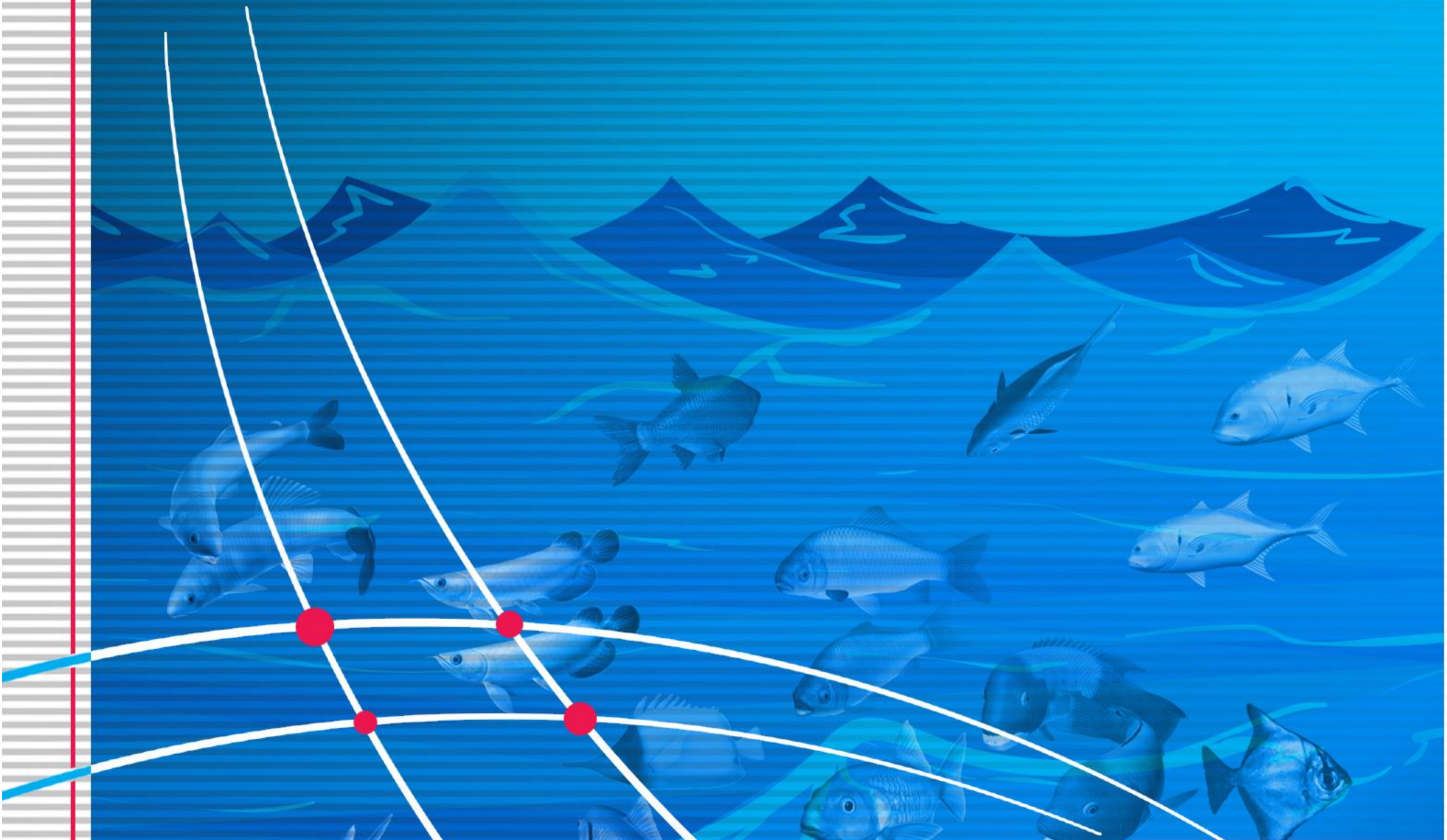
MARINE SCIENCE AND TECHNOLOGY BULLETIN

Volume 11 - Issue 2 - YEAR 2022



e-ISSN: 2147-9666

www.masteb.com
dergipark.org.tr/en/pub/masteb

MARINE SCIENCE AND TECHNOLOGY BULLETIN

2022 • VOLUME: 11 • ISSUE: 2

MARINE SCIENCE AND TECHNOLOGY BULLETIN 2022 • VOLUME 11 • ISSUE 2

T A B L E   O F   C O N T E N T S

MARINE SCIENCE AND TECHNOLOGY BULLETIN 2022 • VOLUME 11 • ISSUE 2

TABLE OF CONTENTS

Mar. Sci. Tech. Bull. (2022) 11(2): 144-157
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1082427

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Fatty acid composition and mRNA expression of fatty acid binding protein genes (*fabp3* and *fabp6*) in rainbow trout fed camelina seed oil (*Camelina sativa*)-based diets

Sinem Keşan[1] • Mehtap Bayır[1*] • Gökhan Arslan[2]

[1] *Atatürk University, Faculty of Agriculture, Department of Agricultural Biotechnology, 25240, Erzurum, Turkey*
[2] *Atatürk University, Faculty of Fisheries, Department of Fisheries and Fish Processing Technology, 25240, Erzurum, Turkey*

ARTICLE INFO

Article History:
Received: 03.03.2022
Received in revised form: 23.03.2022
Accepted: 24.03.2022
Available online: 30.03.2022

Keywords:
Gene expression
Fatty acid composition
Camelina seed oil
Rainbow trout

ABSTRACT

Vegetable lipids such as camelina oil (*Camelina sativa*) are used as alternatives oil sources to fish oil in aquafeeds. In this study, we determined fatty acid-binding protein 3 (*fabp3*) and fatty acid-binding protein 6 (*fabp6*) gene expression and fatty acid composition in the liver and muscle tissue of rainbow trout fed different amounts of dietary camelina seed oil [100% (CO100), 67% (CO67), and 37% (CO33)]. Palmitic acid and oleic acid were identified as the most abundant saturated and monounsaturated fatty acids, respectively, in both tissues across all experimental groups. The highest levels of n-6 polyunsaturated fatty acid (Σn- 6 PUFA) were found in the first biopsy (15^{th} day) taken from fish fed a diet of CO100, while the highest Σn–3 PUFA level was found in the third biopsy (45^{th} day) taken from the same group. The FO100 (fish oil) diet was found to have the highest Σn-3 / n-6 ratio, as well as the highest levels of eicosapentaenoic acid and docosahexaenoic acid. In general, the fatty acid composition of the fish reflected that of their respective diets. The expression of *fabp3* and *fabp6* genes in the muscle of fish fed camelina seed oil were not significantly different from control group. However, *fabp3* gene expression of liver of FO100 group was found to have significantly higher than CO67 and CO33. A difference in hepatic *fabp6* gene expression was also noted in the FO100 group, but was not found to be statistically significant. Growth parameters and survival rate were not affected after the 45 days feeding trial. These results suggest that camelina seed oil can be used as an alternative to fish oil in rainbow trout diet.

Please cite this paper as follows:

Keşan, S., Bayır, M., & Arslan, G. (2022). Fatty acid composition and mRNA expression of fatty acid binding protein genes (*fabp3* and *fabp6*) in rainbow trout fed camelina seed oil (*Camelina sativa*)-based diets. *Marine Science and Technology Bulletin*, *11*(2), 144-157. https://doi.org/10.33714/masteb.1082427

* Corresponding author
E-mail address: mehtap.bayir@atauni.edu.tr (M. Bayır)

Introduction

Nutrigenomics is a multidisciplinary science that combines the fields of nutrition, molecular biology, molecular medicine, genomics, epidemiology and bioinformatics. It uses transcriptomics, proteomics, and metabolomics to explore how dietary ingredients can affect the balance between health and disease, and to identify the effects of nutrients on gene expression, transcription activity and the heterogenous response of gene variants (Mutch et al., 2005). Advances in these technologies have led to a better understanding of the underlying mechanisms of animal nutrition, and could lead to the development of healthier and more economic animal products with reduced risk of disease (Mutch et al., 2005).

Rainbow trout (*Oncorhynchus mykiss*) is the most widely cultured cold freshwater fish in the world, and total production of Rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) are 3.284 million tonnes in 2018 (FAO, 2020). *α*-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA, linoleic acid (LA), and arachidonic acid (ARA) are important for healthy daily life and activity (Baysal, 2004). Aquafeeds containing sufficient essential fatty acids (FAs) are of great importance, particularly during the pre-breeding development of female fish, egg production, and in the early stages of fish development (Çetinkaya, 1995; Bayır, 2011). Deficiencies of these FAs in fish feed can lead to a decrease in hatching rate, and may cause disorders in the embryos. Fatty acid-binding protein FABPs are small (12-15 kDa) intracellular proteins that exist as several distinct types, including liver, intestinal, and adipocyte types. They serve an important role in protecting cells from the repercussions of fatty acids (Glatz & van der Vusse, 1996; Niewold et al., 2004).

Naturally-fed animal meat, poultry eggs, and flaxseed oils are rich in n-3 FAs, while n-6 FAs are found in plants such as sunflower, soybean, and cotton (Aydın, 2004). Fish oil is an important source of the essential FAs EPA and DHA, and is therefore used as a component of fish feed. However, the cost of fish oil has been increasing, due in part to its importance in human health, its use in feed for terrestrial animals, and its stable worldwide production. Studies have shown that cheaper vegetable oil (VO) and meal sources can be used as substitutes for fish oil (FO) and fish meal in the fish feed industry, and different sources of oil and meal have been investigated for this purpose. (Torstensen et al., 2000; Caballero et al., 2002; Bell et al., 2001, 2002; Montero et al., 2003, 2005; Izquierdo et al., 2003, 2005; Ganga et al., 2011). Studies conducted with VOs show that alternative oil sources can be used partially or completely as a substitute for FO, without adversely affecting the growth performance of fish, and meet the species growth requirement (Bayır, 2011). The availability of VOs that can be used as suitable alternatives to FO will reduce both the fish feed industry's dependence on FO, and the cost of feed (Mourente & Bell, 2006). Camelina oil (CO; *Camelina sativa*) contains less monounsaturated fatty acids (MUFAs) than peanut or rape oil, but more than flax, soybean, and cotton oil. CO contains a similar concentration of PUFAs to that found in soybean and sunflower oil, which is less than in flax oil, but more than in cotton, peanut, and rape oil (Legendre et al., 1995). The composition of saturated fatty acids (SFAs) and PUFAs in CO is also similar to sunflower oil, but CO contains a significantly higher proportion of n-3 PUFA (Kurt & Seyis, 2008). Previous research has investigated changes in gene expression levels in multiple species of fish that were fed different diets using various VOs instead of FOs (Tocher et al., 2003; Ruyter et al., 2000; Jordal et al., 2005; Zheng et al., 2005; Torstonsen et al., 2009). Fabps are found in tissues that are highly active in FA metabolism, and are involved in lipid uptake, transport, and homeostasis (Bayır et al., 2015). However, no studies have yet determined how a vegetable oil-based diet may alter mRNA levels of the *fabp3* and *fabp6* genes, which encode proteins involved in intracellular transport in rainbow trout. This study aims to investigate changes in expression of *fabp3* and *fabp6* in rainbow trout fed with CO, which are the FAs in intracellular transport, and to determine differences in the FA composition of liver and muscle tissues in these fish.

Material and Methods

Husbandry of Rainbow Trout

A total of 44 rainbow trout (97.45±15.05 g average weight) were weighed and stocked in 12 experimental tanks. Fish were fed commercial trout feed for 15 days twice daily as 2% of the body. The tank water temperature was 16.0°C, and the dissolved oxygen concentration of the water was 9.3 mg/l.

Four experiments were conducted, the first of which was performed using a FO-fed control group. In each of the other three experiments, fish oil feed was replaced with a feed of 33% CO (CO33), 67% CO (CO67), or 100% CO (CO100) (Table 1). Fish were each randomly assigned to one of the four treatment groups at the beginning of the experiment, and were fed to apparent satiation four times daily for 45 days. Working protocols were approved by Atatürk University Local Ethical Committee for Animal Studies. Liver and muscle samples were

Table 1. 5′ → 3′ Sequences of primers (Bayır et al., 2015)

Rainbow trout genes	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Tm (°C)	qPCR efficiency
fabp3	ATGAAGGCTCTGGGTGTGG	TCCTTGCCATCCCACTTCTG	54.8	1.04
fabp6	GGGAAAAAGTTCAAGGCCAC	GCTGGTTCTTTTCAGCACGA	57.4	0.94
ß-actin	CTTCTACAACGAGCTGAGGGT	GGTCTCAAACATGATCTGGGT	57.0	0.93

Table 2. Fatty acid composition of rainbow trout diets

Fatty Acid	Cod Liver Oil (100 %)	Camelina Seed Oil (100%)	Camelina Seed Oil (67%)	Camelina Seed Oil (33%)
14:0	6.6	1.6	3.0	4.7
15:0	0.2	0.3	0.5	0.8
16:0	23.7	11.7	14.3	17.6
17:0	0.3	0.4	0.6	0.9
18:0	4.8	6.2	3.5	4.5
20:0	1.6	0.3	0.8	1.2
22:0	0.9	1.4	1.5	1.5
24:0	0.2	0.1	0.2	0.2
ΣSFA	**40.3**	**22.0**	**24.8**	**31.4**
14:1	0.3	0.1	0.2	0.2
15:1	0.3	0.2	0.5	0.3
16:1	6.5	1.6	3.1	4.6
17:1	0.7	0.2	0.4	0.5
18:1n-9	16.7	14.8	17.1	17.0
20:1n-9	–	–	–	–
22:1	0.5	0.2	0.4	0.5
24:1n-9	0.2	0.1	0.1	0.2
ΣMUFA	**25.3**	**17.2**	**21.7**	**23.3**
18:2n-6	6.5	18,5	14.5	11.4
18:3n-6	0.2	0.1	0.2	0.3
20:2n-6	1.5	8.6	6.2	4.1
20:3n-6	0.1	0.1	_	0.1
20:4n-6	0.8	0.7	0.6	0.6
22:2n-6	0.2	0.1	0.1	0.2
22:4n-6	0.1	0.2	0.1	0.1
Σn-6 PUFA	**9.3**	**28.2**	**21.7**	**16.8**
18:3n-3	2.0	24.2	17.3	10.0
18:4n-3	0.1	0.1	0.1	0.1
20:3n-3	0.3	1,1	0.9	0.6
20:4n-3	0.1	0,3	0.5	0.2
22:5n-3	0.3	0.2	0.4	0.5
20:5n-3	8.4	2.3	4.5	6.2
22:6n-3	13.8	4.5	8.3	11.0
Σn-3 PUFA	**25.1**	**32.6**	**31.8**	**28.6**
EPA+DHA	**22.2**	**6.7**	**12.7**	**17.2**
Σn-3/Σn-6 PUFA	**2.7**	**1.2**	**1.5**	**1.7**

taken from the fish four times (15th, 30th, and 45th days of the experiment) throughout the trial. At the end of the trial the fish were euthanized, and their liver and muscle weights recorded. The muscle and liver samples intended for study of mRNA expression were stored in RNAlater at −80°C until RNA isolation could be performed. The other samples were frozen immediately in liquid nitrogen and stored at −80°C until their FA composition could be analyzed. At the end of the study, growth parameters of rainbow trout were calculated as follow:

$$SGR(\%day^{-1}) = 100 \times \frac{\ln(Final\ weight) - \ln(Initial\ weight)}{Feeding\ days} \quad (1)$$

$$WG(\%) = 100 \times \frac{Final\ weight - Initial\ weight}{Initial\ weight} \quad (2)$$

$$SR(\%) = 100 \times \frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} \quad (3)$$

where *SGR* is the specific growth rate, *WG* is the weight gain, *SR* is the survival rate.

Lipid and Fatty Acid Analysis

Lipid analysis was performed using the Folch et al. (1957) method for lipid extraction. Accordingly, the liver and muscle tissue samples of fish from each tank were homogenized with a ratio of 2:1 v/v chloroform/methanol containing 0.01% (w/v) butylated hydroxytoluene (Sigma, ≥99.0% gas chromatography). The organic solvent was allowed to evaporate under a nitrogen stream before the lipid content was determined gravimetrically.

Table 3. Fatty acid composition of 100% fish oil and 100% camelina seed in muscle tissue (mean±SD)

Fatty acids	Initial	100% Fish Oil			100% Camelina Seed Oil		
		First sample (15th day)	*Second sample (30th day)*	*Third sample (45th day)*	*First sample (15th day)*	*Second sample (30th day)*	*Third sample (45th day)*
14:0	2.0 ± 0.2	3.0 ± 0.1^a	2.8 ± 0.0^a	3.0 ± 0.2^a	2.0 ± 0.0^b	1.9 ± 0.0^b	1.7 ± 0.0^b
15:0	0.3 ± 0.0	0.4 ± 0.0^a	0.5 ± 0.0^a	0.5 ± 0.0^a	0.2 ± 0.0^b	0.4 ± 0.0^b	0.3 ± 0.0^b
16:0	15.6 ± 0.3	17.6 ± 0.2^a	21.0 ± 0.3^a	21.4 ± 0.1^a	16.7 ± 0.1^a	17.9 ± 0.5^b	19.1 ± 0.1b
17:0	0.5 ± 0.0	0.4 ± 0.0^a	0.5 ± 0.0^a	0.5 ± 0.0^a	0.4 ± 0.0^a	0.5 ± 0.0^a	0.4 ± 0.0^b
18:0	4.7 ± 0.3	4.7 ± 0.1^a	5.0 ± 0.1^a	4.8 ± 0.3^a	4.5 ± 0.0^a	5.2 ± 0.1^a	4.9 ± 0.1^a
20:0	0.6 ± 0.1	0.5 ± 0.0^b	0.5 ± 0.0^b	0.6 ± 0.0^b	0.8 ± 0.0^a	0.7 ± 0.0^a	0.7 ± 0.0^a
22:0	1.0 ± 0.0	0.9 ± 0.1^b	0.6 ± 0.1^a	0.5 ± 0.0^b	1.0 ± 0.0^a	0.6 ± 0.1^a	0.7 ± 0.1^a
24:0	0.4 ± 0.1	0.7 ± 0.0^a	0.4 ± 0.0^b	0.3 ± 0.0^b	0.3 ± 0.0^b	0.5 ± 0.1^a	0.4 ± 0.2^a
ΣSFA	**25.0 ± 0.5**	**28.3 ± 0.2^a**	**31.4 ± 0.2^a**	**31.5 ± 0.2^a**	**25.9 ± 0.2^b**	**27.7 ± 0.6^b**	**28.2 ± 0.3^b**
14:1	0.1 ± 0.0	0.1 ± 0.0^a	0.1 ± 0.0^a	0.1 ± 0.0^a	0.1 ± 0.0^a	0.2 ± 0.1^a	0.1 ± 0.0^a
15:1	0.3 ± 0.1	0.3 ± 0.0^a	0.2 ± 0.1^b	0.2 ± 0.0^b	0.3 ± 0.1^a	0.3 ± 0.1^a	0.3 ± 0.1^a
16:1	2.9 ± 0.3	3.9 ± 0.1^a	5.9 ± 0.3^a	5.3 ± 0.2^a	3.2 ± 0.1^b	3.3 ± 0.2^b	3.1 ± 0.1^b
17:1	0.3 ± 0.0	0.4 ± 0.0^a	0.4 ± 0.0^a	0.4 ± 0.0^a	0.3 ± 0.0^b	0.3 ± 0.0^b	0.3 ± 0.0^b
18:1n-9	26.2 ± 0.2	22.7 ± 0.1^b	24.0 ± 0.1^a	20.7 ± 0.5^a	24.8 ± 0.2^a	20.6 ± 0.4^b	19.9 ± 0.3^a
20:1n-9	0.4 ± 0.0	0.2 ± 0.0^a	0.2 ± 0.0^a	0.1 ± 0.0^a	0.2 ± 0.0^a	0.2 ± 0.0^a	_
22:1	0.4 ± 0.0	0.6 ± 0.0^a	0.5 ± 0.0^a	0.5 ± 0.0^a	0.4 ± 0.0^b	0.2 ± 0.0^b	0.5 ± 0.0^a
24:1n-9	0.4 ± 0.1	0.6 ± 0.1^a	0.5 ± 0.1^b	0.1 ± 0.0^a	0.3 ± 0.0^b	1.1 ± 0.2^a	0.2 ± 0.0^a
ΣMUFA	**30.9 ± 0.4**	**28.8 ± 0.1^b**	**31.8 ± 0.3^a**	**27.5 ± 0.4^a**	**29.5 ± 0.2^a**	**26.2 ± 0.4^b**	**24.4 ± 0.3^b**
18:2n-6	12.9 ± 0.4	10.6 ± 0.1^b	8.0 ± 0.1^b	8.1 ± 0.2^b	12.4 ± 0.1^a	11.1 ± 0.4^a	11.4 ± 0.1^a
18:3n-6	0.2 ± 0.0	0.3 ± 0.1^a	0.2 ± 0.0^a	0.1 ± 0.0^b	0.2 ± 0.0^a	0.2 ± 0.0^a	0.3 ± 0.1^a
20:2n-6	2.0 ± 0.2	1.7 ± 0.0^b	1.8 ± 0.0^b	1.5 ± 0.1^b	3.3 ± 0.1^a	2.8 ± 0.2^a	3.5 ± 0.1^a
20:3n-6	0.2 ± 0.1	0.3 ± 0.0^a	0.3 ± 0.0^a	0.2 ± 0.0^b	0.1 ± 0.0^b	0.1 ± 0.0^b	0.3 ± 0.0^a
20:4n-6	1.1 ± 0.0	1.1 ± 0.0^a	1.1 ± 0.0^a	1.0 ± 0.0^a	0.9 ± 0.0^a	1.0 ± 0.0^a	1.0 ± 0.0^a
22:2n-6	0.2 ± 0.0	0.3 ± 0.0^a	0.1 ± 0.0^a	0.1 ± 0.0^a	0.2 ± 0.0^b	0.1 ± 0.0^a	0.1 ± 0.0^a
22:4n-6	0.1 ± 0.0	0.2 ± 0.0^a	0.1 ± 0.0^b	0.1 ± 0.0^b	0.1 ± 0.0^b	0.4 ± 0.1^a	0.1 ± 0.0^b
Σn-6 PUFA	**16.7 ± 0.4**	**14.4 ± 0.1^b**	**11.6 ± 0.2^b**	**11.0 ± 0.1^b**	**17.3 ± 0.1^a**	**15.8 ± 0.6^a**	**16.5 ± 0.1^a**
18:3n-3	2.9 ± 0.3	2.4 ± 0.0^b	2.4 ± 0.0^b	3.1 ± 0.1^b	6.0 ± 0.1^a	7.3 ± 0.1^a	7.4 ± 0.3^a
18:4n-3	0.3 ± 0.0	0.2 ± 0.0^a	0.2 ± 0.0^a	0.1 ± 0.0^b	0.2 ± 0.0^a	0.2 ± 0.0^a	0.2 ± 0.0^a
20:3n-3	0.8 ± 0.1	0.9 ± 0.0^b	0.7 ± 0.0^b	0.6 ± 0.0^b	1.1 ± 0.0^a	1.0 ± 0.0^a	1.2 ± 0.0^a
20:4n-3	0.6 ± 0.0	0.6 ± 0.0^a	0.5 ± 0.0^b	0.4 ± 0.0^b	0.6 ± 0.0^a	0.6 ± 0.0^a	0.6 ± 0.0^a
20:5n-3	4.3 ± 0.2	1.4 ± 0.0^a	3.7 ± 0.0^a	4.3 ± 0.1^a	0.9 ± 0.0^b	3.6 ± 0.1^a	3.3 ± 0.0^b
22:5n-3	1.1 ± 0.1	4.4 ± 0.2^a	0.9 ± 0.1^a	1.0 ± 0.0^a	3.4 ± 0.1^b	0.8 ± 0.1^a	0.8 ± 0.0^a
22:6n-3	17.8 ± 0.3	18.7 ± 0.3^a	16.7 ± 0.1^a	20.3 ± 0.2^a	15.3 ± 0.2^b	16.7 ± 0.7^a	17.4 ± 0.5^b
Σn-3 PUFA	**27.7 ± 0.4**	**28.6 ± 0.4^a**	**25.2 ± 0.2^b**	**29.8 ± 0.4^b**	**27.4 ± 0.2^b**	**30.3 ± 0.7^a**	**30.8 ± 0.4a**
EPA+DHA	**22.0 ± 0.3**	**23.1 ± 0.4^a**	**20.5 ± 0.1^a**	**24.6 ± 0.3^a**	**18.7 ± 0.2^b**	**20.3 ± 0.8^a**	**20.7 ± 0.5^b**
Σn-3/Σn-6 PUFA	**1.7 ± 0.0**	**2.0 ± 0.0^a**	**2.2 ± 0.0^a**	**2.7 ± 0.1^a**	**1.6 ± 0.0^b**	**1.9 ± 0.1^b**	**1.9 ± 0.0^b**

Note: SD is the standard deviation, n=6. Different letters indicate groups that differ from each other (p<0.05)

Table 4. Fatty acid composition of 67% and 33% camelina seed in muscle tissue (mean±SD)

67% Camelina Oil				33% Camelina Seed Oil			
Fatty acids	*Initial*	*First sample (15th day)*	*Second sample (30th day)*	*Fatty acids*	*Initial*	*First sample (15th day)*	*Second sample (30th day)*
14:0	2.0 ± 0.2	2.4 ± 0.1^{b}	2.2 ± 0.1^{b}	2.3 ± 0.2^{b}	2.7 ± 0.1^{b}	2.6 ± 0.0^{b}	2.6 ± 0.1^{b}
15:0	0.3 ± 0.0	0.4 ± 0.0^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	0.4 ± 0.0^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}
16:0	15.6 ± 0.3	16.1 ± 0.1^{b}	19.3 ± 0.4^{b}	18.9 ± 0.4^{b}	16.5 ± 0.1^{b}	19.2 ± 0.1^{b}	20.7 ± 0.1^{b}
17:0	0.5 ± 0.0	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.4 ± 0.0^{b}	0.5 ± 0.1^{a}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}
18:0	4.7 ± 0.3	4.6 ± 0.0^{a}	5.3 ± 0.2^{a}	4.4 ± 1.0^{a}	4.4 ± 0.2^{c}	5.0 ± 0.1^{b}	5.0 ± 0.1^{a}
20:0	0.6 ± 0.1	0.9 ± 0.0^{a}	0.7 ± 0.0^{a}	0.6 ± 0.0^{a}	0.7 ± 0.1^{b}	0.6 ± 0.0^{b}	0.5 ± 0.0^{b}
22:0	1.0 ± 0.0	1.3 ± 0.2^{a}	0.7 ± 0.1^{a}	0.7 ± 0.0^{a}	1.2 ± 0.0^{ab}	0.7 ± 0.1^{a}	0.6 ± 0.0^{b}
24:0	0.4 ± 0.1	0.4 ± 0.1^{b}	0.3 ± 0.1^{b}	0.4 ± 0.2^{a}	0.3 ± 0.0^{c}	0.2 ± 0.0^{c}	0.4 ± 0.1^{a}
ΣSFA	**25.0 ± 0.5**	**26.5 ± 0.5^{b}**	**29.4 ± 0.9^{b}**	**27.9 ± 1.0^{b}**	**26.7 ± 0.2^{b}**	**29.2 ± 0.1^{b}**	**30.7 ± 0.1^{a}**
14:1	0.1 ± 0.0	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
15:1	0.3 ± 0.1	0.3 ± 0.0^{a}	0.1 ± 0.0^{b}	0.3 ± 0.1^{a}	0.3 ± 0.0^{a}	0.1 ± 0.0^{b}	0.5 ± 0.1^{a}
16:1	2.9 ± 0.3	3.3 ± 0.2^{b}	3.9 ± 0.2^{b}	4.5 ± 1.0^{b}	4.0 ± 0.5^{a}	4.6 ± 0.1^{b}	4.0 ± 0.1^{c}
17:1	0.3 ± 0.0	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.1^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}
18:1n-9	26.2 ± 0.2	25.0 ± 0.4^{a}	22.2 ± 0.6^{b}	23.2 ± 0.5^{a}	27.1 ± 0.3^{a}	23.5 ± 0.3^{b}	20.8 ± 0.1^{b}
20:1n-9	0.4 ± 0.0	0.2 ± 0.0^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.1 ± 0.0^{a}
22:1	0.4 ± 0.0	0.4 ± 0.0^{b}	0.5 ± 0.0^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}
24:1n-9	0.4 ± 0.1	0.4 ± 0.2^{b}	0.4 ± 0.0^{b}	0.6 ± 0.5^{a}	0.3 ± 0.0^{c}	0.2 ± 0.1^{c}	0.4 ± 0.1^{b}
ΣMUFA	**30.9 ± 0.4**	**30.2 ± 0.4^{a}**	**27.7 ± 0.8^{b}**	**29.3 ± 0.6^{a}**	**32.8 ± 0.8^{a}**	**29.8 ± 0.3^{b}**	**26.8 ± 0.2^{c}**
18:2n-6	12.9 ± 0.4	11.6 ± 0.3^{a}	10.9 ± 0.1^{a}	12.9 ± 0.8^{a}	12.4 ± 0.1^{a}	9.5 ± 0.1^{b}	10.6 ± 0.1^{b}
18:3n-6	0.2 ± 0.0	0.2 ± 0.0^{b}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.1 ± 0.0^{c}	0.2 ± 0.0^{a}	0.1 ± 0.0^{b}
20:2n-6	2.0 ± 0.2	3.5 ± 0.3^{a}	2.7 ± 0.0^{a}	2.6 ± 0.0^{a}	2.8 ± 0.1^{b}	2.3 ± 0.0^{b}	2.0 ± 0.1^{b}
20:3n-6	0.2 ± 0.1	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.1 ± 0.0^{b}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}	0.2 ± 0.0^{a}
20:4n-6	1.1 ± 0.0	0.9 ± 0.0^{b}	1.0 ± 0.1^{a}	0.8 ± 0.1^{b}	0.8 ± 0.0^{c}	1.1 ± 0.0^{a}	1.0 ± 0.0^{a}
22:2n-6	0.2 ± 0.0	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
22:4n-6	0.1 ± 0.0	0.1 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
Σn-6 PUFA	**16.7 ± 0.4**	**16.6 ± 0.1^{a}**	**15.2 ± 0.1^{a}**	**16.9 ± 0.8^{a}**	**16.8 ± 0.2^{a}**	**13.5 ± 0.1^{c}**	**14.0 ± 0.1^{b}**
18:3n-3	2.9 ± 0.3	5.7 ± 0.3^{a}	5.4 ± 0.2^{a}	5.6 ± 0.4^{a}	4.1 ± 0.1^{b}	4.0 ± 0.0^{b}	4.4 ± 0.1^{b}
18:4n-3	0.3 ± 0.0	0.3 ± 0.0^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{a}	0.1 ± 0.0^{a}
20:3n-3	0.8 ± 0.1	1.0 ± 0.0^{b}	1.0 ± 0.0^{b}	1.0 ± 0.1^{b}	1.0 ± 0.0^{a}	0.8 ± 0.0^{a}	0.9 ± 0.0^{b}
20:4n-3	0.6 ± 0.0	0.5 ± 0.0^{b}	0.7 ± 0.0^{a}	0.5 ± 0.0^{a}	0.4 ± 0.0^{c}	0.5 ± 0.0^{b}	0.5 ± 0.0^{a}
20:5n-3	4.3 ± 0.2	1.0 ± 0.1^{b}	3.4 ± 0.0^{a}	3.2 ± 0.2^{b}	0.8 ± 0.0^{c}	4.0 ± 0.1^{a}	3.8 ± 0.1^{b}
22:5n-3	1.1 ± 0.1	3.8 ± 0.2^{b}	0.9 ± 0.0^{a}	0.9 ± 0.2^{a}	3.4 ± 0.2^{c}	1.0 ± 0.1^{a}	0.9 ± 0.1^{b}
22:6n-3	17.8 ± 0.3	14.6 ± 0.3^{b}	16.0 ± 0.3^{a}	14.5 ± 0.3^{b}	13.7 ± 0.7^{c}	16.7 ± 0.3^{a}	18.0 ± 0.3^{b}
Σn-3 PUFA	**27.7 ± 0.4**	**26.7 ± 0.4^{b}**	**27.6 ± 0.2^{a}**	**25.8 ± 0.4^{b}**	**23.6 ± 0.8^{c}**	**27.4 ± 0.5^{a}**	**28.6 ± 0.3^{a}**
EPA+DHA	**22.0 ± 0.3**	**18.4 ± 0.2^{b}**	**19.4 ± 0.3^{b}**	**17.6 ± 0.2^{b}**	**17.0 ± 0.9^{b}**	**20.7 ± 0.4^{a}**	**21.8 ± 0.3^{b}**
Σn-3/Σn-6 PUFA	**1.7 ± 0.0**	**1.6 ± 0.0^{b}**	**1.8 ± 0.0^{b}**	**1.5 ± 0.1^{b}**	**1.4 ± 0.1^{c}**	**2.0 ± 0.0^{a}**	**2.0 ± 0.0^{b}**

Note: SD is the standard deviation, n=6. Different letters indicate groups that differ from each other ($p<0.05$)

Preparation of Fatty Acid Methyl Esters (FAMEs)

The crude lipids obtained from the samples were weighed and transferred to clean tubes, to which 1.5 ml of 2 M NaOH was added. The saponification process was performed by filling the tubes with nitrogen gas and subjecting them to a temperature of 80°C for 1 hour. After cooling the samples, 2 ml of BF_3 (25% of brontrifluoride methanol) was then added, and the tubes were again filled with nitrogen and kept at 80°C for 30 minutes. After the incubation period, the samples were allowed to cool again. 1 ml of hexane was added and the samples were vortexed; following this, 1 ml of ultrapure water was added and the samples were vortexed again. The hexane layer in each tube was then taken and transferred to a new tube containing sodium sulphate. After adding another 1 ml of hexane and vortexed again, the upper hexane layer of each tube was transferred to 2 ml GC vials, which were then filled with nitrogen (Metcalfe & Schmitz, 1961). Finally, the vials were placed on the Hewlett Packard Agilent 6890 N model gas chromatography (GC) for analysis of FAME (Bayır, 2011).

Table 5. Fatty acid composition of 100% fish oil and 100% camelina seed in liver tissue(mean±SD)

Fatty Acids	Initial	100% Fish Oil			100% Camelina Seed Oil		
		First sample (15th day)	*Second sample (30th day)*	*Third sample (45th day)*	*First sample (15th day)*	*Second sample (30th day)*	*Third sample (45th day)*
14:0	1.0 ± 0.0	2.8 ± 0.2ᵃ	2.8 ± 0.0ᵃ	3.0 ± 0.2ᵃ	1.1 ± 0.0ᵇ	1.0 ± 0.0ᵇ	1.0 ± 0.0ᵇ
15:0	0.2 ± 0.0	0.4 ± 0.0ᵃ	0.5 ± 0.0ᵃ	0.5 ± 0.0ᵃ	0.2 ± 0.0ᵃᵇ	0.2 ± 0.0ᵇ	0.2 ± 0.0ᵇ
16:0	16.8 ± 0.1	17.7 ± 0.1ᵃ	21.0 ± 0.3ᵃ	21.4 ± 0.1ᵃ	15.4 ± 0.2ᶜ	16.4 ± 0.4ᵃ	14.1 ± 0.4ᵃ
17:0	0.4 ± 0.0	0.4 ± 0.0ᵃ	0.5 ± 0.0ᵃ	0.5 ± 0.0ᵃ	0.3 ± 0.0ᵃ	0.5 ± 0.0ᵃ	0.3 ± 0.0ᵇ
18:0	7.2 ± 0.2	4.7 ± 0.1ᵃ	5.0 ± 0.1ᵇ	4.8 ± 0.3ᵇ	5.6 ± 0.3ᵃᵇ	7.8 ± 0.1ᵃ	6.0 ± 0.0ᵃ
20:0	0.2 ± 0.0	0.5 ± 0.0ᵃ	0.5 ± 0.0ᵃ	0.6 ± 0.0ᵃ	0.4 ± 0.0ᵃ	0.3 ± 0.0ᵃ	0.3 ± 0.0ᵃ
22:0	0.3 ± 0.0	0.9 ± 0.1ᵃ	0.6 ± 0.1ᵃ	0.5 ± 0.0ᵃ	0.5 ± 0.0ᵃ	0.3 ± 0.0ᵃ	0.3 ± 0.0ᵃ
24:0	0.4 ± 0.1	0.6 ± 0.0ᵃ	0.4 ± 0.0ᵇ	0.3 ± 0.0ᵇ	0.5 ± 0.1ᵇ	0.3 ± 0.0ᵈ	0.5 ± 0.1ᵃ
ΣSFA	**26.5 ± 0.2**	**28.2 ± 0.2ᵃ**	**31.4 ± 0.2ᵃ**	**31.5 ± 0.2ᵃ**	**24.0 ± 0.3ᵇ**	**26.8 ± 0.5ᵇ**	**22.7 ± 0.5ᵇ**
14:1	0.1 ± 0.0	0.1 ± 0.0ᵃ	0.1 ± 0.0ᵃ	0.1 ± 0.0ᵃ	0.1 ± 0.0ᵃ	_	0.1 ± 0.0ᵃ
15:1	0.2 ± 0.0	0.3 ± 0.0ᵃ	0.2 ± 0.1ᵃ	0.2 ± 0.0ᵃ	0.2 ± 0.0ᵃ	0.1 ± 0.0ᵃ	0.1 ± 0.0ᵇ
16:1	2.5 ± 0.1	3.9 ± 0.1ᵇ	5.9 ± 0.3ᵃ	5.3 ± 0.2ᵇ	3.4 ± 0.1ᵈ	2.4 ± 0.4ᵇ	3.0 ± 0.2ᶜ
17:1	0.2 ± 0.0	0.4 ± 0.0ᵃ	0.4 ± 0.0ᵃ	0.4 ± 0.0ᵇ	0.3 ± 0.0ᵃᵇ	0.4 ± 0.0ᵃ	0.3 ± 0.0ᶜ
18:1n-9	21.2 ± 0.3	22.8 ± 0.0ᵇ	24.0 ± 0.1ᵃ	20.7 ± 0.5ᵇ	21.6 ± 0.1ᵈ	17.2 ± 0.1ᶜ	19.9 ± 1.1ᵈ
20:1n-9	0.1 ± 0.0	0.2 ± 0.0ᵃ	0.2 ± 0.0ᵃ	0.1 ± 0.0ᵃ	0.2 ± 0.1ᵃ	0.1 ± 0.0ᵃ	_
22:1	0.5 ± 0.0	0.6 ± 0.0ᵇ	0.5 ± 0.0ᵇ	0.5 ± 0.0ᵇ	0.6 ± 0.0ᶜ	0.7 ± 0.0ᵇ	0.7 ± 0.0ᵃᵇ
24:1n-9	0.2 ± 0.1	0.6 ± 0.1ᵃ	0.5 ± 0.1ᵃ	0.3 ± 0.3ᵃ	0.2 ± 0.0ᵇᶜ	0.2 ± 0.1ᵇ	0.5 ± 0.1ᵃ
ΣMUFA	**25.0 ± 0.4**	**28.9 ± 0.2ᵃ**	**31.8 ± 0.3ᵃ**	**27.5 ± 0.4ᵃ**	**26.7 ± 0.2ᵇ**	**21.2 ± 0.5ᵇ**	**24.7 ± 1.1ᵇ**
18:2n-6	9.2 ± 0.2	10.6 ± 0.1ᵃ	8.0 ± 0.1ᵃ	8.1 ± 0.2ᵃ	8.1 ± 0.3ᵃ	6.8 ± 0.1ᶜ	6.2 ± 0.5ᵇ
18:3n-6	0.2 ± 0.0	0.3 ± 0.1ᵃ	0.2 ± 0.0ᵃ	0.1 ± 0.0ᵃ	0.2 ± 0.0ᶜ	0.1 ± 0.0ᵇ	0.1 ± 0.0ᵇ
20:2n-6	1.6 ± 0.1	1.6 ± 0.1ᵇ	1.8 ± 0.0ᵇ	1.5 ± 0.1ᵇ	4.3 ± 0.1ᵃ	1.8 ± 0.1ᵇ	2.8 ± 0.2ᵃ
20:3n-6	0.1 ± 0.1	0.3 ± 0.0ᵃ	0.3 ± 0.0ᵃ	0.2 ± 0.0ᵃ	0.2 ± 0.0ᶜ	0.1 ± 0.0ᶜ	0.2 ± 0.0ᶜ
20:4n-6	2.9 ± 0.1	1.1 ± 0.0ᵇ	1.1 ± 0.0ᵇ	1.0 ± 0.0ᵇ	2.0 ± 0.1ᵇ	4.1 ± 0.1ᵃ	3.8 ± 0.1ᵃ
22:2n-6	0.5 ± 0.1	0.3 ± 0.0ᵇ	0.1 ± 0.0ᵇ	0.1 ± 0.0ᵇ	0.3 ± 0.0ᵇ	0.9 ± 0.0ᵃ	0.8 ± 0.0ᵃ
22:4n-6	0.2 ± 0.1	0.2 ± 0.0ᵇ	0.1 ± 0.0ᵃ	0.1 ± 0.0ᵃ	0.1 ± 0.0ᵇ	0.1 ± 0.0ᶜ	0.1 ± 0.0ᵃ
Σn-6 PUFA	**14.7 ± 0.2**	**14.4 ± 0.1ᵇ**	**11.6 ± 0.2ᵃ**	**11.0 ± 0.1ᵇ**	**15.2 ± 0.3ᵃ**	**14.1 ± 0.0ᵇ**	**14.0 ± 0.3ᵃ**
18:3n-3	1.2 ± 0.2	2.4 ± 0.0ᵃ	2.4 ± 0.0ᵃ	3.1 ± 0.1ᵃ	4.0 ± 0.0ᵃ	2.2 ± 0.1ᵃ	3.3 ± 0.2ᵃ
18:4n-3	0.2 ± 0.0	0.2 ± 0.0ᵃ	0.2 ± 0.0ᵃ	0.1 ± 0.0ᵇ	0.1 ± 0.0ᵃ	0.2 ± 0.0ᵇ	0.2 ± 0.0ᵃ
20:3n-3	1.4 ± 0.0	0.6 ± 0.0ᵃ	0.7 ± 0.0ᵇ	0.6 ± 0.0ᵇ	1.9 ± 0.0ᵃ	1.4 ± 0.0ᵇ	1.8 ± 0.1ᵃ
20:4n-3	1.0 ± 0.0	0.8 ± 0.4ᵃ	0.5 ± 0.0ᵇ	0.4 ± 0.0ᵇ	1.4 ± 0.0ᵇ	1.4 ± 0.1ᵇ	1.1 ± 0.0ᵇ
20:5n-3	3.6 ± 0.1	1.4 ± 0.1ᵃ	3.7 ± 0.0ᵇ	4.3 ± 0.1ᵃ	0.8 ± 0.0ᵇ	5.5 ± 0.1ᵃ	4.8 ± 0.2ᵃ
22:5n-3	1.1 ± 0.1	4.4 ± 0.2ᵃ	0.9 ± 0.1ᵃ	1.0 ± 0.0ᵇ	3.1 ± 0.1ᵃ	1.1 ± 0.0ᵇ	1.1 ± 0.0ᵇ
22:6n-3	25.3 ± 0.2	18.7 ± 0.3ᵇ	16.7 ± 0.1ᵇ	20.3 ± 0.2ᵇ	22.8 ± 0.2ᵇ	26.0 ± 0.5ᵇᶜ	26.5 ± 0.6ᵃ
Σn-3 PUFA	**33.9 ± 0.4**	**28.5 ± 0.3ᵇ**	**25.2 ± 0.2ᵇ**	**29.8 ± 0.4ᵇ**	**34.2 ± 0.2ᵃ**	**37.8 ± 0.7ᵃ**	**38.7 ± 0.7ᵃ**
EPA+DHA	**28.9 ± 0.3**	**23.1 ± 0.4ᵇ**	**20.5 ± 0.1ᵇ**	**24.6 ± 0.3ᵇ**	**25.9 ± 0.2ᵃ**	**31.5 ± 0.7ᵃ**	**31.4 ± 0.5ᵃ**
Σn-3/Σn-6 PUFA	**2.3 ± 0.1**	**2.0 ± 0.0ᵇ**	**2.2 ± 0.0ᵇ**	**2.7 ± 0.1ᵃ**	**2.2 ± 0.1ᵃ**	**2.7 ± 0.0ᵃ**	**2.8 ± 0.1ᵃ**

Note: SD is the standard deviation, n=6. Different letters indicate groups that differ from each other ($p<0.05$)

Reverse Transcriptase and Quantitative Polymerase Chain Reaction (RT-qPCR) Analysis

For this study, six fish were anesthetized with 0.2% tricaine methanesulfonate (Finquel MS-222, Argent Chemical Laboratories, Redmond, WA, USA), and liver and muscle samples of approximately 0.5 g were taken. These samples were stored in 2 ml RNAlater and transferred to the Molecular Biology Laboratory of Department of Agricultural Biotechnology, Atatürk University, Erzurum, Turkey, where they were stored at -80°C until analysis could be performed. After the isolation of total RNA (TRIzol method, Life Technologies), agarose gel electrophoresis and nanodrop were used to determine the quality of total RNA and the total RNA concentration, respectively. Before cDNA synthesis, RNA was treated with DNase (DNase I Deoxiribonuclease I, Amplification Grade Cat No: 18068-015) to eliminate genomic DNA contamination. Omniscript Reverse Transcription kit (Qiagen, Düsseldorf, Germany) was used for cDNA synthesis from a total of 2 µg of RNA from each tissue (Vélez-Calabria et al., 2021). The steady-state levels of rainbow trout *fabp6* and *fabp3* mRNA transcripts for liver and muscle tissues were assayed using RT-qPCR as outlined by Bustin et al. (2005). A Rotor-Gene 6000 thermal cycler system (Qiagen GmbH, Düsseldorf, Germany) and a QuantiTect SYBR Green PCR kit (Qiagen) were used for RT-qPCR analyses according to the manufacturers' instructions. For RT-qPCR, initial denaturation occurred at 95.0°C for 15 min, and was followed by 40 cycles consisting of denaturation (at 95.0°C for 20 s), primer annealing (for 30 s) and elongation (at 72.0°C for 30 s). To calculate normalized steady-state levels of *fabp* mRNA transcripts in rainbow trout liver and muscle tissues, the mean copy number of *fabp* mRNA transcripts was divided by the mean copy number of the reference gene (ß-actin, which is constitutively expressed at approximately the same steady-state levels in all tissues) (Torstensen et al., 2009; Anderson & Elizur, 2012). SPSS (Version 10.0) was used for statistical analysis. Differences in gene expression between the study groups seemed significant as a result of the two-way ANOVA test were subjected to Duncan's multiple comparison test (SPSS, 2011).

Results and Discussion

Nutrigenomics of Rainbow Trout fabp3 and fabp6 Genes

The aim of this study is to investigate the effect of camelina oil as a replacement of fish oil in rainbow trout diets for to evaluate fatty acid composition, gene expression of fatty acids binding protein genes (*fabp3* and *fabp6*) of liver and muscle tissues and investigate the growth parameters.

Results showed that hepatic *fabp6* gene expression levels were significantly higher ($p<0.05$) in the CO67 (25.15±0.46) experimental group than in the CO100 (22.76±0.87) and CO33 (23.19±0.66) groups, but were not significantly higher than expression levels in the FO (23.76 ± 0.52) group. Statistical analysis showed that differences in hepatic expression of *fabp6* between the CO33, CO100 and FO groups were not significant. Similarly, differences in expression of the *fabp6* gene in muscle tissue were not found to be significant across the experimental groups [FO (23.76±0.52), CO100 (22.52±0.46), CO67 (23.27±0.09), CO33 (22.48±0.58)].

Hepatic *fabp3* gene expression levels were found to be significantly higher in the FO group (24.46±0.52) than in the CO100 group (22.71±0.87) ($p<0.05$). Hepatic expression was lower in the CO67 group (23.44±0.52) than in the CO33 group (22.76±0.46), but this difference was found to be statistically insignificant. Muscle *fabp3* gene expression levels were found to be higher in the FO group (22.78±0.52) than in all three of the CO-fed groups [CO67 (22.55±0.52), CO100 (23.76±0.87) and CO33 (23.76±0.46)], but the difference was very slight, and was found to be insignificant as a result of statistical analysis.

The mRNA expression levels of *fabp3* and *fabp6* genes in liver and muscle tissues, and indication of effects of replacing dietary FO with 33%, 67%, and 100% CO mix is presented here in rainbow trout. It was observed that muscle and liver tissues fatty acid composition analysed for gene expression analysis reflected the dietary fatty acid composition in this study.

Intracellular lipid chaperones, known as FABPs, are a group of molecules that regulate lipid response in cells, and are strongly associated with metabolic and inflammatory pathways (Furuhashi & Hotamisligil, 2008). Free FAs are transported into the cell by protein transporters and *fabp*s from the plasma membrane. Tissues such as liver and muscle have rapid fat metabolism, and have high *fabp* levels in proportion to their FA intake and use (Storch & Corsico, 2008). Fish convert ALA (18:3 n-3) to DHA (22:6 n-3) with EPA and LA (18:2 n-6) to ARA (20:4 n-6) cannot be synthesized by vertebrates, and are therefore referred to as "essential" FAs (Kanazawa et al., 1979; Sargent et al., 2002). They can be converted to n-3 and n-6 HUFA by desaturation, elongation, and alternative chains (Sargent et al., 2002; Nakamura & Nara, 2004). In fish, it has been shown that an increase in 18:2n-6 and 18:3n-3 FAs increases desaturation activity for the production of HUFAs. In this study, however, the low levels of *fabp3* gene expression and highs levels of *fabp6* gene expression were found in the muscle of fish fed a FO-rich diet (Tocher et al., 2002; Ling et al., 2006).

Bayır et al. (2015) used nucleotide sequence databases (*fabp1, 2, 3, 6, 7, 10* and *11*) and phylogenetic analysis to determine the mRNA transcripts encoded by 14 different *fabp* genes in rainbow trout, and reported that most of these genes are duplications resulting from teleost-specific whole genome duplication (WGD) events. However, the *fabp3* and *fabp6* genes were found as single copies in the rainbow trout genome. Steady-state transcript levels differ between *fabp3 and fabp6* genes. While some *fabp* transcripts such as *fabp3* are found large quantities in many tissues, others (such as *fapb6*) are limited to a few tissues. In the study, levels of *fabp3* and *fabp6* mRNA expression in fish fed CO-based diets were very similar to those found in fish fed FO-based diets. No adverse effects were found on FA metabolism in the muscle and liver.

Table 6. Fatty acid composition of 67% and 33% camelina seed in liver tissue(mean±SD)

Fatty Acids	Initial	33 % Camelina Seed Oil			67% Camelina Seed Oil		
		First sample (15th day)	*Second sample (30th day)*	*Third sample (45th day)*	*First sample (15th day)*	*Second sample (30th day)*	*Third sample (45th day)*
14:0	2.0 ± 0.2	2.7 ± 0.1[b]	2.6 ± 0.0[b]	2.6 ± 0.1[b]	2.4 ± 0.1[b]	2.2 ± 0.1[b]	2.3 ± 0.2[b]
15:0	0.3 ± 0.0	0.4 ± 0.0[a]	0.4 ± 0.0[b]	0.4 ± 0.0[b]	0.4 ± 0.0[a]	0.4 ± 0.0[b]	0.4 ± 0.0[b]
16:0	15.6 ± 0.3	16.5 ± 0.1[b]	19.2 ± 0.1[b]	20.7 ± 0.1[b]	16.1 ± 0.1[b]	19.3 ± 0.4[b]	18.9 ± 0.4[b]
17:0	0.5 ± 0.0	0.5 ± 0.1[a]	0.5 ± 0.0[a]	0.5 ± 0.0[a]	0.5 ± 0.0[a]	0.5 ± 0.0[a]	0.4 ± 0.0[b]
18:0	4.7 ± 0.3	4.4 ± 0.2[c]	5.0 ± 0.1[b]	5.0 ± 0.1[a]	4.6 ± 0.0[a]	5.3 ± 0.2[a]	4.4 ± 1.0[a]
20:0	0.6 ± 0.1	0.7 ± 0.1[b]	0.6 ± 0.0[b]	0.5 ± 0.0[b]	0.9 ± 0.0[a]	0.7 ± 0.0[a]	0.6 ± 0.0[a]
22:0	1.0 ± 0.0	1.2 ± 0.0[ab]	0.7 ± 0.1[a]	0.6 ± 0.0[b]	1.3 ± 0.2[a]	0.7 ± 0.1[a]	0.7 ± 0.0[a]
24:0	0.4 ± 0.1	0.3 ± 0.0[c]	0.2 ± 0.0[c]	0.4 ± 0.1[a]	0.4 ± 0.1[b]	0.3 ± 0.1[b]	0.4 ± 0.2[a]
ΣSFA	**25.0 ± 0.5**	**26.7 ± 0.2[b]**	**29.2 ± 0.1[b]**	**30.7 ± 0.1[a]**	**26.5 ± 0.5[b]**	**29.4 ± 0.9[b]**	**27.9 ± 1.0[b]**
14:1	0.1 ± 0.0	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.0[a]
15:1	0.3 ± 0.1	0.3 ± 0.0[a]	0.1 ± 0.0[b]	0.5 ± 0.1[a]	0.3 ± 0.0[a]	0.1 ± 0.0[b]	0.3 ± 0.1[a]
16:1	2.9 ± 0.3	4.0 ± 0.5[a]	4.6 ± 0.1[b]	4.0 ± 0.1[c]	3.3 ± 0.2[a]	3.9 ± 0.2[b]	4.5 ± 1.0[b]
17:1	0.3 ± 0.0	0.4 ± 0.0[a]	0.4 ± 0.0[a]	0.4 ± 0.0[a]	0.4 ± 0.0[a]	0.4 ± 0.0[a]	0.4 ± 0.1[a]
18:1n-9	26.2 ± 0.2	27.1 ± 0.3[a]	23.5 ± 0.3[b]	20.8 ± 0.1[b]	25.0 ± 0.4[a]	22.2 ± 0.6[b]	23.2 ± 0.5[a]
20:1n-9	0.4 ± 0.0	0.2 ± 0.0[a]	0.2 ± 0.0[a]	0.1 ± 0.0[a]	0.2 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[a]
22:1	0.4 ± 0.0	0.4 ± 0.0[b]	0.5 ± 0.0[a]	0.5 ± 0.0[a]	0.4 ± 0.0[b]	0.5 ± 0.0[a]	0.4 ± 0.0[b]
24:1n-9	0.4 ± 0.1	0.3 ± 0.0[c]	0.2 ± 0.1[c]	0.4 ± 0.1[b]	0.4 ± 0.2[b]	0.4 ± 0.0[b]	0.6 ± 0.5[a]
ΣMUFA	**30.9 ± 0.4**	**32.8 ± 0.8[a]**	**29.8 ± 0.3[b]**	**26.8 ± 0.2[c]**	**30.2 ± 0.4[a]**	**27.7 ± 0.8[b]**	**29.3 ± 0.6[a]**
18:2n-6	12.9 ± 0.4	12.4 ± 0.1[a]	9.5 ± 0.1[b]	10.6 ± 0.1[b]	11.6 ± 0.3[a]	10.9 ± 0.1[a]	12.9 ± 0.8[a]
18:3n-6	0.2 ± 0.0	0.1 ± 0.0[c]	0.2 ± 0.0[a]	0.1 ± 0.0[b]	0.2 ± 0.0[b]	0.2 ± 0.0[a]	0.2 ± 0.0[a]
20:2n-6	2.0 ± 0.2	2.8 ± 0.1[b]	2.3 ± 0.0[b]	2.0 ± 0.1[b]	3.5 ± 0.3[a]	2.7 ± 0.0[a]	2.6 ± 0.0[a]
20:3n-6	0.2 ± 0.1	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.2 ± 0.0[a]	0.2 ± 0.0[b]	0.2 ± 0.0[b]	0.1 ± 0.0[a]
20:4n-6	1.1 ± 0.0	0.8 ± 0.0[c]	1.1 ± 0.0[a]	1.0 ± 0.0[a]	0.9 ± 0.0[b]	1.0 ± 0.1[a]	0.8 ± 0.1[b]
22:2n-6	0.2 ± 0.0	0.2 ± 0.0[b]	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.2 ± 0.0[b]	0.1 ± 0.0[a]	0.1 ± 0.0[a]
22:4n-6	0.1 ± 0.0	0.1 ± 0.0[b]	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[a]	0.1 ± 0.0[a]
Σn-6 PUFA	**16.7 ± 0.4**	**16.8 ± 0.2[a]**	**13.5 ± 0.1[c]**	**14.0 ± 0.1[b]**	**16.6 ± 0.1[a]**	**15.2 ± 0.1[a]**	**16.9 ± 0.8[a]**
18:3n-3	2.9 ± 0.3	4.1 ± 0.1[b]	4.0 ± 0.0[b]	4.4 ± 0.1[b]	5.7 ± 0.3[a]	5.4 ± 0.2[a]	5.6 ± 0.4[a]
18:4n-3	0.3 ± 0.0	0.2 ± 0.0[b]	0.2 ± 0.0[a]	0.1 ± 0.0[a]	0.3 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[a]
20:3n-3	0.8 ± 0.1	1.0 ± 0.0[a]	0.8 ± 0.0[a]	0.9 ± 0.0[b]	1.0 ± 0.0[b]	1.0 ± 0.0[b]	1.0 ± 0.1[b]
20:4n-3	0.6 ± 0.0	0.4 ± 0.0[c]	0.5 ± 0.0[b]	0.5 ± 0.0[a]	0.5 ± 0.0[b]	0.7 ± 0.0[a]	0.5 ± 0.0[a]
20:5n-3	4.3 ± 0.2	0.8 ± 0.0[c]	4.0 ± 0.1[a]	3.8 ± 0.1[b]	1.0 ± 0.1[b]	3.4 ± 0.0[a]	3.2 ± 0.2[b]
22:5n-3	1.1 ± 0.1	3.4 ± 0.2[c]	1.0 ± 0.1[a]	0.9 ± 0.1[b]	3.8 ± 0.2[b]	0.9 ± 0.0[a]	0.9 ± 0.2[a]
22:6n-3	17.8 ± 0.3	13.7 ± 0.7[c]	16.7 ± 0.3[a]	18.0 ± 0.3[b]	14.6 ± 0.3[b]	16.0 ± 0.3[a]	14.5 ± 0.3[b]
Σn-3 PUFA	**27.7 ± 0.4**	**23.6 ± 0.8[c]**	**27.4 ± 0.5[a]**	**28.6 ± 0.3[a]**	**26.7 ± 0.4[b]**	**27.6 ± 0.2[a]**	**25.8 ± 0.4[b]**
EPA+DHA	**22.0 ± 0.3**	**17.0 ± 0.9[b]**	**20.7 ± 0.4[a]**	**21.8 ± 0.3[b]**	**18.4 ± 0.2[b]**	**19.4 ± 0.3[b]**	**17.6 ± 0.2[b]**
Σn-3/Σn-6 PUFA	**1.7 ± 0.0**	**1.4 ± 0.1[c]**	**2.0 ± 0.0[a]**	**2.0 ± 0.0[b]**	**1.6 ± 0.0[b]**	**1.8 ± 0.0[b]**	**1.5 ± 0.1[b]**

Note: SD is the standard deviation, n=6. Different letters indicate groups that differ from each other ($p<0.05$)

Table 7. Growth parameters of rainbow trout fed camelina seed oil (*Camelina sativa*)-based diet(mean±SD)

Diets	Weight Gain Rate (%)	Specific Growth Rate (%)	Feed Conversion Rate (%)	Survival Rate (%)
FO100	261.80 ± 11.25[a]	2.16 ± 0,56[a]	0.98±0.08[a]	100[a]
CO100	254.65 ± 23.82[a]	2.06 ±0.15[a]	0.97±0.03[a]	100[a]
CO67	250.27 ± 23.70[a]	1.99±0.13[a]	0.97±0.01[a]	100[a]
CO33	252.42 ± 24.91[a]	2.19±0.15[a]	0.96±0.07[a]	100[a]

Note: SD is the standard deviation, n=6. Different letters indicate groups that differ from each other ($p<0.05$)

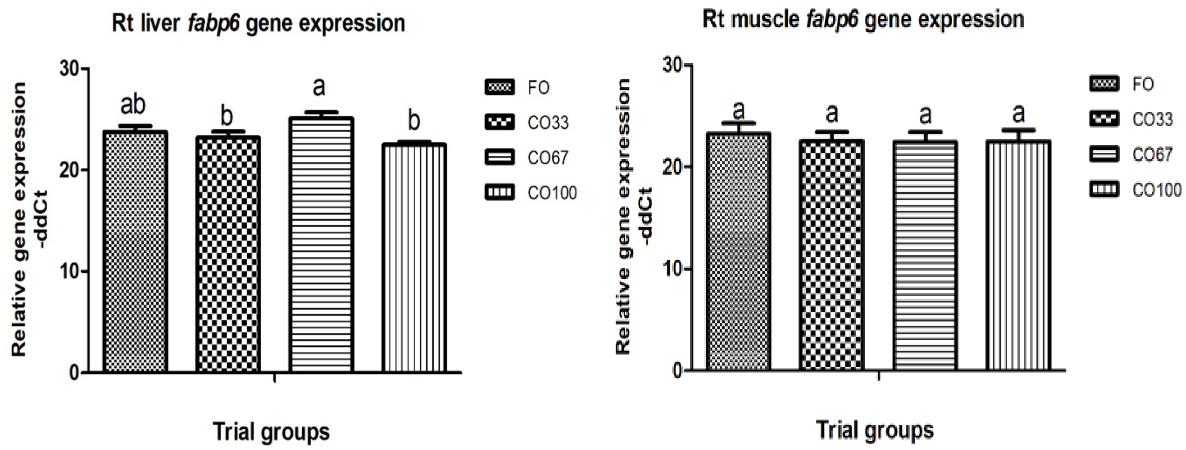


Figure 1. Relative gene expression (−ΔΔCt) for *fabp6* gene expression in liver and muscle of fish fed different lipid contain diets. Bars represent the difference in least square mean relative expression (ΔCt camelina oil (different amount) −ΔCt fish oil) ± SE.

