EVALUATION OF TRACE-METAL CONTAMINATION IN THE NORTHEASTERN ADRIATIC COASTAL WATERS USING THE SEAGRASS *POSIDONIA OCEANICA*

OCENITEV ONESNAŽENOSTI OBALNIH VODA SEVEROVZHODNEGA JADRANA S KOVINAMI OB POMOČI POZEJDONKE *POSIDONIA OCEANICA*

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Ključne besede: morska trava, posegi, *Posidonia oceanica*, onesnaženost s kovinami, Jadransko morje

ABSTRACT

Seagrasses appear relevant bioindicators of metallic contamination in coastal waters. *Posidonia oceanica* leaves were collected at six locations along the north and east coast of the Adriatic Sea selected on the basis of the presence of different types and levels of human-induced pressures. Concentration of seven trace-metals (Ag, As, Cd, Cu, Hg, Ni and Pb), as well as the Methyl-Mercury content, were determined. The level of contamination is low at the pristine site of Lavdara (Cu: 4.73 ± 0.01 μg/g; Hg: 0.054 ± 0.003 μg/g; Met-Hg: 0.015 ± 0.000 μg/g; Ni: 21.40 ± 0.63 μg/g; Pb: 0.90 ± 0.10 μg/g) and higher along the Slovenian coast (Cu: 11.83 ± 0.22 μg/g, Hg : 0.092 ± 0.004 μg/g ; Met-Hg: 0.027 ± 0.004 μg/g; Ni: 40.07 ± 2.08 μg/g, Pb: 1.50 ± 0.06 μg/g). Generally, Mercury and the associated trace metals contents are relatively high in *P. oceanica* collected in the Northern Adriatic region. This is due not only to the strong anthropogenic impact (ports of Trieste and Koper, former chloralkaline plant – PVC near Seget Donji) but also to the geological characteristics of the hinterland (former cinnabar ore in Slovenia). This preliminary study confirms the capability of *P. oceanica* to record trace metal in relation with human-induced pressures, as well as with geological background of the coast.

IZVLEČEK

Morske trave so se izkazale kot nadvse ustrezni bioindikatorji onesnaženosti s kovinami v obrežnih vodah. Na šestih lokacijah ob severni in vzhodni obali Jadranskega morja so avtorji nabrali liste pozejdonke *Posidonia oceanica*, in sicer glede na različne vrste in ravni človekovih posegov v tamkajšnje okolje. Ugotovljene so bile koncentracije sedmih kovin (Ag, As, Cd, Cu, Hg, Ni in Pb) in tudi metil živega srebra. Raven onesnaženosti je bila nizka na neokrnjeni lokaciji ob otoku Lavdara (Cu: 4,73 ± 0,01 μg/g; Hg: 0,054 ± 0,003 μg/g; Met-Hg: 0,015 ± 0,000 μg/g; Ni: 21,40 ± 0,63 μg/g; Pb: 0,90 ± 0,10 μg/g) in višja ob slovenski obali (Cu: 11,83 ± 0,22 μg/g, Hg : 0,092 ± 0,004 μg/g ; Met-Hg: 0,027 ± 0,004 μg/g; Ni: 40,07 ± 2,08 μg/g, Pb: 1,50 ± 0,06 μg/g). Na splošno je bila vsebnost živega srebra in z njim povezanimi kovinami razmeroma visoka v pozejdonki *P. oceanica*, ki so jo nabrali v območju severnega Jadrana, vendar pa razloga za takšno vsebnost ne gre iskati le v antropogenih vplivih (koprskega in tržaškega pristanišča in nekdanje tovarne PVC pri Segetu Donjem), marveč tudi v geoloških značilnostih zaledja (nekdanja proizvodnja cinobra v Sloveniji). Pričujoča študija potrjuje sposobnost pozejdonke *P. oceanica*, da beleži sledi kovin, povezanih z antropogenimi vplivi in geološkim zaledjem obale.

