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Research Paper

# The Impact of Sea Transportation on Environmental Health

Iksiroh El Husna[1], Nenik Kholilah[2], Sarifuddin[3], Anissofiah Azise Wijinurhayati[1]*, Widya Putri Idayatma[1]

[1] Politeknik Ilmu Pelayaran Semarang, Indonesia
[2]Universitas Diponegoro, Indonesia
[3]Politeknik Pelayaran Sumatera Barat, Indonesia

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**Abstract**

Discharging untreated ballast water into harbor waters often contains pathogenic bacteria, posing a potential threat as pollutants to the environment and fishery products. This study assesses the bacteriological impact of ballast water disposal on harbor waters and fishery products. Comparative analyses, utilizing both morphological and molecular examinations, are conducted to determine the presence of pathogenic bacteria according to the BWM Convention standards. The findings of morphological research on ballast water, harbor waters, and captured fish reveal similarities in the types of pathogenic bacteria, specifically the presence of V. cholerae and E. coli bacteria. Similarly, the molecular examination confirms the presence of the same pathogenic bacteria in both water and ballast samples, namely V. harveyi and V. parahaemolyticus. Additionally, other pathogenic bacteria are identified in the ballast water, including V. fortis and V. alginolyticus. Notably, there is no evidence of V. cholerae bacteria contribution from the river. Applying the BWM Convention is crucial and requires the attention of stakeholders to safeguard the maritime environment.

**Keywords** *Ballast Water, Fish, Maritime Environment, BWM Convention*

## INTRODUCTION

As a maritime nation and a world axis, Indonesia has a long history that predates the formation of the Republic of Indonesia. Maritime achievements originated in the Majapahit Kingdom era (Witjaksana, 2017). Indonesia has a coastline stretching over 81,290 kilometers, making it the second country globally with the longest coastline after Canada (Ministry of Energy and Mineral Resources, 2009). Two-thirds of Indonesia's territory comprises oceans, harboring abundant marine resources. Mapped coral reefs span approximately 25,000 square kilometers. Around 8,500 fish species, 555 seaweed species, and 950 coral reef organisms exist. The Indonesian seas, teeming with abundant marine fish resources, contribute to 37% of the world's fish species (Putri, 2020). This abundant potential necessitates conservation and sustainable management. Therefore, taking preventive measures to minimize the influx of pollutants into Indonesian waters is not only wise but also imperative.

Ships, as maritime transport vessels, make significant contributions to the global economy due to their high cargo capacity and cost-effectiveness compared to other transportation means. The International Chamber of Shipping asserts that the maritime transport industry is responsible for transporting approximately 90% of traded commodities worldwide, with 80% being exports and imports. This sentiment is echoed by Erga et al. (2019).

During voyages without cargo, ships require ballast water for stability (Apetroaei et al., 2018; ClearSeas, 2017). Ballast water also adjusts the ship's draft, ensuring adequate submerged weight (USDA, 2021). Ballast water can consist of fresh water, but more commonly, it is drawn from departure port waters (Lv et al., 2022) and discharged in arrival port waters (Carne7 et al., 2017).


Corresponding author's email: anissofiah@pip-semarang.ac.id

However, ballast water poses environmental concerns (Apetroaei et al., 2018; Hess-Erga et al., 2019; Zhu et al., 2020), affecting aquatic habitats, organisms, health, and economic activities (Wanga et al., 2020).

Several studies report that discharging untreated ballast water into ports has worrisome consequences, such as introducing foreign species, including bacteria. El-Husna et al. (2017) found Vibrio cholerae, Escherichia coli, and Enterococcus intestinalis in ballast water, associated with microbial presence in coastal environments. In their research at Tanjung Emas Port, Semarang, El-Husna et al. (2022) identified nine types of pathogenic bacteria, including V. cholerae at levels ranging from 0 to 15,000 CFU (colony forming units), E. coli ranging from 0 to 13,000 CFU, and E. intestinalis ranging from 0 to 7,000 CFU. These counts exceeded the limits set by Table 1 of the D-2 Ballast Water Management Convention Standards (IMO, 2019).

