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  - o Sonuçlar
  - o Etik Standartlara Uyum
    - a) Yazarların Katkıları
    - b) Çıkar Çatışması
    - c) Hayvanların Refahına İlişkin Beyan
    - d) İnsan Hakları Beyanı
  - o Kaynaklar
  - o Çizelge(ler) (metinde uygun konumda)
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Makale boyunca A4 boyutundaki kağıdın tüm kenarlarında çift aralıklı ve 25 mm kenar boşluklu referanslar, tablo başlıkları ve şekil başlıkları dahil olmak üzere 12 puntoluk bir yazı tipi kullanın (Times New Roman). Sayfanın bütün yönlerinde 25 mm'lik kenar boşlukları kullanın. Metin tek sütun formatında olmalıdır Yazarların şablon dosyalarını aşağıdaki bağlantılardan indirmeleri önerilir:

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Lütfen makale için yazarların katkılarını sağlayın. Ad ve soyadlarının ilk harflerini kullanın (örneğin; Yazar MO çalışmayı tasarladı, MF makalenin ilk taslağını yazdı, AF istatistiksel analizleri gerçekleştirdi ve yönetti. Tüm yazarlar son makaleyi okudu ve onayladı.).

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Mevcut herhangi bir çıkar çatışması burada verilmelidir. Çatışma yoksa, yazarlar şunları belirtmelidir:
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Çalışmada hayvan kullanılmışsa; Araştırma için kullanılan hayvanların refahına saygı gösterilmelidir. Hayvanlar üzerindeki deneyleri bildirirken, yazarlar aşağıdaki ifadeyi belirtmelidir:
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d) İnsan Hakları Beyanı
İnsan katılımcıları içeren çalışmaları bildirirken, yazarlar aşağıdaki ifadeyi eklemelidir:
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KAYNAKLAR

<u>Metinde Alıntı;</u>
Lütfen metinde geçen her bir atıfın kaynaklar listesinde de sunulduğundan emin olun. Metindeki literatürü kronolojik olarak, ardından bu örnekler gibi alfabetik sırayla belirtin "(Elp vd., 2018; Biswas vd., 2016; Elp ve Osmanoğlu, 2019)". Atıfta bulunulan kaynak bir cümlenin konusuysa, parantez içinde yalnızca tarih verilmelidir. Bu örnek gibi biçimlendirilmiştir: "Durmaz (2007) .... etkinliğini araştırmıştır".
• Tek yazar: yazarın soyadı ve yayın yılı (Elp, 2017)
• İki yazar: hem yazarların soyadları hem de yayın yılı (Adem ve Elp, 2017)

• Üç veya daha fazla yazar: birinci yazarın soyadı ve ardından "ve diğerleri". ve Elp et al., 2018 yayın yılı)

Kaynaklar Listesinde Alıntı;
Kaynaklar önce alfabetik olarak sıralanmalı ve daha sonra makalenin sonunda kronolojik olarak sıralanmalıdır. Aynı yazar (lar) dan aynı yıl içinde birden fazla kaynak yayın tarihinden (2016a) sonra yerleştirilen a, b, c vb. Harflerle belirtilmelidir. Çevrimiçi olarak yayınlanan makalelerin, kitapların, çok yazarlı kitapların ve makalelerin alıntıları aşağıdaki örneklere uygun olmalıdır:

Makale:
Adem, S. S., & Elp, M. (2017). Muscle spindle and comparison of fish muscle spindle with other vertebrates. Alinteri Journal of Agriculture Sciences, 32(2): 113-117

Durmaz, Y. (2007). Vitamin E (alpha-tocopherol) production by the marine microalgae Nannochloropsis oculata (Eustigmatophyceae) in nitrogen limitation. Aquaculture, 272(4): 717-722.

Elderwish, N., M., Taştan, Y. & Sönmez, A. Y., (2019). Türkiye'nin batı karadeniz kıyı sularındaki ağır metal birikimin mevsimsel olarak incelenmesi. Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi, 5(2): 1-8.

Elp, M., Osmanoğlu, M. İ., Kadak, A. E., & Turan, D., (2018). Characteristics of Capoeta oguzelii, a new species of cyprinid fish from the Ezine Stream, Black Sea basin, Turkey (Teleostei: Cyprinidae). Zoology in the Middle East. 64(2): 102–111. https://doi.org/10.1080/09397140.2018.1442295

Sönmez, A. Y., Kale, S., Özdemir, R. C. & Kadak, A. E. (2018). An adaptive neuro-fuzzy inference system (ANFIS) to predict of cadmium (Cd) concentration in the Filyos River, Turkey. Turkish Journal of Fisheries and Aquatic Sciences, 18(12): 1333-1343. https://doi.org/10.4194/1303-2712-v18_12_01

Kitap:
Brown, C., Laland, K. & Krause, J. (Eds.) (2011). Fish Cognition and Behavior. 2nd ed. Oxford, UK: Wiley-Blackwell. 472p.

Kitap bölümü:
Langston, W. J. (1990). Toxic effects of metals and the incidence of marine ecosystems, pp. 102-122. In: Furness, R. W. (Ed.), Rainbow Heavy Metals in the Marine Environment. New York, USA: CRC Press. 256p.
Vassallo, A. I. & Mora, M. S. (2007). Interspecific scaling and ontogenetic growth patterns of the skull in living and fossil ctenomyid and octodontid rodents (Caviomorpha: Octodontoidea).pp. 945-968. In: Kelt, D. A., Lessa, E., Salazar-Bravo, J. A., Patton, J. L. (Eds.), The Quintessential Naturalist: Honoring the Life and Legacy of Oliver P. Pearson. 1st ed. Berkeley, CA, USA: University of California Press. 981p.

Tez:
Elp, M. (2002). Koçköprü baraj gölü'nde (Van) yaşayan siraz *(Capoeta capoeta*, Guldensteadt, 1772) ve inci kefali (*Chalcalburnus tarichi*, Pallas, 1811) populasyonları üzerine bir araştırma. Ph.D. Thesis. İstanbul University, İstanbul, Turkey.

Konferans bildirimleri:
Notev, E. & Uzunova, S. (2008). A new biological method for water quality improvement. Proceedings of the 2nd Conference of Small and Decentralized Water and Wastewater Treatment Plants, Greece, pp. 487-492.

Enstitü yayınları:
FAO. (2016). The State of World Fisheries and Aquaculture: Contributing to food security and nutrition for all. Rome. 200 pp.

Rapor:
FAO. (2018). Report of the ninth session of the Sub-Committee on Aquaculture. FAO Fisheries and Aquaculture Report No. 1188. Rome, Italy.

İnternet kaynakları:
Froese, R. & Pauly, D. (Eds.) (2018). FishBase. World Wide Web electronic publication. Retrieved on January 11, 2018 from http://www.fishbase.org.

TurkStat. (2019). Fishery Statistics. Retrieved on December 28, 2019 from http://www.turkstat.gov.tr/

Çizelge(ler)
Arapça olarak numaralandırılmış çizelgeler, üstte kısa bir açıklayıcı başlık ile ayrı sayfalarda yer almalıdır. Dipnotları çizelge gövdesinin altındaki tablolara yerleştirin ve bunları küçük harflerle (veya anlamlılık değerleri ve diğer istatistiksel veriler için yıldız işaretleriyle) belirtin. Dikey kurallardan kaçının. Çizelgelerde sunulan veriler, makalenin başka bir yerinde açıklanan sonuçları tekrar etmemelidir.

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  b) Conflict of Interest
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REFERENCES

<u>Citation in text;</u>
Please ensure that each reference cited in the text is also presented in the reference list. Cite literature in the text in chronological, followed by alphabetical order like these examples "(Elp et al., 2018; Biswas et al., 2016; Elp and Osmanoğlu, 2019)". If the cited reference is the subject of a sentence, only the date should be given in parentheses. Formatted like this example: "Durmaz (2007) investigated the efficacy of…".

• Single author: the author's surname and the year of publication (Elp, 2017)
• Two authors: both authors' surnames and the year of publication (Adem and Elp, 2017)
• Three or more authors: first author's surname followed by "et al." and the year of publication Elp et al., 2018)

Citation in the reference list;
References should be listed first alphabetically and then further sorted chronologically at the end of the article. More than one reference from the same author(s) in the same year must be identified by the letters a, b, c, etc. placed after the year of publication (2016a). The citation of articles, books, multi-author books and articles published online should conform to the following examples:

Article:
Adem, S. S., & Elp, M. (2017). Muscle spindle and comparison of fish muscle spindle with other vertebrates. Alinteri Journal of Agriculture Sciences, 32(2): 113-117

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Book:
Brown, C., Laland, K. & Krause, J. (Eds.) (2011). Fish Cognition and Behavior. 2nd ed. Oxford, UK: WileyBlackwell. 472p.

Chapter:
Langston, W. J. (1990). Toxic effects of metals and the incidence of marine ecosystems, pp. 102-l22. In: Furness, R. W. (Eds.), Rainbow Heavy Metals in the Marine Environment. New York, USA: CRC Press. 256p.

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Conference Proceedings:
Notev, E. & Uzunova, S. (2008). A new biological method for water quality improvement. Proceedings of the 2nd Conference of Small and Decentralized Water and Wastewater Treatment Plants, Greece, pp. 487-492.

Institution Publication:
FAO. (2016). The State of World Fisheries and Aquaculture: Contributing to food security and nutrition for all. Rome. 200 pp.

Report:
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TurkStat. (2019). Fishery Statistics. Retrieved on December 28, 2019 from http://www.turkstat.gov.tr/

Table(s)
Tables, numbered in Arabic, should be in separate pages with a short descriptive title at the top. Place footnotes to tables below the table body and indicate them with superscript lowercase letters (or asterisks for significance values and other statistical data). Avoid vertical rules. The data presented in tables should not duplicate results described elsewhere in the article.

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All illustrations should be labelled as 'Figure' and numbered in consecutive Arabic numbers, Figure 1, Figure 2 etc. in the text. If panels of a figure are labelled (a, b, etc.) use the same case when referring to these panels in the text. Figures are recommended to be in electronic formats such as PNG, JPEG. TIFF (min. 300 dpi) should be also arranged in available dimensions. All figures or tables should be presented in the body of the text. Font sizes size should be from 9 to 11 points.

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Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2023; 9(1): 1-6 Araştırma Makalesi/Research Article

Chromosomal analysis of *Sander lucioperca* (L., 1758) (Perciformes: Percidae) from Turkey

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[2]Kırşehir Ahi Evran Üniversitesi, Fen Edebiyat Fakültesi, Moleküler Biyoloji ve Genetik Bölümü, Kırşehir/Türkiye
[3]Kırşehir Ahi Evran Üniversitesi, Sağlık Bilimleri Fakültesi, Beslenme ve Diyetetik Bölümü, Kırşehir/Türkiye

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- Constitutive heterochromatin region
- Nucleolus organizer region

Abstract

Sander lucioperca (pikeperch) is a percid fish species of high commercial value and potential for being aquaculture in Turkey. However, karyological studies are deficient for population of Turkey. So, the aim of this study is to carry out diploid chromosome number, karyotype formula, fundamental arm number and chromosomal banding properties (with C-banding and Ag-NOR staining) of *S. lucioperca*. Specimens of *S. lucioperca* were captured from Konya, Turkey and alive specimens carried to the laboratory. Chromosome obtaining was provided by using air-drying technique from the head kidney. Chromosome slides were prepared and banding procedures were applied. Result of the analysis, diploid chromosome number was found as 48 and karyotype of the pikeperch consist of 32 biarmed and 16 uniarmed chromosomes. Constitutive heterochromatin regions were observed on the pericentromeres of some of the chromosomes. Ag-NORs were determined on one pair of submetacentric chromosome. This report is the first that determines chromosomal properties of *S. lucioperca* from Turkey. This study may contribute the cytogenetic information of this species.

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INTRODUCTION

Totally 384 fish species distribute in the inland waters of Turkey. A total of 32 species have been introduced deliberately or accidentally (Çiçek et al., 2020). The family Percidae has three subfamilies, these are Percinae, Luciopercinae and Etheostomatinae. The subfamily Luciopercinae Jordan and Evermann, 1896 has three valid genera (*Sander*, *Zingel* and *Romanichthys*) and 10 valid species. The genus *Sander* Oken, 1817 belongs to the subfamily Luciopercinae. This genus has five species named as *Sander canadensis*, *S. lucioperca*, *S. marinus*, *S. vitreus* and *S. volgensis* (Fricke et al., 2021). From this species, *S. lucioperca* is a fish species originating from Europe and was introduced into the inland waters of Turkey since 1950's (Küçük, 2012). Pikeperch is distributed widely and highly a popular fish in Turkey with good export prospects (Ablak & Yılmaz, 2004; Küçük, 2012). Turkey is listed as one of the top pikeperch producers (Küçük, 2012). Otherwise, this piscivorous fish has destructive effects on the native fish taxa especially on the endemic and restricted species (Küçük, 2012). Endemic fish species of Turkey like *Alburnus akili* and *Pseudophoxinus handlirchii* are extinct. One of the reasons for this extinction is the introduction of predatory *S. lucioperca* in Eğirdir and Beyşehir Lakes (Küçük, 2012). Also, the population of *Pseudophoxinus anatolicus* is extinct due to the introduction of *S. lucioperca* in Beyşehir Lake (Sasi, 2011).

The methodologies used for chromosomal obtaining and karyotype analysis have been developed so much in the last years. Fish cytogenetic is a useful area in cytotaxonomy, fish breeding in aquaculture, in phylogenetic studies and detecting variations within and among the populations (Martins et al., 2011).

Cytogenetic studies especially in endemic freshwater fish species are very popular in Turkey (Karasu-Ayata et al., 2021; Unal-Karakuş, 2021). Also this studies have been carried out on the Turkey populations of conventional distributed fish species like *Carassius auratus* (Ölmez-Aydın & Kuru, 2001), *Gobius paganellus* (Ergene-Gözükara & Çavaş, 2002), *Oncorhynchus mykiss* (Örs, 2003), *Alburnoides bipunctatus* (Kılıç-Demirok & Ünlü, 2004), *Anguilla anguilla* (Turan et al., 2005), *Silurus glanis* (Aydın, 2005), *Pseudorosbora parva* (Karasu-Ayata et al., 2016), *Cyprinus carpio* (Unal & Gaffaroğlu, 2016), *Rhodeus amarus* (Karasu-Ayata et al., 2021) and *Esox lucius* (Arslan & Alpaslan, 2020). Above mentioned studies were conducted in the determination of the diploid chromosome number, chromosome morphology and conventional chromosomal banding techniques (especially C-banding and silver staining of NORs). Some cytogenetic studies available from different countries in *S. lucioperca* (Ráb et al., 1987; Mirinargesi et al., 2007; Jankun et al., 2014) to date. However, cytogenetics of *S. lucioperca* population from

Turkey have not been studied. It is necessary to study *Sander lucioperca*'s chromosomal characteristics since the Turkish population has not been studied and it harms Anatolian endemic species in particular. So, the aim of this study is to determine cytogenetic properties of *S. lucioperca* with conventional cytogenetic techniques.

MATERIALS AND METHODS

Seven specimens (four females, three males) of *S. lucioperca* were collected from Kayabaşı Stream, Beyşehir, Konya, Turkey (37°30′N, 31°31′E) by electrofishing. The individuals were carried alive to the laboratory and kept in well aerated aquarium until analysis. The fishes were treated with the guidelines of the local ethics committee of Kırşehir Ahi Evran University (Protocol Number: 68429034/05). The air-drying technique of Bertollo et al. (2015) was performed on the head kidney for chromosome preparation. The fish were injected intraperitoneally with 0.1% colchicine solution (1 ml per 100 g body weight) and kept in aerated aquaria for 2 h. Then the head kidneys of the specimens were removed and placed in hypotonic KCl solution (0.075 M) for 40 min at 37 °C. After this step, the cell suspension was centrifuged for 10 min at 1200 rpm, after which the supernatant was discarded. The cells were fixed with 5 ml fixative solution (3:1, methanol: glacial acetic acid) for 30 min at 4 °C. Then the cells were centrifuged, and supernatant was discarded again. These last two steps were repeated two to three times. The cell suspensions were then dropped onto cleaned slides. Air-dried slides were stained by 10% Giemsa for 20 min. Then slides were rinsed with distilled water and allowed to dry at room temperature. 10 to 20 slides were prepared from each specimen. All analysed specimens are deposited in the Genetic Laboratory of Kırşehir Ahi Evran University, Turkey (MGSUMKA 300-307).

The C-banding technique of Sumner (1972) was performed for determining constitutive heterochromatin regions whereas Ag-staining technique of Howell & Black (1980) was followed for determining NORs. For C-banding, slides were treated with 0.2 N HCl for 30 min at room temperature, then rinsed with distilled water and air-dried. The slides were then incubated with 5% $Ba(OH)_2$ for 15-20 min at 37 °C, followed by rinsing and drying. Slides were incubated with 2 × SSC for 2 h at 70 °C and rinsed and dried once again. Then slides were stained by 10% Giemsa for 30 min. For Ag-staining, two drops of colloidal developer and four drops of 50% $AgNO_3$ solution were added onto the slides. The coverslip was used to cover the slide and then placed in an incubator at 70 °C. When the slide colour changed to golden brown, the coverslip was removed. Then slide was rinsed and dried.

The chromosome slides were scanned via Leica DM3000 research light microscope (Leica Microsystems, GmbH, Germany) and photographs of metaphases were taken under AKAS software (Argenit Mikrosistem, Turkey). At least 10 metaphases were examined per individual. Karyotypes were arranged manually. Chromosomes were measured by a digital calliper. Chromosomes were classified according to Levan et al. (1964). For calculating fundamental arm number (FN) meta-submetacentrics were taken as biarmed whereas subtelo-acrocentrics were considered as uniarmed.

RESULTS

The diploid chromosome number of *S. lucioperca* was 2n = 48 (Figure 1a). Karyotype was consisted of one pair of metacentric, 15 pairs of submetacentrics and eight pairs of subtelo-acrocentric chromosomes (Figure 1b). FN was calculated as 80. The largest chromosome in the karyotype was a submetacentric. Morphologically differentiated sex chromosomes were not detected. Constitutive heterochromatin regions were observed on the pericentromeres of some of the chromosomes (Figure 1c). Moreover, heterochromatic blocs were determined on three pair of chromosomes (second and third submetacentric pairs and fifth subtelo-acrocentric pair) (Figure 1d). Also, some C-bands were observed on the long arms of six chromosome pairs (fourth and thirteenth submetacentric and first, second, third and seventh subtelo-acrocentric) (Figure 1d). Otherwise, Ag-NORs were determined on the terminal regions of the short arms of fifth submetacentric chromosome pair (Figure 1e, f). One of this NOR had a weaker signal compared to another. Also, on some silver-stained metaphases only one Ag-NOR was observed.

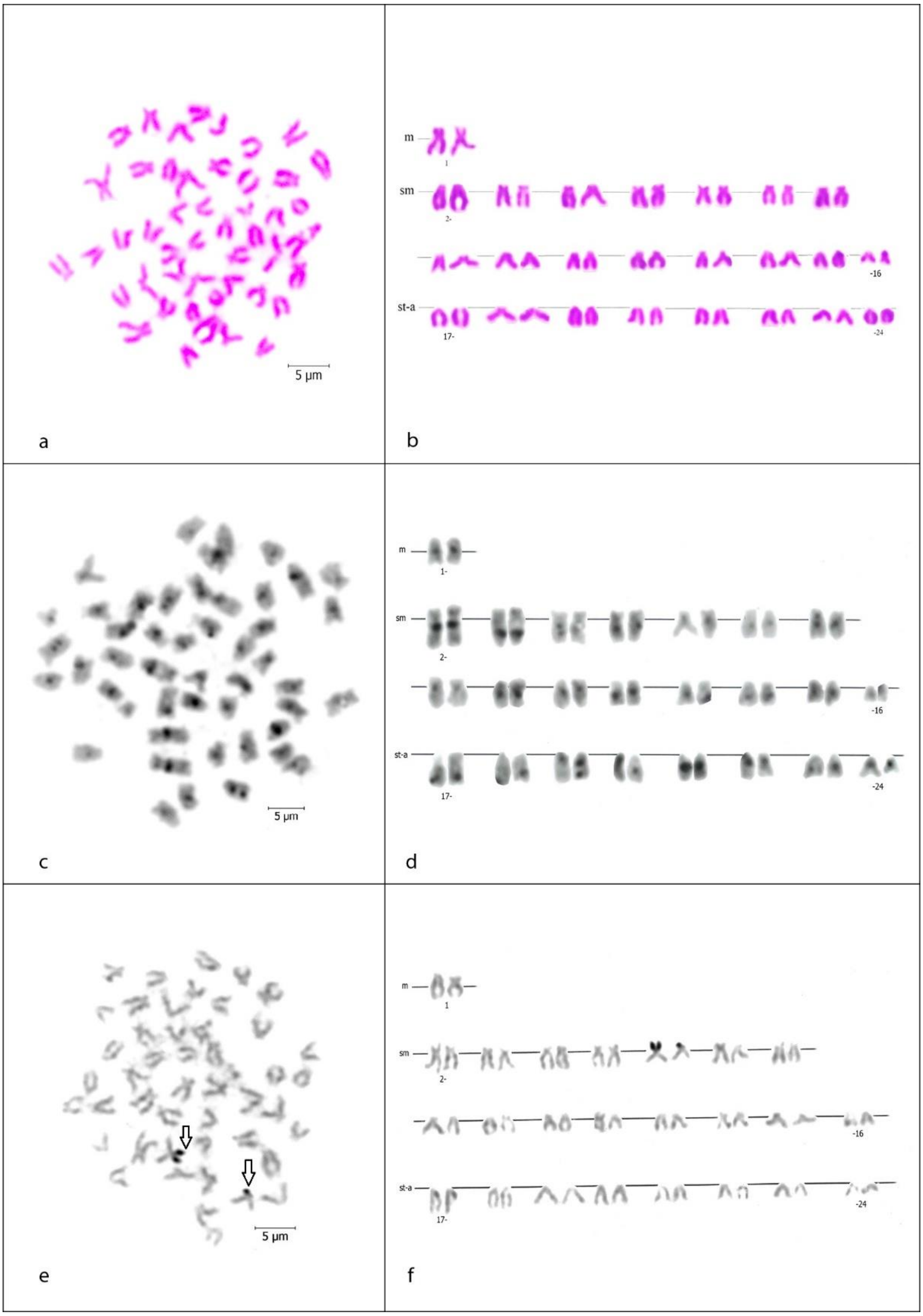

Figure 1. Giemsa stained metaphase (a), arranged karyotype (b), C-banded metaphase (c), arranged karyotype (d), silver stained metaphase (e) and arranged karyotype (f) of *Sander lucioperca*. Arrows indicate the Ag-NORs. Scale bar = 5 µm.

DISCUSSION

Although the advances in the cytogenetics have been developed the basic features of karyotypes have been observed under the conventional staining's (Martins et al. 2011). In this context, the determination of diploid number, chromosome morphology and FN are most popular in fish species (Martins et al., 2011). The diploid chromosome numbers 2n = 48 have been reported in all studied species of Percidae (Arai, 2011). In this regard, 2n of *S. lucioperca* is determined as 48 in this study as reported by Ráb et al. (1987), Mirinargesi et al. (2007), Arai (2011) and Jankun et al. (2014) from different countries. Arai (2011) reported that chromosome morphologies and FN's show some differences between the species of Percidae. Ráb et al. (1987) suggested that karyology of percid chromosomal evolution has been connected with rearrangements of the centromere position rather than chromosome number change. Percid karyotypes are dominated by submeta and subtelo-acrocentric chromosomes (Suciu & Ráb, 1992) as observed in this study. Chromosome morphology of *S. lucioperca* in this study is the same with the reports of Ráb et al. (1987) and Jankun et al. (2014). However, chromosome morphologies of Turkey population of *S. lucioperca* is different from South Caspian Sea (Mirinargesi et al., 2007) and Hungary (Arai, 2011) populations. The number of biarmed chromosomes of South Caspian Sea (Mirinargesi et al., 2007) and Hungary (Arai, 2011) populations are less than this study. So, FN's of this populations are lower than this study. The differences on these studies should be the result of chromosome contraction. The largest chromosome pair in the karyotype was submetacentric in this study however no information about the largest chromosome was given in previous *S. lucioperca* studies.

From the other four species of the genus *Sander* only *S. volgensis* Ráb et al. (1987) and *S. vitreus* (Arai, 2011) have been studied karyologically. The 2n of this species is the same with populations of *S. lucioperca*. Also, the karyotype of *S. volgensis* Ráb et al. (1987) is the same with this study. However, *S. vitreus* (Arai, 2011) differs from *S. lucioperca* about having all chromosomes as subtelo-acrocentric. From the same subfamily Luciopercinae, *Zingel zingel* and *Zingel streber* (Ráb et al., 1987) have the same diploid chromosome number as *S. lucioperca*. But the number of biarmed chromosomes of this species (Ráb et al., 1987) are less than *S. lucioperca*. Moreover, from the subfamily Percinae, *Perca fluviatilis* (Ráb et al., 1987) and *Percarina demidoffi* (Suciu & Ráb, 1992) have the same diploid chromosome number as *S. lucioperca*. Ráb et al. (1987) reported 30 biarmed chromosomes in *P. fluviatilis* whereas 32 biarmed chromosomes were determined in this study. Otherwise, *P. demidoffi* has 28 biarmed chromosomes and half of them were categorized as metacentrics (Suciu & Ráb, 1992). In this study only one pair of metacentric was observed. One small pair of metacentric was concluded as a percid marker chromosome in *P. demidoffi* (Suciu & Ráb, 1992) but in our karyotypes of *S. lucioperca* metacentric pair is not a small chromosome pair (medium-sized). A pair of large submetacentric and a pair of small metacentric in six percid species including *S. lucioperca* was reported as a marker chromosome by Ráb et al. (1987). This marker submetacentric pair is observed in this study too.

Otherwise, morphologically differentiated sex chromosomes were not determined in *S. lucioperca* like the other studies in percids (Ráb et al., 1987; Klinkhardt & Buuk, 1991; Jankun et al., 2014).

