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METAL ACCUMULATION IN A BIOLOGICAL INDICATOR (*POSIDONIA OCEANICA*) FROM THE MONTENEGRIN COAST

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ABSTRACT

Posidonia oceanica L. Delile was collected along the Montenegrin coast in the period from autumn 2005 until the spring of 2006, or two seasons (autumn, spring), at eight locations. Four samples were collected at locations that are under strong influence of the open sea and the other four sites are located in an enclosed Boka Kotorska bay, or on sites that are differently exposed to the negative anthropogenic influence. Metal contents (Fe, Mn, Zn, Cu, Cd, Ni, Pb, Co and Cr) in *P. oceanica* were determined by atomic absorption spectrometry (AAS) and the difference between the seasons was analyzed. After analysis of the correlation coefficient between metals in sea grass *P. oceanica* it was concluded that between some pairs of metals synergistic effect exists, such as exponential dependence between Fe and Pb concentrations of metal pair from the fall 2005. At the same time antagonistic effect was observed in a number of pairs of metals. Also, the analysis of biological concentration factors led to the conclusion that sea grass *P. oceanica* can be a good bio indicator for some metals as pollutants of sea water.

Keywords: Trace metals, *Posidonia oceanica*, Southeastern Adriatic Sea, Montenegro

INTRODUCTION

Many toxic and bioaccumulative pollutants are found in only trace amounts in sea water, and often at elevated levels in seagrass. Trace metals are regarded as serious pollutants of the marine environment because of their toxicity and persistence, their difficult biodegradability and tendency to concentrate in aquatic organisms (Conti et al. 2010). The concentration of heavy metals in macroalge is used for monitoring of bioacumulative pollutants levels in the marine environment, and heavy metals are part of them. Seagrass have relatively long life cycle and can provide information of environmental pollution during the prolonged period of time. Settlements of sea grass *Posidonia oceanica* L. Delile makes the most typical biocoenosis in the Mediterranean. The *P. oceanica* meadows are one of the most diverse marine communities, which have a crucial role in the ecology of the Mediterranean. It is the only marine phanerogrm capable of forming durable structures, it may absorb metals directly from the water columnor and from interstitial water in sediments (Lafabrie et al. 2007) and has a high capacity for concentrating chemical pollutants and to accumulate trace metals occurring in the environment during its life cycle (Calmet et al. 1988, Malea & Horitonidis, 1989). In fact, in the last 10 years *P. oceanica* has been used as a biological »recorder« of marine environmental quality (Lafabrie et al. 2007, 2008). The capability of *P. oceanica* to concentrate a range of pollutants such as trace metals has been clearly established (Capiomont et al. 2000, Pergent-Martini & Pergent, 2000, Campanella et al. 2001, Ferrat et al. 2003, Lafabrie et al. 2007).

Underwater meadows which *P. oceanica* creates are significant not only for the production of organic matter and oxygen, but also because they are habitat, food and shelter for many marine organisms. In addition, this species have a very important role in retain of sediment, reducing the movement of water and preventing erosion of the sea bottom. The coastal part of continental shelf is the most sensetive, as it receives large amounts of contaminants introduced by domestic, industrial and agricultural activities, directly or via rivers or through atmospheric deposition (Zoller & Hushan, 2000, Usero et al. 2005). Because of eutrophication, extracting of sand, heavy metals contamination and other oil derivatives pollution, damage and withdrawal meadows of sea grass occurs. Because of their attachment to the sediment by roots and their nutrition through roots too, this species of sea grass is also suitable indicator of sediment pollution. In the Mediterranean Sea, the endemic species *Posidonia oceanica* (L.) Delile is often considered a useful metal bio-indicator (Ferrat et al. 2003, Lafabrie et al. 2007).

The aim of the present work was: i) to measure heavy metal concentrations in seagrass *P. oceanica*, from Montenegrian coast in order to assess the degree of metal pollution in the area as represents, ii) summary of correlation coefficient (r) between metals in seagrass and concentration factor (CF), and iii) to evaluate the relevance of *Posidonia oceanica* as a metal bio-indicator.