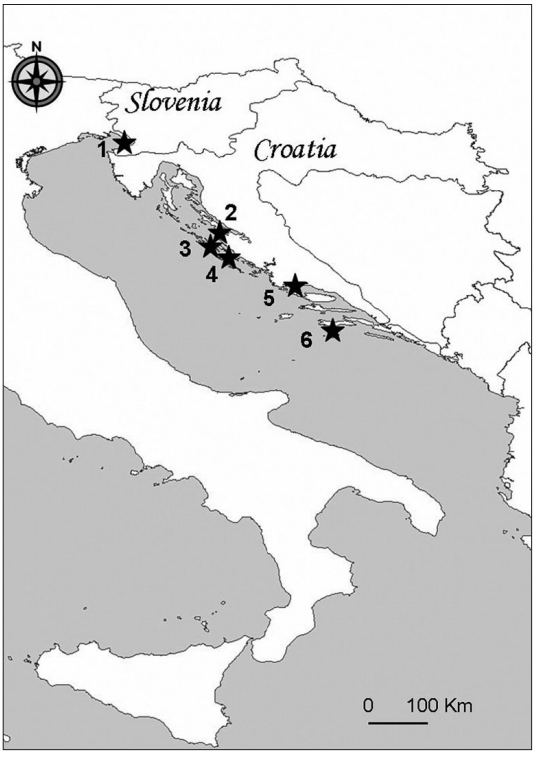
1. INTRODUCTION

In spite of its exceptional biodiversity (Galil 2007), the Mediterranean Sea is one of the most threatened seas in the world (PAM/Plan Bleu 2005). Indeed, the Mediterranean basin is subject to important human-induced pressures. Human activities along the coasts are responsible for the release of, among other things, contaminants (organic and inorganic). The Adriatic Sea, situated between the eastern coast of Italy and Croatia, is subject to both growing tourism pressure and industrial expansion. Study of trace-metals appears to be necessary to evaluate anthropogenic contamination of the coastal environment.

Seagrasses, and *Posidonia oceanica* (L.) Delile in particular, are important bioindicators of metallic contamination in coastal waters (Pergent-Martini and Pergent 2000). *P. oceanica* bioaccumulates and concentrates trace-metals in relation to their concentration in the water column (Lafabrie *et al*., 2007).

The aim of this study is to evaluate the metallic contamination (seven trace-metals Ag, As, Cd, Cu, Hg, Ni and Pb, as well as the Methyl-Mercury content) of *P. oceanica* leaves at different locations, selected on the basis of different types and levels of human-induced pressures, in the Northern and Eastern Adriatic.

2. MATERIAL AND METHODS



Samples of *P. oceanica* leaves were collected at six sites along the north and east coast of the Adriatic Sea (Figure 1). These sites are characterized by different types and levels of human activities.

Figure 1: Location of studied sites in the Northern and Eastern Adriatic Sea (1: Izola (Izo); 2: Zadar (Zdr); 3: Brbinjšćica (Brb); 4: Lavdara (Lav); 5: Seget Donji (SgD); 6: Island Vlašnik (IsV))

Slika 1: Preučevane lokacije v severnem in vzhodnem Jadranu (1: Izola (Izo); 2: Zadar (Zdr); 3: Brbinjšćica (Brb); 4: Lavdara (Lav); 5: Seget Donji (SgD); 6: otok Vlašnik (IsV))