C-banding and silver staining are the most popular chromosomal banding techniques in fish species (Martins et al., 2011). C-banding reveals the constitutive heterochromatin regions concerning the repeated DNAs whereas silver staining detects the active ribosomal sites named as NORs (Arslan & Alpaslan, 2020; Martins et al., 2011; Karasu-Ayata et al., 2021; Unal-Karakuş, 2021). The determination of Ag-NOR number and location, and the location of C-bands have been studied in many fish species from Turkey (Arslan & Alpaslan, 2020; Karasu-Ayata et al., 2021; Unal-Karakuş, 2021). This chromosomal banding features are usually contribute to fish cytotaxonomy (Unal-Karakuş, 2021). As our knowledge, silver staining on the chromosomes of *S. lucioperca* was not applied in the previous cytogenetic studies (Ráb et al., 1987; Mirinargesi et al., 2007; Arai, 2011; Jankun et al., 2014). Only, Ráb et al. (1987) reported a pair of large satellited submetacentric (i.e., NORs carrying) chromosome after Giemsa staining in *S. lucioperca*. It was stated that achromatic regions on the end of its short arms were corresponded to the NOR (Ráb et al., 1987). In this silver-stained karyotype study, Ag-NORs were on the terminal regions of the short arms of middle-sized submetacentric chromosomes as stated by Ráb et al. (1987). Also, the same situation about NOR was reported for *S. volgensis* too (Ráb et al., 1987). So, it is similar in this respect to *S. lucioperca*. No studies have been reported on the Ag-NORs in the other species of the genus *Sander*. One of the most studied percid species, *P. fluviatilis* (Mayr et al., 1985; Klinkhardt & Buuk, 1991) is similar to *S. lucioperca* about having a single Ag-NOR. However, the location of Ag-NORs were on subtelocentric chromosomes (Mayr et al., 1985; Klinkhardt & Buuk, 1991) whereas they were localized on submetacentric chromosomes in this study. Reported heteromorphism between the two NORs in *P. fluviatilis* (Mayr et al., 1985) were observed in this study too. Additionally, observed one Ag-NOR on some silver-stained metaphases of *P. fluviatilis* (Mayr et al., 1985) was detected in some silver-stained metaphases of *S. lucioperca*. Moreover, *P. demidoffi* (Suciu & Ráb, 1992) is like *S. lucioperca* in having single Ag-NOR. However, about the location on the subtelo-acrocentric chromosomes (Suciu & Ráb, 1992) it seems different from *S. lucioperca*.

Otherwise, Jankun et al. (2014) reported the C-banded karyotype of *S. lucioperca* from Poland. In their report the Poland population had no centromeric C-bands. However, there are some centromeric C-bands in the Turkey population. C-bands on the long arms were reported for eight chromosome pairs in Poland population (Jankun et al., 2014) whereas this C-bands are on six chromosome pairs in our study. Heterochromatic blocs were determined on two chromosome pairs in Poland population (Jankun et al., 2014) whereas they were on three pair of chromosomes in Turkey population. There is no C-band information on the other studies of *S. lucioperca* (Ráb et al., 1987; Mirinargesi et al., 2007) and also other species of the genus *Sander* (Ráb et al., 1987), so they cannot be compared with this study.

As compared with samples of other populations of *S. lucioperca*, this study has better resolution results (Giemsa, C-banded and, Ag-NOR metaphases) according to the sensitivity of the method. Chromosome formula is not usually change among the populations of fish (Gaffaroğlu et al., 2013). However, chromosomal banding results should show differences among the populations. Especially number and location of the Ag-NORs are very polymorphic.

CONCLUSION

The karyological investigation of *S. lucioperca* was determined via basic genetics methods. Also, determination of chromosomal banding properties and associated karyotypes were characterized for the first time for Turkey population. Outcomes of the study provide a suitable resource for new cytotaxonomically projects.

COMPLIANCE WITH ETHICAL STANDARDS

a) Authors' Contributions

M. G., S. U. K., and M. K. A.: Designed the study and interpreted data

S. U. K.: Performed the survey work.

M. K. A.: Drafted the paper.

b) Conflict of Interests:

The authors declare that there is no conflict of interest.

c) Statement on the Welfare of Animals:

All procedures used in exeriments involving animals (fish) were in compliance with the "Kırşehir Ahi Evran University Ethical Committee's (numbered 68429034/05)" ethical standards.

d) Statement of Human Rights

This study does not involve human participants.

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Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2023; 9(1): 7-11 Araştırma Makalesi/Research Article

Effect of Selenomethionine and Concentation on Growth and *Chlorophyll-a* of *Scenedesmus quadricauda*

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Abstract

Selenium is an essential element for all living organisms. Since its deficiency can cause health Selenium is an essential element that plays an important role in the growth and development of all living organisms. However, the correct dose must be used for all living things. Otherwise, high selenium concentrations can cause toxic effects. In this study, the effect of adding selenomethionine (SeMet), an organic form of selenium at different concentrations of 50, 75, 100, and 250 mg/L, to the culture medium of the freshwater green microalgae *Scenedesmus quadricauda*, on microalgae growth and chlorophyll-a content was investigated. The results obtained after the study; It has been observed that the presence of high seleniummethionine in microalgae culture may cause toxic effects, but when 50mg/L is used as an effective dose, it positively supports the cell count as 64±4.9 x106 cells/mL and the chlorophyll-a pigment content of 0.593±0.1µg/L.

Selenometiyonin ve Konsantrasyonun *Scenedesmus quadricauda*'nın Büyüme ve *Klorofil-a* Üzerine Etkisi

Makale Bilgisi

Alınış tarihi:
02/12/2022
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13/06/2023

Anahtar Kelimeler:
- Selenometiyonin
- Selenyum
- *Scenedesmus*
- *Klorofil-a*

Öz

Selenyum, tüm canlı organizmaların büyüme ve gelişiminde önemli rol oynayan esansiyel bir elementtir. Ancak tüm canlılar için doğru dozun kullanımı gerekmektedir. Aksi takdirde yüksek selenyum konsantrasyonları toksik etkiye sebep olabilmektedir. Bu çalışmada, tatlı su yeşil mikroalgi *Scenedesmus quadricauda*'nın kültür ortamına 50, 75, 100 ve 250 mg/L olmak üzere farklı konsantrasyonlarda selenyumun organik formu olan selenometiyonin(SeMet) eklenmesinin mikroalg büyümesi ve klorofil-a içeriği üzerindeki etkisi araştırılmıştır. Yapılan çalışmanın ardından elde edilen sonuçlar; mikroalg kültüründe yüksek selenyummetiyonin varlığının toksik etkiye neden olabileceği ancak efektif doz olarak 50mg/L kullanıldığında hücre sayısını 64±4,9 x106 hücre/mL ve klorofil-a pigment içeriğini 0,593±0,1µg/L olmak üzere pozitif yönde desteklediği görülmüştür.

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INTRODUCTION

Selenium (Se) is an essential trace element that functions as the 21st amino acid selenocysteine (Sec) in a defined set of proteins, which is very important in animals and plants, especially the human body (Qian et al., 2019). Selenium affects organisms in a dose-dependent manner, being an important element that is beneficial at low concentrations and toxic at high concentrations. Organically, it is found in the form of selenocysteine in animal foods, while it is found in the form of Selenomethionine (SeMet) in plant foods (Kangalgil and Yardimci, 2017). In aquatic ecosystems, microalgae have begun to be recognized as vector organisms for Se transfer. It has been investigated that microalgae can accumulate selenium in their metabolism by exposure to a selenium-containing environment. However, what kind of changes in the morphological and physiological properties of microalgae produced in the presence of selenium have not been fully revealed yet. Green algae are accepted as target organisms in Se bioaccumulation studies. Therefore, the obtained biomass, product amount, and enriched pigment content of freshwater microalgae grown in a selenium-enriched nutrient medium have the potential to be used as food, cosmetics, fish feeding in aquaculture, inclusion in the food chain in feed areas or as biofertilizer to enrich the soil.

In this study, the effect of adding different concentrations of Selenomethionine (SeMet), the organic form of selenium, to the culture medium on the growth and chlorophyll-a content of *Scenedesmus quadricauda* was evaluated. Investigation of the effect of selenium addition on microalgae pigments and in terms of obtaining natural pigment contents from selenium-enriched microalgae biomass will be evaluated in line with the results to be obtained.

MATERIAL AND METHODS

The Scenedesmus quadricauda were obtained from Ege University Fisheries Faculty Aquaculture Department, Plankton Culture Laboratory (Figure 1).

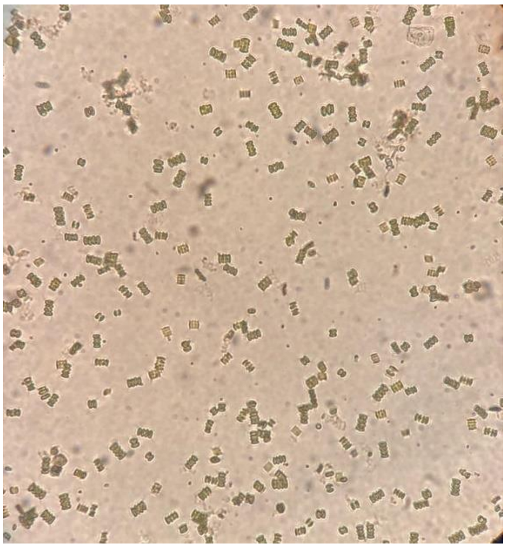

Figure 1. Light microscope image of *Scenedemus quadricauda.*

Microalgae Culture Condition

The preferred nutrient medium for the culture of *Scenedesmus quadricauda* was The Bold Basal Medium (BBM). For the culture, the flasks were illuminated with LED (Philips TLM 40W/54RS) lighting for 24 hours, and the ambient conditions were kept constant at 24°C with the air conditioning system. In addition, an aeration system was added to prevent biofilm formation, but no CO2 was added to the flasks. Different concentrations of SeMet were added to the found culture medium and cultured in a 1 L flask at concentrations of 50 mg/L, 75 mg/L, 100 mg/L, and 250 mg/L.

Number of Cells

The daily cell count of Scenedesmus quadricauda was followed. As the culture entered the stationary phase at the end of the 11th day, the trial set was terminated. Cell numbers were determined using a Neubauer hematocytometer with a light microscope (Nikon 250, Japan). The specific growth rates (μ) were calculated using the formula below.

$$\mu = \frac{\ln x_2 - \ln x_1}{t_2 - t_1}$$

X2 and X1 represent the culture densities at t2 and t1 times.

Chlorophyll-a Analysis

Chlorophyll-a analysis for *Scenedesmus quadricauda* was performed according to the spectrophotometric method. After taking 5 mg of dried sample and treated with 5 ml of methanol (Merck 100%, Germany). The cells were homogenized with an Ika (Ultra Turrax T25) brand homogenizer for 5 minutes and then ultrasonically (Sonorex) at 70°C for 10 minutes. After the extract obtained is separated by centrifugation (Elektromag M 615 P) at 3500 rpm. The samples were read at 666 nm wavelengths using the spectrophotometer (Boe co, S-20 VS, England) and the chlorphyll -a values were calculated with the below formula. Analyzes were performed in triplicate.

Chlorophyll-a(mg/g) = 13.9 x A_{666} (Sanchez et al., 2005)

(A_{666}: absorbance reading at 666 nm)

Statistical analysis

All cultivation experiments and pigment analysis were performed in triplicate and all data are expressed as the mean with standard deviation. Results were analyzed by one-way ANOVA with significance level at P≤0.05 and Tukey’s multiple comparison test was performed by using SPSS Statistics 23 (IBM, US).

RESULTS AND DISCUSSION

Cultural Densities

The initial culture density of *Scenedesmus quadricauda* in the study was 4x10^{6}cells/mL (Figure 2). The highest cell count has achieved at 64±4.9 x10^{6}cells/mL on day 8 when used 50mg/L SeMet concentration in the culture medium. 75mg SeMet concentration reached higher levels than the control group ($p<0.05$). When it was used 100 mg/L and 250 mg/L SeMet concentrations, there were negative affected on growth *S. quadricauda*. As a result of, SeMet to use in the medium more than 100 mg cause a significant growth difference of *S. quadricauda* ($p>0.05$). For this reason, trial sets were terminated before the SeMet concentration caused a toxic effect.

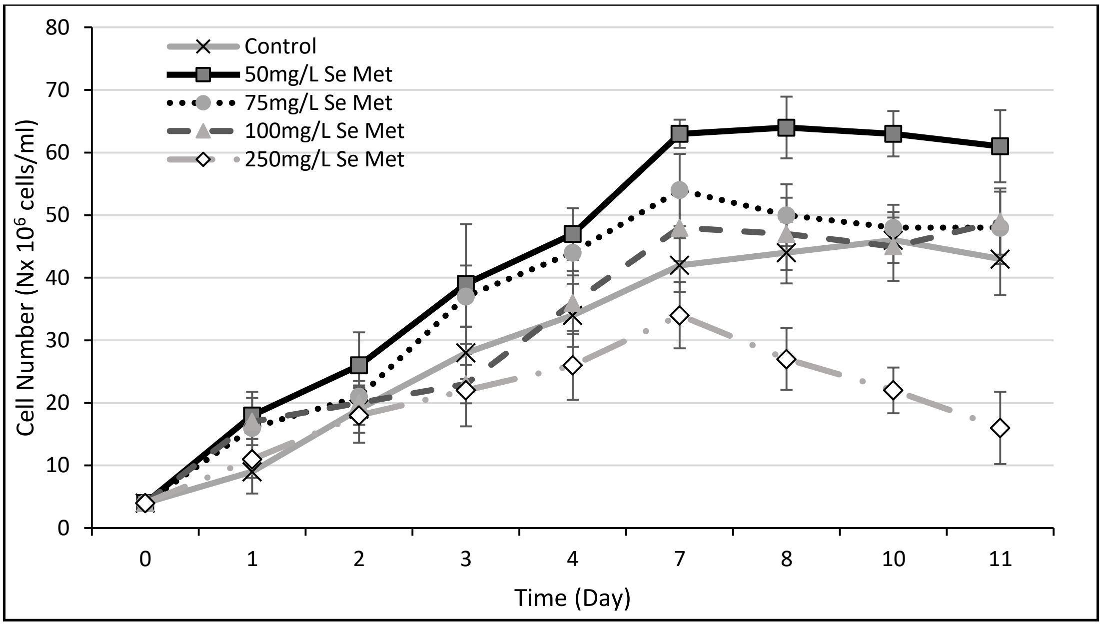

Figure 2. Cell density of *Scenedesmus quadricauda* at 11 days of production by addition of SeMet.

Chlorophyll-a Amounts

In the pigment analysis, chlorophyll values were measured every 3 days and the average results were taken. In all cultures, the highest chlorophyll value was observed in 0.593±0.1µg/L, and 50mg/L SeMet addition (Figure 3). The highest chlorophyll value for the control group was measured as 0.436±0.09µg/L. Therefore, it is seen that the addition of a maximum of 100 mg/L not a higher concentration of SeMet did cause a difference in the amount of chlorophyll per cell compared to the control group ($p<0.05$).

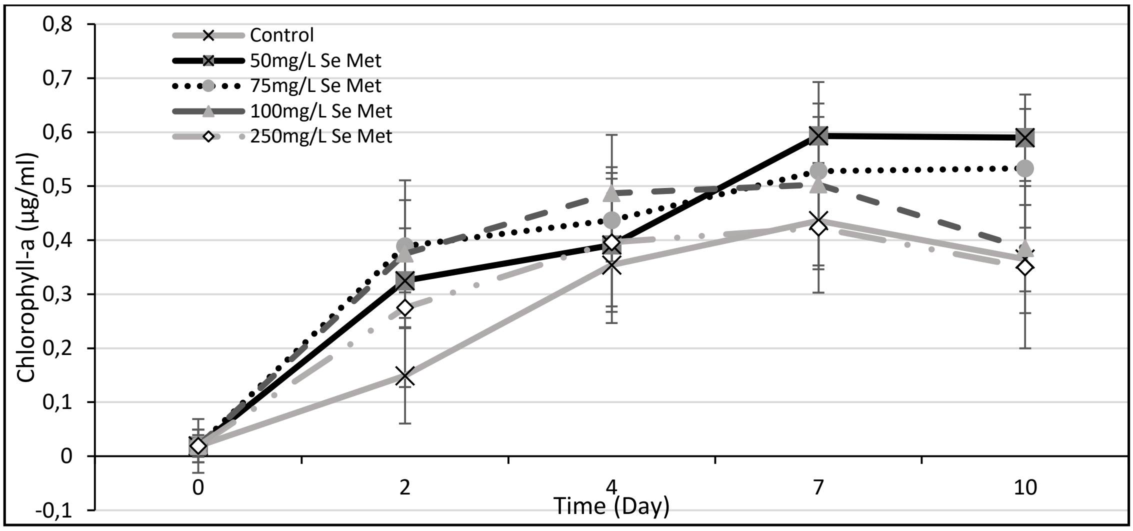

Figure 3. Chlorophyll-a values determined from the production of *Scenedesmus quadricauda* with the addition of SeMet.

Information on the presence and possible effects of selenium in microalgae culture media is limited. However, the effects of inorganic forms of selenium on growth rate and photosynthetic pigment (such as chlorophyll-a, and β-carotene) production in various microalgae species vary depending on the dose of selenium used. First, while the dose plays an active role, it is also an important factor in whether the form used is organic or inorganic. In the study, the effect of organic se form on growth dynamics and chlorophyll-a amount was investigated since it is known that the toxic effect of organic se form is less (Demircan,2022). The results obtained showed that the presence of SeMet in *Scenedesmus quadricauda* culture medium was effective at 50mg/L to 75mg/L depending on the dose of use.

Pigments are one of the important metabolites for microalgae. Culture conditions that encourage pigment levels of microalgae, which have many uses in the biotechnological field thanks to their various pigment contents, have been studied for a long time. In line with the results obtained from the literature studies, the effects of selenium on various pigment substances during the culture of microalgae species are discussed. For example, While the effect of *Dunaliella salina* on the presence of beta-carotene in the 21-day period followed by the production of selenium was limited, the results obtained did not find a significant effect of the presence of selenium on pigment substances (Constantinescu-Aruxandei et al., 2019). During the example, the promotion of selenium has been observed. However, in previous studies, it has also been encountered that the inorganic form of Selenium has no effect or has a toxic effect. However, in another study by Sun et al., (2014) a steady increase in the amount of chlorophyll-a was observed in the cultivation of Chlorella vulgaris, and the inorganic form of selenium was used at a concentration of 75mg/L. Similarly, *Spirulina platensis* was used in the study conducted by Chen et al. 2008 and the addition of selenium during its production showed a stimulating effect on the chlorophyll content. It has been reported that the chlorophyll content reaches its maximum value (29.3 mg / g) on the 3rd day with the addition of 40 mg / L Se at the effective dose. In another study prepared by Pronina et al., 2001, it was reported that sodium selenite supplementation increased from 1.04 to 1.10 compared to the control group with the inclusion of 50mg/L. Therefore, in this study, findings parallel to the references compared for pigment sources were obtained and the effect of selenium addition on the chlorophyll-a content of *Scenedesmus quadricauda* was found to be positive. In addition, as the most recent example, by Demircan, (2022) in the study in which SeMet was added to *Scenedesmus dimorphus* culture medium when the effect of growth conditions and chlorophyll-a content was examined, approximately 1.5 times positive effect was reported in the flask containing 50mg/L SeMet compared to the control group.

When the experiment was evaluated, it was observed that the SeMet form positively promoted growth and chlorophyll-a content when used at an effective dose for the freshwater green microalgae *Scenedesmus quadricauda.*

CONCLUSION

According to our study results, 50, 75 and 100mg/L SeMet concentrations added to the *S. quadricauda* culture medium stimulated cell growth, while 250mg/L caused a toxic effect and reduced the cell number to a lower level than the control group. For this reason, it has been observed that selenium supplementation can be effective at different values for each species, but levels below 100mg/L have a positive effect. Thus, it was concluded that the potential of using selenium methionine form in the culture medium is positive in increasing the microalgae biomass and enriching the pigment contents.

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Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2023; 9(1): 12-24 Araştırma Makalesi/Research Article

An Assessment of the Cultivation Potential and Suitability for Human Consumption of Mediterranean Mussels (*Mytilus galloprovincialis* Lamarck, 1819) from the Yalova Coast of the Marmara Sea

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- Marmara Sea

Abstract

This study aimed to determine into potential suitability of juvenile Mediterranean mussel (*Mytilus galloprovincialis*) for cultivation and human consumption collected from various stations (Kapaklı, Çınarcık, Koru, and Deveboynu) along the Yalova coast of the South Marmara Sea during the spring season. Specifically, meat yield, condition index, moisture content, density of fouling organisms on the shells, and heavy metal concentrations (copper, zinc, mercury, cadmium, lead, and arsenic) were analysed. The average meat yields for Çınarcık, Deveboynu, Kapaklı, and Koru were found to be 24.51±2.96, 21.72±3.92, 23.75±2.31, and 21.54±4.01, respectively. Similarly, the average condition index were found to be 7.29±1.16, 6.02±0.97, 7.30±0.77, and 5.94±1.39 for Çınarcık, Deveboynu, Kapaklı, and Koru, respectively. The average shell component index for Çınarcık, Deveboynu, Kapaklı, and Koru were detected that 72.06±2.75, 74.39±4.13, 69.99±2.55, and 74.68±4.54, respectively. Additionally, the average moisture contents were found to be 82.63±1.95, 83.34±3.23, 84.02±1.82, and 83.40±4.17 for Çınarcık, Deveboynu, Kapaklı, and Koru, respectively. The statistical analysis revealed significant differences ($p<0.05$) in these parameters among the stations. Moreover, the quantities of competitive and fouling organisms, such as polychaetes and barnacles, were found to be highest in the Deveboynu and Koru stations. It has been determined that copper, zinc, mercury, and lead levels of the mussels are suitable for human consumption in all stations. However, arsenic values exceeded legal limits in Çınarcık, Koru, and Deveboynu, and cadmium values exceeded legal limits in all stations.

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INTRODUCTION

Food and Agriculture Organization of the United Nations report that aquaculture will play a crucial role in meeting the food needs of the world's population, which is expected to reach 9 billion by 2050 (FAO, 2015). Therefore, it is predicted that the growth in the world's aquaculture industry will continue to increase at an accelerated pace (FAO, 2015). While there are many economically valuable bivalve species such as mussels, oysters, scallops, and cockles that are rich in essential amino acids, unsaturated fatty acids, vitamins, minerals, and antioxidant substances along the coasts of Turkey, country's aquaculture production is focused on fish farming. Therefore the production of bivalve species is well below their potential (Yildiz et al., 2011). Additionally, there are no other species grown besides Mediterranean mussels. The production of Mediterranean mussels, which was 3 tons in 2015, rose to 907 tons in 2018 and 4.585 tons in 2021 (TUIK, 2022). The production potential of Mediterranean mussels along the coasts of Turkey is much higher than these amounts. There are many mussel farms that have recently been established and have received preliminary permits in the Marmara Sea, Çanakkale Strait, and around Izmir (Yildiz et al., 2023). In particular, in newly established mussel farms, a large number of juveniles are required, and initially, they are collected from natural beds rather than using collectors. The quality of the juvenile mussels collected from nature directly affects the quality of the product that will be sold in the market. Therefore, it is essential to establish specific criteria to determine the quality of the juvenile mussels. Meat yield and condition index are important criteria that determine the quality and marketing characteristics of mussels (Yildiz et al., 2021). The density of fouling organisms attached to the mussels is another factor that determines their commercial quality (Sievers et al., 2014; Forrest & Atalah, 2017).

Heavy metals can be formed from discharge or leakage from various anthropogenic activities in aquatic environments (Freije, 2015). The concentration of heavy metals in aquatic organisms is higher than in water, and they can accumulate biologically in aquatic organisms up to levels that can cause physiological damage in humans through the food chain (Raposo et al., 2009; Stanković et al., 2011). Since mussels feed by filtering seawater, this accumulation can be even higher. According to the Republic of Turkey Ministry of Agriculture and Rural Affairs Aquaculture Regulation (Republic of Turkey Ministry of Agriculture and Forestry, 2002), which is in line with internationally accepted criteria, the chemical acceptable values for bivalves, including mussels, are 0.5 mg/kg for mercury, 0.1 mg/kg for cadmium, 1.5 mg/kg for lead, 20.0 mg/kg for copper, 50.0 mg/kg for zinc, and 1.0 mg/kg for arsenic.

The overall aim of this study is to determine and evaluate the criteria for the usability of mussels collected from different stations for consumption and aquaculture systems. In this scope, in the Yalova coast of the Marmara Sea in different stations the meat yields, condition index, heavy metal contents (Cu, Zn, Hg, Cd, Pb, and As of juvenile mussels, and densities of organisms attached to the mussels were compared.

MATERIALS AND METHODS

Sampling Area

Mediterranean mussel samples were collected by hand from four stations (Kapaklı, Çınarcık, Koru and Deveboynu) in Yalova, Marmara Sea, Turkey in April 2021 (40° 27' 612"N, 28° 58' 061" E, 40° 38' 794"N, 29° 07' 049" E, 40° 39' 444"N, 29° 09' 271" E, 40° 39' 294"N, 29° 02' 707" E, respectively) (Figure 1). Three kilograms of mussels were collected from 1 to 2 m depth and rocky area each station and bring to the laboratory for analysis. The shells of all mussels were macroscopically examined. The area covered by fouling organisms on the surface of the mussel shells was determined (0 = no occurrence, 1 = 20% coverage, 2 = 40% coverage, 3 = 60% coverage, 4 = 80% coverage, and 5 = full coverage), and the type of fouling organism was recorded (Lök & Acarli, 2006). The fouling organisms adhering to the mussels were then removed using a knife, and the meat of the mussels was separated from the shells and stored in deep freeze.

