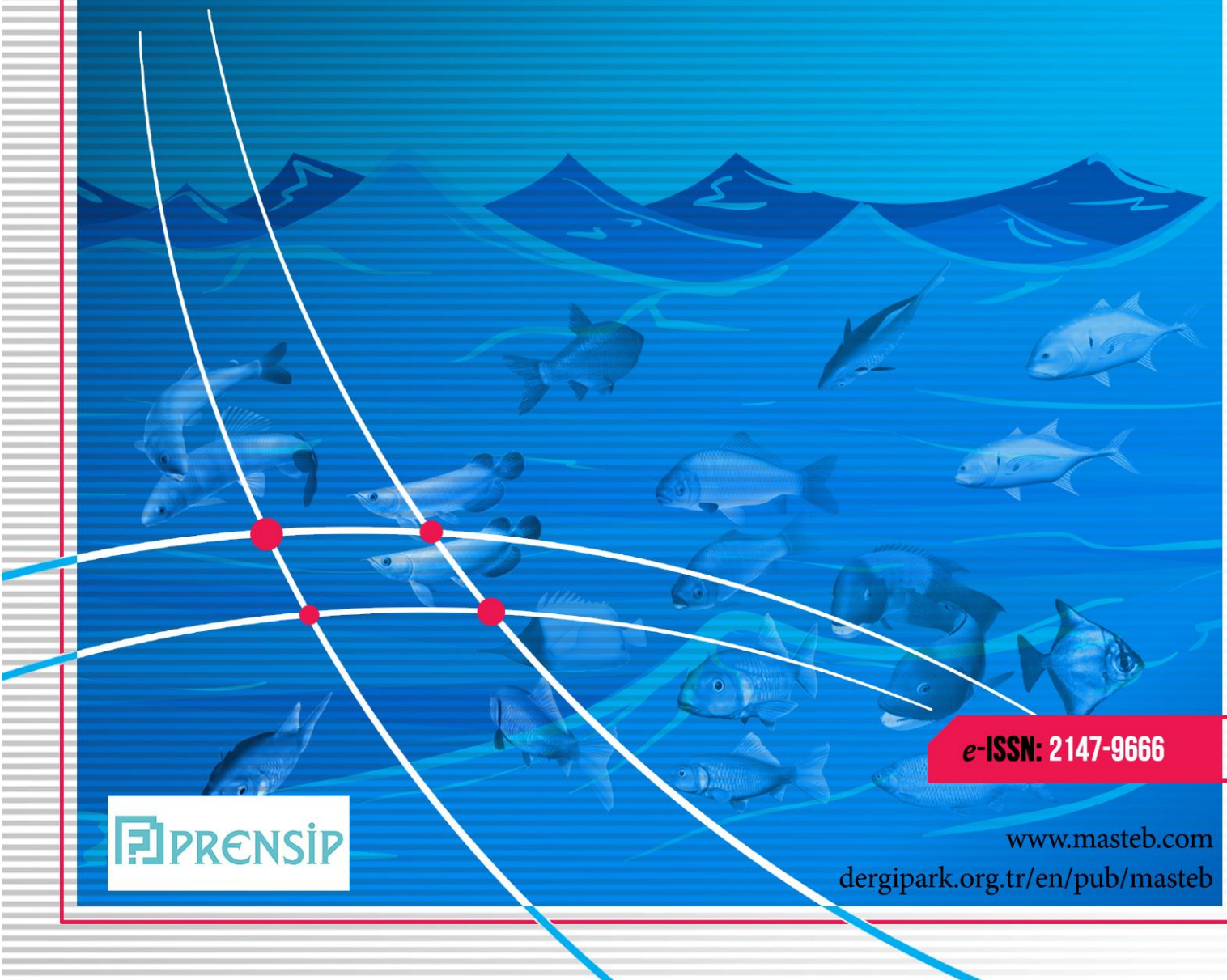
MARINE SCIENCE AND TECHNOLOGY BULLETIN

Volume 13 - Issue 3 - YEAR 2024



e-ISSN: 2147-9666

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MARINE SCIENCE AND TECHNOLOGY BULLETIN

2024 • VOLUME: 13 • ISSUE: 3

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While the peer-reviewed journal literature should be accessible online without cost to readers, it is not costless to produce. However, experiments show that the overall costs of providing open access to this literature are far lower than the costs of traditional forms of dissemination. With such an opportunity to save money and expand the scope of dissemination at the same time, there is today a strong incentive for professional associations, universities, libraries, foundations, and others to embrace open access as a means of advancing their missions. Achieving open access will require new cost recovery models and financing mechanisms, but the significantly lower overall cost of dissemination is a reason to be confident that the goal is attainable and not merely preferable or utopian.

To achieve open access to scholarly journal literature, we recommend two complementary strategies.

I. Self-Archiving: First, scholars need the tools and assistance to deposit their refereed journal articles in open electronic archives, a practice commonly called, self-archiving. When these archives conform to standards created by the Open Archives Initiative, then search engines and other tools can treat the separate archives as one. Users then need not know which archives exist or where they are located in order to find and make use of their contents.

II. Open-access Journals: Second, scholars need the means to launch a new generation of journals committed to open access, and to help existing journals that elect to make the transition to open access. Because journal articles should be disseminated as widely as possible, these new journals will no longer invoke copyright to restrict access to and use of the material they publish. Instead, they will use copyright and other tools to ensure permanent open access to all the articles they publish. Because price is a barrier to access, these new journals will not charge subscription or access fees, and will turn to other methods for covering their expenses. There are many alternative sources of funds for this purpose, including the foundations and governments that fund research, the universities and laboratories that employ researchers, endowments set up by discipline or institution, friends of the cause of open access, profits from the sale of add-ons to the basic texts, funds freed up by the demise or cancellation of journals charging traditional subscription or access fees, or even contributions from the researchers themselves. There is no need to favor one of these solutions over the others for all disciplines or nations, and no need to stop looking for other, creative alternatives.

Open access to peer-reviewed journal literature is the goal. Self-archiving (I.) and a new generation of open-access journals (II.) are the ways to attain this goal. They are not only direct and effective means to this end, they are within the reach of scholars themselves, immediately, and need not wait on changes brought about by markets or legislation. While we endorse the two strategies just outlined, we also encourage experimentation with further ways to make the transition from the present methods of dissemination to open access. Flexibility, experimentation, and adaptation to local circumstances are the best ways to assure that progress in diverse settings will be rapid, secure, and long-lived.

The Open Society Institute, the foundation network founded by philanthropist George Soros, is committed to providing initial help and funding to realize this goal. It will use its resources and influence to extend and promote institutional self-archiving, to launch new open-access journals, and to help an open-access journal system become economically self-sustaining. While the Open Society Institute's commitment and resources are substantial, this initiative is very much in need of other organizations to lend their effort and resources.

We invite governments, universities, libraries, journal editors, publishers, foundations, learned societies, professional associations, and individual scholars who share our vision to join us in the task of removing the barriers to open access and building a future in which research and education in every part of the world are that much more free to flourish.

For various reasons, this kind of free and unrestricted online availability, which we will call open access, has so far been limited to small portions of the journal literature. But even in these limited collections, many different initiatives have shown that open access is economically feasible, that it gives readers extraordinary power to find and make use of relevant literature, and that it gives authors and their works vast and measurable new visibility, readership, and impact. To secure these benefits for all, we call on all interested institutions and individuals to help open up access to the rest of this literature and remove the barriers, especially the price barriers, that stand in the way. The more who join the effort to advance this cause, the sooner we will all enjoy the benefits of open access.

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The studies submitted to *Marine Science and Technology Bulletin* are first evaluated by the editor. At this stage, studies that are not in line with the aim and scope of the journal, are weak in terms of language and narrative rules in English contain scientifically critical mistakes, are not original worthy and cannot meet publication policies are rejected. Authors of rejected studies will be notified within one month at the latest from the date of submission. Eligible studies are sent to the field editor to which the study is relevant for pre-evaluation.

2. Pre-Evaluation Process

In the pre-evaluation process, the field editors examine the studies, introduction and literature, methods, findings, results, evaluation and discussion sections in detail in terms of journal publication policies, scope and authenticity of study. Study which is not suitable as a result of this examination is returned to the author with the field editor's evaluation report within four weeks at the

latest. The studies which are suitable for the journal are passed to the referee process.

3. Referee Process

The studies are sent to the referees according to their content and the expertise of the referees. The field editor examining the study may propose at least two referees from the pool of Marine Science and Technology Bulletin Advisory Board or referee pool according to their field of expertise or may propose a new referee appropriate to the field of study.

The editors evaluate the referee's suggestions coming from the field editor and the studies are submitted to the referees. Referees are obliged to guarantee that they will not share any process or document about the study they are evaluating.

4. Referee Evaluation Process

The period given to the referee for the evaluation process is 15 days. Proposals for corrections from referees or editors must be completed by the authors within 1 month according to the "correction instruction".

Referees can decide on the suitability of the study by reviewing the corrections and may also request multiple corrections if necessary.

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Referee evaluations are based in general on the originality of the studies, the method used, and the conformity with the ethical rules, the consistent presentation of the findings and results, and the examination of the literature.

This review is based on the following elements:

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4. Evaluation and discussion: The evaluation report includes the opinion on the subject based on findings, relevance to research questions and hypotheses, generalizability and applicability.

5. Conclusion and suggestions: The evaluation report contains the opinion on the contributions to the literature, future studies and recommendations for the applications in the area.

6. Style and narration: The evaluation report includes compatibility of the headline with the content, appropriate use of English in the study, and references in accordance with the language of the study and APA rules.

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Mar. Sci. Tech. Bull. (2024) 13(3): 175-182
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1514540

Marine Science and Technology Bulletin

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RESEARCH ARTICLE

Heavy metal distributions of macroalgal species from the Mersin inner bay, Türkiye

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ARTICLE INFO

Article History:
Received: 11.07.2024
Received in revised form: 05.08.2024
Accepted: 15.08.2024
Available online: 30.09.2024

Keywords:
Trace elements
Bioaccumulation
Marine organisms
Health risk assessment
Mediterranean Sea

ABSTRACT

The bio-accumulation of heavy metals in macroalgae poses significant risks to marine ecosystems and human health. In this study, heavy metal distributions of macroalgae and potential health risk assessment in the Mersin inner Bay were determined from macroalgae (*Stypopodium schimperi*, *Padina pavonica*, *Laurencia obtusa, Dictyota* sp., *Sargassum vulgare, Jania rubens, Halopithys* sp., *Osmundea* sp., *Halopithys incurve, Ulva intestinalis, Entromorpha flexuosa*, *Ulva linza*) samples collected between February and September 2021. The levels of heavy metals were found as 1.24-5.93 g/kg for aluminum, 0.61-5.26 g/kg for iron, 1.17-7.61 mg/kg for cobalt, 0.03-0.21 mg/kg for cadmium, 4.26-43.10 mg/kg for chromium, 0.28-437.52 mg/kg for cupper, 17.90-139.08 mg/kg for manganese, 2.78-79.25 mg/kg for nickel, 0.75-3.49 mg/kg for lead and 1.71-70.34 mg/kg for zinc, respectively. The potential risk assessment of macroalgal species using the calculated total cancer risk (TCR) suggested high carcinogenic risk from the carcinogenic chromium, copper and nickel (TCR>1.00E-04).

Please cite this paper as follows:

Özbay, Ö., & Akçay, İ. (2024). Heavy metal distributions of macroalgal species from the Mersin inner bay, Türkiye. *Marine Science and Technology Bulletin*, *13*(3), 175-182. https://doi.org/10.33714/masteb.1514540

Introduction

Coastal marine regions are adversely affected by human-induced pressures. The development of cities along the coasts and industrialization are the main causes of anthropogenic contaminants in these ecosystems (Bouri et al., 2021; Rahhou et al., 2023). Heavy metals, one of the certain kinds of contaminants, are naturally available in aquatic ecosystems. In order to assess heavy metal pollution in aquatic ecosystems, it is important to distinguish between anthropogenic heavy metal pollution and background levels (Akcali & Kucuksezgin, 2011). It is well known that anthropogenic and non-anthropogenic inputs into the aquatic environments will eventually accumulate in water column, sediments and organisms (Bibak et al., 2020).

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Heavy metals that accumulate in marine organisms are strongly link to metal contamination (Alkan et al., 2016; Jeong & Ra, 2022). Many marine organisms are widely used for bio-monitoring studies to assess the degree of contamination due to anthropogenic pressures (Akcali & Kucuksezgin, 2011; Hao et al., 2019; Rakib et al., 2021; Jeong & Ra, 2022). Additionally, bio-monitoring studies are of critical importance since some marine organisms are used as food resources for humans due to their high content of polysaccharides, polyunsaturated fatty acids, minerals and vitamins (Jeong & Ra, 2022). It was estimated that there are 150 macroalgal species which are consumed as human food (Barrow, 2007; Mohamed & Hasan, 2023).

Mersin Bay is located at the Northeastern (NE) Mediterranean (Figure 1). Previous studies showed the development of eutrophication and sedimentary heavy metal pollution in the Mersin inner bay due to anthropogenic pressures (Akcay et al., 2022; Özbay & Akçay, 2023; Akçay, 2023; Özbay, 2024). However, only a few studies have been conducted on the distributions of heavy metals in the most common macroalgal species along the coastal areas of the Mersin Bay (Alp et al., 2012; Börekçi et al., 2021). Therefore, this study focuses on the heavy metal levels of the collected macroalgal species in the Mersin Bay.

Heavy metal pollution poses a significant threat to macroalgae and the broader marine environment. By understanding the sources and effects of heavy metals, and implementing effective monitoring and mitigation strategies, it is possible to protect marine ecosystems for ensuring the health and sustainability of our seas. The main objective of this study was to determine concentrations of heavy metals in the macroalgae samples collected from the Mersin Bay, located at the NE Mediterranean Sea. Due to the adverse health effects of heavy metals for humans (Doshi et al., 2008; Cheng et al., 2019), assessment of potential health risk due to consumption of the collected species as human food was also determined in the present study.

Material and Methods

In order to determine distributions of heavy metals [aluminum (Al), iron (Fe), cobalt (Co), cadmium (Cd), chromium (Cr), cupper (Cu), manganese (Mn), nickel (Ni), lead (Pb), zinc (Zn)] for the collected macroalgal species, 6 stations were visited along the coastal regions of the Mersin inner Bay (Figure 1). The list of macroalgal species was presented in Table 1.

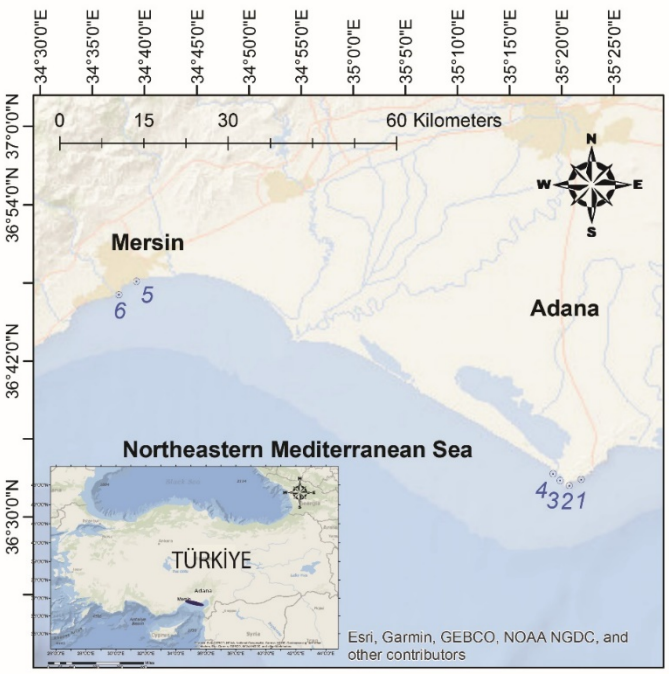

Figure 1. Study area

Table 1. The macroalgal species collected from the Mersin inner bay

Station	Macroalgae
1	*Stypopodium schimperi*
	Padina pavonica
	Laurencia obtusa
2	*Dictyota* sp.
	Sargassum vulgare
	Jania rubens
3	*Halopithys* sp.
4	*Osmundea* sp.
	Halopithys incurva
5	*Ulva intestinalis*
6	*Entromorpha flexuosa*
	Ulva linza

Macroalgal species, obtained between February and September 2021, were collected by hand and washed by seawater. The obtained samples were put in the polyethylene bags and stored at –20°C till the analysis of heavy metals. In the laboratory, the collected macroalgae samples were dried and homogenized. Then, concentrations of heavy metals were by using Inductively Coupled Plasma Mass Spectrometer (ICP-MS -AGILENT 7850 Model) according to method described by the study of Alp et al. (2012).

Health risk of heavy metals was assessed by the calculation of Hazard Quotient through ingestion pathway ($HQ_{ing.}$) and Total Cancer Risk (TCR) (USEPA, 2002, 2014; Magni et al., 2021; Özbay & Akçay, 2023).

Results and Discussion

Macroalgal species are good bio-indicators for the assessment of heavy metal pollution due to their high capacity for bioaccumulation (Akcali & Kucuksezgin, 2011; Hao et al., 2019; Rakib et al., 2021; Jeong & Ra, 2022; El-Mahrouk et al., 2023). There were some studies carried out to determine heavy metals contents in macroalgae of the Mediterranean coasts. However, studies performed in the Mersin inner Bay are very limited. Previous studies performed in the study region showed that concentrations of heavy metals displayed spatio-temporal variability (Alp et al., 2012; Börekçi et al., 2021). The heavy metal concentrations of the collected macroalgae samples varied between 1.24-5.93 g/kg for Al, 0.61-5.26 g/kg for Fe, 1.17-7.61 mg/kg for Co, 0.03-0.21 mg/kg for Cd, 4.26-43.10 mg/kg for Cr, 0.28-437.52 mg/kg for Cu, 17.90-139.08 mg/kg for Mn, 2.78-79.25 mg/kg for Ni, 0.75-3.49 mg/kg for Pb and 1.71-70.34 mg/kg for Zn, respectively (Table 2). It should be noted that maximum concentrations of heavy metals Co, Cr, Fe, Mn and Ni were recorded in the eastern section of the Mersin Bay for the macroalgae *Stypopodium schimperi* whilst the Cd, Cu and Zn contents were higher for the macroalgae *Halopithys incurva*, strongly suggested high bioaccumulation capacity of these macroalgal species for heavy metals.

Previous studies showed heavy metal pollution in sediments and macroalgae of the Mersin inner bay due to natural processes and anthropogenic pressures (Yalçın et al., 2013; Özbay & Akçay, 2023; Özbay, 2024; Akçay, 2023, 2024). The Ni enrichment in the Mersin inner bay is probably due to natural weathering and erosion of rocks (Yalcın et al., 2013; Akçay, 2024). It was also reported that large Cr mineral deposits and the use of pesticide and fertilizer with high Cr, Ni and Mn contents in the study region enhanced heavy metals pollution in the coastal sites of the Mersin Bay (Tuncel et al., 2007; Alp et al., 2012; Özbay, 2024). All these natural and human-induced pressures increased the concentration of heavy metals in the collected macroalgal samples from the Mersin inner bay. Furthermore, comparison of heavy metal concentrations in macroalgae along different coastal regions in the Mediterranean Sea showed that the concentrations of heavy metals Al, Cr, Cu, Fe and Mn were higher along the coastal areas of the Mersin Bay (Table 2). In the recent studies performed in the Mersin Bay also showed that the macroalgae species has high metal contents due to anthropogenic inputs (Alp et al., 2012; Börekçi et al., 2021).

It was reported that there are 150 macroalgal species which are consumed as food resources for humans due to their high content of polysaccharides, polyunsaturated fatty acids, minerals and vitamins (Barrow, 2007; Jeong & Ra, 2022; Mohamed & Hasan, 2023). Furthermore, about 15–20 edible algae strains are marketed for food consumption in Europe (Polat & Ozogul, 2013; Ozgun & Turan, 2015). Previous studies indicated that though some macroalgal species, (*Ulva* sp., *Entromorpha* sp., *Laurencia obtusa*) are edible, *Padina pavonica* species are non-edible (Çolakoğlu & Ak, 2017; Caf et al., 2019; Tuzen et al., 2009). The studied macroalgal species collected from the Mersin inner bay is of great importance for their edibility. In the present study, therefore, health risk assessment of heavy metals for the collected macroalgae samples was determined by the calculation of $HQ_{ing.}$ and TCR. The calculated $HQ_{ing.}$ values, less than 1 indicated non-carcinogenic risk (safe for human health), while the values, greater than 1, showed carcinogenic risk. For the TCR values, a TCR value, less than 1.00E-06, indicates no significant health hazards, but a TCR value, higher than 1.00E-04, indicates high carcinogenic risk. A TCR values, varied between 1.00E-06 and 1.00E-04, suggests tolerable carcinogenic risk (Magni et al., 2021; Özbay & Akçay, 2023). The calculated values were presented in Table 3. The study findings showed that though all the calculated $HQ_{ing.}$ values were less than 1, the calculated TCR values ranged from 7.64E-09 for adults to 9.51E-03 for children (Table 3). The high values calculated for the heavy metals Cu and Ni for adults, and Cr, Cu and Ni for children (TCR>1.00E-04) strongly suggested high carcinogenic risk for adults and children. In a recent study, Akçay (2024) reported health hazards for Cr and Ni by studying heavy metals content of *Enteromorpha* sp. in the Mersin inner Bay. Therefore, all these findings suggested that the consumption of these macroalgae species from the coastal regions as human food or animal feed may cause health hazards.

Human-induced metal contamination has been experienced in the Mersin Bay with levels and sources potentially changing over time. This study revealed that regular monitoring is necessary to track these changes and assess the effectiveness of mitigation measures. Establishing a regular monitoring schedule, comparing current data with historical records, and implementing recommended measures based on the findings ensure ongoing protection of the marine environment.

Table 2. Heavy metal contents in macroalgae from the Mersin inner bay (mg/kg)

Region	Macroalgae	Al*	Fe*	Co	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Reference
Mersin Bay coastal regions, Türkiye	*Stypopodium schimperi*	5.92	5.26	7.61	0.11	43.10	303.52	139.08	79.25	1.57	30.66	This study
	Padina pavonica	3.86	2.84	3.21	0.10	16.62	5.34	89.07	33.58	2.88	12.82	
	Laurencia obtusa	3.26	2.75	2.52	0.14	16.81	4.35	82.76	13.70	2.27	8.93	
	Dictyota sp.	3.19	1.45	2.04	0.07	10.35	4.19	62.12	5.30	1.68	6.16	
	Sargassum vulgare	1.34	0.61	1.32	0.04	4.26	2.05	17.90	4.48	0.75	4.85	
	Jania rubens	1.24	0.77	1.17	0.04	5.30	0.28	28.22	2.78	1.13	1.71	
	Halopithys sp.	1.69	0.87	1.49	0.03	4.95	18.96	58.33	4.92	1.39	6.40	
	Osmundea sp.	3.40	2.15	2.12	0.14	14.30	3.01	51.72	9.09	1.77	7.98	
	Halopithys incurva	4.67	3.87	4.29	0.21	14.93	437.52	98.79	21.29	3.28	70.34	
	Ulva intestinalis	4.28	1.95	1.72	0.13	12.94	5.30	45.23	12.81	2.16	22.92	
	Entromorpha flexuosa	5.93	3.09	3.06	0.14	20.19	6.38	50.11	19.90	1.53	15.92	
	Ulva linza	5.44	2.70	2.46	0.15	17.25	8.36	64.39	20.70	3.49	59.80	
South Adriatic Sea, Italy	*Ulva lactuca*	-	0.34	-	0.20	-	12.07	-	-	0.84	127.27	Storelli et al. (2001)
	Entromorpha prolifera	-	0.39	-	0.72	-	10.33	-	-	1.15	58.79	
Eastern Aegean coastal regions, Türkiye	*Ulva* sp.	-	0.128-0.308	-	0.015-0.057	0.78 -4.78	6.49-13.90	-	-	0.001-0.006	-	Akcali and Kucuksezgin (2011)
	Enterophmorpha sp.	-	0.205-0.440	-	0.013 -0.044	3.15 -9.63	4.43-13.20	-	-	0.001-0.010	-	
	Padina pavonica	-	0.039-0.299	-	0.018-0.148	0.73 -3.19	3.73 - 8.22	-	-	0.001-0.005	-	
Mediterranean Sea, Mersin, Türkiye	*Ulva* sp.	0.05	0.20	-	0.03	3.07	1.02	7.84	7.58	0.81	4.59	Alp et al. (2012)
	Enterophmorpha sp.	0.18	0.65	-	0.03	4.02	1.65	14.62	16.77	1.04	6.42	
Mediterranean Sea, Egypt	*Entromorpha prolifera*	-	0.86	4.60	0.09	1.02	4.09	15.55	5.70	5.61	102.82	El Zokm et al. (2021)
	Ulva linza	-	1.54	7.83	1.04	0.85	0.28	23.34	9.16	10.82	88.19	
	Jania rubens	-	1.36	29.30	3.10	0.84	3.30	56.25	30.00	35.60	48.80	
	Sargassum vulgare	-	0.26	6.70	1.85	1.64	18.00	15.00	14.80	9.00	129.05	
	Padina pavonica	-	0.94	10.52	1.35	0.95	6.68	89.65	12.64	13.36	109.79	
Mediterranean Sea, Mersin, Türkiye	*Ulva rigada*	0.66-1.65	-	-	-	95.96-195.65	-	88.61-224.08	-	-	-	Börekçi et al. (2021)
Mediterranean Sea, Morocco	*Ulva lactuca*	-	0.12-0.42	-	-	0.90-1.00	1.90-7.40	25.50-164	4.00-4.50	2.70-4.80	8.52-10.20	Rahhou et al. (2023)

Note: *The units of Al and Fe concentrations are g/kg

Table 3. Health risk assessment for the macroalgae from the Mersin inner Bay

$HQ_{ing.}$		ADULT										CHILD								
Station	**Macroalgae**	**Co**	**Cd**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**		**Co**	**Cd**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
1	*Stypopodium schimperi*	3.04E-02	1.32E-04	1.72E-02	1.82E-02	9.01E-03	1.19E-03	4.75E-03	1.34E-02	1.23E-04		3.24E-01	1.41E-03	1.84E-01	1.94E-01	9.61E-02	1.27E-02	5.07E-02	1.43E-01	1.31E-03
1	*Padina pavonica*	1.28E-02	1.20E-04	6.64E-03	3.20E-04	4.86E-03	7.63E-04	2.01E-03	2.47E-02	5.12E-05		1.37E-01	1.28E-03	7.08E-02	3.41E-03	5.19E-02	8.13E-03	2.15E-02	2.63E-01	5.46E-04
1	*Laurencia obtusa*	1.01E-02	1.68E-04	6.72E-03	2.61E-04	4.71E-03	7.09E-04	8.21E-04	1.94E-02	3.57E-05		1.07E-01	1.79E-03	7.16E-02	2.78E-03	5.02E-02	7.56E-03	8.76E-03	2.07E-01	3.81E-04
2	*Dictyota* sp.	8.15E-03	8.39E-05	4.14E-03	2.51E-04	2.48E-03	5.32E-04	3.18E-04	1.44E-02	2.46E-05		8.69E-02	8.95E-04	4.41E-02	2.68E-03	2.65E-02	5.67E-03	3.39E-03	1.53E-01	2.63E-04
2	*Sargassum vulgare*	5.27E-03	4.79E-05	1.70E-03	1.23E-04	1.04E-03	1.53E-04	2.68E-04	6.42E-03	1.94E-05		5.63E-02	5.11E-04	1.82E-02	1.31E-03	1.11E-02	1.63E-03	2.86E-03	6.85E-02	2.07E-04
2	*Jania rubens*	4.67E-03	4.79E-05	2.12E-03	1.68E-05	1.32E-03	2.42E-04	1.67E-04	9.67E-03	6.83E-06		4.99E-02	5.11E-04	2.26E-02	1.79E-04	1.41E-02	2.58E-03	1.78E-03	1.03E-01	7.29E-05
3	*Halopithys* sp.	5.95E-03	3.60E-05	1.98E-03	1.14E-03	1.49E-03	4.99E-04	2.95E-04	1.19E-02	2.56E-05		6.35E-02	3.84E-04	2.11E-02	1.21E-02	1.59E-02	5.33E-03	3.15E-03	1.27E-01	2.73E-04
4	*Osmundea* sp.	8.47E-03	1.68E-04	5.71E-03	1.80E-04	3.68E-03	4.43E-04	5.45E-04	1.52E-02	3.19E-05		9.04E-02	1.79E-03	6.09E-02	1.92E-03	3.93E-02	4.72E-03	5.81E-03	1.62E-01	3.40E-04
4	*Halopithys incurva*	1.71E-02	2.52E-04	5.97E-03	2.62E-02	6.63E-03	8.46E-04	1.28E-03	2.81E-02	2.81E-04		1.83E-01	2.68E-03	6.36E-02	2.80E-01	7.07E-02	9.02E-03	1.36E-02	3.00E-01	3.00E-03
5	*Ulva intestinalis*	6.87E-03	1.56E-04	5.17E-03	3.18E-04	3.34E-03	3.87E-04	7.68E-04	1.85E-02	9.16E-05		7.33E-02	1.66E-03	5.51E-02	3.39E-03	3.56E-02	4.13E-03	8.19E-03	1.97E-01	9.77E-04
6	*Entromorpha flexuosa*	1.22E-02	1.68E-04	8.07E-03	3.82E-04	5.29E-03	4.29E-04	1.19E-03	1.31E-02	6.36E-05		1.30E-01	1.79E-03	8.60E-02	4.08E-03	5.64E-02	4.58E-03	1.27E-02	1.40E-01	6.78E-04
6	*Ulva linza*	9.83E-03	1.80E-04	6.89E-03	5.01E-04	4.62E-03	5.51E-04	1.24E-03	2.99E-02	2.39E-04		1.05E-01	1.92E-03	7.35E-02	5.34E-03	4.93E-02	5.88E-03	1.32E-02	3.19E-01	2.55E-03

TCR.		ADULT										CHILD								
Station	**Macroalgae**	**Co**	**Cd**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**		**Co**	**Cd**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
1	*Stypopodium schimperi*		6.61E-08	2.59E-05	6.18E-04			1.61E-04	1.60E-08				7.05E-07	2.76E-04	6.60E-03			1.72E-03	1.71E-07	
1	*Padina pavonica*		6.01E-08	9.98E-06	1.09E-05			6.84E-05	2.93E-08				6.41E-07	1.06E-04	1.16E-04			7.30E-04	3.13E-07	
1	*Laurencia obtusa*		8.41E-08	1.01E-05	8.86E-06			2.79E-05	2.31E-08				8.97E-07	1.08E-04	9.45E-05			2.98E-04	2.47E-07	
2	*Dictyota sp.*		4.20E-08	6.22E-06	8.54E-06			1.08E-05	1.71E-08				4.48E-07	6.63E-05	9.11E-05			1.15E-04	1.83E-07	
2	*Sargassum vulgare*		2.40E-08	2.56E-06	4.18E-06			9.13E-06	7.64E-09				2.56E-07	2.73E-05	4.46E-05			9.74E-05	8.15E-08	
2	*Jania rubens*		2.40E-08	3.18E-06	5.71E-07			5.66E-06	1.15E-08				2.56E-07	3.39E-05	6.09E-06			6.04E-05	1.23E-07	
3	*Halopithys sp.*		1.80E-08	2.97E-06	3.86E-05			1.00E-05	1.42E-08				1.92E-07	3.17E-05	4.12E-04			1.07E-04	1.51E-07	
4	*Osmundea sp.*		8.41E-08	8.59E-06	6.13E-06			1.85E-05	1.80E-08				8.97E-07	9.16E-05	6.54E-05			1.98E-04	1.92E-07	
4	*Halopithys incurva*		1.26E-07	8.97E-06	8.92E-04			4.34E-05	3.34E-08				1.35E-06	9.56E-05	9.51E-03			4.63E-04	3.56E-07	
5	*Ulva intestinalis*		7.81E-08	7.77E-06	1.08E-05			2.61E-05	2.20E-08				8.33E-07	8.29E-05	1.15E-04			2.78E-04	2.35E-07	
6	*Entromorpha flexuosa*		8.41E-08	1.21E-05	1.30E-05			4.05E-05	1.56E-08				8.97E-07	1.29E-04	1.39E-04			4.33E-04	1.66E-07	
6	*Ulva linza*		9.01E-08	1.04E-05	1.70E-05			4.22E-05	3.56E-08				9.61E-07	1.10E-04	1.82E-04			4.50E-04	3.79E-07	

$HQ_{ing.}$< 1; non-carcinogenic risk	$HQ_{ing.}$≥ 1; potential carcinogenic	TCR<1.00E-06; No significant health hazards	1.00E-06 <TCR< 1.00E-04; Acceptable carcinogenic risk	TCR>1.00E-04; High risk of carcinogenesis

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Conclusion

In the present study, heavy metal distributions of macroalgae and potential health risk assessment along the coastal regions of the Mersin Bay were determined. The finding of this study showed that the concentrations of Al, Cr, Cu, Fe and Mn were higher in macroalgal species in the Mersin Bay, showing high bioaccumulation of these heavy metals. Furthermore, the high TCR values for the adults and children living in the study region showed potential carcinogenic risk from the carcinogenic Cr, Cu and Ni. Continuous bio-monitoring and proactive measures based on these studies are essential for safeguarding marine ecosystems and human health from the adverse effects of metal pollution.