MATERIALS AND METHODS

Sampling locations, sampling method, sample preparation and trace metals analyses

Samples were collected at eight selected locations in this Adriatic coastal area: Sveta Stasija, Kukuljina and Herceg Novi in the semi-enclosed Boka Kotorska Bay, and on the coastline of open sea at Mamula, Žanjice, Bigova, Budva and Bar (Figure 1), situated in the proximity of different geochemical, hydrological and human impacts.

At the same time and place, about 350 g of the fresh *P. oceanica* samples and two litres of seawater from the bottom were collected at a depth of

7 ± 1 m. The *P. oceanica* samples were washed very thoroughly, rinsed with ultrapure water, frozen, lyophilized and reduced to powder, dissolved and analyzed. Preparation of dissolved biota samples (approximately 0.5 g) for trace metal analysis was performed as follows: the powder was digested with a mixture of 7 mL concentrated HNO_3 (65% Merck, Suprapur) and 2 mL H_2O_2 (30% Merck, Suprapur). To ensure the quality control and accuracy of the applied analytical procedure for the determination of heavy metals in the seagrass, certified reference materials, IAEA 140 (*Fucus* Sample), were also digested and analyzed.

All the seawater samples, from the bottom, were collected at the same time as the biota samples at all the studied locations. The water samples were analyzed after filtration and acidification with nitric acid ($pH \leq 2$) for the metals determination, immediately in the days after the sampling. The pre-concentration technique was applied for the sea water analysis following the solvent extraction technique (Murakami & Takada, 1992) prior to analysis by GF-AAS (Fe, Mn, Zn, Cu, Cd, Ni, Pb and Co) by Perkin-Elmer, 4100ZL, with Zeeman background correction and Hg and As were measured following a form of CV AAS procedure (Lafabrie et al. 2008) using a Perkin–Elmer Hydride System coupled to an atomic absorption spectrometry (AAS-PerkinElmer, AAnalyst 200).

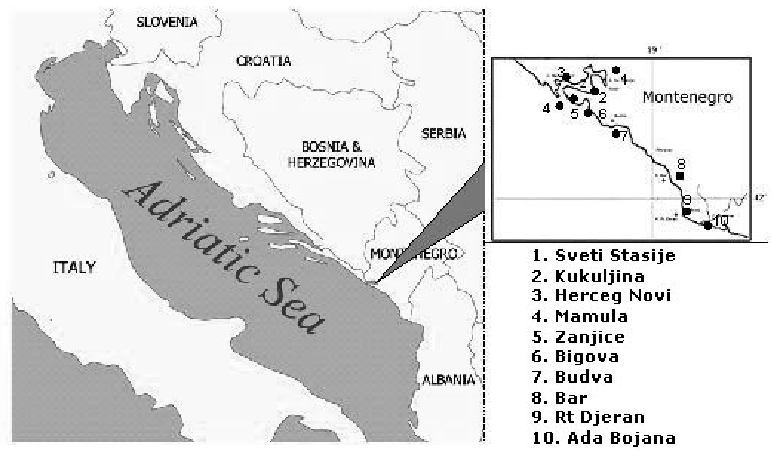

Figure 1. Map of investigated positions

The accuracy of the methods was checked with three calibration standards prepared in our laboratory from standard solutions of 1000 mg L^{-1} (Merck) and a seawater matrix was used for the preparation of the Fe, Mn, Zn, Cu, Cd, Ni, Pb and Co standards. These standards were analyzed directly after solvent extraction as mentioned above. Ultrapure water (18.2 MΩ cm) from a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all the aqueous solutions. All employed mineral acids and oxidants (HNO_3, H_2O_2 and HCl) were of the highest quality (Suprapure, Merck, Darmstadt, Germany).

All the results of the investigated elements in seagrass are expressed in dry weight (dw). To check for contamination, procedural blanks were analyzed after every five samples. The recovery of metals in standard reference materials was in the range of 82-115 % of the certified total concentrations. This was indicated by results of triplicate measurements for the all samples. No correction was applied to the obtained data.