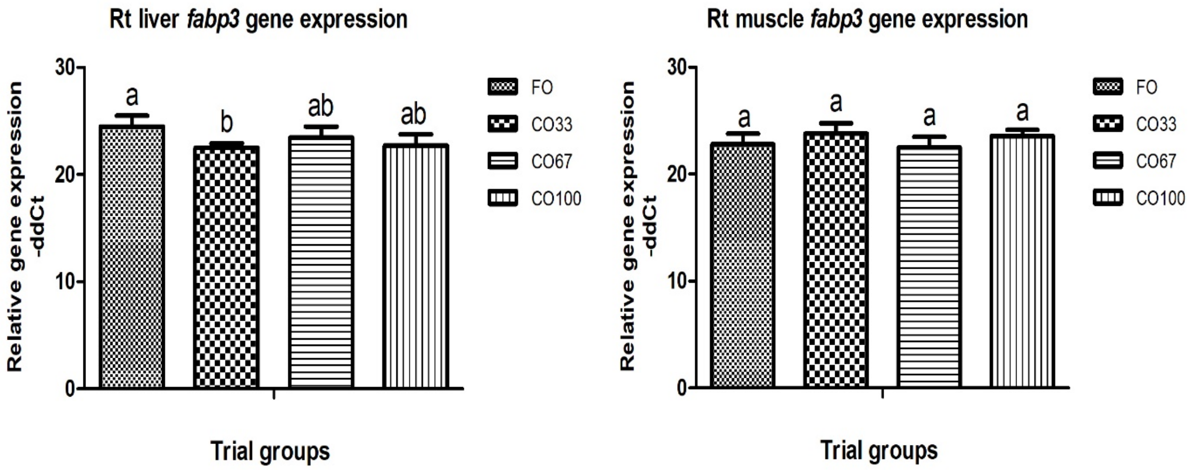


Figure 2. Relative gene expression (−ΔΔCt) for *fabp3* gene expression in liver and muscle of fish fed different lipid contain diets. Bars represent the difference in least square mean relative expression (ΔCt camelina oil (different amount) −ΔCt fish oil) ± SE.

In general, as the energy requirements of a fish increase, its use of lipids also increases (Tocher et al., 2003). For example, the same literature reported that the quantities of ΣSFA, ARA, and DHA increased during the larval development period of many fish species. In this study, it was observed that the levels of ΣSFA increased in all trial groups compared to the initial value. As SFA is a carcass tissue element (Wiegant et al., 1996), levels will increase with the growth of the fish. 16:0 is used as a source of metabolic energy during fish growth, and was found to be the dominant FA of the ΣSFAs, and it also overlaps with many studies such as Mohamed & Al-Sabahi, (2011), Henderson & Tocher (1987), Vázquez et al. (1994), Sargent et al. (1999a), Luo et al. (2009), and Bayır et al. (2010).

Of the MUFAs, oleic acid (OA; C18: 1) and palmitoleic acid (C16: 1) are the predominant FAs that account for approximately 67.69% and 22.85% of ΣMUFA, respectively (Yanar et al., 2006). In this study, C18:1 was found to be the most abundant FA overall, while palmitoleic acid (C16:1) was the most abundant in liver and muscle tissues in the experimental groups, followed closely by oleic acid (C18:1). At the first sampling, the CO100, CO67, and CO33 groups were found to have significantly higher ΣMUFA content than the fish fed the control diet; while at the end of the trial, the CO67 group had more ΣMUFA content than any of the other groups. As CO is rich in C18:1, these findings are likely to reflect the FA composition of the diets.

The lipid content of fish meat mainly consists of triglycerides and long-chain polyunsaturated fatty acids (PUFA). Like all vertebrates, fish require three FAs from PUFAs for normal growth, development, and reproduction (Piedecausa et al., 2007). It has been shown that the essential FA composition changes depending on the molecular size of the lipids present in the fish's diet (Watanabe, 1982; Ensminger et al., 1990; Greene & Selivonchick, 1990; Tucker, 1998).

The levels of n-6 HUFA in fish muscle and liver tissue were found to be directly correlated to the quantity of LA in their diet, with the highest amount of n-6 HUFA found in the CO100 group and the lowest in the FO100 group.

Finally, the levels of EPA and DHA were determined across the experimental groups, with the highest quantities found in fish in the FO100 group. It is well recognized that marine and freshwater fish have different requirements for essential FAs; while marine fish require high quantities of HUFAs in the n-3 series, freshwater fish have an additional requirement of FAs in the n-6 series. These FAs enable effective growth and feed utilization rate, which reduces the levels of protein required in the fish feed (Sowizral et al., 1990; Sargent et al., 1999b; Rinchart et al., 2007).

From the results of this study, it can therefore be concluded that CO can be used as an alternative oil source to FO in rainbow trout diets. This is supported by the lack of significant difference in expression of the *fabp3* and *fabp6* genes in rainbow trout muscle tissue between fish fed CO-based diets, compared to those fed FO-based diets. This suggests that the fundamental molecular lipid metabolism was not negatively impacted by the change in diet. However, rainbow trout should be fed a finishing diet prepared with FO for the last months before being prepared for consumption (Bordignon et al., 2020), which would restore the majority of the FA content of the fish.

The effects of feeding camelina seed oil on the weight gain, specific growth rate, feed conversion rate, and survival rate are presented in Table 7. Weight gain rate of rainbow trout were 261.80±11.25, 254.65±23.82, 250.27±23.70, and 252.42±24.91 in FO100, CO100, CO67, and CO33, respectively. Feed conversion rate of rainbow trout were 0.98±0.08, 0.97±0.03, 0.97±0.01, and 0.96±0.07 in FO100, CO100, CO67, and CO33, respectively. Weight gain, specific growth rate, feed conversion rate, and survival rate decreased in the fish fed cameline seed oil-based diets compared to control group, but the differences was not significantly important among groups. The survival rates were 100% in all groups. In this study, it was observed that the use of camelina seed oil completely or partially replace of fish oil did not have a negative effect on rainbow trout growth rates and survival rate. Many studies have been carried out in which various vegetable oils have been tested in trout and these oils have provided good growth in fish (Torstensen et al., 2000; Bell et al., 2001, 2002; Grisdale-Helland et al., 2002). Fish meal and fish oil, which are the main ingredients of feed in aquaculture, may be insufficient due to the rapid development of fish farming, and this causes the search for alternative oil sources (Olsen et al., 2003). Studies with vegetable oils show that alternative oil sources can be used to partially or completely replace fish oil and meet the fatty acid needs of the species to be grown without adversely affecting the growth performance of the fish (Dernekbaşı & Karayücel, 2010). The availability of vegetable oils that can replace with fish oil will both eliminate the dependence on fish oil and reduce the cost of feed (Mourente & Bell, 2006). Camelina oil has high levels of PUFA compared to other plant oil (Ni Eidhin et al., 2003). N-3 PUFA and LA are essential dietary components for ensuring optimum fish growth (Chou & Shiau, 1999). The growth performance of salmonids, most notably the offspring of anadrome salmon and trout, is increased when they are fed a diet containing PUFAs, particularly ALA (Sargent et al., 1989). ALA, DHA, and EPA are therefore essential for the growth and development of rainbow trout juveniles. Vegetable oils are poor in n-3 HUFA, but rich in C18 PUFAs, particularly LA, ALA, and OA (Ganga et al., 2011). This was confirmed by the high levels of C18 FA in the liver and muscle tissues of fish fed CO-based diets in this study. Camelina seed oil has potential as a lipid source in diets for rainbow trout replace FO.

Conclusion

In the study, levels of *fabp3* and *fabp6* mRNA expression in fish fed camelina seed oil-based diets were very similar to found in fish fed fish oil -based diets. When the fish meat was evaluated in terms of fatty acid composition, it was observed that the n-3/n-6 ratio was lower in the group fed with camelina seed oil as the oil source. However, it was observed that feeding with camelina seed oil did not have a negative effect on growth parameters and survival rate. For this reason, it is thought that camelina seed oil can be replace with fish oil in rainbow trout feeds, but it would be much more appropriate to apply a fish oil-based final feeding regimen, especially prepared with a high amount of long chain PUFA.

Compliance With Ethical Standards

Authors' Contributions

This manuscript is produced from Sinem Keşan's master thesis. Author SK took part in the literature review and writing of manuscript as well as performing the trial phase and laboratory studies of the study. MB designed the study and wrote the first draft of the manuscript, GA performed and managed statistical analyses. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

Ethics committee approval was not required at the time this study was conducted. Working protocols were approved by Atatürk University Local Ethical Committee for Animal Studies. The studies have been approved by Atatürk University research ethics committee and have been performed in accordance with the ethical standards of Ataturk University and its later amendments or comparable ethical standards.

References

Anderson, K. C., & Elizur, A. (2012). Hepatic reference gene selection in adult and juvenile female Atlantic salmon at normal and elevated temperatures. *BMC Research Notes*, *5*, 21. https://doi.org/10.1186/1756-0500-5-21

Aydın, A. (2004). Sağlığımız ve omega-3 yağ asitleri. *Sağlıkta ve Hastalıkta Beslenme Sempozyum Dizisi*, *41*, 181-189.

Bayır, A., Sirkecioğlu, A. N., Aras, N. M., Aksakal, E., Haliloğlu, H. İ., & Bayır, M. (2010). Fatty acids of neutral and phospholipids of three endangered trout: *Salmo trutta caspius* Kessler, *Salmo trutta labrax* Pallas and *Salmo trutta macrostigma* Dumeril. *Food Chemistry*, *119*(3), 1050-1056. https://doi.org/10.1016/j.foodchem.2009.07.064

Bayır, M. (2011). *Effect of dietary lipid sources on fatty acid pattern, growth and starvation response indicated by antioxidant enzymes in brown trout (Salmo trutta)* [Ph.D. Thesis, Atatürk University].

Bayır, M., Bayır, A., & Wright, J. M. (2015). Divergent spatial regulation of duplicated fatty acid-binding protein (*fabp*) genes in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *14*, 26-32, https://doi.org/10.1016/j.cbd.2015.02.002

Baysal, A. (2004). *Beslenme*. Hatipoğlu Yayınevi.

Bell, J. G., Henderson, R. J., Tocher, D. R., McGhee, F., Dick, J. R., Porter, A., Smullen, R., & Sargent, J. R. (2002). Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects tissue fatty acid compositions and hepatic fatty acid metabolism. *The Journal of Nutrition*, *132*(2), 222-230. https://doi.org/10.1093/jn/132.2.222

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., & Sargent, J.R. (2001). Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *The Journal of Nutrition*, *131*(5), 1535-1543. https://doi.org/10.1093/jn/131.5.1535

Bordignon, F., Martínez-Llorens, S., Trocino, A., Jover-Cerdá, M., & Tomás-Vidal, A. (2020). Recovery of fatty acid composition in Mediterranean yellowtail (*Seriola dumerili*, Risso 1810) fed a fish-oil finishing diet. *International Journal of Molecular Sciences*, *21*(14), 4871. https://doi.org/10.3390/ijms21144871

Bustin, S. A. Benes, V., Nolan, T., & Pfaffl, M. W. (2005). Quantitative real-time RT-PCR – a perspective. *Journal of Molecular Endocrinology*, *34*(3), 597-601. https://doi.org/10.1677/jme.1.01755

Caballero, M. J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M., & Izquierdo, M. S. (2002). Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology on rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, *214*(1-4), 253-271. https://doi.org/10.1016/S0044-8486(01)00852-3

Çetinkaya, O. (1995). *Balık Besleme*. Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Yayın No: 9.

Chou, B. -S., & Shiau, S. -Y. (1999). Both n-6 and n-3 fatty acids are required for maximal growth of juvenile hybrid tilapia. *North American Journal of Aquaculture*, *61*(1), 13-20. https://doi.org/10.1577/1548-8454(1999)061%3C0013:BNANFA%3E2.0.CO;2

Dernekbaşı, S., & Karayücel, İ. (2010). *Balık yemlerinde kanola yağının kullanımı* [Use of canola oil in fish feeds]. *Journal of FisheriesSciences.com*, *4*(4), 469-479. https://doi.org/10.3153/jfscom.2010051

Ensminger, M. E., Oldfield, J. E., & Heinemann, W. W. (1990). *Feeds and Nutrition*. The Ensminger Publishing Company.

FAO. (2020). *The State of World Fisheries and Aquaculture 2020. Sustainability in action*. FAO. https://doi.org/10.4060/ca9229en

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, *226*(1), 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5

Furuhashi, M., Hotamisligil, G.S. (2008) Fatty acid-binding proteins: role in metabolic diseases andpotential as drug targets. *Nature Reviews: Drug Discovery*, *7*(6), 489–503. https://doi.org/10.1038/nrd2589

Ganga, R., Montero D., Bell, J. G., Atalah, E., Ganuza, E., Vega-Orellana, O., Tort, L., Acerete, L., Afonso, J. M., Benitez-Sanatana, T., Vaquero, A. F., & Izquierdo, M. (2011). Stress response in sea bream (*Sparus aurata*) held under crowded conditions and fed diets containing linseed and/or soybean oil. *Aquaculture*, *311*(1-4), 215-223. https://doi.org/10.1016/j.aquaculture.2010.11.050

Glatz, J. F., & van der Vusse, G. J. (1996). Cellular fatty acid-binding proteins: their function and physiological significance. *Progress in Lipid Research*, *35*, 243-282. https://doi.org/10.1016/S0163-7827(96)00006-9

Greene, D. H. S., & Selivonchick, D. P. (1990). Effects of dietary vegetable, animal and marine lipids on muscle lipid and hematology of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *89*(2), 165-182. https://doi.org/10.1016/0044-8486(90)90308-A

Grisdale-Helland, B., Ruyter, B., Rosenlund, G., Obach, A., Helland, S. J., Sandberg, M. G., Standal, H., & Røsjø, C. (2002). Influence of high contents of dietary soybean oil on growth, feed utilization, tissue fatty acid composition, heart histology and Standard oxygen consumption of Atlantic salmon (*Salmo salar*) raised at two temperatures. *Aquaculture*, *207(3-4)*, 311–329. https://doi.org/10.1016/S0044-8486(01)00743-8

Henderson, R. J., & Tocher, D. R. (1987). The lipid composition and biochemistry of freshwater fish. *Progress in Lipid Research*, *26*(4), 281–347 https://doi.org/10.1016/0163-7827(87)90002-6

Izquierdo, M. S., Montero, D., Robaina, L., Caballero, M. J., Rosenlund, G., & Ginés, R. (2005). Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. *Aquaculture*, *250*(1-2), 431-444. https://doi.org/10.1016/j.aquaculture.2004.12.001

Izquierdo, M. S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L., & Rosenlund, G. (2003). Dietary lipid sources for seabream and seabass: Growth performance, tissue composition and flesh quality. *Aquaculture Nutrition*, *9*(6), 397-407. https://doi.org/10.1046/j.1365-2095.2003.00270.x

Jordal, A. -E. O., Torstensen, B. E., Tsoi, S., Tocher, D. R., Lall, S. P., & Douglas, S. (2005). Small scale cDNA microarray analysis of expression of genes for lipid metabolism in liver of Atlantic salmon (*Salmo salar* L.) effect of dietary rapeseed oil replacement. *The Journal Nutrition*, *135*(10), 2355–2361. https://doi.org/10.1093/jn/135.10.2355

Kanazawa, A., Teshima, S., Tokiwa, S., Kayama, M., & Hirata, M. (1979). Essential fatty acid in the diet of prawn II. Effect of docosahexaenoic acid on growth. *Bulletin of the Japanese Society for the Science of Fish*, *45*(9), 1151-1153. https://doi.org/10.2331/suisan.45.1151

Kurt, O., & Seyis, F. (2008). *Alternatif yağ bitkisi: Ketencik (Camelina sativa (L.) Crantz)* [An alternative oilseed crop: camelina [*Camelina sativa* (L.) Crantz]]. *Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi*, *23*(2), 116-120.

Legendre, M., Kerdcuhen, N., Corraze, G., & Bergot, P. (1995). Larval rearing of an African catfish *Heterobranchus longifilis* (Teleostei, Clariidae): effect of dietary lipids on growth, survival and fatty acid composition of fry. *Aquatic Living Resources*, *8*, 355-363.

Ling, S., Hashim, R. Kolkovski, S., & Shu-Chien A. C. (2006). Effect of varying dietary lipid and protein levels on growth and reproductive performance of female swordtails *Xiphophorus helleri* (Poeciliidae). *Aquaculture Research*, *37*(13), 1267-1275. https://doi.org/10.1111/j.1365-2109.2006.01554.x

Luo, B. Z., Tan, X. Y, Wang, V. M., & Fan, Q. X. (2009). Effects of long-term starvation on body weight and body composition of juvenile channel catfish, *Ictalurus punctatus*, with special emphasis on amino acid and fatty acid changes. *Journal of Applied Ichthyology*, *25*(2), 184–189. https://doi.org/10.1111/j.1439-0426.2009.01216.x

Metcalfe, L. D., & Schmitz, A. A. (1961). The rapid preparation of fatty acid esters for gas chromatographic analysis. *Analytical Chemistry*, *33*(3), 363-364. https://doi.org/10.1021/ac60171a016

Mohamed, E. H. A., & Al-Sabahi, J. N. (2011). Fatty acids content and profile of common commercial Nile fishes in Sudan. *International Journal of Advanced Research in Agriculture*, *1*(2), 18-22.

Montero, D., Kalinowski, T., Caballero, M. J., Obach, A., Tort, L., Robaina, L., & Izquierdo, M. S. (2005). Effect of dietary vegetable lipid sources in gilthead sea bream (*Sparus aurata*) immune status and stress resistance. *Cahiers Options Méditerranéennes*, *63*, 103-112.

Montero, D., Kalinowski, T., Obach, A., Robaina, L., Tort, L., Caballero, M. J., & Izquierdo, M. S. (2003). Vegetable lipid sources for gilthead sea bream (*Sparus aurata*): Effects on fish health. *Aquaculture*, *225*(1-4), 353-370. https://doi.org/10.1016/S0044-8486(03)00301-6

Mourente, G., & Bell, J. G. (2006). Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over a long-term growth study: Effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. *Comparative Biochemistry and Physiology. Part B*, *145*(3-4), 389-399. https://doi.org/10.1016/j.cbpb.2006.08.012

Mutch, D. M., Wahli, W., & Williamson, G. (2005). Nutrigenomics and nutrigenetics: the emerging faces of nutrition. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *19*(12), 1602–1616. https://doi.org/10.1096/fj.05-3911rev

Nakamura, M. T., & Nara, T. Y. (2004). Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. *Annual review of nutrition*, *24*, 345–376. https://doi.org/10.1146/annurev.nutr.24.121803.063211

Ni Eidhin, D., Burke, J., & O'Beirne, D. (2003). Oxidative stability of ω3-rich camelina oil and camelina oil-based spread compared with plant and fish oils and sunflower spread. *Journal of Food Science*, *68*(1), 345–353. https://doi.org/10.1111/j.1365-2621.2003.tb14163.x

Niewold, T.A., Meinen M., & van der Meulen, J. (2004). Plasma intestinal fatty acid binding protein (I-FABP) concentrations increase following intestinal ischemia in pigs. *Research in Veterinary Science*, *77*(1), 89-91. https://doi.org/10.1016/j.rvsc.2004.02.006

Olsen, R. E., Dragnes, B. T., Myklebust, R., & Ringo, E. (2003). Effect of soybean oil and soybean lecithin on intestinal lipid composition and lipid droplet accumulation of rainbow trout, *Oncorhynchus mykiss* Walbaum. *Fish Physiology and Biochemistry*, *29*,181-192. https://doi.org/10.1023/B:FISH.0000045708.67760.43

Piedecausa, M. A., Mazon, M. J., Garcia, B., & Hernandez, M. D. (2007). Effects of total replacement of fish oil by vegetable oils in the diets of sharpsnout seabream (*Diplodus puntazzo*), *Aquaculture*, *263*(1-4), 211-219. https://doi.org/10.1016/j.aquaculture.2006.09.039

Rinchard, J., Czesny, S., & Dabrowski, K. (2007). Influence of lipid class and fatty acid deficiency on survival, growth and fatty acid composition in rainbow trout juveniles. *Aquaculture*, *264*(1-4), 363-371. https://doi.org/10.1016/j.aquaculture.2006.11.024

Ruyter, B., Roesjoe, C., Maesoeval, K., Einen, O., & Thomassen, M. S. (2000) Influence of dietary n-3 fatty acids on the desaturation and elongation of [1-14C] 18:2 n-6 and [1-14C] 18:3 n-3 in Atlantic salmon hepatocytes. *Fish Physiology and Biochemistry*, *23*, 151-158. https://doi.org/10.1023/A:1007893317923

Sargent, J. R., Henderson, R. J., Tocher, D. R. (1989). The lipids. In Halver, J. (Ed.), *Fish nutrition* (2nd ed.) (pp. 153-218). Academic Press.

Sargent, J. R., Tocher, D. R., & Bell, J. G. (2002). The lipids. In Halver, J. E., & Hardy, R. W. (Eds.), *Fish nutrition* (pp. 181-257) (3rd ed.). Academic.

Sargent, J., Mcevoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., & Tocher, D. (1999a). Lipid nutrition of marine fish during early development: Current status and future directions, *Aquaculture, 179*(1-4), 217-229. https://doi.org/10.1016/S0044-8486(99)00191-X

Sargent, J., Bell, G., Mcevoy, L., Tocher, D., & Estevaz, A. (1999b). Recent development in the essential fatty acid nutrition in fish, *Aquaculture*, *177*(1-4), 191-199. https://doi.org/10.1016/S0044-8486(99)00083-6

Sowizral, K. C., Rumsay, G. L., & Kinsella, J. E. (1990). Effect of dietary α-linolenic acid on n−3 fatty acids of rainbow trout lipids. *Lipids*, *25*, 246-253.

SPSS. (2011). IBM SPSS statistics for window, version 20,0 Armonk, NY.

Storch, J., & Corsico, B. (2008). The emerging functions and mechanisms of mammalian fatty acid-binding proteins. *Annual Review of Nutrition*, *28*, 73-95. https://doi.org/10.1146/annurev.nutr.27.061406.093710

Tocher, D. R., Bell, J. G., Dick, J. R., & Crampton, V. O. (2003). Effects of dietary vegetable oil on Atlantic salmon hepatocyte fatty acid desaturation and liver fatty acid compositions. *Lipids*, *38*(7), 723–732. https://doi.org/10.1007/s11745-003-1120-y

Tocher, D. R., Fonseca-Madrigal, J., Bell, J. G., Dick, J. R., Henderson R. J., & Sargent, J. R. (2002). Effects of diets containing linseed oil o n fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes in Atlantic salmon (*Salmo salar*). *Fish Physiology and*

Biochemistry, *26*, 157-170. https://doi.org/10.1023/A:1025416731014

Torstensen, B. E., Lie, Ø., & Frøyland, L. (2000). Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.)--effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. *Lipids, 35*(6), 653-664. https://doi.org/10.1007/s11745-000-0570-6

Torstensen, B. E., Nanton, A., Olsvik, P. A., Sundvold, H., & Stubhaug, I. (2009). Gene expression of fatty acid transport proteins (cd36 and FATP) and ß-oxidation-related genes in Atlantic salmon (*Salmo salar* L.) fed fish oil or vegetable oil. *Aquaculture Nutrition*, *15*(4), 440–451. https://doi.org/10.1111/j.1365-2095.2008.00609.x

Tucker, J. W. Jr. (1998). *Marine Fish Culture*. Kluwer Academic Publishers.

Vázquez, R., Gonzalez, S., Rodriguez, A., & Mourente, G. (1994). Biochemical composition and fatty acid content of fertilized eggs, yolk sac stage larvae and first feeding larvae of the Senegal sole (*Solea senegalensis* Kaup). *Aquaculture*, *119*(2-3), 273-286. https://doi.org/10.1016/0044-8486(94)90182-1

Vélez-Calabria, G., Peñaranda, D. S., Jover-Cerdá, M., Llorens, S. M., & Tomás-Vidal, A. (2021). Successful inclusion of high vegetable protein sources in feed for rainbow trout without decrement in intestinal health. *Animals*, *11*(12), 3577. https://doi.org/10.3390/ani11123577

Watanabe, T. (1982). Lipid nutrition in fish. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *73*(1), 3-15. https://doi.org/10.1016/0305-0491(82)90196-1

Wiegand, M. D. (1996). Utilization of yolk fatty acids by goldfish embryos and larvae. *Fish Physiology and Biochemistry*, *15*(1), 21-27. https://doi.org/10.1007/bf01874834

Yanar, Y., Büyükcapar, H., Yanar, M., & Göcer, M. (2006). Effects of carotenoids from red pepper and marigold flower on pigmentation, sensory properties and fatty acid composition of rainbow trout. *Food Chemistry*, *100*(1), 326-330. https://doi.org/10.1016/j.foodchem.2005.09.056

Zheng, X. Z., Torstensen, B. E., Tocher, D. R., Dick, J. R., Henderson, R. J., & Bell, J. G. (2005) Environmental and dietary influences on highly unsaturated fatty acid biosynthesis and expression of fatty acyl desaturase and elongase genes in liver of Atlantic salmon (*Salmo salar*). *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1734*(1), 13-24. https://doi.org/10.1016/j.bbalip.2005.01.006

Mar. Sci. Tech. Bull. (2022) 11(2): 158-168
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1079480

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Analyzing marine engineering curriculum from the perspective of the sustainable development goals

Burak Zincir[1]*

[1] *Istanbul Technical University, Maritime Faculty, Department of Marine Engineering, 34940, Istanbul, Turkey*

ARTICLE INFO

Article History:
Received: 26.02.2022
Received in revised form: 20.03.2022
Accepted: 20.03.2022
Available online: 30.03.2022

Keywords:
Curriculum
Marine engineering
Maritime education and training
Maritime transportation
Sustainable development goals

ABSTRACT

The aim of this study is to answer the questions, what are the relevancies of sustainable development goals (SDGs) and the marine engineering curriculum, and what areas should be improved to achieve a curriculum that supports sustainable marine engineering education. This study is the first study that analyzes all courses at the existing marine engineering curriculum of a university from the perspective of SDGs. Five stepped methodology is applied, which are understanding the content of all SDGs and relevance with maritime transportation, examining the marine engineering curriculum of ITU Maritime Faculty, examining each course catalog and weekly course plan, comparing the content of the SDGs with the content of the courses to determine the relevancy, and find strong and weak sides of the marine engineering curriculum from the aspect of the SDGs. According to the study findings, the top three relevant SDGs to the marine engineering curriculum are SDG4 – Quality education, SDG12 – Responsible consumption and production, and SDG8 – Decent work and economic growth with the percentages of 50%, 18%, and 13%, respectively. On the other hand, the least relevant three SDGs are SDG17 – Partnerships for the goals, SDG2 – Zero hunger, SDG1 – No poverty, and SDG15 – Life on land with the percentages of 1%, 2%, and 3% for the remaining two, respectively. In addition to the curriculum relevancy, IMO Agenda topics are examined and linked with SDGs. According to this examination, the marine engineering curriculum should be improved to achieve sustainable development-based marine engineering education. Moreover, maritime education and training should be changed from the STCW-based structure to the SD-based structure for a modern and sustainable marine engineering curriculum.

Please cite this paper as follows:

Zincir, B. (2022). Analyzing marine engineering curriculum from the perspective of the sustainable development goals. *Marine Science and Technology Bulletin*, *11*(2),158-168. https://doi.org/10.33714/masteb.1079480

* Corresponding author
E-mail address: bzincir@itu.edu.tr (B. Zincir)

Introduction

Sustainable development is a popular term worldwide nowadays, but the basis is formed in the 1980s. The definition of sustainable development is 'development that meets the needs of the present without compromising the ability of future generations to meet their own needs', according to the World Commission on Environment and Development (WECD) (UN, 1987). The main elements of sustainable development are determined as social equity, economic growth, and environmental protection. Figure 1 shows three pillars of sustainable development which are the foundation of the further agendas for sustainable development.

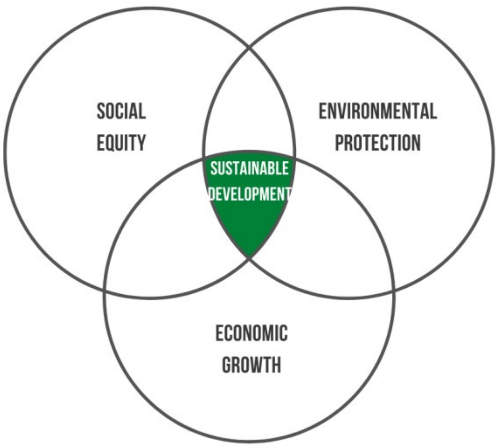


Figure 1. Three pillars of the sustainable development

To achieve and maintain sustainable development, the United Nations (UN) has been working on various declarations and agendas in history. The progress of the studies of the UN for sustainable development is shown in Figure 2. The first milestone of the works of the UN is the adoption of Agenda 21 at the Earth Summit in June 1992 (UN, 2022a). This agenda contains a comprehensive plan to promote and spread global cooperation for sustainable development. The next step is the Millennium Declaration at the Millennium Summit in September 2000. The Millennium Declaration describes 8 Millennium Development Goals (MDGs) with 18 targets under the three pillars of sustainable development (Rowihil & Farag, 2021). The MDGs are for fifteen years between 2000 and 2015. To improve the MDGs, the Johannesburg Declaration on Sustainable Development and the Plan of Implementation was adopted in 2002 at the World Summit on Sustainable Development. In June 2012, the Member States of the UN adopted the Future We Want as the outcome document of the UN Conference on Sustainable Development. This document is the start of the development process of Sustainable Development Goals (SDGs). And the last milestone of the works of the UN is the adoption of the 2030 Agenda for Sustainable Development in September 2015. This agenda includes 17 SDGs and 169 targets for people, the planet, and prosperity (UN, 2022b). The SDGs aim to build on MDGs to improve lacking sides for sustainable development. The 2030 Agenda determines goals and targets to achieve a higher standard of sustainable development by working on poverty, inequality, social, economic, and environmental preservation between 2015 and 2030 (Di Vaio et al., 2021).

Maritime transportation with its all stakeholders is one of the important elements of global sustainable development (Wang et al., 2020). Maritime transportation carries 90% of global trade (OECD, 2022) in the most efficient way if cargo ton per mile is considered (Inal & Deniz, 2021). Since maritime transportation is the major actor of global trade, it has links with almost all SDGs. International Maritime Organization (IMO) is also one of the UN organizations that support sustainable development and indicates that global sustainable development is directly connected to the sustainable development of maritime transportation (UNCTAD, 2019). Moreover, IMO worked on a concept is named "a sustainable maritime transport system" in 2013 and under this concept one of the focus areas is "Education and Training in Maritime Professions, and Support for Seafarers" (Rowihil & Farag, 2021). The main element of sustainable maritime transportation is high-quality maritime education and training (MET) which provides safe and effective action to the sea conditions (Ölçer et al., 2017).

Marine engineering education and training is one of essential elements in the MET. There are various engine parts, systems, and operations in the engine room that the marine engineers have to be aware of. Furthermore, new technologies are developed, international maritime regulations are evolved, and the way of thinking is started to change. The marine engineering curriculum should adapt to the new technologies, regulations, and ideas. For instance, the previous studies by Zincir & Deniz (2018) and Inal et al. (2021) focus on a course proposal to the marine engineering curriculum about alternative fuels and hybrid propulsion technologies. Dere et al. (2017) and Kandemir et al. (2018) have studies on effectively including engine room simulators at teaching and assessment methods. Two other studies are focused on distance/online learning courses/curricula (Michaeli, 2016; Balagiu & Sandiuc, 2020).

Sustainable development at maritime transportation is a recent idea that should be considered in the marine engineering curriculum. However, there is only one study as to the best of

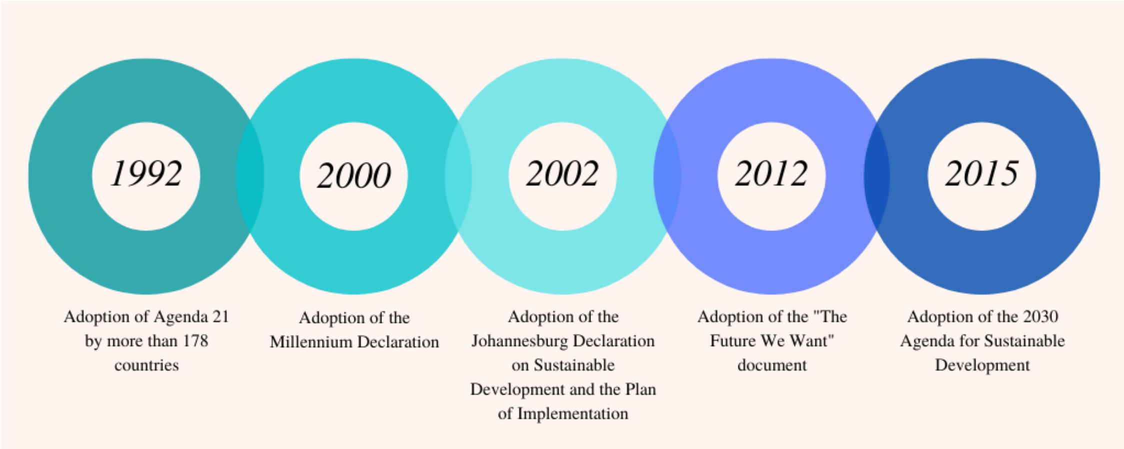


Figure 2. Progress of the studies for the sustainable development

author's knowledge that targeted maritime education and sustainable development topic (Aakre, 2021). The study aims to look at maritime education and sustainable development in Japan from a Norwegian perspective. There is a gap in the maritime education-sustainable development area and the purpose of this study is to fill the gap and contribute to the literature.

The main research question of this paper is what are the relevancy of SDGs and the marine engineering curriculum of Istanbul Technical University Maritime Faculty (ITU MF) and what areas should be improved to achieve a curriculum that supports sustainable marine engineering education. This study is the first study that analyzes all courses at the existing marine engineering curriculum of a university from the perspective of SDGs.

The marine engineering curriculum of ITU MF is used in this study. The relationship between SDGs and curriculum courses is examined and the relevancy percentage of each SDG is found. The discussion was made on the strong and weak sides of the curriculum considering SDGs and how to improve the existing STCW-based marine engineering curriculum to a sustainable development-based modern curriculum. The study shows that the marine engineering curriculum of ITU MF supports some SDGs more and some SDGs less. The findings indicate that some improvements should be made on the existing curriculum for the modernization of the marine engineering curriculum from an STCW-based structure to a more sustainable development-based (SD) structure.

Methodology

This section explains each step of the methodology. The details of the steps are shown in Figure 3. The study is done in five steps. The study starts with understanding the content of all SDGs and their relevance with maritime transportation. The next step of the study is to examine the updated marine engineering curriculum of ITU MF in 2021. ITU MF is the oldest maritime university in Turkey and has graduated many students since 1884. The faculty is inspected by European Maritime Safety Agency (EMSA) every five years. One of the roles of EMSA is inspecting maritime education and certification in non-EU countries (EMSA, 2022). After then, course catalog and weekly course plan of each course in the marine engineering curriculum of ITU MF are examined. The comparison of the content of the SDGs with the content of the courses in the marine engineering curriculum is the fourth step of the study. The final step of the study is to find the strong and weak sides of the marine engineering curriculum by considering the SDGs. Which SDGs are supported more and which SDGs are supported less is shown at the end of the study.

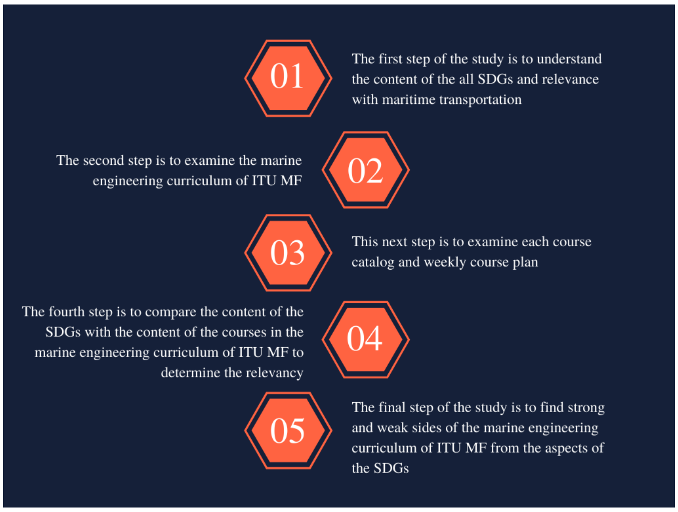


Figure 3. Steps of the study

Examination of the Marine Engineering Curriculum

Examination of the marine engineering curriculum section contains the analysis of the relationship between SDGs and maritime transportation and the relevancy of SDGs and marine engineering education.

Table 1. SDGs and their relevancy with maritime transportation

SDG No	SDGs	Description (UN, 2022a)	Relevancy (Carpenter et al., 2021)
1	No poverty	End poverty in all its forms everywhere	Partially/Directly
2	Zero hunger	End hunger, achieve food security and improved nutrition and promote sustainable agriculture	Partially
3	Good health and well-being	Ensure healthy lives and promote well-being for all at all ages	Partially/Directly
4	Quality education	Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all	Directly
5	Gender equality	Achieve gender equality and empower all woman and girls	Partially/Directly
6	Clean water and sanitation	Ensure availability and sustainable management of water and sanitation for all	Directly
7	Affordable and clean energy	Ensure access to affordable, reliable, sustainable and modern energy for all	Directly
8	Decent work and economic growth	Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all	Directly
9	Industry, innovation and infrastructure	Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation	Non
10	Reduced inequalities	Reduce inequality within and among countries	Non
11	Sustainable cities and communities	Make cities and human settlements inclusive, safe, resilient and sustainable	Partially
12	Responsible consumption and production	Ensure sustainable consumption and production patterns	Directly
13	Climate action	Take urgent action to combat climate change and its impacts	Directly
14	Life below water	Conserve and sustainably use the oceans, seas and marine resources for sustainable development	Directly
15	Life on land	Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss	Partially
16	Peace, justice and strong institutions	Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions at all levels	Directly
17	Partnerships for the goals	Strengthen the means of implementation and revitalize the global partnership for sustainable development	Directly

Sustainable development goals and maritime transportation

The 2030 Agenda of the UN contains 17 SDGs and 169 various targets under these SDGs. The SDGs are described with different colors and symbols which are shown in Figure 4. The SDGs are included in the main three pillars, social equity, economic growth, and environmental protection.

Table 1 contains the name and description of seventeen SDGs and their relevance with maritime transportation. There are not many studies in the literature that discuss the relevancy of the SDGs with maritime transportation. The study of Carpenter et al. (2021) is the only one as the author's knowledge, and it is used in this study. According to the derived information from the found study, SDG 9 – Industry, innovation and infrastructure and SDG 10 – Reduced inequalities are not relevant to maritime transportation. On the

Table 2. Marine engineering curriculum of ITU MF

Fall	Spring
1st Year	
Physics I	Physics II
Physics I Laboratory	Physics II Laboratory
General Chemistry I	Mathematics II
General Chemistry I Laboratory	Numerical Analysis of Engineering Systems
Mathematics I	Computer Aided Technical Drawing
Material Science and Manufacturing Methods	History of Turkish Revolution II
Introduction to Marine Engineering Ethics	Turkish II
Maritime Safety I	Maritime Safety II
Turkish I	Maritime English I
History of Turkish Revolution I	Academic Advising
2nd Year	
Engineering Mathematics	Probability and Statistics
Introduction to Scientific & Engineering Computing	Thermodynamics
Engineering Mechanics	Hydraulic and Pneumatics
Workshop	Fundamentals of Electronics
Naval Architecture and Stability	Fluid Mechanics
Marine Auxiliary Machinery I	Electrotechnics
Ship Emergency Response	Marine Diesel Engine I
Basic Swimming Skills	Elective Course
Elective Course	Elective Course
3rd Year	
Automatic Control Systems	Long Term Sea Training
Heat Transfer	
Marine Boilers and Operation	
Marine Diesel Engines II	
Ship Machinery Operation and Maintenance	
Engine Room Simulator (ERS) I	
Maritime Rules and Regulations	
Maritime Law	
Elective Course	
4th Year	
Marine Auxiliary Machinery II	Operation of Steam and Gas Turbines
Marine Electrotechnics	Engine Room Simulator (ERS) II
Marine Diesel Engines III	Marine Engineering Design II
Maritime English II	Economics
Elective Course	Elective Course
Elective Course	Elective Course

other hand, SDG 2 – Zero hunger, SDG 11 – Sustainable cities and communities, and SDG 15 – Life on land have partial relevancy with maritime transportation. SDG 1 – No poverty, SDG 3 – Good health and well-being, and SDG 5 – Gender equality have partial or direct relevancy with maritime transportation according to the targets included in these SDGs. The remaining SDGs have direct relevance with maritime transportation. This shows that 52.94% of the SDGs are directly relevant to maritime transportation, 17.64% are partially/directly relevant, 17.64% are partially relevant and 11.76% are non-relevant. It is obvious that maritime transportation is essential for the SDGs.

Sustainable development goals and marine engineering education

In this section, marine engineering courses of ITU MF are examined and which courses meet with the topics and targets of which SDGs are determined. Each course catalog and weekly course plan are checked to determine the relevancy with the SDGs. Table 2 shows the marine engineering curriculum of ITU

MF. This curriculum is the newest curriculum which was updated in 2021. In the first year, marine engineering students get courses on basic sciences and some introductory courses to engineering. In the second year of the curriculum, besides basic science courses, there are fundamental engineering courses such as “Thermodynamics” and “Fluid Mechanics”. There are also vocational courses in the second year of the curriculum for instance “Marine Auxiliary Machinery I” and “Marine Diesel Engine I”. Moreover, there are “Elective Courses” that the students can choose according to their interests. The fall semester of the third year contains one fundamental engineering course, “Heat Transfer”, vocational courses on marine engineering and maritime, and “Elective Courses”. The spring semester of the third year is the “Long Term Sea Training”. The marine engineering students do intern for six months on commercial ships worldwide. The students get intensive vocational courses and some “Elective Courses” at the last year of the curriculum and become marine engineers after the completion of the program.

Table 3. Keywords for each SDG

SDGs	Keywords
SDG1	Economy, social security, disasters
SDG2	Social security, disasters
SDG3	Safety, physical education, health, disaster
SDG4	Practical skill, manual skill, language skill, academic skill
SDG5	Rules, regulations, law, ethics
SDG6	Water, sea, sanitation, ecology, environment, disasters
SDG7	Energy, energy generation, energy efficiency
SDG8	Ethics, safety, advising, rules, regulations, law, economy, finance, human resource
SDG9	-
SDG10	-
SDG11	Law, environment, society, safety, quality
SDG12	Energy efficiency, consumption theory, design
SDG13	Environment, disasters, innovative technologies, clean energy
SDG14	Disasters, rules, regulations, environment, ecology
SDG15	Environment, disasters
SDG16	Advising, rules, regulations, law, economy, ethics, disasters
SDG17	Advising

The next step of the study is to match the marine engineering courses with the relevant SDGs. The matching of the courses with SDGs is determined according to the related keywords with each SDG. A similar approach was used at the study of Aleixo et al. (2020). The keywords are shown in Table 3 and the matching is shown in Table 4. The marine engineering course number in the curriculum is 112 which is the sum of compulsory given courses at maritime faculty (53) and allowed elective courses from other faculties (59) for marine engineering students.

Figure 4. Sustainable development goals (UN, 2022c)

Figure 5 shows the percentages of the relevant courses with SDGs. The courses, “Economics” and two elective courses “Social Security Law” and “Disaster Awareness” are related to SDG1 – No poverty. 3% of the total courses in the curriculum are related to this SDG. The elective courses, “Social Security Law” and “Disaster Awareness”, are in line with SDG2 – Zero hunger, which is only 2% of the total courses in the curriculum. SDG3 – Good health and well-being is supported by three courses of the department and four elective courses which correspond to 6% of the total courses in the curriculum. The targets of SDG4 – Quality education is in line with twenty-two courses of the department and thirty-four elective courses that equal 50% of the total courses in the curriculum. SDG4 is the most relevant goal with the courses of the marine engineering curriculum. Quality education is given by such as laboratories, workshop courses, swimming pool, etc. that include exercises and applications. SDG5 – Gender equality, SDG6 – Clean water and sanitation, and SDG7 – Affordable and clean energy are relevant with four, nine, and nine courses which are 4%, 9%, and 9%, respectively. SDG8 – Decent work and economic growth is the third most relevant SDG with the curriculum courses. It is linked with fourteen courses, seven of them are courses of the department and seven of them are elective courses which correspond to 13% of the total courses.

Table 4. Marine engineering courses relevant with the SDGs

SDGs	Marine Engineering Courses
SDG1	Economics / Elective Course
SDG2	Elective Course
SDG3	Maritime Safety I / Maritime Safety II / Basic Swimming Skills / Elective Course
SDG4	Physics I Lab. / General Chemistry I Lab. / Material Science and Manufacturing Methods / Maritime Safety I / Physics II Lab. / Maritime Safety II / Maritime English I / Academic Advising / Introduction to Scientific & Engineering Computing / Workshop / Ship Emergency Response / Basic Swimming Skills / Fundamentals of Electronics / Automatic Control Systems / Ship Machinery Operation and Maintenance / Engine Room Simulator I / Long Term Sea Training / Marine Electrotechnics / Marine Engineering Design I / Maritime English II / Engine Room Simulator II / Marine Engineering Design II / Elective Course
SDG5	Academic Advising / Maritime Rules and Regulations / Elective Course
SDG6	Marine Auxiliary Machinery I / Long Term Sea Training / Marine Auxiliary Machinery II / Elective Course
SDG7	Thermodynamics / Marine Diesel Engines I / Heat Transfer / Marine Diesel Engines II / Marine Diesel Engines III / Operation of Steam and Gas Turbines / Elective Course
SDG8	Introduction to Marine Engineering and Ethics / Maritime Safety I / Maritime Safety II / Academic Advising / Maritime Rules and Regulations / Maritime Law / Economics / Elective Course
SDG9	-
SDG10	-
SDG11	Elective Course
SDG12	Marine Auxiliary Machinery I / Thermodynamics / Fluid Mechanics / Marine Diesel Engines I / Heat Transfer / Marine Diesel Engines II / Engine Room Simulator I / Long Term Sea Training / Marine Auxiliary Machinery II / Marine Diesel Engines III / Marine Engineering Design I / Operation of Steam and Gas Turbines / Engine Room Simulator II / Marine Engineering Design II / Elective Course
SDG13	Maritime Rules and Regulations / Long Term Sea Training / Elective Course
SDG14	Ship Emergency Response / Engine Room Simulator I / Maritime Rules and Regulations / Long Term Sea Training / Engine Room Simulator II / Elective Course
SDG15	Elective Course
SDG16	Introduction to Marine Engineering and Ethics / Academic Advising / Maritime Rules and Regulations / Maritime Law / Economics / Elective Course
SDG17	Elective Course

Table 5. IMO Agenda topics between 2020-2022 and covering SDGs

IMO Topics	Covering SDGs
Safety of navigation, communication and search and rescue	SDG11, SDG14, SDG17
Ship design and construction	SDG12, SDG17
Pollution prevention and response	SDG6, SDG13, SDG14, SDG15, SDG17
Ship systems and equipment	SDG12, SDG17
Human element, training and watchkeeping	SDG3, SDG4, SDG17
Carriage of cargoes and containers	SDG8, SDG17
Reduction of greenhouse gas emissions from ships	SDG7, SDG13, SDG15, SDG17
Implementation of IMO instruments	SDG16, SDG17

Table 6. STCW-based and SD-based MET (Rowihil & Farag, 2021)

STCW-based MET	SD-based MET
Curriculum content with minimum requirements for the competence	Aim to reach higher standards at the education
Related to shipboard skills and knowledge	Related to interaction with ship, society, and environment
Aim to improve skills and knowledge (psychomotor and cognitive areas)	Aim to improve skills, knowledge, and values (psychomotor, cognitive, and affective areas)
Reactive (focus on respond to situation on ships)	Proactive (focus on anticipation and acting)
Trainee centered education	Institutional centered education
Vocational education	Academic and critical
Career shift of the individual is disregarded	Career shift of the individual is regarded

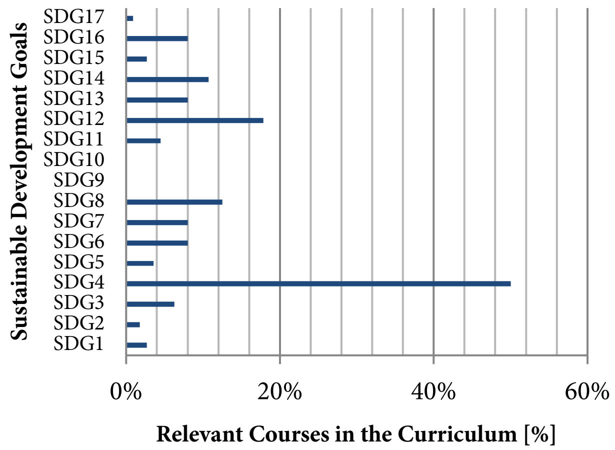


Figure 5. Relevant SDGs-courses in the marine engineering curriculum

SDG9 – Industry, innovation and infrastructure and SDG10 – Reduced inequality are not relevant with the curriculum courses. SDG11 – Sustainable cities and communities is in line with five elective courses which are "Occupational Safety and Health Law", "Environment and Society", "Quality and Safety Management Systems", "Marine Insurance", and "Occupational Health and Safety". The courses correspond to 4% of the total courses in the curriculum. The second most relevant SDG with the courses in the curriculum is SDG12 – Responsible consumption and production. This SDG is relevant with twenty courses which equal 18% of the curriculum. SDG13 – Climate action is one of the important SDGs, but it is only relevant with nine courses, two compulsory and seven elective courses, that corresponds to 8% of the curriculum. SDG14 – Life below water, SDG15 – Life on land, and SDG16 – Peace, justice and strong institutions relevant with twelve, three, and nine courses that equal to 11%, 3%, and 8% of the courses in the curriculum, respectively. The least relevant SDG is SDG17 – Partnerships for the goals is relevant to one elective course, "International Relations and Globalization", which equals approximately 1% of the total curriculum. Lastly, it is observed that twenty-four courses, equal to 21% of the courses at the curriculum, are not related to any SDGs.

Results

This section discusses the strong and weak sides of the marine engineering curriculum of ITU MF. The previous steps of the study show that the curriculum is strong at SDG4 – Quality education, SDG12 – Responsible consumption and production, and SDG8 – Decent work and economic growth with the relevancy of 50%, 18%, and 13%, respectively. After these SDGs, the curriculum is relevant to SDG14 – Life below water by 11%. The remaining SDGs are covered below 10% of the curriculum courses. The least covered SDGs by the curriculum courses are SDG17 – Partnerships for the goals, SDG2 – Zero hunger, SDG1 – No poverty, and SDG15 – Life on land with 1%, 2%, 3%, and 3%, respectively. The findings showed that 1 course is relevant to nine SDGs, 5 courses are relevant to 6 SDGs, 2 courses contain topics from 5 SDGs and another 2 courses include topics from 4 SDGs. 12 courses are relevant to 3 SDGs and 18 courses are pertinent to 2 SDGs. 24 courses are not relevant to any SDGs, and the remaining 49 courses include topics from 1 SDG. The analysis showed that the marine engineering curriculum should be improved by updating the content of the courses with the related topics which are in line with the targets of SDGs.

Discussion

IMO is one of the agencies of the United Nations and determines the minimum standards for international maritime transportation. There are Maritime Safety Committee (MSC) and Marine Environment Protection Committee (MEPC) under IMO. Under these main committees, there are sub-committees that are working groups on different focus areas. IMO prepares its yearly agenda and what topics are discussed at that year. Table 5 shows IMO Agenda between 2020 and 2022 that sub-committees discussed or will discuss on these topics (IMO, 2022). The Agenda shows the focus areas of IMO and the marine engineering education has to be in parallel with IMO focus areas. The topics are linked with related SDGs to understand the status of the marine engineering curriculum of ITU MF. All topics are covered by SDG17 – Partnerships for the goals since the reason for arranging working group meetings is to establish strong partnerships to achieve the targets.

Safety of navigation, communication and search and rescue is one of the IMO topics that are covered by SDG11, SDG14, and SDG17. These SDGs are related to 4%, 11%, and 1%, respectively, at the marine engineering curriculum. SDG14 is moderately met by the curriculum. On the other hand, SDG11 and SDG17 are weakly met and the curriculum should be updated to increase the number of courses related to these SDGs. Since SDG17 covers all topics, no comments will be made on the remaining topics.

Ship design and construction topic is covered by SDG12 and SDG17 which correspond to 18% and 1%, respectively, of the curriculum. SDG12 is almost strongly met by the curriculum. Because responsible consumption is required on ships, therefore this is supported by given courses at the curriculum.

Pollution prevention and response topic is related to SDG6, SDG13, SDG14, SDG15, and SDG17 which correspond to 8%, 8%, 11%, 3%, and 1%, respectively, of the curriculum. SDG6, SDG13, and SDG14 are moderately met by the curriculum, but the percentage of SDG15 has to be improved by adding new courses to the curriculum on life on land.

The fourth topic is ship systems and equipment that is covered by SDG12 and SDG17. This topic has the same SDGs to ship design and construction topic. This topic is almost strongly met by the marine engineering curriculum.

The human element, training and watchkeeping topic is related to SDG3, SDG4, and SDG17 with the curriculum percentages of 6%, 50%, and 1%, respectively. SDG4 is supported strongly by the marine engineering curriculum. Nevertheless, SDG3 – Good health and well-being should be supported by new course additions to the curriculum.

Carriage of cargoes and containers is covered by SDG8 and SDG17 which correspond to 13% and 1%, respectively, of the curriculum. SDG8 – Decent work and economic growth is moderately supported by the curriculum courses.

The seventh topic is the reduction of greenhouse gas emissions from ships, which is one of the important topics for IMO, which is covered by SDG7, SDG13, SDG15, and SDG17. These SDGs are 8%, 8%, 3%, and 1% relevant respectively to the courses at the marine engineering curriculum. The curriculum has to be improved to adequately meet with the SDG7 – Affordable and clean energy, SDG13 – Climate action, SDG15 – Life on land, and SDG17 – Partnerships for the goals.

The last agenda topic is the implementation of IMO instruments that is covered by SDG16 and SDG17. SDG16 – Peace, justice and strong institutions relevant to the 8% of the curriculum. New courses have to be added to the curriculum to increase the percentage of relevancy.

The curriculums at the MET are prepared according to the Standards of Training Certification and Watchkeeping (STCW) Convention to comply with the minimum requirements of competencies for marine engineers or marine officers. Therefore, the MET at the maritime universities is STCW-based. The world is changing and maritime universities have to keep up with the change. If sustainable development (SD) is considered at the MET, some updates should be made on the MET curriculum for improvement. Differences between STCW-based and SD-based MET are shown in Table 6. The curriculum should include higher standards rather than minimum requirements of the competencies of STCW. The content of the curriculum should focus on link and interaction with ship, society, and environment and should aim to improve psychomotor, cognitive, and affective areas of the individuals. The courses at the curriculum should improve the anticipation and acting skills of the individuals and should care about the possibility of career shift of the graduated persons. The MET should be more academic and critical, and rather than individual-centered it should be institutional-centered. In addition to these, the MET should include knowledge and understanding of environmental protection, social and economic wellbeing not only in the present but also at the future generations (Mkpandiok & Ukpai, 2017). These properties of SD-based MET have to be considered to improve the marine engineering curriculum of ITU MF and to make the curriculum more supportive of the SDGs.

On contrary, despite SD-based MET will improve maritime higher education, there are still challenges to be applied. Although the importance of SD is well-known, not only MET but also all higher education has not adapted SD into the education system (Aleixo et al., 2018). The positive thing is there are some case applications in the higher education curriculum, but it has not spread to higher education institutions yet (Barth & Rieckmann, 2012). This study reveals that the marine engineering curriculum of ITU MF is not completely in line with SDGs. This finding is similar to the finding of the study of Mkpandiok & Ukpai (2017) which was done for MET in Nigeria. Some courses contain SDG or SDGs in their content, however more clear and integrated course contents are required to meet SDGs which is also stated in the study of Aleixo et al. (2020). With the Covid-19 pandemic, the education system has been in transition from conventional face-to-face education to more online education. New online education programs and systems can speed up the integration of SDGs to MET as is also discussed in a previous study (Dyagileva et al., 2020).

Conclusion

This paper aims to answer the question, what are the relevancy of SDGs and the marine engineering curriculum of ITU MF, and what areas should be improved to achieve a curriculum that supports sustainable marine engineering education. This study is the first study that analyzes all courses at the existing marine engineering curriculum of a university from the perspective of SDGs. Five stepped methodology is applied, which are understanding the content of all SDGs and relevance with maritime transportation, examining the marine engineering curriculum of ITU MF, examining each course catalog and weekly course plan, comparing the content of the SDGs with the content of the courses to determine the relevancy, and find strong and weak sides of the marine engineering curriculum from the aspect of the SDGs.

According to the study findings, the top three relevant SDGs to the marine engineering curriculum are SDG4 – Quality education, SDG12 – Responsible consumption and production, and SDG8 – Decent work and economic growth with the percentages of 50%, 18%, and 13%, respectively. On the other hand, the least relevant three SDGs are SDG17 – Partnerships for the goals, SDG2 – Zero hunger, SDG1 – No poverty, and SDG15 – Life on land with the percentages of 1%, 2%, and 3% for the remaining two, respectively. In addition to the curriculum relevancy, IMO Agenda topics are examined and linked with SDGs. According to this examination, the marine engineering curriculum should be improved for met a higher percentage with SDG3, SDG7, SDG11, SDG13, SDG15, SDG16, and SDG17. Moreover, the MET should be changed from the STCW-based structure to the SD-based structure for a modern and sustainable marine engineering curriculum.

This study sheds light on future studies about the modernization of the marine engineering curriculum for including a more SD-based structure. Course proposals can be done to improve the curriculum for further studies. Also, the study can be adapted for the maritime transportation engineering curriculum of ITU MF for future study.

Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

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

References

Aakre, B. M. (2021). Maritime education and sustainable development in Japan, a Norwegian perspective. *Quest for Technical Pedagogy*, *23*, 1-24.

