Asia-Pacific Journal of Science and Technology :Volume :30 .Issue :02 .Article ID :.APST-30-02-13. Research Article

APST

Asia-Pacific Journal of Science and Technology

https//:www.tci-thaijo.org/index.php/APST/index

Published by Research Department,
Khon Kaen University, Thailand

Snapshot sample of gastropods and bacterial antagonistic activity in areas of a mangrove ecosystem prone to disturbance from the Iloilo River, Iloilo, Philippines

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Received 31 March 2024
Revised 10 April 2024
Accepted 5 July 2024

Abstract

Mangrove ecosystems and their macro and microorganisms are particularly vulnerable to anthropogenic activities. Using a snapshot sampling strategy, this study assessed mangrove-associated gastropods and used the agar plug method to screen the antagonism of bacterial isolates against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 from a mangrove ecosystem most prone to human disturbance at the Iloilo River, Iloilo, Philippines. The present study recorded largely consistent physicochemical parameters (water and soil) with pre-existing data for this area and documented the presence of four gastropod taxa: *Cerithidea* sp., *Chicoreus* sp., *Littoraria* sp., and *Nerita* sp. The results also showed that two bacterial isolates (Soil Isolate 10 and Mangrove Isolate 1) had antagonistic activity against *S. aureus* ATCC 25923, and four isolates (Mangrove-Isolate 1, Soil Isolate 8, Gastropod Isolate 8, and Mangrove Isolate 15) had antagonistic activity against *E. coli* ATCC 25922. The researchers hypothesize that anthropogenic activities lead to increased rates of antagonistic relationships in the microbiota, but a comparative study is needed to confirm this. The work presented herein offers a preliminary baseline both for monitoring mangrove-associated gastropods along the Iloilo River, and for screening the antagonistic activities of bacteria in this human-perturbed mangrove ecosystem.

Keywords: Rapid assessment, Antagonistic activity, Mangrove-associated Gastropods, Mangrove ecosystem

1. Introduction

Mangrove forests are essential for maintaining biodiversity in aquatic and marine environments [1]. A component of that biodiversity is gastropods, which use mangrove forests as food sources and for shelter during spawning [2]. Given their diverse trophic roles as detritivores, herbivores, fungivores, and carnivores [3], gastropods are essential for maintaining mangrove ecosystem balance as bioturbators [4]. Microorganisms are another foundational element of mangrove biodiversity. Mangrove bacteria are rich sources of bioactive secondary metabolites that can be cytotoxic, antifungal, and antiviral in their activity, among others [5]. Antagonistic bacterial activity can be revealed through their inhibitory effects on other bacteria [6]. Importantly, gastropods and bacteria are linked; the former are known to have bacterial associates that inhibit *Escherichia coli* and *Bacillus cereus* [7].

In the Philippines, various factors, but mainly anthropogenic activities, have caused damage to mangrove forests. Urbanization, agricultural conversion, pond construction, and timber cutting all negatively affect the extent and quality of Philippine mangroves [8]. Furthermore, to date, the literature contains limited to no data about mangrove-associated gastropod diversity, nor has there been extensive screening of bacterial isolates with antagonistic activities in mangroves prone to human disturbance. The researchers hypothesize that these anthropogenic activities can result in greater rates of antagonistic microbiota interactions within the area of mangrove ecosystem prone to human disturbance.

The work presented here focuses on the gastropod and bacterial fauna of a mangrove ecosystem prone to human disturbance at Iloilo River, Iloilo, Philippines. Specifically, this study aims to: (1) conduct a rapid assessment of mangrove-associated gastropods present in areas of the mangrove ecosystem prone to human disturbance, and (2) screen for antagonistic activity in isolates of mangrove-resident bacteria against *Escherichia coli* and *Staphylococcus aureus*.

2. Materials and methods

2.1 Study site

Iloilo River is an estuarine ecosystem in the southern portion of Iloilo City in the Central islands of the Philippines, between latitudes N 10°41'35" and 10º42'30" and longitudes E 122º30'' and 122º35''. Located along the 11-km stretch of the river are the domestic port, fuel bulk depots, fuel-based electric powergenerator plant, fishponds, hospitals, private residential houses, and several commercial and food establishments. The principal tributaries of the Iloilo River are the Calajunan and Dungon Creeks. These creeks also receive domestic and commercial wastes, leachates from garbage disposal sites, agricultural runoff, and commercial and industrial wastes [9]. In the present study, the sample collection was restricted to the mangrove area of Esplanade 5 along 29 Senator Efraim P. Trenas Boulevard, La Paz, Iloilo City. This area was selected because it is among the areas most prone to human disturbance, as indicated by the Iloilo City Agriculture Office. See Figure 1 for images of food waste and other garbage directly dumped into the mangrove area.

