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Trace metals assessment in seawater, sediment and seagrasses Posidonia oceanica (L.) Delile and Cymodocea nodosa (Ucria) Asch. from the Montenegrin coast

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ABSTRACT

This paper reports on the content of heavy metals Cu, Zn, Mn, Pb, Cd and Hg in the seawater, sediment and sea grasses *Posidonia oceanica* and *Cymodocea nodosa* sampled from five locations along the Montenegrin coast in summer 2016. The pollution status was evaluated by using indexes such as CF, BCF, BSAF, MPI and PLI. Bigger values of PLI and MPI indexes were determined for sediment and sea grasses sampled on the locations within Boka Kotorska Bay comparing to the locations which are under influence of the open sea. Location Sv. Stasije was the most polluted while Žanjice was the least polluted location. Correlation analysis of the content of heavy metals in seawater, sediment and both sea grasses showed that Cu, Zn, Mn, Cd and Hg in *Posidonia oceanica* originate from the sediment, while Pb originates from the water column. Mn and Cd in *Cymodocea nodosa* originate from sediment, while Cu, Zn and Hg come from natural or anthropogenic sources. Therefore, *Posidonia oceanica* can be used as a bioindicator for Cu, Zn, Cd, Mn, and Hg content in sediment and Pb in seawater, while *Cymodocea nodosa* can be used as a bioindicator of Mn and Cd content in the sediment.

Keywords: *Posidonia oceanica*, *Cymodoceea nodosa*, heavy metals, seawater, sediment

INTRODUCTION

Environmental pollution is a growing problem at the global level that is directly caused by anthropogenic factors. Among the many types of pollution, marine pollution stands out, which is especially recognized issue within EU Marine Strategy Framework

Directive (MSFD 2008/56/EC). The Mediterranean Sea is surrounded by three continents and is subject to pronounced anthropogenic influences due to limited water exchange (Durrieu de Madron *et al.*, 2011). The northernmost branch of the Mediterranean Sea is the Adriatic Sea, which partly belongs to Montenegro, as its internal and territorial sea (Radojičić, 2008).

The Montenegrin marine ecosystem is threatened by various negative impacts which contribute to the pollution of the area, especially heavy metal contamination. Negative impacts that effect the sea pollution are increasing intensive urbanization of the coastal area, large influx of sewage and industrial waste waters and agricultural activities (Joksimovic *et al.*, 2018).

Due to its toxicity, persistence, poor biodegradability and tendency to concentrate in aquatic orgranisms, trace metals are considered as pollutants of the marine environment (Lafabrie *et al.*, 2007; Conti *et al*., 2010). The increased values of heavy metal concentration can cause serious issues in marine ecosystems, resulting in decrease of species diversity and increase in levels of toxic elements entering the food chain (He *et al.*, 1998). The bioaccumulation and toxic properties of trace metals largely depend both on their own characteristics and environmental conditions, which control the bioavailability of these metals (Moiseenko & Gashkina, 2020).

In order to precisely assess the level of heavy metal pollution of marine ecosystems, it is needed to conduct the analysis of the heavy metal content not only in living organisms, but also of the abiotic components. The use of biological species in the monitoring of marine environment quality permits the evaluation of the biologically available levels of pollutants in the ecosystem on the effects on pollutants on living organisms. The analysis of environmental matrices such as water or sediment provides a picture of the total pollution load rather than of that fraction of direct ecotoxicological relevance (i. e. the bioavailable forms) (Akcali & Kucuksezgin, 2011). Biomonitoring cannot replace chemical monitoring but it integrates them and provides unique contribution in determination of pollutants and its toxicity. This lead to an increase in the number of studies related to the correlation analysis of the concentrations of seawater, sediment and one or more sea grasses (Khaled *et al.*, 2014; Bonanno *et al.*, 2017; Bonanno & Borg, 2018; Malea *et al.*, 2019).

P. oceanica, endemic species of the Mediterranean, plays an important role in the ecology of the Mediterranean and mostly occur in shallow and sheltered coastal waters anchored in sand or mud bottoms. It meadows serve a spawning area, habitat or hunting territory for many plant and animal species. It may absorb trace elements directly from the water column and/or from interstitial water in sediments (Lafabrie *et al.*, 2007) and has a high capacity to accumulate trace metals and concentrate pollutants occurring in the environment (Di Leo *et al.*, 2013). Therefore, seagrass *Posidonica oceanica* (L.) has been used as an indicator of the degree of marine ecosystem pollution in the Mediterranean for several decades (Di Leo *et al.*, 2013; El Zrelli *et al.*, 2017; Bertini *et al.*, 2019). In recent years, given that it is the most widespread sea grass in Montenegro, *P. oceanica* has been the subject of research by many Montenegrin and other scientists from the region (Joksimović & Stanković, 2012; Jović, 2013; Stanković *et al.*, 2014; Stanković *et al.*, 2015).

