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Differences in shrimp pond bottom soil properties and bacterial load between acute hepatopancreatic necrosis disease (AHPND)-infected ponds and AHPND-free ponds and their relation to AHPND

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Abstract

This study investigated the disparities in soil characteristics and pathogenic bacteria prevalence between shrimp ponds affected by acute hepatopancreatic necrosis disease (AHPND) and unaffected ponds, alongside examining the spatial distribution of soil attributes in flat-oriented pond soil strata. Using Pearson correlation and logistic regression analyses, relationships among variables and indicators associated with AHPND prevalence were discerned, leading to the formulation of a predictive model for AHPND occurrence. Soil samples were collected from distinct locations and depths within ponds across three southern provinces of Thailand's Andaman Seaboard. The analysis revealed significantly higher concentrations of several variables, including SOD, TIC, NO_2^--N, Ca, Mn, Cu, Zn, and specified Vibrio strains, in AHPND-afflicted ponds, especially at 0–5 cm depth. A prominent differentiation was the escalated concentration of easily decomposable organic matter (EDOM) within infected ponds, implicating potential soil and water quality deterioration alongside heightened shrimp susceptibility to AHPND. Correlational analysis showed links

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between bacterial densities and organic matter groupings, trace elements, exchangeable bases, and soil pH, in AHPND-infected ponds. The logistic regression model encapsulated three soil variables (TOC, Mg, and Mn) and one pathogen variable (*V. parahaemolyticus*) and furnished an equation to estimate the log (odds) of AHPND occurrence, facilitating better understanding and potential forecasting of AHPND prevalence in shrimp cultivation environments.

KEYWORDS
acute hepatopancreatic necrosis disease, AHPND, correlation, logistic regression, pond bottom soil, shrimp pond, *Vibrio parahaemolyticus*

1 | INTRODUCTION

Amidst the multitude of diseases afflicting the global shrimp aquaculture sector, acute hepatopancreatic necrosis disease (AHPND), also known as early mortality syndrome (EMS), induced by various bacterial strains such as *Vibrio parahaemolyticus*, *V. harveyi* (Kondo et al., 2015), *V. owensii* (Liu et al., 2015), *V. punensis* (Restrepo et al., 2018), *V. campbellii* (Dong et al., 2017), and *Shewanella* sp. (Wechprasit et al., 2019), emerges as a formidable challenge. These bacteria synthesize toxins, specifically PirAB (the virulent PirAB-like toxin gene), which wreak havoc on the shrimp's hepatopancreas, ultimately resulting in mortality rates ranging from 40% to 100% (Hong et al., 2016) over a culture period spanning from 30 to 96 days (FAO, 2013; Peña et al., 2015; Tendencia & Estilo, 2017).

The inception of this disease was first documented in 2009 within southern China and further in Hainan Island during 2010. The epidemic swiftly extended its grasp to Vietnam and Malaysia in the same year before pervading Thailand's eastern region in 2011 and intensifying across Thailand in 2012 (Zorriehzahra & Banaederakhshan, 2015). Moreover, the disease proliferated to other shrimp-cultivating nations, including the Philippines and Mexico, in 2013 (Nunan et al., 2014; Peña et al., 2015), and marked its presence in the United States in 2017, with the first case of AHPND infection being identified in whiteleg shrimp in Texas (Dhar et al., 2019).

Various strategic interventions have been posited for the prevention and management of this disease, encompassing the breeding of disease-resistant shrimp strains, the production of disease-free shrimp juveniles, and the management of the pond milieu, particularly focusing on the soil and water quality. This necessitates the maintenance of superior water and soil quality and the mitigation of the accumulation of easily decomposable organic matter (EDOM) and other residual substances within the ponds, which might detrimentally impact the pond ecosystem and shrimp growth. Among the preventive measures employed extensively by shrimp farmers in Thailand, the deployment of diverse microorganisms for varied objectives such as hastening the decomposition of organic matter within ponds and utilizing probiotics (Primphon et al., 2016; Ranjan, 2023) has gained considerable traction.

However, it has been noted that the abovementioned methods of shrimp disease prevention have not achieved universal success across all farms. While disease management through antibiotic drugs combined with other practices may yield positive outcomes for certain farms (Barman et al., 2013; Tinwongger, 2021), instances of complete losses, reaching 100%, have still been documented in this region as well as other regions of Thailand and worldwide (Alune, 2020; Kumar et al., 2021).

The quality of pond soil is hypothesized to be intricately linked with the incidence of AHPND, either directly or indirectly, given its role as a substrate for waste emanating from aquaculture activities such as leftover feed, aquatic animal waste, algae, and microorganism debris, inclusive of eroded soil (Barik et al., 2018; Hopkins et al., 1994; Saraswathy et al., 2019). These materials constitute a substantial portion of the organic substances present within the pond. A myriad of reactions and processes like precipitation, dissolution, oxidation, reduction, adsorption, cation exchange, sedimentation, decomposition, and diffusion transpire at the bottom areas of aquaculture ponds, particularly at the water-soil interface (Boyd, 1995). Such processes interplay between the pond bottom soil (sludge or sediment plus the original soil) and the overlying water; thus, the quality of the sediment at the pond bottom significantly affects water quality, the concentration of disease pathogens, and the growth trajectory of the cultured shrimp (Boyd & Phu, 2018; Mahajan & Billore, 2014).

Presently, there exists a paucity of comprehensive research elucidating the relationship between the quality of pond soil and the onset of diseases or other pertinent aspects when juxtaposed with water quality within the ponds (Boyd, 1995; Ranjan, 2023; Siddique et al., 2012). Given that the pathogen implicated in AHPND is a bacterium, it is plausible that the density of pathogens within the pond is interlinked with the pond environment, particularly the accumulation of EDOM and other factors within the pond soil that may foster or support the proliferation of *V. parahaemolyticus* and other pathogenic bacteria instrumental in the dissemination of AHPND infection.

Therefore, the objectives of the present study encompass the examination of disparities in soil properties and the quantity of *Vibrio parahaemolyticus* bacteria and other pathogenic bacteria within ponds harboring AHPND-infected shrimp and those identified as normal ponds (elucidated as "normal ponds" in Section 2.2 Farm Survey). The study endeavors to delineate the distribution patterns of soil properties and bacterial quantities horizontally, identify soil and bacterial variables correlated with the emergence of AHPND, and construct a logistic regression model to predict the likelihood of AHPND occurrence predicated on soil properties and bacterial variables pertinent to AHPND selected from an array of 27 variables. This investigation aims to improve the understanding of the soil environment system of pond bottoms, which would underpin the precise management of shrimp cultivation and pond soil, especially in managing organic substances and other materials accumulated within the ponds. These differences are anticipated among two distinct groups of marine shrimp farmers, potentially impacting soil quality, water quality, and shrimp health, ultimately engendering the onset of AHPND in the group of ponds that are most afflicted.

2 | MATERIALS AND METHODS

This section outlines the research design and methodology, systematically progressing through each phase of the study. First, the details of the selection and characterization of the research (sampling) sites are elaborated, followed by an explanation of the farm surveys conducted to classify the pond groups. Subsequently, the methodology for collecting bottom pond soil samples is explained, which forms the basis for our bacterial analyses, with a particular focus on the assessment of Vibrio bacterial content in the pond bottom soil. Lastly, the data analysis techniques employed, including correlation and logistic regression analysis and modeling, are presented. This structured approach ensures a comprehensive understanding of the research processes, from site selection through data analysis, and is consistent with standard empirical research methodologies in the field.

2.1 | Sampling sites

Shrimp pond bottom soil samples were collected from a total of 10 marine shrimp farms in each province (Phuket, Phangnga, and Krabi), including five ponds affected by AHPND and five AHPND-free ponds. This resulted in a combined collection from 30 shrimp farms situated along the coastline of the three Andaman provinces, as depicted in Figure 1.

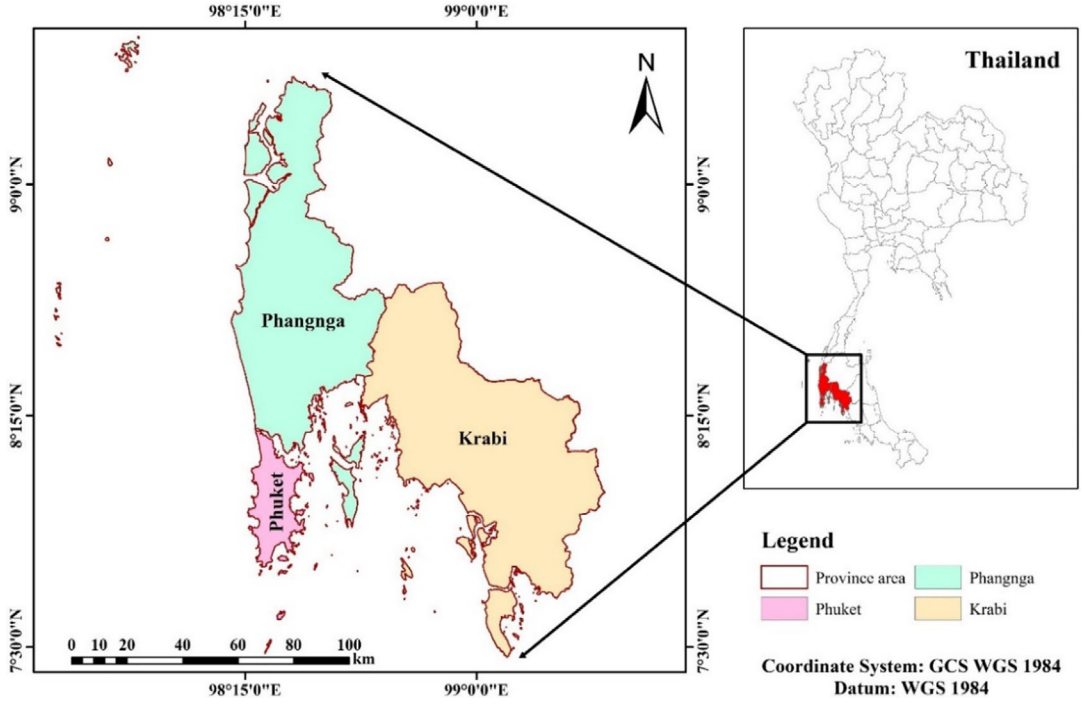

FIGURE 1 Location of Krabi, Phangnga, and Phuket Provinces in southern Thailand used in shrimp pond bottom soil sampling.

2.2 | Farm survey

An empirical examination was undertaken to ascertain adherence to the stipulated prerequisites for both categorizations of pond groups. The parameters were as follows:

1. The grow-out phase for both groups of ponds was mandated to be between the first and second months after stocking (frequent AHPND occurrences).
2. The designation of the control pond (AHPND-uninfected) necessitated a history devoid of AHPND infection either prior to the survey or for a minimum continuum of three successive years sans AHPND infection.
3. Verification of AHPND infection within respective ponds was thoroughly performed utilizing polymerase chain reaction (PCR) thermal cycling methods at the Department of Fisheries' to conclusively classify them as infected domains.
4. The procurement of bottom soil samples was thoroughly executed in a state of water saturation or after immediate drainage (in case of emergency harvest), ensuring the preservation of the integrity and authenticity of the samples for ensuing analytical evaluations.

2.3 | Pond bottom soil sampling

Bottom soil samples were collected from two predetermined locations within rectangular shrimp ponds, ranging in size from 4800 to 6400 m^2. Site A was located at the edge of the sediment pile situated in the center of the pond, where the size of the sediment pile differs from one pond to another. Site B was positioned halfway between the

outer edge of the sediment pile and the pond's perimeter. Thus, the distance between Sites A and B varies across ponds based on their respective sizes, yet this distance is proportionally consistent across all ponds. In each specified locale on the pond bed, eight distinct points were selected for sampling, arrayed in a circular or circumferential pattern to facilitate the combination of a composite sample, as explained in Figures 2 and 3.

