

Application of the TRIX for water quality assessment along the Montenegrin coast

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ABSTRACT

Determination of trophic state of the Adriatic Sea along the Montenegrin coast was performed through calculation of the trophic index (TRIX), based on a linear combination of the logarithms of four variables: chlorophyll a concentration, total nitrogen (TN), total phosphorus (TP) and the absolute percentage deviation from the oxygen saturation (aD%O).

Water samples were collected monthly from April 2010. to November 2010. at five stations in the Boka Kotorska Bay and at five stations at the open sea along the Montenegrin coast.

TRIX index is scaled from 0 to 10, covering a range of four water quality state (high, good, moderate and degraded). During investigated period average values of the TRIX index were 3.97 in the Boka Kotorska Bay and 3.81 at open sea along the Montenegrin coast, corresponding to the category of good trophic conditions. TRIX index ranging from 0.52 at station MNE-03 (Bar) to 6.39 at station OS-6 (near the mouth of the Bojana River), which indicates that the trophic state of investigated area ranged from the category of high quality and low productivity to category of poor and degraded conditions of sea water and increased productivity.

Introducing logarithmic transformation of the original values of parameters in order to form the trophic index (TRIX) proved to be a good method for evaluation of the trophic state of the Adriatic Sea along the Montenegrin coast. These results indicate that investigated area is in a good trophic state, where natural eutrophication still dominates over eutrophication of an anthropogenic origin.

Keywords : the Montenegrin coast, eutrophication, TRIX index, water quality, the Adriatic Sea

INTRODUCTION

Nutrient enrichment has been one of the leading threats to the health of coastal ecosystems and resources and eutrophication has become a major concern in many parts of

the European Seas (Nixon, 1995). Trophic conditions of European marine areas vary considerably from region to region and within regions (UNESCO, 1988; UNEP/FAO/WHO, 1996).

Ecological indicators are commonly used to provide synoptic information about the state of ecosystems (Marques *et al.*, 2009). The trophic index (TRIX) works like a multivariate tool, which offers next advantages of utilisation:

- (a) the outcome of the index is a single number,
- (b) it is a multimetric index encompassing four variables, which are strongly correlated with primary production: chlorophyll *a*, total nitrogen (TN), total phosphorus (TP) and oxygen saturation (aD%O),
- (c) the environmental variables involved can be measured directly on a routine basis.

The TRIX index was developed by Vollenweider *et al.* (1998) for the coastal area of Emilia-Romagna (northern Adriatic Sea) and was used by Italian authorities on a routine basis to monitor the trophic state of coastal waters of the Adriatic and Tyrrhenian Sea (Doncheva *et al.*, 2003; Giovanardi & Vollenweider, 2004; Bendoricchio & De Boni, 2005; Artioli *et al.*, 2005). The northwestern Adriatic Sea shows the highest TRIX values (> 6), corresponding to eutrophic conditions that could be related to possible anoxia/hypoxia events. The TRIX values progressively decrease along the western Adriatic coast, with values ranging between 5-6 on the Emilia-Romagna coast and values < 5 along the Marche coast. The southern section of the western Adriatic characterizes low TRIX values (< 4), to the contrary of the south-eastern shelves where eutrophic conditions are prevalent as found in previous studies (Marini *et al.*, 2010).

The TRIX index was originally developed for Italian coastal waters, but the need for using the TRIX in the Mediterranean was confirmed by UNEP (2007a, b). However, the applicability of the TRIX in other regions as well as the possibility of developing a general TRIX index for all European coastal and marine areas has to be evaluated. The TRIX index has been applied in the Black Sea (Moncheva *et al.*, 2001; Moncheva *et al.*, 2002; Doncheva *et al.*, 2003; Zaika, 2003; Baytut *et al.*, 2010; Dyatlov *et al.*, 2010; Medinets *et al.*, 2010; Kovalova & Medinets, 2012), the North Sea (EEA, 2001), the Baltic Sea (Vascetta *et al.*, 2004), the Caspian Sea (Nasrollahzadeh *et al.*, 2008), the Aegean Sea (Moncheva *et al.*, 2001; Balkis & Balci, 2010), the Ionian Sea (Nikolaidis *et al.*, 2008), the Marmara Sea (Balci *et al.*, 2012; Balkis *et al.*, 2012), the Mar Menor Lagoon (Spain) and the Mondego estuary (Salas *et al.*, 2008) and the Persian Gulf (Zoriasatein *et al.*, 2013).

