Desalination and Water Treatment
www.deswater.com
doi:10.5004/dwt.2017.21786

98 (2017) 190–195
December

Assessment of microbial and physiochemical quality of ballast water in commercial ships entering Bushehr port, along the Persian Gulf

Farshid Soleimani[a], Sina Dobaradaran[a,b,c,*], Reza Taherkhani[d,e], Reza Saeedi[f], Mohammad Javad Mohammadi[g], Mozhgan Keshtkar[a], Maryam Ghaderi[a], Roghayeh Mirahmadi[a]

[a]*Department of Environmental Health Engineering, Faculty of Health, Bushehr University of Medical Sciences, Bushehr, Iran*
[b]*The Persian Gulf Marine Biotechnology Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran*
[c]*Systems Environmental Health, Oil, Gas and Energy Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran, Tel./Fax +98 7514763448, email: s.dobaradaran@bpums.ac.ir, Sina_dobaradaran@yahoo.com*
[d]*Department of Microbiology and Parasitology, School of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran*
[e]*Persian Gulf Biomedical Research Center, Bushehr University of Medical Sciences, Bushehr, Iran*
[f]*Department of Health Sciences, School of Health, Safety and Environment, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[g]*Abadan School of Medical Sciences, Abadan, Iran*

Received 15 February 2017; Accepted 7 December 2017

ABSTRACT

In the present study to evaluate ballast water impacts on coastal areas, samples were taken from commercial ships entering Bushehr port along the Persian Gulf. Standard methods were used for analyses of total and fecal *coliforms*, Pseudomonas, heterotrophic plate count (HPC), alkalinity, and hardness. Other parameters such as TOC, salinity, EC and TDS were also determined. The levels of TDS, EC, alkalinity, total hardness, salinity and TOC ranged from 3790–14510 mg/L, 5690–21760 µS/cm, 104–191 mg/L $CaCO_3$, 1160–8940 mg/L $CaCO_3$, 33.2–44.98 g/L and 1.9–5.7 mg/L respectively. Twenty-four, thirteen and four samples from 34 collected samples were positive in the case of total *coliforms*, fecal *coliforms* and *E. coli* respectively. All samples contained *Pseudomonas aeruginosa* and *HPC*. Our results showed that ballast water has the potential to change bacteria communities and also can be pathogenic for humans and coral reefs where ships discharge their ballast water.

Keywords: Ballast water; Bushehr; Commercial ships; Microbial quality; Persian Gulf

1. Introduction

The international maritime industry transports approximately 90% of the world's commodities [1]. One of inadvertent subsequences of this traffic is the transport and delivery of organisms from one place to another via ballast water, which is carried by ships to control draft, constancy and trim [2,3]. Propagation of invasive species in new environments through the ballast water discharges of commercial sea traffic is a main concern of the International Maritime Organization [4,5]. Recently, in spite of progress in the understanding of ballast water-mediated transfer and invasion for marine species, micro-organisms have been largely ignored in ballast water research. This problem caused the first IMO invention to appoint resolution A.774 (18) in 1993, following A.868 (20) in 1997, in which it recommends ships to accomplish ballast water exchange in open Ocean [6]. In 2004, the international convention for the control and management of ships ballast water was adopted, with the aim of providing guidelines for ballast water management [7]. Among aquatic organisms, bacteria are the most widespread [8]. Due to their reproductive potency, and persistence, these micro-organisms actively invade coastal ecosystems [9]. Many researchers [10–14] have pointed

*Corresponding author.

out a high durability rate of pathogenic and opportunistic pathogenic bacteria such as *Enterococci*, *Salmonella* spp., *E. coli*, *Mycobacterium* spp, *Clostridium perfingens*, *Pseudomonas aeruginosa*, *Pseudomonas putrefaciens*, *Vibrio algynolyticus*, *Vibrio cholerae*, and *Vibrio* spp in the samples of ballast water. The main aims of this study were to determine the microbial quality including total and fecal *coliforms*, *Pseudomonas aeruginosa*, and heterotrophic plate count (HPC) bacteria as well as physicochemical quality of ballast water in commercial ships entering Bushehr port, along the Persian Gulf.

