Indian Journal of Geo Marine Sciences
Vol. 54 (03), March 2025, pp. 147-155
DOI: 10.56042/ijms.v54i03.14882

Research Article

Sublethal effect of organophosphorus pesticides on the gut flora of *Litopenaeus vannamei* (Boone, 1931), Whiteleg shrimp

A S Dake[a], A S Sanaye[a,b], S V Sanaye*[,c] & R A Sreepada[a]

[a]Aquaculture Laboratory, Biological Oceanography Division, CSIR-National Institute of Oceanography, Dona Paula, Goa – 403 004, India

[b]Analytical & Environmental Science Division, CSIR-Central Salt & Marine Chemical Research Institute, Gijubhai Badheka Marg, Bhavnagar, Gujarat – 364 002, India

[c]Mangrove & Marine Biodiversity Conservation Foundation of Maharashtra, 302, 3rd floor, Wakefield House, Ballard Pier, Fort, Mumbai, Maharashtra – 400 001, India

*[E-mail ID: sushant.sanaye@gmail.com]

Received 06 November 2024; revised 06 February 2025

During the present study, the sublethal effects of two organophosphorus pesticides, chlorpyrifos (CPF) and dimethoate (DMT), on the gut flora of *Litopenaeus vannamei* (Boone, 1931), Whiteleg shrimp juveniles were studied for 21 days. Bacterial species such as *Alteromonas* sp., *Bacillus* sp., *Demequina* sp., *Micrococcus* sp., *Pseudomonas* sp., *Pseudoalteromonas* sp., *Pseudoruegeria* sp., *Staphylococcus* sp., *Shewanella* sp., and *Vibrio* sp. were reported from the guts of shrimps exposed to pesticides and in control groups. Bacteria such as *Demequina flava*, *D. globuliformis* and *Pseudoruegeria* sp. were isolated for the first time from the gut flora of *L. vannamei*. During the 21 Days of Exposure (DoE), a total of 17 different bacterial strains were identified from the control group; however, 10 and 12 strains were identified from the CPF- and DMT-treated groups, respectively. The bacterial diversity quantified by the Shannon diversity index (H') in CPF- and DMT-exposed shrimps was observed to decrease over the exposure period compared to the control. Pesticide exposure leads to a decrease in valuable microbes and an increase in harmful microbes in the gut microflora of shrimp. Therefore, this may result in reduced growth, weakened health, increased susceptibility to diseases, and a decrease in the production of high-quality shrimp.

[**Keywords**: Chlorpyrifos, Dimethoate, Gut flora, *Litopenaeus vannamei*, *Vibrio*]

Introduction

The increased use of organophosphorus (OP) pesticides on crops near riverine and estuarine areas posed a potential risk of toxicity to aquatic organisms due to agricultural runoff and spray drift[1,2]. Therefore, the detrimental effects of pesticide toxicity on commercially important crustacean species (shrimps and freshwater prawns) have been studied previously by several researchers[3-6].

The *Litopenaeus vannamei* (Boone, 1931), Whiteleg shrimp, is the most important aquaculture species worldwide, and the farming of *L. vannamei* globally contributed to ~53 % of total shrimp and prawn production[7]. They can grow and survive at lower water salinities, even in inland freshwater areas[8].

Microbiota interactions in the gut are essential in promoting and maintaining the host's health. They increase the uptake of amino acids, sugars and fatty acids, which is essential for the growth of the host[9]. The inflexion of gut microbiota helps increase shrimp production and improve disease control by providing prebiotics, probiotics, and synbiotics[10]. Knowledge of the bacterial ecology of the crustacean gut is helpful for both advancing hatchery management and farm production to maximise productivity and protection of shrimps as safe food[10,11]. The natural intestinal flora of different penaeid shrimp species, such as *L. vannamei*, *Penaeus monodon*, and *P. merguiensis*, has been previously studied[12-16]. Compared to reports on the bacterial gut flora of fish[17], there is still a need for more knowledge on the gut flora of shrimps.

Physiological stress and environmental factors during aquaculture practices also affect the shrimp gut flora[12]. Additionally, the negative effects of organophosphate and organochlorine pesticides on microbial communities may indirectly affect higher trophic levels[18]. Pesticide-induced changes in the gut microbiota composition of crustaceans have been reported previously[19,20]. For instance, chronic imidacloprid exposure in *L. vannamei* resulted in oxidative stress, reduced growth, immune suppression,

and notable shifts in gut microbial communities, favouring pathogenic taxa and disrupting network stability[21]. Similarly, thiamethoxam exposure was shown to impair microbial diversity and alter host transcriptomic responses related to immunity and detoxification[22]. Dietary deltamethrin caused intestinal tissue damage, dysbiosis, and increased vulnerability to pathogens in shrimp[23]. Comparable effects have also been observed in other crustaceans; for example, imidacloprid exposure in red claw crayfish and Chinese mitten crab led to microbiota shifts and oxidative stress[24,25].

Furthermore, due to the rapid expansion of shrimp farming in coastal and inland areas, and the potential risk of pesticide entry into adjacent aquaculture ponds, there is a need to assess the effects of pesticides on shrimp physiology. In the absence of comprehensive data on pesticide-induced changes in gut flora of commercially important shrimp species, the present study was undertaken to determine the variation in gut microflora of *L. vannamei* when exposed to sublethal concentrations of two OPs; chlorpyrifos (CPF) and dimethoate (DMT). The outcome of this study has practical implications, as shrimp growth depends on interactions with beneficial gut bacteria, which may be disrupted due to pesticide exposure. At the same time, outbreaks of bacterial diseases may occur due to pathogenic bacteria, as shrimp become lethargic under pesticide exposure.

