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Research Article / Araştırma Makalesi

DETECTION OF FECAL INDICATOR BACTERIA IN SHIP BALLAST WATER

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ABSTRACT

Nowadays, discharge of ballast water is recognized as one of the greatest threats to the biodiversity of Sea of Marmara. In addition, transfer of pathogenic bacteria from ships' ballast water to ecosystem and coastal waters of Marmara Sea has negative impacts. In this study, presence of fecal indicator bacteria (FIB) and *Salmonella* spp. were investigated in the ships' ballast water coming from different marine environments of Burgas-Bulgaria, Susa-Tunisia and Misratah-Libya to Marmara Sea. Membrane filtration method was used for the detection of FIB and *Salmonella* spp. in the samples. Based on the experimental results, presence of fecal indicator bacteria and *Salmonella* spp. were found to be over the International Maritime Organization (IMO) limit values. It is concluded that the uptake of ballast water from these areas and discharging to Marmara Sea could pose a serious threat to the coastal ecosystems and human health. Thus, development of national ballast water management procedure is urgently needed, especially for the future of the Marmara Sea.
Keywords: Ballast water, fecal indicator bacteria, *Salmonella* spp., Marmara Sea.

BALAST SUYUNDA BULUNAN FEKAL İNDİKATÖR BAKTERİLERİN BELİRLENMESİ

ÖZ

Günümüzde gemilerin farklı deniz/okyanuslardan aldıkları balast sularını Marmara Denizine basması, Marmara Denizi'nin biyolojik çeşitliliği için büyük tehditler oluşturmaktadır. Ayrıca, gemilerin balast suları ile taşınan patojen bakteriler, Marmara Denizi'nin kıyı kesimlerini ve ekosistemini olumsuz bir şekilde etkilemektedir. Bu çalışmada, Burgaz-Bulgaristan, Susa-Tunus ve Misratah-Libya gibi farklı deniz ortamlarından balast sularını alarak Marmara Denizi'ne gelen gemilerin balast sularından alınan örnekler içerisindeki Fekal İndikatör Bakteri ve *Salmonella* spp. varlıkları incelenmiştir. Fekal İndikatör Bakteri ve *Salmonella* spp. varlığını tespit edebilmek amacıyla membran filtrasyon metodu kullanılmıştır. Yapılan deneysel çalışmalar sonucunda, Fekal İndikatör Bakteri ve *Salmonella* spp. miktarları Uluslararası Denizcilik Örgütü tarafından belirlenen standartların üzerinde olduğu tespit edilmiştir. Sonuç olarak, bu bölgelerden alınan balast sularının Marmara Denizi'ne basılması; kıyı kesimleri, deniz ekosistemi ve insan sağlığı için tehdit oluşturabileceği ve aynı zamanda Marmara Denizi geleceği için, acilen Ulusal balast suyu yönetim prosedürünün geliştirilmesi gerekliliği sonucuna varılmıştır.
Anahtar Sözcükler: Balast suyu, fekal indikatör bakteri, *Salmonella* spp., Marmara Denizi.

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1. INTRODUCTION

The occurrence and distribution of pathogenic bacteria in marine environments may a major threat to global biodiversity and sustainable uses of marine resources. The effect of pathogenic bacteria on marine biodiversity is generally irreversible, and it can be nearly impossible to eliminate them once established [1,2]. There is an increasing global demand for prevent the risk of spreading of pathogenic bacteria. Especially, pathogenic bacteria are transferred globally by ships' ballast tanks to aquatic environments [3]. It is estimated that 100.000 pathogenic and dominant species are transported between marine and freshwater ecosystems on the earth [4,5], and they could disrupt the native ecology. Consequently, biological invasions adversely affect the marine biodiversity, human well-being and economic productivity. The management of introduced pathogenic bacteria is to adopt the necessary comprehensive approach and coordinated action by national and territorial governments [6]. In February 2004, International Maritime Organization (IMO) was implemented International Convention for the Control and Management of Ships' Ballast Water and Sediments to control the spread of non-native species into new ports [7]. The convention will enter into force 12 months after 30 countries with combined merchant fleets constituting 35% of the gross tonnage of the world's merchant shipping have signed the Convention. According to the Convention there are two basic methods: Ballast water exchange (BWE-D1) and Ballast water treatment (BWT-D2). BWT is an effective method for preventing the spread of non-native species than BWE [8]. Implementation of D2 standard should provide following point:

- less than 10 viable organisms per m^3 for organisms $\geq 50\mu$ in minimum dimension,
- less than 10 viable organisms per ml for $10\mu \leq$ organisms $< 50\mu$ in minimum dimension,
- less than 1 colony forming unit (cfu) per 100 ml or less than 1 cfu per 1-gram zooplankton samples for toxicogenic Vibrio cholera,
- less than 250 cfu per 100 ml for *Escherichia coli (E. coli)*,
- less than 100 cfu per 100 ml for Enterococci.

Salmonella spp. is generally present at high densities of indicator organisms [9,10]. However, identification of *Salmonella* spp. in the lack of indicators of fecal pollution has been studied [11,12]. Various epidemiological studies have proved an increasing relative risk of gastroenteritis for bathers exposed to as few as 20 cfu $(100\ ml)^{-1}$ [13] or 35 cfu $(100\ ml)^{-1}$ [14,15] of fecal streptococci.

The marine ecosystems of Marmara Sea are to exposed to severe environmental degradation related to heavy marine traffic, and correspondingly the introduction of alien species are increased. Previous studies indicated that approximately 23 million tons of ballast water are discharged from ships every year in Turkish ports. In 2010, 15 million tons of ballast water is being discharged in the Sea of Marmara. Especially, Marmara Sea is vulnerable due to the increase of pollution from the ships' ballast water as well as the heavy shipping traffic coming from various marine environments to Marmara Sea. Marmara Sea is the most important shipping trade routes from the Black Sea countries to the Europe [16]. Altuğ et al. reported that the ships coming from various marine environments to the Marmara Sea carry a potential bacteriological risk for this region. In their study, 38 bacterial species were reported in the ballast water samples and 27 of them pathogenic bacteria [16]. The increase of the pollution level in the Sea of Marmara significantly affect the biodiversity of the Black Sea and the Aegean Sea. Kalkan and Altuğ determined that the presence of indicator bacteria of the seawater samples taken from coastal areas of the Güllük Bay of the Aegean Sea were found higher than the standard limits [17].

In the present study, the count of fecal indicator bacteria (FIB) and *Salmonella* spp. were investigated in the ships' ballast water which coming from various marine environments to the Sea of Marmara. The possible negative effects of these bacteria in study area were also discussed.

2. MATERIAL AND METHODS

The ballast water samples were directly taken from ballast tanks of bulk ships which voyages in the lines of Burgas-Zeyport, Susa-Tuzla Shipyard and Misratah-Derince. All the samplings were taken during the ships discharge the ballast water to Marmara Ports. The origins of the ballast water were shown in Fig. 1. One ballast tank per ship was sampled. Three water samples were taken from each tank. The all water samples were collected in sterile plastic containers, stored in the dark at 4 °C and immediately transferred to the laboratory. The samples were processed according to the protocols recommended in IMO Convention (ANNEX-3 Resolution MEPC.173 (58)).

Figure 1. The points which the ballast samples are taken in various country.

Membrane filtration method was used to determine the presence of living organisms in the ballast water samples. Ballast water sample is passed through a sterile membrane filter (0.45 µm pore size) in the membrane filtration (Fig. 2). Microorganisms are retained on the filter. The filter placed on a suitable sterile Petri dish and incubated according to media given in Table 1. After the adequate incubation period, the colonies are counted on the surface of the filter and the number of colonies in the original sample was determined. The results were then reported as the number of colonies per 100 ml of the ballast water. To ensure acceptable results, the samples were properly diluted as ballast water contained high numbers of FIB [18].

Table 1. Required nutrient pads and incubation times to determine FIB and *Salmonella* spp.