2. Material and methods

Ballast water samples were collected from commercial ships entering Bushehr port, along the Persian Gulf from 34 ports around the world (see Fig. 1) between 15 February and 25 August 2016. Each sample was obtained directly from the manhole of each tank, at a depth of 4 m. Samples were placed in a nice box at 4°C, and transported to the laboratory on the day of sampling.

2.1. Physicochemical parameters

The levels of alkalinity and total hardness were determined by standard methods [15]. The concentration levels of TOC and salinity were determined by using TOC analyzers (Shimadzu, TOC v-csh, Japan) and Salt meter ES-421 respectively. Electrical conductivity (EC) and total dissolved solids (TDS) were determined by using Portable YK- 2001CT.

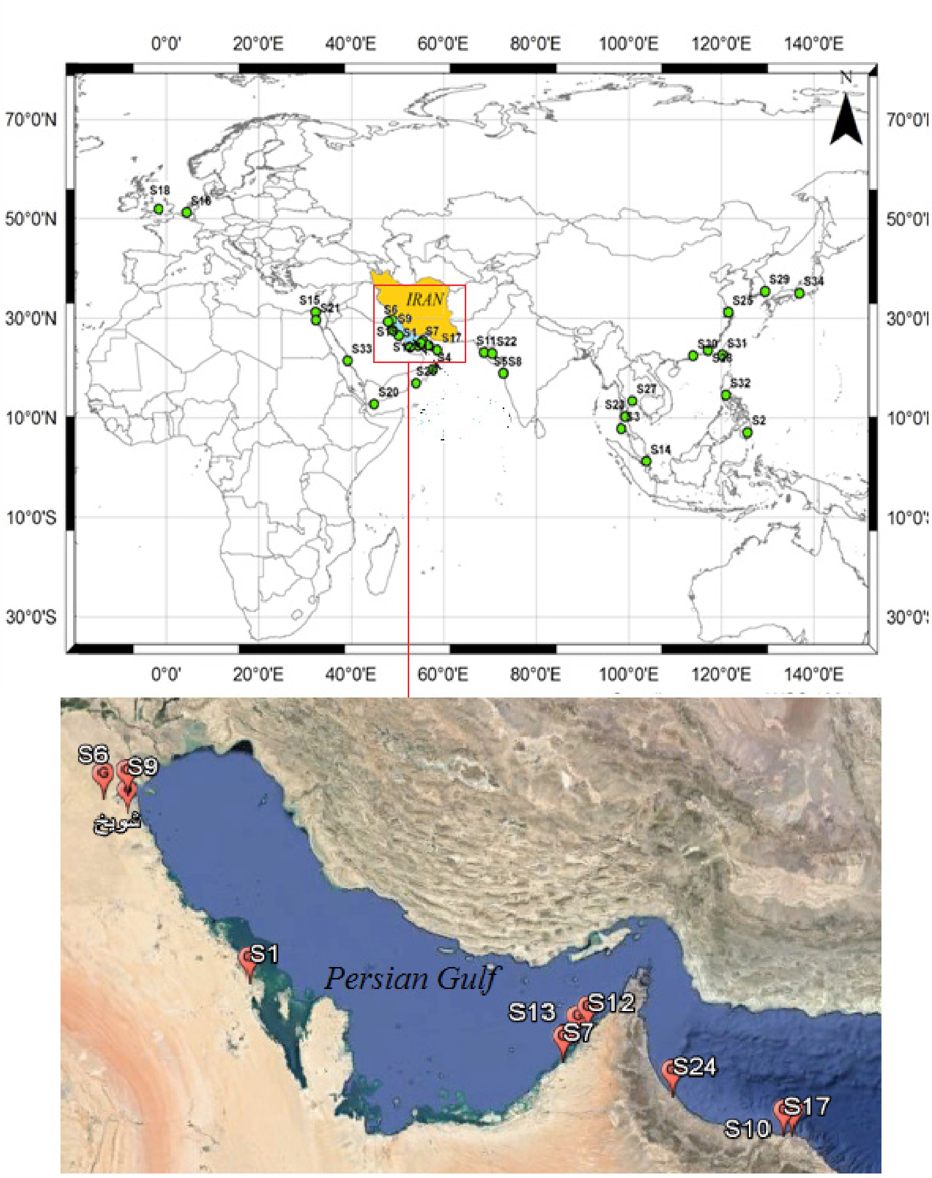

Fig. 1. The geographical location of all ports from which their ships entered Bushehr port.