Figure 1. Map showing the sampling area Marmara Sea

Condition index, meat yield, moisture and shell component index

To assess the condition index (CI), meat yield (MY), moisture content, and shell component index (SCI) of *M. galloprovincialis*, 30 mussel samples were weighed from each station and their meats were removed. The meats were washed with distilled water to eliminate extraneous salt and sand particles and were then placed on an absorbent surface until they were as dry as possible. The shells were dried in an oven at 60 °C until they reached a constant weight (42-72 h), while the soft tissues were freeze-dried. These methods were adapted from previous studies (Freeman 1974; Pekkarinen, 1983; Crosby & Gale 1990; AOAC 2000).

$$MY = \frac{Wet\ meat\ weight\ (g)}{Total\ weight\ (g)} \times 100 \quad (1)$$

$$CI = \frac{Dry\ meat\ weight\ (g)}{Dry\ shell\ weight\ (g)} \times 100 \quad (2)$$

$$SCI = \frac{Wet\ shell\ \ weight\ (g)}{Wet\ shell\ weight\ (g)+Wet\ meat\ weight\ (g)} \times 100 \quad (3)$$

$$Moisture(\%) = \frac{Wet\ meat\ weight(g)-Dry\ meat\ weight(g)}{Wet\ meat\ weight(g)} \times 100 \quad (4)$$

To analyse the heavy metal composition, a 0.5 g of mussel meats (wet weight,ww) were taken from each station and mixed with 10 ml of HNO_3. The mixture was then incinerated using a microwave incinerator. The concentration of heavy metals in the mussel meat was detected according to reference method the NMKL 161 (1998) and NMKL 186 (2007) using inductively coupled plasma optical emission spectrometry (ICP-MS).

Statistical analyses

Statistical analyses were performed using SPSS 19.0 software for Windows. The data was analysed for normality and variance homogeneity using the Kolmogorov-Smirnov and Levene's tests, respectively. As the data showed a normal distribution, the relationship between morphometric components was determined using Pearson correlation. Differences between CI, MY, and SCI of different stations were determined using the one-way analysis of variance (ANOVA) test. Incase the necessary conditions for parametric analyses were not met, the non-parametric Kruskal-Wallis H test was used to compared the differences sampling stations for concentrations of heavy metals.

RESULTS

The results showed that the length of the Mediterranean mussels was 32.19±2.43 mm at Kapaklı station, 30.10±4.05 mm at Çınarcık station, 28.02±4.63 mm at Koru station, and 31.67±2.71 mm at Deveboynu station (Table 1).

Table 1. Morphometric characteristics and meat yield (MY), condition index (CI), moisture and shell component index (SCI) of *M. galloprovincialis* from Kapaklı, Çınarcık, Koru, Deveboynu station (mean±standard deviation)

Station	Length	Width	Height	Weight	MY (%)	CI	Moisture (%)	SCI
Kapaklı	32.19±2.43	18.07±2.21	11.07±1.25	2.40±0.46	23.75±2.31	7.30±0.77	84.02±1.82	69.99±2.55
Çınarcık	30.10±4.05	17.38±2.60	11.23±1.71	1.99±0.61	24.51±2.96	7.29±1.16	82.63±1.95	72.06±2.75
Koru	28.02±4.63	16.41±2.80	10.11±1.85	1.58±0.63	21.54±4.01	5.94±1.39	83.40±4.17	74.68±4.54
Deveboynu	31.67±2.71	18.36±1.37	12.12±1.33	2.18±0.54	21.72±3.92	6.02±0.97	83.34±3.23	74.39±4.13

Fouling organism density

Mussels collected from Deveboynu and Koru stations were grouped as "0" due to the absence of any adhesion in terms of cleanliness, brightness, and fouling organism attachment and density. Mussels collected from Çınarcık station were found to have polychaetes and *Balanus sp.* adhesion on their shells and were categorized as "3" in terms of adhesion density. Mussels collected from Kapaklı station were found to have adhesion of polychaetes, *Balanus sp.* and bryozoans on their shells and were categorized as "4" in terms of adhesion density.

Meat yield, condition index, shell component index and moisture

The results show that there were significant differences ($p<0.05$) in the meat yield (MY), condition index (CI), and shell component index (SCI) values among the stations. The MY, values were 21.54±4.01 %, 21.72±3.92 %, 23.75±2.31 %, and 24.51±2.96 %, CI 5.94±1.39, 6.02±0.97, 7.30±0.77, and 7.29±1.16; and SCI 74.68±4.54, 74.39±4.13, 69.99±2.55, and 72.06±2.75 for Koru, Deveboynu, Kapaklı, and Çınarcık stations, respectively. The moisture content of the mussel meat was detected also 82.63±1.95 %, 83.34±3.23 %, 84.02±1.82 %, and 83.40±4.17 % for Çınarcık, Deveboynu, Kapaklı, and Koru stations, respectively (Table 1).

Heavy metal concentration

Cu, Zn, Hg, Cd, Pb, and As concentrations were measured and presented in Table 2. Among all stations, Zn was the most abundant heavy metal. The highest concentration of Zn was found in the Çınarcık station, while the values for Koru, Deveboynu, and Kapaklı stations were similar. The highest amount of Cu was detected in the Çınarcık station, followed by Deveboynu, Koru, and Kapaklı stations. On the other hand, the As concentration was found to be the lowest in the Kapaklı station, while it was significantly higher in the other stations (Table 6).

Table 2. Pearson correlation of length, width, height, weight, MY, CI, moisture, SCI of *M. galloprovincialis* from Kapaklı station

	Length	**Width**	**Height**	**Weight**	**MY**	**CI**	**Moisture**	**SCI**
Length	0.1							
Width	0.533	1						
Height	0.581	0.877*	1					
Weight	0.887*	0.295	0.420	1				
MY	-0.587	-0.664	-0.560	-0.552	1			
CI	-0.017	-0.517	-0.558	-0.209	0.529	1		
Moisture	-0.388	-0.231	-0.111	-0.061	0.442	-0.442	1	
SCI	0.273	0.705	0.582	0.147	-0.892*	-0.562	-0.483	1

**Correlation is significant at the 0.01 level
*Correlation is significant at the 0.05 level

Statistical analyses

At Kapaklı station, there was a positive correlation between length, width, thickness, and weight, and a negative correlation between MY and SCI (Table 2). At Çınarcık station, there was a positive correlation between length, width, thickness, and weight, and between CI and MY, and a negative correlation between CI and moisture and SCI (Table 3). At Koru station, there was a positive correlation between length, width, thickness, and weight, and between MY and moisture, and a negative correlation between SCI and MY and moisture (Table 4). At Deveboynu station, there was a positive correlation between length, width, thickness, and weight, and between MY and moisture and CI, and a negative correlation between SCI and MY, CI, and moisture (Table 5). Among all stations of heavy metal composition, MY, CI, and SCI were found statistically significant ($p<0.05$).

Table 3. Pearson correlation of length, width, height, weight, MY, CI, moisture, SCI of *M. galloprovincialis* from Çınarcık station

	Length	**Width**	**Height**	**Weight**	**MY**	**CI**	**Moisture**	**SCI**
Length	1							
Width	0.811**	1						
Height	0.865**	0.804**	1					
Weight	0.894**	0.920**	0.896**	1				
MY	0.230	0.091	0.098	0.099	1			
CI	-0.185	-0.339	-0.311	-0.246	0.475*	1		
Moisture	0.126	0.168	0.108	0.088	0.264	-0.577**	1	
SCI	0.037	0.184	0.194	0.139	-0.881**	-0.678**	-0.188	1

**Correlation is significant at the 0.01 level
*Correlation is significant at the 0.05 level

Table 4. Pearson correlation of length, width, height, weight, MY, CI, moisture, SCI of *M. galloprovincialis* from Koru station

	Length	**Width**	**Height**	**Weight**	**MY**	**CI**	**Moisture**	**SCI**
Length	1							
Width	0.897**	1						
Height	0.879**	0.848**	1					
Weight	0.954**	0.889**	0.913**	1				
MY	0.245	0.114	0.169	0.193	1			
CI	-0.127	-0.073	-0.130	-0.090	0.113	1		
Moisture	0.298	0.195	0.241	0.237	0.575**	-0.692**	1	
SCI	-0.277	-0.148	-0.175	-0.241	-0.973**	-0.114	-0.591**	1

**Correlation is significant at the 0.01 level
*Correlation is significant at the 0.05 level

Table 5. Pearson correlation of length, width, height, weight, MY, CI, moisture, SCI of *M. galloprovincialis* from Deveboynu station

	Length	**Width**	**Height**	**Weight**	**MY**	**CI**	**Moisture**	**SCI**
Length	1							
Width	0.634**	1						
Height	0.819**	0.504*	1					
Weight	0.853**	0.656**	0.895**	1				
MY	0.181	0.082	0.380	0.300	1			
CI	0.084	0.346	0.176	0.212	0.689**	1		
Moisture	0.314	-0.144	0.446	0.348	0.611*	-0.108	1	
SCI	-0.265	-0.081	-0.492	-0.406	-0.975**	-0.605*	-0.709**	1

**Correlation is significant at the 0.01 level
*Correlation is significant at the 0.05 level

Table 6. Heavy metal composition (Cu, Zn, Hg, Cd, Pb, As) of *M. galloprovincialis* (mg/kg ww)[l] from Kapaklı, Çınarcık, Koru, Deveboynu station (mean±standard deviation) and limitations of Republic of Turkey Ministry of Agriculture and Forestry (2002)

Heavy metal	Kapaklı	Çınarcık	Koru	Deveboynu	The limitations of Republic of Turkey Ministry of Agriculture and Forestry (2002)
Cu	0.21±0.00	0.99±0.05	0.60±0.05	0.61±0.04	20.0
Zn	15.93±0.04	39.66±0.63	39.03±0.56	37.29±0.64	50.0
Hg	N.D.	N.D.	N.D.	N.D.	0.5
Cd	0.11±0.02	0.19±0,03	0.15±0.02	0.13±0.00	0.1
Pb	0.11±0.03	0.72±0,05	0.57±0.04	0.31±0.05	1.5
As	0.61±0.05	2.59±0.04	2.29±0.04	1.92±0.05	1.0

ND: Not detected.

DISCUSSION

Various factors such as environmental factors (Yıldız et al., 2006; Yildiz & Berber, 2010; Yıldız et al., 2013a, b; Vural et al., 2015; Acarlı et al., 2018), interspecific competition (Bertness and Grosholz, 1985; Okamura, 1986), and epibionts on shells (Arakawa, 1990; Dittman & Robles, 1991; Buschbaum & Saier, 2001) are influential in the growth of bivalves. Biofouling is a significant concern in aquaculture (LeBlanc et al., 2002). Therefore, seed collected from natural beds or spat collectors is thoroughly cleaned to eliminate epifauna and other organisms (Velayudhan et al., 2007). In this study, mussels collected from the Deveboynu and Koru stations were found to be clean in terms of cleanliness, brightness, and fouling organisms; meaning that no biofouling organisms or mud were detected on them. Mussels collected from the Çınarcık station were evaluated as '3' for polychaetes and barnacles, and those collected from the Kapaklı station were evaluated as '4' for polychaetes, barnacles, and *Bugula sp*. Polychaetes and barnacles can potentially compete with mussels by weighing down on the mussel shells and restricting water circulation (LeBlanc et al., 2002). This competition can lead to a decrease in yield because it makes it difficult for mussels to find food. Considering that biofouling organisms on young individuals to be transported to the cultivation area are unwanted despite the possibility of transportation to this area, the preferred stations to be placed in the cultivation system are Koru and Deveboynu.

In general, MY, CI, and SCI are often used as indicators of the feeding status of bivalves (Crosby & Gale, 1990; Yildiz et al., 2011), it's ability to tolerate stress (Mann, 1979), harvest time (Galvao et al., 2015), and reproductive status. MY is also an indicator of meat-to-shell ratio and is therefore an indicator of quality, with higher values generally indicating better quality. It is particularly important for seed collection, a critical step in the mussel production cycle (Macneill et al., 2000). In this study, the lowest MY was observed at Koru station (21.54±4.01%), and the highest at Çınarcık station (24.51±2.96%). Yıldız et al. (2021) found that the MY and CI of *M. galloprovincialis* (83.69 mm in length) in the Çanakkale Strait was 17.79% and 9.33 in April, respectively. Lök et al. (2011) was determined that MY and CI of *M. galloprovincialis* was 27.05% and 11.53 (51.22 mm in length) in the Sinop- Içliman (Black Sea), and 22.63% and 10.47 (62.56 mm in length) in Edincikaltı (Marmara), 18.26% and 9.93 (50.44 mm in length) in Mersin Bay (Aegean Sea), respectively. In this study, Koru and Deveboynu stations were found to have lower MY and CI compared to Çınarcık and Kapaklı stations. Environmental parameters, size, and reproductive period are directly related to CI, MY, and SCI (Acarli et al., 2015; Vural et al., 2015; Acarlı et al., 2018; Kızılkaya et al., 2019; Biandolino et al., 2020; Yıldız et al., 2021). In this study, differences were found between stations ($p<0.05$). Even between neighboring stations, the differences in CI and MY may be attributed to the biological status of the organisms, including their feeding status.

As a result of natural processes and various anthropogenic activities, discharge or leakage can lead to the formation of heavy metals in aquatic environments (Freije, 2015). Concentrations of heavy metals in aquatic organisms are higher than in water and they can biologically accumulate to levels that can cause physiological disturbances in humans through the food chain (Raposo et al., 2009; Stanković et al., 2011). The accumulation rate depends not only on the metal loads present in the environment but also on factors such as temperature, pH, salinity, age, sex, size, and sexual maturity of mussels (Bartolomé et al., 2010; Besada et al., 2014; Richir & Gobert, 2014; Mandich, 2018).

Excessive Cu has a toxic effect on the body and can inhibit the functions of some enzymes (Bajgas, 2000). Cu levels decrease when industrial and urban emissions, fertilizers, algaecides, fungicides, molluscicides, and cyanobacteria in the region decrease (Moffett et al., 1997; Besada et al., 2002; Cheriyan et al., 2015). Cu is found in the greenish-blue pigment (hemocyanin) that carries oxygen in mollusks and crustaceans (Clark, 1992). The influx and efflux of Cu in bivalves vary among species (Cai & Wang, 2019). Republic of Turkey Ministry of Agriculture and Forestry (2002) has specified the maximum value for Cu as 20 mg/kg. Erkan et al. (2011) reported Cu levels in *Ostrea edulis* as 1.075 mg/kg ww in April in the Marmara Sea, while Türk Çulha et al. (2011) found the highest Cu levels in *M. galloprovincialis* as 0.93 mg/kg ww in the spring season (49.62 mm and 9.87 g) in the Marmara Sea. Lök et al. (2010) indicated that Cu concentration of *M. galloprovincialis* in the Çanakkale Strait was determined as between 0.54 mg/kg ww and 0.66 mg/kg ww whereas, Topçuoğlu et al. (2004) reported that Cu levels of *M. galloprovincialis* varied between 94 mg/kg ww and 1.33 mg/kg ww (70-80 mm) among regions in the Marmara Sea. In this study, Cu levels were measured as 0.21 mg/kg ww, 0.99 mg/kg ww, 0.60 mg/kg ww, and 0.61 mg/kg ww in Kapaklı, Çınarcık, Koru, and Deveboynu stations, respectively. These values were well below the limits set by Republic of Turkey Ministry of Agriculture and Forestry (2002).

Zinc (Zn) is involved in the synthesis of several enzymes that influence the uptake of CO_2 and P, photosynthesis, phytoplankton and microalgal growth, and the catabolism of carbohydrates, fats, proteins, and nucleic acids. Zn found in the cell wall of bacteria and diatoms, and the death or decay of these microorganisms can increase the amount of Zn in the upwelling zone where coastal water and seawater mix. Higher amounts of Zn have been reported in mollusks than in fish (Storelli et al., 2000). In the Marmara Sea, the Zn levels in *O. edulis* ranged from 290.114 to 147.62 mg/kg ww throughout the year, with the highest values found in April (Erkan et al., 2011; Özden et al., 2010; Türk Çulha et al., 2011). Topçuoğlu et al. (2004) found that the Zn content in Marmara Sea ranged from 29.16 to 44.79 mg/kg ww in *M. galloprovincialis* (70-80 mm), while Lök et al. (2010) declared that Zn concentration of *M. galloprovincialis* in the Çanakkale Strait was from 32.55 mg/kg ww to 65.61 mg/kg ww. However, Periyasamy et al. (2014) reported 0.34 mg/g dw in *D. incarnatus* and Orban et al. (2007) reported 0.91-1.48 mg/100g ww in *Chamelea gallina*. In the current study, Zn levels in mussels ranged from 15.93 to 39.66 mg/kg ww across all sampling stations, which was within the limits set by Republic of Turkey Ministry of Agriculture and Forestry (2002) of 50 mg/kg. These results suggest that the Zn content in mussels from all sampling stations is suitable for cultivation.

When the limit of Cd in the body is exceeded, it can damage the kidneys and cause chronic toxicity (Abou-Arab et al., 1996; Mol, 2011). Azizi et al. (2018) found a Cd concentration of 0.89 mg/kg in *M. galloprovincialis* in Cala Iris offshore (Northern Morocco) during the winter season. According to Wallace and Luoma (2003), biological detoxification of Cd in bivalves increases Cd concentration depending on the color and age of the organism. Erkan et al. (2011) found Cd concentrations in *O. edulis* to be 0.120 mg/kg ww in April in the Marmara Sea. Cd of *M. galloprovincialis* in the Marmara Sea varied from 0.18 to 0.40 mg/kg ww (70-80 mm) (Topçuoğlu et al. 2004). In addition Lök et al. (2010) found that Cd content of *M. galloprovincialis* in the Çanakkale Strait changed between 0.10 mg/kg and 0.52 mg/kg. Kayhan (2006) reported Cd levels in unpolluted water to range from 0.01-5 µg/L. Industrial and agricultural activities are the main sources of cadmium (Jarup & Akesson, 2009; Obaiah et al., 2020). Sources of cadmium in freshwater and sea water environments include atmospheric deposition, runoff, and direct discharges into water or watersheds (Wright & Welbourn, 1994). In this study, Cd concentrations were measured in Kapaklı, Çınarcık, Koru, and Deveboynu stations as 0.11 mg/kg ww, 0.19 mg/kg ww, 0.15 mg/kg ww, and 0.13 mg/kg ww, respectively. The permissible limit for Cd is 0.1 mg/kg (Republic of Turkey Ministry of Agriculture and Forestry, 2002). The results from all stations in this study were found to exceed the permissible limits. The source of the detected cadmium in the study may have been due to agricultural activities in the areas where the stations were located, or in other words, the rainwater that carried the cadmium to the areas where the mussels were collected.

El Shenawy et al. (2016) stated that high levels of Pb in sediment could be attributed to human activities such as shipbuilding and maintenance, industrial and agricultural discharges, as well as leaded gasoline spills from fishing boats. Excessive amounts of Pb are known to exhibit mutagenic, teratogenic, and carcinogenic effects (Castoldi Anna et al., 2003; Lidsky & Schneider, 2003; García-Lestón et al., 2010; Sharma et al., 2014). Erkan et al. (2011) found Pb levels in *O. edulis* as 0.165 mg/kg ww in April, while Türk Çulha et al. (2011) measured the highest Pb levels in *M. galloprovincialis* (49.62 mm and 9.87 g) in the spring season, with 0.35 mg/kg ww. Topçuoğlu et al. (2004) reported that Pb levels of *M. galloprovincialis* in the Marmara Sea ranged from <0.014 to 0.73 mg/kg ww (70-80 mm). However, Lök et al. (2010) reported that Pb content of *M. galloprovincialis* in the Çanakkale Strait was between 0.22 mg/kg ww and 18.47 mg/kg ww in the Çanakkale Strait. Özden et al. (2009) examined Pb concentrations in *C. gallina* and *Donax trunculus*, which yielded 1.34 mg/kg and 1.32 mg/kg ww, respectively. Prato et al. (2019) measured Pb in *F. glaber* at 0.55 mg/kg ww. The amount of Cd accumulated in the body varies depending on species, age, size, etc. In this study, Pb levels were measured at Kapaklı, Çınarcık, Koru, and Deveboynu stations at 0.11 mg/kg ww, 0.72 mg/kg ww, 0.57 mg/kg ww, and 0.31 mg/kg ww, respectively. The limit value for Pb in this study was determined to be 1.0 mg/kg (Republic Turkey Ministry of Agriculture and Forestry, 2002), and all stations in this study had Pb values below this limit.

Seafood is known to contain high levels of organic arsenic (As) (Han et al., 1998). Inorganic forms of As are carcinogenic and chronic exposure to inorganic As can lead to various health problems by affecting the gastrointestinal, respiratory, skin, liver, cardiovascular, and nervous systems (Mandal & Suzuki, 2002); in addition, it can cause vomiting, diarrhea, anemia, liver damage, and death (Centeno et al., 2002). Özden et al. (2010) reported that the As content in *M. galloprovincialis* varied between 0.070 mg/kg ww (in September) and 1.183 mg/kg ww (in February) in the Marmara Sea (Istanbul) and was 0.150 mg/kg ww (in April). Lök et al. (2010) informed that As content of *M. galloprovincialis* in the Çanakkale Strait changed between 0.03 mg/kg ww and 0.05 mg/kg ww. As values in *C. gallina* and *D. trunculus* varied between 2.64-2.91 mg/kg ww and 1.74-3.45 mg/kg ww, respectively, in winter and summer seasons (Özden & Erkan, 2011). Prato et al. (2019) measured the As content in *F. glaber* to be 6.10 mg/kg ww. Republic of Turkey Ministry of Agriculture and Forestry (2002) reported that the maximum limit for As is 1 mg/kg. In this study, the As content was measured as 0.61 mg/kg ww in Kapaklı, 2.59 mg/kg ww in Çınarcık, 2.29 mg/kg ww in Koru, and 1.92 mg/kg ww in Deveboynu stations, respectively. Generally, As values obtained in studies conducted in the Marmara Sea are above the limit values, including those found in this study, except for the Kapaklı station. The entry of anthropogenic arsenic is mainly from agricultural, forestry, industrial, and mining activities, which contaminate the soil and water (Smith et al., 2003). We believe that the study area is located in regions affected by agricultural activities, and waste resulting from pesticide spraying and mixing with the soil is carried to the natural habitat of mussels through freshwater sources. Mussels are filter-feeding organisms, meaning that they take in organic and inorganic substances present in the environment, and As may accumulate in their bodies as a result.

CONCLUSION

In recent years, there has been a significant increase in commercial activities for the production of mussels through aquaculture in Turkey. However, due to the new structuring of the sector, many problems have been encountered in the production facilities such as facility installation and qualified personnel. Especially during the initial establishment phase of the system, the lack of juvenile mussels is known as the most common problem. Procuring juveniles from other businesses is a widely used method, but currently, it is not considered a sufficient solution for the needs of newly established businesses. Therefore, it is important to determine the conditions of stations in the Marmara Sea for collecting *M. galloprovincialis* juvenile. Therefore, determining the meat quality and heavy metal concentration of *M. galloprovincialis* in the Sea of Marmara in the different stations is important for its aquaculture facilities.

Although it was possible to say that the mussels at all stations are suitable for use in a production system in terms of their condition index, meat yield, fouling organism attachment, and Cu, Zn, Hg, and Pb values, it had been concluded that Çınarcık, Koru, and Deveboynu stations were risky due to the As level found in these stations and the Cd level detected in all stations exceeding the limit value according to Republic of Turkey Ministry of Agriculture and Forestry (2002). Although other parameters was appropriate, the study has concluded that the quality of the products from these stations was not safe for both the juvenile collection and human consumption due to the toxicity of As and Cd.

COMPLIANCE WITH ETHICAL STANDARDS

a) Authors' Contributions

1. S.A.: She designed the study and interpreted the data.

2. P.V.: She carried out the laboratory work.

3. H.Y.: He designed the study and prepared the article.

b) Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper

c) Statement on the Welfare of Animals

Not applicable

d) Statement of Human Rights

Not applicable

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Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2023; 9(1): 25-33 Araştırma Makalesi/Research Article

Determination of Antibacterial and Antibiofilm Activities for Laurel (*Laurus nobilis* L.) Essential Oil Against the Fish Pathogen *Pseudomonas* Species

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Abstract

The essential oil derived from *Laurus nobilis* exhibits a high concentration of bioactive components, imparting various therapeutic characteristics. This study aimed to analyze the chemical composition of the essential oil extracted from *Laurus nobilis* leaves as well as its biological properties, including its antipseudomonal and antibiofilm actions. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that 1,8-cineole (%48.43) and α-terpinyl acetate (14.78) were the major compounds present in the essential oil (EO). While, the minimum inhibitory concentration (MIC) values of *Laurus nobilis* essential oils (LEO) against *P. fluorescens* and *P. putida* were determined as 31.25 µg/mL, it was 62.5 µg/mL for *P. aeruginosa*. LEO, at a MIC level of 31,25µg/mL, exhibited significant inhibition of *Pseudomonas* species biofilm formation except for *P. aeruginosa.* Based on its demonstrated antibacterial and antibiofilm potential, LEO holds promise as a prospective source of antibacterial agents.

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INTRODUCTION

Aquaculture, a rapidly growing sector of agriculture globally, faces various challenges that impede its production growth. Among these challenges, fish diseases caused by a diverse range of infectious organisms pose a significant concern, leading to substantial economic losses for farmers (Francis-Floyd, 2005; Alfred et al., 2020; Assefa and Abunna, 2018). Bacterial infections pose a significant threat to both wild and aquaculture-reared fish. In the aquatic environment, bacteria are commonly present as part of the normal flora, and they typically do not cause disease unless fish are exposed to unfavorable environmental conditions and inadequate husbandry techniques (Tendencia and Lavilla-Pitogo, 2004).