For coastal communities, marine fisheries are crucial for the economy. However, fishing grounds and fishponds are often in proximity to port areas. Inadequate enforcement of regulations on ship ballast water discharge in port waters leads to some vessels disposing of their ballast water in port areas without prior treatment.

## RESEARCH METHOD

This study was conducted in June 2021. Samples were collected from the vicinity of Tanjung Mas Port, Semarang, Central Java, Indonesia. The samples included ballast water from ships (E), the port vicinity environment (P1), Baru River (S2) that flows into Tanjung Emas port, and fish caught by local fishermen (I) (Figure 1). Water samples were gathered in sterile 250 mL buckets and then transferred to sterile 50 mL tubes. These samples were transported to the laboratory for further analysis.

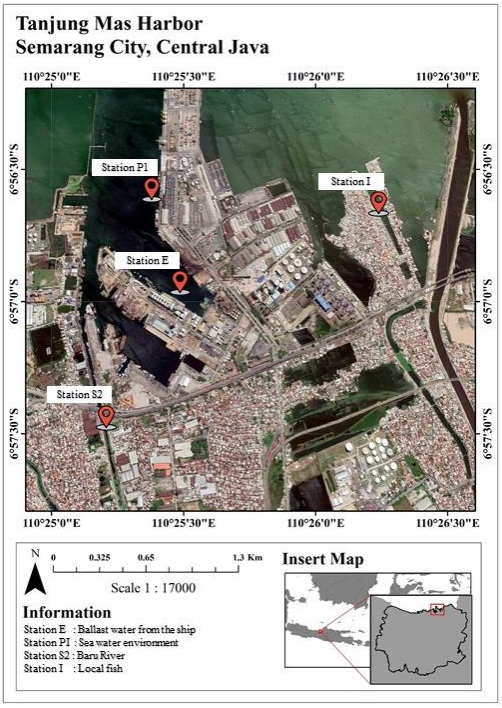


**Figure 1.** The sampling location in the area of Tanjung Mas Port, Semarang, Central Java

### Morphological Analysis

The laboratory research approach employed in this study is a variation of the Altug et al.

(2012) method. The water sample received at the laboratory was serially diluted with NaCl ranging from 10^-0 to 10^-5. TCBS (Thiosulfate-Citrate-Bile Salts-Sucrose) and McConkey agar were utilized as bacterial growth media. Identification of V. cholerae was conducted using the TCBS agar medium. A 0.2 cc sample was obtained, diluted, and then plated on a petri dish with TCBS, followed by incubation at 35°C for 24-48 hours. V. cholerae bacteria were extracted from colonies using a toothpick, transferred to a glass slide, and subjected to V. cholerae anti-sera. The presence of agglutination confirms the presence of V. cholerae.

McConkey agar, a selective Gram-negative medium, was used for bacterial isolation, acting as a differential medium. Diluted samples were inoculated onto McConkey petri plates and incubated at 35°C for 24-48 hours. Identification of Shigella and Salmonella typhi bacteria was achieved by applying bacterial colonies to anti-sera. Differentiation between Shigella and S. typhi bacteria was based on agglutination in each colony. Morphological identification adhered to the guidelines outlined in the World Health Organization's EOC 1669 manual (2003) and the Basic Techniques Guidelines for Health Laboratories, 2nd Edition. The content of ballast water permitted for discharge into the seas is governed by Standard D-2 (Table 1).

**Molecular Analysis**

Bacterial DNA extraction followed the Chelex 10% method. Amplification of the 16S rRNA gene was achieved by combining one µL of template DNA with the primary pair of 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1429R (5'-GGTTACCTTGTTACGACTT-3') primers (ElSamak et al., 2018), each at a concentration of 10 mM. Additionally, 12.5 µL of Thermo Scientific 2X Phire Plant Direct PCR Master Mix and 9.5 µL of ddH2O were added. The PCR process consisted of 40 cycles, encompassing initial denaturation (98°C, 5 min), denaturation (98°C, 5 sec), annealing (55°C, 5 sec), extension (72°C, 1 min), and final extension (72°C, 1 min).

Subsequent visualization of formed DNA bands was conducted through electrophoresis. The nucleotide base sequence was determined using the Sanger Dideoxy Method. Sequencing data were edited using MEGA XI software, and the 16S rDNA primer data were compared with GenBank NCBI records.

