, respectively. The result suggests that integrating 12 tons ha<sup>-1</sup> of vermicompost with HB101 spraying at 10-day intervals represents an effective agronomic strategy for enhancing the growth and yield of bush-type common beans.

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# EVALUATION OF FRUIT QUALITY AND ESSENTIAL OIL EXTRACTED FROM PEEL OF FOUR LIME SPECIES (*Citrus sp.*) GROWN IN AN GIANG PROVINCE, VIETNAM

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## ABSTRACT

The economic value of lime mainly comes from the juice and their essential oils extracted from the peel. This study aimed to evaluate various fruit quality indicators and juice characteristics of four lime species commonly cultivated in An Giang province, Vietnam. The species of Citrus are: *Citrus aurantifolia*, *Citrus latifolia*, *Citrus hystrix*, *Citrus limonia*. The findings indicated that *C. latifolia* exhibited the highest fruit weight, fruit firmness, and brightness, with peel thickness comparable to that of *C. hystrix* and superior to the other species. *C. latifolia* had lower total soluble solids and total titratable acidity levels than *C. hystrix*, but similar vitamin C and ash content, and greater than *C. aurantifolia*, *C. limonia*. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that limonene was the most abundant compound in the essential oils of *C. aurantifolia*, *C. latifolia* and *C. limonia*, while beta-Pinene was the most abundant compound in the essential oil of *C. hystrix*. Although *C. hystrix* demonstrated the highest essential oil recovery efficiency, *C. latifolia* exhibited the greatest antioxidant activity, as evidenced by a DPPH assay value of 96.14%, was noted for having a more prevalent aroma than the other species. Consequently, the extraction of essential oils from the peel and the development of value-added products from the juice of *C. latifolia* appear to be of considerable economic benefit and enhance the value of limes.

**Keywords:** Lime species, citrus sp., fruit quality, essential oil, Vietnam.

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## 1. INTRODUCTION

The annual growth rate of the lime industry is 7% worldwide [1]. Warmer conditions due to climate change, may also lead to less water, more aridity, a condition to which lime trees are well suited, leading to increased production in the future [2]. Lime can be harvested several times a

year because the climate allows the tree to grow continuously. According to CIRAD statistics (2021) [1], Vietnam was one of the largest lime-exporting countries in the world in 2019. In the context of climate change, unstable prices of many agricultural products, reducing the area of ineffective rice is an urgent challenge. Currently,

some localities have switched to lime cultivation to help improve productivity, bring economic efficiency, meet market demand.

The fruit flesh is juicy, delicate, packed with vitamins and goodness, with aroma seeping out through the peel. Lime has been a significant part of diets for its nutritional benefits and natural medicinal advantages, contributing to disease prevention and improved resistance. Its role in purifying the blood naturally has made it a staple in traditional dietary practices [3]. Lime juice is popular and commonly consumed due to its aromatic composition, citric acid, vitamin C, minerals, flavonoids. It has many uses including: (i) popular culinary use to add flavor to dishes in a particular region; (ii) is the main ingredient in many refreshing drinks; (iii) used to preserve processed foods such as sauces and pickles; (iv) used for potential health benefits in some traditional medicine practices, such as strengthening the immune system or aiding digestion; (v) in cosmetics to remove rust and wash hair [3 - 8]. However, citrus growers face substantial challenges during harvesting, post-harvest management, and marketing [9].

Lime is consumed mainly in fresh form or processed into products such as spice powder, juice, cakes, candy, jam. The primary ingredient used in processing is lime juice. The lime pulp and juice make up 48% of the total weight while the remaining parts such as peel, seeds and dehydrated flesh make up about 52% of the total weight [10]. Lime peels could be used as food, nutritional supplements in the food and feed industry because they may include pectin, fiber and numerous biological substances as essential oils, flavonoids, limonoids, alkaloids [11 - 13].

Lime essential oil has a strong citric flavor and is yellow. Compared with orange and lemon essential oils, the yield of lime essential oils is lower than orange but higher than lemon [14]. Lime essential oil yield and composition varies not only by extraction method but also by plant parts and plant origin [15]. Due to its composition, essential oil from different lime species may have slightly different flavors and biological properties.

Additionally, some types of lime may be more sustainable or easier to source than others, which may be something to consider for environmentally conscious consumers. Therefore, comparing fruit quality and essential oils from different lime species, especially local species, can help to identify sources with consistent aroma and medicinal benefits.

Research so far has mainly focused on essential oils from *C. aurantifolia* or *C. hystrix* cultivars while there is little published information on *C. latifolia*, especially less about *C. limonia* as well as other species. Therefore, our study aimed to evaluate some fruit quality indicators, juice composition as well as essential oils extracted from 4 limes species commonly grown in An Giang province, Vietnam.

## 2. RESEARCH METHODS

### 2.1. Material

Fresh limes were harvested from farms in An Giang province, Vietnam, specifically *C. aurantifolia* (Cho Moi commune, Cho Moi district), *C. latifolia* (Binh Hoa commune, Chau Thanh district), *C. limonia* (Vinh Nhuan commune, Chau Thanh district) and *C. hystrix* (Ba Chuc commune, Tri Ton district). After harvesting, the limes were packed in cartons to limit impact damage and transported immediately to the laboratory.

### 2.2. Analytical method

Physical analysis was performed independently on 10 randomly selected fruits of each species per replicate. Measurements were taken at three different locations on each sample. Physica and chemical analysis was analyzed in triplicate.

**Fruit mass (g):** Fruit mass was determined using an Electronic scale (Precisa, Model LS220A, Switzerland).

**Peel-to-fruit mass ratio (%):** After peeling, the mass of the lime peels was measured to calculate the peel ratio in the whole fruit.

**Peel thickness of fruit (mm):** Peel thickness was determined using a Micrometer (Mitutoyo, Model CD-12"AX, Japan).

Color value: The color of fruit was determined using a Colorimeter (Konica Minolta, CR-400, Japan) and was expressed in terms of  $L^*$ ,  $a^*$  and  $b^*$  values.

Fruit firmness ( $\text{kg}\cdot\text{cm}^{-2}$ ): Firmness of fruit was determined using a Texture analyzer (Brookfield, CT3, USA).

Total soluble solids ( $^{\circ}\text{Brix}$ ): TSS were determined using a Brix meter (Atago, Model N-2E, Japan) at room temperature.

Total ash content (%): Total ash content was determined using a Carbolite (Parsons Lane, Model ELF 14B, England).

Total titratable acidity (%): Was determined by the titration method of AOAC (2012) (No. 9720.21) [16].

Vitamin C content ( $\text{mg}/100\text{ g}$ ): Vitamin C content was determined by using the titration method of AOAC (2012) (No. 967.22) [16].

#### *Essential oil yield and composition*

The lime essential oil was extracted according to the method described by Lin *et al.* (2019) [17] with minor modifications: The lime peels were separated from the fruit, cut into 2 cm and added to distilled water (1:3 w:v ratio) for extraction. Hydro distillation was then performed for 3 hours by a Clevenger-type apparatus. The extracted essential oil was dehydrated with anhydrous sodium sulfate and filtered. The essential oil was stored at  $4^{\circ}\text{C}$  and protected from light until used.

Essential oil yield (% v/w): The extraction yield was calculated according to the volume of essential oil and mass of raw material [18].

Essential oil composition: The chemical composition of the essential oil was analyzed using gas chromatography-mass spectrometry (GC-MS). Separation was performed on a DB-5MS capillary column ( $30\text{ m} \times 0.32\text{ mm i.d.}$ , film thickness  $0.25\text{ }\mu\text{m}$ ). The injector temperature was maintained at  $250^{\circ}\text{C}$  and the injection was carried out in split mode (split ratio 20:1). Helium was employed as the carrier gas under linear velocity control at a pressure of 15.8 kPa, with a total flow of 35.3 mL/min and a column flow of 1.54 mL/min. The

oven temperature was programmed as follows: initial temperature  $50^{\circ}\text{C}$  (held for 2 min), then increased at a rate of  $10^{\circ}\text{C}/\text{min}$  to  $310^{\circ}\text{C}$  and held for 10 min, giving a total run time of 38 min. The ion source and interface temperatures were set at  $200^{\circ}\text{C}$  and  $250^{\circ}\text{C}$ , respectively. Mass spectra were acquired in electron impact (EI) mode, scanning over the range  $m/z$  30 - 450 with a solvent delay of 3 min. The components of the essential oil were identified by comparing their mass spectra with those available in the NIST14 mass spectral library.

### 2.3. Statistical analysis

The evaluation data were implemented by running Microsoft Excel and statistical analysis were done by the Portable Statgraphics Centurion software (version 15.2.11.0, U.S.A.).

## 3. RESULTS AND DISCUSSIONS

### 3.1. Lime fruit characteristic

Figure 1 shows the different appearances of the studied lime species.

*C. aurantifolia*, also known as key lime, was oval or round in shape and relatively small in size, compared to the three other species. The peel was thin, rough and bright green when unripe, which may turn yellow when ripe. The peel of *C. aurantifolia* was not shiny but has a slightly rough texture, with cracks or wrinkles. When cut across the fruit, the inside was juicy, pale yellow or slightly green and contains a lot of juice.

*C. latifolia*, also known as the Persian lime or Tahitian lime, was popular because the fruit was larger and seedless. *C. latifolia* was usually larger than other limes and was round or slightly oval in shape. The peel surface was usually smooth, less rough than other limes and light green when unripe. When cut across the fruit, the inside had light yellow segments, lots of juice, had no seeds.

*C. limonia*, also known as purple flower lemon, was oval and slightly flattened at both ends. The peel was quite thick and rough, often bright green when unripe and turns light yellow when ripe. The outstanding feature of this species was the purple or light purple flowers that appear on the fruit

stem or the tree trunk. When cut across the fruit, the inside had succulent segments and contains a lot of juice.

*C. hystrix*, also known as kafir lime, has a fruit with a rather distinctive shape. The fruit was small, slightly round or oval, with a thick, wrinkled skin that creates a rough, uneven surface. The skin was dark green when young, turns yellow when ripe, had a characteristic, slightly pungent aroma.

When cut across the fruit, the inside was little juice, containing many small segments and seeds.

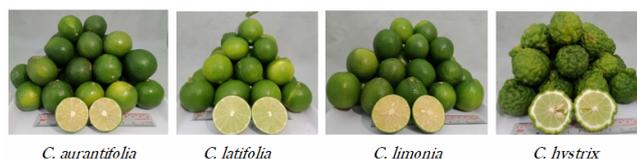


Figure 1. Fruit characteristics of 4 lime species growing in An Giang province, Vietnam

3.2. Physical attributes of lime fruit

Table 1. Physical attributes of 4 lime species in An Giang province, Vietnam

| Lime species           | Fruit mass (g)            | Fruit firmness (kg.cm <sup>-2</sup> ) | Peel: fruit ratio (%)     | Peel thickness (mm)      |
|------------------------|---------------------------|---------------------------------------|---------------------------|--------------------------|
| <i>C. aurantifolia</i> | 54.35 ± 1.56 <sup>b</sup> | 6226.24 ± 479.57 <sup>bc</sup>        | 15.85 ± 0.75 <sup>b</sup> | 1.48 ± 0.11 <sup>b</sup> |
| <i>C. latifolia</i>    | 79.67 ± 8.32 <sup>a</sup> | 8032.85 ± 341.98 <sup>a</sup>         | 14.65 ± 0.50 <sup>c</sup> | 1.66 ± 0.07 <sup>a</sup> |
| <i>C. limonia</i>      | 56.63 ± 3.25 <sup>b</sup> | 6614.43 ± 344.66 <sup>b</sup>         | 15.74 ± 0.20 <sup>b</sup> | 1.51 ± 0.02 <sup>b</sup> |
| <i>C. hystrix</i>      | 50.80 ± 2.79 <sup>b</sup> | 5764.77 ± 356.88 <sup>c</sup>         | 27.72 ± 0.54 <sup>a</sup> | 1.69 ± 0.10 <sup>a</sup> |
| <i>P</i>               | 0.0003                    | 0.0005                                | 0.0000                    | 0.0262                   |

Note: Values with same superscript letter in each column are no significant difference ( $p < 0.05$ ).

The results presented in table 1 show that the *C. latifolia* had the largest fruit weight and was statistically different from the other three species. In addition, fruit firmness increased as fruit mass increased, resulting in *C. latifolia* having the highest fruit firmness and *C. hystrix* having the lowest. In contrast, the peel-to-fruit ratio of limes species was inversely proportional to fruit mass, except for *C. hystrix* due to the presence of nodules on the peel surface. Fruit mass has a positive relationship with juice content, thus larger fruits are generally considered to be of better quality and are sold at higher prices in the market [19].

Peel thickness is one of the criteria considered in quality assessment due to their suitability for long-distance transport. The comparison results presented in table 1 showed that peel thickness varies according to lime species. Among them, *C. hystrix* had the highest peel thickness, followed by *C. latifolia* and there was a statistically significant difference compared to the other two.

The color value of lime is expressed through the indices L\* (brightness), a\* (from green to red), b\* (from blue to yellow), as shown in figure 2. Although *C. latifolia* had the highest brightness due to larger fruit size, statistical analysis found no significant difference in color values among the

four lime species ( $p > 0.05$ ). Limes are typically harvested with green peels due to its chlorophyll pigment. Lime peel turns yellow under enzyme activity like chlorophyllase and at that point fruit loses commercial value [20].

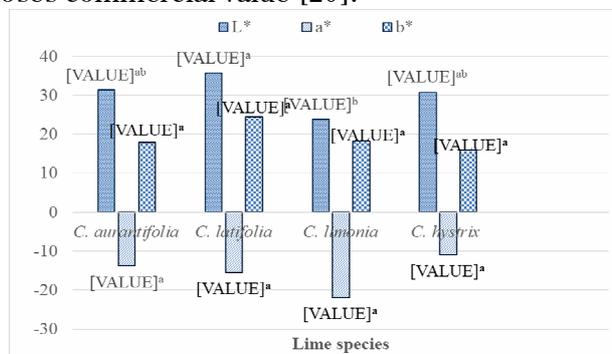


Figure 2. Color value (L\*, a\*, b\*) of 4 lime species in An Giang province, Vietnam

Note: Values with same superscript letter in each attribute are no significant difference ( $p < 0.05$ ).

3.3. Chemical composition of lime juice

Total soluble solids (TSS) and total titratable acidity (TTA) affect the usability of limes in food processing and are presented in table 2. TSS values decreased from *C. hystrix*, *C. latifolia*, *C. aurantifolia* and *C. limonia* to 10.83, 9.83, 9.43, and 8.1°Bx, respectively. In addition, the TTA content of species varied greatly and ranged from 5.90 to 7.79%, with *C.*

*hystrix* having the highest content, followed by *C. limonia*, *C. latifolia*, and *C. aurantifolia* having the lowest content. The TTA value of *C. latifolia* in this study was almost equivalent to the analysis results of *C. latifolia* grown in the Mekong Delta (Vietnam), however, the TSS value was slightly higher,

specifically 9.83°Bx compared to 7.6°Bx [21]. The soluble solids content of citrus may include substances such as sugars, vitamins, free salts as well as organic acids [22]. Therefore, the higher the Brix level of lime, the higher the potential for use in food production.

**Table 2. Chemical composition of juice from 4 lime species in An Giang province, Vietnam**

| Lime species           | TSS (°Bx)                 | TTA (g/100 ml)           | Vitamin C (mg/100 ml)     | Total Ash (%)             |
|------------------------|---------------------------|--------------------------|---------------------------|---------------------------|
| <i>C. aurantifolia</i> | 9.43 ± 0.47 <sup>b</sup>  | 5.90 ± 0.25 <sup>c</sup> | 0.02 ± 0.00 <sup>ab</sup> | 0.40 ± 0.02 <sup>ab</sup> |
| <i>C. latifolia</i>    | 9.83 ± 0.46 <sup>b</sup>  | 6.20 ± 0.04 <sup>b</sup> | 0.03 ± 0.01 <sup>ab</sup> | 0.33 ± 0.07 <sup>b</sup>  |
| <i>C. limonia</i>      | 8.10 ± 0.26 <sup>c</sup>  | 6.40 ± 0.14 <sup>b</sup> | 0.01 ± 0.00 <sup>b</sup>  | 0.31 ± 0.06 <sup>b</sup>  |
| <i>C. hystrix</i>      | 10.83 ± 0.29 <sup>a</sup> | 7.79 ± 0.05 <sup>a</sup> | 0.04 ± 0.00 <sup>a</sup>  | 0.49 ± 0.03 <sup>a</sup>  |
| <i>P</i>               | 0.0002                    | 0.0000                   | 0.0034                    | 0.0654                    |

Note: Values with same superscript letter in each column are no significant difference ( $p < 0.05$ ).

Vitamin C content is important chemical indices of citrus fruits. Although the vitamin C content of *C. hystrix* was the highest, it was not significantly different from the vitamin C content of *C. latifolia* and *C. aurantifolia* (Table 2). Ash content is the minerals and inorganic matter remaining after heating at extremely high temperatures to remove moisture, volatile matter, and all organic materials, so a higher ash content implies that it is a good source of minerals. There was no statistically significant difference in total ash content among lime species in the study ( $p > 0.05$ ). Lemons and limes are similar nutritionally; however, lime juice contains more vitamin C than lemon juice [5].

This results also differ significantly from previously published studies on vitamin C content in citrus fruits [23, 24].

In addition to the difference in species, the content of substances in lime juice is also affected by growing conditions. The study of Naseer *et al.*

(2020) [25] showed that figs harvested in different locations had differences in chemical composition such as TTA, TSS, and vitamin C due to the influence of changing climatic conditions.

### 3.4. Essential oils of lime peel

Although the main use of lime is juice and fruit juice products, lime essential oil is exploited and traded mainly in the food, pharmaceutical, and cosmetic industries [4]. The essential oil extraction yield from four lime species is presented in table 3. The results showed that *C. hystrix* had the highest extraction yield (3.87%) followed by *C. latifolia* (1.78%), *C. limonia* (1.35%). The *C. aurantifolia* had the lowest extraction yield (1.33%) and was statistically different from the others. The essential oil yield of lime is lower than orange but higher than lemon [14]. The extraction yield of essential oil from *C. aurantifolia* in this study was higher than Julaeha *et al.* (2020) [26] but lower than Indriyani *et al.* (2023) [27].

**Table 3. Chemical composition of juice from 4 lime species in An Giang province, Vietnam**

| Lime species           | Extraction yield (% v/w) | DPPH (%)                   |
|------------------------|--------------------------|----------------------------|
| <i>C. aurantifolia</i> | 1.33 ± 0.09 <sup>c</sup> | 93.46 ± 1.19 <sup>ab</sup> |
| <i>C. latifolia</i>    | 1.78 ± 0.04 <sup>b</sup> | 96.14 ± 2.14 <sup>a</sup>  |
| <i>C. limonia</i>      | 1.35 ± 0.05 <sup>c</sup> | 89.26 ± 0.95 <sup>bc</sup> |
| <i>C. hystrix</i>      | 3.87 ± 0.04 <sup>a</sup> | 85.13 ± 2.04 <sup>c</sup>  |
| <i>P</i>               | 0.0000                   | 0.0098                     |

Note: Values with same superscript letter in each attribute are no significant difference ( $p < 0.05$ ).

The overall antioxidant activity of lime essential oil was determined by DPPH (Table 3). The DPPH value represents the ability to inhibit free radicals and is a very important index in evaluating the antioxidant capacity of essential oils. The higher the antioxidant activity of the essential oil, the greater the DPPH value and vice versa. The highest antioxidant activity was found in the essential oils of *C. latifolia* and *C. aurantifolia* with percentages of 96.14 and 93.46 respectively compared to lower values in *C. limonia* (89.26%) and *C. hystrix* (85.13%). Research on the composition and essential oils of 21 citrus varieties showed that all essential oils have the ability to eliminate DPPH free radicals [28].

Essential oils are complex mixtures with concentrations varying from 20 to 60 natural compounds. Their composition will vary significantly depending on, season, geographical location, and fruit ripeness [29]. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that the essential oils of *C. aurantifolia*, *C. latifolia*, *C. limonia*, and *C. hystrix* contained 32, 29, 29, and 28 compounds, respectively. Although limonene was the most abundant component in *C. aurantifolia* (60.73%), *C. latifolia* (47.65%) and *C. limonia* (64.56%), its content was lower in *C. hystrix* (18.56%). Table 4 represents the 5 most common compounds in each essential oil.

The number of compounds as well as the proportion of substances in the essential oil is not a constant fixed number but often has a very large amplitude of variation depending on the distribution area as well as the natural conditions in that area [30]. According to the report of Bora *et al.* (2020) [29], depending on the species, the concentration of limonene in lime essential oil varies from 32% to 98%, which has many important

biological activities such as inhibiting carcinogenic properties and preventing cancer.



Figure 3. The essential oil extracted from 4 lime species in An Giang province, Vietnam

Figure 3 shows the essential oils from the peels of the studied lime species. Sensorily, the essential oils were quite similar except for a distinct difference in odor. The essential oil extracted from the peel of *C. latifolia* has a pleasant aroma and scored highest on the Hedonic scale compared to the essential oils from the remaining species in this study.

4. CONCLUSION

It is concluded from the studies that *C. latifolia* had the best characteristics in terms of fruit weight and firmness, as well as the color and thickness of the peel. The total soluble solids and total titratable acidity content of *C. latifolia* were lower than those of *C. hystrix*, but the vitamin C and ash content were equivalent to those of *C. hystrix* and higher than those of *C. aurantifolia* and *C. limonia*. Although the essential oil extraction yield of *C. latifolia* ranked second, its antioxidant capacity was the strongest its aroma was also more favorable. The main components of *C. latifolia* essential oil were limonene (47.65%),  $\beta$  - Pinene (13.89%) and 3-Carene (13.18%). Therefore, the essential oils from the peel and value-added product from the juice of *C. latifolia* brought significant economic benefits and enhanced its value.

Table 4. Essential oil composition from 4 lime species in An Giang province, Vietnam (\*)

| N <sub>0</sub> | Compound (*)       | Retention time | Concentration (%)      |                     |                   |                   |
|----------------|--------------------|----------------|------------------------|---------------------|-------------------|-------------------|
|                |                    |                | <i>C. aurantifolia</i> | <i>C. latifolia</i> | <i>C. limonia</i> | <i>C. hystrix</i> |
| 1              | Limonene           | 5.36           | 60.73                  | 47.65               | 64.56             | 18.65             |
| 2              | $\beta$ -Pinene    | 4.55           | 6.90                   | 13.89               |                   | 30.13             |
| 3              | $\gamma$ -Terpinen | 5.82           | 15.11                  | -                   | 13.49             | -                 |
| 4              | 3-Carene           | 5.81           | -                      | 13.18               | -                 | -                 |

|                             |                        |       |              |        |       |        |
|-----------------------------|------------------------|-------|--------------|--------|-------|--------|
| 5                           | Sabinene               | 4.47  | -            | -      | -     | 20.20  |
| 6                           | (1S)- $\alpha$ -pinene | 4.55  | -            | -      | 6.56  | -      |
| 8                           | $\beta$ -Myrcene       | 4.70  | 2.84         | (2.15) | 2.92  | (1.70) |
| 12                          | $\alpha$ -Pinene       | 3.87  | 2.48         | -      | -     | -      |
| 15                          | 1R- $\alpha$ -Pinene   | 3.87  | -            | 2.68   | 2.55  | 3.54   |
| 19                          | (R)-(+)-Citronellal    | 7.28  | (0.70)       | -      | -     | 12.24  |
| 23                          | Neryl acetate          | 10.25 | -            | 2.74   | -     | -      |
| <i>Total</i> <sup>(*)</sup> |                        |       | <i>88.06</i> | 80.14  | 90.08 | 84.76  |

Notes: <sup>(\*)</sup>: The data represents only the 5 most common compounds in each essential oil; (...): Numbers in parentheses do not belong to the top 5 highest concentrations in each essential oil; (-): means not detected.

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# NUTRITIONAL AND FUNCTIONAL PROPERTIES OF PROTEIN HYDROLYSATE FROM WHITE LEG SHRIMP HEADS

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## ABSTRACT

White leg shrimp heads from the processing industry were hydrolyzed by Protamex enzyme 0.5% at a temperature of 50°C with different hydrolysis times from 1 hour to 4 hours to obtain four shrimp head protein hydrolysates. The produced protein hydrolysates were characterized by biochemical analysis for protein content and amino acids. Functional properties of shrimp head protein hydrolysates such as solubility, foaming capacity and emulsifying capacity were assessed. Study results showed that the degree of hydrolysis was 31.74% after 4 hours of hydrolysis. The protein hydrolysate powder from white leg shrimp heads had 74.32% protein, 0.85% lipid and 11.18% ash. The protein hydrolysate powder from shrimp heads was found to have high nutritional value with essential amino acids. The amino acids with high contents were glutamic acid, aspartic acid, glycine, leucine, lysine and valine. The solubility of shrimp head protein hydrolysates increased as hydrolysis time increased from 1 hour to 4 hours. The protein hydrolysate with a hydrolysis time of 4 hours had the highest solubility (95.85%) at pH 8. The foaming capacity and emulsifying capacity of shrimp head protein hydrolysates decreased with increasing hydrolysis time. This study suggested that shrimp head protein hydrolysate was a potential ingredient for the food industry.