Pseudomoniasis, also known as *Pseudomonas* septicemia or *Pseudomonas* infection, is a bacterial disease caused by various species within the genus *Pseudomonas*. Several *Pseudomonas* species including *P. anguilliseptica*, *P. baetica*, *P. chlororaphis*, *P. fluorescens*, *P. luteola*, *P. plecoglossicida*, *P. pseudoalcaligenes*, *P. aeruginosa* and *P. putida* have been reported to be pathogenic that cause ulcerative syndrome and hemorrhagic septicemia in various fish species (Austin and Austin, 2012; Algammal et.al., 2020; Eissa, 2010; Bektas and Ayik, 2009).

When a bacterial disease is encountered in farms, one of the effective treatments is the use of antimicrobial agents for years. However, intensive and misuse of antibiotics for preventing and treating bacterial infection have led to the emergence of bacterial resistance, resulting in treatment failures, jeopardizing food safety, and posing environmental concerns (Romero et al., 2012; WHO, 2020). The increase in antimicrobial resistance not only poses challenges in the treatment of bacterial infections in fish but also constitutes a substantial and worldwide public health risk (Sabo and Knezevic, 2019; Insuan and Chahomchuen, 2020). The escalating occurrence of antibiotic resistance has directed researchers attention towards exploring alternative avenues for the treatment of infections (Da Cunha et al., 2018).

Biofilms, consisting of multicellular matrices, facilitate bacterial adhesion to the external environment and represent a contributing factor to microbial drug resistance. Biofilm cells exhibit distinct characteristics compared to planktonic cells, including differences in morphology, physiology, and gene expression. Consequently, it is crucial to invest efforts in identifying novel compounds capable of combating drug-resistant microbes and biofilms to prevent the emergence of resistance (Liu et al., 2022; Lim et al., 2022).

EOs obtained from various aromatic plants contain a diverse array of secondary metabolites and exhibit significant antimicrobial activity by inhibiting bacterial growth. Due to their antimicrobial properties, EOs have the potential to serve as an alternative to antibiotics for the management of infectious fish diseases (Kunová et al., 2021; Gholipourkanani et al., 2019; Nazzaro et al., 2013; Bektas and Ozdal, 2022).

Most EOs are composed of terpenes, terpenoids, and other aromatic and aliphatic constituents with low molecular weights. Not only the natural monoterpenes but also their synthetic forms have various pharmacological properties such as antifungal, antibacterial, antioxidant, anticancer, antibiofilm, antiarrhythmic, anti-aggregating, local anesthetic, antinociceptive, anti-inflammatory, antihistaminic, and anti-spasmodic activities (Loza-Tavera, 1999; Kozioł et al., 2014; Swamy et al., 2016).

The laurel (*Laurus nobilis* L.), an evergreen plant species endemic to Southern Europe and the Mediterranean, is a member of the Lauraceae family. Bay leaves derived from this plant are utilized as a spice in Mediterranean cuisine owing to their aromatic and pleasing scent. Additionally, they have been traditionally employed in medicinal practices for treating diverse infections and find applications in the cosmetics industry (Ramos et al., 2012; Fidan et al., 2019).

The present study was conducted to determine the chemical composition of the EOs of laurel and to evaluate the antibacterial and antibiofilm activities against the fish pathogen *Pseudomonas* species: *P. aeruginosa*, *P. fluorescens*, *and P. putida*.

MATERIAL and METHODS

Plant material

The leaves and fruits of laurel were collected from the trees growing wild in Sinop Region, Turkey (42°02'43.4"N 35°02'27.9"E) during June in 2015. Samples were cleaned and dried in the shade at room temperature.

Extraction of essential oil

About 500 g of dried plant material, comprising leaves and fruits, was crushed and subjected to hydro-distillation using a Clevenger's apparatus. Crushed samples were immersed in water and heated to boiling. The EOs evaporated together with water vapor and passed through the refrigerant before being collected into the condensation flask. Following the separation of the liquid phase, the EOs were collected in a glass vial (Ghalem and Mohamed, 2008; Mazumder et al., 2020).

GC and GC/MS analyses

Analyses were conducted in Eskisehir Anadolu University Medicinal Plants, Drugs and Scientific Research Center (AUBİBAM). Briefly, Hewlett Packard system, HP 5973 Mass Selective Detector System and GC–MS 6890 GC system were used in analyses. Agilent HP innowax column (60 m in length, inner diameter of 0.25 mm, film thickness of 0.25 µm) was used. As a carrier gas, helium was used. The injection temperature was 250 °C and the oven temperature was kept at 60 °C for 10 minutes, then programmed to 220 °C at a rate of 4 °C/min, kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 2 °C/min for 40 minutes. Relative amounts of the characterized components were expressed in percentages, and the retention time (RT) was recorded in minutes (Sevindik et al., 2016).

Microbial Strains

The EOs and their components were tested against fish pathogenic *Pseudomonas* species; *P. aeruginosa* (ATCC 9027), *P. fluorescens* (BC 7324), *and P. putida* (BC 1617). All laboratory stock cultures of the microorganisms were obtained from the Microbiology Laboratory, Department of Food Engineering, Faculty of Agriculture, Atatürk University, Erzurum, Turkey. Bacteria were identified by morphological and biochemical tests, including the assessment of colony morphology, Gram staining, oxidase and catalase activities, indole, citrate utilization, methyl red, gelatin hydrolysis, fermentation of mannitol, sucrose, lactose, fructose, glucose, and urease, and growth at different temperature conditions. Throughout the investigation, bacterial cultures were stored frozen at -86 °C in nutrient broth (NB) containing 20% (v/v) glycerol and utilized as stock cultures.

Determination of antibacterial concentration

To determine the minimum inhibitory concentration (MIC) of LEO, the Broth Dilution Method was utilized (Rath and Priyadarshanee, 2017). This involved mixing varying amounts of the oil with NB, which included a 0.5% dilution of Tween 20, to create a range of concentrations from 7.8-125 µg/ml using a serial dilution approach. The study tested a total of five different concentrations. The experiment involved inoculating 100 µl of overnight culture with approximately $5x10^5$ bacterial cells into wells that contained 100 µl of laurel oil at varying concentrations. The 96-well plate, which contained a total of 200 µl, was then subjected to shaking and incubation for 24 hours at 37 °C. The point at which bacterial growth was inhibited and no turbidity was observed was recognized as the MIC of the laurel oil against the particular strain of bacteria being tested. Following 24 h incubation, the optical density (OD_{600}) was measured for MIC analysis. In order to calculate the Minimum Bactericidal Concentration (MBC), a 20-µl solution obtained from the final three test wells, which did not exhibit bacterial growth, was inoculated on nutrient agar (NA) plates and incubated overnight at 37 °C.

Antibiofilm effects

Biofilm inhibition assay was tested against *P. aeruginosa* (ATCC 9027), *P. fluorescens* (BC 7324) and *P. putida* (BC 1617) using laurel oil in 96-well culture plates. The bacterial strains were incubated in 10 mL of Tryptic soy broth (TSB) that contained 1% glucose and were maintained at 37 °C for a period of 24 h. This followed by the preparation of dilutions equivalent to 0.5 McFarland standard value. In each well of plates, 90 µL of growth medium (TSB with 1% glucose), 100 µL of laurel oil (final concentrations of 7.8-125 µg/ml), and 10 µL of test bacterial dilutions were mixed. In the positive controls, 10 µL of the bacterial dilutions were mixed with 190 µL of growth medium, while negative control contained only growth medium. After 24 h-incubation at 37 °C, the 96-well plate was washed with distilled water three times to remove unattached planktonic cells and the remaining adherent sessile cells were dyed with 200 µL of 0.4% crystal violet for 30 min, then the excess dye was poured and the wells were washed three-times with distilled water. The remaining dyed biofilm was suspended in 200 µL of 70% ethanol for 30 min at room temperature. OD of the wells were read at 570 nm by microplate reader (Thermo Scientific Inc., Multiscan GO, Finland) (Bai et al., 2019). Biofilm inhibition was calculated by the following formula.

Biofilm inhibition (%) = [(Control OD570nm – Test OD570nm) / Control OD570nm] x 100

RESULTS AND DISCUSSION

All the strains demonstrated the characteristic phenotypic traits commonly observed in fluorescent pseudomonads, including the production of a water-soluble yellow-green or yellow-brown pigment known as pyoverdine. While all the strains tested in this study were Gram-negative, motile, rod-shaped, oxidase, catalase, citrate, mannitol, fructose, and glucose positive, they were indole, methyl red, sucrose, lactose, and urease negative. *P. aeruginosa* is differentiated from the other strains by its ability to grow at 42 °C and is also recognized by its characteristic colony morphology. *P. fluorescens* and *P. aeruginosa* differ from *P. putida* in their ability to hydrolyze gelatin.

Chemical Composition of the laurel

The GC/MS analysis resulted in the identification of 15 components in LEO, which collectively accounted for 87.04% of the total composition of the analyzed EOs. Analysis of the LEO revealed 1,8-cineole or eucalyptol (%48.43) and α-terpinyl acetate (14.78) as the major compounds with the highest peaks (Figure 1).

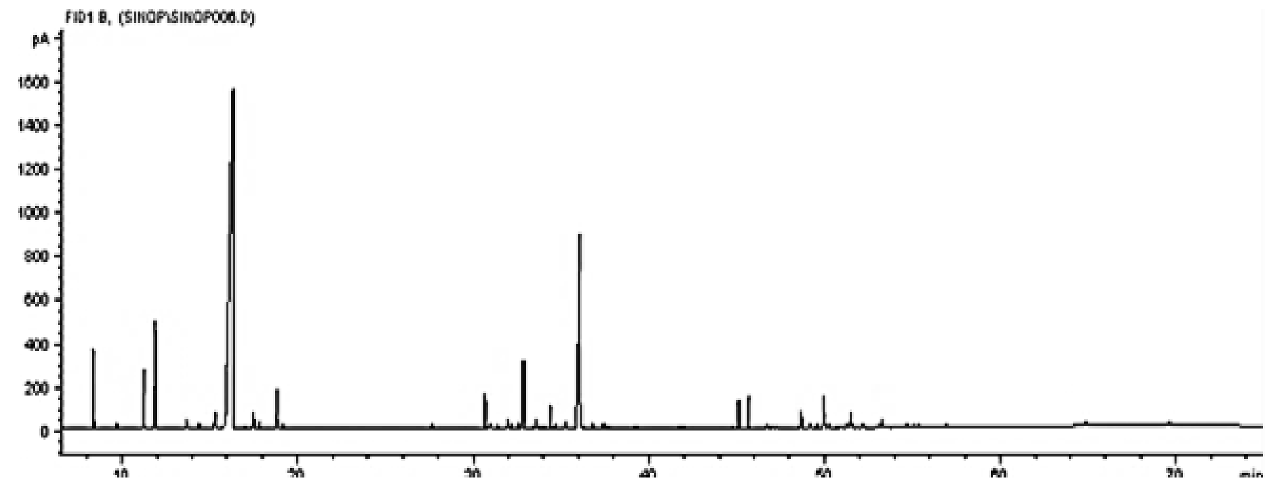

Figure 1. Gas chromatogram of the essential oil from laurel

The retention time and percentage composition of the identified compounds of laurel EOs are presented in Table 1.

Table 1. Essential oil composition of laurel

RT (min)	Component	Quantity (%)
16.35	1,8-cineole	48.43
36.03	α-terpinyl acetate	14.78
11.95	sabinene	5.68
32.84	terpinen-4-ol	2.95
11.31	β-pinen	2.73
8.40	α-pinene	2.59
18.88	p-cymene	1.67
15.36	limonen	1.34
30.63	linalool	1.23
45.10	caryophyllene oxide	1.20
45.63	methyl eugenol	1.18
49.91	eugenol	1.15
36.06	α-terpineol	0.82
48.62	Spathulenol	0.66
51.48	β-eudesmol	0.63
Total		87.04

RT: Retention time

The present study demonstrated that the EO from laurel involved eucalyptol and α-terpinyl acetate as the primary constituents. Similarly, Ramos et al. (2012) reported the major components of bay laurel as eucalyptol (27.2%), α-terpinenyl acetate (10.2%), linalool (8.4%), methyleugenol (5.4%), sabinene (4.0%) and carvacrol (3.2%). Bay laurel EOs were reported to contain 1,8-cineole (60.72%), α -terpinene (12.53%), sabinene (12.12%), and α -pinene (6.11%) as major constituents (Dadalioglu et al., 2004). Taban et al. (2018) reported the main chemical components in EOs obtained with different extraction methods from laurel as eucalyptol (34.4–50.0%), α-terpinenyl acetate (14.9–18.8%), terpinene-4-ol (4.7–6.0%), and sabinene (4.9–5.9%).

While the major components of laurel oil identified in this study were comparable to findings from various other studies, variations in the quantities of these essential oils were observed. These differences are likely influenced by several factors, including diverse seasons, regions, cultivation conditions, stages of maturity, harvesting methods, and extraction techniques (Arumugam et al., 2016; Swamy et al., 2016).

1,8-Cineole, referred to as eucalyptol, is a bicyclic terpenoid present in essential oils derived from diverse plant species and is known to possess antimicrobial properties (Mączka et al., 2021; Moo et al., 2021). 1.8-cineole was reported as the major component in the essential oil of laurel from Morocco (Derwich et al., 2009). Mazumder et al. (2020) reported significant antibacterial activities of 1,8-cineole, obtained from *Eucalyptus maculate* as a major compound, together with the crude EOs, against the fish pathogens *Aeromonas hydrophila and A. jandae*.

In this study, another main component of laurel oil was determined to be α-Terpinyl acetate (α-TA) with a ratio of 14.78%. α-Terpinyl acetate which could be found in some EOs bearing plants, is an organic, volatile monoterpene ester. Vaičiulytė et al. (2021) reported that α-TA EO has high antimicrobial activity against fungi but lower activity against bacteria.

The antibacterial activity of Laurel essential oil

In order to assess the antimicrobial efficacy of LEO against the tested *Pseudomonas* pathogens, the MIC and MBC values were determined. The findings revealed diverse effects of the essential oil on the bacterial strains under investigation (Table 2). Based on their characteristics as bactericides (close to to 1) or bacteriostatics (greater than 4), antibiotics are categorized using the MBC/MIC ratio. Table 2 displays MBC values that were higher than MIC. All of the isolates had an MBC/MIC ratio of 2. The MBC/MIC ratio of LEO was equivalent to 2.0, which clearly suggests that LEO had a bactericidal impact on Pseudomonas species.

Through our investigation, it was discovered that LEO possess diverse anti-pseudomonal properties as evaluated for antibacterial activity. Notably, LEO exhibited greater efficacy against *P. fluorescens* and *P. putida* compared to *P. aeruginosa*, suggesting that *P. aeruginosa* exhibited higher resistance to LEO when compared to the other strains.

Table 2. Antibacterial parameters (MIC and MBC µg/mL) of the essential oil of the *L. nobilis*

Bacteria	MIC	MBC	MBC/MIC
P. aeruginosa	62.5	125	2
P. putida	31.25	6.25	2
P. fluorescens	31.25	62.5	2

Consistent with the findings of this study, Řebíčková et al. (2020) observed limited inhibitory effects of LEO against *P. aeruginosa*, which could potentially be attributed to the notable lipid-degrading capability of *P. aeruginosa* (Ozdal et al., 2017).

Goudjil et al. (2015) reported variable bacteriostatic properties of the LEO against different strains. MIC values for *Salmonella enterica* and *Klebsiella pneumoniae* were reported as 0.2 and 0.11 mg/mL, respectively.

LEO was tested for its antimicrobial activity against four different bacterial species, including Gram-positive bacteria (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Antibacterial activity against all tested pathogens varied from 1250 to 2250 µg/mL (Santoyo et al., 2006). Gram-positive bacteria tend to exhibit higher susceptibility to EOs compared to Gram-negative bacteria, primarily due to the presence of an additional outer membrane in Gram-negative bacteria, which provides an enhanced protective barrier for the cytoplasmic membrane against antimicrobial substances such as EOs (Caputo et al., 2017). In the investigation conducted by Ertürk (2006), MIC of *L. nobilis* ethanolic extract against the tested bacteria, including *B. subtilis*, *Staphylococcus aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa*, was determined to be 5 mg/mL using agar dilution methods (Ertürk, 2006).

Dadalioğlu et al. (2004) showed that LEO (5-80 µL/mL) exhibited antibacterial activity against *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *S. aureus*. Unlikely, Jacobo et al. (2022) also reported that the MIC and MBC of LEO was 12.36 mg/mL for *S. aureus*, *E. coli*, and *Enterococcus faecalis*. Snuossi et al. (2016) reported varying antibacterial activities of LEO against *A. hydrophila, Staphylococcus spp., Vibrio alginolyticus, Enterobacter cloacae, K. ornithinolytica, K. oxytoca and Serratia odorifera*. MIC values for the bacteria, have been reported ranging from 0.05 to 0.39 mg/mL.

Antimicrobial effect of an EO is attributed to its chemical makeup, particularly the functional groups present in its main constituents such as alcohols, phenols, and aldehydes, as well as the synergistic interactions among these compounds. Previous investigations have demonstrated the antimicrobial activity of hydrocarbon and oxygenated monoterpenes, including 1,8-cineole, linalool, -terpineol, and terpinen-4-ol (Caputo et al., 2017; Badawy et al., 2019). The -OH groups of bioactive chemicals positioned at the meta and ortho locations are responsible for the antibacterial properties of essential oils. The cytoplasmic membrane of bacterial cells can interact with these -OH groups, causing cell death and disintegration (Shahbazi, 2019).

Antibiofilm Activity of the Essential Oil

Biofilms pose challenges in water treatment systems and particularly impact various sectors of the food industry, such as brewing, dairy processing, fresh produce, poultry processing, and meat processing, as evidenced by studies conducted by Chen et al. (2007) and Lu et al. (2022). Bacteria in biofilms can be up to 1000 times more resistant to antibiotics than their planktonic counterparts. (Simoes et al., 2009).

Pseudomonas is an opportunistic pathogen with a high level of vitality that is widely distributed in the water, air, soil, and food supply (Osman et al., 2019). Species such as *P. aeruginosa*, *P. fluorescens*, and *P. putida* are known capable of forming biofilms (Iseppi et al., 2020).

P. fluorescens and *P. putida* biofilms were found to be suppressed by the anti-biofilm activity of LEO at sub-MIC doses. Our findings show that *P. fluorescens* and *P. putida* are more vulnerable to LEO than *P. aeruginosa*. When LEO at concentrations of 7.81, 15.62, 31.25, 62.5, and 125 µg/mL were used, 0, 0, 0, 28, and 45% of *P. aeruginosa* biofilm formation was inhibited, respectively (Figure 2). Likewise, the same concentrations of LEO prevented 30, 54, 100, 100, and 100% of biofilm formation by *P. fluorescens*. For *P. putida* at the same concentrations, these values were measured as 24, 77, 100, 100 and 100. As seen in Figure 2, the addition of LEO at the MIC level (31.25 µg/mL) considerably reduced the development of *Pseudomonas* species biofilms except for *P. aeruginosa*.

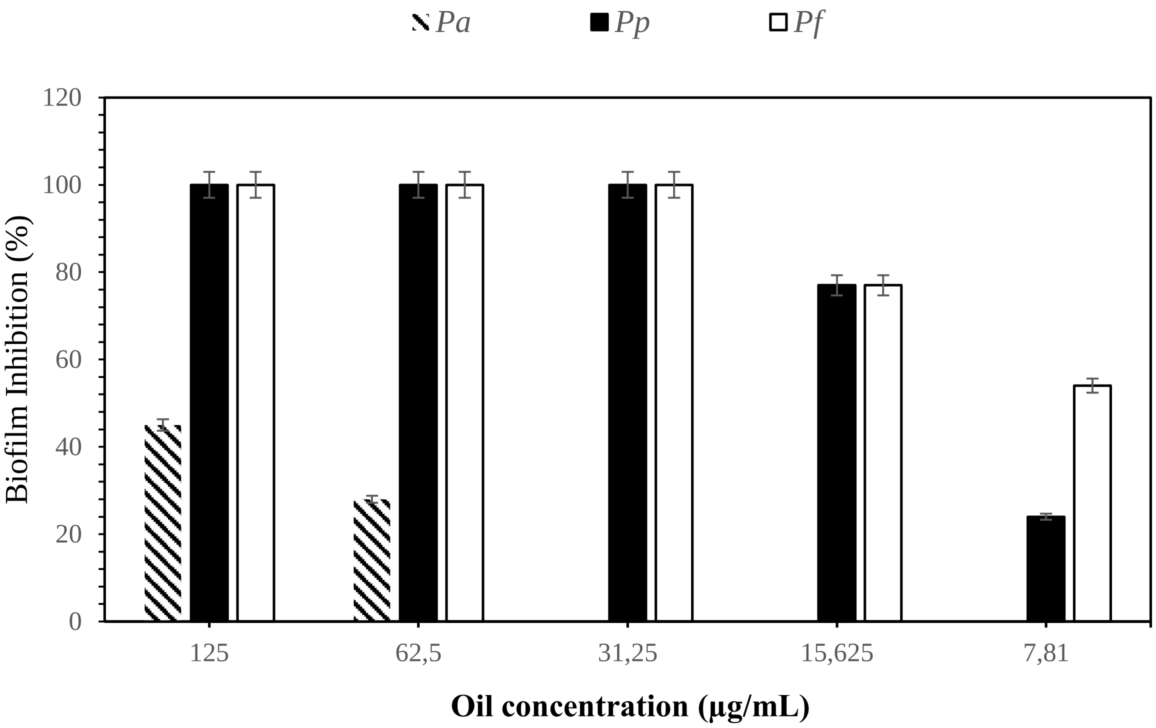

Figure 2. Inhibition of biofilm formation by *P. aeruginosa*, *P. fluorescens*, and *P. putida* using LEO.

The efficacy of LEO in eliminating *S. epidermidis* CIP 444 biofilms was investigated in a prior study, revealing biofilm inhibition rates ranging from 8.5% to 33.75% (Chmit et al., 2014). Merghni et al. (2016) reported that LEO, at a concentration of 1/16 x MIC (0.24 to 1.95 mg/mL), exhibited a biofilm inhibition rate exceeding 70% against *S. aureus* strains. Also, LEO at concentrations starting at 2 MICs inhibited the initial adhesion of *Candida albicans* (Peixoto et al., 2017).

In conclusion, *L. nobilis* EO exhibits antipseudomonal activity and affects the formation of biofilms in *Pseudomonas* species, possibly by interfering with cell wall biosynthesis and membrane ionic permeability, facilitated by the presence of identified monoterpenes and sesquiterpenes.

CONCLUSION

The present study provides evidence that LEO possess both antibacterial and antibiofilm activities against *Pseudomonas* species. Given the emergence of multidrug-resistant strains and the prevalence of biofilm formation, there is a pressing need to identify effective alternatives to combat Pseudomonads, and EOs have emerged as a potential option. Several EOs have been shown to be effective antimicrobials and antibiofilm agents, allowing them to be used in therapeutic formulations either alone or in combination with already established antibiotics. However, further study is necessary to gain a better understanding of the interactions of the biofilm formation phases with the EOs and their components independently. Also, additional acute investigations on volatility and solubility should be conducted in order to boost the antibacterial potential of EOs as a pharmaceutical product.

COMPLIANCE WITH ETHICAL STANDARDS

a) Authors' Contributions

SB & MÖ: Designed the study.

SB & SG: Wrote the first draft of the manuscript.

MÖ, SG & SB: Performed laboratory experiments

(All authors read and approved the final manuscript).

b) 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.

Data Availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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Gemi İnşa Sektörü Açısından Beton Gemilerin Tarihsel Süreci Üzerine Bir Araştırma

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Makale Bilgisi

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Öz

Denizcilik sektöründe, günümüze kadar birçok malzeme çeşidi gemi gövdesi inşasında kullanılmaktadır. En çok bilinen gemi inşa malzemeleri arasında çelik, alüminyum alaşımları, elyaf destekli kompozit ürünler ve ahşap malzemeler bulunmaktadır. Bu çalışmada en yaygın gemi inşa malzemelerin aksine betonun gemi gövdesinde kullanılması ham çelik üretimini de göz önünde bulundurarak tarihsel açıdan incelemektedir. Gemi gövdesi inşasında beton kullanımı 1848 yıllarına kadar dayanmakta, kargo gemisi, yüzer barç, yat, balıkçı tekneleri olmak üzere birçok gemi tipinde kullanılmaktadır. Çelik tedariğinin zor ve maliyetli olduğu, nitelikli iş gücü ihtiyacının tam olarak karşılanamadığı özellikle birinci ve ikinci dünya savaşı döneminde oldukça büyük ölçekli beton gemiler inşa edilmiş fakat bu gemilerin hiçbiri faaliyetlerini günümüze kadar sürdürememiştir. Genellikle inşa edildikten kısa bir süre sonra ana amaçlarının dışında eğitim gemisi, depo, dalgakıran olarak kullanılmış veya batırılmıştır. Çalışmanın beton gemilerle ilgili süreci analiz etmesi, literatürde oldukça az değinilen bu alana katkı sağlamakta ve bu alanda çalışacak akademisyenler, uzmanlar ve denizcilik paydaşları için önemli bir kaynak oluşturmayı hedeflemektedir.

A Study on the Historical Process of Concrete Ships in Terms of Shipbuilding Industry

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Abstract

Diversified types of materials have been utilized in ship hull construction up till the present day. The most well-known shipbuilding materials are steel, aluminium alloys, fibre-reinforced composite products and wood materials. In contrast to the most well-known construction materials, the use of concrete in the ship's hull have been investigated from a historical perspective, taking into account the raw steel production. Concrete ships, whose history dates back to 1848, have been served as cargo ships, floating barges, yachts, fishing boats. Particularly throughout the first and second world wars, quite large-scale concrete ships were built when obtaining raw steel was challenging and expensive also qualified workforce needs were not fully met but none of these ships could continue their activities until today. Soon after concrete ships construction, they were generally utilized as a training ship, warehouse, or breakwater apart from their main purpose or were sunk. The paper's analysis of the process related to concrete ships contributes to rarely mentioned area in the literature, and aims to create a significant resource for academicians, experts and maritime stakeholders who will work in this field.