Compliance With Ethical Standards

Authors' Contributions

ÖÖ: Writing – original draft, Formal analysis, Writing – review and editing

İA: Writing – original draft, Formal analysis, Writing – review and editing

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

Not applicable.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Mar. Sci. Tech. Bull. (2024) 13(3): 183-198
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1522501

Marine Science and Technology Bulletin

https://prensip.gen.tr/

RESEARCH ARTICLE

Isolation, molecular characterization and determination of biological activity of alkalitolerant *Streptomyces* members from Van Lake-Çarpanak Island soil

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ARTICLE INFO

Article History:
Received: 26.07.2024
Received in revised form: 14.08.2024
Accepted: 27.08.2024
Available online: 30.09.2024

Keywords:
Alkalitolerant Streptomyces
Lake Van
Çarpanak Island
16S rRNA gene
Antimicrobial activity
Enzyme production

ABSTRACT

Çarpanak Island is one of the islands in Lake Van, the easternmost and largest lake in Türkiye. In this study, soil samples taken from three different locations of Çarpanak island, Lake Van, were inoculated into 4 different selective isolation media using the dilution plate technique, and 42 actinomycete isolates were stocked. PCR amplification of the 16S rRNA gene of 13 isolates selected according to their morphological differences was performed with 27F and 1525R primers. Phylogenetic trees were constructed with the neighbor-joining algorithm in the MEGA 7.0 software. According to 16S rRNA gene sequence analysis, it was determined that 13 isolates belonged to the genus *Streptomyces*. The antimicrobial activities of 13 isolates against 8 pathogens and the ability of these isolates to produce lipase, amylase, protease and pectinase were determined. Except for CA43 and CA62 isolates, other *Streptomyces* isolates have the ability to produce lipase. Other *Streptomyces* isolates except CA59 and CA94 can synthesize amylase. In addition, 3 of the 13 *Streptomyces* isolates, CA40, CA61 and CA94, do not have the ability to synthesize protease. No isolate is capable of producing pectinase enzyme. As a result of this study, it was observed that most of the *Streptomyces* isolates had higher lipase enzyme production abilities than other enzyme groups.

Please cite this paper as follows:

Veyisoglu, A., Tatar, D., Duyar, H. A., & Tokatli, A. (2024). Isolation, molecular characterization and determination of biological activity of alkalitolerant *Streptomyces* members from Van Lake-Çarpanak Island soil. *Marine Science and Technology Bulletin, 13*(3), 183-198. https://doi.org/10.33714/masteb.1522501

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Introduction

The phylum Actinobacteria is amongst the most prevalent among bacteria, comprising heterogeneous genera of Gram-positive and Gram-variable bacteria, as described by Ventura et al. (2007). The phylum additionally encompasses *Thermoleophilum* sp. (Zarilla & Perry, 1986), *Gardenerella vaginalis* (Gardner & Dukes, 1955), *Saccharomonospora viridis* P101^T (Pati et al., 2009), *Ferrimicrobium acidiphilum* and *Ferrithrix thermotolerans* (Johnson et al., 2009). Actinobacteria are classified according to their oxygen requirements, motility, spore formation, and G+C content (Ensign, 1992). The genome size of Actinobacteria is variable, with values ranging from 0.93 Mb (*Tropheryma whipplei*; Bentley et al., 2003) to 12.7 Mb (*Streptomyces rapamycinicus*; Baranasic et al., 2013). These genomes are typically found in either circular or linear form.

Actinobacteria are found in a variety of ecological niches, including and aquatic (marine and fresh waters) and terrestrial ecosystems, and exhibit a complex life cycle, including the existence of dormant spores or actively growing hyphae (Shivlata & Satyanarayana, 2015).

The genus *Streptomyces* is classified in the family *Streptomycetaceae*, belonging to the suborder *Streptomycineae* (Waksman & Henrici, 1943; Stackebrandt et al., 1997; Kim et al., 2003; Zhi & Stackebrandt, 2009). Currently, the genus contains more than 800 species with validly published names (see https://www.bacterio.net/genus/streptomyces). Members of the genus *Streptomyces* have been identified from different habitats such as soil (Saricaoglu et al., 2014), rhizospheres (Piao et al., 2018; Sujarit et al., 2018), marine sponge (Silva et al., 2016), marine sediment (Veyisoglu & Sahin, 2014), lake sediment (Ray et al., 2016), mangrove sediment (Hu et al., 2012), mangrove soil (Law et al., 2019), desert (Li et al., 2019) and plant tissue (Wang et al., 2018).

It is well established that Actinobacteria are able to thrive in a variety of environments, both normal as well as extreme. These include environments with acidic or alkaline pH, high or low salinity, high radiation, low levels of available moisture or nutrients (Zenova et al., 2011). The diverse physiology and metabolic flexibility of extremophilic and extremotolerant actinobacteria enable them to survive under unfavourable conditions. Alkaliphilic actinobacteria are categorised into three principal groups. The first group is alkaliphilic (optimal growth occurs at pH 10-11), the second group is moderately alkaliphilic (growth occurs at pH 7-10, although poor growth is observed at pH 7.0) and the third group is alkalitolerant actinobacteria (optimal growth occurs at pH 6-11) (Jiang & Xu, 1993). Baldacci (1944) was the first to report alkaliphilicity. Subsequently, Taber (1960) isolated alkaliphilic Actinobacteria from soil. The presence of alkaliphilic and alkalitolerant Actinobacteria has been documented in a range of environments, including deep-sea sediments (Yu et al., 2013), alkaline desert soils (Li et al., 2006), and soda lakes (Groth et al., 1997) (Shivlata & Satyanarayana, 2015).

Alkaliphilic and alkalitolerant actinobacteria have been observed to thrive in high salinity environments, classified as haloalkaliphiles or haloalkalitolerants. Similarly, they are known to inhabit thermobiotic conditions, classified as alkalithermophiles or alkalithermotolerants. Furthermore, halophilic and halotolerant alkalithermophiles and alkalithermotolerant actinobacteria have also been isolated from saline habitats (Zenova et al., 2011; Shivlata & Satyanarayana, 2015). Alkaliphilic *Streptomycetes* are commonly found in soda lakes and alkaline soils (Świecimska et al., 2020). Actinobacteria constitute an important group of microorganisms with an ecological significance. Their involvement extends to a wide range of biological processes, including biogeochemical cycles, bioremediation, biodegradation, and plant growth promotion (Palaniyandi et al., 2013; Cockell et al., 2013; Chen et al., 2015). Moreover, they produce a diverse array of pharmaceutically valuable bioactive compounds (antibiotics, antitumor agents, anti-inflammatory compounds, and enzyme inhibitors) and secrete numerous industrially and clinically significant enzymes.

Following the discovery of streptomycin, the first antituberculosis drug derived from actinobacteria, drug discovery and development programmes have increasingly focused on the identification of antimicrobial agents. A significant number of actinobacterial species have subsequently been investigated for the discovery of clinically valuable compounds. The phylum Actinobacteria includes several genera that include antibiotic-producing species, with the genus *Streptomyces* being of particular importance as an important source of secondary metabolites, especially antibiotics. It is estimated that *Streptomyces* species account for more than 50% of the total known microbial antibiotics, with at least 10,000 examples currently documented (Bull, 2010).

A number of new bioactive compounds with different antimicrobial activities have been identified. These compounds synthesized by various species of the *Streptomyces* genus are albocycline-type macrolides (Gu et al., 2019), benditerpenoic acid (Zhu et al., 2021) and pyrimidomycin (Das et al., 2022). Despite this, bacteria causing nosocomial infections remain a significant public health concern. These bacteria present a

significant public health crisis and health security threat due to the increasing prevalence of antibiotic resistance (Mancuso et al., 2021; Chanama et al., 2023).

Although pharmaceutical companies and research laboratories have access to a wide array of clinical drugs, they remain committed to the search for new therapeutic drugs that can combat microbial pathogens. In order to identify new bioactive compounds with potential pharmaceutical or industrial applications, actinobacteria have been isolated from remote and previously unexplored locations, including deserts (Kurapova et al., 2012), marine environments (Dhakal et al., 2017) and wetlands (Yu et al., 2015). It has been hypothesised that extremophilic actinobacteria may be a source of novel, valuable metabolites containing gene clusters for the synthesis of new biomolecules (Bull, 2010). Consequently, efforts are being made to isolate such organisms from extremely extreme environments.

Lake Van is located in the eastern part of Türkiye's Anatolian peninsula, at approximately 43°E longitude and 38.5°N latitude. The surface area of the lake is 3574 km^2, its volume is 650 km^3, its maximum depth is 450 m and its maximum length is 130 km (Cukur, 2014). In addition to being a closed basin, Lake Van is a salty soda lake and differs from other lakes in the world with its importance. Considering its features and volume, in addition to being the largest lake in Türkiye, it is the largest soda lake and the third largest closed lake in the world (Reimer et al., 2009).

The chemistry of soda lakes is distinctive due to the presence of elevated concentrations of carbonate/bicarbonate in the form of Na_2CO_3 and $NaHCO_3$ (Jones et al., 1998). Soda lakes host a diverse array of microbial communities, and studies on the microbial diversity of these lakes contribute to our understanding of the biology of extreme habitats. At the same time, new microorganisms obtained from these environments and their novel enzymes are of significant interest within the scope of biotechnological applications. Proteases, lipases and cellulases are obtained from alkaliphilic microorganisms in the production of biotechnologically advanced laundry detergents (Horikoshi, 2008). Alkaline protease is also used in the food industry, pharmaceuticals and medical diagnostics (Kanekar et al., 2002). In addition to enzymes, alkaliphilic microorganisms can also be employed for the biodegradation of organic and inorganic pollutants and hydrocarbons (Sorokin et al., 2012; Poyraz & Mutlu, 2017).

The aim of this project is to isolate alkalitolerant *Streptomyces* members obtained from Çarpanak Island in Lake Van, to determine the nucleotide sequences and phylogenetic analysis of the *Streptomyces* isolates obtained by PCR amplification of 16S rRNA gene regions and to determine the antimicrobial activities of the isolates and their capacity to produce enzymes of industrial importance.

Material and Methods

Collection and Storage of Soil Samples

Soil samples taken from three different localities (Table 1) of Çarpanak Island by measuring their coordinates with the eXplorist100 were placed in sterile 50 mL falcon tubes and transferred to the laboratory, dried at room temperature, and then planted in four different alkaline selective isolation media (Table 2) using the dilution plate method. The locality and geographical coordinates of the soil samples are given in Table 1.

Table 1. Locality and geographical coordinates of soil samples

Locality	Geographic Coordinates
From the part of the island close to Lake Van	38°36.366 N 043°05.197 E
From the part of the island far from Lake Van	38°36.531 N 043°05.055 E
One of the regions of the island with dense plant flora	38°36.399 N 043°05.115 E

pH Measurement of Soil Samples

The pH of the soil samples was measured from the saturation mud prepared with the help of a glass electrode pH meter. pH values of soil samples were determined using a 1:2.5 soil:water solution (Sağlam, 1978). For this, 10 g of soil was weighed and transferred into a 50 mL falcon tube, and 25 mL of pure water was added. The falcon tubes were shaken for 1 hour and left to rest for 1 day. The pH values of the samples were measured the next day.

Actinobacteria Isolation

Dilution plate technique was used to isolate actinomycetes. 1 g of the soil samples was weighed and placed in bottles containing 9 mL of sterile Ringer's solution containing glass beads. In this way, the 10^{-1} solutions prepared for each soil sample were shaken gently for 45 minutes to separate the spores and mycelia of actinomycetes attached to the soil colloids from the colloids. Then, these 10^{-1} dilutions were kept in a water bath set at 60°C for 45 minutes in order to reduce contamination that could be caused by vegetative forms, and the 10^{-1} dilutions of

each soil sample were mixed with a vortex mixer (Fisons Sientifiv Appartus Ltd., Loughborough, Leicesteshire, England, UK) and made homogeneous. 1 mL soil dilution was taken under aseptic conditions with an automatic pipette (1000: Axygen Inc, 33210 Central Avenue, Union City, CA 94587 USA) and placed in glass tubes containing 9 mL of sterile Ringer's solution, and in this way, 10^{-2} soil dilutions were obtained. This process was repeated and 10^{-3} sediment dilutions were prepared. 0.1 mL of soil solutions were taken from each of the 10^{-2} and 10^{-3} dilutions with an automatic pipette, placed on the surfaces of isolation plates with antibiotics added in different concentrations as mentioned above, and inoculated by the smear plate method with a sterile loop. 3 plates were prepared for each dilution and incubated at 28°C for 25-30 days.

Selection, Purification and Stocking of Isolates

Actinomycete strains were selected from the isolation plates that were incubated in selective isolation media for 21-30 days, taking into account their colony morphologies. The selected strains were inoculated onto the surface of malt extract-yeast extract (ISP2) (Shirling & Gottlieb, 1966) supplemented with cycloheximide (50 µg/ml) by the line sowing method to ensure a single colony, and were incubated at 28°C for 20 days. The isolates selected considering the colony morphology were numbered, pure cultures were made, and transferred to autoclaved screw cap tubes containing 25% glycerol with the help of sterile toothpicks and stored at -20°C.

Determining pH Range

Tolerance ranges were determined at pH 4, 5, 6, 7, 8, 9, 10, 11, and 12 for 20 isolates selected according to color groups. The study employed glucose yeast extract-malt extract agar. The pH values of the agar were adjusted with 0.1 M NaOH and 0.1 M HCl. The inoculation of the isolates into the culture medium was conducted using the point inoculation method with the aid of an automatic pipette. For each isolate, 1.5 mL of Ringer's solution was added to screw-capped bottles and sterilised by autoclaving at 121°C for 15 minutes. The spore and substrate mycelia of the isolates were transferred to small glass vials containing a ringer under aseptic conditions with the help of a sterile loop. This was done to facilitate the point inoculation procedure, which was then carried out using an automatic pipette and a bacterial solution that had been homogenised by mixing with a vortex.

Genomic DNA Isolation

Genomic DNA isolation of 17 out of 20 isolates selected according to color groups was performed with the Invitrogen PureLink Genomic DNA Mini Kit.

Amplification of the 16S rRNA Gene Region

Primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525r (5'-AAGGAGGTGWTCCARCC-3') were used for amplification of the 16S rRNA gene region of the test isolates. PCR products were checked using agarose gel electrophoresis (1%, w/v) (Table 3).

Table 2. List of selective media used

No	Medium	Antibiotics
1	Humic acid vitamin agar (Hayakawa & Nonomuraea, 1987)	Cycloheximide (50 µg/ml), Nalidixic acid (10 µg/ml)
2	Starch-casein agar (Küster & Williams, 1964)	Nystatin (50 µg/ml), Cycloheximide (50 µg/ml)
3	SM3 agar-Gauze's agar (Tan et al., 2006)	Nystatin (50 µg/ml), Rifampicin (5 µg/ml)
4	R2A agar (Reasoner & Geldreich, 1985)	Nystatin (50 µg/ml), Cycloheximide (50 µg/ml)

Table 3. Nucleotide sequences of primers used in 16S rRNA gene region studies

Primer Code	Sequence (5'-3')	Length	Reference
518F	CCAGCAGCCGCGGTAAT	17	Buchholz-Cleven et al. (1997)
800R	TACCAGGGTATCTAATCC	18	Chun (1995)
MG5F	AAACTCAAAGGAATTGACGG	20	Chun (1995)

Sequencing of purified 16S rRNA gene region PCR products was performed by Macrogen Inc., Netherlands, using two forward and one reverse primer.

Analysis of 16S rRNA Sequence Data and Phylogenetic Dendrogram Creation

After the 16S rRNA gene region sequence analysis of the isolates was completed, the sequence data obtained were combined with the ChromasPro 1.7.5 program and using the global alignment algorithms available in Ezbiocloud Server (Chalita et al., 2024; see https://eztaxon-e.ezbiocloud.net/). 16S rRNA nucleotide similarity with the most closely related organisms was determined. MEGA 7 program was used for phylogenetic analysis, and the CLUSTAL_W (Kumar et al., 2016) option of the same program was used for alignment. Neighbor Joining (Saitou & Nei, 1987) method and Kimura-2 (Kimura, 1980) phylogenetic distance matrix were used to draw phylogenetic dendrograms. Bootstrap analyzes of the created phylogenetic trees (Felsenstein, 1985) were performed with 1000 replicates.

Evaluation of Antimicrobial Activities

The isolates were examined for their ability to inhibit the growth of eight pathogenic organisms, including three Gram-positive and three Gram-negative bacteria and two fungi (Williams et al., 1983) (Table 4). The inoculation of the isolates into the culture medium was carried out by the point inoculation method using an automatic pipette. For each isolate, 1.5 mL of Ringer's solution was added to screw-capped bottles and sterilised by autoclaving at 121°C for 15 minutes. The spore and substrate mycelia of the isolates grown on glucose yeast extract-malt extract agar medium at 28°C were transferred to small glass vials containing a Ringer solution under aseptic conditions with the help of a sterile loop.

Subsequently, 7 µL of the bacterial solutions, which were homogenised by mixing with a vortex, were taken with an automatic pipette and inoculated by the point inoculation method in groups of 5 onto the modified Bennett's agar surface (Jones, 1949) without the addition of antibiotics. The inoculated plates were incubated at 28°C for 72 hours. At the end of the incubation period, 3-5 mL of chloroform was poured onto the developing colonies with the help of a sterile syringe. The lid of the petri plates was kept half-open for 45 minutes to allow the chloroform to evaporate. The pathogen test organisms, each of which had been cultivated in 5% nutrient agar for 2 days, were inoculated onto the colonies that had been killed in this way by the spread plate method. The inhibition zones formed around the inoculated plates after 48 hours of incubation at 37°C were measured.

Table 4. Pathogenic organisms selected for antimicrobial activity testing

Number	Pathogenic Organisms
1	*Bacillus subtilis* ATCC 6633
2	*Staphylococcus aureus* ATCC 25923
3	*Enterococcus faecalis* ATCC 29212
4	*Escherichia coli* ATCC 25922
5	*Klebsiella pneumoniae* ATCC 70060
6	*Pseudomonas aeruginosa* ATCC 27853
7	*Aspergillus niger* ATCC 16404
8	*Candida albicans* ATCC 1023

Examination of Lipase Producing Abilities

Lipase activity was determined according to the ability of the isolates to hydrolyze Tween 20. For this purpose, 5 g peptone (Merck, Germany), 2.5 g NaCl (Merck, Germany), 0.05 g $CaCl_2 \times 2H_2O$ (Merck, Germany), 10 g agar and 5 mL Tween 20 (Merck, Germany) in 500 mL distilled water. Then, a single colony was taken from the pure colonies of the isolates in the GYM and they were inoculated in the medium in a spot manner. The media were incubated at 28°C for 5 days. The formation of a zone with a frosted glass appearance around the planting line after incubation was considered positive (Kumar et al., 2012).

Examination of Amylase Producing Abilities

The amylase activity of the isolates was determined by adding 1% lugol to the petri dishes after 7 days of incubation in starch agar medium (1% starch, 0.3% NaCl, 0.1% KH_2PO_4, 2% agar). Lugol dyes the medium in a dark color and creates a transparent zone in the area where amylase production occurs. The presence of activity was determined by measuring the transparent zone formed around the actinobacteria (Fossi et al., 2009).

Examination of Protease Production Abilities

The protease production abilities of the isolates were determined by measuring the transparent zone formed around the actinobacteria after 7 days of incubation in a medium containing 1% casein, 0.5% yeast extract, and 2% agar (Mohamedin, 1999).

Examination of Pectinase Producing Abilities

Pectinase production abilities of the isolates were determined by measuring the transparent zone around the actinobacteria colonies at the end of 7 days of incubation in the medium containing 1% pectin, 0.3% $(NH_4)_2HPO_4$, 0.2% KH_2PO_4, 0.3% K_2HPO_4, 0.01% $MgSO_4$, 2% agar (Raju & Divakar, 2013).

Results and Discussion

Soil samples were taken from three different localities of Çarpanak Island by measuring their coordinates with the eXplorist 100. Dried soil samples were inoculated into four different alkaline selective isolation media using the dilution plate method.

The pH of the soil samples was measured from the saturation mud prepared with the help of a glass electrode pH meter. pH values of soil samples were determined using a 1:2.5 soil:water solution (Sağlam, 1978). For this, 10 grams of soil was weighed and transferred into a 50 mL falcon tube, and 25 mL of pure water was added. The falcon tubes were shaken for 1 hour and left to rest for 1 day. Three repeated measurements were then made. The average of three measurements is given in Table 5.

Table 5. pH values of soil samples

Locality	Soil pH
From the part of the island close to Lake Van	6.93
From the part of the island far from Lake Van	7.20
One of the regions of the island with dense plant flora	6.88

Soil samples brought to the laboratory were placed in petri dishes and left to dry at room temperature until studied. The dilution plate technique was used to isolate *Streptomyces*.

Actinomycete strains were selected from the isolation plates that were incubated in selective isolation media for 21-30 days, taking into account their colony morphologies. The selected isolates were numbered and pure cultures were made and transferred to autoclaved screw cap tubes containing 25% glycerol with the help of sterile toothpicks and stored at -20°C. 42 isolates were selected and stocked according to colony morphology. Four different media were used as selective isolation media in the study. The highest number of isolates were isolated from R2A medium. 17 isolates were isolated from R2A agar, 14 isolates from starch casein agar, 6 isolates from humic acid-vitamin agar, and 5 isolates from SM3 agar. R2A medium was found to be more efficient than other media we used in isolation from tested soils. Meklat et al. (2020) performed isolation of Alkalitolerant actinobacteria from algerian saharan soils. 29 actinobacterial strains were selectively isolated by the dilution plate method on Complex Medium agar (Chun et al., 2000). Arayes et al. (2022) isolated a haloalkalitolerant *Streptomyces* from Um-Risha Lake in Egypt. Isolation of *Streptomyces* was performed by the spread plate technique using starch casein agar medium (SCA) (Küster & Williams, 1964).

The graph showing the distribution of isolates obtained from the selective isolation media used is given in Figure 1.

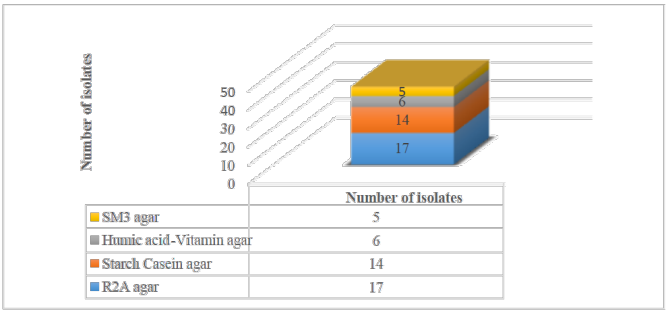

Figure 1. Graph showing the distribution of isolates obtained from the selective isolation media used

Isolation and subculture of some isolates were performed and petri dishes are shown in Figure 2. When the locality distributions of the isolates obtained are examined, it is seen that 18 isolates were obtained from the 2nd locality, 16 isolates from the 3rd locality and 8 isolates from the 1st locality. The distribution of isolates according to localities is given in Figure 3.

Studies continued with 13 isolates selected based on colony morphology differences among the 42 isolates obtained. Glucose yeast extract-malt extract agar was used in the study. The pH values of the agar (4, 5, 6, 7, 8, 9, 10, 11, 12, respectively) were adjusted with 0.1 M NaOH and 0.1 M HCl buffers. The development of 13 spot cultured isolates at each pH value was evaluated. The results are given in Table 6. When the results were evaluated, isolates coded CA43, CA46, CA62, CA68 and CA75 showed growth between pH 4 and 10, while CA59 and CA94 showed growth between pH 4 and 11. The remaining isolates grew between pH 4 and 12. When the pH results were evaluated, it was concluded that the isolates were alkalitolerant, not alkaliphilic.