**Keywords:** *Functional property, nutritional property, protein hydrolysate, white leg shrimp head.*

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## 1. INTRODUCTION

White leg shrimp is one of the most popular crustaceans. The shrimp processing industry in Vietnam generates a large amount of shrimp waste. About 35 - 45% by weight of shrimp raw material is discarded as waste depending on the species and processing method [1]. These wastes spoil rapidly, thus causing environmental problems. The most important waste material in shrimp processing industries is head, which contains valuable proteins. Mizani *et al.* (2005) studied the hydrolysis of shrimp heads using Alcalase to obtain protein hydrolysates [2]. The enzymatic hydrolysis of shrimp heads generated the hydrolysates with a high protein content and a low fat content [3, 4]. Gunasekaran *et al.* (2015) indicated that the optimal hydrolysis conditions for maximum degree of hydrolysis were pH 8.2,

temperature of 45.4°C and enzyme/substrate ratio of 1.8% [5]. Utilization of shrimp wastes from the processing industry contributes to reducing environmental pollution and also produces valuable protein hydrolysate. Enzymes from microbial sources such as Alcalase, Protamex, Flavourzyme, Neutrase are efficient in the hydrolysis of proteins. Protamex was chosen for the hydrolysis of shrimp heads in this study because it is known to produce a non-bitter hydrolysate [6]. Protein hydrolysis consists of the cleavage of protein molecules into peptides and amino acids. The enzymatic hydrolysis of fish protein generates the protein hydrolysates, which not only have the high nutritional value but also have the necessary functional properties. Fish protein hydrolysates have potential applications as functional ingredients in different foods because of

their important properties such as solubility, foaming capacity and emulsifying capacity. Studies indicate that the degree of hydrolysis or hydrolysis time influences the functional properties of the protein hydrolysates [7, 8, 9, 10]. The protein hydrolysate with desirable functionality can be produced by adjusting the hydrolysis time [8]. The protein hydrolysates have a high content of amino acids and are used as available sources of protein for humans due to their good functional properties. The protein hydrolysates from fishery by-products have potential for application in the food industry [3].

This study aimed to produce the protein hydrolysate from white leg shrimp heads and to determine the nutritional and functional properties such as solubility, foaming capacity and emulsifying capacity of the protein hydrolysate.

## 2. MATERIALS AND METHODS

### 2.1. White leg shrimp head

White leg shrimp (*Litopenaeus vannamei*) heads were provided by Nha Trang seafood company, Vietnam. The shrimp heads were placed in ice with a shrimp head/ice ratio of 1/1 (w/w) and transported to the laboratory at Nha Trang University. They were thawed and ground in a grinder through a 3 mm plate. The minced materials were packed in plastic bags (0.5 kg per unit), frozen and stored at -20°C.

### 2.2. Enzyme Protamex

Protamex (CAS 9001-92-7) is a *Bacillus* protease complex developed for the hydrolysis of food proteins. Protamex was produced by Novozymes A/S (Bagsvaerd Denmark). The optimal working conditions for Protamex are reported to be at a pH of 5.5 - 7.5 and at a temperature of 35 - 60°C. Protamex has a declared activity of 1.5 Anson Units/g.

### 2.3. Preparation of protein hydrolysate from white leg shrimp heads

The minced shrimp heads were thawed at 4°C overnight and added with 1 volume of distilled water. The mixture was heated at a temperature of 90°C for 30 min to inactivate the endogenous

hydrolyzing enzyme. After heating, the mixture was cooled in a water bath until it reached a temperature of 50°C. The hydrolysis of shrimp heads was then carried out using Protamex with 0.5% of the weight of shrimp heads (w/w) at 50°C and the natural pH (6.8) for 1 hour, 2 hours, 3 hours, 4 hours. After hydrolysis, the enzyme was inactivated by heating in a water bath at 90°C for 15 min. The mixture was then filtered through a mesh with a 1 mm hole size to remove the solids. The filtrate was centrifuged at 7,000 rpm for 20 min using a centrifuge to separate the liquid protein hydrolysate and the sludge. The liquid protein hydrolysate was spray-dried using a spray dryer to obtain the protein hydrolysate powder. The spray drying of protein hydrolysate was carried out with an inlet air temperature of 150°C and a maltodextrin concentration of 8% (w/w). Protein hydrolysate powders from shrimp heads were stored in sealed plastic bags at 4°C. The experiments were carried out in triplicate.

### 2.4. Analysis methods

The moisture content was determined according to TCVN 3700-90 [11]. Ash content was measured according to TCVN 5105:2009 [12]. Lipid content was determined according to Folch *et al.* (1957) [13]. The total protein content of shrimp heads was calculated according to the following formula [2]:

$$\text{Total protein} = (\text{total nitrogen} - \text{chitin nitrogen}) \times 6.25.$$

The total nitrogen and chitin nitrogen contents of shrimp heads were measured by the Kjeldahl method according to TCVN 3705-90 [14]. The percentage of chitin present in shrimp heads was determined according to the method of Mizani *et al.* (2005) [2]. Briefly, 1 g shrimp head was deproteinised by adding 20 mL of a 5% NaOH solution and incubating for 2 hours at 100°C. The suspension was filtered, and the precipitate was washed with deionised water. The demineralisation step was accomplished by adding 20 mL of 5% HCl for 2 hours at room temperature, followed by filtering the suspension and washing the precipitate (chitin) with deionised water and

then oven drying at 105°C. Finally, the percentage of chitin was determined according to the following formula:

Percentage of chitin = (weight of the chitin/initial weight of the sample) x 100.

Amino acid composition was determined according to Raftani *et al.* (2016) [15]. Sample preparation was performed by hydrolysis with 6N HCl at 110°C for 12 hours and derivatisation using phenyl isothiocyanate prior to HPLC (Knauer, Germany) analysis. The total amino acids were analyzed by the Pico Tag method using a Pico Tag column (3.9 x 150 mm) at a flow rate of 1 mL min with UV detection.

The degree of hydrolysis (DH) is defined as the ratio between the number of broken peptide bonds and the total number of peptide bonds per mass unit. The degree of hydrolysis was determined according to Nguyen *et al.* (2011) [16].

The solubility of protein hydrolysate was determined according to Synowiecki and Al-Khateeb (2000) [17]. 0.2 g of the protein hydrolysate was suspended in 20 ml of distilled water. The pH of the mixture was adjusted to 2, 4, 6, 8 using 0.1 M solutions of HCl or NaOH. After standing for 10 min at room temperature, the mixtures were then centrifuged at 12,000 g for 15 min. The supernatants were diluted to 50 ml with water, and 20 ml of aliquots were used for Kjeldahl analysis. The percentage of solubility (X) was expressed as:

$X (\%) = (\text{Nitrogen content in the supernatant}/\text{Nitrogen in the hydrolysate}) \times 100\%$ .

Foaming capacity (FC) was determined according to Amiza *et al.* (2012) [8]. The protein hydrolysate (3 g) was dispersed in 100 ml of distilled water and the mixture was homogenized for 1 min using a homogenizer at high speed. The mixture was then poured into a 250 ml graduated cylinder and the total volume was read. Foaming capability was expressed as a percentage of volume increase upon whipping. Foaming capability was calculated as follows:

$FC\% = (\text{Volume after homogenisation} - \text{Volume before homogenisation}) \times 100/\text{Volume before homogenization}$ .

Emulsification capacity (EC) was determined according to Diniz and Martin (1997) [9]. Protein hydrolysate samples (0.5 g) and 30 ml of vegetable oil were added to 60 ml of NaCl solution (30 g/l) and mixed using a homogenizer at 9,500 rpm for 30 min. After this period, another 30 ml of oil were added over 1.5 min and mixed for a further 30 seconds. The mixture was transferred to centrifuge tubes, held in a water bath at 85°C for 15 min, then centrifuged at 3,000 rpm for 30 min. Emulsification capacity was calculated by the following equation:

$$EC \text{ (ml/g)} = (V_A - V_R)/W_S$$

Where:  $V_A$  was the volume of oil added to form an emulsion (ml),  $V_R$  was the volume of oil released after centrifugation (ml),  $W_S$  was the weight of the sample (g).

### 2.5. Statistical analysis

The experiments were carried out in triplicate. The data obtained were subjected to one way analysis of variance (ANOVA), followed by the Duncan's multiple range test to determine the significant difference between samples at  $p < 0.05$  level using the SPSS program (SPSS Version 16.0).

## 3. RESULTS AND DISCUSSION

### 3.1. Proximate composition of shrimp head

The chemical composition of white leg shrimp head is shown in table 1.

**Table 1. Proximate composition of white leg shrimp head**  
(based on the wet weight)

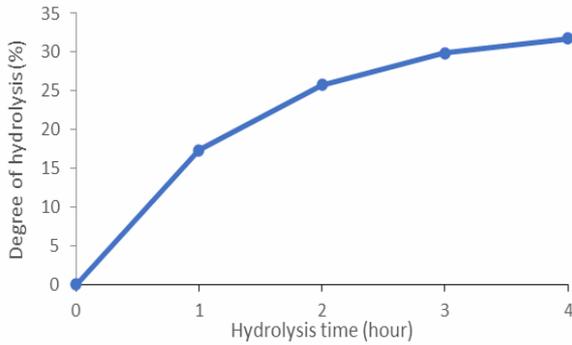
| Chemical composition | Content (%)  |
|----------------------|--------------|
| Moisture             | 75.36 ± 0.34 |
| Protein              | 11.56 ± 0.27 |
| Chitin               | 2.05 ± 0.18  |
| Lipid                | 1.64 ± 0.16  |
| Ash                  | 7.51 ± 0.21  |

The white leg shrimp head had a high protein content of 11.56%, chitin content of 2.05%, low lipid content of 1.64% and ash content of 7.51%. According to Babu *et al.* (2008) [18], four different types of shrimp head waste had moisture contents of 67.4 - 77%, protein contents of 11.2 - 13.7%, chitin contents of 1.7 - 2.9%, lipid content of 1.2 - 1.7% and ash content of 6 - 10.5%. The results showed that

white leg shrimp heads were a good source of protein, which can be used for the production of protein hydrolysate.

**3.2. Degree of hydrolysis**

The degree of hydrolysis during enzymatic hydrolysis of white leg shrimp heads is presented in figure 1.



**Figure 1. The degree of hydrolysis of shrimp head protein hydrolysate**

Research results showed that the degree of hydrolysis increased with hydrolysis time. The degree of hydrolysis increased rapidly in the first hour of hydrolysis, then the degree of hydrolysis increased slowly. After 4 hours of hydrolysis of white leg shrimp heads, the degree was 31.74%. The hydrolysis curve exhibited an initial fast reaction. In the initial period of hydrolysis, a large number of peptide bonds were cleaved, leading to an increase of soluble peptides in the reaction mixture. The shape of the hydrolysis curve in this study was similar to those previously published for herring [19], salmon head [20], sardine by-products [21].

**3.3. Proximate composition of protein hydrolysate from white leg shrimp heads**

The proximate composition of shrimp head protein hydrolysate after 4 hours of hydrolysis is indicated in table 2.

**Table 2. Proximate composition of protein hydrolysate powder from white leg shrimp heads**

| Proximate composition | Content (%)  |
|-----------------------|--------------|
| Moisture              | 7.54 ± 0.28  |
| Protein               | 74.32 ± 0.35 |
| Lipid                 | 0.85 ± 0.21  |
| Ash                   | 11.18 ± 0.26 |

The protein content of protein hydrolysate from white leg shrimp (*Litopenaeus vannamei*) heads was 74.32%, which was higher than those reported for protein hydrolysate from black tiger shrimp (*Penaeus monodon*) heads (48.12 - 64.73%) by Randriamahatody *et al.* (2011) [3] but lower than that reported for protein hydrolysate from shrimp waste (75.05%) by Veeranjanyulu *et al.* (2013) [1]. The lipid content of shrimp head protein hydrolysate in this study was low (0.85%). The small amount of lipid in the protein hydrolysate was in accordance with a previous study on shrimp head hydrolysates where less than 1% of lipids were quantified [22]. The low lipid content might enhance the stability of the hydrolysate towards lipid oxidation. The ash content of protein hydrolysate from white leg shrimp heads was 11.18%. A previous study reported that the moisture, protein, lipid and ash contents of shrimp waste protein hydrolysate were 8.56%, 75.05%, 2.65% and 14.17%, respectively [1]. The difference in chemical composition of protein hydrolysates could be due to differences in proximate composition of raw material and hydrolysis conditions (enzyme type, temperature, pH...).

**3.4. Amino acid composition of the protein hydrolysate from white leg shrimp heads**

The total amino acid compositions of protein hydrolysate from white leg shrimp heads after 4 hours of hydrolysis are presented in table 3.

**Table 3. Total amino acid composition of protein hydrolysate from white leg shrimp heads**

| Amino acid composition | Content (g/100 g dry weight) |
|------------------------|------------------------------|
| Histidine              | 1.62 ± 0.07                  |
| Isoleucine             | 2.61 ± 0.12                  |
| Leucine                | 4.27 ± 0.16                  |
| Lysine                 | 3.92 ± 0.22                  |
| Methionine             | 1.55 ± 0.05                  |

|   |              |
|---|--------------|
| Phenylalanine                               | 2.54 ± 0.14  |
| Threonine                                   | 2.03 ± 0.08  |
| Valine                                      | 3.48 ± 0.21  |
| Essential amino acids (EAA)                 | 22.02 ± 0.27 |
| Alanine                                     | 3.23 ± 0.13  |
| Arginine                                    | 3.42 ± 0.06  |
| Aspartic acid                               | 5.16 ± 0.17  |
| Glutamic acid                               | 6.35 ± 0.24  |
| Glycine                                     | 4.68 ± 0.21  |
| Proline                                     | 3.15 ± 0.18  |
| Serine                                      | 2.88 ± 0.12  |
| Tyrosine                                    | 2.65 ± 0.16  |
| Non essential amino acids (EAA)             | 31.52 ± 0.25 |
| Total amino acids                           | 53.54 ± 0.32 |
| Essential amino acids/Total amino acids (%) | 41.13 ± 0.27 |

The protein hydrolysate obtained from white leg shrimp heads had a total amino acid content of 53.54 g/100 g dry weight and an essential amino acid content of 22.02 g/100 g dry weight. The ratio of essential amino acids to total amino acids was 41.13%. The shrimp head protein hydrolysate showed high contents of glutamic acid, aspartic acid, glycine, leucine, lysine and valine. Among amino acids, glutamic acid, aspartic acid, and glycine were flavour enhancers that might account for the good taste. Dey and Dora (2014) also showed that the content of glycine, valine, leucine and lysine tended to predominate in shrimp waste hydrolysates [4]. Ruttanapornvareesakul *et al.* (2005) have reported that the amino acid with high content in shrimp head protein hydrolysate was glutamic acid and the limiting amino acid was methionine [22]. The protein hydrolysate from white leg shrimp heads was found to have high nutritional value and could be used in human diets.

### 3.5. Functional properties of the protein hydrolysate from white leg shrimp heads

#### 3.5.1. Solubility

The solubility of proteins is one of the most important parameters for protein functionality. The high solubility of protein hydrolysates indicates potential applications in food systems [8]. The

solubility of shrimp head protein hydrolysates was measured in the pH range 2 - 8 and shown in figure 2.

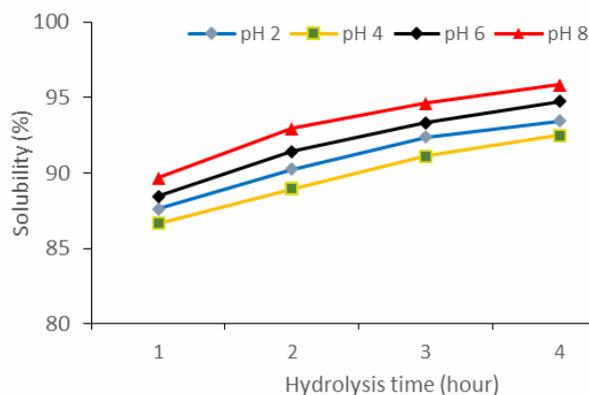


Figure 2. Solubility of shrimp head protein hydrolysate

The study results showed that the solubility of shrimp head protein hydrolysates increased when hydrolysis time increased. The protein hydrolysate with a hydrolysis time of 4 hours showed the highest solubility. The maximum solubility value was presented at pH 8 and the minimum value at pH 4. At pH 8, the shrimp head protein hydrolysates with hydrolysis times of 1 hour, 2 hours, 3 hours and 4 hours had the solubility of 89.67%, 92.94%, 94.63% and 95.85%, respectively.

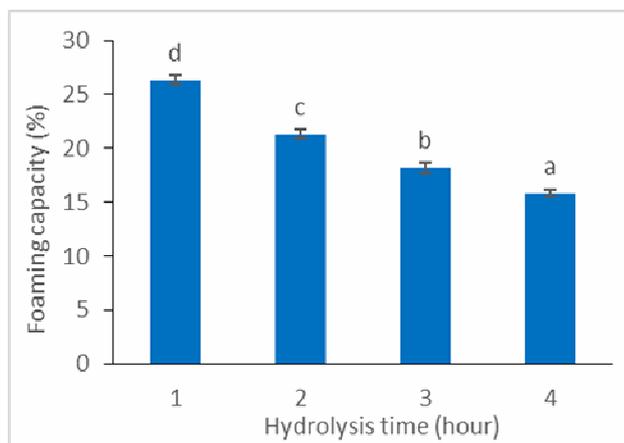
Similar results were reported for the protein hydrolysates from salmon by-products. The

solubility of salmon by-product hydrolysates also increased with increasing degree of hydrolysis [20]. The increase in solubility of the hydrolysates with increased hydrolysis time may be due to the cleavage of proteins into smaller peptides that have increased solubility [21]. The smaller peptides from proteins are expected to have proportionally more polar residues, with the ability to form hydrogen bonds with water and increasing solubility [20].

Protein hydrolysates from white leg shrimp heads display low solubility at their isoelectric point (pH 4). The minimum solubility within the region of isoelectric points was reported by Synowiecki and Al-Khateeb (2000) [17] for shrimp shell protein hydrolysate. Latorres *et al.* (2018) also showed that minimum solubility values were presented at pH 4 for protein hydrolysates from white leg shrimp muscle [23]. The influence of pH on the solubility of protein hydrolysates could be attributed to the net charge of peptides that increases when pH moves away from the isoelectric point.

### 3.5.2. Foaming capacity

The foaming capacity of shrimp head protein hydrolysates is presented in figure 3.



**Figure 3. Foaming capacity of shrimp head protein hydrolysates.**

*Mean values with different superscript letters are significantly different ( $p < 0.05$ ).*

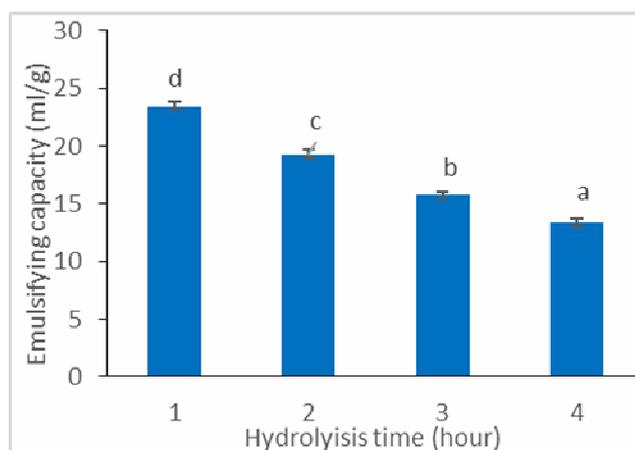
The foaming capacity of shrimp head protein hydrolysates decreased when hydrolysis time increased. With hydrolysis times of 1 hour, 2 hours, 3 hours, 4 hours, the foaming capacity of

shrimp head protein hydrolysates was 26.34%, 21.32%, 18.15%, and 15.83%, respectively. The similar trend of foaming capacity was also observed for the protein hydrolysates from yellow stripe trevally muscle [7], cobia frame [8], sardine by-product [21], and shrimp muscle [23].

The decrease in foaming capacity with an extensive hydrolysis process may be due to the formation of smaller peptides with less surface activity, which would hinder the formation of a stable film around the gas bubbles [8]. Further, the decrease in foaming capacity is also due to the reduction of hydrophobicity of the hydrolysate during hydrolysis [21].

### 3.5.3. Emulsifying capacity

Figure 4 shows the emulsifying capacity of shrimp head protein hydrolysates with different hydrolysis times.



**Figure 4. Emulsifying capacity of shrimp head protein hydrolysates.**

*Mean values with different superscript letters are significantly different ( $p < 0.05$ ).*

The emulsifying capacity of shrimp head protein hydrolysates decreased with increasing hydrolysis time. The emulsifying capacity of protein hydrolysates with hydrolysis times of 1 hour, 2 hours, 3 hours, 4 hours was 23.45 ml/g, 19.26 ml/g, 15.75 ml/g, 13.37 ml/g, respectively. Hydrolysates with short hydrolysis times had high emulsifying capacity, and extensive hydrolysis resulted in a decrease in emulsifying property. A similar trend was reported for protein hydrolysates from shrimp muscle by Latorres *et al.* (2018) [23] and sardine by-products by Souissi *et al.* (2007)

[21], who showed that the protein hydrolysates from sardine by-products with degrees of hydrolysis of 6.62%, 9.31%, and 10.16% had emulsifying capacities of 20 ml/g, 15.2 ml/g, and 10.8 ml/g, respectively.

The hydrolysates with a longer hydrolysis time had poorer emulsifying capacity due to their small peptide size. Longer hydrolysis time will lead to the presence of smaller peptides, which are less effective in stabilizing emulsions. Moreover, the diminishing in emulsifying capacity with an extensive hydrolysis process is also due to the reduction of hydrophobicity of the hydrolysate during hydrolysis [21].

#### 4. CONCLUSION

The results of the present study indicated that the protein hydrolysate from white leg shrimp heads had a high nutritional value with a ratio of essential amino acids to total amino acids of 41.13%. The protein hydrolysate with a hydrolysis time of 4 hours showed the highest solubility (95.85%) at pH 8. The foaming capacity and emulsifying capacity of shrimp head protein hydrolysate with a hydrolysis time of 1 hour were the highest (26.34% and 23.45 ml/g, respectively). The protein hydrolysate from white leg shrimp heads had good functional properties. Thus, shrimp head protein hydrolysate is a source of high quality proteins that can be used as protein ingredients for food.

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# NEW PROVINCIAL RECORD AND EXPANDED DESCRIPTION OF *Hemiphyllodactylus nahangensis* DO, PHAM, PHAN, LE, ZIEGLER & NGUYEN, 2020 FROM THAI NGUYEN PROVINCE, VIETNAM

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## ABSTRACT

*Hemiphyllodactylus nahangensis* was originally described in 2020 from three specimens collected in Tuyen Quang province, northern Vietnam. During a recent herpetofaunal survey in the Nam Xuan Lac Species and Habitat Conservation Area, Thai Nguyen province, nine additional *Hemiphyllodactylus* individuals were discovered in secondary limestone karst forests at elevations above 800 m a.s.l. Morphological examination revealed that these specimens match the original description, with small body size (SVL 39.6 - 41.9 mm in males, 40.6 - 45.5 mm in females), head longer than wide and slightly concave, enlarged chin and first sublabial scales, cloacal spurs in both sexes, dark coloration on the dorsum and head, a light postorbital stripe extending to the forelimb, a fork-like postsacral marking. Phylogenetic analyses confirmed their identity, grouping all specimens with *H. nahangensis* with strong support (100%) and genetic divergences ranging from 1.6% to 5.4%. These results provide the first confirmed record of *H. nahangensis* from Thai Nguyen province, thereby extending the known distribution of this species by approximately 30 km and contributing novel data for its conservation and taxonomy.

**Keywords:** *Distribution, new record, Hemiphyllodactylus, taxonomy, conservation.*

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## 1. INTRODUCTION

The genus *Hemiphyllodactylus* (Bleeker) currently contains 69 species [1] widely distributed across southern India and Sri Lanka, through Indochina and Southeast Asia to the Western Pacific [2]. In Vietnam, the number of *Hemiphyllodactylus* (Bleeker, 1860) species has increased in recent years as a result of intensified herpetological surveys and molecular analyses, particularly in karst habitats [3]. During our field

survey in Nam Xuan Lac Habitat and Species Conservation Area, Thai Nguyen province, nine specimens were collected that were assignable to the genus *Hemiphyllodactylus* (Bleeker). Based on morphological and phylogenetic analyses they were confirmed to be representatives of *Hemiphyllodactylus nahangensis*, a recently described species by Do *et al.* (2020) [4] from the limestone karst forest of Trung Phin, Na Hang district, Tuyen Quang province in northern

Vietnam. The new record of this species in Thai Nguyen province is approximately 30 kilometres southeast from the type locality. In addition, we provide novel morphological and genetic data, enhancing our understanding of the species' distribution within Vietnam's limestone systems and aiding in assessing its diversity and conservation status.

**2. MATERIALS AND METHODS**

**2.1. Sampling**

During a survey in Nam Xuan Lac Habitat and Species Conservation Area (Figure 1), Thai Nguyen province, Vietnam, we collected two adult males, six females and a juvenile on September 8<sup>th</sup> and 9<sup>th</sup>, 2023 from 19:00 to 23:30. They were found on the wall of the main gate at the Binh Trai Ranger Station surrounded by many small shrubs and few small trees. The specimens were deposited in the collection of the Vietnam National University of Forestry (VNUF), Ha Noi, Vietnam.



**Figure 1. Map showing the distribution records of *Hemiphyllodactylus nahangensis* in Vietnam: Tuyen Quang (1); Thai Nguyen (2).**

**2.2. Morphological analysis**

Morphological data were recorded using Olympus SZ61 dissecting microscope.