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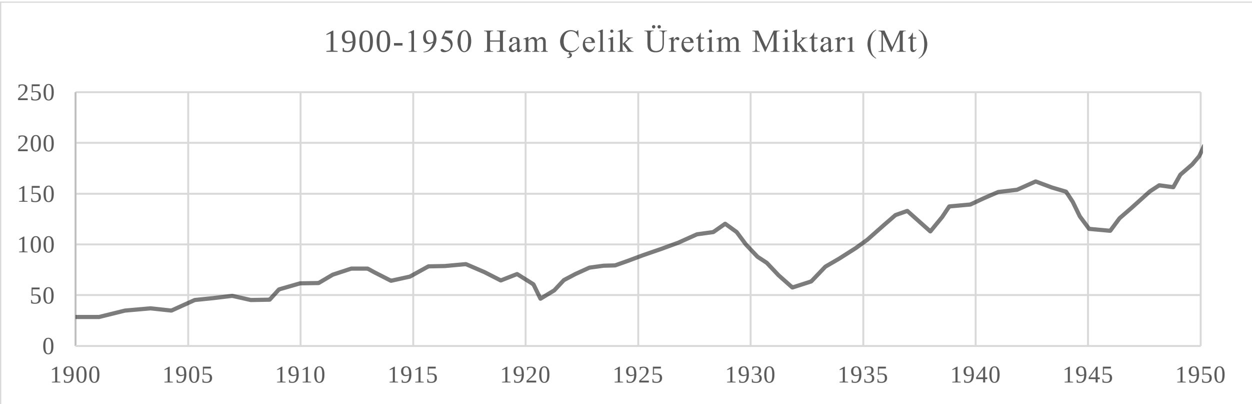
GİRİŞ

Gemi İnşa Sanayi ve Çelik Kullanımı

Alüminyum alaşımları, elyaf destekli kompozit ürünler ve ahşap gibi birçok malzeme gemi inşaatı gövde üretiminde kullanılmasına rağmen, üretim ve tüketim açısından sanayileşmenin ve gelişmişliğin sembollerinden biri olan çelik, gemi inşaatı sektöründe gemi gövde üretim aşamasında kullanılan en önemli malzemedir. 1798 Vauquelin tarafından ortaya konan krom parçası ile özellikle paslanmaz çeliğin ana bileşeni oluşturulmaktadır (Özsoysal ve Ünsan, 2005; Cobb, 2010). Gemi inşa sektöründe gemi gövdesinde kullanılacak ana maddede üç temel özellik aranmaktadır. Malzemenin mukavemeti, malzemenin zaman içinde korozyon gibi değişime karşı direnci ve yorulma dayanımı bu üç temel özelliği oluşturmaktadır (Mandal, 2017).

Korozyona karşı direnç, yüksek yorulma dayanımı gibi birçok mekanik üstünlüğe sahip olan çelik, büyük miktarlarda kaynaklama işlemiyle birlikte gemi gövde yapısı inşaatında kullanılmaktadır (Imai, 2008). Düşük maliyetinin yanında, kaynak yapılarak bloklar halinde imalata olanak sağlaması çeliği ön plana çıkarmaktadır. Ayrıca, çeliğin yapısında birçok element bulunduğundan elementlerin bileşiklerdeki oranları değiştirilerek süneklik, korozyona karşı direnç, şekillendirilme gibi özellikler açısından isteğe göre daha fazla üstünlük sağlanılabilmektedir (Mandal, 2017). Alaşım çeliği üzerine ilk çalışmalar Michael Faraday tarafından yapılmıştır ve ilerleyen yıllarda korozyon, mukavemet açısından güçlü özellikleri içinde barından güçlü çelik alaşımları elde edilmiştir (Cobb, 2010). 19 yüzyılın ikinci yarısından itibaren demir ve çelik gemilerin inşası artarak devam etmekte ve günümüzde halen ana maddelerin başında gelmektedir (Rogers, 2009). Çelik gemilerin inşaatının sürdürebilirliğini sağlayan ham çelik üretimi ile ilgili 1900-1950 arası sayısal bilgiler Şekil 1'de ifade edilmektedir.



Şekil 1. 1900-1950 yılları arası küresel ham çelik üretim miktarları (Price ve diğer., 1998).

Dalgalanmalara rağmen, belirlenen yıllarda çelik üretimin düzenli olarak arttığı görülmektedir. Özellikle artan çelik üretimin talebi tam karşılayamaması veya maliyetlerin yüksek olması gibi durumlar göz önünde bulundurulduğunda bu dönemlerde askeri amaçlı hizmetleri karşılamak adına birçok beton gemi inşa edilmiştir. 1950'den sonra günümüze kadar ilerleyen yıllarda ham çelik üretimi artarak devam etmektedir. Ham çelik üretimi 1950 yılları ile 2021 yılları arasındaki değişimi Tablo 1'de günümüze doğru ilerlendiğinde daha kısa aralıklarla olacak şekilde sayısal verilerle detaylı bir şekilde ifade edilmektedir.

Tablo 1. 1950-2021 yılları arası küresel ham çelik üretim miktarları (Worldsteel 1997, 2022).

Yıl	1950	1955	1960	1965	1970	1975	1980	1985	1990	1995	1997
Üretim Miktarı (Mt)	189	270	347	456	595	644	717	719	770	753	799
Yıl	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Üretim Miktarı (Mt)	850	852	905	971	1063	1148	1250	1350	1345	1241	1435
Yıl	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Üretim Miktarı (Mt)	850	852	905	971	1063	1148	1250	1350	1345	1241	1435

Tablo 1'de ifade edilen yıllar beş yıllık aralıklarla incelendiğinde en büyük pozitif değişim 1950-1955, 2000-2005 ve 1960-1965 yılları arasında sırasıyla %7,4, %6,2 ve %5,6'dır. Negatif yönde düşüş dünya genelinde sadece 1990-1995 yılları arasında %0,5 oranında gerçekleşmektedir. Miktar açısından 1435 Mt ile 2021'de en yüksek ham çelik üretimi gerçekleşmiştir (Worldsteel, 2022). Ham çelik üretiminin istisnalar dışında sürekli artış göstermesine rağmen özellikle I. ve II. Dünya Savaşı döneminde Birleşik Devletler Denizcilik Komisyonu U.*S. Maritime Commission* tarafından beton gemilerin üretimine karar verilmiştir.

Beton Gemiler

Beton gemilerin dizaynı ve inşası 19. yüzyılın ilk yarısının sonlarına kadar dayanmaktadır (Liu ve MacDonald, 1977). 1848 yılında inşa edilen ilk beton gemi 1914-1887 yılları arasında yaşayan Fransız Joseph Louis Lambot tarafından gerçekleştirilmiştir ve Lambot tarafından gerçekleştirilen bu teknoloji ile birçok tekne inşa edilmiştir (Key, 1970; Whang, 1972; Özsoysal ve Ünsan, 2008; Topçu ve Bahadırlı, 2010). Şekil 2'de Joseph Louis Lambot ile ilk inşa ettiği beton gemi gösterilmektedir.

Şekil 2. Joseph Louis Lambot ve ilk beton gemi (Maisonlambot, 2022).

İlerleyen yıllarda Lambot tarafından ortaya konan benzer tekniklerle birlikte Zeemeeuw adlı beton gemi Gabellini and Boon tarafından 1887 yılında inşa edilmiştir. Beton üzerine yaşanan gelişmelerle birlikte daha önce inşa edilenlerden farklı olarak oldukça hafif ilk güçlendirilmiş "*reinforced"* betonarme gemi 1902 yılında inşa edilmiş ve ek olarak ilk öngerilmeli "*prestressed*" beton gemi 1943 yılında denize indirilmiştir. Ferrocement, güçlendirilmiş ve öngerilmeli beton çeşitleri başlıca betonarme tiplerini oluşturmaktadır. Gemi inşa sanayinde betonun hammadde olarak kullanılmasını ferrocement tekne yapımını, maliyetlerinin düşük olması, karmaşık bir yapıya sahip olamaması, üretim kolaylığı ve çeliğe göre daha kolayca şekil alabilmesi, bakım-tutum maliyetlerinin düşük ve aralıklarının uzun olması, dayanıklı yapısı ve yüksek basınç dayanımı, korozyona karşı dayanımı, inşasında deneyimli personele olan ihtiyacın az olması tetiklemektedir. Ferrocement yapısında tel örgü oluşturmak amacıyla %5 ile %8 arası hacimde ve %15'i aşan ağırlıkta çelik barındıran 1,5'e 1 ile 2'ye 1 oranında değişen kum ve çimento harcının karışımından oluşmaktadır (Key, 1970; Whang, 1972; Brauer, 1973; Desayi ve Prasanna Kumar, 1977; Liu ve MacDonald, 1977; Lavache, 1978; Arslan, 2007; Özsoysal ve Ünsan, 2008; Topçu ve Bahadırlı, 2010). Bu duruma karşın, betonun ağırlığı, düşük yüzerlik profili ve herhangi bir çarpışma anında küçük parçalara bölünecek gevrek yapısından dolayı 19. yüzyılın başlarında gemi inşasında demir ana madde olarak tanıtılmaktadır (Liu ve MacDonald, 1977).

Betonun çeliğe olan üstünlükleri olarak, yoğunluk açısından çeliğin yoğunluğu yaklaşık olarak 7,85 kg/dm^3 iken, betonun 2,25 ile 2,4 kg/dm^3 arasında değişmektedir. Bu durum yaklaşık olarak aynı hacim için 3 kat ağırlıktan kazanım sağlamaktadır. Ek olarak, çeliğin belirli sıcaklıklar altında bükülme; belirli kuvvetlerin etkilemesi sonucu ayrılma, bölünme eğiliminin olması; kolay paslanması ve pahalı bir şekilde elde edilmesi çeliğin kullanımını belirlenen dönemde kısıtlamaktadır (Liu ve MacDonald, 1977; İnşaat Mühendisleri Odası, 2022). Diğer alternatif inşaat malzemelerinden biri olan ahşabın aksine kurtlara ve mantarlara karşı daha dayanıklıdır (Brauer, 1973; Topçu ve Bahadırlı, 2010). Betonun yangına karşı dayanıklı olması, titreşimi çeliğe göre daha az iletmesi, dönem şartlarında çeliğinin özelikle tamirinin zor yapılması, nitelikli personel gerektirmesi ve yüksek bakım masrafları oluşturması dikkat çekmektedir (Brauer, 1973; Liu ve MacDonald, 1977). Buna karşın beton gemilerin inşa edilme ve işletilme zorluğu; kalın gövde yapılarından kaynaklı düşük kargo kapasitesi bu tip gemilerin önündeki potansiyel engellerin başında gelmektedir (Roth, 2016; Stilwell, 2021).

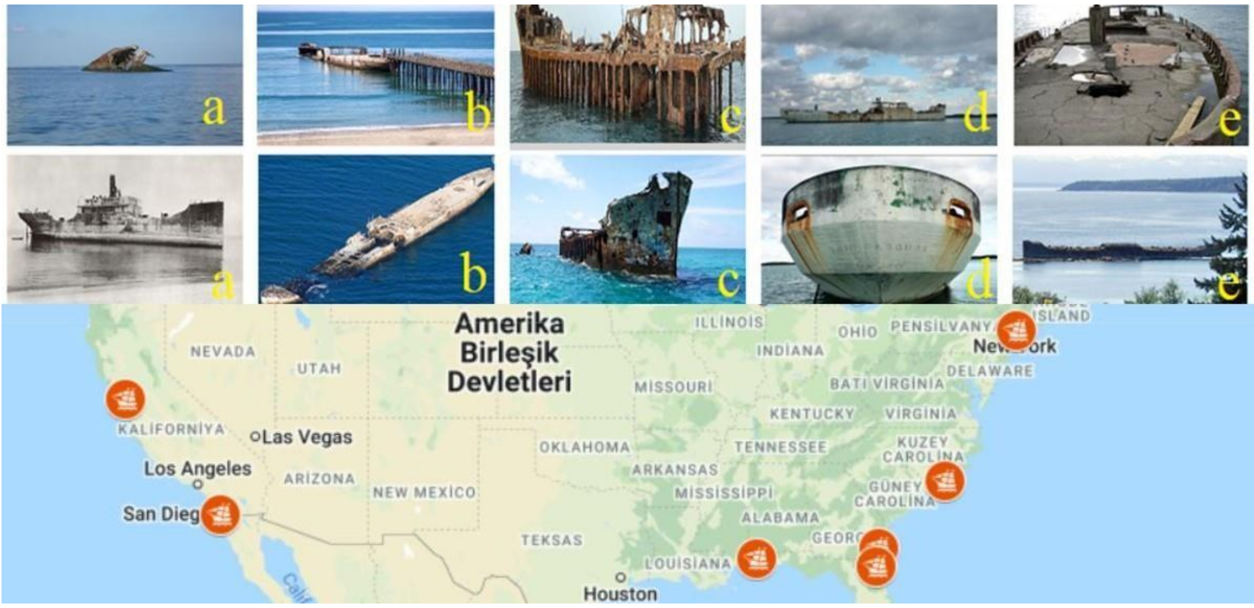
Birleşik Devletler Denizcilik Komisyonu tarafından 36 tane beton gemi şeker taşımak gibi çeşitli amaçları karşılamak adına inşa edilmiştir. İnşaatı gerçekleştiği dönem incelendiğinde, özellikle I. Dünya Savaşında (WWI) çelik krizi yaşanmakta, var olan çelik üretimi, ihtiyacı tam olarak karşılayamamakta ve nitelik işgücü oldukça düşük seviyedir. WWI sürecinde gemi ve mavna inşasında hafif beton kullanımını görülmektedir (Arslan, 2007). Yaşanan krizi fırsata çevirmek adına çelikten daha ucuz ve kolayca elde edilme potansiyelleri göz önünde bulundurularak 12 adet beton geminin WWI döneminde inşa edilmesine karar verilmiştir. Bu beton gemilerin yapısında betonun güçlendirilmesini sağlayan çelik çubuklar bulunmaktadır (Topçu ve Bahadırlı, 2010; Roth, 2016; Stilwell, 2021). İnşa edilen 12 beton geminin adları, tipleri, inşaatın gerçekleştiği yerler, denize indiriliş tarihleri (DIT), boyut, ağırlık ve makine güç değerleri ve son durumları hakkında detaylı bilgi Tablo 2'de belirtilmektedir.

Tablo 2. 1918-1921 yılları arasında denize indirilen beton gemiler (Roth, 2016; Wrecksite, 2022; Concreteships, 2022).

Gemi Adı/Gemi Tipi	İnşa Yeri	Denize İndiriliş Tarihi	Teknik Özellikler Boyutsal Özellik (L*B*T/D) (m) Ağırlık (W/DWT/GRT) (ton) Makine Gücü (HP/NHP)	Detaylar
S.S. Atlantus (Buharlı yük gemisi)	Brunswick, GA	Kasım, 1918	76,2*13,7*6,7(T); 2500 (W); 1520 (HP)	Fırtına kaynaklı battı ve Cape May, NJ Sunset plajında kalıntıları görülmektedir.
S. S. Cape Fear1* (Buharlı yük gemisi)	Wilmington, NC	1919	86,00*14,02*8.61(D); 3590 (DWT); 1520 (HP)	1920'de çatışma olayından kaynaklı battı.
S. S. Cuyamaca3* (Petrol Tankeri)	San Diego, CA	1920	132*16,45*8,10 (T); 7500 (DWT); 359 NHP	Petrol barcına dönüştürüldü ve 1926'da söküme gönderildi.
S. S. Dinsmore4* (Petrol Tankeri)	Jacksonville, FL	Haziran, 1920	128*16,5*11 (D); 6144 (GRT); 598 NHP	1932'de söküme gönderildi.
S. S. Latham2* (Petrol Tankeri)	Mobile, AL	Mayıs, 1920	125,7*16.48*10,97 (D); 6826(GRT); 359 NHP	1926'da söküme gönderildi ve petrol depolama tankına dönüştürüldü.
S. S. Moffit4*	Jacksonville, FL	Aralık, 1920	128*16,5*11 (D); 6144 (GRT); 598 NHP	Petrol barcına dönüştürüldü.
S. S. Palo Alto5* (Petrol Tankeri)	Oakland, CA	Mayıs, 1919	128,02*16,46*10,67 (D); 6144 (GRT); 359 NHP	Uzun süreler San Francisco körfezinde bekletildi. İskeleye bağlanarak içerisinde yemek, dans ve yüzme gibi etkinlikler için bölümler inşa edildi. İşletme yapan şirket battı ve gemi sonraki yıllarda fırtına kaynaklı battı. Gerekli ekipmanlar alınarak sadece balıkçı iskelesi olarak bırakıldı.
S. S. Peralta5* (Petrol Tankeri)	Oakland, CA	Şubat, 1921	128,02*16,46*10,67 (D); 6144 (GRT); 359 NHP	1924'te Sardalye konserve imalathanesine dönüştürüldü. 1958'de Powell River içinde kereste ve kütük depolaması için dalga kıran görevi görmektedir.
S. S. Polias (Acil Durum Müdahale Gemisi)	New York, NY	Aralık, 1918	81,4*14,2*8,11 (T)	Kömür taşımacılığında kullanıldı. Fırtınalar ve kasırga sonucu battı MA eyaletinde Clyde Limanında kalıntıları bulunmaktadır.
S. S. San Pasqual3* (Petrol Tankeri)	San Diego, CA	Haziran, 1920	132*16,45*8,10 (T); 7500 (DWT); 359 NHP	1921'de Fırtınada gemi yaralandı, 1924'de Gemi ihtiyaçları mağazası olarak Santiego, Cuba'da kullanıldı. 1932'de söküme gitti ve onarım gemisi olarak kullanıldı WWII döneminde Askeri silahlarla donatıldı Küba Devriminde *Cuban Revolution* hapishane olarak kullanıldı. Balıkçılık yarışmaları gibi birçok spor faaliyetlerde kullanıldı ve sonunda otele dönüştürüldü.
S. S. Sapona1* (Buharlı yük gemisi)	Wilmington, NC	Ocak, 1920	86,00*14,02*8.61(D); 3590 (DWT); 1520 (HP)	1924 yasak yıllarında alkol ürünleri için yüzen depo olarak kullanıldı Bahama Bimini Adasının güneyinde 5 metre su batmış şekilde bulunmaktadır. Dalgıçlar ve balıklar için oldukça cezbedicidir.
S. S. Selma2* (Petrol Tankeri)	Mobile, AL	Haziran 1919	125,7*16.48*10,97 (D); 6826(GRT); 359 NHP	Genleştirilmiş şeyl agregası, taşıyıcı hafif beton yapımında ilk kez bu gemide kullanıldı. 1920 yılında Tampico, FL'de bir rıhtıma çarptı. Devlet tarafından sökümüne karar verildi. Son olarak 1922'de Texas Pelican Adasında kazılan kanalda bırakıldı.

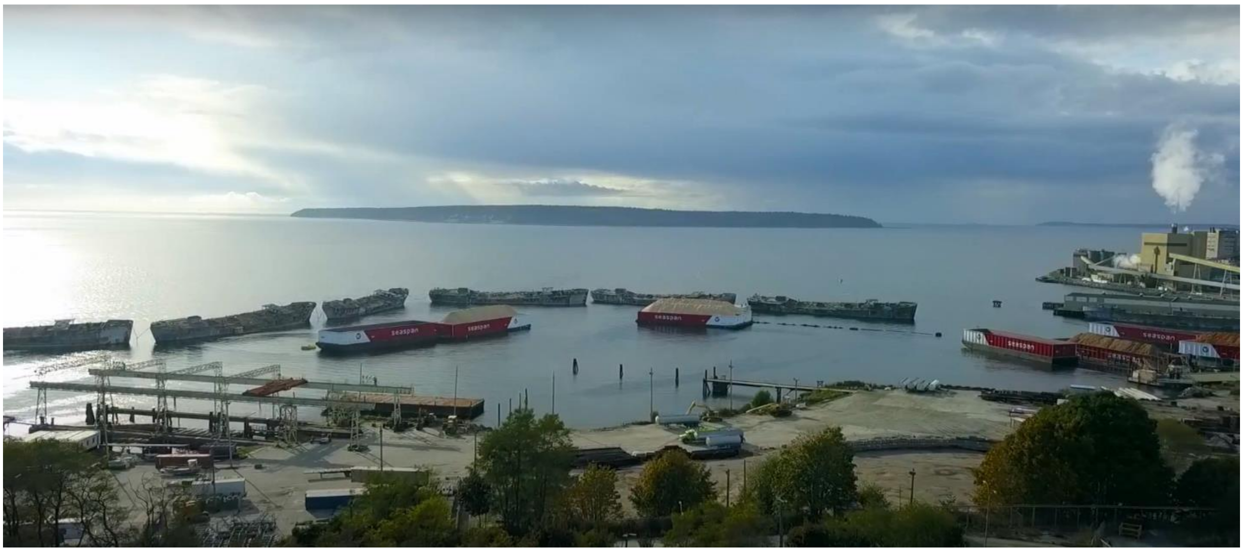
*Üst indis olarak ifade rakamlardaki gemiler birinin kardeş gemisi "*sister ship*" olarak inşa edilmiştir.

1918-1921 tarihleri arasında denize indirilen betonarme gemiler için Tablo 2'deki veriler incelendiğinde genellikle yük ve petrol tankeri üzerine gemi inşaatı gerçekleşmektedir. Özellikle Selma petrol tankerinde uygulanan, yapay hafif agreganın geliştirilmesi beton gemilerin inşasını hızlandırmaktadır (Yolcu ve Girgin, 2007). İşletilme süreleri oldukça kısa olan bu gemilerin bazıları eğitim gemisi, depo gibi farklı amaçlarda kullanılmak üzere dönüşüme gitmişlerdir. Bunların dışındaki gemilerde gerekli malzemelerin sökümü gerçekleştikten sonra iskele, dalga kıran veya dalgıçlar için dalış alanı olarak kullanılmaktadır. Gemilerin günümüze kadar ulaşan görselleri ve inşaat alanları Şekil 3'te ifade edilmektedir.



Şekil 3. SS Atlantus (a), S. S. Palo Alto (b), S. S. Sapona (c), S. S. San Pasqual (d), S. S. Peralta (e) gemileri ve inşa bölgeleri (Atlasobscure, 2022).

WWI ve II. Dünya savaşı (WWII) sürecinde inşaatı gerçekleşen beton gemilerin bazıları Kanada'nın batısında bulunan British Columbia bölgesinde bulunmaktadır. 1948 ve 1966 yılında ana amacı kâğıt fabrikası olan bir tesis tarafından satın alınmakta ve bu gemiler içerindeki ana makine gibi sabit teçhizatlarının çoğu alındıktan sonra dalga kıran ve baraj gibi su tutulmasını sağlayarak kereste ve kütük depolanmasına yardımcı olmaktadır. Şekil 4'te beton gemilerin konumları ve kâğıt fabrikasına sağladığı dalga kıran hattı ifade edilmektedir (McAskill ve Heere, 2004).



Şekil 4. Powell River içinde dalga kıran ve baraj görevi gören beton gemiler görülmektedir.

"*The Hulks*" olarak tanımlanan Şekil 4'teki beton gemilerin 7 tanesini WWII buharlı gemisi, iki tanesini WWII barcı ve son olarak 1 tanesi WWI buharlı gemisi oluşturmaktadır. Halen yüzer durumda olan 11 geminin 10'u bu bölgede bulunmaktadır (Campbell, 2013). Fakat bu gemilerde yapısal bozulmalardan kaynaklı yağmur suları beton gemilerin içerisine girmektedir. Görevlerinin devamlılığını sağlamak, bu durumu engellemek ve tekrar eski su çekimlerini geri kazanmak adına pompalar aracılığıyla belirli süreçlerde su tahliyesi yapılmaktadır (McAskill ve Heere, 2004).

WWI elde edilen tüm deneyimler göz önünde bulundurularak, inşa edilen ve var olan cevher gemileri ile çelik tedariğinin sürdürebilir şekilde gerçekleştirilmesi planlanmaktadır. Fakat 1941 yılında çelik levha krizin ortaya çıkması özellikle vergilendirme sürecinde yaşanan zorluklar çelik gemi imalatını durdurmuş, beton ve ahşap teknelerin üretimini tekrar gündeme getirmektedir. Böylece WWII döneminde Birleşik Devletler Denizcilik Komisyonu inşaatında hafif beton kullanılacak gemi ve barçların sırasıyla 24 ve 58 adet inşasına karar vermektedir (Key, 1970; Lane, 2001; McAskill ve Heere, 2004; Yolcu ve Girgin, 2007). Askeri amaçlar doğrultusunda inşaatı gerçekleşen bu gemilerin ve barçların bazılarının inşaatı bitmesine rağmen hiç işletilmeden servis dışı bırakılmıştır (McAskill ve Heere, 2004). Tablo 3'te WWII sürecinde inşaatı gerçekleşen beton gemilerin adları, denize indiriliş tarihleri (DIT), inşa bitiş tarihleri (IBT) ile son durumları hakkında bilgiler ifade edilmektedir (McAskill ve Heere, 2004; Concreteships, 2022).

Tablo 3. 1943-1944 yılları arasında denize indirilen beton gemiler (Concreteships, 2022).

