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Associations between the Presence of Bacteria and the Physico-Chemical Parameters of Water in Peat Swamp Forest, Paddy Field and Oil Palm Plantation in North Selangor, Malaysia

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ABSTRACT

This study determines the associations between the presence of bacteria and water physico-chemical parameters in peat swamp forest, paddy field and oil palm plantation in north Selangor, Malaysia. Sampling of bacteria and water physico-chemical parameters were conducted from four sites in the peat swamp forest, two sites each in paddy field and oil palm plantations. Oil palm plantation recorded the highest bacterial diversity (Shannon's H = 3.3713) and richness ($I_{Margalef}$ = 11.5955), while peat swamp forest showed highest bacterial evenness (Pielou's e = 0.9526). A total of 3,421 bacterial isolates from 39 bacterial species were obtained, which comprised of 11 Gram-positive and 28 Gram-negative bacteria. The highest number of bacteria was recorded in the oil palm plantation (1,552 isolates from 38 species), followed by the paddy field (1,191 isolates from 30 species) and the peat swamp forest (678 isolates from 22 species). In general, the most abundant bacteria was *Escherichia coli* (333 isolates; 9.73 %), followed by *Salmonella* spp. (288 isolates; 8.42 %), and *Streptococcus agalactiae* (252 isolates; 7.37 %). Moreover, *E. coli* recorded the highest isolated bacterium in the peat swamp forest (10.47%), paddy field (10.66%) and the oil palm plantation (8.7%). Inconsistent association was observed between the water physico-chemical parameters and the presence of bacteria in all studied habitats. However, multivariate analyses showed that water temperature, NH_3-N, Cl_2, DO, EC, SO_4 and PO_4 were able to influence

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the bacterial presence. This study showed that oil palm plantation and paddy field had the highest number of isolates, species, and bacterial concentrations due to the extensive anthropogenic activities in these areas.

Keywords: Association, bacteria, north Selangor peat swamp forest, water physico-chemical parameters

INTRODUCTION

The north Selangor peat swamp forest (NSPSF) is located on a flat coastal plain in the northern part of Selangor, Malaysia. The peat soil of NSPSF has high content of organic materials in various stages of decomposition, highly acidic with pH as low as 3.5, and contains limited amounts of nutrients but high carbon content (Global Environment Centre [GEC], 2014; Sule et al., 2016; Yule & Gomez, 2008). Currently, the NSPSF is the most extensively cleared peat swamp forest in Peninsular Malaysia. This is partly due to the proximity to the Integrated Agricultural Development Project, a paddy production scheme, which is the single biggest agricultural investment in the state. The NSPSF also suffers large-scale conversion to oil palm plantations, which is presently the biggest threat to peat swamp forests in the Southeast Asian region (Koh et al., 2011).

Numerous studies revealed a diverse microbial flora in Malaysian peat swamps (Jackson et al., 2009; Yule & Gomez, 2008). Jackson et al. (2009) employed molecular techniques to study the microbial communities of the peat sediment in NSPSF and found that the microbial communities are dominated by Acidobacteria and Crenarchaeota, with Archaea is limited to, but dominating the deeper samples. Moreover, they also revealed the lack of methanogenic bacteria in the microbial communities of NSPSF. This was supported by stable C isotope analyses of the peat which revealed depleted values of ^{13}C (Yule & Gomez, 2008). Fish from the same peat swamp also recorded lower ^{13}C values than those from freshwaters, indicating that the bacteria that respired carbon were being assimilated throughout the aquatic food web (Yule & Gomez, 2008). Moreover, gut analyses showed the most abundant invertebrates (mayfly and chironomid larvae) mainly ingested fine particulate organic matters that were largely composed of bacteria. These in turn were eaten by carnivorous invertebrates and fishes (Yule & Gomez, 2008). The importance of these is that the bacteria form the base of the peat swamp food web, illustrating a relationship between fish and bacteria in water and sediment.

However, previous studies only focussed on the dominant peat sediment bacterial communities and the leaf-degrading bacteria using molecular techniques and C isotope analyses. Studies on the diversity and distribution of bacterial species in sediment, water and fishes in peat swamp that are usually used as bio-indicators are generally lacking. Thus, this study determines the bacterial abundance and diversity in peat swamp forest, paddy field and oil palm plantation in north Selangor, while at the same time identifies the impact of water physico-chemical parameters on their presence.

MATERIALS AND METHODS

Study Area

NSPSF consisted of four forest reserves, namely the Raja Musa, Sungai Karang, Bukit Belata Extension and Sungai Dusun/ Wildlife Reserves. There was a stretch of paddy fields in NSPSF, which is one of the primary rice granary area in Malaysia, covering an area of 18980 hectares. An extensive area of the peat swamp forest has been converted to oil palm plantation, but remained a part of the NSPSF (GEC, 2014; Sule, 2016; Sule et al., 2016). The flora of NSPSF consisted of very tolerant tree species with relatively low diversity. The main sources of water entering the NSPSF were the rainfall and occasional water overflow from the Bernam River, Kuala Selangor. The NSPSF has a mean annual rainfall ranging from 1359 to 2480 mm, a mean temperature of 27°C and a mean relative humidity of 79.3%. The rainfall varies with distinct seasons of the year, wet/ rainy seasons (March-April and October-November) and relatively dry seasons (January-February and May-September) (GEC, 2014).

Eight sampling sites, comprising four sites in the peat swamp, and two each in the paddy field and oil palm plantation were selected. The sites were located within the Kampung Sungai Sireh area, Tanjong Karang, such that all peat swamp sites were on one side separated from the paddy field and oil palm plantation sites by the Tengi River, Tanjong Karang, along its entire length (Figure 1). Samplings were done thrice for water physicho-chemical analyses, fish collection and bacterial examination during the dry month (June 2015), relatively high rainfall month (October 2015) and a moderately dry month (January, 2016). A total of 24 sampling points, three points at each sampling site, were selected for this study.

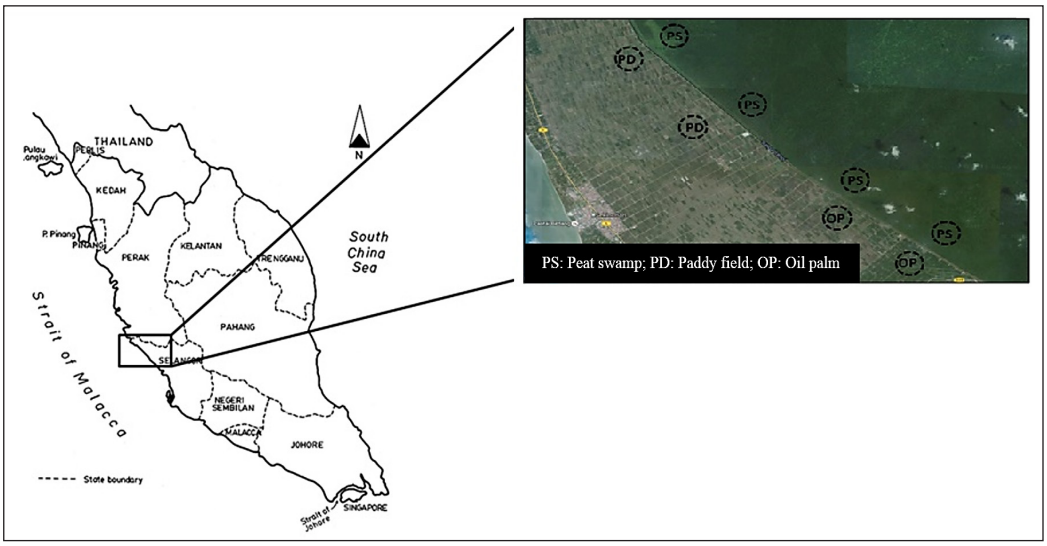

Figure 1. Map of Peninsular Malaysia with enlarged view of north Selangor peat swamp forest and study sites

Peat Swamp Forest

Four sampling sites in peat swamp forest were separated at ~ 200 m. Site 1 was located at 3° 34' 40.5444'' N, 101° 7' 0.4152'' E; site 2 at 3° 33' 7.1712'' N, 101° 8' 36.15'' E; site 3 at 3° 31' 9.4188'' N, 101° 10' 43.7736'' E; and site 4 at 3° 29' 45.7692'' N, 101° 12' 37.8864'' E. Site 1 had undergone significant logging, and dominated by shrubs with only very few trees present. During the third visit to the site, we noticed further degradation, where majority had been cleared and burnt. A major dumpsite was within the proximity of the site. Sites 2 and 3 had also undergone some logging, but had more vegetation cover than site 1. Site 4 appeared to be pristine, preserved in its natural form and untouched. The site was almost completely covered with vegetation, with many mature dipterocarp and other trees. It was relatively hidden and difficult to access.