The extraction of soil samples was carried out utilizing a manually operated core sampler, with a diameter of 4 cm, procured from Royal Eijkelkamp Company, The Netherlands (Catalog No. 04.15.SA). Stratified segments of surface soil were harvested at specified depths of 0–5 and 5–10 cm from the eight delineated points at each site and

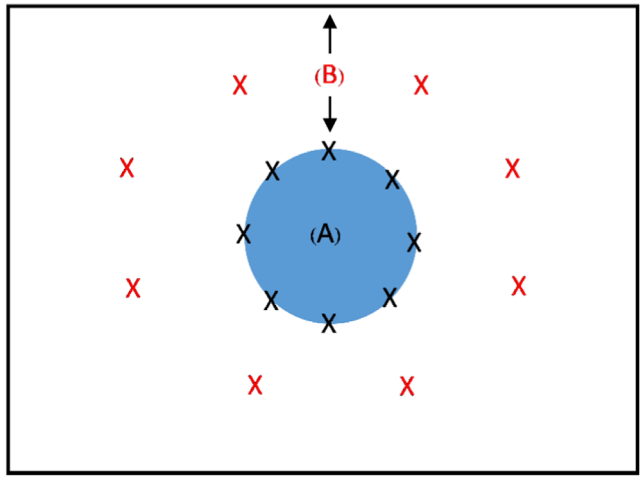

FIGURE 2 Site of bottom soil sample collection: (a) the middle of the pond (periphery of the sediment pile); and (b) the middle of the periphery of the sediment pile and the edge of the pond.

FIGURE 3 Bottom soil collection between the periphery of the sediment pile in the middle of the pond and the pond edge (Site B).

were subsequently aggregated to formulate a composite sample. The acquired samples were conscientiously preserved at cool temperatures until their conveyance to the designated soil and microbial laboratory for further analysis.

Pond soil sampling did not coincide with the end of the production cycle. Instead, sampling focused on ponds afflicted with diseases during the critical first to second months, aligning with the peak incidence period of AHPND, despite occasional reports in the third month. Similarly, aged ponds without AHPND were also sampled to facilitate a direct comparison, isolating the impact of varying management practices on soil properties and pathogen levels from potential differences attributable to the duration of cultivation. This approach ensures that observed disparities stem from management decisions rather than differences in cultivation periods, which might otherwise lead to an accumulation of organic substances.

The methodology for collecting soil samples from ponds with AHPND was twofold:

1. Periodic random shrimp sampling by farmers for inspection at the local Department of Fisheries (DOF) Laboratory—where not all ponds are covered under each farm's quota—led to the identification of AHPND through PCR tests, even in the absence of visible symptoms. Ponds confirmed to have AHPND were earmarked for soil sampling by researchers upon notification from the DOF.
2. Farmers submitted shrimp samples for PCR testing at the DOF Laboratory upon observing signs of illness. AHPND confirmation prompted the DOF to instruct researchers to collect soil samples from affected ponds.

It is crucial to note that soil sampling was exclusively conducted in conditions where the soil was either waterlogged or fresh after water discharge. Ponds that had been drained and had dry soil were excluded to avoid potential alterations in soil characteristics and pathogen quantities that could arise from transitioning from waterlogged to dry conditions and exposure to sunlight.

2.4 | Pond bottom soil and bacterial analysis

For the determination of sediment oxygen demand (SOD), ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N), and bacteria detection and characterization (total bacteria, total Vibrio, *V. parahaemolyticus*, *V. harveyi*, *V. vulnificus*, and *V. alginolyticus*), all samples were promptly analyzed in the laboratory.

Soil and bacteria parameters initially measured on a wet weight basis needed to be converted to a dry weight basis. This conversion was achieved by multiplying the wet weight of soil samples by the soil moisture factor, using the following equation:

$$\text{Soil moisture factor} = \text{weight of dry soil (g)} / \text{weight of wet soil (g)}.$$

here, the weight of dry soil (g) is determined by weighing wet soils that had been dried at 105°C for 24 h.

The residual wet soil samples were subsequently subjected to a process of air-drying, followed by accurate crushing and sieving operations. These sieving processes were executed through meshes with defined apertures of 10, 35, and 60, corresponding to dimensions of 2000, 500, and 125 μm, respectively. The methodologies employed for these operations are succinctly outlined in Table 1.

2.5 | Vibrio bacterial content in pond bottom soil

Quantities of total bacteria, total Vibrio, *V. parahaemolyticus*, *V. harveyi*, *V. vulnificus*, and *V. alginolyticus* were determined in samples of pond bottom soil. The analysis of bacterial counts was conducted at the Krabi

TABLE 1 Soil and bacteria parameters, methods, and references.

Soil and bacteria parameters	Methods	References
1. Sediment oxygen demand (SOD)	Modified water BOD method	Department of Fisheries (2008)
2. Organic matter (OM)	Wet combustion (Walkley & Black)	Nelson and Sommers (1996)
3. Total carbon (TC)	Dry combustion	Nelson and Sommers (1996)
4. Total organic carbon (TOC)	Dry combustion	Nelson and Sommers (1996)
5. Total inorganic carbon (TIC)	Dry combustion	Goh and Mermut (2008), Loeppert and Inskeep (1996)
6. Total nitrogen (TN)	Kjeldahl method	Bremner (1996), Rutherford et al. (2008), Tan (2005)
7. Ammonia-nitrogen (NH_3 - N)	3% NaCl extracting solution (Indophenol blue method)	Modified Chuan and Sugahara (1984), Maynard et al. (2008)
8. Nitrite-nitrogen (NO_2^- - N)	3% NaCl extracting solution (Diazotization method)	Modified Chuan and Sugahara (1984), Strickland and Parsons (1972)
9. Nitrate-nitrogen (NO_3^- - N)	3% NaCl extracting solution (Szechrome NAS method)	Modified Chuan and Sugahara (1984), Polysciences (2023)
10. Extractable phosphorus	Bray II extracting solution	Tan (2005), Kuo (1996)
11. Exchangeable Ca, Mg, and K	NH_4OAc method	Tan (2005)
12. Extractable Fe, Mn, Cu, and Zn	DTPA method	Gambrell (1996), Loeppert and Inskeep (1996), Reed and Martens (1996), Tan (2005)
13. Total sulfur (TS)	Dry combustion	Tabatabai (1996)
14. Soil pH	1:1 ratio of air-dried soil: water	Thomas (1996)
15. Lime requirement (LR)	Modified Adams-Evans method	Boyd (1995), Sims (1996)
16. Soil texture	Hydrometer method	Boyd (1995), Tan (2005), Kroetsch and Wang (2008)
17. Total bacteria	Spread plate method	Modified Steubing (1993), Maturin and Peeler (2001)
18. Total Vibrio	Spread plate method	Modified Steubing (1993), Kaysner et al. (2004)
19. *Vibrio harveyi*	Spread plate method	Modified Steubing (1993), Kaysner et al. (2004)
20. *V. vulnificus*	Spread plate method	Modified Steubing (1993), Kaysner et al. (2004)
21. *V. parahaemolyticus*	Spread plate method	Modified Steubing (1993), Kaysner et al. (2004)
22. *V. parahaemolyticus* (AHPND/EMS)	Multiplex PCR	Modified Steubing (1993), Tinwongger et al. (2014)
23. *V. alginolyticus*	Spread plate method	Modified Steubing (1993), Kaysner et al. (2004)

Note: All ponds identified as AHPND-infected ponds were tested with a PCR Thermal Cycler.

Coastal Aquaculture Research and Development Center, Department of Fisheries, using the methods outlined in Table 1. To confirm the presence of AHPND infection caused by *V. parahaemolyticus* strains producing hepatopancreatic toxins in diseased marine shrimp ponds, the PCR technique was employed, following the in-house methods from Tinwongger et al. (2014) and soil extraction by modified Steubing (1993) (using a Vortex Mixer for 1 minute).

2.6 | Data analysis: Correlation, and logistic regression analysis and modeling

The study examined disparities in soil properties and the quantity of Vibrio parahaemolyticus bacteria and other pathogenic bacteria between ponds harboring AHPND-infected shrimp and those identified as normal ponds. It delineated distribution patterns of soil properties and bacterial quantities, identified correlated variables, and constructed a logistic regression model predicting AHPND occurrence based on selected soil and bacterial variables, aiming to enhance the understanding of pond bottom soil environments for precise shrimp cultivation management, particularly in organic substance and material accumulation management, potentially impacting soil and water quality as well as shrimp health. Therefore, the following data analysis techniques were carried out:

First, hypotheses concerning the mean values of both pond groups were tested using the Student's *t*-test, and the disparities in areas and depths of the pond floor in individual groups of ponds were tested with the *F*-test. These two processes involved verifying several key assumptions critical for the validity of the tests, including the normal distribution of data, equal variance (*t*-test) and homogeneity of variances (*F*-test), independence of samples, continuity of data, and random sampling across both groups. To satisfy the prerequisites for the t-distribution and F-distribution, particularly for bacterial data, a natural logarithm (ln) transformation was applied. This step ensured the adherence of the data to the necessary assumptions, facilitating reliable and accurate *t*-test and *F*-test analyses.

Then, a correlation analysis was conducted to examine relationships among soil and bacterial variables in both pond groups. Before performing the correlation analysis, the assumptions of the Pearson correlation were assessed, including a linear relationship, normality, absence of outliers, and preliminary checks.

Afterwards, the relationship among soil and bacterial variables on AHPND occurrence in marine shrimp ponds was performed using a logistic regression model instead of a multiple linear regression model, which is normally inadequate for binary responses because it can permit values less than zero and greater than one (Kaps & Lamberson, 2009). A logistic regression model was developed to handle this problem. It uses a transformation (called a logit), which forces the prediction equation to predict a value between 0 and 1. The logistic regression equation predicts the natural log of odds for a subject being in one category or another (Cody & Smith, 1997). The regression coefficient describes a function of the mean (a function of the probabilities) rather than the mean itself. The particular function is the logarithm of the odds. The interpretation of logistics regression coefficients is made in terms of statements about odds and odds ratios (Ramsey & Schafer, 2002).

Logistic regression equation:

$$\log\left(\frac{P}{1-P}\right) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_n x_n.$$

where *p* represents the probability of the outcome, β_0 is the intercept, and β_1 to βn are the coefficients associated with each explanatory variable x_1 to x_n. The dependent variable is the logarithm of the odds, which is the logarithm of the ratio of two probabilities: the probability that a disease outbreak will occur and the probability that it will not occur. The logarithm of the odds {log[P/(1-P)]} is related in a linear manner to the potential explanatory variables. Where there is no available theoretical model, explanatory variables are usually selected through some specific techniques such as backward, forward or stepwise regression with different criteria to include or to reject an explanatory variable. The maximum likelihood method is used to estimate the coefficients β_1 to βn in the logistic regression (Leung & Tran, 2000).

Subsequently, logistic regression represents a variant of nonlinear regression, distinguished from ordinary regression by the fact that scatterplots and residual plots hold limited value in this context. This is primarily because of the binary nature of the response variable, which allows for only two possible outcomes. Consequently, the need to assess nonconstant variance and identify outliers is rendered superfluous (Ramsey & Schafer, 2002). Nevertheless, it remains imperative to examine the assumptions underpinning logistic regression before embarking on the analysis (Stoltzfus, 2011).