Terminology follows Luu *et al.* (2024) [2] Measurements were taken with a Mitutoyo digital caliper to the nearest 0.1 mm on the left side of the body when possible. The following character abbreviations and descriptions were used. SVL: Snout-vent length (from the tip of snout to the vent); TaL: Tail length (from the vent to the tip of the tail, original and regenerated); TrunkL: Trunk

length (from the posterior margin of the forelimb at its insertion point on the body to the anterior margin of the hindlimb insertion point on the body); HL: Head length (from the posterior margin of the retroarticular process of the lower jaw to the tip of the snout); HW: Head width (measured at the angle of the jaws); ED: Eye diameter (the greatest horizontal diameter of the eyeball); SnEye: Snout-eye length (from anteriormost margin of the eyeball to the tip of snout); NarEye: Nares-eye length (from the anterior margin of the eyeball to the posterior

margin of the external nares); SnW: Internarial width (measured between the nares across the rostrum). The following meristic characters were used. Chin: Chin scales (the number of scales contacting the infralabials and mental from the juncture of the second and third infralabials on the left side to the juncture of the second and third infralabials on the right side); CN: Circumnasal scales (the number of scales abutting the external naris, exclusive of the rostral and first supralabial); IS: The number of scales between the supranasals; SL: Supralabial scales (number of enlarged scales bordering the mouth on the upper jaw from the rostral to a point in line with the posterior margin of the orbit); IL: Infralabial scales (the number of enlarged scales bordering the mouth on the lower jaw from the mental to a point in line with the posterior margin of the orbit); VS: Ventral scales (the number of longitudinal ventral scales at mid-body contained within one eye diameter); DS: Dorsal scales (the number of longitudinal dorsal scales at mid-body contained within one eye diameter); lamellae formulae determined as the number of U-shaped, subdigital lamellae (split and single) on the digital pads on digits 2 - 5 of the hands (FingLm) and feet (ToeLm); SL1F: The number of subdigital lamellae wider than long on the first finger; SL1T: The number of subdigital lamellae wider than long on the first toe; the total number of preloacal and femoral pores (i.e. the contiguous or discontinuous rows of femoral and preloacal pore-bearing scales); CloacS: The number of cloacal spurs.

### 2.3. Molecular analysis

We analyzed 1,038 base pairs of the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene from 50 specimens of Clade 6 from Agung *et al.* (2021) [5], downloaded from GenBank. Two newly sequenced specimens from Thai Nguyen province, Vietnam, were added to the dataset for phylogenetic analysis. *Hemiphyllodactylusharterti* (*H. harterti* as an out-group species) was used to root the phylogenetic tree following Grismer *et al.* (2013) [6].

Genomic DNA was extracted from ethanol-preserved liver tissue using the DNeasy Blood and

Tissue Kit (Qiagen, Germany). A fragment of the ND2 gene was amplified using the primer pair ND2f101A (5'-CAACAGAAGCCACAACAAAAT-3') and HemiR (5'-GAAGAAGAGGCTTGKAGGCT-3') [7]. The PCR volume consisted of 20  $\mu$ l (1  $\mu$ l each primer 10 mM, 7  $\mu$ l water (dH<sub>2</sub>O), 10  $\mu$ l of Taq mastermix 2X and 1  $\mu$ l DNA template (50 - 100 ng)). Amplification conditions were: Initial denaturation at 95°C for 5 min; 42 cycles of 95°C for 30 s, 50°C for 45 s and 72°C for 60 s; a final extension at 72°C for 6 min.

PCR products were visualized on a 1.2% agarose gel using a 100 bp marker in 1 x TAE buffer, stained with RedSafe (iNtRON, Korea), and photographed under UV light with a GelDoc system (Quantum CX5, Vilber, France). Successfully amplified products were purified using the innuPREP Gel Extraction Kit (Analytik Jena, Germany) and sequenced bidirectionally by 1 st BASE (Malaysia). Sequences were aligned using MUSCLE implemented in MEGA 11 [8].

### 2.4. Phylogenetic analysis

A Maximum likelihood (ML) analysis was conducted to infer phylogenetic relationships among the aligned sequences. The ML tree was generated using the IQ-TREE web server [9, 10], with the best-fit substitution model determined by ModelFinder [11] under the Bayesian Information Criterion (BIC). The TIM+F+G4 model was selected as the most appropriate model of sequence evolution. Node support was assessed using 1,000 ultrafast bootstrap (UFB) replicates [12], values  $\geq 95$  were considered to indicate strong support [13].

## 3. RESULTS AND DISCUSSION

### 3.1. Morphological analysis

Two adult males, snout-vent length (SVL) 39.6 - 41.9 mm (mean  $\pm$  SD: 40.8  $\pm$  1.2 mm), tail length (TaL) 30.3 - 36.9 mm (mean  $\pm$  SD: 33.6  $\pm$  3.3 mm), body elongate (TrunkL) 19.3 - 22.3 mm (mean  $\pm$  SD: 20.8  $\pm$  1.5 mm); head longer than wide, Head length (HL) 11.3 - 11.4 mm (mean  $\pm$  SD: 11.4  $\pm$  0.0 mm), Head width (HW) 7.0 - 7.2 mm (mean  $\pm$  SD: 7.1  $\pm$  0.1 mm) not markedly depressed; eye diameter (ED) 2.3 - 2.5 mm (mean  $\pm$  SD: 2.4  $\pm$  0.1 mm); NarEye length

(NarEye) 3.1 - 3.2 mm (mean  $\pm$  SD: 3.2  $\pm$  0.0 mm), snout - eye length (SnEye) 3.9 - 4.0 mm (mean  $\pm$  SD: 4.0  $\pm$  0.1 mm), internarial distance (SnW) 1.5 - 1.8 mm (mean  $\pm$  SD: 1.7  $\pm$  0.2 mm).

Six adult females, snout-vent length (SVL) 40.6 - 45.5 mm (mean  $\pm$  SD: 42.4  $\pm$  1.8 mm) 40.6, tail length (TaL) 32.0 - 40.0 mm (mean  $\pm$  SD: 35.4  $\pm$  2.9 mm), body elongate (TrunkL) 18.9 - 22.1 mm (mean  $\pm$  SD: 20.4  $\pm$  1.3 mm); Head length (HL) 10.3 - 12.2 mm (mean  $\pm$  SD: 11.3  $\pm$  0.6 mm), Head width (HW) 7.3 - 8.1 mm (mean  $\pm$  SD: 7.7  $\pm$  0.3 mm); eye large, eye diameter (ED) 2.1 - 2.6 mm (mean  $\pm$  SD: 2.3  $\pm$  0.2 mm), NarEye 2.1 - 3.8 mm (mean  $\pm$  SD: 3.1  $\pm$  0.5 mm), SnEye 3.6 - 4.1 mm (mean  $\pm$  SD: 3.8  $\pm$  0.2 mm), SnW 1.3 - 1.7 mm (mean  $\pm$  SD: 1.5  $\pm$  0.1 mm).

Pupils vertical; rostral wider than high; ear oval shaped, small equal approximately 28% eye; surrounded by supranasals, rostral, first supralabial; supranasals separated from each other by three small granular internasals. Supralabials 9 - 12; infralabials 9 - 12, gradually decreased in size towards angle of jaw, 8 - 12 chin scales, touching internal edge of infralabials and mental between the juncture of the second and third infralabials on each side of the head. Dorsal scales small, granular, in 15 - 20 rows at midbody; ventral scales smooth, larger than dorsal scales, subcircle, in 8 - 11 longitudinal rows at midbody. Pads of digits II-V each with large triangular lamella, digital formula 3 - 7 (forefoot) and 4 - 8 (hindfoot); lamellae 1 - 5 on first fingers, 2 - 3 on first toes. Femoropreloacal pores series continuous in male

samples, 10 - 13 in total; cloacal spur single on each side (Table 1).

3.1.1. Colour in life (Figure 2)

Ground colour of dorsum, head and limbs blown and densely mottled with indistinct black markings. The presence of a dark postorbital stripe extending onto at least the neck. Dark dorsolateral stripe on trunk, upper zone of flank with light spots, clear in all specimens; Tail subcaudal enlarged, plate-like markings in all specimens.

3.1.2. Remarks

The newly collected specimens from Thai Nguyen province morphologically match the type series of *Hemiphyllodactylus nahangensis* [4]. However, our specimens exhibit slight differences compared to the original description. Male specimens from Thai Nguyen province are slightly smaller than the holotype (maximum SVL 41.9 mm vs. 43.6 mm), while female specimens are slightly larger on average (mean SVL 43.7 mm vs. 41.4 mm) and have more subdigital lamellae on the 4<sup>th</sup> toe (SL1T) (vs. 3 in the type series), 3 - 6 (2 FingLm) (vs. 3 - 4 in the type series), 4 - 7 (3 FingLm) (vs. 4 - 5 in the type series), 5 - 7 (4 FingLm) (vs. 5 in the type series), 4 - 6 (5 FingLm) (vs. 5 in in the type series); more lamelar formulae foot 4 - 6 (2 ToeLm) (vs. 4 in the type series), 5 - 7 (3 ToeLm) (vs. 5 in the type series), 4 - 8 (4 ToeLm) (vs. 5 in in the type series), 4 - 6 (5 ToeLm) (vs. 5 in the type series); fewer femoropreloacal pores (maximum 13 versus 24 in the type series); more chin scales (maximum 12 vs. 9 in the type series).

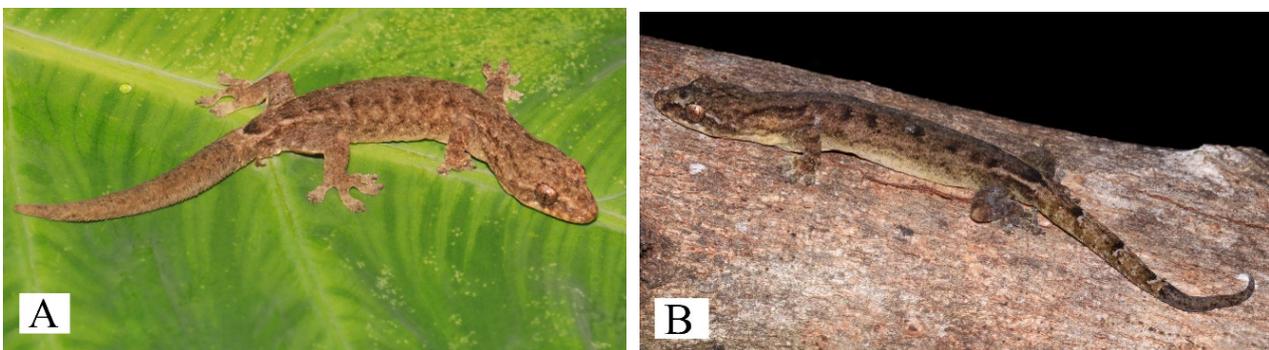


Figure 2. Dorsal views of *Hemiphyllodactylus nahangensis* in life from Thai Nguyen province: A) Adult female (VNUF R.2023.49), B) Adult female (VNUF R.2023.44) Photos: Luu Quang Vinh

**Table 1a. Morphological characters of juvenile and male specimens *Hemiphyllodactylus nahangensis* from Thai Nguyen province, Vietnam (measurements in mm, other abbreviations defined in the text, the mark "-" shows data not available)**

|   | VNUF<br>R.2023.43 | VNUF<br>R.2023.45 | VNUF R.2023.46 | Min - Max<br>(Mean ± SD) |
|---|-------------------|-------------------|----------------|--------------------------|
| Sex   | Juvenile          | Male              | Male           |                          |
| SVL   | 18.5              | 39.6              | 41.9           | 39.6 - 41.9 (40.8 ± 1.2) |
| TrunkL                                      | 7.8               | 19.3              | 22.3           | 19.3 - 22.3 (20.8 ± 1.5) |
| TaL   | 11.9              | 30.3              | 36.9           | 30.3 - 36.9 (33.6 ± 3.3) |
| HL  | 5.5               | 11.3              | 11.4           | 11.3 - 11.4 (11.4 ± 0.0) |
| HW  | 3.9               | 7.2               | 7.0            | 7.0 - 7.2 (7.1 ± 0.1)    |
| ED  | 1.2               | 2.3               | 2.5            | 2.3 - 2.5 (2.4 ± 0.1)    |
| SnEye                                       | 1.8               | 3.9               | 4.0            | 3.9 - 4.0 (4.0 ± 0.1)    |
| NarEye                                      | 1.4               | 3.1               | 3.2            | 3.1 - 3.2 (3.2 ± 0.0)    |
| SnW   | 0.6               | 1.5               | 1.8            | 1.5 - 1.8 (1.7 ± 0.2)    |
| TrunkL/SVL                                  | 0.4               | 0.5               | 0.5            | -                        |
| HW/HL                                       | 0.7               | 0.6               | 0.6            | -                        |
| SnEye/HL                                    | 0.3               | 0.4               | 0.4            | -                        |
| HL/SVL                                      | 0.3               | 0.3               | 0.3            | -                        |
| HW/SVL                                      | 0.2               | 0.2               | 0.2            | -                        |
| HW/HL                                       | 0.7               | 0.6               | 0.6            | -                        |
| NarEye/HL                                   | 0.3               | 0.3               | 0.3            | -                        |
| ED/HL                                       | 0.2               | 0.2               | 0.2            | -                        |
| ED/SnEye                                    | 0.7               | 0.6               | 0.6            | -                        |
| SnW/HL                                      | 0.1               | 0.1               | 0.2            | -                        |
| ED/NarEye                                   | 0.9               | 0.7               | 0.8            | -                        |
| SnW/HW                                      | 0.15              | 0.26              | 0.21           | -                        |
| Chin scales                                 | 9                 | 10                | 12             | 10 - 12 (11 ± 1.0)       |
| CN  | 3/3               | 3/3               | 3/3            | -                        |
| IS  | 3                 | 3                 | 3              | -                        |
| SL (left/right)                             | 11/10             | 11/10             | 9/10           | 9 - 11                   |
| IL (left/right)                             | 11/10             | 10/10             | 11/9           | 9 - 11                   |
| VS  | 11                | 10                | 10             | -                        |
| DS  | 16                | 20                | 20             | -                        |
| Lamelar formulae hands 2-5 (FingLm) (left)  | 4 - 5 - 5 - 5     | 3 - 4 - 4 - 5     | 4 - 5 - 5 - 4  | 3 - 5                    |
| Lamelar formulae hands 2-5 (FingLm) (right) | 4 - 5 - 5 - 4     | 3 - 4 - 5 - 5     | 4 - 5 - 6 - 5  | 3 - 6                    |
| Lamelar formulae hands 2-5 (ToeLm) (left)   | 4 - 5 - 5 - 5     | 6 - 7 - 8 - 5     | 5 - 6 - 6 - 5  | 5 - 8                    |
| Lamelar formulae hands 2-5 (ToeLm) (right)  | 0                 | 6 - 6 - 6 - 4     | 5 - 6 - 6 - 5  | 4 - 6                    |
| SL1F  | 1/1               | 5/4               | 1/1            | 1 - 5                    |
| SL1T  | 2/0               | 3/3               | 3/2            | 2 - 3                    |
| Total femoroprecloacal pores                | -                 | 10                | 13             | 10 - 13                  |
| CloacS                                      | -                 | 1/1               | 1/1            | -                        |

**Table 1b. Morphological characters of female specimens of *Hemiphyllodactylus nahangensis* from Thai Nguyen province, Vietnam (measurements in mm, other abbreviations defined in the text, the mark “-” shows data not available)**

|                 | VNUF<br>R.2023.44 | VNUF<br>R.2023.47 | VNUF<br>R.2023.48 | VNUF<br>R.2023.49 | VNUF<br>R.2023.50 | VNUF<br>R.2023.51 | Min-Max<br>(Mean ± SD)      |
|-----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-----------------------------|
| Sex             | Female            | Female            | Female            | Female            | Female            | Female            |                             |
| SVL             | 45.5              | 40.6              | 41.65             | 44.0              | 41.8              | 40.7              | 40.6 - 45.5<br>(42.4 ± 1.8) |
| TrunkL          | 22.1              | 18.9              | 19.30             | 21.3              | 19.2              | 21.3              | 18.9 - 22.1<br>(20.4 ± 1.3) |
| TaL             | 35.3              | 32.0              | 40.0              | 24.3*             | 21.1*             | 34.4              | 32.0 - 40.0<br>(35.4 ± 2.9) |
| HL              | 12.2              | 12.0              | 11.0              | 10.3              | 11.1              | 11.4              | 10.3 - 12.2<br>(11.3 ± 0.6) |
| HW              | 8.1               | 8.0               | 7.5               | 7.8               | 7.7               | 7.3               | 7.3 - 8.1<br>(7.7 ± 0.3)    |
| ED              | 2.6               | 2.2               | 2.4               | 2.2               | 2.1               | 2.3               | 2.1 - 2.6<br>(2.3 ± 0.2)    |
| SnEye           | 4.1               | 3.6               | 3.6               | 3.8               | 3.6               | 4.0               | 3.6 - 4.1<br>(3.8 ± 0.2)    |
| NarEye          | 3.8               | 3.0               | 3.1               | 3.2               | 2.1               | 3.2               | 2.1 - 3.8<br>(3.1 ± 0.5)    |
| SnW             | 1.5               | 1.4               | 1.3               | 1.5               | 1.7               | 1.3               | 1.3 - 1.7<br>(1.5 ± 0.1)    |
| TrunkL/SVL      | 0.5               | 0.5               | 0.5               | 0.5               | 0.5               | 0.5               | -                           |
| HW/HL           | 0.7               | 0.7               | 0.7               | 0.7               | 0.7               | 0.6               | -                           |
| SnEye/HL        | 0.3               | 0.3               | 0.3               | 0.4               | 0.3               | 0.4               | -                           |
| HL/SVL          | 0.3               | 0.3               | 0.3               | 0.2               | 0.3               | 0.3               | -                           |
| HW/SVL          | 0.2               | 0.2               | 0.2               | 0.2               | 0.2               | 0.2               | -                           |
| HW/HL           | 0.7               | 0.7               | 0.7               | 0.8               | 0.7               | 0.6               | -                           |
| NarEye/HL       | 0.3               | 0.3               | 0.3               | 0.3               | 0.2               | 0.3               | -                           |
| ED/HL           | 0.2               | 0.2               | 0.2               | 0.2               | 0.2               | 0.2               | -                           |
| ED/SnEye        | 0.6               | 0.6               | 0.7               | 0.6               | 0.6               | 0.6               | -                           |
| SnW/HL          | 0.1               | 0.1               | 0.1               | 0.1               | 0.2               | 0.1               | -                           |
| ED/NarEye       | 0.7               | 0.7               | 0.8               | 0.7               | 1.0               | 0.7               | -                           |
| SnW/HW          | 0.2               | 0.2               | 0.2               | 0.2               | 0.2               | 0.2               | -                           |
| Chin scales     | 11                | 10                | 9                 | 9                 | 8                 | 9                 | 8 - 11<br>(9.3 ± 0.9)       |
| CN              | 3/3               | 3/4               | 3/3               | 3/3               | 3/3               | 3/3               | 3 - 4                       |
| IS              | 3                 | 2                 | 3                 | 3                 | 3                 | 3                 | 2 - 3                       |
| SL (left/right) | 11/10             | 11/11             | 11/12             | 10/10             | 10/10             | 11/12             | 10 - 12<br>(9.0 ± 0.6)      |
| IL (left/right) | 11/10             | 12/10             | 9/10              | 9/9               | 9/10              | 10/9              | 9 - 12<br>(17.0 ± 1.4)      |
| VS              | 10                | 9                 | 9                 | 9                 | 8                 | 9                 | 8 - 10                      |
| DS              | 18                | 18                | 19                | 16                | 15                | 16                | 15 - 19                     |

|  |               |               |               |               |               |               |       |
|--|---------------|---------------|---------------|---------------|---------------|---------------|-------|
| Lamellar formulae hands 2-5 (FingLm) (left)  | 4 - 5 - 6 - 5 | 4 - 5 - 5 - 5 | 4 - 4 - 5 - 4 | 6 - 6 - 6 - 5 | 4 - 5 - 5 - 5 | 5 - 6 - 7 - 6 | 4 - 7 |
| Lamellar formulae hands 2-5 (FingLm) (right) | 6 - 7 - 6 - 5 | 4 - 5 - 5 - 5 | 3 - 4 - 5 - 4 | 5 - 5 - 6 - 5 | 5 - 5 - 5 - 5 | 6 - 6 - 7 - 5 | 3 - 7 |
| Lamellar formulae hands 2-5 (ToeLm) (left)   | 5 - 7 - 6 - 5 | 5 - 6 - 6 - 5 | 6 - 6 - 6 - 5 | 5 - 5 - 4 - 5 | 5 - 6 - 6 - 6 | 5 - 7 - 6 - 6 | 4 - 7 |
| Lamellar formulae hands 2-5 (ToeLm) (right)  | 5 - 7 - 6 - 6 | 5 - 6 - 6 - 5 | 4 - 5 - 6 - 5 | 4 - 5 - 5 - 5 | 5 - 6 - 6 - 6 | 6 - 7 - 7 - 6 | 4 - 7 |
| SL1F   | 3/3           | 2/2           | 3/3           | 3/3           | 3/3           | 3/3           | 2 - 3 |
| SL1T   | 4/4           | 2/2           | 4/4           | 3/3           | 4/4           | 4/4           | 2 - 4 |
| Total femoroprecloacal pores                 | -             | -             | -             | -             | -             | -             | -     |
| CloacS                                       | 1/1           | 1/1           | 1/1           | 1/1           | 1/1           | 1/1           | 1     |

\*: Regenerated tail

3.2. Phylogenetic analysis

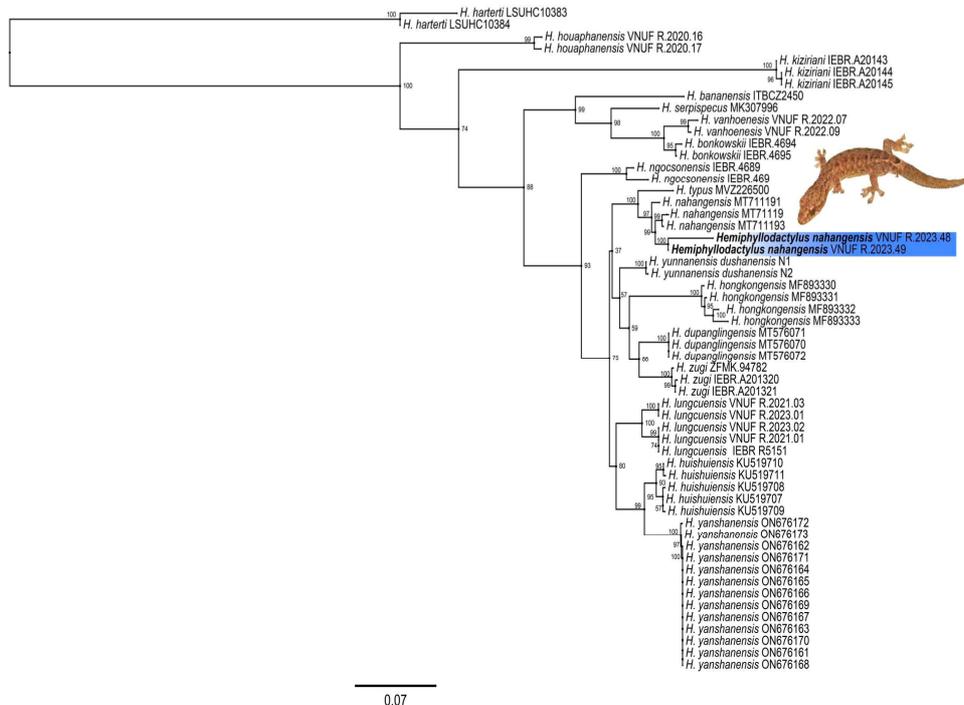


Figure 3. Maximum Likelihood phylogeny showing *Hemiphyllodactylus nahangensis* within Clade 6 of Agung *et al.* (2021) [5]

The Maximum Likelihood (ML) indicated the newly collected specimens in Thai Nguyen province formed a monophyletic sister lineage to *Hemiphyllodactylus nahangensis* with high bootstrap support (100) (Figure 3). The genetic distance between two representative specimens is 3.28%. However, one specimen (VNUF R.2023.49) exhibits genetic distance from the original samples (1.6 - 2.2%), whereas another (VNUF R.2023.48)

shows a larger distance (4.6 - 5.4%). The distance values between *H. nahangensis* and other species in the genus *Hemiphyllodactylus* ranges from 4.0 - 7.1% (*H. typus*, *H. lungcuensis*, *H. yunnanensis dushanensis*, *H. zugii*, *H. ngocsonensis*, *H. dupanglingensis*, *H. huishuiensis*, *H. hongkongensis*, *H. yanshanensis*) and 13.4 - 25.7% (*H. vanhoensis*, *H. bonkowskii*, *H. serpispecus*, *H. bananensis*, *H. houaphanensis*, *H. harterti*).