Gemi Adı/Gemi Tipi	DIT/IBT	Detaylar
S.S. P. M. Anderson	Mayıs, 1944 (DIT)	Powell River içinde dalga kıran görevi görmektedir.
S.S. John Aspdin	Mayıs, 1944 (DIT)	Kasırgaya yakalandı ve sonrasında yüzer ambar olarak kullanıldı. 1948 yılında körfez içinde karaya oturarak battı
S.S. Henri Le Chatelier	Ocak, 1944 (DIT)	Powell River içinde dalga kıran görevi görmektedir.
S.S. Armand Considere	Mayıs, 1944 (DIT)	Askeri amaçla kullanıldıktan sonra, yüzer ambar olarak kullanıldı. Powell River içinde dalga kıran görevi görmektedir.
S.S. William Foster Cowham	Kasım, 1944 (IBT)	Yüzer ambar olarak kullanıldı. 1948 yılında kısmen batırıldı ve 8 diğer betonarme gemi gibi dalgakıran olarak Kiptopeke, Lower Chesapeake Körfezinde kullanıldı.
S.S. Edwin Clarence Eckel	1944 (IBT)	Mühimmat ve patlayıcılar taşırken fırtınaya yakalandı. Onarım maliyetleri çok yüksek olması sebebiyle 1947 yılında batırıldı.
S.S. John Grant	Haziran 1944 (IBT)	Askeri eğitim gemisi ve yüzer ambar olarak kullanıldı. 1948 yılında batırıldı ve Kiptopeke'de dalgakıran olarak kullanıldı.
S.S. Francois Hennebique	Ağustos, 1944 (IBT)	Batırıldıktan sonra iskele olarak kullanıldı.
S.S. Richard Lewis Humphrey	Mart, 1944 (IBT)	Kahve taşımacılığı döneminde fırtınada hasar görmüş ve 1945 yılında hurda olarak satılmıştır.
S.S. Albert Kahn	Ekim, 1944 (IBT)	Askeri amaçlı yüzer ambar olarak kullanıldı. 1947 yılında alabora oldu ve seyir problemi oluşturmaması için batırıldı.
S.S. Robert Whitman Lesley	Kasım, 1944 (IBT)	Askeri eğitim gemisi olarak kullanıldı. 1948 yılında Kiptopeke de dalgakıran olarak kullanıldı.
S.S. Richard Kidder Meade	Mart, 1944 (IBT)	1948 yılında Kiptopeke de dalgakıran olarak kullanıldı.
S.S. Thaddeus Merriman	Eylül, 1944 (DIT)	1950 yılında Powell River içinde dalga kıran görevi görmektedir
S.S. C. W. Pasley	Ağustos, 1944 (DIT)	İskele olarak kullanılma başlandı
S.S. Willard A. Pollard	Aralık, 1944 (IBT)	Yüzer ambar amaçlı kullanıldı. 1948 yılında batırıldı ve Kiptopeke de dalgakıran olarak kullanıldı.
S.S. David O. Saylor	Kasım, 1943 (DIT)	Betonarme yapısı denizcilik açısından uygun değildi. Normandiya kıyılarında Dalgakıran oluşturmak için batırıldı.
S.S. Willis A. Slater	Ocak, 1944 (IBT)	Askeri eğitim gemisi olarak kullanıldı. 1948 yılında Kiptopeke de dalgakıran olarak kullanıldı.
S.S. John Smeaton	Kasım, 1943 (DIT)	Seker ticaretinde ve sonrasında yüzer ambar amaçlı kullanıldı. 1948 yılında Powell River içinde dalga kıran görevi görmektedir
S.S. Arthur Newell Talbot	Temmuz, 1943 (DIT)	Askeri eğitim gemisi olarak kullanıldı. 1948 yılında Kiptopeke de dalgakıran olarak kullanıldı.
S.S. Edwin Thatcher	Kasım, 1944 (DIT)	Yüzer ambar olarak kullanıldı. 1948 yılında batırıldı ve Kiptopeke de dalgakıran olarak kullanıldı.
S.S. L. J. Vicat	Ocak, 1944 (DIT)	Yüzer ambar olarak kullanıldı. 1948 yılında Powell River içinde dalga kıran görevi görmektedir
S.S. Emile N. Vidal	Eylül, 1944 (DIT)	Yüzer ambar olarak kullanıldı. Barça dönüştürüldü ve 1960 yıllarında Powell River içinde dalga kıran görevi görmektedir
S.S. Vitruvius	Aralık, 1943 (DIT)	Seker ticaretinde kullanıldı. Normandiya kıyılarında dalgakıran oluşturmak için batırıldı.
S.S. Leonard Chase Wason	Kasım, 1944 (DIT)	Askeri eğitim gemisi olarak kullanıldı. 1948 yılında Kiptopeke'de dalgakıran olarak kullanıldı.

WWII sürecinde Tampa, FL bölgesinde inşaatı gerçekleşen ve 102,53 m boya, 16,45 m genişliğe, 10,66 m derinliğe, 4690 GRT kapasiteye sahip beton gemiler tüm detaylarıyla birlikte Tablo 3'te ifade edilmektedir. Çeşitli dezavantajlar ve savaşın sona ermesi göz önünde bulundurularak, inşaatlarından kısa bir süre sonra, hizmet dışı bırakılmıştır. Bu yüzden işletilme ömürleri sadece birkaç ayı kapsayacak şekilde kısa sürmüştür (Key, 1970; Bain III ve diğer., 1998; McAskill ve Heere, 2004; Sakı, 2021). Süreç sonunda 24 buharlı kargo gemisinin 17 tanesi yüzer ambar haline getirilmiş ve bazıları eğitim gemisi olarak kullanılmıştır (Lane, 2001). Ayrıca bu gemilerden 9'u Chesapeake Körfezinde sert hava koşullarını engellemek adına Kiptopeke sahilinde dalgakıran olarak kullanılmaktadır. Şekil 5'te 9 beton geminin dalgakıran olarak konumlanması görsel olarak ifade edilmektedir (Key, 1970, Bain III ve diğer., 1998; Sakı, 2021).

Şekil 5. Kiptopeke sahilinde dalgakıran amaçlı konumlandıran beton gemiler görülmektedir.

Şekil 5'de belirtilen beton gemiler dalgakıran görevlerinin yanında farklı türden balıkların yaşamı için önemli bir yuva merkezi olmaktadır. Ayrıca, Virginia'da düzenlenen balık etiketleme yarışma programları için kaynak oluşturmaktadır (Bain III ve diğer., 1998). Özellikle inşaatı aşamasında yaşanan gecikmeler, zorluklar ile birlikte diğer problemler beton gemi kavramının sürdürebilirliğini sağlamasını oldukça çıkmaza sokmaktadır (Lane, 2001). Buna rağmen, WWII ardından birçok beton yüzer barçlar işleme ve depolama tesisi olarak inşa edilmiştir. Askeri uygulamalarının yanında çeşitli ticari amaçlı beton gemilerde inşa edilmiştir. Özellikle WWI döneminde Alman denizaltı saldırılarından kaynaklı çok sayıda ticaret filosunun kaybı deniz taşımacılığını aksatmıştır (Domingo, 1998; Özsoysal ve Ünsan, 2008). Tekrar ticari canlandırmak adına ve askeri alandaki çelik ihtiyacını sekteye uğratmamak için beton gibi kuvvetlendirilmiş alternatif gemi gövde malzemeleri kullanılmaktadır. 1918 yılında The Mirotres adlı gemi İspanya'da inşa edilen ilk ticari betondan yapılmış gemi olmuştur (Domingo, 1998). Ticari gemi uygulamaların yanında ilerleyen yıllarda yat, balıkçı teknesi olarak da ferrocement malzemeden üretilmiş beton gemiler bulunmaktadır. Özellikle 1970 yıllarında ferrocement malzeme ile ilgili gemi inşaatı üzerine Avustralya, İngiltere, Yeni Zelanda, Sovyet Sosyalist Cumhuriyetler Birliği (SSCB), Çin, Tayland, Vietnam, İran, Güney Afrika, Fransa ve Kore ve Amerika Birleşik Devletleri önem vermektedir. SSCB dışındaki ülkeler daha çok 20 metre altındaki trol tekneleri gibi gemi uygulamalarına yer vermekte çünkü beton gemilerin, 10 metreyi aşan uygulamalarda oldukça düşük operasyonel performansa sahiptir. Buna karşın bükme, gerilim, darbe ve yorulma testleri genişletilmiş metal ile güçlendirilmiş ferrocement bot üzerine uygulandığında, özellikle darbe direnci ve çatlak kontrolü açısından oldukça fayda sağlamıştır (Iorns ve Watson, 1977). Diğer ülkelerin küçük ölçekli uygulamaların aksine SSCB yük kapasitesi ve çatlama üzerine testler yaparak iç sularda ve ticari amaçlı gemilerde güçlendirilmiş beton gemilerin kullanılabilirliği araştırılmıştır (Whang, 1972; Key, 1970). Beton gemilerin ticari uygulamaları günümüzde bulunmamasına rağmen her yıl ABD'de Amerikan İnşaat Mühendisleri Birliği *American Society of Civil Engineers* (ASCE) tarafından ferrocement kanolar yapılmakta ve her yıl belirli zamanlarda üniversiteler arası yarışma yapılmaktadır. Türkiye'de de benzer etkinlik geleneksel olarak üniversiteler arası yapılmaktadır.

SONUÇ VE ÖNERİLER

Günümüzde inşaatı gerçekleşen ticari ve askeri gemilerde beton malzeme gemi gövdesinde kullanılmamasına karşın, ilk inşaatın gerçekleştiği 1841 yılından itibaren özellikle WWI ve WWII kapsayan önemli bir süreçte gemi inşaatı gündeminde bulunmaktadır. Belirtilen dönemde var olan çelik üretimi ve tedariği, çelik ile ilgili politikalar ve çelik malzeme üzerinde çalışacak deneyimli personelin bulunmaması gibi zorluklar betonu esas elan gemi gövdelerin inşasının önünü açmaktadır. Ticari ve askeri amaçlar gibi farklı bir alan doğrultusunda inşaatı gerçekleşen bu gemilerin operasyon süreleri oldukça kısa veya hiç olmamıştır. İşletilmelerine son verildikten sonra genellikle dalgakıran, depo ve iskele olarak kullanılmış veya çeşitli deniz canlılarına yaşam alanı sağlaması açısından batırılmıştır. İlerleyen süreçlerde betonun tekrar gemi gövdesinde tercih edilip edilmeyeceğini zaman gösterecektir. Bu çalışma 20 yüzyılın ilk yarısında özellikle beton gemilere olan eğilimleri nedenleri ile belirtmesi açısından bu alanda çalışacak akademisyenler, uzmanlar ve sektör temsilcileri için önemli bir kaynak oluşturacaktır.

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Use of Microalgae and its Importance in Türkiye and Worldwide

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Abstract

The increasing global environmental pollution, rising energy consumption, and global warming, which are important problems worldwide, have led countries to seek different solutions in environmental issues. Türkiye and other countries are making efforts to continue living in a healthier environment economically and socially while meeting the increasing energy demand and rising environmental pollution. Renewable technologies are being developed and produced. Therefore, sustainable ecology and sustainable green economy have started to take the top spot on the global agenda. For this reason, the use of microalgae in environmental applications is increasing rapidly, and microalgae technology is being rapidly developed. Photobioreactors are currently at the forefront of microalgae production. Accordingly, microalgae are being used in many different areas of biotechnological and technical applications, such as health, food, cosmetics, pharmaceutical production, wastewater treatment, heavy metal removal from the environment, and animal feed. In addition, the production of biofuels based on microalgae is also attracting attention. Therefore, microalgae are creating potential alternatives to coal, petroleum, and natural gas. In this sense, it seems inevitable that microalgae will be one of the main energy sources in the future, and a green revolution will take place with the development of microalgae technology. This study aims to reflect the current situation of algae and microalgae used and that can be used in biotechnology, along with new applications and necessary considerations in Türkiye, as well as in the world.

Mikroalg kullanımı ve Türkiye ile Dünya'daki Önemi

Makale Bilgisi

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Anahtar Kelimeler:
- Mikroalg
- Fotobiyoreaktör
- Atık Su Arıtımı
- Biyoyakıt
- Çevre

Öz

Küresel çevre kirliliğinin artması, enerji tüketiminin artışı ve küresel ısınma, dünya genelinde önemli sorunlar olup, ülkeler çevre sorunlarında farklı çözümler aramaktadır. Türkiye ve diğer ülkeler artan enerji talebini ve artan çevre kirliliğini karşılayarak daha sağlıklı bir çevrede ekonomik ve sosyal olarak yaşamaya devam etme çabası içindedir. Yenilenebilir teknolojiler geliştirilmekte ve üretilmektedir. Bu nedenle, sürdürülebilir ekoloji ve sürdürülebilir yeşil ekonomi küresel gündemin en önemli konularından biri haline gelmiştir. Bu nedenle, mikroalglerin çevresel uygulamalarda kullanımı hızla artmakta ve mikroalg teknolojisi hızla geliştirilmektedir. Fotobioreaktörler şu anda mikroalg üretiminde öncü konumdadır. Buna göre, mikroalgler sağlık, gıda, kozmetik, ilaç üretimi, atıksu arıtımı, çevreden ağır metal çıkarımı ve hayvan yemi gibi biyoteknolojik ve teknik uygulamaların birçok farklı alanında kullanılmaktadır. Ayrıca, mikroalg temelli biyoyakıt üretimi de dikkat çekmektedir. Bu nedenle, mikroalgler kömür, petrol ve doğal gaz gibi potansiyel alternatifler yaratmaktadır. Bu bağlamda, mikroalglerin gelecekte ana enerji kaynaklarından biri olacağı ve mikroalg teknolojisinin geliştirilmesiyle yeşil bir devrimin yaşanacağı kaçınılmaz görünmektedir. Bu çalışma, Türkiye'de ve dünyada biyoteknolojide kullanılan alg ve mikroalglerin mevcut durumunu, yeni uygulamaları ve gerekli düşünceleri yansıtmayı amaçlamaktadır.

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INTRODUCTION

There are two different methods used in microalgae production: open (external) systems and closed (internal) systems (Suali & Sarbatly 2012).

a) Open Systems (Open Ponds)

Open (external) system microalgae production is carried out in non-mixed and mixed type (channel) open ponds (Figure 1.; Figure 2.) (Suali & Sarbatly 2012). It is known that microalgae cultivation in open ponds started in the 1950s (Pawlowki et al., 2014; Kargın, 2020). Large-scale bioreactors and channel ponds are commonly used in these types of microalgae cultures (Christenson & Sims 2011; Rawat et al., 2013; Kargın, 2020). Since the channel (raceway) ponds are open, the ion concentration in the culture water increases due to evaporation. Therefore, microalgae production is negatively affected (Rawat et al., 2013). In addition, easily contaminable microorganisms can negatively affect production in open systems (Bahadar & Khan 2013). Factors such as temperature changes during the day and seasonal variations also affect production. As the density of microalgae increases, the amount of CO_2 in the culture medium decreases, which also negatively affects production (Kargın, 2020).

Figure 1. Outdoor Production Systems (Anonymous, 2022a).

Figure 2. Channel (Raceway) Type Pond Systems (Kumar & Mohan 2014)

b) Closed Systems (Photobioreactors)

In closed photobioreactors, there are tubular, flat, and spiral (Figure 3.) models with continuous circulation of the culture medium and stirring during the production process (Demirbas, 2010).

Figure 3. Large Bag Production Systems (Gezici, 2012)

Tubular photobioreactors are commonly designed as horizontal, vertical, and spiral (Figure 4.; Figure 5.) bioreactors in microalgae production (Khan et al., 2009). The production materials of tubular photobioreactors are made of glass and plastic tubes (Chisti, 2007). Tubes can be produced vertically, horizontally, spirally, and inclined (Chisti, 2007; Rawat et al., 2013).

Figure 4. Flat-panel photobioreactor production system (Kükdamar & Tokuç 2015).

Figure 5. Horizontal (flat) production system in photobioreactor (Gezici, 2012).

When comparing closed type photobioreactors with open ponds, advantages include the ability to control temperature, pH, and light, the ability to continuously and easily mix, minimal losses due to evaporation, higher microalgae production quantities, predictability of production, and high-quality production. However, the construction costs for closed systems are considerably higher than those for open ponds. Since microalgae can adhere to surfaces, culturing on large surface areas can make it difficult to control temperature and CO_2 levels. Stirrers are not necessary in flat-panel photobioreactors, as the gas distributor system located at the bottom of the reactor serves as the stirrer. Air-lift photobioreactors are preferred bioreactors for microalgae production due to their easy design and low cost. Flow columns are also present in photobioreactors made of glass and plastic to facilitate vertical flow.

Application areas of microalgae products

Metabolites (phototoxins) generally refer to small molecules and represent intermediate products of metabolism. Secondary metabolites are chemical compounds directly related to the normal growth, development, or reproduction of organisms. The importance of studying these organisms for pharmaceutical purposes has been demonstrated by the discovery of new agents through physical techniques and biomedical applications of chemical prototypes (Dos Santos et al., 2005).

Microalgae-derived metabolites are a promising group of bioactive molecules for biotechnological applications. Bioactive metabolites are an important source, especially for cytotoxic agents. These types of compounds are mainly produced by dinoflagellates and cyanobacteria, especially in marine or freshwater environments with harmful algal blooms. Excessive growth of dinoflagellates can cause red tides and color changes in the sea, while cyanobacteria can cause eutrophication in lakes, especially due to increased nitrogen and phosphorus (Lipton, 2003). Many microalgal metabolites have different biological activity and chemical structure. Research on the effects of algae on human health and other beneficial effects is increasing (Guedes et al., 2011).

Metabolites stored in algae cells are also used in various sectors such as food, health, animal feed, fertilizer, organic food coloring, and cosmetics. They are also used in wastewater treatment because of their ability to bind to metals (Gökpınar et al., 2013).

Since the necessary climate conditions and nutrient elements for the production of algae, which are considered to meet the raw material requirements of some energy sources, are available in Türkiye, it is seen that our country is suitable for economic algae production. When considering only thermal power plants that operate with natural gas, microalgae are an inexhaustible source due to CO_2 being their main nutrient (Ulukardeşler & Ulusoy 2012). Maximizing the utilization of existing resources and minimizing waste and losses is necessary, within a framework that includes not only water resources but also all economic and social resources to gain value (Mostafa et al., 2012).

a. Food

The use of microalgae by humans dates back to ancient times, and it is known that in China, they used *Nostoc* as a food source to survive during times of famine (Figure 6).

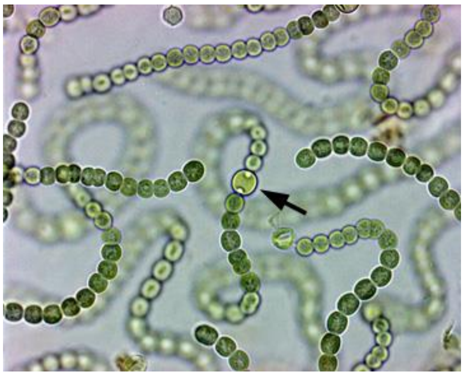

Figure 6. *Nostoc* genus (Anonymous, 2022b).

Hunger, which is becoming an increasingly problematic issue worldwide, affects 1 in 10 individuals, and this ratio is gradually increasing. Particularly, protein and energy deficiency are major reasons for health problems and premature death (FAO, 2017; Muslu & Gökçay 2020). The United Nations predicts that the global population could reach 9.7 billion in 2050. While many people still lack access to food, alternative sources of nutrition must be explored, and existing production must be increased to meet the needs of the growing population (UN DESA, 2019; Muslu & Gökçay 2020).

Commercial microalgae applications are primarily controlled by four strains: *Arthrospira*, *Chlorella*, *Dunaliella salina*, *Aphanizomenon* and *Arthrospira* species (Figure 7) are used in human nutrition due to their high protein content and nutritional value (Rangel-Yagui et al., 2004; Soletto et al., 2005).

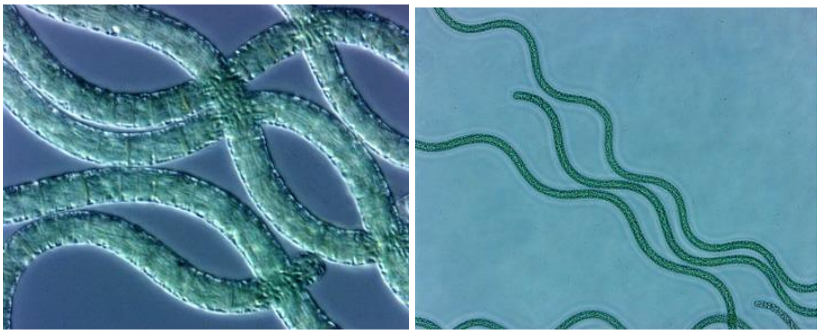

Figure 7. *Arthrospira* genus (Anonymous, 2022c).

Microalgae for human nutrition are currently marketed in various forms such as tablets, capsules, and liquids. They are also added to pasta, snack foods, candy bars, gum, and beverages (Table 1.) (Yamaguchi, 1997; Liang et al., 2004; Sasa et al., 2020).

Table 1. Some studies conducted with microalgae addition (Sasa et al., 2020).

Microalgae	**The food to which it is added**
Spirulina sp.	Yogurt Muffin Cookie Biscuit Bread Gluten-free bread Pasta
Chlorella sp.	Cheese Cookie Bread Oil/water emulsions
Dunaliella sp.	Bread Pasta

According to the data of the Food and Agriculture Organization of the United Nations in 2016, microalgae production reached 89,000 tons in 11 countries. However, China reported a production of 88,600 tons in the same year. Many microalgae species are used as dietary supplements, including *Spirulina* spp., *Chlorella* spp. and *Haematococcus pluvialis*, among others. It is believed that production may exceed what is reported in production reports (FAO, 2018; Muslu & Gökçay 2020). The U.S. Food and Drug Administration (FDA) refers to any organism, substance, or chemical that is safe for human consumption as "Generally Recognized as Safe (GRAS)" for all individuals. Algae such as *Dunaliella bardawil, Chlamydomonas reinhardtii, Auxenochlorella protothecoides, Chlorella vulgaris, Arthrospira platensis,* and *Euglena gracilis* and their derivatives are considered GRAS (Muslu & Gökçay 2020). The amino acid contents of the algae considered GRAS are given in Table 2 (Muslu & Gökçay 2020; Torres-Tiji et al., 2020).

Table 2. Amino Acid Content of GRAS Algae (Muslu & Gökçay 2020; Torres-Tiji et al., 2020).

Amino Acid	**Species of Algae**					
	A. platensis	*C. reinhardtii*	*A. protothecoids*	*C. vulgaris*	*D. bardawil*	*E. gracilis*
Alanine	9,5	8,8	6,2	7,9	7,3	15,8
Arginine	7,3	7,2	13,4	6,4	7,3	3,4
Aspartic Acid	11,8	9,7	7,1	9	10,4	7,1
Cysteine	0,9	-	1,6	1,4	1,2	0,2
Glutamic acid	10,3	11,3	10,3	11,6	12,7	9,5
Glycine	5,7	5,7	5,5	5,8	5,5	7
Histidine	2,2	2,3	3	2	1,8	2,2
Isoleucine	6,7	4,4	3,7	3,8	4,2	0,2
Leucine	9,8	9,8	5,6	8,8	11	3,7
Lysine	4,8	6,6	4,9	8,4	7	4,9
Methionine	2,5	2,7	2,1	2,2	2,3	0
Phenylalanine	5,3	5,6	5,5	5	5,8	0,9
Proline	4,2	5,6	5,6	4,8	3,3	0
Serine	5,1	4,3	5,1	4,1	5,4	10,6
Threonine	6,2	5,1	4,9	4,8	5,4	4,5
Tryptophan	0,3	2,8	0,5	2,1	0,7	1,7
Tyrosine	5,3	4,3	4,7	3,4	3,7	0,7
Valin	7,1	6,5	5,2	5,5	5,8	8

Microalgae are widely used in a variety of applications, ranging from aquaculture to pet and livestock feed. Around 30% of global algae production is used for animal feed, with more than 50% of current Arthrospira production used as a dietary supplement (Becker, 2004).

b. Agriculture

The world's energy demand is increasing parallel to the growing population. Various solutions are being researched and new regulations are being developed in Türkiye and other countries to meet this demand. Regulations highlight the importance of environmentally friendly, sustainable, and renewable energy sources (Karakaş et al., 2014).

In recent years, the advantages and disadvantages of bioenergy sources have been discussed in terms of their effectiveness in sustainability. The use of oilseed plants (OSP) in biofuel production has been emphasized in this context. The interest in these plants has been increased by their widespread use in various sectors.

In Türkiye, sunflower is the top oilseed crop produced in 2019 with approximately 1.9 million tons, followed by cottonseed with 1.32 million tons, rapeseed with 180 thousand tons, soybean with 150 thousand tons, sesame with 16.9 thousand tons, and flaxseed with 21.9 thousand tons. Sunflower has the largest cultivation area with 675.9 thousand hectares, followed by cotton with 477.9 thousand hectares, rapeseed with 52.5 thousand hectares, soybean with 35.3 thousand hectares, sesame with 24.9 thousand hectares, and flaxseed with 15.9 thousand hectares. Accordingly, in 2019, approximately 3.6 million tons of oilseeds were produced on an area of about 1.28 million hectares in Türkiye. The total domestic usage value of sunflower is 4.7

million tons, and 2.8 million tons are met by imports. The domestic consumption value of soybean is 2.6 million tons, and a large portion of 2.4 million tons is provided by imports (TÜİK, 2020).

Despite its potential for YTB production, Türkiye's YTB production is insufficient. As a result, it becomes difficult for the biodiesel sector to access YTB as a valuable raw material, and the increase in raw material prices under market conditions negatively affects the sector's competitiveness (Hatunoğlu, 2010). Due to the difficulties in obtaining biofuels from agricultural products, alternative sources are being preferred. Algae are considered the most efficient product in terms of oil content compared to other oilseeds and are the fastest-growing source of biofuels worldwide (Demirbaş & Demirbaş 2011).

Environmental factors such as air and water pollution, soil depletion, and declining biodiversity contribute to degradation. Synthetic chemicals, agricultural pesticides, and fertilizers pollute soil, water, and air, harming both the environment and human health (Horrigan et al., 2002).

Heterocystic cyanobacteria are known for their ability to fix atmospheric nitrogen. The productivity of most tropical rice fields is attributed to the nitrogen-fixing activities of cyanobacteria. In order to increase soil fertility, the inoculation of cyanobacteria has been successfully achieved. Recently, it has been reported that cyanobacteria with nitrogen fixation ability dominate desert soils. It is believed that this significantly contributes to the fertility of desert soils and eventually facilitates the vegetation of deserts (Mahdi et al., 2010).