Aleixo, A. M., Azeiteiro, U. M., & Leal, S. (2018). The implementation of sustainability practices in Portuguese higher education institutions. *International Journal of Sustainability in Higher Education*, *19*(1), 146-178, https://doi.org/10.1108/IJSHE-02-2017-0016

Aleixo, A. M., Azeiteiro, U. M., & Leal, S. (2020). Are the sustainable development goals being implemented in the Portuguese higher education formative offer? *International Journal of Sustainability in Higher Education*, *21*(2), 336-352. https://doi.org/10.1108/IJSHE-04-2019-0150

Balagiu, A., & Sandiuc, C. (2020). Developing an online course for marine engineering. *Scientific Bulletin of Naval Academy*, *XXIII*, 282-286. https://doi.org/10.21279/1454-864X-20-I1-040

Barth, M., & Rieckmann, M. (2012). Academic staff development as a catalyst of curriculum change towards education for sustainable development: An output perspective. *Journal of Cleaner Production*, *26*, 28-36, https://doi.org/10.1016/j.jclepro.2011.12.011

Carpenter, A., Skinner, J. A., & Johansson, T. M. (2021). Conclusions: Connecting sustainable development goals to the maritime domain. In Carpenter, A., Johansson, T. M., & Skinner, J. A. (Eds.), *Sustainability in the Maritime Domain* (pp. 489-507). Springer. https://doi.org/10.1007/978-3-030-69325-1_22

Dere, C., Zincir, B., & Deniz, C. (2017). Usage of simulator as an energy efficient operation of main engine practice. *Proceedings of the 13th International Conference on Engine Room Simulators,* Ukraine, pp. 202-208.

Di Vaio, A., Varriale, L., Lekakou, M., & Stefanidaki, E. (2021). Cruise and container shipping companies: a comparative analysis of sustainable development goals through environmental sustainability disclosure. *Maritime Policy & Management*, *48*(2), 184-212. https://doi.org/10.1080/03088839.2020.1754480

European Maritime Safety Agency (EMSA). (2022). *Overview*. Retrieved on December 12, 2021, from https://european-union.europa.eu/institutions-law-budget/institutions-and-bodies/institutions-and-bodies-profiles/emsa_en

Inal, O. B., & Deniz, C. (2021). Emission analysis of LNG fuelled molten carbonate fuel cell system for a chemical tanker ship: a case study. *Marine Science Technology Bulletin, 10*(2), 118-133. https://doi.org/10.33714/masteb.827195

Inal, O. B., Dere, C., Zincir, B., & Deniz, C. (2021). Hybrid propulsion and alternative fuels education in the course of decarbonised shipping. *Australian Journal of Maritime & Ocean Affairs, In press*. https://doi.org/10.1080/18366503.2021.1940475

International Maritime Organization (IMO). (2022). *Meeting Summaries and Schedule*. Retrieved on January 8, 2022, from https://www.imo.org/en/MediaCentre/MeetingSummaries/Pages/default.aspx

Kandemir, C., Soner, O., & Celik, M. (2018). Proposing a practical training assessment technique to adopt simulators into marine engineering education. *WMU Journal of Maritime Affairs, 17*(1), 1-15. https://doi.org/10.1007/s13437-018-0137-4

Michaeli, J. G. (2016). Developing a distance learning curriculum for marine engineering education. *Proceedings of the 2016 ASEE Annual Conference & Exposition,* Louisiana, USA, 9p. https://doi.org/10.18260/p.26722

Mkpandiok, A., & Ukpai, U. E. (2017). Managing maritime education and training for the attainment of sustainable development goals in Nigeria. *World Educators Forum*, *9*(1), 1-15.

OECD. (2022). *Ocean Shipping and Shipbuilding*. Retrieved on March 18, 2022, from https://www.oecd.org/ocean/topics/ocean-shipping/#:~:text=The%20main%20transport%20mode%20for,transport%20arteries%20for%20global%20trade

Ölçer, A. I., Ballini, F., Kitada, M., & Dalaklis, D. (2017). Development of a holistic maritime energy management programme at the postgraduate level: the case of WMU. *Proceedings of the 11th annual International Technology, Education and Development (INTED 2017) Conference*, Spain, pp. 1426-1432.

Rowihil, M. S., & Farag, Y. B. A. (2021). Sustainable development in maritime education and training; trends, challenges and the way forward. Strathprints, *Preprint*. https://strathprints.strath.ac.uk/77215/

United Nations (UN). (2022a). *The 17 Goals*. Retrieved on December 10, 2021, from https://sdgs.un.org/goals

United Nations (UN). (2022b). *Transforming our world: the 2030 Agenda for Sustainable Development*. Retrieved on December 10, 2021, from https://sdgs.un.org/2030agenda

United Nations (UN). (2022c). *Communication materials*. Retrieved on December 10, 2021, from https://www.un.org/sustainabledevelopment/news/communications-material/

United Nations Conference on Trade and Development (UNCTAD). (2019). *Review of Maritime Transport 2019*. Retrieved on December 11, 2021, from https://unctad.org/

United Nations General Assembly (UN). (1987). *Development and international economic co-operation: environment. Report of the world commission on environment and development: our common future*. Retrieved on December 10, 2021, from https://sswm.info/sites/default/files/reference_attachments/UN%20WCED%201987%20Brundtland%20Report.pdf

Wang, X., Yuen, K. F., Wong, Y. D., & Li, K. X. (2020). How can the maritime industry meet sustainable development goals? An analysis of sustainability reports from the social entrepreneurship perspective. *Transportation Research Part D: Transport and Environment*, *78*, 102173. https://doi.org/10.1016/j.trd.2019.11.002

Zincir, B., & Deniz, C. (2018). A course proposal for the training of marine engineering students about alternative fuels, related systems, and operation. *Proceedings of the 19th Annual General Assembly (IAMU AGA 2018)*, Spain, pp. 37-45.

Mar. Sci. Tech. Bull. (2022) 11(2): 169-178
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1053864

Marine Science and Technology Bulletin

REVIEW ARTICLE

Design criteria for the floating walkways and pontoons considering the extreme climatic conditions

Sami M. Ayyad[1*]

[1] *Amman Arab University (AAU), Faculty of Engineering, Civil Engineering Department; Jordan Street–Mubis, 11953, Amman, Jordan*

ARTICLE INFO

Article History:
Received: 13.01.2022
Received in revised form: 16.05.2022
Accepted: 16.05.2022
Available online: 15.06.2022

Keywords:
Identification
Manufacturing
Transportation
Installation
Construction

ABSTRACT

The study has addressed design criteria about how the industry of the floating reinforcement concrete precast (pontoons) is installed in the factory with the combinations of utility, electricity services, and internet service. The pontoon bridges are successfully installed on the road for transport. The installation process for pontoons is successfully attempted in a balanced situation above the surface of the sea to the resistance of floating precast (pontoons) to any ambient effects such as weather conditions, the movement of the waves, or any other effects. This study has found new inspirations to identify the pontoon bridges of the future in spite of the fact that several new ideas are already presented by the most imaginative designers. A detailed explanation regarding the procedures of mold preparation, reinforcement, and use of expanded polystyrene, casting, and the pontoon installation is presented. The findings of the study may help engineers in providing firm considerations to the technical details of the construction process while considering the significance of extreme climatic conditions in various regions.

Please cite this paper as follows:

Ayyad, S. M. (2022). Design criteria for the floating walkways and pontoons considering the extreme climatic conditions. *Marine Science and Technology Bulletin*, *11*(2), 169-178. https://doi.org/10.33714/masteb.1053864

Introduction

Floating structures that are large-scale are majorly classified as either pontoons or semisubmersibles. Pontoons are basically floating slabs with low depth-to-width ratios that are placed in calm seas along the shore, inside a cove or lagoon, or where breakwaters and other protective structures may be built to shield the structure from high waves and surges (Wang & Wang, 2015) Moreover, in the history of floating structures, a prominent position is also enjoyed by the floating bridges. Floating walkways are a linear pontoon system formed from numerous hinged flotation modules and supported by a boat

* Corresponding author
E-mail address: samiayyad@aau.edu.jo (S. M. Ayyad)

ramp lane during tides, according to the Queensland Government's Department of Transport and Major Highways (2015). It is usually without a gangway or access bridge and is immediately attached to a cast in situ concrete abutment. Floating bridges generally serve as a structure of last resort which can only be used for marshes, ponds, bogs, or other areas that are too damp to trail bridges that are traditional and less costly.

According to Wang & Wang (2015), the earliest floating bridges of notes were created in 480 BC during the invasion of Greece by the Persian king Xerxes. In addition, the history of floating bridges can go back up to 2000 BC, according to Watanabe & Utsunomiya (2003); however, only in recent decades where floating bridges are being used in modern infrastructures. According to Chandler (2021), there are currently twenty floating bridges around the world, and out of them, four are only present in Washington. Important trends in the development of floating bridges and other floating structures have been discussed by Wang & Wang (2015). The latest floating bridge constructed in 2007 is in Dubai, which is constructed across the Dubai creek and connected Bur Dubai with the Deira section of Dubai.

Neese (2002) mentioned that the best water-resistant material that can provide firm support to a floating structure is the one whose specific gravity is lower than water. Besides, a reliable floating structure is one that is developed from a small section of plywood fixed to floating logs which is reinforced through concrete pontoons. However, the complex structure and size of floating walkways majorly depend on the environment in which it is being placed. These types of bridges are rare and are generally found in a few locations. However, in the majority of locations, boardwalk, puncheon, and traditional bridge are often preferred.

Pontoons, on the other hand, serve as a temporary fixed floating structure and are used as a pathway for commuters to reach the surrounding water. It consists of a gangway ramp style that is developed and designed to match to shore. Floating pontoons are constructed focusing on the tropical waterways, which experience high and low floods (season). They are made to survive through severe loading conditions such as high and low floods while ensuring to meet unique requirements such as; function, safety, and structure. Such designs are further important in mitigating and protecting the tropical environment, which serves as a source of retaining the cultural heritage (Igwe & Ajoko, 2020). The manufacturing of pontoons is based on different materials, dimensions, and weights in marine construction to enable them to lift large weights. It has been broadly accepted that pontoon bridges are only available in the field of military engineering (Derewenko et al., 2011). However, these bridges are also seen for swamps and crossing rivers and, therefore, are considered as obsolete and folkloristic works.

Pontoons are widely used for different purposes, such as timber pontoons, concrete pontoons, and metal pontoons (steel and aluminum). It is a common dilemma that the theory and technique of the pontoon bridges are not taught in the universities (Zhang et al., 2010). Most structural designers do not think that they have an opportunity to tackle such situations. On the contrary, the pontoon bridges are of significant importance to installing new structural applications, whereas it presents a rapid, economical, and simple solution to cross water sheets (Tattoni, 2007).

According to Australian Standard Guidelines (2001) for design marinas, concrete pontoons are present in different dimensions, such as concourse pontoons up to (6 m wide, 1 m wide central service channel, 20 m long, and 1.5 m height), superyacht pontoons up to (4 m wide, 20 m long, 1.5 m height), premier pontoons (3 m wide, 15 m long, 1.2 m height), and superyacht fingers pontoons (2 m wide, 20 m long, 1.2 m height) (Australian Standard Guidelines, 2001). The quality assurance system ISO 9001:2000 is used in the fabrication of concrete pontoon, which is manufactured through preparing the mold, reinforcing installation, using expanded polystyrene, and casting of concrete (Planning and Design Guidelines for Small Craft Harbors, 2000). Several studies focused on the structures and construction of floating walkways, which include the evolutionary development of floating structures and pontoons. For instance, Morra (2012) conducted a study and provided details related to the processes involved in the construction of floating structures and pontoons. The study included information regarding the previous characteristics of floating structures, and technological developments, along with the need that brought about these changes. The study was central in outlining different issues that are significant in the advancement of the floating structures. Another study was conducted by Rapo (1981) and provided significant details in relation to the facilities and different types of floating structures. However, according to the study, different circumstances and possible damages are caused by severe damages in the docking. The study suggested block loading, ship strength, and dock stability as the major sources of preventing increased damage.

In general, floating bridges are balanced vertically by pontoons. A number of elements should be considered in the design of floating bridges. The structural eigen properties of floating bridges are influenced by several parameters, which include the cross-section properties, end connections, curvature of the curved bridges, pontoon types, and the number of pontoons. The environmental loads, including the wave current, wind, and tidal forces, excite the dynamic responses of the bridge under different types of loads. Fluid-structure interaction is certainly important in the case of a tsunami or extreme wave impact loads from the environment.

The idea of a self-floating reinforced concrete precast bridge is a novel idea to be installed in the shipyard based on tugging and assembling in the selected locations. Another important idea is to support a pontoon bridge by a railway line crossing the Bering Strait. Therefore, this study has presented the steps to manufacture and install the Floating Reinforcement Concrete Precast considering the extreme climatic conditions. The development of curved floating bridges considers conventional onshore arch bridges that are conceptualized as vertically curved and axially compressed structural systems. The elevation, as a curved structure, is as critical as the span length. In addition, the rise-to-span ratio is a fundamental indicator of the structural properties of a curved bridge. As a curved structure, the rise is a critical parameter that is as important as the span length.

Moreover, the rise-to-span ratio is a key indicator of the structural properties of a curved bridge. Theoretically, the elbow structure only forms a compressive force along the center of the mass line. Although it is virtually impossible due to complex load combinations, it is possible to study the perfect rate of increase to achieve a perfect internal power distribution. In the case of a floating bridge, due to local constraints, it is not possible to design the lift conversion ratio too much. On the other hand, shallow arches are believed to cause significant horizontal movement of the subsistence and have the disadvantages of high deformation and low rigidity. It is not yet known whether the horizontally curved floating bridge has such a disadvantage when it is centuries old. In this article, a relatively low rate of elevation and selection is chosen for a curved floating bridge, and the response and internal forces are investigated by changing the boundary conditions (BCs) and span. It is believed that the distribution of the pontoon is in the coastal sea of Singapore. The water depth is very shallow. Therefore, the waveform characteristics of deep-sea pontoons are also different. In addition, the cross-section of the bridge was developed to meet high traffic flow requirements (three lanes each way), and the span of the bridge was relatively short. In this case, the horizontal stiffness of the bridge is expected to be high. Therefore, a lower rate of increase is studied at an early stage to see if arc activity can be achieved. However, when compared to previous research, these new properties of floating bridges are relatively new; therefore, there is a need to explore these properties of floating bridges.

Experimental Investigation of Pontoon Bridges

Design Development

The design standard is that the structure of the reservoir must be stable and capable of carrying personnel and materials. The design should ensure stability and minimize the impact of registration because the pontoon bridge is supported by two concrete sinkers. The aim of the design is to maximize the cargo (that is, cargo space) that the floating ship can effectively carry. Finally, the project should strike a balance between this optimization and the construction cost of the pontoon, given the weight of the pontoon. The scope of the design is to develop and use ordinary carbon steel as a point for a landing dock. Gangway made of steel pipe with a wooden bottom/floor, with a mound base as an anchor on the shore and an offshore roll on the floor of the lagoon. The concept of design will be limited to floating rules and production descriptions. The design will mainly include the pontoon and the gangway (gangway), and the strength analysis of the material will only be done on the components of the pontoon. The material used in the construction of the pontoon pier shall be steel. Building components and non-building components must be cold-formed to meet the requirements of AISI S240 Chapter A3 and AISI S220 Chapter A4. Organizing members should be limited to category 33 (230) and category 50 (340), while non-structured members should be limited to category 33 (230). Corrosion protection should meet the same standards.

Preparing the Mold

Mold plates are made of smooth mold purpose steel as they are installed based on required tolerances to meet all structures measured. The fixing bolts are tightened, and all holes are plugged with welding and grounded smooth to clean the mold surfaces and treat them with oil (Figures 1 and 2).

Figure 1. Preparation of the mold

Figure 2. Phases of mold preparation

Reinforcing Installation

The reinforced concrete industry provides increased significance to use higher strength reinforcing steel for specific applications (Morra, 2012). Relief of congestion is the prime factor toward this interest, specifically in constructions assembled in a high seismic design category. Construction efficiencies or high-strength concrete enhance other areas where a high-strength bar enables reinforced concrete is required to be utilized for more inspiring applications. Today, Grade 60 steel is used within the concrete design and construction along with the occasional but increasing use of Grade 75. It has been notified that different grade of steel can be used for large projects with higher grades and a minimum grade for ordinary tasks for more inspiring structures. The increase in costs is generally observed for higher grades; therefore, the material is used in small quantities. In the actual scenario, small concrete members are usually permitted by higher grades that are associated with the space issues for reinforcement placement. The steel acts as a central supporting material, although the steel usually comprises merely a few percent of the total reinforced concrete volume. The cutting and bending, the cost of the steel, and the forming of the deformed bars are included in the form's installation. The general attempt is represented by the cost-saving factor of using the minimal reinforcement and the concrete, which reflects typical unit costs for the two materials.

All reinforcing mesh and steel bars of stainless steel to resist corrosion (side mesh, intermediate wall mesh, intermediate wall corner and vertical rebar, sidewall upper long rebar, deck mesh) are included in the pontoon installation. The minimum yield stress of steel bars is 500 MPa, which must be cleaned completely of lubricate and any dirt to use plastic spacers and checked all installed reinforcing. It is not allowed to mix stainless and galvanized when attaching seaflex to anchors.

The importance of Grade 500 steel allows for reinforcement bars in all international and national building codes and standards. All the current codes restrict the allowable design strength of reinforcement to 80 Ksi (MPa) from a design standpoint. An economy can also be accomplished using Grade 500 steel rather than commonly accessible Grade 415 bars in the market. One of the main advantages of using Grade 500 is to replace the steel congestion in the foundation mat and at the beam-column joints. On the contrary, the development length differs for Grade 500 steel as compared to Grade 415 steel. Higher-strength concrete and better engineering judgments are required for achieving maximum benefits from Grade 500 steel.

Using Expanded Polystyrene (EPS)

The manufacturing of buoys, floats, and pontoons is commonly observed through expanded polystyrene (EPS) due to its excellent physical properties as it has better resilience and buoyancy and is light in weight. Archimedes' principles are used for buoys, floats, and pontoons to displace their own fluid weight. There is no standard shape, pattern, or design for pontoons, floats, and buoys, whereas it is simply customized on the basis of the application nature. It is not essential that EPS is used to manufacture buoys, but pontoons are manufactured with high densities either with proof coating or without coating (Figure 3).

Center of buoyancy

A floating object creates a water displacement; the center of gravity of the displaced volume of water is called the center of buoyancy. The center of buoyancy is important because the resulting upward force engages here. The position of this center of buoyancy can be calculated for the different shapes, assuming a center or evenly distributed load on the floating body.

Rectangular body

The center of buoyancy for a rectangular body is:

$$B_{rec} = \frac{1}{2} d \tag{1}$$

B_{rec} = center of buoyancy measured from water surface [m]

Triangular body

The center of gravity for a triangular body is:

$$\mathcal{Z} = \frac{S_y}{A} \tag{2}$$

$\mathcal{Z}$ = position center of gravity [m]
S_y = static moment in the x-y area [m^3]
A = area [m^2]

$$A_{tri} = \frac{1}{2}\, w, h \tag{3}$$

A_{tri} = area triangle [m^2]
w = width of floating body (m)
h = height of floating body (m)

$$S_y = \frac{1}{3} w.h^2 \tag{4}$$

S_y= static moment in the x-y area [m^3]
w = width of floating body (m)
h = height of floating body (m)

Substituting these formulas into the formula of the center of gravity results in:

$$\mathcal{Z} = \frac{2}{3}\, h \tag{5}$$

The center of gravity of an equilateral triangular object is seen from the base, at one-third of the height.

The center of buoyancy for a triangular body is then:

$$B_{tri} = \frac{1}{3} d \tag{6}$$

B_{tri} = center of buoyancy measured from water surface [m]

Cylindrical body

The center of gravity for a half-cylindrical body is:

$$\mathcal{Z} = \frac{4.r}{3\pi} \tag{7}$$

$\mathcal{Z}$ = position center of gravity [m]
r = radius (m)

This is approximately 0,42 times the radius (0,42r).
The center of buoyancy for a half-cylindrical body is then:

$$B_{cyt} = \frac{4.r}{3\pi} \approx 0{,}42r \tag{8}$$

B_{cyt}Center of buoyancy measured from water surface [m]

This formula only applies to a cylindrical body that is half fully underwater. Otherwise, the Simpson method should be used to determine the center of gravity and buoyancy.

Figure 3. Use of expanded polystyrene

Features of EPS Pontoons

Stable

High-quality expanded polystyrene materials are used to form these products. The main reason for using these high-quality materials is that it floats on water and will not crumble once an individual board on them.

Durable

EPS is lightweight and durable to withstand any amount of weight, specifically for pontoons. Due to its stability, individuals in huge numbers can stand on it regardless of any fear of falling off.

High compressive strength

The manufacturing process of pontoons is meticulously attempted to ensure that it is tough and robust, to a certain extent, to withstand extreme climatic conditions. The manufacturing process of pontoon EPS is two-fold. Steam is used to expand the raw beads by creating pre-puff beads that are cured in large bags before mold preparation. The pre-puff beads are steamed to form a block that is anywhere from 37-1/2″ – 54″ x 49″ x 121-1/2″-220″ once in the mold. Afterward, the placement of blocks is made on the storage floor based on the adequate time length before being cut into assorted sizes and shapes. To meet specific project needs, blocks are manufactured and produced in several densities.

EPS size and density

There are different block sizes available for state-of-the-art Hirsch mold anywhere from 37-1/2″ – 54″ x 49″ x 121-1/2″ – 220″. The most common cut sizes available are 48 inches X 96 inches and 24 inches X 96 inches, even though pontoon does not stock any foam. Following specifications are used in the manufacturing process of pontoons.

Table 1. EPS requirements

Items	Dimensions
Thickness	1/4″ to 54″
Lengths	Up to 216″ (18′)
Widths	Up to 48″
Densities	75 lb to 2.85 lbs per cubic foot

During the pontoons manufacturing, all the ducts are needed for the installations of utility, electricity services, and Internet service. The EPS blocks were used as filling and casting molds inside the pontoon, and the bottom was treated to resist marine borers by using polyurea coating and after reinforcing installation, which allowed to lift off the readymade EPS into the mold.

Casting

Before proceeding to the casting process, the area of casting is examined since an equally leveled casting is required. Check the height position of deck mesh before casting is checked; the strength of concrete is durable (45 N/mm. s), containing cement, superplasticizer, Micro-Silica, and plastic fibers. Color pigment impregnation should be added to concrete, whereas water-resistant silicone impregnation is for further treatment. Casting must be done very carefully to prevent any risk considering the correct positioning of EPS upon completion of the casting process, which includes pouring the pontoon deck to the bottom of the service duct and lifting the service duct (Figure 4). The concrete is then compacted by using vibrating rods, linear and vibrating beam deck surface, and integrated air-operated side mold vibrators to increase the quality of pontoon sides. All side and deck structures of the pontoon are made uniform and continuous. Next anti-slip graining is being made by using mild brushing. The poured concrete is then protected with water curing and plastic covering, which supply the pontoons with seaflex mooring tubes.

Figure 4. Service duct lifting

Concrete Sinkers and Seaflex Mooring System

The system is effective as it solved many problems in pontoons design & installation, specifically the efforts made by the experienced divers. It used to moor the pontoons to the seabed to keep the pontoon in its place regardless of the wave's movement or the difference in water level consisting of seaflex and rope. Seaflex is regarded as an active part of the mooring, and it does not cover all the distance from anchor blocks to the pontoon. Always pre-tensioned at the lowest water level, adjusting for water level changes and taking care of forces. The number of seaflex is calculated by the attachment distance and the length of the pontoon (Figure 5).

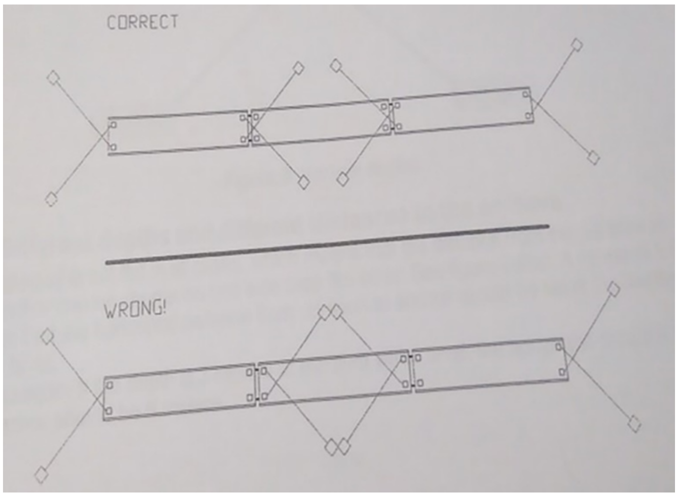


Figure 5. Placement of seaflex

The seaflex mooring is placed at a horizontal angle of 45 degrees, as this angle depends on the direction of the main force. The vertical angle between the mooring line and the seabed does not exceed 40° at the highest water level because if the angles increase the stability of pontoons, it will decrease (Figure 6).

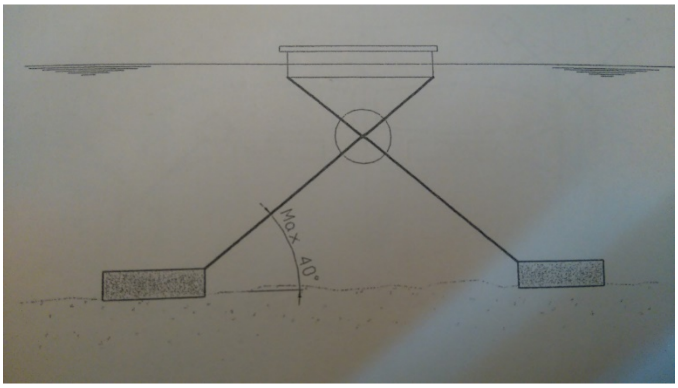


Figure 6. Right vertical angle between the mooring line and the seabed

When the depth increases further out from land, the distance between the pontoon and anchors will increase (Figure 7).

If the seabed is not flat, the horizontal distance between the pontoon and the anchor will be twice the medium water level. For instance, when the water depth was 3 m, the horizontal distance between the pontoon and the anchor was equal to 6 m.

From the Pythagoras theorem, the distance from the anchor is found to the rope, which was the hypotenuse of the depth, and the horizontal distance to the anchor equals the root of (3^2+6^2)=6.7 m (Figure 8).

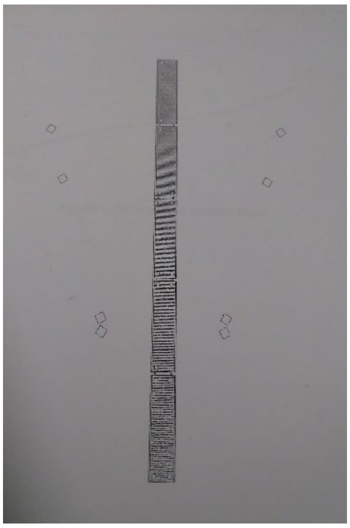


Figure 7. Deeper further out from the shore

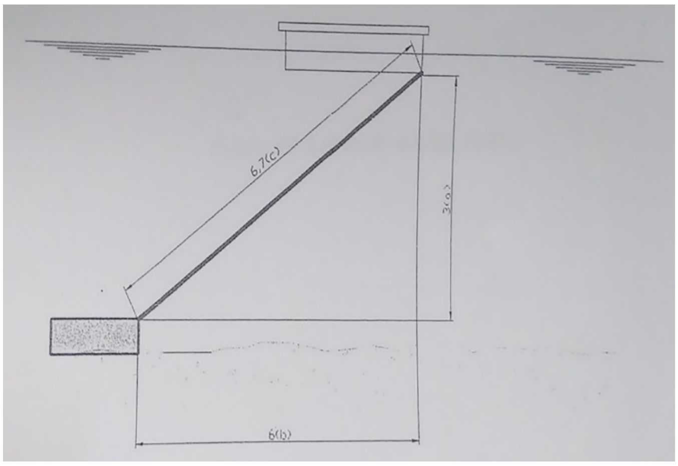


Figure 8. Hypotenuse calculation

The type of rope used is different according to the breaking load of rope and the number of seaflex rubber hawsers such as square plait polyester rope. Seaflex is always pre-tensioned 30% at the lowest water level even if the pontoon is moved (re-installed). The seaflex is pre-tensioned according to its length when left in the factory. This system is safe, strong, no effect on sea beds, easy to handle, low corrosion, simple to install, low maintenance, keeps the pontoon in the right place, handles any variation in water level, no bottom erosion, and no damage to the marine ecosystem.

Anchors

Anchors are normal dead weight from reinforced concrete on the land, expressed in tons, and it shall be the same number of rubber hawsers in the seaflex. Therefore, if a seaflex is used with 4 rubber hawsers, the weight of the anchor will be 4 tons or more. The weight of the anchor depends on the type of seabed; if the bottom is soft, it will be easier for the anchor to skin down, and a lighter anchor can be used. If there are small stones in the seabed and the friction is very low, a heavier anchor will be used. If the anchor is 10 tons, two 5 tons anchors can be connected together instead of 10 tons.

The Pontoon Installation

All pontoons contain service channels, an underwater lighting system, electricity and water outlets, illuminated stainless steel mooring bollards, led lighting, safety equipment, service buildings, and a sewage sanitation pump-out system. The chafing between the ropes is removed because they are crossing each other under the pontoon so that the ropes can be fixed by swapping. This shows that the rope that is under moves and lays over the other rope. Some pontoons, such as superyacht fingers, are fixed to walkways with flexible joints. When the moorings are moved, the free depth for boats is increased.

To install the pontoon, each one is lifted, pontoons and anchors (sinkers) are transported by trailers or barges, and sometimes by tag boats. The inner corners of the pontoon are moored to the land, and the pontoon is moored temporarily in its position with a lighter anchor. Permanent anchors are placed, and the anchor with seaflex attached to the rope is loaded on the boat. The anchor is lowered slowly down by a winch, and the diver checks if the anchor in the correct position. The rope is connected to the pontoon. The seaflex is pre-tensioned. This system is available for all the pontoon units.

In cross-border surveillance, fiber optic sensors were used to monitor possible damage. The bridge passed the above test. No voltage damage or excess buoyancy was found. A space scanner was also used to test the displacement of bridge elements under the load of the above-mentioned vehicle. As part of the reservoir's work, many other tests have been performed, such as the use of strain gauges to measure the load when testing on a pile of sand with the appropriate shape, various tests of technology for the production of other components, and strength testing of internal nodes. Current research shows that polymer composites and movable composite bridge structures can be used in the construction of military bridges. The use of composite materials in military and civilian bridges has unique advantages because these materials have a special strength compared to steel and aluminum and have the best corrosion resistance to atmospheric resistance during storage. Therefore, polymers have a bright future in bridge construction. The example of a new floating bridge confirms the usefulness of polymer composites in bridge construction. This also proves the positive impact of the collaboration between the Technical University and the Polish Army's design and research departments.

Conclusion

The study findings showed it is evident that the pontoon bridges are not just a military device or folkloristic curiosity, but they also represent the effective and economical solutions for crossing large sections of even deep water. The findings also suggested that the length of the pontoon bridges is not restricted by structural or technological problems. It is concluded that blocks of EPS comprise different dimensions. According to its original rubber length, seaflex is always pre-tensioned. Seaflex system shows better stability for pontoons as it is manufactured faster compared to any other system, is gentle to the environment, and is the most modern method for mooring a pontoon bridge. The weights for pontoons differ up to 75 tons, resulting in pontoons as strong and carrying large weights. There will be a substantial impact of inclusive access to the population and economy.

To implement such requirements to floating structures, there are still restrictions because of variable tide heights and dynamic motions, while there is clear guidance for buildings. Conflicting requirements are required for operating a terminal, such as minimal lighting levels regardless of glare so as not to intervene in the navigation platform. This review has sought to present some of the design tactics for using the improved accessibility for a waiting area and pontoon and lighting, stimulated by the input of a user group and an access consultant. On the contrary, it does not offer guidance about how to design to obey the pre-requisites mentioned within the regulatory standards.

Recommendations

The pontoons are designed for 50 years of service life. The area of casting must be checked. Fixing of mold and steel must be checked. The bottom of EPS blocks must be treated. The casting must be done very carefully. Cracks and holes must be repaired. Lifting the service duct must be careful in all pontoons-built services ducts. The curing operations must be done every day. Transportation of pontoons and anchors must be careful. The seaflex system is safer than other systems because it is an elastic and environmentally friendly mooring solution for any floating application such as docks/pontoons, floating wave attenuators, and buoys. It is unrivaled in its ability to keep it stable and secure even in locations exposed to extreme weather conditions.

From the construction response, it can be concluded that the flexible end connection will be better compared to the vertical and horizontal bending differences. In terms of axial force and horizontal bending moment, the short bridge is better, while the vertical bending moment of a long bridge is relatively small. In further design and optimization, it is recommended to increase the horizontal rigidity of long-term working conditions in the bridge, but vertical rigidity can be reduced. For the curved bridge, this is not an ideal load condition when the axial compression effect is still excellent. However, short bridges show more flexible effects than long bridges. Therefore, a higher rate of increase in peer-to-peer bonds should be considered to discuss the best rate of increase in curved floating bridges, and the related N-M effects will be further investigated.

Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

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

References

Australian Standard Guidelines. (n.d.). Guidelines for design marinas, AS3962-2001 Concrete work instruction RIL 149-1995

Chandler, N. (2011). How Floating Bridges Work. 13 September 2011. HowStuffWorks.com. Retrieved on May 18, 2022, from https://science.howstuffworks.com/engineering/structural/floating-bridge.htm

Derewenko, A., Niezgoda, T., Kosiuczenko, K., & Bogusz, P. (2011). Cassette pontoon bridge of high mobility. *Transport Problems*, *6*, 97-103.

Igwe, I. S., & Ajoko, T. J. (2020). Analysis and design of steel a floating pontoon jetty for use in the coastal waters of Nigeria. *European Journal of Engineering and Technology Research*, *5*(9), 1013-1021.

Morra, T. (2012). *The Evolutionary Development of Floating Dry Docks*. East Carolina University.

Neese, J. (2002). Floating trail bridges and docks. USDA Forest Service, Technology and Development Program. Retrieved on July 2002, from https://www.fs.fed.us/t-d/pubs/pdfpubs/pdf02232812/pdf02232812dpi72.pdf

Planning and Design Guidelines for Small Craft Harbors. (2000). American Society of Civil Engineers.

Queensland Government's Department of Transport and Major Highways (2015). *Manual: Design Criteria for Floating Walkways and Pontoons*. Department of Transport and Major Highways. Retrieved on May 18, 2022, from https://www.tmr.qld.gov.au/

Rapo, B. (1981). Dry Docking of Loaded or Partially Loaded Ships. *Proceedings of the Pan-American Institute of Naval Engineering International Congress*, USA.

Tattoni, S. (2007). Functional refurbishment of a pontoon bridge. *Proceedings of the 4th International Conference on the Conceptual Approach to Structural Design*, Venezia, pp. 27-29.

Wang, C. M., & Wang, B. T. (2015). Great Ideas Float to the Top. In Wang, C., & Wang, B. (Eds.), *Large Floating Structures* (pp. 1-36). Ocean Engineering & Oceanography, vol 3. Springer, Singapore. https://doi.org/10.1007/978-981-287-137-4_1

Watanabe, E., & Utsunomiya, T. (2003). Analysis and design of floating bridges. *Progress in Structural Engineering and Materials*, *5*(3), 127-144. https://doi.org/10.1002/pse.151

Zhang, J., Miao, G. P., Zhou, Z. W., Chen, H., & Lin, Z. M. (2010). Simulink based simulation of the behavior of a ribbon pontoon bridge. *Journal of Marine Science and Application*, *9*(3), 328-333.

Mar. Sci. Tech. Bull. (2022) 11(2): 179-186
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1107277

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Effect of mucilage pollution on ship cooling system: A case study

Hasan Bora Usluer[1] • Emir Ejder[2] • Bugra Arda Zincir[1,2*] • Yasin Arslanoğlu[2]

[1] *Galatasaray University, Maritime Vocational School, Department of Marine Engines and Machinery, Beşiktaş 34349, Istanbul, Turkey*
[2] *Istanbul Technical University, Maritime Faculty, Department of Maritime Transportation and Management Engineering, Tuzla 34940, Istanbul, Turkey*

ARTICLE INFO

Article History:
Received: 21.04.2022
Received in revised form: 16.05.2022
Accepted: 16.05.2022
Available online: 20.06.2022

Keywords:
Energy efficiency
Marine pollution
Mucilage
Turkish straits

ABSTRACT

In recent years, ecological degradation has increased and drawn many environmental problems with it. One of the outcomes is the mucilage problem, which directly affects tourism, fishing, and especially the maritime sector. The recent mucilage outbreak in the Sea of Marmara has brought attention to these environmental disaster-related studies. However, there are only a few studies about mucilage's effect on maritime transportation. In this context, the impact of mucilage on a marine vessel is examined within the scope of the study. One of the major negative effects of mucilage on a vessel is, its cooling system. Due to the mucosal formation of mucilage, it clogs the filters and reduces the cooling effect of seawater. In our paper, a case study was made, and data was obtained from a simulator. The seawater filter in front of the seawater pump was clogged by 0%, 30%, 45%, and 60% during the simulated case studies. 0% is the base condition that is not clogged by the mucilage while the other three cases are partially clogged. A comparison study was made according to the data gathered from the simulator. While the filter is clogged by 60% main engine is in slow down condition, but during 30% and 45% of cases, pumps tried to compensate cooling effect by increasing the seawater flow. However, enough cooling effects on the main engine jacket water, air cooler, lubrication oil cooler, steam condenser, and air compressor were not provided. Moreover, the reduced cooling effect increases fuel oil consumption which leads to higher CO_2 emissions. These results show that mucilage not only affects fishing, and tourism but also affects the environment by increasing the fuel oil consumption of a vessel.

Please cite this paper as follows:

Usluer, H. B., Ejder, E., Zincir, B. A., & Arslanoğlu, Y. (2022). Effect of mucilage pollution on ship cooling system: A case study. *Marine Science and Technology Bulletin*, *11*(2), 179-186. https://doi.org/10.33714/masteb.1107277

* Corresponding author
E-mail address: bazincir@gsu.edu.tr (B. A. Zincir)

Introduction

The rise in global warming is increasing sea surface temperatures and changing the properties of seawater. Due to the changes in the marine ecosystem, the biological structure of the seas has changed. With increasing seawater temperature, insufficient treatment of domestic and industrial wastes, and excessive fishing, sea algae in the area grows extremely and causes mucilage (Ozdelice et al., 2021). Mucilage, marine snow, or sea snot is a mucosal organic substance that can be found in the sea and is a product of sea algae (Precali et al., 2005). Recently, this phenomenon occurred in the Sea of Marmara, and mucilage concentration increased. An excessive amount of mucilage crippled the marine environment and also clogged fishers' nets (Hekimoğlu & Gazioğlu, 2021).

According to Topçu & Öztürk (2021), the latest mucilage outbreak occurred in the 2020 autumn and lasted until the summer of 2021. After the mucilage pandemic in the Marmara Sea in 2020, several studies were undertaken. A study is made by Hekimoğlu & Gazioğlu (2021) about mucilage issues in closed seas and the Sea of Marmara. In the study, the impact of mucilage on industries, the environment, fishing, and tourism is investigated. In addition, intense mucilage production in the Sea of Marmara is investigated. According to the authors, the marine ecosystem has altered as a result of the mucilage outbreak, and previously unknown species have taken over. Also, Acarlı et al. (2021), Özalp (2021) and Ozdelice et al. (2021) made similar studies on changing ecosystems due to mucilage outbreaks. Mucilage outbreaks in the Dardanelles impact surface organisms and endanger the coral ecosystem, which is located 39-51 meters down in the water (Özalp, 2021).

Tas et al. (2021) stated that mucilage has been a big concern in the Sea of Marmara since 2004, and it has gotten much worse since then. Mucilage events and potentially hazardous species in the Marmara Sea were investigated as part of the analysis. The study looked at phytoplankton composition and physicochemical factors from January 2004 to December 2007. In addition, another research on mucilage events and their consequences on cyanobacteria habitat was made in 2008 by obtaining seawater samples from the Sea of Marmara. According to their findings, cyanobacteria development peaked in November 2007 in both the Gulf of Bandirma and the Erdek (Alicli et al., 2020). However, cyanobacteria formation was the lowest in the Gulf of Bandirma in February 2008 and Erdek in May 2007. Furthermore, cyanobacteria abundance rose in the top layers of the water, but it was limited in the deeper levels because the density of mucilage changes with depth, temperature, and pollution of the water (Tüfekçi et al., 2010). Also, in a study made by Yentur et al. (2013), it was reported that precipitation, wind speed, and sudden temperature changes affect mucilage formation.

Considering the intended use of seawater for ships, it plays an important role in the ship's cooling system. Another drawback mucilage brought into our lives is its negative effects on marine vessels, which are highlighted by Uflaz et al. (2021). A large amount of mucilage clogs the sea chest filters by preventing the liquid entry of the cooling system used to cool the ship machinery groups. It causes inefficiency by affecting the cooling system components and getting the ship machinery to operate with low performance. Thus, fuel oil consumption and exhaust emissions increase. Considering the International Maritime Organization's (IMO) recent targets of reducing Greenhouse gases (GHG), inefficient ship operation is not desired.

Material and Methods

Various studies were made about mucilage incidents in the Sea of Marmara, especially on its ecological effects, tourism, and fishing. However, there is a vast gap in the effect of mucilage on shipping. In this paper, a short review of the impact of mucilage on pumps, filters, and heat exchangers is examined. Furthermore, the malfunction of the ship's main engine and auxiliary systems due to lack of cooling is investigated.

General Overview of Cooling Systems on Ships

Due to the working principles of the machinery systems functioning on the ships, they need cooling to reduce the heat generated. The section where seawater is used for cooling water systems is supplied is defined as sea chests. Sea chests are designed as both high and low sea chests. According to the level of the water in which the ship is cruising, one sea chest is kept open, and the other is kept ready to be used as clean. Thus, when marine pollution or living organisms contaminate the filters in the sea chests, the cooling process is continued by using the spare. Conventional or central cooling systems are widely used in the maritime industry.

Conventional cooling water systems

In the conventional seawater system, seawater is preferred as the coolant in the main engine (M.E.) jacket water coolers, air coolers, lube oil (L.O.) coolers, auxiliary engines (A.E.) coolers, air condition (A.C.), and refrigeration. The types of coolers in the system are generally tubular-type coolers.

Seawater pumps on the cooling water system provide water circulation.

Central cooling water systems

The central cooling water system includes low-temperature freshwater (LTFW) coolers and a high-temperature freshwater (HTFW) cooler. Due to the system principle, seawater is only used for cooling in the LTFW cooler. However apart from cooling, seawater is used for freshwater production, ballast operations, and fire system. Cooling of engine room system components such as M.E. air coolers, L.O. coolers, A.E. coolers, A.C., refrigeration, and HTFW coolers is carried out with low-temperature freshwater from LTFW coolers. The central cooling water system and its components are demonstrated in Figure 1 briefly.

According to the circuit diagram in Figure 1, there are LTFW pumps in the system. The task of these pumps is to circulate the low-temperature fresh water in the system and feed the other coolers. In addition, an expansion tank is placed in the system to eliminate water leaks that may occur in the system and prevent the water from expanding due to the increase in temperature and damaging the system components. The main purpose of the LTFW coolers, which are the main components of the central system and perform the cooling of all coolers, is to keep the system temperatures at the optimum levels determined by the manufacturers. Another vital piece of equipment in the system is the HTFW coolers. HTFW's task is to provide cooling of the main engine jacket water. HTFW pumps supply water circulation to keep the cooling efficiency of the main engine at maximum. Together with the pre-heating system placed on the HTFW coolers, the main engine keeps the jacket waters warm at the standby position of the vessel and ensures that it is ready for operation. To get maximum efficiency from the system's features, freshwater is produced from seawater in the fresh water generator by using the waste heat of the HTFW that completes the cooling process in the main engine.

Engine Room Simulator

The purpose of the engine room simulator (ERS), including actual ship features, is to make realistic analyses of the main engine and other systems in maritime training processes. ERS is also often preferred for examining and testing targeted situations used in academic studies (Rubio et al., 2018; Stavinuk et al., 2021). ERS was used for a fuel-water emulsion operating system to reduce exhaust gas emissions for a ship engine (Laskowski et al., 2015). In addition, another study examined the effect of shaft generators on ship energy efficiency using ERS (Yutuc, 2020). Furthermore, ERS systems are widely preferred in academic studies in the field of education as well as technical studies (Shen et al., 2017; Ivanov et al., 2020; Dere et al., 2022).

Case Study

In this study, the impact levels of cooling water and other mechanical systems due to mucilage of a ship using narrow waterways were simulated using ERS developed by Kongsberg. The Kongsberg ERS used in the study was approved by Det Norske Veritas (DNV), which is a classification society. Specifications of the ship used in the Kongsberg ERS can be seen in Table 1.

Table 1. Specifications of the ship

Ship Type	Container
Cylinder bore	840 mm
Piston stroke	2,400 mm
Cylinders	12 pcs
Maximum continuous rating	48,600 kW
Corresponding speed	102 RPM
Mean indicated pressure	17.9 bar
Scavenge air pressure	2.4 bar
Turbocharger speed	9,500 RPM
Specific fuel oil consumption	171 g/kW
Draught	12.6 m
Deadweight	55,000 tonnes
Design Speed	25 knots

During the case study, four scenarios are observed using the ERS. The four scenarios are 0% (no pollution), 30%, 45%, and 60% pollution in the filter of the seawater pump. The percentages represent the clogging of the filter in terms of flow rate. In the base case, the scenario flow rate is 2130.16 ton/h and decreased to 871.58 ton/h and 609.28 ton/h at 30% and 45%, respectively.

First, the pressure and temperature values of the engine systems (pumps and other equipment) are observed in the case of 0% pollution. In the next stage, the pollution level is increased by 30%. Thus, changing values under different conditions can be analyzed when contamination starts and progresses. In another scenario, the pollution has increased to 45%. Critical temperature and pressure values are obtained for the ship with this pollution rate, and its effects can be monitored. Finally, 60% of pollution, which is the highest point, is applied, and system efficiency is examined.

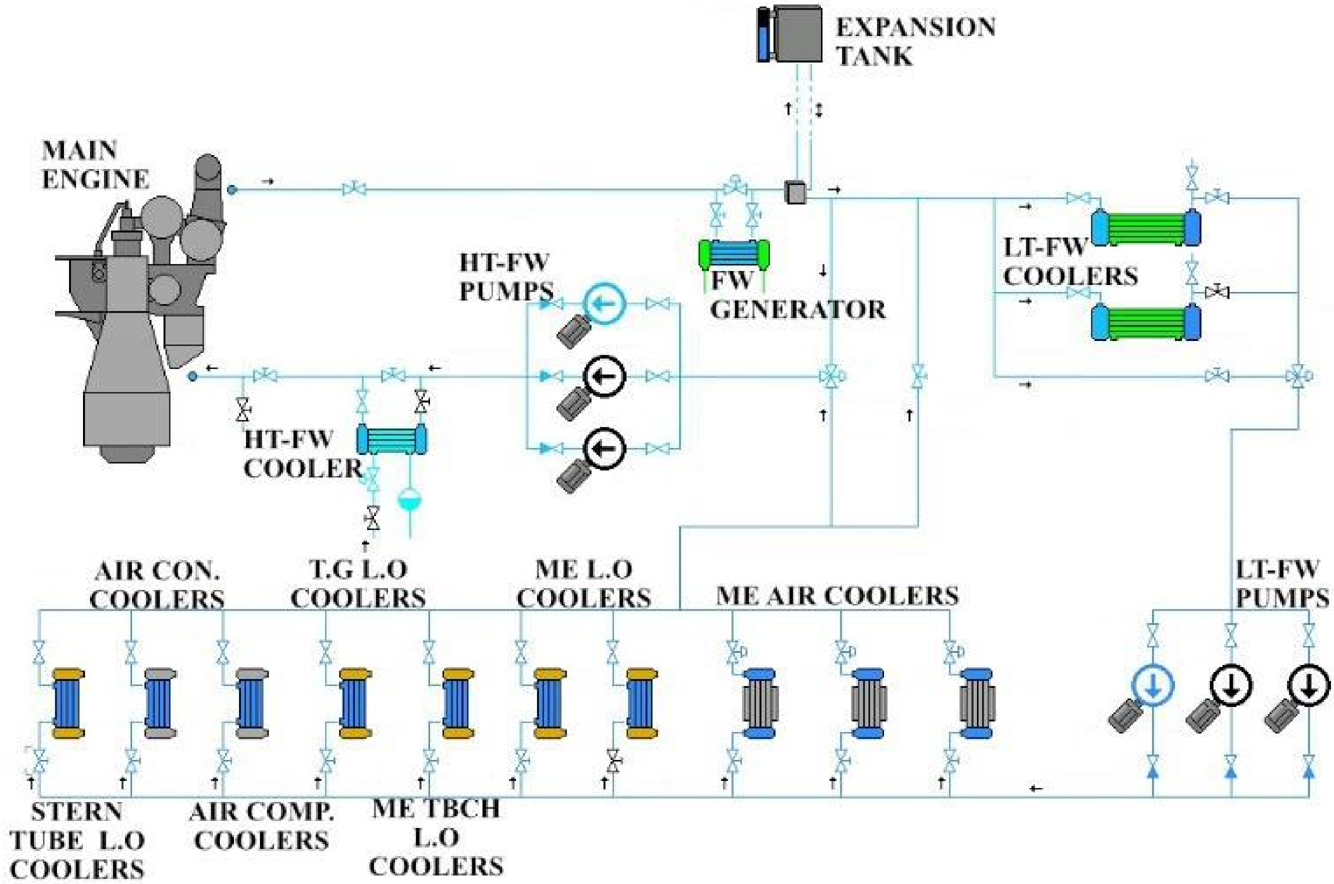


Figure 1. Central cooling water system

Results and Discussion

The data gathered from the simulator are shown in Figures 2-4. The results indicate that the ship has been affected by the increased mucilage formation in the Sea of Marmara. 60% of pollution scenarios are not included in the figures. That is because seconds after the scenario was created and clogging occurred, the main engine automatically slowed down itself due to high freshwater temperatures leaving the LTFW cooler.

The first remarkable change occurred in the high suction sea chest filter, as can be seen in Figure 2. The pressure difference in the filter has increased by a quiet margin, which indicates that the filter is partially clogged. Low suction sea chest filter pressure was zero since the high suction sea chest valve was open and the low suction sea chest valve was closed. Even though the seawater temperature has not changed, LTFW cooler seawater outlet temperature is increased because of insufficient seawater flow due to a clogged filter, which also demonstrates the reason for increased freshwater temperature leaving the LTFW cooler. Another indicator of a clogged filter is lower seawater pump discharge pressure and main seawater supply pressure. Although the 30% and 45% cases pressure are barely changed, a change between the base and 30% cases can be seen in Figure 2.

As shown in Figure 3, the clogged filters directly increase the freshwater outlet temperature of the LTFW cooler and affect the M.E. air cooler, M.E. L.O. cooler, M.E. turbocharger (TBCH) L.O. cooler, air compressor cooler, and stern tube L.O. cooler. M.E. air cooler freshwater outlet temperature is 38.22°C in the base scenario, 43.47°C in 30%, and 52.08°C in the 45% scenario. This increase also affected the M.E. air cooler air outlet temperature and the M.E. scavenge temperature. The same principle applies to the M.E. L.O. cooler; the freshwater temperature of the cooler increases and the L.O. outlet temperature is increased from 40.10°C to 46.67°C and 53.99°C, for 30% and 45%, respectively. Another affected component is the M.E. TBCH L.O. cooler. As shown in Figure 3, M.E. TBCH L.O. cooler freshwater, both inlet, and outlet temperature are increased. Moreover, the freshwater temperature of the air compressor rises from 32.56°C to 38.47°C and 46.96°C for 30% and 45% scenarios. The final component affected by the freshwater leaving the LTFW cooler is the stern tube L.O. cooler. In the base case scenario, the L.O. in the stern tube L.O. cooler is 32.78°C, while in 30% and 45%, it increases to 38.56°C and 46.54°C, respectively.

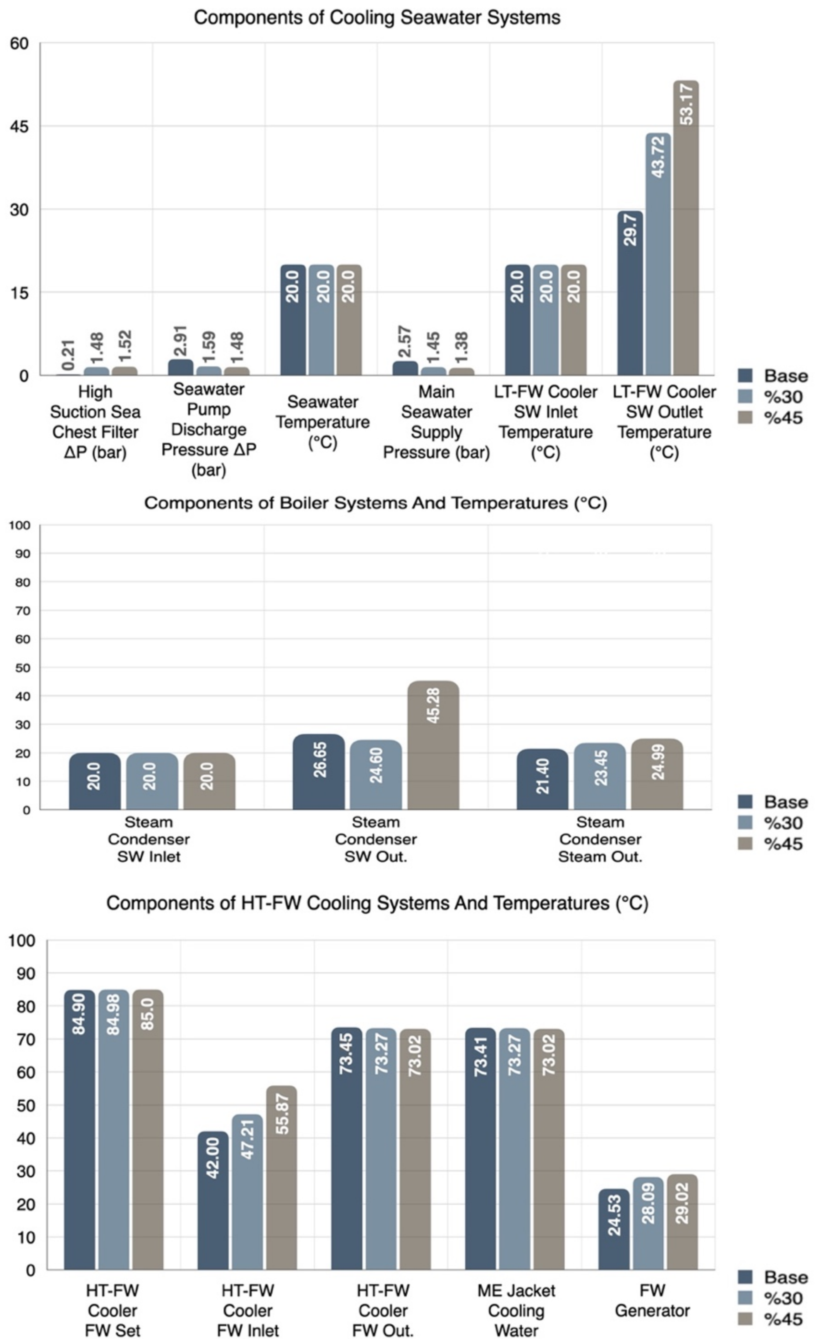


Figure 2. Effects of different pollution scenarios on ship machinery systems

HTFW is another affected cooling system. As shown in Figure 2, only the HTFW cooler inlet temperature and freshwater generator temperature are changed. However, HTFW cooler freshwater temperature and M.E. jacket water are not changed. The reason for the unchanged temperature is a mixture of LTFW and fresh water, leaving the freshwater generator mixed with a three-way valve, and then the mixture is heated in a preheater. By decreasing the load of the preheater, HTFW temperature can be decreased. On the other hand, HTFW cooler inlet temperature increases as the LTFW temperature increases.

In Figure 4, diesel generator (DG) shaft power for each scenario can be seen. In the base case, power output is 1501.4 kW, and in %30 and %45 it is decreased to 1454.18 kW and 1440.86 kW, respectively. It indicates that to generate the same power higher fuel consumption is needed.

Conclusion

A simulator study has been made about the effects of clogged seawater filters and pumps on a ship's energy efficiency and cooling system. Even though the temperature of the seawater used for cooling has not changed, the freshwater temperature

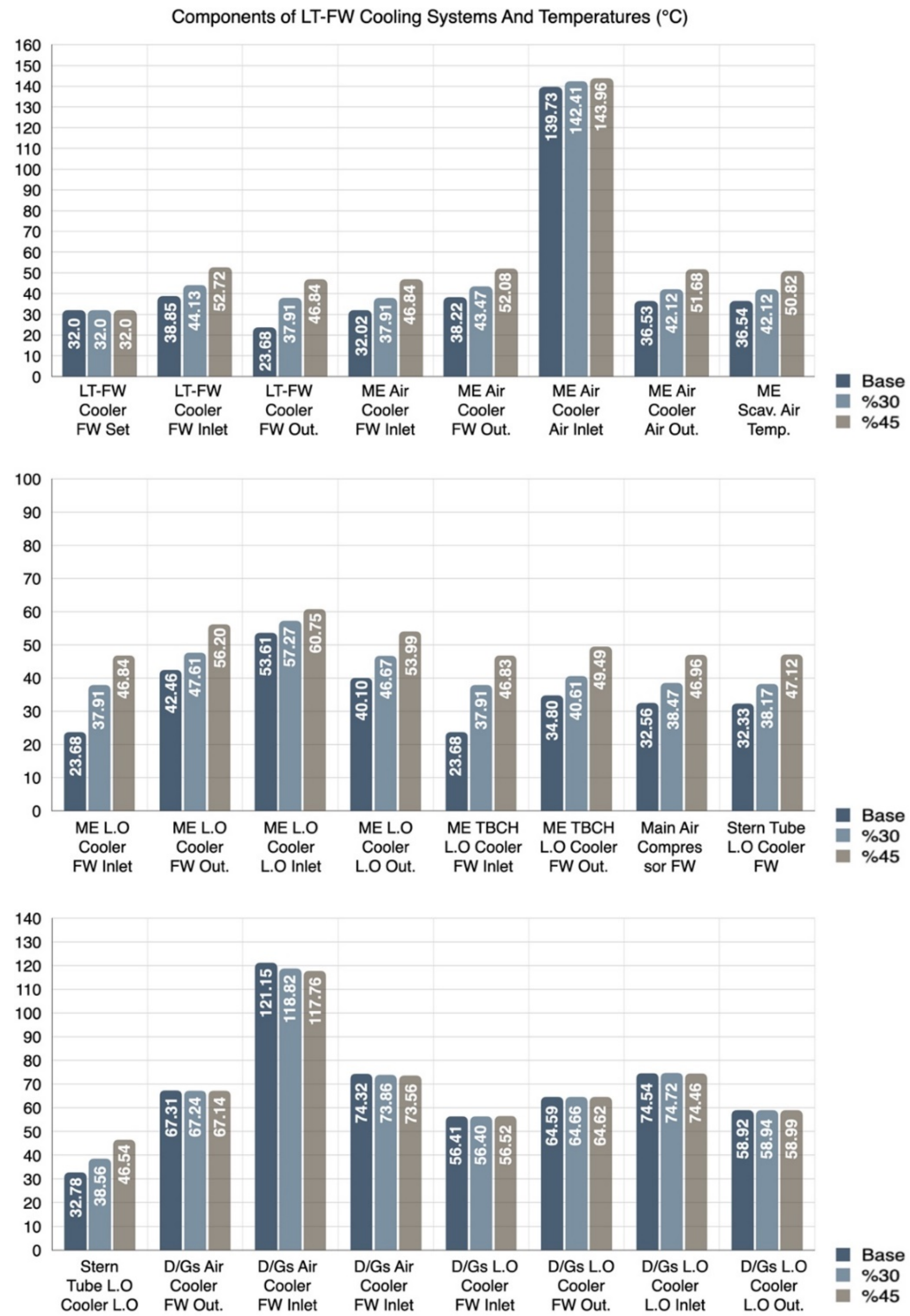


Figure 3. Effects of different pollution scenarios on ship machinery systems

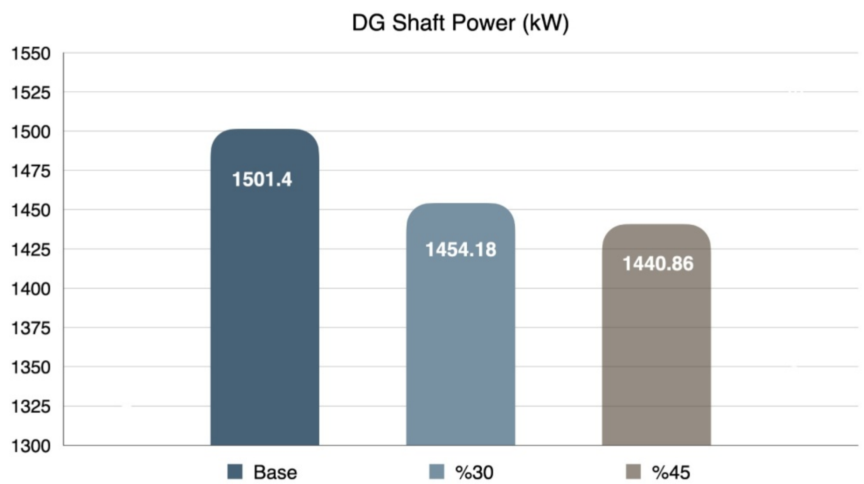


Figure 4. Diesel generator shaft power output

increased due to the decrease in the flow rate. Consequently, the increased freshwater temperature harms cooling systems and machinery. After the clogging in the sea chest filter reaches 60%, the main engine slows down itself, which can be defined as the critical point. Deficiencies in the cooling system result in inefficient operation and higher fuel oil consumption. A notable finding from the study is that high M.E. scavenge air temperature. High M.E. scavenge air temperature increases the fuel oil consumption since the combustion in the cylinder is not optimal. Besides that, seawater pump load is increased to compensate for the low seawater flow, and freshwater pumps also increase the load to compensate for the temperature difference. Thus, electrical consumption by the pumps is increased. Considering the latest regulations regarding the ship-based emissions, ship machinery should not operate at low efficiency. Current effects of the mucilage formation are mainly on the cooling system, but the further analysis should be made on the ships passing through the Sea of Marmara to investigate the other effects of the mucilage. The limitation of the study is the effect of mucilage on the cooling system, but its effect on the boiler and fresh water generator is excluded.