Material and Methods

Experimental shrimps and rearing conditions

Healthy post larvae (PL14; total length: 14.4±0.5 mm; wet weight: 60±5 mg) of the Whiteleg shrimp, *L. vannamei*, obtained from a commercial shrimp hatchery (Skyline Aqua Hatchery, Kumta, Karnataka, India) were reared at Aquaculture Laboratory, CSIR-NIO, Goa (India). The Post Larvae (PLs) were reared in filtered seawater in an 800 L fibre-reinforced plastic tank and acclimatised to 30 ppt salinity for 30 days with a photoperiod of 14 h light to 10 h dark. Water quality parameters were analysed according to the methods described in APHA[26]. The rearing tank was supplied with oxygenated water continuously, with stable water quality parameters such as temperature (28.5±0.2 °C), salinity (30±0.5 g/L), DO (5.96±0.6 mg/L), pH (7.90±0.30) and NO_2–N (< 0.06 mg/L). During rearing, shrimps were fed thrice daily with commercial shrimp pellet feed (CP-Aquaculture, India; proximate composition, 38 – 40 % protein; 5 % lipids and 3 % fiber). Uneaten feed and sloughed exoskeletons were removed by siphoning daily to prevent infection.

Chemicals

The commercial grades of OP pesticides, chlorpyrifos (CPF) (diethoxy-sulfanylidene- (3,5,6-trichloropyridin-2-yl) oxy-λ5-phosphane) and dimethoate (DMT) (O, O-dimethyl S-[2-(methy-lamino)-2-oxoethyl] dithiophosphate) were used for the present study. The CPF and DMT commercial grades have Effective Concentrations (EC) of 20 % and 30 %, respectively. These pesticides obtained from the local market under the trade names 'PYRIBAN' (AIMCO Pesticides Ltd., Ratnagiri, India) and 'TAFGOR' (Rallis India Ltd., Akola, India), respectively, were further diluted with deionised water to prepare stock solutions.

Sublethal experimental set-up

After acclimatisation, juveniles of *L. vannamei* were randomly selected for a sublethal experiment. For this purpose, 30 days of laboratory-reared, active and healthy uniform-size juveniles (total length: 52.4±3.4 mm; wet weight: 1.73±0.6 g) without any signs of stress or any visual symptoms of the disease were selected. Before the sublethal exposure experiment, a 96-h acute toxicity test was conducted on *L. vannamei* juveniles. Juveniles were exposed to different nominal concentrations of CPF as 0.4, 0.8, 1.2, 1.4, 1.8 and 2 µg/L, whereas DMT concentrations were 200, 300, 400, 500, 600 and 800 µg/L[(refs. 5-6)]. After the acute toxicity experiment, the 96 h LC_{50} values of CPF and DMT were calculated as 1.40 µg/L and 558.23 µg/L, respectively. For the sublethal (SL) experiment, shrimps were exposed to a single concentration of CPF ($1/6^{th}$ of the LC_{50}; 0.23 µg/L) and DMT ($1/6^{th}$ of the LC_{50}; 93.04 µg/L), including a control (without pesticide) in duplicate. The $1/6^{th}$ values of both OPs were selected by calculating LC_{10} as well as LC_1 values, which were found to be well below the observed Lowest Observed Effect Concentration (LOEC) and the No Observed Effect Concentration (NOEC). The exact amount of CPF present in each SL concentration, however, was not ascertained quantitatively. For this purpose, 21 L glass aquaria containing 15 L filtered seawater with a stocking density of 70 juveniles per aquaria were used. A sublethal exposure experiment was conducted for 21 days. During the experiment, shrimps were fed with the pellet feed at the rate of 4 % of body weight in three parts daily. All essential water quality

parameters are within the optimal range for the rearing of *L. vannamei*. During the entire experimental period, excretory material and uneaten feed were removed by siphoning, and the same pesticide concentration was added except for the control.

Collection of the gut and its homogenisation

During 21 days of sublethal exposure, gut samples were collected from *L. vannamei* on the 7th, 14th, and 21st day, on the basis recommended by sublethal toxicity for a short term up to 21 days of exposure[27]. Every seventh day, 3-4 shrimps were randomly taken from each glass aquarium and euthanised in an ice bath for 5 – 10 min. Each individual was surface-sterilised by immersion in 70 % ethanol for the 30 sec[28]. The guts of each exposed and control shrimps were aseptically dissected from the body and pooled into respective sterile test tubes marked as CPF, DMT, and Control (CNT) for homogenisation. The homogenisation solution was prepared in 2 ml of 0.9 % saline solution under sterile conditions. The homogenised solutions were further diluted in a 1:10 ratio (1 ml homogenised solution: 9 ml of 0.9 % NaCl sterile solution) by Bergey's manual method[29]. All the procedures were carried out in a sterile condition to minimise bacterial contamination.

Isolation and molecular identification of bacteria

The spread plate method was used, and 0.1 ml of the gut homogenate sample was inoculated in duplicate on nutrient agar media containing 50 % seawater[26]. The inoculated plates were incubated for 24 h at 35±1 °C. After incubation, developed colonies were characterised by colony morphology and Gram staining. For further study, the isolated colonies were stored in glycerol and kept at -80 °C. The DNA was extracted using the GenElute bacterial genomic DNA kits method (Sigma-Aldrich, USA). The DNA was amplified using the PCR (Polymerase Chain Reaction) technique. Amplification was carried out using 96-well thermal cyclers (Applied Biosystems, CA). For the initial denaturation at 95 °C/3 min, for 30 cycles, the reaction was set as 95 °C/1 min, 45 °C/ 45 sec, 72 °C/1 min, and final extension at 72 °C/2 min. The 16s rRNA sequencing was performed on gut samples from CPF-, DMT- and CNT-exposed *L. vannamei*. The universal primer used for sequencing was 27F 5'AGAAGTTTGATCCTGGCTCAG-3' and 1392R 5'- GGTTACCTTGTTACGACTT-3'[refs. 30,31]. The final volume of the PCR reaction was 25 µl, comprising Ready mix Taq PCR reagent mix (12.5 µl), forward and reverse primer (1.5 µl each), Template DNA (3 µl), and PCR reagent water (6.5 µl). After PCR, product quality was analysed by electrophoresis on 1 % agarose gel and stained with ethidium bromide. The products that showed bands on the gel were further purified using Wizard SV gel and a PCR clean-up system (Promega, USA). The ABI 3730 (48 capillary) electrophoresis instrument verified the purified products.