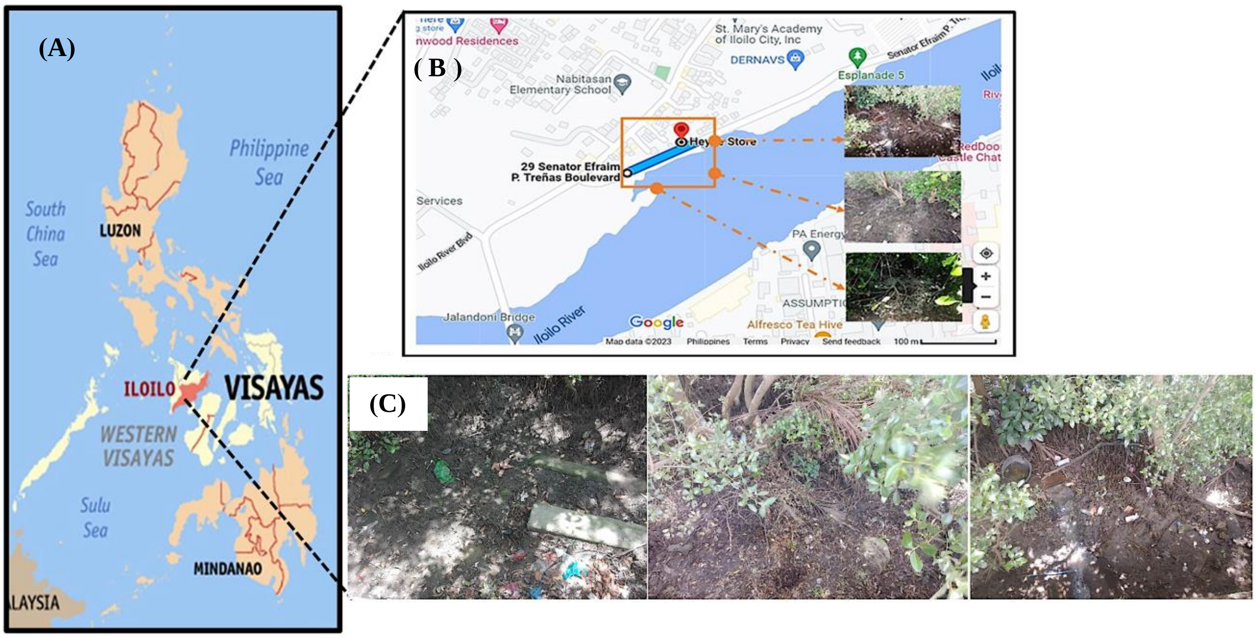

Figure 1 Study area on Iloilo River, Iloilo, Philippines. (A) location of Iloilo (Orange polygon) within the Philippines; (B) mangrove sampling site (orange square) at the interface of Iloilo City and the Iloilo River, Philippines; (C) human trash in the mangrove area.

2.2 Sample preparation, collection, and identification

Three different samples were prepared and collected on January 12, 2023, from the mangrove area of Esplanade 5 near Gaisano La Paz, along Brgy. Nabitasan, La Paz, Iloilo City, Iloilo, Philippines. The gastropod samples were gathered from a 100-m line transect established in the mangrove vegetation, and removed individually from substrates, roots, stems, and leaves during low tide, and pooled for analysis. Individual gastropods were thoroughly cleaned to facilitate their identification [10]: brushed the shell exterior while avoiding the surface scratches, samples were then boiled, followed by the removal of the body using a hook, and then air-dried for long-term storage. Genus-level gastropod identification was confirmed by experts from the Southeast Asian Fisheries Development Center (SEAFDEC) FishWorld museum. To collect gastropod-associated bacteria, the pooled soft tissues of all gastropod samples regardless of genus were extracted [11].

To collect mangrove tree-associated bacteria, a swab sampling technique was modified from that of Ramesh and Mohanraju was used [12]. Specifically, samples were gathered from the mangrove trees [*A. marina* and *S. alba* (Figure 2, species identification confirmed by Dr. Edgar Hortillosa from the Department of Marine Biology, Iloilo State University of Science and Technology)] using a sterile swab stored post-swabbing in a sterile container of 0.1% peptone solution. To collect mangrove soil-associated bacteria, soil samples were randomly collected within the line transect up to a depth of 10 cm [13, 14] pooled into a single sample that weighed approximately 1kg, and stored in a sterilized plastic bag for same-day analysis, and the remaining soil samples were submitted to Regional Soil Laboratory 6 for soil nutrient profile analysis including potassium content (K), phosphorus content (P),organic matter (OM) and pH [15]. All samples were maintained at 4°C during the collection and transportation from the field site to the Biology Laboratory, Western Institute of Technology, for analysis. For the in-situ water analyses, the salinity and temperature of water were measured using a refractometer and glass thermometer, respectively.