Table 2. Genetic p-distances in ND2 gene of *Hemiphyllodactylus* species. Intraspecific p-distance of *Hemiphyllodactylus nahangensis* was highlighted

|    |                                   | 1      | 2      | 3      | 4      | 5      | 6      | 7      | 8      | 9      | 10     | 11     | 12     | 13     | 14     | 15     | 16     | 17     |
|----|-----------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1  | <i>H. nahangensis</i>             | 0.0328 |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| 2  | <i>H. vanhoensis</i>              | 0.1358 | 0.0006 |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| 3  | <i>H. houaphanensis</i>           | 0.1605 | 0.1683 | 0.0006 |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| 4  | <i>H. bonkowskii</i>              | 0.1341 | 0.0253 | 0.1629 | 0.0029 |        |        |        |        |        |        |        |        |        |        |        |        |        |
| 5  | <i>H. banaensis</i>               | 0.1495 | 0.1275 | 0.1914 | 0.1297 | 0.0000 |        |        |        |        |        |        |        |        |        |        |        |        |
| 6  | <i>H. harterti</i>                | 0.2568 | 0.2569 | 0.2642 | 0.2646 | 0.3043 | 0.0213 |        |        |        |        |        |        |        |        |        |        |        |
| 7  | <i>H. hongkongensis</i>           | 0.0714 | 0.1447 | 0.1714 | 0.1457 | 0.1634 | 0.2752 | 0.0099 |        |        |        |        |        |        |        |        |        |        |
| 8  | <i>H. huishuiensis</i>            | 0.0603 | 0.1504 | 0.1716 | 0.1542 | 0.1567 | 0.2893 | 0.0794 | 0.0068 |        |        |        |        |        |        |        |        |        |
| 9  | <i>H. kiziriani</i>               | 0.2017 | 0.1994 | 0.2037 | 0.2000 | 0.2268 | 0.2960 | 0.1999 | 0.2047 | 0.0027 |        |        |        |        |        |        |        |        |
| 10 | <i>H. dupanglingensis</i>         | 0.0576 | 0.1551 | 0.1707 | 0.1526 | 0.1694 | 0.2781 | 0.0788 | 0.0709 | 0.2028 | 0.0000 |        |        |        |        |        |        |        |
| 11 | <i>H. ngocsonensis</i>            | 0.0693 | 0.1310 | 0.1685 | 0.1279 | 0.1451 | 0.2696 | 0.0922 | 0.0775 | 0.1932 | 0.0756 | 0.0115 |        |        |        |        |        |        |
| 12 | <i>H. serpispecus</i>             | 0.1369 | 0.0832 | 0.1806 | 0.0747 | 0.1225 | 0.2575 | 0.1639 | 0.1537 | 0.2136 | 0.1664 | 0.1257 | 0.0000 |        |        |        |        |        |
| 13 | <i>H. typus</i>                   | 0.0397 | 0.1560 | 0.1789 | 0.1462 | 0.1523 | 0.2787 | 0.0893 | 0.0743 | 0.2123 | 0.0725 | 0.0876 | 0.1563 | 0.0000 |        |        |        |        |
| 14 | <i>H. yanshanensis</i>            | 0.0713 | 0.1457 | 0.1695 | 0.1516 | 0.1706 | 0.2596 | 0.0939 | 0.0442 | 0.2216 | 0.0856 | 0.0847 | 0.1473 | 0.0825 | 0.0003 |        |        |        |
| 15 | <i>H. yunnanensis dushanensis</i> | 0.0541 | 0.1404 | 0.1777 | 0.1469 | 0.1728 | 0.2910 | 0.0709 | 0.0638 | 0.2050 | 0.0515 | 0.0790 | 0.1599 | 0.0631 | 0.0826 | 0.0009 |        |        |
| 16 | <i>H. zugii</i>                   | 0.0578 | 0.1408 | 0.1614 | 0.1399 | 0.1601 | 0.2705 | 0.0739 | 0.0697 | 0.1992 | 0.0479 | 0.0752 | 0.1500 | 0.0728 | 0.0812 | 0.0577 | 0.0024 |        |
| 17 | <i>H. lungcuensis</i>             | 0.0448 | 0.1363 | 0.1580 | 0.1382 | 0.1580 | 0.2716 | 0.0695 | 0.0529 | 0.2023 | 0.0625 | 0.0662 | 0.1437 | 0.0559 | 0.0678 | 0.0476 | 0.0675 | 0.0102 |



Figure 4. Habitat of *Hemiphyllodactylus nahangensis* in Nam Xuan Lac Species and Habitat Conservation Area, Thai Nguyen province

Specimens of *Hemiphyllodactylus nahangensis* was found on primary forest on limestone karst terrain at night between 19:00 and 23:00. Most of the specimens were collected on the wall of the main

gate at the Binh Trai Ranger Station, one individual on a branch of tree, approximately 1.5 - 2 meter above the ground the entrance of a karstic cave.

4. CONCLUSION

These newly collected specimens of *Hemiphyllodactylus nahangensis* from Thai Nguyen province are morphologically and phylogenetically consistent with the type series from Tuyen Quang province, northern Vietnam. This is the first record of the species from the Nam Xuan Lac Species and Habitat Conservation Area as well as in Thai Nguyen province and it is approximately 30 km southeast of the type locality. Although the specimens were found in a secondary forest within a protected area, ongoing mineral exploitation and extraction continues to threaten the surrounding habitat.

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# STRATEGIC DEVELOPMENT OF ECOTOURISM IN THE PROTECTION FOREST MANAGEMENT BOARD OF CAM LAM - CAM RANH - KHANH SON, KHANH HOA PROVINCE: AN APPROACH FROM SWOT, TOWS AND QSPM ANALYSIS

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## ABSTRACT

This paper presents the results of a study on ecotourism development in the Cam Lam - Cam Ranh - Khanh Son Protection Forest Management Board in Khanh Hoa province aims to identify and evaluate priority strategies for effective and sustainable ecotourism development. The research employs methodologies including field surveys, stakeholder consultations, SWOT analysis, the development of a Quantitative Strategic Planning Matrix (QSPM). Through SWOT and QSPM analysis, internal factors (strengths, weaknesses) and external factors (opportunities, threats) were identified, leading to the formulation of appropriate development strategies. Based on QSPM analysis, nine proposed strategies were clearly differentiated by their level of attractiveness, reflecting feasibility, resource mobilization potential, alignment with local conditions. The SO1 strategy- "Developing unique ecotourism products linked to natural and cultural resources" - achieved the highest attractiveness score (2.68 points), followed by WO1 - "Upgrading ecotourism infrastructure and services" (2.65 points), SO2 - strengthening public-private partnerships to exploit the forest environment (2.41 points), and ST1 - Master plan, develop brand positioning strategy and communication for ecotourism destinations (2.40 points). These strategies are recommended for short-term priority implementation. Medium-low-scoring strategies (1.86 - 2.34 points), such as digital transformation, community training and improving coordination mechanisms, require mid-term roadmaps, while lower-scoring strategies (< 1.5 points) - like conservation monitoring, seasonal diversification - should be integrated into long-term plans to ensure sustainable ecotourism development. The study contributes proposes directions for responsible, resource - conserving ecotourism development.

**Keywords:** *Ecotourism, SWOT, QSPM, protection forest, Khanh Hoa.*

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## 1. INTRODUCTION

Tourism is currently one of the fastest-growing sectors worldwide, making a significant contribution to global GDP and increasingly meeting human needs. According to the General Statistics Office (2025) [1], as of June 2025, Vietnam welcomed 10,664,608 international visitors, an increase of 20.7% compared to the

same period in 2024. In this context, ecotourism has emerged as a nature - based form of tourism that promotes conscious conservation of natural and cultural resources, causes less environmental impact, and brings benefits to local communities [2]. Ecotourism emphasizes the integration of conservation, community involvement, sustainable tourism such as: Minimizing negative

environmental impacts, raising environmental and cultural awareness, providing positive experiences for visitors, delivering direct financial benefits for conservation, supporting local communities [3]. Ecotourism can have profound impacts on economic development and natural resource conservation [4] helps strengthen the connection between tourism and conservation, enabling tourism to effectively support the protection of natural resources and meet long-term ecological needs [5]. Ecotourism plays a key role in global biodiversity conservation strategies. It not only raises public awareness but also generates net positive impacts on the economy, society, and the environment [6].

At present, numerous studies have been conducted to evaluate the factors influencing sustainable ecotourism development [7, 8] and have applied SWOT analysis and the construction of the Quantitative Strategic Planning Matrix (QSPM) to identify sustainable ecotourism development strategies [9]. The advantage of SWOT analysis lies in its ability to assess internal weaknesses and external threats. It is considered useful, straightforward, cost-effective [10]. The TOWS matrix is an extension of the SWOT analysis that helps link analytical insights into feasible groups of strategic options. Many studies have applied this model to develop strategies for ecotourism development [11 - 14]. The Quantitative Strategic Planning Matrix (QSPM) is a quantitative tool used to select the most appropriate strategies for sustainable ecotourism development [15 - 17]. Priority strategies for development include enhancing multi-stakeholder participation, developing marketing and communication strategies based on information technology applications, managing tourism infrastructure, and implementing capacity-building programs [18]. Leveraging natural, historical, climatic, environmental potential, as well as transportation advantages, to gain policy support [19].

According to Vietnam's national tourism development strategy and master plan to 2030, with a vision to 2045, Vietnam is divided into six tourism regions. Among them, the South Central

Coast is identified as a key strategic area, prioritized for investment in scenic landscape planning and the development of ecotourism alongside marine and island tourism. One of the main priorities in this region is the promotion of ecotourism products linked to agriculture and culture [20]. With the advantage of a rich ecosystem, protection forests play an important role not only in maintaining ecological balance but also as a potential foundation for ecotourism development. The integration of tourism with forest conservation is increasingly emphasized in strategies for green economic growth and climate change adaptation. The Protection Forest Management Board of Cam Lam – Cam Ranh – Khanh Son in Khanh Hoa province is home to a distinctive forest ecosystem, featuring abundant natural landscapes and rich indigenous cultural resources. However, ecotourism development activities in this protection forest area still face many limitations, largely spontaneous, ecotourism products have not been adequately explored. There is also a lack of strategic direction and effective long-term management mechanisms for development in this area. This study was conducted by analyzing internal and external factors using the SWOT model, combined with TOWS analysis to formulate strategies, prioritizing them through the QSPM Matrix.

## **2. RESEARCH METHODOLOGY**

### **2.1. Study area**

The Protection Forest Management Board of Cam Lam - Cam Ranh - Khanh Son is located in 6 communes and wards including: Tay Khanh Son, Khanh Son, Dong Khanh Son, Cam Hiep, Cam An, Ba Ngoi ward. The area lies between 108°47'03" to 109°05'56" East longitude and 11°53'57" to 12°09'05" North latitude, with a total natural area of 30,212.3 hectares. Of this, 22,470.19 hectares are designated as protection forest and 7,671.55 hectares as production forest. The terrain of the area is diverse and highly fragmented, consisting of high mountain ranges and mid-elevation hills. This topography offers favorable conditions for the development of various ecotourism activities such as mountain climbing, forest trekking, and terrain-

based experiences. The climate is characterized as tropical monsoon, with a dry season extending from January to August and a rainy season concentrated between September and December. The dry season, especially from January to April, provides ideal weather conditions for outdoor tourism. The complex terrain, combined with spatial climatic variation, has resulted in a rich and diverse forest ecosystem. This contributes significantly to the region's potential for sustainable forestry and ecotourism development.

## **2.2. Methods**

### *2.2.1. Secondary data collection and field surveys*

#### *- Secondary data collection and field surveys*

Secondary data included reports on forest protection and management, natural conditions, socio-economic characteristics collected from various sources, previous reports, plans, research findings related to the current status of ecotourism development in the area. Field surveys were conducted in forest management units and local communities. These surveys also served to verify the data collected during interviews. Information from field sites, combined with secondary data and in-depth interviews, provided important inputs for the analysis and strategy development.

#### *- Interview method*

The main information providers included relevant management agencies at the provincial and local (commune) levels, forest owners. These stakeholders were selected to provide information, perspectives, assessments on the current situation, influencing factors, the level of impact on sustainable ecotourism development. Specifically, the interview participants consisted of: 9 experts, 18 government and forest protection officers and forest owners. The interviews were conducted using two approaches: Direct (in-person) interviews and online questionnaires designed through an internet-based platform. The questionnaire primarily used a five-point Likert scale ranging from "Strongly disagree" to "Strongly agree." The responses were coded for quantitative analysis and used in data processing.

### *2.2.2. SWOT analysis method for identifying strategic factors*

The SWOT analysis (Strengths - Weaknesses - Opportunities - Threats) was applied to identify both internal (strengths and weaknesses) and external (opportunities and threats) strategic factors influencing the potential for ecotourism development in the study area [21]. These factors were identified based on secondary data sources, expert, government and forest protection officers interviews. After collection, the SWOT elements were standardized and categorized into thematic groups as the foundation for developing the Internal Factors Evaluation (IFE) matrix and the External Factors Evaluation (EFE) matrix, which support quantitative strategic planning.

The Internal Factor Evaluation (IFE) matrix is used to analyze the internal environment of raw water infrastructure components, by evaluating strengths and weaknesses. While the External Factor Evaluation Matrix (EFE) analyzes the external factors used to evaluate opportunities and threats. After identifying the internal and external factors, the weight can be assigned to each factor, ranging from 0.0 to 1.0, depending on significance in its contribution or inhibition to the development of tourism. The total number of Internal factors is 1, as well as the total number of external factors. Zero means the least important or unrelated and one shows the most important or very related. Next, each factor was rated on a scale from 1 to 4 based on its effectiveness. Each factor was then scored on a scale of 1 to 4, where: 1 represents a fundamental weakness; 2 indicates a minor weakness; 3 denotes a strength, and 4 reflects a significant strength.

The total final score for the IFEM was calculated by summing the weighted scores of all internal factors and the EFEM was calculated by summing the weighted scores of all external factors. For instance, a strength with a weight of 0.03 and a score of 3 would yield a final score of 0.09. The total final score for the IFEM was calculated by summing the weighted scores of all internal factors, below 2.5 indicates that weaknesses outweigh strengths, above 2.5

suggests that strengths outweigh weaknesses. The total final score for the EFEM, where a total score below 2.5 signifies that threats surpass opportunities, while a score above 2.5 reflects the dominance of opportunities over threats.

2.2.3. TOWS matrix analysis and strategy grouping

The TOWS method, introduced by Heinz Wehrich [22], helps convert SWOT results from qualitative insights into actionable strategies by systematically combining factor pairs: S-O (Strengths-Opportunities): Using strengths to take

advantage of opportunities; W-O (Weaknesses-Opportunities): Improving weaknesses to capture opportunities; S-T (Strengths-Threats): Using strengths to overcome threats; W-T (Weaknesses-Threats): Minimizing weaknesses to reduce external risks. Based on these combinations, strategy groups were formulated and coded (e.g., SO1, WO2, ST3, WT1) to facilitate comparison and analysis in subsequent steps. This coding system creates a direct link between strategic orientation (TOWS) and quantitative analysis in the QSPM matrix.

| EXTERNAL FACTORS | INTERNAL FACTORS   |  |
|------------------|--|--|
|                  | STRENGTHS  | WEAKNESSES   |
| OPPORTUNITIES    | <b>SO</b><br><i>Use strengths to take advantage of opportunities</i> | <b>WO</b><br><i>Overcome weaknesses by using opportunities</i> |
| THREATS          | <b>ST</b><br><i>Use strengths to avoid threats</i>                   | <b>WT</b><br><i>Minimize weaknesses and avoid threats</i>      |

Figure 1. TOWS matrix diagram

2.2.4. Quantitative strategic planning matrix (QSPM)

In QSPM, each strategy is quantitatively assessed based on how well it aligns with the internal and external factors identified in the IFE and EFE matrices. The integration of TOWS and QSPM ensures a systematic approach to strategic planning and supports the selection of the most suitable development options based on quantitative evaluation. To identify and prioritize sustainable ecotourism development strategies, the QSPM method is applied. QSPM allows for a comparative quantitative analysis of strategic alternatives by evaluating the relative attractiveness of each strategy in relation to internal (Strengths and Weaknesses) and external (Opportunities and Threats) environmental factors. The QSPM uses input from the IFE, EFE, SWOT matrices along with the proposed strategic

options to objectively evaluate the most feasible strategies. Each strategy is scored based on an Attractiveness Score (AS) ranging from 0 to 4: 0 = not relevant to the strategy; 1 - 2 = weak to moderate relevance; 3 - 4 = strong to very strong relevance. The Total Attractiveness Score (TAS) for each strategy is calculated by multiplying the weight of each factor by its AS (TAS = Weight x AS). By summing all TAS values, the total attractiveness of each strategy is determined. The strategy with the highest total score is considered the most appropriate for implementation.

3. RESULTS AND DISCUSSION

3.1. SWOT analysis results in ecotourism development

The strengths, weaknesses, opportunities, threats (SWOT factors) related to ecotourism development are summarized in table 1 below.

**Table 1. SWOT snalysis**

| Strengths (S)   | Opportunities (O)  |
|---|--|
| <p>(S1) Diverse and attractive natural resources: The area has well-preserved forest ecosystems, beautiful natural landscapes, varied topography, which are highly suitable for ecotourism and nature-based experiences.</p> <p>(S2) Unique local cultural resources: The presence of ethnic communities such as the Raglai and Cham Hroi provides rich cultural heritage. Combined with forest and agricultural production models, the area has high potential for developing nature-based tourism, ecotourism, and community-based tourism.</p> <p>(S3) Clear development orientation from the Management Board: The Management Board has established a clear plan for ecotourism development based on legal regulations and sustainable development principles, with strong focus on biodiversity conservation.</p> <p>(S4) Skilled human resources in conservation: The staff of the Management Board possess good expertise in nature conservation, biodiversity, wildlife, forming a strong foundation for developing ecotourism linked with conservation goals.</p> <p>(S5) Strategic geographical location: This area is located between major tourism centers in the province such as Lam Dong, Dak Lak. It is connected to key transport routes like National Highway 1A, is close to Airport, seaports, popular coastal tourism zones. This offers favorable conditions to attract domestic and international tourists and to promote integrated forest-sea-cultural tourism in Khanh Hoa.</p> <p>(S6) Popular existing trekking and leisure routes: The route from Ham Leo Bridge to Ta Giang grassland is well-known among visitors and attracts a stable number of tourists (about 50 - 100 visitors per week).</p> | <p>(O1) National and local tourism policies: National strategies, laws, and development programs have identified tourism as a key economic sector, with ecotourism being prioritized. There are also clear regulations on how to prepare ecotourism development plans for protected and protection forests, which serve as an important legal basis.</p> <p>(O2) Interest and investment potential from tourism enterprises: Many travel agencies and tourism companies are willing to invest or partner through forest environment leases. This presents a strong opportunity to attract investment and develop forest-based ecotourism in the near future.</p> <p>(O3) Potential for community livelihood development and increasing demand for ecotourism: Nature-based and community-based travel experiences are becoming more popular. Many tourists prefer destinations close to nature such as forests, streams, waterfalls for relaxation and exploration, creating potential to attract both domestic and international visitors.</p> <p>(O4) Regional transportation infrastructure development: Upgrades to roads and highways - such as the North-South Expressway and inter-district roads (e.g., Lap Dinh - Suoi Mon) - have improved travel time and access to the protection forest area, making tourism development more feasible.</p> <p>(O5) Regional connectivity and integrated tourism routes: The area is well-positioned to connect with nearby attractions such as Truong Xuan hot springs, Nui Chua National Park, Binh Ba Island. Developing joint tourism routes will enhance competitiveness and improve the visitor experience.</p> |

| Weaknesses (W)  | Threats (T)   |
|---|---|
| <p>(W1) Limited quality and quantity of tourism human resources: Although the Management Board staff have strong conservation expertise, they lack experience in tourism management and business operations. Many local tour guides are untrained.</p> <p>(W2) Limited knowledge and capacity in local communities: While local people are interested in participating in tourism, many still face economic difficulties and lack the skills required for tourism services, especially in communication, customer service, environmental awareness.</p> <p>(W3) Poor infrastructure and tourism services: There is a lack of basic facilities such as eco-lodges, rest stops, signage, restrooms, electricity, water, communication systems, standard food services. Tourism planning is not yet well integrated, financial resources for infrastructure investment are limited, affecting service quality.</p> <p>(W4) Weak regional linkages and tourism routes: Local transport infrastructure is still underdeveloped. Many roads are in poor condition, making it difficult to access potential tourist sites. There is no well-established ecotourism network, tourism products are still limited.</p> <p>(W5) Lack of effective information and tourism promotion: Information systems, signage, digital tools, websites, digital maps, promotional content and communication strategies at tourism sites are outdated and not user-friendly.</p> <p>(W6) Unregulated and spontaneous tourism activities: Several tourism activities in natural areas such as Valy Stream, Ta Gu Waterfall, Ta Giang trekking routes are operating without unified management or regulation.</p> | <p>(T1) Climate change and extreme weather conditions: Heavy rains, droughts, landslides, flash floods, and forest fire risks directly affect tourism activities, especially during the rainy season. These events can interrupt tourism services and negatively impact forest ecosystems.</p> <p>(T2) Risks in balancing conservation and tourism: Ecotourism development requires a balance between natural resource conservation and tourism exploitation. Without proper control and guidance, tourism may cause negative impacts on forest ecosystems and natural heritage values, including waste, noise, landscape degradation.</p> <p>(T3) Increasing competition among tourist destinations: The Southern Khanh Hoa region is under pressure from more developed destinations like Nui Chua National Park, Hon Ba, coastal resorts. This requires a unique and clear strategy to highlight ecological and cultural values and create special, competitive tourism products.</p> <p>(T4) High seasonality of ecotourism: Tourism activities heavily depend on weather conditions. However, the Management Board has not yet developed effective strategies to diversify tourism products or extend the tourist season throughout the year.</p> <p>(T5) Uneven public awareness of ecotourism: Some local communities and authorities still consider tourism as a secondary activity and lack a strategic approach. This could lead to unplanned tourism development, inefficient use of resources, potential conflicts of interest.</p> |

Based on the SWOT analysis table (Table 1), it can be seen that this study area has diverse forest ecosystems, rich indigenous cultural resources, and a strategic geographic location - providing a strong foundation for developing ecotourism. Clear strategic direction, professional conservation staff, and some popular trekking routes (e.g., Ta

Giang, Ta Gu Waterfall, Valy Stream,...) demonstrate the area's high development potential (S1-S6). In addition, favorable national and local policies, increasing interest from tourism enterprises, and trends toward nature-based experiences further support growth opportunities (O1-O5). However, internal weaknesses such as

limited tourism workforce capacity, inadequate infrastructure, weak destination linkages, and spontaneous tourism activities remain significant barriers (W1–W6). External threats including climate change, environmental risks, strong competition, seasonality, uneven awareness of ecotourism (T1–T5) highlight the need for a sustainable and well-adapted development

strategy.

### 3.2. Evaluation results of IFEM and EFEM

#### 3.2.1. EFEM (External factors evaluation matrix)

The EFEM summarizes 13 key factors related to opportunities and threats that influence ecotourism development in the study area.

**Table 2. External factors evaluation matrix (EFEM)**

| Factors  | Weight | Rating | Weighted Score |
|--|--------|--------|----------------|
| Opportunities (O)  |        |        |                |
| (O1) National and local policies supporting ecotourism development                               | 0.13   | 3.80   | 0.49           |
| (O3) Community livelihood potential and rising demand for ecotourism and community-based tourism | 0.12   | 3.73   | 0.46           |
| (O2) Interest and potential investment from tourism enterprises                                  | 0.10   | 3.73   | 0.38           |
| (O4) Regional transportation infrastructure development  | 0.09   | 3.80   | 0.36           |
| (O5) Regional connectivity and integrated tourism routes   | 0.10   | 3.33   | 0.33           |
| Total  | 0.55   | 3.20   | 2.00           |
| Threats (T)  |        |        |                |
| (T1) Climate change and extreme weather conditions   | 0.11   | 3.87   | 0.44           |
| (T2) Risks in balancing conservation and tourism   | 0.10   | 3.73   | 0.38           |
| (T3) Increasing competition among tourist destinations   | 0.08   | 3.13   | 0.26           |
| (T4) High seasonality of ecotourism  | 0.08   | 2.67   | 0.21           |
| (T5) Uneven public awareness of ecotourism   | 0.07   | 2.47   | 0.18           |
| Total  | 0.45   | 2.70   | 1.20           |
| Total (EFEM)   | 1.00   | 18.57  | 3.20           |

The analysis of the EFEM shows a total score of 3.20 out of 4.00, indicating that the macro-environment and tourism sector currently offer favorable conditions for developing ecotourism in this area. Notably, opportunities (O) play a dominant role, contributing 2.0 points, which is significantly higher than the 1.2 points from

threats (T). Among the opportunities, the two most influential factors are: (O1) A supportive policy framework and legal environment (weight: 0.13; rating: 3.80); (O3) Rapidly increasing market demand for ecotourism and community-based tourism post-Covid-19 (weight: 0.12; rating: 3.73). On the other hand, while the threats group (T) is

less dominant, it still reflects several potential risks that should not be overlooked. In particular: Among the identified threats, climate change and extreme weather received the highest score (weight = 0.11; rating = 3.87), suggesting limited adaptive capacity. This suggests that climate risks remain a major barrier and should be prioritized in planning. Other concerns such as conservation risks (T2) and low public awareness of ecotourism (T5) may hinder tourism development unless addressed through targeted communication, education campaigns, proper regulatory

mechanisms. With an EFEM score above 2.5, it can be concluded that this area is in a very positive position to respond to external factors, especially in leveraging supportive policies, market trends, investment opportunities, community participation.

*3.2.2. Internal factor evaluation matrix (IFEM)*

The IFEM was developed based on 20 key internal factors, including both strengths (S) and weaknesses (W), which influence the ability to plan and manage sustainable ecotourism. The detailed results of the IFEM are shown in table 3.

**Table 3. Internal factor evaluation matrix (IFEM)**

| Factors  | Weight | Rating | Weighted Score |
|--|--------|--------|----------------|
| <b>Strengths (S)</b>   |        |        |                |
| (S1) Diverse and attractive natural resources                | 0.10   | 3.87   | 0.38           |
| (S2) Unique local cultural resources                         | 0.10   | 3.80   | 0.36           |
| (S5) Strategic geographical location                         | 0.08   | 3.73   | 0.32           |
| (S3) Clear development orientation from the Management Board | 0.08   | 3.73   | 0.31           |
| (S6) Popular existing trekking and leisure routes            | 0.09   | 3.07   | 0.29           |
| (S4) Skilled human resources in conservation                 | 0.06   | 3.07   | 0.19           |
| Total  | 0.52   | 21.27  | 1.85           |
| <b>Weaknesses (W)</b>  |        |        |                |
| (W3) Poor infrastructure and tourism services                | 0.09   | 3.87   | 0.34           |
| (W5) Lack of effective information and tourism promotion     | 0.08   | 3.40   | 0.29           |
| (W6) Unregulated and spontaneous tourism activities          | 0.08   | 3.60   | 0.27           |
| (W2) Limited knowledge and capacity in local communities     | 0.08   | 3.07   | 0.24           |
| (W1) Limited quality and quantity of tourism human resources | 0.08   | 3.00   | 0.23           |
| (W4) Weak regional linkages and tourism routes               | 0.08   | 2.67   | 0.22           |
| Total  | 0.48   | 19.60  | 1.58           |
| Total IFEM   | 1.00   | 40.87  | 3.43           |

The result shows that, total IFEM score of 3.43 out of 4.00, highlights that the organization possesses a moderately strong internal foundation

for promoting ecotourism, especially when its existing advantages are properly capitalized upon and current limitations are addressed

systematically. Key internal strengths contributing to this score include the presence of diverse ecosystems and scenic landscapes (S1), as well as the rich cultural heritage of local ethnic communities (S2). Additionally, a clear development orientation (S3) and the area's strategic location (S5) both contribute significantly to the region's potential. These factors form the groundwork for developing tourism models that integrate ecological preservation with community involvement and cultural appreciation. Despite these advantages, the organization still faces internal challenges. Notably, infrastructure remains underdeveloped (W3), promotional

activities are limited in reach and effectiveness (W5), certain tourism activities are being conducted without proper regulation or planning (W6). If left unaddressed, these weaknesses may impede the shift toward a sustainable and well-managed ecotourism framework.