The aim of this study is to apply the TRIX index to assess the trophic state of the Adriatic Sea along the Montenegrin coast.

The TRIX index provides uniform criteria for the classification of the Italian coastal waters (Pettine *et al.*, 2007). Investigating the applicability of the TRIX index along the Montenegrin coast was also intended to indicate whether TRIX can be used as an universal index of eutrophication or the scaling of the TRIX should be region specific.

MATERIALS AND METHODS

The sampling stations are situated in coastal waters of Montenegro, which is located in the south eastern part of the Adriatic Sea. Water samples were taken monthly from April 2010 to November 2010 at five stations in the Boka Kotorska Bay and at five stations at the open sea along the Montenegrin coast (Figure 1.). The geographic longitude, latitude and depth of investigated stations are presented in Table 1.

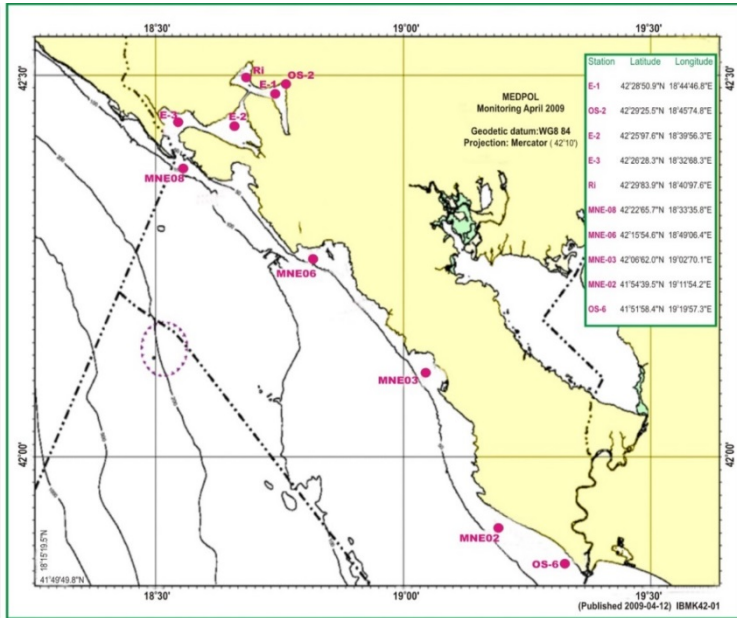


Figure 1. Study area with investigated stations

The trophic index (TRIX) used in the present work has been given by the following formulation (Vollenweider et al. 1998):

$$\text{TRIX} = [\log(\text{Chl}a \cdot (\text{D}\%)_{\text{O}_2} \cdot \text{TN} \cdot \text{TP})] - (-1.5)/1.2$$

Where:

- Chl-*a* - Chlorophyll-*a* concentration (mg/m³)
- (D%)_{O₂} - The % deviation of the oxygen concentration from saturation conditions (abs [100-%O])
- TN - Total nitrogen (μmol/l)
- TP - Total phosphorus (μmol/l)

TRIX index is scaled from 0 to 10 which corresponds to range of water quality (high, good, moderate and poor and degraded) and to the categories of water trophic state oligotrophic, mesotrophic, eutrophic and hypertrophic (Vollenweider *et al.*, 1998).