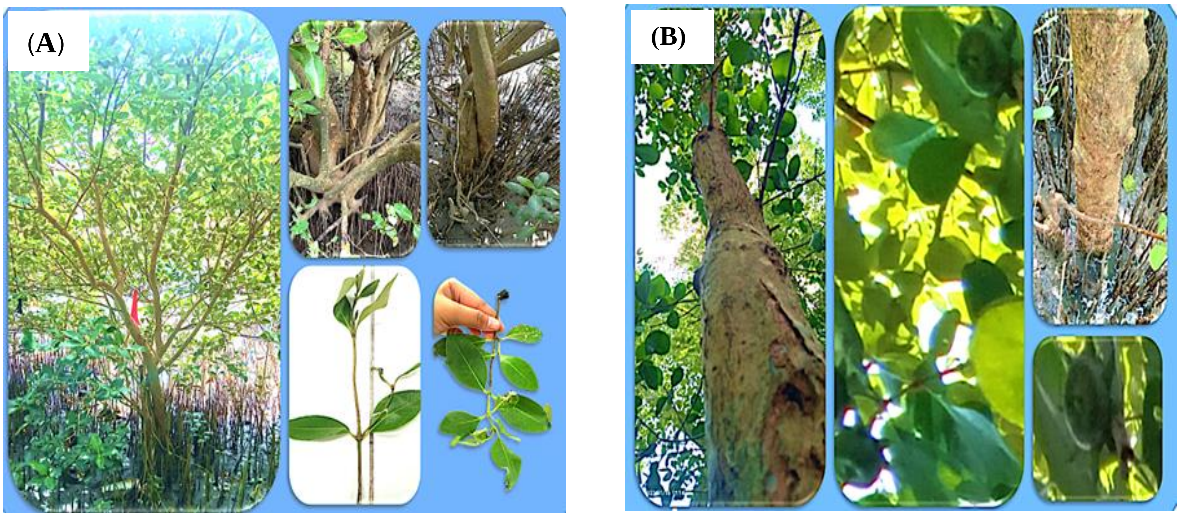

Figure 2 Mangroves Species Morphology. (A) *Avicenia marina*; (B) *Soneratia alba*.

2.3 Bacterial assessment

The Nutrient Agar (Scharlau) was prepared according to the manufacturer's instructions. The sterilization condition was set to 121°C at 15 psi for 15 min [16], then serially diluted different samples, wherein 1 gram of samples was used and transferred in the first tube with diluent for the soil and gastropod samples. For mangrove swab suspension, one mL was used and transferred in the first tube with diluent and continued until the desired dilution was reached. The last two dilutions ($10^{-9}$and 10^{-10}) were selected for the spread plate technique [17]. The samples were incubated for 24 hours at 37˚C. The colony with a prominent colony color, which indicates potential bioactive pigment-producing bacteria from the initial agar plates, was selected for isolation [18]. The light microscopy with oil immersion through a Gram staining process was used to confirm a pure culture [17].

2.4 Antagonistic activity and colony morphology

The isolated bacterial colonies were tested for their ability to inhibit the growth of *S. aureus* ATCC 25923 as a representative of Gram-positive (+) bacteria and *E. coli* ATCC 25922 as a representative of Gram-negative (–) bacteria. Colony cultures of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were provided by the Bureau of Fisheries and Aquatic Resources. The agar plug method was used to transfer the bacterial isolates to the plates with indicator bacteria [19], and incubated the plates at 37 °C for 24 hrs. After incubation, a ruler was used to measure any clear zones that formed around the agar plug to evaluate the degree of inhibition [20]. All antagonistic tests were conducted in three replicates for each isolate. The preliminary characterization was analyzed using Bergey's Manual of Determinative Bacteriology. The bacterial isolates with antagonistic activity against indicator bacteria were categorized based on their (1) Gram-stain reaction and (2) morphological characterization of isolates grown on agar based on their colony margin, texture, color, elevation, and shape [17].

2.5 Statistical Analysis

The gastropod relative abundance was expressed as a percentage of the number of individuals in a genus relative to the number of individuals in all genera from the pooled samples. The frequency (counting the number of genera present in the collected samples) and cumulative frequency (expressed in percentage) were calculated using Microsoft Excel (v16.0) and visualized the results using a Pareto Chart Plot. To evaluate antagonistic activity of the samples, analysis of variance (ANOVA) followed by Tukey's test was used for *E. coli* ATCC 25922 [SI8, GI8, MI1, and MI15 data points in three replicates each], and Independent Sample T-tests for *S. aureus* ATCC 25923 [SI10 and MI15 data points, in three replicates each]. The IBM SPSS Statistics 20 was used to complete the two antagonistic activity analyses.

3. Results

3.1 Physicochemical parameters

The results of the physicochemical analysis showed that the soil samples were highest in potassium (K) content, followed by phosphorus, and lastly, organic matter (mean±SD:2138.49±36 ppm, 15.13±0.43 ppm, and 5.19±0.26 %, respectively). Mean soil sample pH was 7.10±0.15 (Table 1). In the water analysis of the sampling site, the results showed that the salinity is brackish (24.17±3.11 ppt) with a temperature of 28.63±0.28 °C.

Table 1 Soil nutrient analysis

Soil Nutrient Contents	Potassium (K) ppm	Phosphorus (P) ppm	Organic Matter (OM) %	pH
Soil Sample*	2138.49±36	15.13±0.43	5.19±0.26	7.10±0.15

**Values are mean ± SD of three determinations.*

3.2 Gastropod Abundance

The present study identified four gastropod genera from 88 individual samples: *Cerithidea* sp., *Chicoreus* sp., *Littoraria* sp., and *Nerita* sp. Photographs of each genus are provided in Figure 3, and in situ photographs provided in Figure 4 (some gastropods were collected from mud; these are not shown in the latter figure).

Figure 5 shows the frequency and cumulative frequency (%) of the sampled gastropod genera. *Cerithidea* sp. is the most abundant gastropod that we sampled, with a frequency of 48 and a relative abundance of 55%. The three other sampled gastropod genera were far less abundant (*Littoraria* sp.: frequency = 24, relative abundance = 27%; *Nerita* sp.: frequency = 10, relative abundance = 11%; and *Chicoreus* sp.: frequency = 6, relative abundance = 7%). The red line in Figure 5 indicates that the cumulative frequency of 80% of the gastropod species in the Iloilo River is represented by the first two genera on the chart, *Cerithidea* and *Littoraria*.