2.2. Microbial parameters

All microbial indicator analyses including total *coliforms*, fecal *coliforms*, *Pseudomonas aeruginosa, E.coli* as well as *HPC* bacteria were done according to standard methods [15]. Lactose broth (Merck, Germany), Brilliant Green-broth (Merck, Germany) and EC broth (Merck, Germany) were employed to determine the most probable number (MPN/100 ml) of total *coliforms* and fecal *coliforms*. Asparagine broth (Biomark, India) and Acetamide broth (Micro master, India) were employed to determine the most probable number (MPN/100 ml) of *Pseudomonas aeruginosa*, using a five-tube multiple-dilution technique. R2A agar (Merck, Germany) using the spread plate technique was used for analyzing of the colony forming unit (CFU) per ml of *HPC* bacteria, and a colony counter (Scan 100 France) was used for colony enumeration. Additionally, MacConkey agar (Merck, Germany) media and PCR techniques were used for the isolation and identification of *E. coli* bacteria.

3. Result and discussion

The measured physicochemical parameters of ballast water samples in commercial ships are presented in Table 1. TDS and EC analyses showed that the concentra-

Table 1
Physicochemical parameters of examined ballast water samples from different ports around the world (n = 34)

Location of harvesting ballast water	Stations	TDS (mg/L)	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Salinity (g/L)	EC (μS/cm)	TOC (mg/L)
Dammam – Saudi Arabia	S1	8927	5020	132	38.682	14170	2.5
Davao – Philippines	S2	7685	4000	130	39.582	11546	3.5
Phuket – Thailand	S3	11542	3880	128	35.282	16890	2.9
Duqm – Oman	S4	5378	2800	136	41.9	7985	1.9
Jawaharlal Nehru Port – India	S5	3790	3200	156	34.182	5690	3.1
Kuwait – Kuwait	S6	12231	3480	124	40.58	16800	2
Jebel ali – Emirate	S7	13180	4720	152	41.5	19290	2.6
Mumbai – India	S8	6745	5760	116	43.98	10050	3.4
Shuhaikh – Kuwait	S9	4980	2880	150	38.4	7865	2.4
Hamriyah – Emirate	S10	9659	1680	128	40.4	14675	2.5
Kandla port – India	S11	7531	1160	134	41.38	12375	3.1
Ajman port – Emirate	S12	4813	7680	140	40.38	6540	2.7
Mina rashid – Emirate	S13	6135	8230	154	35.86	9087	3
Singapore – Singapore	S14	10863	8940	164	39.3	15893	3.2
Port said – Egypt	S15	13752	7430	149	39.6	19430	4
Antwerp – Belgium	S16	11545	8045	153	37.58	17347	5.7
Muscat – Oman	S17	13140	8590	161	34.28	19342	2.8
Portsmouth – U.K	S18	10170	7995	159	40..82	14350	4.9
Basra – Iraq	S19	11090	3450	191	33.2	16105	3.2
Aden – Yemen	S20	8170	6730	169	36.46	12150	2.8
Suez – Egypt	S21	6520	8120	188	36.2	10670	3.5
Navlakhi – India	S22	12050	6870	152	38.78	17705	4
Bangkonk – Thailand	S23	14510	8795	175	39.5	21760	2.9
Sohar – Oman	S24	10055	4390	162	37.68	15435	3
Shanghai – Chain	S25	10730	8080	104	39.582	17540	3.1
Salalah – Oman	S26	9740	7640	160	40.98	15910	2.7
Laemchabang – Thailand	S27	8130	7490	156	36.81	13795	3.2
Hong kong – Chain	S28	7435	8154	144	38.55	11780	3.2
Busan – Korea	S29	10700	7400	168	41.38	16740	2.9
Shenzhen – Chain	S30	9135	7840	144	36.882	14650	3.3
Kaohsiung – Taiwan	S31	8130	7320	162	40.14	13450	2.8
Manila – Philippines	S32	10700	7850	170	37.98	15660	2.6
Jeddah – Saudi Arabia	S33	8600	6700	165	38.74	12360	2
Nagoya – Japan	S34	9950	7870	138	37.62	14510	2.9
Mean		9344.4	6182	150.4	38.59	14104.3	3.04
Std. deviation		2653	2240.6	19.06	2.52	3812.45	0.91