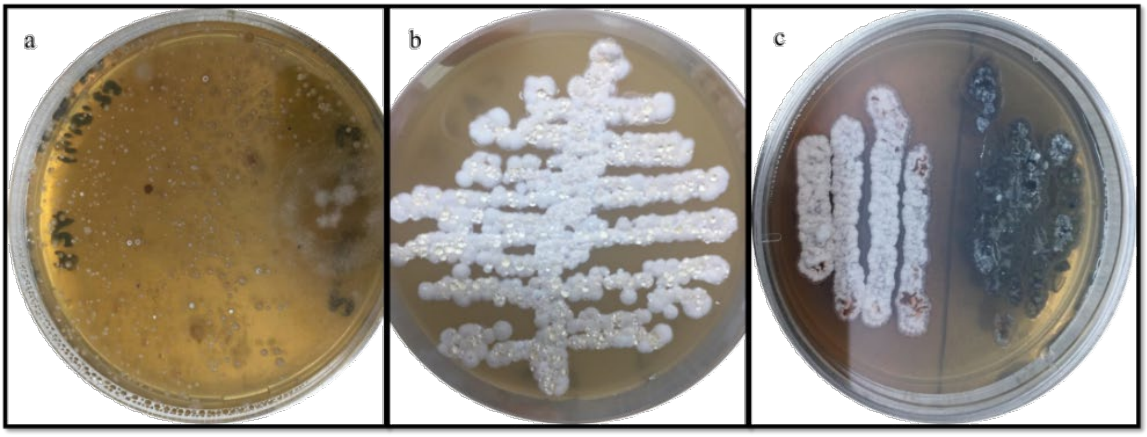

Figure 2. a) Isolation of 1st soil sample obtained from Çarpanak Island (R2A Agar). **b)** Growth of isolate CA46 in ISP2 medium. **c)** Growth of isolates CA94 (left side) and CA40 (right side) in ISP2 medium

Table 6. Development of isolates at different pH values

No	Organism	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11	pH 12
1	CA36	+	+	+	+	+	+	+	+	+
2	CA40	+	+	+	+	+	+	+	+	+
3	CA43	+	+	+	+	+	+	+	-	-
4	CA49	+	+	+	+	+	+	+	-	-
5	CA56	+	+	+	+	+	+	+	+	+
6	CA59	+	+	+	+	+	+	+	+	-
7	CA61	+	+	+	+	+	+	+	+	+
8	CA62	+	+	+	+	+	+	+	-	-
9	CA68	+	+	+	+	+	+	+	-	-
10	CA75	+	+	+	+	+	+	+	-	-
11	CA76	+	+	+	+	+	+	+	+	+
12	CA77	+	+	+	+	+	+	+	+	+
13	CA94	+	+	+	+	+	+	+	+	-

Note: "+" indicate growth; "–" indicate no growth.

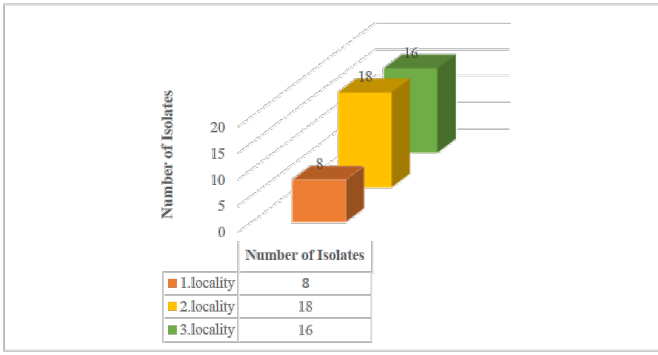

Figure 3. Graph showing the distribution of isolates according to localities

Genomics of 13 isolates, selected according to their colony morphology differences among the 42 isolates obtained, were performed with the Invitrogen PureLink Genomic DNA Mini Kit. The 16S rRNA gene region of 13 isolates, whose genomic DNA was isolated, was amplified in Gradient PCR with 27F and 1525R universal primers. The DNA region, amplified after PCR, with an average size of 1500 base pairs, was visualized on agarose gel electrophoresis on the Mini Lumi imaging device.

16S rRNA gene sequence analysis was performed with 3 primers, 518F, MG5F and 800R, of the 13 isolates for which 16S rRNA gene region PCR amplifications were performed. After the 16S rRNA gene sequence analysis of the isolates was completed, the sequence data obtained were combined with the ChromasPro 1.7.5 program and the sequence data of the most closely related species in international databases were used using Ezbiocloud Server (Chalita et al., 2024; see https://eztaxon-e.ezbiocloud.net/) and their % similarity was determined. According to the sequence analysis results, all 13 isolates were determined to belong to the *Streptomyces* genus (Table 7). The obtained 16S rRNA gene nucleotide sequences have been deposited in GenBank and their accession numbers are given in Table 7.

Table 7. Phylogenetic similarity of test organisms of the *Streptomyces* genus with their closest type species according to 16S rRNA sequence results

No	Strain Code	GenBank No	Closest Type	% Similarity- Nucleotide Difference
1.	**CA36**	PP352073	*Streptomyces exfoliates* NRRL B-2924^T	100% - 0/1447
2.	**CA40**	PP352075	*Streptomyces rectiviolaceus* NRRL B-16374^T	99.31% - 10/1448
3.	**CA43**	PP352171	*Streptomyces sanglieri* NBRC 100784^T	98.96% - 15/1448
4.	**CA49**	PP352271	*Streptomyces himalayensis subsp. Himalayensis* PSKA28^T	95.76% - 60/1414
5.	**CA56**	PP352628	*Streptomyces ambofaciens* ATCC 23877^T	99.45% - 8/1449
6.	**CA59**	PP352629	*Streptomyces seymenliensis* B1041^T	99.10% - 13/1449
7.	**CA61**	PP352630	*Streptomyces aureoverticillatus* NRRL B-3326^T	99.45% - 8/1448
8.	**CA62**	PP352631	*Streptomyces ureilyticus* YC419^T	99.59% - 6/1448
9.	**CA68**	PP352689	*Streptomyces angustmyceticus* NRRL B-2347^T	99.86% - 2/1451
10.	**CA75**	PP352913	*Streptomyces arenae* ISP 5293^T	97.53% -35/1417
11.	**CA76**	PP352914	*Streptomyces ambofaciens* ATCC 23877^T	99.72% - 4/1448
12.	**CA77**	PP353666	*Streptomyces rosealbus* YIM 31634^T	99.78% - 3/1388
13.	**CA94**	PP353683	*Streptomyces violarus* NBRC 13104^T	98.76% - 18/1446

According to 16S rRNA gene region nucleotide sequence analysis, 13 isolates belonging to the *Streptomyces* genus show 95.76% to 100% similarity to their closest type species. CA36 isolate was 100% with its closest type species, *Streptomyces exfoliates* NRRL B-2924^T, CA68 isolate was 99.86% with its closest type species, *Streptomyces angustmyceticus* NRRL B-2347^T, CA76 isolate was 99.72% with its closest type species, *Streptomyces ambofaciens* ATCC 23877^T, CA77 isolate was 99.78% with *Streptomyces rosealbus* YIM 31634^T, the closest type species to the isolate, CA62 isolate was 99.59% to the closest type species, *Streptomyces ureilyticus* YC419^T, CA56 isolate was 99.45% to the closest type species, *Streptomyces ambofaciens* ATCC 23877^T, CA61 isolate was 99.45% with *Streptomyces aureoverticillatus* NRRL B-3326^T has similarity, and CA40 isolate has 99.31% similarity with its closest type, *Streptomyces rectiviolaceus* NRRL B-16374^T. CA59 isolate was 99.10% with its closest type species, *Streptomyces seymenliensis* B1041^T, CA43 isolate was 98.96% with its closest type species, *Streptomyces sanglieri* NBRC 100784^T, CA94 isolate was 98.76% with its closest type species, *Streptomyces violarus* NBRC 13104^T, and CA75 isolate was 97.53% with *Streptomyces arenae* ISP 5293^T, and CA49 isolate was 95.76% with the closest type species, *Streptomyces himalayensis* subsp. *himalayensis* PSKA28^T has similarity.

For the identification of new species, the 16S rRNA gene similarity value has been recommended to be below 97% (Stackebrandt & Goebel, 1994), but recently this value has been increased to the range of 98.7-99% (Stackebrandt & Ebers, 2006).

The neighbor-joining (Saitou & Nei, 1987) method was used to draw the phylogenetic dendrogram containing 13 isolates of the *Streptomyces* genus. Additionally, bootstrap analyses of the phylogenetic tree created using the Kimura-2 phylogenetic distance matrix (Felsenstein, 1985) were performed with 1000 replicates (Figure 4).

A total of 13 isolates were examined for their ability to inhibit the growth of eight pathogenic organisms, including three Gram-positive and three Gram-negative bacteria and two fungi. The *Streptomyces* sp. CA61 and *Streptomyces* sp. CA94 isolates demonstrated the most promising antimicrobial activity against five different pathogens.

The isolate coded CA36 showed antimicrobial activity against four different pathogenic microorganisms. While isolates coded CA56, CA68 and CA76 showed antimicrobial activity against three different pathogenic microorganisms, isolates coded CA62 and CA75 showed antimicrobial activity against two pathogenic microorganisms. While the isolate coded CA40 showed antimicrobial activity against a single pathogenic microorganism, isolates coded CA43, CA49, CA59 and CA77 did not show antimicrobial activity against any of the eight pathogens used in the study. Additionally, it was determined that none of the isolates tested showed activity against the fungi. The petri dish image of the zone diameters of the antimicrobial activity of some isolates against pathogenic microorganisms is given in Figure 5 and Table 8.

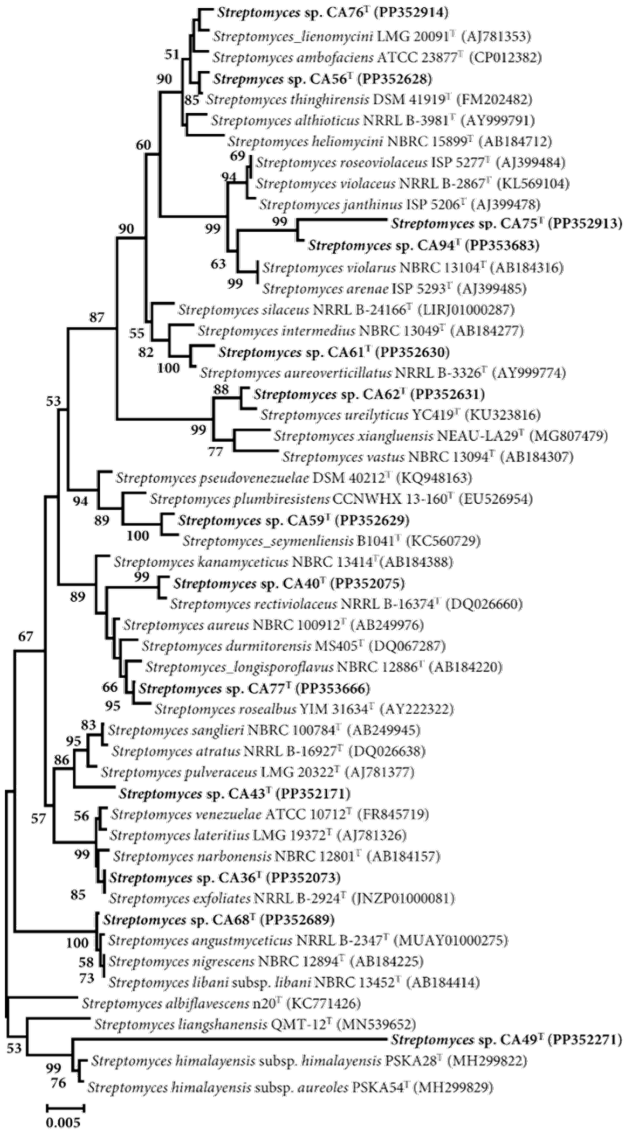

Figure 4 Phylogenetic dendrogram of isolates of the Streptomyces genus based on 16S rRNA gene sequence analysis. In the tree drawn according to the neighbor-joining algorithm, bootstrap values were given for branch points that were supported over 50%. Bar, 0.005

Tween 20 agar was used to test the lipase-producing abilities of the isolates. The zones formed around the isolates, which were sown as dots on petri dishes, as a result of 5-day incubation, were evaluated. When their lipase production abilities were examined, 11 isolates, except CA43 and CA62, had the ability to produce lipase.

The most researched and most efficient sources of lipases are animal, plant and microbial (bacterial, fungal and yeast) sources. However, one bacterial phylum that shows good potential for lipase production is actinobacteria (Mansour et al., 2015; Arumugam et al., 2017; Hamedi et al., 2019; Sharma & Thakur, 2020). Among the lipase-producing actinobacteria, the most studied is the *Streptomyces* genus (Nithya et al., 2018; Panyachanakul et al., 2020).

The amylase activity of the isolates was evaluated on starch agar medium. Spot-seeded isolates were incubated for seven days. After incubation, 1% lugol was added to the petri dishes. The formation of a transparent zone in the area of amylase production was evaluated. While two isolates did not produce amylase, 11 isolates produced amylase.

Studies on amylases are largely of fungal origin, but actinobacteria have also been shown to be good amylase producers. Soil has been an excellent source for obtaining such bacteria. For example, Thampi & Bhai (2017) isolated three species of amylase-producing actinobacteria from rhizosphere soil samples of black pepper plants.

The protease production abilities of the isolates were evaluated by measuring the transparent zones formed around the isolates. While three isolates did not produce protease, ten isolates were determined to produce protease.

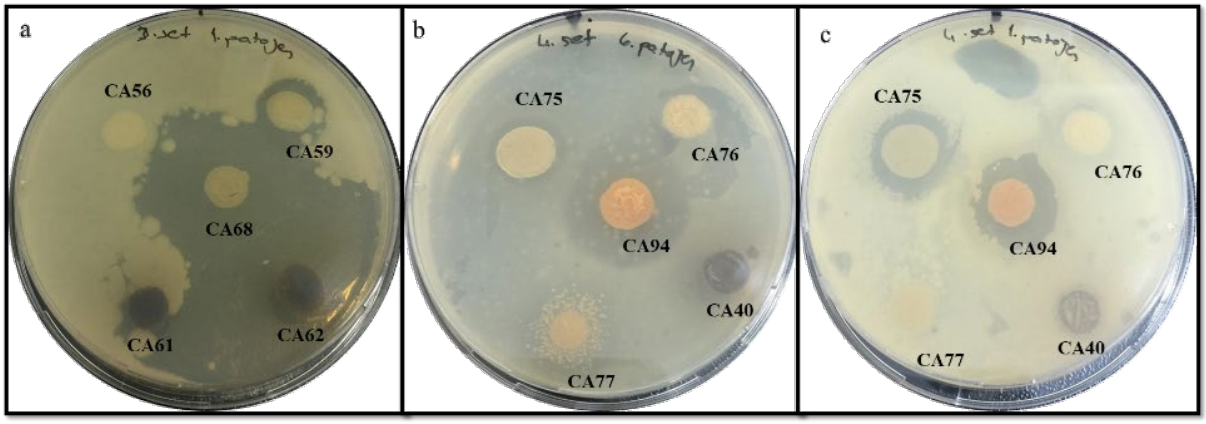

Figure 5. Antimicrobial activity test. **a)** *Bacillus subtilis* ATCC 6633^T-Group 1. **b)** *Pseudomonas aeruginosa* ATCC 27853^T. **c)** *Bacillus subtilis* ATCC 6633^T-Group 2

Table 8. Zone diameters (mm) of the antimicrobial activity of the isolates against pathogenic microorganisms

No	Organism	*B. subtilis*	*S. aureus*	*E. faecalis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*A. niger*	*C. albicans*
1	**CA36**	30	20	-	14	20	-	-	-
2	**CA40**	-	-	-	-	30	-	-	-
3	**CA43**	-	-	-	-	-	-	-	-
4	**CA49**	-	-	-	-	-	-	-	-
5	**CA56**	14	14	-	-	50	-	-	-
6	**CA59**	-	-	-	-	-	-	-	-
7	**CA61**	54	54	40	20	40	-	-	-
8	**CA62**	-	10	-	-	40	-	-	-
9	**CA68**	44	30	-	-	30	-	-	-
10	**CA75**	14	-	-	-	-	20	-	-
11	**CA76**	20	-	16	10	-	14	-	-
12	**CA77**	-	-	-	-	-	-	-	-
13	**CA94**	20	20	16	-	20	26	-	-

Microbial production is an ideal alternative to meet industrial needs. Within the microbiota, the *Bacillus* genus is mainly used for enzyme production. In addition, another new microbial source studied is Actinobacteria members, which are widely used in the production of bioactive compounds such as cellulase and amylase. These are currently under investigation because extremophilic actinobacteria are known to produce thermostable proteases and mesophilic actinobacteria such as *Streptomyces* are known to produce pronase 7 M (*S. griseus*) and fradiase (*S. fradiae*), which are in commercial use (McCarthy et al., 1985; Flores-Gallegos & Nava-Reyna, 2019).

The pectinase production abilities of the isolates were evaluated by measuring the transparent zones formed around the isolates. In the study, it was observed that none of the isolates produced pectinase. Table 9 presents the lipase, amylase, protease and pectinase production abilities of the isolates. The commercial applications of this enzyme include juice purification, vegetable purification, wastewater treatment, degumming, defoaming agent, and fermentation accelerator in the tea and coffee industry. Additionally, it is used as an analytical tool for plant product evaluation (Beulah et al., 2015; Oumer & Abate, 2018).

An alternative and powerful source of pectinase has been identified in actinomycetes, which are known for their production of bioactive compounds and are used for the production of antibiotics (Kumar & Sharma, 2012). In recent studies, it has been reported that *Streptomyces thermocarboxydus* produces thermostable pectinases, which are active at high temperatures and are gaining increasing importance. At elevated temperatures, the process is accelerated, which contributes to economic gain (Priyanka, 2019).

Table 9. Abilities of isolates to produce lipase, amylase, protease and pectinase

No	Organism	Lipase	Amylase	Protease	Pectinase
1	**CA36**	+++	+	+	-
2	**CA40**	+++	+	-	-
3	**CA43**	-	+	+	-
4	**CA49**	+++	+	+	-
5	**CA56**	+++	+	+	-
6	**CA59**	+++	-	+	-
7	**CA61**	+++	+	-	-
8	**CA62**	-	+	+	-
9	**CA68**	+++	+	+	-
10	**CA75**	+++	+	+	-
11	**CA76**	+++	+	+	-
12	**CA77**	+++	+	+	-
13	**CA94**	+++	-	-	-

Note: Symbols: (-) No growth; (+) Little growth; (++) Moderate growth; (+++) Excellent growth.

Conclusion

According to 16S rRNA gene region nucleotide sequence analysis, *Streptomyces* spp. isolates CA49, CA75, CA94 and CA43 show similarities between 95.76% and 98.96% to their closest type species. All genotypic, chemotaxonomic and phenotypic analyzes of these four isolates, which are likely to be new species, will be completed and introduced to the literature in the near future. Members of *Streptomyces* are the most

common microorganisms that have the function of breaking down organic compounds through enzyme activities. Therefore, industrially and agriculturally important enzymes detailed characterization of the industrially important enzymes produced by these isolates to be introduced into the literature is required.

Compliance With Ethical Standards

Authors' Contributions

AV: Conceptualization, Writing – original draft, Investigation, Formal analysis
DT: Writing – original draft, Investigation, Formal analysis
HAD: Investigation, Formal analysis
AT: Investigation, Formal analysis
All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants and/or animals performed by any of the authors. Formal consent is not required in this study.

Funding

This research was supported by Sinop University, project no SHMYO-1901-23-001.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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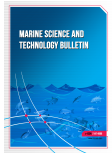
Mar. Sci. Tech. Bull. (2024) 13(3): 199-214
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1509128



Marine Science and Technology Bulletin

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RESEARCH ARTICLE

Exploring communication barriers in bridge-teams: An innovative fuzzy-Bayesian approach

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ARTICLE INFO

Article History:
Received: 02.07.2024
Received in revised form: 28.08.2024
Accepted: 08.09.2024
Available online: 30.09.2024

Keywords:
Bayesian network
Bridge team
Collaboration
Communication barriers
Navigation

ABSTRACT

The bridge teams on merchant vessels have a grave responsibility to guarantee the safe navigation and management of ships in the critical waterways of the world. In addition to maintaining effective communication between external stations (other ships-Vessel Traffic Service), it is crucial to ensure continuous internal collaboration among the bridge team in order to fulfil this important task to the highest standard. Nevertheless, the challenging working conditions and harsh environmental factors may impede the uninterrupted flow of information between bridge teams and disrupt the communication. Communication issues among team members are frequently mentioned as a root cause in maritime accident investigation reports. The aim of this research is to propose a novel model for identifying the factors that may cause to inadequate communication among bridge team members, employing a fuzzy Bayesian network (FBN) approach. As indicated by the findings, attitudinal and behavioural barriers exert a greater influence (43.3%) on communication than language barriers (41.5%), representing the most significant factors affecting communication. Environmental barriers and cultural barriers, on the other hand, have comparatively less impact, at 38.7% and 31.2%, respectively. The sensivity analysis also revealed that the root nodes exhibiting the highest degree of impacts were cultural barriers (31.2%), age differences (20.6%), and workplace issues (20.2%). The findings suggest that bridge communication refresher training programs are essential for the mitigation of the aforementioned barriers, and are expected to lead to the development of new strategies for the overcoming of these communication barriers.

Please cite this paper as follows:

Turna, İ., & Öztürk, O. B. (2024). Exploring communication barriers in bridge-teams: An innovative fuzzy-Bayesian approach. *Marine Science and Technology Bulletin*, *13*(3), 199-214. https://doi.org/10.33714/masteb.1509128

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Introduction

The major maritime decision-making mechanisms such as International Maritime Organization (IMO) and the European Maritime Safety Agency (EMSA) are working to make maritime transportation safer, more ecologically friendly, and more efficient. A review of contemporary advancements in maritime engineering reveals a number of innovative solutions, particularly in the domains of energy efficiency and environmental protection, which are aligned with the IMO's 2030 and 2050 targets for reducing greenhouse gas emissions from ships (Mallouppas & Yfantis 2021; Islam Rony et al., 2023). In spite of technological advances, the maritime transport industry is still primarily a human-centred sector (Mallam et al., 2020; Wu et al., 2022). A review of the relevant literature on maritime accident investigations reveals that the human factor is identified as the primary root cause of accidents (Yıldırım et al., 2019; Shi et al., 2021; Paolo et al., 2021; EMSA, 2023). It is well known that seafarers work in harsh conditions; fatigue, static electricity, motion, noise and vibration have a negative impact on these key workers at their working environment. Despite these challenging conditions, seafarers shall complete their daily tasks as a well-organized team in strong coordination. It is of the utmost importance that those responsible for the ship's navigation and management, in particular the master and deck officers, work in harmony as a team in order to ensure the safety of navigation and thus, the protection of the environment. The notion of bridge team management (BTM) appears in maritime literature as a concept that has grown in importance in recent years, and critical positions such as the master, officers, lookout and helmsman are defined as members of this team (Aylward et al., 2020; Cavaleiro et al., 2020; UK Chamber of Shipping, 2020; Danielsen et al., 2022).

Communication, defined as a two-way process that involves the exchange of information, thoughts, and comments between the speaker and the listener, is the most critical factor influencing team cohesion (Sutter & Strassmair, 2009; Gervits et al., 2016; Yusof et al., 2020). A considerable number of studies have demonstrated a correlation between high levels of solidarity, collaboration, and harmony within organizational units and the efficacy of communication (Halis, 2000; Crant, 2000; Butchibabu et al., 2016). However, a multitude of factors may obstruct the efficacy of communication, and restrict members of a group from communicating and understanding each other clearly (Gürüz & Eğinli, 2008). It is a well-documented fact that communication issues are frequently cited as a cause of human error in maritime accidents and risks (Sotiralis et al., 2016; Kee et al., 2017; Zhang et al., 2019; Coraddu et al., 2020; Tunçel & Arslan, 2022, Güzel et al., 2023).

It is the responsibility of the bridge team to maintain the navigational safety of the ship (ICS, 2022), and this team is in charge of the most vital tasks in ship navigation and manoeuvring such as position fixing and course altering especially in restricted waters. The team is primarily composed of deck crew, and may also include a maritime pilot, who may be invited to join the team on a temporary basis, in order to provide local expertise and experience of the navigational hazards of the waterways. Team members shall comply with various conventions and policies that govern their responsibilities, including Convention on the International Regulations for Preventing Collisions at Sea (COLREG), the International Convention on Maritime Search and Rescue (SAR), and the International Convention for the Safety of Life at Sea (SOLAS). In this context, effective communication between the bridge team is of paramount importance for the successful completion of these challenging tasks.

Despite the abundance of studies that have identified communication problems in maritime accidents, it is notable that interrelations between the variables responsible for communication problems have been inadequately addressed. The aim of this research is to determine the variables that impede communication between bridge team members, and to determine the relationships between these variables and their respective influences, using the FBN approach. A systematic review of existing literature revealed a number of studies investigating communication difficulties in various occupational settings. Notably, no studies were identified that examined communication challenges specifically within the context of bridge teams on ships, to the best of our knowledge.

The current study consists of four sections. The introduction section places particular emphasis on the importance of effective communication within the bridge team, as evidenced by the analysis of maritime accident investigation reports. The following section, designated as "Methodology," will elucidate the flowchart of the study and the Fuzzy Bayesian Network method employed. Additionally, this section will present information concerning the experts involved in the study and the procedures utilized for their evaluations. The results and discussion are presented in the third section of the study, and finally the fourth chapter presents the study's conclusions and limitations, as well as priorities and advice for minimizing communication issues within the bridge team.

Material and Methods

Bayesian Network

The Bayes network is a dynamic and effective graphical model for revealing the probabilistic relationships between variables (Chang et al., 2021; Aydın & Kamal, 2022). The approach aids in the analysing and explaining of the sequence of complex interactions between system variables, allowing variables' impact to be accurately evaluated (Yang et al., 2008). For this reason, it is considered that this technique can reveal all the causes of inadequate bridge communication and the weights of the factors that can contribute to failure in this process. This section describes the Fuzzy-Bayesian network approach, as well as the methodology's conceptual structure as shown in Figure 1.

BNs are comprised of qualitative and quantitative components. The qualitative part comprises a network structure called a Directed Acyclic Graph (DAG) (Rostamabadi et al., 2019). The network consisted of nodes and directed arcs. The quantitative part of the BN is created using a number of conditional probability distributions. The arcs describe the variables' probabilistic causal connection, and Conditional Probability Tables (CPTs) are attached to the nodes to illustrate conditional dependencies (Yazdi & Kabir, 2017). In BNs, if an arrow begins from a node, that node is referred to as the parent node and the node to which the arrows point is referred to as the child node. The Root nodes are nodes that have no parents, whereas the leaf nodes are nodes that have no children. The inference presumption of the BN approach relies on Bayes probability theory. The following equations demonstrate the inference algorithms (Mahadevan et al., 2001).

The joint probability distribution of a set of variables $N = \{X_1, X_2, X_3 \ldots, X_n\}$ can be expressed as Eq. (1):

$$P(X_1, X_2, \ldots, X_n) = \prod_{i=1}^{n} P\left(X_i \setminus P_a(X_i)\right) \quad (1)$$

Based on Zarei et al. (2019), The parent set of variables is denoted by $P_a(X_i)$. Eq. (2) expresses the posterior probability of the parent node X_j under the scenario of the child node Xi:

$$P\left(x_j \setminus Xi\right) = \frac{P(x_i, x_j)}{P(x_i)} \quad (2)$$

Determining the probability of the root nodes is a critical step in achieving meaningful results from the BN structure. The CPTs and the marginal probability of the root nodes can be created based on statistical data, expert judgment, or a mix of the two (Chen et al., 2022). In accordance with the objectives of this study, a survey was conducted among the bridge team members of merchant ships, during which communication problems that negatively affect collaboration on the bridge were identified. These survey results were also used to ascertain the marginal probability of root nodes in the Bayesian network. Figure 2 illustrates the rank distribution of the bridge team members who participated in the study.

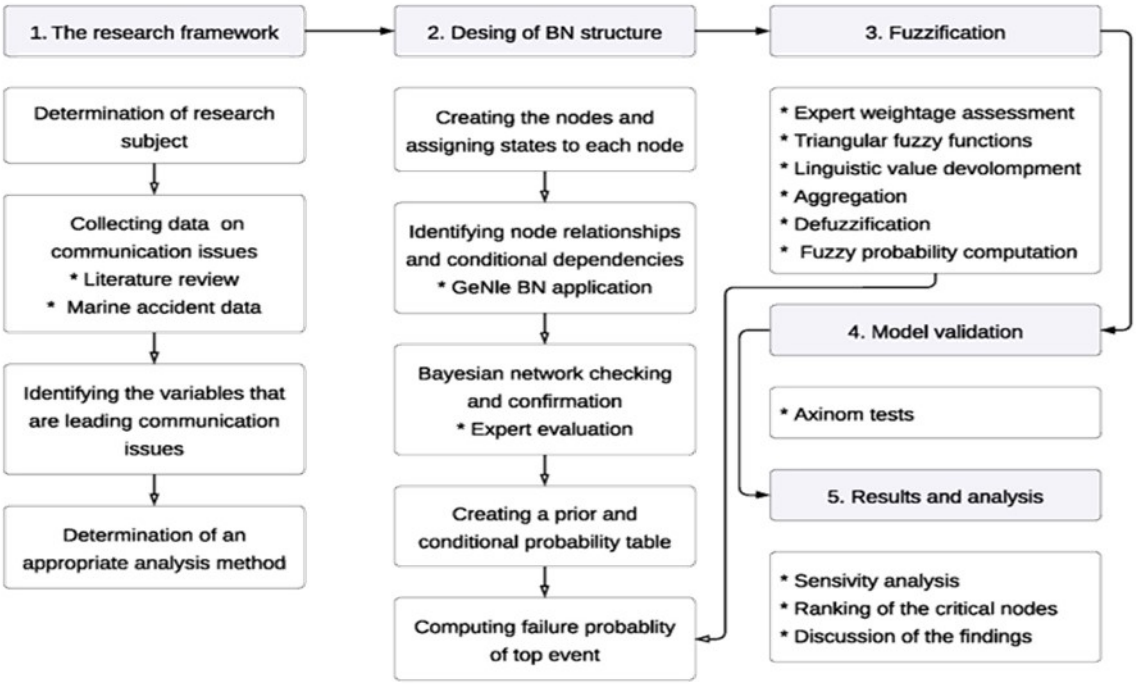

Figure 1. The research flow chart

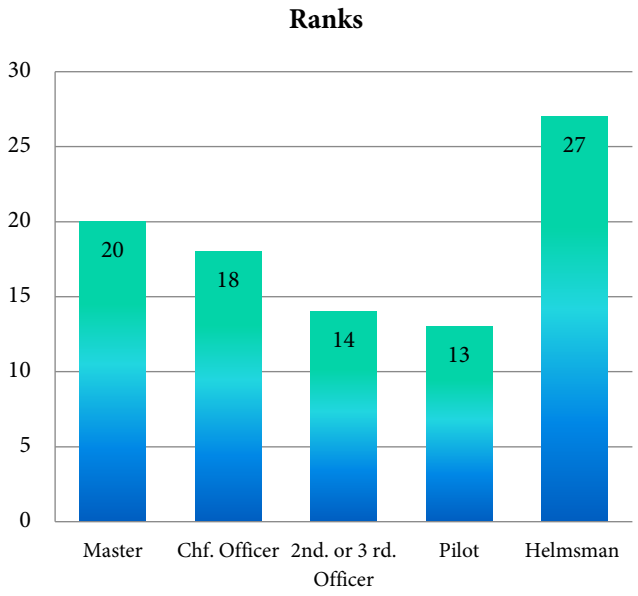

Figure 2. Distribution of participants of the survey

Prior Probabilities for a Node with Multiple Parents

The variables that have negative effects on communication have been categorized in BN structure. The final version of the BN network was determined in consultation with a heterogeneous expert group. Where the number of nodes used in the BN network is limited, experts are able to determine the probability directly, utilizing their expertise and knowledge. The assessment of probability combinations becomes more challenging when there are a large number of probabilities to be evaluated, particularly when the nodes have more than one parent, as is the case of the current study. To decrease complexity, the decomposition approach is used in this paper, which allows experts to extract the CPT by evaluating each parent node independently. The decomposition approach helps experts elicit the CPT more efficiently while reducing subjective prejudices (Wang et al., 2010, 2011; Ping et al., 2018).