Although the sea grass *Cymodocea nodosa* (Ucria) Asch. meets the conditions for usage for the monitoring of the marine ecosystem (Bonnano & Di Martino, 2016) such as: wide distribution, sensitivity to natural and anthropogenic influences, ease sampling and identification, it is less used as an indicator of heavy metal pollution compared to *P. oceanica* (Papathanasiou *et al.*, 2016, Bonnano & Borg, 2018). This sea grass was the topic of only few researches regarding the heavy metal concentrations and pollution of Montenegrin marine ecosystem (Mačić, 2001; Mačić & Sekulić, 2001).

The main aim of this study was to determine the concentration of Cu, Zn, Mn, Pb, Cd and Hg in seawater, sediments, and sea grasses *P. oceanica* and *C. nodosa* sampled from five locations along Montenegrin coast and assess the pollution level using different indexes. The obtained results were compared with the findings of the similar researches done both within Adriatic and Mediterranean region.

MATERIAL AND METHODS

Sampling and sample preparation

The samples of seawater, sediment and sea grasses *P. oceanica* and *C. nodosa* were collected from five locations along Montenegrin coast in summer 2016. Samples of seawater were collected above the sea grasses meadows. The sediment was hand sampled by diving. Samples of whole sea grasses (leaves, roots and rhizomes) were collected by diving.

The geographical positions of material sampling are presented in Fig. 1.

Samples of seawater were stored in dark bottles of 1l volume which were marked and conserved with 2 ml HNO_3 *(>68%, PrimarPlus-Trace analysis grade, Fisher Chemical*).

The upper 10 cm of the surface sediment samples were placed in polypropylene bags and stored in the freezer before transferring to the laboratory. The homogenization of the sample was done by conning and quartering, after which the samples were frozen and freeze-dried at -40 °C during 48 h in a freeze-dryer *(CHRIST, Alpha 2-4 LD plus, Germany*). The samples were sieved and the fraction less than 63 µm was used for the analysis of heavy metals. After sieving, the amount of sediment samples collected at Žukovica and Buljarica was not sufficient to perform analyzes, so they were excluded from the process of further processing. Analysis of sediment samples was performed on material collected at the following locations: Žanjice, Sv. Marko and Sv. Stasije.

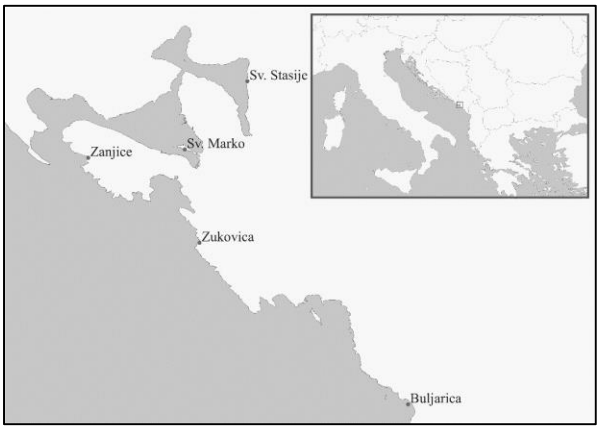

Figure 1. Geographical positions of the sampled materials

After collecting, the sea grasses samples were washed with Milli-Q water, drained and stored in the polypropylene bags and stored in the freezer. The analysis were performed on a homogenized sample obtained from the whole sea grass plant (leaves, roots and rhizomes). The samples were then freeze-dried at at -40 °C during 48 h in a freeze-dryer (*CHRIST*, *Alpha 2-4 LD plus*, *Germany*). The obtained dry samples were weighed and then kept in the desiccator until the analysis

Chemical analysis

The Cu, Zn, Mn, Pb and Cd concentrations in seawater were analyzed by using an inductively coupled plasma–optical emission spectrophotometry, ICP-OES, Spectro Arcos.

Hg levels were determined by using direct mercury analyzer (DMA-80, Milestone). The obtained results of the investigated elements in seawater are expressed in µg/l.

Before the performed analysis, the prepared sediment samples (0.2 g) were digested in a closed vessel microwave digestion system using mineral acids and oxidants, 5 ml of HNO_3 *(>68%, PrimarPlus-Trace analysis grade, Fisher Chemical)*, 2 ml of HF (47-51%, *Superpure for trace analysis, Carlo Erba*) and 2 ml of H_2O_2 (*>30%, Analytical reagent grade, Fisher Chemical*). The microwave digestion was performed in two steps, first with these reagents and the second after adding 10 ml 4% H_3BO_3 (*99,97% Trace metal basis, Sigma Aldrich*). After the digestion, another 10 ml of 4% H_3BO_3 was added to the samples. The digested samples were diluted using Milli-Q water and for each batch of analysis two blank digests were prepared in the same way. The mineralized samples were analyzed for Cu, Zn, Mn, Pb, and Hg content by using flame and hydride vapor generation atomic absorption spectrometer (F-AAS, HVG-AAS, Shimadzu A7000).