Table 1. Geographic position and depth and sampling depth of investigated stations

	Position	Depth (m)	Sampling depth (m)	Geographic latitude	Geographic longitude
Boka Bay	E-1 (Kotor)	34	0, 15, 30	42° 28' 50.9" N	18° 44' 46.8" E
	OS-2 (Orahovac)	22	0, 10, 20	42° 29' 25.5" N	18° 45' 74.8" E
	E-2 (Tivat)	39	0, 20, 36	42° 25' 97.6" N	18° 39' 53.6" E
	E-3 (Herceg- Novi)	43	0, 20, 40	42° 26' 28.3" N	18° 32' 68.3" E
	Ri (Risan)	28	0, 13, 25	42° 29' 83.9" N	18° 40' 97.6" E
Open sea	MNE-08 (Mamula)	86	0, 40, 80	42° 22' 65.7" N	18° 33' 35.8" E
	MNE-06 (Budva)	47	0, 20, 45	42° 15' 54.6" N	18° 49' 06.4"E
	MNE-03 (Bar)	42	0, 20, 40	42° 06' 62.0" N	19° 02' 70.1" E
	MNE-02 (Ulcinj)	30	0 14, 27	41° 54' 39.5" N	19° 11' 54.2" E
	OS-6 (Mouth of Bojana)	12	0, 5, 10	41° 51' 58.4" N	19° 19' 57.3" E

Table 2. The TRIX index scale (Pettine *et al.*, 2007, Vollenweider *et al.*, 1998)

TRIX value	State water quality	Level of eutrophication	Trophic state
0<TRIX<4	high	low	oligotrophic
4<TRIX<5	good	medium	mesotrophic
5<TRIX<6	moderate	high	eutrophic
6<TRIX<10	poor and degraded	elevated	hipertrophic

Chlorophyll *a* concentration was calculated according to APHA (1995). Oxygen concentrations were measured *in situ* by the use of an oxygen meter. Nutrients were estimated by standard spectrophotometric methods (Parsons *et al.*, 1985). Transparency, additional factor in determining the quality of sea water was measured with a Secchi disk.

Statistics 7 was used for statistical analyses of data. Biplot for the studied elements was obtained by using S-Plus statistical programme.

RESULTS

Source data for the TRIX index assessment along the Montenegrin coast were the results of regular observations of TN, TP, chlorophyll *a* and saturation of O₂ (%), which are presented on Figure 2. a-d, respectively.

Concentration of total nitrogen varied widely, from 1.58 µmol/l at station MNE-06 (Budva) to 39.69 µmol/l at station MNE-02 (Ulcinj) (Figure 2. a), while the concentration of total phosphorus ranged from the value below detection to 2.35 µmol/l at station OS-2 (Orahovac) (Figure 2. b).

The phytoplankton biomass, expressed as chlorophyll *a* (chl *a*) at the investigated stations ranged from 0.07 to 2.09 mg/m³. The maximum value of chlorophyll *a* was recorded in inner part of the Boka Kotorska Bay, at station E-1 (Kotor), while on the open sea along the Montenegrin coast the highest value of 1.39 mg/m³ was noted at station OS-6 (Mouth of the Bojana River) (Figure 2. c)

Oxygen saturation generally ranging over 80-131 %, with the exception at stations MNE-06 (Budva) and OS-6 (Mouth of the Bojana River) where were noted minimum value of 65% and 73.29 %, respectively. The maximum value of oxygen saturation was recorded in inner part of the Boka Kotorska Bay, at stations OS-2 (Orahovac) and Ri (Risan) (Figure 2. d).

Transparency ranged between 4m and 25 m. The minimum value of transparency was noted at stations E-1 (Kotor), OS-2 (Orahovac) and E-3 (Herceg Novi), while the maximum value was recorded at station MNE-06 (Budva) (Figure 3).

The TRIX values along the Montenegrin coastal waters varied in wide range, from 0.52 at station MNE-03 (Bar) to 6.39 at the station OS-6 (Mouth of the Bojana River). The highest value of the TRIX index in the Boka Kotorska Bay was 5.79, at station OS-2 (Orahovac) (Figure 4).

The median values of the TRIX index at stations in the Boka Kotorska Bay including stations MNE-06 (Budva), MNE-02 (Ulcinj) and OS-6 (Mouth of the Bojana River) were between 4-5, while at stations MNE-08 (Mamula) and MNE-03 (Bar) were between 3-4 (Figure 4).

