relatively short period, particularly within the first 48 h. However, regular sanitation and system management are essential to minimize biofilm formation and maintain long-term depuration efficiency.

4 Materials and Methods

4.1 Study area

Live black clams (*Villorita cyprinoides*) were collected from Varapuzha region (10°04′34.85″ N, 76°16′00.33″ E) of Ernakulam District, Kerala, India (Figure 5). This site is an oligohaline zone of the Cochin backwater estuary with salinity recorded at 0.03‰ during the wet season and 0.05‰ during the dry season (Abhilash et al., 2012).

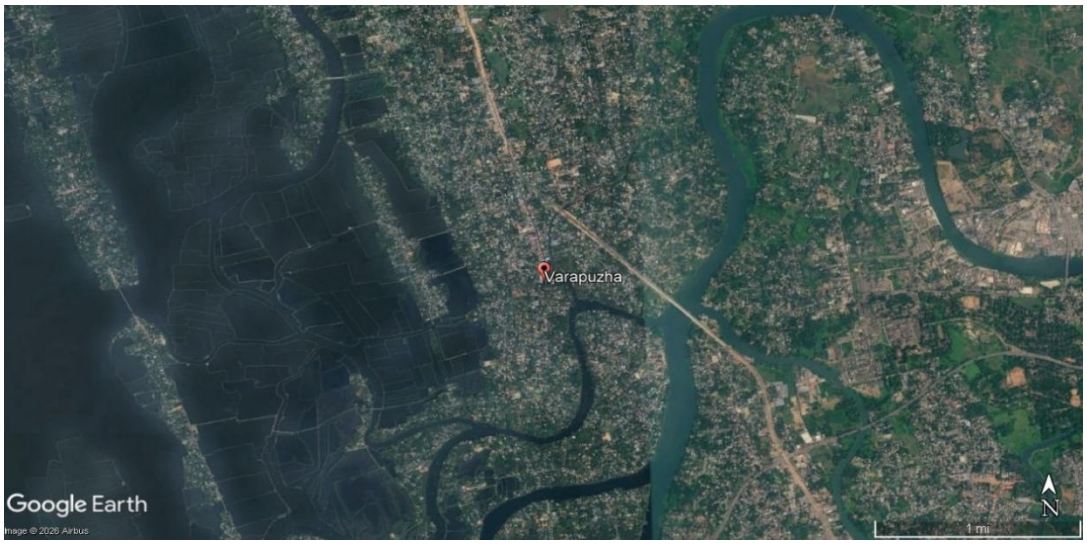

Figure 5 Map showing the sampling site at Varapuzha, Kerala, India

4.2 Collection of shellfish samples

The samples were used for bacteriological analysis and depuration experiments.

Bacteriological analyses were performed on shellfish samples to determine the initial microbial load and to monitor changes during the depuration process. Samples were collected at different depuration intervals (0, 6, 12, 24, 48, and 72 h). Total Coliforms (TC) and Faecal Coliforms (FC) were enumerated using the Most Probable Number (MPN) method, while Total Heterotrophic Bacteria (THB) and *Vibrio* spp. were determined using the total plate count method following standard microbiological procedures (Cappuccino and Sherman, 2014; APHA, 2017). Biofilm samples were also collected from the inner walls of the depuration tank at 0, 24, 48, and 72 h using sterile swabs and analyzed for TC, FC, THB, and *Vibrio* spp. using the same procedures.

4.3.1 Enumeration of Total Coliforms (TC)

Most Probable Number (MPN) method using lactose broth as the medium was used to enumerate total coliform load in the shellfish sample. Ten grams of bivalve tissue is aseptically weighed and homogenized in 90mL of sterile distilled water using tissue homogenizer (Masticator, Spain). From this homogenate 3×10 mL samples were transferred to 10 mL double strength lactose broth. 3×1 mL samples were transferred to 9 mL single strength lactose broth and 3×0.1 mL into 9.9 single strength lactose broth. Tubes were incubated at 37 ºC and observed for growth and gas production in the Durham tubes kept inverted in the test tubes. Gas production after 24 to 48 hours is considered positive for the presence of coliforms. The number of positive tubes in each dilution (10 mL, 1 mL, and 0.1 mL) was recorded and referred to *McCardy's* MPN table to estimate the total coliform load.

4.3.2 Enumeration of Faecal Coliforms (FC)

The tissue was processed as mentioned in the above section 4.3.1, and incubated at 44.5 °C to enumerate faecal coliform in the sample. After the incubation (growth and gas production) positive tubes in each dilution were recorded and referred to McCardy's MPN table to estimate the faecal coliform load.