Compliance With Ethical Standards

Authors' Contributions

Author HBU and YA designed the study, BAZ wrote the first draft of the manuscript, EE performed and managed statistical analyses. 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.

References

Acarli, D., Acarli, S., & Kale, S. (2021). The effects of mucilage event on the population of critically endangered *Pinna nobilis* (Linnaeus 1758) in Ocaklar Bay (Marmara Sea, Turkey). *Acta Natura et Scientia*, *2*(2), 148-158. https://doi.org/10.29329/actanatsci.2021.350.09

Alicli, B. T., Polat, S., & Ozdelice, N. B. (2020). Temporal variations in the abundance of picoplanktonic *Synechococcus* (Cyanobacteria) during a mucilage event in the Gulfs of Bandırma and Erdek. *Estuarine, Coastal and Shelf Science*, *233*, 106513. https://doi.org/10.1016/j.ecss.2019.106513

Dere, C., Zincir, B., Inal, O. M., & Deniz, C. (2022). Investigation of the adverse effects of slow steaming operations for ships. *Proceedings of the Institution of Mechanical Engineers, Part M: Journal of Engineering for the Maritime Environment.* In press. https://doi.org/10.1177/14750902221074191

Hekimoğlu, B. S., & Gazioğlu, C. (2021). Mucilage problem in the semi-enclosed seas: Recent outbreak in the Sea of Marmara. *International Journal of Environment and Geoinformatics*, *8*(4), 402-413. https://doi.org/10.30897/ijegeo.955739

Ivanov, A., Kolosov, I., Danyk, V., Voronenko, S., Lebedenko, Y., & Rudakova, H. (2020). Design of multifunction simulator for engine room personnel training. *Informatyka, Automatyka, Pomiary W Gospodarce I Ochronie Srodowiska*, *10*(2), 62-69. https://doi.org/10.35784/iapgos.1617

Laskowski, R., Chybowski, L., & Gawdzinska, K. (2015). An engine room simulator as a tool for environmental education of marine engineers. In Rocha, A., Correia, A. M., Costanzo, S., & Reis, L. P. (Eds.), *New Contributions in Information Systems and Technologies* (pp. 311-322). Springer. https://doi.org/10.1007/978-3-319-16528-8

Özalp, H. B. (2021). First massive mucilage event observed in deep waters of Çanakkale Strait (Dardanelles) Turkey. *Journal of the Black Sea/Mediterranean Environment, 27*(1), 49-66.

Ozdelice, N. B., Durmuş, T., & Balcı, M. (2021). A preliminary study on the intense pelagic and benthic mucilage phenomenon observed in the Sea of Marmara. *International Journal of Environment and Geoinformatics*, *8*(4), 414-422. https://doi.org/10.30897/ijegeo.954787

Precali, R., Giani, M., Marini, M., Grilli, F., Ferrari, C. R., Pecar, O., & Paschini, E. (2005). Mucilaginous aggregates in the northern Adriatic in the period 1999-2002: Typology and distribution. *Science of the Total Environment*, *353*(1-3), 10-23, https://doi.org/10.1016/j.scitotenv.2005.09.066

Rubio, J. A. P., Vera-Garcia, F., Grau, J. H., Camara, J. M., & Hernandez, D. A. (2018). Marine diesel engine failure simulator based on thermodynamic model. *Applied Thermal Engineering*, *144*, 982-995. https://doi.org/10.1016/j.applthermaleng.2018.08.096

Shen, H., Zhang, J., & Cao, H. (2017). Research of marine engine room 3-D visual simulation system for the training of marine engineers. *Journal of Applied Science and Engineering*, *20*(2), 229-242. https://doi.org/10.6180/jase.2017.20.2.11

Stavinuk, T., Lalic, B., Mikulcic, J. Z., & Sundov, M. (2021). Simulation modelling of marine diesel engine cooling system. *Transactions on Maritime Science*, *10*(1), 112-125. https://doi.org/10.7225/toms.v10.n01.008

Tas, S., Kus, D., & Yılmaz, I. N. (2020). Temporal variations in phytoplankton composition in the northeastern Sea of Marmara: Potentially toxic species and mucilage event. *Mediterranean Marine Science*, *21*(3), 668-683. https://doi.org/10.12681/mms.22562

Topçu, N. E., & Öztürk, B. (2021). The impact of the massive mucilage outbreak in the Sea of Marmara on gorgonians of Prince Islands: A qualitative assessment. *Journal of the Black Sea/Mediterranean Environment*, *27*(2), 270-278.

Tüfekçi, V., Balkis, N., Beken, Ç., Ediger, D., & Mantikci, M. (2010). Phytoplankton composition and environmental conditions of the mucilage event in the Sea of Marmara. *Turkish Journal of Biology*, *34*(2), 199-210. https://doi.org/10.3906/biy-0812-1

Uflaz, E., Akyüz, E., Bolat, P., & Arslan, Ö. (2021). Investigation of the effects of mucilage on maritime operation. *Journal of the Black Sea/Mediterranean Environment*, *27*(2), 140-153.

Yentur, R. E., Buyukates, Y., Ozen, O., & Altin, A. (2013). The environmental and socio-economical effects of a biologic problem: Mucilage. *Marine Science and Technology Bulletin, 2*(2), 13-15.

Yutuc, W. (2020). Advancement in emerging technologies and engineering applications. In Saw, C. L. (Ed.), *An Investigation on the Overall Efficiency of a Ship with Shaft Generator Using an Engine Room Simulator*. *Springer Singapore*. https://doi.org/10.1007/978-981-15-0002-2_26

Mar. Sci. Tech. Bull. (2022) 11(2): 187-193
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1060608

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Development of a water quality index for Lake Aygır in Bitlis, Turkey

Asude Çavuş[1*] • Fazıl Şen[1]

[1] *Van Yüzüncü Yıl University, Faculty of Fisheries, Department of Basic Sciences, 65000, Van, Turkey*

ARTICLE INFO

Article History:
Received: 20.01.2022
Received in revised form: 24.03.2022
Accepted: 26.05.2022
Available online: 20.06.2022

Keywords:
Water quality management
Spatial analysis
Aggregation function
Lake Van Basin

ABSTRACT

Water quality indices help to develop correct policies using water quality data. In this study, a useful and reliable method was determined for water quality management of Lake Aygır. For this, monthly water quality measurements were made from Lake Aygır between May 2015 and May 2016. Expert opinions and literature were used in the selection of parameters. Temperature, dissolved oxygen, electrical conductivity, pH, ammonia, alkalinity, hardness, fluorine, and arsenic parameters were selected for the calculation of the water quality index of Lake Aygır for drinking (AG-$WQI_{drinking}$), as it is more effective on human health. Temperature, dissolved oxygen, electrical conductivity, pH, ammonia, alkalinity, hardness, and turbidity were selected for the calculation of the water quality index of Lake Aygır for fisheries (AG-$WQI_{fisheries}$) as they are the parameters to which fish is most affected. Water quality indices of Lake Aygır were found AG-$WQI_{drinking}$ as 149.41 and AG-$WQI_{fisheries}$ as 76.11. The most effective parameters were hardness and alkalinity in the AG-$WQI_{drinking}$ calculations; and ammonia and dissolved oxygen in the AG-$WQI_{fisheries}$ calculations. Hence, it expresses numerically the suitability of Lake Aygır in terms of drinking and fishing.

Please cite this paper as follows:

Çavuş, A., & Şen, F. (2022). Development of a water quality index for Lake Aygır in Bitlis, Turkey. *Marine Science and Technology Bulletin*, *11*(2), 187-193. https://doi.org/10.33714/masteb.1060608

Introduction

Water is a material that covers three-quarters of the earth, and it is an indispensable source of life for living things (Bulum, 2015). Lakes and reservoirs are major resources as they contain approximately 90% of the world's surface freshwater (Karmakar & Musthafa, 2013). The water quality of the lakes is the center of human and economic development. For this reason, the assessment and prediction of water quality levels are crucial for social and economic development (Li et al., 2016). Water quality is affected by natural and human effects (Gray, 1994). In general, the most important natural impacts in a basin

* Corresponding author
E-mail address: a.gultekin@yyu.edu.tr (A. Çavuş)

are geological, hydrological, and climatic factors (Scheffer & van Nes, 2007), while anthropogenic effects stem from agricultural irrigation, industrial pollution, and municipal use, among other factors (Cosgrove & Rijsberman, 2000).

Conventional water quality assessment can be a complex application as many parameters are individually evaluated. At the same time, determining water quality is an expensive and time-consuming process (Poonam et al., 2013). Moreover, many efforts have been made to manage water quality (Karmakar & Musthafa, 2013). Additionally, water quality indices (WQI) are approaches that greatly reduce data volume and simplify the expression of water quality status (Poonam et al., 2013). Therefore, involvement with the use and development of WQI methods, in general, is increasing all over the world (Ou et al., 2014). However, each water source has its own characteristics, so water quality parameters vary according to the source. Thus, the relative weight values of the parameters for each source should be determined in WQI calculations. Otherwise, consistent results may not be obtained (Alver, 2019).

Typically, Lake Aygır is an important part of locals' total potable and irrigation water supply. Therefore, the main objective of this study was to create a water quality index (WQI) for water quality evaluation and classification in Lake Aygır.

Material and Methods

Study Area

Lake Aygır (Bitlis) is in the Lake Van Basin, Bitlis, Turkey. The lake, which has an area of approximately 1.4 km^2 and a maximum depth of 43.4 m, is located at an altitude of 1938 m. The lake has been used markedly for the purposes of drinking, fisheries, recreation, and irrigation. In addition, the lake water is stored by means of a regulator and used as a reservoir (Güllü & Güzel, 2006; Elp et al., 2014; Doğu & Deniz, 2015; Çavuş, 2018) (Figure 1).

Water Quality Analysis

Water samples were taken monthly between May 2015 and May 2016. The samples were taken from the surface (-20 cm), -15 m depth, and lake bottom with a Nansen bottle.

To determine the quality of water samples, chlorine (Cl) and salinity by Mohr-Knudsen method, calcium (Ca), magnesium (Mg), total hardness by EDTA method, carbonate (CO_3), bicarbonate (HCO_3), total alkalinity by hydrogen chloride (HCl) titration method were analyzed by titrimetric analysis solutions (Greenberg et al., 1992), total suspended solids (TSS), aluminum (Al), chromium (Cr), cyanide (CN), ammonium (NH_4), ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), sulphate (SO_4), orthophosphate (o-PO_4), potassium (K), zinc (Zn), copper (Cu), fluorine (F), manganese (Mn), silver (Ag), boron (B), nickel (Ni), cobalt (Co), bromine (Br), iodine (I), molybdenum (Mo), iron (Fe), silicon (Si), chemical oxygen demand (COD) parameters with a HACH DR 5000 Spectrophotometer (HACH, 2010), arsenic (As) and cadmium (Cd) with ICP-OES spectrometer; boron (B) with ICP-MS spectrometer; sodium (Na) with AAS spectrometer (Thompson & Wood, 1982; Hill et al., 1995; Morales-Rubio & De la Guardia, 1999; Kmiecik et al., 2016). In addition, fecal coliform was determined using a membrane filter set (TS EN ISO 9308-1) (TSE, 2014; Tekbaş & Oğur, 2005).

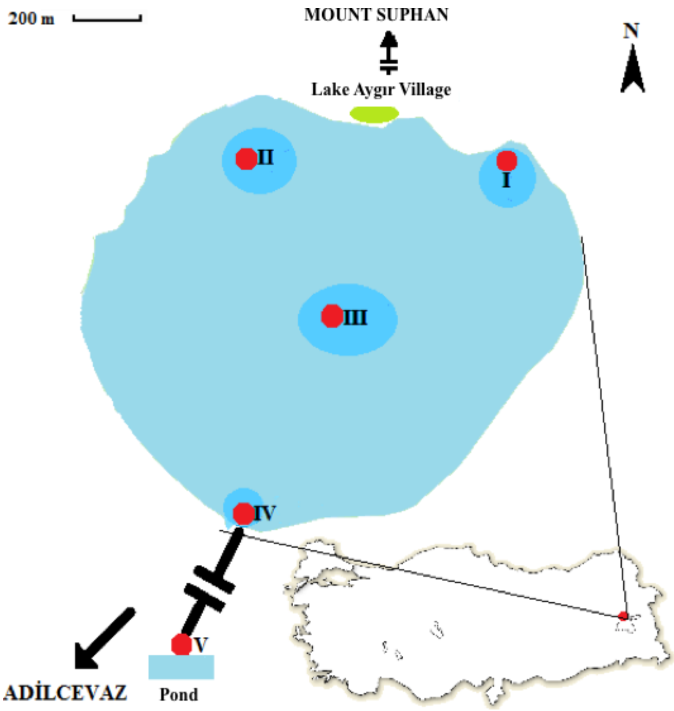


Figure 1. Location of Lake Aygır and sampling points; I. station (38° 50′ 18.00″ N, 42° 49′ 29.7″ E), II. station (38° 50′ 28.56″ N, 42° 49′ 14.82″ E), III. station (38° 50′ 6.66″ N, 42° 50′ 22.92″ E), IV. station (38° 49′ 54.36″ N, 42° 49′ 10.98″ E), V. station (38° 49′ 18.12″ N, 42° 50′ 11.7″ E).

Calculation of WQI

The water quality index has been developed using Horton (1965), Brown et al. (1970), Brown et al. (1972), and Ravikumar et al. (2013). The formulae are as follows:

$$AG - WQI = \sum SI_i \tag{1}$$

$$SI_i = W_i q_i \tag{1.1}$$

$$q_i = \frac{C_i}{S_i} \times 100 \quad (1.2)$$

$$W_i = \frac{w_i}{\sum w_i} \quad (1.3)$$

SI$_i$: water quality sub-index; *W$_i$*: relative weight; *q$_i$*: quality rating scale; *C$_i$*: concentration in the water sample; *S$_i$*: standard value set by legislation, literature, or experts. They are EEC and WHO (Tebbutt, 1998), WPC (2004), TSE (2005 and 2014), WHC (2005), APRAR (2006), Emre & Kürüm (2007), and PWTC (2014) for drinking and fisheries. *w$_i$*: weighting. Each of the physical and chemical parameters was selected by means of experts and literature. They should be weighted by the weight of their importance for their intended use from 2 to 5.

Finally, the calculated water quality index values are classified into categories as excellent (<50), good (<100-50≤), poor (<200-100≤), very poor (<300-200≤), unsuitable for using purpose (≥300) (Ravikumar et al., 2013).

$$Ew_i = \frac{SI_i}{WQI} \times 100 \quad (2)$$

In addition, the effective weights (Ew$_i$) of each water quality parameter were calculated to determine the water quality parameter that had the greatest impact on the WQI results. where Ew_i is the effective weight of the *i*th parameter (Equation 2). W_i is compared with Ew_i, reflecting the importance of each parameter with respect to other parameters used in WQI calculations (Şener et al., 2017).

Results

In this study, analyses were carried out monthly between May 2015 and May 2016 from the Aygır Lake and the irrigation pool. Water quality parameters used in AG-WQIs were found as DO: 0.41-13.14 g.L^{-1}, EC: 222.30-507.40 µS.cm^{-1}.

Average water quality parameters were found as oxygen saturation: 76.6%, SPC: 435.0 µS.cm^{-1}, salinity: 0.21‰, TDS: 0.2881 g.L^{-1}, transparency: 5.8 m, Cl: 17.3 mg.L^{-1}, Ca: 54.3 mg.L^{-1}, Mg: 40.6 mg.L^{-1}, CO_3: 9.8 mg.L^{-1}, HCO_3: 256.9 mg.L^{-1}, NO_2: 17.8 µg.L^{-1}, NO_3: 1.2 mg.L^{-1}, NH_4: 0.06 mg.L^{-1}, SO_4: 11.7 mg.L^{-1}, o-PO_4: 21.7 µg.L^{-1}, K: 1.72 mg.L^{-1}, Al: 1.13 µg.L^{-1}, Zn: 0.22 µg.L^{-1}, Cu: 3.7 µg.L^{-1}, Mn: 3.2 µg.L^{-1}, Ag: 0.15 µg.L^{-1}, Cd: 1.1 µg.L^{-1}, Pb: 0.21 mg.L^{-1}, B: 0.01 mg.L^{-1}, Br: 0.12 mg.L^{-1}, I: 0.16 mg.L^{-1}, Na: 27.0 mg.L^{-1}, Mo: 1.69 mg.L^{-1}, CN: 1.19 µg.L^{-1}, Si: 5.74 mg.L^{-1}, SiO_2: 24.27 mg.L^{-1}, TSS: 3.8 mg.L^{-1}, and fecal coliform: 0.5 colony.100 mL^{-1}. Cr, Co, Ni, COD and BOD were not found in water samples. Total Fe (0.018 mg.L^{-1}). was found only in IV. station.

All values in water samples were interpreted and evaluated by experts. Temperature, DO, EC, pH, NH_3, alkalinity, hardness, F, and As parameters were selected for AG-WQI$_{drinking}$ as drinking water. As a result, Lake Aygır, used as drinking water, has been found to be of medium quality with 149.41 (Table 1).

Table 1. Water quality index developed for drinking water

Parameter	Unit	Weight (w_i)	Relative weight (W_i)	Desirable limit* (S_i)	Min-Max	Mean±SE (C_i)	q_i	SI_i	Ew_i (%)
Temperature	°C	2	0.06	25	3.7-21.5	9.9±0.5	39.59	2.40	1.6
DO	mg.L^{-1}	3	0.09	8.0	0.41-13.14	8.15±0.4	101.88	9.26	6.2
EC	µS.cm^{-1}	3	0.09	2000	222.3-507.4	353.05±4.1	17.65	1.60	1.1
pH		4	0.12	9.20	7.03-8.73	8.14±0.06	88.44	10.72	7.2
NH_3	mg.L^{-1}	4	0.12	0.50	0-0.19	0.05±0.01	10.0	1.21	0.8
Alkalinity	mg.L^{-1}	3	0.09	50	120-310	235.08±2.9	470.2	42.7	28.6
Hardness	mg.L^{-1}	4	0.12	50	173.3-443.3	302.66±4.79	605.3	73.4	49.2
Fluorine	µg.L^{-1}	5	0.15	1500	0.31-1.82	0.56±0.05	37.33	5.66	3.8
Arsenic	µg.L^{-1}	5	0.15	10	0-43.76	1.59±0.35	15.93	2.41	1.6
Total		33	1.00						
AG-WQI$_{drinking}$								**149.38**	

Note: *The desirable limits were determined according to EEC and WHO (Tebbutt, 1998), WPC (2004), TSE (2005), and WHC (2005).

Table 2. Water quality index developed for fisheries

Parameter	Unit	Weight (w_i)	Relative weight (W_i)	Desirable limit* (S_i)	Min-Max	Mean±SE (C_i)	q_i	SI_i	E_{Wi} (%)
Temperature	°C	5	0.20	21.5	3.7-21.5	9.9±0.5	46.03	9.21	12.1
DO	$mg.L^{-1}$	4	0.16	9.0	0.41-13.14	8.15±0.4	90.56	14.49	19.0
EC	$\mu S.cm^{-1}$	2	0.08	2000	222.3-507.4	353.05±4.1	17.65	1.41	1.9
pH		2	0.08	9.0	7.03-8.73	8.14±0.06	90.41	7.23	9.5
NH_3	$mg.L^{-1}$	4	0.16	0.025	0-0.19	0.05±0.01	198.13	31.70	41.7
Alkalinity	$mg.L^{-1}$	2	0.08	400	120-310	235.08±2.9	58.77	4.70	6.2
Hardness	$mg.L^{-1}$	2	0.08	400	173.3-443.3	302.66±4.79	75.66	6.05	7.9
Turbidity	NTU	4	0.16	10	0.2-7.2	0.82±0.1	8.23	1.32	1.7
Total		25	1.00						
AG-WQI$_{fisheries}$								**76.11**	

Note: * The desirable limits were determined according to APRAR (2006), Emre & Kürüm (2007), and PWTC (2014).

Temperature, DO, electrical conductivity, pH, NH_3, alkalinity, hardness, and turbidity parameters were selected for AG-WQI$_{fisheries}$ for fisheries. As a result, Lake Aygır has been found to be of good quality, with 76.11 in terms of fisheries (Table 2).

The order of anions was HCO_3>Cl>SO_4>CO_3>NO_3>F>I>Br>PO_4>NO_2>CN and cations were Ca>Mg>Na>K>NH_4>Cu>As>Al>Cd>Zn>Ag>Fe≥Cr≥Ni in water samples. The results showed that the highest effective weight value belonged to the hardness (49.2%) and alkalinity (28.6%) parameters compared to the others for drinking. Generally, there is a strong relationship between the major ion contents of water samples and the water-rock interaction (Şener et al., 2017). There was a directly proportional correlation between the effective and the relative weights in terms of temperature, DO, and pH. F and As parameters with high relative weight showed low effective weight (Table 1). This observation was primarily due to the very low concentrations of these measured parameters in water samples. According to calculations, the highest effective weight values belonged to NH_3 and DO parameters with 41.7% and 19.0%, respectively, in the AG-WQI$_{fisheries}$ calculations. There was a directly proportional correlation between the effective and the relative weights in terms of EC. Turbidity with secondary relative weight showed low effective weight (Table 2). This observation was primarily due to the low concentrations of turbidity in water samples.

Discussion

Lake Aygır, used as drinking water, has been found to be of medium quality (Table 1). Our AG-WQI$_{drinking}$ study results are similar to those found by Karakaya & Evrendilek (2010). Lake Aygır has been found to be of good quality, with 76.11 in terms of fisheries (Table 2). Our AG-WQI$_{fisheries}$ study results are similar to those found by Imneisi & Aydin (2016) and Çavuş & Şen (2020).

Water quality indices have some limitations. For example, it may not contain enough information about the actual quality status of the water. Therefore, the parameters were chosen for the AG-WQI. In addition, many uses of water quality data cannot be covered by an index. For this, separate indices such as AG-WQI$_{drinking}$ and AG-WQI$_{fisheries}$ have been developed for each use. However, WQI has more advantages than disadvantages. An index is not a complex forecast model for technical and scientific applications. Thus, it is a useful tool for communicating water quality information to the public and legal decision-makers (McClelland, 1974; Kumar & Dua, 2009).

While Lake Aygır is not polluted and has a suitable quality in terms of drinking, use, fishing and irrigation, it has been determined that it could be adversely affected by the settlements and agricultural activities around it (Çavuş & Şen, 2023).

Scientists doing research on water resources as a priority in Turkey can create a water quality index with the national water quality criteria. The data obtained at the end of the study were compared with the regulations and standards regarding drinking and fisheries (Tebbutt, 1998; WPC, 2004; TSE, 2005; TSE, 2014; WHC, 2005; APRAR, 2006; Emre & Kürüm, 2007; PWTC, 2014). Selected national legislations are "Regulation on Water for Human Consumption", "Water-Water for Human Consumption", and "Regulation on Surface Water Quality Management" for drinking water. The national legislation for fisheries is "Regulation on the Protection and Improvement of Waters Where Trout and Carp Fish Live" and "Water Quality

Criteria for Trout Breeding". In the absence of national water quality criteria, they can use international quality criteria (Tebbutt, 1998). For WQI, meetings can be held with people specializing in water quality, and the data can be presented and discussed. While creating the water quality index, selecting the priority parameters for the basin is an important step in the development of the index. Another important step is the selection of parameters for the intended use. It is very important to have a single method and a single quality scale in choosing WQI. Thus, comparisons between results can be made easily. This WQI should help develop local water management strategies.

The developed index is not in agreement with the other many indices that can be applied for the water quality assessment because the selected parameters in indices are different from each other.

Conclusion

The locals are engaged in agriculture and animal husbandry as well as aquaculture and fishing. It has become one of the most beautiful recreation areas of the region with a trout facility and trout restaurant in the Lake Aygır village and location of the lake. No industrial establishment was found in the region. The water of the lake is transferred to the pool for irrigation in the spring and summer months. Besides, it has been solving the water needs of Aydınlar Town since about 2008. Therefore, the water level of Aygır Lake is gradually decreasing. In order to prevent such changes that will affect the water quality, other water sources can be found in the town. Farmers can be encouraged to use technological irrigation systems that consume less water.

Considering all these observations and AG-WQI, we can say that Lake Aygır is not polluted and is suitable for drinking and fishing. For this suitable water resource, necessary protection measures should be taken regarding the use of lake water. Hence, the AG-WQI can be used in the evaluation of all freshwater resources in the Lake Van Basin.

Acknowledgements

This study was produced from the data of doctoral thesis of the first author supervised by the second author. The thesis was supported by YYÜ Scientific Research Projects Directorate with the project number 2015-FBE-D185.

Compliance With Ethical Standards

Authors' Contributions

FŞ contributed substantially to the conception and design of the study, interpretation of the results. He provided critical revision of the article, and final approval of the version to publish. AÇ contributed substantially to the acquisition of data. She drafted of the article. AÇ and FŞ agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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.

References

Alver, A. (2019). Evaluation of conventional drinking water treatment plant efficiency according to water quality index and health risk assessment. *Environmental Science and Pollution Research*, *26*(26), 27225-27238.

APRAR. (2006). *Application principles regarding the aquaculture regulation (2006/1)*. Ministry of Agriculture and Rural Affairs, Ankara, Turkey.

Brown, R. M., McClelland, N. I., Deininger, R. A., & O'Connor, M. F. (1972). A water quality index—crashing the psychological barrier (pp. 173-182). In W. A. Thomas (Ed.), *Indicators of environmental quality*. Springer.

Brown, R. M., McClelland, N. I., Deininger, R. A., & Tozer, R. G. (1970). A water quality index: Do we dare? *Water Sewage Works*, *117*(10), 339–343.

Bulum, Ö. B. (2015). A study on water quality criteria of Bendimahi Stream in Van, Turkey [MSc. Thesis. Van Yüzüncü Yıl University].

Çavuş, A., & Şen, F. (2020). Application of CCME WQI to assess surface water quality under Turkish national legislations: Lake Aygır. *Avrupa Bilim ve Teknoloji Dergisi*, (19), 836-842.

Çavuş, A., & Şen, F. (2023). Chemical and microbiological properties of Lake Aygır in Turkey and usage of drinking, fisheries, and irrigation. *Brazilian Journal of Biology*, *83*, e244494. https://doi.org/10.1590/1519-6984.244494

Çavuş, A. (2018). An investigation on water quality and management of Aygır Lake, Van [Ph.D. Thesis. Van Yüzüncü Yıl University].

Cosgrove, W. J., & Rijsberman, F. R. (Eds.). (2000). *World water vision: Making water everybody's business*. Routledge.

Doğu, A. F., & Deniz, O. (2015). Morphologic features and tourism facilities of Aygır Lake. *The Journal of International Social Research*, *8*(41), 692-702.

Elp, M., Şen, F., & Atıcı, A. A. (2014). The distribution area of tarek (*Alburnus tarichi* (Guldenstaedtii, 1814)) in the Van Lake Basin, Turkey. *Yuzuncu Yıl University Journal of Agricultural Sciences*, *24*(3), 228-232. https://doi.org/10.29133/yyutbd.236277

Emre, Y., & Kürüm, V. (2007). *Havuz ve kafeslerde alabalık yetiştiriciliği*. Ofset Hazırlık Basım Evi.

Gray, N. F. (1994). *Drinking water quality: Problems and solutions*. John Wiley Sons.

Greenberg, A. E., Clesceri, L. S., & Eaton, A. D. (1992). *Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, WEF*. American Public Health Association.

Güllü, K., & Güzel, Ş. (2006). Cage farming feasibility and growth performance of rainbow trout, *Oncorhynchus mykiss*, in the Dam Lake of Adilcevaz-Bitlis Hydroelectric Power Plant. *Yuzuncu Yıl University Journal of Agricultural Sciences*, *16*(2), 81-85.

HACH. (2005). DR 5000 Spectrometer Procedures Manuel. Retrieved on March 09, 2018, from https://ca.hach.com/dr-5000-uv-vis-spectrophotometer/product-downloads?id=14534087101

Hill, S. J., Brown, A., Rivas, C., Sparkes, S., & Ebdon, L. (1995). 16. High performance liquid chromatography—Isotope dilution-inductively coupled plasma—mass spectrometry for lead and tin speciation in environmental samples. In *Techniques and Instrumentation in Analytical Chemistry* (pp. 411-434). Elsevier. https://doi.org/10.1016/S0167-9244(06)80017-X

Horton, R. K. (1965). An index number system for rating water quality. *Journal of Water Pollution Control Federation*, *37*(3), 300-306.

Imneisi, I. B., & Aydin, M. (2016). Water quality index (WQI) for main source of drinking water (Karaçomak Dam) in Kastamonu City, Turkey. *Journal of Environmental Analytical Toxicology*, *6*(407), 2161-0525. https://doi.org/10.4172/2161-0525.1000407

Karakaya, N., & Evrendilek, F. (2010). Water quality time series for Big Melen Stream (Turkey): Its decomposition analysis and comparison to upstream. *Environmental Monitoring and Assessment*, *165*(1), 125-136. https://doi.org/10.1007/s10661-009-0932-7

Karmakar, S., & Musthafa, O. M. (2013). Lakes and reservoirs: Pollution. In S. A. Jorgensen (Ed.), *Encyclopedia of Environmental Management* (pp. 1576-1587). Taylor & Francis.

Kmiecik, E., Tomaszewska, B., Wątor, K., & Bodzek, M. (2016). Selected problems with boron determination in water treatment processes. Part I: Comparison of the reference methods for ICP-MS and ICP-OES determinations. *Environmental Science and Pollution Research*, *23*(12), 11658-11667. https://doi.org/10.1007%2Fs11356-016-6328-7

Kumar, A., & Dua, A. (2009). Water quality index for assessment of water quality of river Ravi at Madhopur (India). *Global Journal of Environmental Sciences*, *8*(1), 49-57. https://doi.org/10.4314/gjes.v8i1.50824

Li, R., Zou, Z., & An, Y. (2016). Water quality assessment in Qu River based on fuzzy water pollution index method. *Journal of Environmental Sciences*, *50*, 87-92. https://doi.org/10.1016/j.jes.2016.03.030

McClelland, N. I. (1974). Water quality index application in the Kansas River Basin. (Vol. 74, No. 1). US Environmental Protection Agency-Region VII.

Morales-Rubio, A., & De la Guardia, M. (1999). Applications in environmental analysis. *Analytical Spectroscopy Library*, *9*, 309-341.

Ou, H. S., Wei, C. H., Deng, Y., Gao, N. Y., Ren, Y., & Hu, Y. (2014). Principal component analysis to assess the efficiency and mechanism for enhanced coagulation of natural algae-laden water using a novel dual coagulant system. *Environmental Science and Pollution Research*, *21*(3), 2122-2131. https://doi.org/10.1007/s11356-013-2077-z

Poonam, T., Tanushree, B., & Sukalyan, C. (2013). Water quality indices—important tools for water quality assessment: A review. *International Journal of Advances in Chemistry*, *1*(1), 15-28. https://doi.org/10.5121/ijac.2015.1102

PWTC. (2014). *Regulation on the Protection and Improvement of the Waters inhabited by Trout and Carp Fish*. Official newspaper date: 12.01.2014, number: 28880. Ministry of

Forestry and Water Management, and Ministry of Food, Agriculture and Livestock. Ankara, Turkey.

Ravikumar, P., Mehmood, M. A., & Somashekar, R. K. (2013). Water quality index to determine the surface water quality of Sankey Tank and Mallathahalli Lake, Bangalore Urban District, Karnataka, India. *Applied Water Science*, *3*(1), 247-261. https://doi.org/10.1007/s13201-013-0077-2

Scheffer, M., & van Nes, E. H. (2007). Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia*, *584*, 455-466. Springer. https://doi.org/10.1007/s10750- 007-0616-7

Şener, Ş., Şener, E., & Davraz, A. (2017). Evaluation of water quality using water quality index (WQI) method and GIS in Aksu River (SW-Turkey). *Science of the Total Environment*, *584-585*, 131-144. https://doi.org/10.1016/j.scitotenv.2017.01.102

Tebbutt, T. H. Y. (1998). *Principles of water quality control* (Fifth Edition). Elsevier.

Tekbaş, F., & Oğur, R. (2005). Temel su analiz teknikleri. In *GATA Halk Sağlığı* (pp. 19-27). AD Yayınları.

Thompson, M., & Wood, S. (1982). Atomic absorption spectrometry. In E. J. Cantle (Ed.), *Water and effluents* (pp. 67-94). Elsevier.

TSE. (2005). *TS 266, Water-Water for Human Consumption.* Turkish Standardization Institute, Industry and Trade Ministry. Ankara, Turkey.

TSE. (2014). *TS EN ISO 9308-1, Water quality- Detection and enumeration of Escherichia coli and coliform bacteria Part-1 Membran filtration method.* Turkish Standardization Institute, Ankara, Turkey.

WHC. (2005). *Regulation on Water for Human Consumption.* Official Gazette number: 25730. Ministry of Health. Ankara, Turkey.

WPC. (2004). *Water Pollution Control Regulation.* Official Gazette Date: 31.12.2004, number: 25687. Ministry of Environment and Forest, Ankara, Turkey.

Mar. Sci. Tech. Bull. (2022) 11(2): 194-201
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1100624

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Effects of nitrogen and phosphorus concentrations on the growth and lipid accumulation of microalgae *Scenedesmus obliquus*

Leyla Uslu[1]* • Oya Işık[1] • Yasemin Barış[1] • Selin Sayın[2]

[1] *Çukurova University, Fisheries Faculty, Basic Science Department, 01330, Adana, Turkey*
[2] *İskenderun Technical University, Faculty of Marine Sciences and Technology İskenderun, 31200, Hatay, Turkey*

ARTICLE INFO

Article History:
Received: 08.04.2022
Received in revised form: 18.05.2022
Accepted: 19.05.2022
Available online: 20.06.2022

Keywords:
Growth
Lipid
Scenedesmus obliquus
Nitrogen concentration
Phosphorus concentration

ABSTRACT

In the study, *Scenedesmus obliquus* green algae was cultivated under laboratory conditions at 21±2°C, 16:8 (light:dark) photoperiod and continuous aeration in different nitrogen and phosphorus ratio nutrient medium and its growth was determined. Dry weight, cell density (optical density) and chlorophyll *a* and *b* were used to determine the growth of the algae. The best growth was determined in the group consisting of 30 ml $NaNO_3$+10 ml PO_4. The amount of biomass obtained was determined as 1.549 gL^{-1} in this group. The lowest values were the group containing 5 ml $NaNO_3$+5 ml PO_4. With the decrease in the amount of nitrogen in the medium, an increase in the amount of carotene and a decrease in the amount of chl *a* and *b* were detected. The highest lipid values were determined as 36.7% in the group containing 5 ml $NaNO_3$+5 ml PO_4 and 36.2% in the group containing 5 ml $NaNO_3$+10 ml PO_4.

Please cite this paper as follows:

Uslu, L., Işık, O., Barış, Y., & Sayın, S. (2022). Effects of nitrogen and phosphorus concentrations on the growth and lipid accumulation of microalgae *Scenedesmus obliquus*. *Marine Science and Technology Bulletin*, *11*(2), 194-201. https://doi.org/10.33714/masteb.1100624

Introduction

It is important to cultivate microalgae species that have commercial value in the field of algae biotechnology due to the metabolites they produce. High quality vitamins, essential unsaturated fatty acids and amino acids can be synthesized from microalgae groups. Nutrient elements, light, temperature and pH have important roles in the growth of algae. However, it is known that the most important factor in the lipid production of microalgae is the nutritional regime, especially such as nitrogen (N) and phosphorus (P) limitations (Sugimoto et al., 2008). Microalgae cells increase some of their metabolites

* Corresponding author
E-mail address: hizarcil@cu.edu.tr (L. Uslu)

under stress. The N atom influences production and accumulation carbohydrates and lipids in the cell. N restriction is associated with cellular fatty acids and growth, causing a decrease in cell number and chl-*a* amounts, and an increase in organic carbon compounds such as lipids. One of the renewable, non-toxic biodiesel fuel sources as an energy source is microalgae biomass. *Scenedesmus obliquus* is a freshwater species and is considered as a biodiesel source (Mandal & Mallick, 2009). In addition to having high biomass, it has been reported in the literature that it contains high lipid (Mandal & Mallick, 2009).

Hundreds of microalgae strains with high lipid content have been screened and their lipid production metabolism has been characterized and reported (Sheehan et al., 1998). There are many studies dealing with the amount and quality of lipids in the cell can be altered because of changes in growth conditions such as temperature and light intensity or nutrient medium properties, N concentration, phosphates and iron (Illman et al., 2000; Liu et al., 2008).

Nutrient concentration is a key factor in the coupling system of biodiesel production. In this article, the effects of N and P nutrient concentrations on the growth and lipid accumulation characteristics of *S. obliquus* were examined.

Material and Methods

Microalgae Cultivation Conditions

Scenedesmus obliquus (UTEX # 393), a member of the green algae (Chlorophyceae) group, was used in this study. The experiment was set up in the Algal Biotechnology Laboratory with constant light (40 µmol photon $m^{-2}s^{-1}$) and a 16:8-hour light and dark cycle and a constant temperature (21±2°C) using the semi-continuous culture method. Illumination was measured with a radiation sensor LI-COR (LI-250). The experimental study was carried out in Bold Basal medium (BBM), in 2L glass flasks and with 10% inoculation intensities, and the culture was continuously stirred by air. In the experiment, two main elements (N and P) that affect the growth of algae but contribute to lipid production were selected and used at various concentrations. A total of 6 groups were formed with the combinations of the different ratios of N and P concentrations (Table 1) (Concentration in Final Medium: 10 ml $NaNO_3$=2.94*10^{-3}mM, 10 ml K_2HPO_4=4.31*10^{-4}mM, 10 ml KH_2PO_4=1.29*10^{-3}mM).

Table 1. Trial groups and quantities used in the study

Trial Groups	Quantity Used
1. Trial group	10 ml $NaNO_3$, 10 ml PO_4
2. Trial group	5 ml $NaNO_3$, 5 ml PO_4
3. Trial group	30 ml $NaNO_3$, 10 ml PO_4
4. Trial group	30 ml $NaNO_3$, 5 ml PO_4
5. Trial group	10 ml $NaNO_3$, 5 ml PO_4
6. Trial group	5 ml $NaNO_3$, 10 ml PO_4

Note: All applications were made in triplicate.

Cell Density (OD) and Dry Weight

Cell density (OD) was checked daily by a visible spectrophotometer UV-Vis SP-3000 nano at 665 nm (Kaewkannetra et al., 2012). Regression equation was created according to the regression curve formed between dry weight and OD and the amount of dry weight was calculated according to equation 1 (Yue & Chen, 2005).

$$Dry\ weight\ (gL^{-1})x = 1.22OD_{665} + 0.0001 \quad (1)$$

Chlorophyll and Carotene Analysis

The chlorophyll and carotene were measured at three days intervals. Five milliliters of each *S. obliquus* culture were taken and filtered using Whatman GF/CTM, 1.2 µm, UK. For chlorophyll extraction, the filters were put in 15 mL glass tubes to which 10 mL of 95% ethanol /water mixture was added on it, and left in the refrigerator (+4°C) in the dark for 24 hours. At the end of the extraction period, the upper clear part was removed and absorption values were measured at 470, 649 and 665 nm in the visible spectrophotometer. Using the formula below, chl-*a*, chl-*b* and total carotene amounts were calculated (Sartory & Grobbelaar, 1984).

$$Chl\ a = 13.7 \times OD_{665} - 5.76 \times OD_{649} \quad (2)$$

$$Chl\ b = 23.96 * OD_{649} - 7.32 * OD_{665} \quad (3)$$

$$Carotene = \frac{(1.00 \times OD470 - 2.05 \times Chl\ a)}{245} \quad (4)$$

Lipid and Protein Analysis

At the end of the experiment, all of the remaining cultures were harvested for 10 minutes with the help of refrigerated centrifuge (Heraeus, Suprafuge 22) at 7500 rpm rotation speed.

The biomass obtained was dried at 55°C for protein and total lipid analysis. Protein analysis was performed according to the Kjeldahl method (Williams, 1984). Total lipid values were calculated according to the percentage of dry biomass. Total lipid analysis was made according to the method applied by Bligh & Dyer (1959).

Statistical Analysis

The data were analyzed by statistical analysis using IBM SPSS-12 and the graphs were drawn by Microsoft Excel (2010 Microsoft Corporation, USA) program. Two-way analysis of variance (ANOVA) was used to test the effects of N and P limitation of the culture on lipid, protein, cell density OD, chl *a* and *b*, carotene and dry weight. When differences were found in two-way ANOVA, Duncan multiple comparison test (HSD), (SPSS) of one-way ANOVA (Version 12.0, SPSS, Chicago, IL) was used (Zar, 1999).

Results

In the study, *S. obliquus* was cultured under laboratory conditions and their growth were determined by performing continuous aeration in nutrient media containing different ratios of N and P at 21±2°C, and 16:8 (light: dark) photo period. Growth and pigment production (chlorophyll *a*, *b* and carotenoid) of *S. obliquus* were followed over time.

The growth of the *S. obliquus* was followed over a period of three weeks (Figure 1). In the experiment, the initial cell density OD values of all groups were statistically similar with value of 0.294±0.003. Considering the cell density OD values of the groups on the last day, the best result was obtained in the group containing 30ml $NaNO_3$+10ml PO_4 and 30ml $NaNO_3$+5ml PO_4. The results were statistically significant ($P<0.05$) compared to other groups. The last day cell density OD in these groups were 1.297±0.005 and 1.270±0.3, respectively. The lowest cell density OD values were found for group containing 5ml $NaNO_3$+5ml PO_4, and this value was determined as 1.119±0.008. Cell density OD of the treatment groups was shown below (Figure 1). Likewise, the highest amount of dry matter was determined in groups containing 30 ml of $NaNO_3$ ($P<0.05$) (Table 2). As can be seen from the Table 2, the best growth was observed in the group containing 30ml $NaNO_3$+10ml PO_4. The lowest biomass values were in the group containing 5ml $NaNO_3$+5ml PO_4. The highest biomass was determined as 1.583 and 1.549 gL^{-1} in the groups containing 30ml $NaNO_3$. The lowest biomass was determined as 1.366 gL^{-1} in the group containing 5ml $NaNO_3$+5ml PO_4 ($P<0.05$).

The last day biomass, cell density OD, chl *a*, chl *b* and carotene amounts of *S. obliquus* at 40 $\mu molm^{-2}s^{-1}$ photon light intensity and different ratios of nutrients are summarized in Table 2. It was determined that the amount of carotene increased and the amount of chlorophyll decreased with the decrease of N in the nutrient medium. The highest amount of carotene was determined in the group including 5ml $NaNO_3$+ 5ml PO_4 with the lowest biomass and the lowest chlorophyll value.

Nutrient limitation leads to an increase in the content of lipids but decrease in the content of protein in cells of *S. obliquus*. Reducing the concentration of the N resulted in an approximately 2.5-fold increase in the cellular lipid content of cells. However, P deficiency in the growth medium did not cause an increase in lipid content. Protein and lipid content of the experimental groups were summarized in Figure 2 and Figure 3. As indicated in Table 2, the highest biomass and

Table 2. Last day biomass, optical density, chlorophyll *a*, *b* and carotene values of *S. obliquus* in different N and P ratios

Trial Groups	Biomass (gL^{-1})	Cell density OD	Chl *a* (μgmL^{-1})	Chl *b* (μgmL^{-1})	Carotene (μgmL^{-1})
10 ml $NaNO_3$, 10 ml PO_4	$1.404±0.1^b$	$1.151±0.2^b$	$2.152±0.02^b$	$0.301±0.02^b$	$1.089±0.04^b$
5 ml $NaNO_3$, 5 ml PO_4	$1.366±0.2^c$	$1.119±0.3^c$	$0.953.5±0.02^d$	$0.102±0.004^d$	$1.203±0.03^a$
30 ml $NaNO_3$, 10 ml PO_4	$1.583±0.2^a$	$1.297±0.2^a$	$2.410.5±0.03^a$	$0.357±0.002^a$	$0.651±0.02^c$
30 ml $NaNO_3$, 5 ml PO_4	$1.549±0.3^a$	$1.270±0.3^a$	$2.145±0.001^b$	$0.318±0.001^b$	$0.649±0.001^c$
10 ml $NaNO_3$, 5 ml PO_4	$1.405±0.2^b$	$1.152±0.1^b$	$1.028±0,01^c$	$0.153±0.002^c$	$1.094±0.03^b$
5 ml $NaNO_3$, 10 ml PO_4	$1.401±0.1^b$	$1.148±0.1^b$	$1.017±0.02^c$	$0.168±0.001^c$	$1.181±0.002^a$

Note: Values are means (n=3). Means in a row without a common superscript letter differ ($p<0.05$) as analyzed by one-way ANOVA and the Duncan test.

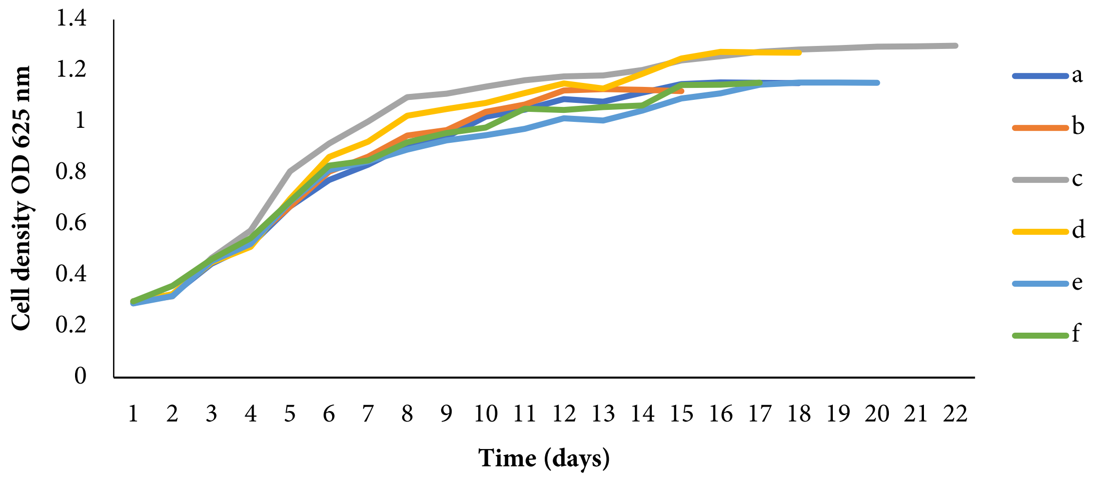


Figure 1. Optical density values of treatment groups. **a:**10 ml $NaNO_3$, 10 ml PO_4, **b:** 5 ml $NaNO_3$, 5 ml PO_4, **c**: 30 ml $NaNO_3$, 10 ml PO_4, **d**: 30 ml $NaNO_3$, 5 ml PO_4, **e:**10 ml $NaNO_3$, 5 ml PO_4, **f:** 5 ml $NaNO_3$, 10 ml PO_4 (mean values, n=3).

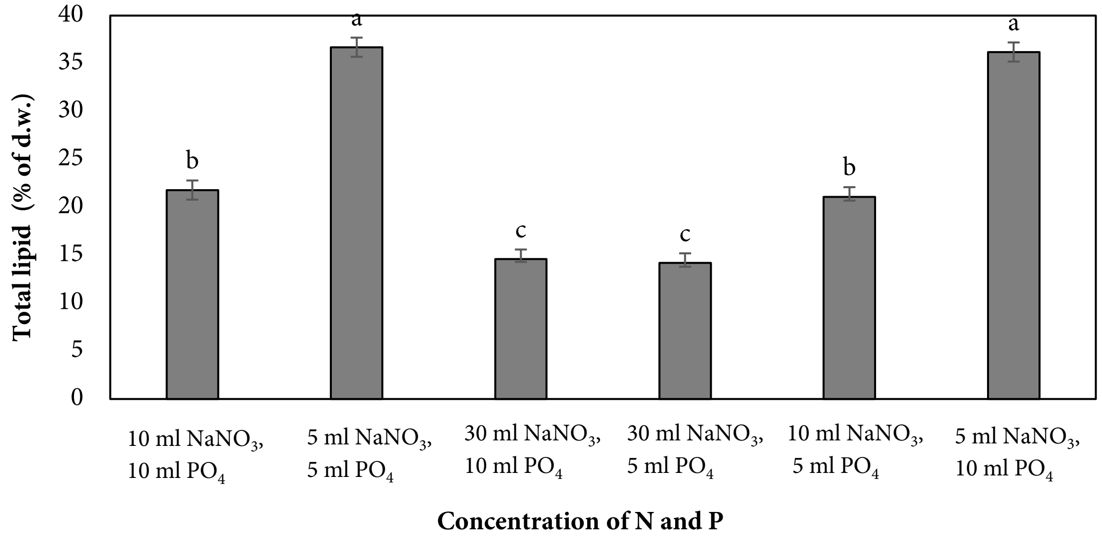


Figure 2. Total lipid production of *S. obliquus* in response to different N and P concentrations. Each error bar represents mean±standard error triplicates (n=3). Mean values different letters (a-c) are significantly different at P<0.05, one-way ANOVA.

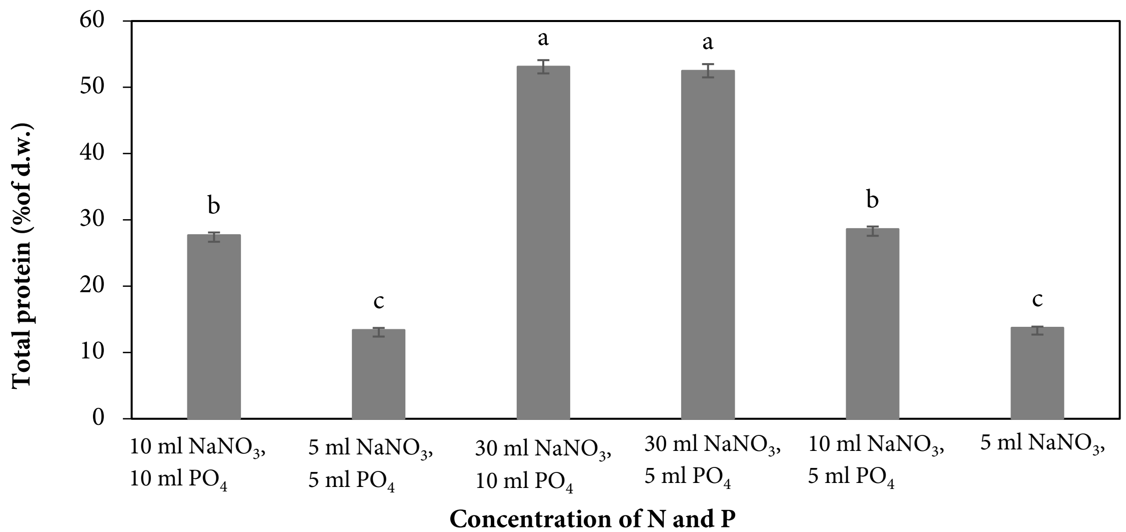


Figure 3. Total protein production of *S. obliquus* in response to different N and P concentration. Each error bar represents mean±standard error triplicates (n=3). Mean values different letters (a-c) are significantly different at P<0.05, one-way ANOVA.

optical density was obtained in the groups containing 30ml $NaNO_3$. As seen in Figure 2 and Figure 3, the amount of lipid and protein varied depending on the N amount in the culture medium which caused the lipid ratio to increase as the N amount decreased. The highest lipid values were determined as 36.7% in the group containing 5ml $NaNO_3$+5ml PO_4 and 36.2% in the group containing 5ml $NaNO_3$+10ml PO_4 ($P<0.05$). Protein contents were found to be the lowest in the groups with the highest lipid values. The highest protein contents were found in the groups containing 30ml $NaNO_3$ ($P<0.05$).

Discussion

Despite advances in algal biotechnology, various difficulties are encountered in the culture of microalgae species. The main purpose in the production of phototrophic organisms is generally to provide a continuous culture at optimal cell density. During the cultivation of an algae species in the outdoor environment, various environmental factors change greatly both daily and seasonally, requiring the cells in the culture to constantly react to these conditions. Biochemical composition of biomass depends on growth conditions such as environmental factors, nutrient environment, temperature, salinity, pH, light (Sukenik, 1991).

Microalgae *S. obliquus* is an excellent species for biodiesel production in terms of their important fatty acids (Abd El Baky et al., 2012; Gouveia & Oliveira, 2009). Limiting elements such as N and P that are necessary for algae growth can increase lipid content (Abd El Baky et al., 2012; El-Sheekh et al., 2013; Ho et al., 2012; Mandal & Mallick, 2009). The nitrogen element, which plays a role in the structure of many macromolecules, especially proteins, is one of the most important elements for microalgae. Limiting N in the growing medium causes a decrease in some metabolites such as protein, while it also causes an increase in some metabolites such as lipid and carbohydrate. It also slows down growth (Simionato et al., 2013, Li et al., 2012). In this study, it was determined that N depletion caused significant changes in the lipid content of *S. obliquus* ($P<0.05$), but P deficiency had no effect. Similar results have been observed in many microalgal species such as *Chlorella* (Illman et al., 2000) and *Botryococcus braunii* (Dayananda et al., 2007). Lipid contents increased in *Nannochloropsis oculata*, *Chlorella vulgaris* and *Chaetoceros muelleri* in N-deficient culture media (McGinnis et al., 1997; Mutlu et al., 2011). Damiani et al. (2010) cultured *Haematococcus pluvialis* under different stress conditions (high light and high light-N deficiency) and stated that the total lipid increased from 15% to 32.99% in N deficiency and high light. Anand & Arumugam (2015) reported that they observed a 2.27- fold increase in lipid for *Scenedesmus quadricauda* in the N deficient growth medium. Kamalanathan et al. (2016) reported that physiological changes are more pronounced in N deficiency in *Chlamydomonas reinhardtii*. They also reported that the photosynthetic performance of *C. reinhardtii* showed large changes under N limitation but relatively did not change with P starvation. They reported that lipid concentrations per cell were at least 2.4 times higher in groups with N deficiency and the amount of protein was lower than in the control group. Mutlu et al. (2011) cultured *C. vulgaris* in a medium containing nitrogen and phosphorus at different concentrations in their study. The lipid value was highest in the group with N deficiency, the amount of lipid did not increase much in the group with P deficiency. They also reported the highest amount of protein in the control group. Pancha et al. (2014) cultured *Scenedesmus* sp. in a nutrient medium containing different levels of nitrogen. They reported that the lipid content of *Scenedesmus* sp. was much higher in N-free environment than in BG-11 medium. Uslu et al. (2011) cultured *Spriulina* in nutrient medium containing different amounts of N. They reported the highest lipid (17.05%) in the group cultured under N deficiency and the highest protein (67.4%) in the control group.

Overall, they found that N deficiency was more effective on lipid and protein levels than P deficiency. In this study, as in many studies, N restriction increased the cellular lipid ratio by decreasing the growth rate. However, in groups with N deficiency, the biomass amounts are not very low. Groups with increased lipid content can be evaluated economically.

Microalgal biomass is very important in lipid studies. The aim of the studies is to keep both lipid and biomass amounts of algae at high levels. In this study, the decrease in the amount of N in the nutrient medium caused an increase in cellular lipid and a decrease in biomass. In the present study, the highest biomass was found in the groups containing 30ml $NaNO_3$, while the lowest lipid ratio was determined in these groups. N deficiencies stated that the amount of biomass decreases, which was consistent with the results of many studies (Adenan et al. 2016, Uslu et al. 2011, Mutlu et al. 2011). Adenan et al. (2016) reported that in *Chlorella* sp. and *Chaetoceros* sp., when N deficiency was applied, the lipid ratio increased, but the amount of biomass, protein and carbohydrate decreased.

In many studies investigating the effects of N restriction on metabolites in microalgae cultures, it was observed that the ratio of organic carbon compounds such as lipids increased, while decreases in cell number and chl a were found. However, yellowing of culture colors has been observed due to increases in carotene content (Shifrin & Chisholm 1981; Sukenik et al. 1989).

Uslu et al. (2020) cultured *Isochrysis affinis galbana* in nutrient medium containing different amounts of N in tubular and panel systems. In the study, they reported that the chlorophyll content decreased and the total carotene content increased in 50% N (-) cultures. The highest OD values were found in control group, whilst the lowest OD was obtained in the 50% N (-) group. It was concluded that the OD was lower in the N deficient groups as in other parameters. In this study, chlorophyll content decreased in cultures with N deficiency and total carotene content increased. Likewise, the highest OD was determined in the groups with the highest N content.

Conclusion

The first studies in the field of algal biotechnology were generally carried out with species with high protein content and easily cultivable. However, the discovery of algae that produces valuable metabolites other than protein has also continued. *Scenedesmus obliquus* is one of the important species in the field of microalgal biotechnology due to its metabolites accumulated in the cell. In our study, the best growth and protein content was found in the group containing 30 ml of $NaNO_3$, while the lipid ratio was found in the groups containing 5 ml of $NaNO_3$. If *S. obliquus* is to be used as a source of biodiesel, it is recommended to use groups containing 5 ml of $NaNO_3$ with a lipid ratio of 36%. As a result of the study, we can say that *S. obliquus* is a potential algae species to be used for biodiesel purposes.

Acknowledgements

We gratefully acknowledge the research funding provided for this project (Project No: FBA-2018-11155) by the Scientific Research Projects Unit at Cukurova University for their financial support.

Compliance With Ethical Standards

Authors' Contributions

LU planned, analyzed data, conducted experiments and wrote all parts of manuscript, OI helped in finalizing research theme and objectives, YB guided experiments, SS helped in writing results and discussion.

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.

References

Abd El Baky, H. H., El-Baroty, G. S., Bouaid, A., Martinez, M., & Aracil, J. (2012). Enhancement of lipid accumulation in *Scenedesmus obliquus* by optimizing CO_2 and Fe^{3+} levels for biodiesel production. *Bioresource Technology*, *119*, 429-432. https://doi.org/10.1016/j.biortech.2012.05.104

Adenan, N. S., Yusoff, F. M., Medipally, S. R., & Shariff, M. (2016). Enhancement of lipid production in two marine microalgae under different levels of nitrogen and phosphorus deficiency. *Journal of Environmental Biology*, *37*(4 Spec No), 669-676.

Anand, J., & Arumugam, M. (2015). Enhanced lipid accumulation and biomass yield of *Scenedesmus quadricauda* under nitrogen starved condition. *Bioresource Technology*, *188*, 190-194. https://doi.org/10.1016/j.biortech.2014.12.097

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911-917. https://doi.org/10.1139/o59-099

Damiani, M. C., Popovich, C. A., Constenla, D., & Leonardi, P. I. (2010). Lipid analysis in *Haematococcus pluvialis* to assess its potential use as a biodiesel feedstock. *Bioresource Technology*, *101*(11), 3801-3807. https://doi.org/10.1016/j.biortech.2009.12.136

Dayananda, C., Sarada, R., Kumar, V., & Ravishankar, G. A. (2007). Isolation and characterization of hydrocarbon producing green alga *Botryococcus braunii* from Indian freshwater bodies. *Electronic Journal of Biotechnology*, *10*(1), 78-91.