Data analysis

The certified values and analysis results of the reference material are given in Table 1. The analytical precision, measured as the relative standard deviations for Ni, Co and Hg, were routinely under or around 10%, but higher than 10% for Cd, Pb and As. The average analytical standard errors observed for the rest of elements measured certified material, in the *Fucus* samples, were below 10%. Correlations between the metal concentrations in *P. oceanica* were performed by analysis of the Pearson's correlations.

Table 1. Analysis of certified reference materials IAEA 140 (Fucus Sample): certified values and found values (mean ± S.D.)

Elements	IAEA 140 (*Fucus* Sample)	
	Certified	Found
	(mg kg^{-1} dw)	
Ni	3.79 ± 0.41	4.10 ± 0.31
Co	0.83 ± 0.13	0.95 ± 0.09
Pb	2.19 ± 0.28	1.87 ± 0.11
As	44.3 ± 2.1	47.10 ±3.4
Cd	0.54 ± 0.04	0.65 ± 0.03
Hg	0.038 ± 0.006	0.037 ± 0.009
Fe	1256 ± 36	1240 ± 27
Mn	56.1 ± 2.4	56.9 ± 1.7
Zn	47.3 ±2.0	46.5 ± 1.4
Cu	5.05 ± 0.28	4.8 ± 0.30

RESULTS AND DISCUSSION

Marine water

The measured trace element concentrations in the samples of sea water from the bottom in the fall 2005 and spring 2006 are listed in Table 2. The levels of Fe, Cu, Co, Ni, Cd, Mn, Zn, As, Hg and Pb were relatively high (up to 53.4 μg L^{-1} Fe, 6.3 μg L^{-1} Cu, 10.4 μg L^{-1} Co, 7.4 μg L^{-1} Ni, 6.20 μg L^{-1} As, 10.6 μg L^{-1} Cd, 23.0 μg L^{-1} Mn, 20.7 μg L^{-1} Zn, 2.0 μg L^{-1} Hg and 27 μg L^{-1} Pb). In the Venice lagoon and southern Adriatic of Italy such a high levels of elements in sea water were found (Giusti &. Zhang, 2002, Manfra & Accornero, 2005). The concentration of Cd was below the detection limit at the location Budva. The relative standard deviation of replicate analyses of each sample was within 10-30%.

Table 2. Total metal concentrations in the bottom sea water from the fall 2005/spring 2006 (μg L^{-1})

Fall 2005	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Hg	Pb
Sv.Stasija	21.4	35.56	3.92	6.41	5.35	7.13	2.50	2.50	0.92	5.7
Kukuljina	19.0	14.63	5.66	4.90	3.10	5.01	2.90	2.70	0.40	27.0
H. Novi	19.0	23.52	10.45	5.88	4.38	8.77	2.60	6.20	0.98	3.92
Žanjice	22.8	19.60	4.53	4.99	6.31	20.70	2.90	3.30	0.40	2.60
Mamula	19.0	4.90	6.53	3.92	3.46	5.19	2.40	n.d	0.68	18.6
Bigova	23.0	53.41	2.61	4.41	4.92	19.11	2.40	1.90	0.54	26.9
Budva	19.0	24.50	3.92	3.92	2.19	4.96	2.50	n.d.	0.62	2.94
Bar	20.3	8.96	4.28	4.28	8.03	15.09	2.70	8.1	1.28	26.4
Spring 2006	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Cd**	**Hg**	**Pb**
Sv.Stasija	6.40	23.57	4.44	5.55	4.87	10.19	1.10	1.0	1.03	2.6
Kukuljina	9.2	41,26	5.99	6.46	3.14	6.96	1.05	n.d.	1.03	10.7
H. Novi	9.8	13.57	5.0	5.55	2.96	9.42	1.05	n.d	0.90	1.8
Žanjice	5.05	34.29	4.44	6.67	5.56	13.75	n.d	n.d.	1.20	0.6
Mamula	5.6	8.05	6.46	5.07	6.27	4.71	1.10	n.d.	0.68	0.7
Bigova	6.0	3.79	1.84	3.51	2.82	3.79	1.0	n.d.	1.03	2.1
Budva	5.4	7.84	6.46	7.38	0.94	7.84	1.0	n.d.	2.0	1.9
Bar	4.79	4.62	5.56	6.67	3.33	4.62	1.10	n.d.	0.90	1.2