Statistical analysis

The Shannon diversity index (H') was used to quantify the bacterial diversity of different treatment groups (CNT, CPF and DMT), and further data were analysed using the pairwise *t*-test for differences in bacterial diversity. Statistical analysis was performed using computer-based GraphPad PRISM 9 and Past 4.03 software. A comprehensive heatmap of all identified bacterial genera was generated using the R package Pheatmap v1.0.12.

Results

During the whole experimental period of 21 DoE, all the water quality parameters were within the optimum range for the rearing of *L. vannamei*. The developed bacterial colony numbers (cfu/ml) on the CNT, CPF, and DMT groups of different DoE were recorded (Table 1) and showed a substantial decrease in colony numbers in the samples of shrimp gut exposed to CPF and DMT compared to CNT. The sequences used for this study were deposited in the NCBI GenBank database, and accession numbers were acquired. Further, the number of identified bacterial strains of different DoE shrimp, including CNT (17), CPF (10), and DMT (12), were classified.

Table 1 — Results of bacterial colonies from the gut of Whiteleg shrimp (*Litopenaeus vannamei*) after 7, 14 and 21 days of exposure to CPF, DMT and control group

Treatment	Days of exposure	No. of colonies	Total nos. of organism (CFU/ml)
Control	7	58	5.8×10^3
	14	64	6.4×10^3
	21	52	5.2×10^3
CPF	7	35	3.5×10^3
	14	32	3.2×10^3
	21	28	2.8×10^3
DMT	7	40	4×10^3
	14	38	3.8×10^3
	21	36	3.6×10^3

CPF = Chlorpyrifos; DMT = Dimethoate

After 7 DoE, the identified bacteria from the gut of CNT shrimps showed the presence of *Bacillus cereus* (8.33 %), *Pseudoalteromonas* sp. (8.33 %), *Pseudomonas aeruginosa* (8.33 %), *Staphylococcus epidermidis* (8.33 %), *Vibrio* sp. (25 %), *V. alginolyticus* (25 %), *V. neocaledonicus* (8.33 %), and *V. parahaemolyticus* (8.33 %). On the other hand, the identified bacteria in the gut of CPF-exposed shrimps include *Vibrio* sp. (18.18 %), *V. alginolyticus* (9.09 %), *V. natriegens* (9.09 %) and *V. parahaemolyticus* (63.63 %). However, in DMT-exposed shrimps, the bacterial species include *Pseudoalteromonas flavipulchra* (10 %), *Vibrio* sp. (40 %), *V. mytili* (10 %), *V. neocaledonicus* (10 %), *V. owensii* (10 %) and *V. parahaemolyticus* (20 %) as shown in Figure 1(a).

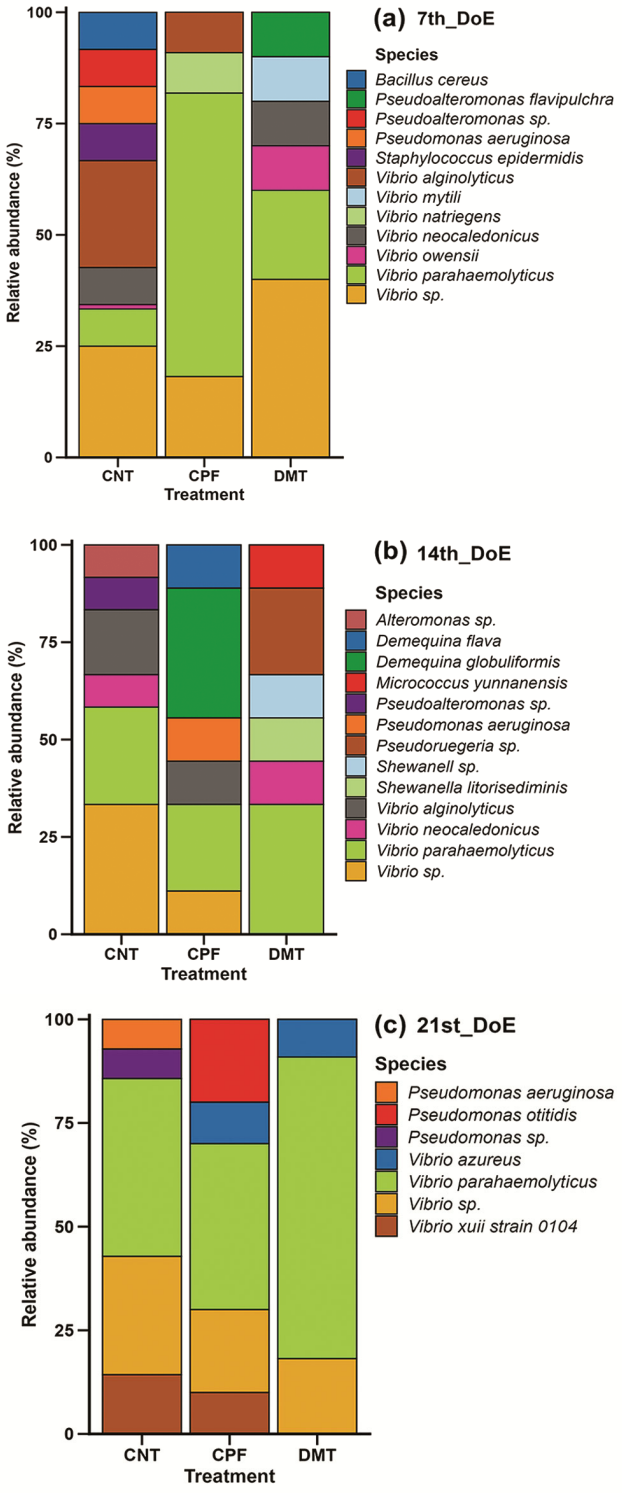

Fig. 1 — Relative abundance of gut flora in the intestine of *L. vannamei* after (a) 7th, (b) 14th, and (c) 21st DoE to CNT, CPF and DMT

After 14 DoE, the identified bacterial species from the gut of CNT shrimps include *Alteromonas* sp. (8.33 %), *Pseudoalteromonas* sp. (8.33 %), *Vibrio* sp. (33.33 %), *V. alginolyticus* (16.66 %), *V. neocaledonicus* (8.33 %), and *V. parahaemolyticus* (25 %). In contrast, the identified bacteria in the gut of CPF-exposed shrimps include *Demequina flava* (11.11 %), *Demequina globuliformis* (33.33 %), *Pseudomonas aeruginosa* (11.11 %), *V. alginolyticus* (11.11 %), *Vibrio* sp., (11.11 %), and *V. parahaemolyticus* (22.22 %). However, in DMT-exposed shrimps, the identified bacterial species include *Micrococcus yunnanensis* (11.11 %), *Pseudoruegeria* sp. (22.22 %), *Shewanella litorisediminis* (11.11 %), *Shewanella* sp. (11.11 %), *V. neocaledonicus* (11.11 %), and *V. parahaemolyticus* (33.33 %) as shown in Figure 1(b).