*3.2.3. Results of strategic group analysis based on TOWS matrix*

Based on the identification of 6 strengths, 6 weaknesses, 5 opportunities, and 6 threats, the study proposes four strategic groups using the TOWS matrix, as follows.

**Table 4. Analysis of SWOT and TOWS strategic matrix of the study area**

|                 | Opportunities (O)  | Threats (T)   |
|-----------------|--|---|
| Strengths (S)   | <p>1. SO1 - Develop distinctive ecotourism products focused on nature-based experiences and local culture (S1, S2, S5 + O1, O3): Take advantage of rich natural resources, unique indigenous culture, and a favorable location to create eco-friendly tourism products that combine nature-based experiences with local cultural values. These products will meet the growing trend of green, ecological, community-based tourism.</p> <p>2. SO2 - Promote investment and partnerships for sustainable forest-based tourism development (S1, S2, S3 + O1, O2, O5): Based on the clear development orientation and interest from tourism businesses, this strategy focuses on strengthening public - private partnerships (PPPs) to improve infrastructure, lease forest areas for ecotourism activities, and develop inter-regional tour routes.</p> | <p>1. ST1 - Develop an integrated plan and strategic branding for ecotourism destination marketing (S1, S2, S5, S6 + T3, T5): Leverage the strategic location and popular trekking routes to design a professional marketing strategy that clearly positions the destination brand around forest-based, cultural, adventure, agritourism themes.</p> <p>2. ST2 - Strengthen monitoring and regulation for sustainable ecotourism development (S3, S4 + T2): Combine conservation expertise and tourism planning capacity to strictly manage tourism activities, ensuring that forest ecosystems and biodiversity are not negatively impacted.</p> |
| Weaknesses (W). | <p>1. WO1 - Upgrade infrastructure and service quality for ecotourism (W1, W3, W4 + O2, O4, O5): Address weaknesses in infrastructure, tourism services, regional connectivity by utilizing existing transportation investment and policy support, improving accessibility to potential ecotourism sites.</p> <p>2. WO2 - Build local capacity and raise</p>   | <p>1. WT1 - Improve coordination, management, and control of unplanned tourism development (W1, W6, W5 + T1, T2, T5): Establish a clear coordination mechanism between local authorities, the management board, communities to control</p>  |

|  |  |   |
|--|--|---|
|  | <p>community awareness (W1, W2 + O3): Enhance tourism skills for staff and local residents through training programs, encourage their participation in conservation-linked community-based tourism activities to support sustainable livelihoods.</p> <p>3. WO3 - Apply digital technology and promote digital transformation in ecotourism management and marketing (W5 + O1, O2, O3): Leverage policy support and investment trends to adopt digital tools such as smart guide systems, interactive maps, websites, and digital media platforms, aiming to improve tourism management, enhance visitor experiences, attract responsible investors.</p> | <p>unregulated tourism activities, strengthen risk management, promote guided, responsible development.</p> <p>2. WT2 - Diversify tourism products and reduce seasonality (W3, W4 + T4): Develop seasonal tourism products such as cultural experiences, dry-season trekking, agro-tourism to extend visitor stay and attract tourists throughout the year.</p> |
|--|--|---|

Based on the results of the SWOT matrix, the study has built a system of ecotourism development strategies using the TOWS matrix method. The table shows that 9 groups of strategies have been proposed, including 3 SO strategies, 2 ST strategies, 2 WO strategies and 2 WT strategies.

The SO (Strengths - Opportunities) strategy group takes advantage of internal advantages to maximize development opportunities. Strategy SO1 emphasizes the development of unique ecotourism products associated with natural experiences and indigenous culture - a direction in line with the current green tourism trend. Strategies SO2 and SO3 aim to attract investment and strengthen public-private partnership in exploiting forest resources, while applying digital transformation to destination management and promotion, in line with the policy of transforming the growth model in the tourism industry.

The WO (Weaknesses - Opportunities) strategy group focuses on overcoming weaknesses by taking advantage of policy and investment opportunities. The WO1 and WO2 strategies propose solutions to upgrade infrastructure,

tourism services and train local human resources - two factors that are bottlenecks in tourism development in the region. Notably, the WO3 strategy proposes the application of digital technology, smart maps and digital media platforms as a new direction to improve the tourist experience and optimize management.

The ST (Strength-Threat) strategy group exploits internal resources to effectively respond to challenges from competition, uncontrolled development and climate change, including ST1 and ST2. The WT (Weakness-Threat) strategy group aims to prevent risks and overcome long-term limitations. The SO and WO strategies have important strategic significance and can be prioritized for implementation in the early stages, while the ST and WT strategy groups play a complementary role in controlling risks and maintaining long-term stability for the development process.

### 3.3. Results of proposed strategies through QSPM matrix

The results of the QSPM quantitative matrix analysis for selection through the total attractiveness score are as follows.

**Table 5. QSPM analysis results for proposed strategies**

| Strategies   | Total (AS) | Total (TAS) |
|--|------------|-------------|
| 1. SO1 – Developing unique ecotourism products to experience nature associated with indigenous culture (S1, S2, S5 + O1, O2, O3) | 25.0       | 2.68        |

|   |      |      |
|---|------|------|
| 2. WO1 – Upgrade infrastructure and quality of ecotourism services (S1,S2, S5, S6 + T2,T3, T4)                                    | 28.5 | 2.65 |
| 3. SO2 – Calling for investment and cooperation in exploiting forest environment (S1, S2, S5, S6 + T2, T3, T4)                    | 23.0 | 2.41 |
| 4. ST1 – Master plan, develop brand positioning strategy and communication for ecotourism destinations (S1, S2, S5, S6 + T3, T5)  | 25.7 | 2.40 |
| 5. WO3 – Applying digital technology and digital transformation in tourism management and promotion (W1, W4, W5, W6 + O1, O2, O3) | 25.0 | 2.34 |
| 6. WT1 – Improve coordination, management and mitigation mechanisms for spontaneous development (W1, W5, W6 + T1, T2, T5)         | 26.5 | 1.89 |
| 7. WO2 – Training human resources and raising community awareness (W1, W2 + O3)   | 16.0 | 1.86 |
| 8. ST3 – Diversify products and reduce seasonality in tourism (W3, W4 + T4)   | 12.0 | 1.09 |
| 9. ST2 – Strengthen monitoring and control of sustainable ecotourism development (S3, S4 + T2)                                    | 10.5 | 1.03 |

The results of the QSPM matrix analysis show that the attractiveness of ecotourism development strategies is clearly differentiated, with total scores ranging from 1.01 to 2.68. The strategy with the highest score is SO1 (2.68 points) - focusing on developing experiential tourism products associated with local resources and culture. In contrast, the strategy with the lowest score is ST2 (1.03 points) - aiming at strengthen monitoring and control of sustainable ecotourism development. The highest rated strategies include: SO1 (2.68 points): Focusing on developing specific ecotourism products based on natural resources and local culture, developing ecotourism products integrating indigenous cultural values; WO1 (2.65 points) – focusing on upgrading infrastructure and service quality – is a practical and feasible solution, reflecting the urgent need to improve access and visitor experience; meanwhile, SO2 (2.41 points) - strengthening public-private partnerships to exploit the forest environment - shows the potential to expand ecotourism investment through partnership mechanisms and taking advantage of existing forests; ST1 (2.40 points) - master plan, develop brand positioning strategy and communication for ecotourism destinations; This is a group of highly feasible strategies, suitable for current visitor needs and orienting ecotourism development according to market

trends. In general, the high-priority strategies all focus on three key groups: (i) Specific products - Developing ecotourism products integrating indigenous cultural values(ii) Infrastructure and services, (iii) Master planning and brand positioning and (iv) Encouraging investment and collaboration in sustainable forest environment exploitation. This is an important foundation for creating a competitive, sustainable and community-engaging ecotourism ecosystem. From the above analysis results, the authors propose to prioritize the implementation of strategies SO1, WO1, SO2, ST1 in the initial phase, while building a roadmap to integrate medium - and long-term strategies to ensure comprehensive and harmonious development between tourism growth and conservation of natural and cultural resources.

Although some strategies play an important role in sustainable ecotourism development, they are rated as lower attractive, such as: WO3 (2.34 points): Applying digital technology in managing and promoting ecotourism. WT1 (1.86 points): Perfecting the coordination, management and control mechanism for spontaneous development. Strategies in the low-medium group are mainly related to governance systems, technology platforms and management coordination capacity, which require time, budget and strong policy commitment to operate effectively.

The group of strategies with low total attractiveness includes: ST2, ST3, WO2, WT1 (1.03 - 1.89 points): Focus on training, conservation, impact control and reducing seasonality. Despite their low attractiveness scores, these strategies play a role in protecting the resource and cultural foundations – core conditions for sustainable ecotourism development. Integrating these solutions into the institutional framework and medium-to-long-term plans will contribute to stabilizing the ecosystem and enhancing the community's autonomy. Capacity-building programs should be implemented to provide training and enhance local skills in resource management and service development. Moreover, encouraging small-scale community entrepreneurship, such as homestays, traditional cuisine, and handicraft production, can strengthen local livelihoods while reinforcing cultural identity within ecotourism development. In addition, ecotourism development requires concrete safeguards linked to climate-risk scenarios. Adaptive measures addressing drought, flooding and soil erosion, or wildfire risks would help ensure that ecotourism is aligned with climate resilience and long-term conservation objectives.

#### **4. CONCLUSIONS AND RECOMMENDATIONS**

The research results show that the application of the SWOT model combined with QSPM helps to quantify the attractiveness of ecotourism development strategies in a systematic and objective manner. The study proposed and evaluated 9 local ecotourism development strategies. The results showed a clear differentiation in the attractiveness between the strategies. Among the 9 proposed strategies, the strategies in the SO and WO groups such as developing specific ecotourism products - integrating indigenous cultural values(ii) Infrastructure and services (SO1), upgrading infrastructure and services (WO1), attracting public-private investment (SO2), planning destination brands (ST1) were assessed to be highly attractive, suitable for priority implementation in the first phase. In contrast, strategies related to training, risk management

and seasonal product diversification, although having lower scores, are of long-term significance and require a more appropriate roadmap and resources for effective implementation in the medium and long term.

On that basis, the study recommends that prioritize the development of specific ecotourism products associated with local resources and culture, while implementing communication strategies - brand positioning and upgrading infrastructure, integrating long-term strategies on management, conservation, community training in the next phase to ensure sustainable ecotourism development. However, the strategies in this study are mainly consulted by experts and managers, forest managers and forest owners so the analysis can be expanded to different target groups (tourists, businesses,...) to determine the level of consensus and feasibility of each strategic orientation.

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# VIETNAMESE MEKONG DELTA SURFACE WATER POLLUTION: A COMPREHENSIVE REVIEW

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## ABSTRACT

The Vietnamese Mekong Delta (VMD), a socio-economically vital region, depends heavily on its surface water. This review synthesizes 2013 - 2025 knowledge on VMD's polluted surface water parameters, their progression, sources, current management, and proposes future research and mitigation. Findings reveal persistent pollution by organic matter (BOD, COD), nutrients (NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub>-N, PO<sub>4</sub><sup>3-</sup>-P), heavy metals/metalloids (As, Mn, Fe, Al, Cr, Hg), and microbial contaminants (*E. coli*, total coliforms), often exceeding national standards. Major drivers include untreated domestic/industrial wastewater, extensive agricultural/aquaculture non-point pollution, geogenic factors like arsenic mobilization and acid sulfate soil leaching, and saline intrusion worsened by climate change and upstream developments. Regulatory frameworks (e.g., Law on Environmental Protection 2020, Resolution 120/NQ-CP) and monitoring by national agencies and the Mekong River Commission underpin current management. However, the coexistence of multiple pollutant types underscores systemic weaknesses in wastewater control and diffuse source management, further complicated by the delta's complex hydrology. There is an urgent need for integrated, adaptive, and well-enforced management strategies that address both local pressures and transboundary influences while incorporating climate change adaptation. Future research should particularly focus on knowledge gaps, data management systems, emerging contaminants, and the real-world effectiveness of mitigation policies to ensure the sustainable protection of the VMD's surface waters.

**Keywords:** *Vietnamese Mekong Delta, surface water quality, polluted parameters, pollution sources, water management.*

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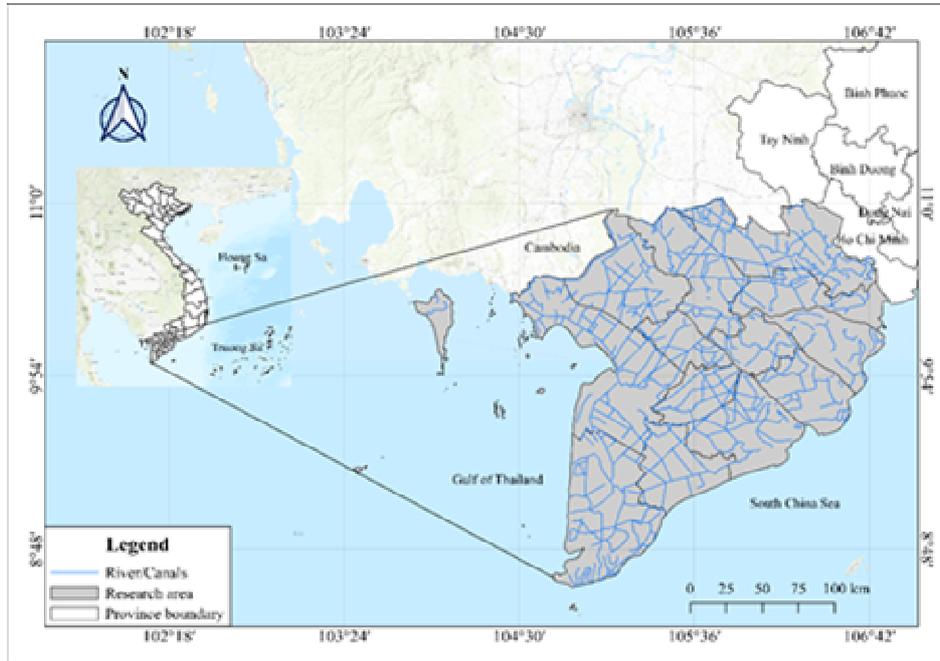
## 1. INTRODUCTION

The Vietnamese Mekong Delta (VMD), encompassing 13 provinces in Vietnam's southernmost region, is a socio-ecological system of profound national and international importance. Often referred to as Vietnam's "rice bowl," it accounts for approximately 50% of the country's rice production and 70% of its aquaculture output, significantly bolstering the national Gross Domestic product (around 27 - 33%) and food

security [1, 2]. These surface waters are indispensable for domestic water supply, agricultural irrigation, aquaculture, industrial processes, and navigation. The region's intricate hydrological network comprising the Tien and Hau Rivers, dense canal systems, a monsoonal climate, and significant tidal influence, inherently renders the VMD susceptible to the widespread dispersion and accumulation of pollutants [3]. Pollutants introduced can readily spread, tidal

actions can trap contaminants within canal networks, and seasonal flow variations alter dilution capacities, demanding sophisticated and integrated management approaches compared to

simpler riverine systems. Figure 1 shows the administrative map of the VMD, illustrating the river-canal network and major pollution hotspots.



**Figure 1. Administrative map and main river network of VMD**

Surface water pollution has escalated into a critical global environmental challenge, posing substantial threats to human health, aquatic ecosystem integrity, and sustainable economic development. Deltas in Southeast Asia, including the VMD, are particularly vulnerable due to high population densities, rapid urbanization and industrialization, intensified agriculture and aquaculture, and the escalating impacts of climate change such as sea-level rise and altered hydrological patterns [4]. The degradation of water quality in these vital regions can lead to far-reaching consequences, including undermining food production, increasing waterborne diseases, and diminishing overall environmental resilience. Over the past few decades, the surface water quality in the VMD has become a subject of increasing concern, with consistent reports of contamination by a diverse array of pollutants [5, 6]. This pollution poses significant threats to public health, with studies linking poor water quality to health risks such as diarrheal diseases, agricultural productivity, the vital aquaculture sector, and the delta's aquatic ecosystems [7 - 9]. The narrative is shifting from

isolated incidents to recognition of a systemic issue driven by cumulative impacts from multiple, often inadequately regulated sources across provinces [6].

The primary purpose of this review is to synthesize and critically evaluate studies published between 2013 and 2025 on surface water pollution in the VMD. The specific aims are multifaceted: firstly, to document the key polluted parameters, their concentration ranges, and compares them with Vietnamese water quality standards. Secondly, this review seeks to analyze the spatial distribution and temporal progression of these pollutants within the VMD during the specified review period. Thirdly, it aims to identify and discuss the major natural and anthropogenic sources contributing to surface water pollution in the region. Furthermore, the manuscript intends to review current water quality management practices, encompassing policies, regulatory frameworks, monitoring efforts, and the roles of various stakeholders. Finally, this review identify critical knowledge gaps and methodological limitations, proposing directions for future research and management, such as improved data

management, enhanced monitoring, and adaptive cross-sectoral solutions. The focus remains on surface waters, considering groundwater only where it directly affects surface water quality in the VMD.

## 2. MATERIALS AND METHODS

### 2.1. Data collection strategy

The literature search for this review was conducted systematically to identify relevant publications from the period 2013 to 2025. Major scientific databases, including Scopus, Web of Science and Google Scholar, were primary sources. Additionally, reports from reputable international and national organizations such as the Mekong River Commission (MRC), the World Bank, and Vietnamese governmental agencies (where peer-reviewed or widely cited in academic literature and relevant to surface water pollution parameters) were considered.

Inclusion criteria for selected articles were: (i) publication between 2013 and 2025 (or latest available data within this timeframe); (ii) focus on surface water quality within the geographical boundaries of the Vietnamese Mekong Delta; (iii) reporting of quantitative data on specific polluted parameters; (iv) peer-reviewed journal articles, or highly credible reports from recognized institutions. Studies focusing solely on groundwater without direct linkage to surface water quality, air pollution, soil contamination (unless directly impacting surface water), or regions outside the VMD were excluded.

### 2.2. Data analysis and synthesis

Data pertaining to polluted parameters, their measured concentrations, observed spatiotemporal trends, identified pollution sources, and current management approaches were meticulously extracted from the selected articles and reports. This information was then synthesized through a qualitative comparison of findings across different studies, geographical areas within the VMD and water body types.

The assessment of pollution levels was benchmarked against the Vietnamese National Technical Regulations on Surface Water Quality,

primarily QCVN 08-MT:2015/BTNMT and has been superseded by QCVN 08:2023/BTNMT, considering the specified limits for different water use classifications [10, 11]. Insights from source apportionment techniques reported in the literature, such as Principal Component Analysis (PCA), were reviewed to understand the relative contributions of different pollution origins [12].

The synthesis process involved a critical evaluation of the consistency and variability of findings across the reviewed studies. This considered potential differences in analytical methodologies, sampling frequencies and durations, specific geographical locations of sampling sites, and the range of parameters investigated. Such critical assessment is necessary to develop a nuanced understanding of the VMD's overall surface water quality status, acknowledging any uncertainties or areas where data might be conflicting or sparse. For example, comparing results from an early study like Wilbers *et al.* (2014) [13] with more recent provincial assessments such as Giao and Ly (2023) [14] requires careful consideration of potential temporal changes in pollution loads and drivers, as well as differences in the specific scope and focus of each study.

## 3. RESULTS AND DISCUSSION

### 3.1. Progression of surface water quality focusing on polluted parameters

Studies conducted between 2013 and 2025 consistently highlight a range of pollutants impairing surface water quality in the Vietnamese Mekong Delta (VMD). These primarily include organic matter, indicated by elevated Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Organic Carbon (TOC), largely originating from untreated domestic and industrial wastewater, as well as agricultural and aquaculture runoff [5, 14]. Reported COD values range from 10.19 to 75.0 mg/L, with some sites exceeding 100 mg/L [15], while TOC concentrations vary between 3.19 and 16.55 mg/L, and in some cases significantly exceed allowable thresholds [14]. Excessive nutrient concentrations, particularly nitrogenous

compounds like ammonium ( $\text{NH}_4^+\text{-N}$ ) and nitrite ( $\text{NO}_2^-\text{-N}$ ), and phosphorus compounds such as orthophosphate ( $\text{PO}_4^{3-}\text{-P}$ ), are also common, primarily stemming from agricultural fertilizers, aquaculture effluents, and domestic sewage [7, 16]. Wilbers *et al.* (2014) [13] recorded ammonium concentrations substantially above typical guideline values, Giao and Ly (2023) [14] reported ammonium ( $0.04 \pm 0.01 - 4.05 \pm 0.70$  mg/L) and nitrite concentrations ( $0.01 \pm 0.00 - 0.46 \pm 0.66$  mg/L) in Soc Trang periodically exceeding QCVN 08-MT:2015/BTNMT limits. Several heavy metals and metalloids, including arsenic (As), manganese (Mn), iron (Fe), aluminum (Al), chromium (Cr), and mercury (Hg), have been detected at concerning levels, often exceeding Vietnamese standards and WHO guidelines [10, 17]. Arsenic, in particular, presents a complex issue due to both natural geogenic sources and potential anthropogenic inputs [13, 18]. Widespread microbial contamination, evidenced by high counts of *Escherichia coli* and total coliforms, indicates significant fecal pollution from untreated sewage and animal waste [19, 20]. Physical and general parameters such as pH, turbidity, Total Suspended Solids (TSS), and persistently low Dissolved Oxygen (DO) also frequently deviate from acceptable ranges, with low DO being a major concern for aquatic life [5]. Electrical Conductivity (EC) is highly variable but can exceed 500 mS/m, and salinity ranges from 0 to 22‰, particularly elevated in coastal and estuarine areas during the dry season due to saline intrusion [15]. The surface waters of the VMD often exhibit a complex pollution issues with the simultaneous presence of these varied contaminants underscoring systemic deficiencies in wastewater management and control of diffuse pollution.

Pollution levels in the VMD demonstrate considerable spatial and temporal variability. Spatially, canals and inland waterways often show higher concentrations of organic matter and nutrients than the main rivers such as the Tien and Hau, while urbanized and aquaculture-

intensive areas (e.g., Can Tho City) consistently emerge as hotspots with elevated organic pollutants, nutrients, and microbial contaminants [21]. Coastal zones tend to contain higher levels of metals and salts due to saline intrusion and geological influences. Temporally, seasonal dynamics are evident: during the wet season, turbidity, TSS, BOD, COD, nutrients ( $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ ,  $\text{PO}_4^{3-}\text{-P}$ ), and fecal coliforms increase due to surface runoff from agriculture and domestic sources; whereas the dry season shows elevated salinity and EC from seawater intrusion, and reduced dilution concentrating pollutants. pH values also tend to decrease in the rainy season due to leaching from acid sulfate soils [22]. Tidal influences further modulate pollutant transport and accumulation [15]. The intricate hydrogeology means arsenic mobilized in groundwater can affect surface waters, complicating source distinction [23].

Assessing definitive long-term trends (2013-2025) is challenging. Mekong River Commission (MRC) reports generally describe mainstream water quality as “good” or “excellent,” but these assessments mainly reflect conditions at mainstream stations and focus on health-related parameters. In contrast, provincial monitoring in local canals and tributaries frequently records exceedances of DO, BOD, and occasional EC due to salinity, highlighting more severe pollution at the local scale [8, 24, 25]. This explains the apparent discrepancy between MRC classifications and provincial data. Decreasing trends in suspended alluvium and water levels in the Tien and Hau rivers have also been reported [12]. Despite inter-annual variability in parameters like salinity, core pollution indicators such as organic matter, nutrients, and microbial contaminants show persistent elevation, suggesting dominant local anthropogenic pressures. Arsenic contamination, linked to geogenic sources, historical herbicide use, and potentially ongoing inputs, remains a persistent challenge, with surface water concentrations sometimes exceeding WHO guidelines [17].