In the effort to predict the likelihood of AHPND occurrence in marine shrimp ponds, a comprehensive statistical methodology was utilized to evaluate a variety of potential models. The selection process incorporated several key criteria for determining a model's efficacy in predicting AHPND. These included the −2 Log Likelihood (−2 LOG L) and the Score test, both of which assess the significance of independent variables using a Chi-squared distribution. Additionally, Akaike's information criterion (AIC) and the Schwarz criterion (SC) were employed, both of which account for the model's complexity by adjusting for the number of explanatory variables and the sample size, respectively. These metrics are essential for model comparison and are particularly adept at handling issues of multicollinearity, with lower values suggesting a more accurate model fit. Furthermore, sequential variable selection methods such as forward, backward, and stepwise selection were applied to identify the most predictive model. These techniques, as discussed by Cody and Smith (1997) and Ramsey and Schafer (2002), played a crucial role in finalizing the optimal logistic regression model. The goal was to accurately forecast the odds of AHPND manifestation, with the response variable (*Y*) representing the disease occurrence and the explanatory variables (*X*) encompassing relevant soil and bacterial population factors. This strategic approach facilitated the identification of the most suitable model for predicting AHPND in shrimp ponds, underpinning the importance of rigorous statistical analysis in ecological research.

The statistical analyses were performed utilizing the SAS statistical software, specifically version Education Analytical Suite 9.4.

3 | RESULTS AND DISCUSSION

3.1 | Variances in soil characteristics and bacterial pathogen quantities between AHPND-afflicted and unafflicted ponds

The examination of variances in soil characteristics and the presence of disease-causing bacteria in pond soil between AHPND-infected and noninfected ponds has revealed significant disparities in several soil and bacterial variables. These distinctions underline the unique characteristics of AHPND-afflicted ponds, with higher values observed in multiple variables within these infected ponds when compared with their noninfected counterparts. Specifically, these variables include SOD, total inorganic carbon (TIC), nitrite-nitrogen (NO_2^--N), extractable phosphorus (P), calcium (Ca), manganese (Mn), copper (Cu), zinc (Zn), total Vibrio, *V. harveyi*, *V. vulnificus*, *V. parahaemolyticus*, and *V. alginolyticus*. These elevated values were predominantly concentrated in the central region of the ponds, as indicated by the superscript letters in Table 2.

Among these variables, EDOM exhibited a particularly clear and substantial contrast between AHPND-infected and non-infected ponds, as inferred from SOD measurements. It was observed that the average SOD values in noninfected and infected ponds were 3334 and 5420 mg/kg, respectively ($p < 0.01$) (Table 2). This discrepancy suggests differential pond management practices, particularly in terms of handling organic matter within these aquatic environments, between the two groups of marine shrimp farmers. Consequently, ponds afflicted by AHPND disease tended to accumulate higher levels of organic substances compared with their noninfected counterparts. Such an accumulation of organic matter in pond soil has the dual effect of augmenting oxygen demand while creating favorable anaerobic conditions (Sonnenholzner & Boyd, 2000). The EDOM variable represents a pivotal factor that exerts direct and indirect influence over various aspects, including water quality, soil quality, shrimp growth, and health. It is also anticipated to have a direct or indirect impact on the incidence of AHPND infection.

Moreover, the elevated levels of EDOM in AHPND-infected ponds correlated with increased quantities of Mn, Cu, and Zn in these diseased ponds compared with the noninfected ones ($p < 0.01$). This phenomenon can be attributed to the affinity of organic matter to bind with trace elements, forming intricate complexes (Giacalone et al., 2005; Marchand et al., 2011). The decomposition of organic matter releases these elements into the environment (Mahajan & Billore, 2014). Therefore, areas characterized by substantial organic matter accumulation exhibit

TABLE 2 Minimum, maximum, mean (± standard deviation) values, and mean comparison of pond bottom soil properties and bacterial count in 15 uninfected ponds and 15 AHPND-infected ponds at two areas and depths of the pond floor during the first 2 months of the grow-out period in three provinces, southern Thailand.

Pond bottom soil and bacteria parameters	Depth (cm)	Uninfected ponds		AHPND-infected ponds	
		Min-max	Mean ± SD	Min-max	Mean ± SD
1. Sediment oxygen demand (mg/kg)					
Middle	0–5	863–8260	3334 ± 2029[b]	1602–10,015	5420 ± 2453[a]
	5–10	1–5908	1697 ± 1529	483–4583	2345 ± 1161
Between the middle and edge	0–5	352–6398	2384 ± 1734	298–4465	2459 ± 1165
	5–10	504–3977	1637 ± 1043	62–3093	1677 ± 944
2. Organic matter (%)					
Middle	0–5	0.05–3.21	1.17 ± 0.89	0.31–3.42	1.19 ± 0.74
	5–10	0.1–3.88	1.23 ± 1.02	0.23–4.00	1.05 ± 0.97
Between the middle and edge	0–5	0.18–2.33	0.91 ± 0.63	0.08–2.29	0.78 ± 0.57
	5–10	0.23–2.90	1.12 ± 0.73	0–3.13	1.01 ± 0.88
3. Total carbon (%)					
Middle	0–5	0.26–3.15	1.24 ± 0.83	0.40–4.49	1.58 ± 1.00
	5–10	0.1–3.88	1.23 ± 1.02	0.23–4.00	1.05 ± 0.97
Between the middle and edge	0–5	0.24–2.67	1.08 ± 0.74	0.17–4.78	1.16 ± 1.07
	5–10	0.19–2.80	1.01 ± 0.75	0.09–3.07	0.89 ± 0.78
4. Total organic carbon (%)					
Middle	0–5	0.23–3.15	1.09 ± 0.73	0.27–1.85	0.88 ± 0.37
	5–10	0.15–3.13	1.13 ± 0.87	0.24–2.17	0.83 ± 0.52
Between the middle and edge	0–5	0.19–2.54	0.91 ± 0.65	0.15–1.64	0.66 ± 0.37
	5–10	0.19–2.80	1.01 ± 0.75	0.09–1.76	0.72 ± 0.55
5. Total inorganic carbon (%)					
Middle	0–5	0–1.065	0.151 ± 0.275[b]	0–3.734	0.705 ± 1.000[a]
	5–10	0–0.060	0.006 ± 0.017[b]	0–1.026	0.163 ± 0.279[a]
Between the middle and edge	0–5	0–0.875	0.167 ± 0.265	0–4.466	0.498 ± 1.130
	5–10	0–0.030	0.003 ± 0.009	0–1.771	0.165 ± 0.457
6. Total nitrogen, TN (%)					
Middle	0–5	0.03–0.21	0.07 ± 0.05	0.06–0.21	0.10 ± 0.05
	5–10	0.02–0.11	0.05 ± 0.03	0.03–0.15	0.07 ± 0.03
Between the middle and edge	0–5	0.02–0.15	0.06 ± 0.03	0.02–0.11	0.07 ± 0.02
	5–10	0.03–0.09	0.05 ± 0.02	0.02–0.07	0.05 ± 0.02
7. Ammonia-nitrogen (mg/kg)					
Middle	0–5	5–98	22.22 ± 25.53	4–282	56.37 ± 67.88
	5–10	1.49–83.88	18.08 ± 21.40	2.09–78	24.86 ± 22.23
Between the middle and edge	0–5	0.61–40.56	9.06 ± 10.08	2–69	12.61 ± 16.75
	5–10	1.38–13.32	5.79 ± 3.38	1.09–52.23	10.58 ± 13.38
8. Nitrite-nitrogen (mg/kg)					
Middle	0–5	0–0.124	0.034 ± 0.038[b]	0–1.152	0.288 ± 0.428[a]
	5–10	0–0.123	0.025 ± 0.034[b]	0–0.611	0.124 ± 0.184[a]

TABLE 2 (Continued)

Pond bottom soil and bacteria parameters	Depth (cm)	Uninfected ponds		AHPND-infected ponds	
		Min-max	Mean ± SD	Min-max	Mean ± SD
Between the middle and edge	0–5	0–0.161	0.041 ± 0.052	0–0.816	0.132 ± 0.200
	5–10	0–0.082	0.021 ± 0.027	0–0.167	0.035 ± 0.052
9. Nitrate-nitrogen (mg/kg)					
Middle	0–5	0–0.07	0.014 ± 0.026	0–0.013	0.001 ± 0.003
	5–10	0–0.164	0.026 ± 0.057	0–0.120	0.020 ± 0.040
Between the middle and edge	0–5	0–0.121	0.018 ± 0.034	0–0.030	0.003 ± 0.009
	5–10	0–0.020	0.004 ± 0.008	0–0.105	0.016 ± 0.036
10. Extractable P (mg/kg)					
Middle	0–5	49–439	178 ± 111	38–483	201 ± 110
	5–10	8–279	57 ± 66[b]	2–270	120 ± 75[a]
Between the middle and edge	0–5	28–390	184 ± 104	34–379	152 ± 122
	5–10	0–203	60 ± 55	3–351	88 ± 112
11. Calcium (mg/kg)					
Middle	0–5	318–3964	1702 ± 1187[b]	1116–6633	3349 ± 1473[a]
	5–10	234–3973	1095 ± 1090[b]	228–7721	2469 ± 1798[a]
Between the middle and edge	0–5	313–5104	2134 ± 1600	294–6514	2786 ± 1850
	5–10	183–5879	1322 ± 1515	149–1127	1952 ± 1873
12. Magnesium (mg/kg)					
Middle	0–5	265–1162	610 ± 279	187–1555	824 ± 319
	5–10	159–1106	551 ± 296	147–1896	717 ± 399
Between the middle and edge	0–5	245–944	551 ± 234	335–1234	646 ± 199
	5–10	270–1191	553 ± 266	149–1127	635 ± 272
13. Potassium (mg/kg)					
Middle	0–5	28–571	216 ± 167	68–576	296 ± 134
	5–10	5–517	166 ± 154	7–370	245 ± 113
Between the middle and edge	0–5	62–523	205 ± 138	29–355	209 ± 92
	5–10	5–583	209 ± 168	9–415	173 ± 124
14. Iron (mg/kg)					
Middle	0–5	9–225	64 ± 70	9–91	37 ± 27
	5–10	3–308	91 ± 101	2–242	60 ± 72
Between the middle and edge	0–5	5–117	33 ± 31	2–235	37 ± 60
	5–10	3–208	75 ± 77	1–292	123 ± 109
15. Manganese (mg/kg)					
Middle	0–5	0.22–12.54	3.77 ± 2.99[b]	0.30–17.86	8.61 ± 5.32[a]
	5–10	0.04–6.87	2.45 ± 1.89[b]	0.27–11.47	5.26 ± 3.99[a]
Between the middle and edge	0–5	0.32–12.25	3.09 ± 2.93	0.31–15.73	5.14 ± 4.86
	5–10	0.20–5.57	2.20 ± 1.44	0.29–39.83	6.76 ± 10.54
16. Copper (mg/kg)					
Middle	0–5	0.10–15.40	4.04 ± 3.96[b]	1.74–32.35	10.56 ± 9.95[a]
	5–10	0.07–2.08	0.75 ± 0.61[b]	0.38–7.74	2.29 ± 2.05[a]

(Continues)

TABLE 2 (Continued)