The prepared sea grasses samples, 0.5 g of *Posidonia oceanica* sample and 0.4 g of *Cymodocea nodosa* sample, were digested in a closed vessel microwave digestion system using 5 ml of HNO_3 *(>68%, PrimarPlus-Trace analysis grade, Fisher Chemical)* and 2 ml of H_2O_2 (*>30%, Analytical reagent grade, Fisher Chemical*) . The digested samples were diluted using Milli-Q water and for each batch of analysis two blank digests were prepared in the same way. The mineralized samples were analyzed for Cu, Zn, Mn, Cd and Hg content by using flame and hydride vapor generation atomic absorption spectrometer (F-AAS, HVG-AAS, Shimadzu A7000). The analyses of Pb in sea grasses and Cd in sediment were performed by a graphite furnace atomic absorption spectrophotometry (Agilent Technologies 240Z AA, GTA 120). The obtained results of the investigated elements in sediments and sea grass are expressed in mg/kg of sample dry weight (dw). The accuracy of the analytical procedure was checked using the certified reference material, IAEA 158 (Marine sediment) and IAEA 140 (Sea grass), which were also digested and analyzed together with the samples.

Statistical analysis

The level of contamination of sediment with the heavy metals was calculated by using concentration factor (CF) (equation 1) proposed by Tomlinson *et al.,* (1980):

$$CF= C_{metal} / C_{Background\ level} \qquad (1)$$

The following scale (Håkanson, 1980) is used to describe the metal contamination level:

CF< 1 –low metal contamination level
1 ≤CF≤ 3- moderate metal contamination level
3 ≤ CF≤ 6 - moderately high metal contamination level
CF≥ 6 - high metal contamination level

Pollution load index (PLI) is used to calculate the magnitude of the contamination of sediment with heavy metals (Tomlinson *et al.*, 1980). This parameter (equation 2) can be expressed as:

$$PLI = (CF1 \times CF2 \times \ldots\ldots \times CFn)^{1/n} \qquad (2)$$

where CF is the concentration factor, while n is the number of metals. PLI value higher than 1 indicates at the existence of pollution, while the value lower than 1 indicated that there is no pollution load.

The efficiency of metal bioaccumulation of *P. oceanica* and *C. nodosa* were evaluated by calculating the bioconcentration factor (BCF) and biota-sediment factor (BSAF) which are defined as ratio between metal concentration in the organism and in seawater (Geyer *et al.*, 2000) and sediment (Lafabrie *et al*., 2007), respectively. According to Chiou (2002), the BCF values above 1000 are considered as high bioconcentration level. The BCF values between 250 and 1000 are considered as moderate bioconcentration level, while BCF values lower than 250 present low bioconcentration level. Higher BSAF levels present the higher bioaccumulation capability of the investigated organism (sea grass).

The overall metal contents at all locations were evaluated using the metal pollution index (MPI). It was calculated by using the following formula (equation 3):

$$MPI = (Cf_1 \ x \ Cf_2 \ x \ Cf_3 \ x \ ...x \ Cf_n)^{1/n} \quad (3)$$

where Cf_1 is the first metal concentration value, Cf_2 is the second metal concentration value, Cf_3 is the third metal concentration value, etc. while Cf_n is the value of the concentration of *n* metal, while *n* is the number of metal (Usero *et al*, 2005). MPI values lower than 1 indicated that ecosystem is not polluted, while MPI values higher than 1 indicate the polluted ecosystem.

The analysis of the obtained results was performed by using Microsoft Excel 2013. Correlation analysis of the heavy metal concentrations in seawater, sediment and both sea grasses was done using Pearson correlation coefficient (r) in the programme TIBCO Statistica 13.5 (TIBCO Software Inc. 2018).

RESULTS AND DISCUSSION

The concentrations of investigated heavy metals (Cu, Zn, Mn, Pb, Cd, Hg) in seawater samples are summarized in Tab. 2. Concentrations of Cd and Hg were under the detection limit.

Comparing the obtained results of the research with the recommended criteria for sea water quality established by the US Environmental Protection Agency (US EPA, 2009), it was noticed that the concentration of Pb in seawater was above the MAC (5.6 µg/l) in all investigated locations. localities. Cu concentration from Sv. Marko was below the MAC (3.1µg/l), while the concentrations of this metal from Sv. Stasije, Buljarica, Žukovica and Žanjica were above MAC. Zn concentrations at all locations were below the MAC (81µg/l).

Concentrations of Cu, Zn, Mn and Pb in the seawater samples are significantly higher compared to the data obtained for the same metals in the previous researches of the heavy metal content in seawater of Montenegrin coast (Mačić, 2001; Mačić & Sekulić, 2001; Mihajlović *et al*., 2002; Joksimović *et al*., 2011; Joksimović & Stanković, 2012). The concentrations of Zn and Pb in this study are lower than the concentrations of these metals in the seawater in Croatia (Komar *et al*., 2017).

The high content of heavy metals in the seawater samples can be explained by the increased impact of various anthropogenic activities that take place along the Montenegrin coast during the summer months, when sampling was performed, such as discharge of untreated municipal wastewater from households and touristic facilities into the sea and intensive traffic along the coast during the touristic season (Joksimović *et al*., 2016).