El-Sheekh, M., Abomohra, A. E. F., & Hanelt, D. (2013). Optimization of biomass and fatty acid productivity of *Scenedesmus obliquus* as a promising microalga for biodiesel production. *World Journal of Microbiology and Biotechnology*, *29*(5), 915-922. https://doi.org/10.1007/s11274-012-1248-2

Gouveia, L., & Oliveira, A. C. (2009). Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology and Biotechnology*, *36*(2), 269-274. https://doi.org/10.1007/s10295-008-0495-6

Ho, S. H., Chen, C. Y., & Chang, J. S. (2012). Effect of light intensity and nitrogen starvation on CO_2 fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. *Bioresource Technology*, *113*, 244-252. https://doi.org/10.1016/j.biortech.2011.11.133

Illman, A. M., Scragg, A. H., & Shales, S. W. (2000). Increase in Chlorella strains calorific values when grown in low nitrogen medium. *Enzyme and Microbial Technology*, *27*(8), 631-635. https://doi.org/10.1016/S0141-0229(00)00266-0

Kaewkannetra, P., Enmak, P., & Chiu, T. (2012). The effect of CO_2 and salinity on the cultivation of *Scenedesmus obliquus* for biodiesel production. *Biotechnology and Bioprocess Engineering*, *17*(3), 591-597. https://doi.org/10.1007/s12257-011-0533-5

Kamalanathan, M., Pierangelini, M., Shearman, L. A., Gleadow, R., & Beardall, J. (2016). Impacts of nitrogen and phosphorus starvation on the physiology of *Chlamydomonas reinhardtii*. *Journal of Applied Phycology*, *28*(3), 1509-1520. https://doi.org/10.1007/s10811-015-0726-y

Li, Y., Fei, X., & Deng, X. (2012). Novel molecular insights into nitrogen starvation-induced triacylglycerols accumulation revealed by differential gene expression analysis in green algae *Micractinium pusillum*. *Biomass and Bioenergy*, *42*, 199-211. https://doi.org/10.1016/j.biombioe.2012.03.010

Liu, Z. Y., Wang, G. C., & Zhou, B. C. (2008). Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. *Bioresource Technology*, *99*(11), 4717-4722. https://doi.org/10.1016/j.biortech.2007.09.073

Mandal, S., & Mallick, N. (2009). Microalga *Scenedesmus obliquus* as a potential source for biodiesel production. *Applied Microbiology and Biotechnology*, *84*(2), 281-291. https://doi.org/10.1007/s00253-009-1935-6

McGinnis, K. M., Dempster, T. A., & Sommerfeld, M. R. (1997). Characterization of the growth and lipid content of the diatom *Chaetoceros muelleri*. *Journal of Applied Phycology*, *9*(1), 19-24. https://doi.org/10.1023/A:1007972214462

Mutlu, Y. B., Işık, O., Uslu, L., Koç, K., & Durmaz, Y. (2011). The effects of nitrogen and phosphorus deficiencies and nitrite addition on the lipid content of *Chlorella vulgaris* (Chlorophyceae). *African Journal of Biotechnology*, *10*(3), 453-456.

Pancha, I., Chokshi, K., George, B., Ghosh, T., Paliwal, C., Maurya, R., & Mishra, S. (2014). Nitrogen stress triggered biochemical and morphological changes in the microalgae *Scenedesmus* sp. CCNM 1077. *Bioresource Technology*, *156*, 146-154. https://doi.org/10.1016/j.biortech.2014.01.025

Sartory, D. P., & Grobbelaar, J. U. (1984). Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia*, *114*(3), 177-187. https://doi.org/10.1007/BF00031869

Sheehan, J., Dunahay, T., Benemann, J., & Roessler, P. (1998). *Look back at the US department of energy's aquatic species program: biodiesel from algae; close-out report* (No. NREL/TP-580-24190). National Renewable Energy Lab., Golden, CO.(US). https://doi.org/10.2172/15003040

Shifrin, N. S., & Chisholm, S. W. (1981). Phytoplankton lipids: interspecific differences and effects of nitrate, silicate and light-dark cycles. *Journal of Phycology*, *17*(4), 374-384. https://doi.org/10.1111/j.1529-8817.1981.tb00865.x

Simionato, D., Block, M. A., La Rocca, N., Jouhet, J., Maréchal, E., Finazzi, G., & Morosinotto, T. (2013). The response of *Nannochloropsis gaditana* to nitrogen starvation includes de novo biosynthesis of triacylglycerols, a decrease of chloroplast galactolipids, and reorganization of the photosynthetic apparatus. *Eukaryotic Cell*, *12*(5), 665-676. https://doi.org/10.1128/EC.00363-12

Sugimoto, K., Midorikawa, T., Tsuzuki, M., & Sato, N. (2008). Upregulation of PG synthesis on sulfur-starvation for PS I in *Chlamydomonas*. *Biochemical and Biophysical Research Communications*, *369*(2), 660-665. https://doi.org/10.1016/j.bbrc.2008.02.058

Sukenik, A. (1991). Ecophysiological considerations in the optimization of eicosapentaenoic acid production by *Nannochloropsis* sp. (Eustigmatophyceae). *Bioresource Technology*, *35*(3), 263-269. https://doi.org/10.1016/0960-8524(91)90123-2

Sukenik, A., Carmeli, Y., & Berner, T. (1989). Regulation of fatty acid composition by irradiance level in the eustigmatophyte *Nannochloropsis* sp. *Journal of Phycology*, *25*(4), 686-692. https://doi.org/10.1111/j.0022-3646.1989.00686.x

Uslu, L., Işık, O., Koç, K., & Göksan, T. (2011). The effects of nitrogen deficiencies on the lipid and protein contents of *Spirulina platensis*. *African Journal of Biotechnology*, *10*(3), 386-389.

Uslu, L., Işık, O. & Cimen, B. A. (2020). The effect of nitrogen deficiency on the growth and lipid content of *Isochrysis affinis galbana* in two photobioreactor systems (PBR): Tubular and flat panel. *Journal of Agricultural Sciences*, *26*(3), 282-289. https://doi.org/10.15832/ankutbd.526989

Williams, S. (1984). *Official methods of analysis* (No. 630.24 A8 1984). Association of Official Analytical Chemists.

Yue, L., & Chen, W. (2005). Isolation and determination of cultural characteristics of a new highly CO_2 tolerant fresh water microalgae. *Energy Conversion and Management*, *46*(11-12), 1868-1876. https://doi.org/10.1016/j.enconman.2004.10.010

Zar, J. H. (1999). *Biostatistical Analysis*. 4th ed. Prentice Hall.

Mar. Sci. Tech. Bull. (2022) 11(2): 202-211
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1101875

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Preliminary observation on microplastic contamination in the Scombridae species from coastal waters of Pakistan

Farzana Yousuf[1] • Levent Bat[2] • Ayşah Öztekin[2] • Qadeer Mohammad Ali[3] • Quratulan Ahmed[3*] • Iqra Shaikh[3]

[1] *University of Karachi, Department of Zoology, 75270, Karachi, Pakistan*
[2] *Sinop University, Fisheries Faculty, Department of Hydrobiology, 57000, Sinop, Turkey*
[3] *The Marine Reference Collection and Resources Centre, 75270, Karachi, Pakistan*

ARTICLE INFO

Article History:
Received: 11.04.2022
Received in revised form: 29.05.2022
Accepted: 29.05.2022
Available online: 21.06.2022

Keywords:
Arabian Sea
Karachi
Microplastic
Pakistan
Scombridae

ABSTRACT

Microplastics are one of the major pollution problems nowadays, have been found in both marine environments and various fish species worldwide. In this study, the presence of microplastics in the digestive systems and the gills of 6 species from the Scombridae family on the coast of Karachi in Pakistan was investigated. A total of 336 fish were examined for the presence of microplastic in gills and the digestive systems. Microplastics were detected in digestive systems and gills in 11.11%-19.51% and 58.62%-85.71% of total individuals, respectively. The number of microplastics varied from 0.19 to 1.12 items.ind^{-1} in digestive system and 1.5 to 7.04 items.ind^{-1} in gill. Fibre was dominant in both gills (98.67-99.17%) and digestive systems (100%). More extensive and further investigations are needed on microplastic contamination of the biota on the Pakistan coast.

Please cite this paper as follows:

Yousuf, F., Bat, L., Öztekin, A., Ali, Q. M., Ahmed, Q., & Shaikh, I. (2022). Preliminary observation on microplastic contamination in the Scombridae species from coastal waters of Pakistan. *Marine Science and Technology Bulletin*, *11*(2), 202-211. https://doi.org/10.33714/masteb.1101875

Introduction

Microplastics are commonly defined as plastic particles less than 5 mm in size (Arthur et al., 2009; Hidalgo-Ruz et al., 2012) that have been found in marine environments (Ivar do Sul et al., 2014; Song et al., 2015; Isobe et al., 2017; Cincinelli et al., 2019) and in marine fish (Neves et al., 2015; Bellas et al., 2016; Karbalaei et al., 2019; Sparks & Immelman, 2020) all over the world. Microplastics' abilities to absorb various contaminants from surrounding environment and they are also a source of

* Corresponding author
E-mail address: quratulanahmed_ku@yahoo.com (Q. Ahmed)

toxic chemicals added as ingredients during manufacturing (Mato et al., 2001; Teuten et al., 2009; Oehlmann et al., 2009). So, they raised significant concern on their role as a vector for transferring harmful contaminants into the aquatic environment (Brennecke et al., 2016; Jaafar et al., 2021).

Microplastics have been detected in various aquatic organisms, including zooplankton, invertebrates, fish, and marine mammals (Li et al., 2015; Neves et al., 2015; Sun et al., 2017; Güven et al., 2017; Thushari et al., 2017; Aytan et al., 2022a). Laboratory studies have also revealed that microplastics are ingested by several organisms from various trophic levels (Farrel & Nelson, 2013; Messinetti et al., 2017; LeMoine et al., 2018; De Felice et al., 2019).

Fish are generally used as biomonitors to determine the health of aquatic ecosystems (Joy & Death, 2002; Zrnčić et al., 2013). The investigations on the presence of microplastics in fish have gained momentum and results of investigations showed that microplastics have been detected both in the digestive systems and in various tissues and organs in fish, recently (Neves et al., 2015; Mizraji et al., 2017; Abbasi et al., 2018; Koongolla et al., 2020). Several pathways have been suggested for the uptake routes of microplastics in fish and one of the uptake pathways in fish is generally considered as ingestion (Confusing with food, accidental ingestion, transfer with the food chain) (Roch et al., 2020). Filter deposit feeders are non-selective feeders that are prone to unintentionally consuming more inorganic material, on the other hand, predators, choose their prey deliberately and mostly do not have other paths of uptake, so microplastic ingestion is usually due to bioaccumulation (Murray & Cowie, 2011; Wesch et al., 2016). The gill of fish is the most physiologically diverse, anatomically complicated, and multifunctional organ (gas exchange, ion regulation, osmoregulation, hormone synthesis, immunological defense, acid–base balance and ammonia excretion) (Rombough, 2007; Olson, 2011; Secombes & Wang, 2012). The microplastics in gills were retained in this organ during water filtration, causing physical injury to the gills as well as reduced respiratory efficiency, which can lead to hypoxia and be fatal (Barboza et al., 2020).

The family of Scombridae is some of the world's most popular food fishes. Their importance as tertiary consumers in the marine food web. Scombrids are pelagic-neritic (open-ocean) fish that live mostly in tropical and subtropical oceans (Froese & Pauly, 2021). Pakistan has a significant Scombrids fish industry (Ahmed et al., 2018). Pakistan's fishing industry is vital to the country's economy. Scombrids fish support fisheries on a large scale and make up almost one-fifth of the total marine fish catch in Pakistan (Ahmed et al., 2016; FAO, 2019). Karachi is situated in the Sindh province and industrial and commercial center of the Pakistan. The marine ecosystem is severely impacted by environmental deterioration throughout Pakistan's coastline, notably in the Karachi city harbor areas (Qaimkhani, 2018). The harbor's location puts it in close proximity to important shipping routes. The harbor handles over 90% of Pakistan's fish and seafood catch and exports. Increased marine pollution in Karachi's coastal area has come from the city's industrialization and economic growth (Ahmed & Bat, 2015). The investigations on microplastic pollution in Pakistan are very limited and the presence of microplastics has been reported from sea water, sediments, and beaches (Balasubramaniam & Phillott, 2016; Irfan et al., 2020a, 2020b; Ahmed et al., 2022) and there is no information about the contamination of microplastics in fish. This study aims to detect the presence of microplastics in digestive systems and gills of the commercial fish from Scombridae family in Karachi-Pakistan.

Material and Methods

Karachi Fish Harbour is located in Karachi, Sindh, Pakistan, in the northeastern border of the Arabian Sea, between 24°50'54.71" N and 66°58'38.68" E (Fig. 1). The harbor's location makes it close to the main shipping routes as well as the main commercial and industrial areas.

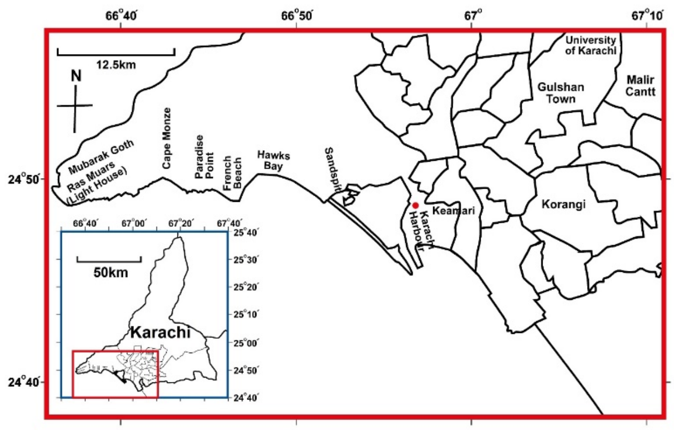


Figure 1. Karachi Fish Harbour

A total of 336 fish from 6 species of the Scombridae family were obtained from fishermen fishing in Karachi Fish Harbour in 2021. Detailed information about the species used in the research is given in the Table 1. The fish samples were transported to the laboratory in ice boxes. Fish samples were washed with distilled water and removed from foreign particles and then frozen -20°C until analysis.

Table 1. Taxonomic and common name, environment, feeding behavior, trophic level and IUCN status of species (Froese & Pauly, 2021; IUCN, 2022).

Species	Common name	Environment	Feeding behaviour	Trophic level	IUCN status*
Rastrelliger kanagurta (Cuvier, 1816)	Indian mackerel	pelagic-neritic	macroplankton such as larval shrimps and fish	3.2	DD
Scomberomorus commerson (Lacepède, 1800)	Narrow-barred Spanish mackerel	pelagic-neritic	small fishes, squids and penaeid shrimps	4.5	NT
Scomberomorus guttatus (Bloch & Schneider, 1801)	Indo-Pacific king mackerel	pelagic-neritic	small fishes, squids and crustaceans	4.3	DD
Euthynnus affinis (Cantor, 1849)	Kawakawa	pelagic-neritic	small fishes, squids, crustaceans, and zooplankton.	4.5	LC
Katsuwonus pelamis (Linnaeus, 1758)	Skipjack tuna	pelagic-oceanic	fishes, crustaceans, cephalopods, and molluscs	4.4	LC
Thunnus tonggol (Bleeker, 1851)	Longtail tuna	pelagic-neritic	fishes, cephalopods, and crustaceans	4.5	DD

Note: *DD: Data Deficiency; NT: Near Threatened; LC: Least Concern

All fish dissect, digestive systems and gills were removed from each fish separately. Digestive systems and gills were placed in glass containers separately and added hydrogen peroxide (H_2O_2, 30%) to eliminate organic matter. After that the solutions were filtered on fine-mesh filters (55 µm pore size) and filters placed in petri dishes for microscopic examination. Zeiss Stemi SV6 stereomicroscope used for examination. To prevent contamination special precautions were taken. All, working surfaces and equipment were cleaned with distilled water, cotton laboratory coats and gloves were worn continually. The filters and solutions were covered with aluminium foils during all procedures to diminish airborne contamination.

The number of microplastic in each fish was counted and categorized to type of microplastics (fibre and fragment). The percentage occurrence of microplastic and the mean microplastic amount in all fish and only fish with contaminated microplastic were determined for each species. The relationships between the number of microplastics and the length of fish were assessed with Spearman correlation analysis in IBM SPSS software.

Results

A total of 336 fish from 6 species were examined for presence of microplastic in the gills and the digestive systems. 250 specimens (74.4%) were contained microplastics in their digestive system or gill (Table 2). More microplastics were found in the gills than in the digestive systems in all analysed fish species.

The minimum and maximum percentages of microplastics in digestive systems of the analysed species were among 11.11% in *E. affinis* and 19.51% in *R. kanagurta*. The highest number of microplastic in digestive system was observed in *T. tonggol* (1.12 items.ind^{-1}) and followed by *K. pelamis* (0.93 items.ind^{-1}), *E. affinis* (0.80 items.ind^{-1}), *S. commerson* (0.76 items.ind^{-1}), *S. guttatus* (0.41 items.ind^{-1}) and *R. kanagurta* (0.19 items.ind^{-1}) respectively. Generally, there was a weak positive correlation between the number of microplastics and fish length except *R. kanagurta*, *S. commerson* and *S. guttatus* (*E. affinis*: R=0.37 p=0.006; *K. pelamis*: R=0.31, p=0.044; *T. tonggol*: R=0.34, p=0.016).

The minimum and maximum occurrence percentage of microplastics in gills of the analysed species were among 58.62% in *S. commerson* and 85.71% in *R. kanagurta*. The highest number of microplastics in gills was observed in *T. tonggol* (7.04 items.ind^{-1}) and followed by *E. affinis* (6.98 items.ind^{-1}), *K. pelamis* (6.19 items.ind^{-1}), *S. commerson* (3.98 items.ind^{-1}), *S. guttatus* (3.49 items.ind^{-1}) and *R. kanagurta* (1.5 items.ind^{-1}) respectively.

Two different types of microplastics were found during the study. Fibre was dominant in the gills (98.67% in *E. affinis*-99.17% in *R.kanagurta*) and a low percentage of fragment was also found (0.83% in *R.kanagurta*-1.33% in *E. affinis*). In digestive systems only fibre was found (Figure 2).

Table 2. The percentage occurrence and number of microplastics in gills and digestive systems of Scombridae species

Species	No.	Mean size (SD)	Microplastic					
			Gill			Digestive system		
			%	Mean¹	Mean²	%	Mean¹	Mean²
R. kanagurta	82	20.73 (2.60)	59.76	1.5	2.44	19.51	0.19	1
S. commerson	58	43.76 (2.05)	58.62	3.98	6.79	18.96	0.76	4
S. guttatus	51	42.92 (3.49)	78.43	3.49	4.45	11.76	0.41	3.5
E. affinis	54	57.44 (1.06)	85.18	6.98	8.20	11.11	0.80	7.17
K. pelamis	42	51.43 (1.87)	80.95	6.19	7.65	14.29	0.93	6.5
T. tonggol	49	52.43 (3.28)	85.71	7.04	8.21	16.33	1.12	6.87

Note: %: of individual with microplastic, Mean¹: mean microplastic number of all investigated fish, Mean²: mean microplastic number of only contaminated fish

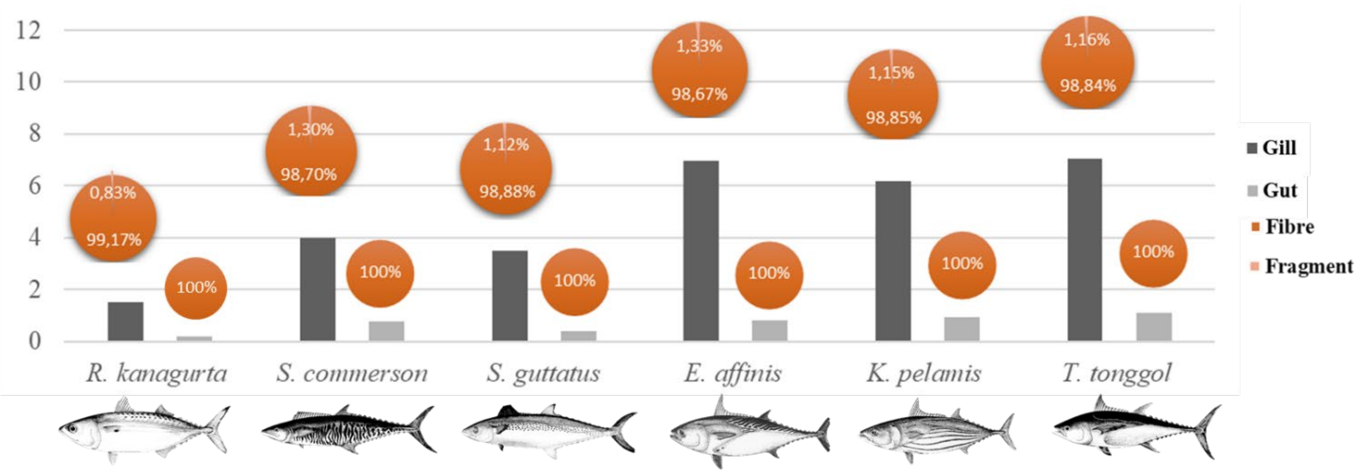


Figure 2. Types and abundance of microplastics in gill and digestive system from the species of the Scombridae family

Discussion

The amount of research on microplastic pollution in the aquatic environment of the Pakistan is quite a few (Balasubramaniam & Phillott, 2016; Irfan et al., 2020a, 2020b; Ahmed et al., 2022). The presence of plastic spherules in fish was one of the first articles on this issue (Carpenter et al., 1972). Investigations showed that microplastics were found in the digestive systems and in various tissues and organs in fish (Mizraji et al., 2017; Abbasi et al., 2018; Su et al., 2019; Koongolla et al., 2020). In the current study, microplastics were detected in both digestive system and gills of 6 different commercial fish species in Karachi, where there is a lack of data on a regional basis. As a result of the study, the number of microplastic in the gills was found to be higher than the digestive systems for all specimens.

Microplastics were detected in digestive systems 11.11%-19.51% of total individuals and the number of microplastics in digestive systems varied from 0.19 to 1.12 items.ind^{-1}. The results of the present study are similar to the investigation carried out on Scombrids by various researchers in different environments, the percentage occurrence of plastic in gastrointestinal tract was found between 0-71% and the number of plastic particles were 0-6.71 items.ind^{-1} (Table 3).

Various factors may have caused the presence of plastic in the digestive systems of fish. Plastic particles may be consumed intentionally mistaking for food, or unintentionally, or they can be passed via trophic transfer (from prey to predator) and

Table 3. Microplastic presence in digestive systems of Scombridae species from different environments

Species	Location	Abundance items.ind^{-1} (% of individual with microplastic)	Type	References
S. japonicus	Portuguese	0.57±1.04 (31%)	Fibre, Fragment	Neves et al. (2015)
S. scombrus		0.46±0.78 (31%)		
S. japonicus	Mediterranean, Turkey	6.71 (71%)		Güven et al. (2017)
S. japonicus	Peru	0.03 (3.3%)	Fragment	Ory et al. (2018)
S. japoicus	Hangzhou Bay and Yangtze Estuary, China	0.8±0.8 (56%)	Fibre, Fragment	Su et al. (2019)
R. kanagurta	Kochi, Arabian Sea, India	(0-55%)		James et al. (2020)
Scomber spp.	Moroccan Atlantic shelf	(27%)		Maaghloud et al. (2020)
Rastrelliger sp.	Southeast coast of India	(0%)		James et al. (2021)
R. kanagurta				
S. sarda	Black Sea, Turkey	4 (70%)	Fibre, Fragment	Aytan et al. (2022b)
R. kanagurta, S. commerson, S. guttatus, E. affinis, K. pelamis, T. tonggol	Karachi-Pakistan	0.19-1.12 (11.11%-19.51%)	Fibre	This study

drinking has also been identified as a potential source of microplastic consumption, particularly for big marine fish (Ory et al., 2017; Athey et al., 2020; Roch et al., 2020). Ingestion of plastic can cause various adverse effects such as deterioration of feeding capacity, digestive system blockage, starvation and death, poor quality of life and reproductive capacity and plastics can adsorb and concentrate potentially harmful toxic compounds from the aquatic environment (Gregory, 2009; Wright et al., 2013).

In present study, microplastics were detected in gills 58.62%-85.71% of total individuals and number of microplastics in gills varied from 1.50 to 7.04 items.ind^{-1}. Research on the presence of microplastic in gills of fish is relatively limited (Abbasi et al., 2018; Su et al., 2019; Huang et al., 2020; Koongla et al., 2020; Lin et al., 2020; Jaafar et al., 2021). In Scombridae species, the frequency rate and number of microplastics in gills were found similar in Yangtze Estuary-China [*S.japonius*: 2.4±2.0 items.ind^{-1} (%78 of total fish) (Su et al., 2019)] with present study and Barboza et al. (2020) was found lower (*S.colias*: 0.7±1.0 items.ind^{-1}) than present study in Northwest (NW) Portuguese coastal waters. In this study microplastic abundance in gills higher than the digestive systems. Similarly, the microplastic abundance reported as higher than in gills than digestive systems in Persian Gulf by Abbasi et al. (2018) in Malaysia by Jaafar et al. (2021). but contrary more microplastic was found in digestive systems than gills in China (Su et al., 2019; Koonglla et al., 2020; Lin et al., 2020) and the Northeast Atlantic Ocean (Barboza et al., 2020). The results can be showed variations due to the species, habitats, surrounding environments and gill's structures. Therefore, the uptake of microplastics via gills depend on microplastic size, and morphology of the gills (Collard et al., 2017; Barboza et al., 2020). Microplastics found in the gills are due to their keeping in the gills during filtration of water (Barboza et al., 2020), so microplastic content in water affects plastic uptake of gills. Microplastics can cause physical harm to the gills as well as reduced respiratory efficiency, which can be fatal (Barboza et al., 2020).

Fibre dominant shape of microplastic in the present study like many other study (Su et al., 2019; Lin et al., 2020; Aytan et al., 2022b). Recently published article on microplastic contamination of Pakistan coast (including Karachi), the high amount of microplastics has been detected in surface water and sediments, and these are mostly fibres (>%99) (Ahmed et al., 2022). Several factors were affected the plastic uptake, and one of them microplastic concentration in the water. Therefore, the fibre density found in fish caught in the region supported by the Ahmed et al. (2022). Washing machine effluent is discharged into the local sewer system, which plays a crucial role in the fate and transportation of fibres into the marine environment due to the huge number of fibres released when clothing is washed (Napper & Thompson, 2016). Karachi Fish Harbour is near the major commercial sectors and various industrial districts of Karachi, Sindh, Pakistan. Furthermore, because of the region's intensive agricultural, household, and urbanization activity, the harbour may receive enormous amounts of untreated agricultural and domestic sewage (Ahmed et al., 2015). Therefore, it was stated that every day, 450 million gallons of untreated water is poured into the sea in Karachi and other coastal cities during the apex court hearing in December 2017, because no sewage treatment plants are operational (Qaimkhani, 2018).

Conclusion

Microplastics are in fact a global issue. Aside from the increasingly well-known challenges that ocean litter poses to the environment, coastal communities, and marine industries, there is now hard evidence that it poses a large-scale and substantial threat to the well-being of wild marine species.

In this study, a total of 336 fish from Scombridae family were examined for the presence of microplastic in gills and digestive systems. 74.4% of fish were contaminated with microplastics in their digestive system or gill and fibre was dominant. The risk of bioaccumulation is significant in long-lived species and for those who use fish as food. Many studies have shown that microplastics are more present in the digestive tract than in fish meat. Therefore, the fish should be cleaned thoroughly by washing with plenty of water before consumption.

There is a very limited number of investigations on microplastic contamination both marine environment and biota in Pakistan coast, so more extensive and further research are needed.

Compliance With Ethical Standards

Authors' Contributions

Author QA Conception and designed the study, FY data acquisition, LB and AÖ wrote the first draft and critical revision of the manuscript, QMA technical or material support, IS data analysis and interpretation. 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, ethics committee approval is not required.

References

Abbasi, S., Soltani, N., Keshavarzi, B., Moore, F., Turner, A., &, M. (2018). Microplastics in different tissues of Hassanaghaei fish and prawn from the Musa Estuary, Persian Gulf. *Chemosphere*, *205*, 80-87. https://doi.org/10.1016/j.chemosphere.2018.04.076

Ahmed, Q., & Bat, L. (2015). Heavy metal levels in *Euthynnus affinis* (Cantor 1849) Kawakawa fish marketed at Karachi Fish Harbour, Pakistan and potential risk to human health. *Journal of the Black Sea/Mediterranean Environment*, *21*(1), 35-44.

Ahmed, Q., Ali, Q. M., Bat, L., Öztekin, A., Memon, S., & Baloch, A. (2022). Preliminary study on abundance of microplastic in sediments and water samples along the coast of Pakistan (Sindh and Balochistan)-Northern Arabian Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, *22*(SI). *In press*. https://doi.org/10.4194/TRJFAS19998

Ahmed, Q., Bat, L., & Yousuf, F. (2015). Heavy metals in *Terapon puta* (Cuvier, 1829) from Karachi coasts, Pakistan. *Journal of Marine Biology*, *2015*, 132768. https://doi.org/10.1155/2015/132768

Ahmed, Q., Bat, L., Öztekin, A., & Ali, Q. M. (2018). A review on studies of heavy metal determination in mackerel and tuna (Family-Scombridae) fishes. *Journal of Anatolian Environmental and Animal Sciences*, *3*(3), 107-123. https://doi.org/10.35229/jaes.425382

Ahmed, Q., Bilgin, S., & Bat, L. (2016). Length based growth estimation of most commercially important Scombridae from offshore water of Pakistan Coast in the Arabian Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, *16*, 155-167. https://doi.org/10.4194/1303-2712-v16_1_16

Arthur, C., Baker, J., & Bamford, H. (2009). Proceedings of the international research workshop on the occurrence, effects, and fate of microplastic marine debris. NOAA marine debris program. Technical memorandum NOS-OR&R-30.

Athey, S. N., Albotra, S. D., Gordon, C. A., Monteleone, B., Seaton, P., Andrady, A. L., Taylor, A. R., & Brander, S. M. (2020). Trophic transfer of microplastics in an estuarine food chain and the effects of a sorbed legacy pollutant *Limnology and Oceanography Letters*, *5*(1), 154-162. https://doi.org/10.1002/lol2.10130

Aytan, U., Esensoy, F. B., & Senturk, Y. (2022a). Microplastic ingestion and egestion by copepods in the Black Sea. *Science of The Total Environment*, *806*, 150921. https://doi.org/10.1016/j.scitotenv.2021.150921

Aytan, U., Esensoy, F. B., Senturk, Y., Arifoğlu, E., Karaoğlu, K., Ceylan, Y., & Valente, A. (2022b). Plastic occurrence in commercial fish species of the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, *22*(SI), *In press*. http://doi.org/10.4194/TRJFAS20504

Balasubramaniam, M., & Phillott, A. D. (2016). Preliminary observations of microplastics from beaches in the Indian ocean. *Indian Ocean Turtle Newsletter*, *23*, 13-16.

Barboza, L. G. A., Lopes, C., Oliveira, P., Bessa, F., Otero, V., Henriques, B., Raimundo, J., Caetano, M., Vale, C., & Guilhermino, L. (2020). Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. *Science of The Total Environment*, *717*, 134625. https://doi.org/10.1016/j.scitotenv.2019.134625

Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., & Martínez-Gómez, C. (2016). Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. *Marine pollution bulletin*, *109*(1), 55-60. https://doi.org/10.1016/j.marpolbul.2016.06.026

Brennecke, D., Duarte, B., Paiva, F., Caçador, I., & Canning-Clode, J. (2016). Microplastics as vector for heavy metal contamination from the marine environment. *Estuarine, Coastal and Shelf Science*, *178*, 189-195. https://doi.org/10.1016/j.ecss.2015.12.003

Carpenter, E. J., Anderson, S. J., Harvey, G. R., Miklas, H. P., & Peck, B. B. (1972). Polystyrene spherules in coastal waters. *Science*, *178*(4062), 749-750. https://doi.org/10.1126/science.178.4062.749

Cincinelli, A., Martellini, T., Guerranti, C., Scopetani, C., Chelazzi, D., & Giarrizzo, T. (2019). A potpourri of microplastics in the sea surface and water column of the Mediterranean Sea. *Trends in Analytical Chemistry*, *110*, 321-326. https://doi.org/10.1016/j.trac.2018.10.026

Collard, F., Gilbert, B., Eppe, G., Roos, L., Compère, P., Das, K., & Parmentier, E. (2017). Morphology of the filtration apparatus of three planktivorous fishes and relation with ingested anthropogenic particles. *Marine Pollution Bulletin*, *116*(1-2), 182-191. https://doi.org/10.1016/j.marpolbul.2016.12.067

De Felice, B., Sabatini, V., Antenucci, S., Gattoni, G., Santo, N., Bacchetta, R., Ortenzi, M., & Parolini, M. (2019). Polystyrene microplastics ingestion induced behavioral effects to the cladoceran *Daphnia magna*. *Chemosphere*, *231*, 423-431. https://doi.org/10.1016/j.chemosphere.2019.05.115

FAO. (2019). Global capture production quantity (1950-2019) Retrieved on April 11, 2022, from https://www.fao.org/fishery/statistics-query/en/capture/capture_quantity

Farrell, P., & Nelson, K. (2013). Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). *Environmental Pollution*, *177*, 1-3. https://doi.org/10.1016/j.envpol.2013.01.046

Froese, R., & Pauly, D. (Eds.). (2021). FishBase. World Wide Web electronic publication. www.fishbase.org, (08/2021)

Gregory, M. R. (2009). Environmental implications of plastic debris in marine settings—entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1526), 2013-2025. https://doi.org/10.1098/rstb.2008.0265

Güven, O., Gökdağ, K., Jovanović, B., & Kıdeyş, A. E. (2017). Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. *Environmental Pollution*, *223*, 286-294. https://doi.org/10.1016/j.envpol.2017.01.025

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environmental Science & Technology*, *46*(6), 3060-3075. https://doi.org/10.1021/es2031505

Huang, J. S., Koongolla, J. B., Li, H. X., Lin, L., Pan, Y. F., Liu, S., He, W. H., Maharana, D., & Xu, X. R. (2020). Microplastic accumulation in fish from Zhanjiang mangrove wetland, South China. *Science of The Total Environment*, *708*, 134839. https://doi.org/10.1016/j.scitotenv.2019.134839

Irfan, M., Qadir, A., Mumtaz, M., & Ahmad, S. R. (2020a). An unintended challenge of microplastic pollution in the urban surface water system of Lahore, Pakistan. *Environmental Science and Pollution Research*, *27*(14), 16718-16730. https://doi.org/10.1007/s11356-020-08114-7

Irfan, T., Khalid, S., Taneez, M., & Hashmi, M. Z. (2020b). Plastic driven pollution in Pakistan: the first evidence of environmental exposure to microplastic in sediments and water of Rawal Lake. *Environmental Science and Pollution Research*, *27*, 15083-15092. https://doi.org/10.1007/s11356-020-07833-1

Isobe, A., Uchiyama-Matsumoto, K., Uchida, K., & Tokai, T. (2017). Microplastics in the Southern Ocean. *Marine Pollution Bulletin*, *114*(1), 623-626. https://doi.org/10.1016/j.marpolbul.2016.09.037

Ivar do Sul, J. A., Costa, M. F., & Fillmann, G. (2014). Microplastics in the pelagic environment around oceanic islands of the Western Tropical Atlantic Ocean. *Water, Air, & Soil Pollution*, *225*(7), 1-13. https://doi.org/10.1007/s11270-014-2004-z

Jaafar, N., Azfaralariff, A., Musa, S. M., Mohamed, M., Yusoff, A. H., & Lazim, A. M. (2021). Occurrence, distribution and characteristics of microplastics in gastrointestinal tract and gills of commercial marine fish from Malaysia. *Science of The Total Environment*, *799*, 149457. https://doi.org/10.1016/j.scitotenv.2021.149457

James, K., Vasant, K., Padua, S., Gopinath, V., Abilash, K. S., Jeyabaskaran, R., Babu, A., & John, S. (2020). An assessment of microplastics in the ecosystem and selected commercially important fishes off Kochi, southeastern Arabian Sea, India. *Marine Pollution Bulletin*, *154*, 111027. https://doi.org/10.1016/j.marpolbul.2020.111027

James, K., Vasant, K., Sikkander Batcha, SM, S.B., Padua, S., Jeyabaskaran, R., Thirumalaiselvan, S., Vineetha, G., & Benjamin, L. V. (2021). Seasonal variability in the distribution of microplastics in the coastal ecosystems and in some commercially important fishes of the Gulf of Mannar and Palk Bay, Southeast coast of India. *Regional Studies in Marine Science*, *41*, 101558. https://doi.org/10.1016/j.rsma.2020.101558

Joy, M. K., & Death, R. G. (2002). Predictive modelling of freshwater fish as a biomonitoring tool in New Zealand. *Freshwater Biology*, *47*(11), 2261-2275. https://doi.org/10.1046/j.1365-2427.2002.00954.x

Karbalaei, S., Golieskardi, A., Hamzah, H. B., Abdulwahid, S., Hanachi, P., Walker, T. R., & Karami, A. (2019). Abundance and characteristics of microplastics in commercial marine fish from Malaysia. *Marine Pollution Bulletin*, *148*, 5-15. https://doi.org/10.1016/j.marpolbul.2019.07.072

Koongolla, J. B., Lin, L., Pan, Y. F., Yang, C. P., Sun, D. R., Liu, S., Xu, X. R., Maharana, D., Huang, J. S., & Li, H. X. (2020). Occurrence of microplastics in gastrointestinal tracts and gills of fish from Beibu Gulf, South China Sea. *Environmental Pollution*, *258*, 113734. https://doi.org/10.1016/j.envpol.2019.113734

LeMoine, C. M., Kelleher, B. M., Lagarde, R., Northam, C., Elebute, O. O., & Cassone, B. J. (2018). Transcriptional effects of polyethylene microplastics ingestion in developing zebrafish (*Danio rerio*). *Environmental Pollution*, *243*, 591-600. https://doi.org/10.1016/j.envpol.2018.08.084

Li, J., Yang, D., Li, L., Jabeen, K., & Shi, H. (2015). Microplastics in commercial bivalves from China. *Environmental Pollution*, *207*, 190-195. https://doi.org/10.1016/j.envpol.2015.09.018

Lin, L., Ma, L. S., Li, H. X., Pan, Y. F., Liu, S., Zhang, L., Peng, J. P., Fok, L., Xu, X. R., & He, W. H. (2020). Low level of microplastic contamination in wild fish from an urban estuary. *Marine Pollution Bulletin*, *160*, 111650. https://doi.org/10.1016/j.marpolbul.2020.111650

Maaghloud, H., Houssa, R., Ouansafi, S., Bellali, F., El Bouqdaoui, K., Charouki, N., & Fahde, A. (2020). Ingestion of microplastics by pelagic fish from the

Moroccan Central Atlantic coast. *Environmental Pollution*, *261*, 114194. https://doi.org/10.1016/j.envpol.2020.114194

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., & Kaminuma, T. (2001). Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environmental Science & Technology*, *35*(2), 318-324. https://doi.org/10.1021/es0010498

Messinetti, S., Mercurio, S., Parolini, M., Sugni, M., & Pennati, R. (2018). Effects of polystyrene microplastics on early stages of two marine invertebrates with different feeding strategies. *Environmental Pollution*, *237*, 1080-1087. https://doi.org/10.1016/j.envpol.2017.11.030

Mizraji, R., Ahrendt, C., Perez-Venegas, D., Vargas, J., Pulgar, J., Aldana, M., Ojeda, F. P., Duarte, C., & Galbán-Malagón, C. (2017). Is the feeding type related with the content of microplastics in intertidal fish gut?. *Marine Pollution Bulletin*, *116*(1-2), 498-500. https://doi.org/10.1016/j.marpolbul.2017.01.008

Murray, F., & Cowie, P. R. (2011). Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). *Marine Pollution Bulletin*, *62*(6), 1207-1217. https://doi.org/10.1016/j.marpolbul.2011.03.032

Napper, I. E., & Thompson, R. C. (2016). Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. *Marine Pollution Bulletin*, *112*(1-2), 39-45. https://doi.org/10.1016/j.marpolbul.2016.09.025

Neves, D., Sobral, P., Ferreira, J. L., & Pereira, T. (2015). Ingestion of microplastics by commercial fish off the Portuguese coast. *Marine Pollution Bulletin*, *101*(1), 119-126. https://doi.org/10.1016/j.marpolbul.2015.11.008

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K. O., Wollenberger, L., Santos, E. M., Paull, G. C., Van Look, K. J. W., & Tyler, C. R. (2009). A critical analysis of the biological impacts of plasticizers on wildlife. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1526), 2047-2062. https://doi.org/10.1098/rstb.2008.0242

Olson, K. R. (2011). Design and physiology of arteries and veins: Branchial anatomy. In A. P. Farrell (Ed.), *Encyclopedia of Fish Physiology: From Genome to Environment* (pp. 1095-1103). Academic Press. https://doi.org/10.1016/B978-0-12-374553-8.00048-4

Ory, N. C., Sobral, P., Ferreira, J. L., & Thiel, M. (2017). Amberstripe scad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. *Science of the Total Environment*, *586*, 430-437. https://doi.org/10.1016/j.scitotenv.2017.01.175

Ory, N., Chagnon, C., Felix, F., Fernández, C., Ferreira, J. L., Gallardo, C., Ordóñez, O. G., Henostroza, A., Laaz, E., Mizraji, R., Mojica, H., Haro, V. M., Medina, L. O., Preciado, M., Sobral, P., Urbina, M. A., & Thiel, M. (2018). Low prevalence of microplastic contamination in planktivorous fish species from the southeast Pacific Ocean. *Marine Pollution Bulletin*, *127*, 211-216. https://doi.org/10.1016/j.marpolbul.2017.12.016

Qaimkhani, A. M. (2018). The Marine Litter Action Plan-Status Report (Pakistan), pp.43

Roch, S., Friedrich, C., & Brinker, A. (2020). Uptake routes of microplastics in fishes: practical and theoretical approaches to test existing theories. *Scientific Reports*, *10*(1), 1-12. https://doi.org/10.1038/s41598-020-60630-1

Rombough, P. (2007). The functional ontogeny of the teleost gill: which comes first, gas or ion exchange?. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *148*(4), 732-742. https://doi.org/10.1016/j.cbpa.2007.03.007

Secombes, C. J., & Wang, T. (2012). The innate and adaptive immune system of fish. In B. Austin (Ed.), *Infectious Disease in Aquaculture: Prevention and Control* (pp. 3-68). Woodhead Publishing Series in Food Science, Technology and Nutrition, Woodhead Publishing, https://doi.org/10.1533/9780857095732.1.3

Song, Y. K., Hong, S. H., Jang, M., Han, G. M., & Shim, W. J. (2015). Occurrence and distribution of microplastics in the sea surface microlayer in Jinhae Bay, South Korea. *Archives of Environmental Contamination and Toxicology*, *69*(3), 279-287. https://doi.org/10.1007/s00244-015-0209-9

Sparks, C., & Immelman, S. (2020). Microplastics in offshore fish from the Agulhas Bank, South Africa. *Marine Pollution Bulletin*, *156*, 111216. https://doi.org/10.1016/j.marpolbul.2020.111216

Su, L., Deng, H., Li, B., Chen, Q., Pettigrove, V., Wu, C. & Shi, H. (2019). The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of east China. *Journal of Hazardous*

Materials, *365*, 716-724. https://doi.org/10.1016/j.jhazmat.2018.11.024

Sun, X., Li, Q., Zhu, M., Liang, J., Zheng, S., & Zhao, Y. (2017). Ingestion of microplastics by natural zooplankton groups in the northern South China Sea. *Marine Pollution Bulletin*, *115*(1-2), 217-224. https://doi.org/10.1016/j.marpolbul.2016.12.004

Teuten, E. L., Saquing, J. M., Knappe, D. R., Barlaz, M. A., Jonsson, S., Björn, A., Rowland, S. J., Thompson, R. C., Galloway, T. S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P. H., Tana, T. S., Prudente, M., Boonyatumanond, R., Zakaria, M. P., Akkhavong, K., Ogaa, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., & Takada, H. (2009). Transport and release of chemicals from plastics to the environment and to wildlife. *Philosophical Transactions of The Royal Society B: Biological Sciences*, *364*(1526), 2027-2045. https://doi.org/10.1098/rstb.2008.0284

Thushari, G. G. N., Senevirathna, J. D. M., Yakupitiyage, A., & Chavanich, S. (2017). Effects of microplastics on sessile invertebrates in Thailand: an approach to coastal zone conservation. *Marine Pollution Bulletin*, *124*(1), 349-355. https://doi.org/10.1016/j.marpolbul.2017.06.010

Wesch, C., Bredimus, K., Paulus, M., & Klein, R. (2016). Towards the suitable monitoring of ingestion of microplastics by marine biota: A review. *Environmental pollution*, *218*, 1200-1208. https://doi.org/10.1016/j.envpol.2016.08.076

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution*, *178*, 483-492. https://doi.org/10.1016/j.envpol.2013.02.031

Zrnčić, S., Oraić, D., Ćaleta, M., Mihaljević, Ž., Zanella, D., & Bilandžić, N. (2013). Biomonitoring of heavy metals in fish from the Danube River. *Environmental Monitoring and Assessment*, *185*(2), 1189-1198. https://doi.org/10.1007/s10661-012-2625-x

Mar. Sci. Tech. Bull. (2022) 11(2): 212-220
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1106896

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Carotenoid composition and investigation of the antioxidant activity of *Phormidium* sp.

Yaşar Aluç[1]*

[1] *Kırıkkale University, Scientific and Technological Research Application and Research Center, Kırıkkale, Turkey*

ARTICLE INFO

Article History:
Received: 21.04.2022
Received in revised form: 26.05.2022
Accepted: 02.06.2022
Available online: 22.06.2022

Keywords:
Microalgae
Phormidium sp.
Carotenoids
HPLC analysis
DPPH and FRAP assays

ABSTRACT

Microalgae metabolites are used for health, feed additives, cosmetic industries, food and biodiesel production. *Phormidium* species have an important position in medical studies because they contain essential components. In this study, carotenoid profile and content were analyzed using the HPLC method. Antioxidant activities for *Phormidium* sp. were determined using DPPH and FRAP assays. BHT and ASC were used as control samples in antioxidant assays. The method used to resolve a number of carotenoids from saponified *Phormidium* sp. proved acceptable separation, as evidenced by retention factor (k) values of 0.54 to 3.83 and separation factor (α) values greater than 1. Main carotenoids were dominated by the two main derivatives, all-trans form of lutein 41.35% (1.25 mg/g) and 9- or 9'-cis-β-carotene 36.43% (1.10 mg/g). Auroxanthin and cis neoxanthin were identified as epoxy-containing compounds. It is also understood that considering the DPPH assay, the extract of *Phormidium* sp. (IC50:127.6 mg/L) exhibited clearly low radical scavenging activity compared to the standards ASC (IC50: 0.02 mg/L) and BHT (IC50: 0.19 mg/L). In the FRAP antioxidant experiment, the mean ASC and BHT equivalent amounts were determined as 828.6 and 124.6 mg/L, respectively. Quantitatively, *Phormidium* sp. was predominated by cis-Lutein as a major constituent, being 41.35% (3.02 mg/g) in total carotenoids (Tc). The antioxidant capacity of *Phormidium* sp. that considering the DPPH and FRAP were compared to control standards were showed considerably low effects.

Please cite this paper as follows:

Aluç, Y. (2022). Carotenoid composition and investigation of the antioxidant activity of *Phormidium* sp. *Marine Science and Technology Bulletin*, *11*(2), 212-220. https://doi.org/10.33714/masteb.1106896

* Corresponding author
E-mail address: alucyasar@kku.edu.tr (Y. Aluç)

Introduction

Microalgae are single-celled microscopic organisms capable of producing bioactive compounds and are the primary producers of the aquatic food chain. Microalgae are microorganisms that can preserve complex organic compounds in their bodies and release them out of the cell with the help of sunlight (Chacon-Lee & Gonzalez-Marino, 2010; Batista et al., 2013). Carotenoids of structural and functional roles are indispensable in photosynthetic life. They prevent the formation of photooxidative damage by providing the transfer of excess energy from the photosynthetic apparatus (Stange, 2016). Reactive oxygen species (ROS) are a natural biochemical product involved in the aerobic metabolism processes of the cell (Hancock et al., 2001). Increased exposure to ROS causes disruption of redox signaling and control known as oxidative stress, resulting in cell membrane, enzyme, and DNA fragmentation as a result (Zuluaga et al., 2017). New therapeutic molecules that can block oxidative stress are very important in the prevention and improvement of chronic disease due to oxidative stress. Because of their antioxidant properties, carotenoids have been suggested for the prevention of chronic diseases. Current studies show that carotenoids prevent oxidation of cholesterol, proteins or DNA by scavenging free radicals and reducing stress-induced by ROS (Rao & Rao, 2007; Khansari et al., 2009; Gong & Bassi, 2016).

The antioxidant properties of carotenoids are explained by the scavenging of singlet molecular oxygen and peroxyl radicals in general, due to the mixture of various isomers in their structures and their chemical formation with other bioactive compounds (Niyogi et al., 1997). It is known that natural carotenoid isomers are more active for direct human consumption than their synthetic counterparts dominated by all-trans compounds (Patrick, 2001).

Rodríguez-Meizoso et al. (2008) detected various carotene groups such as β-carotene, lutein, violaxanthin and neoxanthin by extraction with different temperatures and solvents in their study with *Phormidium* species and stated that they showed high antioxidant activity. In a different study, Rodrigues et al. (2015) investigated the carotene profile and antioxidant effect of *Phormidium autumnale,* as a result, 24 carotenoids, 3 phycobiliproteins, and 2 chlorophylls of this species were identified (Rodrigues et al., 2015). The high antioxidant effect of *Phormidium* species has been demonstrated in many studies (Soni et al., 2008; Shanab et al., 2012; Chatterjee & Bhattacharjee, 2014).

The potential of different species, which may differ in the carotenoid compositions of many algae species, is being investigated. Comparisons are used to understand the local environments of different growing conditions over similar or identical species. For this purpose, it focused on elucidating the profile of carotenoids and antioxidant activity performance of the microalgae *Phormidium* sp. isolated from Kapulukaya Reservoir (Kirikkale, Turkey).

Material and Methods

Phormidium sp. were isolated from freshwater samples of the River Kızılırmak in Kırıkkale Province (Turkey). Basal Bold Medium (BBM) (Bischoff & Bold, 1963) was selected for the growth of *Phormidium* sp. using illumination of 16:8 h light-dark cycle of 4000 lux light intensity under temperature conditions kept at 25±1°C. Centrifugated biomass at 3000 rpm for 10 min. was lyophilized for 48 hours at -83°C and 1.33 Pa (Richmond & Hu, 2013) and stored at –80°C until used.

Extraction and Profiling of Carotenoids

The details of both the extraction procedure (Chen et al., 1991) and the analysis of carotenoids were given in a previous study by Aluç et al. (2018). Briefly; 0.1 g of the microalgae sample was stirred for 1 hour using 3 mL of a hexane-ethanol-acetone-toluene mixture (10:6:7:7, v/v). 1 mL of 40% methanolic KOH was added to wait 16 hours to achieve saponification. Then hexane and 10% sodium sulfate were added. The carotenoids were then allowed to separate under dim light and nitrogen gas. Collected extracts dried by evaporation were subjected to HPLC analysis in mobile phase solvent. Two mobile phases (A and B) of methanol–acetonitrile-water (84:14:2, v/v/v) and methylene chloride (100%) with an arranged flow rate of 0.6 mL/min were used in a gradient manner from 100% A and 0%.

Antioxidant Capacity Assays

For the extraction of antioxidants, the microalgae biomass (0.1 g) was mixed with 1 ml methanol-toluene (3:1) in a volumetric flask. Following homogenization, insoluble biomass was separated from the supernatant by centrifugation at 13500 rpm for 10 min. (Chen et al., 1991).

DPPH (2,2-Diphenyl-1-picrylhydrazyl) activity of the *Phormidium* sp. extract solutions was determined based on the method described by Blois (1958). Volumes of 62.5 μL from each extract solution prepared with methanol at concentrations of 2, 10, 25, 50, 100 μg/mL, respectively, were added to a mixture of methanol (125 μL) and DPPH (62.5 μL) and then left for incubation in the dark at room temperature. The reaction values measured at 515 nm against methanol extract as blank were used to calculate the percentage radical scavenging activity as below.

So that the control included DPPH and methanol while sample consisted of microalgae extract, DPPH and methanol. The EC_{50} values which were calculated from the plot of scavenging activity against the concentration of sample indicated the half-maximal potency of microalgal extract to scavenge DPPH radicals. For the Ferric Reducing Antioxidant Power Assay (FRAP), a 1 ml of *Phormidium* sp. extract solutions prepared at graded concentrations (2, 5, 10, 25, 50, 100 µg/mL) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (200 µL, 1%) and left for incubation at 50°C for 20 min. Trichloroacetic acid (TCA, 10%, 200 µL) was added to the mixture and centrifuged at 3750 rpm for 10 min. (Oyaizu, 1986). A 125 µL of the upper layer was separated and mixed with 25 µL distilled water and 20 µL $FeCl_3$ (0.1%). The absorbance of the mixture was measured at 665 nm by a UV-spectrophotometer.

Statistical Analysis

Means of data were obtained from triplicate analysis during HPLC analysis. Results from antioxidant assays were obtained via triplicate experiments. Relationships between antioxidant activity and concentrations of algal extracts and standards were depicted by regression analysis. Stepwise multiple regression analysis was used to STATISTICA (version 7) statistical software.

Results and Discussion

Identification of Carotenoids

The method proved an acceptable separation as inferred from the retention factor (k) ranging between (0.54-3.83) (Liu et al., 2004; Inbaraj et al., 2006). They were all positively identified based on retention time and Q ratio values of standards designation which was tentative and made by the comparison of retention time and absorption spectra values given in the literature (Tables 1 and 2) (Inbaraj et al., 2006; Aluç et al., 2018). For the identification of carotenoids in *Phormidium* sp. and the photoisomerized standards were subjected to HPLC analysis. Carotenoids spectral characteristics of *Phormidium* sp. samples were designated. The chromatogram of the extract solution from the *Phormidium* sp. cells revealed 16 resolution peaks assigned to carotenoids (Figure 1 and Table 1). All-*trans* forms of lutein (0.023 mg/g), zeaxanthin (0.074 mg/g), β-cryptoxanthin (0.005 mg/g), α-carotene (0.000 mg/g) and β-carotene (0.321 mg/g) were assigned to peaks 6, 8, 11, 13 and 14, respectively (Figure 1 and Tables 1 and 2). The *Phormidium* sp. eliquates contained 3.005 mg/g of total carotenoids (TC), dominated by the two main derivatives, trans and *cis* forms of cis-lutein 41.35% (1.25 mg/g) and 9-or 9'-cis-β-carotene 36.43% (1.10 mg/g), All-trans-zeaxanthin 2.43% (0.074 mg/g), was represented by a small amount in Tc (Table 2).

Wojtasiewicz & Ston-Egiert (2016) identified the *Phormidium* sp. (CCNP 1317) species obtained from the Pomeranian lakes and investigated pigment composition under optimized growing conditions by cultivating. Total carotenoid content was found to be around 15.80 mg/m^3 under optimized conditions. The corresponding values of β-carotene and zeaxanthin were around 14.66 mg/m^3 and 1.44 mg/m^3 (Wojtasiewicz & Ston-Egiert, 2016). In another study, Morone et al. (2020) identified the *Phormidium* sp. (LEGE 05292) compounds consisted of three xanthophylls, lutein (28.65 µg/g), canthaxanthin (23.48 µg/g), echinenone (105.81 µg/g) and one carotene β-carotene (62.94 µg/g) total carotenoid content was found 215.88 µg/g. All of these findings are much less than those obtained in our study.

Various factors play a role in the biosynthesis of carotenoids and the distribution of their derivatives in algae. Although it varies species-specific (Gong & Bassi, 2016), it can also differ in different environments and conditions of the same species (Ho et al., 2014). Additionally, variations may arise due to different culture conditions, as noted in studies for direct determination of carotenoids or those applying stress conditions to potentially increase carotenoid content (Goiris et al., 2012; Rodrigues et al., 2015; González-López et al., 2018).

Antioxidant Capacity

One of the most important features of photosynthetic organisms such as algae for human needs is based on the antioxidant potential of free radical extracts, which are produced by the physiological and biochemical processes of cells. In this study, the methods to measure antioxidant activity represented the DPPH and FRAP the single electron transfer reactions (ET). The antioxidant activities of the algal extract determined by DPPH and FRAP assays were evaluated against those of BHT (BHT or its chemical name 2,6-di-tert-butyl-p-cresol (DBPC) is a synthetic phenolic antioxidant widely used as a food additive) (Leclercq et al., 2000). The advantage of the DPPH method is that DPPH is a stable radical that is often used to measure the antioxidant activity of plant extracts. The DPPH method can be used as samples or solid solutions and is not specific for certain antioxidant components. In addition, this method is simple, accurate, fast and inexpensive to test the ability of components to capture radical compounds (Sri Mariani, 2018). The advantages of the FRAP method are that it is fast and inexpensive, the reagents used are very simple, and there are no special tools used to calculate total antioxidants. The FRAP method is a plant antioxidant test method. The FRAP method was used to measure the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} (Tahir et al., 2018). However, both methods have disadvantages. The DPPH method can only be used to measure antioxidants that are soluble in organic solvents, especially alcohol, and are very sensitive to light, oxygen, pH and solvent type. On the other hand, the FRAP method cannot measure antioxidants with thiol groups (including -SH) such as glutathione (Putri et al., 2020).

Table 1. Retention time, retention factor (k), separation factor (α), peak purity and resolution of carotenoids in *Phormidium* sp.

Peak no.	Compound	Retention time (min)	k^a	α^b	Peak purity (%)	Resolution
1	Auroxanthin	8.89	0.00	0.00	98.1	0.00
2	Auroxanthin	10.47	0.18	0.00	89.2	4.14
3	Cis-neoxanthin	13.73	0.54	3.07	97.5	8.34
4	13-or-13'-cis lutein	20.57	1.31	2.41	99.7	18.53
5	cis-lutein	22.63	1.54	1.18	99.7	4.61
6	All-trans-lutein	24.42	1.75	1.13	99.9	3.74
7	Cis-lutein	25.19	1.83	1.05	95.4	1.31
8	All-trans-zeaxanthin	26.19	1.94	1.06	99.9	1.67
9	9-or-9'-cis-lutein	27.66	2.11	1.09	99.7	3.08
10	Cis-lutein	31.05	2.49	1.18	98.2	6.97
11	All-trans-beta-cryptoxanthin	33.36	2.75	1.10	98.6	4.67
12	13-or-13'-cis-beta-carotene	36.92	3.15	1.15	92.6	6.71
13	All-trans-alfa-carotene	37.31	3.19	1.01	99.3	0.42
14	All-trans-beta-carotene	41.31	3.64	1.14	99.8	4.64
15	9-or 9'-cis-alfa-carotene	41.92	3.71	1.02	99.6	1.33
16	9-or 9'-cis-beta-carotene	42.924	3.83	1.03	99.9	2.15

Note: a: retention factor; b: selectivity (separation factor).