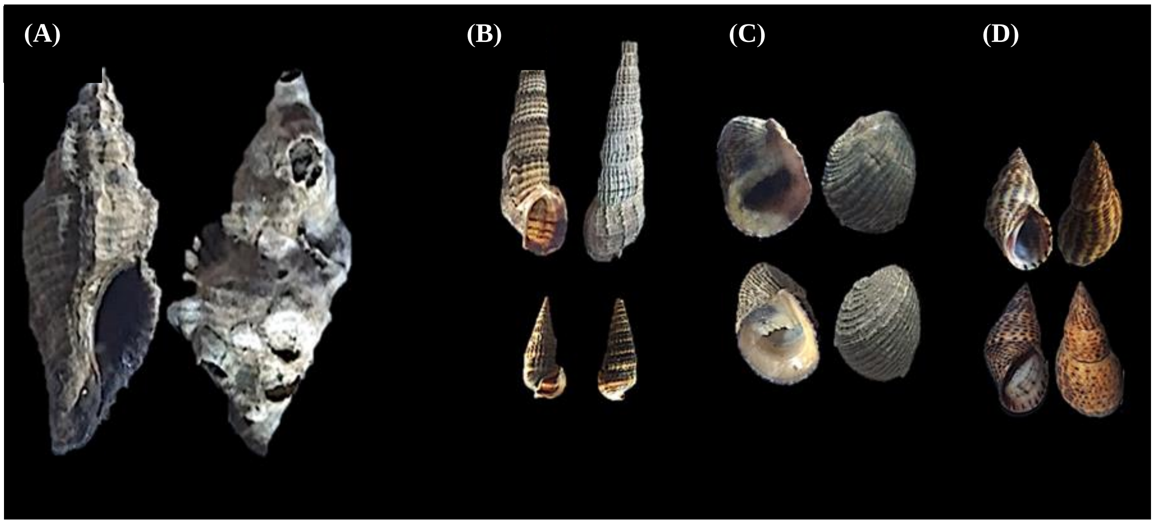

Figure 3 Photographs of gastropods genera sampled from the Iloilo River, Iloilo, Philippines ventral (left) and dorsal (right) view. (A) *Chicoreus* sp.*;* (B) *Cerithidea* sp.*;* (C) *Nerita* sp.*;* and (D) *Littoraria* sp.

Figure 4 In situ photographs of gastropods from the Iloilo River, Iloilo, Philippines.

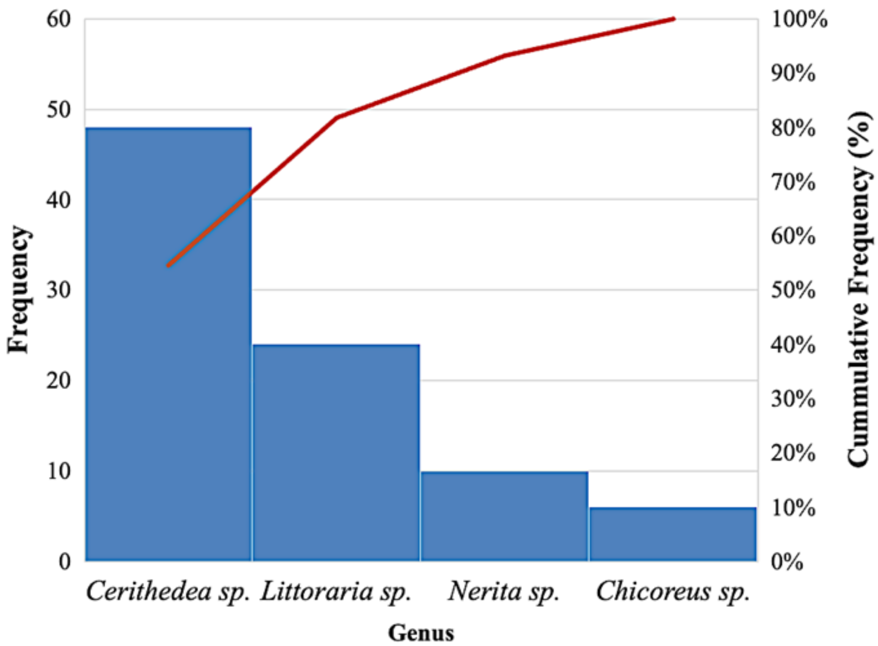

Figure 5 Pareto Chart Plot showing frequency and cumulative frequency of gastropod genera found in Esplanade 5 Gaisano La Paz, along Brgy. Nabitasan, La Paz, Iloilo City, Iloilo, Philippines.

3.3 Antagonistic activities of isolates

A total of 54 bacterial isolates, with 18 each derived from mangroves, gastropods, and soil were tested for antagonistic activity (Table 2). Isolates with antagonistic activity (indicated by a "+") are shown in red text in Table 1. Of all the isolates, only two (Soil Isolate 10 and Mangrove Isolate 1) showed antagonistic activity against *S. aureus* ATCC 25923, while four isolates (Mangrove Isolate 1, Mangrove Isolate 15, Soil Isolate 8, and Gastropod Isolate 8) showed antagonistic activity against *E. coli* ATCC 25922.