Assume that node *N* has *k* states (S_1, S_2, …, S_k) with n(n≥2) parents ($T^{(1)}$, $T^{(2)}$,… $T^{(j)}$, … $T^{(n)}$). The parent node T(j) has m states, which $T_1^{(j)}, T_2^{(j)}, \ldots T_m^{(j)}$ (J=1, …, m). Thus, the prior probability of each state of N under the various state combinations of its parent nodes can be described as:

$$P(N = S_i \mid T^{(1)} = T_u^{(1)}, T^{(2)} = T_u^{(2)}, \ldots, T^{(n)} = T_u^{(n)})\ i = 1, 2, \ldots, k;\ u = 1, 2, \ldots, m \quad (3)$$

When a node A has two parents B and C, its conditional probability on B and C can be approximated by means of:

$$P(A \backslash B, C) = \alpha P(A \backslash B) P(A \backslash C) \quad (4)$$

The normalizing constant (α) ensures that:

$$\sum_{\alpha \in A} P(\alpha \backslash B, C) = 1 \quad (5)$$

Fuzzification

Language expressions that are unclear are translated into exact numerical expressions using linguistic variables. Fuzzy numbers, which generate values ranging from 0 to 1, indicate expert judgment uncertainty, whereas linguistic expressions express uncertain language expressions. The literature presents a variety of membership functions, most of which use triangle and trapezoidal functions. In maritime studies, triangular or trapezoidal fuzzy numbers are commonly used for assessing linguistic variables. The triangle membership function (TMF) is widely used because of its simplicity of use and accuracy in converting exact numbers to fuzzy numbers (Kamal et al., 2020; Akan & Bayar, 2022). Equation (6) illustrates the membership function of triangular fuzzy numbers. The triangular membership function (TMF) is used in this study because of its simplicity of use and accuracy.

$$\mu_{\tilde{A}}(x) = \begin{cases} 0, x \le a_1 \\ \frac{(x-a_1)}{(a_2-a_1)}, a_1 \le x \le a_2 \\ \frac{(a_3-x)}{(a_3-a_2)}, a_2 \le x \le a_3 \\ 0, x \ge a_3 \end{cases} \quad (6)$$

The equation for the triangular fuzzy number E (a1, a2, a3) is presented below:

$$X = \frac{\int_{a1}^{a2} \frac{x-a_1}{a_2-a_1} x\, dx + \int_{a2}^{a3} \frac{a_3-x}{a_3-a_2} x\, dx}{\int_{a1}^{a2} \frac{x-a_1}{a_2-a_1} x\, dx + \int_{a2}^{a3} \frac{a_3-x}{a_3-a_2} x\, dx} = \frac{1}{3}(a_1 + a_2 + a_3) \quad (7)$$

A higher level of precision can be achieved by splitting smaller probability ranges and applying the experts' probability decision to the fuzzy number. To evaluate nodes with unclear conditional probability, a seven-term linguistic scale was employed as shown in Table 1 (Rajakarunakaran et al., 2015).

Table 1. Linguistic scale

Linguistic terms	a_1	a_2	a_3
Very high (VH)	0.92	0.96	1.0
High (H)	0.81	0.87	0.93
Mildly high (MH)	0.63	0.73	0.83
Medium (M)	0.35	0.50	0.65
Mildly low (ML)	0.17	0.27	0.37
Low (L)	0.07	0.13	0.19
Very low (VL)	0.00	0.04	0.08

Expert Elicitation and Aggregation

Makridakis & Winkler (1983) and Clemen & Winkler (1999) highlights the negative marginal values associated with the large number of experts. Five experts have been selected who can assist with variable identification and the development of the Bayesian network at the highest level. Experts' risk perception differs due to variances in their knowledge structure and skills. A weighing process has been employed at this step, taking into account the positions, operational experience, and educational degrees of the chosen experts. Risk perceptions differ due to variances in the knowledge structure and skills of experts. Each expert's decision weight has been assessed by four objective criteria: professional position, competency, service time, and education level. Each parameter is ranked from 1 to 5. The decision weights of the experts chosen for this study were calculated using the criteria shown in Table 1. Table 2 provides the details for the experts as well as the weighting procedure calculations.

Table 3 indicates that the five experts are the Editor in chief, the Accident surveyor, the Senior pilot, the Communications consultant, and the Senior lecturer. For example, the chief editor is a communication expert with 11 years of experience in the field, demonstrating expertise across a range of media outlets. He provides consultancy services to companies as an experienced communication professional and also works as a field editor in a publishing organization. The senior lecturer who is a PhD-qualified maritime educator with 11 years of teaching experience and is an ocean goingmaster, is responsible for teaching maritime communication and maritime English.

Table 2. Weighting criteria of the experts

Attribute	Classification	Weighting Score (WS)
Occupational position	Marine accident surveyor	5
	Editor in chief	4
	Senior marine pilot	3
	Communication consultant	2
	Senior lecturer	1
Competency	Senior pilot	5
	Ocean going master (STCW II/2)	4
	Communications professional	3
	Chief officer & Chief engineer	2
	2nd officer	1
Service time	≥ 15	5
	11-15	4
	6-10	3
	3-5	2
	≤ 2	1
Educational level	Doctorate (PhD)	5
	Master of Science (MSc)	4
	Bachelor (BSc)	3
	Junior college	2
	High school	1

Table 3. Experts' background and decision weights

Expert	Profession title/WS	Competency/WS	Service time/WS	Education level/WS	TWS	Score (75)
E1	Editor in chief/4	Comm. Pro/3	11/4	BSc/3	14	0.186
E2	Accident surveyor/5	Master/4	16/5	PhD/5	19	0.253
E3	Senior pilot/3	Senior pilot/5	9/3	PhD/5	16	0.213
E4	Comm. consultant/2	Master/4	10/3	BSc/3	12	0.160
E5	Senior lecturer/1	Master/4	11/4	PhD/5	14	0.186

To aggregate judgments of these expert group, the Similarity Aggregation Method (SAM) proposed by Hsu & Chen (1996) was employed. The methodological framework is presented as follows:

E1, E2 : It represents a pair of expert opinions.
SUV (E1, E2) : The degree of agreement (similarity level) between two different expert opinions,
S (E1, E2) : It indicates the level of similarity between two fuzzy numbers
AA (EU) : It denotes the average degree of agreement among experts
RA (EU) : It refers experts' relative level of agreement
CC (EU) : Consensus coefficient level of the experts
RAG : It describes the aggregated outcome of the expert decisions.

Step (1): The level of similarity of judgements of the experts are determined. If the opinions of E1 and E2 experts are expressed by E1 = (a1, a2, a3) and E2 = (b1, b2, b3), The following equation illustrates the similarity function between expert E1 and expert E2.

$$S(E_1, E_2) = 1 - \frac{1}{3}\sum_{i=1}^{3}\lceil a1 - b1 \rceil \quad (8)$$

Step (2): The average agreement (AA) of M experts can be calculated as follows.

$$\text{AA}\,(E_U) = \frac{1}{M-1}\sum_{\substack{i=1 \\ n\neq m}}^{M} i\,S(E_1, E_2) \quad (9)$$

Step (3): The Relative Agreement Degree (RA) of M experts can be determined as follows.

$$RA(E_U) = \frac{\text{AA}\,(E_U)}{\sum_1^M \text{AA}\,(E_U)} \quad (10)$$

Step (4): The equation that follows can be used to figure out the consensus coefficient of M experts.

$$CC\,(E_U) = \beta.w\,(E_U) + (1 - \beta).\ RA(E_U) \quad (11)$$

Step (5): The equation below is used to aggregate expert opinions.

$$R_{AG} = CC_1 x E_1 + CC_2 x E_2 + \cdots CC_{M1} x E_M \quad (12)$$

Defuzzification

To derive an inference from the Bayes network, the fuzzy numbers must be converted into crisp numbers (Aydin et al., 2024). Multiple methods for this conversion process have been provided in the literature, including the centre of sums, weighted average, centroid method, maximum membership degree, and centre of the largest area (Wang, 1997). The centre-of-area approach was adopted in this study because of its simplicity and versatility. This eliminates data corruption and leads to more accurate analysis. Equations (13) and (14) were employed to convert fuzzy numbers into crisp numbers.

Defuzzification equation:

$$X^* = \frac{\int \mu_j(x)\,dx}{\int \mu_j(x)} \quad (13)$$

For triangular fuzzy numbers;

$$\widetilde{A} = (a_1, a_2, a_3)$$

$$X = \frac{\int_{a_1}^{a_2}\frac{x-a_1}{a_2-a_1}x\,dx + \int\int_{a_2}^{a_3}\frac{a_3-x}{a_3-a_2}x\,dx}{\int_{a_1}^{a_2}\frac{x-a_1}{a_2-a_1}dx + \int\int_{a_2}^{a_3}\frac{a_3-x}{a_3-a_2}dx} = \frac{1}{3}(a_1 + a_2 + a_3) \quad (14)$$

Application of the Method to Bridge Team Communication Issues

It is evident that the safety of merchant ship port operations and navigation is highly dependent on the existence of a collaborative cooperative environment on board. In this section, the FBN approach was employed to simulate the potential barriers that may emerge during communication among bridge teams in these operations. Prior to implementing the proposed methodology, it is essential to identify the variables that hinder communication within the bridge team. A review of the literature revealed that studies on communication difficulties in different sectors have been conducted, but to the best of our knowledge, no study on the communication problems of the bridge team on ships has been found (Erven, 2002; John et al., 2013, 2016; Rani, 2016; Kapur, 2018; White et al., 2018; Salvation, 2019; Tunçel & Arslan, 2022; Güzel et al., 2023).

The following phase reviewed maritime accident investigation reports published in recent years by the Maritime Accident Investigation Branch (MAIB), Marine Casualty Investigation Board (MCIB), Japan Transport Safety Board (JTSB), and National Transportation Safety Board (NTSB) to identify bridge-team communication failures in collision-type accidents. The Bayesian network creation process commenced following the identification of the variables. Before commencing the interviews, the experts were instructed about the goal of the study, the Bayes network method, and the process for exposing probabilities. The data on bridge team

communication issues were shared with the appointed experts, and the variables included in the framework were debated based on their structure. Before commencing the interviews, the experts were instructed about the goal of the study, the Bayes network method, and the process for exposing probabilities. The data pertaining to the communication issues encountered by bridge teams were presented to experts, and the specific variables included in the proposed framework were discussed. A Bayes network-based model was created using the academic software package GeNle 3.0 (Bayes Fusion LLC, 2021). The variables were connected in the BN in hierarchical order based on previous studies (Kapur, 2018; Salvation, 2019; Çakır & Kamal, 2021). The final version of the BN was established through the consensus of the experts. The marginal probabilities of the root nodes in the BN were calculated using data collected from a web-based survey of bridge team members. Before employing the software, the opinions of experts, represented in linguistic terms, were converted into triangular fuzzy sets. Expert opinions were reconciled using Hsu & Chen's (1996) similarity aggregation approach. Figure 3 illustrates the posterior probability for all nodes. The nodes in the BN are assigned colors according to their hierarchical order.

Table 4 describes the nodes that may cause bridge-team communication issues. The nodes of the network are based on the literature, expert judgments, and accident reports (MAIB, 2015, 2020; BSU, 2019; UEIM, 2019; JTSB, 2020; NTSB, 2020; MCIB, 2022; USCG, 2022).

Prior to entering the data in the GeNIe program, the expert views collected in linguistic form were fuzzified using the triangular fuzzy members listed in Table 1. Due to limited space, Tables 5 and 6 only provide expert verbal evaluations of the node "Language barriers" and conditional probabilities for the node "Personal barriers".

Validity of the Method

In the final step, the model that was created will be validated. Validation is an essential component of the BN model since it offers reasonable confidence in the findings. Based on the literature, there are various methods to assess the validity of the created model. Three axioms should be satisfied using a frequently utilized approach, which is also applied in this paper (Zhang et al., 2013; Chang et al., 2021). Axiom 1 states that increasing or decreasing the prior probability of each parent node by a particular percentage must result in a meaningful and significant rise or drop in the posterior probabilities of the linked child nodes (Table 7).

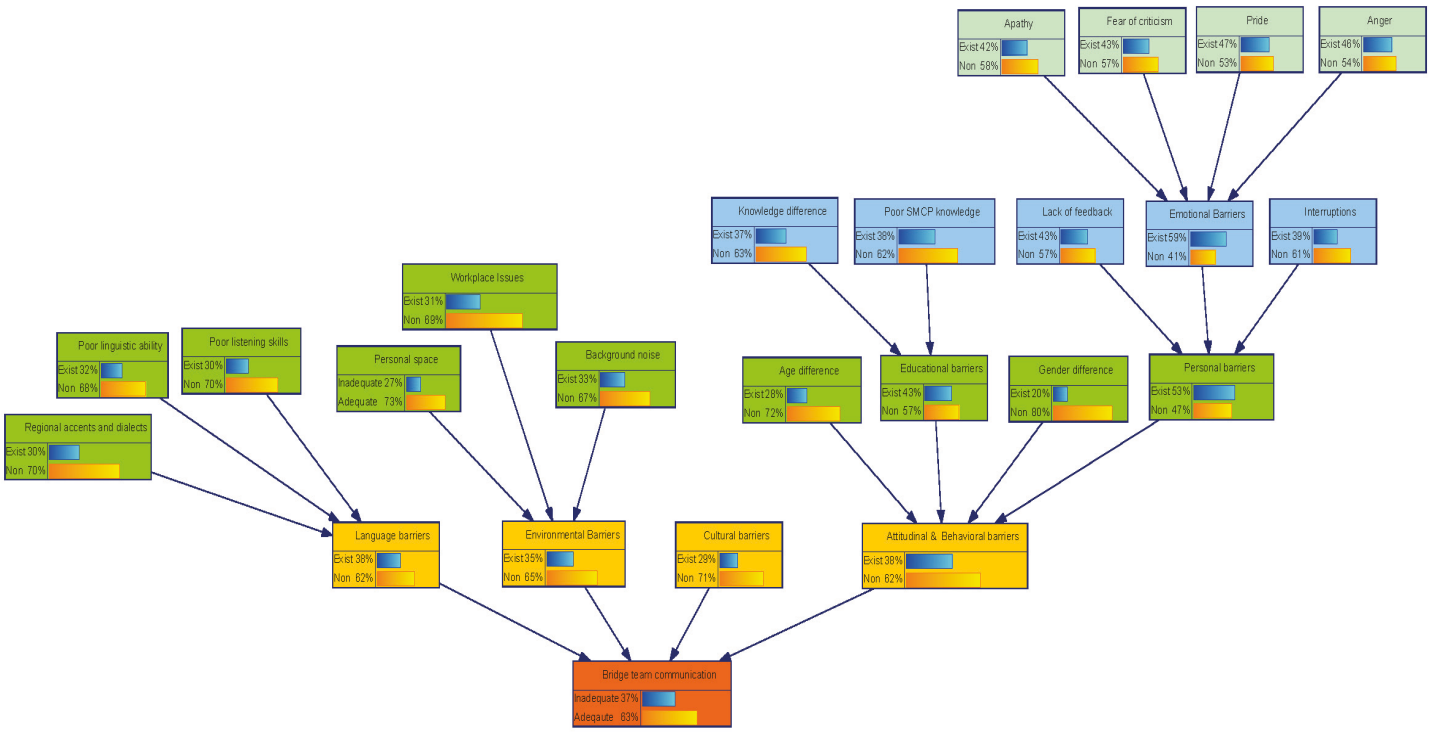

Figure 3. Nodes of bridge team communication issues in the FBN approach

Table 4. Details of the nodes

Node Description	Node Condition	Failure Reference	Definition
Bridge team communication	Leaf	AIR	The term is used to describe the process of exchanging information and instructions in a coordinated manner among the members of a ship's bridge team.
Language barriers	Parent	LR, AIR, EJ	It refers to the obstacles or challenges that arise when individuals or groups cannot effectively communicate due to the lack of a shared or common language.
Environmental barriers	Parent	LR	The term refers to obstacles in the physical environment that hinder people's ability to participate fully in activities or access services.
Cultural barriers	Root	LR	The term encompasses the discrepancies in norms, values, traditions, and communication styles that can impede comprehension and efficacious interaction between individuals from disparate cultural backgrounds.
Attitudinal & Behavioural barriers	Child	AIR, EJ	It refers obstacles that stem from people's attitudes and behaviors rather than physical or systemic issues.
Regional accents and dialects	Root	AIR	The term denotes a variation in the manner of linguistic expression, contingent upon the geographical region or area from which the speaker hails.
Poor linguistic ability	Root	LR	The term refers a lack of aptitude in the acquisition of language.
Poor listening skills	Root	EJ	The term is used to describe an individual's limited capacity to decode and interpret verbal messages and nonverbal cues, such as tone of voice, facial expressions, and physical posture.
Personal space	Root	LR	The term denotes the immediate physical environment of an individual, which may evoke feelings of encroachment and discomfort when perceived as a threat.
Workplace issues	Root	LR	The term is used to describe the difficulties encountered by employees in their professional environments.
Background noise	Root	LR	The term which is defined as any sound that is not the primary sound being monitored is a form of noise pollution. .
Age difference	Root	LR	It refers to the amount by which ages are different.
Educational barriers	Parent	EJ	It denotes obstacles that arise from differences in knowledge, skills, or educational background
Gender difference	Root	LR	The term is used to describe the range of behaviours and attitudes that are associated with being female or male.
Personal barriers	Parent	LR, EJ	The term refers to obstacles that stem from individual characteristics, affecting one's ability to communicate or participate effectively.
Knowledge difference	Root	EJ	The term denotes the level of information or awareness that an individual possesses regarding a specific topic area.
Poor SMCP knowledge	Root	AIR, EJ	The term denotes the low level of information that an individual possesses regarding SMCP.
Lack of feedback	Root	AIR	The term denotes the insufficient transmission of evaluative or corrective information regarding an action, event, or process to the original or controlling source.
Emotional barriers	Parent	LR, EJ	The term refers to communication and interaction barriers arising from an individual's emotional state.
Interruptions	Root	EJ	The term refers to disruptions during communication that break the flow of conversation, leading to potential misunderstandings and loss of key information.
Apathy	Root	EJ	It refers to lack of feeling or emotion
Fear of criticism	Root	LR, EJ	It refers to the apprehension of articulating disapproval of an individual or entity due to perceived imperfections or shortcomings.
Pride	Root	EJ	It can be defined as a feeling of positive affect associated with the accomplishment or acquisition of something perceived as beneficial.
Anger	Root	EJ	It signifies a powerful inclination to inflict harm or exhibit malevolence as a consequence of an unjust or unkind occurrence.

Note: AIR: Accident Investigation Report, LR: Literature Review, EJ: Expert judgment, SMCP: Standard Marine Communication Phrases

Table 5. Verbal evaluations by experts and fuzzy possibility scores (FPS) for State A of the node "Language barriers"

Language barriers	States		Evaluations for states A					(FPS)
	States A	*States B*	*Expert 1*	*Expert 2*	*Expert 3*	*Expert 4*	*Expert 5*	
Poor listening skills	Exist	Non	H	MH	H	H	H	0.896
Regional accents and dialects	Exist	Non	ML	M	M	ML	ML	0.858
Poor linguistic ability	Exist	Non	M	VH	VH	H	VH	0.903

Table 6. Conditional probabilities for the personal barriers' node

Emotional barriers	Exist				Non			
Interruptions	*Exist*		*Non*		*Exist*		*Non*	
Lack of feedback	*Exist*	*Non*	*Exist*	*Non*	*Exist*	*Non*	*Exist*	*Non*
Exist	0.998	0.940	0.828	0.091	0.984	0.577	0.293	0.008
Non	0.001	0.059	0.171	0.908	0.015	0.422	0.706	0.991

Table 7. Test of Axiom 1 for the node "Environmental barriers"

Condition	Root Nodes	Parent node
Exist	Workplace issues	Environmental barriers
	Prior %	34.8
	100 %	71.1
	0 %	18.8
Exist	Background noise	Environmental barriers
	Prior %	34.8
	100 %	53.5
	0 %	25.7
Exist	Personal space	Environmental barriers
	Prior %	34.8
	100 %	67.0
	0 %	22.8

Table 8. Axiom Test 3 for the node "Emotional barriers"

Parent nodes				Child node	Percentage change
Apathy (%)	*Fear of criticism (%)*	*Pride (%)*	*Anger (%)*	*Emotional barriers (%)*	
41.5	42.7	46.9	46.4	59.1	0%
100	42.7	46.9	46.4	81.9	22.8%
41.5	100	46.9	46.4	81.1	22.0%
41.5	42.7	100	46.4	80.1	21.0%
41.5	42.7	46.9	100	76.8	17.7%
100	100	100	100	99.9	40.8%

According to the Axiom 2, different levels of increase in a parent node's prior probability should have the coherent effect on the child node. Figure 4 shows the change in probability for the child node 'Environmental barriers' as a result of alterations to its parent variables 'Personal space', 'Workplace issues', and 'Background noise'. When examining child nodes with more than one parent, the combined influence of parents should be greater than the effect of each parent individually, according to Axiom 3 (Table 8).

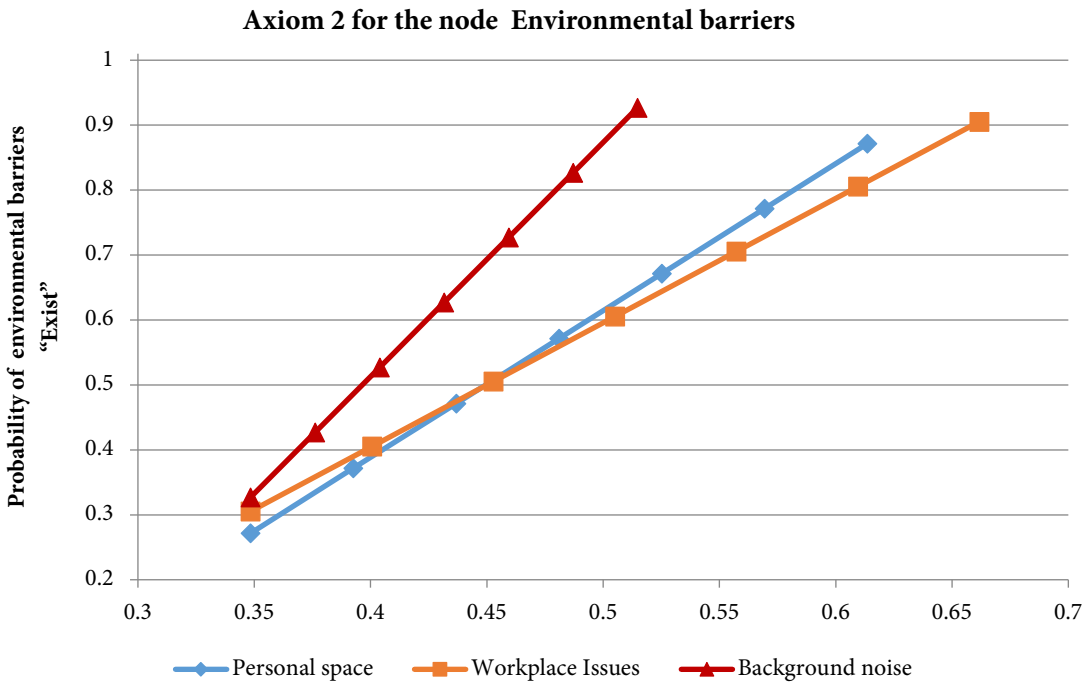

Figure 4. Test of Axiom 2 for the node "Environmental barriers"

Table 9. Sensivity analysis results of the top fifteen node's

Nodes affecting bridge-team communication	Condition	Prior (%)	Change 0%	Change 100%	Effect (%)
Attitudinal & behavioural barriers	Child	38.3	20.8	64.1	43.3
Language barriers	Parent	38.4	21.5	63.0	41.5
Environmental barriers	Parent	34.8	23.9	62.6	38.7
Cultural barriers	Root	28.6	28.5	59.7	31.2
Age difference	Root	27.7	31.7	52.3	20.6
Workplace issues	Root	30.5	31.2	51.4	20.2
Poor linguistic ability	Root	32.1	31.1	50.6	19.5
Poor listening skills	Root	30.2	31.7	50.5	18.8
Gender difference	Root	19.6	33.8	52.2	18.4
Personal space	Root	27.1	32.8	49.9	17.1
Personal barriers	Parent	52.5	28.4	45.5	17.1
Regional accents and dialects	Root	29.9	33.4	46.9	13.5
Educational barriers	Parent	43.3	32.4	44.0	11.6
Background noise	Root	32.6	33.9	44.6	10.7
Interruptions	Root	39.1	33.5	43.5	10.0

Table 10. Sensivity analysis results of the last three node's

Nodes affecting bridge-team communication	Condition 1st	Prior (%)	Change 0%	Change 100%	Effect (%)
Apathy	Exist	41.5	36.7	38.4	1.7
Pride	Exist	46.9	36.5	38.3	1.8
Fear of criticism	Exist	42.7	36.6	38.5	1.9

Sensivity Analysis

Sensivity analysis is a crucial technique in probabilistic assessment, employed to ascertain the behavioural patterns of a given BN model. This technique enables the discovery of discrepancies in the created model. Sensivity analysis determines which variables in the network have the greatest influence on the target node. The prior probability numbers for the variables in the network are changed during the Sensivity analysis, allowing the influence of each node on the target node to be explored. It also allows the network's preventative activities to be uncovered (Zhang et al., 2014). In this study, Sensivity analyses were conducted for all root and intermediate nodes, and the findings are presented in Tables 9 and 10.

Results and Discussion

According to the Figure 3, results, among the 92 seafarers engaged in various duties within the bridge teams of merchant ships, the probability of communication difficulties among bridge crew members was calculated to be 37%. The probability of the root causes identified as potential barriers to bridge communication was calculated, and the subsequent findings yielded the highest marginal probability ratio for emotional barriers (59%), pride (47%), and anger (46%). Furthermore, the root causes with the lowest marginal probability ratio were found to be "Gender difference, with a probability of 20%, "Personnel space", with a probability of 27%, and "Age difference", with a probability of 28%.

Based on the Sensivity analysis, the most significant three root nodes on bridge communication failures were identified as "Cultural barriers (31.2%)", "Age differences (20.6%)", and "Workplace issues (20.2%)". Moreover, the least effective root nodes of bridge communication barriers are the "Apathy (1.7%)", "Pride (1.8%)", and "Fear of criticism (1.9%)", respectively. It was revealed that "Attitudinal and behavioural barriers" had the highest impact (43.3%) on the leaf node, "Bridge team communication". Considering the Sensivity analysis results, it is observed that "Language barriers (41.5%)" and "Environmental barriers (38.7%)" have the most substantial impact on the occurrence of bridge-team communication issues after "Attitudinal and behavioural barriers". Upon examination of the intermediate nodes designated "Attitudinal & behavioural barriers," "Language barriers," "Environmental barriers," and "Cultural barriers," which impact the "Bridge team communication" leaf node in Figure 3, it becomes evident that the cultural barrier node exhibits the lowest efficacy, with an effect of 31.2%.