Paddy Field

Two sampling sites in paddy field were also separated by ~ 200 m, and were ~ 500 m away from the peat swamp forest sites. Site 1 was located at 3° 34' 15.4164'' N, 101° 6' 43.2864'' E and site 2 at 3° 32' 40.8012'' N, 101° 8' 23.892'' E. These sites were typically artificial freshwater swamps dominated by the swamp grass with shrubs at the bank. The entire site areas were completely exposed to direct sunlight without vegetation cover. These sites were converted from the peat swamp forest as evident in the peat characteristics retained in the soil and water. The sites were characterised by the presence of trenches and screens firmly or loosely placed at interval. There were networks of large irrigation canals leading from the paddy sites to the Tengi River. Within the proximity of each site were human dwellings and other agricultural infrastructures. Intense application of insecticides/pesticides was observed during visits to the sites.

Oil Palm Plantation

Two sites within the oil palm plantation were also separated by ~ 200 m, and were ~ 500 m away from the peat swamp forest site. Site 1 was located at 3° 30' 41.7384'' N, 101° 10' 32.2068'' E, and ~ 300 m away from paddy site 2. Meanwhile, site 2 was located at 3° 29' 19.8816'' N, 101° 12' 31.5864'' E. The sites were dominated by the oil palm plants. The sites were flooded with water during each visit but there were irrigation canals leading to the Tengi River at each site. Site 1 was close to human settlement. The water was visibly polluted with domestic effluents and plastics. The irrigation canal in the oil palm plantation site 1 received direct discharge of waste water. Site 2 was relatively further away from human settlement. Although no direct effluent discharge into the canals was observed, the water was visibly polluted. Site 2 was used as a major litter site.

Water and Sediment Samples for Bacteriological Analyses

Prior to the measurement of water physico-chemical parameters, the water and sediment samples for bacteriological analyses were collected in triplicate from each site, totaling

24 samples for each sampling season. A total of 200 mL of the water sample was collected aseptically from 15 cm below the water surface using sterile polyethylene bottles, and immediately placed in an icebox for transportation to the laboratory. About 100 g of the sediment sample was collected from each station using a sterile scoop, transferred into sterile plastic bag, then immediately placed in an icebox and transported to the laboratory for further processing.

Fish Samples for Bacteriological Analyses

Twenty-four fish samples, or three fish from each site were collected using the scoop nets. Collected fish were transferred into a mini aquarium containing water from the site of collection, and immediately transferred to the laboratory for bacteriological analysis. In this study, we used three spot gourami *Trichopodus trichopterus*, as the target fish due to its availability in all of the three sampling areas in this study.

Bacterial Isolation from Water and Sediment

A total of 1 mL of the water sample and 1 g of the sediment sample was serially diluted to 10^{-6} using sterilized deionized water under complete aseptic condition. Briefly, six dilution tubes and petri dishes containing tryptic soy agar (TSA) (Merck, Darmstadt, Germany) were labelled from 1 to 6 accordingly. Nine mL of sterilized deionized water was transferred into each tube with the aid of a sterile pipette. The original water sample was vortexed. Then, 1 mL of the water sample was transferred into tube 1 and the contents were mixed vigorously.

A total of 1 g of the sediment sample was measured by transferring into pre-weighed sterilized dilution tube 1 containing 9 mL of sterilized deionized water and carefully weighed. The content was mixed vigorously. One mL of the solutions was then transferred from tube 1 to tube 2 using a sterile pipette and the contents were mixed vigorously. The same process was repeated for tubes 3, 4, 5 and 6 to give dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} respectively. Plates of TSA were then inoculated with 1 mL of the dilution from water and sediment in triplicate and incubated for 24 - 48 h at 37°C. Colony counts were performed and the results were expressed as CFU/mL and CFU/g of water and sediment respectively.

Bacterial Isolation from Fish

The skin, gill and intestine of the fish were used for bacterial analysis. The collected fish were euthanized with 250 mg/L of Tricane Methanesulfonate (MS 222), according to methods approved by the Institutional Animal Care and Utilization Committee, Universiti Putra Malaysia. A swab was taken from a 1 cm^2 surface area at the right abdomen of the fish. The swab was diluted in 10 mL of sterilized deionized water. The fish surfaces were then swabbed with 90% ethyl alcohol for surface sterilization prior to gill and intestine samplings. The gill was completely removed and macerated in a sterilized ceramic mortar. One gram of the sample was then measured by transferring

into a pre-weighed sterilized dilution tube containing 9 mL of sterilized deionized water and carefully weighed, and the content was mixed vigorously. The intestine was also removed completely, macerated in sterilized mortar, and 1 g was weighted by transferring into a pre-weighed sterilized dilution tube containing 9 mL of sterilized deionized water and carefully weighed, and the content mixed vigorously. All samples were then serially diluted to 10^{-6} using sterilized deionized water as previously described (Al-Harbi, 2003; Al-Harbi & Uddin, 2003). One mL of the dilution was then inoculated on plates of TSA in triplicate and incubated for 24 - 48 h at 37°C. Colony counts were performed and the results were expressed as CFU/cm^2 for skin, and CFU/g for gill and intestine.

Bacterial Identification from Water, Sediment and Fish

All bacterial growth isolates were sub-cultured on nutrient agar (NA) (Merck) for 24 – 48 h at 37°C to obtain pure isolates. All pure isolates were identified for their Gram staining, following characterization of colony size, structure, shape, elevation, edge, surface, opacity and colour. Thereafter, presumptive biochemical identification tests of oxidase, catalase, motility, amylase, gelatinase, lipase, indole, H_2S production, and nitrite reduction were performed.

Identification of isolates to genus or species level was done using Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). In addition, Gram-positive cocci and catalase-negative isolates were identified to species level using API® 20 Strep (bioMérieux, Marcy l'Etoile, France), Gram-positive cocci and catalase-positive isolates were identified using API® 20 Staph (bioMérieux), and Gram-negative rod were identified using API® 20E (bioMérieux).

Following bacterial identification from water, sediment and fish, the bacterial Shannon-Weaver diversity index (*H'*) (Shannon & Weaver, 1963), Pielou's evenness index (*e*) (Pielou, 1969) and Margalef's richness index ($I_{Margalef}$) (Margalef, 1958) were calculated. The indexes readings were pooled from the water, sediment and fish results. For better understanding and comparison, the results were also combined from all three sampling times and presented as mean for each habitat studied.

Water Physico-Chemical Analyses

The dissolved oxygen (DO), electrical conductivity (EC), pH, salinity, temperature, and total dissolved solids (TDS) were measured *in situ* using an YSI 556 MPS probe (YSI Incorporation, NY, USA). A total of 500 mL of water sample was collected in sterilized polyethylene sampling bottle in replicate from 15 cm below the water surface at each sampling site and transferred into an icebox. The samples were immediately transported to the laboratory. The nitrite (NO_3-N), ammonia-nitrogen (NH_3-N), chlorine (Cl_2), sulphate (SO_4), and phosphate (PO_4) concentration were measured using a DR900 Multiparameter Handheld Calorimeter (Hach Company, Loveland, Colorado, USA).