Average values of the TRIX index for investigated period were 3.97 in the Boka Kotorska Bay and 3.81 at open sea along the Montenegrin coast (Table 3). Monthly average values of the TRIX index in the Boka Kotorska Bay ranged from 3.34 at October to 4.58 at June, and at open sea along the Montenegrin coast from 2.68 at October to 4.77 at June (Table 3).

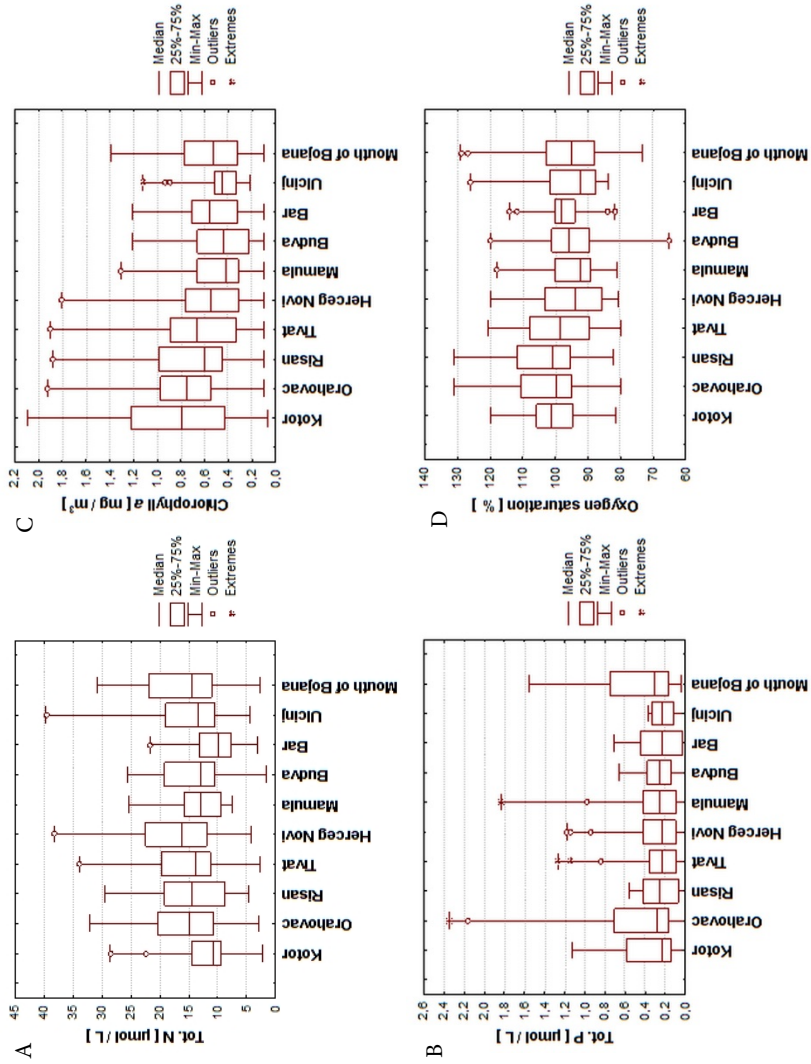


Figure 2. A) Box-plot diagram - Total nitrogen ($\mu\text{mol/l}$); B) Box-plot diagram - Total phosphorus ($\mu\text{mol/l}$); C) Box-plot diagram - Chlorophyll *a* (mg/m^3); D) Box-plot diagram - Saturation of oxygen (%)