Biota

Metal concentrations in marine organism *P. oceanica* at different locations in samples collected in the fall of 2005. and in the spring 2006. are presented in Table 3. Comparing the average values of the examined elements in seagrass, it can be concluded that their content in the *P. oceanica*, whether they are sampled in the fall or spring, approximately are the same, and showed in the following order:

Fe > Mn > Cu > Zn> Pb > Ni > Co> As > Cd > Hg.

The highest concentrations of the investigated elements were in seagrass at locations Sveta Stasija and Kukuljina in the fall 2005. Both locations are in the semi-closed Boka Kotorska Bay. Generally at all locations, concentrations of Pb and Cd were higher in samples from the fall 2005.

Table 3. Trace metal concentrations in P. oceanica from two seasons, F- fall 2005 and S- spring 2006 (mg kg^{-1} dry wt)

Location		Fe	Mn	Zn	Ni	Pb	Cu	Co	As	Cd	Hg
S.Stasija	F	1332	211	110.5	24.8	10.1	8.1	4.1	3.8	2.2	2.61
S.Stasija	S	885	280	47.2	20.9	2.9	4.7	3.3	2.9	0.7	0.31
Kukuljina	F	1700	104	82.5	33.3	10.5	6.8	4.0	7.5	2.6	0.91
Kukuljina	S	1890	140	42.2	30.0	2.5	4.9	4.7	13.6	2.2	0.12
H. Novi	F	825	120	49.0	22.8	8.2	6.2	3.7	2.7	2.9	0.35
H. Novi	S	1250	65.0	39.2	21.5	4.9	5.1	3.9	5.4	1.8	0.40
Mamula	F	750	134	45.2	31.5	6.2	5.9	4.3	1.2	3.9	0.03
Mamula	S	1170	79.0	46.4	37.3	4.0	5.3	4.3	1.5	2.4	0.08
Žanjice	F	1075	118	41.8	31.0	21.5	7.5	4.3	8.0	2.8	0.57
Žanjice	S	775	101	45.0	34.1	5.0	7.1	5.3	3.2	2.6	0.03
Bigova	F	540	137	44.4	39.0	6.9	5.3	4.1	2.0	2.8	0.49
Bigova	S	395	92.8	48.5	39.8	3.7	5.7	4.7	2.5	2.4	0.04
Budva	F	725	106	34.9	24.7	6.2	5.7	3.8	2.7	2.7	3.46
Budva	S	740	180	51.1	29.0	9.5	6.6	5.9	1.9	2.2	0.04
Bar	F	550	385	93.5	36.7	17.1	8.9	6.5	2.5	3.5	1.37
Bar	S	475	215	121	34.6	8.8	6.0	6.9	2.5	4.4	0.38

Seasonal variability

On the basis of experimental data of metal content in sea grass can be concluded that there are some differences in the examined microelements contents in the sea grass from fall of 2005 and spring of 2006. The mean values of the examined microelements in samples of sea grass from the fall of the year 2005. are in the next order (concentrations are expressed in mg kg^{-1} dry wt):

Fe (937.0) > Mn (164.4) > Zn (62.7) > Ni (30.5) > Pb (10.9) > Cu (6.81) > Co (4.36) > As (3.78) > Cd (2.92) > Hg (1.22),

while the mean values for the same examined microelements in sea grass from the spring of the year 2006. are in the next order (concentrations are expressed in mg kg^{-1} dry wt):

Fe (947.5) > Mn (144.1) > Zn (55.0) > Ni (30.9) > Cu (5.67) > Pb (5.17) > Co (4.86) > As (4.18)

Cd (2.33) > Hg (0.17).