After 21 DoE, the bacterial species observed in CNT samples were *Pseudomonas* sp. (7.14 %), *Pseudomonas aeruginosa* (7.14 %), *Vibrio* sp. (28.57 %), *V. xuii* (14.28 %), and *V. parahaemolyticus* (42.85 %). However, in CPF-exposed samples, the bacterial species include *Pseudomonas otitidis* (20 %), *Vibrio* sp. (20 %), *V. azureus* (10 %), *V. parahaemolyticus* (40 %) and *V. xuii* (10 %). The identified bacterial species in DMT-exposed shrimp guts include *Vibrio* sp. (18.18 %), *V. azureus* (9.09 %), and *V. parahaemolyticus* (72.72 %), as shown in Figure 1(c).

During the 21 days of exposure, significant changes in the bacterial diversity quantified with the Shannon diversity index (H') in CPF and DMT treatments compared to the control treatment over time (Table 2). In 7 days, significant differences were observed among the treatment groups. The control (CNT) exhibited the highest diversity (H' = 1.9719), while CPF treatment resulted in a substantial reduction in diversity (H' = 1.0336, $p < 0.001$). Although DMT also showed lower diversity than the

control (H' = 1.6094, $p < 0.001$) group, it maintained significantly higher values than CPF ($p < 0.001$), indicating a less disruptive effect on microbial community structure. By day 14th, no statistically significant differences in bacterial diversity were detected among the treatment groups ($p > 0.05$), suggesting that microbial diversity had stabilised across all the groups. Species richness and evenness appeared resilient to CPF and DMT exposure at this stage. However, on the 21st day, a renewed decline in diversity was evident, particularly in the DMT group. Shannon diversity index values for DMT (H' = 0.7596) were significantly lower than both CPF (H' = 1.4708) and CNT (H' = 1.376) treatments ($p < 0.001$), while no significant difference was found between CPF and CNT ($p = 0.261$). These findings indicate that although CPF initially caused a more severe short-term disruption, prolonged DMT exposure ultimately led to a greater long-term reduction in microbial community.

Table 2 — Pairwise comparison (*t*-test) of Shannon diversity index (H') across treatment groups (CNT, CPF, DMT) at 7th, 14th, and 21st DoE. Values in the rows denoted by a different letter indicate a significant difference

DoE	Treatment comparison	Shannon diversity Index (H')	*P*-value
7th	CNT vs CPF	1.9719[a]; 1.0336[b]	< 0.001
	CNT vs DMT	1.9719[a]; 1.6094[c]	< 0.001
	CPF vs DMT	1.0336[b]; 1.6094[c]	< 0.001
14th	CNT vs CPF	1.6326[a]; 1.677[a]	0.561
	CNT vs DMT	1.6326[a]; 1.670[a]	0.56
	CPF vs DMT	1.677[a]; 1.670[a]	0.999
21st	CNT vs CPF	1.376[a]; 1.4708[a]	0.261
	CNT vs DMT	1.376[a]; 0.7596[c]	< 0.001
	CPF vs DMT	1.4708[b]; 0.7596[c]	< 0.001

The bacterial species *Demequina flava*, *D. globuliformis* and *Pseudoruegeria* sp. are observed for the first time from the gut samples of *L. vannamei.* The present study showed that species such as *Vibrio* sp., *V. alginolyticus*, *V. neocaledonicus* and *V. parahaemolyticus* are most common and less affected by pesticide exposure. The bacterial diversity and numbers from gut samples of CPF- and DMT-exposed *L. vannamei* juveniles are lower than those of control shrimps. During the sublethal exposure of 21 days, the percentage of pathogenic bacteria in CPF- and DMT-exposed shrimps was 70 % and 66 %, respectively; whereas in CNT shrimps, it was 64 %.

The heat map was constructed to present the bacterial abundance of species across the treatment CPF and DMT against the control (Fig. 2). The differences in bacterial abundance were observed between treatment groups in DMT samples on the 14th