**Table 1. Key surface water pollutants and exceedances in the Vietnamese Mekong Delta**

| Polluted Parameter                                | Typical Reported Range (Unit)                                  | Vietnamese Standard (QCVN 08:2023/BTNMT Value & Class/Use) | General Exceedance Status  | Reference |
|---|--|--|--|-----------|
| <b>Organic Matter</b>                             |  |  |  |           |
| BOD   | 2.14 - 75.0 mg/L   | ≤4 to ≤10 mg/L (Class A-C)                                 | Frequently exceeds Class A/B, especially in polluted canals, farming, livestock, landfill, and domestic activities areas | [15]      |
| COD   | 10.19 - 75.0 mg/L (some studies report higher, e.g. >100 mg/L) | ≤10 to ≤20 mg/L (Class A-C)                                | Frequently exceeds Class A/B/C in many areas   | [14]      |
| TOC   | 3.19 - 16.55 mg/L  | ≤4 to ≤8 mg/L (Class A-C)                                  | Often exceeds Class A/B, particularly in Soc Trang study   | [13]      |
| <b>Nutrients</b>                                  |  |  |  |           |
| Ammonium (NH <sub>4</sub> <sup>+</sup> -N)        | 0.04 - 14.7 mg/L   | 0.3 mg/L; ≤0.6 to ≤2.0 mg/L (Total Nitrogen for Class A-C) | Frequently exceeds 0.3 mg/L limit and contributes to high TN, especially in urban and aquaculture zones                  | [12]      |
| Nitrite (NO <sub>2</sub> <sup>-</sup> -N)         | 0.01 - 1.10 mg/L   | 0.05 mg/L  | Often exceeds limit, particularly in aquaculture, industrial areas and seasonal changes                                  | [21]      |
| Orthophosphate (PO <sub>4</sub> <sup>3-</sup> -P) | 0.01 - >1 mg/L (specific high values vary)                     | ≤0.1 to ≤0.5 mg/L (TP Class A-C)                           | Often exceeds Class A/B limits, contributing to high TP, linked to aquaculture and agriculture                           | [6]       |
| <b>Heavy Metals/Metalloids</b>                    |  |  |  |           |
| Arsenic (As)                                      | <1 - 44.1 µg/L   | 0.01 mg/L (10 µg/L)  | Frequently exceeds in certain area and studies   | [12]      |
| Manganese (Mn)                                    | <10 - 1,659.7 µg/L   | 0.1 mg/L (100 µg/L)  | Often exceeds limit, particularly in canals and areas with soil leaching   | [12]      |
| Iron (Fe)   | 0.1 - 17.0 mg/L  | 0.5 mg/L   | Commonly exceeds limit, especially in rainy season or areas with acid sulfate soils                                      | [12]      |
| Aluminum (Al)                                     | Up to 14.5 mg/L  | -  | High levels reported, linked to soil leaching  | [12]      |
| <b>Microbial</b>                                  |  |  |  |           |
| <i>E. coli</i>                                    | Up to 87,000 CFU/100 mL  | 20 MPN or CFU/100 mL (Table 1)                             | Consistently and significantly exceeds limit across VMD  | [12]      |
| Total Coliforms                                   | Up to  | ≤1,000 to ≤7,500   | Consistently and significantly   | [12, 13]  |

|                          |  |   |  |          |
|--------------------------|--|---|--|----------|
|                          | 2,500,000<br>CFU/100 mL;<br>17,924 - 23,919<br>MPN/100 mL              | CFU or<br>MPN/100 mL<br>(Class A-C)                             | exceeds limits across VMD  |          |
| Physical/General         |  |   |  |          |
| Dissolved<br>Oxygen (DO) | 0.48 - < 6.0<br>mg/L (often <<br>4 or < 5)                             | ≥6.0 to ≥ 4.0<br>mg/L (Class A-<br>C)                           | Frequently below levels for<br>healthy aquatic life (Class<br>A/B), widespread issue | [6]      |
| TSS                      | Up to 225<br>mg/L (some<br>studies >100<br>mg/L)                       | ≤ 25 to ≤ 100<br>mg/L (Class A-<br>B, rivers)                   | Often exceeds Class A/B,<br>especially in dry/rainy season<br>depending on location  | [14]     |
| EC/Salinity              | Highly variable,<br>> 500 mS/m in<br>saline areas;<br>Salinity 0 - 22‰ | No direct limit,<br>but Cl <sup>-</sup> ≤ 250<br>mg/L (Table 1) | High in coastal areas, dry<br>season, impacting freshwater<br>use                    | [14, 13] |

**3.2. Sources of surface water pollution**

The contamination of surface waters in the Vietnamese Mekong Delta (VMD) originates from a complex interplay of anthropogenic activities and natural processes. With regard to organic pollution, the main sources include untreated or insufficiently treated domestic wastewater, particularly from densely populated urban areas and canal-side settlements, which discharge large loads of organic matter (BOD, COD) into surface waters [13, 14]. Additionally, industrial effluents from food processing, textile, and manufacturing sectors contribute organic pollutants, suspended solids, and chemicals due to inadequate wastewater treatment [26, 27].

In terms of nutrient pollution, agricultural runoff and aquaculture discharges are the two dominant sources. The widespread use of nitrogen- and phosphorus-based fertilizers increases NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N and PO<sub>4</sub><sup>3-</sup>-P concentrations in surface waters [16], while uneaten feed and waste from aquaculture ponds further increase nutrient loads [15]. Pesticides applied to crops can also be transported into water bodies via runoff and leaching [6]. Regarding heavy metal pollution, both geogenic and anthropogenic sources are significant. Metals such as As, Fe, Mn, and Hg frequently exceed Vietnamese and WHO limits, originating from

acid sulfate soils, mining operations, and industrial or urban effluents [27]. Microbial pollution remains widespread, primarily arising from domestic wastewater and livestock effluents that are either untreated or treated below standard levels. Elevated *E. coli* and total coliform counts indicate widespread fecal contamination [16]. For instance, Ho Chi Minh City, a major urban agglomeration influencing the northern VMD, reportedly treats only a small fraction of its total wastewater, implying substantial untreated discharges [28].

Natural and geogenic sources also play a role in VMD's surface water pollution. The geology of the delta contributes to background levels of certain pollutants, with leaching of metals like aluminum, iron, and manganese occurring from soils, particularly the extensive acid sulfate soils (ASS) when disturbed or drained [13]. Arsenic is naturally present in Mekong Delta sediments, and its release into groundwater, potentially exacerbated by over-extraction, can lead to its transfer into surface waters through groundwater-surface water interactions [18]. Saline intrusion, a major water quality concern particularly during the dry season, is worsened by sea-level rise, reduced upstream freshwater flows, and land subsidence, leading to elevated salinity and rendering water unsuitable for many uses [1].

Multivariate statistical techniques like Principal Component Analysis (PCA) have been employed to identify dominant factors influencing surface water quality, consistently pointing to urbanization and domestic wastewater (associated with coliforms,  $\text{NH}_4^+\text{-N}$ ,  $\text{PO}_4^{3-}\text{-P}$ ), agricultural and aquaculture activities (linked to nutrients and organic loading), industrial activities, and natural processes like metal leaching and tidal influences as key source categories [12, 13, 16]. Wilbers *et al.* (2014) [13] reported that these four factors explaining 85% of the total variance in their dataset. However, a significant challenge persists in quantitatively apportioning pollutant loads from specific sub-sectors, hindering the development of highly targeted mitigation strategies [16]. The critical distinction between point sources (e.g., industrial outfalls, municipal discharges) and non-

point sources (e.g., agricultural runoff) is vital for management, as the diffuse nature of non-point pollution presents a far more complex challenge requiring different approaches like best management practices and landscape-level planning.

In addition to these conventional sources, emerging contaminants (ECs) such as pharmaceuticals, personal care products, and microplastics have recently been recognized as supplementary pollution sources in the Mekong Delta. These substances primarily derived from domestic wastewater, aquaculture inputs, and agricultural chemicals are persistent, bioaccumulative, and poorly removed by conventional treatment systems, posing an emerging risk that warrants systematic monitoring and management [29].

**Table 2. Major identified sources of surface water pollution and associated key pollutants in the VMD**

| Pollution Source Category  | Key Associated Pollutants   | Reference |
|----------------------------|---|-----------|
| Domestic Wastewater        | BOD, COD, TOC, $\text{NH}_4^+\text{-N}$ , $\text{NO}_2^-\text{-N}$ , $\text{PO}_4^{3-}\text{-P}$ , Total Coliforms, E. coli | [13, 14]  |
| Industrial Effluent        | BOD, COD, TSS, Heavy Metals, Chemicals, Nutrients   | [27]      |
| Agricultural Runoff        | Nutrients (N, P from fertilizers), Pesticides, Herbicides, TSS, BOD   | [6, 16]   |
| Aquaculture Effluents      | Organic Matter (BOD, COD), Nutrients (N, P from feed/waste), TSS, potential antibiotics/chemicals                           | [15]      |
| Acid Sulfate Soil Leaching | Low pH, Acidity, Al, Fe, Mn, other metals   | [13]      |
| Saline Intrusion           | High EC, $\text{Cl}^-$ , $\text{Na}^+$ , and TDS  | [14, 15]  |
| Natural Geogenic           | Arsenic and other metals  | [17]      |
| Tourism Activities         | Organic matter, Nutrients, Surfactants, Solid waste   | [22]      |

**3.3. Current management practices of stakeholders**

The management of surface water quality in the VMD involves a multi-layered approach, with the Law on Environmental Protection (LEP) serving as the fundamental legal framework, outlining clear requirements and responsibilities

for water resource protection. A major policy shift is marked by Resolution 120/NQ-CP on “Sustainable and Climate-Resilient Development of the Mekong Delta” which promotes a development model that respects natural laws and adapts to changing conditions, emphasizing integrated solutions and enhanced international

cooperation [30]. National Technical Regulations on Surface Water Quality (QCVN), such as QCVN 08-MT:2015/BTNMT was widely used during the review period, and has been superseded by QCVN 08:2023/BTNMT, provide specific standards, classifying water quality for various uses [10, 11]. Effective management necessitates a multi-scalar governance approach, but ensuring coherence and coordination across these diverse scales, particularly in aligning national objectives with provincial capacities and addressing transboundary issues, remains a significant hurdle.

Implemented pollution control measures include significant investments in hydraulic infrastructure like dykes and sluice gates, primarily for flood and salinity control, which also influence pollutant transport [31]. Within VMD provinces, various wastewater treatment plant technologies are employed, but overall capacity is insufficient, often due to high costs and limited local operational capacity. Various projects also promote climate-smart agricultural techniques and improved aquaculture practices aimed at reducing pollution [32].

**Table 3. Overview of key management policies, stakeholders, and initiatives for surface water quality in the VMD**

| Management Aspect                   | Key Policies/Initiatives/Stakeholders   | Objectives/Outcomes/Challenges  |
|-------------------------------------|---|---|
| Legal/Policy Framework              | Law on Environmental Protection (esp. 2020); Resolution 120/NQ-CP (2017); National Technical Regulations (QCVN 08:2023/BTNMT) | Strengthen legal basis for pollution control; Promote sustainable, climate-resilient development; Set water quality standards. Challenges in enforcement, translating policy into on-the-ground action, coordination.     |
| Monitoring & Assessment             | MONRE & Provincial DONREs; Mekong River Commission (MRC) Water Quality Monitoring Network (WQMN)                              | Provide data for planning, assessment, and compliance checking; Early warning of pollution events. Resource constraints, harmonization of methods, data sharing gaps, coverage limitations.                               |
| Infrastructure Development          | Government investments; International development partner support (e.g., World Bank) for dykes, sluices, WWTPs.               | Control salinity and floods; Treat domestic and industrial wastewater. High costs, low WWTP coverage and operational effectiveness, selection of appropriate technology.  |
| Agricultural/Aquacultural Practices | MARD; Promotion of Best Management Practices (BMPs), VietGAP, climate-smart agriculture/aquaculture; Farmer cooperatives.     | Reduce agrochemical runoff; Improve water use efficiency; Minimize waste from aquaculture. Low adoption rates of BMPs without adequate support/incentives, economic viability concerns for farmers.                       |
| International Cooperation           | MRC; Bilateral partnerships (e.g., Netherlands, Australia); Multilateral development banks.                                   | Address transboundary water issues; Provide funding and technical expertise for water projects. Ensuring alignment with national priorities, sustainability of interventions, effective coordination of donor activities. |

**3.4. Management Practice Solutions**

Addressing the VMD’s complex surface water pollution requires a multi-pronged strategy. Strengthening policy and enforcement is fundamental, including robust implementation of

the Law on Environmental Protection 2020 and Resolution 120/NQ-CP [30, 33], supported by adequate resources and stricter penalties. Promoting sustainable agricultural and aquacultural Best Management Practices (BMPs)

is vital for non-point source control. This involves wider adoption of agricultural BMPs (e.g., Integrated Pest Management, optimized fertilizer use) and sustainable aquaculture practices (e.g., improved feed management, Recirculation Aquaculture Systems). Success hinges on providing farmers with technical support, training, credit, and incentives.

Technological innovations and Nature-Based Solutions (NBS) offer significant opportunities to improve wastewater and runoff management. A clear differentiation between centralized and decentralized wastewater treatment systems is crucial to ensure appropriate technological application for diverse socioeconomic contexts. Centralized treatment facilities are suitable for urban centers and industrial zones, while decentralized and low-cost systems such as constructed wetlands or compact technologies (e.g., membrane bioreactors, MBR) are more applicable to rural and peri-urban areas. Integrating NBS (wetland restoration, mangrove rehabilitation, vegetative buffer strips) can enhance pollutant removal, support biodiversity, and increase climate resilience. Strengthening real-time monitoring networks and data-sharing platforms, particularly for emerging contaminants, is essential to support adaptive management and evidence-based policy decisions.

Implementation of BMPs in agriculture and aquaculture should focus on optimizing fertilizer and feed use, improving effluent management, and minimizing chemical inputs through ecological farming approaches. Successful adoption requires government support mechanisms (technical assistance, training programs, and financial incentives) to enable farmers and producers to scale up sustainable practices. Beyond local measures, addressing transboundary pressures requires enhanced cooperation through the Mekong River Commission (MRC) and bilateral frameworks to jointly manage upstream impacts on sediment, salinity, and pollutant transport. Ultimately, an integrated governance approach combining technological innovation, ecological restoration,

and institutional collaboration is essential for long-term water quality improvement and sustainable resource management in the VMD.

### **3.5. Knowledge Gaps and Future Research Directions**

Despite extensive research, significant knowledge gaps still hinder effective surface water management in the Vietnamese Mekong Delta (VMD). While multivariate analyses like PCA have clarified major pollution sources, they are limited in quantifying overlapping contributions. Future studies should adopt advanced analytical tools such as stable isotope tracing, mass balance modeling, and integrated simulation frameworks to achieve more precise source apportionment and predict combined hydrological, climatic, and anthropogenic impacts. These models would provide strategic insights for adaptive planning and policy-making. Enhancing stakeholder participation, data-sharing mechanisms, and transboundary cooperation through the Mekong River Commission (MRC) remains vital, as local actions alone cannot mitigate basin-wide pressures.

At present, numerous strategies and management interventions have been proposed to improve surface water quality in the VMD, encompassing both engineered and nature-based approaches. However, comprehensive and independent evaluations of their real-world effectiveness remain limited. Most existing studies have primarily focused on identifying pollution sources and proposing mitigation options, while quantitative assessments of the performance, scalability, and long-term sustainability of these measures are still lacking. Moreover, despite the growing body of research on surface water quality in the VMD, no study to date has systematically addressed data management and integration for surface water monitoring, which is essential for supporting long-term assessment and decision-making. Therefore, future research should prioritize systematic evaluations and comparative analyses of implemented solutions, providing robust scientific evidence to guide policy development, investment decisions, and sustainable water resource management in the region.

#### 4. CONCLUSION

The Vietnamese Mekong Delta's surface waters face persistent and complex pollution from a confluence of domestic, industrial, agricultural, and natural sources, frequently exceeding national standards and threatening regional sustainability. This review has synthesized the critical parameters, identified key pollution drivers, and examined current management frameworks, revealing systemic challenges despite ongoing efforts. Addressing this pressing issue necessitates a concerted, multi-pronged approach. Key strategic directions include strengthening policy enforcement and integrated water resource management, alongside significant upgrades to wastewater treatment infrastructure, embracing both centralized and decentralized solutions. Crucially, the widespread adoption of sustainable Best Management Practices in agriculture and aquaculture, supported by robust mechanisms, is vital for mitigating non-point source pollution. Fostering technological innovation, deploying Nature-Based Solutions, enhancing comprehensive monitoring, and ensuring active community participation will further bolster these efforts. Ultimately, the long-term health of the VMD's waters also hinges on effective transboundary cooperation to manage upstream influences. A sustained, collaborative commitment from all stakeholders, integrating scientific understanding with adaptive policy and practice, is paramount to safeguarding these vital water resources for future generations.

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# CRITICAL ASSESSMENT OF IRRIGATION INFRASTRUCTURE AND ITS USE IN MANAGING WATER RESOURCES DURING PERIODS OF DROUGHT AND SEAWATER INTRUSION IN THE VIETNAMESE MEKONG DELTA

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## ABSTRACT

Despite significant investment in the construction of an irrigation system (IS) in the Vietnamese Mekong Delta (VMD), a lack of operational integration across the delta limits its effectiveness in terms of managing water supply and water quality for agriculture, aquaculture and domestic needs, especially in relation to changes in water flow in the Mekong River due to upstream dams and other development, and in relation to salt intrusion from the sea. This study evaluates the operational effectiveness of irrigation infrastructure, with a focus on the types of sluice gate structures currently used in the VMD. Opinions from 77 experts were collected and analyzed to identify the strengths and weaknesses of different operational modes and types of sluice gate structures currently used in the VMD. The research findings can be used as a basis for proposing solutions to enhance the operational effectiveness of the IS in the VMD in the context of climate change and sea-level rise.

**Keywords:** *Vietnamese Mekong Delta, irrigation system, climate change, salt intrusion.*

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## 1. INTRODUCTION

The Vietnamese Mekong Delta (VMD) is a key agricultural production area of Vietnam. This region not only provides agricultural and aquaculture products for the country but also for export. In recent years, the region has been severely affected by saline intrusion, causing significant impacts on agriculture, aquaculture and people's lives [1]. While significant investments have been made in developing the irrigation system (IS) in the VMD over the years, these developments have been inconsistent. The Bay Nui region, primarily located in An Giang and Kien Giang (new An Giang) provinces, has received relatively limited investment in irrigation

infrastructure due to the higher associated costs. Additionally, the inter-regional IS in the VMD remains underdeveloped, hindering effective water regulation [2]. Several irrigation projects, including water storage and regulation systems on rivers and small to medium-sized reservoirs, are currently being considered [3]. The impacts of reservoir systems, socioeconomic development, and proposed water diversion projects in upstream Mekong countries might affect the annual flow regime in the VMD [4]; and recently, the Funan-Techo canal project in Cambodia has attracted significant attention. Notable impacts in recent years include a decreasing frequency of small floods, increasingly severe reductions in dry

season flows, growing water scarcity, and deeper saltwater intrusion, which has adversely affected water supply for various sectors.

An Artificial Neural Network (ANN) model was applied to forecast salinity levels in Long My district, Hau Giang province (now Xa Phien commune, Can tho city) [5]. Preliminary results indicated that saltwater intrusion has impacted water management for agriculture, with salinity levels and frequency varying spatially. Calibration and validation results showed that simulated salinity levels were reasonably consistent with measured values, particularly for short-term forecasts. The research findings provide valuable information for policymakers to develop appropriate adaptation measures for different regions within the study area. Other studies on sluice gate operation under drought and salinity conditions in Tien Giang province (now Dong Thap)[6] and the operation of irrigation systems in salinity-affected areas, as exemplified by Vung Liem district, Vinh Long province [7], have evaluated the effectiveness of irrigation system operation and proposed solutions for water scarcity conditions.

The primary objective of this study is to assess the current state of irrigation system operation,

with a particular focus on sluice-gate management for agricultural and domestic purposes. This research involves a comprehensive review of existing literature and a survey of experts in the VMD to: (1) evaluate the current practices of sluice-gate operation; (2) identify the associated advantages and challenges.

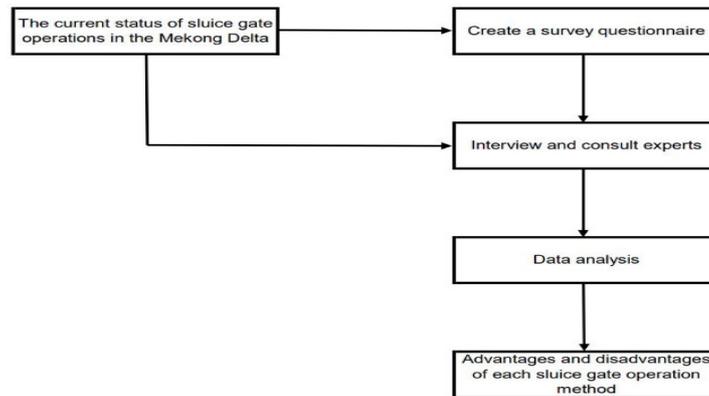
**2. METHODOLOGY**

**2.1. Land use in study area**

The most common use of land in the study area is for rice production, probably followed by aquaculture, mainly catfish culture in freshwater areas upstream, and shrimp culture in coastal areas. Fruit orchards and vegetable production are also common. In addition, the VMD has a population of over 20 million people, and thus there is a significant demand for clean fresh water for domestic purposes. However, the relatively high population density and the lack of urban and industrial waste water treatment also results in serious water pollution in many parts of the VMD.

**2.2. Research diagram**

The implementation steps are shown in figure 1.



**Figure 1. Research diagram**

**2.3. Expert interview**

This study conducted an online survey of experts in the irrigation sector or related fields in the VMD, categorized into three regions: (1) Upper region (An Giang, Dong Thap); (2) Central region (Can Tho, Vinh Long, Hau Giang); (3) Coastal region (remaining provinces except Long An now Tay Ninh).

The online survey consisted of 40 multiple-choice questions and 5 open questions on 1) current practices of sluice-gate operation, 2) the advantages and disadvantages of these practices. The survey questionnaire was sent online to irrigation experts working in provincial and district departments of agriculture and rural development, environment and natural resources. The results of

the survey were tabulated and analysed with Microsoft Excel.

Of the 77 experts who responded, 31% had less than 10 years of experience, 55% had 10 - 20 years of experience, and the remaining 14% had over 20 years of experience (Figure 2).

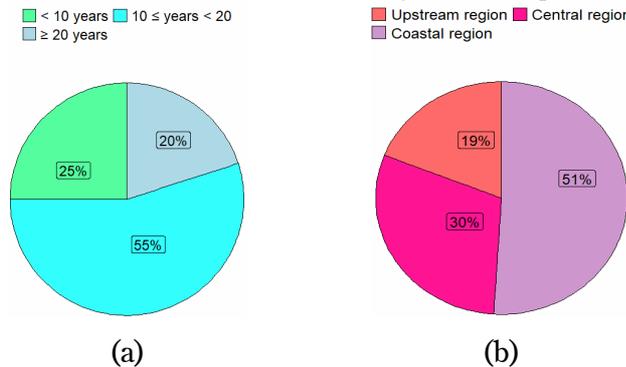


Figure 2. Number of years of experience of experts (a), regional distribution (b)

3. RESULTS AND DISCUSSION

3.1. Current status of irrigation system operation

According to a comprehensive report by relevant agencies on water storage in the VMD, the Ministry of Natural Resources and Environment MONRE (2019) indicated that water storage in the delta has been implemented for a long time, primarily for agricultural purposes. However, these projects often serve specific objectives of individual sectors or regions and lack a comprehensive, inter-sectoral perspective with a long-term vision. Moreover, these projects and studies have not paid sufficient attention to the geological and geomorphological characteristics of the VMD, nor have they demonstrated the connection between surface water and groundwater in the region. Therefore, there is an urgent need to conduct comprehensive research on water storage solutions in the VMD based on a long-term vision and aligned with the spirit of Resolution No. 120/NQ-CP of November 17, 2017, on "Sustainable development of the Mekong Delta to adapt to climate change", with a focus on inter-regional and inter-sectoral cooperation. The research results will serve as valuable data for policymakers in making decisions regarding water storage in the VMD in the coming period [8, 9].

Broadly, sluice-gates currently used in the VMD are of three kinds, automated only, manual only, or automatic with a manual override ('combined'). Current automatic gates that open and close according to tidal fluctuations (dependent

on the water level difference between upstream and downstream sides of the sluice) have many limitations which will be covered in greater detail later in this paper. Therefore, the sluice operation system needs to be upgraded towards proactive gate operation and an automated operation connection system - supervisory control and data acquisition (SCADA) (exemplified by the Cai Lon - Cai Be sluice system, some sluices in Kien Giang (now An Giang province) and An Giang provinces). Automation in sluice operation is a modern technical solution, helping to save costs, and enable timely and synchronized intervention in the region's irrigation system.

Practical experience has shown that constructed infrastructure requires appropriate management, operation, maintenance to ensure its effective and sustainable utilization. This has been evidenced by data on the current state of infrastructure, the complex hydro-meteorological conditions of the VMD further underscore the need for suitable irrigation system management processes. Automation and modernization are inevitable trends, the project "Modernization of the irrigation system to serve agricultural transformation and sustainable development in the VMD's ecological sub-regions" has been approved under Decision No. 633/QD-TTg, dated May 12, 2020 [10].

The survey results summarized in figure 3, present an overview of different sluice-gate operation types in three representative sub-regions of the VMD: Kien Giang (now An Giang province), Vinh Long and Dong Thap provinces.

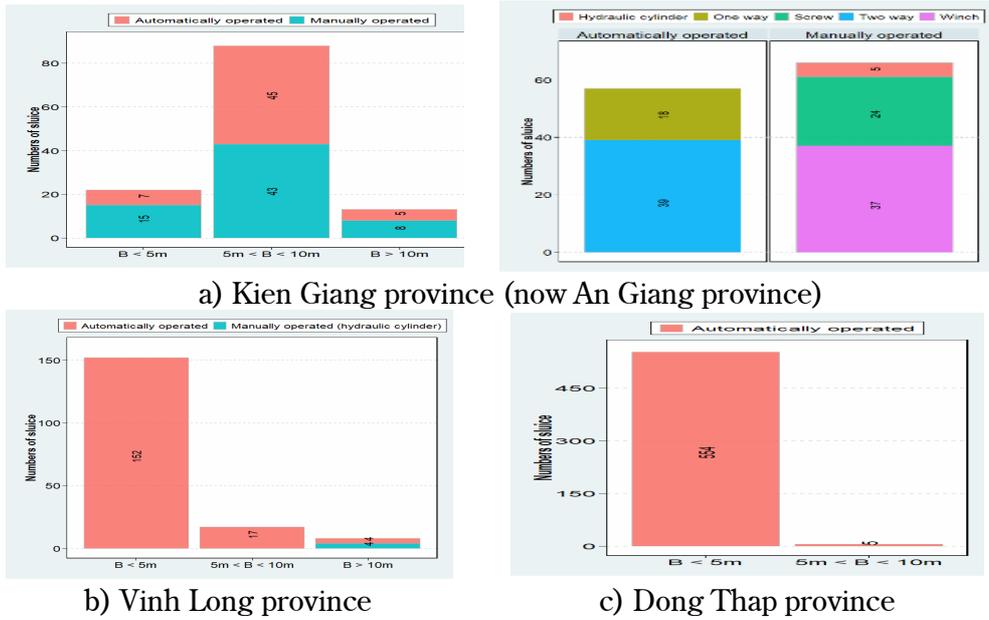


Figure 3. Number and percentage of sluice-gate operation modes by regions

Automatically operated sluice-gates are predominant in Vinh Long and Dong Thap provinces (upper and central regions). Most sluices in these regions have a narrow width ( $B < 5$  m) and are equipped with flat steel gates that automatically open and close according to the tide. In Kien Giang province (now An Giang province)

(coastal region), sluices tend to be of medium width ( $5 \text{ m} < B < 10 \text{ m}$ ) and the proportion of automatically operated sluices is lower compared to manually operated ones.