It can be noticed that the mean contents of Mn, Cu and Zn are registered in a higher concentrations in the samples from the fall 2005, than in the spring samples from 2006, which is a consequence of metabolic activation induced by temperature and light exposure. Also for these elements is characteristic that their content in the plants are gradually decreased in spring samples (Zwart, 2005), while the contents of Fe, Ni, Co and As are approximately the same, whether they are from the fall or spring samples. Values of Co are approximately the same in samples of water and sea grass, regardless of the season, which leads to the conclusion that the Co in the alga comes primarily through absorption from sea water, which comes from the atmosphere (Morel & Price, 2003). Concentrations of Fe are approximately the same in the fall and spring samples of sea grass. The concentrations of Zn in the spring samples are evenly, except the position Bar, where the mean value was 46.2 mg kg^{-1} dry wt. Approximately the same concentrations of Zn in almost all spring samples of sea grass, leads to the conclusion that the calculated mean value of 46.2 mg kg^{-1} for Zn, could be a natural concentration for this essential element in the sea grass *P. oceanica.* Also values for Zn obtained from the positions St. Stasija and Bar in the sea grass from the fall period, in relation to the values registered for the same element in the same positions in the spring, leads to the conclusion that the accumulation of Zn in sea grass are also the result of anthropogenic influence, which is obvious if mean values for Zn are compared the sea water-sea grass, for the seasons fall of 2005. and spring of 2006. For the content of Cu can notice that its concentration depending on the sampling site does not change significantly. Spring samples of sea grass contain a greater concentration of Cu then in the fall, and range from 4.68-7.10 mg kg^{-1} dry wt, with the mean value 5.8 mg kg^{-1} dry wt. In samples from the fall concentration of Cu ranged in the interval from 5.3 to 8.9 mg kg^{-1} dry wt, with the mean content of 6.8 mg kg^{-1} dry wt. Approximate mean value of the concentration of copper in sea grass, in comparison with open sea-closed sea, and in comparison with the fall-spring seasons, points to the necessity of this element in the cycle of growth and development of sea grass. Increased concentration of Cu in the sea grass samples from the fall, points to the physiological and biochemical necessity of this element in the fall period (Zwart, 2005). At the same time, monitoring of Cu concentration in seawater, points that the absorption of copper is done primarily through the roots. Increased concentration of Cu on sampling site Sv. Stasija (8.1 mg kg^{-1} dry wt) in Kotor Bay and Bar (8.9 mg kg^{-1} dry wt) at open sea, in the fall period, in relation to the mean value and in relation to the other sampling sites, points to the anthropogenic influence.

What is very worrying is a very high concentration of Pb found in all sea grass samples examined from all the sampling sites in relation to the literature data (Ancora et al. 2004, Ledent et al. 1995, Morillo et al. 2005, Lafabrie et al. 2007). Especially sea grass samples are contaminated from Sv. Stasija, Kukuljina and H. Novi in the Bay and at locations Žanjice and Bar, at the open sea area. Pb content is much higher in samples from the fall 2005, than in samples from the spring 2006. The highest content of Pb was detected at location Žanjice 21.5 mg kg^{-1} dry wt in the fall of 2005, what can be primarily explained by pollution that is caused with human factor. Pb concentrations in seagrass from the Bay can be explained by a negligible influence of sea currents and calm water in this part of the Bay, large amounts of Pb, whose origin is from aero pollution, car exhaust gases (Di Tullio et al. 1993), whose content in sea water is considerable (7.84 mg L^{-1} in Bigova, Table 2), fall to the bottom, where deposit through years in the sediment (Finden &

Tipping, 1984) and seagrass naturally absorbs Pb from sea water and sediment (Fritioff, 2005, Lafabrie et al. 2007). We concluded that the source of pollution is automobile exhaust gases, which in themselves contain Pb, and that due to rain comes to the marine ecosystem (Di Tullio et al. 1993). Five times higher content of Pb can be explained comparing the values obtained for the same elements, but in samples of sea water: as higher content of lead is detected in the fall samples of sea water, logical consequence is higher concentration of lead in samples of sea grass in the fall due to absorption, through root from the sediment and through leaves from sea water (Joksimović & Stanković, 2011). It is known that the alkaline pH value makes Pb insoluble and unavailable for alga, and that concludes that certainly other factors affect the bioavailability of Pb (Zwart, 2005). In some plants phosphates can control mechanisms, or reduce toxicity of Pb or control its binding to plant, so we can assume that the concentration of Pb depends on the level of phosphate in sea water (Favero et al. 1996).