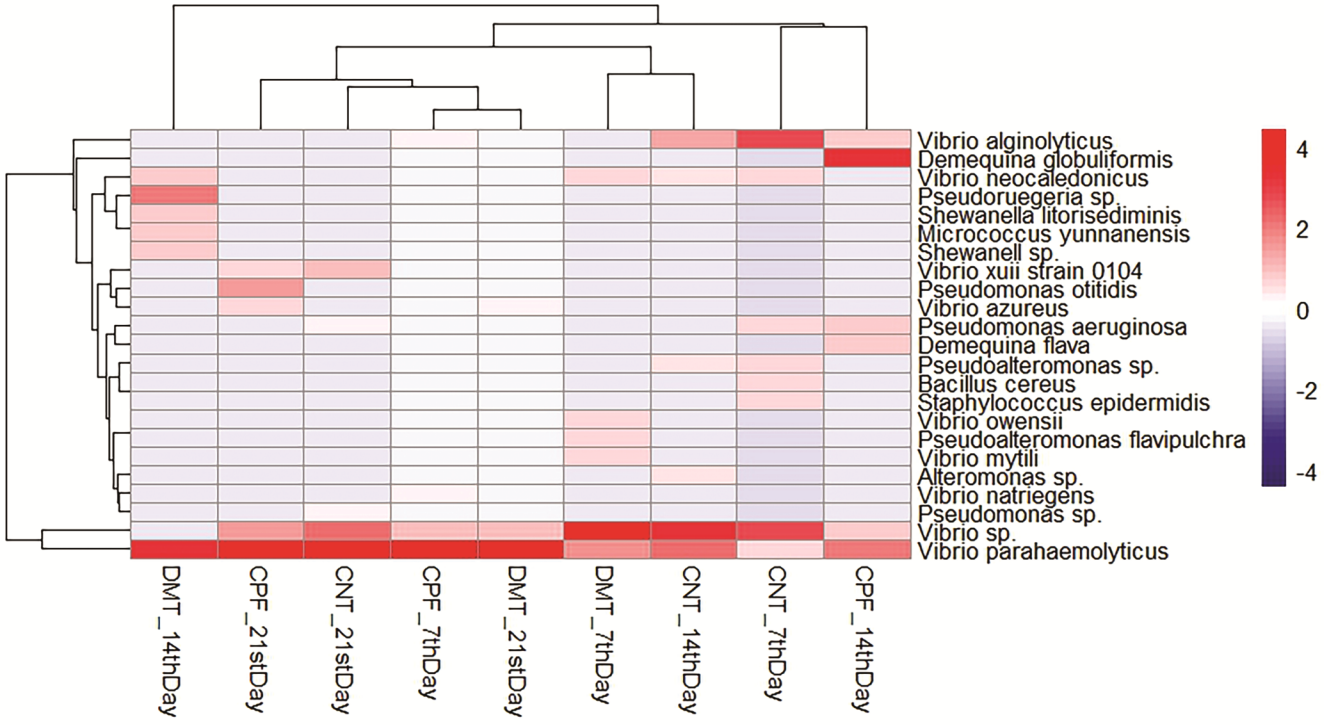

Fig. 2 — Heat map representing the shift in bacterial community composition across different treatment groups (CNT, CPF, DMT) and time points (7th DoE, 14th DoE, 21st DoE)

DoE, where species such as *V. parahaemolyticus* and *Pseudoruegeria* sp. showed higher abundance compared to control groups. However, hierarchical clustering revealed that the *DMT* group became more similar over time, forming a distinct cluster by the 14th DoE. Meanwhile, the control (CNT) group samples remained more consistent across time points, indicating stability in bacterial abundance. The species *V. parahaemolyticus* was abundant in all groups, notably at the 21st DoE, suggesting these bacteria may respond strongly to the treated group on the 21st DoE. However, the predominance of *V. parahaemolyticus* is observed in pesticide treatment across the exposure time.

Discussion

The development of intestinal microflora is a gradual process. The variation in environmental factors such as temperature, salinity, trophic level, and host phylogeny may affect the gut microbial community of aquatic animals[12-16,32,33]. Furthermore, it depends on the feed intake, hormone secretion, nutrient absorption and the form of proteins and digestive enzymes[14,36,37]. Knowledge of shrimp gut microflora interactions with shrimps has often helped increase production and profits in the shrimp farming industry by developing commercial feeds and disease control policies[34]. At standard conditions, the intestinal bacterial communities of *Penaeus monodon* (Black tiger shrimp), and *L. vannamei* (Pacific whiteleg shrimp) are similar, but *L. vannamei* is resistant to the pathogen as compared to *P. monodon*[35]. Considering the increased farming of *L. vannamei* in the shrimp aquaculture sector in Asian countries, the potential for pesticide pollution in estuarine and farming areas has been studied[5-6]. The present study was conducted to examine the effects of sublethal concentrations of two OP pesticides (CPF and DMT) on the intestinal microflora of *L. vannamei* juveniles. Although a few reports have highlighted pesticide-induced alterations in the gut microflora of crustaceans, previous studies have shown that long-term dietary exposure to deltamethrin, a pyrethroid pesticide, impairs growth and damages intestinal health in *L. vannamei*[23]. Although structurally different from organophosphates, deltamethrin has a similar effect on gut integrity, indicating that sublethal exposure to various pesticide classes can harm shrimp intestinal health. Likewise, imidacloprid, a neonicotinoid, was found to alter gut microbiota, disrupt biochemical balance, and decrease growth performance in *L. vannamei*[21]. Furthermore, evidence from thiamethoxam exposure showed significant changes in intestinal microbial communities, emphasising the gut's role as a sensitive indicator of xenobiotic toxicity[22]. Comparable effects have also been observed in other crustaceans. In the Chinese mitten crab (*E. sinensis*), exposure to glyphosate and glufosinate resulted in reduced microbial diversity and disrupted host-microbiota interactions[19,20]. In the red claw crayfish (*C. quadricarinatus*), exposure to imidacloprid triggered toxic responses that extended beyond the gut, affecting host immune signalling and physiological processes[24]. Collectively, these findings highlight that various pesticides, even at sublethal levels, can harm gut microbial communities and host health in shrimp and other crustaceans regardless of their chemical class. Similar bacteriotoxic effects of two OPs (CPF and DMT) decreased bacterial diversity and abundance compared to CNT shrimps after 21 DoE have been reported in this study.

In the present study, bacterial diversity of *L. vannamei* gut flora has resulted in genera such as *Alteromonas*, *Bacillus*, *Demequina*, *Pseudomonas*, *Pseudoalteromonas*, *Pseudoruegeria*, *Vibrio*, *Shewanella*, and *Staphylococcus*, which accords with the previous studies that have been reported, from the guts of shrimp species[10,12-14]. The strain belongs to *Vibrio* sp. and *Pseudoalteromonas* sp., which are considered normal flora of the digestive system of shrimp[12,13,36,37], and *Vibrio* is the most dominant during the present study. However, in the current study, bacterial species such as *Demequina flava*, *D. globuliformis*, and *Pseudoruegeria* sp. are observed for the first time in the gut samples of *L. vannamei*.