The outcomes of this study are in line with the results presented by Tunçel & Arslan (2022), as one of the greatest threats to communication on board is a lack of training, which we recognize as an educational barrier. Similarly, "Language barriers" node was determined to be the second most adverse barrier influencing bridge-team communication (41.5%) in the present study. Moreover, in the other study conducted by Güzel et al. (2023), the potential causes of communication failure during cargo operations and proposed countermeasures are discussed in detail. Although the research was conducted on the cargo operations of a cargo ship, the variables identified as affecting communication problems in cargo operations, including poor listening skills, insufficient knowledge of maritime English, and distraction and noise, are similar to those identified in the present study as root causes of communication problems. These include poor listening skills, poor SMCP knowledge, poor linguistic ability, and background noise. The study revealed that language barriers constitute a significant communication barrier in the context of cargo operations. This finding aligns with the results of the Sensivity analysis conducted in current study.

As discussed above, several studies have focused on human factors in maritime accidents or operations and highlighted the impact of communication issues (Chauvin et al., 2013; Barić et al., 2018; Yıldırım et al., 2019, Güzel et al., 2023). Marine accident reports highlight the significance of bridge team communication, which is the primary element of Bridge Resource Management (UK Chamber of Shipping, 2020). Based on the created model, the study's findings suggest that to prevent issues with communication, one should first address "Attitudinal and Behavioural barriers" that arise from working together. In terms of navigation safety, the effectiveness of communication within the bridge team is dependent upon a number of variables. The subject of monitoring and evaluation methods for these variables may be a future area of research.

Conclusion

Hybrid fuzzy Bayesian networks are an exceptionally beneficial method for identifying and evaluating the causes of marine accidents. This study explored the communication issues faced by 92 bridge team members, reviewed accident investigation reports, and sought expert opinion. The study examined ship bridge communication barriers, identifying 17 root causes. These root causes were grouped into four intermediate nodes: "Attitudinal & behavioural barriers," "Language barriers," "Environmental barriers," and "Cultural

barriers." The model results revealed that the node with the greatest impact on bridge communication was the "Attitudinal & behavioural barriers" node. It is evident that addressing the key communication barriers identified in this study can significantly reduce the risk of accidents, and therefore should be a priority for all those concerned with safety of navigation.

Upon examination of the underlying factors contributing to the mentioned node (attitudinal and behavioural barriers), it became evident that the node pertaining to age differences exhibited a notable pattern. In this regard, the findings indicated that a significant age discrepancy between the members of the bridge team could potentially impede the flow of communication. This aspect should be recommended and taken into account during the personnel planning stage.

In addition, the following practical implications and suggestions can be drawn from the present study: Firstly, it is recommended that the communication and collaboration skills of the bridge team be evaluated on a regular basis. Prior research has indicated that the flow of information on the bridge can be quantified, with the development of a specific index for this purpose (John et al., 2013). In the event that professionals deem these skills to be inadequate, companies should provide training in communication skills to facilitate development. It is recommended that the obligation to adhere strictly to the standardized communication protocols defined under the SMCP be included in the duties and responsibilities section of the International Safety Management (ISM) manuals of maritime companies. It would be beneficial to implement refresher training programs for both the rating and the officers on this subject.

Secondly, crew satisfaction should be reviewed on a regular basis and its influence on retention rates monitored. It would be prudent to enhance the social facilities on board in order to enhance satisfaction among crew members. It would also be beneficial to prioritize social associations in order to facilitate enhanced communication between the crew.

Thirdly, in light of the considerable diversity of cultural backgrounds among the personnel involved in maritime shipping, it is recommended that shipping companies implement culturally sensitive educational programs designed to foster awareness of cultural differences.

Finally, it is also recommended that companies provide easily accessible psychological support, which can assist in reducing communication barriers by ensuring the crew's emotional well-being. While these decisions fall under the purview of the company's Safety Management System (SMS) and the company's overall safety culture, issues with communication should be addressed in depth, particularly during officer training, to promote good communication.

The generalisability of the results of this study is contingent upon certain limitations. For instance, the study employed expert evaluations as a data source. It is possible that evaluations conducted by experts working on diverse types of ships and engaged in disparate tasks may yield disparate results. In this direction, it is recommended that future studies employ different methods and engage experts working on different types of ships. It is postulated that a bridge communication problem study focusing on ships with a demanding work schedule, particularly chemical tankers and container ships, may yield more divergent results in terms of findings. Additionally, Further research could also examine the potential application of the fuzzy Bayesian network model to other maritime operations where effective communication is of paramount importance, such as cargo and mooring operations.

Compliance With Ethical Standards

Authors' Contributions

İT: Conceptualization, Visualization, Data curation, Formal analysis, Methodology, Investigation, Writing – original draft, Writing – review & editing,

OBÖ: Data curation, Methodology, Writing – review & editing

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

This study was approved by the Social and Human Sciences Ethics Committee of Recep Tayyip Erdoğan University (Ethics approval number: 2023/026, Date: 25/01/2023).

Funding

Not applicable.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Mar. Sci. Tech. Bull. (2024) 13(3): 215-224
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1524081

Marine Science and Technology Bulletin

https://prensip.gen.tr/

RESEARCH ARTICLE

Composition of bivalve community in the coastal waters (0-4 m) of the Çanakkale Strait along with various environmental variables

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ARTICLE INFO

Article History:
Received: 29.07.2024
Received in revised form: 11.09.2024
Accepted: 12.09.2024
Available online: 30.09.2024

Keywords:
Bivalvia
Community structure
Environmental variables
Çanakkale Strait

ABSTRACT

The present study focused on the soft bottom of the coastal waters (0-4 m) in the Çanakkale Strait. The objective was to determine the composition of bivalve species in the area. Sediment samples were collected using a 30×30 cm quadrat system by a SCUBA diver at eight sites between July 2008 and April 2009. The study recorded a total of 2299 individuals belonging to 55 species. The most abundant species in the study area was *Lucinoma borealis*. A significant positive correlation was found between seawater salinity and species diversity (r_s = 0.59; p<0.05). The highest similarity in species diversity between seasons occurred between autumn and summer.

Please cite this paper as follows:

Yazıcı, H., Ateş, A. S., & Acar, S. (2024). Composition of bivalve community in the coastal waters (0-4 m) of the Çanakkale Strait along with various environmental variables. *Marine Science and Technology Bulletin*, *13*(3), 215-224. https://doi.org/10.33714/masteb.1524081

Introduction

The effects of global warming and anthropogenic impacts are increasingly evident in the world's seas and oceans. As a result, macrozoobenthic communities are adapting to current environmental conditions, leading to notable changes in species composition and distribution patterns within these communities. Some communities exhibit greater resilience to environmental impacts (López-Alonso et al., 2022).

The diversity of macrozoobenthos is influenced by various environmental variables, such as sediment particle size, pH, temperature, and nutrient levels. Changes in these factors can significantly impact macrozoobenthic communities, potentially causing substantial harm (Templado, 2011). Spatial and temporal variations in marine biodiversity often mirror the condition of the benthic ecosystem and its response to various disturbances. Species diversity plays a crucial role not only in the establishment of marine protected areas, but also in

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monitoring the impacts of human activities (Rufino et al., 2008). The species composition of marine benthic communities varies with depth, latitude, and longitude (Nybakken, 1998). While these factors are not environmental variables themselves, they often serve as primary structuring factors for communities. However, environmental factors such as sediment type, water temperature, and oxygen levels are closely associated with depth and geographical location (Rufino et al., 2008).

The predominant environmental factor influencing the distribution of marine benthic communities is sediment type. This factor is especially critical in the distribution patterns of bivalves, as demonstrated in studies by Guerra-García & García-Gomez (2004) and Van Hoey et al. (2004). The main factors influencing macrofauna abundance and diversity on soft sea bottoms are tidal strength and sediment type. Marine benthic organisms, particularly those involved in sediment morphodynamics, experience a highly dynamic environment (Magni et al., 2006). It is widely acknowledged that the activities of benthic communities influence seafloor sediments and thereby impact community structure. Previous studies have primarily linked species richness and abundance of macrobenthos to organic matter content and sediment grain size. Bivalves represent a dominant component of the biomass of macrozoobenthic communities found in shallow waters globally. They also play a significant role as bioturbators in coastal habitats (Jaramillo et al., 2008). Bivalves, a significant component of the benthic fauna, were selected for investigation into the correlation between diversity, sediment, and environmental variables (Compton et al., 2008).

In the context of bivalve community studies within the Turkish Straits System, Albayrak (2003) conducted research on bivalves along the shores of the Bosphorus. Subsequently, Albayrak et al. (2004) investigated the bivalve fauna and their ecology in the Sea of Marmara. Although studies in the Çanakkale Strait, another part of the Turkish Straits System, are limited, Palaz & Berber (2005) studied bivalve species in the infralittoral zone of the Çanakkale Strait. More recently, Aslan-Cihangir & Ovalis (2013) explored the ecology and zoogeographic distribution of molluscs, including bivalves, in the Çanakkale Strait. A comprehensive study on bivalve distribution in the Çanakkale Strait has yet to be conducted this study aims to elucidate the seasonal, spatial, and depth-dependent distribution of bivalve species about environmental variables.

Material and Methods

Sediment Samplings

For this study, 8 stations were selected in the Çanakkale Strait (Figure 1). Benthic samples were collected along 3 transects at depths ranging from 0 to 4 meters. Sampling was conducted in July and November 2008, and in February and April 2009, using a SCUBA diver equipped with a 30×30 cm quadrat system. Environmental variables of seawater (salinity, temperature, pH, and dissolved oxygen saturation) were measured in situ using a YSI 556 model MPS. The collected material was preserved in 4% neutralized formaldehyde in 5-liter plastic drums. In the laboratory, sediment samples were washed under pressure using a triple sieve system with mesh sizes of 0.5, 1, and 2 mm. Bivalve material retained on the sieves was subsequently fixed in 70% ethanol in 50 mL tubes.

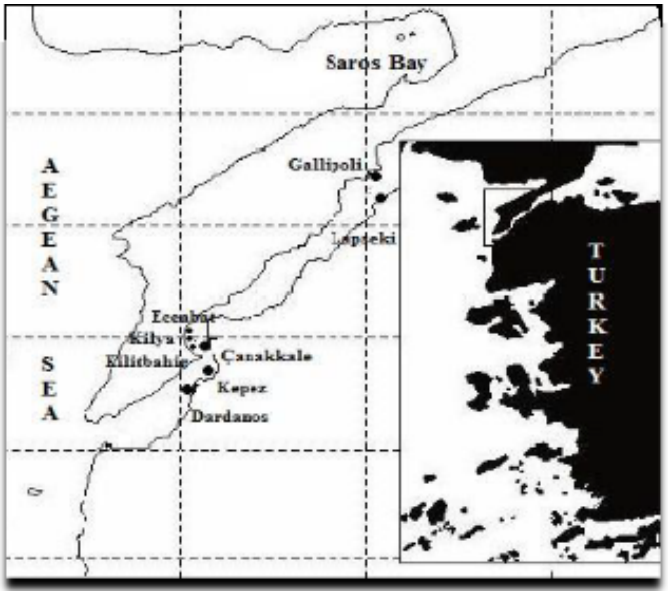

Figure 1. Map showing sampling locations in the Çanakkale Strait. St. 1: Gelibolu, St. 2: Lâpseki, St. 3: Çanakkale, St. 4: Kilya Cove, St. 5: Eceabat, St. 6: Kilitbahir, St. 7: Kepez Port, St. 8: Dardanos.

Granulometric Analysis

A sediment core containing 393 cm^3 of acrylic material was used for particle size analysis of the soft bottom at the sampling sites. A total of 32 samples were collected for analysis from the soft sediments at each sampling point during each sampling period. Particle size analysis of the sediment followed the methodology described by Allen (1997).

Faunistic Analysis

Bivalve specimens were identified using a trinocular stereomicroscope based on the taxonomic descriptions provided by Doğan (2005) and Öztürk et al. (2008). The dominance index (di%) formulated by Bellan-Santini (1969) was employed to calculate bivalve dominance. The correlations between environmental variables and bivalve abundance were assessed using Spearman's rank correlation coefficient (rs) in the PAST 4.02 software. The ecological quality of the study area was determined using the Shannon-Wiener Diversity Index (H′) as described by Shannon & Weaver (1949).

Statistical Analysis

The relationships between several environmental variables, species richness, and abundance were analyzed using Spearman's rank correlation (r_s). The relationship between environmental variables and the number of individuals was determined using a Principal Component Analysis (PCA). The relationship between the dominant species and the environmental variables was determined using a Canonical Correspondence Analysis (CCA). All statistical procedures were executed using SPSS 25.0 software and PAST 4.03 with a significance level set at 95% confidence.

Results

Faunistic Data

A total of 55 species and 2299 individuals belonging to the bivalves were recorded from samples collected in the soft bottoms at depths between 0 and 4 m across 8 different stations in the Çanakkale Strait (Table 1).

In the study, the most common species is *Lucinoma borealis*, representing 13.79% of the total population. This species is followed by *Parvicardium exiguum*, with a dominance value of 12.22%. The species with the lowest number (1 individual, %b=0.04) are *Modiolarca subpicta*, *Mimachlamys varia*, and *Mactra stultorum*. The Shannon-Weaver Diversity Index (H′) values for the seasons were calculated, with the highest H′ value observed in the fall at 3.08. The spring had the lowest H′ value, at 2.49. Overall, the diversity index values for the seasons are generally low. The highest H′ value was recorded at station 7 (H′ = 3.01), while the lowest H′ values were recorded at stations 2 and 4 (H′=1.95).

Water Quality

The environmental variable values of seawater in the research area are presented in Table 2. According to this table, the station with the highest oxygen value (9.79 mg L^{-1}) is station 3 in April 2008, while the station with the lowest oxygen value (3.68 mg L^{-1}) is station 2 in July 2008. The annual mean oxygen concentration in the study area is 7.13±0.59 mg L^{-1}. The highest salinity value (30.5‰) was measured at station 8 in November 2008, while the station with the lowest salinity concentration (23.6‰) was station 2 in April 2009. The highest annual average dissolved oxygen and salinity values were recorded in February 2009 (DO=8.81 mg L^{-1}, salinity=27.61‰), while the lowest were observed in July 2008 (DO=5.76 mg L^{-1}, salinity=23.67‰) (Table 2).

Sediment Structure

A particle size analysis was performed on the samples obtained from the study area. The weighted average particle diameter of the samples was 1027 µm for station 3, 1046 µm for station 7, 342 µm for station 2, 235 µm for station 8, 636 µm for station 6, 364 µm for station 5, 465 µm for station 4, and 437 µm for station 1 (Table 3).

Correlations With Environmental Variables of Species Richness and Abundance

The relationships between several environmental variables, species richness, and abundance were analyzed using Spearman's rank correlation (r_s). The results indicated a moderate negative correlation (r_s =-0.72; p<0.05) between the annual mean temperature values measured at the stations and the number of species. Conversely, there was a moderate positive correlation between annual mean salinity values and the number of species (r_s = 0.59; p<0.05). A moderate positive correlation (r_s = 0.55; p<0.05) was observed between annual pH values and bivalve abundance. Spearman's rank correlation revealed a moderate positive correlation (r_s = 0.46; p<0.05) between the calculated sediment particle size values and the number of species. Conversely, a weak negative correlation (r_s=-0.17; p<0.05) was observed between sediment particle size and bivalve abundance (Table 4, Figure 2). Sediment particle size percentages were calculated for each station and are presented in Table 5. Here, the highest number of species was found on the bottoms with a sand content of approximately 83%, whereas the lowest number of species was observed on the bottoms with a higher sand content. No consistent relationship was observed between sediment particle size and species richness.

Table 1. The total number of individuals (Σ), occurrence frequency (%f), and dominance (%b) values of the bivalve species found in the study area

Species	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7	St. 8	Σ	f %	di %
	SM	*S*	*CSE*	*Si*	*SZ*	*SM*	*CSM*	*SZ*			
Abra alba (Wood W., 1802)	68	2	2	2	5	4	0	4	87	87.5	3.78
Abra prismatica (Montagu, 1808)	5	8	0	2	2	0	2	0	19	62.5	0.83
Acanthocardia paucicosta (Linnaeus, 1758)	2	0	0	0	0	14	0	0	16	25	0.7
Aequipecten opercularis (Linnaeus, 1758)	22	4	1	0	0	4	1	4	36	75	1.57
Anadara corbuloides (Monterosato,1880)	0	0	0	0	0	4	0	0	4	25	0.17
Anodontia fragilis (Philippi, 1836)	9	2	0	0	3	1	2	0	17	62.5	0.74
Arcopagia balaustina (Linnaeus, 1758)	0	0	2	0	0	0	0	0	2	12.5	0.09
Astarte sulcata (Da Costa, 1778)	0	2	3	4	1	21	4	1	36	87.5	1.57
Callista chione (Linnaeus, 1758)	1	2	2	0	0	9	0	5	19	62.5	0.83
Cardites antiquatus (Linnaeus, 1758)	1	0	0	0	0	0	1	4	6	37.5	0.26
Chamelea gallina (Linnaeus, 1758)	3	0	2	7	2	15	4	6	39	87.5	1.7
Clausinella fasciata (da Costa, 1778)	2	2	3	9	3	6	4	2	31	100	1.35
Centrocardita aculeata (Poli, 1795)	0	0	0	0	0	0	1	12	13	25	0.57
Ctena decussata (Costa O. G., 1829)	6	2	4	1	69	22	6	9	119	100	5.18
Donacilla cornea (Poli, 1795)	1	0	0	0	0	0	3	1	5	37.5	0.22
Donax trunculus Linnaeus, 1758	0	0	0	2	0	0	0	1	3	25	0.13
Flexopecten flexuosus (Poli, 1795)	13	0	0	0	0	2	2	1	18	50	0.78
Flexopecten glaber (Linnaeus, 1758)	2	0	0	0	0	0	0	1	3	25	0.13
Gari costulata (Turton, 1822)	4	0	1	2	0	4	2	0	13	62.5	0.57
Gastrochaena dubia (Pennant, 1777)	0	0	0	0	0	150	6	0	156	25	6.79
Gouldia minima (Montagu, 1803)	2	1	4	0	0	23	3	4	37	75	1.61
Irus irus (Linnaeus, 1758)	1	0	0	0	0	9	0	0	10	25	0.43
Kellia suborbicularis (Montagu, 1803)	2	57	17	8	10	20	28	0	142	87.5	6.18
Kurtiella bidentata (Montagu, 1803)	0	0	0	1	1	0	0	0	2	25	0.09
Limaria loscombi (Sowerby G. B. I, 1824)	0	0	0	0	0	2	2	0	4	25	0.17
Loripes lacteus (Lamarck, 1818)	73	51	25	0	22	37	13	8	229	100	9.96
Lucinella divaricata (Linnaeus, 1758)	39	1	3	5	32	13	5	2	100	100	4.35
Lucinoma borealis (Linnaeus, 1767)	132	90	9	7	27	2	14	36	317	100	13.79
Mactra stultorum (Linnaeus, 1758)	0	0	0	1	0	0	0	0	1	12.5	0.04
Mimachlamys varia (Linnaeus, 1758)	0	0	0	0	0	1	0	0	1	12.5	0.04
Modiolarca subpicta (Cantraine, 1835)	0	1	0	0	0	0	0	0	1	12.5	0.04
Modiolula phaseolina (Philippi, 1844)	0	0	0	0	0	2	0	0	2	12.5	0.09
Modiolus barbatus (Linnaeus, 1758)	0	0	0	0	0	0	6	0	6	12.5	0.26
Mytilaster lineatus (Gmelin, 1791)	0	1	7	1	1	8	6	0	24	75	1.04
Mytilus galloprovincialis Lamarck, 1819	2	13	2	3	21	5	4	0	50	87.5	2.17
Nucula nitidosa Winckworth, 1930	0	0	0	0	0	2	0	0	2	12.5	0.09
Nucula nucleus (Linnaeus, 1758)	3	0	0	1	0	1	0	2	7	50	0.3
Nucula sulcata Bronn, 1831	0	0	0	0	0	0	2	0	2	12.5	0.09
Papillicardium papillosum (Poli, 1795)	10	0	0	2	0	5	5	12	34	62.5	1.48
Pecten jacobeus (Linnaeus, 1758)	1	0	0	0	0	0	2	2	5	37.5	0.22
Pitar rudis (Poli, 1795)	6	2	1	1	0	17	7	7	41	87.5	1.78
Polititapes aureus (Gmelin, 1791)	2	0	2	0	0	5	4	1	14	62.5	0.61
Solen marginatus Pulteney, 1799	0	0	2	0	0	0	0	0	2	12.5	0.09
Striarca lactea (Linnaeus, 1758)	0	0	0	3	0	8	3	1	15	50	0.65
Talochlamys multistriata (Poli, 1795)	0	0	0	0	0	2	0	1	3	25	0.13
Tellina pulchella Lamarck, 1818	2	0	2	0	0	4	0	2	10	50	0.43
Tellina tenuis Da Costa, 1778	0	0	2	0	6	0	4	0	12	37.5	0.52
Thracia phaseolina (Lamarck, 1818)	1	0	1	0	0	5	0	0	7	37.5	0.3
Venus verrucosa Linnaeus, 1758	0	0	2	0	0	6	0	0	8	25	0.35

Note: St: Station, SM: Sand with *Mytilus galloprovincialis*, S: Sand, CSE: Coarse sand with *Enteromorpha linza*, Si: Silt, SZ: Sand with *Zostera marina*, CSM: Coarse sand with *Mytilus galloprovincialis*.

Table 2. Seasonal measurements of environmental variable values at the sampling stations

Sampling period	Stations	Variables			
		O_2	T	S	pH
July 2008	St. 1	5.58	25.03	22.8	8.33
	St. 2	3.68	24.57	22.6	8.15
	St. 3	4.19	23.7	23.3	8.21
	St. 4	8.46	26.77	23.1	8.53
	St. 5	7.4	25.6	22.9	8.39
	St. 6	5.16	25.1	23.1	8.31
	St. 7	5.14	24.39	23.5	8.3
	St. 8	6.49	24.36	28.1	8.44
November 2008	St. 1	5.56	16.17	25.5	8.51
	St. 2	3.34	15.7	24.6	8.25
	St. 3	5	15.25	25.6	8.32
	St. 4	5.9	16.3	25.7	8.55
	St. 5	6.01	16.01	25.5	8.46
	St. 6	5.68	16.37	25.6	8.33
	St. 7	5.28	16.22	26.1	8.45
	St. 8	5.83	16.07	30.5	8.7
February 2009	St. 1	9.61	8.87	27.6	7.48
	St. 2	9.65	9.31	27.4	6.4
	St. 3	9.63	9.18	27.8	5.3
	St. 4	9.25	9.24	26.5	7.55
	St. 5	9.56	9.24	27.4	8.09
	St. 6	9.2	9.12	27.6	8.79
	St. 7	5.68	9.65	28.3	5.44
	St. 8	7.94	9.61	28.3	5.13
April 2009	St. 1	8.13	13.1	24.3	6.5
	St. 2	8.72	13.68	23.6	6.85
	St. 3	9.79	14.26	24.4	7.07
	St. 4	7.95	13.5	23.3	6.48
	St. 5	8.9	13.31	24.2	6.52
	St. 6	8.9	13.23	24.3	7.05
	St. 7	8.65	14.1	24.8	6.74
	St. 8	8.04	15.75	28.6	6.88

Note: O_2: Dissolved oxygen (mg L^{-1}), T: Temperature (°C), S: Salinity (‰)

Table 3. Annual average values of several environment variables at the stations

Stations	PS (µm)	DO (mgL^{-1})	S (‰)	T (°C)	pH
St. 1	437±50	7.89±1.23	24.65±1.47	16.45±6.46	7.71±0.92
St. 2	342±83	7.96±1.37	25±1.66	16.04±6.02	7.41±0.93
St. 3	1027±198	7.24±1.82	25.15±1.66	15.95±5.87	7.23±1.40
St. 4	465±346	7.22±1.73	25.05±1.75	15.79±5.92	7.78±0.98
St. 5	364±74	6.34±2.85	24.55±1.79	15.81±5.55	7.87±0.91
St. 6	636±133	7.13±2.57	25.27±1.66	15.59±5.21	8.12±0.75
St. 7	1046±74	6.18±1.43	25.67±1.77	16.09±5.34	7.23±1.42
St. 8	235±390	7.07±0.94	28.87±0.95	16.44±5.24	7.29±1.65

Note: PS: Particle size, DO: Dissolved oxygen, S: Salinity, T: Temperature.

Table 4. Spearman correlation values (r_s; $p<0.05$) between various environmental variables, species richness, and abundance (ind. 0.09 m^{-2})

	PS (µm)	T (°C)	S (‰)	O_2 (mg L^{-1})	pH
Number of species	0.46	—0.72	0.59	—0.26	—0.42
Abundance (ind. 0.09 m^{-2})	—0.17	—0.30	—0.36	0.12	0.55

Note: PS: Particle size, O_2: Dissolved oxygen, S: Salinity, T: Temperature.

Table 5. Particle size, species number, and abundance values for the stations

Station	Particle grain size (%)				
	Gravel	***Sand***	***Silt***	***SN***	***A***
St. 1	6.3	93.22	0.44	33	655
St. 2	2.68	97.23	0.09	22	256
St. 3	10.19	89.76	0.05	27	111
St. 4	8.15	91.64	0.19	23	160
St. 5	0.15	99.59	0.26	19	233
St. 6	16.75	83.11	0.13	40	512
St. 7	26.96	73.01	0.04	33	212
St. 8	0.32	98.91	0.77	31	160

Note: SN: Species number, A: Abundance (ind. 0.09 m^{-2})

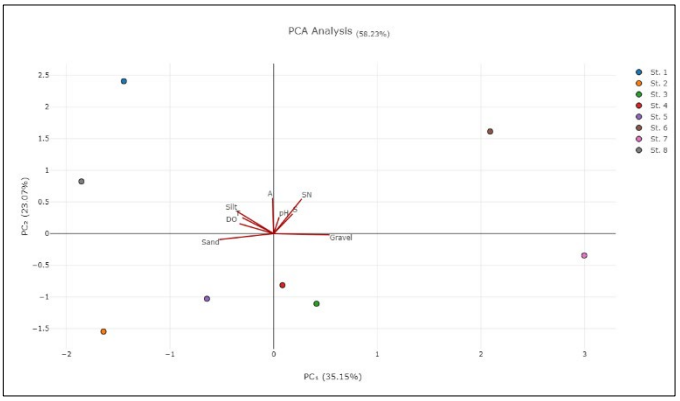

Figure 2. PCA ordination diagram of the spatial mean environmental variables, abundance and species number

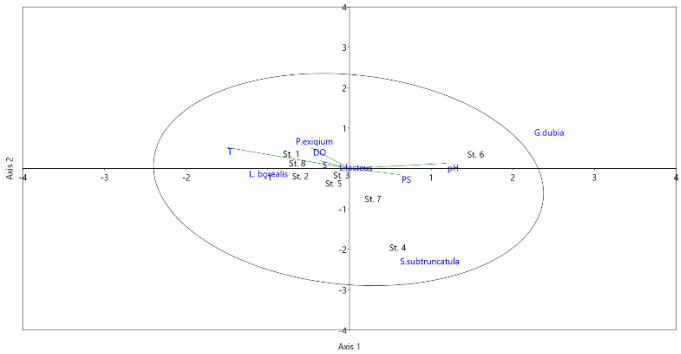

Figure 3. Canonical correlation analysis (CCA) between dominant species and environmental variables

Principal component analysis (PCA) was conducted to investigate the relationship between environmental variables and the species and individual counts of bivalves. The analysis accounts for 58.23% of the model's variance. According to the findings, salinity predominantly influences species richness, whereas pH emerges as the primary environmental determinant affecting the distribution of bivalve individuals. Canonical correspondence analysis (CCA) was conducted to assess the relationship between the most abundant species and environmental variables. Temperature was found to significantly influence the distribution of *Lucinoma borealis*, whereas dissolved oxygen (DO) played a crucial role in the distribution of *Parvicardium exiguum*. *Loripes lacteus* was associated with salinity, while *Spisula subtruncatula* showed correlation with particle size (Figure 3).

Discussion

Significant studies on bivalves in the Çanakkale Strait were conducted by Colombo (1885), Marion (1898), and Pallary (1917). Palaz & Berber (2005) subsequently reported 28 bivalve species from the Çanakkale Strait. More recently, Aslan-Cihangir & Ovalis (2013) documented a total of 80 bivalve species at depths ranging from 10 to 83 meters in the Çanakkale Strait. In their study, Aslan-Cihangir & Ovalis (2013) identified several species (*Nucula sulcata, Nucula nitidosa, Mytilaster lineatus, Anodontia fragilis, Lucinella divericata, Kellia suborbicularis, Kurtiella bidentata, Parvicardium scabrum, Tellina tenuis, Tellina donacina, Arcopagia balaustina, Abra prismatica*) as new records for the Çanakkale Strait. These newly recorded species had also been identified during our study conducted between 2008 and 2009.