Izola (Izo) is located in the Northern Adriatic, in the Gulf of Trieste, along the Slovenian coast between Izola and Koper. This relic meadow grows in shallow water in the area of strong anthropogenic influence due mainly to the nearby port of Trieste. Moreover, cinnabar (red Mercury (II) sulphide) is naturally found in Slovenian hinterland and therefore in the coastal sediments due to mining exploitation. Zadar (Zdr) is located in the Mid Adriatic, in the vicinity of the main sewage outlet (city of 73,000 inhabitants), which also collects rain water run-off. Brbinjšćica (Brb) cove faces open Adriatic Sea. This location is exposed to low anthropogenic influence, since there are no human settlements in the vicinity. However, the cove is used during the summer as a mooring site for small local boats. Lavdara (Lav), an island in the Mid Adriatic, is located in one of the channels between two rows of islands, far from any human activities. Seget Donji (SgD), also in the Mid Adriatic, is situated near the city of Trogir, in an area with a huge human impact. This location is a few kilometres down-current from Kaštela Bay – one of the most polluted spots along the Croatian coast. The location of Vlašnik Island (IsV), in the Southern Adriatic, is oriented towards the open sea and exposed to high water movement. Despite being on the trajectory of a regular everyday ferry connection to Lastovo, this site can be considered as being under a low anthropogenic influence.

The two external leaves of *P. oceanica* (Adult 1 and Adult 2) were collected at 10 m depth, between May 15th and July 15th. Blades were cleaned (epiphytes scraped off), rinsed (ultrapure water) and either lyophilisied (Heto® FD4-85 freeze dryer, HetoHolten A/S) or dried at 30°C to constant weight, before they were reduced to powder. For Hg analyses, 50mg of each sample were weighed in a Teflon digestion vessel CEM® ACV of 100ml (CEM Corporation, USA). 5ml of 69% HNO3 (Normapur) and 1ml of H2O2 30% (Normapur) were added. The vessels were sealed and placed into CEM® MARS 5 chamber (20 minutes at 200°C and 20 minutes of cooling). The content of each vessel was poured into 25ml volumetric flasks and diluted to volume with ultrapure water and then transferred to 60ml polypropylene flasks. Mineralized samples were analysed with a cold vapour atomic absorption spectrometer (CV-AAS – Perkin Elmer®). The standard addition method was applied for calibration. Calibration standards were prepared from a mercury standard solution 1 000 mg·l^{-1}. For Met-Hg, 50mg of each sample were heated for one hour at 95°C with 205 ml potassium hydroxide (10M). When it had cooled at room temperature, 5 ml NaCl (2%) were added. After centrifugation at 1000 g for 10 min, 34 ml of NaCl (2%) and 1ml of K2Cr2O7 (1%) were added to the supernatant. Concentrations of inorganic and organic Mercury in the sample were determined with the same laboratory apparatus, according to the adapted method of Riisgard and Hansen (1990) and Ferrat *et al.* (2002). Ag, As, Cd, Cu, Ni and Pb were analysed by atomic absorption spectrometry with quality assurance procedures at the Laboratory of Rouen/ ETSA (France). The analytic procedure was verified using certified reference materials (*Lagarosiphon major,* CRM 60; Community Bureau of Reference – Commission of the European Communities and TORT-2 Lobster Hepatopancreas Marine Reference Material for Trace Metals, National Research Council of Canada, Ottawa).

3. RESULTS

All mean concentrations of trace metals show significant difference between sites (KW, $p<0.05$ or $p<0.1$). The lowest value is every time significantly different from the highest value (Post Hoc Test, $p<0.05$ or $p<0.10$). Zadar and Izola seem to be the most contaminated sites (maximum values for Ag, Cu, Ni and Pb) and Lavdara the least (minimum values in Ag, Cd, and Cu).

Table 1: Metal concentrations ($\mu g \cdot g^{-1}$ dry wt.) in the blades of adult 1 and 2 leaves of *P. oceanica* (St.: station; mean ± SE; maximum values in bold and minimum values in italic; ** KW, $p<0.05$ and * KW, $p<0.1$)
*Tabela 1: Koncentracije kovin ($\mu g \cdot g^{-1}$ suha wt.) v bilkah pozejdonke P. oceanica (St.: postaja; srednja ± SE; maksimalne vrednosti v krepkem tisku, minimalne v poševnem; ** KW, p<0,05 in * KW, p<0,1)*