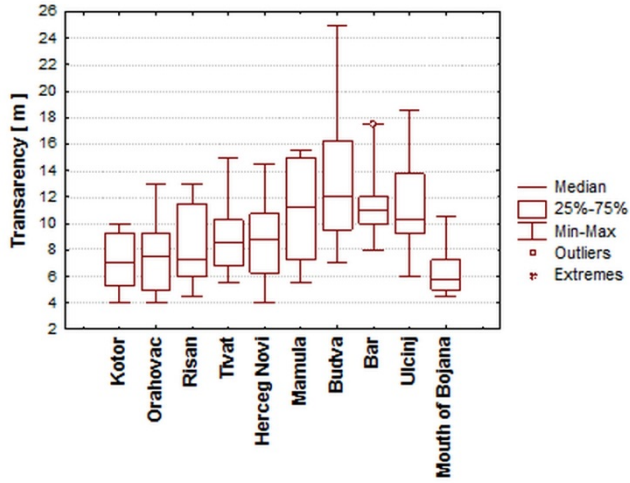


Figure 3. Box-plot diagram - Transparency (m)

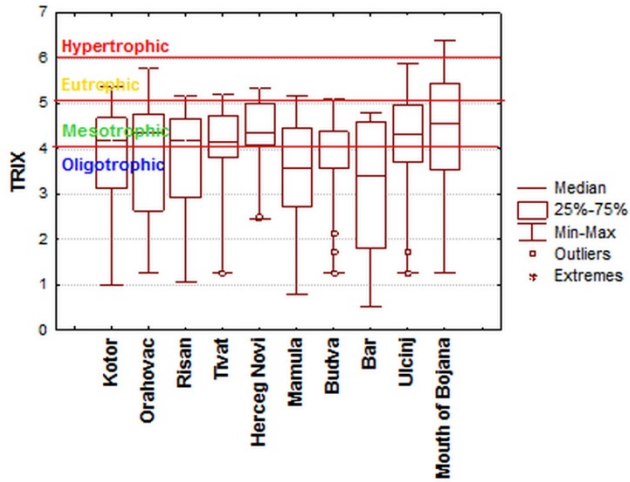


Figure 4. Box-plot diagram - TRIX index (classification according Pettine *et al.*, 2007)

Table3. Number of samples (n), average values (AVG), minimum (Min), maximum (Max), standard deviation (St.dev.) and coefficient of variance (Cv) for the TRIX index

Months	n		AVG		Min		Max		St.dev.		Cv (%)	
	BOKA BAY	OPEN SEA	BOKA BAY	OPEN SEA	BOKA BAY	OPEN SEA	BOKA BAY	OPEN SEA	BOKA BAY	OPEN SEA	BOKA BAY	OPEN SEA
APRIL	15	15	4.30	4.06	2.46	0.78	5.37	5.46	0.69	1.31	0.48	1.70
MAY	15	15	3.47	3.55	1.07	0.52	5.17	5.43	1.21	1.27	1.46	1.62
JUNE	15	15	4.58	4.77	3.53	3.40	5.15	5.91	0.48	0.65	0.23	0.42
JULY	15	15	3.92	3.03	1.72	1.72	5.79	4.30	1.32	0.92	1.75	0.85
AUGUST	15	15	4.25	3.76	2.52	1.72	5.48	5.19	1.05	1.20	1.09	1.44
SEPTEMBER	15	15	3.95	3.97	1.25	1.25	5.39	5.70	1.58	1.31	2.49	1.72
OCTOBER	15	15	3.34	2.68	1.83	1.25	4.86	3.94	0.95	1.03	0.90	1.06
NOVEMBER	15	15	3.95	4.64	1.00	3.66	5.14	6.39	1.06	0.82	1.12	0.68
AVERAGE TRIX VALUE			3.97	3.81								

Principal component analysis (PCA) applied to identify the variables responsible for the trophic state of the system. PCA showed the greatest interaction between the TRIX index and total phosphorus as well as chlorophyll *a* and oxygen. (Figure 5).