During the sublethal exposure of 21 days, the percentage of pathogenic bacteria in CPF- and DMT-exposed shrimps were 70 % and 66 %, respectively; whereas, in CNT shrimps it was 64 %. The percentages of non-pathogenic or/and beneficial bacteria were 36 %, 30 % and 34 % in CNT, CPF- and DMT-exposed shrimps, respectively. The presence of pesticides in rearing water, even at very low concentrations, weakens the shrimp's immune system, thereby leading to mortality. The weakened immune system can even cause an outbreak of infectious diseases[2].

The non-pathogenic/beneficial bacteria such as *Alteromonas* sp., *Bacillus cereus*, *Pseudoalteromonas* sp., *Pseudoalteromonas* sp. strain B25, *Pseudomonas*

aeruginosa, and *Pseudomonas aeruginosa* strain T1 have been isolated from the guts of CNT shrimps. On the other hand, *Demequina flava*, *Demequina globuliformis*, and *Pseudomonas aeruginosa* have been isolated from CPF-exposed shrimps; and *Micrococcus yunnanensis*, *Pseudoalteromonas flavipulchra* and *Pseudoruegeria* sp. have been isolated from DMT-exposed shrimps. The species of *Pseudoalteromonas* were tested as possible probiotics against acute hepatopancreatic necrosis disease, which is caused by *V. parahaemolyticus*[38]. The species of *Pseudoalteromonas* is also used as a probiotic in the rearing of *Seriola lalandi* fish larvae and has been observed to increase survival[39]. The species of *Pseudoruegeria* play an essential role in the assimilation of trehalose sugar, acid production, enzyme activities, and the hydrolysis of surfactants[40]. *Pseudomonas aeruginosa* has potential as a probiotic bacterium and showed antibacterial activity against pathogenic bacteria such as *V. cholerae*, *V. para-haemolyticus*, and *V. alginolyticus* in *P. monodon*[41,42]. Similarly, the *Micrococcus yunnanensis* has a pathogen-inhibitory action[43]. Vidal *et al.*[44] reported that the bacterium, *Bacillus cereus*, showed a high colonising capacity in post-larvae of *L. vannamei*, causing a significant reduction of pathogens, probably by secreting antimicrobial substances, and the competitive exclusion.

Most of the pathogenic bacterial species reported during the present study belong to the genus *Vibrio*. Some species of *Vibrio* are not sensitive to marine pollution, and a previous study on *Vibrio natriegens* exposed to the organophosphate insecticide, Temephos, showed a negative effect[45]. The dominant presence of *Vibrio* sp. in CPF- and DMT-exposed shrimps indicates that sublethal pesticide concentrations negatively impact the gut flora of *L. vannamei* and may inhibit the colonisation of potential non-pathogenic and beneficial bacteria in the shrimp's guts. Alterations in these bacteria due to pesticide toxicity may also change growth and susceptibility to pathogens, and subsequently, the production of this commercially important aquaculture species. Therefore, the results of the present study can be carefully considered when farming *L. vannamei* to bio-monitor pesticide pollution in inland shrimp farms.

Conclusion

The present study reported the effects of two pesticides (CPF and DMT) on the intestinal gut flora of *L. vannamei*, leading to changes in gut flora compared to the control. The bacterial community has been observed to decline in CPF- and DMT-exposed treatments compared to CNT, even at low concentrations. This suggests that CPF and DMT pesticides have a lethal effect on the gut flora of *L. vannamei*, whilst *Vibrio* sp. are less affected by these pesticides, but slowly decrease in abundance as exposure time increases. As *L. vannamei* is the major shrimp species in rapidly expanding farming in low-saline inland areas, the entry of minute amounts of pesticides into aquaculture ponds through adjacent estuarine/riverine water may have severe effects on the growth and health of shrimps. Therefore, biomonitoring of these pesticides through the assessment of gut microflora is necessary to avoid heavy losses in shrimp production. Furthermore, results of the present study obtained in laboratory-reared shrimps may differ in actual farm-reared species due to the use of different types of probiotic formulations, feed additives and vitamins, and different geographical conditions and farming practices. Additionally, some bacterial isolates were not cultured under laboratory conditions. Thus, future studies employing metagenomics for a more comprehensive assessment of shrimp gut microflora are recommended to enhance understanding and management of gut health in aquaculture settings.

Acknowledgements

The authors are grateful to the Director, CSIR-National Institute of Oceanography, Goa (India), for encouragement and facilities.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Conceptualisation: ASD, ASS and SVS; Software and formal analysis: ASD and ASS; Supervision: RAS and ASS; Writing original draft: ASD; and Writing – review and editing: SVS and RAS.

References

1 Guzzella L, Pozzoni F & Giuliano G, Proceedings of International Conference and Special Seminars on Groundwater Quality: Remediation and Protection, (Tübingen, Germany) September 21–25, 1998, pp. 1–3.

2 Roque A, Abad S, Betancourt-Lozano M, de la Parra L M, Baird D, *et al*., Evaluation of the susceptibility of the cultured shrimp *Litopenaeus vannamei* to vibriosis

when orally exposed to the insecticide methyl parathion, *Chemosphere*, 60 (2005) 126–134. https://doi:10.1016/j.chemosphere.2005.01.008

3 Satapornvanit K, Baird D J & Little D C, Laboratory toxicity test and post-exposure feeding inhibition using the giant freshwater prawn *Macrobrachium rosenbergii*, *Chemosphere*, 74 (2009) 1209–1215. https://doi:10.1016/j.chemosphere.2008.11.033

4 Krishnapriya R & Padmaja M, Study on individual and combined toxicity of Quinalphos and Dimethoate on certain neurological aspects of giant fresh water prawn *Macrobrachium rosenbergii* (Deman, 1879), *Int J Sci Resea Public*, 4 (2014) 1–5.

5 Pawar A P, Sanaye S V, Shyama S, Sreepada R A, Bhagat J, *et al*., In vivo DNA damage in gill, haemolymph and muscle cells of whiteleg shrimp *Litopenaeus vannamei* on exposure to organophosphorus pesticide, *Aquacult Environ Interac*, 11 (2019) 75–86. https://doi.org/10.3354/aei00299

6 Pawar A P, Sanaye S V, Shyama S, Sreepada R A & Dake A S, Effects of salinity and temperature on the acute toxicity of the pesticides, dimethoate and chlorpyrifos in post-larvae and juveniles of the whiteleg shrimp, *Aquacult Rep*, 16 (2020) p. 100240. https://doi.org/10.1016/j.aqrep.2019.100240

7 FAO, *The state of world fisheries and aquaculture—meeting the sustainable development goals*, (Food and Agriculture Organization of the United Nations, Rome), 2018, pp. 227.