St.	Ag**	As**	Cd*	Cu**	Ni**	Pb*
Izo	0.13 ± 0.03	0.87 ± 0.03	2.1 ± 0.27	11.83 ± 0.22	**40.07 ± 2.08**	**1.50 ± 0.06**
Zdr	**0.83 ± 0.03**	0.40 ± 0.06	1.74 ± 0.11	**15.17 ± 0.32**	*18.40 ± 0.56*	**1.50 ± 0.15**
Brb	0.30 ± 0.00	**5.3 ± 0.35**	2.44 ± 0.09	7.43 ± 0.33	32.10 ± 0.70	1.23 ± 0.10
Lav	*0.10 ± 0.00*	1.77 ± 0.22	*1.41 ± 0.02*	*4.73 ± 0.09*	21.40 ± 0.62	0.90 ± 0.10
SgD	0.23 ± 0.03	*0.33 ± 0.03*	1.72 ± 0.03	10.57 ± 0.32	20.83 ± 1.37	1.33 ± 0.03
IsV	0.17 ± 0.03	0.67 ± 0.03	**2.75 ± 0.15**	6.20 ± 0.15	22.60 ± 3.51	*0.77 ± 0.15*

The Mercury content was measured at the six sites and Methyl-Mercury in Izola, Brbinjšćica and Lavdara (Table 2). Izola and Seget Donji present the highest values in the total Mercury content and exhibit statistical difference from Brbinjšćica (Post Hoc Test, $p<0.05$ and $p<0.10$). Methyl Mercury concentration in the Gulf of Trieste is twice as high as Lavdara's.

Table 2: Metal concentrations ($\mu g \cdot g^{-1}$ dry wt.) in the blades of adult 1 and 2 leaves of *P. oceanica* (St.: station; mean ± ES; maximum values in bold and minimum values in italic; **KW, $p<0.05$)
*Tabela 2: Koncentracije kovin ($\mu g \cdot g^{-1}$ suha wt.) v bilkah pozejdonke P. oceanica (St.: postaja; srednja ± ES; maksimalne vrednosti v krepkem tisku, minimalne v poševnem; ** KW, p<0,05)*

St.	Hg**	Met-Hg
Izo	**0.092 ± 0.004**	**0.027 ± 0.004**
Zdr	0.066 ± 0.008	
Brb	*0.041 ± 0.003*	0.017 ± 0.003
Lav	0.054 ± 0.003	*0.015 ± 0.000*
SgD	**0.094 ± 0.011**	
IsV	0.074 ± 0.008	

4. DISCUSSION AND CONCLUSION

According to the preliminary quality scale based on values recorded in the North Occidental Mediterranean Sea (Pergent, 2007; Romero *et al*. 2007), the level of contamination for each site can be evaluated (Table 3).

Table 3: Evaluation of the contamination using quality scale (from Pergent, 2007)
Tabela 3: Ocena onesnaženosti z uporabo lestvice kakovosti (po Pergentu, 2007)

	Very low contamination level	Low contamination level	Moderate contamination level	High contamination level	Very high contamination level
Ag	<0.29	0.29 – 045	0.45 – 0.61	0.61 – 0.77	>0.77
Cd	<1.92	1.92 – 2.52	2.52 – 3.16	3.16 – 3.98	>3.98
Hg	<0.035	0.035 – 0.053	0.053 – 0.067	0.067 – 0.092	>0.092
Ni	<18.10	18.10 – 23.32	23.32 – 31.58	31.58 – 55.05	>55.05
Pb	<1.31	1.31 – 1.83	1.83 – 2.42	2.42 – 3.54	>3.54

The level of contamination recorded at Lavdara is generally between "Low contamination" (Ni) and "Very low contamination" (Ag, Cd and Pb) for the five trace metals; it confirms the pristine character of this site. Moreover, this site presents the lowest values for Copper and Methyl-Mercury (Tables 1 and 2). Brbinjšćica is considered as a "Low contaminated" site for all metals, except for Mercury (moderate contamination).