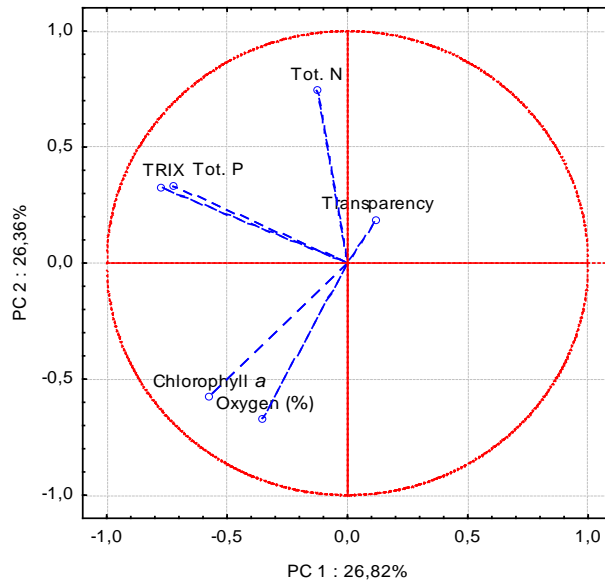


Figure 5. PCA analysis

DISCUSSION

During this investigation median values of TP (total phosphorus) is rather similar at all stations, but TN (total nitrogen) median values differs. According to the criteria of UNEP (1994) and the median values of total nitrogen the investigated area can be described as eutrophic, while according to the median values of total phosphorus the investigated area can be described as oligotrophic. The large excess of N over P is related to phytoplankton demand which can indicate that phosphorus is the limiting factor for primary production at investigated area, which is consistent with findings in the middle Adriatic Sea (Vukadin & Stojanoski (2001), Burić *et al.* (2007). The largest range of concentration of total phosphorus at stations OS-2 (Orahovac) and OS-6 (Mouth of the Bojana River) can indicate an increased nutrient influx in the seawater by significant fresh water from rivers Ljuta and Bojana.

The large range of nutrient concentration at the Boka Kotorska Bay, compared to narrow one at stations at open sea along the Montenegrin coast seem to be related with significant nutrient input by waste water during the tourist season and by fresh water from

numerous water streams and submarine springs along the entire sea coast, particularly in semi-enclosed Kotor and Risan Bays. Nutrient distribution reflects similarly on chlorophyll *a* concentration in the Boka Kotorska Bay than at the open sea along the Montenegrin coast. This indicates a significant relationship between the concentration of nutrients and phytoplankton biomass (chl *a*), which is consistent with the traditional model of phytoplankton being a direct result of increased nutrient loads along coast (Cloern, 2001).

According to the median values of chlorophyll *a* concentration and the criteria of Simboura *et al.* (2005) stations at open sea including station E-3 (Herceg Novi) can be described as low mesotrophic while positions in the Boka Kotorska Bay can be classified as high mesotrophic.

Results indicate that water column along the Montenegrin coast is well-oxygenated, which is consistent with findings of oxygen saturation along Croatian coast (Vidjak *et al.*, 2012). Also, Regner *et al.* (2003) noted that saturation of oxygen, one of the key indicators of the eutrophication level, were generally above 100 % along the Montenegrin coast, which is characterized as for extremely eutrophic water. According to the median values of oxygen saturation and the criteria of UNEP (1994) stations at open sea including station E-3 (Herceg Novi) and E-2 (Tivat) can be described as mesoeutrophic, while stations in inner part of the Boka Kotorska Bay can be classified as eutrophic.

According to the median values of transparency and the criteria of UNEP (1994) stations at the Boka Kotorska Bay including station OS-6 (Mouth of the Bojana River) can be described as mesoeutrophic, while stations at open sea can be classified as oligotrophic. The lowest transparency noticed at station E-1 (Kotor) seem to be correlated to the high concentration of chlorophyll *a*, while at station OS-6 (Mouth of the Bojana River) is probably consequence of a significant fresh water influx from Bojana River.

During this investigation TRIX values varied widely along the Montenegrin coast, corresponding to the trophic states fluctuations from the category of high quality of water and low productivity to category of poor and degraded conditions of water and increased productivity. Values of the TRIX exceeding 6 units are typical for highly productive coastal waters, where the effects of eutrophication determine frequent episodes of anoxia in bottom waters. On the contrary, values lower than 4 TRIX units are associated to coastal waters of low production, while values under 3 TRIX units usually represent the open sea (Vollenweider *et al.*, 1998).