Pond bottom soil and bacteria parameters	Depth (cm)	Uninfected ponds		AHPND-infected ponds	
		Min-max	Mean ± SD	Min-max	Mean ± SD
Between the middle and edge	0–5	0.14–13.19	3.31 ± 3.81	0.34–12.43	4.02 ± 3.84
	5–10	0.05–7.25	0.99 ± 1.80	0.28–8.05	1.62 ± 1.39
17. Zinc (mg/kg)					
Middle	0–5	0.78–10.22	3.76 ± 2.94[b]	1.62–13.47	6.74 ± 3.59[a]
	5–10	0.07–7.71	1.80 ± 2.04	0.45–9.77	3.20 ± 2.34
Between the middle and edge	0–5	1.01–7.10	3.21 ± 1.94	0.87–6.61	3.18 ± 1.92
	5–10	0.14–4.81	1.71 ± 1.48	0.33–9.38	2.85 ± 2.53
18. Total sulfur (%)					
Middle	0–5	0–1.03	0.32 ± 0.32	0.003–1.14	0.24 ± 0.30
	5–10	0.002–1.11	0.40 ± 0.42	0.001–1.78	0.35 ± 0.47
Between the middle and edge	0–5	0.004–1.14	0.27 ± 0.37	0–1.03	0.15 ± 0.27
	5–10	0–1.1	0.35 ± 0.41	0–1.09	0.24 ± 0.32
19. Soil pH					
Middle	0–5	5.41–8.80	7.55 ± 0.79	7.31–8.44	7.85 ± 0.33
	5–10	4.45–8.01	7.15 ± 0.92	5.39–8.62	7.43 ± 0.84
Between the middle and edge	0–5	6.33–8.41	7.59 ± 0.63	7.18–8.42	7.72 ± 0.38
	5–10	4.91–8.29	6.98 ± 0.98	5.43–8.28	7.19 ± 0.74
20. Sand (%)					
Middle	0–5	24–80	52 ± 18	13–79	42 ± 20
	5–10	20–71	50 ± 16	5–74	38 ± 18
Between the middle and edge	0–5	21–82	56 ± 16	15–75	44 ± 18
	5–10	21–71	50 ± 16	3–67	37 ± 18
21. Silt (%)					
Middle	0–5	6–33	18 ± 9	8–41	22 ± 9
	5–10	6–33	20 ± 8	9–37	23 ± 8
Between the middle and edge	0–5	4–31	17 ± 9	10–38	22 ± 9
	5–10	8–37	20 ± 9	12–41	24 ± 9
22. Clay (%)					
Middle	0–5	14–47	30 ± 12	13–69	36 ± 15
	5–10	19–54	31 ± 12	15–86	40 ± 17
Between the middle and edge	0–5	14–44	27 ± 10	15–75	34 ± 14
	5–10	19–50	30 ± 10	20–88	39 ± 16
23. ln_Total bacteria (cfu/g)*					
Middle	0–5	11.82–13.81	12.98 ± 0.71	11.03–15.66	13.18 ± 1.52
	5–10	10.13–14.16	11.78 ± 1.41	9.04–14.97	12.07 ± 1.60
Between the middle and edge	0–5	10.36–14.65	12.49 ± 1.14	10.59–16.12	12.89 ± 1.50
	5–10	10.31–14.31	11.97 ± 1.35	9.09–16.47	12.16 ± 1.91
24. ln_Total Vibrio (cfu/g)*					
Middle	0–5	0–11.32	5.53 ± 3.80[b]	7.12–14.24	9.14 ± 1.84[a]
	5–10	0–12.82	3.53 ± 4.57	0–9.96	4.58 ± 3.58

TABLE 2 (Continued)

Pond bottom soil and bacteria parameters	Depth (cm)	Uninfected ponds		AHPND-infected ponds	
		Min-max	Mean ± SD	Min-max	Mean ± SD
Between the middle and edge	0–5	0–9.49	5.74 ± 3.21	0–9.54	6.14 ± 3.41
	5–10	0–11.75	3.92 ± 3.94	0–10.71	3.27 ± 3.52
25. ln_*V. harveyi* (cfu/g)*					
Middle	0–5	0–0	0 ± 0[b]	0–6.07	1.43 ± 2.48[a]
	5–10	0–0	0 ± 0	0–3.50	0.23 ± 0.90
Between the middle and edge	0–5	0–0	0 ± 0	0–5.81	0.67 ± 1.79
	5–10	0–0	0 ± 0	0–3.50	0.23 ± 0.90
26. ln_*V. vulnificus* (cfu/g)*					
Middle	0–5	0–10.43	6.92 ± 2.47[b]	6.63–14.64	9.04 ± 1.99[a]
	5–10	0–12.01	4.06 ± 3.10	0–9.52	4.56 ± 3.51
Between the middle and edge	0–5	0–14.22	6.99 ± 3.25	4.45–8.95	7.24 ± 1.30
	5–10	0–9.35	4.94 ± 3.49	0–8.79	3.75 ± 3.35
27. ln_*V. parahaemolyticus* (cfu/g)*					
Middle	0–5	0–10.43	4.79 ± 4.24[b]	4.04–11.36	8.22 ± 2.14[a]
	5–10	0–11.19	3.15 ± 4.24	0–11.71	3.94 ± 3.79
Between the middle and edge	0–5	0–11.58	3.87 ± 3.93[b]	5.61–9.13	7.34 ± 1.09[a]
	5–10	0–8.18	2.06 ± 2.82	0–10.48	3.78 ± 3.54
28. ln_*V. alginolyticus* (cfu/g)*					
Middle	0–5	0–9.90	7.25 ± 2.46[b]	5.39–14.80	9.37 ± 2.29[a]
	5–10	0–11.67	5.17 ± 3.96	0–11.96	5.09 ± 3.66
Between the middle and edge	0–5	0–11.03	6.98 ± 2.64	5.61–9.13	7.91 ± 1.52
	5–10	0–9.21	4.62 ± 3.65	0–9.56	4.42 ± 3.55

Note: 1. Differences in the superscript letters horizontally between two means indicate significant differences ($p < 0.01$). 2. *The natural logarithm (ln) transformation was applied to the bacterial data.

elevated levels of these trace elements (Marchand et al., 2011). Furthermore, EDOM also exerts influence over the quantities of disease-causing bacteria (e.g., total Vibrio, *V. harveyi*, *V. vulnificus*, *V. parahaemolyticus*, *and V. alginolyticus*), encompassing not only the pond bottom but also the suspended particulate organic fraction within the overlying water (Alfiansah et al., 2018). In AHPND-infected ponds, where EDOM accumulates at higher levels, the present study noted significantly greater quantities of these bacteria than in noninfected ponds ($p < 0.01$). This relationship arises from the microbial contribution to organic matter degradation (Arndt et al., 2013), and thus, AHPND-infected ponds exhibited augmented bacterial quantities compared with their noninfected counterparts.

Intriguingly, the study also noted significant disparities in the quantities of Ca and TIC between ponds with and without the disease ($p < 0.01$), stemming from higher lime application practices in AHPND-infected ponds. Although soil pH did not exhibit statistically significant differences, it is worth noting that the infected pond group displayed greater mean soil pH values (Table 2). The average Ca content in the soil of diseased and nondiseased ponds was 3349 and 1702 mg/kg, respectively, indicating that the diseased ponds received approximately twice as much lime application. Meanwhile, the average TIC values between the diseased and non-diseased ponds were 0.705% and 0.151%, respectively. On average, the TIC content in the ponds with AHPND infection was almost five times higher. Currently, no reports indicate the impact of excessive lime application on water and soil quality in shrimp ponds, shrimp growth, or disease occurrence. The application of lime in aquaculture ponds, primarily intended to elevate soil

pH, ought to be judiciously based on the actual lime requirements of the soil. In cases where the pond soil exhibits a neutral to slightly acidic pH, the necessity for lime application may be negligible, barring specific objectives like pathogen mitigation or the enhancement of water alkalinity, hardness, and mineral content throughout the cultivation period. This approach not only circumvents unnecessary increases in operational costs but also aligns lime application with the precise needs of the pond environment, ensuring optimal conditions for aquaculture without the undue expenditure.

Furthermore, the examination of phosphorus content in pond soil unveiled higher levels in ponds afflicted by AHPND disease, especially within the central region of the pond at a depth of 5–10 cm ($p < 0.01$). This observation suggests that AHPND-infected ponds in the central pond area amassed greater amounts of organic matter in contrast to their noninfected counterparts. It is important to emphasize that the primary source of phosphorus in aquatic animal feed significantly contributes to the phosphorus input in aquaculture ponds (Dien et al., 2018; Sun & Boyd, 2013). Hence, the breakdown of organic matter results in the release of additional phosphorus, particularly within ponds afflicted by the disease.

Another noteworthy discovery pertains to nitrite-nitrogen content in the soil, which displayed significant differences between ponds with and without AHPND disease, particularly within the central pond area where the infected ponds registered higher levels of nitrite-nitrogen ($p < 0.01$). This discrepancy arises from the increased accumulation of organic matter within the central region of AHPND-infected ponds compared with the noninfected ones. This observation aligns with prior research on the distribution profile of total organic carbon (TOC), NH_4^+-N, and NO_2^--N in sediment within freshwater aquaculture ponds, indicating elevated levels in 0–6 cm of sediment that decline rapidly beyond 6–10 cm (Lu et al., 2016). In the central area of AHPND-infected ponds, the nitrification process transitions from ammonia to nitrite and, in some instances, nitrate. This shift is primarily attributed to the relatively high aeration in these marine shrimp ponds (Barik et al., 2018). Nevertheless, the oxygen levels at the pond bottom, derived from dissolved oxygen in the water, were insufficient for the nitrifying bacteria to convert all nitrite into nitrate. This insufficiency was attributed to the substantial accumulation of organic matter in the area, which demanded a significant amount of oxygen for its breakdown by bacteria, along with other oxidative reactions. Consequently, a considerable portion of the residue remained as nitrite. Ponds with a higher concentration of organic matter exhibited elevated levels of nitrite. As a result, nitrite concentrations were higher in the central areas of the pond compared with the edges. In addition, ponds with disease, particularly in their central regions, showed higher nitrite levels than those without disease, underscoring the impact of organic matter accumulation on the pond's biochemical environment.

Generally, waterlogged soils are characterized by anaerobic conditions, under which the presence of nitrate is often considered negligible. However, the observations from this study reveal that nitrate was infrequently detected in the bottom soil of both diseased and nondiseased pond groups, with only sporadic instances of minimal nitrate traces. This occasional presence of nitrate may be linked to the oxygen levels reaching the pond bottom through water diffusion. Despite the potential for increased dissolved oxygen levels facilitated by aerators, this was insufficient for the complete conversion of nitrite to nitrate across all ponds, with only select ponds showing nitrate presence. This variation suggests that factors such as the effectiveness of the pond's aeration system or the condition of the pond bottom—particularly the extent of organic matter accumulation—played a role in influencing the nitrification process to varying degrees. Moreover, when comparing the average nitrate levels in the bottom soil of both pond groups, it was observed that ponds without disease generally exhibited higher nitrate levels than their diseased counterparts, highlighting a possible link between disease presence and nitrification efficiency.

Regarding the assessment of total organic matter and related organic matter content within the pond bottom soil, encapsulated by variables such as organic matter, total carbon, total nitrogen, total organic carbon, and total sulfur, the study's analysis revealed no statistically significant differences between ponds with and without AHPND disease in any of these total variables (Table 2). This discovery contrasts with the analysis of EOM (easily oxidized matter) content (Avnimelech et al., 2004; Joyni et al., 2011), which yielded congruent results with this study. EDOM, as estimated from SOD, constitutes a subset of organic matter that exerts a profound influence on soil quality, water

quality, bacterial quantities, and the growth of aquatic organisms. In the current study's analysis, it was ascertained that ponds afflicted with AHPND disease demonstrated significantly elevated levels of EDOM compared with their noninfected counterparts ($p < 0.01$). This augmented EDOM content was found to impact other variables, particularly bacterial quantities linked to disease occurrence. The heightened bacterial quantities can be directly linked to the increased EDOM content accumulating within these ponds. These bacteria, in turn, are implicated as direct or indirect contributory factors to the incidence of AHPND infection in the affected group of ponds (Tables 2, 4, and 5).