A detailed study on the bivalve molluscs of the Turkish Aegean coasts was carried out by Doğan (2005). Doğan (2005) reported *Modiolarca subpicta* as the most dominant species in the coastal waters of the Aegean Sea, Türkiye, with a dominance of 7.57%. This study also identified a single specimen of *M. subpicta*. According to Doğan (2005), *Parvicardium exiguum* was the sixth most common species, with a relative abundance of 3.5%. Contrary, in the present study, *P. exiguum* was

recorded as the second most prevalent species, with a relative abundance of 12.22%. The same author noted the highest species richness at stations with sandy bottoms. The species with the highest occurrence frequencies were *Papillicardium papillosum* (66.7%) and *Pitar rudis* (53.33%). Similarly, in this study, *P. rudis* and *P. papillosum* were commonly found in sandy bottoms, with frequencies of 87.5% and 62.5%, respectively.

In Greek waters of the Aegean Sea, Evagelopoulus & Koutsoubas (2008) identified *Loripes lacteus* as the most dominant species on sandy bottoms in shallow waters (0.2–0.5 m) off the coast of Lesvos Island, in the northeastern Aegean Sea, close to the Turkish coast. They reported a salinity range of 33-45‰ for this species. In contrast, salinity values measured in this study ranged between 22‰ and 30‰ annually. This suggests that *L. lacteus* exhibits a relatively wide salinity tolerance. In the western Mediterranean, Urra et al. (2011) found *Spisula subtruncata* to be the most abundant species throughout the year in shallow waters (5 m) of the Alboran Sea. In this study, *S. subtruncata* was most abundant (89 ind. 0.09 m^{-2}) on silt bottoms.

Albayrak et al. (2004) reported the presence of *Parvicardium exiguum* on sandy bottoms at a depth of 1 m, along with *L. lacteus* in sandy, muddy, and gravelly bottoms at the same depth. The same authors also recorded *S. subtruncata* at depths ranging from 1 to 42 m, with water temperatures between 14.8 and 23.4°C and salinity ranging from 19.9 to 37.5 ‰. Papazacharias et al. (1998) also reported *L. lacteus* as the dominant species on silt bottoms along the coast of Kavala.

With regard to the question of family dominance, Doğan (2005) reported Cardiidae and Veneridae as the most dominant families, comprising 9.09% of the dominance on the Aegean Sea coasts. Similarly, in this study, Veneridae was the most dominant family, accounting for 19.09% dominance. A comprehensive study was conducted by Mutlu & Ergev (2012) to observe the variability in bivalve abundance in shallow soft-bottom habitats across different depths and seasons in neritic waters (10-200 m) of the eastern Mediterranean. They identified *Corbula giba* and *Abra alba* as dominant species in sandy substrates of shallow waters up to 25 m depth. Melis et al. (2015) documented high bivalve species diversity at depths ranging from 1 to 12 m off the coast of Mersin (Elaiussa Sebaste) in their archaeological study. They highlighted *L. lacteus* and *Venericardia antiquata* as predominant species specifically at depths between 7 and 11 m. In this study, conducted in shallow waters down to 4 m depth, *Lucinoma borealis* was identified as the commonest species.

Based on statistical data, no significant relationship was found between temperature and species richness, whereas salinity appears to be a significant factor in species distribution. Analysing the relationships between species and individual numbers with environmental variables, a weak negative correlation was observed between annual mean temperature and the number of individuals, a similar weak negative correlation between salinity values and the number of individuals, a moderate positive correlation between pH values and the number of individuals, and a weak positive correlation between average oxygen concentrations and the number of individuals. Changes in pH and dissolved oxygen values from the obtained data exhibit stronger associations with species and individual distributions compared to other variables. Papazacharias et al. (1998) reported *Loripes lacteus* as dominant in the fine sand and silt substrates of Kavala Bay (northern Aegean Sea), with sediment grain sizes ranging from 4 to 1741 µm. In the Çanakkale Strait, which serves as the transition zone between the northern Aegean Sea and the Sea of Marmara, Meriç et al. (2009) reported an average silt content of 46%, a clay content of 9%, and a mud content of 27%. Additionally, they observed bivalve communities in sandy sediments, with distribution ranging from 0 to 9.7%. In this study, granulometric analyses were conducted to assess sediment structure, as sand content plays a crucial role in determining community structure. The sand, mud, and clay contents at the stations were recorded. A moderately positive correlation was found between particle size and species richness, while a weak correlation was observed between particle size and abundance. The station with the highest sand content was identified as Eceabat, with a value of 99.59%, while Kepez Port had the lowest sand content at 73.01%. Accordingly, Eceabat exhibited 19 species and 233 individuals, whereas Kepez Port had 33 species and 212 individuals. Overall, strong currents influenced by prevailing northerly, northwesterly, and southerly winds significantly impact the seabed structure in the study area.

Two environmental variables, sediment particle size and seawater salinity, moderately influence the species richness of bivalve species in the soft substrate of the shallow waters of the Çanakkale Strait. Sediment particle size directly affects bivalve feeding, filtration, and habitat preferences, as bivalve thrive in soft substrates enriched with small particles that offer abundant nutrients (Alexander et al., 1993). Optimal salinity levels are crucial for bivalve populations, as extremes can negatively

impact their abundance. Understanding these factors is essential for biodiversity and habitat management in ecosystems like the Çanakkale Strait, informing conservation strategies and ecosystem management practices.

About to the study area's biodiversity, we found low values for the diversity index (H) and these values range from 1.95 to 3.01. However, according to Simboura & Zenetos (2002), the study area is characterized by low diversity. In the study conducted by Albayrak et al. (2007) in Edremit Gulf, H' values ranged from 2.5 to 5. The lower index values recorded in this study may be attributed to the irregularity of the environmental variables in the study area or the fact that some environmental variable values are lower than those measured in Edremit Bay.

Conclusion

Consequently, efforts were made to contribute to bivalve communities in the coastal waters of Çanakkale Strait. The community structure of bivalve populations on the soft bottoms of these coastal waters, an integral part of the Turkish Straits System, was examined. Based on the data obtained, Çanakkale Strait experiences strong current systems, leading to continuous changes in its seabed structure. These powerful waves and currents contribute to a dynamic alteration in the benthic fauna of the area. This study focused on the shallow waters of the strait ecosystem. Future comprehensive studies conducted at various depths could offer more detailed insights into the community structures formed by species distributed throughout Çanakkale Strait.

Compliance With Ethical Standards

Authors' Contributions

HY: Investigation, Methodology, Writing – review & editing

ASA: Writing – original draft, Writing – review & editing, Supervision

SA: Formal Analysis, Writing – original draft, Writing – review & editing

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

The material of this study was obtained by the financial support of Scientific and Technological Research Council of Türkiye CAYDAG 107Y332 code Project.This study is a part of MSc thesis of the first author.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

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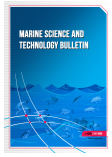
Mar. Sci. Tech. Bull. (2024) 13(3): 225-233
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1515193



Marine Science and Technology Bulletin

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RESEARCH ARTICLE

Temporal volatility of Brent oil prices and interrelation with maritime traffic density in the Turkish Straits during COVID-19 crisis

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ARTICLE INFO

Article History:
Received: 12.07.2024
Received in revised form: 14.09.2024
Accepted: 17.09.2024
Available online: 30.09.2024

Keywords:
Brent petrol
Economic crisis
Marine transport
Pandemic
Shipping industry
Turkish straits

ABSTRACT

This research evaluated variations in Brent oil prices and the interrelation with maritime traffic density in the Turkish Straits during the COVID-19 pandemic. The number of commercial ships that made non-stop over passage through the Turkish Straits in the last 5 years, covering the COVID-19 -and post-pandemic periods with economic instabilities was investigated along with variables of vessel characteristics such as; gross tonnage, size and type of vessel loads. Results of the present study reveal that the maritime traffic density between 2019 and 2023, was influenced by the pandemic crisis, when harsh quarantine measures of lockdown and curfews in the first shock wave. In the aftermath, conflicts between Ukraine and Russia led to economic recession or upheaval with instabilities in Brent oil prices. For the period examined in this study, the number of non-stop over passage vessels and gross tonnages used the Turkish Straits were affected by the pandemic outbreak and Brent oil price variations. The number of vessels decreased by 5.22% from 84,871 to 80,440 during the epidemic in 2020, and by 5.38% from 43.342 to 42.340 during the global recession in 2022. Overall, the number of non-stop over passage vessels using the Turkish Straits between 2019 and 2023 declined by 1.15%, while the gross tonnage and ship length increased by 3.44% and 13.24%, respectively. In total, the number of non-specific tankers (TTA) and those carrying chemicals (TCH) increased by 2.92% and 10.97%, respectively, but a 13.25% decrease was noted for the liquefied petroleum gas (LPG) tankers over the 5 years. Considering that the world trade network is largely dependent on maritime transportation, identifying the changes in maritime transportation with the interrelation of Brent oil during global crises may provide important data for strategy building of best trade management with foresights to world economic crises.

Please cite this paper as follows:

Yiğit, Ü. (2024). Temporal volatility of Brent oil prices and interrelation with maritime traffic density in the Turkish Straits during COVID-19 crisis. *Marine Science and Technology Bulletin*, *13*(3), 225-233. https://doi.org/10.33714/masteb.1515193

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Introduction

Shipping, which forms the backbone of the world trade network, accounts for 80% of total commercial shipments. The capacity of the merchant fleet operating worldwide increased by approximately 43% between 2013 and 2021, reaching approximately 2.1 million deadweight tons in 2021 (Statista, 2024a). It has been seen that global crises affected maritime transportation. Especially during the lockdown period right after the start of the recent COVID-19 pandemic, the disruption of port operations due to the decrease in manpower has also negatively affected the shipping industry. The Covid pandemic, which emerged in early 2020, caused a decrease by approximately 4% in the world maritime trade (UNCTAD, 2021). Ports, which play an important role in maritime transport, connect the trade network between production centers and consumer markets at an international level. Any change in the maritime transport network, such as the closure of ports, the reduction of border crossings, etc., can cause trade disruptions in many countries, thus causing economic shocks. Interruptions in supply chains can cause economic bottlenecks not only regionally but also globally (Verschuur et al., 2022). This has been the case in 2020, when the countries closed borders and reduced human mobility to prevent the spread of the COVID-19 pandemic, that eventually went into a rapid global crisis with economic aftershocks and damage world-wide (March et al., 2021). The Turkish Straits, consisting of Istanbul Strait and the Çanakkale Strait, are important sea passages for international maritime traffic and have an important geo-strategic position. According to 2023 figures, the number of ships passing through the Turkish Straits was recorded at approximately 84,000 (MTI, 2024). In contrast, it is reported that 14,000 ships passed through the Panama Canal (Statista, 2024b) and 22,000 ships through the Suez Canals (Statista, 2024c) in 2022. While the Suez Canal, one of the important sea passage points in the world, is a junction point between Europe and Asia, the Turkish Straits system forms the borders connecting Europe and Asia with the Istanbul and Çanakkale Straits. As oil prices increase, transportation costs will also increase, leading to significant changes in the maritime transportation industry. Contrary to the stable course or even downward trend in oil prices in the 1980s and 1990s, oil prices have reached very high levels today. Until 2004, brent oil prices were relatively stable at an average of $40 per barrel and did not show sharp fluctuations. After 2004, however, prices began to increase sharply, rising above $120 per barrel following the global credit and economic crises that emerged in mid-2008 (UNCTAD, 2010). According to various forecast models made in previous years, it has been predicted that oil prices may vary from $60 per barrel to $160 by the 2020s (TEMS, 2008). It is stated that the high increase in oil prices may have a two-way effect on global economies: The first of these effects is that higher oil prices will reduce consumption and therefore reduce the economic growth rate. This will lead to a slowdown in economies around the world. On the other hand, as the increase in demand for goods and services slows down, the growth in demand for trade and transportation services will also slow down and the momentum of economic flow will decrease (TEMS, 2008).

Considering that the world trade network is largely dependent on maritime transportation, identifying the changes in maritime transportation during global crises may provide important data for maritime trade and management in times of possible future crises. Hence, this study investigated the impacts of brent oil price changes during COVID-19 pandemic period and the global economic recession followed thereafter.

Material and Methods

Study Area

The study covers the Istanbul and Çanakkale Straits, two internationally important waterways in the northwest of Türkiye. The Aegean and Mediterranean are connected to the Black Sea via the Straits. The Marmara Sea, located between the two straits, opens to the Aegean and Mediterranean through the Çanakkale Strait, and to the Black Sea via the Istanbul Strait, which are the borders between the European and Asian continents (Figure 1).

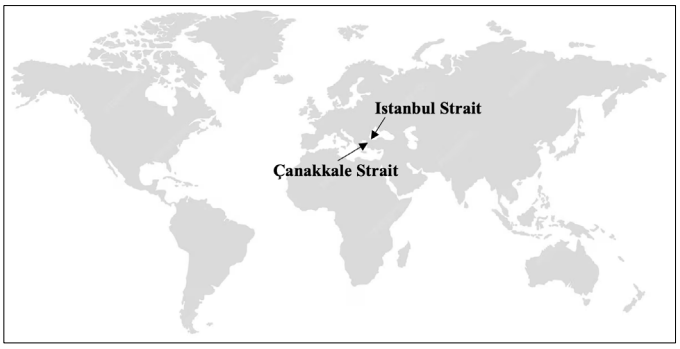

Figure 1. Study area with arrows pointing on locations of Istanbul and Çanakkale Straits

Maritime chokepoints are narrow water passages where the marine traffic is dense. Therefore, these chokepoints provide effective study locations for monitoring specific conditional variations in traffic densities. Among the World's important maritime chokepoints, the two straits of Istanbul and

Çanakkale were used for the monitoring of maritime traffic activities during and after the COVID-19 pandemic, along with possible interrelation of brent oil price variations.

Data Definition and Analyses

The interrelations between variables such as; number of ships, length, total gross tonnage and type of vessels with brent oil price variations were evaluated for the sample period from 2019 to 2023, covering the pandemic crisis, that triggered collapsing global economies in the aftermath with recession and upheaval.

The data evaluated for the variables for ship traffic density with variables of number, length, and total gross tonnage of non-stop over passage vessels through the Turkish Straits over the pandemic years, including the aftermath, have been collected from online statistical panels provided by the Turkish Ministry of Transport and Infrastructure (MTI, 2024). Percent variations for the investigated variables between 2019 and 2023 have been estimated using the following equations (Equations 1-4) as earlier described by Yigit & Kusku (2022), and Yiğit (2024):

$$\%PVsn = \frac{(sn(t)-sn(t-1))}{sn(t-1)} \times 100 \quad (1)$$

where *%PVsn*: percent variation in the number of ships transited; *sn(t)* and *sn(t-1)* are the number of ships transited at time *t* and *t-1*, respectively.

$$\%PVtgt = \frac{(tgt(t)-tgt(t-1))}{tgt(t-1)} \times 100 \quad (2)$$

where *%PVtgt:* percent variation in total gross tonnage of ships transited; *tgt(t)* and *tgt(t-1)* are the total gross tonnages of ships transited at time *t* and *t-1*, respectively (*Note: besides total gross tonnage, vessels lower than 500 GT (gross tonnage, LOA) transited have been assessed in a separate group, using the same equation above*).

$$\%PVloa = \frac{(loa(t)-loa(t-1))}{loa(t-1)} \times 100 \quad (3)$$

where *%PVloa*: percent variation in the LOA of ships transited; *loa(t)* and *loa(t-1)* are the LOA of ships transited at time *t* and *t-1*, respectively; LOA: length over all, vessels longer than 200 m have been assessed.

$$\%PVtt = \frac{(tt(t)-tt(t-1))}{tt(t-1)} \times 100 \quad (4)$$

where *%PVtt*: percent variation in the number of total Tankers transited; *tt(t)* and *tt(t-1)* are total tankers transited at time *t* and *t-1*, respectively.

Tankers transited through the straits have been grouped under 3 classes: (a) TTA (Non-Specific Tankers, indicating tankers of unspecified type), (b) TCH (tanker carrying chemical cargo), (c) LPG (liquefied petroleum gas), and the traffic density has been estimated by using the following equations (Equations 5-7):

$$\%PVtta = \frac{(tta(t)-tta(t-1))}{tta(t-1)} \times 100 \quad (5)$$

where *%PVtta*: percent variation in Non-Specific Tankers (TTA) transited; *tta(t)* and *tta(t-1)* are TTA tankers transited at time *t* and *t-1*, respectively.

$$\%PVtch = \frac{(tch(t)-tch(t-1))}{tch(t-1)} \times 100 \quad (6)$$

where *%PVtch*: percent variation in tankers carrying chemical cargo (TCH) transited; *tch(t)* and *tch(t-1)* are TCH tankers transited at time *t* and *t-1*, respectively.

$$\%PVlpg = \frac{(lpg(t)-lpg(t-1))}{lpg(t-1)} \times 100 \quad (7)$$

where *%PVlpg*: percent variation in Liquefied Petroleum Gas Tankers (LPG) transited; *lpg(t)* and *lpg(t-1)* are LPG tankers transited at time *t* and *t-1*, respectively.

Results

Findings of this study reveal that the maritime traffic density in the Turkish Straits was affected by the pandemic and economic trends of global recession or upheaval periods after the pandemic outbreak, with variations in brent oil prices. With the outbreak of COVID-19 pandemic early 2020, brent oil prices dropped from $64.30 to $41.96 per barrel with a collapse of 34.74% compared to the pre-pandemic year of 2019 (Figure 2). The total number of non-stop over passage vessels used the Turkish Straits also dropped from 84,871 to 80,440, showing a decline by 5.22% with the outbreak of the pandemic (Figure 3). This was also noted for the total gross tonnages, which was reduced from 1,511,206,284 to 1,478,603,748, representing a drop by 2.16% (Figure 4). The total number of non-stop over passage tankers used the Turkish Straits also dropped from 18.800 to 17.807, with a decline by 5,28% (Figure 5).

The transition of Non-Specific Tankers (TTA) and those carrying Liquefied Petroleum Gas (LPG) declined by 10.04% and 2.36%, respectively, whereas tankers carrying chemicals (TCH) however, increased transition through the Straits by 4.62% over the previous non-pandemic year of 2019 (Figures 6-8).

In the post-pandemic year of 2021, brent oil price increased from $41.96 to $70.86 per barrel, that was an increase by 68.88% (Figure 2). This was also a year of recovery period for the Maritime transportation density in the Turkish Straits when the number of non-stop over passage vessels increased from 80.440 to 81.893 (1.81% increase), with total gross tonnages from 1,478,603,748 to 1,530,393,894 (3.50% increase) in 2021 over the previous year (Figure 3, Figure 4).

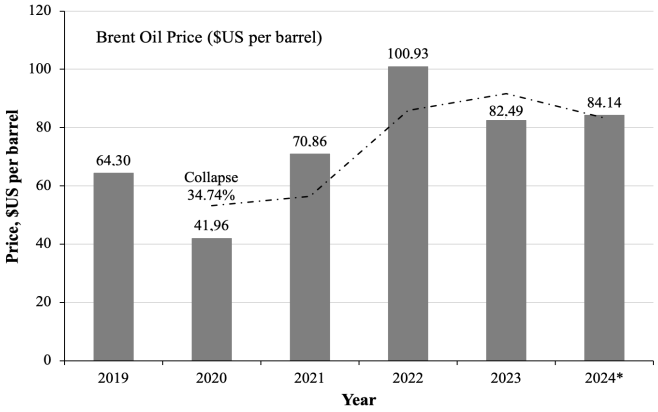

Figure 2. Variations of brent oil prices prior to and over the pandemic crisis and the aftermath from 2019 to 2024. Data for 2024*, as of May; dashed line: Moving average, 2 periods. 2019: pre-pandemic; 2020: pandemic year, 2021: post-pandemic; 2022-2023: global recession and upheaval.

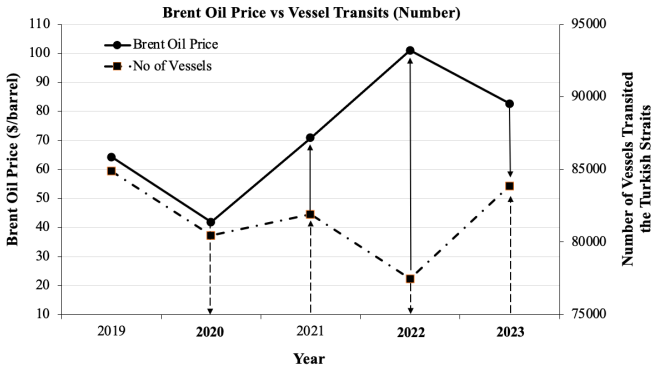

Figure 3. Superimposed figure for the interrelation of brent crude oil price versus number of non-stop over passage vessels used the Turkish Straits prior to and over the pandemic crisis and the aftermath from 2019 to 2023. Vertical arrow lines present drops and increases for brent oil prices (straight line) and non-stop over passage vessel (dashed line). Figure produced by statistical data provided by Statista (2024d) and MTI (2024).

The drop in the transition volume of TTA and LPG tankers continued their decreasing trend by 5.64% and 9.33%, respectively, during the post-pandemic year of 2021, with only increase of TCH tankers by 6.58% (Figures 6-8). However, the total non-stop over passage tankers used the Straits remained in a declining trend of 1.97% over the previous year (Figure 5).

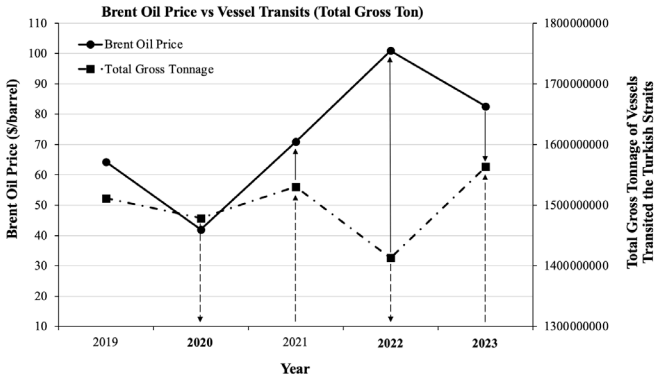

Figure 4. Explanatory overlay for the interrelation of brent crude oil price versus total gross tonnage of non-stop over passage vessels used the Turkish Straits prior to and over the pandemic crisis and the aftermath from 2019 to 2023. Vertical arrow lines present drops and increases for brent oil prices (straight line) and non-stop over passage vessel (dashed line). Figure produced by statistical data provided by Statista (2024d) and MTI (2024).

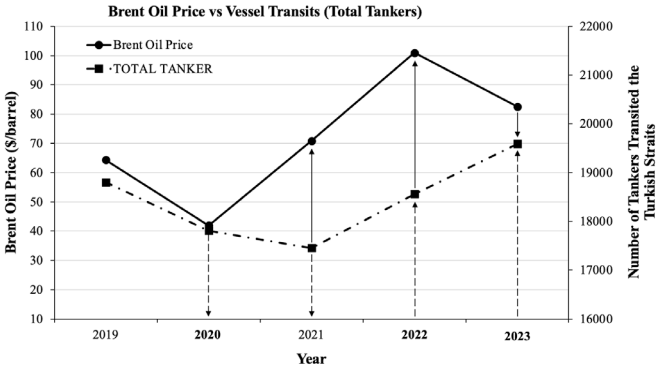

Figure 5. Explanatory overlay for the interrelation of brent crude oil price versus the number of non-stop over passage tankers used the Turkish straits prior to and over the pandemic crisis and the aftermath from 2019 to 2023. Vertical arrow lines present drops and increases for brent oil prices (straight line) and number of non-stop over passage tanker (dashed line). Figure produced by statistical data provided by Statista (2024d) and MTI (2024).

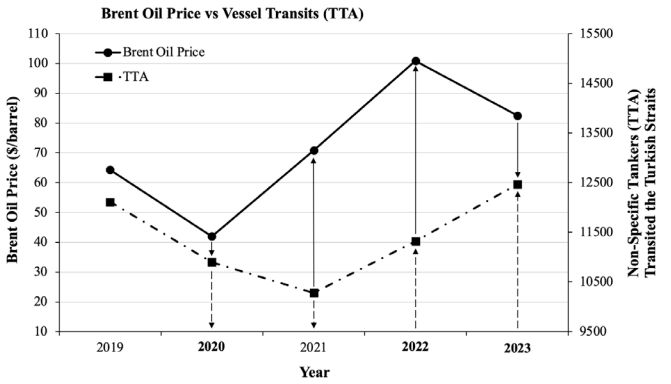

Figure 6. Explanatory overlay for the interrelation of brent crude oil price versus the number of Non-Specific Tankers (TTA) used the Turkish Straits prior to and over the pandemic crisis and the aftermath from 2019 to 2023. Vertical arrow lines present drops and increases for brent oil prices (straight line) and number of non-stop over passage TTA (dashed line). Figure produced by statistical data provided by Statista (2024d) and MTI (2024).

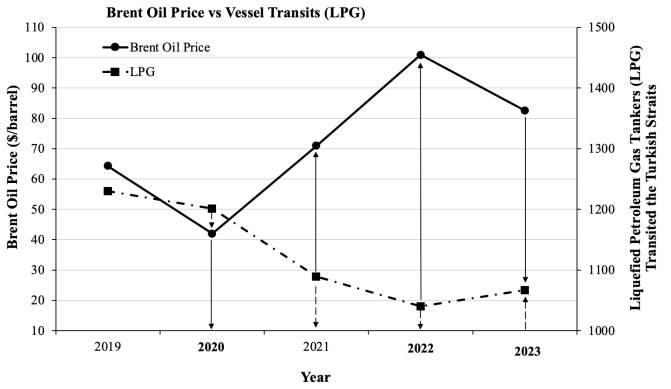

Figure 7. Explanatory overlay for the interrelation of brent crude oil price versus the number of tankers carrying Liquefied Petroleum Gas (LPG) used the Turkish straits prior to and over the pandemic crisis and the aftermath from 2019 to 2023. Vertical arrow lines present drops and increases for brent oil prices (straight line) and number of non-stop over passage LPG (dashed line). Figure produced by statistical data provided by Statista (2024d) and MTI (2024).

The aftermath of pandemic encountered a severe increase of brent oil price rate from $70.86 to $100.93 per barrel, that was an increase by 42.44% in the global recession year of 2022 compared to the previous year. The excessive price increases in brent oil resulted in another declining period for the vessel number from 81,893 to 77,486 (5.38% decline), and total gross tonnages from 1,530,393,894 to 1,413,066,367 (7.67% decline). The total number of non-stop over passage tankers used the Straits, however, increased from 17,456 to 18,557 (6.31% increase), with only declining rate of 4.50% for the LPG carriers over the previous year.

In the global upheaval period of 2023, however, the brent oil price dropped by 18.21% from its highest rate of 100.92 in 2022 to $82.55 per barrel in 2023, which was followed by a flurry of ship traffic density by 8.27% increase from 77,486 to 83,892 vessels, with an increase of total gross tonnages from 1,413,066,367 to 1,563,158,348, representing an increase of 10.62%. This was the year of highest volume for the tankers used the straits with a total number of 19,590, where both TTA and LPG tankers increased by 10.11% and 2.6%, respectively, with only decline for the TCH tankers by 2.24% over the previous year.

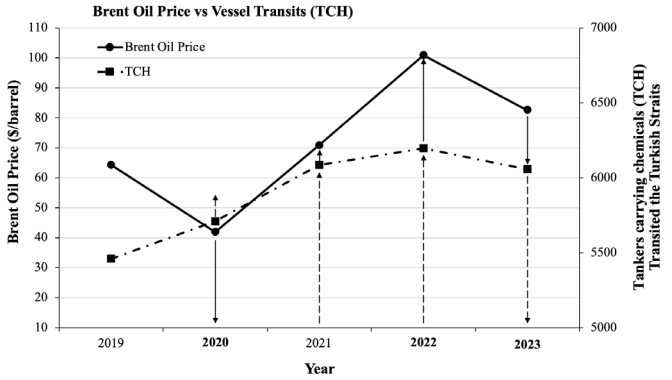

Figure 8. Explanatory overlay for the interrelation of brent crude oil price versus the number of tankers carrying chemicals (TCH) used the Turkish straits prior to and over the pandemic crisis and the aftermath from 2019 to 2023. Vertical arrow lines present drops and increases for brent oil prices (straight line) and number of non-stop over passage TCH (dashed line). Figure produced by statistical data provided by Statista (2024d) and MTI (2024).

In general, the number of non-stop over passage vessels used the Turkish Strait System declined by 1.15% between the investigated year span of 2019-2023, covering the global pandemic crisis with aftermath consequences. However, the total gross tonnage and ship length increased by 3.44% and 13.24%, respectively, since the outbreak of COVID-19 pandemic from 2019 to 2023. The total number of tankers also increased by 4.20% overall, with 2.92% and 10.97% increases for the TTA and TCH tankers, respectively, but 13.25% decline for the LPG tankers over the 5-years of investigation period that was initiated with the outbreak of the global pandemic crisis.

Variations in brent oil prices prior to and over the pandemic crisis with aftermath rates, along with the shipping traffic densities and characteristics of vessels used the Turkish Straits from 2019 to 2024 are given in Table 1.