Figure 4 and 5 present some images of sluice-gate operations in Kien Giang (now An Giang province), Tien Giang, Vinh Long provinces.



Binh Giang 1 sluice-gate - Hon Dat district (Kien Giang (now An Giang province))



Muoi Nen sluice-gate - Cai Lay district (Tien Giang province)

Figure 4. Automatically operated sluice-gates



Vam Ba Lich tidal gate and ferry lock, Chau Thanh district, Kien Giang province (now An Giang province)



Vung Liem sluice-gate (Vinh Long province)  
a) Operation of the gate with hydraulic cylinder



Ong Tho sluice-gate - Go Quao district  
(Kien Giang province now An Giang province)  
b) Screw operation



Nha Bang sluice-gate - Giong Rieng district  
(Kien Giang province now An Giang province)  
c) Operated by winch



Cay Cong sluice - Cai Lay district (Tien Giang province now Dong Thap province)  
d) Operation of the gate with hydraulic cylinder

Figure 5. Manually operated sluices

3.2. Expert survey results

Figure 6 presents the statistics of sluice-gate operation methods based on the survey results. Of the 77 responses, 40 were from experts working in the coastal region, 23 from experts in the central region, 14 from experts in the upper region.

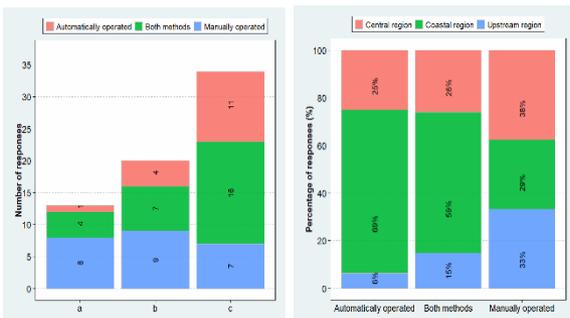


Figure 6. Number and percentage of gate operation modes by regions

3.3. Classification of sluice-gate operation modes

3.3.1. Operation modes

Figure 7 shows the relative proportions of manual, automatic and combined (automatic with a manual override) sluice-gates in upstream, central, coastal regions.

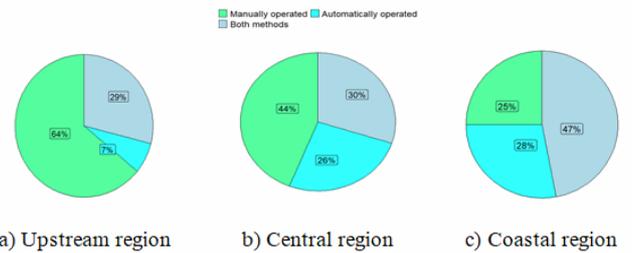


Figure 7. Sluice gate operation methods by regions

Manually operated sluice-gates account for a majority of 44% in the central region and 64% in the upstream region. Automatic operation of sluice gates only accounts for 7; 26; 28% in the upstream, central, coastal regions, respectively. Combined manual and automatic operation accounts for a majority of 47% in the coastal region (Figure 7).

3.3.2. Classification by operation type and gate width

The operation of sluice-gates using winches in the upstream region is mostly for small gates ( $B < 5$  m), accounting for 65%, with the proportion decreasing to 43% in the central region and 23% in the coastal region. The operation of sluice-gates

using winches with medium and large widths ( $B > 10$  m) is very low, being only 4% in both the coastal and central regions. In the upstream region, the operation of opening and closing sluice-gates using winches with a gate width of less than 10 m accounts for 100% (Figure 8).

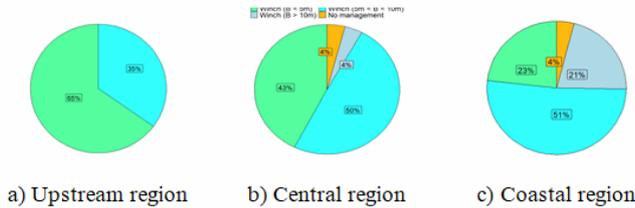


Figure 8. Forced sluice-gate operation by winch

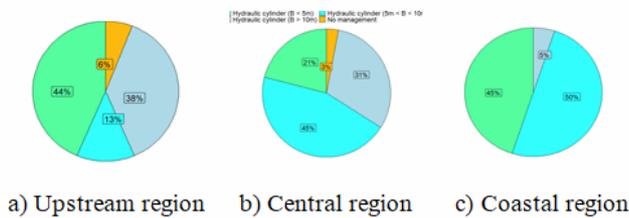


Figure 9. Forced sluice-gate operation using a hydraulic cylinder

Most sluice-gates in coastal areas have a width greater than 10 m (50%) or between 5 and 10 m (45%). In the upstream region, 45% of sluice gates

with a width of less than 5 m are hydraulically operated (Figure 9), and in the central region 45% of sluice gates with a width of 5 m to 10 m are hydraulically operated (Figure 9).

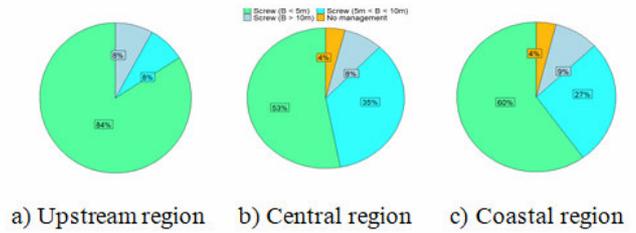


Figure 10. Manual sluice gate operation using a screw mechanism

Over 50% of sluice gates with widths of less than 5 m are operated by a screw mechanism, especially in upstream regions where the proportion reaches up to 84% (Figure 10). For sluice gates of 5 - 10 m in width, the proportion using winches is 35% in the central region, 27% in the coastal region, only 7% in the upstream region (Figure 10). Winch operation for sluice-gates larger than 10 m accounts for approximately 7% in all regions (Figure 10).

3.3.3. Advantages and disadvantages of sluice-gate operation modes

Table 1. Advantages and disadvantages of sluice-gate operation modes

| Areas           |               | Automatically operated sluices  | Manually operated sluices   |
|-----------------|---------------|---|---|
| Upstream region | Advantages    | The operation of tide-operated sluice gates is based on a straightforward water balance mechanism and is budget-friendly.                   | The sluice gate is designed to open and close under all conditions with manual intervention.  |
|                 | Disadvantages | The sluice gate cannot be fully opened due to the water level difference and can only operate according to the tide.                        | + The gate opening and closing time is slow when operated by a winch.<br>+ Screw operation is only applicable to small sluices.<br>+ Hydraulic cylinder operation has a high initial capital cost, requires precise installation, and can only be used where there is a power supply. |
| Central region  | Advantages    | The sluice gate automatically opens and closes utilizing the energy of the water level difference, simplifying operation, requiring minimal | + The sluice gate can be operated flexibly, opening and closing as desired, allowing for proactive water regulation and timely salinity control.<br>+ Winch-operated sluice gates have  |

|                |               |   |  |
|----------------|---------------|---|--|
|                |               | <p>manual intervention, and reducing labor costs.</p>   | <p>low initial investment costs, are easy to operate, require no electricity, and have minimal maintenance requirements.</p> <p>+ Hydraulic operation significantly reduces the time required to open and close the sluice gate.</p>   |
|                | Disadvantages | <p>Operation is slow due to water dependency, lacking proactive inflow and outflow regulation; unable to drain water in case of emergencies, requiring a water level difference, only suitable for culverts with <math>B \leq 10</math> m, small gate openings, long operation time, difficult to maintain and repair; for large-span culverts, the bottom slab must be extended.</p> | <p>+ High initial and operational costs, along with labor and energy requirements, make this option less cost-effective.</p> <p>+ Hydraulic cylinder operation requires high skill levels, complex operation procedures, is prone to failures. Winch operation is time-consuming.</p>  |
| Coastal region | Advantages    | <p>+ Tide-operated systems require minimal labor, are suitable for remote areas without electricity or generators.</p> <p>+ Automated operation reduces manpower and operation time.</p>  | <p>+ Proactive water intake and discharge, suitable for conditions of climate change, saltwater intrusion, large apertures ensuring waterway traffic.</p> <p>+ Hydraulic cylinder operation is easy and convenient.</p> <p>+ Screw operation is cost-effective and easy to install, while winch operation is efficient but slower than hydraulic cylinders.</p> <p>+ Installation of modern equipment allows for remote salinity monitoring, enabling the development of detailed plans for each area.</p> |
|                | Disadvantages | <p>+ Automatic operation is susceptible to tidal fluctuations and strong currents. High operating pressures can damage mechanical components, and sedimentation within the culvert is complex.</p> <p>+ Sedimentation occurs rapidly at the gate location prior to operation, requiring</p>   | <p>+ Hydraulic gates must be larger than 10 meters but are prone to operational failures and require highly skilled operators. The complex operation and infrequent use can lead to damage to mechanical components and generators.</p> <p>+ Manual operation using winches is safe for gates smaller than 10 meters but can lead to sedimentation in the</p>  |

|  |  |  |  |
|--|--|--|--|
|  |  | <p>frequent dredging and cleaning, and making operations highly dependent on water levels.</p> <p>+ The passive nature of the system restricts water traffic and hinders the ability to open the gate for supplementary water supply during extended droughts and salinity periods.</p> <p>+ Causing restrictions on water traffic and difficulties in opening gates to take in additional water during prolonged drought and salinity.</p> <p>+ At the sluice gate location, mud quickly settles. Before operating, it is necessary to dredge and clean the mud, and it depends a lot on the water level.</p> | <p>culvert and make maintenance difficult.</p> <p>+ Screw gates are typically smaller than 8 meters and can easily misalign. Operation requires electricity and continuous maintenance, making it labor-intensive and requiring electrical systems and complex maintenance procedures. In case of a power outage, manual operation is not feasible.</p> <p>+ Hydraulic valves must be larger than 10m, and are prone to problems during operation. Complex operations require highly specialized personnel, and mechanical parts are prone to damage.</p> <p>+ Maintenance and repair work is difficult.</p> <p>+ Operation requires connected electrical system and regular maintenance. In case of power failure (power outage), forced operation cannot be performed.</p> |
|--|--|--|--|

**4. CONCLUDING REMARKS**

The results based on 77 responses from irrigation experts working in the VMD have identified the advantages and disadvantages of sluice gate operation methods, particularly in the three studied regions.

**Advantages:** Manual operation allows for gate opening and closing under all water level conditions, enabling proactive water intake and discharge, making it suitable for conditions of climate change, saltwater intrusion, and large apertures ensuring waterway traffic. Automated operation according to the tide is simple, requires less manpower, and is suitable for remote areas without electricity.

**Disadvantages:** Hydraulic cylinder-based manual operation is costly and requires skilled and trained operators, as well as frequent maintenance of operating equipment. Manual operation using winches and screws takes a long time, limiting the width of the sluice-gate. Proactive operation with slow gate opening and closing times depends on water levels, making it difficult to proactively

regulate water inflow and outflow; it is impossible to drain water in case of emergencies, limiting waterway traffic. Additionally, sedimentation occurs rapidly at the sluice-gate, requiring frequent dredging before operation.

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# CO-EXPOSURE OF MICROPLASTICS AND POLYCHLORINATED BIPHENYLS IN AQUATIC ECOSYSTEMS: A SYSTEMATIC REVIEW OF IMPACT MECHANISMS AND RESEARCH DIRECTIONS

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## ABSTRACT

Microplastics (MPs) and polychlorinated biphenyls (PCBs) pollution represents a major global environmental challenge that requires comprehensive understanding of their combined effects. Recent research shows that MPs can transport PCBs into aquatic organisms, creating complex synergistic interactions that may exceed the toxicity of individual pollutants. This systematic review examines the combined effects of MP-PCB exposure on aquatic organisms based on data from Web of Science, Scopus, PubMed, Science Direct databases. Our analysis revealed that polyethylene and polystyrene were the most studied MP types, with PCB concentrations on MPs ranging from nanograms to micrograms per gram. However, most studies used MP concentrations 2 - 7 orders of magnitude higher than environmental levels, limiting their environmental relevance. Experimental designs have evolved from basic exposure methods to complex models simulating food chain interactions, with exposure durations ranging from acute to chronic timeframes. Bioaccumulation studies demonstrate that MPs act as vectors for PCBs transport, extending PCB residence time by 10 - 20 fold compared to individual exposure. The biomarker assessment systems have expanded to include 33 multidimensional indicators, with antioxidant enzymes and detoxification enzymes showing the highest sensitivity to combined exposure effects. Histological assessments provide additional evidence of severe tissue damage in liver and intestine. Current research faces important limitations including unrealistic exposure scenarios and methodological inconsistencies that reduce environmental relevance. Future research should adopt standardized procedures using environmentally realistic concentrations and integrated multidisciplinary approaches to enhance understanding of impact mechanisms and inform effective management strategies.

**Keywords:** *Microplastics, PCBs, aquatic organisms, combined exposure, biomarkers, bioaccumulation.*

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## 1. INTRODUCTION

Microplastics (MPs) pollution has emerged as one of the most critical environmental challenges of the 21st century [1], requiring urgent scientific attention and management action [2]. MPs are plastic particles smaller than 5 mm in diameter [3, 4] that have been detected in aquatic ecosystems

worldwide, from deep ocean to freshwater lakes and rivers [5]. This widespread distribution demonstrates the global scale of plastic pollution and its potential impacts on aquatic life [6]. Concurrently, polychlorinated biphenyls (PCBs) present another significant environmental concern as persistent industrial chemicals that remain in the environment

despite production bans in many countries [7]. These compounds are characterized by their strong hydrophobicity, environmental persistence, bioaccumulation potential within aquatic food chains [8]. The combination of these two pollutant types creates a complex environmental scenario that demands comprehensive investigation [9].

Recent studies have highlighted a concerning interaction between MPs and PCBs that significantly amplifies environmental risks [10]. MPs can adsorb and transport PCBs within aquatic environments due to their high adsorption affinity for hydrophobic compounds [11]. Marine MPs can accumulate PCBs at concentrations millions of times higher than in surrounding water [12]. When aquatic organisms ingest PCB-contaminated MPs, they face simultaneous exposure to both pollutants, leading to complex biological effects that may exceed those of individual exposures [1]. This co-exposure scenario represents a paradigm shift from traditional single-pollutant risk assessment approaches.

Understanding MPs-PCBs interactions is crucial for several interconnected reasons that affect both environmental science and policy development. First, MPs function as transport vectors for PCBs into organisms, resulting in higher uptake rates and bioavailability compared to PCB-only exposure scenarios [13]. Second, both pollutants cause harmful effects individually, but their combination may produce synergistic, additive, or antagonistic effects that require thorough investigation to distinguish clearly [14]. From a risk management perspective, considering both MPs and associated pollutants simultaneously allows for more accurate assessment of environmental hazards, particularly in ecosystems with multiple co-existing contaminants [15]. This multi-pollutant approach overcomes the limitations of single-substance risk assessments, providing more reliable data to guide ecosystem protection and environmental policy development.

Despite significant research interest over the past decade, understanding of MP-PCB combined effects remains fragmented and lacks systematic

organization [16]. Research on MPs-PCBs co-exposure has not been comprehensively synthesized, creating knowledge gaps that underscore the urgent need for systematic review. Synthesizing existing evidence will clarify interaction mechanisms between MPs and PCBs while establishing a strong scientific foundation for future research directions and effective environmental management strategies [15]. This comprehensive approach is essential for transitioning from experimental research to practical applications in environmental protection and policy development.

Therefore, our study aims to systematically review research on the effects of PCB-MPs co-exposure in aquatic organisms to summarize current knowledge with the intention of better focus future research in this area and fill knowledge gaps. Specifically, this review will synthesize and analyze MPs characteristics and PCB concentrations used in co-exposure studies, evaluate experimental design approaches, assess the vector role of MPs through bioaccumulation data analysis, compile multidimensional biomarker systems for biological impact assessment, identify methodological limitations while proposing future research directions. We expect this systematic review to provide a solid scientific foundation for pollution risk assessment in aquatic environments and assist policymakers in developing effective strategies for PCBs-MPs pollution control.

## **2. METHODS**

This study employed systematic review methodology to synthesize and analyze scientific evidence on combined MPs - PCBs exposure effects on aquatic organisms. Searches were conducted across four major scientific databases including Web of Science, Scopus, PubMed, ScienceDirect from 2013 to 2025 using their native advanced search functions.

The search strategy was built on three main keyword groups connected by Boolean operators (AND/OR). Microplastics-related terms included "microplastic\*", "micro-plastic\*", "nanoplastic\*", "plastic particle\*", "plastic debris", "MP" and "MPs" with wildcard symbols (\*) to capture word

variations. Polychlorinated biphenyls-focused terms comprised "PCB", "polychlorinated biphenyl\*" and "persistent organic pollutant\*". Aquatic organism terms encompassed "aquatic\*", "fish\*", "mussel\*", "shrimp\*", "algae\*", "marine\*" and "freshwater\*" with variants.

To ensure comprehensive coverage, both abbreviated forms (MP, MPs, PCB) and full terms were included, along with hyphenated and non-hyphenated variants (microplastic vs microplastic). Search results were exported and managed using EndNote reference management software with duplicate removal performed automatically and manually verified. In addition to database searches, reference lists from relevant review articles and primary studies were screened to supplement potentially missed literature.

Selection criteria included specific requirements for study inclusion to ensure comprehensive coverage of relevant research. Studies must evaluate combined exposure effects on aquatic organisms, be published in peer-reviewed journals, contain quantitative or qualitative data, be written in English. Studies were excluded if they examined only MPs or PCBs

individually or lacked experimental data. This systematic approach ensured comprehensive coverage of available literature while maintaining quality standards for included studies.

3. RESULTS AND DISCUSSION

3.1. Material selection and contamination profiles

Analysis of MPs-PCBs co-exposure studies reveals significant diversity in research material selection and application, reflecting both methodological development and challenges in simulating realistic environmental conditions. Polyethylene (PE) and polystyrene (PS) dominate studies for clear scientific reasons. PE is preferred as the most common plastic type in marine environments with suitable hydrophobic properties for adsorbing persistent organic pollutants (POPs), while PS, especially in nano form (PS-NPs), enables investigation of cellular penetration mechanisms and molecular-level impacts. However, excessive focus on these two polymers may overlook impacts from other types like PET, PVC and PP that are significantly present in actual environments.

Table 1. Material characteristics and contamination levels in MP-PCB co-exposure studies

| MP type               | Size range   | PCB compounds           | Concentration range | Spiking method | Studies |
|-----------------------|--------------|-------------------------|---------------------|----------------|---------|
| Polystyrene (PS)      |              |                         |                     |                |         |
| PS-NPs                | 80 nm        | PCB-44                  | EC50 = 9 mg/L       | Soaking        | [17]    |
| PS-NPs                | 80 nm        | PCB-77                  | 0.01 mg/L           | Simultaneous   | [8]     |
| PS-NPs                | 80 nm        | PCB-126                 | 0.63 - 2.52 µg/L    | Simultaneous   | [18]    |
| PS                    | 27 - 32 µm   | <sup>14</sup> C-PCB-153 | 4.44 ng + 74 kBq    | Soaking        | [19]    |
| PS                    | 6 - 600 µm   | 10 PCB congeners        | 1.35 µg/congener    | Soaking        | [20]    |
| Polyethylene (PE)     |              |                         |                     |                |         |
| PE                    | 63 - 75 µm   | PCB-153                 | 8389 ± 2930 ng/g    | Simultaneous   | [21]    |
| PE                    | 1 - 4 µm     | 7 PCBs + PBDEs          | 0.62 - 9.66 µg/g    | Food chain     | [22]    |
| PE                    | 150 - 300 µm | PCB-126                 | 4.1 µg/g            | Soaking        | [23]    |
| PE                    | 100 - 500 µm | Aroclor 1254 + 14 PCBs  | 0 - 25% in food     | Soaking        | [24]    |
| PE                    | 212 - 250 µm | 4 PCBs                  | 9.7 - 26.4 µg/g     | Simultaneous   | [25]    |
| Low-Density PE (LDPE) |              |                         |                     |                |         |
| LD-PE                 | 125 - 250 µm | 7 PCBs + PBDEs          | 10 - 140 ng/g lipid | Food mixing    | [26]    |
| LD-PE                 | 125 - 250 µm | PCB-138 + others        | 29.0 ng/g           | Soaking        | [1]     |
| LDPE                  | Various      | 10 PCBs + PAHs + PBDEs  | 1.1 - 17 ng/g       | Natural        | [27]    |

|                   |                         |                                    |                    |                   |      |
|-------------------|-------------------------|------------------------------------|--------------------|-------------------|------|
| LD-PE             | 125 - 250 $\mu\text{m}$ | 7 PCBs + PFAS                      | 7 - 150 ng/g lipid | Soaking           | [13] |
| LDPE & PVC        | Not specified           | $^{14}\text{C}$ -PCB-153 + 13 PCBs | Variable           | Long-term soaking | [28] |
| Other Polymers    |                         |                                    |                    |                   |      |
| PP                | 0.3 - 5.0 mm            | 11 PCBs                            | 500 - 5,000 ng/g   | Soaking           | [3]  |
| PVC               | < 0.3 mm                | Environmental mix                  | Variable           | Natural           | [29] |
| Environmental MPs |                         |                                    |                    |                   |      |
| Mixed types       | Variable                | Environmental PCBs                 | 0.2 - 3.3 ng/g     | Natural           | [30] |

PCB concentrations show extremely large variation across studies, ranging from low-chlorinated congeners to highly toxic dioxin-like compounds used at vastly different concentrations. Low-chlorinated congeners like PCB-44 are used by Zheng *et al.* (2024) [17] at EC50-96h = 9 mg/L, while highly toxic dioxin-like PCBs such as PCB77 and PCB126 are studied at significantly lower concentrations from 0.01 mg/L to 2.52  $\mu\text{g/L}$  [8, 22]. This variation reflects not only toxicity differences among congeners but also methodological inconsistencies, especially when compared to natural environmental PCB concentrations typically at ng/L to  $\mu\text{g/L}$  levels. Marine ecosystem studies tend to use multi-congener PCB mixtures, as demonstrated by van der Hal *et al.* (2020) study with 11 congeners including OSPAR indicator substances at 500 - 5,000 ng/g concentrations, reflecting higher realism since organisms in nature typically encounter multiple substances simultaneously [3].

PCB concentrations adsorbed on MPs create a significant "gap" between realistic and experimental conditions that limits the environmental relevance of current research. While studies using environmental MPs like Herrera *et al.* reported PCB concentrations of only 0.9 - 1.4 ng/g [30], laboratory studies typically use concentrations thousands of times higher, such as 8,389 ng/g in Trinh *et al.* (2024) study [21], making result extrapolation difficult and potentially leading to toxicity mechanism observations that don't occur at realistic environmental concentrations.

The overwhelming bias toward PE and PS represents a fundamental limitation that may significantly underestimate real - world environmental impacts. While these polymers

offer experimental advantages including well - characterized properties and commercial availability, this narrow focus creates critical knowledge gaps regarding PVC, PET, PP which dominate plastic pollution in specific environments such as urban waterways and coastal areas. The 2 - 7 orders of magnitude difference between experimental and environmental concentrations represents not merely a methodological challenge but a systematic failure to prioritize environmental relevance over experimental convenience. This disconnect raises serious questions about the applicability of current findings to actual environmental risk assessment and policy development. The field urgently requires a paradigm shift toward environmental realism, even if this necessitates longer exposure periods and more sensitive analytical methods.

High adsorption efficiency under laboratory conditions, typically above 95% due to lack of competition from dissolved organic matter, absence of biofilm coverage, optimal temperature and pH conditions, differs significantly from natural conditions and biases assessment of MPs vector roles in the environment. PCB spiking methods to MPs show diversity but also reveal limitations regarding environmental realism. Laboratory-based attachment methods dominate experimental studies. The solvent-based technique represents the most prevalent approach in controlled settings. POPs are dissolved in organic solvents such as dimethyl sulfoxide (DMSO), acetone, ethanol, hexane, or methanol. The solution is then mixed with MPss to facilitate surface sorption and interior penetration [13, 17, 19, 20]. The long-term soaking method requires exposure periods exceeding 24 hours. MPs are immersed in POPs-containing solutions until

sorption equilibrium is achieved [28]. These laboratory methods provide excellent experimental control but may not reflect natural contamination processes. Environmental simulation approaches attempt to bridge laboratory control with real-world conditions. The simultaneous exposure method introduces both PCBs and MPs into experimental environments concurrently. This allows natural sorption processes to occur over time [18, 21, 25, 26]. The natural sorption method employs MPss collected directly from marine or riverine systems. These particles have accumulated pollutants through extended environmental exposure [27, 29 - 31]. While environmentally relevant, these methods sacrifice experimental control for realism. Biological transfer methods focus on organism-mediated POPs delivery. The food chain transfer approach utilizes intermediate organisms such as rotifers. These organisms facilitate POPs transfer from MPss to fish [22, 26]. The direct dietary incorporation method involves mixing POPs-contaminated MPss into test fish feed. This approach is simpler but may not reflect natural ingestion patterns. Critical limitations compromise the environmental relevance of current methodologies. Organic solvent soaking achieves high efficiency (96 - 97%) as reported by Norland *et al.* (2021) [19]. However, it produces unnaturally uniform PCB distribution and elevated concentrations. Solvents may alter polymer structure and leave residues that confound results [19, 28]. Naturally exposed MPss offer greater environmental relevance but lack compositional control. Environmental particles are typically weathered and co-sorbed with multiple substances that introduce analytical interference [30, 31]. The simultaneous introduction method employed by Chen *et al.* (2025) [8] attempts to balance realism with control. However, it encounters difficulties in accurately quantifying POPs sorbed onto MPss.