8 Roy L A, Davis D A, Saoud I P, Boyd C A, Pine H J, *et al*., Shrimp culture in inland low salinity waters, *Rev Aquacult*, 2 (2010) 191–208. https://doi.org/10.1111/j.1753-5131.2010.01036.x

9 Nolasco H, Del Monte A, Hinojosa P, Civera-Cerecedo R & Vega-Villasante F, Digestibilidad in vitro de lípidos alimentarios para el camarón, In: Avances en Nutrición Acuicola VIII. VIII Simposium Internacional de Nutrición Acuicola, Universidad Autónoma de Nuevo León, Monterrey, edited by Cruz L, Ricque D, Tapia M, Nieto M G, Villarreal D A, *et al*., (Nuevo León, México), 2006, pp. 377–395.

10 Gainza O, Ramirez C, Ramos A S & Romero J, Intestinal microbiota of white shrimp *Penaeus vannamei* under intensive cultivation conditions in Ecuador, *Microb Ecol*, 75 (2018) 562–568. https://doi.org/10.1007/s00248-017-1066-z

11 Schwarz L, *Vision general del sector acuicolanacional, Ecuador*, FAO Fisheries and Aquaculture Department Rome, 2005.

12 Oxley A P A, Shipton W, Owens L & Mckay D, Bacterial flora from the gut of the wild and cultured banana prawn *Penaeus merguiensis*, *J Appl Microbiol*, 93 (2002) 214–223. https://doi.org/10.1046/j.1365-2672.2002.01673.x

13 Moss S M, LeaMaster B R & Sweeney J N, Relative abundance and species composition of gram-negative, aerobic bacteria associated with the gut of juvenile white shrimp *Litopenaeus vannamei* reared in oligotrophic well water and eutrophic pond water, *J World Aquacult Soc*, 31 (2000) 255–263.

14 Tzuc J T, Escalante D R, Herrera R R, Cortes G G & Ortiz M L A, Microbiota from *Litopenaeus vannamei*: digestive tract microbial community of Pacific white shrimp (*Litopenaeus vannamei*), *SpringerPlus*, 3 (2014) 1–10. https://doi.org/10.1186/2193-1801-3-280

15 Rungrassamee W, Klanchui A, Maibunkaew S, Chaiyapechara S, Jiravanichpaisal P, *et al*., Characterization of intestinal bacteria in wild and domesticated adult black tiger shrimp (*Penaeus monodon*), *PLoS ONE*, 9 (2014) 1–11.

16 Huang Z, Li X, Wang L & Shao Z, Changes in the intestinal bacterial community during the growth of white shrimp, *Litopenaeus vannamei*, Aqua Res, 47 (2014) 1737–1746. https://doi.org/10.1111/are.12628

17 Tanasomwang V & Muroga K, Intestinal microflora of larval and juvenile stages in Japanese flounder (*Paralichthys olivaceus*), *Fish Pathol*, 23 (1988) 77–83. https://doi.org/10.3147/jsfp.23.77

18 DeLorenzo M E, Scott G I & Ross P E, Toxicity of pesticides to aquatic microorganisms: a review, *Environ Toxicol Chem*, 20 (2001) 84–98. https://doi.org/10.1002/etc.5620200108

19 Feng H, Song L, Wu Y, Zhao F, Zhu F, *et al*., Novel insight into the mechanisms of neurotoxicity induced by glufosinate-ammonium via the microbiota-intestine-brain axis in Chinese mitten crab (*Eriocheir sinensis*), *Pestic Biochem Physiol*, (2025) p. 106426. https://doi.org/10.1016/j.pestbp.2024.106426

20 Song Y, Song X, Wu M, Pang Y, Shi A, *et al*., The protective effects of melatonin on survival, immune response, digestive enzymes activities and intestinal microbiota diversity in Chinese mitten crab (*Eriocheir sinensis*) exposed to glyphosate, *Comp Biochem Physiol C Toxicol Pharmacol*, 238 (2020) p. 108845. https://doi.org/10.1016/j.cbpc.2020.108845

21 Fu Z, Han F, Huang K, Zhang J, Qin J G, *et al*., Impact of imidacloprid exposure on the biochemical responses, transcriptome, gut microbiota and growth performance of the Pacific white shrimp (*Litopenaeus vannamei*), *J Hazard Mater*, 424 (2022) p. 127513. https://doi.org/10.1016/j.jhazmat.2021.127513

22 Fu Z, Zhang J, Huang K, Chen L, Li E, *et al*., Combined toxic effects of thiamethoxam on intestinal flora, transcriptome and physiology of the Pacific white shrimp (*Litopenaeus vannamei*), *Sci Total Environ*, 805 (2022) p. 150354. https://doi.org/10.1016/j.scitotenv.2022.154799

23 Qu C, Zhao Y, Liu Y, Wang Y & Dong X, Chronic dietary exposure to deltamethrin impairs growth and intestinal health in Pacific white shrimp (*Litopenaeus vannamei*), *SSRN Preprint*, (2023) p. 4784798. https://doi.org/10.2139/ssrn.4784798

24 Lu Y, Yang G, Jin Y, Liu Y, Zhang L, *et al*., Integration of transcriptome, gut microbiota, and physiology reveals toxic responses of the red claw crayfish (*Cherax quadricarinatus*) to imidacloprid, *J Hazard Mater*, 452 (2024) p. 132711. https://doi.org/10.1016/j.jhazmat.2024.134293

25 Hong X, Liu Y, Chen L, Wang J & Cheng D, Effects of imidacloprid on oxidative stress, detoxification and gut microbiota of Chinese mitten crab (*Eriocheir sinensis*), *Sci Total Environ*, 738 (2020) p. 139924. https://doi.org/10.1016/j.scitotenv.2020.138276

26 APHA, *Standard methods for the examination of water and wastewater*, (American Public Health Association, Washington, DC), 1995.