Concentration of heavy metals in macro alga is used as an indicator of the level of bioavailability from seawater and sediments (Lafabrie, 2007, Joksimović & Stanković, 2011). Macro alga is bio indicator and demonstrate bioavailability of pollutants whose part is heavy metals ions. This ability is a consequence of specific biochemical condition which is determined by binding dissolved metals in sea water, and argues that macro alga can characterize one location for the certain time (Zwart, 2005).

Metals can be classified into three groups:

-metals whose level gradually decreases from the fall to spring, when they reach the lowest values,

-some essential metals algal stem usually accumulates them more from fall to spring.

Obliviously essential metals, Zn and Cu, and toxic metals (Pb, Cd and Hg) belong to groups 1. It is likely that factor responsible for their high concentrations act on both plant metabolism and chemical characteristics of the external algal surface (Zwart, 2005). Hg, Cd and Pb are characterized as the main, while Zn and Cu are temporary parameters for monitoring pollutants of biota. Mechanisms that regulate the absorption of certain metals can rule accumulation of metals in this species and all macro algae in general.

Table 4. Correlation coefficient for metals in sea grass P. oceanica from the fall 2005

Fall 05	Fe	Mn	Zn	Ni	Pb	Cu	Co	As	Cd	Hg
Fe	1									
Mn	-0.32	1								
Zn	0.46	***0.64***	1							
Ni	-0.23	0.34	0.05	1						
Pb	0.14	0.41	0.22	0.24	1					
Cu	0.22	***0.79***	***0.76***	0.06	***0.74***	1				
Co	-0.35	***0.94***	0.45	0.52	0.54	***0.72***	1			
As	***0.76***	-0.27	0.14	0.00	***0.63***	0.30	-0.15	1		
Cd	-0.61	0.31	-0.29	0.38	0.00	-0.05	0.50	-0.50	1	
Hg	0.09	0.14	0.26	-0.44	-0.14	0.17	-0.04	-0.05	-0.51	1

Table 5. Correlation coefficient for metals in sea grass P. oceanica in spring 2006

Spring 06	Fe	Mn	Zn	Ni	Pb	Cu	Co	As	Cd	Hg
Fe	1									
Mn	-0.22	1								
Zn	-0.43	0.43	1							
Ni	-0.36	-0.39	0.26	1						
Pb	-0.51	0.22	***0.61***	0.10	1					
Cu	-0.53	-0.15	0.22	0.41	***0.65***	1				
Co	-0.40	0.10	***0.74***	0.48	***0.81***	***0.69***	1			
As	***0.93***	-0.10	-0.26	-0.23	-0.46	-0.41	-0.15	1		
Cd	-0.34	-0.10	***0.81***	***0.61***	0.59	0.49	***0.89***	-0.12	1	
Hg	0.03	0.35	0.44	-0.60	0.10	-0.45	-0.09	0.04	0.07	1

Seasonal variability and seasonal activation of heavy metals in the alga was statistically presented by correlation analysis (Zwart, 2005). In Table 4. and Table 5. are reported correlation coefficients, r values. Values for the $r \geq 0.5$ are evidence of synergism, and if r is closer to 1.0 it is greater synergetic effect between metal pairs in their absorption by the alga.

The data from Tables 4. and Table 5. can be seen that the synergetic effect, in fall and spring samples of seagrass, occurs in two pairs of metals, such as Fe-As and Co-Cu. It is interesting to note that the synergetic effect, for very toxic metal, Cd occurs much less in samples from the fall, comparing to spring samples where Cd in synergetic effect with Zn, Ni and Co. Based on the literature, regarding the absorption of toxic metals, it is much easier to absorb Cd in relation to the other toxic elements, and has much stronger binding mechanism in the cells of alga, as well as much slower leakage in surrounding environment through leaves and the rhizome (Fritioff, 2005). Co is correlated with essential Mn and Cu metals in the fall samples, and essential Zn and Cu, and toxic Pb in spring samples. In fall samples is very high synergetic effect of essential Mn with Zn, Cu and Pb, but in spring is very high synergetic effect of essential Zn with toxic Pb, Co and Cd elements in *P. oceanica*. The exponential dependence was found between Pb and Fe in the fall samples, Figure 2, which is already proven in previous studies of other authors (Zwart, 2005), but it was not case in the spring samples.