**FINDINGS AND DISCUSSION**

The observations of morphology yielded isolation results indicating that the ballast water contained V. cholerae at 1,300 CFU/100 ml and E. coli at 400 CFU/100 ml in sample E.1. In sample E.2, there were 1,400 CFU/100 ml of V. cholerae and 200 CFU/100 ml of E. coli. Meanwhile, sample E.3 exhibited 1,800 CFU/100 ml of V. cholerae and 200 CFU/100 ml of E. coli. These findings surpass the permissible limits as outlined in the Ballast Water Management (BWM) Convention regulations (Table 1).

**Table 1.** D-2 BWM Convention Performance Standards

| Category | IMO Standart |
|---|---|
| 50 my (Zooplankton) | < 10 off per m3 |
| 10-50 my (Phytoplankton) | < 10 0ff per m3 |
| Bacteria: - Toxicogenic Vibrio cholerae | < 1 cfu/100 ml |
| - E. Coli | < 250 cfu/100 ml |

| | |
|---|---|
| - Intestinal enterococci | < 100 cfu/100 ml |

Pathogenic bacteria were found in the waters surrounding Tanjung Emas Port in Semarang. Specifically, sample P1.1 contained 2,300 CFU/100 ml of V. cholerae and 500 CFU/100 ml of E. coli. Sample P1.3 contained 2,100 CFU/100 ml of V. cholerae and 500 CFU/100 ml of E. coli. Isolation of bacteria from the caught fish conducted by fishermen indicated the presence of V. cholerae with 820 CFU/100 ml in sample I.2 and E. coli with 170 CFU/100 ml. In sample I.3, V. cholerae was present at 710 CFU/100 ml and E. coli at 210 CFU/100 ml. In addition to these two pathogenic bacteria, other pathogenic bacteria were identified in both water and fish samples, including Enterococci, S. typhii, Klebsiella, S. epidermidis, B. subtilis, and Actinobacter. River water samples, however, lacked V. cholerae, S. typhii, S. disenteriae, Klebsiella, and S. epidermidis, while containing E. coli, Enterococci, B. subtilis, and Actinobacter. (Table 2)

**Table 2.** Results of morphological examination of pathogenic microorganisms present in the waters of Tanjung Emas Port and its surrounding fisheries products

| **ID** | **Mount of bacteria CFU/100 ml** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *V. cholerae* | *E. coli* | *Enterococci* | *S. typhii* | *S. desenteriae* | *Klebsiella* | *S. epidermidis* | *B. subtilis* | *Actinobacteria* |
| E.1 | 1.300 | 400 | 0 | 0 | 0 | 600 | 0 | 300 | 0 |
| E.2 | 1.400 | 200 | 0 | 0 | 100 | 400 | 0 | 400 | 0 |
| E.3 | 1.800 | 200 | 0 | 0 | 300 | 500 | 0 | 500 | 0 |
| P1.1 | 2.300 | 300 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P1.2 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P1.3 | 2.100 | 500 | 0 | 0 | 0 | 100 | 0 | 0 | 0 |
| I.1 | 0 | 180 | 0 | 0 | 0 | 40 | 0 | 50 | 0 |
| I.2 | 820 | 170 | 40 | 310 | 0 | 0 | 0 | 0 | 0 |
| I.3 | 710 | 210 | 0 | 150 | 0 | 0 | 0 | 0 | 140 |
| S2.1 | 0 | 31000 | 12000 | 0 | 0 | 0 | 6000 | 4000 | 27000 |
| S2.2 | 0 | 22000 | 500 | 0 | 0 | 3000 | 0 | 6000 | 18000 |
| S2.3 | 0 | 24000 | 13000 | 0 | 0 | 0 | 0 | 3000 | 17000 |

Note: (E: Ballast water, P1: Zone 1, I: Fish)

The results of the molecular analysis of samples from the waters around Tanjung Emas

Semarang and ship ballast water revealed the presence of the same pathogenic bacteria, namely V. harveyi and V. parahaemolyticus. Additionally, in ship ballast water, other pathogenic bacteria were identified, including V. fortis and V. alginolitycus (Table 3).