Statistical Analyses

The data for water physico-chemical parameters were tested for fitness to a normal distribution by the Shapiro-Wilk test, followed by ANOVA and Tukey's pairwise comparisons to test for significant difference of the water physico-chemical data between the three habitats (IBM SPSS, Version 22.0). Statistical significant difference was determined at $P< 0.05$.

In order to identify the relationships between water physico-chemical parameters and bacterial composition, Principal Component Analysis (PCA) was performed using IBM SPSS to reduce variable numbers in the dataset by combining highly correlated variables into factors, while retaining the variability in the data. This was based on the principle that dataset comprised of numerous variables was likely to be redundant if two or more variables were highly correlated with each other. PCA extraction was based on eigenvalues of 1 or greater, which was considered significant. Factor loadings of > 0.75, 0.75–0.50, and 0.50–0.00 were classified as strong, moderate, and weak respectively. Data reduction was performed on the eleven measured water physico-chemical parameters.

Then, water physico-chemical parameters variables that showed variation within the study area were utilized in determining the relationships between water physico-chemical parameters and bacterial composition. The relationships were identified using Canonical Component Analysis (CCA). The CCA was performed using XLSAT add-in for Microsoft Excel (Office 365, Version 2016). The significance of each variable was tested using CCA XLSTAT-ADA with 5000 permutations at a significance level of 5%. Results were presented using canonical biplots and other descriptive statistics.

RESULTS

Bacterial Counts from Water, Sediment and Fish

There was a significant difference ($P<0.05$) between the bacterial concentration in sediments of peat swamp forest, paddy field and oil palm plantation (Table 1). Meanwhile, peat swamp forest had significantly ($P<0.05$) lower bacterial counts in water, fish body surface, gill and intestine. However, no significant difference ($P>0.05$) existed between bacterial counts in water, fish body surface, gill and intestine

Table 1
Bacterial concentration in sediment, water, gill, body surface and intestine of fish

Habitat	Sediment ($\times 10^8$ cfu g^{-1})	Water ($\times 10^8$ cfu mL^{-1})	Fish body surface ($\times 10^8$ cfu cm^{-2})	Fish gill ($\times 10^8$ cfu g^{-1})	Fish intestine ($\times 10^8$ cfu g^{-1})
Peat swamp	0.09 ± 0.02^{aA}	0.59 ± 0.39^{aB}	0.71 ± 0.24^{aB}	1.28 ± 0.36^{aC}	3.87 ± 1.12^{aD}
Paddy field	0.30 ± 0.11^{bA}	1.15 ± 0.40^{bB}	1.16 ± 0.27^{bB}	2.19 ± 0.27^{bC}	5.12 ± 0.93^{bD}
Oil palm	0.48 ± 0.13^{cA}	1.71 ± 0.65^{bB}	1.53 ± 0.54^{bB}	2.35 ± 0.40^{bC}	6.33 ± 1.44^{bD}

Values with different superscript of lower case letters of the same columns are significantly different at $P<0.05$
Values with different superscript of capital letters of the same rows are significantly different at $P<0.05$

between paddy field and oil palm plantation. Moreover, the bacterial counts in sediments of all habitats were significantly ($P<0.05$) lower compared to bacteria counts in water. No significant difference ($P>0.05$) existed between the bacterial counts in water and fish body surface, but significant difference ($P<0.05$) was observed between the bacterial counts in the fish body surface, gill and intestine for all habitats.

Taxonomic Composition of Isolated Bacteria

Bacterial community structure analyses showed that oil palm plantation recorded the highest bacterial diversity (Shannon's $H = 3.3713$) and richness ($I_{Margalef} = 11.5955$), while peat swamp forest showed the highest bacterial evenness (Pielou's $e = 0.9526$) (Table 2).

In general, 39 species of bacteria were isolated throughout the study comprising of 11 Gram-positive and 28 Gram-negative bacteria. The highest number of bacteria was recorded for the oil palm plantation (1,552 isolates from 38 species), followed by the paddy field (1,191 isolates from 30 species), and the peat swamp forest (678 isolates from 22 species). The most abundant bacterial species was *Escherichia coli* (333 isolates; 9.73 %), followed by *Salmonella* spp. (288 isolates; 8.42 %) and *Streptococcus agalactiae* (252 isolates; 7.37 %). Meanwhile the least isolated bacterial species were *Aerococcus urinae* (9 isolates; 0.26 %), *Aeromonas veronii* (9 isolates; 0.26 %), and *Yersinia pseudotuberculosis* (9 isolates; 0.26 %).

The highest number of isolates were recorded in the fish intestine from the peat swamp forest (212 isolates), the paddy field (374 isolates) and the oil palm plantation (484 isolates), while the least number of isolates were recorded in the sediment from peat swamp forest (54 isolates), the paddy field (96 isolates) and the oil palm plantation (118 isolates).

In peat swamp forest, the most isolated bacteria from the sediment and fish intestine were *Bacillus* spp. (13 isolates; 24.07%) and *Salmonella* spp. (21 isolates; 9.91%). However, *E. coli* dominated the isolation of bacteria from water (19 isolates; 14.96%), the fish body surface (17 isolates; 12.78%) and the fish gill (17 isolates; 11.18%). In the paddy field area, *E. coli* was also dominated in the water (32 isolates; 12.50%) and fish gill (28 isolates; 11.11%). However, *Enterococcus pseudoarium*, *Salmonella* spp. and *S. agalactiae* were commonly isolated from the sediment (15 isolates; 15.63%), fish body surface (24 isolates; 11.27%) and fish intestine (37 isolates; 9.89%), respectively. However, for oil palm plantation area, *E. coli* was mostly isolated from the sediment (15 isolates; 12.71%), water (29 isolates; 9.45%), and the fish body surface (33 isolates; 10.61%). Similar isolation rate of *Salmonella* spp. and *S. agalactiae* were recorded in fish gill (31 isolates; 9.34%), while *S. agalactiae* was also dominantly isolated from the fish intestine (42 isolates; 8.68%). The list of isolated bacterial species, their number, percentages, and details of their sources from each habitat are presented in Tables 3 - 6.

Table 2
Bacterial community structure in peat swamp forest, paddy field and oil palm plantation expressed as diversity, evenness and richness index

Habitat	Community structure index		
	Shannon's *H*	Pielou's *e*	$I_{Margalef}$
Peat swamp	2.9445	0.9526	7.4173
Paddy field	3.1826	0.9357	9.4281
Oil palm	3.3713	0.9268	11.5955

Shannon's *H* = Shannon-Weiner diversity index; Pielou's *e* = Pielou's evenness index; $I_{Margalef}$ = Margalef's richness index

Table 3
List of bacteria, abbreviation used, number and percentage of isolates from peat swamp forest, paddy field and oil palm plantation