Reducing CO_2 in the atmosphere can be addressed with the transition to more widespread use of biofuels, nuclear, and renewable energy sources through CO_2 reduction. Microalgae are important for CO_2 fixation and biofuels because they can convert CO_2 (and additional nutrients) to biomass through photosynthesis at much higher rates than traditional biofuel products. This biomass can then be converted to methane or hydrogen through processes facilitated by anaerobic bacteria (Haiduc et al., 2009).

c. Wastewater Treatment

According to UNESCO (2003) data, water usage distribution is 22% in industry, 8% in domestic use, and 70% in agriculture. A significant portion of this water is discharged into the environment as wastewater. Therefore, a modern approach is needed for the treatment of industrial waste. Industrial wastewater mostly contains heavy metals, organic toxins, and surfactants. Waste from the textile, electroplating, and other metal processing industries contain significant amounts of toxic metal ions (Ahluwalia & Goyal 2007). Microalgae can use low-quality water sources such as agricultural runoff or municipal, industrial, or agricultural wastewater as growth media and can be used for the recovery of wastewater (Valverde et al., 2016). The biological uptake of heavy metal ions through various mechanisms such as ion exchange, complex formation, and electrostatic interactions occurs at the micro-scale. The residual nutrients and heavy metal ions in domestic, agricultural, and industrial wastewater are responsible for the pollution of rivers, lakes, and oceans (Michalak & Chojnacka 2002). The fundamental advantage and potential of using algal biomass for the biological removal of heavy metal ions are the sustainability of the process and, therefore, the cost-effectiveness of the method at an industrial scale (O'Connell et al., 2008).

d. Biofuels

In recent years, algal biomass (living and non-living) has been used for the biological removal of heavy metals. Laboratory studies have shown that algal biomass (dead or alive) actively absorbs various heavy metals (Volesky, 2007).

Microalgae, single-cell photosynthetic organisms, have many advantages over other biofuels in terms of higher area efficiency, the ability to use non-arable waters for growth, and the conversion of carbon dioxide and other industrial wastes into useful products (Lohrey & Kochergin 2012). For these reasons, using microalgae for biodiesel production is more attractive both environmentally and economically (Demir, 2015).

Microalgal fuels are not yet commercialized (Chisti et al., 2011). The high production cost is the primary obstacle to the commercialization of microalgal biofuel production (Bahadar & Khan 2013). In the future, microalgal-based biofuel production will become more important in light of the ecological impacts if petroleum reserves become depleted or scarce (Chisti et al., 2011).

e. Alg Flora of Türkiye

The lakes in Türkiye exhibit different structures, formations, and hydrological properties, which also support algal biodiversity in these conditions (Demir et al., 2021). Studies on freshwater algae have been conducted in 275 lakes in Türkiye, and 1363 phytoplankton taxa have been identified. As a new record, 30 *Ochrophyta* taxa were identified. This study also identified 10 genera (*Bitrichia, Chromulina, Ochromonas, Chrysococcus, Kephyrion, Kephyriopsis, Chrysosphaerella, Phacomonas, Pseudotetraëdron, Ducellieria*) as new records for the algae flora of Türkiye (Demir et al., 2021).

Production Status of Microalgae in the World

The oldest known application of microalgae is their use as fertilizers in the Far East. Similar applications were made in some European countries with extensive coastlines in the 12th century. In the 17th century, France and later England began to use microalgae, and by the end of the 18th century, Scotland started using microalgae, with an annual microalgae production of 20,000 tons of dry algae reported. This amount is equivalent to approximately 400,000 tons of fresh algae (Kargın, 2020).

Since the mid-2010s, the importance of microalgae has increased, and the US Department of Energy has pledged $24 million in financial support to three research companies to commercialize algal biofuels (Kargın, 2020). From the 1970s to the mid-1990s, the US National Renewable Energy Laboratory conducted studies on improving the use of microalgae for biodiesel production, and Cyanotech began using *Haematococcus pluvialis* as a source of carotenoids at the end of the 1990s (Deng et al., 2009; Mata et al., 2010). The US Department of Energy discontinued the studies in the mid-1990s due to the decrease in fossil fuel prices.

In 2008, ABDEB started reinvesting in research on biofuels (Deng et al., 2009). Nowadays, various companies are conducting research to commercialize algae-based fuels. For instance, Exxon Mobil has allocated a budget of 600 million dollars for algae fuel production (Chisti et al., 2011). Origin Oil has conducted studies that have reduced production costs to as low as 0.60$/L (Gendy & El-Temtamy 2013). According to a report by Singh & Gu (2010), 78% of companies producing algae fuel are located in the United States, 13% in Europe, and 9% in other regions. In 2009, the United States led the microalgae production sector with a 47% share. Most of the microalgae produced in the United States are used in the pharmaceutical and cosmetic industries, with a smaller portion used for algae-based fuel production. China is in second place in algae production with a 21% share, using all of its production in the food sector. Australia and New Zealand follow with a 14% share, with New Zealand being the most effective in using microalgae for biofuel production. The European Union countries, Argentina, and Brazil follow in the rankings (Figure 8).

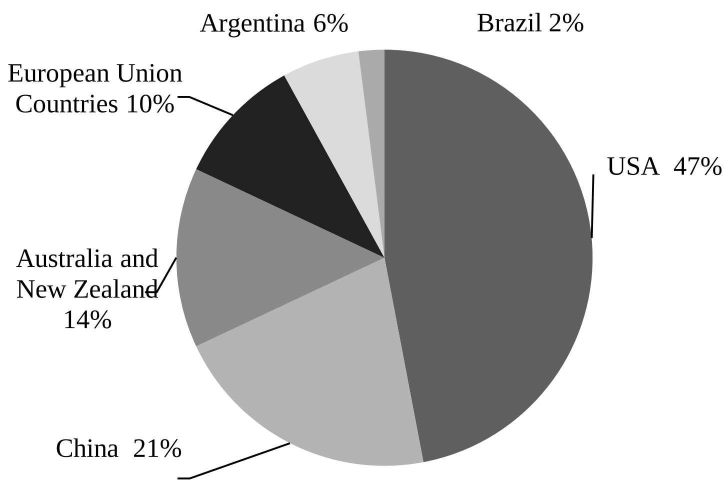

Figure 8. Microalgae production quantities of countries (%) (FAO, 2009).

While microalgae cultivation falls under the definition of aquaculture, serious investments should also be made in other sectors (Energy, Cosmetics, etc.) for microalgae cultivation. The cultivation of microalgae species such as *Spirulina* spp., *Chlorella* spp., *Haematococcus pluvialis*, and *Nannochloropsis* spp. has turned into large-scale commercial production and is produced especially for use as nutritional supplements for humans and other purposes. According to FAO data, countries with large-scale microalgae farms include Australia, the Czech Republic, France, Iceland, India, Israel, Italy, Japan, Malaysia, Myanmar, and the United States (Figure 9, Figure 10, Figure 11, Figure 12, Figure 13).

Figure 9. Green Fuel Tech Aurora Biofuels Gmbh, Arizona, Algae Production Facility (Gezici, 2012).

Figure 10. Algepower Gmbh Algae Production Facility in Manhattan, USA (Gezici, 2012).

Figure 11. Otto Pulz Microalgae Production Facility in Germany (Gezici, 2012).

In recent years, algae production facilities have been developed on a larger scale using open and closed photobioreactors (Figure 2) compared to previous years.

Production Status of Microalgae in Türkiye

Scientific studies related to microalgae in Türkiye are mainly focused on larval feed production and monitoring eutrophication in marine surface waters. Additionally, there are ongoing photobioreactor design studies for food and active substance production at the Department of Biomedical Engineering at Ege University. Microalgal biomass production has begun in Türkiye, particularly at Ege University (Figure 12); however, there is a lack of research on energy production using microalgae.

Figure 12. Ege University Microalgae Production Greenhouse (Gezici, 2012).

In Türkiye, scientific studies on microalgae are mostly carried out for larval feed production and monitoring eutrophication in surface waters. In addition, the Department of Biomedical Engineering at Ege University conducts photobioreactor design studies for food and bioactive substance production. Microalgal biomass production has also started in Türkiye, particularly at Ege University (Figure 12); however, there is not enough research on energy production.

One of the few studies in this field is the TUBITAK-funded "Innovative Approaches to Microalgal Biomass Production" project, which started in April 2010 under the Gebze Institute of Technology. In addition, experiments are carried out to investigate biomass and oil production along with nutrient consumption rates and CO_2 absorption rates by propagating algae samples collected from different natural environments and from the waste water of the Ömerli Municipal Wastewater Treatment Plant at specific intervals. Harvesting, or the successful and cost-effective separation of algae from water, is crucial for the success of this process. None of the commonly used industrial approaches have been economical or suitable for large-scale microalgal production (Asla et al., 2016).

Many private companies in Türkiye are conducting research on microalgae individually or in partnership with the government. One such company is Ege Biotechnology Inc., which produces high yielding microalgal species in its laboratories and has a collection of approximately 30 microalgal species. Algal fuel is produced at the Production Facilities (Figure 13) owned by Ege Biotechnology Inc. in Bergama. Laboratory units were established at the Production Facilities in Bergama of Ege Biotechnology Inc. with support from KOSGEB to determine the conformity of the obtained algal fuel with standards. Another organization supporting this project, the Environmental Research and Application Center (ÇEVMER) at Dokuz Eylül University, will provide contaminated water to be used in microalgae production and will determine the chemical and physical properties of this water and the water after microalgae culture. In addition, it will contribute to oil extraction and the processes involved in algal fuel production (Gezici, 2012).

Figure 13. Ege Biotechnology Inc. Microalgae Production Facility (Gezici, 2012)

DISCUSSION

Finding renewable energy sources is one of the main research topics for scientists. Currently used energy sources are decreasing and the concern about not being able to meet the demand in the coming years is increasing. Therefore, it has become inevitable to find and develop new energy sources, and algae have become the focus of attention. Industrial and domestic waste pollute water and wastewater treatment is becoming increasingly important. In order to find solutions to heavy metal pollution caused by pollution in wastewater, the use of algae is evaluated and their use is becoming more widespread. Additionally, the use of microalgae for energy production reduces wastewater costs, so the use of microalgae in wastewater treatment has also become important.

When the polluting effect of human activity on the environment and the factor of global warming come together, our biodiversity is subject to destruction. Therefore, the current algae culture established to preserve the existing biodiversity is inadequate. In Türkiye, the existence of the Istanbul Microalgae Biotechnology R&D Unit (IMBIYOTAB) at Bogazici University and the algae culture collections belonging to Ege University are not considered sufficient compared to other countries. Although Türkiye's rich algae diversity has been observed in studies on the country's algae flora, the algae culture collection is inadequate. When looking at the studies conducted in our country, most of them consist of laboratory-scale or different modeling of strains studied abroad. This situation does not allow for the observation of positive and negative results that may arise in different strains. In addition, studies conducted under laboratory conditions can only be recorded as scientific studies, as they cannot be commercialized due to problems with finding resources, costs, and insufficient support from public institutions. When looking at the studies conducted in our country over the past quarter-century, there are many valuable academic studies and scientists researching freshwater and saltwater sources, and algae flora. However, when looking at the world, it is seen that we are far behind in terms of time and more studies and support are needed. In this context, a strategy that encourages breakthroughs in energy and other areas that can be realized with R&D and innovative approaches should be implemented to go beyond these goals in Türkiye. When looking at our country's energy policies, increasing economic, environmentally friendly, and renewable energy sources is the most important goal. In this direction, the use of national and environmentally friendly energy sources and the use of algae in fields such as agriculture, industry, cosmetics, and animal feed are of great importance.

CONCLUSION AND RECOMMENDATIONS

The development and widespread use of microalgae for energy production is likely to be an alternative to fossil fuels in the future. Microalgae production for commercial purposes is limited in Türkiye, with most of the research being conducted in laboratory-scale scientific studies. Although many wastewater treatment studies are being carried out with microalgae in the field of environmental engineering, some universities are also working on biofuel production. The benefits of using microalgae for sustainable environment and renewable energy are clear, and microalgae will be a priority microorganism in many areas (environment, energy, and economy) in the future. Therefore, funding should be allocated for algae biotechnology studies in Türkiye, and large-scale algae ponds should be established with the support of public institutions and enterprises under the leadership of trained scientists in suitable areas. Efforts should be made to develop suitable areas and systems to increase production.

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Anonymous(2022c)https://fmp.conncoll.edu/silicasecchidisk/LucidKeys3.5/Keys_v3.5/Carolina35_Key/Media/Html/Arthrospira_Main.html 25/11/2022

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Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2023; 9(1): 54-62 Derleme Makale/Review Article

Mikroplastik Kirliliği ve Tatlısu Ekosistemlerindeki Etkileri

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Öz

Günümüzde kullanılan birçok materyal; ucuz maliyeti, işlenme kolaylığı, dayanıklılığı ve elverişliliği gibi nedenlerle gerek ana hammadde gerekse yan ürün olarak yaygın bir şekilde plastik içermektedir. Bu yaygın kullanım, kaçınılmaz olarak küresel ölçekte toplam plastik üretimini ve buna bağlı olarak atık plastik miktarını gün geçtikçe attırmaktadır. Atık plastikler en nihayetinde doğaya karışarak, birçoğu doğada çözünmediği veya çok geç çözündüğü için, uzun süre mevcudiyet gösterebilmektedir. Bu mevcudiyet, beraberinde önemli sorunlar getirmektedir. Bu sorunların en önemlilerinden birisi ve günümüzde en çok üzerine yoğunlaşılanı; plastiklerin canlı yaşamına olan etkisidir. Sorun teşkil eden en önemli plastik gruplarından biri ise boyutları nedeniyle canlı vücuduna alınması ihtimali yüksek olan mikroplastiklerdir. Mikroplastikler, genel olarak 5 mm'den küçük olan plastikler olarak tanımlanmaktadır. Mikroplastikler, sucul ekosistemlere doğrudan katılabildiği gibi, büyük boyutlardaki plastiklerin doğal süreçler sonucunda parçalanarak dağılmasıyla da ortaya çıkabilmektedir. Bunlar çeşitli yollarla canlıların vücutlarına girerek birtakım fizyolojik ve kimyasal süreçlerle etkileşime girebilmektedir. Nispeten yeni sayılabilecek bu konuda literatürde hatırı sayılır düzeyde bilgi birikimi bulunsa da ilgilendirdiği birçok hususta genel geçer kanılara varabilmek için katedilmesi gereken uzun bir yol vardır. Buradan hareketle bu derleme makalede okuyucuya mevcut literatür verileri ışığında bugüne dek katedilen yolda elde edilen bilgilerin aktarılması ve mikroplastiklerin çevresel etkisini azaltmaya yönelik çalışmalar için ilgili otoritelerin dikkatinin çekilmesi amaçlanmıştır. Bu amaçlara ulaşmak için mikroplastiklerin tarihçesi, sınıflandırılması, kaynakları, doğaya karışması, yayılımı, canlılar tarafından alınımı, canlılara olan etkileri ve çevresel riskleri araştırılmıştır.

Microplastic Pollution and its Effects on Freshwater Ecosystems

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Abstract

Due to its inexpensiveness, ease of processing, durability, and convenience; plastic is widely used either as main or side raw material in many materials used today. This widespread use inevitably increases the total global plastic production and accordingly the amount of plastic waste each day. Waste plastics eventually enter into the environment and last for a long time, as many of them biodegrade slowly or do not biodegrade at all. This presence brings along important problems. One of the most important of these problems and the most focused on today is the effect of plastics on life. One of the most important plastic groups that poses a problem on the other hand is microplastics, which are highly likely to be uptaken into the body due to their size. Microplastics are typically defined as plastics smaller than 5 mm. Microplastics can directly enter into aquatic ecosystems, as well as emerge when large plastics are disintegrated and dispersed as a result of natural events. These can enter the bodies of living beings in various ways and interact with certain physiological and chemical processes. Although there is a considerable amount of knowledge in the literature on this relatively new topic, there is still a vast ground to cover in order to conclude a consensus on many issues it concerns. From this point of view, in this literature review, it was aimed to convey the information obtained so far in the light of the current literature data to the reader and to attract attention of the relevant authorities for the efforts aimed at reducing the environmental impact of microplastics. To reach these aims, microplastics' history, classification, sources, entrance into the nature, spread, uptake by living beings, effects on living beings, and environmental risks are discussed.

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GİRİŞ

Mikroplastikler, özellikle son yıllarda deniz ve tatlı su ekosistemlerinde sık ve her yerde bulunmaları nedeniyle önemli bir çevresel sorun haline gelmiştir. Bu nedenle biyota muhtemelen mikroplastiklerle karşılaşacak ve onlarla etkileşime girecektir. Bununla birlikte, yakın zamana kadar plastik kirliliği üzerine yapılan araştırmaların ana odak noktası deniz ortamı olmuştur. Bu durum son yıllarda değişmeye başlamıştır ve artık dikkatler hem karasal (Lambert vd., 2014; Rillig, 2012) hem de tatlı su ortamlarına (Lambert vd., 2014; Wagner vd., 2014; Eerkes-Medrano vd., 2015) çevrilmiştir. Yapılan bu izleme çalışmalarında nehir kıyıları, yüzey suları ve nehirlerin, göllerin ve rezervuarların tortuları dahil olmak üzere tatlı su sistemlerinde mikroplastikler olarak adlandırılan mikroskobik plastik kalıntılarının miktarını belirlenmiştir (Zbyszewski ve Corcoran, 2011; Klein vd., 2015). Deniz sistemlerine kıyasla çok daha az veri mevcut olmasına rağmen, bu çalışmalar mikroplastiklerin her yerde bulunduğunu ve konsantrasyonların karşılaştırılabilir olduğunu vurgulamaktadır (Klein vd., 2017). İzleme verilerinin yanı sıra, ekotoksikolojik çalışmalar temel olarak çeşitli türler tarafından mikroplastik alımını ve bunların yaşam döngüsü parametreleri üzerindeki etkilerini araştırmıştır (Ogonowski vd., 2016; Green vd., 2016). Çalışmaların çoğunda polietilen ve polistirenin birincil mikroboncukları kısa süreli maruz kalmalar üzerinden yüksek konsantrasyonlarda (Phuong vd., 2016) kullanılmış olsa da, mikroplastiklerin tatlı su ekosistemleri için risk oluşturabileceğine dair bazı kanıtlar vardır (Scherer vd., 2017).

Bu nedenle bu derlemede, mikroplastiklerin tatlısu ortamlarına olası etkileri üzerine genel bir bakış sunulmaya çalışılacaktır.

Mahiyetleri Bakımından Plastikler ve Mikroplastikler

Mikroplastikler, menşei, malzeme türü, parçacık boyutu ve parçacık şekli gibi çok çeşitli bireysel özelliklere sahip heterojen bir kirletici sınıfı olduğundan temel boyutu ile derinlemesine incelenmesi gerekmektedir. Çünkü seri üretimin mühendislik metotları ile birleşen yeni sentetik kimyasalların üretilmesi, plastiği modern zamanların en popüler malzemelerinden biri haline getirmiştir.

Günümüzde plastik malzemelerin başlıca kullanımı kauçuk teknolojisinin gelişmesiyle birlikte 1800'lere kadar dayanmaktadır. Bu alandaki en önemli atılımlardan biri, Charles Goodyear (Stevenson vd., 2008) tarafından doğal kauçuğun vulkanizasyonunun keşfedilmesi olarak görülmektedir. 1800'ler boyunca polistiren (PS) ve polivinil klorür (PVC) dahil olmak üzere sentetik polimerler geliştirmek için bir dizi girişimde bulunulsa da kırılganlıkları ya da şekillerinin bozulması gibi sebeplerden dolayı ticarileşememiştir. Seri üretime giren ilk sentetik polimer, 1909 yılında olmuştur (Vlachopoulos ve Strutt, 2003).

Daha sonra, 1930'larda PVC'nin modern biçimleri, polietilen tereftalat (PET), poliüretan (PUR) ve daha işlenebilir bir polistiren üretilmiştir (Brandsch ve Piringer, 2008). Devam eden süreçte 1950'lerin başlarında yüksek yoğunluklu polietilen (HDPE) ve polipropilen geliştirilmiştir. 1960'larda, malzeme bilimlerindeki ilerlemeler, şekerlerin ve lipidlerin bakteriyel fermantasyonu gibi doğal kaynaklardan (Lambert, 2015) üretilen ve polihidroksialkanoatlar (PHA), polilaktitler (PLA), alifatik polyesterleri içeren plastik malzemelerin geliştirilmesine yol açmıştır. Plastik ve türevlerindeki gelişim ivmesi günümüzde dev bir sektör olmanın ötesinde hayatın her aşamasında nerdeyse yok denecek kadar az kullanım alanı ile çevresel açıdan büyük tehlike arz etmeye başlamıştır.

Plastikler, polimerlere dayalı işlenebilir malzemelerdir (Baner ve Piringer, 2007) ve onları amaca uygun malzemeler haline getirmek için genellikle bir dizi kimyasal katkı maddesi ile işlenirler. Bu bileşikler, malzemelerin özelliklerini ayarlamak ve kullanım amaçlarına uygun hale getirmek için kullanılır. Bu nedenle, polimer sınıflandırmaları içinde plastik malzemeler, birleştirildikleri katkı maddelerinin türüne ve miktarına bağlı olarak yapı ve performans açısından hala farklılık gösterebilir. Daha yakın zamanlarda, teknolojik gelişmeler, artık plastik nanokompozitler üreten nano ölçeklere dayalı yeni element uygulamalarının gelişimini görmüştür (Lambert ve Wagner, 2018).

Bununla birlikte yeni ortaya çıkan ve birçok kaynakta kendini gösteren mikroplastik kavramı plastiklerin çeşitlerinin ve türlerinin yanı sıra boyutları itibari ile sınıflandırılması gerekliliğini de ortaya koymuştur.

"Mikro plastikler" terimi, genellikle en uzun çapı 5 mm'den küçük olan ve çoğu yazar tarafından kullanılan tanım olan plastik parçacıklarını ifade eder (Lambert ve Wagner, 2018).

Mikroplastik teriminin, yalnızca mikrometre boyut aralığında (Andrady, 2011; Browne vd., 2011) partikülleri içerecek şekilde 1 mm'den küçük maddeler olarak yeniden tanımlanması ve 1 ile 2.500 mm arasındaki maddeleri hesaba katmak için 'mezoplastik' teriminin getirilmesi önerilmiştir (GESAMP, 2015). Lambert vd. (2014) makroplastikleri 5 mm'den büyük, mezoplastikleri 1-5 mm, mikroplastikleri 0,1-1 mm arasında ve nanoplastikleri ise 0,1 μm'dan küçük parçacıklar olarak tanımlamıştır. Bununla birlikte, 5 mm'lik üst sınır genel olarak kabul edilmektedir. Çünkü bu boyut, organizmalar tarafından kolayca sindirilebilen bir dizi küçük partikül içerebilir (GESAMP, 2015).

Plastik ve Mikroplastik Kaynakları, Çevreye ve Sulara Taşınımı ve Yayılımı

Plastikler, çok çeşitli kaynaklardan çevreye atık olarak girebilmektedirler. Karasal çöp atma faaliyetleri bugün önemli bir çevresel ve kamusal sorun haline gelmiştir (Seco Pon ve Becherucci, 2012; Njeru, 2006). Buna bağlı olarak atık yönetimi artan dünya nüfusuna karşın çok ciddi bir konu olarak ortada durmaktadır. Dünyanın çeşitli bölgelerindeki atık yönetimi uygulamaları da farklılık göstermektedir (Lambert vd. (2014). Bu da atığın mahiyeti, uygulamalar ve toplumsal bakış açısını ortaya koymaktadır. Toplu plastik parçalarda olduğu gibi, mikroplastikler çevreye birkaç yoldan girebilir. Örneğin, tüketici kozmetiklerinde kullanılan birincil mikroplastikler muhtemelen zengin bölgelerde daha önemlidir (Lambert vd., 2014). Mikroplastikler; (1) Atık su arıtım tesislerinden geçiş, kişisel bakım ürünlerinde mikroplastik kullanımından ya da giysilerin yıkanması sırasında tekstillerden yüzey sularına salınan liflerden, (2) Atık Su Arıtım Tesislerinden tarım arazilerine biyokatıların uygulanması (Nizzetto vd., 2016), (3) yağmur suyu taşma olayları, (4) arızi salınım (örn. lastik aşınması sırasında), (5) endüstriyel ürünlerden veya işlemlerden salınım ve (6) liflerin atmosferik birikimi (Dris vd., 2017) gibi birçok farklı kaynaktan karışabilmektedir. Bitkisel üretim için kullanılan plastik filmler, önemli bir tarımsal emisyon olarak kabul edilir ve bunların kullanımının, tarım topraklarındaki plastik kontaminasyonunun en önemli kaynaklarından biri olduğu düşünülmektedir (Xu vd., 2006a; Brodhagen vd., 2015; Kyrikou ve Briassoulis, 2007).

Topraklı tarımda avantajlı bir yol olarak görülen plastik kullanımı esasen bazı handikapları beraberinde getirir. Nemi muhafaza ederek sulamayı azaltmak, yabancı ot büyümesini azaltmak, toprak besinlerini tutmak ve gübre maliyetlerini düşürmek ve toprak sıcaklığını artırarak mahsul verimini artırmak ve ürünü olumsuz hava koşullarından korumak için bu plastik malzemler kullanılabilmektedir (Klemchuk, 1990; Liu vd., 2014). Fakat bununla birlikte, hava koşulları onları kırılgan hale getirebilir ve malzemenin parçalanmasına neden olarak geri kazanılmasını zorlaştırabilir ve art arda gelen yağış olaylarıyla birleştiğinde, kalıntılar ve parçalanmış parçacıklar biriktikleri toprağa karışmasına neden olabilmektedir (Klemchuk, 1990; Liu vd., 2014; Xu vd., 2006b). Bunlara ek olarak endüstri, sanayi, lastik üretimi, ulaştırma faaliyetleri ve bunun gibi birçok sektörel faaliyetlerden çevreye önemli ölçülerde plastik taşınımı olmaktadır.