**Table 3.** BLAST Results of Bacterial Samples from Tanjung Emas Waters and Ship Ballast Water

| No | Sample Code | Name of the BLAST result | Sample accession number | Similarity | Sequence length (Bp) | Ident (%) | Query Cover (%) |
|---|---|---|---|---|---|---|---|
| 1. | P1.1 | *V. harveyi* | MW996726 | MK138564 | 1439 | 99.79 | 99 |
| 2. | P1.2 | *V. parahaemolyticus* | MZ310503 | MW996732 | 1450 | 99.93 | 99 |
| 3. | E_1.1 | *V. harveyi* | MZ081629 | MN938227 | 1446 | 99.79 | 99 |
| 4. | E_1.2 | *V. parahaemolyticus* | MZ081630 | MK377081 | 1450 | 99.86 | 99 |
| 5. | E_2.1 | *V. fortis* | MZ081631 | MH283811 | 1454 | 99.86 | 99 |
| 6. | E_2.2 | *V. alginolyticus* | MZ081632 | JF784015 | 1451 | 99.86 | 99 |
| 7. | E_3.1 | *V. parahaemolyticus* | MZ081633 | MK308610 | 1451 | 99.72 | 99 |
| 8. | E_3.2 | *V. alginolyticus* | MZ081634 | MT299659 | 1448 | 99.65 | 99 |

Note: (P1: Waters 1, E: Ballast Waters)

The phylogenetic results indicate that the obtained bacterial samples belong to the Vibrio bacterial genus, comprising several species such as V. harveyi, V. parahaemolyticus, V. fortis, and V. alginolyticus (Figure 2), consistent with the Blast results in the previous table.

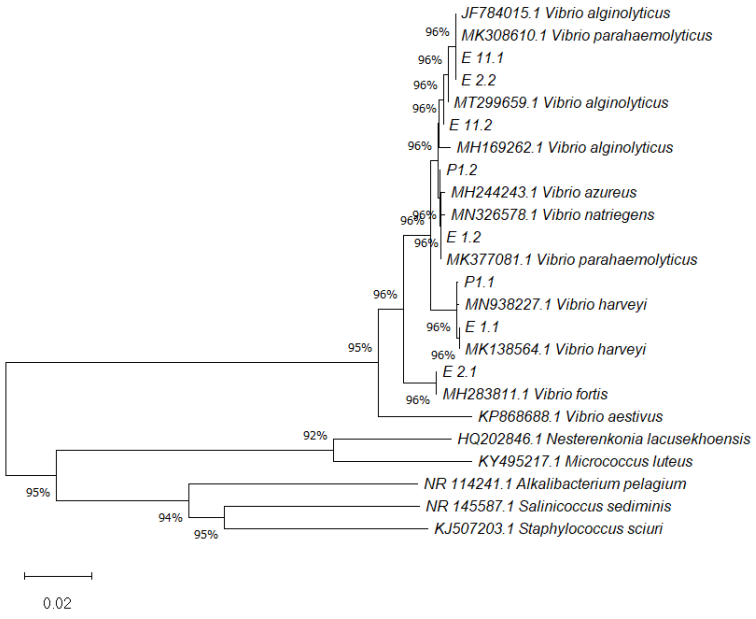


**Figure 2**. Phylogenetic Tree of Bacterial Samples from the Waters around Tanjung Emas Semarang and Ship Ballast Water using the Neighbor-Joining Method, 1000 Bootstrap Replications, Kimura 2-parameter model with Ingroup and Outgroup derived from GeneBank data (www.ncbi.nlm.nih.gov)

## CONCLUSIONS

From the findings, it can be concluded that through morphological and molecular analyses, ballast water has contaminated the waters of the harbor and captured fish. Evidence indicates that samples from both fish and water in the vicinity contain the same pathogenic bacteria as those found in ballast water (in accordance with BWM Convention standards, i.e., V. cholerae and E. coli). However, river water does not contain V. cholerae, implying that the presence of V. cholerae in fish and harbor waters is not attributed to contributions from the river water. The phylogenetic tree further illustrates that the obtained bacterial samples belong to the Vibrio bacterial genus, comprising several species such as V. harveyi, V. parahaemolyticus, V. fortis, and V. alginolyticus.

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