Table 1. Average annual brent oil price ($ per barrel), and non-stop over passage of vessels through the Turkish Straits between 2019 and 2023, with indications to vessel characteristics. Percent variations for all variables to the previous year have been given in parenthesis and italic. Minus (-) in front of percent rates indicate "percent decline". (The year of pandemic outbreak with brent oil price dip in 2020, and the year of global recession with overshoot of brent oil price in 2022 have been highlighted in grey color)

Brent Oil*	**Year**				
	2019	**2020**	**2021**	**2022**	**2023**
$/barrel	64.30	41.96	70.86	100.93	82.49
	(-9.87%)	*(-34.74%)*	*(68.88%)*	*(42.44%)*	*(-18.21%)*
Σ-VN**	84,871	80,440	81,893	77,486	83,892
		(-5.22%)	*(1.81%)*	*(-5.38%)*	*(8.27%)*
Σ-TGT**	1,511,206,284	1,478,603,748	1,530,393,894	1,413,066,367	1,563,158,348
		(-2.16%)	*(3.50)*	*(-7.67)*	*(10.62)*
Σ-LOA**	11,410	12,382	13,161	11,302	12,921
		(8.52)	*(6.29)*	*(-14.13)*	*(14.32)*
Σ-TT**	18,800	17,807	17,456	18,557	19,590
		(-5.28)	*(-1.97)*	*(6.31)*	*(5.57)*
Σ-TTA**	12,112	10,896	10,281	11,321	12,466
		(-10.04)	*(-5.64)*	*(10.12)*	*(10.11)*
Σ-LPG**	1,230	1,201	1,089	1,040	1,067
		(-2.36)	*(-9.33)*	*(-4.50)*	*(2.60)*
Σ-TCH**	5,458	5,710	6,086	6,196	6,057
		(4.62)	*(6.58)*	*(1.81)*	*(-2.24)*

Note: Σ-VN: Total number of non-stop over passage vessels; Σ-TGT: Total number of total gross tonnage; Σ-LOA: Total number of vessels lower than 500 Gross Tonnage (LOA); Σ-TT: Total number of tankers; Σ-TTA: Total number of non-specific tankers; Σ-LPG: Total number of Liquefied Petroleum Gas tankers; Σ-TCH: Total number of chemical cargo tankers; *Statista (2024d); **MTI (2024)

Discussion

According to estimates made ten years ago, it was predicted that oil prices could range between $60 and $160 per barrel by 2020 (TEMS, 2008). It was probably not foreseen when these predictions were made that the COVID-19 pandemic would affect the whole world. Because, the lowest daily closing price of $19.33 per barrel for brent oil was experienced on 21st April during the coronavirus outbreak early 2020 (Statista, 2024e). It has been stated that various factors may have caused the decline in crude oil prices with the emergence of the COVID-19 pandemic. Fan & Li (2015) reported that uncertainties can be triggered by supply and demand imbalances, unexpected shock waves, political changes and financial impacts, which in turn may potentially destabilize the economy and affect the volatility of crude oil prices. It is possible to attribute the sharp decline in oil prices in 2020, when the COVID-19 pandemic began to spread, to the sudden contraction in the trade network due to harsh measures such as lockdowns and border closures since the beginning of the outbreak, which also noted by Boldea et al. (2023).

Overall, our analyses evidence a decline of the ship traffic density in the Turkish Straits during the pandemic, which is in line with March et al. (2021), who reported a decline in global maritime traffic during the COVID-19 pandemic period. Based on the findings in the present study, the total number of vessels (-5.22%) and so the total gross tonnages (-2.16%) used the Turkish Straits reduced as did the prices for brent oil rates (-34.74%). In line with the findings, March et al. (2021) reported remarkable decreases in maritime traffic after March 2020, for Istanbul Strait, along with the Panama Canal, Strait of Gibraltar, Strait of Dover.

Essentially, there has been a gradual increasing trend in the shipping industry for a long time in all maritime activities, including shipping and tourism cruise lines before the pandemic outbreak (Jouffray et al., 2020; Vicente-Cera et al., 2020), with 92% of maritime transport of the Economic Exclusive Zones (Halpern et al., 2019), and the global shipping network was expected to increase between 240% and 1209% by 2050 according to estimates (Sardain et al., 2019). However, changes in maritime transport can be affected by many factors of legal regulations such as marine protected areas, speed restrictions, changes in separation lines, socio-economic

changes, pirate attacks, environmental changes or cultural and political conflicts (Tournadre, 2014; McCauley et al., 2016; Moore et al., 2018). The decline in oil prices at the beginning of 2020 may have triggered an increase in tanker transportation before the decrease in oil demand due to the COVID-19 pandemic (Michail & Melas, 2020; Jefferson, 2020), that was noted for the tanker transport activities in the Turkish Straits in the present study. The total number of non-stop over passage tankers used both the Istanbul and Çanakkale Straits declined from 18,800 to 17,807 with a drop by 5.28% after the outbreak of COVID-19 in 2020. The decline for the TTA tankers were sharper (-10.04%), but only 2.36% reduction was recorded for the LPG tankers used the Turkish Straits during the pandemic in 2020. However, it appears that tankers carrying chemical cargo (TCH) has not been affected by the spread of the COVID-19 pandemic. In contrast, the TCH tanker used the Turkish Straits increased by 4.62 % despite the outbreak of COVID-19 in 2020, compared to the pre-pandemic year of 2019. Hence the TCH tankers were in the front line compared to the others and the situation for the TCH carriers was not as bad as might have been expected, as also reported by UNCTAD (2022). With the outbreak of COVID-19 pandemic, the production of chemicals in China initially slowed down due to lowered demand as an aftershock, some facilities were closed, and some slowed down the production. However, it was stated that some enterprises continued production to meet the slow-motion demands from the world by increasing the amount of supply, hence, the impact on the chemical tanker sector was lower than expected, as there was no problem in the supply of chemical materials (UNCTAD, 2022).

According to UNCTAD (2022) reports, port calls by container vessels lowered by -2.8% over the pre-pandemic year of 2019. Dry bulk and wet bulk carriers slowed by -4.1 and -4.8%, respectively. The decline of dry breakbulk carriers was higher (-7.8%). This was also noted in the present study with larger declines observed among the TTA (-10.04%). Since liquified natural gas (LNG) and liquified petroleum gas (LPG) are utilized for the use in household energy supply or electric power plants, port calls by LNG (-0.2%) and LPG (-3.1%) have been reported to be less affected (UNCTAD, 2022). This was in line with the present study, where the number of LPG carriers transited the Turkish Straits during the pandemic year of 2020 declined only by 2.36%, in close agreement with UNCTAD (2022) reports for the world maritime transportation.

Despite the 68.88% increase of brent oil prices in the post-pandemic period of 2021, there was a slight positive move in ship traffic density in the Turkish Straits. Although the total number of ships and eventually total gross tonnage rate increased by 1.81% and 3.50%, respectively, 5.64% decline was recorded for the transition of TTA tankers and 9.33% for the LPG carriers through the Straits. In contrast however, there was a progressive increase in chemical cargo carriers by 6.58% in 2021 over the pandemic year of 2020. Although the sudden shock waves had eased in the second half of 2020, when the pandemic outbreak emerged, and the world trade network gradually entered a recovery process early 2021, the delays and congestion in the supply chain led to a significant increase in container freight charges, as the recovery process from the pandemic was prolonged and could not respond rapidly to the increasing world demand (UNCTAD, 2021). This may explain the continued decrease in the number of tankers used the Turkish Straits, even though the effects of the pandemic began to wane in 2021.

In the post-pandemic period of late 2021 and early 2022, increased energy prices likely lead to a market pressure, weakening the purchase power of people, as has been reported by TEMS (2008). A rise in oil prices may reduce consumption and economic growth of countries, thus slowing down the world economy. Further, a decline in consumer demand also slows down the demand for trade and transportation services (TEMS, 2008). The main result of the initial rise in energy prices might be attributed to the recovery in energy demand after the easing quarantine measures of lockdown and curfew after the first wave of the pandemic, the subsequent price increase during 2021 was probably influenced by the supply challenges (Kuik et al., 2022). This process has likely intervened an even greater chaos with the conflict arisen between Russia and Ukraine in early 2022. As has been reported earlier by McCauley et al. (2016), Tournadre (2014), and Moore et al. (2018), several factors such as legal regulations socio-economic changes, pirate attacks, cultural and political conflicts may influence maritime transportation activities. With the turn of regional conflict between Ukraine and Russia into a war with Russian invasion, was the second stage of the aftermath of pandemic epidemic with severe increase in brent oil price, reaching over $100 per barrel in 2022. This shape increase of price per barrel was followed another declining period for the total non-stop over passage vessels through the Turkish straits. The tanker transits however, continued further increase by 6.31%, except the LPG carriers. When the brent oil price dropped (-18.21%) to $82.55 per barrel in 2023, the ship traffic density increased further by 8.27%.

Overall, the maritime traffic density in the Turkish Straits declined by 1.15% between 2019 and 2023, covering the global

pandemic crisis with aftermath consequences. However, the total number of tankers increased by 4.20% over the 5-years span from 2019 to 2023. Consequently, it seems that COVID-19 pandemic has less explanatory power over time in explanation of the brent oil price fluctuations, however stronger in interpreting the impacts on maritime transportation density in the Turkish Straits. Further investigations are worth for the understanding the roles of regional conflicts such as the war in Ukraine that may have substituted for the COVID-19 pandemic to a larger extent over the aftermath, as also reported earlier by Amri Amamou & Aguir Bargaoui (2022), and Xing et al. (2023).

Conclusion

In conclusion, analyses in regional scale as in this study, provide important information for the assessment of variations in the blue economy on global scale. The findings of this study show that the COVID-19 outbreak has caused remarkable disruptions and volumetric contraction of the shipping traffic in the Turkish Straits, one of the important sea routes and maritime chokepoints in the World. Further investigations in long-term on temporal variability in the energy market and structural breaks in maritime traffic during world crises are encouraged with attention. Such research approaches may provide early insights into management strategies for the blue economy and marine industry during world crisis periods.

Compliance With Ethical Standards

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

Not applicable.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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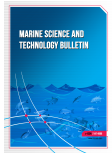
Mar. Sci. Tech. Bull. (2024) 13(3): 234-238
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1524629



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RESEARCH ARTICLE

Effect of vermicompost supplement on rainbow trout performance

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ARTICLE INFO

Article History:
Received: 30.07.2024
Received in revised form: 16.09.2024
Accepted: 22.09.2024
Available online: 30.09.2024

Keywords:
Rainbow trout
Vermicompost
Performance
Feeding

ABSTRACT

Vermicompost is the product of organic waste digestion by earthworms besides aerobic decomposition at ambient temperature. It is a rich composition containing plant nutrients and beneficial microorganisms. Some fishes, especially predatory species such as rainbow trout, need nutritive feed so that they can be healthy; a useful and cheaper additive such as vermicompost can supply this aim. In this study, the effect of different percentages of vermicompost in the diet of rainbow trout was investigated. For this purpose, 100 rainbow trout with an average weight of 120±3 g were distributed in 5 groups in concrete tanks. Their nutrition and physical and chemical water parameters were determined according to the standard. Vermicompost with confirmable analysis was added to their daily feed (2, 4, 6, and 10%). After 2 months, the feed conversion ratio and survival rate were examined. The results showed that there was a significant relationship between the performance of the control group and the treatment groups ($p<0.05$). The data obtained from this experiment indicated that the 10% of vermicompost in the diet was more effective. Therefore, the use of vermicompost supplements can be recommended for the cultivation of rainbow trout.

Please cite this paper as follows:

Parsa, A., & Can, E. (2024). Effect of vermicompost supplement on rainbow trout performance. *Marine Science and Technology Bulletin*, *13*(3), 234-238. https://doi.org/10.33714/masteb.1524629

Introduction

Vermicompost (excrete of earthworms) has been recognized as a potential pond fertilizer because it has superior nutritional quality, contains microbes, and is in ready-for-uptake form. Besides, the vermicompost contains humic acid, which has antibiotic properties and promotes fish gut health, stress management, and immune systems (Musyoka & Nairuti, 2024).

Sustainable aquaculture is one in which the goal is permanence, achieved through the utilization of renewable resources. This leads to the development of the concept of

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organic and natural farming. Among various components of organic and natural farming, vermicomposting is a key component for making compost through earthworms (Chakrabarty et al., 2009).

Fisheries and aquaculture are important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world. Since 2014, more than half of all fish for human consumption came from aquaculture. Its extent, diversification and intensification make aquaculture one of the fastest growing food-producing sectors globally. As a result of the magnitude and intensity of aquaculture production, issues related to its long-term sustainability and environmental impacts have become more pronounced (Kouba et al., 2018; Austin et al., 2022).

Most of the degradable organic waste in the environment can be converted into vermicompost by earthworms. Earthworms can eat these organic wastes and provide fertilizer to the ecosystem. Earthworm excrement contains plant nutrients that are covered in the mucous membrane secreted from the digestive system, and these secretions increase the stability and consistency of excrement, which is used as vermicompost (Karaca, 2010). The earthworm breeding industry has grown considerably due to its role in waste management. The mass production of earthworms and their use in animal feed (using of live or dry earthworms) is important in aquatic animals feeding. The combination of fatty acids and essential amino acids and the presence of omega-3 in this substance make its use different from plant sources (Ng et al., 2001).

Vermicompost contains enzymes such as protease, lipase, amylase, cellulase, and chitinase. These enzymes continue to function in the biodegradation of large molecules in the soil. Vermicompost is also rich in vitamins, antibiotics, and growth hormones. In a study, it has a positive effect on the growth performance of goldfish (Zou et al., 2012). In another study, vermicompost was used as a fertilizer in a fish farm and the results showed that the growth performance and production of phytoplankton and zooplankton in the water tank containing superphosphate fertilizer and vermicompost was significantly different from the control group. This happens due to the presence of various inorganic and organic compounds in vermicompost (Chakrabarty et al., 2010).

Due to the lack of investigation of the effects of vermicompost consumption as a food supplement in the diet of rainbow trout (*Oncorhynchus mykiss*), the initial idea of conducting this study was formed. Also, the above studies showed that consumption of vermicompost can affect some functional factors of fish, so in this study we wanted to investigate the possibility of oral consumption of vermicompost in rainbow trout, the possible anti-nutritional effects of vermicompost and the effect of adding vermicompost in feed on growth rate and feed conversion ratio and survival rate of rainbow trout.

Material and Methods

This research was conducted at the Islamic Azad University in Iran, according to the country's legislation guidelines for the care and use of animals (Ethics approval number: 2916/4/2111, Date: 12/09/2017). All applicable international, national and institutional guidelines for the care and use of animals were followed.

To do this research, 100 rainbow trout with an average body weight of 120±3 g were distributed in 5 groups in tanks (500 l). Physicochemical water parameters including temperature (20±3°C), dissolved oxygen (9 mg/l), and pH (8) were recorded daily. The water flow rate was 120 l/h. The diet based on the formulation of 38% protein, 23% lipid, 11% moisture, 12% ash, and 2050 kJ/kg energy was prepared by extruder in 6 mm size. Fish were exposed to natural photoperiods. After acclimatization for 10 days, vermicompost was purchased from a commercial company with confirmable analysis (Table 1) and it was added to the daily feed (2, 4, 6, and 10%). After 2 months, fish were harvested after anesthesia and their weights were calculated, and then FCR and survival rate were examined.

Table1. Physicochemical properties of vermicompost

Parameters	Amounts
Ash (%)	20
Total nitrogen (%)	2
C/N (%)	10
P (%)	2.15
K (%)	1.2
Ca (%)	0.5
pH	7.8
EC (μ mho/cm)	3.2
Humidity (%)	30

Statistical analyses followed the methods outlined by Minitab (16). Data were tested for normality and homogeneity of variances using Kolmogorov–Smirnov, and then data were analyzed with analysis of variance (ANOVA) to test differences between dietary treatments.

When this test showed significance, individual means were compared using Tukey's test. Significant differences were considered when $p \leq 0.05$.

Results

There is a significant relationship between the amount of feed conversion ratio in different groups and it indicates the reduction of the FCR with the use of vermicompost. The highest number of deaths was for the control group and treatment 1 and the lowest number was for treatment 4. There is a significant relationship between the survival rates in different groups. The results showed that there is a significant relationship between the performance and survival of the control group and the treatment groups ($p<0.05$).

There is a significant relationship between the amounts of FCR in different groups. There is no significant relationship between the control group and the treatment, but there is a significant relationship between the control group and the rest of the treatment groups, and it indicates the reduction of the food conversion ratio with the use of vermicompost ($p<0.05$) (Figure 1).

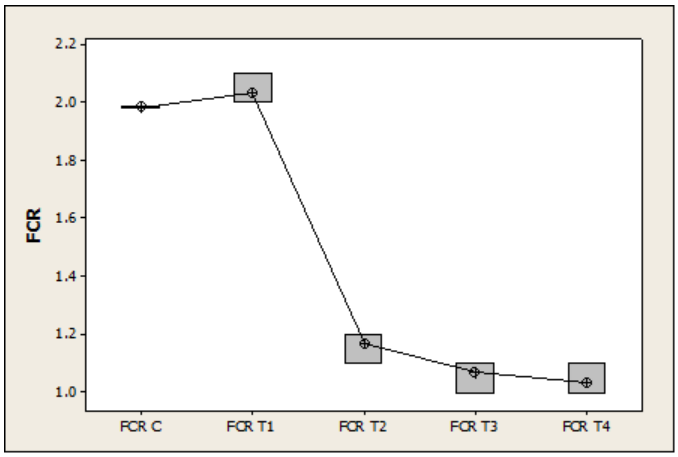

Figure 1. Feed conversion ratio (%) (C=Control, T1=Treatment 1, T2=Treatment 2, T3=Treatment 3, T4=Treatment 4)

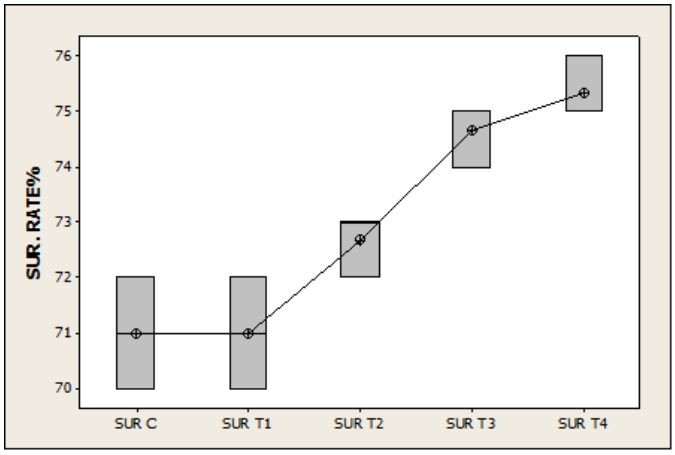

Figure 2. Survival rate (%) (C=Control, T1=Treatment 1, T2=Treatment 2, T3=Treatment 3, T4=Treatment 4)

The results showed that the highest number of deaths was for the control group and treatment 1 (29%) and the lowest number of deaths was for treatments 3 and 4 (25%). There is a significant relationship between survival rate in different groups ($p<0.05$). There is no significant relationship between the control group and treatments 1 and 2, but there is a significant relationship between the control group and the rest of the treatments, and it indicates the effect of the vermicompost dosage on increasing the survival rate (Figure 2).

Discussion

Various researches have been conducted on adding earthworms to the food of different fishes and investigating its effects. They had different and sometimes contradictory results. In a study, by substituting frozen earthworms in rainbow trout food with levels of 0, 25, 50, 75%, no significant difference was observed in final weight among treatments (Pereira & Gomes, 1995). In a study, replacing earthworm (*Eisenia fetida*) powder with amounts of 25 and 50% in the feed of rainbow trout showed that in these amounts, a higher growth rate was created in treatment groups (Velsquez et al., 1991).

Paying attention to the above results makes it difficult to decide on adding earthworms to rainbow trout feed. In these researches, there has not been a discussion about adding vermicompost made from earthworms as a supplement to fish feed, while the secretions of the worm's body surface and the contents of feces from worms can have nutritional effects and are worth investigating. In another research, vermicompost obtained from earthworms was used as a growth stimulant in common carp fish feed and it was found that the quality of fish meat protein increased (Cheng et al., 2012a). The findings of these studies confirm the results of the present study.

When the food material passes through the gut of the earthworm, changes are made in it and the remaining material is rich in nutrients and microbial organisms. Earthworms can transform waste into valuable material through a kind of biological alchemy. The intestinal activity of the worm is like a miniature composting tube that mixes different materials and creates a stable synergistic relationship with the enzymes of the digestive system in the humidity, pH and microbial populations of the intestine in a favorable way and finally, a wonderful by-product is produced which it is called vermicast and it is rich in nitrate, phosphate, and potash, and other micronutrients are excreted from the body along with bacteria (Adhikary, 2012). These components can be suitable and useful for fish, for example, enzymatic components of vermicompost can be effective in increasing the efficiency of digestion of feed and help decrease FCR. Also, other non-living and living compounds of vermicompost can have a positive effect on the

thickness of the intestinal mucosa of fish and increase the efficiency of absorption, increase the population of beneficial bacteria, and increase local and humoral immunity, all can increase fish survival and we can see in the results of this research.

Vermicomposting is a complex biological and ecological process of accelerated bio-oxidation and stabilization of organic material. In contrast to traditional composting, it involves the joint action of earthworms and microorganisms without a thermophilic phase, exhibiting reduced emissions of greenhouse gases (Nigussie et al., 2016). The applicability of this biotechnology has been shown for a wide range of organic matrices. Vermicomposting allows the transformation of potentially problematic organic solid waste into highly valuable end-products vermicompost and biomass of earthworms (Lim et al., 2016). The results of one study showed that adding 6% vermicompost to the diet increases the activities of pepsin, pancreatic protease, amylase and lipase of hepatopancreas and foregut can significantly improve digestibility and work better. Also, using paraffin sections technique to watch the foregut intestinal internal structure of grass carp in each test group showed significantly increase in the intestinal mucosa height, width and thickness of roots (Cheng et al., 2012b). In this research the greatest effect on FCR and survival rate was at the concentration of 10% vermicompost, which considering that no other supplement was added to the fish food and only with vermicompost we observed good results in FCR and survival rate in all groups. It can be concluded that adding vermicompost has not a significant effect on decreasing of nutritional parameters of the diet.

In the present study, the results were in the same direction, and with the increase in the amount of vermicompost, a significant decrease in the feed conversion ratio was observed, which can show the existing nutritional potential and the effect of earthworm body secretions and the compounds in their feces. The feed conversion ratio is one of the most important nutritional factors and the reduction of it in this study can have various reasons, but it can be related to the surface secretions of the worm's body and the enzyme compounds in the feces and the changes made in the worm's food. Probably, the reason for the high death in all groups and the observation of low survival can be related to the high water temperature (20±3°C), and because this situation is also seen in the control group, it does not affection the statistical analysis and is ineffective on the interpretation of the results.

These results showed that, unlike earthworms, the addition of which, especially in high amounts, to the diet of trout caused a decrease in efficiency, in the case of vermicompost, this was not the case, and the highest efficiency was observed in the highest dose (10%).

Conclusion

Considering the presence of various organic and inorganic compounds in vermicompost and their similarity with the nutritional needs of fish and the relative confirmation of this issue by the results of the above research, it is possible to add vermicompost to rainbow trout feed to increase production efficiency and to help to optimize nutrition. Also, vermiwash can be used as a food supplement for rainbow trout and the effects of vermicompost and vermiwash in extruded foods as a binder or attractant in the preparation of fish feed and the effect on the level of bioavailability and safety of fish and the changes in the intestinal microbial flora can be investigated. The data obtained from this experiment indicated that the 10% vermicompost in the diet was more effective and it can be a sustainable method for aquaculture and proves vermicompost to be a promising addition to fish feed for better nutrition and betterment of microflora of fish intestine. The comparison of the effects of adding vermiwash with vermicompost in rainbow trout feed and investigating the effect of adding vermicompost during feed production by extruding method and the effect of adding vermicompost to feed on the rate of fish fecundity and investigating the effect of feeding with vermicompost on the intestinal microbial flora population and effects of vermicompost in performance of carp, mullet, tilapia and crustacean can be suggested. Although we have obtained evidence that vermicompost may be a useful addition to trout feeds, further research into its best uses and potential benefits is warranted.

Acknowledgements

Authors are grateful to everybody helped us in carrying out this research.

Compliance With Ethical Standards

Authors' Contributions

AP: Investigation, Methodology, Data analysis, Writing – original draft

EC: Supervision, Writing – review and editing

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

This research was conducted at the Islamic Azad University in Iran, according to the country's legislation guidelines for the care and use of animals (Ethics approval number: 2916/4/2111, Date: 12/09/2017). All applicable international, national and institutional guidelines for the care and use of animals were followed.

Funding

Not applicable.

Data Availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

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Mar. Sci. Tech. Bull. (2024) 13(3): 239-250
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1488998

Marine Science and Technology Bulletin

https://prensip.gen.tr/

REVIEW ARTICLE

Sustainable approaches in aquaculture: Pharmacological and natural alternatives to antibiotics

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ARTICLE INFO

Article History:
Received: 27.05.2024
Received in revised form: 19.08.2024
Accepted: 26.08.2024
Available online: 30.09.2024

Keywords:
Antibiotic resistance
Aquaculture
Bacteriophages
Probiotics
Vaccination

ABSTRACT

Aquaculture, a vital component of global food production, faces challenges such as antimicrobial residues and resistance due to the extensive use of antibiotics. This review explores sustainable alternatives to antibiotics in aquaculture. Vaccines play a critical role in disease prevention, significantly reducing antibiotic reliance. Phage therapy targets specific bacterial pathogens, offering an environmentally friendly solution, while quorum quenching disrupts bacterial communication, reducing virulence without promoting resistance. Probiotics and prebiotics enhance gut health and disease resistance, with synbiotics showing synergistic effects. Emerging technologies such as parabiotics and postbiotics, along with advances in metagenomics and next-generation sequencing, improve our understanding of microbiomes, leading to more effective disease control strategies. Medicinal plants provide cost-effective, natural antimicrobial and immune-stimulating properties, while nanoparticles degrade antibiotics, reducing pollution. A multifaceted approach that integrates these methods can mitigate antimicrobial resistance risks, ensuring the sustainability of aquaculture. Tailoring strategies to specific environmental conditions, species, and pathogens is crucial, emphasizing the need for continuous development and adaptation to maintain the long-term viability of the aquaculture industry.

Please cite this paper as follows:

Kılıç, N., & Gültekin, G. (2024). Sustainable approaches in aquaculture: Pharmacological and natural alternatives to antibiotics. *Marine Science and Technology Bulletin*, *13*(3), 239-250. https://doi.org/10.33714/masteb.1488998

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Introduction

Aquaculture involves growing aquatic organisms for food in various environments such as freshwater, saltwater, and brackish water. This industry operates in diverse locations, from coastal and riverside areas to land-based systems, and in a range of climates from tropical to temperate. Aquaculture encompasses a wide biodiversity, including seaweeds, mollusks, crustaceans, and various species of fish. Production methods fall into three main categories: extensive (low intensity with natural nutrition), semi-intensive, and intensive (high intensity with artificial nutrition). The use of technologies to manage aquaculture varies based on economy, infrastructure, and species being cultured. Consequently, aquaculture ranges from high-tech international farming of valuable species to low-tech agriculture (Kumar et al., 2018; Bondad-Reantaso et al., 2023). Since the 1980s, aquaculture has expanded rapidly to satisfy rising production demands. According to the FAO, global fisheries and aquaculture production reached a new record high of 214 million tonnes in 2022, driven largely by the growth in aquaculture, which accounted for 46% of the total production. This upward trend is expected to continue, with projections suggesting a 15% increase in global fish production by 2030, reaching approximately 245 million tonnes (FAO, 2023). However, this rapid expansion and globalization have brought challenges, such as the emergence and spread of diseases, necessitating the use of antimicrobials to improve production (Cabello et al., 2013; Schar et al., 2020). New reports indicate that pathogenic agents have undergone changes, reducing the effectiveness of common antimicrobials. Consequently, the doses of antimicrobials used in fish have been increasing over the years to maintain their efficacy (Erkinharju et al., 2021; Duman et al., 2023).

Aquatic organisms can harbor various potentially pathogenic microorganisms. The presence and impact of these pathogens are influenced by factors specific to the host, the pathogen itself, and the environment. Antibiotics frequently used in aquaculture typically target Gram-negative bacteria, as the most common pathogens in this field are usually aerobic, Gram-negative rods (Schar et al., 2020; Petty et al., 2022; Hossain et al., 2022). According to a 2012 study by the Food and Agriculture Organization of the United Nations (FAO), the most used antibiotics in farms are oxytetracycline, florfenicol, and trimethoprim/sulfadiazine (Alday-Sanz et al., 2005). However, the use of antibiotics differs significantly between regions and countries. In developed regions such as Europe, North America, and Japan, antibiotic use is tightly regulated, resulting in lower usage, particularly in countries like Norway and Scotland where the amount of antibiotics used per harvested salmon is minimal. Conversely, in countries like China, Vietnam, Thailand, and Chile, antibiotic use is considerably higher, leading to widespread antimicrobial resistance in pathogens such as *Aeromonas*, *Vibrio*, and *Acinetobacter* species. For instance, China alone uses an estimated 15,000 to 20,000 tons of antibiotics annually, while Thailand's shrimp farming consumes around 600 metric tons, both contributing to significant resistance rates (Preena et al., 2020; Deekshit et al., 2023). Metaphylactic use of antibiotics, treating the entire population rather than individual animals, is generally preferred (Cabello et al., 2013).