Bacteria	Abbrev.	Peat swamp (n = 678)		Paddy field (n = 1,191)		Oil palm (n = 1,552)		Total (N = 3,421)	
		No.	%	No.	%	No.	%	No.	%
Aerococcus urinae	A.uri	-	-	-	-	9	0.58	9	0.26
Aeromonas hydrophila	A.hyd	-	-	23	1.93	31	2.00	54	1.58
Aeromonas veronii	A.ver	-	-	-	-	9	0.58	9	0.26
Bacillus spp.†	Baci	59	8.70	78	6.55	92	5.93	229	6.69
Budvicia aquatica	B.aqu	37	5.46	51	4.28	47	3.03	135	3.95
Citobacter diversus	C.div	-	-	27	2.27	40	2.58	67	1.96
Citrobacter koseri	C.kos	32	4.72	57	4.79	65	4.19	154	4.50
Deinobacter grandis	D.gra	19	2.80	28	2.35	25	1.61	72	2.10
Deinococcus proteolyticus†	D.pro	9	1.33	17	1.43	15	0.97	41	1.20
Deinococcus radiopugnans†	D.rad	-	-	7	0.59	9	0.58	16	0.47
Edwardsiella tarda	E.tar	31	4.57	43	3.61	41	2.64	115	3.36
Enterobacter aerogenes	E.aer	-	-	9	0.76	15	0.97	24	0.70
Enterobacter cloacae	E.clo	11	1.62	31	2.60	37	2.38	79	2.31
Enterococcus cecorum†	E.cec	-	-	19	1.60	25	1.61	44	1.29
Enterococcus faecalis†	E.fae	-	-	13	1.09	19	1.22	32	0.94
Enterococcus pseudoarium†	E.pse	26	3.83	37	3.11	47	3.03	110	3.22
Escherichia coli	E.col	71	10.47	127	10.66	135	8.70	333	9.73
Escherichia coli 1	E.co1	-	-	34	2.85	47	3.03	81	2.37
Flavobacterium aquatile	F.aqu	19	2.80	29	2.43	48	3.09	96	2.81
Klebsiella oxytoca	K.oxy	28	4.13	37	3.11	46	2.96	111	3.24
Klebsiella pneumoniae subsp. *ozaenae*	K.pso	21	3.10	32	2.69	46	2.96	99	2.89
Klebsiella pneumoniae subsp. *pneumoniae*	K.psp	-	-	-	-	13	0.84	13	0.38
Lactococcus lactis subsp. *lactis*†	L.lsl	-	-	-	-	15	0.97	15	0.44

Table 3 *(continue)*

Bacteria	Abbrev.	Peat swamp (n = 678)		Paddy field (n = 1,191)		Oil palm (n = 1,552)		Total (N = 3,421)	
		No.	%	No.	%	No.	%	No.	%
Leuconostoc spp.†	Leuc	-	-	15	1.26	29	1.87	44	1.29
Pantoea spp.	Pant	19	2.80	24	2.02	32	2.06	75	2.19
Pragia fontium	P.fon	27	3.98	37	3.11	42	2.71	106	3.10
Proteus mirabilis	P.mir	16	2.36	23	1.93	31	2.00	70	2.05
Proteus vulgaris	P.vul	14	2.06	19	1.60	29	1.87	62	1.81
Rahnella aquatilis	R.aqu	29	4.28	39	3.27	43	2.77	111	3.24
Salmonella choleraesius subsp. *choleraesius*	S.csc	-	-	-	-	11	0.71	11	0.32
Salmonella enterica	S.ent	-	-	19	1.60	33	2.13	52	1.52
Salmonella spp.	Salm	63	9.29	103	8.65	122	7.86	288	8.42
Serratia fonticola	S.fon	23	3.39	47	3.95	52	3.35	122	3.57
Spirochaeta aurantia	S.aur	19	2.80	-	-	-	-	19	0.56
Staphylococcus aureus subsp. *aureus*†	S.asa	-	-	-	-	15	0.97	15	0.44
Staphylococcus spp.†	Stap	58	8.55	77	6.47	101	6.51	236	6.90
Streptococcus agalactiae†	S.aga	47	6.93	89	7.47	116	7.47	252	7.37
Vibrio cholerae	V.cho	-	-	-	-	11	0.71	11	0.32
Yersinia pseudotuberculosis	Y.pse	-	-	-	-	9	0.58	9	0.26

†: Indicate Gram-positive
-: Absent

Table 4
List of bacteria, abbreviation used, number and percentage of isolates from sediment, water, gill, body surface and intestine of fish in peat swamp forest

Bacteria	Abbrev.	Sediment (n = 54)		Water (n = 127)		Body surface (n = 133)		Gill (n = 152)		Intestine (n = 212)	
		No.	%	No.	%	No.	%	No.	%	No.	%
Bacillus spp.†	Baci	13	24.07	9	7.09	12	9.02	8	5.26	17	8.02
Budvicia aquatica	B.aqu	2	3.70	3	2.36	10	7.52	9	5.92	13	6.13
Citrobacter koseri	C.kos	0	0.00	3	2.36	7	5.26	9	5.92	13	6.13
Deinobacter grandis	D.gra	1	1.85	2	1.57	4	3.01	5	3.29	7	3.30
Deinococcus proteolyticus†	D.pro	0	0.00	2	1.57	3	2.26	2	1.32	2	0.94
Edwardsiela tarda	E.tar	3	5.56	7	5.51	6	4.51	4	2.63	11	5.19
Enterobacter cloacae	E.clo	0	0.00	3	2.36	1	0.75	2	1.32	5	2.36
Enterococcus pseudoarium†	E.pse	1	1.85	4	3.15	5	3.76	5	3.29	11	5.19
Escherichia coli	E.col	5	9.26	19	14.96	17	12.78	17	11.18	13	6.13
Flavobacterium aquatile	F.aqu	2	3.70	3	2.36	4	3.01	6	3.95	4	1.89
Klebsiella oxytoca	K.oxy	1	1.85	6	4.72	4	3.01	5	3.29	12	5.66

Table 4 *(continue)*

Bacteria	Abbrev.	Sediment (n = 54)		Water (n = 127)		Body surface (n = 133)		Gill (n = 152)		Intestine (n = 212)	
		No.	%	No.	%	No.	%	No.	%	No.	%
Klebsiella pneumoniae subsp. *ozaenae*	K.pso	0	0.00	3	2.36	3	2.26	8	5.26	7	3.30
Pantoea spp.	Pant	3	5.56	2	1.57	3	2.26	5	3.29	6	2.83
Pragia fontium	P.fon	5	9.26	12	9.45	5	3.76	2	1.32	3	1.42
Proteus mirabilis	P.mir	0	0.00	3	2.36	4	3.01	3	1.97	6	2.83
Proteus vulgaris	P.vul	2	3.70	2	1.57	2	1.50	4	2.63	4	1.89
Rahnella aquatilis	R.aqu	2	3.70	5	3.94	7	5.26	6	3.95	9	4.25
Salmonella spp.	Salm	4	7.41	13	10.24	11	8.27	14	9.21	21	9.91
Serratia fonticola	S.fon	0	0.00	5	3.94	4	3.01	5	3.29	9	4.25
Spirochaeta aurantia	S.aur	1	1.85	2	1.57	3	2.26	9	5.92	4	1.89
Staphylococcus spp.†	Stap	6	11.11	9	7.09	11	8.27	13	8.55	19	8.96
Streptococcus agalactiae†	S.aga	3	5.56	10	7.87	7	5.26	11	7.24	16	7.55

†: Indicate Gram-positive

Table 5
List of bacteria, abbreviation, number and percentage of isolates from sediment, water, gill, body surface and intestine in paddy field

Bacteria	Abbrev.	Sediment (n = 96)		Water (n = 256)		Body surface (n = 213)		Gill (n = 252)		Intestine (n = 374)	
		No.	%	No.	%	No.	%	No.	%	No.	%
Aeromonas hydrophila	A.hyd	10	10.42	4	1.56	3	1.41	5	1.98	10	2.67
Bacillus spp.†	Baci	4	4.17	15	5.86	13	6.10	18	7.14	22	5.88
Budvicia aquatica	B.aqu	4	4.17	11	4.30	7	3.29	13	5.16	16	4.28
Citobacter diversus	C.div	9	9.38	6	2.34	4	1.88	3	1.19	10	2.67
Citrobacter koseri	C.kos	3	3.13	11	4.30	7	3.29	9	3.57	21	5.61
Deinobacter grandis	D.gra	0	0	6	2.34	4	1.88	6	2.38	9	2.41
Deinococcus proteolyticus†	D.pro	0	0	5	1.95	3	1.41	2	0.79	7	1.87
Deinococcus radiopugnans†	D.rad	2	2.08	3	1.17	2	0.94	1	0.40	1	0.27
Edwardsiela tarda	E.tar	1	1.04	7	2.73	6	2.82	13	5.16	15	4.01
Enterobacter aerogenes	E.aer	1	1.04	2	0.78	1	0.47	1	0.40	4	1.07
Enterobacter cloacae	E.clo	1	1.04	4	1.56	5	2.35	9	3.57	12	3.21
Enterococcus cecorum†	E.cec	0	0	4	1.56	2	0.94	5	1.98	7	1.87
Enterococcus faecalis†	E.fae	1	1.04	3	1.17	1	0.47	4	1.59	5	1.34
Enterococcus pseudoarium†	E.pse	15	15.63	6	2.34	6	2.82	10	3.97	14	3.74
Escherichia coli	E.col	2	2.08	32	12.50	21	9.86	28	11.11	31	8.29
Escherichia coli 1	E.co1	2	2.08	12	4.69	5	2.35	7	2.78	8	2.14