Minimal concentrations of Cu, Zn, Mn and Hg in sediment were recorded for Žanjice, while the minimal concentrations of Pb and Cd were recorded for Sv. Marko. The highest concentrations of Cu, Zn, Mn, Pb and Hg in sediment were recorded at Sv. Stasije, while the highest Cd concentration was recorded for Žanjice (Tab. 3). The maximum values of the heavy metals in the sediment decrease as follows: Mn> Zn> Cu> Pb> Cd> Hg, which is

Table 2. The concentrations of Cu, Zn, Mn, Pb, Cd, Hg in seawater (µg/l)

SEA WATER	Metal concentration (*µg/l*)					
	Cu	Zn	Mn	Pb	Cd	Hg
Žanjice	122	13.3	49.7	25.3	1.36	ND
Sv. Stasije	20.6	24.2	99.7	58.5	ND	ND
Sv. Marko	1.48	1.52	1.21	ND	ND	ND
Žukovica	353	23.8	131	45.2	ND	ND
Buljarica	203	18.0	29.8	22.3	ND	ND
X± S.D	140.02± 144.1	16.6±9.34	62.3±5 2.6	37.8±17. 1	N/A	N/A
CV (%)	103	58	84	45	N/A	N/A

ND- (not detected)
N/A- not applicable

Table 3. Heavy metal concentrations in sediment (mg/kg)

SEDIMENT	Metal concentration (*mg/kg*)					
	Cu	Zn	Mn	Pb	Cd	Hg
Žanjice	11.2	15.2	216	6.3	0.589	0.032
Sv. Stasije	27.1	88.5	373	25.3	0.269	0.163
Sv. Marko	14.7	49.4	353	4.3	0.033	0.113
X ± S.D	17.6±8.57	51±36.7	314±85.5	11.97±11.97	0.297±0.279	0.103±0.066
CV (%)	41	72	27	97	94	64

in accordance with the results of previous research for the Montenegrin coast (Mihajlović & Joksimović, 2002; Joksimović *et al*., 2013; Stanković *et al*., 2014; Stanković *et al*., 2015; Joksimović *et al*., 2019). The same decreasing order was recorded by Rivaro *et al.* (2011) for the coast of Albania and Bonanno and Borg (2018) for the Tyrrhenian Sea.

The concentrations of the investigated heavy metals in the sediment at the location in Boka Kotorska Bay (Sv. Stasija and Sv. Marko) are higher in relation to the concentrations of the metals in the area under the influence of the open sea (Žanjice), which is in accordance with Stanković (2012), Joksimović *et al.*, (2013), Joksimović *et al.*, (2019). The Boka Kotoraska bay is a specific closed system with increased urbanization and various anthropogenic activities where there is almost no change in sea currents and surface movement and distribution of sediment in relation to locations which are under the influence of open sea (Bellafiore, 2011; Perošević-Bajčeta, 2020), so this can be one of the causes of this phenomenon. Also, the deposition of heavy metals in the sediment depends on the composition of the sediment, where the content of heavy metals increases in the fine-grained sediment (Tavakoly Sany *et al.*, 2011; Stanković *et al*., 2015).

The concentration of Cd in sediment for locations Žanjice and Sv. Stasije were above BAC-background values (0.15 mg/kg) according to UNEP Programme of the Mediterranean Sea and Coast and Related Assessment Criteria- IMAP criteria (UNEP/MAP, 2016). The obtained Pb values is sediment were below BAC value for Pb (30 mg/kg), while the Hg concentrations in sediment for Sv. Stasije and Sv. Marko were above the BAC value for Hg (0.045 mg/kg).

According to Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME, 2001) the obtained values of the content of most of the tested heavy metals in the sediment were below the values of ISQG (Interim freshwater Sediment Quality Guidelines) (ISQG Cu =18.7 mg/kg; ISQG Zn=124 mg/kg; ISQG Pb=30.2 mg/kg; ISQG Cd=0.7 mg/kg; ISQG Hg=0.13 mg/kg) which indicates a rare occurrence of negative biological effects on the living organisms. The obtained value of Cu concentration in the sediment sampled from the locality of Sv. Stasije was in the range between the proposed values of ISQG and PEL (Probable Effect Level) (18.7-108 mg/g) indicating the occasional negative biological effects that this heavy metal may have on the organizms bound to the sediment of this area.

The metal concentrations found in sea grasses *P. oceanica* and *C. nodosa* from different locations are presented in Tab. 4. The maximum concentrations of heavy metals in *P.oceanica* are represented in decreasing order as follows: Mn>Zn>Cu>Pb>Cd>Hg. The same declining order of investigated metals was determined by Radonjić (2014) for Montenegrin coast, Bonanno *et al*., (2017), Bonanno & Borg (2018) for the Ionian Sea, Bonanno & Racuia (2018) for Tyrrhenian Sea and Malea *et al*., (2019) for the Aegean Sea. Joksimović *et al.*, (2011) and Stanković *et al*., (2014) found a similar declining order of heavy metals in this sea grass (Mn> Zn> Pb> Cu> Cd> Hg) for the Montenegrin coast, while for the area of the northwestern Mediterranean (Tovar-Sanchez *et al*., 2010; Luy *et al.,* 2012; Lenzi *et al.*, 2013; Di Leo *et al*., 2013), the same decreasing heavy metal order ((Zn>Cu>Pb>Cd> Hg) was found, with the exception of Mn which was not inlcuded in the analysis within these studies.