The site of Izola exhibits "Very high contamination" in Mercury and "High contamination" in Nickel, additionally the values in Copper and Methyl-Mercury are also highest. The impacted site of Zadar presents "Very high contamination" in Silver and "High contamination" in Mercury.

From a general point of view, the Mercury content is relatively high - between "Very high contamination" and "High contamination" - at four of the sites in the researched area: Izola, Zadar, Seget Donji and Vlašnik.

In the Northern Adriatic (Izola), the results seem in line with the geological and geographical situation - presence of cinnabar in the marine sediments (Hylander and Meil 2003, Frančišković-

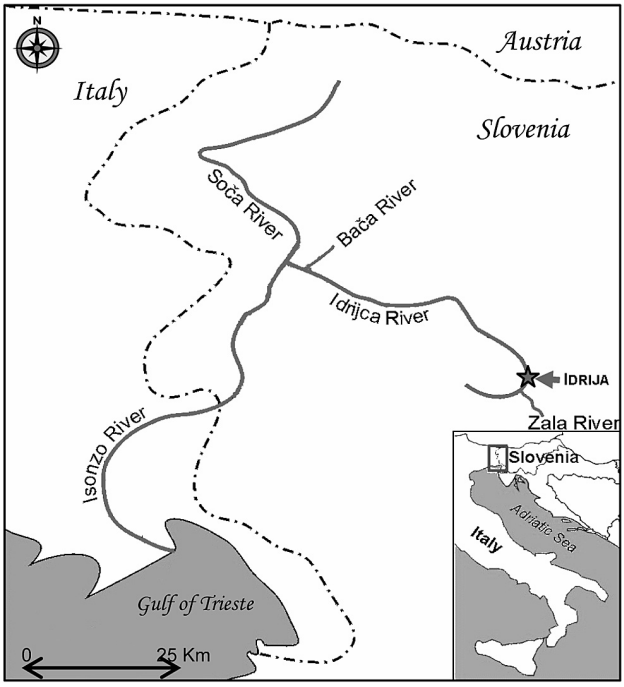


Figure 2: Rivers that flow from former Idrija Hg Mine into the Gulf of Trieste
Slika 2: Reke, ki tečejo iz območja nekdanjega idrijskega rudnika Hg v Tržaški zaliv

Bilinski *et al*., 2005). Indeed, the city of Idrija, in Slovenian hinterland, was the second largest Mercury mine in the world. It has closed in the 1980's, but this area continues to deliver considerable quantities of Mercury to the river system and many kilometres downstream to the Northern Adriatic Sea (Figure 2, Hines *et al.*, 1999, Covelli *et al.*, 2001). Moreover, the high concentration in Nickel observed at the site of Izola could also be related to Mercury extraction (Covelli *et al*., 2001, Frančišković-Bilinski *et al*., 2005).

A general direction of the sea currents in the Adriatic Sea is northwards along its Eastern coast (Poulain, 2001). Although, at local level sea current patterns can be very complex, it is likely that *P. oceanica* meadow in Seget Donji is exposed to an incoming current through the passage between Trogir and Čiovo Island (Figure 3). This current would come from nearby Kaštela Bay – the "black spot" of pollution on the Croatian coast. Today considerable funds and effort are invested in the remediation of the area, but consequences of human activities are still present. Anthropogenic Mercury pollution of the sediments and water column is very high due to the chloralkaline plant (PVC), although it stopped production a decade ago (Kwokal *et al*. 2002, Mikac *et al*. 2006). This would explain the high levels of Mercury in *P. oceanica* leaves in Seget Donji.