The maximum value of the TRIX index noted along the Montenegrin coast was lower than the ones noted in the Black Sea (Baytut *et al.*, 2010; Doncheva *et al.*, 2003; Dyatlov *et al.*, 2010; Kovalova & Medinets, 2012; Moncheva *et al.*, 2002) and higher in comparison to results in the Aegean Sea (Moncheva *et al.*, 2001; Balkis & Balci, 2010), the Ionian Sea (Nikolaidis *et al.*, 2008), the Marmara Sea (Balci *et al.*, 2012; Balkis *et al.*, 2012) and the Adriatic Sea (Vollenweider *et al.*, 1998; Doncheva *et al.*, 2003).

According to the classification of Vollenweider *et al.* (1998) and the average values of the TRIX for investigated period, the Boka Kotorska Bay and open sea along the

Montenegrin coast correspond to the border between the category of oligotrophic and mesotrophic water. The average values of the TRIX were similar as in the northern Adriatic Sea (Pečar & Precali, 2004) and in the Gulf of Trieste (Turk et al., 2007), but lower than the average TRIX at coastal water along Italy (ICRAM, 2000) and in the Varna Bay (Black sea) (Doncheva et al., 2003).

Increased values of the TRIX during the spring and autumn seem to be related with intensity of river discharge and precipitation, while in august with organic matter accumulation from waste water during the tourist season. This is consistent with findings in the Black Sea, where maximal values of the TRIX were most often during spring and autumn (Kovalova & Medinets, 2012).

According to the median values of TRIX index and the classification criteria (Pettine et al., 2007) stations in the Boka Kotorska Bay including stations on open sea along the Montenegrin coast (MNE-06, MNE-02 and OS-6) correspond to the category of good trophic conditions where eutrophication level is medium, while at stations MNE-08 and MNE-03 the median values correspond to high water quality with low level of eutrophication. A gradual trend from west stations at open sea with oligotrophy characteristic to east stations, near Mouth of Bojana with eutrophic characteristic, was observed

TRIX values are very sensitive and any slight change of oxygen, chlorophyll *a*, total nitrogen and total phosphorus concentrations results in changes of index values (Boikova et al., 2008). According to the results of PCA during this investigation period with the greatest influence on the trophic index (TRIX) was total phosphorus (TP). This result is in line with the finding that total phosphorous is the nutrient more associated with eutrophication of the estuary, rather than total nitrogen (Alves et al., 2013). Furthermore, our investigation indicated chlorophyll *a* and oxygen to influence TRIX in our region. According to some previous investigation TRIX was positively correlated to total nitrogen, total phosphorus and chlorophyll *a* in the Edremit Bay (Aegean Sea) (Balkis & Balci, 2010), in the Mar Menor lagoon (Spain) (Salas et al., 2008) and in Gulf of Bandirma (Marmara Sea) (Balkis *et al.*, 2012). The strongest correlation has been observed between TRIX and chlorophyll *a* in the Zmiinyi Island Area (Black Sea) (Kovalova & Medinets, 2012).

CONCLUSION

This study showed that investigated stations in the region differ in water quality and productivity. TRIX indicates the general trophic state and introducing logarithmic transformation of the original values of parameters in order to form trophic index (TRIX) proved to be a good method for evaluation of the trophic state of the Adriatic Sea along the Montenegrin coast. Based on the values of the nutrients, chlorophyll *a*, oxygen saturation, transparency and TRIX index which integrate the previous environmental variables, investigated stations are separated into the three groups: The Boka Kotorska Bay, open sea along the Montenegrin coast and the station OS-6 (Mouth of the Bojana River). At the Boka

Kotorska Bay and station OS-6 (Mouth of the Bojana River), higher concentration of nutrient, chlorophyll a, oxygen saturation and TRIX index and lower transparency, than at the open sea along the Montenegrin coast, were noted.

According to the TRIX index, investigated area generally could be classified as good trophic state, with medium water quality. These results indicate that natural eutrophication is still dominated over eutrophication of an anthropogenic origin in the Adriatic Sea along Montenegrin coast.

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