Table 2 Presence and absence of antagonistic activity of isolates against *E. coli* ATCC 25922 and *S. aureus ATCC* 25923.

Mangrove Isolates	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	Gastropod Isolates	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	Soil Isolates	*S. aureus* ATCC 25923	*E. coli* ATCC 25922
MI 1	-	+	GI 1	-	-	SI 1	-	-
MI 2	-	-	GI 2	-	-	SI 2	-	-
MI 3	-	-	GI 3	-	-	SI 3	-	-
MI 4	-	-	GI 4	-	-	SI 4	-	-
MI 5	-	-	GI 5	-	-	SI 5	-	-
MI 6	-	-	GI 6	-	-	SI 6	-	-
MI 7	-	-	GI 7	-	-	SI 7	-	-
MI 8	-	-	GI 8	-	+	SI 8	-	+
MI 9	-	-	GI 9	-	-	SI 9	-	-
MI 10	-	-	GI 10	-	-	SI 10	+	-
MI 11	-	-	GI 11	-	-	SI 11	-	-
MI 12	-	-	GI 12	-	-	SI 12	-	-
MI 13	-	-	GI 13	-	-	SI 13	-	-
MI 14	-	-	GI 14	-	-	SI 14	-	-
MI 15	+	+	GI 15	-	-	SI 15	-	-
MI 16	-	-	GI 16	-	-	SI 16	-	-
MI 17	-	-	GI 17	-	-	SI 17	-	-
MI 18	-	-	GI 18	-	-	SI 18	-	-

Abbreviations: MI = Mangrove Isolate, GI = Gastropod Isolate, SI = Soil Isolate, (+) with antagonistic activity, and (-) without antagonistic activity.

Figure 6 shows the numerical data of antagonistic activity against *S. aureus* ATCC 25923. Sample Soil Isolate 10 had a relatively large zone of inhibition (21.0±2.05 mm), while that of MI 15 was much smaller (4.33 ±0.47 mm), indicating weaker antagonistic activity of this latter sample against *S. aureus* ATCC 25923. The difference is statistically significant (P<0.05).

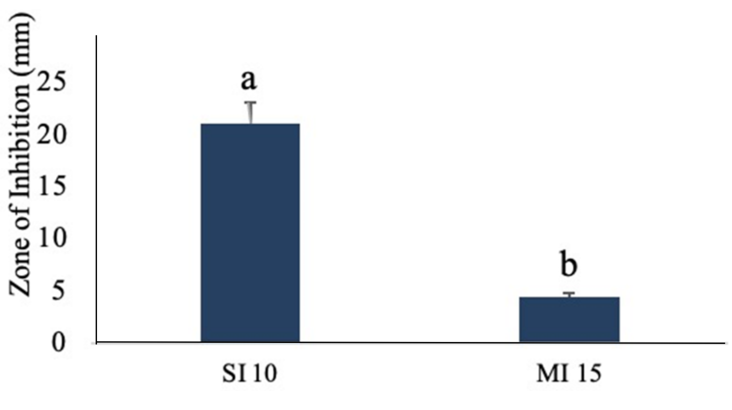

Figure 6 Antagonistic activity against S. aureus ATCC 25923 of selected bacterial isolates from the Iloilo River, Panay Island, Philippines. Abbreviations: MI = Mangrove Isolate, and SI = Soil Isolate.

The four microbial isolates MI 1, SI 8, GI 8, and MI 15 showed variable inhibitory effects on the growth of *E. coli* ATCC 25922, as demonstrated by the zone of inhibition (Figure 7). MI 1 has the widest zone of inhibition (9.33±0.47 mm), indicating the strongest inhibitory activity among the four isolates tested. The other three isolates also showed some degree of inhibitory activity, but their zones of inhibition were smaller than that of MI 1 (4.33±0.47 mm for both SI 8 and MI15, and 3.33±0.47 mm for GI 8). The higher antagonistic activity of MI 1 compared to the other isolates is statistically significant ($P<0.05$).

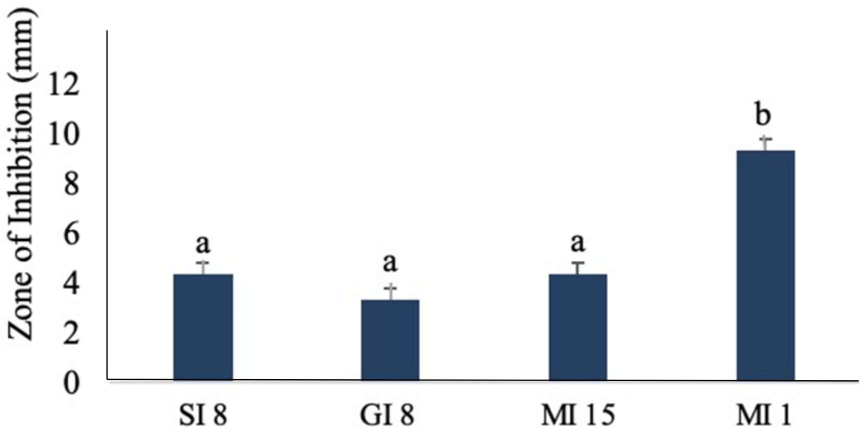

Figure 7 Antagonistic activity against *E. coli* ATCC 25922 of selected bacterial isolates from the Iloilo River, Panay Island, Philippines. Abbreviations: MI = Mangrove Isolate, GI = Gastropod Isolate, and SI = Soil Isolate.