Table 5 *(continue)*

Bacteria	Abbrev.	Sediment (n = 96)		Water (n = 256)		Body surface (n = 213)		Gill (n = 252)		Intestine (n = 374)	
		No.	%	No.	%	No.	%	No.	%	No.	%
Flavobacterium aquatile	F.aqu	3	3.13	6	2.34	5	2.35	11	4.37	5	1.34
Klebsiella oxytoca	K.oxy	0	0	8	3.13	7	3.29	10	3.97	9	2.41
Klebsiella pneumoniae subsp. *ozaenae*	K.pso	0	0	8	3.13	8	3.76	9	3.57	7	1.87
Leuconostoc spp.†	Leuc	2	2.08	6	2.34	2	0.94	2	0.79	5	1.34
Pantoea spp.	Pant	3	3.13	7	2.73	5	2.35	1	0.40	9	2.41
Pragia fontium	P.fon	2	2.08	8	3.13	9	4.23	7	2.78	10	2.67
Proteus mirabilis	P.mir	2	2.08	7	2.73	5	2.35	5	1.98	4	1.07
Proteus vulgaris	P.vul	4	4.17	5	1.95	5	2.35	3	1.19	4	1.07
Rahnella aquatilis	R.aqu	1	1.04	11	4.30	5	2.35	8	3.17	11	2.94
Salmonella enterica	S.ent	11	11.46	8	3.13	3	1.41	2	0.79	5	1.34
Salmonella spp.	Salm	3	3.13	13	5.08	24	11.27	21	8.33	34	9.09
Serratia fonticola	S.fon	6	6.25	14	5.47	11	5.16	7	2.78	12	3.21
Staphylococcus spp.†	Stap	3	3.13	11	4.30	15	7.04	15	5.95	30	8.02
Streptococcus agalactiae†	S.aga	10	10.42	13	5.08	19	8.92	17	6.75	37	9.89

†: Indicate Gram-positive

Table 6
List of bacteria, abbreviation, number and percentage of isolates from sediment, water, gill, body surface and intestine in oil palm plantation

Bacteria	Abbrev.	Sediment (n = 118)		Water (n = 307)		Surface (n = 311)		Gill (n = 332)		Intestine (n = 484)	
		No.	%	No.	%	No.	%	No.	%	No.	%
Aerococcus urinae	A.uri	0	0	6	1.95	1	0.32	0	0.00	2	0.41
Aeromonas hydrophila	A.hyd	1	0.85	8	2.61	4	1.29	7	2.11	11	2.27
Aeromonas veronii	A.ver	0	0	3	0.98	2	0.64	0	0.00	4	0.83
Bacillus spp.†	Baci	13	11.02	18	5.86	16	5.14	19	5.72	26	5.37
Budvicia aquatica	B.aqu	9	7.63	11	3.58	12	3.86	5	1.51	10	2.07
Citobacter diversus	C.div	3	2.54	6	1.95	10	3.22	7	2.11	14	2.89
Citrobacter koseri	C.kos	12	10.17	9	2.93	12	3.86	15	4.52	17	3.51
Deinobacter grandis	D.gra	1	0.85	3	0.98	5	1.61	6	1.81	10	2.07
Deinococcus proteolyticus†	D.pro	0	0.00	4	1.30	1	0.32	2	0.60	8	1.65
Deinococcus radiopugnans†	D.rad	0	0	2	0.65	1	0.32	1	0.30	5	1.03
Edwardsiella tarda	E.tar	1	0.85	7	2.28	9	2.89	8	2.41	16	3.31
Enterobacter aerogenes	E.aer	0	0	4	1.30	3	0.96	1	0.30	7	1.45
Enterobacter cloacae	E.clo	2	1.69	9	2.93	7	2.25	6	1.81	13	2.69

Table 6 *(continue)*

Bacteria	Abbrev.	Sediment (n = 118)		Water (n = 307)		Surface (n = 311)		Gill (n = 332)		Intestine (n = 484)	
		No.	%	No.	%	No.	%	No.	%	No.	%
Enterococcus cecorum†	E.cec	2	1.69	5	1.63	4	1.29	6	1.81	8	1.65
Enterococcus faecalis†	E.fae	0	0	3	0.98	1	0.32	4	1.20	11	2.27
Enterococcus pseudoarium†	E.pse	3	2.54	7	2.28	9	2.89	13	3.92	15	3.10
Escherichia coli	E.col	15	12.71	29	9.45	33	10.61	26	7.83	32	6.61
Escherichia coli 1	E.co1	0	0	13	4.23	9	2.89	14	4.22	11	2.27
Flavobacterium aquatile	F.aqu	5	4.24	9	2.93	12	3.86	11	3.31	11	2.27
Klebsiella oxytoca	K.oxy	2	1.69	8	2.61	12	3.86	7	2.11	17	3.51
Klebsiella pneumoniae subsp. *ozaenae*	K.pso	4	3.39	9	2.93	11	3.54	10	3.01	12	2.48
Klebsiella pneumoniae subsp. *pneumoniae*	K.psp	0	0.00	4	1.30	3	0.96	2	0.60	4	0.83
Lactococcus lactis subsp. *lactis*†	L.lsl	0	0	2	0.65	1	0.32	3	0.90	9	1.86
Leuconostoc spp.†	Leuc	1	0.85	8	2.61	6	1.93	5	1.51	9	1.86
Pantoea spp.	Pant	2	1.69	6	1.95	7	2.25	5	1.51	12	2.48
Pragia fontium	P.fon	4	3.39	8	2.61	7	2.25	10	3.01	13	2.69
Proteus mirabilis	P.mir	1	0.85	5	1.63	10	3.22	6	1.81	9	1.86
Proteus vulgaris	P.vul	1	0.85	6	1.95	5	1.61	7	2.11	10	2.07
Rahnella aquatilis	R.aqu	4	3.39	9	2.93	7	2.25	13	3.92	10	2.07
Salmonella choleraesius subsp. *choleraesius*	S.csc	0	0	5	1.63	2	0.64	1	0.30	3	0.62
Salmonella enterica	S.ent	0	0	5	1.63	7	2.25	10	3.01	11	2.27
Salmonella spp.	Salm	12	10.17	19	6.19	26	8.36	31	9.34	34	7.02
Serratia fonticola	S.fon	9	7.63	11	3.58	12	3.86	7	2.11	13	2.69
Staphylococcus aureus subsp. *aureus*†	S.asa	0	0	5	1.63	3	0.96	2	0.60	5	1.03
Staphylococcus spp.†	Stap	7	5.93	20	6.51	16	5.14	27	8.13	31	6.40
Streptococcus agalactiae†	S.aga	4	3.39	16	5.21	23	7.40	31	9.34	42	8.68
Vibrio cholerae	V.cho	0	0	3	0.98	1	0.32	2	0.60	5	1.03
Yersinia pseudotuberculosis	Y.pse	0	0	2	0.65	1	0.32	2	0.60	4	0.83

†: Indicate Gram-positive

Water Physico-Chemical Parameters

No significant difference ($P>0.05$) was observed for water temperature of the three habitats (Table 7). Peat swamp forest significantly ($P<0.05$) had the lowest water pH (3.68 ± 0.25) and DO (0.59 ± 0.17 mg L^{-1}), compared with paddy field and oil palm plantation. Higher mean of EC (204.85 ± 29.70 µS cm^{-1}) and TDS (0.13 ± 0.02 g L^{-1}) were recorded in the peat swamp forest,

but not significantly different (P>0.05) from the oil palm plantation. Peat swamp forest recorded highest salinity (0.05 ± 0.04 ppt) which was not significantly different (P>0.05) from the paddy field, but significantly (P<0.05) higher from oil palm plantation area. The highest NH_3-N (0.46 ± 0.04 mg L^{-1}) concentration was measured in the oil palm plantation area. The reading was significantly (P<0.05) higher compared to the peat swamp forest, but not for the paddy field. The lowest NO_3-N (0.002 ± 0.003 mg L^{-1}) was recorded in the peat swamp forest. However, the reading was not significantly different (P>0.05) from oil palm plantation, but significantly (P<0.05) lower compared to paddy field. Moreover, peat swamp forest recorded significantly (P<0.05) higher PO_4 (1.94 ± 0.20 mg L^{-1}), SO_4 (28.92 ± 20.40 mg L^{-1}) and Cl_2 (0.92 ± 0.36 mg L^{-1}) compared to the paddy field and oil palm plantation, respectively.