Finally, about the remaining soil variables and bacteria, no statistically significant differences were identified between ponds without AHPND disease and those afflicted by AHPND ($p > 0.05$) (Table 2).

It is essential to emphasize that *Vibrio parahaemolyticus* (AHPND) was detected in pond bottom soil samples, ranging from the surface level down to a depth of 10 cm. The bacteria may potentially penetrate even deeper, given that our samples were collected within the 0–10 cm depth range. Thus, when preparing the pond post-AHPND infection, careful consideration should be given to the eradication of bacteria within the soil to a depth of at least 10 cm, if not deeper.

3.2 | Disparities in horizontal distribution of pond bottom soil characteristics and bacterial pathogen quantities in AHPND-afflicted and unafflicted ponds

The findings of this study can be synthesized into two distinct groups concerning the horizontal distribution patterns of substances and pathogens within the pond soil:

Group 1: This group exhibits parallel distribution characteristics in the horizontal direction across both pond categories. In the central region of the pond, values were consistently higher in comparison with the area situated between the central zone and the pond periphery. Furthermore, the average values at the soil surface (0–5 cm) surpassed those in the deeper soil layer (5–10 cm). Variables falling into this category encompassed SOD, TIC in uninfected ponds, total nitrogen (TN) in infected ponds, ammonia-nitrogen (NH_3-N), phosphorus (P) as per Rana et al. (2017), potassium (K) in infected ponds, copper (Cu), zinc (Zn), total bacteria in uninfected ponds, and total Vibrio in infected ponds, along with *V. vulnificus*, *V. parahaemolyticus*, and *V. alginolyticus*.

The distribution patterns of soil characteristics and bacterial pathogens within this group are intimately linked to the highest concentrations of EDOM, gauged through SOD, predominantly located at the pond's center. This phenomenon arises from the influence of aerators generating circular water flows, thereby facilitating the settlement of organic and inorganic particles within the central pond region. Thus, the central area of the pond exhibits heightened accumulations of substances at the soil surface (at a depth of 0–5 cm) when compared with deeper layers. This pattern extends to the quantity of pathogens in the central region, following the same trend. Furthermore, pathogen quantities diminish with increasing depth, mirroring the trends observed in SOD (or EDOM content) values that also decline with depth. The interaction of copper (Cu) and zinc (Zn) with organic matter results in the formation of intricate compounds, consequently yielding a distribution pattern akin to that of SOD values across the horizontal plane.

In the case of EDOM, it encompasses diverse mineral constituents. Upon decomposition, these minerals yield augmented quantities of specific minerals within the specified area. Additionally, the central pond region displays a higher accumulation of small-sized inorganic sediments relative to the pond periphery. According to Borisover and Davis (2015), these inorganic sediments can hold different elements on their surfaces. This led to higher levels of NH_3-N (both groups), Cu, and Zn (infected group) in the samples than what was seen in the area between the pond's center and edges (Table 3). As for TN and NH_3-N values originating from the decomposition of organic nitrogen compounds, the central area of the pond exhibits higher levels compared with the region between the center and the edges of the pond. Moreover, these values are found to be higher at the upper soil level compared with the lower soil level. These observations are related to the accumulation of EDOM in the pond (Lu et al., 2016). This is because nitrogen is a crucial component of organic matter, especially in shrimp feed (Chaikaew et al., 2019; Dien et al., 2018; Sun & Boyd, 2013).

TABLE 3 Mean comparison of pond bottom soil properties and bacterial abundance at various areas and depths in 15 uninfected ponds and 15 AHPND-infected ponds from three provinces in southern Thailand.

Pond bottom soil and bacteria parameters	Pond types	Middle		Between the middle and edge	
		0–5 cm	5–10 cm	0–5 cm	5–10 cm
1. Sediment oxygen demand (mg/kg)	Uninfected	3334 ± 2029^{a}	1697 ± 1529^{b}	2384 ± 1734^{ab}	1637 ± 10,432^{b}
	Infected	5420 ± 2453^{a}	2345 ± 1161^{b}	2459 ± 1165^{b}	1677 ± 944^{b}
2. Organic matter (%)	Uninfected	1.17 ± 0.89	1.23 ± 1.02	0.91 ± 0.63	1.12 ± 0.73
	Infected	1.19 ± 0.74	1.05 ± 0.97	0.78 ± 0.57	1.01 ± 0.88
3. Total carbon (%)	Uninfected	1.24 ± 0.83	1.14 ± 0.87	1.08 ± 0.74	1.01 ± 0.75
	Infected	1.58 ± 1.00	0.99 ± 0.53	1.16 ± 1.07	0.89 ± 0.78
4. Total organic carbon (%)	Uninfected	1.09 ± 0.73	1.13 ± 0.87	0.91 ± 0.65	1.01 ± 0.75
	Infected	0.88 ± 0.37	0.83 ± 0.52	0.66 ± 0.37	0.72 ± 0.55
5. Total inorganic carbon (%)	Uninfected	0.151 ± 0.275^{a}	0.006 ± 0.017^{b}	0.167 ± 0.265^{a}	0.003 ± 0.009^{b}
	Infected	0.705 ± 1.000	0.163 ± 0.279	0.498 ± 1.130	0.165 ± 0.457
6. Total nitrogen (%)	Uninfected	0.07 ± 0.05	0.05 ± 0.03	0.06 ± 0.03	0.05 ± 0.02
	Infected	0.10 ± 0.05^{a}	0.07 ± 0.03^{b}	0.07 ± 0.02^{b}	0.05 ± 0.02^{b}
7. Ammonia-nitrogen (mg/kg)	Uninfected	22.22 ± 25.53^{a}	18.08 ± 21.40^{ab}	9.06 ± 10.08^{b}	5.79 ± 3.38^{b}
	Infected	56.37 ± 67.88^{a}	24.86 ± 22.23^{b}	12.61 ± 16.75^{b}	10.58 ± 13.38^{b}
8. Nitrite-nitrogen (mg/kg)	Uninfected	0.034 ± 0.038	0.025 ± 0.034	0.041 ± 0.052	0.021 ± 0.027
	Infected	0.288 ± 0.428	0.124 ± 0.184	0.132 ± 0.200	0.035 ± 0.052
9. Nitrate-nitrogen (mg/kg)	Uninfected	0.014 ± 0.026	0.026 ± 0.057	0.018 ± 0.034	0.004 ± 0.008
	Infected	0.001 ± 0.003	0.020 ± 0.040	0.003 ± 0.009	0.016 ± 0.036
10. Extractable phosphorus (mg/kg)	Uninfected	178 ± 111^{a}	57 ± 66^{b}	184 ± 104^{a}	60 ± 55^{b}
	Infected	201 ± 110^{a}	120 ± 75^{ab}	152 ± 122^{ab}	88 ± 112^{b}
11. Calcium (mg/kg)	Uninfected	1702 ± 1187	1095 ± 1090	2134 ± 1600	1322 ± 1515
	Infected	3349 ± 1473	2469 ± 1798	2786 ± 1850	1952 ± 1873
12. Magnesium (mg/kg)	Uninfected	610 ± 279	551 ± 296	551 ± 234	553 ± 266
	Infected	824 ± 319	717 ± 399	646 ± 199	635 ± 272
13. Potassium (mg/kg)	Uninfected	216 ± 167	166 ± 154	205 ± 138	209 ± 168
	Infected	296 ± 134^{a}	245 ± 113^{ab}	209 ± 92^{ab}	173 ± 124^{b}
14. Iron (mg/kg)	Uninfected	64 ± 70	91 ± 101	33 ± 31	75 ± 77
	Infected	37 ± 27	60 ± 72	37 ± 60	123 ± 109
15. Manganese ((mg/kg)	Uninfected	3.77 ± 2.99	2.45 ± 1.89	3.09 ± 2.93	2.20 ± 1.44
	Infected	8.61 ± 5.32	5.26 ± 3.99	5.14 ± 4.86	6.76 ± 10.54
16. Copper (mg/kg)	Uninfected	4.04 ± 3.96^{a}	0.75 ± 0.61^{b}	3.31 ± 3.81^{a}	0.99 ± 1.80^{b}
	Infected	10.56 ± 9.95^{a}	2.29 ± 2.05^{b}	4.02 ± 3.84^{b}	1.62 ± 1.39^{b}
17. Zinc (mg/kg)	Uninfected	3.76 ± 2.94^{a}	1.80 ± 2.04^{b}	3.21 ± 1.94^{ab}	1.71 ± 1.48^{b}
	Infected	6.74 ± 3.59^{a}	3.20 ± 2.34^{b}	3.18 ± 1.92^{b}	2.85 ± 2.53^{b}
18. Total sulfur (%)	Uninfected	0.32 ± 0.32	0.40 ± 0.42	0.27 ± 0.37	0.35 ± 0.41
	Infected	0.24 ± 0.30	0.35 ± 0.47	0.15 ± 0.27	0.24 ± 0.32
19. Soil pH	Uninfected	7.55 ± 0.79	7.15 ± 0.92	7.72 ± 0.38	7.19 ± 0.74
	Infected	7.85 ± 0.33^{a}	7.43 ± 0.84^{ab}	7.59 ± 0.63^{a}	6.98 ± 0.98^{b}
20. Sand (%)	Uninfected	52 ± 18	50 ± 16	56 ± 16	50 ± 16
	Infected	42 ± 20	38 ± 18	44 ± 18	37 ± 18

TABLE 3 (Continued)

Pond bottom soil and bacteria parameters	Pond types	Middle		Between the middle and edge	
		0–5 cm	5–10 cm	0–5 cm	5–10 cm
21. Silt (%)	Uninfected	18 ± 9	20 ± 8	17 ± 9	20 ± 9
	Infected	22 ± 9	23 ± 8	22 ± 9	24 ± 9
22. Clay (%)	Uninfected	30 ± 12	31 ± 12	27 ± 10	30 ± 10
	Infected	36 ± 15	40 ± 17	34 ± 14	39 ± 16
23. ln_Total bacteria (cfu/g)*	Uninfected	12.98 ± 0.71^{a}	11.78 ± 1.41^{b}	12.49 ± 1.14^{ab}	11.97 ± 1.35^{b}
	Infected	13.18 ± 1.52	12.07 ± 1.60	12.89 ± 1.50	12.16 ± 1.91
24. ln_Total Vibrio (cfu/g)*	Uninfected	5.53 ± 3.80	3.53 ± 4.57	5.74 ± 3.21	3.92 ± 3.94
	Infected	9.14 ± 1.84^{a}	4.58 ± 3.58^{bc}	6.14 ± 3.41^{b}	3.27 ± 3.52^{c}
25. ln_*V. harveyi* (cfu/g)*	Uninfected	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Infected	1.43 ± 2.48	0.23 ± 0.90	0.67 ± 1.79	0.23 ± 0.90
26. ln_*V. vulnificus* (cfu/g)*	Uninfected	6.92 ± 2.47^{a}	4.06 ± 3.10^{b}	6.99 ± 3.25^{a}	4.94 ± 3.49^{ab}
	Infected	9.04 ± 1.99^{a}	4.56 ± 3.51^{b}	7.24 ± 1.30^{a}	3.75 ± 3.35^{b}
27. ln_*V. parahaemolyticus* (cfu/g)*	Uninfected	4.79 ± 4.24	3.15 ± 4.24	3.87 ± 3.93	2.06 ± 2.82
	Infected	8.22 ± 2.14^{a}	3.94 ± 3.79^{b}	7.34 ± 1.09^{a}	3.78 ± 3.54^{b}
28. ln_*V. alginolyticus* (cfu/g)*	Uninfected	7.25 ± 2.46	5.17 ± 3.96	6.98 ± 2.64	4.62 ± 3.65
	Infected	9.37 ± 2.29^{a}	5.09 ± 3.66^{b}	7.91 ± 1.52^{a}	4.42 ± 3.55^{b}

Note: 1. Mean (± standard deviation) 2. Differences in the superscript letters horizontally indicate significant differences ($p < 0.01$). 3. *The natural logarithm (ln) transformation was applied to the bacterial data.