All the isolates show the same texture (matte and brittle), color (milky white), and elevation (flat). MI 1 and SI 10 have smooth margins, while the other isolates, GI 8, S I8, and MI 15, have lobate margins. The soil isolates 8 and 10 are Gram-negative bacteria, the same Gram reaction as the Gastropod Isolate 8. The mangrove samples (MI 1 and MI 15) are Gram-positive (Table 3).

Table 3 Colony morphology of bacterial isolates from gastropod (G), mangrove (m) and soil (s) taken from Esplanade 5, Gaisano La Paz, along Brgy. Nabitasan, La Paz, Iloilo City, Iloilo, Philippines

Isolate	Shape	Margin	Color	Elevation	Texture	Gram Stain Reaction
GI 8	Spindle	Lobate	Milky	Flat	Matte and brittle	-
MI 1	Round	Smooth	Milky	Flat	Matte and brittle	+
MI 15	Irregular	Lobate	Milky	Flat	Matte and brittle	+
SI 8	Round	Lobate	Milky	Flat	Matte and brittle	-
SI 10	Round	Smooth	Milky	Flat	Shiny and viscous	-

Abbreviations: MI = Mangrove Isolate, GI = Gastropod Isolate, and SI = Soil Isolate.

4. Discussion

4.1 Physicochemical parameters

The physicochemical parameters recorded in the present study are comparable to previous studies conducted in the Iloilo River. The recorded surface water temperature (28.63±0.28 °C) was within the range set by DENR for a Class C Water Body (25–32 °C), which is a standard reference limit. The water salinity measurement (24.17±3.11 ppt) is almost the same as the minimum surface salinity (23.3 ppt) recorded by Palla et al. [21]. The percentage of soil organic matter that we documented (5.19±0.26%) is also comparable to that of Palla et al. [21], who reported a value of 6.5%. However, the researchers cannot conclude that human disturbance had no impact on the physicochemical parameters of the Iloilo River mangrove ecosystem, because no concrete data is available in the literature indicating the timeline when human perturbation started as a basis of comparison.

4.2 Gastropod abundance

Most gastropods are found in mangrove forests, where they are present in muddy or submerged soil, or attached to stems or roots of mangrove trees [2]. Mangrove ecosystems offer gastropods shelter, spawning sites, and food sources [2]. In addition, the leaf litter that undergoes biomass decomposition becomes food for the gastropods [22].

Consistent with prior literature, the present study identified four gastropod genera from 88 individual samples: *Cerithidea* sp., *Littoraria* sp., *Nerita* sp., and *Chicoreus* sp. (Figure 4). Uy-Bagarinao [23], documented the genera *Cerithidea*, and *Nerita* in mangrove-derived aquaculture ponds in Dumangas, Iloilo, while Cañada et al. [24] documented *Littoraria*, *Nerita*, and *Cerithidea* in the Mangrove Forests of Casiguran, Aurora [24]. *Nerita*, *Chicoreus*, and *Littoraria* were some of the gastropod genera sampled from intertidal areas of Ajuy, Iloilo by Arabaca et al. [25]. The latter two genera were also detected by Velasco et al. [26] on the protected mangroves of the Balingasay River. Similar results were found in the study of Pogado et al. [27] wherein, *Littoraria* and *Nerita* are also included in the genera of gastropods found in the island and fringing mangrove forests in Calatagan, Batangas. Furthermore, In the study of Degamon et al. [28], some of the genera of gastropods were found in the mangrove forest of Barangay Nabago namely *Littoraria*, *Cerithidea*, and *Nerita*. Lastly, in the study of Dolorosa et al. [29], some genera of gastropods encountered in the Iwahig River-Estuary, Palawan were *Littoraria*, *Nerita*, and *Cerithidea*.

4.3 Antagonistic activities

Mangrove ecosystems harbor abundant bacteria that are rich sources of secondary metabolites with a wide range of medical and social applications. These bioactive bacterial compounds include antimicrobial agents, antitumor agents, insecticides, vitamins, immunosuppressants, and immune modulators. Mangrove-inhabiting bacteria can recycle nutrients, produce, and consume gases that impact the world's climate, eliminate pollutants, treat waste produced by humans, and control biological pests of plants and animals. However, unlike in other environments, mangrove microbial diversity remains inadequately described both phylogenetically and functionally [30].