Exposure to antibiotics at subtherapeutic levels can lead to the development of resistant mutants. Once resistance develops in a bacterial strain, it can spread to other species and strains via gene transfer (Nesse & Simm, 2018). As a result, managing common bacterial diseases in aquaculture, such as furunculosis and edwardsiellosis, is becoming increasingly challenging due to the rise in antimicrobial resistance (AMR) (WHO, 2006). Although minimized levels of antibiotics in aquaculture environments can originate from human wastewater, most are directly used in aquaculture (Santos & Ramos, 2018). Technologies are being developed to remove these molecules in wastewater treatment before they are released into the environment (Salgueiro et al., 2020; Bondad-Reantaso et al., 2023).

Antimicrobial residues in food and animal products present significant socio-economic and public health challenges. Key health effects include the development of AMR, disruption of normal intestinal flora, allergies, anaphylactic shock, nephropathy, bone marrow depression, carcinogenicity, teratogenicity, and mutagenicity (Arsène et al., 2022). The indiscriminate use of antimicrobials contributes to these residues and their adverse health impacts. Therefore, control measures are necessary to reduce antibiotic use and protect consumers (Okocha et al., 2018).

According to the FAO/OIE/WHO expert consultation on aquaculture held in Seoul, South Korea, from June 13-16, 2006, the hazards of antimicrobial use are summarised as antimicrobial residues and antimicrobial resistance (AMR) (WHO, 2006). The report concludes that AMR is a more serious problem because it transcends phylogenetic and geographic boundaries, can spread among aquatic bacteria and animal and human pathogens. For example, resistance to aquatic animal pathogens can render fish disease treatments ineffective and may be transferred to animal and human

pathogens, complicating treatment. Another issue with antimicrobial use in aquaculture is that treatment is usually administered through feed, unlike in terrestrial environments. This method can affect the efficiency of treatment by reducing the feed intake of sick animals, and unused feed with medication may remain in sediments, where resistant bacteria can be selected, increasing the resistance pool in the aquatic environment. Therefore, finding alternatives to antibiotic use is vital for the sustainability of the sector (Bondad-Reantaso et al., 2023).

Antibiotic residues in aquaculture products are one of the most common reasons for product returns in major fish markets such as the European Union (EU), the United States (US), and Japan. This situation often results in the destruction of products and significant economic losses for exporting countries. In most aquaculture-producing countries, residue monitoring is conducted according to international market requirements. Many countries strive to comply with EU standards, particularly since the EU is a major importer of seafood. The EU uses the most sensitive detection methods for chemicals banned in aquaculture. However, differences in permitted aquaculture pesticides and maximum residue limits exist among producing countries (Karunasagar, 2020).

The spread of AMR can occur in aquatic environments, such as wastewater from hospitals and farms, through irrigation water, or through its use in animal farms. Wild animals and birds traveling long distances can also carry resistant bacteria to different environments. Therefore, combating antimicrobial resistance requires considering both aquaculture and environmental factors (Zeballos-Gross et al., 2021). Controlling the use of antibiotics in aquaculture not only helps reduce global antibiotic resistance, but also includes alternative strategies, such as strengthening the immunity of aquatic organisms and reducing the virulence (FAO, 2019).

The aim of this review is to examine the scope of problems related to antimicrobial use and the importance of AMR in aquaculture, review the existing literature, evaluate antibiotic use and alternative treatment methods such as vaccination, bacteriophages, bacteriocins, use of probiotics and prebiotics, quorum quenching, chicken egg yolk antibody, nanoparticles, recombinant proteins, and medicinal plant derivatives.

Antibiotics in Aquaculture

Aquaculture faces significant challenges due to disease outbreaks often caused by poor environmental conditions, such as improper farm management and malnutrition. This leads to secondary bacterial infections that require antibiotics for therapeutic, preventive, and metaphylactic purposes (Cabello et al., 2013). Common antibiotics include tetracycline, oxytetracycline, oxolinic acid, amoxicillin, flumequine, sarafloxacin, enrofloxacin, erythromycin, sulfadimethoxine, ormethoprim, and florfenicol (FAO, 2019).

Most antibiotics used in aquaculture enter the environment intact, causing horizontal gene transfer due to residues in polluted water, biofilms, and bacteriophages (Watts et al., 2017). These residues pose post-treatment risks (Cabello et al., 2013). The use in ornamental fish can lead to resistant bacterial strains and facilitate their global spread (Watts et al., 2017).

Tetracyclines, such as oxytetracycline, are widely used in countries like the United States and Norway due to their broad-spectrum activity and affordability. However, overuse has led to significant resistance issues (Grossman, 2016). In Norway, the usage of antibiotics is tightly controlled, and the amount used per ton of harvested fish is less than 1 gram, largely due to effective vaccination programs and stringent regulations (Sommerset et al., 2005). In contrast, Chile has reported higher usage, with up to 500 grams of antibiotics per ton of harvested salmon, contributing to the emergence of resistant strains of *Piscirickettsia salmonis* (Cabello et al., 2013). This high usage rate reflects the reliance on antibiotics in the absence of similarly stringent regulations and effective alternative disease management strategies. In Türkiye, antibiotics like oxytetracycline and florfenicol are commonly used in aquaculture. The use of oxytetracycline is widespread due to its cost-effectiveness and broad-spectrum activity, but there are concerns about increasing resistance, especially among Vibrio and Aeromonas species (Duman et al., 2023). European Union countries, including Scotland, have strict regulations on antibiotic use in aquaculture, favoring vaccination and other preventive measures. For example, the use of quinolones and fluoroquinolones is heavily restricted and generally reserved for human medicine to avoid cross-resistance issues (Bondad-Reantaso et al., 2023). In Vietnam, high levels of antibiotic use in aquaculture have been linked to the development of multi-drug resistant Aeromonas species, posing significant challenges for disease management (Lulijwa et al., 2020). The country continues to struggle with implementing effective regulations to control antibiotic use in aquaculture. Nitrofurans, such as furazolidone, are banned in many countries due to their carcinogenic risks, yet they have been historically used in some regions where regulatory oversight is less stringent (Antunes et al., 2006). Similarly, the use of rifampicin in aquaculture is

limited due to resistance issues and potential impacts on human health (Lulijwa et al., 2020).

Alternatives to Antibiotics

In recent years, the rapid expansion of the industry has led to increased disease outbreaks, challenging the sustainability of production. The misuse of antimicrobial agents, leading to antibiotic-resistant genes (ARGs), has prompted the exploration of alternatives (Dowling et al., 2013; Bondad-Reantaso et al., 2023).

Vaccinations

Vaccines made from weakened or inactivated pathogens, are crucial for reducing fish diseases and antibiotic use in aquaculture. In Norwegian salmon farming, annual antibiotic use dropped from 50,000 kg in 1987 to less than 2,000 kg in 1997 due to vaccination strategies (Sommerset et al., 2005; Rodger, 2016).

Fish vaccination began in 1942 with a vaccine for *Aeromonas salmonicida* in *Oncorhynchus clarkii* trout. Advances in biotechnology and immunology have since led to many vaccines that combat bacterial and viral diseases in aquaculture (Adams, 2019). Most vaccines use inactivated microorganisms administered by immersion or injection (Ma et al., 2019). Whole-cell killed vaccines effectively target extracellular bacteria, while intracellular bacteria require vaccines that stimulate a cellular immune response (Munang'andu, 2018).

A stronger antibody response can be achieved by using live vaccines. Due to their ability to proliferate or enter the host, these vaccines are administered orally or by immersion, providing both innate and adaptive immunity (Ma et al., 2019). Modified live vaccines are prepared from viruses or bacteria with attenuated virulence. They are obtained through chemical or physical processes, culture under abnormal conditions, or natural low virulence against target species (Adams, 2019). Molecular manipulations that lack virulence are also used to create weakening vaccines. This approach is useful for DNA viruses such as herpesviruses, as well as *Streptococcus spp.* and *Edwardsiella spp.* (Ma et al., 2019).

Injectable vaccines containing adjuvants and multiple antigens to protect against different diseases are used in commercial farms, particularly in Atlantic salmon (*Salmo salar*) (Sommerset et al., 2005). Furthermore, autogenous vaccines offer flexibility in cost and production, faster distribution, and the possibility of application during outbreaks (Adams, 2019).

Modern technological approaches to vaccine production, such as targeting specific pathogen components, recombinant technology, or DNA/RNA particle vaccines, are also believed to produce higher immunity. These vaccines use only antigenic components, eliminating replication risks in the host or environment (Hansson et al., 2000). Immunogenic components can be isolated from the pathogen or produced using specific proteins. *Escherichia coli* expression vectors have been used in Norway to combat infectious pancreatic necrosis (IPN) in salmonids by generating plasmids with protective antigen genes (Ma et al., 2019). These vaccines can be freeze-dried, but they are costly due to the need for effective adjuvants and multiple booster shots, leading to a weaker immune response (Adams, 2019).

Virus-like particles (VLPs) are advanced subunit vaccines formed by viral capsid proteins that mimic viruses without genomic material, preventing replication. They enhance immune responses and have shown experimental effectiveness against certain diseases (Noad & Roy, 2003).

Recently, nucleic acid vaccines for aquaculture have been developed that provide strong cellular and humoral immunity. DNA and RNA vaccines are easy to produce, safe, and cost-effective (Ma et al., 2019). DNA vaccines, made in bacterial cells with expression plasmids, offer cross-protection (Adams, 2019). A DNA vaccine for infectious hematopoietic necrosis virus (IHNV) is licensed in Canada. RNA-based vaccines, including non-amplifying and self-amplifying mRNA, show promise for human and animal use (Pardi et al., 2018).

In terms of country-specific regulations, several vaccines are allowed and widely used in aquaculture. In Norway, vaccines against infectious pancreatic necrosis (IPN) and salmonid alphavirus (SAV) are extensively utilized in Atlantic salmon farming (Erkinharju et al., 2021). Canada has licensed a DNA vaccine for infectious hematopoietic necrosis virus (IHNV), which is applied to various salmonid species (Adams, 2019). In the European Union, vaccines against *Aeromonas salmonicida* and *Yersinia ruckeri* are commonly used in trout farming to prevent furunculosis and enteric redmouth disease, respectively (Bondad-Reantaso et al., 2023). The United States employs vaccines for *Streptococcus iniae* and *Vibrio* spp. in tilapia and catfish aquaculture (Ma et al., 2019). In Japan, vaccines for *Edwardsiella tarda* and *Vibrio anguillarum* are used in yellowtail (*Seriola quinqueradiata*) and other marine species (FAO, 2020). Türkiye uses vaccines against *Lactococcus*

garvieae and *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*) farming to combat streptococcosis and enteric redmouth disease (Duman et al., 2023).

Bacteriophages

Bacteriophages are viruses that infect and lyse bacterial cells, particularly lytic phages that disrupt bacterial metabolism. Discovered in the early 1900s, they were initially considered a solution for bacterial diseases, but interest declined with the advent of antibiotics. However, with increasing antibiotic resistance, phage therapy is gaining attention. Phages are the most abundant microorganisms on Earth, especially in marine and freshwater environments, where they can survive for several weeks to over 5 to 7 months (Silva et al., 2014). Generally, phage survival is not affected by pH, salinity, temperature, or organic matter. Bacteriophages can integrate into host DNA as prophages or exist as replicons, as seen with *Vibrio* spp. (Choudhury et al., 2016). Phage therapy has been used to control bacterial infections successfully, with multiple phage therapy proving more effective than single treatments. Reports highlight the use of phage therapy against the Vibrionaceae family. Phages can control the most destructive bacteria, such as *Vibrio harveyi*, which infects mollusks, crustaceans, starfish, and fish (Bondad-Reantaso et al., 2023).

Phage-host interactions are complex, involving gene transfer and varying virulence, affecting genetic traits and relationships. Bacterial hosts can carry prophage-encoded virulence factors, altering virulence and enabling anti-virulence therapy (Defoirdt, 2014). Phage resistance can lead to resistant bacterial strains and increased phage genome size. Resistance to one phage can make bacteria sensitive to others, and some phages remain pathogenic. Some phages may have mutually beneficial relationships with their hosts. While phage therapies are promising alternatives to traditional treatments, more research is needed for widespread use (Żaczek et al., 2020).

The use of phages against *Lactococcus garvieae* was first introduced in Japan in 1999, and since then, various phages have been applied to control bacterial pathogens such as *Vibrio*, *Flavobacterium*, *Edwardsiella*, and *Aeromonas* species across different regions. A notable phage-based product, CuSTuS®, has been launched by ACD Pharmaceuticals in Norway, targeting *Yersinia ruckeri* in salmonids with considerable success. Similarly, Proteon Pharmaceuticals has introduced BAFADOR®, a phage cocktail that targets *Pseudomonas* and *Aeromonas* infections. These products exemplify the growing adoption and regulatory approval of phage therapy in aquaculture, particularly in Europe (Kalatzis, 2019).

Quorum Quenching

Quorum quenching (QQ) encompasses processes that inhibit quorum sensing (QS), which regulates gene expression by monitoring the density of the bacterial population. Many bacteria use QS signals to coordinate diverse behaviors through microorganism-microorganism interactions in various environments. Quorum quenching involves multiple phenomena and mechanisms, including enzymes, chemical compounds, and different mechanisms of action such as QS signal degradation and competitive inhibition. All major steps of the QS pathway can be affected, including synthesis, diffusion, and perception of QS signals. Therefore, QQ has been used for biocontrol of aquatic diseases (Grandclement et al., 2016).

Surface-attached bacteria form biofilms embedded in a hydrogel matrix, making them more resistant than planktonic forms and potentially more resistant to antibiotics. These biofilms interact with QS, a communication method that enables bacteria to communicate. QS uses signaling molecules called autoinducers, which are small, diffusible molecules. Autoinducers activate genes that control various functions such as biofilm formation, virulence, invasion, and dissemination. Blocking QS with QQ can potentially stop gene expression that controls virulence (Grandclément et al., 2016; Bondad-Reantaso et al., 2023).

Antimicrobial Peptides

Antimicrobial peptides (AMPs) are gaining attention in aquaculture as a sustainable alternative to antibiotics, particularly effective against drug-resistant pathogens. Derived from various natural sources such as bacteria, fungi, plants, and also vertebrates, AMPs disrupt cell membranes, making it difficult for pathogens to develop resistance. Fish, which are highly dependent on innate immunity, are significant sources of AMP. Although their application in aquaculture has not yet advanced due to production cost and stability problems, advances in synthetic peptide design may improve their feasibility (Pant et al., 2023).

Bacteriocins

Bacteriocins are bioactive compounds produced by bacteria and are suggested as antibiotic alternatives in aquaculture. These ribosome-synthesized, low-molecular-weight peptides (20-60 amino acids) are encoded in chromosomes or extrachromosomal elements. They have antimicrobial properties, inhibiting or killing various microorganisms.

Bacteriocins are environmentally friendly, biodegradable, non-harmful to hosts, antagonistic to harmful intestinal pathogens, and promote beneficial bacteria (Nayak et al., 2021).

Probiotics

Recent studies have indicated that aquaculture products' healthy and balanced gut microbiome reduces the risk of disease and stress in growing conditions by optimizing nutrient digestion (Diwan et al., 2021; Yilmaz et al., 2022). Disrupted microbiomes are often associated with disease states and scientists as biomarkers to identify pathological problems (Romero et al., 2014). Some types of bacteria are common in healthy animals, while other types increase significantly increase in infected animals. This suggests that the microbiota of diseased animals is more affected by environmental factors and stress, leading to difficulties in regulating the digestive microbiota. A healthy gut microbiome prevents diseases in host organisms, while its disruption can lead to the presence and infections. For example, higher densities of *Aeromonas* bacteria were observed in diseased fish compared to healthy fish, suggesting that a balanced microbiome inhibits the pathogenicity of *Aeromonas* (Li et al., 2016).

Probiotics are live, non-pathogenic microorganisms that positively impact microbiomes and are widely used and commercially available worldwide. They are used to improve microbial balance, especially in the gastrointestinal tract, and primarily consist of yeasts and bacteria such as *Lactobacillus* and *Bifidobacterium* species, often added to foods as dietary supplements (Diwan et al., 2021). Probiotics have been scientifically demonstrated to be effective in preventing and treating various medical conditions, especially in improving gut health. These beneficial microorganisms improve health by inhibiting harmful bacteria through various mechanisms. Probiotic effects in the intestines of aquatic animals occur through pathways such as inhibition of pathogen adhesion, production of antimicrobial components, modulation of the immune system, strengthening of barrier function and lowering of luminal pH. In particular, lowering intestinal pH can alter the host immune response by reducing the colonization and invasion of pathogens (Bondad-Reantaso et al., 2023).

Probiotics benefit aquatic animals by synthesizing essential nutrients like unsaturated fatty acids and vitamin B12 (Diwan et al., 2021). *Bacillus* probiotics also improve the environment by assimilating organic pollutants like ammonia and nitrites, reducing stress and toxicity. These probiotics also compete with opportunistic pathogens for access to nutrients, thus preventing the bacterial pathogens present in the same ecosystem from becoming harmful to aquaculture. This is especially true for many *Vibrio* species, which contribute significantly to the health of aquatic animals (Bondad-Reantaso et al., 2023).

Lactobacillus spp. and *Bacillus* spp. are the most commonly used probiotic species. *Lactobacillus* species help improve the gut flora in fish and optimize nutrient absorption by increasing digestive enzymes (Verschuere et al., 2000). Additionally, *Lactobacillus* species act as competitive inhibitors against pathogenic bacteria, contributing to disease prevention (Nayak, 2010). *Bacillus* spp. are robust spore-producing bacteria that can be added to fish feed. These bacteria establish themselves in the gut, preventing the proliferation of harmful bacteria and improving water quality (Moriarty, 1998). One of the most significant features of *Bacillus* species is their resistance to high temperatures and digestive processes, making them easily incorporated into feed formulations (Dawood et al., 2019). Other probiotics such as *Pediococcus* spp. and *Enterococcus* spp. are also used in aquaculture. These species modulate the immune system of fish, enhancing resistance to infections (Nikoskelainen et al., 2003). *Pediococcus* species are particularly effective in cold-water fish, helping to protect these fish from diseases even at low temperatures (Vendrell et al., 2008).

Prebiotics

Prebiotics are non-living food supplements, typically a family of carbohydrates consisting of oligosaccharides, that are generally not digested by the host but can be digested by certain populations of gut bacteria (Goh et al., 2022). These ingredients act as selective substrates for bacteria, specifically promoting the growth of beneficial gut bacteria. This modification causes specific changes of the intestinal flora, thereby improving the overall well-being and health of the host (Yilmaz et al., 2022).

Commonly used prebiotics in aquaculture include inulin, fructooligosaccharides (FOS), and mannan oligosaccharides (MOS). These compounds serve as substrates for beneficial microorganisms like *Bifidobacteria* and *Lactobacillus*, which can outcompete pathogenic bacteria in the gastrointestinal tract, leading to enhanced health and growth performance of aquatic species (Goh et al., 2022). For instance, inulin has been extensively studied for its effects on fish and shellfish. In turbot larvae, dietary inclusion of inulin has been shown to increase the relative mass of the gastrointestinal tract and promote the proliferation of beneficial *Bacillus* species, while concurrently reducing harmful *Vibrio* species (Mahious et al., 2006). Similarly, mannan oligosaccharides have been used to enhance

the growth performance, feed efficiency, and immune responses in species such as rainbow trout and European sea bass. These prebiotics modulate the gut microbiota, leading to improved resistance against common pathogens like *Streptococcus* and *Mycobacterium* (Staykov et al., 2007; Torrecillas et al., 2007).

The beneficial effects of probiotic bacteria can be enhanced when used together with prebiotics and synbiotics. Synbiotics consist of a combination of probiotics and prebiotics, containing difficult-to-digest fibers that support the growth of beneficial commensal bacteria in the intestine. The beneficial effects provided by this combination arise from the by-products of the fermentation of commensal bacteria, which have the capacity to modulate the immune system. In particular, synbiotics can stimulate the immune systems of aquatic animals at both systemic and local levels through immunosaccharides (Diwan et al., 2021).

New research highlights the importance of parabiotics (dead probiotic cells) and postbiotics (probiotic culture supernatants) in the microbiome and disease formation (Goh et al., 2022). Metagenomic techniques and next-generation sequencing (NGS) now allow the identification of previously unculturable bacterial species, enhancing our understanding of microbiomes. This opens new research areas for antibiotic alternatives, better microbiome control, and improved health of aquatic organisms (Diwan et al., 2021).

Egg Yolk Immunoglobulin

Chicken egg yolk immunoglobulin (IgY) is a cost-effective antibody for passive immunization, produced in large quantities through chicken immunization. IgY has been successfully used in humans, livestock, and aquatic animals, offering greater stability than IgG (Baloch et al., 2015). It effectively controls various bacterial and viral pathogens in aquaculture, such as *Vibrio harveyi*, *V. anguillarum*, and *Aeromonas salmonicida* (Bondad-Reantaso et al., 2023). IgY can be administered by injection, immersion, or oral administration, providing passive immunity and enhancing resistance to disease in fish and shrimp (Gan et al., 2015; Winkelbach et al., 2015).

Nanoparticles

Nanoparticles are being used in aquaculture to address antibiotic issues by leveraging their production of reactive oxygen species to degrade antibiotics, thus mitigating pollution. For instance, titanium dioxide nanoparticles exhibit photocatalytic properties, killing a wide range of microorganisms including bacteria, fungi, and viruses (Foster et al., 2011). Furthermore, iron nanoparticles can break down toxic compounds in water, demonstrating the potential for antibiotic degradation (Majumder & Dash, 2017). This innovative approach improves the sustainability of aquaculture by reducing the use of traditional antibiotics (Fajardo et al., 2022).

Medicinal Plants

Medicinal plants and their derivatives have gained attention as antibiotic alternatives in aquaculture due to their low cost, ease of preparation, and minimal side effects (Tadese et al., 2022). These plants, including herbs, spices, seaweed, and traditional Chinese medicines, contain active ingredients such as polysaccharides, steroids, and secondary metabolites (Citarasu, 2010). They possess antimicrobial properties and stimulate both innate and specific immunity, increasing resistance to pathogens. Common immunostimulants derived from microbial cell walls include β-glucans, alginates, and polysaccharides, typically administered by bait or bath immersion (Harikrishnan et al., 2011; Tadese et al., 2022).

In recent years, a wide variety of medicinal plants have been studied for their effectiveness in aquaculture. For instance, *Euphorbia hirta* has been shown to improve resistance to *Aeromonas hydrophila* in sharptooth catfish (*Clarias gariepinus*) (Sheikhlar et al., 2017). *Ocimum sanctum*, commonly known as holy basil, has demonstrated immunostimulatory effects in *Oreochromis mossambicus*. Rosemary (*Rosmarinus officinalis*) has been used as a treatment against *Streptococcus iniae* in tilapia (*Oreochromis sp.*) (Abutbul et al., 2004; Tadese et al., 2022).

Furthermore, dietary administration of water hyacinth (*Eichhornia crassipes*) leaf extracts has been found to enhance innate immune parameters, antioxidant defense, and disease resistance in rainbow trout (*Oncorhynchus mykiss*) (Rufchaei et al., 2020). The use of *Coriandrum sativum* extract in diets has also shown positive effects on growth and immunity in fish (Farsani et al., 2019).

Recombinant Proteins

Recombinant proteins show great promise in combating infectious diseases in aquaculture (Mohammadzadeh et al., 2022). Recombinant hepcidin has demonstrated preventive and therapeutic effects against *Flavobacterium columnare* in grass carp by regulating iron distribution and immune gene expression (Wang et al., 2016). Another example is recombinant AHL-lactonase from *Bacillus sp.*, which reduced

mortality in common carp infected with *Aeromonas hydrophila* (Chen et al., 2010). Additionally, a vibrio phage recombinant endolysin expressed in *E. coli* effectively lysed *Vibrio parahaemolyticus* (Melo-López et al., 2021).

Conclusion

Aquaculture has faced significant challenges in recent years, including disease outbreaks and antimicrobial resistance, threatening its sustainability. This situation requires the investigation of alternative antimicrobial strategies. Various methods have emerged as potential solutions. Vaccines, for example, play a crucial role in disease prevention and control, effectively reducing antibiotic use, as demonstrated in Norwegian salmon farming. However, vaccines are generally limited to valuable fish species due to high costs and logistical difficulties. Recent technological advances have made oral and immersion vaccines more viable, though further research on their effectiveness is needed. Biological solutions such as probiotics and prebiotics offer significant opportunities to increase disease resistance by supporting the health of the microbiome. These microorganisms enhance intestinal health and the overall well-being of aquatic animals. Innovative methods such as IgY provide passive immunity, while phage therapy targets bacterial pathogens without harming the environment. Natural products like herbal treatments and immunostimulants offer low-cost, environmentally friendly alternatives. Although these strategies present a holistic approach to prevention and control in aquaculture, their effectiveness varies depending on environmental conditions, species, and pathogen characteristics. Therefore, it is crucial to tailor strategies to local ecosystem needs, recognizing that these methods work synergistically and require continuous development and adaptation. Integrating the most effective and sustainable methods, while considering the risks, limitations, and potential benefits of each strategy, is critical for the future of aquaculture.

Compliance With Ethical Standards

Authors' Contributions

NK: Conceptualization, Writing – original draft, Writing – review and editing, Supervision

GG: Writing – original draft, Data curation, Visualization

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

Not applicable.

Data Availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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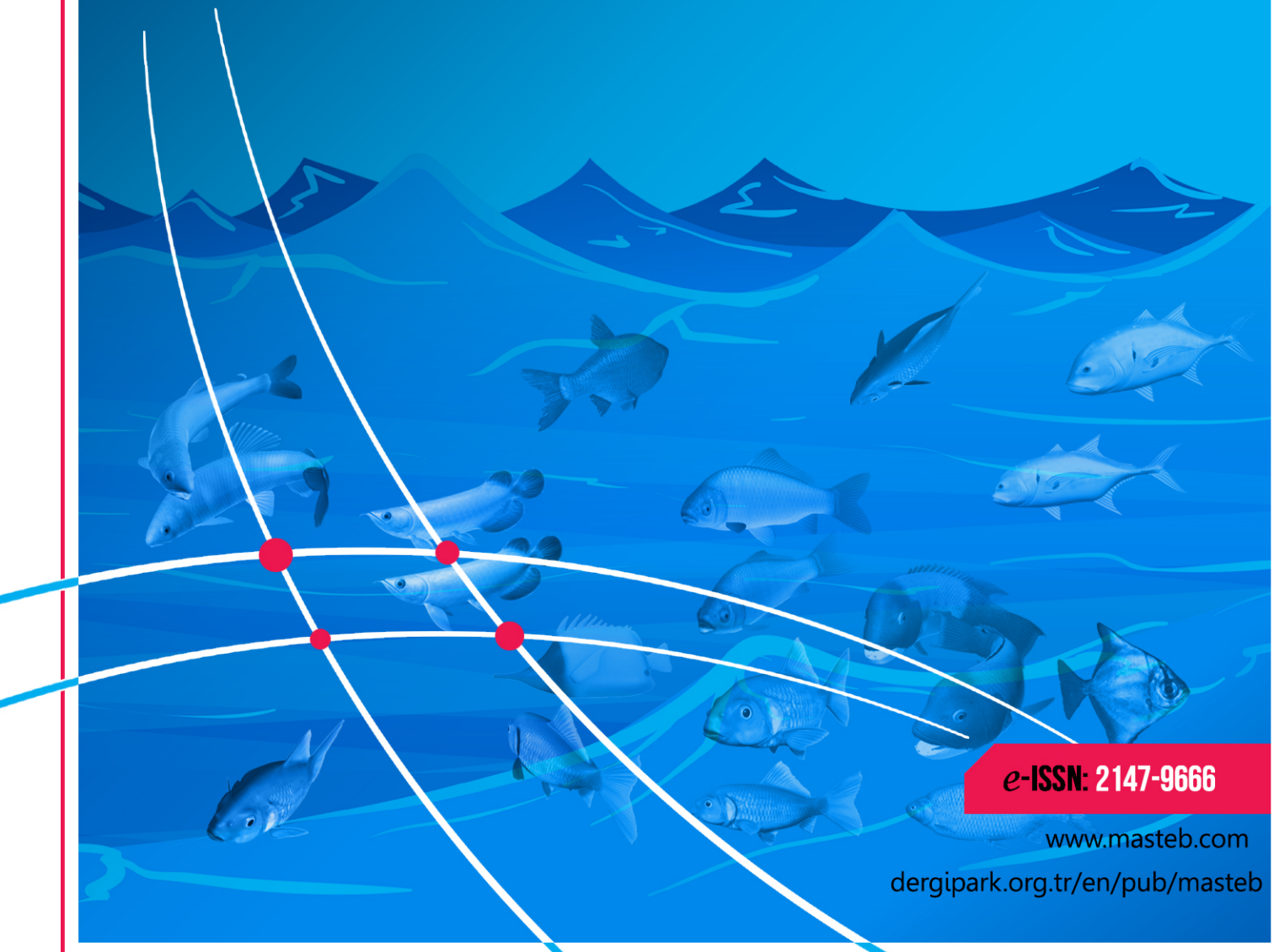

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