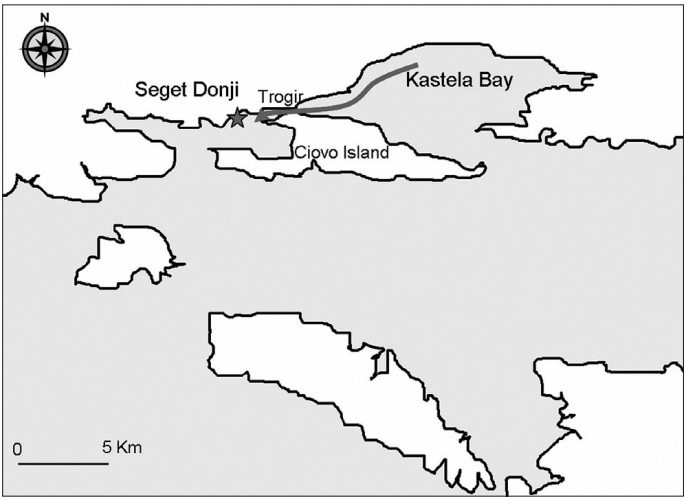


Figure 3: Incoming current from Kaštela Bay to Seget Donji
Slika 3: Morski tok, ki se vije iz Kaštelanskega zaliva proti Segetu Donjem

High Mercury contamination measured in *P. oceanica* at Vlašnik Island (Lastovo Archipelago Natural Park) requires further investigation. Although this area should be considered as not highly impacted by human activities, the nearby small port of Ubli on Lastovo Island – nowadays used for regular daily ferry traffic – was used for decades as a military base. If this could cause high mercury levels, remains to be elucidated.

This preliminary study along the north and east coast of the Adriatic Sea confirms the capability of *P. oceanica* to record trace metal in relation to human-induced pressures as well as to the geological background of the coast (Pergent-Martini *et al*. 1998, Lafabrie *et*

al. 2007) and it highlights two aspects. Indeed, it is interesting to stress that even though immediate activities that yielded Mercury contamination (cinnabar ore in Slovenia, chloralkaline plant in Croatia) ceased to operate decades ago, Mercury is still present in biogeochemical cycling. Moreover, comparing with other sites in the Mediterranean Sea (Grauby *et al*. 1991), the Arsenic contamination values appear very high in the Northeastern Adriatic Sea.

5. SUMMARY

The aim of this study is to evaluate the metallic contamination (seven trace-metals Ag, As, Cd, Cu, Hg, Ni and Pb, as well as the Methyl-Mercury content) of *P. oceanica* leaves at different locations in the Northern and Eastern Adriatic. Samples were collected at six sites, characterised by different types and levels of human-induced pressures, along Slovenian and Croatian coast, from Izola to Vlašnik Island. Concentrations of total and organic Mercury were determined with cold vapour atomic absorption spectrometer (CV-AS – Perkin Elmer®), according to the adapted method of Ferrat *et al*., 2002 and Riisgard and Hansen, 1990. Ag, As, Cd, Cu, Ni and Pb were analysed by atomic absorption spectrometry with quality assurance procedures at the Laboratory of Rouen/ ETSA (France). The level of contamination is low at the pristine site of Lavdara (Ag: 0.10 ± 0.00 μg/g; Cu: 4.73 ± 0.01 μg/g; Hg: 0.054 ± 0.003 μg/g; Met-Hg: 0.015 ± 0.000 μg/g; Ni: 21.40 ± 0.63 μg/g; Pb: 0.90 ± 0.10 μg/g) and higher along the Slovenian coast (Cu: 11.83 ± 0.22 μg/g, Hg : 0.092 ± 0.004 μg/g ; Met-Hg: 0.027 ± 0.004 μg/g; Ni: 40.07 ± 2.08 μg/g, Pb: 1.50 ± 0.06 μg/g) and in Zadar (Ag: 0.83 ± 0.03 μg/g; Cu: 15.17 ± 0.32 μg/g; Hg: 0.066 ± 0.008 μg/g; Pb: 1.50 ± 0.15 μg/g). Generally, Mercury and the associated trace metals contents are relatively high in *P. oceanica* collected in the Northern Adriatic region. This is due not only to the strong anthropogenic impact (ports of Trieste and Koper) but also to the geological characteristics of the hinterland (former cinnabar ore in Slovenia). According to a preliminary quality scale based on values recorded in the North Occidental Mediterranean Sea, the level of contamination in Mercury for Seget Donji is also very high. Indeed, *P. oceanica* meadow at Seget Donji is exposed to an incoming current through the passage between Trogir and Čiovo Island. This current would come from nearby Kaštela Bay – the "black spot" of pollution on the Croatian coast. Anthropogenic Mercury pollution of the sediments and water column is very high due to the chloralkaline plant (PVC), although it stopped production a decade ago. This preliminary study confirms the capability of *P. oceanica* to record trace metal in relation with human-induced pressures, as well as with geological background of the coast.