Group 2: In contrast to Group 1, this group exhibits uniform distribution patterns both horizontally and vertically across both sets of shrimp ponds. The variables encompass organic matter (OM), total carbon (TC), TOC, total sulfur (TS), nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), as well as fractions of sand, silt, and clay (Table 3).

3.3 | Correlation matrix of all variables in AHPND-afflicted and unafflicted ponds

The results of the correlation analysis, comprising correlation coefficients (*r* values), are organized into a comprehensive correlation matrix encompassing all 27 variables, which includes 22 soil variables and 5 pathogen variables. These findings are delineated in Table 4 (noninfected ponds) and Table 5 (AHPND-infected ponds).

Distinct correlation patterns emerged exclusively within the group of ponds afflicted by the AHPND infection. There were positive relationships found between the group of pathogen variables related to the disease and exchangeable bases, trace elements, organic matter or organic-related variables, and soil pH (shown in Table 5 by a red rectangle). These correlation patterns remained absent in the group of normal ponds (Table 4). The rationale for these findings is elucidated as follows:

First, the group of pathogen variables demonstrated a positive correlation with the cluster of organic matter or organic-related variables, a phenomenon consistent with the findings of Xue et al. (2018). Xue et al. reported that soil nutrient properties, such as total carbon, TN, and phosphorus, exhibited relationships with microbial abundance. This positive correlation stems from the fact that organic matter serves as a nutrient source for bacterial pathogens. Consequently, when the quantity of EDOM accumulates at higher levels, the population of bacterial pathogens responsible for diseases in pond soil increases. Particularly, the quantity of *V. parahaemolyticus*, the causative agent of

TABLE 4 Correlation matrix of pond bottom soil variables and the number of bacteria in the bottom soil of the 15 AHPND-uninfected shrimp ponds in three provinces of southern Thailand.

	SOD	OM	TC	TOC	TIC	TN	NH_3-N	NO_2-N	NO_3-N	TS	Ca	Mg	K	Fe	Mn	Cu	Zn	P	pH	Sand	Silt	Clay	ln_TB	ln_TV	ln_*V.vul*	ln_*V.para*	ln_*V.algi*
SOD	1.00																										
OM	0.4592 0.0002	1.00																									
TC	0.4991 <0.0001	0.8033 <0.0001	1.00																								
TOC	0.3967 0.0017	0.8149 <0.0001	0.9667 <0.0001	1.00																							
TIC	0.4863 <0.0001	0.1370 0.2965	0.3454 0.0069	0.0936 0.4770	1.00																						
TN	0.6854 <0.0001	0.4698 0.0002	0.4125 0.0011	0.2938 0.0227	0.5273 <0.0001	1.00																					
NH_3-N	0.2724 0.0352	-0.0566 0.6678	-0.0594 0.6523	-0.1000 0.4473	0.1358 0.3009	0.2758 0.0329	1.00																				
NO_2-N	-0.0268 0.8767	-0.0532 0.6866	0.2580 0.0466	-0.2219 0.0885	-0.1900 0.1460	-0.1932 0.1391	0.1151 0.3812	1.00																			
NO_3-N	-0.0292 0.8249	0.3782 0.0029	0.3189 0.0130	0.3236 0.0117	0.0543 0.6802	0.1887 0.1489	-0.0514 0.6966	0.0354 0.7885	1.00																		
TS	0.4585 0.0002	0.6690 <0.0001	0.8621 <0.0001	0.9085 <0.0001	0.0223 0.8658	0.1593 0.2242	-0.0990 0.4519	-0.2172 0.0955	0.1233 0.3478	1.00																	
Ca	0.3423 0.0074	0.1783 0.1728	0.1222 0.2458	0.0760 0.5637	0.3131 0.0149	0.2769 0.0322	-0.0176 0.8936	0.3804 0.0027	0.0311 0.8138	0.0477 0.7173	1.00																
Mg	0.6316 <0.0001	0.6151 <0.0001	0.4050 0.0013	0.3753 0.0031	0.1993 0.1269	0.5707 <0.0001	0.0284 0.8296	-0.0083 0.9498	0.0480 0.7159	0.2975 0.0210	0.2760 0.0328	1.00															
K	0.2163 0.0138	-0.0641 0.6268	-0.2179 0.0945	-0.2383 0.0463	0.0995 0.4492	0.4545 0.0003	0.2986 0.0243	0.1814 0.4408	-0.1564 0.2328	-0.3002 0.0198	0.1578 0.2285	0.5128 <0.0001	1.00														
Fe	0.3677 0.0039	0.7372 <0.0001	0.5654 <0.0001	0.6462 <0.0001	-0.1702 0.1936	0.1709 0.1917	-0.1084 0.4097	-0.0258 0.8448	0.0753 0.5675	0.6372 <0.0001	-0.0383 0.7715	0.5682 <0.0001	-0.1186 0.3668	1.00													
Mn	0.6175 <0.0001	0.3664 0.0040	0.4205 0.0008	0.2920 0.0236	0.5646 <0.0001	0.6083 <0.0001	-0.0380 0.7729	-0.2472 0.0569	-0.0311 0.8134	0.2737 0.0344	0.3811 0.0027	0.4152 0.0010	0.1589 0.2253	0.1394 0.2881	1.00												
Cu	0.5610 <0.0001	0.0116 0.9301	0.0368 0.7804	-0.0869 0.5090	0.4616 0.0002	0.5209 <0.0001	0.1361 0.2999	0.0932 0.4788	-0.0066 0.9603	-0.1452 0.2683	0.4340 0.0005	0.3808 0.0027	0.3073 0.0169	-0.1173 0.3721	0.5582 <0.0001	1.00											
Zn	0.5470 <0.0001	0.3525 0.0057	0.4030 0.0014	0.3311 0.0098	0.3537 0.0056	0.5868 <0.0001	0.0222 0.8664	-0.0673 0.6095	0.2625 0.0428	0.2045 0.1171	0.3443 0.0071	0.6086 <0.0001	0.2832 0.0284	0.2034 0.1191	0.5773 <0.0001	0.6585 <0.0001	1.00										
P	0.2984 0.0206	-0.1972 0.1309	0.1307 0.3194	0.0589 0.6551	0.2925 0.0233	0.0933 0.4783	0.0746 0.5709	-0.1348 0.3044	-0.0166 0.9001	0.0895 0.4964	0.2873 0.0260	0.1049 0.4251	0.1357 0.3011	-0.2489 0.0552	0.1687 0.1977	0.3695 0.0037	0.4797 0.0001	1.00									
pH	0.1530 0.2430	-0.1597 0.2229	-0.1403 0.2850	-0.2136 0.1013	0.2373 0.0679	0.1624 0.2151	0.1869 0.1528	0.0438 0.7398	-0.0330 0.8026	-0.2268 0.0814	0.1877 0.1509	-0.0045 0.9725	0.3215 0.0122	-0.3575 0.0050	-0.0181 0.8907	0.1378 0.2937	0.1537 0.2410	0.3669 0.0039	1.00								
Sand	-0.1481 0.2587	-0.1917 0.1423	0.0964 0.4637	0.0889 0.4996	0.0491 0.7092	-0.3389 0.0081	0.0718 0.5854	0.0848 0.5194	-0.0363 0.7829	0.1769 0.1764	-0.0826 0.5305	-0.5636 <0.0001	-0.4566 0.0002	-0.2902 0.0245	-0.1977 0.1299	-0.2139 0.1008	-0.1968 0.1318	0.1754 0.1800	0.1783 0.1729	1.00							
Silt	0.2049 0.1164	0.1636 0.2116	-0.1987 0.1280	-0.1768 0.1766	-0.1246 0.3428	0.1560 0.2340	-0.1870 0.1525	0.1519 0.2465	-0.1433 0.2749	-0.1663 0.2042	0.3023 0.0189	0.4662 0.0002	0.2883 0.0255	0.2348 0.0710	0.2654 0.0404	0.3556 0.0053	0.2051 0.1160	-0.1909 0.1441	0.0582 0.6586	-0.6500 <0.0001	1.00						
Clay	0.3324 0.0095	0.2951 0.0221	0.0862 0.5125	0.0578 0.6610	0.4315 0.0006	0.1236 0.3469	0.1862 0.1544	-0.0833 0.5268	-0.1858 0.1552	0.0354 0.7884	0.1129 0.3903	0.5989 <0.0001	0.5618 <0.0001	0.2767 0.0324	0.2088 0.1094	0.1246 0.3427	0.0715 0.5871	-0.1466 0.2636	-0.1382 0.2925	-0.6982 <0.0001	0.3959 0.0017	1.00					
ln_TB	0.1048 0.4257	0.1593 0.2240	-0.0429 0.7450	-0.0707 0.5915	0.0922 0.4833	0.0431 0.7439	-0.0084 0.9490	0.3021 0.0190	0.1964 0.1325	-0.1242 0.3444	0.0922 0.4837	0.0324 0.8058	-0.0168 0.8987	0.0062 0.9626	-0.0474 0.7189	0.0562 0.6699	0.0135 0.9185	0.0455 0.7298	0.1852 0.1567	0.0019 0.9685	0.0342 0.7955	-0.0030 0.9821	1.00				
ln_TV	0.2516 0.0523	0.1776 0.1747	0.1126 0.3917	0.1698 0.1948	-0.1844 0.1585	0.0440 0.7386	0.0589 0.6551	0.2227 0.0872	0.1976 0.1302	0.2273 0.0808	-0.0175 0.8943	0.1326 0.3125	-0.0957 0.9658	0.1667 0.2031	-0.2067 0.1131	0.0914 0.4874	0.1265 0.3357	0.2091 0.1089	0.2051 0.1160	0.3110 0.0156	-0.0677 0.6070	-0.1404 0.2847	0.3605 0.0047	1.00			
ln_*V. vul*	0.1185 0.3672	0.1308 0.3193	0.0116 0.9298	0.0722 0.5837	-0.2194 0.0921	-0.0528 0.6886	-0.0065 0.9607	0.1869 0.1528	0.2604 0.0445	0.0695 0.5976	0.0654 0.6195	0.0835 0.5259	-0.1046 0.4262	0.1190 0.3651	-0.1792 0.1707	0.0491 0.7096	0.1704 0.1931	0.3201 0.0127	0.2450 0.0592	0.2063 0.1137	0.1077 0.4127	-0.2892 0.0250	0.4095 0.0012	0.7049 <0.0001	1.00		
ln_*V.para*	0.0411 0.7552	0.3217 0.0122	0.0726 0.5815	0.1224 0.3517	-0.1662 0.2044	0.0389 0.7677	-0.0831 0.5278	0.2082 0.1103	0.5011 <0.0001	0.0040 0.9761	-0.0015 0.9909	0.1052 0.4239	-0.0289 0.8267	0.2460 0.0582	-0.1453 0.2681	-0.0528 0.6888	0.1388 0.2903	0.0643 0.6254	0.1349 0.3040	-0.0064 0.9612	0.0115 0.9307	-0.1328 0.3116	0.5584 <0.0001	0.5302 <0.0001	0.5925 <0.0001	1.00	
ln_*V.algi*	0.0254 0.8471	-0.0055 0.9666	-0.0346 0.7930	0.0161 0.9028	-0.1936 0.1383	-0.1568 0.2316	-0.0076 0.9540	0.1215 0.3553	0.3036 0.0184	0.0088 0.9467	-0.1634 0.2121	-0.0737 0.5759	-0.1265 0.3353	0.0472 0.7203	-0.2284 0.0792	-0.0068 0.9588	0.1197 0.3621	0.2918 0.0237	0.2034 0.1190	0.3184 0.0132	-0.1630 0.2135	-0.4323 0.0006	0.4630 0.0002	0.7441 <0.0001	0.7891 <0.0001	0.6770 <0.0001	1.00

Note: 1. ln = Natural log transformation. 2. In Table 4, the upper numbers represent the correlation coefficients (R), and the lower numbers are *p*-values. 3. Cell highlights indicate the statistical significance of correlation coefficients. 4. Specific colors represent groups of soil and bacteria variables (in a column): Yellow = Group of organic substances or relation, Light orange = Group of exchangeable cations, Light blue = Group of extractable trace elements, Light gold = Phosphorus, Green = Group of soil textures, and Gray = Group of bacteria.