Table 7
Comparison of water quality parameters between the three different environments

	Peat swamp		Paddy field		Oil palm	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Temp (°C)	31.62 ± 2.70[a]	27.80 – 35.70	30.65 ± 2.23[a]	28.30 – 33.80	30.71 ± 1.80[a]	28.10 – 33.10
pH (1 – 14)	3.68 ± 0.25[a]	3.28 – 4.10	4.96 ± 0.46[b]	4.26 – 5.57	5.61 ± 0.62[b]	4.62 – 6.17
DO (mg L^{-1})	0.59 ± 0.17[a]	0.36 – 0.86	3.16 ± 0.56[b]	2.49 – 3.89	3.58 ± 0.30[b]	3.17 – 3.97
EC (µS cm^{-1})	204.85 ± 29.70[a]	155.70 – 271.00	92.94 ± 41.57[b]	61.00 – 156.03	108.12 ± 99.82[ab]	32.00 – 241.90
TDS (g L^{-1})	0.13 ± 0.02[a]	0.10 – 0.17	0.06 ± 0.03[b]	0.04 – 0.10	0.07 ± 0.07[ab]	0.02 – 0.18
Salinity (ppt)	0.05 ± 0.04[a]	0.01 – 0.12	0.02 ± 0.01[a]	0.01 – 0.03	0.01 ± 0.00[b]	0.01 – 0.12
NH_3-N (mg L^{-1})	0.35 ± 0.08[a]	0.23 – 0.45	0.45 ± 0.03[b]	0.41 – 0.50	0.46 ± 0.04[b]	0.39 – 0.50
NO_3-N (mg L^{-1})	0.002 ± 0.003[a]	0.000 – 0.008	0.005 ± 0.004[b]	0.000 – 0.009	0.003 ± 0.005[ab]	0.000 – 0.012
PO_4 (mg L^{-1})	1.94 ± 0.20[a]	1.56 – 2.30	0.37 ± 0.12[b]	0.22 – 0.56	0.38 ± 0.10[b]	0.28 – 0.56
SO_4 (mg L^{-1})	28.92 ± 20.40[a]	11.00 – 68.00	3.33 ± 1.21[b]	2.00 – 5.00	5.83 ± 3.19[b]	2.00 – 11.00
Cl_2 (mg L^{-1})	0.92 ± 0.36[a]	0.46 – 1.60	0.63 ± 0.72[b]	0.10 – 2.00	0.21 ± 0.10[b]	0.05 – 0.31

Comparison is between mean ± SD along the same row. Values with different superscript letters are significantly different at P <0.05

PCA and CCA of Water Physico-Chemical Parameters

For each habitat, PCA produced two axes that cumulatively explained the 88.25%, 97.70%, and 83.60% variations of water physico-chemical parameters in the habitats, respectively (Table 8). Out of the 11 water physico-chemical parameters evaluated, only four parameters were retained in each habitat. The water temperature, EC, NH_3-N, and Cl_2 were retained in peat swamp forest; the NH_3-N, PO_4, SO_4, and Cl_2 were retained

in paddy field; while the temperature, DO, EC, and SO_4 were retained in the oil palm plantation. Generally, factor loadings were classified as strong (> 0.75), moderate (0.75–0.50), or weak (0.50–0.00).

All four variables of water physico-chemical parameters of each habitat from the PCA were retained by CCA (Table 9). These variables were significant contributors to the variation in CCA's ordination. Four ordination axes were generated for the CCA in each habitat. The cumulative percentages for the first and second ordination axes were 44.26% and 71.52%, 39.68% and 76.58%, and 51.67% and 79.53%, for the peat swamp forest, paddy field, and the oil palm plantation, respectively. Only the first two axes are reported here, since these axes contributed the most to the ordination.

Table 8
Principal component loadings from principal component analysis of water quality parameters from peat swamp forest, paddy field and oil palm plantation

	Peat Swamp		Paddy Field		Oil Palm	
	C1	C2	C1	C2	C1	C2
Eigenvalue	2.501	1.029	2.763	1.145	1.754	1.590
Percentage variance explained	62.535	25.714	69.086	28.613	43.858	39.738
Cumulative variance explained	62.535	88.249	69.086	97.699	43.858	83.597
Temperature (°C)	0.875	0.195			-0.097	**0.840**
DO (mg L^{-1})					**0.873**	-0.324
EC (µS cm^{-1})	0.048	**0.994**			**0.911**	0.167
NH_3-N (mg L^{-1})	**0.949**	0.016	**0.971**	0.168		
PO_4 (mg L^{-1})			**0.977**	-0.110		
SO_4 (mg L^{-1})			-0.060	**0.996**	0.100	**0.911**
Cl_2 (mg L^{-1})	**0.913**	-0.052	**0.929**	-0.336		

Strong loadings > 0.70 in **bold**

Associations between Bacterial Presence and Water Physico-Chemical Parameters

The CCA ordination diagram showing the relationship between bacterial presence and water physico-chemical parameters in the peat swamp forest is presented in Figure 2. As revealed by the length of the vector, Cl_2 was the most important parameter influencing bacterial presence. This was followed by EC, NH_3-N and temperature. Bacterial species such as *Klebsiella oxytoca*, *Pantoea* spp., *Staphylococcus* spp., *Pragia fontium*, *Proteus vulgaris*, *Salmonella* spp., and *Klebsiella pneumoniae* showed positive correlation with the water parameters. In the same vein, species such as *E. coli*, *Flavobacterium aquatile* and *Proteus mirabilis* showed positive correlation with Cl_2. On the other hand, the species such as *Deinococcus preoteolyticus*, *Edwardsiella tarda*, *Enterococcus pseudoarium*, *Rahnella aquatilis* were negatively correlated with all the water physico-chemical parameters.

Table 9
Canonical correspondence analysis summary statistics of physico-chemical parameters of water

	Peat swamp				Paddy field				Oil palm			
	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
Eigenvalue	0.062	0.038	0.027	0.012	0.107	0.100	0.047	0.016	0.051	0.027	0.015	0.005
Constrained inertia (%)	44.261	27.260	19.578	8.901	39.677	36.904	17.477	5.942	51.673	27.856	14.962	5.509
Cumulative %	44.261	71.521	91.099	100.000	39.677	76.581	94.058	100.000	51.673	79.529	94.491	100.000
Regression coefficients:												
Temperature (°C)	1.209	1.107	0.771	0.110					0.039	0.039	1.088	0.313
DO (mg L^{-1})									0.723	0.266	1.228	-0.726
EC (µS cm^{-1})	0.421	-0.709	-0.169	-0.635					0.427	0.417	-0.647	0.948
NH_3-N (mg L^{-1})	-0.115	0.440	-2.485	-0.184	-1.828	0.241	0.066	3.335				
PO_4 (mg L^{-1})					-2.561	1.181	0.549	-2.799				
SO_4 (mg L^{-1})					1.140	-1.566	-0.209	-1.052	-0.008	0.379	-0.404	-1.004
Cl_2 (mg L^{-1})	-0.528	-1.563	1.521	0.997	4.795	-2.309	0.292	-0.837				