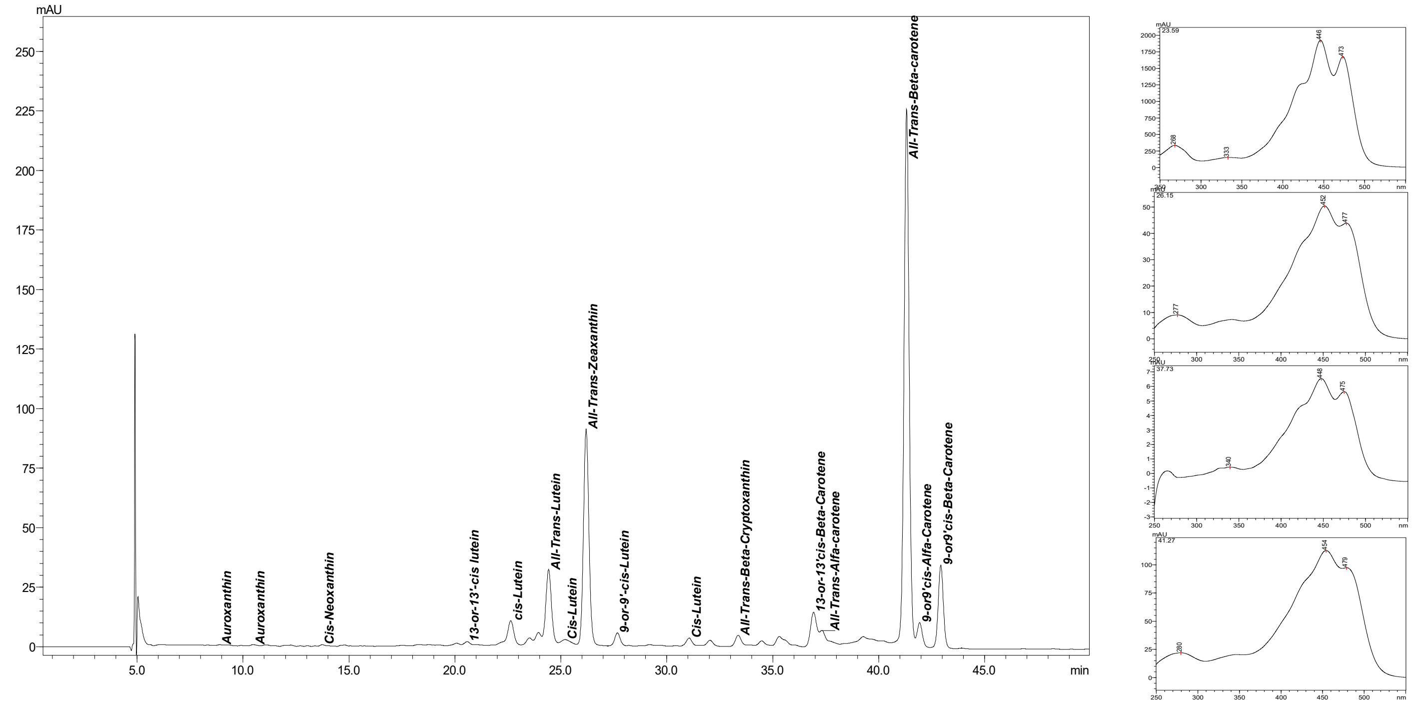


Figure 1. The HPLC chromatogram of the carotenoids obtained from *Phormidium* sp. (left) and the profiles of all-trans carotenoids (right)

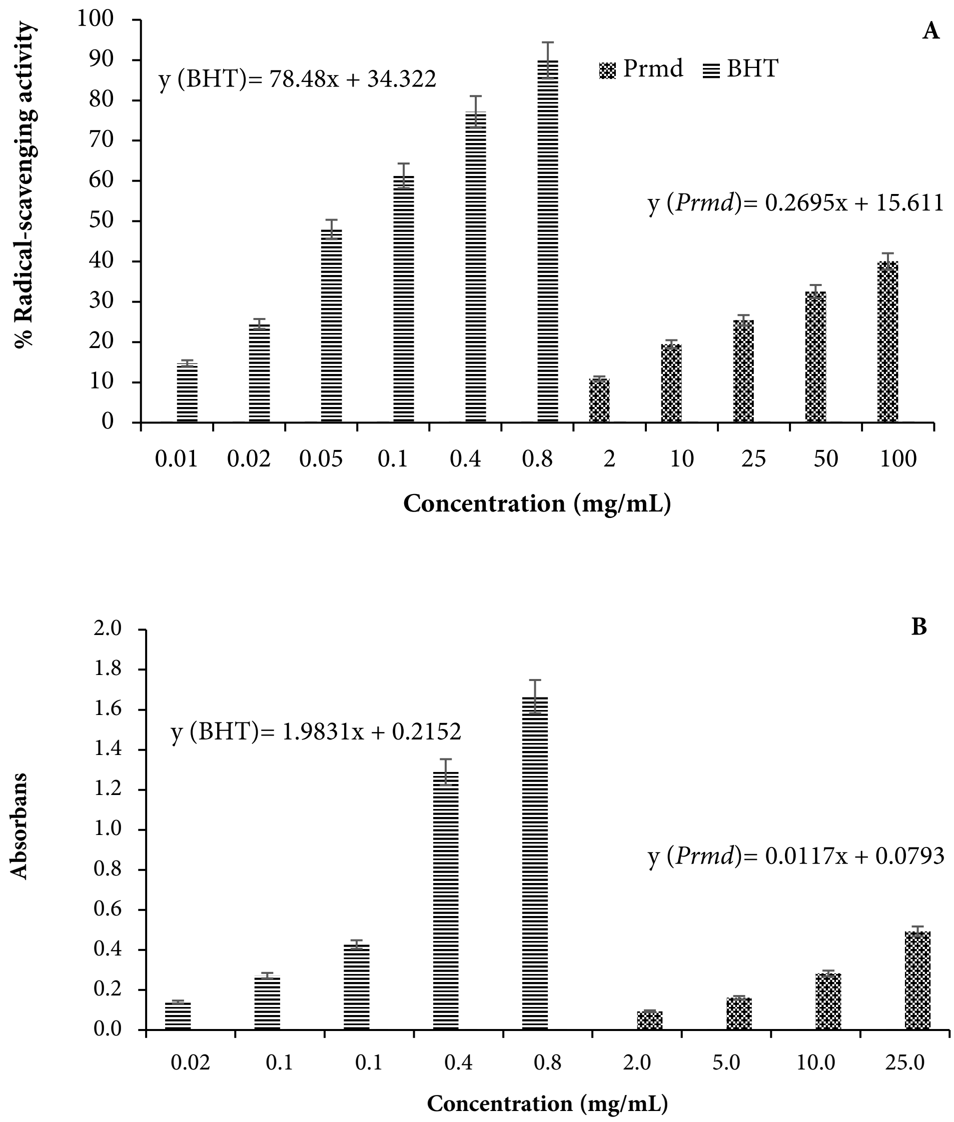


Figure 2. Collective display of DPPH radical sweeping (A) and FRAP inhibition (B) effect of *Phormidium* sp. extract. Column legends and x-axis titles are shown in the graph

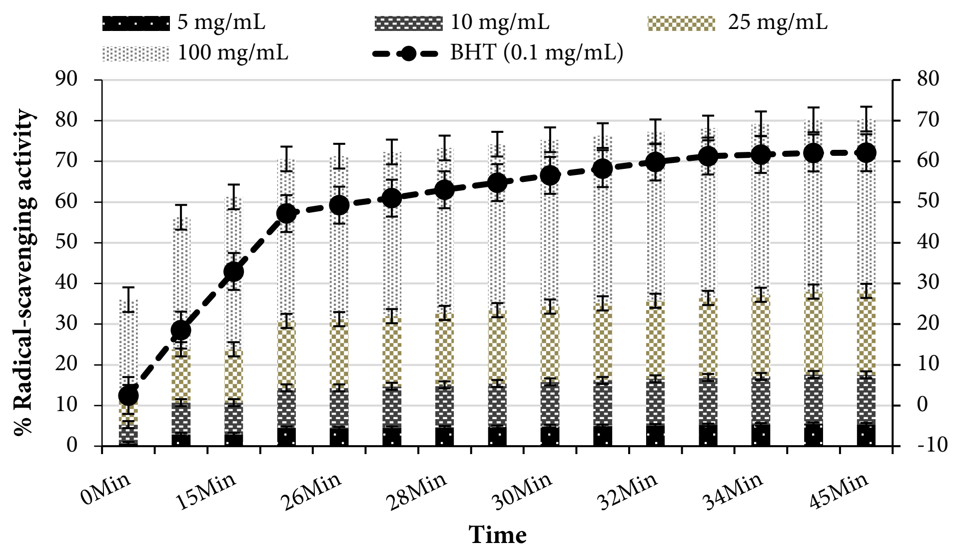


Figure 3. % Radical-scavenging activity of BHT and *Phormidium* sp. in a time-dependent manner

Table 2. Assignment data for all-trans and cis forms of carotenoids and their amounts (mg/g) in *Phormidium* sp.

Peak no.	Compound	Retention time (min.)	λ (nm, inline)				λ (nm, reported)				Q-ratio[e]	Amount (mg/g)
1	Auroxanthin	8.89	-	-	424	-	-	398	422[b]	-	0.35	0.000
2	Auroxanthin	10.47	-	-	424	-	-	398	422[b]	-	0.32	0.000
3	Cis-neoxanthin	13.73	-	396	416	-	-	411	429	459[c]	0.26	0.000
4	13-or-13'-cis lutein	20.57	-	-	443	459	-	415	440	464[b]	0.22	0.006
5	cis-lutein	22.63	339	-	-	458	331	-	440	467[a]	0.31	0.023
6	All-trans-lutein	24.42	-	-	451	477	-	423	446	470[d]	0.18	1.249
7	Cis-lutein	25.19	344	-	451	-	344	427	452	476[b]	0.15	0.000
8	All-trans-zeaxanthin	26.19	-	-	452	478	-	-	452	477[a]	0.19	0.074
9	9-or-9'-cis-lutein	27.66	-	-	460	-	332	416	440	470[b]	0.12	0.049
10	Cis-lutein	31.05	344	-	447	469	332	416	440	470[b]	0.14	0.100
11	All-trans-beta-cryptoxanthin	33.36	345	-	452	480		414	450	476[a]	0.16	0.005
12	13-or-13'-cis-beta-carotene	36.92	342	-	447	469	344	422	452	476[b]	0.12	0.079
13	All-trans-alfa-carotene	37.31	343	-	447	471	344	426	449	476[b]	0.11	0.000
14	All-trans-beta-carotene	41.31	-	-	454	480	350	430	458	482[b]	0.13	0.321
15	9-or 9'-cis-alfa-carotene	41.92	-	-	445	469	344	421	446	470[b]	0.15	0.000
16	9-or 9'-cis-beta-carotene	42.924	-	344	449	474	344	428	452	476[b]	0.17	1.101
Total												**3.031**

Note: a: A gradient mobile phase of methanol-acetonitrile-water (84:14:2, v/v/v) and methylene chloride (from 100:0, v/v to 45:55, v/v) was used by Aluç et al. (2018); b: A gradient mobile phase of methanol-acetonitrile-water (84:14:2, v/v/v) and methylene chloride (from 100:0, v/v to 45:55, v/v) was used by Inbaraj et al.(2006); c: A gradient mobile phase of methanol-2-propanol (99:1, v/v) and methylene chloride (from 100:0, v/v to 70:30, v/v)was used by Chen et al. (2004); d: A gradient mobile phase of methanol-2-propanol (99:1, v/v) and methylene chloride (from 100:0, v/v to 70:30, v/v) was used by Liu et al. (2004); e: Q-ratio is defined as the height ratio of the cis peak to the main absorption peak.

The IC50 values calculated of DPPH activity *Phormidium* sp. extract (IC50 127.60 mg/mL) and BHT equivalent values (14.32 µmol/g DW) as given in Table 3. The results of DPPH scavenging activity (%) of *Phormidium* sp. extract and both standard solutions were presented with positive and significant correlations in the DPPH and FRAP assays (Figures 2A and 2B). In addition, in Figure 3, the % radical scavenging activity of BHT and *Phormidium* sp. were given in a time-dependent manner. DPPH cleaning capacity in the literature; IC50 values of cyanobacterial species such as *Synechocystis*, *Oscillatoria*, and *Phormidium* have reported antioxidant values of 56.79-83.08 g/mL. As for our data, the IC50 value (IC50 127.60 mg/mL) was not as low as those reported in previous studies for other cyanobacterial species. It is also obviously understood that considering the DPPH assay, the extract of *Phormidium* sp. exhibited low activity compared to the BHT. Considerably high amounts of extracts (i.e., 100 mg/mL) reached only a maximum of 40.05% scavenging ability whereas standard of BHT (IC50: 0.19 mg/mL) had strong scavenging effects around (89.2%) despite their small amounts i.e., 0.8 mg/mL. The FRAP assay also generated similar results; the effect of *Phormidium* sp. extract being the lowest (i.e., 0.49 at 25 mg/mL) followed by BHT (0.43 at 0.1 mg/mL). The DPPH and FRAP methods were tested statistically using a paired T-test with a 95% confidence level to determine whether there was a significant difference in IC50 values. Results of the paired test showed an insignificant value ($p < 0.01$); This means that there is a significant difference in the antioxidant activity value of BHT and *Phormidium* sp. using the DPPH and FRAP methods. Based on the research of Maesaroh et al. (2018), the DPPH method has been shown to be more effective and efficient than the FRAP method. This is because the FRAP method is less sensitive to samples than the

DPPH method. In general, these two methods affect each other and may even be interchangeable (Maesaroh et al., 2018).

Table 3. IC50 values of *Phormidium* sp. and standards (BHT) in DPPH assay, and equivalent BHT of *Phormidium* sp. in both assays

Parameters	DPPH	FRAP
Phormidium sp. IC_{50} (mg/mL)	127.60	25.105
BHT Equivalent (μmol/g DW)	14.32	38.59

Babu & Wu (2008) applied a β-carotene-linoleate assay to identify the antioxidation activity of *M. aeruginosa, C. raciborskii, Oscillatoria* sp., *B. braunii* and were shown that the extracts of all four tested species exhibited positive antioxidation activity. They proposed in their studies that algal species produce a natural BHT that demonstrates antioxidant activity similar to that of synthetic BHT. In general, algae display two main types of defense mechanisms against ROS. These are defined as enzymatic and non-enzymatic antioxidant systems. It has been reported that the non-enzymatic antioxidant mechanism studied in *Ulva fenestrata* protects the cell from photooxidation by changes in the lipid composition of the cell under different radiations (Khotimchenko & Yakovleva, 2004). The enzymatic mechanisms studied in *Nodularia*, *Microcystis*, and *Anabaena* showed increases in ascorbate peroxidase and superoxide dismutase activity at high irradiance (Canini et al., 1996). Benedetti et al. (2010) reported the phycobiliprotein found in *Aphanizomenon flos-aquae*, a commercial microalgae species, is 10 times lower than that obtained from *Phormidium autumnale*.

A wide variety of factors play a role in the biosynthesis of carotenoids. Although species-specific varies, it can also differ in different environments and conditions of the same species. In addition, studies for the direct determination of carotenoids can reveal different variations due to stress conditions applied to potentially increase the carotenoid content and different media and culture conditions of the same species. The amount of total carotenoids that the string of a local species, *Phormidium* sp., contained could be considered as satisfactorily, relying upon comparisons to other species of cyanobacteria species. Considering that many other bioactive compounds are present in microalgal cells very high domination of lutein and β-carotene in the composition of total carotenoids ascertained for carotenoids, chlorophylls and phycocyanins that indicating the potential as a renewable source of these pigments.

Conclusion

Phormidium species can be considered an exciting crop for discovering bioactive compounds. Algae biotechnology can be made more proficient by getting over the disadvantages of traditional systems with advanced attempts that will also take into account some other factors such as high sustainable production potential.

Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

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

References

Aluç, Y., Başaran Kankılıç, G., & Tüzün, İ. (2018). Determination of carotenoids in two algae species from the saline water of Kapulukaya reservoir by HPLC. *Journal of Liquid Chromatography & Related Technologies*, *41*(2), 93-100. https://doi.org/10.1080/10826076.2017.1418376

Babu, B., & Wu, J. T. (2008). Production of natural butylated hydroxytoluene as an antioxidant by freshwater phytoplankton. *Journal of Phycology*, *44*(6), 1447-1454. https://doi.org/10.1111/j.1529-8817.2008.00596.x

Batista, A. P., Gouveia, L., Bandarra, N. M., Franco, J. M., & Raymundo, A. (2013). Comparison of microalgal biomass profiles as novel functional ingredient for food products. *Algal Research*, *2*(2), 164-173. https://doi.org/10.1016/j.algal.2013.01.004

Benedetti, S., Benvenuti, F., Scoglio, S., & Canestrari, F. (2010). Oxygen radical absorbance capacity of phycocyanin and phycocyanobilin from the food supplement Aphanizomenon flos-aquae. *Journal of Medicinal Food*, *13*(1), 223-227. https://doi.org/10.1089/jmf.2008.0257

Bischoff, H. W., & Bold, H. C. (1963). Some soil algae from Enchanted Rock and related algal species. Phycological Studies IV. University of Texas Publication.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199-1200. https://doi.org/10.1038/1811199a0

Canini, A., Albertano, P., Leonardi, D., Di Somma, D., & Grilli Caiola, M. (1996). Superoxide dismutase in cyanobacteria of the Baltic Sea. *Algological Studies/Archiv für Hydrobiologie, Supplement Volumes*, *83*, 129-143. https://doi.org/10.1127/algol_stud/83/1996/129

Chacon-Lee, T. L., & Gonzalez-Marino, G. E. (2010). Microalgae for "healthy" foods-possibilities and challenges. *Comprehensive Reviews in Food Science and Food Safety*, *9*(6), 655-675. https://doi.org/10.1111/j.1541-4337.2010.00132.x

Chatterjee, D., & Bhattacharjee, P. (2014). Supercritical carbon dioxide extraction of antioxidant rich fraction from *Phormidium valderianum*: Optimization of experimental process parameters. *Algal Research*, *3*, 49-54. https://doi.org/10.1016/j.algal.2013.11.014

Chen, B. H., Yang, S. H., & Han, L. H. (1991). Characterization of major carotenoids in water convolvulus (*Ipomoea aquatica*) by open-column, thin-layer and high-performance liquid chromatography. *Journal of Chromatography A*, *543*, 147-155. https://doi.org/10.1016/S0021-9673(01)95763-2

Goiris, K., Muylaert, K., Fraeye, I., Foubert, I., & Cooman, L. D. (2012). Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *Journal of Applied Phycology*, *24*(6), 1477–1486. https://doi.org/10.1007/s10811-012-9804-6

Gong, M., & Bassi, A. (2016). Carotenoids from microalgae: A review of recent developments. *Biotechnology Advances*, *34*(8), 1396-1412. https://doi.org/10.1016/j.biotechadv.2016.10.005

González-López, C., Camacho-Rodríguez, J., López-Rosales, L., García-Camacho, F., & Molina-Grima], E. (2018). Maximizing carotenoid extraction from microalgae used as food additives and determined by liquid chromatography (HPLC). *Food Chemistry*, *15*, 257, 316-324. https://doi.org/10.1016/j.foodchem.2018.02.154

Hancock, J. T., R. Desikan, and S. J. Neill. (2001). Role of reactive oxygen species in cell signalling pathways. *Biochemical Society Transactions*, *29*(2), 345-349. https://doi.org/10.1042/0300-5127:0290345

Ho, S. H., Chan, M. C., Liu, C. C., Chen, C. Y., & Chang, J. S. (2014). Enhancing lutein productivity of an indigenous microalga *Scenedesmus obliquus* FSP-3 using light-related strategies. *Bioresource Technology*, *152*(1), 275-282. https://doi.org/10.1016/j.biortech.2013.11.031

Inbaraj, B. S., Chien, J. T., & Chen, B. H. (2006). Improved high performance liquid chromatographic method for determination of carotenoids in the microalga *Chlorella pyrenoidosa*. *Journal of Chromatography A*, *1102*(1-2), 193-199. https://doi.org/10.1016/j.chroma.2005.10.055

Khansari, N., Shakiba, Y., & Mahmoudi, M. (2009). Chronic inflammation and oxidative stress as a major cause of age- related diseases and cancer. *Recent Patents on Inflammation & Allergy Drug Discovery*, *3*(1), 73–80. https://doi.org/10.2174/187221309787158371

Khotimchenko, S. V., & Yakovleva, I. M. (2004). Effect of solar irradiance on lipids of the green alga *Ulva fenestrata* Postels *et* Ruprecht. *Botanica Marina*, *47*(5), 395-401. https://doi.org/10.1515/bot.2004.050

Leclercq, C., Arcella, D., & Turrini, A. (2000). Estimates of the theoretical maximum daily intake of erythorbic acid, gallates, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in Italy: A stepwise approach. *Food and Chemical Toxicology*, *38*(12), 1075-1084. https://doi.org/10.1016/s0278-6915(00)00106-x

Liu, H. L., Kao, T. H., & Chen, B. H. (2004). Determination of Carotenoids in the Chinese medical herb jiao-gu-lan (*Gynostemma pentaphyllum* MAKINO) by liquid chromatography. *Chromatographia*, *60*(7/8), 411-417. https://doi.org/10.1365/s10337-004-0418-2

Maesaroh, K., Kurnia, D., & Anshori, J. A. (2018). Perbandingan metode uji aktivitas antioksidan DPPH, FRAP dan FIC terhadap asam askorbat, asam galat dan kuersetin. *Chimica et Natura Acta*, *6*(2), 93-100. https://doi.org/https://doi.org/10.24198/cna.v6.n2.19049

Morone, J., Lopes, G., Preto, M., Vasconcelos, V., & Martins, R. (2020). Exploitation of filamentous and picoplanktonic cyanobacteria for cosmetic applications: Potential to improve skin structure and preserve dermal matrix components. *Marine Drugs*, *18*(9), 486. https://doi.org/10.3390/md18090486

Niyogi, K. K., Björkman, O., & Grossman, A. R. (1997). The roles of specific xanthophylls in photoprotection. *Proceedings of the National Academy of Sciences*, *94*(25), 14162-14167. https://doi.org/10.1073/pnas.94.25.14162

Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics*, *44*(6), 307-315. https://doi.org/10.5264/eiyogakuzashi.44.307

Patrick, L. (2001). Beta carotene: The controversy continues. *Alternative Medicine Review: A Journal of Clinical Therapeutic*, *5*(6), 530-545.

Putri, M. D., Arumasi, A., Kurniaty, N. (2020). Review artikel: Uji aktivitas antioksidan ekstrak daging buah semangka dan albedo semangka (*Citrullus lanatus*) dengan metode DPPH dan FRAP. *Prosiding Farmasi*, *6*(2), 992-997. https://doi.org/10.29313/.v6i2.24206

Rao, A. V., & Rao, L. G. (2007). Carotenoids and human health. *Pharmacological Research*, *55*(3), 207–216. https://doi.org/10.1016/j.phrs.2007.01.012

Richmond, A., & Hu, Q. (Eds.) (2013). *Handbook of microalgal culture: Biotechnology and applied phycology*. Wiley-Blackwell Publishing.

Rodrigues, D. B., Menezes, C. R., Mercadante, A. Z., Jacob-Lopes, E., & Zepka, L. Q. (2015). Bioactive pigments from microalgae *Phormidium autumnale*. *Food Research International*, *77*, 273-279. https://doi.org/10.1016/j.foodres.2015.04.027

Rodríguez-Meizoso, I., Jaime, L., Santoyo, S., Cifuentes, A., Reina, G. B., Señoráns, F., & Ibáñez, E. (2008). Pressurized fluid extraction of bioactive compounds from *Phormidium* species. *Journal of Agricultural and Food Chemistry*, *56*(10), 3517-3523. https://doi.org/10.1021/jf703719p

Shanab, S. M. M., Mostafa, S. S. M., Shalaby, E. A., & Mahmoud, G. I. (2012). Aqueous extracts of microalgae exhibit antioxidant and anticancer activities. *Asian Pacific Journal of Tropical Biomedicine*, *2*(8), 608-615. https://doi.org/10.1016/s2221-1691(12)60106-3

Soni, B., Trivedi, U. B., & Madamwar, D. (2008). A novel method of single step hydrophobic interaction chromatography for the purification of phycocyanin from *Phormidium* fragile and its characterization for antioxidant property. *Bioresource Technology*, *99*(1), 188-194. https://doi.org/10.1016/j.biortech.2006.11.010

Sri Mariani, N. R. d. S. (2018). Antioxidant activity test of watermelon (*Citrullus lanatus*) fruit extracts. *Jurnal Akademika Kimia*, *7*(2), 96-101.

Stange, C. (Ed.) (2016). *Carotenoids in Nature: Biosynthesis, Regulation and Function*. Springer.

Tahir, M., Heluth, A. C., & Widiastuti, H. (2018). Uji aktivitas antioksidan ekstrak buah semangka (*Citrullus lanatus*) dengan metode FRAP. *As-Syifaa Jurnal Farmasi*, *8*(1), 31-38. https://doi.org/10.33096/jifa.v8i1.155

Wojtasiewicz, B., & Ston-Egiert, J. (2016). Bio-optical characterization of selected cyanobacteria strains present in marine and freshwater ecosystems. *Journal of Applied Phycology*, *28*, 2299-2314. https://doi.org/10.1007/s10811-015-0774-3

Zuluaga, M., Gueguen, V., Pavon-Djavid, G., & Letourneur, D. (2017). Carotenoids from microalgae to block oxidative stress. *Bioimpacts*, 7(1), 1–3. https://doi.org/10.15171%2Fbi.2017.01

Mar. Sci. Tech. Bull. (2022) 11(2): 221-230
e–ISSN: 2147–9666
info@masteb.com
dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1091600

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Determination of shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets cooked at different combinations of temperatures using the sous vide technique

Pakize Çalış Erümit[1] • Pınar Oğuzhan Yıldız[1*]

[1] *Atatürk University, Faculty of Fisheries, Department of Seafood Processing Technology, 25240, Erzurum, Turkey*

ARTICLE INFO

Article History:
Received: 22.03.2022
Received in revised form: 30.05.2022
Accepted: 01.06.2022
Available online: 22.06.2022

Keywords:
Rainbow trout
Sous vide
Shelf life
Temperature
Cooking

ABSTRACT

This study aimed to determine the shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets, which were applied sous vide technique at different temperatures (55, 65 and 70°C) and stored at 4±1°C for 18 days. The fillets were subjected to chemical (total volatile basic nitrogen, thiobarbituric acid reacting substances and pH), microbiological (total aerobic mesophilic, psychrophilic bacteria and yeast-mold) and sensory analyzes (appearance, texture, odor and color). The results showed that the total number of mesophilic aerobic and psychrophilic bacteria did not exceed the limit values during the storage period in the groups treated with sous vide. In addition, temperature and storage time had a statistically significant ($p<0.01$) effect on all bacterial groups. The control group samples had higher TVB-N, TBARS and pH values during the storage period compared to the samples with the sous vide technique. When the sensory analysis results were examined, it was found that the most liked group among all groups, except for the odor parameter, was the group that was cooked at 55°C during storage time. As a result of the analysis, it was determined that the sous vide technique applied to the fish had a positive effect on the shelf life, and the most suitable cooking temperature and time were 15 minutes at 70°C.

Please cite this paper as follows:

Çalış Erümit, P., & Oğuzhan Yıldız, P. (2022). Determination of shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets cooked at different combinations of temperatures using the sous vide technique. *Marine Science and Technology Bulletin*, *11*(2), 221-230. https://doi.org/10.33714/masteb.1091600

* Corresponding author
E-mail address: pinaroguzhan@atauni.edu.tr (P. Oğuzhan Yıldız)

Introduction

Seafood is a foodstuff that contains very high nutritional value and contains both fat and water-soluble vitamins and essential amino acids, which are rich in omega-3 fatty acids (EPA and DHA), and have an important role in human health (Valenzuela & Valenzuela, 2013; Ceylan & Ünal Şengör, 2017; Korkmaz et al., 2020).

Despite its high nutritional value, seafood is one of the foodstuffs that tend to deteriorate quickly due to the loose connective tissue and the polyunsaturated fatty acids they contain. Therefore, if it is not to be consumed immediately, seafood should be preserved using appropriate processing techniques following harvesting (Korkmaz et al., 2020). Many preservation methods have been applied throughout history in order to maintain the freshness of seafood, increase product variety to extend the consumption period. The main purpose here is to extend the shelf life of the fish, as well as to supply the fish for consumption outside the hunting and consumption season. For this purpose, many methods such as drying, salting, smoking, cooling, freezing, surimi, canning, and marinating technology are used to preserve fish. In addition to these, alternative methods have also been found to be applicable in the processing of fish (Anonymous, 2021).

The sous vide technique was firstly developed in France and it means "under vacuum". It is the process of cooking a product by vacuum packaging it in heat-resistant bags and pasteurizing it in a water bath under controlled temperature and time. Sous vide technology emerges as a preservation technique used to increase the shelf life of the product and to obtain products that have not lost their nutritional components (Aksoy & Mete, 2017; Yıldız & Yılmaz, 2020; İlyasoğlu, 2021).

In this technique, aerobic microorganism growth and oxidation is able to be controlled by vacuum packaging of the product by absorbing the oxygen in the package. It is aimed to obtain a product with a long shelf life by applying vacuum packaging and heat treatment in tandem to the product. In this aspect, it is also preferred commonly by schools, hospitals, factories, hotels, etc. that provide mass catering (Mol & Özturan, 2009; Baltalı & Akoğlan Kozak, 2021). This technique has advantages in terms of applying to products such as meat, chicken and fish that are prone to spoilage, being made ready for consumption easily, being suitable for flavoring along with other products, maintaining microbiological development under control with vacuum packaging, and resulting in products with a long shelf life. In addition, it is reported that it preserves products' original taste for a long time, improves their sensory properties positively, and provides healthy and nutritious cooking, which is more beneficial than traditional cooking (Creed & Reeve, 1998; Baldwin, 2012; Ceylan & Ünal Şengör, 2017; Yıldız & Yılmaz, 2020).

The application of the sous-vide technique to seafood is a cooking method that is not widely known in Turkey, but has become increasingly important in the world in recent years. This study investigated increasing the shelf life by preserving the quality of the product. It is a different option for the developing food industry in our country, and in this context, a contribution will be made to the food industry. In addition, the effect of this technique on the nutritional quality of rainbow trout fillets will be determined and the sensory properties will be improved compared to other classical methods. This study aimed to determine the changes in the quality and shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets, which were kept cold and applied sous vide technique at different combinations of temperatures.

Material and Methods

Material

Rainbow trout fillets (168 pieces) an average of 250±25 g weight, were obtained from Atatürk University Fisheries Faculty and brought to our faculty's processing laboratory in accordance with the cold supply chain conditions. Trouts were thoroughly washed with water, then the head, viscera, and skin were removed and the skinless fillets were obtained and vacuum packaging was performed.

Method

Sous-Vide

For the sous vide process, the trout fillets were heat-treated at different temperatures (55, 65 and 70°C) for 15 minutes. The cooking process was applied in a water bath (double boiler) until cooking was observed in both parts of the fillets. The time and temperature of the application have been determined by preliminary studies. The control group that did not any heat treatment. After the cooking process was completed, the bags were immersed in a bucket containing 1/3 water and 2/3 ice and kept for 30 minutes and cooled rapidly. Then, they were stored at 4±1°C for 18 days, and the samples were subjected to chemical, sensory and microbiological analyzes on certain days (0, 3, 6, 9, 12, 15 and 18 days) of storage.

Microbiological Analysis

For microbiological analysis, 25 g of fish samples were taken into a sterile stomacher bag and 225 ml of sterile saline solution was added and homogenized in a stomacher device (Lab Stomacher Blender 400-BA 7021 Seward Medical, England). Plate Count Agar was used for the analysis of total aerobic mesophilic and psychrophilic bacteria and the media were incubated for 2 days at 30°C and 10 days at 4°C, respectively. The Potato Dextrose Agar medium was used for yeast-mold enumeration and incubated at 25°C for 5 days (Gökalp et al., 2001).

Chemical Analysis

100 ml of distilled water was added to 10 g of the sample and the pH value was determined after homogenizing for 1 minute (Gökalp et al., 2001).

The TBARS value was made according to the method used by Lemon (1975) and Kılıç & Richards (2003). 7.5% trichloroacetic acid (TCA) was added to the 2 g sample and filtered through filter paper after homogenization. TBA reagent was added to the filtrate and kept in a water bath at 100°C for approximately 40 minutes. Then, it was taken from the water bath and allowed to cool, and a reading was made in the spectrophotometer (530 nm). TBARS value was calculated according to the following equation (1):

$$TBARS = \left(\left({}^{Abs}/_{k(0.006)}\right) \times {}^{2}/_{1000 \times 6.8}\right) \times \frac{1000}{sample\ weight} \qquad (1)$$

The method proposed by Malle & Tao (1987) was used to determine the TVB-N value. 7.5% (v/v) trichloroacetic acid (TCA) was added to 40 grams of sample, homogenized, centrifuged and filtered through filter paper. 10% NaOH (w/v) was added to the obtained filtrate and placed in the distillation device until the final volume was approximately 50 ml. The obtained distillate was titrated with 0.1 N H_2SO_4 solution and the TVB-N value was calculated using equation (2):

$$TVB - N\left(\frac{mg}{100g}\right) = n \times 16.8\ mg\ nitrogen \qquad (2)$$

Sensory Analysis

The sensory analysis of fish samples was carried out by a panelist group of 10 using a hedonic type scale consisting of appearance, texture, odor and color parameters. The panelists evaluated the samples over 5 points and scored as 1: very bad, 2: bad, 3: normal, 4: good, and 5: very good (Huss, 1995).

Statistical Analysis

This study was carried out according to an entirely random trial plan consisting of control groups of 3 different temperatures (55, 65, and 70°C) and 3 replications. The results obtained were subjected to statistical analysis and the Duncan multiple comparison test was applied to averages that were found to be significant.

Results and Discussion

Microbiological Results

The microbiological analysis results of rainbow trout fillets applied sous vide technique at different temperatures (55, 65, and 70°C) during cold storage (4±1°C) are given in Table 1.

The acceptable limit for total aerobic mesophilic bacteria in fresh fish was reported as 6 log cfu/g by Anonymous (2022). The total number of mesophilic aerobic was found to be lower in the samples with the sous vide technique compared to the control group samples. Total aerobic mesophilic bacteria count of rainbow trout fillets ranged from 2.00-10.53 log cfu/g. The effect of the storage time on the bacterial counts was significant and as the time increased, a significant increase was detected in the bacterial counts in all groups ($p<0.01$). The highest total number of aerobic mesophilic bacteria was observed in the control group with a value of 7.15±2.43 log cfu/g, while the lowest was found in rainbow trout fillets cooked with the sous vide technique at 70°C with a value of 2.33±0.48 log cfu/g. It was observed that the applied heat treatment and vacuum packaging had a positive effect on slowing the bacterial growth of the samples. In particular, it was observed that bacterial growth slowed down significantly as the temperature increased. In the study conducted by Şişmanlar Altıkaya (2016), it was reported that the number of mesophilic aerobic bacteria in total in zander fish (*Sander lucioperca* Linnaeus, 1758) was 6.49, 5.24, and 1.60 log cfu/g on the 42^{nd} day of storage in the samples which had sous vide method applied at 60°C, 70°C, and 80°C, respectively. Nyati (2000) reported that the total number of mesophilic bacteria reached the level of 5 log cfu/g during 5 weeks of storage at 3°C in fish fillets that were treated with sous vide technique for 2 minutes at 70°C. Mol et al. (2012) reported that the total number of aerobic mesophilic bacteria decreased with the sous vide cooking method in a study conducted with whiting (*Merlangius merlangus euxınusi* Nordman, 1840) prepared with the sous vide method. According to Coşansu et al. (2011), the number of mesophilic aerobic bacteria in bonito fish (*Sarda sarda*, Bloch, 1793) with lemon juice and kept at

4±1°C by applying the sous vide technique for 10 minutes at 70°C remained at the acceptable limit values (6 log cfu/g) until the 35th day. Diaz et al. (2011) reported yeast-mold growth in their study with salmon (*Salmo salar*) fillets. Jeya Shakila et al. (2012) reported that the total number of mesophilic bacteria reached the level of 3 log cfu/g after 6 weeks of storage in cobia fish cakes in which 20 minutes of sous vide was applied at 95°C. These results were consistent with the findings of our study.

Psychrotrophic bacteria count of rainbow trout fillets ranged from 2.00-10.83 log cfu/g. Psychrotrophic bacteria was found to be lower in the samples with the sous vide technique compared to the control group samples. The highest number of psychrotrophic bacteria was observed in the control group with a value of 7.46±2.51 log cfu/g, while the lowest was found in samples cooked with the sous vide technique at 70°C with a value of 2.42±0.58 log cfu/g. As the degree of heat treatment applied increased, the number of psychrotrophic bacteria decreased. González-Fandos et al. (2005) found that the groups treated with sous vide at 90°C for 5 minutes (2°C storage) and at 65°C for 10 minutes (2-10°C storage) gave better results. The psychrophilic aerobic bacterial load of raw bonito and whiting was 2.72±0.03 log cfu/g, and 2.61±0.06 log cfu/g, respectively, while it was >1.00±0 log cfu/g in both sous vide-treated fish samples (Özturan, 2009). Garcia-Linares et al. (2004) found that psychrophilic bacteria levels in both fish species were 4-5 log cfu/g in their study with trout and salmon. Kato et al. (2017) emphasized that the numbers of psychrophilic bacteria remained within the limits recommended by the Brazilian legislation during storage, in a study conducted with tambaqui (*Colossoma macropomum*) in which sous vide treatment was applied at 65°C for 12.5 minutes. Bozova & İzci (2021) determined significant increases in total psychrophilic aerobic bacteria counts during storage in meagre (*Argyrosomus regius*) treated with rosemary and thyme extracts and subjected to sous vide treatment. The data obtained in this study are in agreement with the study data reported by the researchers.

Table 1. Microbiological analysis results of rainbow trout fillets applied sous vide technique at different temperatures (log cfu/g)

Microbiological Analysis	Storage time (days)	Groups			
		Control	55°C	65°C	70°C
Total aerobic mesophilic bacteria	0	3.06±0.06[a]	2.00±0.00[a]	2.00±0.00[a]	2.00±0.00[a]
	3	5.16±0.23[a]	2.00±0.00[a]	2.00±0.00[a]	2.00±0.00[a]
	6	6.42±0.30[a]	2.27±0.10[b]	2.08±0.11[b]	2.00±0.00[a]
	9	7.30±0.16[a]	3.04±0.09[c]	2.58±0.12[c]	2.00±0.00[a]
	12	8.03±0.11[a]	3.69±0.25[d]	3.07±0.08[d]	2.20±0.14[b]
	15	9.54±0.29[a]	4.07±0.10[e]	3.26±0.07[e]	2.92±0.13[c]
	18	10.53±0.21[a]	4.93±0.10[f]	3.54±0.12[e]	3.16±0.10[a]
Psychrophilic bacteria	0	3.20±0.05[a]	2.00±0.00[a]	2.00±0.00[a]	2.00±0.00[a]
	3	5.38±0.21[b]	2.00±0.00[a]	2.00±0.00[a]	2.00±0.00[a]
	6	6.69±0.27[c]	2.61±0.14[b]	2.25±0.06[a]	2.00±0.00[a]
	9	7.66±0.09[d]	3.41±0.24[c]	2.60±0.16[a]	2.00±0.00[a]
	12	8.37±0.13[e]	3.94±0.09[d]	3.23±0.22[a]	2.37±0.19[b]
	15	10.07±0.14[f]	4.19±0.17[e]	4.04±0.07[a]	3.26±0.07[c]
	18	10.83±0.15[g]	5.29±0.11[f]	3.91±0.14[a]	3.32±0.11[c]
Yeast-mold	0	2.00±0.00[a]	2.00±0.00[a]	2.00±0.00[a]	2.00±0.00[a]
	3	2.29±0.14[b]	2.00±0.00[a]	2.00±0.00[a]	2.00±0.00[a]
	6	3.11±0.11[c]	2.00±0.00[a]	2.00±0.00[a]	2.00±0.00[a]
	9	4.27±0.16[d]	2.13±0.08[a]	2.00±0.00[a]	2.00±0.00[a]
	12	5.35±0.28[e]	2.54±0.09[b]	2.34±0.20[a]	2.00±0.00[a]
	15	7.02±0.09[f]	2.93±0.13[c]	2.74±0.31[b]	2.30±0.14[b]
	18	6.61±0.18[g]	3.82±0.13[d]	2.69±0.34[b]	2.52±0.27[c]

Note: Means shown with different letters are statistically different from each other ($p<0.05$).

Table 2. Chemical analysis results of rainbow trout fillets applied sous vide technique at different temperatures

Chemical analysis	Storage time (days)	Groups			
		Control	55°C	65°C	70°C
TVB-N	0	14.95±0.07[a]	12.61±0.33[a]	11.65±0.20[a]	10.88±0,19[a]
	3	15.24±0.21[a]	13.25±0.12[b]	12.12±0.18[b]	11.37±0,16[b]
	6	16.29±0.12[b]	14.21±0.26[c]	12.81±0.24[c]	11.04±0.06[ab]
	9	17.57±0.12[c]	15.48±0.37[d]	14.47±0.31[d]	13.71±0.52[c]
	12	18.62±0.28[d]	16.95±0.06[e]	15.63±0.14[e]	15.11±0.12[d]
	15	21.92±0.14[e]	20.21±0.33[f]	17.13±0.10[f]	17.04±0.04[e]
	18	24.27±0.29[f]	21.17±0.17[g]	20.07±0.11[g]	19.48±0.24[f]
TBARS	0	2.55±0.13[a]	1.72±0.14[a]	1.13±0.09[a]	0.87±0,12[a]
	3	3.48±0.15[b]	2.26±0.14[b]	0.96±0.09[a]	0.54±0.12[b]
	6	4.66±0.23[c]	2.84±0.08[c]	1.81±0.14[b]	1.13±0.14[c]
	9	5.54±0.23[d]	3.24±0.09[d]	2.25±0.15[c]	1.61±0.17[d]
	12	6.83±0.12[e]	4.23±0.22[e]	2.92±0.14[d]	2.60±0.16[e]
	15	7.25±0.18[f]	5.05±0.15[f]	3.36±0.26[e]	3.12±0.10[f]
	18	7.57±0.17[g]	5.57±0.14[g]	4.47±0.09[f]	4.05±0.14[g]
pH	0	6.35±0.19[ab]	6,53±0,00[b]	6,46±0,02[c]	6.45±0.00[c]
	3	6.10±0.06[b]	6,76±0,09[a]	6,73±0,03[ab]	6.44±0.00[c]
	6	6.15±0.06[ab]	6,38±0,05[a]	6,22±0,10[b]	6.69±0.00[c]
	9	6.28±0.09[abc]	6.15±0.06[c]	6.15±0.06[a]	6.38±0.08[bc]
	12	6.49±0.14[c]	6.15±0.06[b]	6.15±0.06[a]	6.15±0.08[a]
	15	6.30±0.09[abc]	6.15±0.06[b]	6.15±0.06[c]	6.29±0.06[b]
	18	6.20±0.04[ab]	6.15±0.06[b]	6.15±0.06[ab]	6.46±0.04[d]

Note: Means shown with different letters are statistically different from each other ($p<0.05$)

While the number of yeast-mold was determined as 2.00 log cfu/g in all groups, it increased until the end of storage. The highest yeast-mold count was found in the control group with a value of 4.38±1.92 log cfu/g, while the lowest was observed in the samples cooked at 70°C, with a value of 2.11±0.22 log cfu/g. Gürel İnanlı & Yaz (2020), determined the number of yeast-mold in *Luciobarbus esocinus* (Heckel, 1843) as 3.93±0.29 log cfu/g in the control group, 4.09±0.08 log cfu/g in the group cooked at 43°C for 20 minutes and 81±0.47 log cfu/g in the group cooked at 56°C for 10 minutes. Diaz et al. (2011) reported yeast-mold growth in their study with salmon (*Salmo salar*) fillets. In the study conducted by Pongsetkul & Benjakul (2021) with dried sour-salt fermented torpedo scad fish, yeast-mold was not detected in any of the samples. It has been determined that storage time and application processes have a significant effect on yeast and mold numbers.

Chemical Results

The chemical analysis results of rainbow trout fillets applied sous vide technique at different temperatures (55, 65 and 70°C) during cold storage (4±1°C) are given in Table 2.

The TBARS value, which was 2.55 μ mol MA/kg at the beginning of storage (day 0) in the control group samples, was determined as 1.73, 1.13 and 0.88 μ mol MA/kg in the samples applied with the sous vide technique at different temperatures (55, 65 and 70°C), respectively. It was determined that the increase in cooking temperatures of the samples decreased the TBARS value. In our study, it was observed that the TBARS value determined in the heat-treated samples was in parallel with the data in the literature. It increased in parallel with the storage period and at the end of the storage period as well, with a value of 7.57 µ mol MA/kg being obtained from the control group, 5.57 µ mol MA/kg from the 55°C group, 4.47 µ mol MA/kg from the 65°C group and 4.05 µ mol MA/kg from the 70°C group. According to the TBARS value results obtained during storage, it was observed that the fillets in the control group were close to the consumption limit value from the 15th day, but did not exceed the consumption limit value of 8 µmol MA/kg. It was determined that the results, which did not exceed

Table 3. Sensory analysis results of rainbow trout fillets applied sous vide technique at different temperatures

Microbiological Analysis	Storage time (days)	Groups			
		Control	55°C	65°C	70°C
	0	4.67±0.57^{c}	5.00±0.00^{e}	3.67±0.57^{cd}	4.00±0.00^{cd}
	3	3.33±0.57^{b}	4.33±0.57^{cd}	4.33±0.57^{e}	4.33±0.57^{d}
	6	2.66±0.57^{b}	4.00±1.00^{cd}	3.67±0.57^{cd}	3.66±0.57^{abc}
Appearance	9	2.66±0.57^{b}	3.33±0.57b^{c}	3.33±0.57^{abc}	3.00±0.00^{ab}
	12	1.66±0.57^{a}	2.66±0.57^{ab}	3.00±0.00^{ab}	2.66±0.57^{a}
	15	1.33±0.57^{a}	2.66±0.57^{ab}	2.66±0.57^{ab}	3.00±1.00^{ab}
	18	1.00±0.00^{a}	1.66±0.57^{a}	2.33±0.57^{a}	2.66±0.57^{a}
	0	4.66±0.57^{e}	5.00±0.00^{e}	3.66±0.57^{bc}	3.66±0.57^{ab}
	3	3.33±0.57^{d}	4.33±0.57^{cd}	4.33±0.57^{bc}	4.33±0.57^{b}
	6	2.33±0.57^{bc}	4.00±1.00^{cd}	3.66±0.57^{e}	3.66±0.57^{ab}
Texture	9	2.66±0.57^{cd}	3.33±0.57^{bc}	3.33±0.57^{abc}	3.00±0.00^{a}
	12	1.66±0.57^{ab}	2.66±0.57^{ab}	3.00±0.00^{ab}	2.66±0.57^{a}
	15	1.00±0.00^{a}	2.66±0.57^{ab}	2.66±0.57^{ab}	3.00±1.00^{a}
	18	1.00±0.00^{a}	1.66±0.57^{a}	2.33±0.57^{a}	2.66±0.57^{a}
	0	4.67±0.57^{e}	4.67±0.57^{e}	3.66±0.57^{d}	4.00±0.00^{cd}
	3	3.33±0.57^{d}	4.33±0.57^{cd}	4.33±0.57^{d}	4.33±0.57^{d}
	6	2.00±0.00^{c}	3.66±0,57^{bcd}	3.00±0.00^{abc}	3.66±0.57^{abc}
Odor	9	2.00±0.00^{c}	3.33±0.57^{bc}	3.33±0.57b^{c}	3.00±0.00^{ab}
	12	1.66±0.57^{ab}	2.66±0.57^{ab}	3.00±0.00^{abc}	2.66±0.57^{a}
	15	1.33±0.57^{ab}	2.66±0.57^{ab}	2.66±0.57^{ab}	3.00±1.00^{ab}
	18	1.00±0.00^{a}	1.66±0.57^{a}	2.33±0.57^{a}	2.66±0.57^{a}
	0	4.67±0.57^{e}	4.67±0.57^{e}	3.33±0.57^{b}	3.33±.1.15^{ab}
	3	3.33±0.57^{d}	4.33±0.57^{cd}	4.33±0.57^{c}	4.33±0.57^{c}
	6	6,42±0,02^{c}	3.66±0.57^{bcd}	3.00±0.00^{ab}	3.66±0.57^{ab}
Color	9	2.00±0.00^{c}	3.33±0.57^{bc}	3.33±0.57^{b}	3.00±0.00^{ab}
	12	2.00±0.00^{ab}	2.66±0.57^{ab}	3.00±0.00^{ab}	2.66±0.57^{a}
	15	1.66±0.57^{ab}	2.66±0.57^{ab}	2.66±0.57^{ab}	3.00±1.00^{ab}
	18	1.33±0.57^{a}	1.66±0.57^{a}	2.33±0.57^{a}	2.66±0.57^{a}

Note: Means shown with different letters are statistically different from each other ($p<0.05$)

the limit value during storage in the heat-treated groups, remained within consumable values. The highest mean (5.42±1.84 mol MA/kg) was observed in the control group, while the lowest average (1.99±1.24 mol MA/kg) was found in rainbow trout fillets that were sous-vide cooked at 70°C. However, no significant difference was observed between the fillets cooked at all three temperatures (55, 65 and 70 °C) and the values were different from each other. Jeya Shakila et al. (2012) observed that the TBARS values were well below the limit values after 12 weeks of storage in cobia fish cakes in which sous vide was applied for 20 minutes at 95°C. According to Diaz et al. (2011), TBARS value was observed as 1.10±0.39 mg MDA/kg at week 0, 2.06 mg MDA/kg at week 5, and 2.30 mg MDA/kg at week 10 in salmon fillets (*Salmo salar*) treated with sous vide technology. TBARS value showed differences in the studies according to the application temperature and storage time.

It increased in parallel with the storage period and at the end of the storage period as well, with a value of 14.95 mg/100 g being obtained from the control group, 12.61 mg/100 g from the 55°C group, 11.65 mg/100 g from the 65°C group and 10.89 mg/100 from the 70°C group. According to the TVB-N value results obtained during storage, none of the sous vide applied groups, including the control group, exceeded the TVB-N limit value. The highest TVB-N value results were observed in the control group (18.41±3.33 mg/100 g), while the lowest was found in trout fillets that were cooked sous vide (14.09±3.15 mg/100 g) at 70°C. No significant difference was observed between the fillets cooked at all three temperatures and the values were different from each other. Ramos et al. (2016) determined that the TVB-N value in tambaqui (*Colossoma macropomum*) fish treated with spices and cooked in sous vide was lower than in plain fish fillets treated with sous vide, with the TVB-N value being 11.17 mg/100 g in the control group samples and 8.38 mg/100g in the spice group. It was found that the highest TVB-N value was observed as 30.80 mg N/100 g in sea bass (*Dicentrarchus labrax*) treated with sous vide technology by adding laurel at the end of the storage period (Bolat et al., 2019). In another study conducted by Ceylan & Ünal Şengör (2019) on sea bass fillets (*Dicentrarchus labrax*, Linnaeus, 1758), the TVB-N value in the first week of storage was 21.2 mg/100 g in the control group, 20.79 mg/100 g in the group with dried basil, and garlic added, and 20.52 mg/100g in the group with dried basil, garlic, and dill added. In another study conducted with rainbow trout, the TVB-N value was 15.80±0.69 mg/100g on the 40th day of storage in the sous vide group, and it was 15.13±0.00 mg/100g in the sous vide group treated with ground sage. (Çetinkaya, 2020).

At the beginning of storage, the lowest pH value was found in the control group (6.35), and the highest was found in the samples of the sous vide technique with the highest temperature (70°C) (7.00). On the 18th day of storage, the lowest pH value was found in the control group (6.21) samples, and the highest pH value was found in the samples which were cooked at 70°C using the sous vide method (6.47). Wan et al. (2019) reported that largemouth bass (*Micropterus salmoides*) cooked with the sous vide group showed an increased pH tendency when compared to the control group without any cooking process. The results of the study were consistent with the findings we obtained. del Pulgar et al. (2012) emphasized that the pH increase of fish during cooking was attributed to the formation of disulfide bonds during the cooking process. Seyyar (2015) reported that the pH value of untreated trout was 6.56 in rainbow trout fillets cooked with the sous vide cooking method, while the samples cooked with the sous vide method at different temperatures and cooking levels varied between 6.63-7.00. Ramos et al. (2016) reported that the pH value of tambaqui fish cooked with the sous vide method, which was 5.52±0.51, was significantly lower (6.34±0.04) compared to raw fish.

Sensory Results

The sensory analysis results of rainbow trout fillets applied sous vide technique at different temperatures (55, 65, and 70°C) during cold storage (4±1°C) are given in Table 3.

According to the results of the sensory analysis, a decrease was observed in all sample groups in parallel with the storage and the difference between the groups was found to be statistically significant ($p<0.05$). In terms of sensory parameters, the most liked group, excluding smell, was the group in which the sous vide technique was applied at 55°C. In general, it was determined that the groups in which sous vide cooking was applied were preferred more in terms of all parameters. It has been emphasized that the applied heat treatment time, temperature and storage conditions are determinative in improving the shelf life of the product sensorially (Gonzalez-Fandos et al., 2004). Prevention of aroma loss in sous vide technique a better sensory quality can be achieved with components that give flavor and smell to the fish. It has been reported that sous vide samples treated with rosemary and thyme extracts were more appreciated by the panelists in terms of all sensory parameters than the samples applied without any (Bozova, 2020). Şişmanlar Altıkaya (2016) reported that the group in which the sous vide technique was applied at 80°C got the highest score in terms of sensory parameters for pike perch fillets. Bolat et al. (2019) determined that there was a decrease in parallel with storage in all sample groups of sea bass fish (*Dicentrarchus labrax*) that they applied sous vide cooking by adding ground laurel (LS) and ginger (CS). It was determined that the sensory analysis results reported by the researchers were consistent with the data obtained in our study. Pongsetkul & Benjakul (2022) reported that the group in which 30 minutes of sous vide cooking at 40°C got the highest approval in terms of sensory parameters. The difference in applied temperature and time causes differences between studies.

Conclusion

It was concluded that the sous vide technique slowed the growth of microorganisms in rainbow trout fillets, TVB-N and TBARS values were lower in the sous vide applied groups compared to the control group, and the pH value was highest in the sous vide cooking method at 70°C, and in the fillet group at 3 different temperatures. It has been determined that the sous vide cooking technique extends the storage time. In terms of sensory parameters, it was determined that the groups that applied sous vide cooking were more appreciated. In the light of all these analyses, it was determined that high-temperature application was effective on chemical and microbiological properties. It has been concluded that the vacuum packaged cooking method can be applied to rainbow trout, and we believe that it is also important to evaluate fish meat in this way, both to extend the shelf life and to provide an economic contribution by presenting it to the consumer as an alternative to the consumption of ready-made food.

Acknowledgements

This work was supported by the Ataturk University, Unit of Research Projects under Grant number (FYL-2021-9096).

Compliance With Ethical Standards

Authors' Contributions

This work was produced from the master thesis prepared by the first author under the supervision of the second author. POY designed the study. Both authors read and approved the final version of the article.

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.

References

Aksoy, M., & Mete, E. (2017). Sous vide yöntemiyle pişirilen dana bonfilenin dokusal analizi. The *Journal of Social Science*, *4*(13), 521-530.

Anonymous (2022). Gıda maddeleri için mikrobiyolojik kriterler. Retrieved on May 06, 2022, from https://www.tarimorman.gov.tr/GKGM/Belgeler/Veteriner%20Hizmetleri/hayvanSinirKontrol/SuudiArabistan_Mevzuat%C4%B1/Gida_Maddeleri_icin_Mikrobiyolojik_Kriterler.pdf

Anonymous. (2021). Su ürünleri muhafaza yöntemleri. Retrieved on June 11, 2021, from https://docplayer.biz.tr/16074013-Su-urunleri-muhafaza-yontemleri-doc-dr-abdullah-oksuz-doc-dr-senol-guzel.html.

Baldwin, D. E. (2012). Sous vide cooking: A review. *International Journal of Gastronomy Food Science, 1*, 15-30. https://doi.org/10.1016/j.ijgfs.2011.11.002

Baltalı, B., & Akoğlan Kozak, M. (2021). Sous-Vide Tekniğinin Pişirme Süreci Kapsamında Değerlendirilmesi. *Aydın Gastronomy, 5*(1), 13-33. https://doi.org/10.17932/IAU.GASTRONOMY.2017.016/gastronomy_v05i1002

Bolat, Y., Genç, İ. Y., Tunca, Y., & Demirayak, M. (2019). Effect of laurel (*Laurus nobilis)* and curcuma (*Curcuma longa*) on microbiological, chemical and sensory changes in vacuum packed sous-vide European sea bass (*Dicentrarchus labrax*) under chilled conditions. *Food Science and Technology Campinas, 39*(1), 159-165. https://doi.org/10.1590/fst.41217

Bozova, B. (2020). *Bitki özütlerinin sous vide uygulanmasında sarıağız balığı (Argyrosomus regius) filetolarının kalite özelliklerine etkisi* [Effects of plant extracts on the quality of sous vide meagre (*Argyosomus regius*) fillets]. [Ph.D. Thesis. Isparta Uygulamalı Bilimler University].

Bozova, B., & İzci, L. (2021). Effects of plant extracts on the quality of sous vide meagre (*Argyrosomus regius*) fillets. *Acta Aquatica Turcica*, *17*(2), 255-266. https://doi.org/10.22392/actaquatr.798584

Çetinkaya, S. (2020). The effects of sous-vide cooking method on rainbow trout by adding natural antioxidant effective sage: basic quality criteria. *Natural and Engineering Sciences,* *5*(3), 167-183. https://doi.org/10.28978/nesciences.832987

Ceylan, Z., & Ünal Şengör, G. F. (2017). Sous vide teknolojisi ile muamele edilen balıkların kalite parametrelerinin incelenmesi. *Turkish Journal of Aquatic Sciences*, *32*(1), 8-20. https://doi.org/10.18864/TJAS201702

Coşansu, S., Mol, S., Alakavuk, D. U., & Özturan, S. (2011). The effect of lemon juice on bonito (*Sarda sarda*, Bloch,1793) preserved by sous vide packaging. *International Journal of Food Science and Technology, 46*, 395-401. https://doi.org/10.1111/j.1365-2621.2010.02507.x

Creed, P. G., & Reeve, W. (1998). Principles and applications of sous vide processed foods. In S. Ghazala (Ed.), *Sous Vide and Cook Chill Processing for the Food Industry* (pp. 25-56). Aspen Publishers, Inc.

del Pulgar, J. S., Gázquez, A., & Ruiz-Carrascal, J. (2012). Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. *Meat Science, 90*(3), 828–835. https://doi.org/10.1016/j.meatsci.2011.11.024

Diaz, P., Nieto, G., Banon, S., & Garrido, M. D. (2009). Determination of shelf life of sous vide salmon (*Salmo salar*) based on sensory attributes. *Journal of Food Science, 74,* 371-376. https://doi.org/10.1111/j.1750-3841.2009.01317.x

Garcia-Linares, M. C., Gonzalez-Fandos, E., Garcia-Arias, M. T., & Garcia-Fernandez, M. C. (2004). Microbiological and nutritional quality of sous vide or traditionally processed fish: Influence of fat content. *Journal of Food Quality*, *27*(5), 371-387. https://doi.org/10.1111/j.1745-4557.2004.00676.x

Gökalp, H. Y., Kaya, M., Zorba, O., & Tülek, Y. (2001). *Et ve ürünlerinde kalite kontrolü ve laboratuvar uygulama kılavuzu*. Erzurum: Atatürk Üniversitesi Ziraat Fakültesi Yayını 268 p.

González-Fandos, E., García-Linares, M. C., Villarino-Rodríguez, A., García-Arías, M. T., & García-Fernández, M. C. (2004). Evaluation of the microbial safety and sensory quality of rainbow trout (*Oncorhynchus mykiss*) processed by the sous vide method. *Food Microbiology, 21,* 193-201. https://doi.org/10.1016/S0740-0020(03)00053-4

González-Fandos, E., Villarino-Rodriguez, A., Garcia-Linares, M. C., Garcia-Arias, M. T., Garcia-Fernandez, M. C. (2005). Microbiological safety and sensory characteristics of salmon slices processed by the sous vide method. *Food Control, 16,* 77-85. https://doi.org/10.1016/j.foodcont.2003.11.011

Gürel İnanlı, A., & Yaz, Y. (2020). Assessment of chemical, microbiological and sensory quality of Sous vide cooked *Luciobarbus esocinus* (Heckel, 1843) during chilled storage. *Progress in Nutrition*, *22*(2), 617-625. https://doi.org/10.23751/pn.v22i2.9316

Huss, H. H. (1995). Quality and Quality Changes in Fresh Fish. FAO Fisheries Technical Paper- 348, Rome, Italy.