AHPND disease in marine shrimp, rises in tandem. Second, a significant correlation ($r = 0.4336$, $p < 0.0005$) between SOD and the quantity of *V. parahaemolyticus* in infected ponds is observed in Table 5, a correlation absent in ponds without the disease. This discrepancy is attributed to the lower accumulation of organic matter in ponds devoid of the disease.

Third, the cluster of bacterial pathogen variables also exhibited a positive correlation with exchangeable bases, specifically calcium (Ca) and potassium (K). This association can be explained by the fact that both elements are constituents of organic matter. As organic matter decomposes, it releases these elements in areas characterized by high organic matter content. In the case of Ca, ponds with infections displayed a greater usage of lime compared with noninfected ponds, leading to elevated accumulations, particularly in the central pond areas. Consequently, a positive correlation with the quantity of pathogens in the pond ensues. Then, analogous to exchangeable bases, the positive correlation observed between the quantity of pathogens and trace elements adheres to a similar pattern. Furthermore, specific trace elements, especially copper (Cu) and zinc (Zn), exhibit a propensity to form complex structures with organic matter. Hence, heightened organic matter accumulation results in the complexation of these elements with organic matter, consequently amplifying the pathogen population.

Subsequently, the cluster of pathogen variables evinces a positive correlation with pH values. This phenomenon is attributed to the dominance of bacteria in microbial activity at intermediate and higher pH levels (Brady & Weil, 2000). Most soil microorganisms, particularly soil bacteria, thrive optimally within a pH range of 7–8 (Boyd et al., 2002). Therefore, the adjustment of pond soil pH to higher levels in infected ponds (because of increased lime usage in ponds with infections) potentially leads to a rise in the quantity of *V. parahaemolyticus* and other bacterial pathogens. This, in turn, contributes to the development of AHPND infection in marine shrimp. The correlation

TABLE 5 Correlation matrix of pond bottom soil variables and the number of bacteria in the bottom soil of the 15 AHPND-infected shrimp ponds in three provinces of southern Thailand.

	SOD	OM	TC	TOC	TIC	TN	NH_3-N	NO_2-N	NO_3-N	TS	Ca	Mg	K	Fe	Mn	Cu	Zn	P	pH	Sand	Silt	Clay	ln_TB	ln_TV	ln_*V.vul*	ln_*V.para*	ln_*V.algi*
SOD	1.00																										
OM	0.2721 0.0354	1.00																									
TC	0.2194 0.0921	0.3439 0.0071	1.00																								
TOC	0.3378 0.0083	0.7982 <0.0001	0.4213 0.0008	1.00																							
TIC	0.0519 0.6935	-0.0689 0.6010	0.8619 <0.0001	-0.0967 0.4621	1.00																						
TN	0.5767 <0.0001	0.4818 <0.0001	0.52696 <0.0001	0.5553 <0.0001	0.2678 0.0386	1.00																					
NH_3-N	0.2290 0.0785	0.0121 0.9271	0.2362 0.0692	0.0817 0.5351	0.2136 0.1013	0.5833 <0.0001	1.00																				
NO_2-N	0.5374 <0.0001	0.1798 0.1691	-0.0050 0.9700	0.1616 0.2175	-0.0960 0.4665	0.3494 0.0062	0.1820 0.1640	1.00																			
NO_3-N	-0.2887 0.0253	0.1049 0.4253	-0.0542 0.6806	0.09444 0.4729	-0.1123 0.3929	-0.0722 0.5835	-0.03951 0.7644	0.0214 0.8711	1.00																		
TS	0.2778 0.0317	0.7932 <0.0001	0.2305 0.0764	0.8067 <0.0001	-0.1981 0.1293	0.4177 0.0089	0.0014 0.9918	0.0473 0.7195	0.0273 0.8361	1.00																	
Ca	0.2699 0.0370	0.0374 0.7764	0.3879 0.0022	0.0741 0.5738	0.3842 0.0024	0.3613 0.0046	0.2369 0.0684	0.2082 0.1104	-0.0209 0.8795	-0.0709 0.5902	1.00																
Mg	0.2683 0.0382	0.7679 <0.0001	0.3349 0.0089	0.7421 <0.0001	-0.0473 0.7194	0.6018 <0.0001	0.1686 0.1978	0.1405 0.2844	0.0828 0.5296	0.7024 <0.0001	0.30661 0.0172	1.00															
K	0.3465 0.0067	0.2005 0.1246	0.1167 0.3745	0.1999 0.1258	0.0163 0.9014	0.4991 <0.0001	0.3549 0.0054	0.3134 0.0148	-0.0358 0.7859	0.0221 0.8671	0.39715 0.0017	0.46503 0.0002	1.00														
Fe	0.0315 0.8112	0.5497 <0.0001	0.1389 0.2899	0.6556 <0.0001	-0.2141 0.1005	0.1298 0.3231	-0.1219 0.3535	-0.0500 0.7045	0.2336 0.0725	0.7060 <0.0001	-0.14951 0.2542	0.49579 <0.0001	-0.2240 0.0853	1.00													
Mn	0.3146 0.0143	0.1518 0.2469	0.0152 0.9080	0.1781 0.1735	-0.0828 0.5295	0.1086 0.4087	-0.0214 0.8712	0.0561 0.6706	-0.0935 0.4772	0.1888 0.1486	0.12108 0.3568	0.27957 0.0305	0.1456 0.2670	0.3275 0.0106	1.00												
Cu	0.5799 <0.0001	0.0487 0.7119	0.6641 0.6266	0.0249 0.8500	0.0564 0.6685	0.4306 0.0006	0.5202 <0.0001	0.4463 0.0004	-0.1091 0.4066	-0.1273 0.3324	0.19178 0.1421	0.0945 0.5210	0.39550 0.0018	-0.1176 0.3711	0.2678 0.0386	1.00											
Zn	0.5336 <0.0001	0.2317 0.0748	0.3956 0.0018	0.2893 0.0250	0.2723 0.0353	0.5527 <0.0001	0.3863 0.0023	0.3601 0.0047	-0.1266 0.3351	0.1881 0.1500	0.15780 0.2285	0.2889 0.0252	0.3575 0.0050	0.0823 0.5317	0.4340 0.0005	0.5778 <0.0001	1.00										
P	0.2044 0.1173	0.0926 0.4816	0.5769 <0.0001	0.1726 0.1872	0.5363 <0.0001	0.3443 0.0071	0.2684 0.0381	0.0966 0.4626	0.0506 0.7009	-0.0575 0.6625	0.46483 0.0002	0.0310 0.8141	0.1411 0.2822	-0.1647 0.2086	-0.1431 0.2753	0.1674 0.2011	0.4277 0.0007	1.00									
pH	0.1468 0.2632	-0.2039 0.1181	0.1901 0.1458	-0.2306 0.0763	0.3374 0.0084	0.2140 0.1006	0.2022 0.1213	0.1605 0.2206	-0.0983 0.4550	-0.42213 0.0008	0.47146 0.0001	-0.1793 0.1705	0.2723 0.0353	-0.3565 <0.001	-0.4578 0.0002	0.2089 0.1092	-0.0881 0.5032	0.4711 0.0001	1.00								
Sand	0.0331 0.8020	0.1247 0.3425	0.4405 0.0004	0.1975 0.1304	0.3729 0.0033	-0.0383 0.7715	-0.2209 0.0899	-0.1950 0.1355	-0.1704 0.1930	0.1397 0.2872	0.05786 0.6606	-0.1492 0.2552	-0.4411 0.0004	0.0924 0.4827	-0.1818 0.1645	-0.2887 0.0253	0.0212 0.8722	0.3075 0.0169	0.1440 0.2723	1.00							
Silt	-0.1115 0.3965	-0.0938 0.4760	-0.3630 0.0044	-0.2419 0.0626	-0.2632 0.0422	-0.1334 0.3096	0.1089 0.4432	-0.0281 0.8313	0.1694 0.1957	-0.0790 0.5441	-0.1254 0.3399	0.0639 0.6278	0.1489 0.2562	-0.0232 0.8804	0.2441 0.0414	0.0370 0.7790	0.0174 0.8953	-0.2380 0.0671	-0.2704 0.0366	-0.5410 <0.0001	1.00						
Clay	0.0230 0.8617	-0.0952 0.4696	-0.3107 0.0157	-0.0950 0.4704	-0.2878 0.0258	0.1191 0.3649	0.1995 0.1265	0.2455 0.0587	0.1014 0.4410	-0.1202 0.3602	0.0010 0.9938	0.1359 0.3004	0.4337 0.0005	-0.0972 0.4601	0.0586 0.6566	0.3172 0.0135	-0.0380 0.7730	-0.2270 0.0811	-0.0125 0.9246	-0.8693 <0.0001	0.6550 0.6766	1.00					
ln_TB	0.1078 0.4124	-0.0413 0.7539	-0.0177 0.8933	-0.0228 0.8626	-0.0066 0.9598	0.2059 0.1146	0.2898 0.0287	0.3536 0.0056	0.0865 0.5110	-0.2460 0.0581	0.3659 0.0040	0.1675 0.2007	0.4109 0.0011	-0.2665 0.0096	-0.1846 0.1580	0.3091 0.0162	-0.0260 0.8435	0.1409 0.2829	0.4065 0.0013	-0.3545 0.0054	-0.2427 0.0617	0.5644 <0.0001	1.00				
ln_TV	0.5081 <0.0001	0.3369 0.0085	0.4612 0.0002	0.4075 0.0012	0.2782 0.0313	0.4887 <0.0001	0.2740 0.0341	0.2919 0.0236	-0.0353 0.7887	0.2213 0.0893	0.3524 0.0058	0.2619 0.0433	0.1583 0.2272	0.2051 0.1159	-0.0320 0.8082	0.3365 0.0086	0.3526 0.0057	0.4825 <0.0001	0.2759 0.0329	0.3481 0.0064	-0.1853 0.1563	-0.3069 0.0171	0.0141 0.9150	1.00			
ln_*V. vul*	0.5732 <0.0001	0.2941 0.0226	0.3799 0.0028	0.3368 0.0085	0.2286 0.0790	0.4713 0.0001	0.2504 0.0536	0.4149 0.0010	-0.1410 0.2826	0.1251 0.3407	0.4402 0.0004	0.2411 0.0635	0.2280 0.0797	0.0391 0.7668	0.0112 0.9325	0.4311 0.0006	0.3501 0.0061	0.3935 0.0019	0.3761 0.0031	0.3112 0.0155	-0.3027 0.0188	-0.1968 0.1318	0.2242 0.0850	0.7969 <0.0001	1.00		
ln_*V.para*	0.4336 0.0005	0.2196 0.0918	0.2935 0.0228	0.2649 0.0408	0.1741 0.1835	0.3509 0.0060	0.1531 0.2430	0.3917 0.0020	-0.0862 0.5124	0.0758 0.5650	0.4571 0.0002	0.2479 0.0562	0.2028 0.1201	0.1017 0.4396	-0.0494 0.7077	0.2710 0.0362	0.2018 0.1221	0.3352 0.0089	0.2594 0.0453	0.1058 0.4209	-0.2373 0.0679	0.0147 0.9114	0.3582 0.0049	0.7027 <0.0001	0.7812 <0.0001	1.00	
ln_*V.algi*	0.4933 <0.0001	0.2551 0.0491	0.2537 0.0505	0.2582 0.0464	0.1340 0.3075	0.3637 0.0043	0.1731 0.1859	0.4222 0.0008	-0.0818 0.5343	-0.0039 0.9762	0.4366 0.0005	0.2196 0.0919	0.3396 0.0079	-0.0810 0.5382	-0.0559 0.6714	0.3565 0.0052	0.2080 0.1108	0.3488 0.0063	0.3987 0.0016	0.0955 0.4680	-0.1829 0.1620	-0.0041 0.9751	0.3773 0.0030	0.6173 <0.0001	0.7490 <0.0001	0.8008 <0.0001	1.00

Note: 1. In = Natural log (ln) transformation. 2. In Table 5, the upper numbers represent the correlation coefficients (R), and the lower numbers are p-values. 3. Cell highlights indicate the statistical significance of correlation coefficients. 4. Specific colors represent groups of soil and bacteria variables (in a column): Yellow = Group of organic substances or relation, Light orange = Group of exchangeable cations, Light blue = Group of extractable trace elements, Light gold = Phosphorus, Green = Group of soil textures, and Gray = Group of bacteria.