Microorganism	Nutrient Pad Sets	Incubation time
Enterococci	Azide	36±2 °C; 40–48 h
***Salmonella* spp.**	Bismuth Sulfite	36±2 °C; 40–48 h
***E. coli* and other coliforms**	m-Endo	36±2 °C; 18–24 h
Fecal coliforms	m-FC	44,5±0,5 °C; 18–24 h

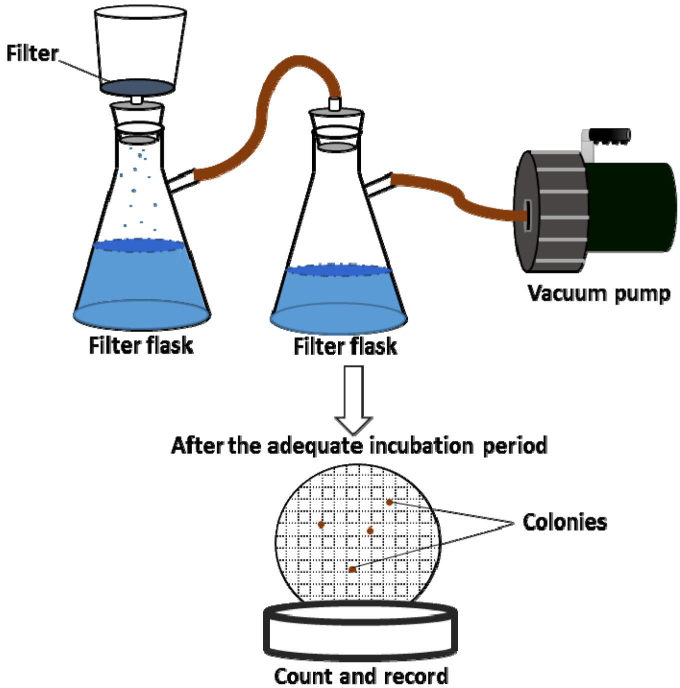

Figure 2. Schematic representation of the membrane filtration method and colony count.

3. RESULTS

The ballast water samples were directly collected from ballast tanks of bulk ships which voyage in the different lines during the discharging to the Marmara Ports.

In the analysis of the ballast water samples, the presence of FIB and *Salmonella* spp. were identified in the ballast water samples. The presence of FIB commonly associated with pathogenic bacteria, viruses, and protozoans that also live in human and animal digestive systems. Abundance and distribution of FIB in ballast water are necessary to know their potential threat to human health and ecosystem health [19–21].

Enterococci concentrations in the sample taken from the ship which came from Burgas were found higher than those of the ships coming from Tunisia and Libya (Fig 3-4). The presence of enterococci in water shows the direct evidence of fecal pollution from warm-blooded animals and their presence indicates the possible presence of pathogens [22,23]. Also, the counts of the enterococci of the ballast water samples was found to be 600 times for Burgas, 42 times for Susa and 26 times for Misratah higher than limit values of the International Maritime Organization (IMO). Exposure to enterococci can adversely affects of humans such as throat symptoms and respiratory and gastrointestinal illnesses [24–27].

E. coli is generally considered harmless and to be a part of the bacterial flora of mammals and birds. However, *E. coli* O157:H7 strain is a certain pathogenic form of *E. coli*. The results presented in this paper demonstrate that the *E. coli* and fecal coliforms were not observed in the ballast water samples (Fig 5-6). On the other hand, Altuğ et al. reported significantly higher levels of *E. coli* O157:H7 in the ships' ballast water coming from different regions of the world to the Sea of Marmara [16].

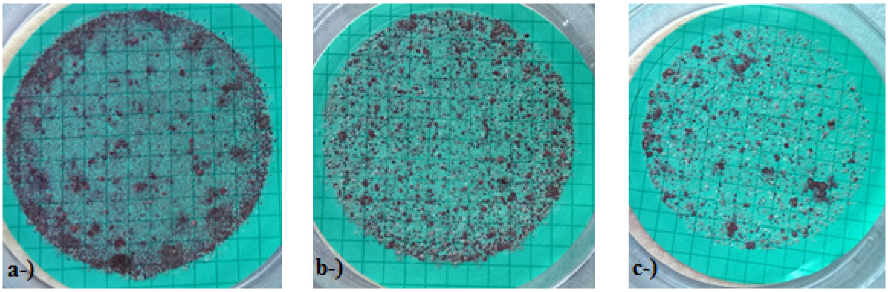

Figure 3. Abundance of enterococci in Azide media (a-Burgaz-pure b- Misratah- 10 times diluted c- Susa 50 times diluted).