İlyasoğlu, İ. (2021). Moleküler gastronomi uygulamaları: Sous vide yöntemi. *Aydın Gastronomy, 5*(2), 157-166. https://doi.org/10.17932/IAU.GASTRONOMY.2017.016/gastronomy_v05i2006

Jeya Shakila, R., Jeyasekaran, G., Vijayakumar, A., & Sukumar, D. (2012). Microbiological quality of sous-vide cook chill fish cakes during chilled storage (3°C). *International Journal of Food Science & Technology, 44*(11), 2120-2126. https://doi.org/10.1111/j.1365-2621.2009.02047.x

Kato, H. C. A., Peixoto Joele, M. R. S., Sousa, C. L., Ribeiro, S. C. A., & Lourenço, L. F. H. (2017). Evaluation of the shelf life of tambaqui fillet processed by the sous vide method. *Journal of Aquatic Food Product Technology*, *10*(26), 1144-1156. https://doi.org/10.1080/10498850.2014.986593

Kılıç, B., & Richards, M. P. (2003). Lipid oxidation in poultry döner kebab: pro-oxidative and anti-oxidative factors. *Journal of Food Science, 68*(2), 686-689.

Korkmaz, A. Ş. Arpa, H., Üstündağ, E., Genç, E., & Yanar, Y. (2020). Current situation and future in the aquaculture industry. *Proceedings of the Türkiye Ziraat Mühendisliği IX. Technical Congress*, Turkey, pp. 279-298.

Lemon, D. W. (1975). *An improved TBA test for rancidity, new series circular*. No: 51. Halifax.

Malle, P., & Tao, S. H. (1987). Rapid quantitative determination of trimethylamine using steam distillation. *Journal of Food Protection*, *50*(9), 756-760.

Mol, S., & Özturan, S. (2009). Sous-vide teknolojisi ve su ürünlerindeki uygulamalar. *Journal of FisheriesSciences.com,* *3*(1), 68-75. https://doi.org/10.3153/jfscom.2009010

Mol, S., Özturan, S., & Coşansu, S. (2012). Determination of the quality and shelf life of sous vide packaged whiting (*Merlangius merlangus euxınusi* Nordman, 1840) stored at cold (4 °C) and temperature abuse (12°C). *Journal of Food Processing and Preservation*, *36*, 497-503. https://doi.org/10.1111/j.1745-4549.2011.00616.x

Nyati, H. (2000). An evaluation of the effect of storage and processing temperatures on the microbiological status of sous vide extended shelf-life products. *Food Control, 11*, 471-476. https://doi.org/10.1016/S0956-7135(00)00013-X

Özturan, S. (2009). *Vakum ambalajda pişirilmiş (sous-vide) balıkta kalite ve raf ömrünün belirlenmesi* [Determination of the quality and shelf life of cooked fish in vacuum package (Sous vide)] [Master Thesis. İstanbul University].

Pongsetkul, J., & Benjakul, S. (2022). Impact of sous vide cooking on quality and shelf-life of dried sour-salted fish. *Journal of Food Processing and Preservation*, *46*, e16142. https://doi.org/10.1111/jfpp.16142

Ramos, F. C. P., Lúcia, F. H. L., Joele, M. R. S. P., & Consuelo, L. S. C. A. (2016). Tambaqui (*Colossoma macropomum*) sous vide: Characterization and quality parameters, *Semina: Ciências Agrárias, Londrina*, *1*, 117-130. https://doi.org/10.5433/1679-0359.2016v37n1p117

Seyyar, E. (2015). *Sous-vide yöntemi ile pişirilen alabalık filetolarında heterosiklik aromatik amin oluşumu ve bisfenol-a migrasyon düzeyinin belirlenmesi* [Determination of the formation of heterocyclic aromatic amines and the migration of bisphenol-A in trout fillets cooked by sous-vide] [Master Thesis. Atatürk University].

Şişmanlar Altıkaya, E. (2016). *Farklı sıcaklıklarda sous vide uygulanmış sudak balığının (Sander lucioperca, Linnaeus, 1758) raf ömrü üzerine bir araştırma* [An investigation of shelf life of zander fish (*Sander lucioperca* Linnaeus, 1758) applied sous vide in different temperatures] [Master Thesis. Recep Tayyip Erdoğan University].

Valenzuela, A., & Valenzuela, R. (2013). Omega-3 docosa hexaenoic acid (DHA) and mood disorders: Why and how to provide supplementation. In N. Kocabasoglu (Ed.), *Mood Disorders* (pp. 242-261). IntechOpen.

Yıldız, M., & Yılmaz, M. (2020). Sous vide technique in Turkish literature. *Journal of Tourism and Gastronomy Studies*, *8*(3), 2318-2336. https://doi.org/10.21325/jotags.2020.662

Mar. Sci. Tech. Bull. (2022) 11(2): 231-235
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1121995

Marine Science and Technology Bulletin

SHORT COMMUNICATION

First record of the diatom *Nitzschia navis-varingica* in the Sea of Marmara

Elif Eker-Develi[1] • Ahmet Erkan Kideys[2*]

[1] *Mersin University, Faculty of Education, Department of Mathematics and Science Education, Mersin, Turkey*
[2] *Middle East Technical University, Institute of Marine Sciences, PO Box 28, Erdemli 33731, Mersin, Turkey*

ARTICLE INFO

Article History:
Received: 26.05.2022
Received in revised form: 21.06.2022
Accepted: 21.06.2022
Available online: 22.06.2022

Keywords:
Nitzschia navis-varingica
Marmara Sea
Mucilage event
Diatom
Non-indigenous

ABSTRACT

We report the first occurrence of a diatom species, *Nitzschia navis-varingica* in the Sea of Marmara. Previously, this species was observed in the Mediterranean Sea in 2016. Samples were collected from the coast of Darıca, Istanbul, Turkey, on 22 June 2021 during a large-scale mucilage event. The species was isolated, cultured under laboratory conditions, and investigated with scanning electron microscopy and light microscopy.

Please cite this paper as follows:

Eker-Develi, E., & Kideys, A. E. (2022). First record of the diatom *Nitzschia navis-varingica* in the Sea of Marmara. *Marine Science and Technology Bulletin*, *11*(2), 231-235. https://doi.org/10.33714/masteb.1121995

Introduction

The diatom *Nitzschia navis-varingica* Lundholm & Moestrup was first identified in Vietnam waters in 1997 (Kotaki et al., 2000) and later observed in Japan and the Philippines (Kotaki et al., 2004), Australia and New Zealand (Chiovitti et al., 2005), Thailand (Romero et al., 2008) and Malaysia (Suriyanti & Usup, 2015). The first report of this species in an area outside the Western Pacific was from the Mediterranean Sea in 2016, when Ayaz et al. (2018) observed it off Erdemli along the southern Turkish coast. This species is generally reported to dominate in benthic, brackish environments (Kotaki et al., 2004; Romero et al., 2012) at salinities ranging from 5 to 33 (Lundholm & Moestrup, 2000; Tan et al., 2016). However, the Mediterranean Sea strain (Strain no: MED_01, Accession number: MW315998) grows at higher salinities of 38 (Eker-Develi et al., 2020).

The purpose of the present study is to report for the first time the presence of the diatom *N. navis-varingica* in the Sea of Marmara.

* Corresponding author
E-mail address: kideys@gmail.com (A. E. Kideys)

Material and Methods

By dipping a plastic container of 10-liter volume, a seawater sample was obtained on 22 June 2021 from the shore where mucilage accumulation was dense in the Sea of Marmara located at Darıca, Istanbul (40.75 N, 29.39 E, Fig. 1). The temperature and salinity of the seawater were 23.2°C and 23.7, respectively (Hanna HI98319 salinity tester).

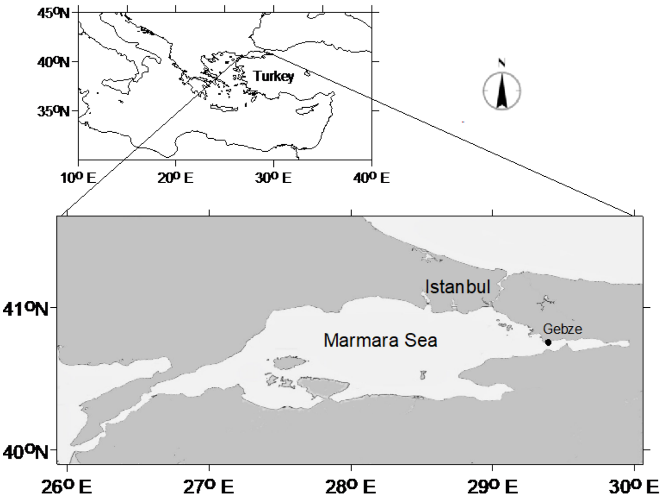


Figure 1. Sampling area for the *Nitzschia navis-varingica* on 22 June 2021.

In the laboratory, during the initial observation of the sample under the microscope, the phytoplankton species identified included one species of the dinoflagellate (i.e., *Prorocentrum micans*) and several species of diatoms (i.e., *Cylindrotheca closterium, Pleurosigma sp., Skeletonema sp. Licmophora sp., Dithylum brightweli* and several unidentified pennates). F/20 Medium was added to the seawater sample when cell numbers of the microalgae were observed to increase. *N. navis-varingica* cells were selected with thinned glass Pasteur pipettes. Cultures were grown under a 12h:12h light-dark cycle at 20°C temperature and ~20-30 μmol photons s^{-1} irradiance in 100 ml Erlenmeyer flasks.

Results and Discussion

Cell sizes of 50 individuals were measured from a three-week old culture with a light microscope (Nikon Eclipse TS100) at 1000X magnification. For scanning electron microscope (SEM) images, samples were first washed with distilled water, acid cleaned, re-washed, and filtered through 0.2 μm cellulose acetate filters. Filters were coated with gold using a Quorum Q150R Sputter Coater and examined with a field emission scanning electron microscope (Quanta 650 Field Emission SEM).

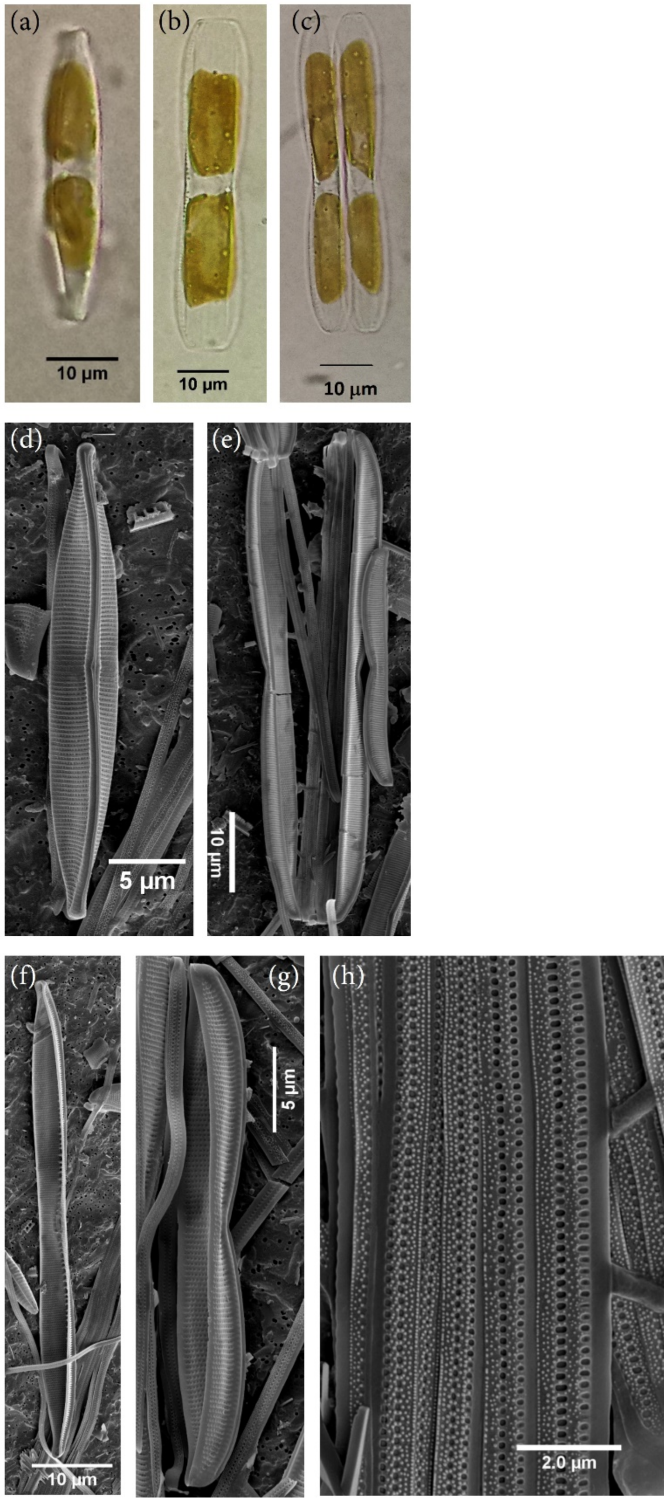


Figure 2. Light microscope (a, b, c) and SEM (d, e, f, g, h) images of *N. navis-varingica*. (a) valve view, (b) girdle view, (c) girdle view of doublet cells, (d) valve view showing the central nodule, (e) girdle view with open bands and two cells having different sizes, (f) internal view showing fibulae and interstriae, (g) girdle view with areolae, arrow shows an open band with two rows of areolae (h) cingular bands.

N. navis-varingica was observed for the first time in the Sea of Marmara in this study. Previously, it was recorded in the Mediterranean Sea in samples isolated in 2016 (Ayaz et al., 2018). However, this species must have been introduced to the Mediterranean Sea before 2011 since its picture was observed in a culture sample from 2011 (unpublished data of Eker-Develi). DNA sequence analysis may show the differences between the Mediterranean and the Marmara Sea strains.

From the world's oceans, the neurotoxin Domoic acid is known to be produced by only two species of approximately 900 species of *Nitzschia*, namely *N. bizertensis* Smida, Lundholm, Sakka and Mabrouk and *N. navis-varingica* (which is the subject of this paper) (Lundholm & Moestrup, 2000; Bouchouicha-Smida et al., 2015) besides 28 out of 58 *Pseudo-nitzschia* species (Bates et al., 2019; Dong et al., 2020; Chen et al., 2021; Olesen et al., 2021) as well as by the red alga *Chondria armata* (Bates et al., 2019).

Domoic acid production of *N. navis-varingica* in different regions has been demonstrated by several studies (Kotaki et al., 2000, 2004, 2005, 2008; Romero et al., 2012; Tan et al., 2016). While in most of these studies, *N. navis-varingica* is shown to produce domoic acid, some other investigations reported production of the closely related isodomoic acid A and/or isodomoic acid B (Bates et al., 2018). A few non-toxic strains have also been recorded from the Philippines (Kotaki et al., 2005) and Malaysia (Suriyanti & Usup, 2015). Domoic acid was not detected in the Mediterranean Sea strain, for which the presence of isodomoic acid was not screened (Eker-Develi et al., 2020). The toxicity of *N. navis-varingica* has been suggested to pass from parent to daughter strains (Romero et al., 2011; Bates et al., 2018).

We have not yet undertaken any study on its toxins; however, toxicity analysis will also elucidate its potential risks to the newly introduced regions. The Sea of Marmara is an essential area for mussel production, both from the capture fishery and mariculture (over 4 thousand tons from Turkish waters; https://data.tuik.gov.tr/Bulten/Index?p=37252&dil=2). Therefore, studying the toxicity of this species is necessary as there are also health implications concerning human consumption. The Sea of Marmara is already jeopardized by the large-scale mucilage events (Aktan et al., 2008; Tüfekçi et al., 2010; Balkıs et al., 2011; Balkıs-Ozdelice et al., 2021; Savun-Hekimoğlu & Gazioğlu, 2021) and mass occurrence of another toxic species may further aggravate ongoing environmental problems in this sensitive ecosystem. The toxicity of this species and its interaction with other dominant phytoplankton species in the environment should be investigated to understand the potential relation of *N. navis-varingica* in such mucilage events.

The size range of this species from an isolate in a shrimp culture in Vietnam were 45-55 µm long and 9-11 µm wide in girdle view (n= ~30; Lundholm & Moestrup, 2000) which are narrower than our respective measurements of 21-56 µm and the 4-13 µm here for the Sea of Marmara isolate.

This species may have also been introduced to the vulnerable Black Sea ecosystem. Since this species is unknown by many investigators, it is possible that it was overlooked by many taxonomists or recorded only as a pennate diatom species.

Compliance With Ethical Standards

Authors' Contributions

EED: Analysis and Investigation, Methodology, Conceptualisation, Writing, Original draft, Review.

AEK: Sampling, Writing, Conceptualisation, Data curation, Validation, Review.

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.

References

Aktan, Y., Dede A., & Çiftçi P. S. (2008). Mucilage event associated with diatoms and dinoflagellates in Sea of Marmara, Turkey. *Harmful Algae News*, *36*, 1-3.

Ayaz, F., Eker-Develi, E., & Sahin, M. (2018). First report of *Nitzschia navis-varingica* in the Mediterranean Sea and growth stimulatory effects of *Nitzschia navis-varingica*, *Chrysochromulina alifera* and *Heterocapsa pygmaea* on different mammalian cell types. *Molecular Biology Reports*, *45*, 571–579. https://doi.org/10.1007/s11033-018-4195-7

Balkıs, N., Atabay, H., Turetgen, I., Albayrak, S., Balkıs, H., & Tüfekçi, V. (2011). Role of single-celled organisms in mucilage formation on the shores of Buyukada Island (the Sea of Marmara). *Journal of the Marine Biological Association of the United Kingdom*, *91*(4), 771–781. https://doi.org/10.1017/S0025315410000081

Balkis-Ozdelice, N., Durmus, T., & Balci, M. (2021). A preliminary study on the intense pelagic and benthic mucilage phenomenon observed in the Sea of Marmara. *International Journal of Environment and Geoinformatics*, *8*(4), 414-422. https://doi.org/10.30897/ijegeo.954787

Bates, S. S., Hubbard, K. A., Lundholm, N., Montresor, M., & Leaw, C. P. (2018). *Pseudo-nitzschia*, *Nitzschia*, and domoic acid: New research since 2011. *Harmful Algae*, *79*, 3–43. https://doi.org/10.1016/j.hal.2018.06.001

Bates, S. S., Lundholm, N., Hubbard, K. A., & Montresor, M. (2019). Toxic and harmful marine diatoms. In J. Seckbach, & G. Richard (Eds.), *Diatoms: Fundamentals and Applications* (pp. 389-434). Scrivener Publishing. https://doi.org/https://doi.org/10.1002/9781119370741.ch17

Bouchouicha Smida, D., Lundholm, N., Sahraoui, I., Lambert, C., Hadj Mabrouk, H., & Sakka Hlaili, A. (2015). Detection of domoic acid in *Mytilus galloprovincialis* and *Ostrea edulis* linked to the presence of *Nitzschia bizertensis* in Bizerte Lagoon (SW Mediterranean) *Estuarine Coastal and Shelf Sciences, 165*, 270–278.

Chen, X. M., Pang, J. X., Huang, C. X., Lundholm, N., Teng, S. T., Li, A., & Li, Y. (2020). Two new and nontoxigenic *Pseudo-nitzschia* species (Bacillariophyceae) from Chinese southeast coastal waters. *Journal of Phycology*, *344*, 335–344. https://doi.org/10.1111/jpy.13101

Chiovitti, A., Harper, R. E., Willis, A., Bacic, A., Mulvaney, P., Wetherbee, R. (2005). Variations in the substituted 3-linked mannans closely associated with the silicified walls of diatoms. *Journal of Phycology*, *41*(6), 1154–1161. https://doi.org/10.1111/j.1529-8817.2005.00140.x

Dong, H. C., Lundholm, N., Teng, S. T., Li, A., Wang, C., Hu, Y., & Li, Y. (2020). Occurrence of *Pseudo-nitzschia* species and associated domoic acid production along the Guangdong coast, South China Sea. *Harmful Algae*, *98*, 101899. https://doi.org/10.1016/j.hal.2020.101899

Eker-Develi, E., Konucu, M., & Tekdal, D. (2020). Analysis of domoic acid within *Nitzschia navis-varingia* and other three *Pseudo-nitzschia* species isolated from the northeastern Mediterranean Sea. In Z. Selamoğlu, & M. Sevindik (Eds.), *International Eurasian Conference on Biotechnology and Biochemistry*, Turkey, pp. 799-806.

Kotaki, Y., Furio, E. F., Satake, M., Lundholm, N., Katayama, T., Koike, K., Fulgueras, V. P., Bajarias, F. A., Takata, Y., Kobayashi, K., Sato, S., Fukuyo, Y., & Kodama, M. (2005). Production of isodomoic acids A and B as major toxin components of a pennate diatom *Nitzschia navis-varingica*. *Toxicon*, *46*(8), 946–953. https://doi.org/10.1016/j.toxicon.2005.09.004

Kotaki, Y., Koike, K., Yoshida, M., Thuoc, C. V., Huyen, N. T. M., Hoi, N. C., Fukuyo Y., & Kodama M. (2000). Domoic acid production in *Nitzschia* sp. (Bacillariophyceae) isolated from a shrimp-culture pond in do son, Vietnam. *Journal of Phycology*, *36*, 1057–1060.

Kotaki, Y., Lundholm, N., Katayama, T., Furio, E. F., Romero, M. L., Relox, J. R., Yasumoto, T., Naoki, H., Hirose, M. Y., Thanh, T. D., Thuoc, C. V., Huyen, N. T. M., Thu, P. T., Takata, Y., Kodama, M., & Fukuyo, Y. (2008). ASP toxins of pennate diatoms and bacterial effects on the variation in toxin composition. In Ø. Moestrup (Ed.), *Proceedings of the 12th International Conference on Harmful Algae*, Denmark, pp. 300-302.

Kotaki, Y., Lundholm, N., Onodera, H., Obayashi, K., Bajarıas, F. F. A., Furio, E., Iwataki, M., Fukuyo, Y., & Kodama, M. (2004). Wide distribution of *Nitzschia navis-varingica* a new domoic acid-producing benthic diatom found in Vietnam. *Fisheries Science*, *70*(1), 28–32. https://doi.org/10.1111/j.1444-2906.2003.00766.x

Lundholm, N., & Moestrup, Ø. (2000). Morphology of the marine diatom *Nitzschia navis-varingica*, sp. nov. (Bacillariophyceae), another producer of the neurotoxin domoic acid. *Journal of Phycology*, *36*, 1162–1174. https://doi.org/10.1016/j.hal.2016.11.003

Olesen, A. J., Leithoff, A., Altenburger, A., Krock, B., Beszteri, B., Eggers, S. L., & Lundholm, N. (2021). First evidence of the toxin domoic acid in Antarctic diatom species. *Toxins*, *13*, 93. https://doi.org/10.3390/toxins13020093

Romero, M. L. J., Kotaki, Y., Lundholm, N., Thoha, H., Ogawa, H., Relox, J. R., Terada, R., Takeda, S., Takata, Y., Haraguchi, K., Endo, T., Lim, P.-T., Kodama, M., & Fukuyo, Y. (2011). Unique amnesic shellfish toxin composition found in the South East Asian diatom *Nitzschia navis-varingica*. *Harmful Algae, 10*, 456–462.

Romero, M. L. J., Kotaki, Y., Relox, J. R., Lundholm, N., Takata, Y., Kodama, M., & Fukuyo, Y. (2012). Two new ASP toxin production types in strains of *Nitzschia navis-varingica* from the Philippines. *Coastal Marine Science*, *35*(1), 67–69.

Romero, M. L. J., Lirdwitayaprasit, T., Kotaki, Y., Lundholm, N., Relox Jr, R. J., Furio, E. F., Terada, R., Yokoyama, T., Kodama, M., & Fukuyo, Y. (2008). Isolation of ASP toxin producing *Nitzschia* from Thailand. *Marine Research in Indonesia*, *33*(2), 225–228. https://doi.org/10.14203/mri.v33i2.498

Savun-Hekimoğlu, B., & Gazioğlu, C. (2021). Mucilage problem in the semi-enclosed seas: Recent outburst in the Sea of Marmara. *International Journal of Environment and Geoinformatics*, *8*(4), 402-413. https://doi.org/10.30897/ijegeo.955739

Suriyanti, S. N. P., & Usup, G. (2015). First report of the toxigenic *Nitzschia navis-varingica* (Bacillariophyceae) isolated from Tebrau Straits, Johor, Malaysia. *Toxicon*, *108*, 257–263. https://doi.org/10.1016/j.toxicon.2015.10.017

Tan, S. N., Teng, S. T., Lim, H. C., Kotaki, Y., Bates, S. S., Leaw, C. P., & Lim, P. T. (2016). Diatom *Nitzschia navis-varingica* (Bacillariophyceae) and its domoic acid production from the mangrove environments of Malaysia. *Harmful Algae*, *60*, 139–149. https://doi.org/10.1016/j.hal.2016.11.003

Tüfekçi, V., Balkıs, N., Beken, C.P., Ediger, D., & Mantıkçı, M. (2010). Phytoplankton composition and environmental conditions of a mucilage event in the Sea of Marmara. *Turkish Journal of Biology*, *34*, 199–210. https://doi.org/10.3906/biy-0812-1

Mar. Sci. Tech. Bull. (2022) 11(2): 236-245
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1103640

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Effect of contamination in cooling water line on emissions and equipment of vessels

Munir Suner[1]* • Tankut Yildiz[2]

[1] *Tarsus University, Enginering Faculty, Mechanical Engineeing Department, Takbaş Tarsus, Mersin, Turkey*
[2] *Henkel-Adhessive Technologies IMEA Region Dangerous Goods Manager Tepeüstü Umraniye,İstanbul, Turkey*

ARTICLE INFO

Article History:
Received: 15.04.2022
Received in revised form: 21.05.2022
Accepted: 28.05.2022
Available online: 23.06.2022

Keywords:
Energy efficiency
Pollution effect
Main engine cooling systems
Emission
Vibration
Ship
Funnel
Safety valve
Fouling

ABSTRACT

Emission is a threat to all living things. Despite all the conferences on climate change, emissions could not be reduced. On the contrary, its effect continues to increase. Ships use fossil-based energy and they are widely used vehicles in transportation. This paper provides an analysis of emission in ship main engine and auxiliary machinery. In addition, the effect of contamination on safety valve of ship and funnel is illustrated clearly. All data used in this study were taken from the ship during the 79-day cruise. When the pollution factor was eliminated, the average NO_x and SO_x and total emissions from the cylinder jackets, seawater circuit, scavenger circuit, freshwater circuit decreased significantly. The average revolution of main engine increased by 20% after cleaning. The results of vibration due to contamination were found to be collapse and broke up of the cylinder safety valve of main engine, and insulation layer of funnel of ship was collapsed. Only due to the contamination of the jackets and cylinders of main engine, the amount of energy losses before the ship goes aground increased by 37.48%. But this decreased by 20.83% just after the cleaning procedures were carried out. In addition, the sea circuit of main engine was contaminated at different rates on ship simulator. The actual data is in consistence with the data obtained from the ship simulator.

Please cite this paper as follows:

Suner, M., & Yildiz, T. (2022). Effect of contamination in cooling water line on emissions and equipment of vessels. *Marine Science and Technology Bulletin*, *11*(2), 236-245. https://doi.org/10.33714/masteb.1103640

* Corresponding author
E-mail address: msuner@tarsus.edu.tr (M. Suner)

Introduction

Emission is a huge problem for all living creatures. As a result of the emission, important consequences such as drought and extinction of many species occur. Ships, which provide a very important part of transportation in the world, use fossil energy sources and these gigantic vehicles have a significant share in emissions.

Since energy resources are limited, energy efficiency must be high, however energy efficiency in ships is considerably low. Thermal and mechanical energy losses in ships are quite high and this must be minimized especially in the significant parts such as circuits, main engine and auxiliary machines. One of the most important reasons of energy lose is pollution that causes an increase in friction losses and prevents heat transfer in cooling and heating systems. As a result, more energy and fuel consumption occur and this increases the emissions to the environment. As it is known, emissions are harmful to all living organisms and sometimes it may get into chemical reactions with the air in the atmosphere which may be the reason of dangerous results. However, appropriate precautions are not taken. Emissions from fuel oil and diesel are high. Even a small increase in the efficiency of ship systems is important in reducing emissions. Fuel consumption reduction can be achieved by using the waste heat recovery system (Larsen et al., 2015). Engine maintenance can be taken as an uncertainty factor in emissions and energy consumption from ships (Moreno-Gutiérrez et al., 2015). The most significant issues in mechanic systems are component life and using them with high efficiency. However, despite all the intensive researches and development, energy efficiency is still very low, and safety of systems is vital. Effects of contamination often encountered in engines are the primary negative factors within this scope. The materials used in the construction of fluid systems such as piping systems, filters, pumps, valves, ship's hull should be able to resist wear and corrosion in order to decrease fouling, which will consequently help to reduce the effect of friction and lead to less energy consumption. Mechanical systems, operators and operation methods are as important as ship design and they are the factors resulting in energy losses on vessels. The quality and nature of the materials that are used in ships is very important for system efficiency and they all affect the energy loss. Erosion of ship materials leads to contamination and causes blockages in the system circuit. Sea organisms grow up on pipe surfaces, and filters in time cause a fall in pressure in the circuit in time. In places where there is fluid at high temperatures, in contact with sea water and in places where electrical failures appear, corrosion takes place due to contamination. One of the main reasons for energy loss is turbulence which is quite common problem in ships and in the various circuits of ships. Contamination with the turbulence increases losses due to coefficiency of friction. Turbulence not only causes energy losses, but also creates vibration which can give rise to corrosion and fatigue. Long-term results of vibration on the whole system are breaks due to fatigue which will result in unusable components in some systems. When wear and corrosion increase, they lead to clogged circuits even in operative piping systems. In addition, the pump inlet and outlet valves can lead to cavitation causing excessive loss of pressure.

The concept of energy efficiency in maritime transportation may be classified under several headings and subheadings such as speed optimization, setting the course of the vessel according to the weather conditions, maintenance of the boat and machinery equipment. Turbulence, corrosion, contamination and vibration are some of the principal factors which affect the flow of substances, turning them into major factors which influence energy efficiency. Although each of them causes different effects at various levels, they are occasionally observed to occur simultaneously and they are therefore likely to affect efficiency in different ways.

The sea water cooling system is a system that takes sea water at 20^{o}C and cools the fresh water in the central heating system. The main engine cooling system is the system in which fresh water is cooled with sea water at the center of the cooling system. The main engine cooling system consists of cooling device, oil cooling device, turbine oil cylinder, main engine piston cooling, main engine jacket water system.

When the literature is searched, many studies such as pollution Mechanism (Kuruneru et al., 2016) pollution effect (Kuosa et al., 2007; Wu & Xiao, 2011; D'Amico et al., 2015; Qureshi et al., 2016; Pan et al., 2016; Suner & Yildiz, 2017), heat transfer (Karakasli et al., 2016; Wang & Wu, 2017), emission (Corbet et al., 2009; Kontovas et al., 2011; Khondaker et al., 2016; Giannopoulos, 2017; Dere et al., 2020), cooling system (Trujillo et al., 2011; Fontanesi et al., 2013; Badak et al., 2016), Fatigue (Nielsen et, 2011), vibration (Lin et al., 2009; Gravalos et al., 2013), system collapse, corrosion (Hattori & Kitagawa, 2010), energy efficiency (Jafarzadeh & Utne, 2014; Dimitrios et al., 2016; Yalcin et al., 2016; Inal et al., 2020; Dere et al., 2022), wastewater from shipbuilding (Akanlar et al., 2011) will be seen.

As can be seen from the literature, there is no such study on the effects of contamination on emissions, engine efficiency, vibration, safety valve and the funnel of vessel. These topics need to be investigated to make up for the deficiency in the literature. In this study analyzed the effects of contamination on the emission, on the main engine revolution, heat transfer through the cylinder jackets of main engine, safety valve, funnel of vessel and it has been displayed by real data have been verified through simulator. The observation is performed using more than 400 real data by the author. All data and photographs have been taken from M/V Infinity in 2014 and operated by Makro Maritime Lines.

Material and Methods

In this study, the effect of impurities in cooling water on the safety valve and funnel of the ship has been investigated by taking the actual data (6 times per day basis) on the ship for three months. The daily average values of the main engine cooling sea water temperature values, fresh water temperature, the main engine cylinder jackets temperature values, and daily variations based on these calculations were shown graphically. Following, average values for before agrounding, after agrounding and after cleaning cooler were determined. The heat transfer variations were tabulated. Considering these average values, temperature changes in cylinder jackets cycle were determined. Finally, these results were examined by increasing the contamination rates on ITU KONGSBERG NORCONTROL SIMULATOR, whose model is Sulzer RTA84C and it is a Full-Mission Engine Room Simulator.

In this study, to what extent the safety valve is influenced by impurities in the cooling water of the main engine was measured based on actual data recorded six times on a daily basis by the author from the ship. It has been demonstrated how the heat transfer is diminished by increase of the contamination. The temperature rising in cylinder jackets after decreasing in heat transfer and consequently the fall of the revolution number in the first cylinder of the main engine to dramatical levels have been shown on graphics depending on actual data. The heat changes after cooler cleaning have also been indicated graphically. Changes in levels during vibration resulting from the increase of contamination on the first cylinder of the main engine and finally collapse of the safety valve due to vibration were analyzed. Subsequently, possible results of the same affects were compared in the simulator. In addition, the problems in funnel due to contamination caused by the waste coming out from the funnel were put forward. The accuracy of these were explained in the simulator.

As it is known, the vibrations on vessels originate from those unbalanced forces and momentum. Those local vibrations and global vibrations which occur all over the vessels cause various problems. The most significant one of those problems is dynamic fatigue. Dynamic fatigue occurs on machines and their equipment exposed to vibration for a long period of time causes various incidents which are likely to pave the way to failures and malfunctions such as fractures and disjuncture.

Contamination can be defined as the case of material being scattered around or keeping material in improper places rather than the places where they belong to and it plays a primary role which hinders the efficient management. If those problematic areas and factors are considered thoroughly, it can be seen that it results in energy losses, malfunctions and shortened life of materials.

The engine room, the freight, cargo, exhaust gases leakage from the working engines, sea water, oil, grease and fuel and similar substances as well as many other sources may create contamination. It may also lead to a variety of problems such as blockage of the cycles, blockage of heat transfer surfaces, mechanical, pneumatic or hydraulic components and seizure of a machine. Although it is possible to decrease the effects of some of these contamination problems, it is impossible to avoid contamination and its negative effects completely.

Results and Discussion

All data used and related photographs were taken from the M/V Infinity ship from 2013 to April 2014. Contamination effect will be examined in the following sections with photographs and data. The rates and values affecting the heat of the cylinder jacket for the duration of 79 days, sailing of 79 days, sailing of the M/V Infinity were used including rotation speed of the main engine (revolutions/per minute), the temperatures of the jacket cooling water before and after the towers (Celsius). These rates and values were obtained by taking the arithmetical averages of rates and values recorded every day on the basis of six shifts. The general rates and values were recorded as follows; from 10/22/ 2013 to 11/24/2013 (case1), from 11/24/2013 to-01/03/2014 (case2), 01/03/2014 to 04/04/2014 (case3). The average rate values of the Main Engine Jacket Water of the four cylinders were obtained from three different situations. According to these findings, the required heat transfer increased significantly just before and after the ship ran aground, and decreased significantly just after the final cleaning process was completed.

The M/V Infinity is stranded offshore Hudeidah – Yemen Republic on 11/24/ 2013. Although the vessel did not face any serious damage, due to the sandy bottom of the sea, extreme amounts of contamination was observed within the sea water cycles after a short while. Because both the shallow water and the deep water sea chests were opened during the maritime accident and as a result there was a rise in temperatures of the main engine jacket. Consequently, the number of revolutions of the main engine had to be decreased, so duration of sailing was extended. Furthermore, their exposure to the negative effects of contamination and high heat shortened the life span of the machines and their parts which were being used for a long time. The towers of the main engine cooling water system were cleaned on January 3rd, 2014. The serpentines were stabbed with iron bars, therefore the obstructions were removed and opened. All of the average daily rates of temperature of the jacket of cylinder 1 are illustrated in the Figure 1, which were observed from 11/22/2013 to 4/6/2014. The percentages of the increases in the rates seen at various two cylinders are as follows: cylinder one 6.324%, cylinder two 3.754%. A considerable amount of decrease is observed at average rates of temperatures for two cylinders when the values taken before and after the cleaning operation was fulfilled. The average values for each cylinder are estimated to be as follows: cylinder one 1.446%, cylinder two - 2.638%. The contamination prevents heat transfer.

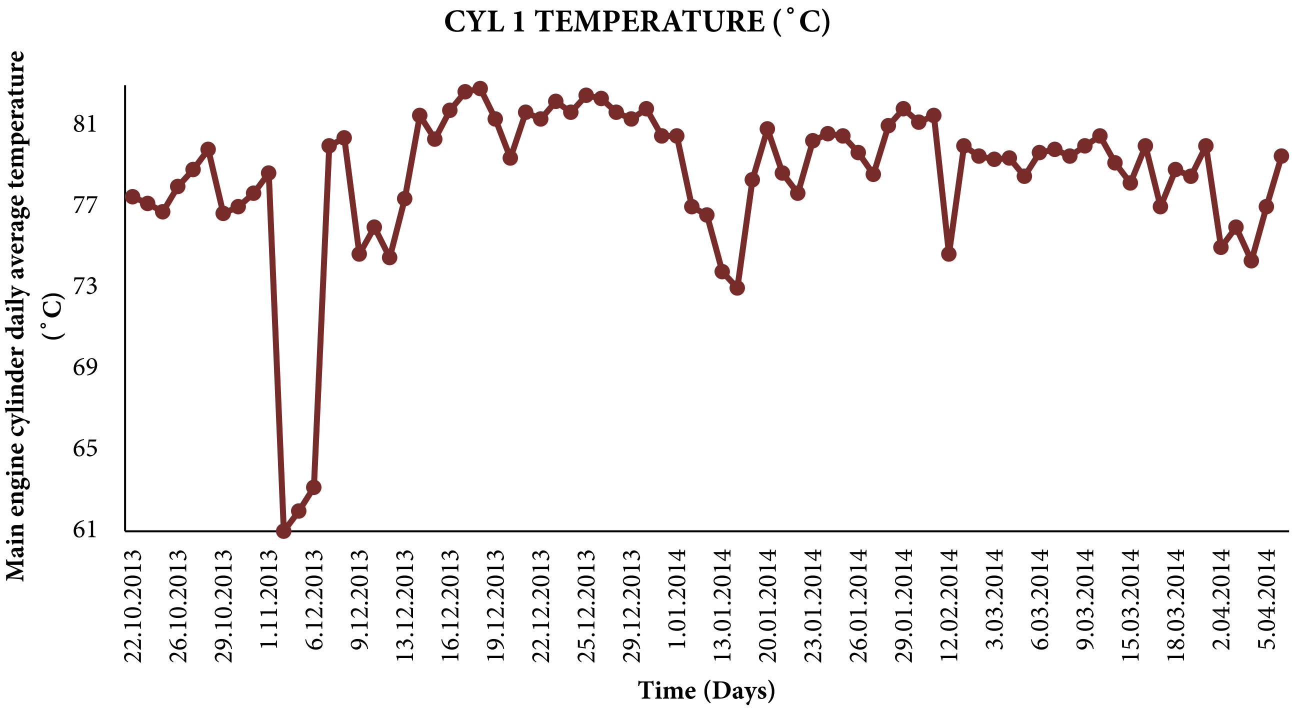


Figure 1. The daily average temperature of the jacket water of cylinder 1 of main engine.

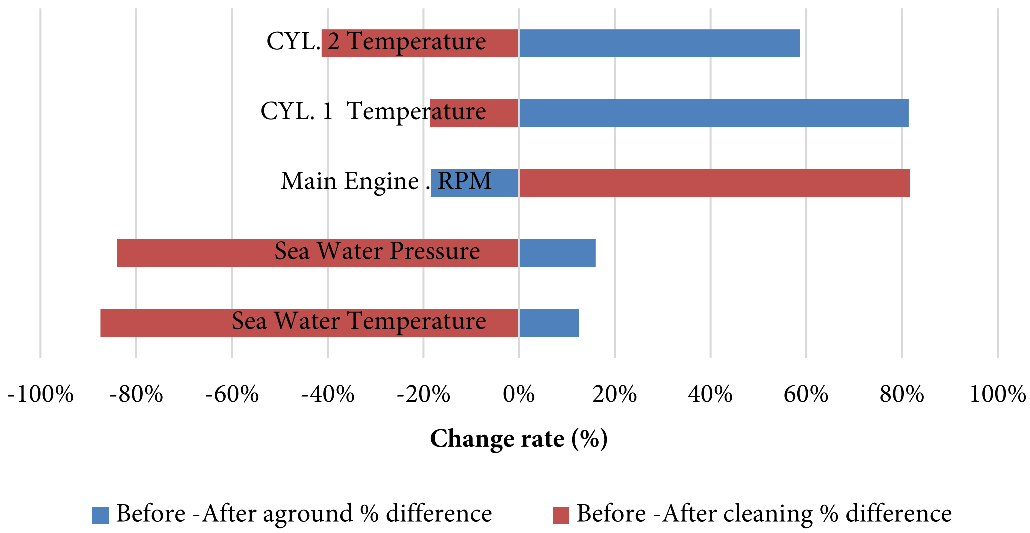


Figure 2. Average changes in the factors affecting the main engine revolution

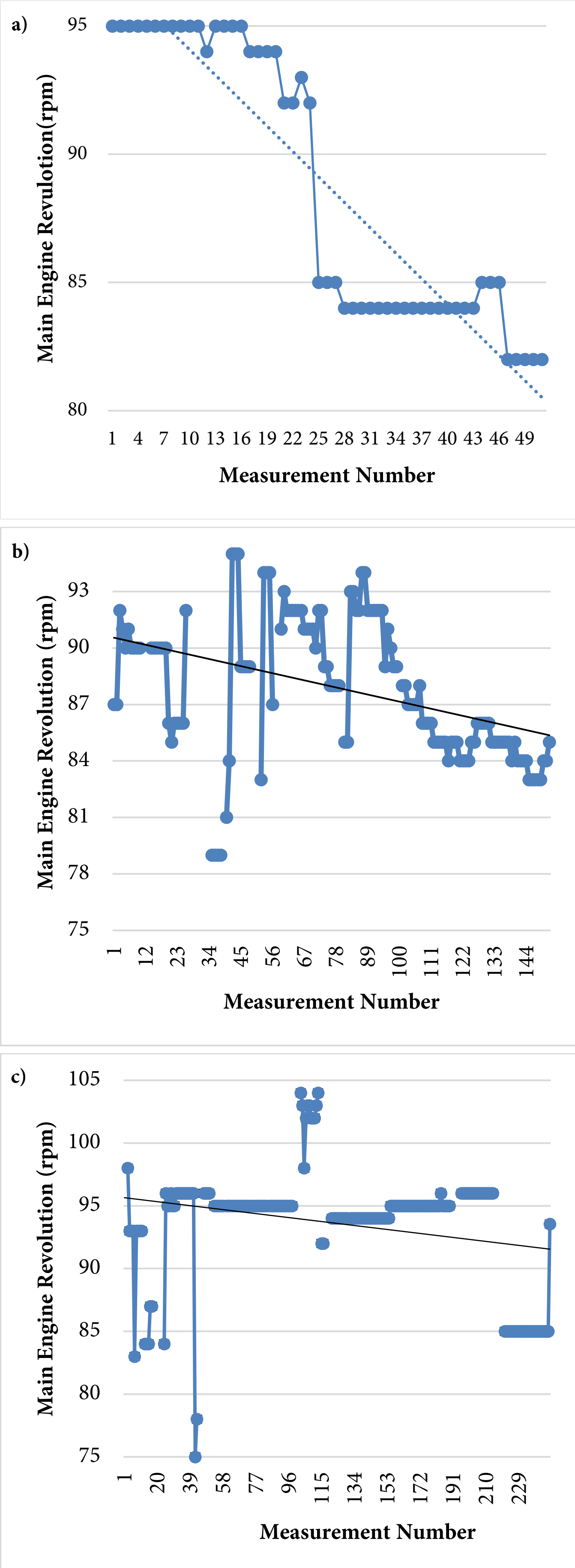


Figure 3. Revolution of main engine; a) Before aground, b) After aground, c) After cleaning 22 October to April 6

The general average rates of those three cases are illustrated in Figure 2. The first case occurred on October 22, 2013 before the ship went aground. The second case was after the ship went aground until the cleaning procedure. The third case was after the cleaning until April 6.

Average temperature of the sea water circuit increased by the average rate of 2.217% before and just after aground. Before and after cleaning, it decreased by rate of 15.470%. This circuit average pressure increased by the average rate of 8.653% before and just after aground. Before and after cleaning, it decreased by rate of 45.475%. The reason of this change is impurities in sea water circuit. In addition, it has led to significant changes in heat transfer and pressure losses.

The revolutionary change of main engine of the vessel for three cases is shown in Figure 3. As it is shown in Figure 3a before aground, Figure 3b after aground and Figure 3c after cleaning, the revolution of main engine decreased to critical numbers both before aground and just after aground. The effects of contamination and vibration on main engine revolution have been determined and illustrated. The revolution of main engine average decreased by the average rate of 1.451% before and just after aground. However, before and after cleaning, it increased by rate of 4.668%.

It is known, the safety valve mechanism which runs on the pressure of spring is utilized to provide pressure relief in such cases in order to avoid excessive and dangerous increase of pressure power which occurs in the cylinders during fuel combustion. While the M/V Infinity was going through the Suez Canal on the date of October 26th, 2013, the safety valves of the number 1 and 2 cylinders remained open due to being exposed to contamination for a long period of time and a very serious accident was hardly avoided. While going through the Suez Canal, the safety valve of main engine started to open as soon as the vessel started sailing. At the beginning of the incident, a slight gas leakage was observed at the safety valves between the compression and exhaust periods. The captain continued sailing thinking that they could deal with the problem when they arrived at the anchorage place approximately in two hours' time. One hour later, a considerable amount of increase was noticed at the leakage when the engine revolution decreased by about 22%. On the other hand, as the fuel-oil used had not been separated regularly, that seemed to be a usual condition but the opened safety valve did not close this time. However, we had to go on sailing as we were in the canal. On the other hand, it would be dangerous to sail as the safety valve remained open. The second captain kept on sailing thinking that it would close soon, but as the revolutions increased, the safety valve started to blow out smoke due to ingenerated flames and as a result of the combustion inside the valve, it started to break into pieces and scatter these glowing metal pieces around. Upon the vessel started sailing by the command of the captain, first pressurized exhaust gases, afterwards flames which ingenerated during combustion began to blow out from the safety valves belonging to the cylinder number 1. Finally, those glowing pieces metals which were broken from the safety valve were scattered around as seen in Figure 4. Thanks to various lucky and narrow escapes, there were not any serious injuries and accidents as a result of having to stop the vessel urgently in the middle of the Suez Canal. The vessel might have gone aground. Fortunately, this incident was avoided by the coil users who were on board by coincidence. They secured the vessels punctually by using the ship's tackles and running rigging. Luckily, there was nobody around the engine during the incident, therefore nobody was injured. And again, while those broken pieces were being scattered, none of them fell on risky areas such as electrical equipment. However, the authorities fined the vessel for 30.000 $. Additionally, some extra charges had to be paid for those coil users and tugboats. Although contamination seems to be a simple concept at the first sight, consequences of neglecting the contamination proves clearly what a significant issue it is as understood from the incident.

Figure 4. The safety valve of the cylinder number 1

The other major factor that led the valve to remain opened was undoubtedly impurities in the fuel. The fuel was separated only once, then the service tank was directly fueled up. As the fuel was not clean, it caused the valve jamming. Other reasons for that incident could be heat and pressures generated during combustion and vibrations occurred while sailing.

The most important damage of the vibration on ships is that vibration causes dynamic fatigue. Those vessel engines and parts which are exposed to vibration for a long period of time are likely to be broken and come off. Moreover, this may cause serious deficiencies in the performance of machines as well as some serious accidents and problems. Then, the number of revolutions of the main engine of the M/V Infinity was not able to exceed certain numbers due to the increase in the jacket temperatures and the fact that the vessel had to sail at some critical revolutions to maintain satisfactory performance. The critical revolution is related to the construction of the vessel, being independent from the manufacturer of the engine, and the values regarding the revolutions of engine are determined by some special tests. Briefly, critical revolution is the number of revolutions as the engine is working at the frequency when vibrations on the ship are at their maximum levels.

Figure 5. The funnel loop some parts of which fell of and uncovered for repairs

Figure 6. The parts which fell off funnel loop

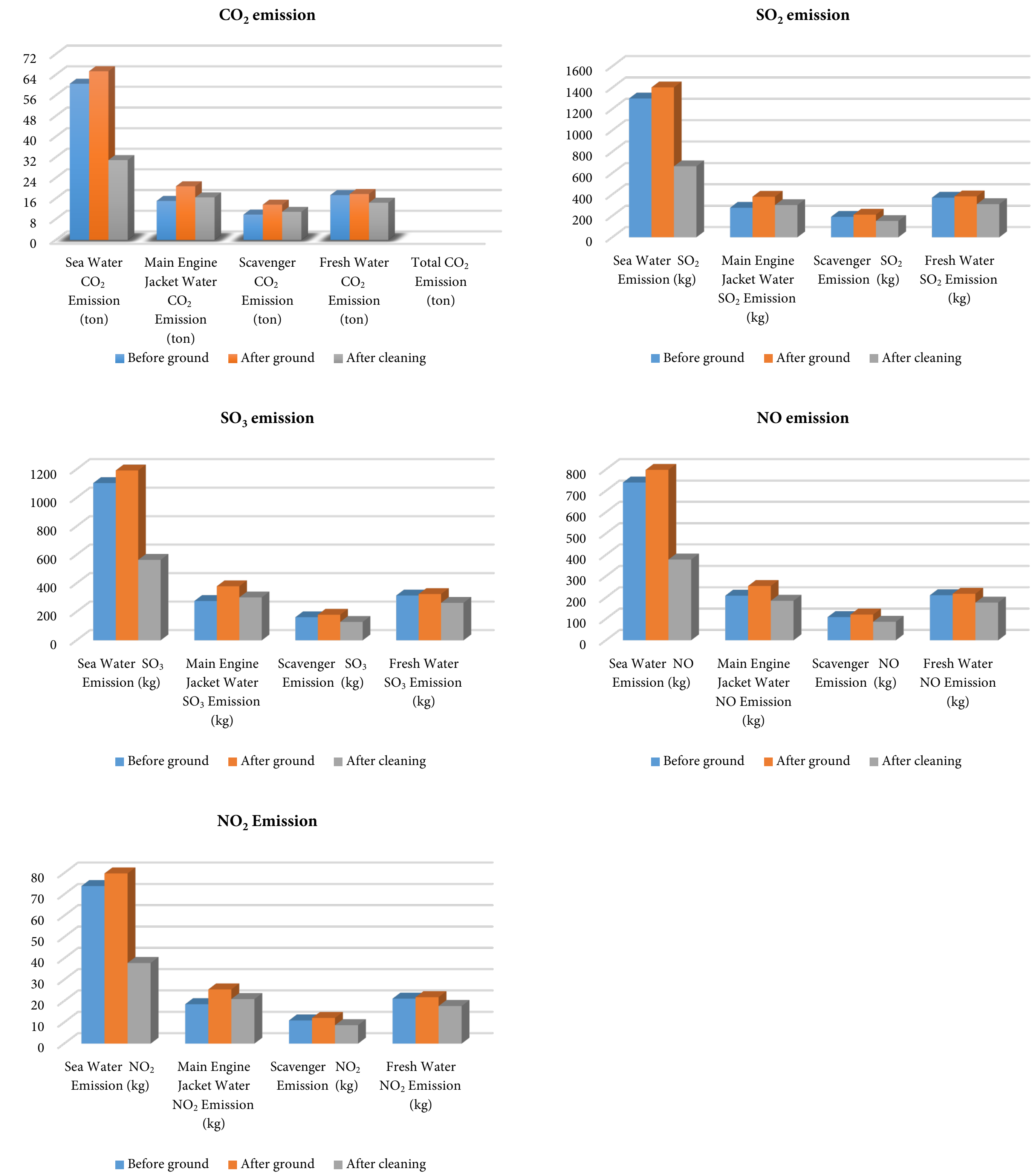


Figure 7. Emission change; a) CO_2 emissions, b) SO_2 emissions, c) SO_3 emissions, d) NO emissions, e) NO_2 emissions

As result of M/V Infinity's engine operating at critical revolutions for a long time while sailing, some damages such as fractures, dents and collapses occurred on the outer covering of the funnel loop, which caused heat insulation. In Figure 5, the funnel, some parts of which were dented and collapsed due to being exposed to excessive vibration and the funnel loop uncovered to get repaired are shown. Figure 6 shows some of those pieces of fractures.

Casualties regarding seafarers as well as the possibility of ship equipment damages were among the most common risks in accidents. Several precautions may be taken to avoid such risks and accidents. For instance, the vessel should not sail while her engines are working at critical maintenance of the dynamic absorbers of vibration dampers should be performed regularly and all sorts of preventive measures should be taken by means of visual inspections of those engines and their equipment which are likely to be affected negatively and damaged.

Table 1. Changes in systems of vessel

Systems	Difference before - after agrounding (1-2%)	Difference after agrounding- after cleaning process (2-3%)
Sea Water Temp. (°C)	+2.21	-15.47
Sea Water Pressure (bar)	+8.65	-45.47
Main Engine Revolution (RPM)	-1.45	+6.44
Cylinder 1 Temp. (°C)	+6.32	-1.44
Cylinder 2 Temp. (°C)	+3.75	-2.63
Cylinders of Jacket Heat (kw)	-37.48	+20.83

The sea circuit of main engine was contaminated with debris in simulator by amounts of 10%, 20%, 30% and 50%. Before sea water circuit was polluted, sea water flow rate was 1038.300 ton/h, and heat transfer was 22 823 kw. When the contamination amount is 10%, seawater heat transfer circuit increases to 23100 kwh and valve openings rise from amount of 44.100% to amount of 45.250%. When the sea water circuit contamination rate is 20%, flow rate is 23.050 ton/h, and heat transfer is 12600 kwh respectively. Also, at this rate of contamination, the jackets of main engine start heating and at the amount of 30%, the jackets of main engine go over heating. When the sea water circuit contamination is 50%, heat transfer is 22600 kwh, and flow rate is 1438 ton/h. These results illustrate to us the actual measured data, that, if the system temperature exceeds a certain value, there will be expansion in the cylinder, blocking, the cylinder oil burning and deadlock occur. Thus, the cylinder temperature change must remain between 70ºC and 80ºC. In the system, the cooling water flow rate and so the heat transfer increases, therefore, revolutions of the main engine are reduced automatically or manually. The effect of contamination was examined in the simulator. As the impact of contamination increases, the circuit pressure losses increase, the alarm rings, the main engine revolution undergoes heavy running position. This resulted in all freshwater cooling not being sufficiently cooled by the cooler. When the lower temperatures rise to higher temperatures, it cannot be cooled and the temperature of jacket rises. Thus, the main engine revolution has to be reduced manually.

Contamination Effect on Emission

The changes in CO_2, SO_2, SO_3, NO and NO_2 emission emissions occurring in the circuits of the main engine cooling system on the ship due to pollution are shown in Figure 7. As can be seen from the graphics, after the circuit cleaning in all circuits, emissions have decreased due to pollution. The increase in emissions due to pollution in the seawater circuit of the main engine cooling system is higher than the increase in all other circuits.

Conclusion

The results show that the main engine seawater circuit is seen as the place where the impact of pollution is the greatest. The fuel consumption caused by the increase in pollution in all circuits and the emissions emitted by the ship increased accordingly. Especially carbon dioxide and nitrogen monoxide emissions are quite high. After cleaning the main engine cooling circuit, emissions were significantly reduced. Changes in the systems of vessel due to contamination are shown in Table 1. Due to effect of contamination, the average revolution of main engine was decreased by 1.45% before and just after aground. Before and after cleaning, it increased by rate of 4.67%, and just after aground, it increased by 6.44%. Cylinder jackets temperature increased due to impurities. Only due to the contamination of the jackets and cylinders of main engine, the amount of energy losses before the ship went aground increased by 37.48%, but decreased by 20.83% just after the cleaning procedures are carried out. The average temperature of jacket cylinder one increased by 6.32%, that of cylinder two was by 3.75%. When the values taken before and after the cleaning operation was fulfilled, the change in average values for each cylinder were estimated to be as follows: cylinder one 1.44%, cylinder two as 2.638%. The contamination prevents heat transfer. The sea water circuit average temperature increased by 2.21% before and just after aground. Before and after cleaning, it decreased by 15.47%. This circuit average pressure increased by 8.65% before and just after aground, and before and after cleaning, it decreased by 45.47%. Revolution of main engine decreased to critical numbers and kept working for a long time. In addition, the effect of contamination on the main engine, revolution of main engine, and the other effect are in consistence with the data obtained from the ship simulator. The results clearly show that contamination not only affects the performance of the system and emission, but also leads to the collapse of the systems and danger.

Acknowledgements

All data, images and photos were compiled by the author during the internship from the M/V Infinity Ship. So, thanks to the sailors and the owner of Makro Maritime Lines Limited.

Compliance With Ethical Standards

Authors' Contributions

Both authors have contributed equally to this paper.

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.

References

Akanlar, F. T., Celebi, U. B., & Vardar, N. (2011). The importance of wastewater treatment in shipbuilding industry. *International Journal of Global Warming*. *3*(1-2), 103-115.

Badak, M. U., & Mert, S. O. (2016). Exergetic simulation and performance assessment of 1-1 shell and tube heat exchangers. *International Journal of Exergy*, *21*(3), 261-276.

Corbett, J. J., Wang, H., & Winebrake, J. J. (2009). The effectiveness and costs of speed reductions on emissions from international shipping. *Transportation Research Part D: Transport and Environment*, *14*(8), 593–598. https://doi.org/10.1016/j.trd.2009.08.005

D'Amico, M., Zampilli, M., Laranci, P., D'Alessandro, B., Bidini, G., & Fantozzi, F. (2015). Measuring injectors fouling in internal combustion engines through imaging. *Energy Procedia*, *82*, 9-16. https://doi.org/10.1016/j.egypro.2015.11.873

Dere, C., & Deniz, C. (2020). Effect analysis on energy efficiency enhancement of controlled cylinder liner temperatures in marine diesel engines with model based approach. *Energy Conversion and Management*, *220*, 113015. https://doi.org/10.1016/j.enconman.2020.113015

Dere, C., Zincir, B., Inal, O. B., & Deniz, C. (2022). Investigation of the adverse effects of slow steaming operations for ships. *Proceedings of the Institution of Mechanical Engineers: Part M: Journal of Engineering for the Maritime Environment*, *In press*. https://doi.org/10.1177/14750902221074191

Dimitrios, T., Hountalas, D. T., Mavropoulos, G. C., Katsanos, C., Daniolos, S., Dolaptzis, L., & Mastorakis, N. (2016). Potential for efficiency improvement of four-stroke marine diesel gensets by utilisation of exhaust gas energy. *International Journal of Global Warming*, *10*(1-3), 133-157 https://doi.org/10.1504/IJGW.2016.077910

Fontanesi, S., & Giacopini, M. (2013). Multiphase CFD–CHT optimization of the cooling jacket and FEM analysis of the engine head of a V6 diesel engine. *Applied Thermal Engineering*, *52*(2), 293-303. https://doi.org/10.1016/j.applthermaleng.2012.12.005

Giannopoulos, G. A. (2017). Transport sector adaptation: actions and prospects. *International Journal of Global Warming*, *13*(3-4), 371-381.

Gravalos, L., Loutridis, S., Moshou, D., Gialamas, T., Kateris, D., Tsiropoulos, Z., Xyradakis, P. (2013). Detection of fuel type on a spark ignition engine from engine vibration behavior. *Applied Thermal Engineering*, *54*(1), 171-175.

Hattori S., & Kitagawa, T. (2010). Cavitation erosion resistance of cast iron and nonferrous metals based on database and comparison with carbon steel data. *Wear*, *269*(5–6), 443-448.

Inal, O. B., & Deniz, C. (2020). Emission analysis of LNG fuelled molten carbonate fuel cell system for a chemical tanker ship: a case study. *Marine Science and Technology Bulletin*, *10*(2), 118–133. https://doi.org/10.33714/masteb.827195

Jafarzadeh, S., & Utne, I. B. (2014) A framework to bridge the energy efficiency gap in shipping. *Energy*, *69*, 603-612. https://doi.org/10.1016/j.energy.2014.03.056

Karakasli, E., Oztop, H. F., & Hepbasli, A. (2016). Performance assessment of a polyclinic heating and cooling system in a hospital building. International *Journal of Exergy*, *21*(1), 70-86.