The present work involved only preliminary screening, which is the initial part of identifying the existence of isolates with positive antagonistic activities against the test microorganisms. In this study, six bacterial isolates were documented with antagonistic activity against the two test bacteria. Several prior studies have shown that mangroves can be a source of bioactive compounds. For instance, Apurillo et al. [31], isolated 73 mangrove fungal endophytes from Leyte and Samar Islands in the eastern Philippines and found them capable of producing different metabolites with potential pharmaceutical applications. Another study in Los Baños, Laguna, Philippines revealed three mangrove-soil bacterial isolates (*Bacillus altitudinis*, *B. subtilis*, and *B. thuringiensis*) with antagonistic effects against the four tested strains of *Ralstonia solanacearum* [32]. Furthermore, Tabao and Monsalud [33], recovered five cellulase-producing bacterial strains from soils collected at various mangrove sites in the Philippines. Similar results were found in other studies conducted in other countries such as, in the study of Indupalli et al. [34], an actinobacterium was isolated from the Nizampatnam mangrove ecosystem of Andhra Pradesh, India. It produced the compound methoxy ethyl cinnamate (ethyl(E)-3-(4-methoxyphenyl acrylate (R1)), which exhibited higher antimicrobial potential against *Staphylococcus aureus*, *B. megaterium*, and *Candida albicans*. Furthermore, Wijaya et al. [7] gathered samples of the gastropods *Certhideopsilla alata*, *Cerithidea quoyii*, *Cassidula aurisfelis*, *Cassidula nucleus*, and *Telescopium telescopium* from the Mangrove Education Park in Tugu, Semarang, which yielded 61 bacterial isolates of which 13 inhibited *E. coli* and 8 inhibited *B. cereus*.

5. Conclusion

The present preliminary, snapshot study recorded four gastropod genera (*Chicoreus* sp., *Cerithidea* sp., *Nerita* sp., and *Littoraria* sp.) in the mangroves of the Iloilo River, which suffers from ongoing human disturbance. Our measurements of physicochemical parameters (water and soil) are largely consistent with pre-existing data for this area. Additionally, we recorded two bacterial isolates (Soil Isolate 10 and Mangrove Isolate 1) with antagonistic activities against *S. aureus* ATCC 25923, and four isolates with antagonistic activities (Mangrove-Isolate 1, Soil Isolate 8, Gastropod Isolate 8, and Mangrove Isolate 15) against *E. coli* ATCC 25922. These data suggest that anthropogenic activities have not measurably aggravated the ecological condition of the area. However, limited data are available in the literature regarding gastropods in the Iloilo River; thus, before-after comparisons are impossible. Further study is needed to monitor the mangrove ecosystem since this is the first study conducted about the mangrove-associated gastropod at Iloilo River and screening of antagonistic activities of the bacteria in the mangrove ecosystem prone to human disturbance.

6. Acknowledgements

We thank the Biological Laboratory of the Department of Sciences, Western Institute of Technology for assistance with laboratory apparatus, reagents, and other equipment. Our gratitude to the Department of Agriculture, Western Visayas, Regional Soil Laboratory for their assistance with soil analysis. The Southeast Asian Fisheries Development Center (SEAFDEC) FishWorld museum, and Dr. Edgar Hortillosa from the Department of Marine Biology at the Iloilo State University of Science and Technology, kindly confirmed the initial identification of our gastropod and mangrove samples, respectively. The Bureau of Fisheries and Aquatic Resources provided the *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 cultures. To Dr. Adam G. Clause from the Department of Herpetology at San Diego Natural History Museum and Dr. Sam Dragga from the Texas Tech University for proofreading our manuscript during the copyediting stage of publication, for which we are thankful.

7. References

[1] Adame MF, Connolly RM, Turschwell MP, Lovelock CE, Fatoyinbo T, Lagomasino D, et al. Future carbon emissions from global mangrove forest loss. Glob Chang Biol. 2021;27(12):2856–66.

[2] Octavina C, Defira CN, Ulfah M, Purnawan S, Dwiriansyah R. Community structure of Gastropods in mangrove ecosystem of Pulo Sarok Village, Aceh Singkil Regency, Indonesia. IOP Conf Ser Earth Environ Sci. 2019;348(1):012005.

[3] Zvonareva S, Kantor Y, Li X, Britayev T. Long-term monitoring of Gastropoda (Mollusca) fauna in planted mangroves in central Vietnam. Zool Stud. 2015;54(1).

[4] Purnama MF, Prayitno SB, Muskananfola MR, Suryanti S. Tropical gastropod density and diversity in the mangrove forest of Totobo Village, Southeast Sulawesi, Indonesia. Biodiversitas. 2024;25(4).

[5] Ancheeva E, Daletos G, Proksch P. Lead compounds from mangrove-associated microorganisms. Mar Drugs. 2018;16(9):319.

[6] Feichtmayer J, Deng L, Griebler C. Antagonistic microbial interactions: Contributions and potential applications for controlling pathogens in the aquatic systems. Front Microbiol. 2017;8.

[7] Wijayaa PA, Pringgeniesb D. Antibacterial activity of gastropod association bacteria from mangrove ecosystem against Bacillus cereus and Escherichia coli and its potency of application for Belanak Fish (*Mugil subviridis*). J Fish Mar Res. 2021;5(1):15–21.

[8] Garcia KB, Malabrigo PL Jr, Gevaña DT. Philippines' mangrove ecosystem: Status, threats and conservation. In: Mangrove Ecosystems of Asia. New York, NY: Springer New York; 2014. p. 81–94.

[9] Taberna HS Jr, Nillos MGG, Pahila IG, Arban JPB. Distribution and geochemical behaviour of heavy metals (Cr, Cu, Ni and Pb) in Iloilo River estuarine sediments. AES Bioflux. 2015;7(1):11-19.

[10] Norcio, C. U., and Flores, A. 2019. Marine gastropods and bivalves of Biri, Northern Samar. J Bio Innov. 8(3): 319-329

[11] Periyasamy N, Srinivasan M, Balakrishnan S. Antimicrobial activities of the tissue extract of Babylonia spirata Linnaeus, 1758 (Mollusca: Gastropoda) from Thazhanguda, southeast coast of India. Asian Pac J Trop Biomed. 2012;2(1):36–40.