Table 4. Heavy metal concentration in *P. oceanica* and *C. nodosa* (mg/kg)

P. oceanica	Metal concentration (*mg/kg*)					
	Cu	Zn	Mn	Pb	Cd	Hg
Žanjice	13.5	42.9	123	1.90	1.46	0.034
Sv. Stasije	26.4	111	301	18.6	0.970	0.206
Sv. Marko	17.3	69.4	278	20.9	0.864	0.094
Žukovica	12.6	35.1	185	0.523	1.86	0.021
Buljarica	19.4	65.4	311	0.814	2.97	0.192
X ± S.D	17.8 ±5.53	64.8 ±29.7	239 ±82	8.55 ±10.3	1.62 ±0.9	0.109 ±0.1
CV (%)	31	46	34	120	52	79

C.nodosa						
Žanjice	5.47	25.6	60.2	N/A	0.585	0.011
Sv. Stasije	7.20	26.5	157	N/A	0.459	0.004
Sv. Marko	9.62	23.9	101	N/A	0.391	0.014
Žukovica	6.48	19.4	43.0	N/A	0.555	0.009
X ± SD	7.19±1.77	23.85±3.16	90.3±50.7	N/A	0.498±0.089	0.010±0.004
CV (%)	25	13	56	N/A	18	44

N/A- not applicable

The following decreasing series of highest detected concentrations of heavy metals was found for C. *nodosa* Mn> Zn> Cu> Cd> Hg. The same distribution of heavy metals was determined in previous studies of heavy metal content in *C. nodosa* from the area of the Boka Kotorska Bay (Mačić, 2001), the central part of the Croatian coast (Lušić, 2016), Tyrrhenian (Bonanno & Di Martino, 2016; Bonanno & Raccuia, 2018) and the Ionian Sea (Bonanno et al., 2017; Bonanno & Borg, 2018; Bonanno *et al.,* 2020).

Since there is no scale of background concentrations of heavy metals for Montenegrin coast, for the calculation of contamination factor by heavy metals in sediment, the average background metal concentrations in Earth crust by Rivaro *et al.*, (2011) were used. The values of concentration factors are shown in the Tab.5.

Table 5. Values of the contamination factor (CF)

Location	Žanjice	Sv. Stasije	Sv. Marko	Average background concentration (Rivaro *et al*, 2011)
CF metal				
CF_{Cu}	0.25	0.60	0.33	45
CF_{Zn}	0.16	0.93	0.52	95
CF_{Mn}	0.48	0.83	0.78	450
CF_{Pb}	0.32	1.27	0.22	20
CF_{Cd}	2.0	0.90	0.11	0.30
CF_{Hg}	0.11	0.54	0.38	0.30

Low values of contamination factor were found for Cu, Zn, Mg and Hg from Žanjice, Sv. Stasije and Sv. Marko and for Cd from Sv. Stasije and Pb from Žanjice. Also, low values of this factor were also found for Pb and Cd from Sv. Marko. Obtained moderate values of contamination factors for Cd from Žanjice (CF = 2.0) and Pb from Sv. Stasije (CF = 1.27) indicate that these heavy metals, apart from natural sources, also originate from anthropogenic sources (Kljakovič-Gašpič *et al.*, 2008).

The bioconcentration and biota-sediment accumulation indexes (BCF and BSAF) are shown for both seagrass species in Tab. 6 and 7, respectively. The metal which presents the highest BCF for *P. oceanica* is Zn from Sv. Marko while Pb from Žukovica is the metal with lowest one. The metal which presents the highest BCF for *C. nodosa* is Mn from Sv. Marko while Cu from Žukovica presents the lowest one. The highest BSAF is found for Cd from Sv. Marko for both seagrasses, while the lowest BSAF is found for Mn from all investigated locations. High values of BCF indexes obtained for Cu, Zn and Mn in both sea grasses indicate at their ability to accumulate heavy metals which classifies them as good bioindicators of marine ecosystems pollution. Comparing the values of the accumulation capacity, it can be concluded that *P. oceanica* has a higher capacity compared to C. *nodosa*. This is in accordance with the researches done by Bonnano *et al.* (2017) and Bonanno & Borg (2018). Greater sediment mobility and hyper accumulation capacity was observed for Cd in *P. oceanica* and *C. nodosa* for Sv. Marko. This indicates that both sea grasses can be used as bioindicators of Cd metal in sediment, especially in conditions when this metal is present in higher concentration.

The values of Pearson 's correlation coefficient (r) of the investigated heavy metals in seawater and sea grasses of *P. oceanica* and *C. nodosa* are shown in Tab. 8.