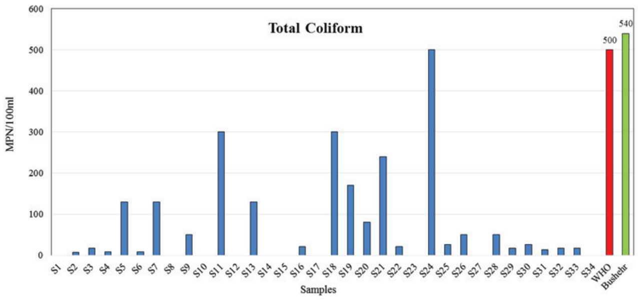

Fig. 2. Comparison of total *coliform* levels in the ballast water samples with value of total *coliform* levels in coastal areas of Bushehr port and WHO guideline for natural natatorium.

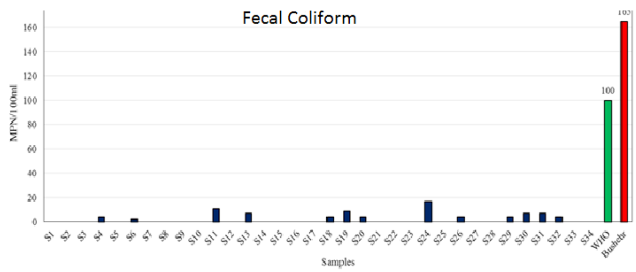

Fig. 3. Comparison of fecal *coliform* levels in the ballast water samples with value of fecal *coliform* level in coastal areas of Bushehr port and WHO guideline for natural natatorium.

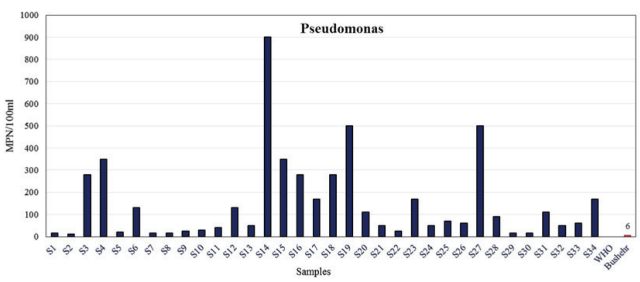

Fig. 4. Comparison of *Pseudomonas aeruginosa* levels in the ballast water samples with value of *Pseudomonas aeruginosa* levels in coastal areas of Bushehr port and WHO guideline for natural natatorium.

tion levels of these parameters ranged from 3790–14510 mg/L and 5690–21760 µS/cm, respectively. The concentration levels of alkalinity and total hardness ranged from 104 to 191 and 1160 to 8940 mg/L $CaCO_3$, respectively. The concentration levels of salinity and TOC in ballast water samples ranged from 33.2 to 44.98 g/L and 1.9 to 5.7 mg/L, respectively.

The results of microbial measurements of ballast water samples are presented in Table 2. Twenty-four of all 34 ballast water samples (70.58%) contained total *coliform* bacteria (ranging from 7 to 500 MPN/100 ml), and 13 samples (38.2%) contained fecal *coliform* bacteria (ranging from 2 to 17 MPN/100 ml). Four samples (11.76%) of ballast water samples were positive for *E. coli*. All of the 34 ballast water samples contained *Pseudomonas aeruginosa* and *HPC* bacteria (ranging from 4 to 900 MPN/100 ml and 3100 to 8930 CFU/ml respectively).

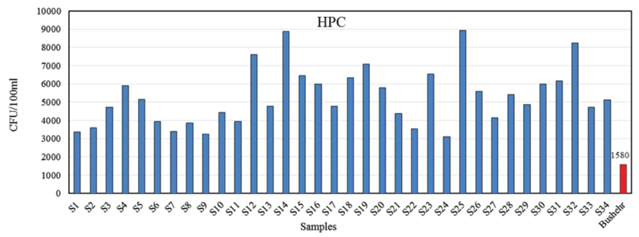

Fig. 5. Comparison of *HPC* levels in the ballast water samples with value of *HPC* levels in coastal areas of Bushehr port.