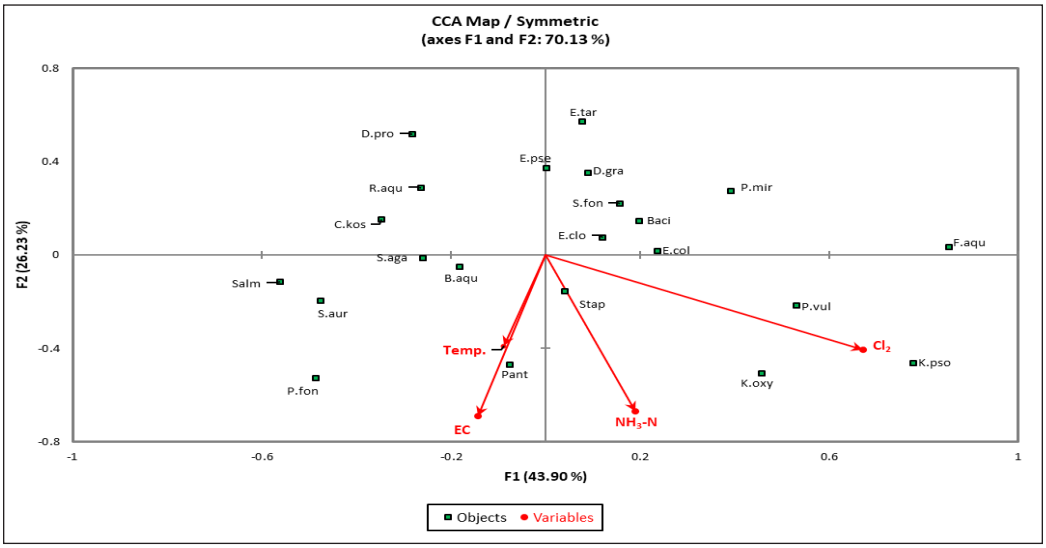

Figure 2. Canonical correspondence analysis ordination diagram showing the effect of water physico-chemical parameters on bacteria presence in peat swamp forest

Figure 3 presents the effects of water physico-chemical parameters on the bacterial presence in the paddy field. SO_4 is the most important parameter influencing the composition. Meanwhile, NH_3-N, PO_4 and Cl_2 displayed the same level of importance based on the vector lengths. Briefly, SO_4 was positively correlated with the species

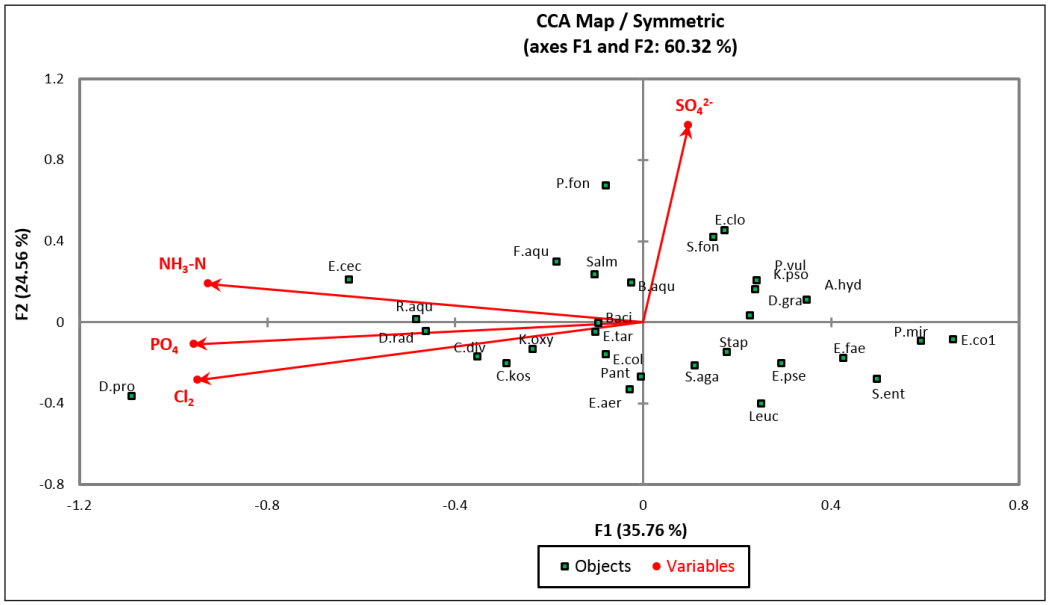

Figure 3. Canonical correspondence analysis ordination diagram showing the effect of water physico-chemical parameters on bacteria presence in paddy field

such as *P. fontium*, *F. aquatile, Serratia fonticola*, *Enterobacter cloacae*, *P. vulgaris*, and *Aeromonas hydrophila*, but negatively correlated with the bacterial species such as *E. coli*, *Leuconostoc* spp., *Streptococcus agalactiae*, *Staphylococcus* spp., and *Enterococcus faecalis*. However, NH_3-N, PO_4 and Cl_2 were positively correlated with *R. aquatilis*, *Deinococcus radiopugnans*, *D. proteolyticus*, *Enterococcus cecorum*, *Citrobacter diversus*, *Citrobacter koseri* and *K. oxytoca*, but negatively correlated with *S. agalactiae*, *Staphylococcus* spp., *P. vulgaris*, *P. mirabilis* and *Leuconostoc* spp.

In oil palm plantation, DO was the most important factor influencing bacterial composition, followed by SO_4, temperature and EC (Figure 4). DO and EC were positively correlated with *E. aerogenes*, *Budvicia aquatica*, *K. oxytoca*, *E. coli*, *E. tarda*, *Salmonella* spp., and *A. urinae*, but negatively correlated with *R. aquatilis*, *Leuconostoc* spp., *Staphylococcus* spp. and *P. vulgaris*. Meanwhile, temperature and SO_4 showed positive correlation with *Pantoea* spp., *Vibro cholera*, *S. agalactiae*, *F. aquatile*, *P. mirabilis*, *P. fontium*, and *E. faecalis*, but negatively correlated with *D. grandis*, *A. hydrophila*, *Bacillus* spp., *A. veronii*, *Salmonella* spp. and *E. coli*.

DISCUSSION

This study represents the first effort to study the influence of water physico-chemical parameters on bacterial presence in water, sediment and fish in the peat swamp forest, paddy field and oil palm plantation in north Selangor, Malaysia. Previous studies focused mainly on peat sediment microbial community (Mishra et al., 2014; Yule,

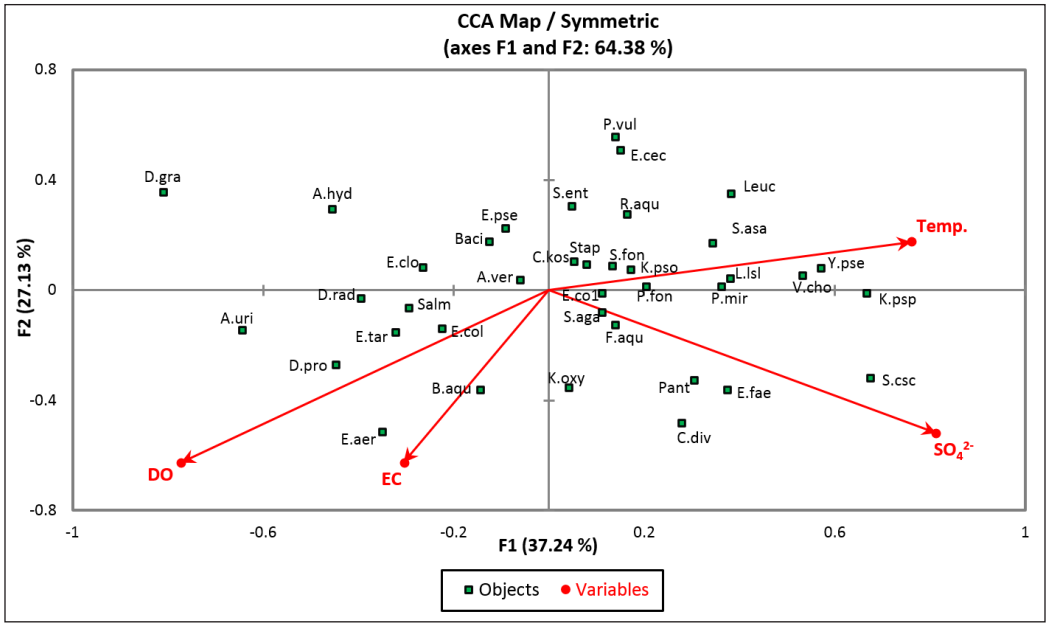

Figure 4. Canonical correspondence analysis ordination diagram showing the effect of water physico-chemical parameters on bacteria presence in oil palm plantation