Khondaker A. N., Rahman S. M., Khan R. A., Malik, K., & Muhyedeen M. A. R. (2016). Management of greenhouse gas emissions from maritime operations - challenges and mitigation opportunities. *International Journal of Global Warming*, *9*(3), 306-336.

Kontovas, C., & Psaraftis, H. N. (2011). Reduction of emissions along the maritime intermodal container chain: operational models and policies. *Maritime Policy & Management*, *38*(4), 451–469. https://doi.org/10.1080/03088839.2011.588262

Kuosa, M., Kaikko, J., & Koskelainen, L. (2007). The impact of heat exchanger fouling on the optimum operation and maintenance of the Stirling engine. *Applied Thermal Engineering*, *27*(10), 1671-1676.

Kuruneru, S. T. W., Sauret, E., Saha, S. C., & Gu, Y. T. (2016). Numerical investigation of the temporal evolution of particulate fouling in metal foams for air-cooled heat exchangers. *Applied Energy*, *184*, 531-547. https://doi.org/10.1016/j.apenergy.2016.10.044

Larsen, U., Pierobon, L., Baldi, F., Haglind, F., & Ivarsson, A. (2015). Development of a model for the prediction of the fuel consumption and nitrogen oxides emission trade-off for large ships. *Energy*, *80*, 545-555. https://doi.org/10.1016/j.energy.2014.12.009

Lin, T. R., Pan, J., O'Shea, P. J, & Mechefske, C. K. (2009). A study of vibration and vibration control of ship structures. *Marine Structures*, *22*(4), 730-743. https://doi.org/10.1016/j.marstruc.2009.06.004

Moreno-Gutiérrez, J., Calderay, F., Saborido, N., Boile, M., Valero, R. R., & Durán-Grados, V. (2015). Methodologies for estimating shipping emissions and energy consumption: A comparative analysis of current methods, *Energy*, *86*(C), 603-616. https://doi.org/10.1016/j.energy.2015.04.083

Nielsen, U. D., Jensen, J. J., Pedersen, P. T., & Ito, Y. (2011). Onboard monitoring of fatigue damage rates in the hull girder. *Marine Structures*, *24*(2), 182-206. https://doi.org/10.1016/j.marstruc.2011.03.003

Pan, M., Bulatov, I., & Smith, R. (2016). Improving heat recovery in retrofitting heat exchanger networks with heat transfer intensification, pressure drop constraint and fouling mitigation. *Applied Energy*, *161*, 611-626. https://doi.org/10.1016/j.apenergy.2015.09.073

Qureshi, B. A., & Zubair, S. M. (2016). Predicting the impact of heat exchanger fouling in power systems. *Energy*; *107*(15), 595-602. https://doi.org/10.1016/j.energy.2016.04.032

Suner, M., & Yildiz, T. (2016). Pollution effects onboard and its generated solution for minimized pollution effect. In P. Grammelis (Ed.), *Energy Transportation and Global Warming* (pp. 851-866). https://doi.org/10.1007/978-3-319-30127-3_63

Trujillo, E. C., Jiménez-Espadafor, F. J., Villanueva, B., & García, M. T. (2011). Methodology for the estimation of cylinder inner surface temperature in an air-cooled engine. *Applied Thermal Engineering*, *31*(8–9), 1474-1481. https://doi.org/10.1016/j.applthermaleng.2011.01.025

Wang, W., & Wu, F. (2017). Exergy destruction analysis of heat exchanger in waste heat recovery system in Kroll process. *International Journal of Exergy*, *22*(1), 89-101.

Wu, S., & Xiao, L. (2011). Comparative study of the effect of fouling on heat exchangers performance based on the first and second laws of thermodynamics. *International Journal of Exergy*, *9*(1), 1-20. https://doi.org/10.1504/IJEX.2011.041427

Yalcin, E., Sogut, M. Z., & Karakoc, H. (2016). Examination of performance indicators' effects based on propulsion parameters in a turboprop engine. *International Journal of Exergy*, *21*(2), 186-201. https://doi.org/10.1504/IJEX.2016.078925

Mar. Sci. Tech. Bull. (2022) 11(2): 246-258
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1108314

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Assessment of heavy metals contamination in fish cultured in selected private fishponds and associated public health risk concerns, Dar es Salaam, Tanzania

Leopord Sibomana Leonard[1*] • Anesi Mahenge[1] • Nehemia Christopher Mudara[2]

[1] *Ardhi University, School of Environmental Science and Technology, P.O.BOX 35176, Dar es Salaam, Tanzania*
[2] *Tanzania Mining Commission, Department of Mining Inspection and Environment, P.O.BOX 9222, Dodoma, Tanzania*

ARTICLE INFO

Article History:
Received: 26.04.2022
Received in revised form: 19.06.2022
Accepted: 21.06.2022
Available online: 24.06.2022

Keywords:
Assessment
Contamination
Dar es Salaam
Fish-Ponds
Metals
Risk

ABSTRACT

Environmental pollution caused by the increase of heavy metals concentration in aquatic and terrestrial environments is a growing global concern due to their nature and toxicity. This paper aimed to undertake an assessment of the quality of fish cultured in individual-owned fishponds in Dar es Salaam city and their associated health risks. Data collection involved sampling and quantification of the quality of two species of fish, which were African catfish (*Clarias gariepinus)* and Nile tilapia (*Oreochromis niloticus*), from three selected fish ponds in Dar es Salaam and chemical analysis involved heavy metals analysis in gills, fins, guts, and muscles. The concentrations of heavy metals were analyzed using Atomic Absorption Spectrophotometer (AAS). Results of this study indicated that the concentrations of trace metals in fish tissues varied considerably. The fish gills had higher concentrations of Cr, Zn, Cu, and Pb than the fins and guts, while muscles had the lowest concentrations of heavy metals in all fish species. A highly significant difference in the heavy metal concentrations measured in both catfish and tilapia tissues was observed with a P value of less than 0.05. Individual risk assessment showed that there was a minimal risk caused by the concentrations of Cr, Zn, and Cu upon consumption of fish; however, the combined effect was higher caused by the high concentration of Pb in fish organs. Monitoring of fish quality in privately owned fish ponds is recommended to safeguard consumers.

Please cite this paper as follows:

Leonard, L. S., Mahenge, A., & Mudara, N. C. (2022). Assessment of heavy metals contamination in fish cultured in selected private fishponds and associated public health risk concerns, Dar es Salaam, Tanzania. *Marine Science and Technology Bulletin*, *11*(2), 246-258. https://doi.org/10.33714/masteb.1108314

* Corresponding author
E-mail address: siboditz1@gmail.com (L. S. Leonard)

Introduction

Environmental pollution, population growth, and degradation of natural resources are among the top global concerns (Shaker et al., 2018). Directly or indirectly, pollution affects the aquatic ecosystem and, ultimately, human health (Kosygin et al., 2007; Shaker et al., 2018; Imlani et al., 2022). Among major pollutants, heavy metals pollution is a growing global concern due to their possible toxicity, long biological half-life, non-biodegradability, and bioaccumulation properties. They can also enter the human body through inhalation, ingestion, and dermal (Resma et al., 2020; Leonard & Mahengea, 2022). The presence of a low concentration of heavy metals such as Zn, Fe, Mn, Cu, Co, and Cr provides a key role in the biochemical process in many organisms (Darko et al., 2016) and is thus classified as essential (Resma et al., 2020). However, they can cause toxicity effects when they become available in high concentrations (Akoto et al., 2014). A low concentration of Fe is required for red blood cell production (Akoto et al., 2014); however, a high concentration of Fe and Mn could lead to pathological events, including iron oxides in Parkinson's diseases (Matusch et al., 2010). A high concentration of heavy metals like Cu can cause liver damage, and Zn reduces immune function.

Metals such as As, Hg, Pb, and Cd are toxic even at low concentrations and have no important functions in humans and thus are classified as non-essential or toxic metals (Resma et al., 2020). The concentration of Pb reduces cognitive development and intellectual performance in children (Darko et al., 2016), cause renal tumors and increases blood pressure in adults, and causes gastrointestinal disorders and liver impairments (Akoto et al., 2014); while Cd causes kidney dysfunctions, osteomalacia and reproductive deficiencies (Akoto et al., 2014). Elevated concentration of heavy metals in the aquatic environment may cause disorders in fish growth and reproduction (Darko et al., 2016) as well as histo-pathological alterations in the liver, skin, spleen gills, and kidney (Vitek et al., 2007), decreasing the plasticity of the cardiorespiratory responses, and hence reducing the survival chances of fish under hypoxic conditions in their wild environments (Leonard & Mahengea, 2022, Monteiro et al., 2013).

Fish farming plays a vital role in global food security, which is being practiced in most countries, including Tanzania, and provides millions of employments and billions of dollars to the country (URT, 2019). However, due to surface water pollution in urban areas, which used to provide natural habitats and food for fish, there is a gradual shift to the use of privately owned fish ponds which need a supply of food supplements, including factory-made feeds and farm-made feeds (Resma et al., 2020; Leonard & Mahengea, 2022). Such commercial fish feeds contain an elevated concentration of trace elements, including Pb, Cd, Cr, Cu, and Zn (Sarkar et al., 2022). Thus, there is a growing concern about the quality of fish and fish feeds used in aquaculture, which can affect consumers (Mohamad et al., 2017). Furthermore, environmental pollution in Tanzania, like other countries in the world, is reported to be polluted by heavy metals, especially in the aquatic environments located near and or within the urban areas (Mwegoha & Kihampa, 2010; Leonard et al., 2012; Leonard & Mahengea, 2022) that can also affect aquatic organisms including fish. A previous study in Dar es Salaam indicated that the quality of water from fish ponds had an elevated concentration of heavy metals (Leonard & Mahengea, 2022).

Freshwater fish demand in Tanzania, both rural and urban areas, is high that has attracted individuals to invest in aquaculture (Leonard & Mahengea, 2022), yet this demand has not been met by the current supplier because a part of the catch is exported that accounts to 10% value of the national exports contributing to 2.7% of the gross domestic product (URT, 2019). Freshwater fish farming in Dar es Salaam involves the Nile tilapia (*O. niloticus*) and the African catfish (*C. gariepinus*) (Deloitte, 2015), which have a high potential and are popular in local and international markets, hence the focus of this study.

Tilapia production in Tanzania ascended from 2856 metric tons worth TZS 12.8 billion in 2010 to 3118 metric tons worth TZS 18.7 billion in 2015 (URT, 2015) and according to the Ministry of Agriculture, Livestock, and Fisheries (MALF, 2015). The annual production of fish is 389,459.4 metric tons, and the per capita consumption is 8.2 kg. The annual export amounts to 38,114 metric tons, which yielded 15.6 billion TZS (URT, 2019). Fishponds scaled from 19,039 in 2010 to 21,300 in 2015 (Rukanda, 2016), while fish farmers increased from 16,284 in 2010 to 19,395 in 2015 (URT, 2015). Freshwater fish farming has recently become a popular source of income and business opportunity in Dar es Salaam (Leonard & Mahengea, 2022), attracting individuals to invest in fish farming. According to MALF (2015), there are more than 50 freshwater fish farms in Dar es Salaam City, with over 130 fishponds (Kyelu, 2016). However, low knowledge of water quality and fish quality was observed as a limiting factor in Tanzania (Rukanda, 2016; Leonard & Mahengea, 2022).

Monitoring fish tissue contamination provides information on any toxic pollutants in fish, which may be harmful to consumers, but also identify fish parts that can be consumed with minimal risk, hence protecting public health and the environment (Mohamad et al., 2017; Kumari & Maiti, 2019).

Fish consumption is the major route through which heavy metals accumulated in fish tissue get into the food chain and hence into the human body (Akoto et al., 2014). Various studies suggest that the rate at which heavy metals intake by fish in a contaminated environment depends on various factors, including exposure period and concentration of heavy metals. Thus, assessing heavy metals in fish parts helps to establish the direct transfer of such metals to humans through fish consumption (Akoto et al., 2014, Imlani et al., 2022). To our understanding, no study has been done to investigate heavy metals in fish grown in privately owned fish ponds in the study area. Thus, the main interest of this study was to establish the potential health risk concerns associated with heavy metals through ingesting fish grown in privately owned fish ponds by estimating daily intake (EDI) and health risk index (HRI) from a single and combined heavy metals including Cadmium (Cd), Lead (Pb), Copper (Cu), Zinc (Zn) and Chromium (Cr).

Material and Methods

Description of the Study Area

The study area is located within Dar es Salaam city, which is the largest city and economic capital in Tanzania, hosting over 10% of the country's population. Most industries, government offices, diplomatic missions, and non-governmental organizations are located in this city. It is the largest city in East Africa and the fifth city largest in Africa. The city is located at 6°48´S, 39°17´E, and covers a total area of 1493 km^2. The study location was chosen based on the availability of prospective fish farming systems, both extensive and intense, as well as respondents' willingness to participate. Thus, the study involved three fish farming ponds located in three municipalities, including Kinondoni, Ilala, and Kigamboni municipal councils, as shown in Figure 1.

Fish Sampling

Two types of fish were sampled using multi-mesh gill nets using stratified random sampling from three sampling points, which included African catfish and Nile tilapia. Three sampling campaign was conducted in the interval of two weeks. Fresh sampled fish were washed using fresh water to remove any mud or debris (Kumari & Maiti, 2019). Each sampled fish was weighed to obtain the total mass of each fish sampled, which ranged between 500 g to 1000 g, and then kept in plastic bags, transported to Ardhi University in the laboratory of School of Environmental Science and Technology, where they were frozen at -20°C until the instant of preparation and analysis.

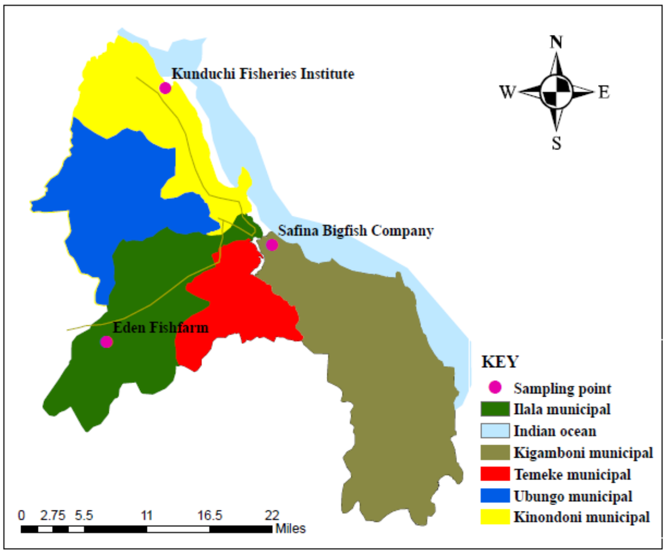


Figure 1. Location of sampled fishponds in Dar es Salaam city

Sample Preparation

Sampled fish were cleaned using distilled water, thawed, and carefully dissected using sterilized scissors, knives, and plastic forceps to elude metal pollution caused by the equipment in the laboratory. Fish organs were separated into four specimens, including muscles, gills, fins, and guts, and then each sample was taken into a microwave oven set at 103–105°C for 24 hours, where they reached a constant dry weight, followed by grinding into a fine powder using pestle and mortar.

All equipment used was rinsed and sterilized to avoid post-contamination. Before usage, the plastic and glasses were cleaned and steeped in 2% HNO_3 overnight, then rinsed three times with distilled water. Reagents used include: Hydrochloric acid (HCl), Nitric acid (HNO_3), Potassium iodide (KI), and Hydrogen Peroxide (H_2O_2) used as oxidants, as explained by Kumari & Maiti (2019). A sample for physical analysis was prepared by taking 2.0 g of each fish organ (gills, fins, guts, and muscles) and placed in a beaker, followed by the addition of 20 ml of distilled water. The mixture was well stirred with a glass rod for about 10 minutes before the analysis of physical parameters.

Samples for heavy metal analysis were prepared by taking 0.5 g of the prepared fine powder into a graduated test tube. For each test tube, 2 ml of the aqua regia (1:3 (Conc. HCl&HNO_3) was added, put in a hot air oven set at 103-105 °C for an hour, followed by the addition of 3 ml of H_2O_2 (35%), then digested at 90°C for 2 hours till the clear solution was attained. Then, all samples were left for cooling at room temperature. Next, 10 ml of distilled water was poured into each test tube to make a dilution and dissolve heavy metals in the water and left to settle overnight then, and the samples were filtered using Whatman filter paper (40) to make them clear and ready for analysis using Atomic Absorption Spectrophotometer. Samples were then allowed to cool at ambient temperature, followed by the addition of 10 ml of distilled water for dilution and dissolution of heavy metals in water, and left to settle overnight. Samples were then filtered using Whatman filter paper (40) for clarity clean ready for laboratory analysis using an Atomic Absorption Spectrophotometer as also described by previous studies (Kumari & Maiti, 2019).

Fish Feed Sampling

Fish feed samples were also collected from the same farmers who volunteered to provide fish samples, stored in polyethylene plastic bags, and transported to Ardhi University. The samples were then dried at 80°C for 24 hrs. in an oven and left to cool, followed by grinding using mortar and pestle available in the School of Environmental Science and Technology at Ardhi University. Two grams of samples were prepared in a graduated test tube (Mannzhi et al., 2021). For each test tube, 2 ml of the aqua regia (1:3) (Conc. HCl&HNO_3) was added, put in a hot air oven set at 103-105°C for an hour, followed by the addition of 3 ml of H_2O_2 (35%), then digested at 90°C for 2 hours till the clear solution was attained. Then, all samples were left for cooling at room temperature. 10 ml of distilled water was poured into each test tube to make a dilution and dissolve heavy metals in water and left to settle overnight; then, the samples were filtered using Whatman filter paper (40) to make them clear and ready for analysis using Atomic Absorption Spectrophotometer.

Laboratory Analysis

The concentrations of heavy metals were analyzed using Atomic Absorption Spectrophotometer with Parking Elmer AS 800 Auto-sampler to determine the concentrations of Pb, Cu Zn, and Cr. Heavy metal concentrations that were read in (mg/L) from AAS were converted into mg/kg through equation 1.

$$C\left(\frac{mg}{kg}\right) = \frac{C \times V}{M} \times 1000 \quad (1)$$

where: C is the metal concentration (mg/L) in the solution digested, V is the volume obtained after digestion, M is the mass of the sample (g) to be tested, and 1000 is the conversion factor from g to kg.

Health Risk Assessment

Health risk assessments resulting from consumption of the sampled fish types were established by applying a number of recommended parameters by the US EPA, such as estimated daily intake of metals (EDI) and target hazard quotient (THQ).

Calculating Estimated Daily Intake (EDI)

This was established by taking the mean concentration of heavy metals in mg/kg fresh and the average consumption of fish, which was established from the study done by Wenaty et al. (2018) and Maurya & Malik (2019) presented in equation 2.

$$EDI = \frac{C_{element} \times D_{food\ intake}}{B_{average\ weight}} \quad (2)$$

where: $C_{element}$: the average element concentration in fish, $D_{food\ intake}$: the daily fish consumption rate (0.0192 kg/person/day equivalent to 7 kg/person.year), $B_{average\ weight}$: the average body weight of the adult person.

Target hazard quotient (THQ)

In order to establish carcinogenic risk resulting from consuming fish was calculated using USEPA (2011) guidelines as provided in Maliki & Maurya (2015), equation 3.

$$THQ = \frac{Efr \times ED \times FIR \times C}{BW \times RfD \times ATn} \times 0^{-3} \quad (3)$$

where: *Efr*=Exposure frequency (365days/year), *ED*=Exposure duration (65.5 years), which is life expectancy in Tanzania, FIR=Fish ingestion rate (7 kg/person.year), C=Metal cocentration, BW=Average body weight of an adult (70 kg), *RfD*=Reference dose as established by US EPA (2011), *ATn*=The average exposure time for non-carcinogens (365 days x no of exposure 65.5 years).

Furthermore, since the exposure to two or more heavy metals concentration may cause additive and or interactive effects, the total HRI of heavy metals for specific fish organ was also treated as the arithmetic sum of the specific metal HRI (Zheng et al., 2007; Akoto et al., 2014).

$$Total\ HRI = HRI_{metal_1} + HRI_{metal_2} + HRI_{metal_3} + \cdots HRI_{metal_n} \quad (4)$$

Data analysis

The results obtained from laboratory analysis were subjected to statistical analysis using various tools; a descriptive tool was used to determine the mean, standard deviations, and coefficient of variation, while the Pearson correlation coefficient was done to establish the dependence of heavy metals in fish organs. The nearer the coefficient to one (1) indicated a stronger correlation between variables, and the nearer to -1 indicated a decrease in a linear relationship (Kumari & Maiti, 2019). Non-parametric test using One-Way ANOVA was used to establish if there was a significant difference between heavy metals among fish parts, and the $p<0.05$ was considered significant (Akoto et al., 2014; Imlani et al., 2022). Principal Component Analysis (PCA) with Varimax rotation was used to reduce the large multi-dimensional dataset to a small number of new variables that accounted for at least 75% of the total variance (Leonard & Mahengea, 2022). Also, Hierarchical Cluster Analysis (HCA), using average linkage between groups, was used to assess the similarities and differences between fish organs and identify possible patterns in distributions of measured data.

Results and Discussion

The Quality of Fish Feed Used in Individually Maintained Fishponds

The results of this study revealed that fish feeds employed in all sampled fish farms were obtained from one supplier in two forms, powdery and pellets forms; thus, only one sample of each type was analyzed. Results from laboratory analysis indicated that fish feed in the form of pellets contained a higher concentration of heavy metals compared to that of powdery form. All fish feeds contained, to some extent, the concentration of Pb, Cu, Cr, and Zn. The concentration of Cu ranged from 16.2 mg/kg in the powdery form to 31.62 mg/kg in pellets, which is above the 30 mg/kg recommended maximum limit by FAO, and Zn concentration ranged from 32 mg/kg in the powdery form to 46 mg/kg in pellets that is above 30 mg/kg recommended by FAO and WHO. In addition, the concentration of Pb ranged from 6.3 mg/kg in the powdery form to 6.44 mg/kg in pellets, which were both above 1.5-2 mg/kg as recommended by WHO food safety guidelines and Cr concentration ranged from 1.3 mg/kg in the powdery form to 1.5 mg/kg in pellets that were below 12-13 mg/kg as recommended by USFDA guideline. The presence of trace elements in fish feeds is likely to influence the level of heavy metals in fish parts, hence bioaccumulation (Cohen et al., 1993).

The Concentration of Heavy Metals in Fish Parts

The concentration of heavy metals (Cr, Zn, Cu, and Pb) analyzed in tilapia and catfish from different parts, including gills, fins, guts, and muscles, are presented in Table 1. Except for Zn and Pb, all concentrations of Cr and Cu were within food safety guidelines (FSG) as proposed by different agencies. The two fish species had the lowest concentrations in muscles, while the highest concentrations of metals were observed in gills, possibly because gills are in direct contact with water (Maurya & Malik, 2019; Kumari & Maiti, 2019). The average concentration of Cr ranged from 0.001 mg/kg in muscles obtained from tilapia to 0.022 mg/kg in gills of the same fish, which is below the recommended USFDA 12-13 mg/kg food safety guidelines.

The concentration of Zn ranged from 13.65 mg/kg in muscles of catfish to 40.5 mg/kg in gills of tilapia, which is above 30 mg/kg FAO recommended guidelines. 18 out of 24 samples, equivalent to 75% of all samples from different fish parts, had elevated concentrations of Zn above recommended FAO guidelines, coinciding with previous studies (Kumari & Maiti, 2019). The concentration of Cu ranged from 1.28 mg/kg in muscles of catfish to 4.12 mg/kg in gills of tilapia; however, all concentrations of Cu from all samples were within recommended WHO food safety guidelines, while the concentration of Pb ranged from 1.09 mg/kg in muscles of catfish to 2.95 mg/kg in gills of tilapia that is above 1.5-2 mg/kg WHO food safety guidelines. These findings showed that fish had some levels of Pb, which might pose health effects if consumed (Akoto et al., 2014; Kumari & Maiti, 2019). Generally, 7 out of 24 samples, equivalent to 29.2%, had elevated Pb concentrations above WHO recommended food safety guidelines.

The concentrations of trace elements like Cu and Zn in recommended levels are essential for body growth (Mapenzi et al., 2019). People consume fish as a source of food and protein (Mapenzi et al., 2019); however, if the concentration of trace elements exceeds the allowable limits can pose serious health effects (Akoto et al., 2014; Kumari & Maiti, 2019). The concentrations of trace elements vary in fish parts and from one type to another depending on the ability of fish to bio accumulate and consumption habits (Mapenzi et al., 2019). For example, the concentrations of trace elements were higher in catfish compared to that of tilapia that were obtained from lake Rukwa and attributed to the reasons that catfish consume other small fish of different species, including tilapia, which feeds on phytoplankton (Nzeve et al., 2014; Mapenzi et al., 2019). Carnivores have the ability to accumulate heavy metals in all organs, including gills, muscles, and livers, compared to herbivores (Maurya & Malik, 2019) which coincides with the findings of this study (Table 1).

Table 1. Average and Standard Deviations (Mean±SD) of heavy metal concentration analyzed in some fish organs sampled in individually owned ponds, Dar es Salaam

Location	Fish Species	Organs	Cr (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Pb (mg/kg)
L1	Tilapia	Gills	0.003±0.001	34.88±5.091	3.15±0.58	2.16±0.318
		Fins	0.0015±0.0005	33.07±1.51	2.06±0.33	2.07±0.08
		Guts	0.0014±0.0005	30.76±2.068	3.033±0.21	1.96±0.13
		Muscles	0.001±0.00008	16.75±1.41	3.29±0.25	1.67±0.12
	Catfish	Gills	0.006±0.0015	36.05±1.30	2.89±0.36	1.46±0.04
		Fins	0.002±0.0005	32.08±0.66	2.17±0.17	1.12±0.1
		Guts	0.002±0.0006	35.86±5.26	3.46±0.51	1.63±0.24
		Muscles	0.002±0.0005	13.65±1.22	1.28±0.23	1.09±0.08
L2	Tilapia	Gills	0.003±0.002	35.76±5.34	3.71±0.59	2.86±0.42
		Fins	0.003±0.0001	31.79±1.43	2.88±0.20	1.82±0.13
		Guts	0.002±0.0001	32.85±2.40	3.15±0.21	1.71±0.16
		Muscles	0.001±0.00007	21.86±1.64	3.41±0.25	2.09±0.46
	Catfish	Gills	0.004±0.0005	34.36±1.65	3.41±0.24	2.11±0.05
		Fins	0.002±0.001	34.14±1.34	1.55±0.11	1.09±0.065
		Guts	0.003±0.0006	38.51±5.71	2.29±0.34	1.35±0.23
		Muscles	0.002±0.0005	21.40±2.63	2.07±0.55	1.09±0.08
L3	Tilapia	Gills	0.022±0.016	40.50±5.90	4.12±0.60	2.95±0.45
		Fins	0.002±0.0004	33.69±1.34	3.07±0.16	1.64±0.06
		Guts	0.002±0.0002	30.78±2.12	2.88±0.19	1.63±0.13
		Muscles	0.003±0.0005	17.54±1.33	3.76±0.32	1.86±0.24
	Catfish	Gills	0.004±0.001	36.60±0.32	3.97±0.25	2.25±0.06
		Fins	0.003±0.0005	33.26±0.21	2.26±0.16	1.11±0.05
		Guts	0.002±0.0003	39.49±5.78	3.96±0.59	1.25±0.19
		Muscles	0.002±0.0005	20.87±2.23	1.33±0.12	1.21±0.13
FSG (Food Safety Guidelines, mg/kg)			**12.0-13.0 (USFDA, 1993)**	**30.0 (FAO, 1983)**	**3 (WHO, 1995)**	**1.5-2.0 (WHO, 1995)**

Table 2. Pearson correlation between heavy metals in fish organs

Heavy Metals	Cr	Zn	Cu	Pb
Cr	1			
Zn	0.366481	1		
Cu	0.356376	0.368952	1	
Pb	**0.5124**	0.308934	**0.693594**	1

Table 3. The results from ANOVA single factor indicated no significant difference between tilapia and catfish in accumulating heavy metals

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.030764	1	0.031	0.00004	**0.995**	5.987
Within Groups	4306.888	6	717.8147			
Total	4306.919	7				

Table 4. Results from Principal Component Analysis (PCA) of trace elements analyzed in fish organs from individually owned fishponds in Dar es Salaam, Tanzania

Location	Fish Type	Fish Organ	PCA 1	PCA 2	PCA 3	PCA 4
L1	Tilapia	Gills	**0.55054**	0.29437	0.77749	-0.71028
		Fins	0.30797	-0.78218	2.0355	-1.0901
		Guts	0.010971	0.2861	0.42235	-0.8095
		Muscles	-1.8192	**1.1471**	-0.67438	-0.04197
	Catfish	Gills	**0.70007**	-0.41978	-0.67235	0.8717
		Fins	0.1765	-1.1349	-0.56439	0.16016
		Guts	**0.67875**	0.27161	-1.0131	-0.41939
		Muscles	-2.2367	-1.0773	0.57774	0.80782
L2	Tilapia	Gills	**0.67049**	**1.2077**	1.817	-1.4644
		Fins	0.14436	-0.00381	0.2703	-0.24194
		Guts	0.284	0.15756	-0.38256	-0.4247
		Muscles	-1.1497	**1.2255**	0.24378	-0.69173
	Catfish	Gills	**0.48383**	0.56332	0.2933	-0.31642
		Fins	0.4423	-1.9021	0.2053	0.051454
		Guts	**1.018**	-1.2316	-0.13638	-0.03715
		Muscles	-1.2197	-0.67944	-0.5033	0.59187
L3	Tilapia	Gills	**1.2923**	**1.4248**	**1.4881**	3.8425
		Fins	0.39314	-0.00851	-0.45387	-0.38724
		Guts	0.011837	-0.05335	-0.21948	-0.28418
		Muscles	-1.713	**1.6935**	-0.82693	0.34852
	Catfish	Gills	**0.77992**	**1.0971**	-0.11146	-0.50052
		Fins	0.33114	-1.1111	-0.71338	0.42721
		Guts	**1.1547**	0.38426	-2.6769	-0.0852
		Muscles	-1.2926	-1.3489	0.81763	0.40341

Table 5. Eigenvalue and variance from principal component analysis

PCA	Eigenvalue	% Variance
1	58.4377	98.568
2	0.731589	1.234
3	0.117406	0.19803
4	1.17E-05	1.98E-05

Results of this study indicated that there were variations of heavy metals concentration in fish parts, with muscles accumulating the lowest concentration while the highest concentrations were observed in the gills of both fish species, which is also similar to what Kumari & Maiti (2019) reported. The sum of all analyzed metals i.e., Cr, Zn, Cu and Pb in the fish parts indicated that the concentration was in the order of gills > fins > guts > muscles for tilapia i.e., 130 > 112.1 > 108.8 > 72.2 mg/kg while for catfish were in the order of gills > guts > fins > muscles in mg/kg i.e., 123.1 > 127.8 > 108.8 > 64 mg/kg. Maurya & Malik (2019) and Kumari & Maiti (2019) obtained similar findings whereby gills accumulated more heavy metals compared to those muscles, which are in agreement with the findings of this study.

Statistical Results from Analyzed Heavy Metals in Fish Parts

Pearson correlation between heavy metal concentrations in fish parts indicated that most heavy metals were weak to moderate correlated. For example, Cu was moderately correlated with Pb by r=0.69, Cr was correlated with Pb by r=0.51, while Cr was weakly correlated with Zn and Cu. Also, there was a weak correlation between Zn and Pb, as summarized in Table 2, indicating variation in fish organs' ability to accumulate heavy metals (Mahjoub et al., 2020). In addition, there was a strong correlation between heavy metals in tilapia and catfish with r=0.9, indicating that both fish have the ability to accumulate heavy metals in their organs. The results from ANOVA-single factor analysis showed that there was no significant difference between the ability of tilapia and catfish to accumulate heavy metals as the p-value was greater than 0.05, p=0.99, as shown in Table 3; thus, all fish types have the ability to accumulate heavy metals in their organs.

Principal Component Analysis (PCA)

Results from Principal Component Analysis (PCA) which analyzed inter-element correlations (Kumari & Maiti, 2019), indicated that PC1 provided 98.6% variance (Table 4) and is explained by the higher concentration of trace elements in gills of both fish types and guts of catfish as shown in Table 5 caused by the concentration of Zn as presented in Table 6. This indicates that fish accumulates more heavy metals in gills and least in muscles regardless of their species which is similar as reported in previous studies (Akoto et al., 2014; Kumari & Maiti, 2019).

Hierarchical Clustering Paired Group (UPGMA)

Hierarchical clustering paired group (UPGMA) indicates that at 18 distances, 5 groups of fish organs were created (Figure 2). A group of muscles is clearly distinguished from the rest of the groups, while, at 4 distances, more clear groups are further formed, including that of gills, fins, and guts. This further confirms that the muscles of fish accumulated the lowest concentration of metals compared to that in gills, fins, and guts, as also presented in Figure 3, showing three clear clusters of fish organs.

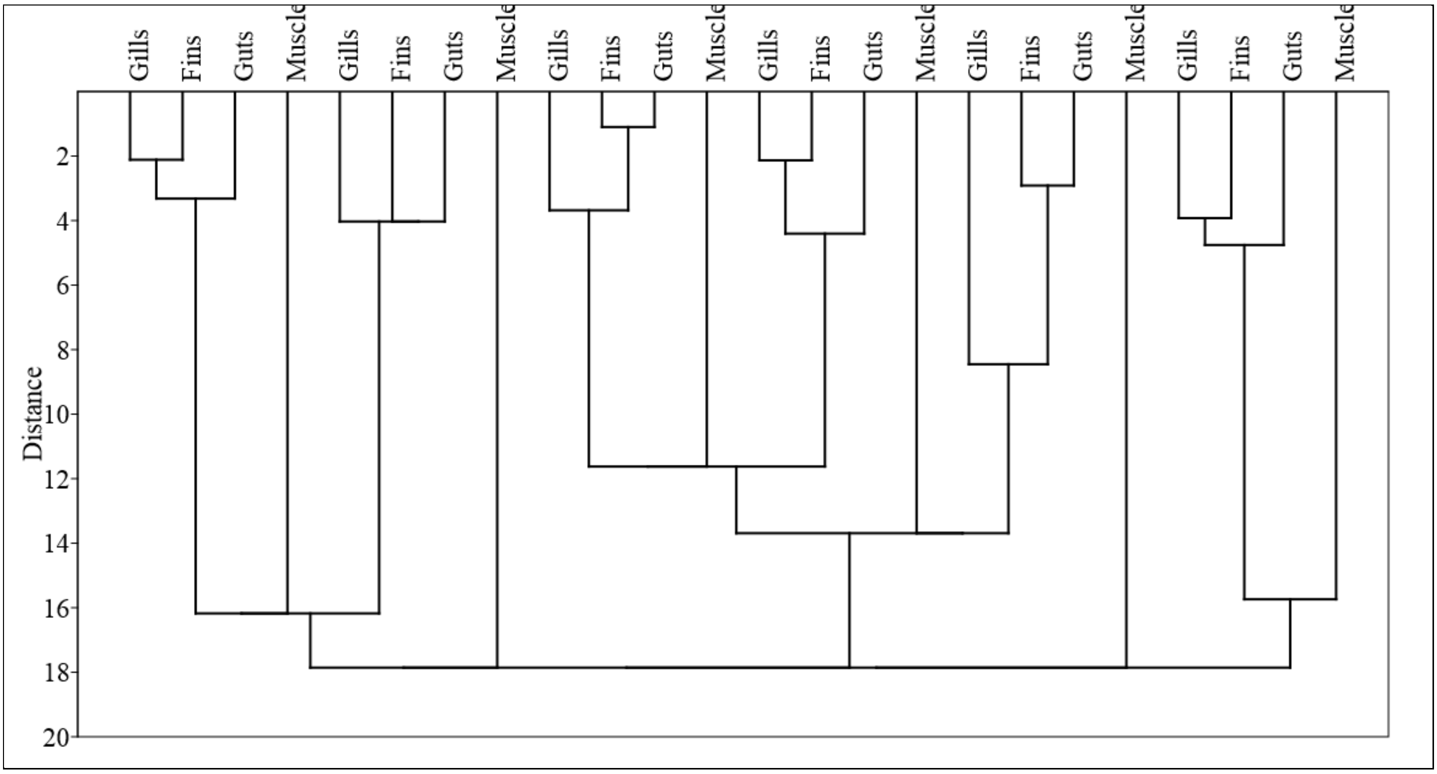


Figure 2. Hierarchical clustering paired group (UPGMA) using classical method among heavy metal concentrations in fish organs

Table 6. Loadings from principal component analysis

Heavy Metals	PCA 1	PCA 2	PCA 3	PCA 4
Cr	0.000199	0.001466	0.003526	0.99999
Zn	**0.99894**	-0.04606	-5.71E-05	-0.00013
Cu	0.040669	0.88262	-0.46832	0.000349
Pb	0.02162	0.46781	0.88355	-0.00381

Table 7. Results from estimated daily intake (EDI) and target hazard quotient (THQ) for different trace elements from consuming tilapia and catfish grown in privately-owned ponds

Fish type		Estimated Daily Intake (E_f x E_D x F_{IR} x C_f x C_m) /(W_{AB} xT_A)				Hazard Index (∑THQs)	Health Risk Index (HRI=EDI/RfD)			
		Cr	*Zn*	*Cu*	*Pb*		*Cr*	*Zn*	*Cu*	*Pb*
Tilapia	Gills	8.2x10^{-7}	0.0096	0.0009	0.0006	0.083	0.362	0.105	0.534	2.138
	Fins	4.1 x10^{-7}	0.0091	0.0006	0.0006	0.072	0.181	0.100	0.349	1.398
	Guts	3.8 x10^{-7}	0.0084	0.0008	0.0005	0.075	0.169	0.093	0.515	2.058
	Muscles	2.7 x10^{-7}	0.0046	0.0009	0.0005	0.061	0.121	0.051	0.558	2.233
Catfish	Gills	1.6 x10^{-6}	0.0099	0.0008	0.0004	0.073	0.724	0.109	0.490	1.961
	Fins	5.49 x10^{-7}	0.0088	0.0006	0.0003	0.059	0.241	0.097	0.368	1.473
	Guts	5.49 x10^{-7}	0.0098	0.0009	0.0004	0.079	0.241	0.108	0.587	2.348
	Muscles	5.49 x10^{-7}	0.0037	0.0004	0.0003	0.036	0.241	0.041	0.217	0.869
Tilapia	Gills	8.23 x10^{-7}	0.0098	0.0010	0.0008	0.097	0.362	0.108	0.629	2.518
	Fins	8.23 x10^{-7}	0.0087	0.0008	0.0005	0.074	0.362	0.096	0.489	1.954
	Guts	5.49 x10^{-7}	0.0090	0.0009	0.0005	0.075	0.241	0.099	0.534	2.138
	Muscles	2.7 x10^{-7}	0.0060	0.0009	0.0006	0.072	0.121	0.066	0.578	2.314
Catfish	Gills	1.1 x10^{-6}	0.0094	0.0009	0.0006	0.084	0.483	0.104	0.578	2.314
	Fins	5.49 x10^{-7}	0.0094	0.0004	0.0003	0.057	0.241	0.103	0.263	1.052
	Guts	8.23 x10^{-7}	0.0106	0.0006	0.0004	0.069	0.362	0.116	0.388	1.554
	Muscles	5.49 x10^{-7}	0.0059	0.0006	0.0003	0.049	0.241	0.065	0.351	1.405
Tilapia	Gills	6.03 x10^{-6}	0.0111	0.0011	0.0008	0.109	2.654	0.122	0.699	2.796
	Fins	5.49 x10^{-7}	0.0092	0.0008	0.0004	0.074	0.241	0.102	0.521	2.083
	Guts	5.49 x10^{-7}	0.0084	0.0008	0.0004	0.070	0.241	0.093	0.489	1.954
	Muscles	8.23 x10^{-7}	0.0048	0.0010	0.0005	0.067	0.362	0.053	0.638	2.551
Catfish	Gills	1.1 x10^{-6}	0.0100	0.0011	0.0006	0.092	0.483	0.110	0.673	2.694
	Fins	8.23 x10^{-7}	0.0091	0.0006	0.0003	0.061	0.362	0.100	0.383	1.534
	Guts	5.49 x10^{-7}	0.0108	0.0011	0.0003	0.080	0.241	0.119	0.672	2.687
	Muscles	5.49 x10^{-7}	0.0057	0.0004	0.0003	0.045	0.241	0.063	0.226	0.903

Health Risk Assessment

The results of human health risks from consuming fish grown in privately owned fishponds are summarized in Table 7. The estimated daily intake of all heavy metals in bodies was in the rank of Zn > Cu > Pb > Cr, which is in agreement with previous studies done by Maurya & Malik (2019). The EDI of Cr was the lowest in all four heavy metals ranging from 2.74 x 10^{-7} to 6.03 x 10^{-6} mg/kg, while the highest EDI was obtained in Zn, which ranged from 0.0037 to 0.011 mg/kg. The hazard index (HI) of all heavy metals ranged from 0.036 to 0.11, and the lowest was observed in muscles while the maximum was observed in gills, as shown in Table 7. However, the health risk index was in the order of Zn < Cr < Cu < Pb, as detailed in Table 7. The HRI for Cr, Zn, and Cu except for gills in tilapia were less

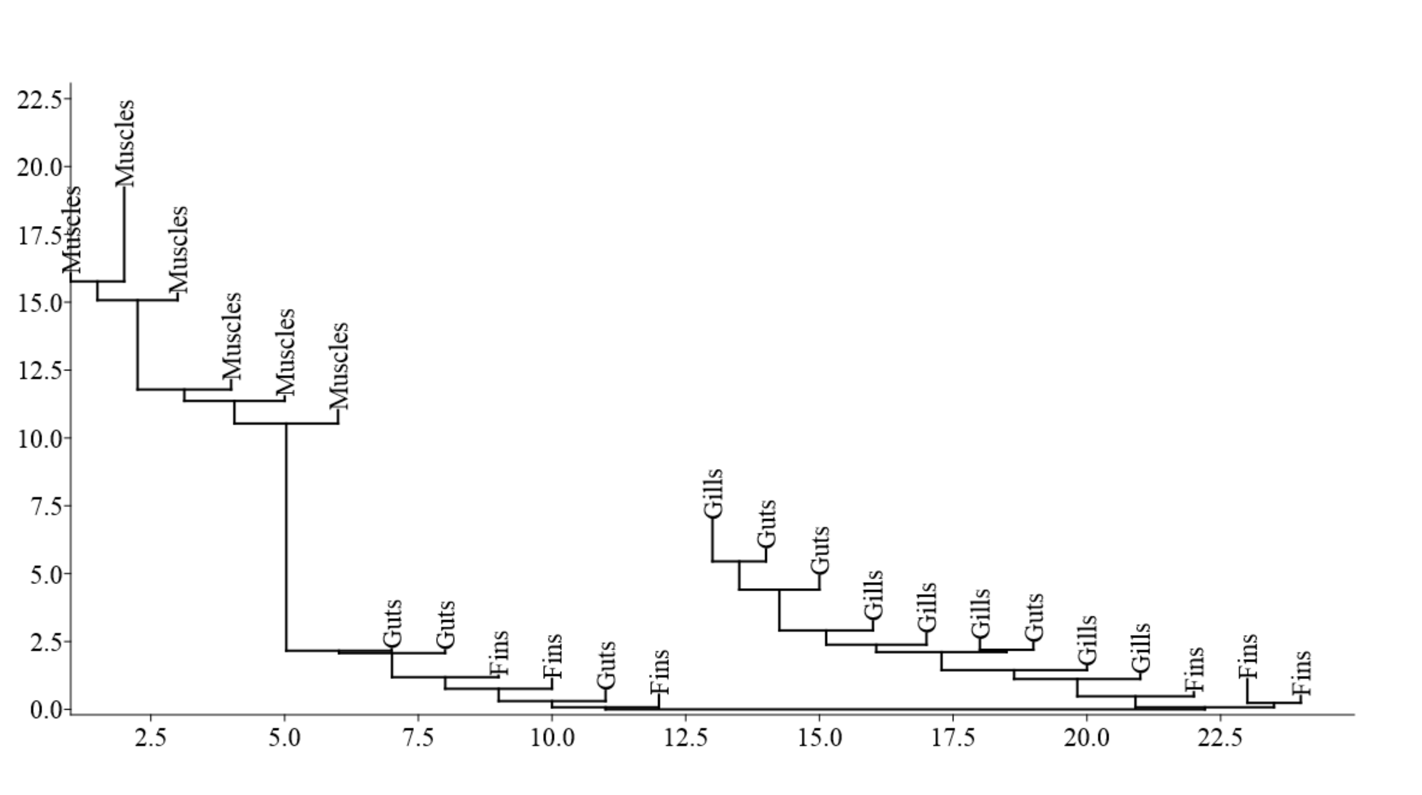


Figure 3. Hierarchical clustering paired group (UPGMA) using neighbor-joining clustering among heavy metal concentrations in fish organs

than one, indicating that humans would not experience any significant health risk caused by the Zn, Cr, and Cu concentrations in fish organs; however, they are likely to experience significant health effects caused by Pb as the HRI>1 across all fish organs. This indicates that humans consuming fish grown in privately owned fishponds are likely to experience effects of Pb concentration, including a reduction in cognitive development and intellectual performance in children (Darko et al., 2016), causing renal tumors and increased blood pressure in adults, cause gastrointestinal disorders and liver impairments (Akoto et al., 2014; Kumari & Maiti, 2019).

The findings of this study coincide with a study done by Kumari & Maiti (2019) in India and Mahjoub et al. (2020) in Morocco, who also established HR>1 for Pb in sampled fish and the concentration of Pb was significantly higher compared to other trace elements. Trace metals bioaccumulation is associated with fish type, type of heavy metals, age, and chemical characteristics of water (Mahjoub et al., 2020). Thus, consuming contaminated fish with non-essential elements especially trace elements, may pose a health risk to humans due to their resistance to degradation and their accumulation in the living organisms (Mahjoub et al., 2020).

Conclusion and Recommendation

This study concludes that there are considerable levels of heavy metals in fish grown in privately owned fish ponds, especially in tilapia and catfish; however, the concentrations vary from one fish organ to another in the order of muscles<fins<guts<gills. The levels of Cr, Cu, and Zn were within the permissible limit in almost all fish organs (gills, fins, guts, and muscles). However, the concentration of Pb was above the permissible limit across all fish organs, which portrays the possibility of carcinogens, especially when consuming fish gills and guts. The HRI indicated that there were no significant human health effects caused by the individual concentration of Cr, Zn, and Cu since HRI<1; however, the HRI>1 was caused by Pb concentrations across all fish organs, indicating that there would be some combined effects of all heavy metals to human health, and thus posing a possible health risk to consumers in the studied area. Since heavy metals have a tendency of bioaccumulation in body tissues due to the biodegradability of toxic metals, needed actions should be considered to minimize metal supplements in fish feeds, and frequent monitoring of fish feeds and fish quality grown in privately owned fish ponds is recommended so as to safeguard consumers. This study should be regarded as an open gate for the Tanzania National Bureau of Standard (TBS) to guide the fish farmers and consumers to accentuate public health by establishing relevant permissible limits and conducting spot checks to establish the quality of fish grown in privately owned fishponds.

Acknowledgements

Strong cooperation provided by fish pond owners in Dar es Salaam, relevant permissions from local government authorities in Dar es Salaam, and SEST laboratory staff during analytical works and the working environment at Ardhi University are highly appreciated.

Compliance With Ethical Standards

Authors' Contributions

LSL: Study design, data evaluation, statistical analysis, article writing

AM: Article reviewing and editing

NCM: Sampling, handling, transportation of the samples, and laboratory analysis

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

This research received no specific grant from any funding agency in the public, commercial or non-profit organization.

References

Akoto, O., Eshunu, B. F., Darko, G., & Adei, E. (2014). Concentrations and Health risk assessment of heavy metals in fish from the Fosu Lagoon. *International Journal of Environmental Research*, *8*(2), 403-410. https://doi.org/10.22059/ijer.2014.731

Cohen, A. S., Bills, R., Cocquyt, C. Z., & Caljon, A. G. (1993). The impact of sediment pollution on biodiversity in Lake Tanganyika. *Conservation Biology*, *7*(3), 667-677. https://doi.org/10.1046/j.1523-1739.1993.07030667.x

Darko, G., Azanu, D., & Logo, N. K. (2016). Accumulation of toxic metals in fish raised from sewage-fed aquaculture and estimated health risks associated with their consumption. *Cogent Environmental Science*, *2(1)*, 1190116. https://doi.org/10.1080/23311843.2016.1190116

Deloitte. (2015). Market study on the aquaculture sector in East Africa.

FAO. (1983). Compilation of legal limits for hazardous substances in fish and fishery products.

Imlani, A. H., Tastan, Y., Tahiluddin, A. B., Bilen, S., Jumah, Y. U., & Sonmez, A. Y. (2022). Preliminary determination of heavy metals in sediments, water and some macroinvertebrates in Tawi-Tawi Bay, Philippines. *Marine Science and Technology Bulletin*, *11*(1), 113-122. https://doi.org/10.33714/masteb.1070711

Kosygin, L., Dhamendra, H., & Gyaneshwari, R. (2007). Pollution status and conservation strategies of Moirang River, Manipur with a note on its aquatic bio-resources. *Journal of Environmental Biology*, *28*(3), 669–673.

Kumari, P., & Maiti, S. K. (2019). Health risk assessment of lead, mercury, and other metal(loids): A potential threat to the population consuming fish inhabiting, a lentic ecosystem in Steel City (Jamshedpur), India. *Human and Ecological Risk Assessment: An International Journal*, *25*(8), 2174-2192. https://doi.org/10.1080/10807039.2018.1495055

Kyelu, A. (2016). *Analysis of socio-economic and environmental effects of urban fish farming in Dar es Salaam, Tanzania*. [Master's Thesis. Sokoine University of Agriculture].

Leonard, L. S., & Mahengea, A. (2022). Assessment of water quality from privately owned fish ponds used for aquaculture in Dar es Salaam, Tanzania. *Applied Journal of Environmental Engineering Science*, *8*(1), 20-33. https://doi.org/10.48422/IMIST.PRSM/ajees-v8i1.29831

Leonard, L. S., Mwegoha, W. J. S., & Kihampa, C. (2012). Heavy metal pollution and urban agriculture in Msimbazi River Valley: Health risk and public awareness. *International Journal of Plant, Animal and Environmental Sciences*, *2*(2), 107-118.

Mahjoub, M., Maadoudi, M. E. I., & Smiri,Y. (2020). Metallic contamination of the muscles of three fish species from the Moulouya River (Lower Moulouya, Eastern Morocco). *International Journal of Ecology*, *2020*, 8824535. https://doi.org/10.1155/2020/8824535

MALF. (2015). Annual Fisheries Statistics, Fisheries Statistics. The United Republic of Tanzania, Ministry of Agriculture livestock and Fisheries Ministry of Agriculture, Livestock and Fisheries.

Maliki, D. S., & Maurya, P. K. (2015). Heavy metal concentration in water, sediment, and tissues of fish species (*Heteropneustis fossilis* and *Puntius ticto*) from Kali River, India. *Toxicological & Environmental Chemistry*, *96*(8), 1195-1206. https://doi.org/10.1080/02772248.2015.1015296

Mannzhi, M. P., Edokpayi, J. N., Durowoju, O. S., Gumbo, J., & Odiyo, J. O. (2021). Assessment of selected trace metals in fish feeds, pond water and edible muscles of *Oreochromis mossambicus* and the evaluation of human health risk associated with its consumption in Vhembe district of Limpopo Province, South Africa. *Toxicology Reports*, *8*, 705-717. https://doi.org/10.1016/j.toxrep.2021.03.018

Mapenzi, L. L., Shimba, M. J., Moto, E. A, Maghembe, R. S., & Mmochi, A. J. (2019). Heavy metals bio-accumulation in tilapia and catfish species in lake Rukwa ecosystem Tanzania. *Journal of Geochemical Exploration*, *208*, 106413. https://doi.org/10.1016/j.gexplo.2019.106413

Matusch, A., Depboylu, C., Palm, C., Wu, B., Gunter, U., Hoglinger, G. U., Schafer, M. K. H., & Becker, J. S. (2010). Cerebral bioimaging of Cu, Fe, Zn and Mn in the MPTP mouse model of Parkinson's disease using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), *Journal of the American Society for Mass Spectrometry*, *21*(1), 161-171. https://doi.org/10.1016/j.jasms.2009.09.022

Maurya, P. K., & Malik, D. S. (2019). Bioaccumulation of heavy metals in tissues of selected fish species from Ganga River, India, and risk assessment for human health. *Human and Ecological Risk Assessment: An International Journal*, *25*(4), 905-923. https://doi.org/10.1080/10807039.2018.1456897

Mohamad, N. A., Mohamadin, M. I., Ahmad, W. A. R. W., & Sahari, N. (2017). Heavy metals concentrations in catfish (*Clarius gariepinus*) from three different farms in Sarawak, Malaysia. *Scientific International Lahore*, *29*(1), 51-55.

Monteiro, D. A., Rantin, F. T., & Kalinin, A. L. (2013). Dietary intake of inorganic mercury: bioaccumulation and oxidative stress parameters in the neotropical fish *Hoplias malabaricus*. *Ecotoxicology*, *22*, 446-456. https://doi.org/10.1007/s10646-012-1038-5

Mwegoha, W. J. S., & Kihampa, C. (2010). Heavy metal contamination in agricultural soils and water in Dar es Salaam city, Tanzania. *African Journal of Environmental Science and Technology, 4*(11), 763-769.

Nzeve, J. K., Njuguna, S. G., & Kitur, E. C. (2014). Bioaccumulation of Heavy metals in *Clarias gariepinus* and *Oreochromis spirulus* Niger from Masinga Reservoir, Kenya. *Journal of Environmental Science, Toxicology and Food Technology, 8*(10), 58-63.

Resma, N. S., Meaze, A. M. H., Hossain, S., Khandaker, M. U., Kamal, M., & Deb, N. (2020). The presence of toxic metals in popular farmed fish species and estimation of health risks through their consumption. *Physics Open, 5*, 100052. https://doi.org/10.1016/j.physo.2020.100052

Rukanda, J. J. (2016). Evaluation of aquaculture developments in Tanzania. Nations University Fisheries Training Programme, Iceland (final project). http://www.unuftp.is/static/ferrows/document/janeth16aprt.pdf

Rukanda, J. J. (2018). Evaluation of aquaculture development in Tanzania. Nations University Fisheries Training Programme, Iceland [final project]. https://www.grocentre.is/static/gro/publication/353/document/janeth16aprf.pdf

Sarkar, M. M., Rohani, M. F., & Hossain, M. A. R. (2022). Evaluation of heavy metal contamination in some selected commercial fish feeds used in Bangladesh. *Biological Trace Element Research*, *200*, 844–854. https://doi.org/10.1007/s12011-021-02692-4

Shaker, I. M., Elnady, M. A., Abdel-Wahed, R. K., & Soliman, M. A. M. (2018). Assessment of heavy metals concentration in water, sediment and fish under different management systems in earthen ponds. *Egyptian Journal of Aquatic Biology and Fisheries, 22*(1), 25-39. https://doi.org/10.21608/ejabf.2018.7704

URT. (2015). Ministry of Agriculture, Livestock and Fisheries, Fisheries Development Division, Annual Fisheries Statistics Report, 2015-2016.

URT. (2019). Ministry of Livestock and Fisheries. Livestock and Fisheries Commodity Value Chain Briefs.

USEPA. (2011). Exposure Factors Handbook: National Center for Environmental Assessment, Washington, DC; EPA/600/R-09/052F. Available from the National Technical Information Service, Springfield, VA, and online at https://www.epa.gov/ncea/efh

USFDA. (1993). Guidance documents for trace elements in seafood. Washington DC: US Food and Drug Administration

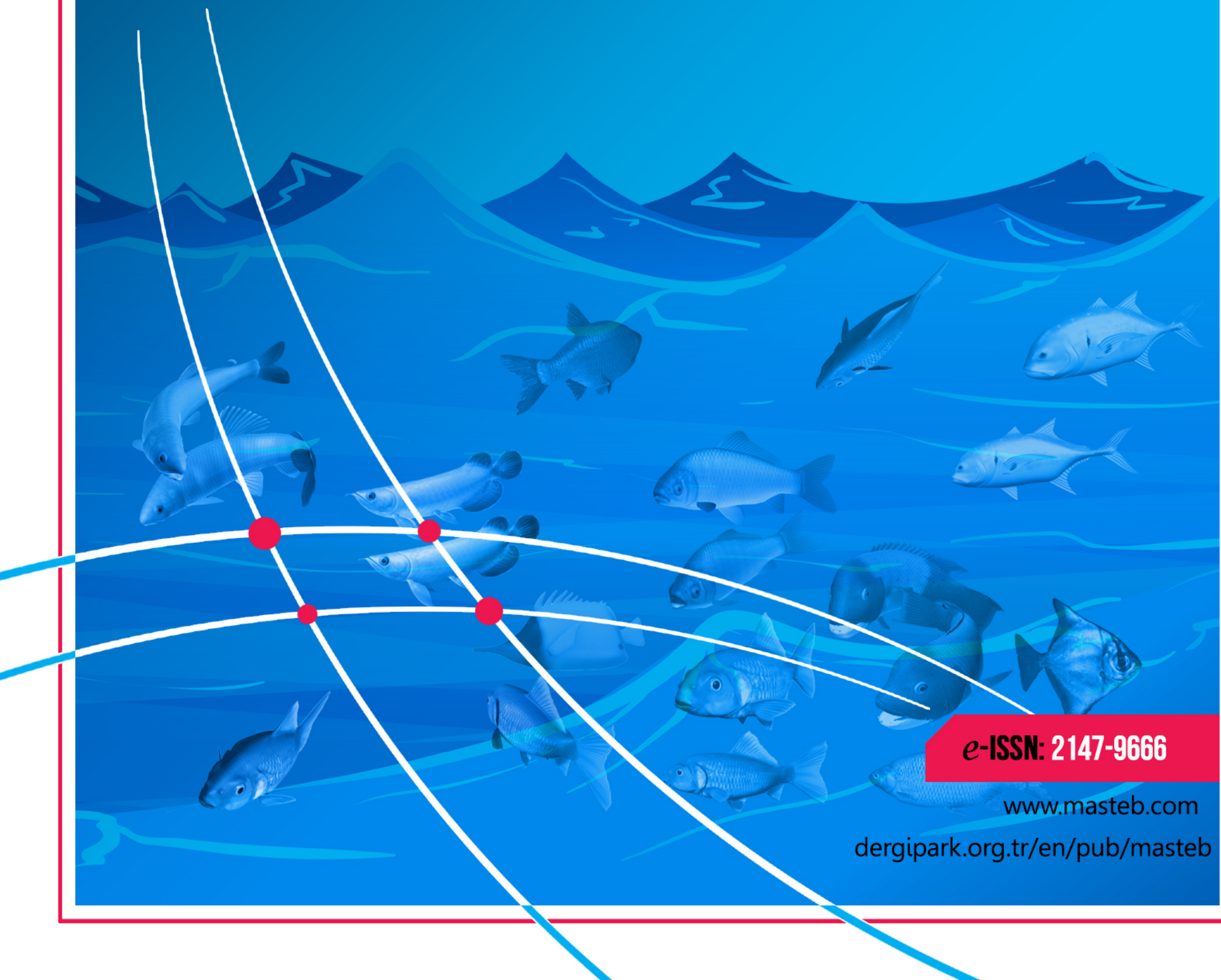
Vitek, T., Spurný, P., Mareš, J., & Zikova, A. (2007). Heavy metal contamination of the Loučka River water ecosystem. *Acta Veterinaria Brno*, *76*(1), 149-154.

Wenaty, A., Mabiki, F., Chove, B., & Mdegela, R. (2018). Fish consumers preferences, quantities of fish consumed and factors affecting fish eating habits: A case of Lake Victoria in Tanzania. *International Journal of Fisheries and Aquatic Studies*, *6*(6), 247-252.

WHO. (1995). The Food Safety (General Food Hygiene) Regulations 1995.

Zheng, N., Wang, O., Zhang, X, Zheng, D., Zhang, Z., & Zhang, S. (2007). Population health risk due to dietary intake of heavy metals in the industrial area of Huludao City, China. *Science of The Total Environment*, *387*(1-3), 96-104. https://doi.org/10.1016/j.scitotenv.2007.07.044

MARINE SCIENCE AND TECHNOLOGY BULLETIN



e-ISSN: 2147-9666

www.masteb.com

dergipark.org.tr/en/pub/masteb