27 Prusty A K, Kohli M P S, Sahu N P, Pal A K, Saharan N, *et al*., Effect of short term exposure of fenvalerate on biochemical and haematological responses in *Labeo rohita* (Hamilton) fingerlings, *Pestic Biochem Physiol*, 100 (2011) 124-129. https://doi.org/10.1016/j.pestbp.2011.02.010

28 Rungrassamee W, Klanchui A, Chaiyapechara S, Maibunkaew S, Tangphatsornruang S, *et al*., Bacterial population in

intestines of the black tiger shrimp (*Penaeus monodon*) under different growth stages, *PLoS ONE*, 8 (2013) p. e60802. https://doi.org/10.1371/journal.pone.0060802

29 Holt J G, Krieg N R, Sneath P H A, Staley J T & Williams S T, Facultatively anaerobic gram-negative rods, In: *Bergey's Manual of Determinative Bacteriology*, 9th edn, (Williams and Wilkins, Baltimore), 1986, pp. 175–289.

30 Navarrete P, Magne F, Mardones P, Riveros M, Opazo R, *et al.*, Molecular analysis of intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*), *Microbiol Ecol*, 71 (2010) 148–156. https://doi.org/10.1111/j.1574-6941.2009.00769.x

31 Tanu, Deobagkar D, Khandeparker R, Sreepada R A, Sanaye S V, *et al*., A study on bacteria associated with the intestinal tract of farmed yellow seahorse, *Hippocampus kuda* (Bleeker, 1852): characterization and extracellular enzymes, *Aquacult Res*, 43 (2012) 386–394. https://doi.org/10.1111/j.1365-2109.2011.02841.x

32 Denev S, Staykov Y, Moutafchieva R & Beev G, Microbial ecology of the gastrointestinal tract of fish and the potential application of probiotics and prebiotics in finfish aquaculture, *Int Aquat Res*, 1 (2009) 1–29.

33 Sullam K E, Essinger S D, Lozupone C A, O'Connor M P, Rosen G L, *et al*., Environmental and ecological factors that shape the gut bacterial communities of fish: A meta-analysis, *Mol Ecol*, 21 (2012) 3363–3378. https://doi.org/10.1111/j.1365-294X.2012.05552.x

34 Holt C C, Bass D, Stentoford G D & van der Giezen M, Understanding the role of the shrimp gut microbiome in health and disease, *J Invert Pathol*, 186 (2021) p. 107387. https://doi.org/10.1016/j.jip.2020.107387

35 Rungrassamee W, Klanchui A, Maibunkaew S & Karoonuthaisiri N, Bacterial dynamics in intestines of the black tiger shrimp and the Pacific white shrimp during *Vibrio harveyi* exposure, *J Invert Pathol*, 133 (2016) 12–19. https://doi.org/10.1016/j.jip.2015.11.004

36 Gomez-Gil B, Evaluation of the susceptibility of the cultured shrimp *Litopenaeus vannamei* to vibriosis when orally exposed to the insecticide methyl parathion, *Chemosphere*, 60 (2005) 126–134. https://doi.org/10.1016/j.chemosphere.2005.01.008

37 Esiobu N & Yamazaki K, Analysis of bacteria associated with the gut of healthy wild penaeid shrimps: A step towards effective probiotics in aquaculture, *J Aqua Tropics*, 18 (2003) 275–286.

38 Wang X, Li E, Xiong Z, Chen K, Na Y, *et al*., Low salinity decreases the tolerance to two pesticides, beta-cypermethrin and acephate, of white-leg shrimp, *Litopenaeus vannamei*, *J Aqua Res Develop*, 4 (2013) 1–5. https://doi.org/10.4172/2155-9546.1000190

39 Leyton Y, Sayes C, Mejias C, Abarca M, Wilson R, *et al*., Increased larval survival of *Seriola lalandi* using *Pseudoalteromonas* sp. as probiotics, *Rev Biol Mar Oceanogr*, 52 (2017) 95–101. http://dx.doi.org/10.4067/S0718-19572017000100007

40 Hyun D W, Shin N R, Kim M S, Kim P S, Kim J Y, *et al.*, *Pseudoruegeria haliotis* sp. nov., isolated from the gut of the abalone *Haliotis discus hannai*, *Int J Syst Evol Microbiol*, 63 (2013) 4626–4632. https://doi.org/10.1099/ijs.0.053892-0

41 Hadi Z F, Che R S, Hassan M D, Mohd S K & Ehsan R F, Isolation and identification of bacteria microflora of white shrimp, *Litopenaeus vannamei*, with antagonistic properties against *Vibrio* species, *Asian J Animal Veter Adv*, 8 (2013) 293–300. https://doi.org/10.3923/ajava.2013.293.300

42 Ariole C N & Anyanwu N G, Efficiency of indigenous *Pseudomonas aeruginosa* as biocontrol agent against *Vibrio* infection in shrimp (*Penaeus monodon*) culture, *Int J Aqua*, 7 (3) (2017) 15–22. https://doi.org/10.5376/ija.2017.07.0003

43 Ghosh K, Mukherjee A, Dutta D, Banerjee S, Breines E M, *et al.*, Endosymbiotic pathogen-inhibitory gut bacteria in three Indian major carps under polyculture system: A step toward making a probiotics consortium, *Aquacult Fish*, 6 (2) (2020) 192–204. https://doi.org/10.1016/j.aaf.2020.03.009

44 Vidal J M A, Cruz Pessoa M N, Santos F L D, Mendes P P & Mendes E S, Probiotic potential of *Bacillus cereus* against *Vibrio* sp. in post larvae shrimps, *Rev Caatinga Mossoro*, 31 (2018) 495–530.

45 Low-Goh N K, Edwards P S, Frost S & Thomas M P, The effect of abate on the growth of *Vibrio natriegens* and *Chlorella vulgaris*, *Int J Environ Stud*, 17 (1981) 135–139. https://doi.org/10.1080/00207238108709898