2010). The bacterial load from this study was generally high, which was attributed to the relatively high ambient temperature of the aquatic systems, resulting from the direct exposure to sunlight following extensive land clearing and conversion of the natural peat forest. High ambient temperature in water bodies has been reported to support the growth of many mesophilic bacteria in natural ecosystems (Ismail et al., 2016). The bacterial load varied in the peat swamp forest, paddy field and oil palm plantation. Oil palm plantation had higher load of bacteria, ranging from 0.48×10^8 cfu mL^{-1} in water to 6.33×10^8 cfu g^{-1} in fish intestine. This was expected due to a relatively lower water quality of the oil palm plantation, resulting from waste and domestic effluent discharge as observed during the sampling time. High waste input affected the water physico-chemical parameters and provided an ideal environment for bacteria growth. It is apparent that the bacterial abundance of any water body is a direct reflection of the environmental condition due to anthropogenic disturbances (Al-Harbi & Uddin, 2003; Mishra et al., 2014). On the other hand, the peat swamp had lower bacteria load, ranging from 0.09×10^8 cfu mL^{-1} in water to 3.87×10^8 cfu g^{-1} in fish intestine. Moreover, in terms of the bacterial community structure, the bacterial diversity and richness in the peat swamp forest also showed the lowest compared to the paddy field and oil palm plantation. The lower abundance of bacteria in the peat swamp forest could be due to acidity of the habitat, which was believed to impede microbial activities (United Nations Development Programme [UNDP], 2006; Whitten et al., 2000).

The results also revealed a clear pattern variation of the bacterial load in the sediment, water, fish body surface, gill and intestine. The bacterial abundance in sediment of all habitats were significantly lower. This finding is contrary to the finding by Al-Harbi and Uddin (2003). They reported a higher abundance of bacteria in sediment compared to the water in an aquaculture pond, which might be due to high accumulation of nutrient in the pond settlement, resulting from the excessive feces of the fish and leftover fish feed. Moreover, anthropogenic disturbances and other natural events may also influence and contribute to increase the bacterial load in water (Amal et al., 2010a; Ismail et al., 2016). The bacterial loads of water and fish body surface for all habitats were not significantly different. Fish is surrounded by water, hence there is continuous interaction of the bacteria present in the water with the skin microflora. The bacteria from the water also enters the gut through the mouth and gill, thus influencing the microbial flora in the gill and intestine (Austin & Austin, 2012). However, the significantly higher bacterial load in the fish gill and intestine, compared to water may be as a result of increased metabolic activity due to the high ambient temperature of the habitats (Al-Harbi & Uddin, 2003). In addition, the bacteria input from the surrounding polluted water, the digestive tract of fish are generally colonized by assemblages

of microorganisms known as the gut microbiota. These microbiota are important in maintaining gut integrity, stepping up immunity and disease resistance, and aiding food digestion (Sullam et al., 2012). Bacterial composition in the intestines of fish are therefore not just dependent on the water in which the fish lives, but a combination of the input from the surrounding water, the gut microbiota and the dissolved organic matters that is part of the fish diet. The ingested organic matters dissolved or in suspension in the fish gut are substrate for microorganism growth, which can enhance bacterial population in the fish intestines (Zhao et al., 2012).

Higher number of bacteria isolates were recorded in the oil palm plantation and paddy field compared to peat swamp. Beside the peat characteristics impeding microbial activity in peat swamp, agricultural and domestic effluents discharged into the oil palm and paddy field enrich the water bodies, thus enhancing bacterial growth (Lee et al., 2010). Similarly, a wide range of bacterial taxa was isolated during this study. However, the most abundant species were *E. coli*, *Salmonella* spp. and *S. agalactiae*. *Escherichia coli* was a common environmental bacteria (Centers for Disease Control and Prevention [CDC], 2018), and in this study, this species was dominant in the water in all habitats and also on fish body surface and gill in the peat swamp forest, in fish gill in the paddy field, and on the fish body surface in the oil palm plantation. Besides that, *Salmonella* spp. was the dominant species in fish intestine in the peat swamp forest and on the fish surface in the paddy field. This pathogen was responsible as one of the most common foodborne infections in human (World Health Organization [WHO], 2018). Several pathogenic bacteria such as *E. faecalis*, *A. hydrophila*, *E. tarda*, *E. cloacae*, *K. pneumoniae*, *F. aquatile* and *V. cholera* were also isolated during this study. For instance, *S. agalactiae* has been reported to cause disease in cultured fish in various type of water body and also in human (Amal et al., 2008, 2010b; Chau et al., 2017), while *A. hydrophila* was responsible for tail and fin rot in fish (Dias et al., 2016), and wound infection in human (Vally et al., 2004).

Studies detailing the influence of water physico-chemical parameters and other natural events on bacterial composition in their natural habitat are well limited, as most studies are focused on fish cultured environments (Al-Harbi & Uddin, 2003, 2005). However, water physico-chemical parameters, nutrients and toxicants have been previously reported to influence bacteria density (Aisyhah et al., 2015; Gorlach-Lira et al., 2013). In this study, the peat swamp forest (conductivity, NH_3-N, temperature and Cl_2), paddy field (SO_4, NH_3-N, PO_4 and Cl_2), and oil palm plantation (DO, conductivity, SO_4 and temperature) showed varying water physico-chemical parameters that influenced the bacterial composition. However, the water conductivity, NH_3-N, temperature, Cl_2 and SO_4 consistently showed their importance in at least two of the studied habitats. The influence of temperature in this study is more

pronounced due to the high temperature, which is regarded as optimum temperature for growth of several mesophilic bacteria (Boyd & Tucker, 1998; Zamri-Saad et al., 2014). Similarly observed in this study, the NH_3-N, SO_4, PO_4, DO, EC and Cl_2 concentration in the water bodies is mostly influenced by anthropogenic chemical, nutrient and waste inputs, such as fertilizer, organic manure and domestic or industrial waste discharge (Mishra et al., 2014; Zhong et al., 2010). Thus, it was believed that high concentration, diversity and composition of bacteria from biotic and abiotic factors in the paddy field and oil palm plantation as observed in this study, reflected to the utilization of fertilizers and human anthropogenic influences. Through this inputs, dissolved organic matter becomes freely available for microbial communities, thus enhancing their growth, composition and diversity (Farrar et al., 2003; Wardle et al., 2004). Consistent with our findings, several studies reported the increasing microbial diversity as a result of increased anthropogenic inputs (Gorlach-Lira et al. 2013; Mishra et al. 2014; Zhong et al. 2010).

CONCLUSION

This study revealed that various bacteria were isolated from the water, sediment, and fish collected from the peat swamp forest, paddy field and oil palm plantation in north Selangor. In addition, the water physico-chemical parameters such as temperature, NH_3-N, Cl_2, DO, EC, SO_4 and PO_4 were all important in influencing the bacterial presence and composition in all of the studied habitats. Moreover, high bacterial load and community structure in the oil palm plantation and paddy field, compared to the peat swamp forest in indicative of pollution due to anthropogenic inputs of fertilizer, nutrients and waste in the area.

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