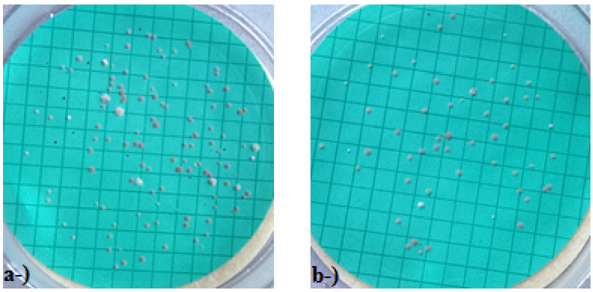

Figure 4. Abundance of enterococci in Azide media (a-Burgaz b- Misratah, Both samples, 50 times diluted).

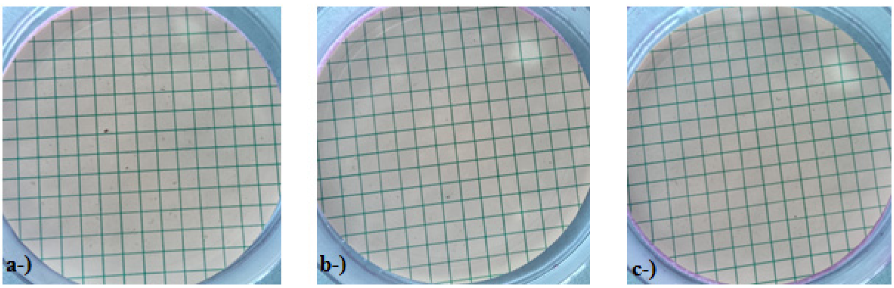

Figure 5. Abundance of fecal coliforms in m-FC media (a-Burgaz b- Misratah c- Susa, All samples are pure).

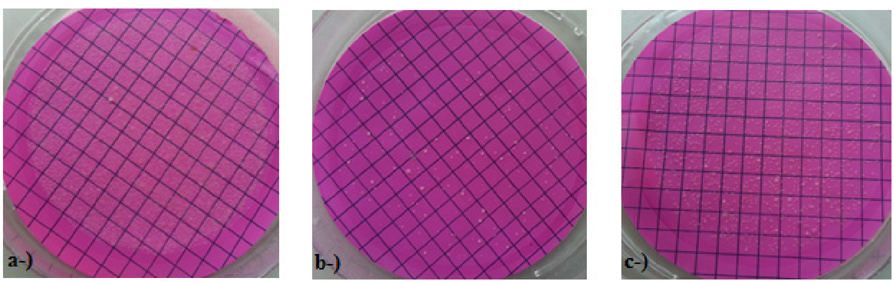

Figure 6. Abundance of *E. coli* and total coliforms in m-FC media (a-Burgaz b- Misratah c- Susa, All samples are pure).

The presence of *Salmonella* spp. in ballast water that could pose a risk to marine environments as an indicator of fecal pollution, such as total coliforms and fecal coliforms. *Salmonella* spp. has shown a high resistance to a large variety of stresses associated to environmental changes [28]. *Salmonella* spp. has been isolated from seawater, molluscs and other seafood products in marine environments [29–35]. The highest total number *Salmonella* spp. was determined in ballast water samples (Fig 7). There is no information related to the level of *Salmonella* spp. and toxicity or influences on marine environment in international guidelines. It is observed that the Ballast Water Convention is insufficient in terms of counting the identified bacteria. The count of FIB and *Salmonella* spp. were shown in Fig. 8. *E. coli* and *f*ecal coliforms were not detected in the ballast water samples.