Particle characteristics further complicate experimental interpretation. MPs size ranges from 70 nm to 5 mm, creating significant challenges for result comparison. Nanoparticles exhibit fundamentally different impact mechanisms compared to microparticles. Lu *et al.* (2016) [32]

reported findings contrary to theoretical predictions. PS MPss of 5  $\mu\text{m}$  induced greater oxidative stress than 70 nm nanoparticles. This may result from intestinal blockage mechanisms and reduced nutrient absorption. Most studies utilize "pristine" MPss in perfect spherical form. These differ substantially from environmental MPss characterized by irregular morphology, rough surfaces, biofilm adhesion [29]. This disparity limits the applicability of laboratory findings to environmental scenarios.

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### 3.2. Experimental design and exposure protocols

Co-exposure study designs reflect the maturation of this research field from initial simple methods to complex models simulating realistic conditions, demonstrating growing sophistication in experimental approaches. Food exposure has emerged as the preferred method due to precise dose control capability and high realism in simulating the primary exposure route in nature. Notably, Bøgevik *et al.* (2023) [22] developed the most complex system with a three-level model from MPs to rotifers to cod larvae, enabling tracking of bioaccumulation across multiple trophic levels and simulating realistic transfer through food chains, despite requiring strict control of environmental variables. Water exposure, though less common, remains primarily used for small organisms or mechanistic studies, with Lu *et al.* (2016) [32] using broad concentration gradients from 20 to 2,000  $\mu\text{g/L}$  to

determine dose-response relationships, while from 0.2 to 2 mg/L to match realistic environmental conditions. Chen *et al.* (2025) [8] applied lower concentrations

**Table 2. Exposure protocols and experimental conditions in co-exposure studies**

| Test species                                | Exposure route      | MP conc.                                     | PCB conc.                          | Duration                         | Ref  |
|---|---------------------|--|------------------------------------|----------------------------------|------|
| Algae                                       |                     |  |                                    |                                  |      |
| <i>Chlorella vulgaris</i>                   | Water               | 0.5 - 50 mg/L (acute); 1 - 10 mg/L (chronic) | PCB-44: EC50 = 9 mg/L              | 96 hours; 21 days                | [17] |
| Fish - Early Life                           |                     |  |                                    |                                  |      |
| <i>Danio rerio</i> (embryo)                 | Water               | 0.1 - 10 µg/mL NPS                           | PCB-126: 0.63 - 2.52 µg/L          | 6 - 240 hours post-fertilization | [18] |
| <i>Gadus morhua</i> (larvae)                | Food chain          | 10 mg MP/125 mL rotifer                      | PCB mix + PBDE                     | 2 - 30 days post-hatch           | [22] |
| <i>Clupea harengus</i> (larvae)             | Tube feeding        | 1 - 200 particles/fish                       | <sup>14</sup> C-PCB-153: 74 kBq    | 24 hours                         | [19] |
| Fish - Juvenile/Adult                       |                     |  |                                    |                                  |      |
| <i>Danio rerio</i> (adult)                  | Water               | 20 - 2,000 µg/L                              | PCB-free                           | 7 days + 21 days                 | [32] |
| <i>Danio rerio</i> (food study)             | Food                | 2 - 4% in diet                               | Multi-contaminants: 7 - 150 ng/g   | 21 days                          | [33] |
| <i>Danio rerio</i> (depuration)             | Food                | 40% PE microspheres                          | PCBs on PE                         | 9 weeks                          | [25] |
| <i>Megalobrama amblycephala</i>             | Water               | 0.2 - 2 mg/L                                 | PCB-77: 0.01 mg/L                  | 7 days                           | [6]  |
| <i>Gadus morhua</i> (juvenile)              | Food                | 1% PE in food                                | PCB-126: 4.1 µg/g MP               | 34 days                          | [15] |
| <i>Dicentrarchus labrax</i> (environmental) | Food                | 10% in food                                  | Environmental PCBs: 0.9 - 1.4 ng/g | 60 days                          | [16] |
| <i>Dicentrarchus labrax</i> (lab)           | Food                | 2 - 4% in diet                               | PCBs: 10 - 140 ng/g lipid          | 80 days + 51 days                | [20] |
| <i>Carassius auratus</i>                    | Food pellets        | 0 - 25% w/w                                  | Aroclor 1254 + 14 PCBs             | 1 feeding + 5 days               | [24] |
| <i>Siganus rivulatus</i>                    | Food pellets        | 0.015 g MP/fish/day                          | PCB mix: 500-5,000 ng/g            | 12 days                          | [3]  |
| Invertebrates                               |                     |  |                                    |                                  |      |
| <i>Meretrix lyrata</i> (clam)               | Water + sediment    | 500 mg/L                                     | PCB-153: 8389 ng/g MP              | 28 days                          | [21] |
| <i>Macrobrachium rosenbergii</i>            | Water + sediment    | 500 mg/L                                     | PCB-153: 8389 ng/g MP              | 28 days                          | [21] |
| <i>Nephrops norvegicus</i>                  | Gelatin food        | 155 mg MP/pellet                             | 1.35 µg/congener                   | 21 days + 7 days                 | [20] |
| <i>In vitro</i> studies                     |                     |  |                                    |                                  |      |
| <i>Arenicola marina</i>                     | <i>In vitro</i> gut | 0.005 - 0.019 g/mL                           | <sup>14</sup> C-PCB-153            | 28 days                          | [28] |
| <i>Gadus morhua</i>                         | <i>In vitro</i> gut | 0.005 - 0.019 g/mL                           | <sup>14</sup> C-PCB-153            | 28 days                          | [28] |

Exposure duration shows increasing diversity across studies, reflecting growing recognition of chronic exposure importance in detecting subtle and long-term effects that acute studies might miss. From acute studies lasting 24 to 96 hours like Zheng *et al.* (2024) [17], many chronic study designs with exposure times exceeding three months have emerged, such as Granby *et al.* (2024) [13] with 98 days accumulation and 56 days depuration. Particularly, some studies like Rummel *et al.* (2016) [25] extended depuration monitoring up to 63 days to fully observe PCBs kinetics in organisms. This temporal evolution reflects scientists' increasing understanding that chronic, low-level exposure may be more environmentally relevant than acute, high-dose scenarios.

MPs concentrations in experimental studies reflect evolving understanding of environmental realism and experimental feasibility. Early research prioritized detectable effects over environmental relevance. Rummel *et al.* (2016) [25] employed extremely high concentrations of 40% w/w to guarantee observable impacts. This approach ensured measurable results but poorly represented natural conditions. Subsequent investigations have progressively adopted more realistic concentration ranges. Granby *et al.* (2024) [13] reduced concentrations to 2 - 4% in their experimental design. Peda *et al.* (2016) [29] further decreased levels to 0.1%, approaching concentrations potentially encountered in natural environments. This methodological evolution demonstrates growing recognition that environmental relevance is essential for meaningful toxicological assessment. PCB concentrations exhibit substantial variation depending on research objectives and target environmental conditions. Mechanistic studies often require specialized approaches for precise quantification. Mohamed and Koelmans. (2019) utilized radioactively labeled PCBs with 74 kBq activity to investigate sorption mechanisms [28]. This approach enables accurate tracking of PCB behavior during MPs interactions. Environmental simulation studies target concentrations representative of contaminated ecosystems. Trinh *et al.* (2024) [21] employed PCB-

153 concentrations of 4,195 ng/L in aqueous media. These levels reflect conditions found in heavily polluted marine and freshwater systems. The wide concentration range demonstrates the ongoing challenge of balancing experimental sensitivity with environmental relevance in POPs-MPs research.

Species diversity studied includes 15 different species ranging from unicellular algae (*Chlorella vulgaris*) to adult fish, showing efforts to expand understanding of impacts across various organism groups with different ecological roles. Among fish, zebrafish (*Danio rerio*) is commonly studied due to its status as a standard model organism with fully sequenced genome and high laboratory adaptability, alongside Atlantic cod (*Gadus morhua*) and European sea bass (*Dicentrarchus labrax*) representing commercially important species, several invertebrates including white clam (*Meretrix lyrata*), giant freshwater prawn (*Macrobrachium rosenbergii*), marine lugworm (*Arenicola marina*) selected for their high representativeness and ecological-economic value [1, 18, 21, 22].

Study designs have become significantly more detailed and comprehensive over time, evolving from simple studies with only negative controls to complex factorial designs like those by Hu *et al.* (2024) [18] and Rainieri *et al.* (2018) [1] that established complete groups including negative controls, solvent controls, individual MPs, individual PCBs, combined exposure, enabling precise analysis of interactive effects.

The evolution from simple exposure protocols to complex food-chain models represents genuine scientific progress, yet reveals concerning inconsistencies in approach selection. The predominance of zebrafish studies, while methodologically sound, may inadequately represent ecological diversity and species-specific vulnerabilities in natural ecosystems. More critically, the persistent gap between experimental convenience and environmental realism suggests that many current findings may have limited predictive value for ecosystem protection. Future research must prioritize ecologically relevant species combinations and exposure scenarios that

reflect actual contamination patterns rather than laboratory feasibility.

### 3.3. Vector transport mechanisms and bioaccumulation dynamics

Bioaccumulation of MPs and PCBs in aquatic organisms has emerged as a significant global concern, reflecting environmental pollution levels and potential risks to food webs with variations in both extent and mechanisms. The accumulation process is primarily influenced by three key factors: Species-specific characteristics, MPs particle size, types of POPs. The capability of aquatic organisms to accumulate MPs varies significantly, as demonstrated by Trinh *et al.* (2024) [21] who reported white clams accumulating MPs at notably high concentrations of  $323 \pm 311$  particles/individual after 28 days of exposure, indicative of their active filter-feeding behavior, while giant freshwater prawns under identical conditions accumulated substantially fewer particles ( $20 \pm 16$  particles/individual). Regarding PCBs, the accumulation process demonstrates notable organ selectivity, aligning with the lipophilic characteristics of these compounds, as shown by Bøgevik *et al.* (2023) [22] who observed pronounced selective accumulation of PCB-126 in Atlantic cod liver reaching concentrations of 301.667 pg/g, approximately 140-fold higher than those in muscle tissues 2.157 pg/g. Alarmingly, van der Hal *et al.* (2020) [3] found PCB-194 concentrations in rabbitfish muscle tissue reaching 200 ng/g within merely two weeks of exposure, four times exceeding the European Union (EU) -established safety threshold of 50 ng/g. MPs size significantly influences accumulation dynamics. Lu *et al.* (2016) [32] demonstrated that 5  $\mu\text{m}$  MPs in zebrafish primarily accumulated in the intestine, liver, gills, whereas larger 20  $\mu\text{m}$  MPs were limited to the gills and intestines due to penetration constraints. At the nanoscale, Zheng *et al.* (2024) [17] revealed that 80 nm polystyrene nanoparticles (PS-NPs) altered cell membrane properties by reducing surface hydrophobicity by 19.8%, enhancing synergistic toxic effects in conjunction with PCB-44.

Research into the vector role of MPs has undergone a fundamental paradigm shift following significant findings that conclusively refuted the previously held "cleaning hypothesis". Rummel *et al.* (2016) [25] demonstrated that MPs exacerbate PCB pollution by significantly reducing PCB elimination rates, with rate constants ( $k_2$ ) dropping markedly from 0.0076 - 0.0178  $\text{day}^{-1}$  to 0.0008 - 0.0081  $\text{day}^{-1}$ , consequently extending PCBs half-lives dramatically from 39 - 92 days to 86 - 866 days, suggesting that PCBs could persist in organisms 10 - 20 times longer than typically expected. Although MPs can facilitate PCBs transport into organisms, the actual efficiency of transfer remains limited, as demonstrated by Grigorakis *et al.* (2018) [24] who found that PCBs bioavailability from MPs was merely 13.36%, considerably lower than the 51.64% bioavailability from natural dietary sources. Mohamed and Koelmans (2019) [28] developed more complex models demonstrating that PCBs exchanges between MPs and biological fluids are reversible, two-phase processes, with bioavailability ranging from 14 - 42% in marine worms and 45 - 83% in fish.

The vector role of MPs remains controversial due to conflicting experimental outcomes that likely stem from varying exposure durations, MP sizes and types, physiological differences among test organisms. Studies by Chen *et al.* (2025) [8] and Hu *et al.* (2024) [18] demonstrated toxicity enhancement effects of MPs combined with PCBs, increasing toxicity 1.2 - 2.4-fold compared to PCB exposure alone, while conversely, Norland *et al.* (2021) [19] detected no measurable transfer of PCBs from MPs to fish tissues within a 24 hours exposure period. Investigations of realistic environmental MPs by Herrera *et al.* (2022) [30] and Rochman *et al.* (2013) [27] revealed significantly higher toxicity of marine MPs compared to pristine MPs, attributed to prolonged adsorption of additives and pollutant mixtures from seawater. Specifically, Rochman *et al.* (2013) [31] recorded increased PCB concentrations (1.2-fold) in medaka liver exposed to marine MPs relative to controls, accompanied by severe hepatic stress indicators such as glycogen

depletion and lipid degeneration, emphasizing cumulative impacts arising from both PCBs and additional co-occurring toxic substances.

The definitive refutation of the "cleaning hypothesis" represents one of the most significant scientific advances in microplastics research, with profound implications for environmental risk assessment. However, the conflicting evidence regarding vector efficiency reveals a critical weakness in current research design. The 10 - 20 fold increase in PCB persistence demonstrated by Rummel *et al.* (2016) [25] fundamentally alters our understanding of pollutant kinetics in aquatic organisms, yet this finding lacks consistent replication across species and exposure scenarios. Our assessment indicates that experimental

variability likely stems from inadequate standardization of critical parameters including particle weathering, biofilm formation, digestive environment simulation. The field requires immediate development of standardized protocols that account for these variables to resolve current contradictions and enable reliable risk predictions for environmental management.

**3.4. Biological response indicators and toxicity assessment**

Biomarker use in MPs-PCBs co-exposure assessment has evolved from single indicators to comprehensive multidimensional systems with 33 different biomarkers, reflecting the complexity of toxicity mechanisms and the need for multidimensional biological impact assessment.

**Table 3. Biological response indicators used in MP-PCB toxicity assessment**

| Category           | Indicators   | Target tissue     | Measurement method            | Key studies         |
|--------------------|--|-------------------|-------------------------------|---------------------|
| Oxidative stress   | SOD, CAT, MDA  | Liver, whole body | Enzyme/chemical analysis      | [8, 17, 32]         |
| Detoxification     | CYP1A/CYP1A1, EROD, GST                                    | Liver             | qPCR, enzyme analysis         | [1, 13, 18, 23, 31] |
| Inflammation       | IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , IL-10           | Liver, intestine  | qRT-PCR                       | [8, 26]             |
| Liver histology    | Glycogen depletion, lipid degeneration, cell necrosis, HSI | Liver             | H&E staining, morphometry     | [26, 31]            |
| Intestinal health  | Villi structure, mucus cells, inflammation                 | Intestine         | Histological analysis         | [8, 29]             |
| Skin histology     | Epidermal thickness, club cells, mucus cells               | Skin              | Special staining, morphometry | [22]                |
| Microbiota         | Firmicutes, Proteobacteria, Actinobacteriota               | Gut               | 16S rRNA sequencing           | [8]                 |
| Development        | Mortality, malformations, heart rate                       | Embryo/larvae     | Morphological observation     | [18]                |
| Growth, metabolism | Growth rate, condition factor, lipids, fatty acids         | Whole body, liver | Weighing, NMR, biochemistry   | [20, 21, 25]        |
| Cellular function  | Cell density, cycle, hydrophobicity                        | Cells/medium      | Flow cytometry, contact angle | [17]                |
| Photosynthesis     | psbA, rbcL, rbcS, chlorophyll a/b                          | Algal cells       | RT-qPCR, spectroscopy         | [17]                |

Antioxidant enzymes play central roles in toxicity assessment, with superoxide dismutase (SOD) and catalase (CAT) appearing consistently across multiple studies as MPs and nanoplastics cause oxidative stress through several mechanisms including direct cell membrane interactions, inflammatory response activation, acting as PCBs transport vectors [8, 17, 32]. Malondialdehyde (MDA) provides direct evidence of oxidative damage as the end product of lipid peroxidation processes, however measurement methods lack standardization with studies using different approaches from traditional spectrophotometry to modern enzyme immunoassays [8, 17].

Detoxification enzymes serve as highly sensitive indicators for MP-POPs exposure assessment. CYP1A and CYP1A1 represent the most frequently utilized enzymes in current research. These enzymes are induced through the aryl hydrocarbon receptor (AhR) pathway. This mechanism is highly conserved across vertebrate species, from fish to mammals [1, 18, 23, 31]. However, substantial interspecies variation in enzyme induction levels presents significant challenges for standardized assessment. Bogevik *et al.* (2023) [23] demonstrated significant CYP1A expression increases in Atlantic cod liver following MPs-POPs exposure. In contrast, Rainieri *et al.* (2018) [1] observed only slight changes in zebrafish under similar experimental conditions. This disparate response pattern highlights the critical need for standardizing assessment methods across different species to enable meaningful comparisons in MPs toxicology research.

Histological indicators reflect actual tissue damage with primary focus on liver (4 indicators), intestine (3 indicators), skin (2 indicators), providing direct evidence of structural and functional impairment. Rochman *et al.* (2013) [31] first demonstrated severe histological impacts of combined exposure including glycogen depletion (74% of fish affected), lipid degeneration (47% of fish), single cell necrosis (11% of fish), showing liver function decline that could lead to systemic metabolic disorders. In the intestine, which serves as the first barrier contacting MPs and PCBs through

food, Chen *et al.* (2025) [8] and Peda *et al.* (2016) [29] recorded severe damage to intestinal villi structure with atrophy and desquamation that not only affects nutrient absorption capacity but also increases intestinal permeability, creating conditions for deeper MPs and PCBs penetration. A major limitation in histological assessment is that most studies only use basic H&E staining, with only Bogevik *et al.* (2023) [22] applying specific staining methods like Alcian Blue and PAS to assess secretory cell function comprehensively.

Recent advances in biomarker development have expanded assessment capabilities beyond traditional enzyme measurements to comprehensive multi-system evaluations. Chen *et al.* (2025) [8] pioneered inflammatory response assessment through multi-gene cytokine panels including pro-inflammatory markers (IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ ) and anti-inflammatory indicators (IL-10). Results demonstrated significant increases in pro-inflammatory cytokines in combined exposure groups while IL-10 remained unchanged, suggesting immune response imbalance that could lead to tissue damage, immune dysfunction, increased cancer risk, reproductive impacts. Hu *et al.* (2024) [18] utilized zebrafish models to evaluate developmental toxicity, with mortality rates and developmental abnormalities serving as the most sensitive biomarkers. Their findings revealed that PCB-126 reduced the protective efficacy of chorion membranes against nanoplastics. Species-specific biomarkers have been developed by Zheng *et al.* (2024) [17] for *Chlorella vulgaris* using photosynthetic genes (psbA, rbcL, rbcS) and cellular indices, where surface hydrophobicity decreased by 19.8%, indicating altered cell membrane properties affecting metabolic processes. Chen *et al.* (2024) [8] also pioneered microbiome analysis using 16S rRNA sequencing to assess intestinal microbiota, finding decreased Actinobacteriota and Firmicutes (beneficial bacteria) accompanied by increased Proteobacteria (associated with dysbiosis). These findings demonstrate broad impacts on microbial ecosystems and establish new research directions linking MPs-PCBs pollution to intestinal health.

The expansion from single biomarkers to 33-indicator systems represents remarkable analytical sophistication, yet reveals a concerning lack of standardization that hampers inter-study comparison and field application. While the diversity of biomarkers demonstrates the multi-system impacts of MP-PCB co-exposure, the absence of core indicator sets prevents development of standardized toxicity assessment protocols essential for regulatory applications. Our analysis identifies oxidative stress and detoxification enzymes as the most promising universal indicators, showing consistent responses across species and exposure scenarios. However, the field's future advancement requires establishing hierarchical biomarker frameworks with essential core indicators supplemented by species-specific markers. The emerging integration of omics approaches with traditional biomarkers presents unprecedented opportunities for mechanistic understanding, but success depends on developing standardized protocols that balance analytical comprehensiveness with practical applicability for environmental monitoring and risk assessment.

#### 4. RESEARCH LIMITATIONS AND FUTURE DIRECTIONS

Our comprehensive analysis reveals three fundamental challenges that critically constrain the field's advancement toward environmental relevance and policy application. These limitations represent not merely technical constraints but systematic research choices that prioritize experimental convenience over environmental protection needs.

The most critical limitation identified is the persistent disconnect between laboratory exposure conditions and environmental reality. Current studies employ MP concentrations 2 - 7 orders of magnitude higher than natural environment levels, creating an unbridgeable gap between experimental findings and real-world risk assessment. While environmental MP concentrations typically range from 0.001 - 0.1 mg/L [34], experimental studies routinely use 10 - 500 mg/L to ensure observable effects within manageable timeframes. This disparity

fundamentally undermines the environmental predictive value of current research and raises serious questions about resource allocation in environmental science. The second critical challenge lies in systematic methodological inconsistencies that prevent meaningful data synthesis and evidence accumulation. Our analysis reveals multiple analytical approaches for MP detection and PCB quantification, each with different sensitivity and accuracy profiles, creating incomparable datasets across research groups [35]. The absence of standardized protocols represents not merely an oversight but a failure of scientific coordination that fragments knowledge advancement and impedes evidence-based policy development. Additionally, current research exhibits significant taxonomic bias toward model organisms, particularly zebrafish, while underrepresenting ecologically and economically important species groups. This bias limits our understanding of ecosystem-level impacts and vulnerability patterns across different aquatic communities, potentially leading to inadequate protection of sensitive species and habitats.

Based on this critical assessment, we propose that MP-PCB research must undergo fundamental transformation from exploratory science to applied environmental protection. This transformation requires strategic reorientation across four interconnected domains that collectively address current limitations while establishing foundations for policy-relevant research. Future research must prioritize environmental relevance over experimental convenience, accepting longer exposure periods and investing in more sensitive analytical methods to detect effects at realistic concentrations, as recommended by Koelmans *et al.* (2019) for improving MPs research quality [34]. This shift requires coordinated funding support for advanced analytical capabilities and extended experimental timelines. Simultaneously, the field urgently requires development and adoption of standardized operating procedures for MP-PCB research, potentially adapting existing Organisation for Economic Co-operation and Development (OECD) guidelines for aquatic

organism toxicity testing [36] or following the blueprint proposed by Kotta *et al.* (2022) for ideal MPs effect studies [37]. These standards should encompass particle preparation, exposure protocols, analytical methods, data reporting formats to enable meaningful meta-analyses and evidence synthesis, addressing the nonalignment issues identified by Koelmans *et al.* (2020) [38]. Research design must also shift from single-species laboratory studies to multi-species, multi-trophic approaches that capture ecological complexity and food web interactions, following successful models demonstrated by Bøgevik *et al.* (2023) [22] in their three-trophic level exposure system. This includes incorporating environmental stressors, seasonal variations, and habitat-specific conditions that influence pollutant fate and effects, as emphasized by Thompson *et al.* (2024) in their comprehensive assessment of twenty years of MPs research [39]. Furthermore, advancing from current disciplinary silos to integrated approaches combining analytical chemistry, toxicology, ecology, and environmental modeling will enable holistic understanding of MP-PCB interactions and their ecosystem consequences, consistent with recommendations by De Ruijter *et al.* (2020) for quality criteria in MPs effect studies [40]. These strategic transformations require coordinated efforts across funding agencies, research institutions, and regulatory bodies to align research priorities with environmental protection needs. Success depends on recognizing that current methodological limitations are not inevitable technical constraints but represent strategic choices that can be redirected toward environmental relevance and policy applicability.

## 5. CONCLUSION

This systematic review provides the first comprehensive critical analysis of MP-PCB co-exposure research, revealing both significant scientific advances and fundamental limitations that constrain environmental application. Our analysis demonstrates that MPs function as effective vectors for PCB transport into aquatic organisms, extending pollutant residence time 10 -

20 fold compared to individual exposure scenarios. The development of 33 - biomarker assessment systems represents remarkable analytical sophistication, with antioxidant and detoxification enzymes emerging as the most sensitive and reliable indicators of combined exposure effects. However, our critical assessment reveals that the field suffers from three fundamental flaws that undermine environmental relevance: Systematic use of unrealistic exposure concentrations, methodological fragmentation preventing data synthesis, and taxonomic bias limiting ecosystem applicability. These limitations represent strategic research choices rather than inevitable technical constraints, indicating that transformation toward environmental relevance is both necessary and achievable. This review definitively refutes the MP "cleaning" hypothesis while establishing the "pollution exacerbation" paradigm for PCB transport. We provide the first critical framework for transitioning from descriptive research to predictive environmental science, establishing strategic priorities for achieving policy-relevant outcomes. Our proposed transformation requires four strategic shifts: environmental realism mandate, methodological standardization initiative, ecosystem-relevant research design, and transdisciplinary integration. These transformations will enable transition from experimental observation to environmental prediction, supporting evidence-based policy development and effective ecosystem protection. The synthesis presented here represents a critical juncture for MP-PCB research, demanding strategic redirection toward environmental relevance and societal benefit. Success in implementing these transformations will determine whether this research field contributes meaningfully to environmental protection or remains confined to academic exploration without real-world impact.

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