High positive values of the correlation coefficient were determined for Zn-Mn ($r = 0.80$, $p < 0.05$), Zn-Pb ($r = 0.86$, $p < 0.05$) and Mn-Pb (r = 0, 81, $p < 0.05$) in seawater. A strong positive correlation was obtained for Pb

Table 6. Values of bioconcentration factor (BCF) for *P. oceanica* and *C. nodosa*

	BCF	Cu	Zn	Mn	Pb	Cd
P. oceanica	Žanjice	1.1×10^2	3.2×10^3	2.4×10^3	2.5×10^2	1.0×10^3
	Sv. Stasije	1.2×10^3	4.5×10^3	3.0×10^3	3.1×10^2	/
	Sv. Marko	1.1×10^4	4.5×10^4	2.2×10^3	/	/
	Žukovica	3.5×10^1	1.4×10^3	1.4×10^3	1.1×10^1	N/A
	Buljarica	9.5×10^1	3.6×10^3	1.0×10^4	3.6×10^2	N/A

	BCF	Cu	Zn	Mn	Pb	Cd
C. nodosa	Žanjice	4.4×10^1	1.9×10^3	1.2×10^3	/	4.3×10^2
	Sv. Stasije	1.0×10^3	1.0×10^3	1.5×10^3	/	/
	Sv. Marko	1.5×10^4	1.5×10^4	8.3×10^4	/	/
	Žukovica	1.8×10^1	8.1×10^2	3.2×10^2	/	/

/-BCF was not calculated

Table 7. Values of biota-sediment accumulation factor (BSAF) for *P. oceanica* and *C. nodosa*

Location	Žanjice		Sv. Stasije		Sv. Marko	
$BSAF_{metal}$	*P. oceanica*	*C. nodosa*	*P. oceanica*	*C. nodosa*	*P. oceanica*	*C. nodosa*
$BSAF_{Cu}$	1.2	0.5	1	0.3	1.2	0.7
$BSAF_{Zn}$	2.8	1.7	1.3	0.3	1.4	0.5
$BSAF_{Mn}$	0.9	0.3	0.8	0.4	0.8	0.3
$BSAF_{Pb}$	0.3	/	0.7	/	4.9	/
$BSAF_{Cd}$	2.5	1	3.6	1.7	26.2	11.8
$BSAF_{Hg}$	1.1	0.3	1.3	0.02	0.8	0.1

/-BSAF was not calculated

Table 8. Pearson correlation coefficient ($p < 0.05$) measuring the relationship between seawater trace metal concentrations and relationships between trace metal concentrations in sea water and sea grasses

Sea water	Cu	Zn	Mn	Pb
Cu	1.00			
Zn	0.14	1.00		
Mn	0.32	***0.80****	1.00	
Pb	-0.22	***0.86****	***0.81****	1.00
Sea water-P.oceanica	Cu	Zn	Mn	Pb
Cu	***-0.73****	***-0.78****	0.51	***-0.77****
Zn	0.42	0.42	0.21	0.50
Mn	-0.04	0.003	-0.39	0.28
Pb	0.51	0.57	0.09	***0.78****
Sea water-C. nodosa	Cu	Zn	Mn	
Cu	-0.53	***-0,85****	***-0,77****	
Zn	-0.63	-0.18	0.06	
Mn	-0.58	-0.42	-0.11	

*High correlation values (p < 0,05)

in seawater and *P. oceanica* (r = 0.78 p <0.05). High negative values of the correlation coefficient was observed for the content of Cu-Cu (r = -0.73), Cu-Zn (r = -0.78), Cu-Pb (r = -0.77) in seawater and *P. oceanica*, as well as for the content Zn-Zn (r = -0.85) and Zn-Mn (r = -0.77) in seawater and *C. nodosa*.

The results of the correlation analysis of the investigated heavy metals in the sediment and sea grasses *P. oceanica* and *C. nodosa* are presented in Tab. 9. Statistically significant positive correlations were recorded for Cu-Zn, Cu-Hg, as well as Mn-Hg in the sea grass *P. oceanica*. Also, a statistically significant positive correlation was observed for Zn-Mn in sediment and *C. nodosa* (r = 0.99, p <0.05). High positive correlations were recorded for many heavy metals in sediment, *P. oceanica* and *C. nodosa* as shown in Tab. 9.

There is no positive correlation between the content of Mn, Cu, Zn, Cd and Hg in seawater and *P. oceanica* and *C. nodosa*, which is in accordance with many different studies (Mačić & Sekulić (2001), Bonanno & Di Martino 2016, Bonanno & Di Martino 2017, Bonnano & Borg 2018, Bonnano & Raccuia 2018). The lack of correlation of the content of most heavy metals in seawater and both sea grasses can be explained by the fact that these metals enter the seagrasses mainly by absorption from sediment by roots (Bonanno & Borg, 2018).

The correlation of Cu-Zn, Cu-Hg, Zn-Mn, Zn-Hg and Mn-Hg in the sediment indicates at the geochemical association of these elements and their input from predominately anthropogenic sources (*Stanković et al.,* 2014; Stanković *et al.,* 2015; Joksimović *et al.,* 2019).