In a previous study, Pereira et al. (2014) reported that ships had discharged ballast water with high level of salinity, *E. coli* and total *coliforms* into the Amazon River. Also they reported that temperature was one of the main factors that affect surviving rates of microorganisms in ballast water [16]. In another study, Buzoleva et al. (2012) reported that ballast waters coming into Vladivostok from Japanese ports were less polluted than from the Chinese ports, which were characterized as dirty in summer [8]. In the Burkholder et al. (2007) study, at least one of the four pathogenic bacteria including *Listeria monocytogenes*, *E. coli*, *Mycobacterium* spp., *Pseudomonas aeruginosa* were detected in 48% of the ballast tanks, but toxigenic strains of *Vibrio cholera* were not detected in the ballast water samples [14].

The results showed that the ballast water discharged into the coastal areas of Bushehr port contained bacteria that have the potential to be pathogenic for humans and coastal ecosystems. Results of this study indicated that microbial parameters measured including total and fecal *coliform* bacteria were lower in the ballast water samples compared with the levels of total and fecal *coliforms* discharge into coastal waters of Bushehr port [17] as well as WHO guidelines [18,19]. But in the case of *Pseudomonas aeruginosa* bacteria, all of the ballast water samples contained higher levels of *Pseudomonas aeruginosa* compared with the levels of *Pseudomonas aeruginosa* bacteria of coastal waters of Bushehr port [17] and WHO guidelines [18,19]. Heterotrophic plate count (HPC) bacteria levels were also higher in all of the ballast water samples compared with levels of coastal waters of Bushehr port [17] as well as WHO guidelines [18,19].

In Table 3 some previous reports on microbial levels in different seas around the world are presented.

As shown in Table 2, the total and fecal *coliform* levels in waters of the Caspian Sea were 235 and 60 MPN/100 ml [20]. Also, the total and fecal *coliform* levels in waters of Black sea were 1151 and 168 CFU/100 ml [21]. The total and fecal *coliform* levels in coastal areas of Bushehr port were 540 and 165.6 MPN/100 ml [20].

4. Conclusions

The results showed that the total *coliform*, fecal *coliform*, *Pseudomonas aeruginosa* and *HPC* bacteria levels in ballast water samples ranged from 0–500, 0–17, 4–900 MPN/100 ml and 3100–8930 CFU/ml respectively. Also, four samples contained *E. coli*. EC and TDS ranged from 5690–21760 µS/cm and

Table 2
Microbial parameters of examined ballast water samples from different ports around the world (n = 34)

Location of harvesting ballast water	Stations	Total *coliform* (MPN/100 ml)	Fecal *coliform* (MPN/100 ml)	*E.coli*	*HPC* (CFU/ml)	*Pseudomonas aeruginosa* (MPN/100 ml)
Dammam – Saudi Arabia	S1	0	0	–	3350	17
Davao – Philippines	S2	7	0	–	3600	11
Phuket – Thailand	S3	17	0	–	4720	280
Duqm – Oman	S4	8	4	+	5900	350
Jawaharlal Nehru port – India	S5	130	0	–	5140	21
Kuwait – Kuwait	S6	9	2	+	3950	130
Jebel Ali – Emirate	S7	130	0	–	3380	17
Mumbai – India	S8	0	0	–	3850	17
Shuhaikh – Kuwait	S9	50	0	–	3245	26
Hamriyah – Emirate	S10	0	0	–	4430	30
Kandla port – India	S11	300	11	–	3950	40
Ajman port – Emirate	S12	0	0	–	7600	130
Mina Rashid – Emirate	S13	130	7	–	4770	50
Singapore – Singapore	S14	0	0	–	8890	900
Port said – Egypt	S15	0	0	–	6440	350
Antwerp – Belgium	S16	21	0	–	5990	280
Muscat – Oman	S17	0	0	–	4780	170
Portsmouth – UK	S18	300	4	–	6350	280
Basra – Iraq	S19	170	9	–	7100	500
Aden – Yemen	S20	80	4	–	5780	110
Suez – Egypt	S21	240	0	–	4370	50
Navlakhi – India	S22	21	0	–	3550	26
Bangkok – Thailand	S23	0	0	–	6530	170
Sohar – Oman	S24	500	17	–	3100	50
Shanghai – China	S25	26	0	–	8930	70
Salalah – Oman	S26	50	4	+	5600	60
LaemChabang – Thailand	S27	0	0	–	4140	500
Hong Kong – China	S28	50	0	–	5410	90
Busan – Korea	S29	17	4	–	4870	17
Shenzhen – China	S30	26	7	–	5990	17
Kaohsiung – Taiwan	S31	14	7	–	6170	110
Manila – Philippines	S32	17	4	+	8240	50
Jeddah – Saudi Arabia	S33	17	0	–	4710	60
Nagoya – Japan	S34	0	0	–	5120	170
Mean		68.52	2.47	–	5292.5	151.44
Std. deviation		111.83	3.95	–	1555.36	187.18
WHO guidelines for natural natatorium		500	100	100	–	0
Value of microbial level incoastal waters of Bushehr port		540[a]	165.56[a]		1580[a]	6[a]