POVZETEK

Namen pričujoče študije je bil oceniti kontaminacijo (s kovinami Ag, As, Cd, Cu, Hg, Ni in Pb in tudi vsebnost metil živega srebra) bilk pozejdonke *P. oceanica* na različnih krajih

v severnem in vzhodnem Jadranu. Primerki pozejdonke so bili nabrani na šestih lokacijah (značilnih po različnih tipih in ravneh človekovih posegov) vzdolž slovenske in hrvaške obale vse od Izole do otoka Vlašnik. Koncentracije celotnega in organskega živega srebra so bile ugotovljene s hladnim parnim atomskim absorpcijskim spektrometrom (CV-AS – Perkin Elmer®), in sicer po metodi Ferrat *et al*., 2002 in Riisgard in Hansen, 1990. Ag, As, Cd, Cu, Ni in Pb so bili analizirani z atomsko absorpcijsko spektrometrijo in s kakovostnimi postopki Laboratorija v Rouenu/ETSA, Francija).

Raven onesnaženosti je bila nizka na neokrnjeni lokaciji ob otočku Lavdara (Ag: 0,10 ± 0,00 μg/g; Cu: 4,73 ± 0,01 μg/g; Hg: 0,054 ± 0,003 μg/g; Met-Hg: 0,015 ± 0,000 μg/g; Ni: 21,40 ± 0,63 μg/g; Pb: 0,90 ± 0,10 μg/g) in višja ob slovenski obali (Cu: 11,83 ± 0,22 μg/g, Hg : 0,092 ± 0,004 μg/g ; Met-Hg: 0,027 ± 0,004 μg/g; Ni: 40,07 ± 2,08 μg/g, Pb: 1,50 ± 0,06 μg/g) in v Zadru (Ag: 0,83 ± 0,03 μg/g; Cu: 15.17 ± 0.32 μg/g; Hg: 0.066 ± 0.008 μg/g; Pb: 1.50 ± 0.15 μg/g). Na splošno je bila vsebnost živega srebra in z njim povezanimi kovinami razmeroma visoka v pozejdonki *P. Oceanica*, nabrani v območju severnega Jadrana, vendar pa razloga za takšno vsebnost ne gre iskati le v antropogenih vplivih (koprskega in tržaškega pristanišča in nekdanje tovarne PVC pri Segetu Donjem), marveč tudi v geoloških značilnostih zaledja (nekdanja proizvodnja cinobra v Sloveniji). Glede na preliminarno lestvico kakovosti, zabeleženo v severozahodnem Sredozemskem morju, je raven kontaminacije v živem srebru pri Segetu Donjem tudi zelo visoka. Vsekakor pa drži, da je travnik pozejdonke *P. oceanica* pri Segetu Donjem izpostavljen morskemu toku, ki priteka skozi preliv med Trogirjem in otokom Čiovo, in sicer iz Kaštelanskega zaliva, ki je znan kot "črna točka" onesnaževanja v obalnem hrvaškem morju. Antropogeno onesnaževanje usedlin in vodnega stolpca z živim srebrom je visoko zaradi tovarne PVC, pa čeprav je nehala obratovati že pred desetletjem. Pričujoča študija potrjuje sposobnost pozejdonke *P. oceanica*, da beleži sledi kovin, povezanih z antropogenimi vplivi in tudi z obalnim geološkim zaledjem.

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