The correlation of Hg in the sediment with Cu, Zn, Mn, Pb and Hg in *P. oceanica* indicates a common origin and mechanism of absorption from sediment This is in accordance with the results of Lafabrie *et al.* (2007) and Di Leo *et al.* (2013), while according to Stanković *et al.,* 2015, Hg is absorbed from the water column. Also, a strong correlation between Cd content in sediment and *P. oceanica* suggests that this metal originates from sediment (Bonnano *et al*., 2017). High correlations of Cu-Mn, Zn-Mn, and Mn-Mn in sediment and *C. nodosa* indicate a similar geochemical origin of these metals in sediment. Except for Cd, this sea grass is a good accumulator of Mn in sediment as confirmed by Komar *et al.* (2017).

Given the low values of PLI indices which ranged from 0.33 to 0.81 for the investigated locations (Fig.2), it can be concluded that no significant input from anthropogenic sources is present (Joksimović *et al*., 2019).Obtained low values of PLI index for locations Sv. Marko and Žanjice indicate at slight influence of antrogenic activities on the pollution of the investigated locations (Joksimović *et al*. 2019). Sampling site Sv. Stasije is the location lost impacted by pollution.

The metal pollution (MPI>1) was registered for all investigated locations in the study (Fig.3). MPI values for sediment are lower than MPI values for the sea grass *P. oceanica* at the Sv. Stasije and Žanjice, which is in accordance with Stanković *et al*., (2014). Higher values of MPI for seagrass compared to MPI for sediment indicate the process of metal uptake from seawater (Stanković *et al*., 2015). Based on the MPI values obtained for sediment, *P. oceanica* and *C. nodosa*, it can be concluded that Sv. Stasije is the most polluted location while Žanjice are the least polluted location with heavy metals (Stanković *et al*., 2015; Joksimović *et al.,* 2019). Žanjice are located near the open sea with more intensive dynamics of changes in sea currents, reduced

Table 9. Pearson correlation coefficient (($p < 0,05$) measuring the relationship between sediment trace metal concentrations and relationships between trace metal concentrations in sediment and sea grasses

sediment	Cu	Zn	Mn	Pb	Cd	Hg
Cu	1.00					
Zn	0.96*	1.00				
Mn	0.75	0.90*	1.00			
Pb	-0.30	-0.03	0.39	1.00		
Cd	-0.29	-0.54	-0.85*	-0.81*	1.00	
Hg	0.90*	0.98*	0.96*	0.13	-0.67	1.00

P.oceanica	Cu	Zn	Mn	Pb	Cd	Hg
Cu	1.00					
Zn	0.98**	1.00				
Mn	0.82*	0.79*	1.00			
Pb	0.60	0.73	0.52	1.00		
Cd	-0.19	-0.35	0.10	-0.77*	1.00	
Hg	0.91**	0.85*	0.93**	0.38	0.17	1.00

C. nodosa	Cu	Zn	Mn	Cd	Hg
Cu	1.00				
Zn	0.01	1.00			
Mn	0.41	0.69	1.00		
Cd	-0.95**	-0.24	-0.66	1.00	
Hg	0.38	-0.23	-0.53	-0.14	1.00

sediment-P. oceanica	Cu	Zn	Mn	Pb	Cd	Hg
Cu	0.99*	0.98*	0.80*	0.58	-0.53	0.99*
Zn	0.98*	0.99*	0.93*	0.78*	-0.74	0.99*
Mn	0.80*	0.86*	0.99*	0.97*	-0.95*	0.83*
Pb	-0.23	-0.12	0.31	0.59	-0.63	-0.17
Cd	-0.36	-0.46	-0.80*	-0.94*	0.96*	-0.42
Hg	0.93*	0.96*	0.98*	0.87*	-0.84*	0.95*

sediment- C.nodosa	Cu	Zn	Mn	Cd	Hg
Cu	0.11	0.61	0.97*	-0.37	-0.87*
Zn	0.37	0.37	0.99**	-0.60	-0.70
Mn	0.74	-0.05	0.87*	-0.89*	-0.33
Pb	0.90*	-0.94*	-0.08	-0.76*	0.73
Cd	-0.98*	0.57	-0.49	0.99*	-0.20
Hg	0.53	0.21	0.97*	-0.73	-0.57

*High correlation values ($p < 0,05$);

**Values of the statisticaly significant correlation ($p < 0,05$)

anthropogenic influence, as well as lower clay and mud content in the sediment compared to the locations in Boka Kotorska and therefore the lower concentrations of heavy metals and thus lower MPI value were obtained.