[a]Noroozi, et al. 2015

Table 3
Microbial contents of seawater in some previous studies

Sampling points	Total coliforms	Fecal coliforms	References
Caspian Sea	235 (MPN/100 ml)	60 (MPN/100 ml)	[20]
Black Sea	1151 (CFU/100 ml)	168 (CFU/100 ml)	[21]
Caspian Sea	10–2700 (CFU/100 ml)	4–200 (CFU/100 ml)	[22]
Northern Lebanese coast	537 (CFU/100 ml)	237 (CFU/100 ml)	[23]
Persian Gulf- Bushehr port	540 (MPN/100 ml)	165.6 (MPN/100 ml)	[20]

3790–14510 mg/l respectively. The levels of alkalinity and total hardness ranged from 104 to191 and 1160 to 8940 mg/L $CaCO_3$ respectively. Also, the concentration levels of salinity and TOC in ballast water samples ranged from 33.2 to 44.98 g/L and 1.9 to 5.7 mg/L respectively. Ballast waters discharged to coastal areas of Bushehr port contained bacteria that have the potential to be toxic for humans and coastal ecosystems. Ballast water has the potential to change bacteria communities in marine environments and can be pathogenic for humans and coral reefs where ships discharge their ballast water. Therefore authorities need to develop more efficient procedures to evaluate ballast water reporting forms and ballast water quality, as there is potential risk of future invasions for Bushehr coastal ecosystems. Also, more research is needed to characterize the changes that nonindigenous microorganisms including potential pathogens, may cause in receiving waters.

Acknowledgements

The authors are grateful to the Bushehr University of Medical Sciences for their financial support (Grant no. 943-104) and the laboratory staff of the Environmental Health Engineering Department for their cooperation. The funder had no role in study design, data collection analysis, or preparation of the manuscript.