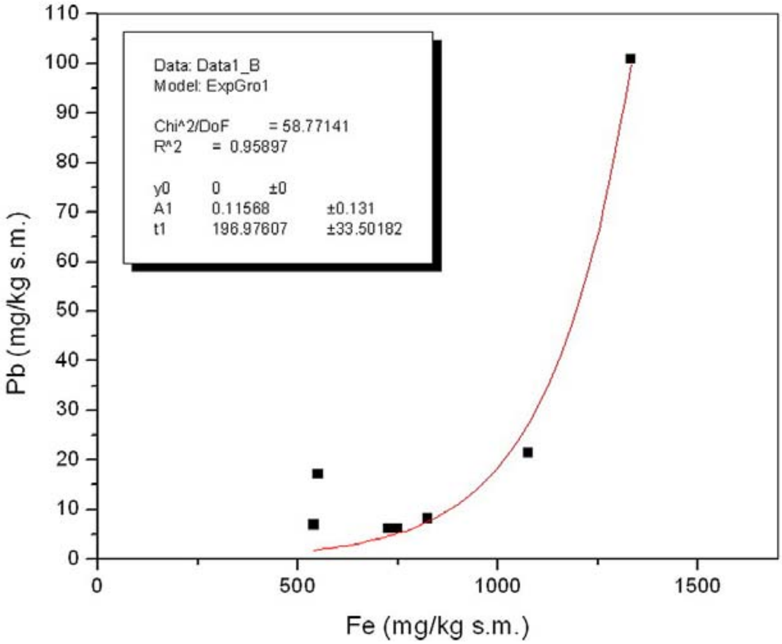

Figure 2. Exponential dependence of Fe concentration related to Pb concentration in P. oceanica sea grass samples in the fall 2005

Based on the results can be noticed that antagonistic effect between examined metals is higher in the sea grass in both examined seasons. A large number of pairs of metals with antagonist effect between them, in this sea grass, probably are a consequence of plants impossibility to absorb them due to the biological, chemical, and physical characteristics of the surrounding seawater (Joksimović & Stanković, 2011). For example, light and nitrogen availability positively affected rates of uptake of Fe, Mn, Zn and Cd in *Ulva fasciata*, and it has been demonstrated that uptake of Cd in *Ulva fasciata* increased with increased ambient concentration of nitrate in the growth medium (Lee & Wang, 2001), but in the case of Ni, in environments with high nutrient levels metal uptake in plants can be inhibited because of complex formation between nutrient and metal ions (Žarković & Blagojević, 2009).

Analysis of biological concentration factor

Analysis of biological concentration factor is one of the main reasons why macro algae *P. oceanica* is bioindicator of heavy metals pollution are given in Table 6. The CF is the ratio between the two variables and should be approximately constant in order to permit comparison of data along the seasonal and environmental gradients.

Table 6. Mean values of biological concentration factor in fall of 2005 and spring of 2006

Element	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Hg	Pb
Fall 05	$8*10^2$	$4.9*10^4$	$9.7*10^2$	$6.6*10^3$	$1.6*10^4$	$8.0*10^3$	$1.4*10^3$	$7.6*10^2$	$2.1*10^2$	$3.0*10^3$
Spring 06	$1.22*10^3$	$8.1*10^4$	$1.11*10^3$	$5.6*10^3$	$2.1*10^4$	$9.2*10^3$	$3.8*10^3$	$8.8\ 10^2$	$1.8*10^2$	$5.3*10^3$

Mean values of CF for examined metals in the fall samples of sea grass are decreased in order:

Fe> Cu > Zn > Ni > Pb > As > Co > Mn > Cd >Hg

Much higher mean values of CF for the same metals were obtained in the spring samples of sea grass in order:

Fe> Cu > Zn > Ni > Pb> As > Mn > Co >Cd >Hg

CF value is directly correlated with dissolved metal concentration in water. The highest mean values of CF for the following metals in this macro alga are in order: Fe> Cu > Zn > Ni > Pb> As and Cd >Hg, whether or not samples are from the fall or spring. Co bioaccumulation is higher in the fall, but Mn bioaccumulation is higher in the spring samples.

In Figure 3. is presented linear relationship of metal bioaccumulations, CF values, in plant and metal concentrations in seawater samples from the same locations. It is obvious that the concentration of examined microelements in sea grass increased from the fall of 2005 to the spring of 2006 (Table 3), but it is not case for the all CF values, such as for the CF values of Ni and Hg. The mean value of CF for Co and Ni are approximately the same for both season, as well as the concentration of these metals in the sea water, which means that their accumulation do not come in the plant during the period fall-spring only from the water.

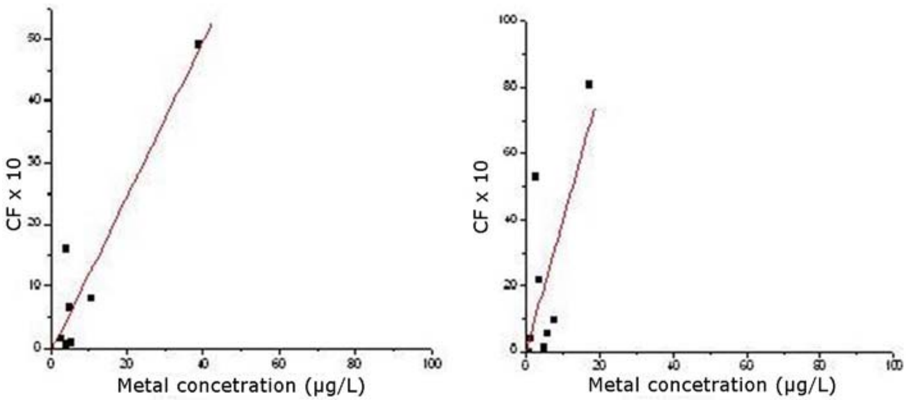

Figure 3. Linear relationship of metal bioaccumulation in seagrass P. oceanica and metal concentrations in seawater from the bottom at the same locations a) in the fall 2005; b) in the spring 2006

Considerably lower concentration of Pb in sea water and *P. oceanica* during the spring, and significantly higher value of CF for Pb in sea grass from the spring samples, leads to the conclusion that the absorption of this element in *P. oceanica* is from the sediments too. Higher values of CF for Cu, Zn and As in the spring regarding to the fall, indicates that the absorption of these elements is from sediments too, because the concentration of these elements in sea water is lower in the spring period then in the fall (Table 2), while their values for CF are higher in the same period. If CF values for Hg is analyzed, apparently coming to releasing of this element from the plant in the fall – spring period, because the concentration of Hg in the average is higher in the spring samples of water. Based on the obtained values of biological concentration factor CF can be concluded that the macro algae *P. oceanica* can be used as bio indicator of marine environment pollution.

Summary

Based on the obtained values of biological concentration factors CFs can be concluded that the macro algae *Posidonia oceanica* L. Delile can be used as bio indicator of marine environment pollution. The highest values of biological concentration factor CF in this seagrass are found for the following metals: Fe> Cu > Zn > Ni > Pb> As, whether it was fall or spring samples. Exponential dependence between the contents of pair Fe-Pb occurs in the fall samples of sea grass, while this is not the case at spring samples.

Thus, the aim of this study is to evaluate the state of metal contamination of the Montenegrin coastline using *P. oceanica* as a bio indicator of the sea water quality. As no data were available on the concentration of metals in sea grass in the coastal area of Montenegro, the results of this study can serve as a baseline against which future anthropogenic effects can be assessed.

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