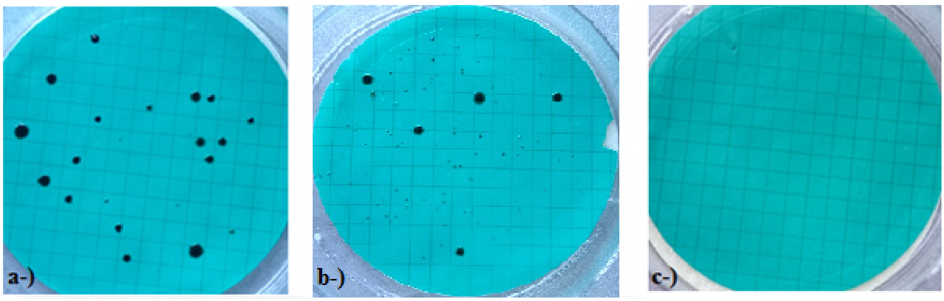

Figure 7. Abundance of *Salmonella* spp. in Bismuth Sulfite media (a- Susa b- Misratah c- Burgaz, All samples, 50 times diluted).

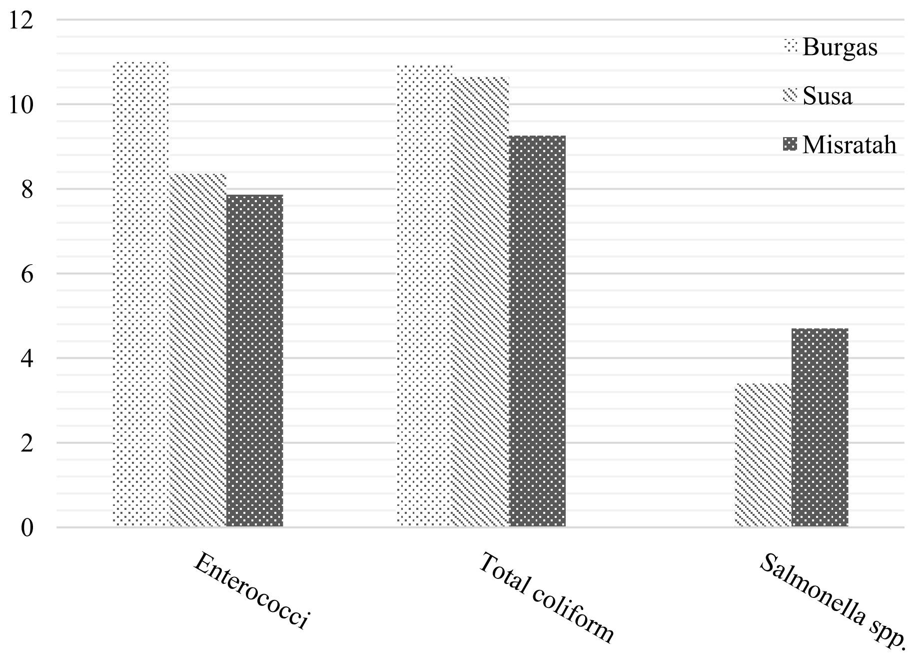

Figure 8. The number of FIB and *Salmonella* spp. in ballast water samples.

The waterborne pathogenic microorganisms at the recreational waters has a strong relationship with the abundance of specific indicator bacteria in the water column. It is known that when indicator bacteria levels are higher than the international standard values, fecal contamination may cause health risks and pathogenic problems [36].

The ballast water coming from these areas to the Marmara Sea carries a risk for marine ecosystem and human health. In addition, pollution rate of the marine and coastal environment should be continuously monitored and kept it within normal ranges.

4. CONCLUSION

In this study, the presence of fecal indicator bacteria and *Salmonella* spp. in the different ships' ballast water samples, coming from different marine areas such as the Burgas-Bulgaria, the Susa-Tunisia, and the Misratah-Libya, was investigated. Membrane filtration method was used to determine the count of fecal indicator bacteria and *Salmonella* spp. While the highest count of fecal indicator bacteria was detected in all ballast water samples, the presence of *Salmonella* spp. only determined in the samples coming from the Susa and the Misratah. The number of fecal indicator bacteria and *Salmonella* spp. in all the samples were found very high values so as to threats the human health. *E. coli* and fecal coliforms were not observed in the ballast water samples.

The better management practices, inspection strategies, and overall awareness are needed related to the impacts of shipping activities and ballast water pollution to protect marine and coastal areas.

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