References

[1] International Maritime Organization (IMO), International shipping facts and figures – information resources on trade, safety, security, environment. (2012), London, U.K. <http://www.imo.org/>.
[2] J.T. Carlton, Trans-oceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water, Oceanogr. Mar. Biol. Annu. Rev., 23 (1985) 313–371.
[3] National Research Council of the National Academies, Assessing the relationship between propagule pressure and invasion risk in ballast water, The National Academies Press, Washington, DC, (2011) 144.
[4] IMO, Guidelines for the control and management of ships' ballast water to minimize the transfer of harmful aquatic organisms and pathogens, International Maritime Organization, Resolution A., 20 (1997) 868.
[5] J.T. Matheickal, S. Raaymakers, In: Proceedings of the 2nd International Ballast Water Treatment R&D Symposium, IMO London, 21–23 July 2003, Global Ballast Monograph Series No., 15 (2003), IMO, London.
[6] E. Joachimsthal, V. Ivanov, S.L. Tay, J.H. Tay, Bacteriological examination of ballast water in Singapore Harbour by flow cytometry with FISH, Mar Pollut Bull., 49 (2004) 334–343.
[7] IMO, International convention for the control and management of ships' ballast water and sediments, (2004).
[8] L. Buzoleva, A. Letyagina, A. Zvyagincev, I. Kashin, Study of microorganisms coming into the port of Vladivostok with ballast water of ships, Russ. J. Biol. Invasions., 3 (2012) 92–100.
[9] K.E. Wommack, R.R. Colwell, Virioplankton: viruses in aquatic ecosystems, Microbiol. Mol. Biol. Rev., 64 (2000) 69–114.
[10] I.T. Knight, C.S. Wells, B. Wiggins, H. Russell, Detection and Enumeration of Fecal Indicators and Pathogens in the Ballast Water of Transoceanic Cargo Vessels Entering the Great Lakes, in Proceedings of the General Meeting of the ASM, Chicago, Illinois. 1999, 546 p.
[11] F.C. Dobbs, A.A. Diallo, M.A. Doblin, L.A. Drake, Pathogens in Ships' Ballast Water and Sediment Residuals, in Proceedings of the Third International Conference on Marine Bioinvasions, March 16–19, La Jolla, California. 29 (2003).
[12] L.A. Drake, R.E. Baier, F.C. Dobbs, M.A. Doblin, Potential Invasion of Microorganisms and Pathogens Via "Interior Hull Fouling": Biofilms Inside Ballast Water Tanks, in Proceedings of the Third International Conference on Marine Bioinvasions, March 16–19, La Jolla, California. 35 (2003).
[13] V. Ivanov, Bacteriological monitoring of ships' ballast water in Singapore and its potential importance for the management of coastal ecosystems, WIT Trans. Biomed. Health., 10 (2006) 59–63.
[14] J.M. Burkholder, G.M. Hallegraeff, G. Melia, A. Cohen, Phytoplankton and bacterial assemblages in ballast water of U.S. military ships as a function of Port of Origin, voyage time, and ocean exchange practices, Harmful Algae., 4 (2007) 486–518.
[15] American Public Health Association Water Environment Federation, Standard methods for the examination of water and wastewater, (1995).
[16] N.N. Pereira, R.C. Botter, R.D. Folena, J.P.F.N. Pereira, A.C. da Cunha, Ballast water: A threat to the Amazon Basin, Mar. Pollut. Bull., 84 (2014) 330–338.
[17] K.V. Noroozi, S. Dobaradaran, S. Mirahmadi, H. Mokhtari, H. Darabi, F. Faraji, Survey of microbiological and chemical quality of the swimming beaches along the Persian Gulf in Bushehr port (2015).
[18] WHO Guidelines for Drinking Water Quality, 4th Ed: World Health Organization Geneva. (2011) ISBN: 978 92 4 154815 1.
[19] European Economic Community. Council directive of 8 december 1975 concerning the quality of bathing water, Official Journal of the European Communities, 1975 No. L31 of February 1976. 1 (76/160/EEC).
[20] R. Nabizadeh, M. Binesh Barahmand, K. Nadafi, A. Mesdaghinia, Qualitative analysis of coastal waters in the Caspian Sea in Guilan Province: Determining the environmental health indicators in swimming areas, J. Mazandaran Univ. Med. Sci., 22 (2012) 41–52.
[21] N. Janelidze, E. Jaiani, N. Lashkhi, A. Tskhvediani, T. Kokashvili, T. Gvarishvili, S. Narodny, Microbial water quality of the Georgian coastal zone of the Black Sea, Mar. Pollut. Bull., 62 (2011) 573–580.
[22] M. Zakaryaee, S. Sefatian, A.A. Saeedi, H. Nasrolahzadeh-Saravi, M. Adel, Microbiological quality of some swimming water in the Caspian Sea in Mazandaran province beaches, Iran, J. Mazandaran Univ. Med. Sci., (JMUMS), 23 (2014).
[23] R. El Fallah, Z. Olama, H. Holail, Marine quality assessment of Northern Lebanese coast: microbiological and chemical characteristics and their impact on the marine ecosystem, Int. J. Curr. Microbiol. App. Sci., 5 (2016) 376–389.