Mikroplastiklerin çevresel matrislerde izolasyonu, özellikle tortular ve topraklar gibi yüksek organik içerikli numunelerle uğraşırken oldukça zorlayıcı olabilir. Benzer şekilde, sentetik polimerlerin spektroskopik olarak tanımlanması, yüksek pigment içerikleri ve partiküllerin ve liflerin ayrışması nedeniyle karmaşıktır. Buna göre, mikroplastiklerin saptanması ve analitik olarak doğrulanması, gelişmiş ekipmana erişim gerektirir (örneğin, mikro-FTIR ve mikro-Raman) (Klein vd., 2017). Yakın zamandaki izleme çalışmaları, deniz ortamlarına benzer şekilde mikroplastiklerin çeşitli tatlı su sistemlerinde her zaman ve her yerde bulunduğunu ortaya koymuştur.

Hiç şüphesiz bu mikroplastiklerin ortama alınması ve çevresel sistem içerisinde dolaşımı ve su kaynklarına taşınımı da çeşitli yollarla olmaktadır.

Ortama giren birçok plastik malzeme sabit kalmamaktadır. Bunun yerine, her birinde farklı kalma süreleri olan çevresel bölümler arasında (örneğin karadan tatlı suya ve tatlı sudan deniz ortamlarına) taşınmaktadır. Örneğin, karadan nehir sistemlerine geçiş, geçerli hava koşullarına, belirli bir nehir alanına olan mesafeye ve arazi örtüsü tipine bağlı olarak değişmektedir (Lambert vd., 2014).

Mikroplastiklerin karadan suya hareketi daha sonra karadan akma veya yol kenarındaki hendeklere dağılma şeklinde gerçekleşebilmektedir. Daha büyük plastiklerle karşılaştırıldığında mikroplastikler, makroplastiklerden daha hızlı bir şekilde çeşitli çevre bölmelerine taşınacakları ve dağıtılacakları için farklı bozunma oranlarına da maruz kalabilirler (Harrison vd., 2014).

Plastik bozunma süreçleriyle ilgili mevcut anlayışımızın çoğu, genellikle foto-,termal veya biyolojik bozunma gibi tek bir mekanizmayı araştıran laboratuvar çalışmalarından elde edilmiştir (Lambert vd., 2013). Bir dizi bozunma mekanizmasının bir arada meydana geldiği çevresel koşullar altında plastiklerin bozunmasına ilişkin sınırlı bilgi bulunmaktadır. Bilginin mevcut olduğu durumlarda, bu çalışmalar ağırlık kaybına, gerilme mukavemetindeki değişikliklere, moleküler yapının bozulmasına ve spesifik polimer türlerini kullanmak için spesifik mikrobiyal suşların tanımlanmasına odaklanma eğiliminde olmuştur. Bozunma süreçleri, araştırılmakta olan bozunma mekanizmasına (örn. termal bozunma) ve oluşturulan deneysel sonuca göre tanımlanır. Aksine, parçacık oluşum hızları genellikle araştırılmaz. Bu önemlidir, çünkü polietilen gibi polimerler kolayca depolimerize olmaz ve genellikle daha küçük parçalara ayrışır. Bu parçalar daha sonra giderek daha küçük parçalara ayrılarak sonunda nanoplastikler (Lambert ve Wagner, 2016a; Lambert ve Wagner, 2016b; Gigault vd., 2016) oluşturur.

Plastik parçalanma oranlarının tahmini basit bir süreç değildir. Matematik ve fizik literatüründe kinetik parçalanma modelleri araştırılmış ve polimer bozunmasının kinetiği, polimer bilimi literatüründe kapsamlı bir şekilde araştırılmıştır. Fakat genel kanaat gereğince yapısı ve şekli itibari ile değişmekle birlikte bu plastik türevlerinin çevresel veya sucul ekosistemde kalıcılık süreleri oldukça uzun ve kirletici boyutları oldukça yüksektir.

Plastik ve Mikroplastiklerin Tatlısu Ekosistemlerine Etkileri

Su ortamına girdikten sonra, plastiklerin hareketliliği ve bozunması, ana materyallerin, farklı boyutlarda parçalanmış parçacıkların ve diğer polimer olmayan bozunma ürünlerinin bir karışımını üretecektir. Buna göre biyota, zaman ve mekanda değişen karmaşık bir plastik ve plastikle ilişkili kimyasal karışımına maruz kalacaktır (Lambert ve Wagner, 2018).

Mikroplastikler çeşitli organizmalar tarafından su ortamından ve tortudan alınabilmektedirler. Bu, doğrudan yutma yoluyla olabilidiği gibi solungaçlar vasıtası ile yani dermal alım şeklinde de olabilmektedir. Tatlı sularda yaşayan zooplanktonlarla ilgili yapılan çalışmalardan birinde; *Bosmina coregoni*, polistiren mikroboncukları (2 ve 6 µm) ve alg kombinasyonlarına maruz kaldığında ikisi arasında ayrım yapmamıştır (Bern, 1990). Aynı çalışma, *Daphnia cucullata*'nın, aynı boyuttaki alg hücreleriyle kombinasyon halinde polistiren mikroboncuklarına (2, 6, 11 ve 19 µm) maruz kaldığında, daha küçük üç boyut sınıfı için benzer filtreleme oranları sergilediğini ortaya koymuştur.

Organizmaların mikroplastik alımı nedeniyle fiziksel strese maruz kalma derecesi parçacık boyutuna bağlıdır. Çünkü tortu veya gıda parçacıklarından daha büyük parçacıkların sindirilmesi daha zor olabilmektedir (Besseling vd., 2013). İlave olarak, partikül şekli de önemli bir parametredir. Çünkü daha iğne benzeri bir şekle sahip partiküller iç ve dış yüzeylere daha kolay tutunabilmektedirler. Mikroplastiklerin dolaylı etkileri, boyutuna ve şekline bağlı olabilen fiziksel tahrişi içerebilir. Daha küçük, daha köşeli parçacıkların yerinden çıkarılması, düz küresel parçacıklardan daha zor olabilir ve solungaçların ve sindirim sisteminin tıkanmasına neden olabilmektedir. Yapılan bir araştırmada, *D. magna*'ya mikroplastik maruziyetinin kronik etkileri değerlendirilmiştir (Ogonowski vd., 2016). İkincil mikroplastiklere (ortalama parçacık boyutu 2,6 µm) maruz kalma, yalnızca çok yüksek mikroplastik seviyelerinde (105.000 parçacık L^{-1}) yüksek mortaliteye, artan kuluçkalar arası süreye ve azalan üremeye neden olduğu ortaya koyulmuştur. Buna karşılık protokolde, karşılık gelen birincil mikroplastiklerde (ortalama parçacık boyutu 4,1 µm) hiçbir etki gözlenmediği bildirilmiştir (Ogonowski vd., 2016).

Bununla birlikte sucul yaşam canlılarından olan midyelerden yengeçlere trofik bir mikroplastik transferinin meydana gelebileceğini öne süren bazı çalışmalar da vardır (Farrell ve Nelson, 2013). Mavi midye, *Mytilus edulis*, 0,5 µm polistiren kürelerine (yaklaşık 1 milyon partikül ml^{-1}) maruz bırakılmış ve yengeçlere (*Carcinus maenas*) yem olarak verilmiştir. Yengeç hemolenfindeki en yüksek partikül konsantrasyonu (15.033 partikül ml^{-1}) 24 saat sonra tespit edilmiş, 21 gün sonra ise bu konsantrasyon midyelere verilen konsantrasyonun %0,027'si olan 267 partikül ml^{-1} olarak rapor edilmiştir. Başka bir çalışma, 1.000, 2.000 ve 10.000 partikül ml^{-1} gibi çok daha düşük konsantrasyonlarda polistiren mikroboncuklar (10 µm) kullanılarak mesoto makro-zooplanktondan mikroplastik transferinin potansiyelini göstermiştir (Setälä vd., 2014). Atılım oranları mevcut olmadığından ve mikroplastik alımı genellikle sindirim sisteminde (yani bir organizmanın dokularında değil, dışında) bulunan parçacıklar olarak tanımlandığından, mikroplastiklerin trofik transferinin aynı zamanda bir biyoakümülasyona mı yoksa biyobüyütmeye mi yol açtığı şimdiye kadar açık değildir.

Bununla birlikte, mikroplastiklerin avdan avcıya kesinlikle aktarılacağı ve bunun belirli durumlarda avcının vücudunda daha uzun süreler boyunca tutulabileceği açıktır.

Buradan hareketle önemli bir merak konusu, organizmaların doğal olarak oluşan mikropartikülleri ne ölçüde tükettiği ve etkilerin mikroplastiklere kıyasla nasıl olduğudur (Scherer vd., 2017). Çünkü doğal olarak oluşan parçacıklar su ekosistemlerinin önemli bir bileşenidir ve konsantrasyon, parçacık boyutu dağılımı, şekli ve kimyasal bileşimi gibi parçacık özellikleri ve ayrıca maruz kalma süresi, bunların sucul topluluklarla etkileşimlerini belirlemede güçlü bir rol oynar (Bilotta ve Brazier, 2008).

Genel olarak, ekosistem işleyişine yönelik daha geniş etkileri belirlemek için hücresel düzeydeki tepkiler ile popülasyon düzeyindeki etkiler arasındaki ilişkilerin anlaşılması önemli olacaktır. Değerlendirilecek noktalar hem biyolojik yönlerle (moleküler hedef, etkilenen uç noktalar) hem de mikroplastiklerin fiziksel ve kimyasal özellikleri gibi parçacık yönleriyle ilgilidir. Mikroplastiklerin biyoyararlanımı ve mikron altı olanların hücrelere penetrasyonu dikkate alınması gereken faktörlerdir (Lambert ve Wagner, 2018).

Bunların yanı sıra sızıntı kimyasalların etkilerinin de ortam açısından değerlendirilmesi konunun izahı açısından önemlidir. Çünkü sızıntı kimyasalları alıcı ortam açısından önemli ölçüde problem oluşturmaktadır.

Başlangıç maddelerinin ve monomerlerin, katalizörlerin, çözücülerin ve plastik malzemelerden sızan katkı maddelerinin çevresel etkilerini değerlendirmek kolay değildir (Muncke, 2009). Sızdırılabilir bileşiklerin karışım bileşimi ve konsantrasyonu, alıcı ortamların fiziksel, kimyasal ve biyolojik koşullarına bağlıdır.

Deiyonize su kullanılarak plastik ürünlerden suda çözünür bileşenlerin süzülmesi, plastiklerin neden olduğu çevresel tehlikelerin profilini çıkarmak için yararlı bir yöntem olarak kabul edilir (Lithner vd., 2012; Lithner vd., 2009). Lithner ve ark. bu tür sızıntı sularını, *D. magna*'ya karşı akut toksisitelerini değerlendirmek için doğrudan toksisite testi yaklaşımında kullanmışlardır (Lithner vd., 2012; Lithner vd., 2009). Örneğin, 10 L kg^{-1} bir sıvı-katı oranı ve 24 saatlik bir maruziyet ile, polivinil klorür, poliüretan ve polikarbonattan gelen sızıntı sularının, en toksik EC_{50} değerlerini 5–69 gr plastik L^{-1} aralığında tespit etmişlerdir (Lithner vd., 2009). Daha yüksek sıvı/katı oranları ve daha uzun süzme süreleri, plastikleştirilmiş PVC ve epoksi reçine ürünlerinden gelen sızıntı sularının 2–235 g plastik L^{-1} EC_{50} değeri ile en toksik etkiyle sonuçlanmıştır (Lithner vd., 2012).

Yapılan başka bir çalışmada; Bejgarn vd. (2015), toz haline getirilen ve üzerinde yapay hava koşulları uygulanan plastiklerin, katı/sıvı oranı 10 olacak şekilde 72 saat süreyle bir deniz kopepdou olan *Nitocra spinipes*'e olan etkilerini araştırmışlardır. Bejgarn vd. (2015), farklı PVC materyallerden gelen sızıntıların toksisitesinin değişkenlik gösterdiğini bulmuş ve paketlemede kullanılan PVC'nın yapay hava koşulları uygulamasından sonra toksik etkisinin arttığını, bahçe hortumunda kullanılan PVC'nin toksik etkisinin ise yapay hava koşulları uygulamasından sonra azaldığını tespit etmişlerdir. Bu çalışma aynı zamanda sızabilen PVC'nin karmaşık bir madde karışımı olduğunu ve ilginç bir şekilde klor içeren kütle parçalarının tanımlanmadığını göstermiştir. Parçalanan ürünlerin ve kimyasal göçün neden olabileceği potansiyel fizikokimyasal çeşitlilikten ötürü bu tarz sızıntıların karakterizasyonu oldukça zor olmaktadır. Gıda ile temas eden maddelerden gelen kimyasal göç ürünlerinin tanımlanması için bir test protokolü geliştirilmiştir. Bu protokol; uçucu, yarı uçucu ve uçucu olmayan maddeleri tespit etmek için isabetli kütle ve formül tahmini yapabilen LC-TOF-MS ve GC-MS teknikleri birleştirilerek üretilmiştir (Bradley vd., 2008; Bradley vd., 2009).

Genel olarak, bu çalışmalarda kullanılan plastik malzemenin sıvı/katı oranı, çevresel izleme çalışmaları sırasında tipik olarak tanımlanandan daha yüksektir. Bununla birlikte, tehlikeli monomerlerden ve katkı maddelerinden imal edilen malzemelere uygulandığında bu tür bir tarama, etkin bir şekilde değiştirilebilmeleri için ilgili bileşiklerin tanımlanmasını kolaylaştırabilir.

Hiç şüphesiz plastik ve türevlerine ilişkin biyolojik etki çalışmalarında en önemli ayaklardan birisini mikrometre altı plastikler oluşturmaktadır. Çünkü mevcut hem boyutları hem de yayılımları açısından daha gelişmiş analiz teknikleri ve ileri düzeyde çalışmalar ile tespiti gerekli olan farklı bir karaktere sahiptirler.

Plastik malzemeler, kullanımlarına bağlı olarak, ekosistem işleyişinde kritik bir rol oynayan bakteri ve mantarlar gibi organizmalar için toksik olabilecek antimikrobiyal maddeler ve nanomalzemeler gibi bileşikler içerebilir. Mikroskobik parçacıkların, sızan katkı maddelerin ve diğer bozunma ürünlerinin bir kombinasyonunun, sucul ve karasal organizmalara karşı mevcut analiz metotlarında tespit edilmesi veya tanımlanması zor olan ince etkilere neden olması mümkündür. Bozunma sırasında mikron altı ve nanometre boyutlarında plastik parçacıkların ortaya çıkması oldukça olasıdır (Lambert vd.., 2014; Andrady, 2011; Lambert ve Wagner, 2016a; Mattsson vd., 2015; Syberg vd., 2015).

Tasarlanmış nanoparçacıklar hücre zarlarını geçebilir ve içselleştirilebilir. Bunlar boyutlarına bağlı olarak endositoz veya fagositoz yolu ile alınabilmektedir (Nowack ve Bucheli, 2007). Hücre içine girdikten sonra tasarlanmış nanoparçacıklar veziküller ve mitokondri içinde depolanır ve bir etki oluşturabilir (Nowack ve Bucheli, 2007). Hücresel tepkiler oksidatif stres, antioksidan aktivite ve sitotoksisiteyi içerir (Oberdörster vd., 2006). Toksisite değerlendirmeleri açısından, nano-boyut aralığındaki mikroplastiklere özgü olabilecek absorpsiyon, dağılım, metabolizma ve boşaltım mekanizmalarının moleküler ve hücresel yollarının ve kinetiklerinin anlaşılmasına ihtiyaç vardır.

Desai vd. (1997) tarafından yapılan bir çalışmada bir polilaktik poliglikolik asit kopolimerinin 100 nm partiküllerinin, aynı malzemeden yapılmış 10 µm partiküllere kıyasla bir in vitro hücre kültüründe hücre içi alımın on kat daha yüksek olduğunu göstermiştir. Tasarlanmış nanoparçacıkların ayrıca memeli ve balık sistemlerinde sitotoksik, genotoksik, enflamatuar ve oksidatif stres tepkileri ürettiği gösterilmiştir (Dhawan vd., 2011).

Handy vd. (2008) tarafından yapılan bir literatür taramasında balıklarda hedef organlar olarak solungaçları, bağırsağı, karaciğeri ve beyni ve ayrıca oksidatif stres, karaciğerde tümör oluşumuyla uyumlu hücresel patolojiler, bazı organa özgü iyon düzenleyici bozukluklar ve vasküler dahil olmak üzere bir dizi toksik etkiyi vurgulamıştır. Bazı plastiklerin karmaşık kimyasal yapısı ve birlikte oluşan kirleticileri absorbe etme yeteneği dikkate alındığında, mikroplastikler için bu uç noktaların deneysel olarak araştırılması oldukça önemlidir.

Plastik ve Türevlerinin Oluşturduğu Çevresel Risklerin Değerlendirilmesi

Çoğu ülkede kimyasal risk değerlendirmeleri, maruz kalma ve etki ölçütü olarak ilgili maddelerin kütle konsantrasyonlarına dayanır. Literatürde, yayılacağı tahmin edilen parçacıkların kütle konsantrasyonları, tasarlanmış nanoparçacıkların risklerini değerlendirmek için kullanılmıştır (Boxall vd., 2007; Musee, 2011). Bu yaklaşımlar, parçacıkların farklı çevresel bölümler arasında transfer olmaksızın eşit olarak dağıldığını varsayar. Bu yaklaşım, Gottschalk vd. (2009) tarafından, geliştirilerek kullanılan farklı bölmeler arasındaki emisyon akışlarını modellemek için transfer katsayılarını ve sedimantasyon hızlarını dahil etmişlerdir. Bununla birlikte, bu tür bir yaklaşım, anlamlı bir maruz kalma değerlendirmesi gerçekleştirmek için birincil mikroplastik üretim seviyeleri, endüstriyel uygulamalar ve kullanımlar, tüketici ürünlerindeki seviyeler, atık su arıtımındaki akıbet, depolama alanlarına deşarj ve çevresel akıbet ve dağılım modellemesi hakkında kapsamlı bilgiler gerektirmektedir. İkincil mikroplastikler için bir maruz kalma değerlendirmesi, izleme verileri gerektirecektir. Ancak saha çalışmalarında bildirilen boyut aralıkları genellikle kullanılan örnekleme teknikleri tarafından kısıtlandığından bu engellenmektedir (GESAMP, 2015).

Mikroplastiklerin kimyasal bileşimlerine dayalı olarak değerlendirilmesi de önemli bir zorluk teşkil eder. Çünkü kimyasal olarak mikroplastikler bir karışım olarak kabul edilir. Kimyasal bileşimine dayalı olarak poliüretan için risk değerlendirmesinin basitleştirilmiş bir örneği Tablo 1'de verilmiştir. Poliüretan esnek köpük şilteler ve araba koltukları için kullanılan ve üç monomerin birleştirilmesinden oluşan, %18'e kadar alev geciktirici içeren bir yapıya sahiptir (Alaee vd., 2003). Karışımın tüm bileşenleri için tahmin edilen çevresel konsantrasyon tahmin edilen etkisiz konsantrasyon oranlarına dayanan örnek bir risk değerlendirmesi daha sonra bir risk katsayısını hesaplamak için kullanılır. Bu özel örnek için risk katsayısı birden küçüktür; ancak bu tür bir değerlendirme, katı parçacıkların fiziksel tahrişinden kaynaklanan potansiyel olumsuz etkileri hesaba katmaz. Bu durumda, tasarlanmış nanoparçacıklarda olduğu gibi mikroplastikler için risk değerlendirmesinin belirli zorluklar içerdiği açıkça ortaya çıkmaktadır (Brennholt vd., 2017).

Tablo 1. Alev geciktirici olarak Tetrabromobisphenol A (TBBPA) içeren esnek Poliüretan köpüğün kimyasal bileşenlerine dayanan farazi bir kimyasal karışım risk değerlendirmesi (birimler mg/L'dir)(Lambert ve Wagner, 2018).

	1. Monomer	2. Monomer	3. Monomer	1. Katkı Maddesi
	Propilen oksit	Etilen oksit	Toluen diizosiyanat	TBBPA
LC_{50} alg	307	502	3,79	0,19
LC_{50} daphnia	188	278	2,61	0,02
LC_{50} balık	45	58	3,91	0,02
TEK (DF=1000)	0,045	0,058	0,003	0,000002
TÇK (çözünmüş bileşik)	0,00067	0,00067	0,00067	0,0000032
$RK_{TÇK/TEK}$	0,015	0,012	0,257	0,160
Karışım RK	0,443			

Bu örnek tablo için LC50 (ortanca ölümcül konsantrasyon), EPI Suite ECOSAR modeli kullanılarak hesaplanmıştır;
DF: Değerlendirme faktörü
TÇK: Tahmin edilen çevresel konsanstrasyon
TEK: Tahmin edilen etkisiz konsantrasyon
RK: Risk katsayısı

Monomerlerin TÇK değerleri ECHA'nın propilen oksite dayalı risk değerlendirmesine dayanmaktadır (European Commission, 2002).

TBBPA'nın TÇK değeri Birleşik Krallık göllerinde ölçülen maksimum konsantrasyonlara dayanmaktadır (Harrad vd., 2009).

Çevresel sistemlerdeki mikroplastiklerin farklı partikül boyutları, bu sistemlerde yaşayan organizmalar için farklı riskler sunmaktadır. Örneğin, küçük planktonla beslenen balık türleri, nano ölçekten 5 mm veya daha büyük mikroplastiklere kadar mikroplastiklerle karşılaşabilir. Balık daha büyük parçacıklardan kaçınabilir, ancak beslenirken küçük parçacıklar yutulabilir. Filtreyle beslenen organizmalar için üst boyut sınırı, belirli bir organizmanın doğal olarak yutacağı parçacıkların boyutuna bağlı olacaktır. Mikroplastiklerin risk değerlendirmesi bu nedenle parçacık boyutuna dayalı olabilir. Arvidsson (2012) tarafından verilen bir örnekten yararlanan Kutucuk 1'de basitleştirilmiş varsayımsal bir durum sunulmaktadır. Bu yaklaşım, o parçacık boyutu aralığındaki en hassas türler için boyut sınıflarına ve parçacık konsantrasyonuna dayalı olarak mikroplastiklerin zararla ilgili eşikleri hakkında bilgi olduğunu varsaymaktadır. Bununla birlikte, çevresel riski tanımlamak için parçacık boyutunun kullanımı o kadar basit olmayabilir. Çünkü mikroplastikler çevrede tek dağılımlı değildir (Lambert ve Wagner, 2018).

Kutucuk 1: Parçacık boyutlarına göre Mikroplastiklere ilişkin risk değerlendirmesi için farazi bir vaka.
Bir gölün, ≤5 mm boyutlarında mikroplastikler için 10.000 parçacık/L TÇK değeri var ve bu parçacıkların TEK değerinin 1.000 parçacık/L olduğu farzediliyor. Ayrıca, alt sınır aynı kabul edilerek TÇK'nin %1'inin 1 mm'den küçük parçacıklardan oluştuğu farzediliyor; buna göre RK, parçacık boyutlarının üst sınırıyla aşağıda verildiği gibi belirlenir:

$$RK_{\text{üst sınır}-\leq 5mm} = \frac{PEC}{PNEC} = \frac{10.000}{1.000} = 10\ (>1)$$

$$RK_{\text{üst sınır}-\leq 1mm} = \frac{PEC}{PNEC} = \frac{100}{1.000} = 0{,}1\ (<1)$$

Daha sonra üst sınırın ayarlanmasıyla risk olup olmadığı belirlenir.

SONUÇ

Bu literatür araştırmasında mikroplastiklerin genel yapıları, çevresel etkileri ile bu parçacıkların sucul ekosistemlerde etkilerine ilişkin değerlendirmeler yapılmıştır. Bu etkilerin miktarı ve mahiyetinin çevresel girdilerin coğrafi dağılımları, kişi başına plastik kullanım miktarları, demografik ve nüfus özelliklerine göre değişiklik gösterdiği ortaya koyulmaktadır (Nguyen Phuc vd., 2011). Tabi artan sanayileşme, nüfus artışı, kentleşme faaliyetleri ve buna bağlı olarak değişen teknolojik gelişmeler nedeniyle çevresel yoğunlaşmalar uzun vadede olumlu ya da olumsuz etkileyebilir. Bu etkileşimin en aza indirilmesindeki en önemli faktörlerden birisi de mevcut altyapıların atık malzemeleri işleme kapasitelerinin artırılması olmaktadır. Mevcut literatür çalışmaları plastik ve türevlerinin çeşitli çeversel ortamlarda dağılımları, taşınımları, parçalanma ve bozunmalarına ilişkin çeşitli çalışmaları içerse de yeterli ve büyük çaplı çalışmalara ihtiyaç vardır. Gelecekteki çalışmalar, mikroplastiklerin çevresel kaderini ve ekolojik etkilerini daha iyi anlamaya odaklanmalıdır. Çünkü sınırlı çalışmalar oldukça değişkenlik gösteren bu olumsuz unsuru açıklamaya henüz yeterli değildir. Mikroplastiklerin maruziyetinin uzun vadeli, ince etkileri hakkında da çok az şey bilinmektedir. Öte yandan bu çalışmaların ekseriyetinin denizel ortama odaklanması tatlı sularda plastik veya mikroplastik kirliliğinin yayılım ve etkilerinin yeterince anlaşılmasını kısıtlamaktadır. Bilimsel çalışmaların tatlısulara da yoğunlaşması kaçınılmaz bir gereklilikdir.

Ayrıca bu tip çevresel sorunların tespiti, giderimi veya önelenmesi gibi etkinliklerin tümü hiç şüphesiz ki politik, sosoyolojik ve teknolojik bir kısım eylemleri gerektirmektedir. Toplumsal eylemler bilinçlenme ve koruma çalışmalarını ele alırken, politik eylemler önleyici mevzuatın oluşturulması ve kararlılıkla uygulanması çalışmalarını üstlenmelidir. Teknolojik faaliyetler ise kirlenen kaynakların aslına uygun şekilde rehabilite edilebilmesi adına tepkiler üretmelidir.

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