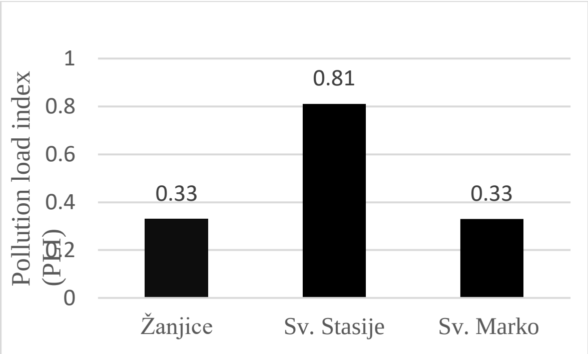

Figure 2. Pollution load index (PLI) for all locations

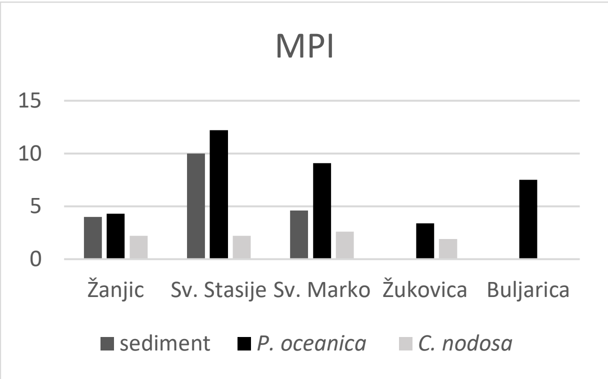

Figure 3. Metal pollution indices (MPI) for all location

CONCLUSIONS

Heavy metal pollution of sea water is present along Montenegrin coast, especially due to the resason that the sampling was done in summer period when the coast is most burdened by various anthropogenic activites. Due to impact of the weak water circulation, specificity of the Boka Kotorska bay and anthropogenic activities, the values of the concentration of heavy metals in sea water, sediment and sea grasses from Boka Kotorska Bay (Sv. Stasije and Sv. Marko) are higher in relation to locations that are under the influence of the open sea (Žukovica, Žanjice and Buljarica).

Average heavy metal concentrations in sediment and *P. oceanica* decrease with the following order: Mn>Zn>Cu>Pb>Cd>Hg. Similar order was also obtained for *C. nodosa* Mn>Zn>Cu>Cd>Hg. PLI values indicate that the basic level of sediment pollution with heavy metal is present. Based on the MPI values, it can be concluded that the higher heavy metals contamination is present in sea grasses than in sediment. Sv. Stasije has the highest MPI values, while Žanjice have the lowest MPI vlaues.

Both seagrasses do not reflect the heavy metal concentration in seawater, except Pb in the case of *P. ocenica*. Correlation analysis of the heavy metal concentration in sea water, sediment and both sea grasses showed that Cu, Zn, Mn, Cd and Hg in *P. oceanica* and Mn and Cd in the case of *C. nodosa* originate from sediment. Seagrasses *P. oceanica* and *C. nodosa* are promising bioindicators of heavy metals in sediments, whereas *P. oceanica* has the higher capacity of heavy metal bioaccumulation (higher values of BCF and BSAF) comparing to *C. nodosa*.

The researches of accumulation of heavy metals in seagrasses will be significant in the upcoming period, especially given the need to establish continuous monitoring of the Montenegrin marine ecosystem using appropriate indicator organisms, as well as the application of legislation governing this area.

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Procjena sadržaja teških metala u morskoj vodi, sedimentu, morskim cvjetnicama *Posidonia oceanica* (L.) Delile i *Cymodocea nodosa* (Ucria) Asch. sa crnogorske obale

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SAŽETAK

Koncentracije, Cu, Zn, Mn, Pb, Cd, i Hg su određene u morskoj vodi, sedimentu i morskim cvjetnicama *Posidonia oceanica* (L). Delile i *Cymodocea nodosa* (Ucria) Asch. uzorkovanih duž crnogorske obale tokom ljeta 2016. godine. Stepen zagađenja teškim metalima je određen koristeći faktore CF, BCF, BSAF, MPI i PLI. Veće vrijednosti PLI i MPI faktora su zabilježene u sedimentu i morskim cvjetnicama unutar zaliava Boke Kotorske u odnosu na lokalitete koji su pod jačim uticajem otvorenog mora. Lokalitet sa većim sadržajem teških metala je Sv. Stasije, dok su Žanjice lokalitet koji je najmanje zagađen teškim metalima. Korelaciona analiza sadržaja teških metala u morskoj vodi, sedimentu i obje cvjetnice je pokazala da Cu, Zn, Mn, Cd i Hg u *Posidonia oceanica* vodi porijeklo iz sedimenta, dok Pb vodi porijeklo iz vodenog stuba. Za *Cymodocea nodosa* je utvrđeno da Mn i Cd vode porijeklo iz sedimenta, dok se za Cu, Zn i Hg u ovoj cvjetnici pretpostvlja da potiču iz prirodnih ili antropogenih izvora. Stoga, *Posidonia oceanica* se može koristiti kao bioindikator zagađenja teškim metalaima za Cu, Zn, Cd, Mn i Hg u sedimentu i Pb u morskoj vodi, dok se *Cymodocea nodosa* može koristiti kao bioindikator za Mn i Cd u sedimentu.

Ključne riječi: *Posidonia oceanica, Cymodocea nodosa*, teški metali, morska voda, sediment