[12] Ramesh C, Mohanraju R. Antibacterial activity of marine bioluminescent bacteria. Indian J Mar Sci. 2017;46(10):2063–74.

[13] Nimnoi P, Pongsilp N. Insights into bacterial communities and diversity of mangrove forest soils along the Upper Gulf of Thailand in response to environmental factors. Biology (Basel). 2022;11(12):1787.

[14] Arabaca MLC, Ilano A, Taunan VO. Diversity of marine macro molluscan gastropods and bivalves in the intertidal areas of Ajuy, Iloilo, Western Visayas, Philippines. IOP Conf Ser Earth Environ Sci. 2022;1118(1):012014.

[15] Billonid J, Padilla P, Muege M, Sumaryo M, Geduspan J. Bacterial community structure, diversity, and fertility of soil with and without press mud in two sites in Panay, Philippines. J Trop Life Sci. 2023;13(1):23–36.

[16] Dawodu OG, Akanbi RB. Isolation and identification of microorganisms associated with automated teller machines on Federal Polytechnic Ede campus. PLoS One. 2021;16(8): e0254658.

[17] Jalani JC, Arshad ZIM, Shaarani SM, Che Man R, Abdul Mudalip SK, Sulaiman SZ. Isolation and characterization of PLA-degrading bacteria from landfill soil at mesophilic temperature. IOP Conf Ser Mater Sci Eng. 2020;991(1):012004.

[18] Lunggani AT, Darmanto YS, Radjasa OK, Sabdono A. Prospective source of antimicrobial compounds from pigment produced by bacteria associated with brown alga (Phaeophyceae) isolated from Karimunjawa Island, Indonesia. IOP Conf Ser Earth Environ Sci. 2018; 116:012088.

[19] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71–9.

[20] Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. Asm.org. [cited 2024 Jul 14]. Available from: https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf

[21] Palla RQ, Campos WL, Campos ADN-. Habitat health of Iloilo River - insights from a study on benthic community structure. Mar Res Indones. 2015;38(1):39–47.

[22] Tavares DS, Maia RC, Rocha-Barreira C, Matthews-Cascon H. Ecological relations between mangrove leaf litter and the spatial distribution of the gastropod *Melampus coffeus* in a fringe mangrove forest. Iheringia Ser Zool. 2015;105(1):35–40.

[23] Uy-Bagarinao T. Biodiversity in mangrove-derived aquaculture ponds Dumangas, Iloilo, Philippines. Philipp J Sci. 2020;150(1).

[24] Cañada MCB, Rotaquio EL Jr, Gallego RBJ. Spatial zonation and diversity of bivalves and gastropods in mangrove forests of Casiguran, Aurora. Open J Ecol. 2021;11(10):645–63.

[25] Arabaca, M., Ilano, A., and Taunan, V. 2022. Diversity of marine macro molluscan gastropods and bivalves in the intertidal areas of Ajuy, Iloilo, Western Visayas, Philippines. IOP Conference Series: Earth and Environmental Science. 1118(1): 012014.

[26] Velasco RU, Lopez ML, Devanadera MK. Gastropod diversity in the riparian zone of Balingasay river, Bolinao, Pangasinan, Philippines. Actamanil. 2018; 66:45–53.

[27] Pogado F, Chavez REC. Diversity and community assembly patterns of gastropods in island and fringing mangrove forests in Calatagan, Batangas, Philippines. J Wetl Biodivers. 2022; 12:7–23.

[28] Degamon LS, Eviota MP, Hugo RL, Bertulfo RE, Odtojan MM, Buenaflor GS, et al. Length – weight relationship of bivalves and gastropods from mangrove forest of Brgy. Nabago, Surigao City, Philippines. IOP Conf Ser Earth Environ Sci. 2023;1250(1):012003.

[29] Dolorosa R, Galon F. Species richness of bivalves and gastropods in Iwahig River-Estuary. Palawan, the Philippines. Int J Fish Aquat Stud. 2014; 2: 207-215.

[30] Thatoi H, Behera BC, Mishra RR, Dutta SK. Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: a review. Ann Microbiol. 2013;63(1):1–19.

[31] Apurillo CCS, Cai L, Cruz TE. Diversity and bioactivities of mangrove fungal endophytes from Leyte and Samar, Philippines. Philipp Sci Lett. 2019; 12:33–48.

[32] Creencia AR, Alcantara EP, Opulencia RB, Diaz MGQ, Monsalud RG. A preliminary screening of Philippine mangrove soil bacteria exhibits suppression of *Ralstonia solanacearum* (Smith) Yabuuchi et al. causing moko disease of banana (Musa acuminata Cavendish subgroup) under laboratory conditions. J Int Soc Southeast Asian Agric Sci. 2022;28(1):135–48.

[33] Monsalud RG, Tabao NSC. Characterization and identification of high cellulase-producing bacterial strains from Philippine mangroves. Philipp J Syst Biol. 2010;4(0).

[34] Indupalli M, Muvva V, Mangamuri U, Munaganti RK, Naragani K. Bioactive compounds from mangrove derived rare actinobacterium *Saccharomonospora oceani* VJDS-3. 3 Biotech. 2018;8(2).