TABLE 6 Summary of stepwise selection.

Summary of stepwise selection							
	Effect			Number	Score	Wald	Pr >ChiSq
Step	Entered	Removed	DF	In	Chi-Square	Chi-Square	
1	Mn		1	1	13.6324		0.0002
2	TOC		1	2	10.0485		0.0015
3	ln_*V. parahaemolyticus*		1	3	11.9699		0.0005
4	Mg		1	4	5.9444		0.0148

analysis results from this study are notably congruent with the findings of Akazawa and Eguchi (2017), who conducted experimental research involving white shrimp cultivation in tanks with pond soil substrates infected by *V. parahaemolyticus* (AHPND/EMS). Their study encompassed seawater with varying pH levels, notably 7.5 and 8.8. Their findings underscored a positive correlation between pathogen quantity and water pH, providing robust support for the present study's results.

Lastly, the correlation analysis between different variables has shown that the amount of EDOM estimated from SOD is different in ponds with the disease and in ponds without it. The EDOM is a variable that significantly influences the pond's internal environment. This is because the quantity of disease pathogens in the pond increases in proportion to the organic matter content (Alfiansah et al., 2018). The accumulation of organic matter not only

TABLE 7 Analysis of maximum likelihood estimates.

The logistic procedure					
Analysis of maximum likelihood estimates					
Parameter	**DF**	**Parameter estimate**	**Standard error**	**Wald Chi-Square**	**Pr > ChiSq**
Intercept	1	−1.5241	0.5942	6.5787	0.0103
TOC	1	−2.4982	0.7843	10.1448	0.0014
Mg	1	0.00275	0.00116	5.5720	0.0183
Mn	1	0.2376	0.0790	9.0385	0.0026
In_*V. parahaemolyticus*	1	0.1913	0.0644	8.8105	0.0030

TABLE 8 Odds ratio estimates.

		95% Wald	
Effect	**Point estimates**	**Confidence**	**Limits**
TOC	0.082	0.018	0.383
Mg	1.003	1.000	1.005
Mn	1.268	1.086	1.481
In_*V. parahaemolyticus*	1.211	1.067	1.374

impacts the pathogen quantity but also affects soil quality, water quality, and the health of shrimp. It is anticipated that this variable could be a direct or indirect factor contributing to AHPND infection in shrimp (Boyd & Phu, 2018). In both diseased and nondiseased ponds, correlation patterns were identified between organic matter (including substances related to organic matter) and both exchangeable cations and extractable trace elements. These positive correlations arise because exchangeable cations and extractable trace elements are integral components of organic matter. As organic matter undergoes decomposition, significant quantities of these groups are released. Additionally, the correlation between bacterial variables and phosphorus levels can be traced back to the accumulation of organic substances on the pond floor. The decomposition process liberates phosphorus, thereby enhancing bacterial proliferation, as highlighted by a blue rectangular perimeter in Tables 4 and 5. This relationship underscores the interconnected dynamics between organic matter decomposition, nutrient release, and bacterial growth in pond ecosystems.

3.4 | Relationship of pond bottom soil and bacterial variables on AHPND development

Logistic regression analysis was employed to discern the relationship between various soil and bacterial variables in the bottom soil of marine shrimp ponds and the incidence of AHPND. This statistical approach facilitated the development of a logistic regression model to predict the likelihood of AHPND occurring in marine shrimp, using regression coefficients to calculate the odds ratio for each independent variable. Excluding variables for lime requirement and *V. harveyi* because of the prevalence of zero observations, a selection of 22 soil and 5 bacterial variables from a total of 30 ponds—comprising both 15 infected and 15 uninfected ponds—underwent a rigorous process of variable and model selection. This process, outlined in Section 2.6, ultimately identified four variables significantly correlated with the occurrence of AHPND: TOC, magnesium (Mg), manganese (Mn), and *V. parahaemolyticus*. These variables represent key groups: TOC for organic matter, Mg for exchangeable cations, Mn for extractable trace elements, and

V. parahaemolyticus for the bacterial variables, thus providing a nuanced understanding of the factors influencing AHPND risk. The statistical software's selection of these variables enables the construction of a predictive model for AHPND occurrence, summarizing the model fitting process in Tables 6–8.

The logistic regression model for predicting the probability of AHPND occurrence in marine shrimp ponds can be constructed as follows:

$$\text{Log (odds of AHPND occurring)} = -1.5241 - 2.4982 \cdot \text{TOC} + 0.00275 \cdot \text{Mg} + 0.2376 \cdot \text{Mn} + 0.1913 \cdot \text{ln\_}V.\ parahaemolyticus.$$

In this equation, the log (odds) value falls between 0 and 1.

Several studies have previously utilized logistic regression models to forecast the probability of disease occurrence in various contexts. These investigations have considered an array of independent variables, encompassing diverse factors such as general management (8 variables), symptom presentation (7 variables), visceral status (7 variables), and environmental factors (9 variables) (Khiem et al., 2020). Furthermore, other research endeavors have delved into farm-level factors (18 variables) and pond-level factors (31 variables) (Boonyawiwat et al., 2017). In addition, specific studies have scrutinized site characteristics (13 variables) and farming systems and practices (18 variables) (Leung et al., 2000).

Researchers have documented several factors associated with AHPND occurrence, including geographical location (district), water depth, weather events, fertilizer usage, the application of probiotics for water treatment, hatchery practices, and the use of minerals and algicides in water treatment protocols (Boonyawiwat et al., 2018). Additionally, factors such as hepatopancreatic atrophy, toughness, pallor, high water temperature, salinity levels, and seedstock quality have been identified as contributors to an elevated risk of AHPND (Khiem et al., 2020).

However, it is imperative to emphasize that the physicochemical attributes of pond bottom soil and the quantities of bacterial pathogens within pond substrates have not been previously documented or included as explanatory variables within a logistic regression model. This underlines the novelty and significance of the present study in shedding light on previously unexplored factors that may influence the occurrence of AHPND in marine shrimp ponds.

4 | CONCLUSIONS

This study delved into the differences in soil attributes and the prevalence of pathogenic bacteria between shrimp ponds impacted by AHPND and those unimpacted, while also exploring the spatial distribution of soil characteristics in flat-oriented pond soil strata. Three key insights have emerged from this study:

The study concludes that ponds afflicted with AHPND exhibit significantly higher levels of EDOM, as measured by SOD, compared with noninfected ponds. This distinction emphasizes differing management practices and highlights that elevated EDOM levels in infected ponds contribute to adverse outcomes, including increased concentrations of *V. parahaemolyticus* and other pathogens, as well as a rise in trace elements because of organic matter accumulation. These elements, in forming complex structures with organic matter, affect soil and water quality, potentially triggering or exacerbating AHPND outbreaks. The findings suggest a targeted assessment of EDOM, rather than total organic content, for effective pond management, as it significantly influences ecosystem health. Additionally, pathogenic bacteria, capable of infiltrating soil up to 10 cm deep, necessitate soil disinfection practices extending beyond this depth for disease control. The observed excessive lime usage in diseased ponds, indicated by higher calcium and total inorganic carbon levels, points to a potential link with AHPND prevalence and calls for a reassessment of lime application practices. Moreover, the sediment distribution pattern influenced by aeration methods, particularly around the pond's center and at shallower depths, suggests a need for strategic placement and use of aerators to mitigate undesirable sediment accumulation. To enhance pond management and disease prevention, we recommend revising lime application rates, adopting comprehensive soil disinfection protocols, and optimizing aeration practices to maintain balanced EDOM levels and prevent pathogen proliferation.

The results of the Pearson correlation analysis (r) among 27 variables of soil and pathogenic variables in shrimp ponds of both groups reveal intriguing findings. It was observed that the correlation patterns between the two pond groups were both similar and different. The different correlations between both pond groups are the positive correlation between disease-related microbial variables and various other groups of variables, including organic matter and related organic matter variables, exchangeable cations variables, extractable trace element variables, and soil pH values. Such distinct correlation patterns were evident only in ponds infected with AHPND. These correlation patterns were absent in ponds that were not affected by the disease. This highlights the impact of EDOM accumulation in pond bottom soils when present in significant quantities. Consequently, it leads to the emergence of correlations between disease-causing bacterial variables and other variable groups.

The relationships observed in the AHPND-infected pond group can be explained as follows: The number of microorganisms increases with the amount of organic matter (readily degradable) within the pond. When the amount of organic matter increases, it leads to an increase in the quantity of extractable trace elements and exchangeable cations. This increase is a consequence of organic matter breaking down, releasing various mineral elements that are components of the structure, or forming complex structures because of interactions between organic matter and trace elements. In the case of calcium, it can also result from the higher application of lime in the disease ponds, which can cause a correlation between the bacterial variable group and calcium.

The correlation between the quantity of microorganisms and soil pH values is a result of the fact that bacteria's activity is impaired, and their quantity is reduced at lower pH values compared with fungi. Therefore, when soil pH is increased, it leads to an increase in microorganism quantity. Based on this study and other works, it is more likely that higher pH contributes to promoting the abundance of *V. parahaemolyticus* (and others), causing AHPND infection.

The results of the logistic regression analysis aimed to identify soil and microorganism variables correlated with the occurrence of AHPND disease. All 27 independent variables were included in this analysis, comprising 22 soil variables and 5 microorganism variables. The program selected four variables that exhibited a relationship with the disease, and these variables were used to create a logistic regression equation to predict the odds of AHPND disease occurrence in pond-raised marine shrimp. The equation incorporates three soil variables: TOC, magnesium (Mg), manganese (Mn), and one microorganism variable: *V. parahaemolyticus*. The resulting logistic regression equation for predicting the odds of AHPND occurrence, where the log (odds) value falls between 0 and 1, is as follows:

$$\text{Log (odds of AHPND occurring)} = -1.5241 - 2.4982 \cdot \text{TOC} + 0.00275 \cdot \text{Mg} + 0.2376 \cdot \text{Mn} + 0.1913 \cdot \text{In\_}V.\ parahaemolyticus.$$

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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