First assessment of butyltins (BuTs) contamination of the Montenegrin coast (Southeast Adriatic): Tributyltin (TBT) poses a threat to the marine ecosystem

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Abstract

This study presents the first assessment of butyltins (BuTs) pollution of the Montenegrin coast. The distribution of tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) was investigated in mussels, sediments and water overlying sediment after the sediment resuspension. The results showed that the investigated sites (marinas, ports, shipyards) are contaminated with BuTs (19-402 ng(Sn)/g in mussels; 43-20641 ng(Sn)/g in sediments; 9-566 ng(Sn)/L in overlying waters). The measured TBT concentrations indicate that toxic effects on marine organisms are expected at most locations. The simultaneous analysis of BuTs and total Sn in sediment cores allowed the assessment of TBT historical input, and it was demonstrated that resuspension of contaminated sediments leads to the release of all BuTs into the water column. This study shows that, despite the ban of TBT-based antifouling paints more than a decade ago, pollution of the marine environment with TBT is still a problem and regular monitoring remains essential.

Keywords: tributyltin (TBT), butyltins (BuTs), pollution, Montenegrin Adriatic coast, sediments, mussels

Tributyltin (TBT), one of the most toxic anthropogenic pollutants intentionally introduced into the aquatic environment, has been used as biocide in antifouling paints since the 1960s (Hoch, 2001). After its high toxicity to various aquatic non-target organisms was established, the use of TBT in antifouling paints was banned in many countries worldwide (Omae, 2003). In 2001, the International Maritime Organization (IMO) established the AFS Convention (International Convention on the Control of Harmful Antifouling Systems on Ships) (IMO, 2008), calling for a global ban on TBT-based paints from 2003. In Europe, the ban was implemented in 2003 by Regulation (EC) No. 782/2003 and 2008 was set as the deadline for the complete ban of their use. Subsequently, a decrease in TBT levels has been observed in European coastal areas (Cacciatore et al., 2018; Guomundsdóttir et al., 2011; Harrison et al., 2020; Langston et al., 2015; Laranjeiro et al., 2018), but TBT levels have still been above concentrations toxic to biota. In the last decade, high levels of TBT have been reported in European seas: Adriatic Sea (Erdelez et al., 2017; Formalewitz et al., 2019; Furdek Turk et al., 2020; Romanelli et al., 2019), North Sea (Eghart et al., 2017), Baltic Sea (Abraham et al., 2017; Filipkowska et al., 2016; Filipkowska and Kowalewska, 2019), Mediterranean and Atlantic coasts (Anastasiou et al., 2016; Concha-Grana et al., 2021; El Ayari et al., 2018) and in the Arctic (Kucklick and Ellisor, 2019; Metelkova et al., 2022). Recently observed TBT pollution was even worse in parts of the world where the use of TBT-based paints is not yet fully regulated (Bandara et al., 2021; Paz-Villarraga et al., 2015; Quintas et al., 2019). The above literature data clearly show that TBT pollution is still a major environmental problem that is far from being solved more than a decade after attempt for its global ban. However, the ban on the use of TBT never became "global" as the restrictions only applied to signatories of the IMO-AFS Convention (91 member states in 2021; Uc-Peraza et al., 2022), EU member states and countries with specific regulation in that sense. To make matters worse, TBT-based antifouling paints were still being manufactured in the Unites States and available on the market in 2021, even in some countries that are signatories of IMO-AFS Convention (Uc-Peraza et al., 2022).

Once TBT has entered the marine environment, it undergoes various processes such as microbiological and photolytic degradation, bioaccumulation by biota (e.g. gastropods, bivalves, fish) and adsorption to suspended particles (Furdek et al., 2012, 2016). The degradation of TBT occurs both in sediments and in the water column, and consists of a stepwise loss of butyl groups, via dibutyltin (DBT) and monobutyltin (MBT) to the inorganic Sn (Furdek et al., 2016, Furdek Turk et al., 2020). This process in the water column is quite rapid (half-lives of several days to weeks; Rodriguez-Gonzalez et al., 2013), and, therefore,

analysis of seawater provides information on the current level of BuTs in the marine environment. On the other hand, butyltins (BuTs) concentrations accumulated in marine organisms, such as mussels, reflect an average pollution of a water column over a longer period of time of about several months (Furdek et al., 2012). Due to its hydrophobic characteristics, TBT has an affinity to adsorb onto the suspended particles in the water column, which settle in the sediments over time. Once in the sediment, TBT degrades slowly, with half-lives of several years to decades (Furdek et al., 2016; Omae, 2003); therefore, TBT content in sediments could provide information on TBT input over a long period of time, from several years to decades. Not only BuTs are highly persistent in sediments, but they can also be released back into bottom waters by diffusion or resuspension of sediments, making sediments a potential source of new pollution to the water column (Furdek Turk et al., 2020; Hoch and Schwesig, 2004; Ruiz et al., 2008). Nevertheless, the field evidence of desorption of BuTs from contaminated sediments are rare (Furdek Turk et al., 2020). Therefore, it is important to determine the status of BuTs pollution of coastal areas not only in the water column but also in sediments, and to assess the risk of BuTs desorption from the contaminated sediments.

The level of TBT pollution in the Adriatic Sea (the northernmost arm of the Mediterranean Sea) has been assessed in several studies, covering the coastal waters of Croatia (Erdelez et al., 2017; Furdek et al., 2012; Furdek Turk et al., 2020), Slovenia (Formalewicz et al., 2019; Milivojevič Nemanič et al., 2009) and Italy (Berto et al., 2007; Formalewicz et al., 2019; Romanelli et al., 2019). All these studies showed that TBT pollution is a significant environmental problem in coastal areas. Regular monitoring of the priority pollutants in Croatian coastal waters carried out under the Water Framework Directive (WFD; Directive 2000/60/EC, 2000) also revealed that TBT levels in water are frequently above the prescribed maximum annual average concentration (0.2 ng/L) (Furdek Turk et al., 2019). However, the level of TBT pollution in the southern part of the Eastern Adriatic that belongs to Montenegro has never been investigated and there are no data on the status of BuTs in this area. There is only one data set for the port of Bar from 2014, when the total BuTs in sediments ranged from 18 to 33 ng(Sn)/g (Romanelli et al., 2019).

The Montenegrin coastline has a length of 294 km, of which 105 km belong to the large fjord-like Boka Kotorska Bay, which is naturally divided into several smaller bays (Kotor, Risan, Tivat and Herceg Novi). The entire coast is under high anthropogenic pressure due to urbanisation and discharge of municipal wastewaters, maritime activities (marine traffic, marinas, shipyards), fishing and tourism (Joksimović et al., 2017, 2020). The studies on the

distribution of ecotoxic metals in mussels, sediments and seagrass (Jokanović et al., 2021; Joksimović et al., 2011, 2020; Radmirović et al., 2021; Stanković et al., 2015) have shown that most of the investigated sites, especially shipyards and urban areas, are contaminated with metals due to significant anthropogenic pressure.

The aims of this work were: i) to assess the level of BuTs pollution along the Montenegrin coast by studying BuTs distribution in mussels and sediments; ii) to assess the extent of BuTs desorption from contaminated sediments into the water column caused by sediment resuspension; and iii) to assess the historical input of BuTs along the Montenegrin Adriatic coast by studying the relationship between total Sn and BuTs in sediments.

Sampling locations included various areas along the Montenegrin coast which are affected by intensive shipping and industrial activities, such as marinas, shipyards and municipal and local ports. Samples of mussels *Mytilus galloprovincialis* (Lamarck, 1819) and sediments were collected in July 2015 from 16 sampling sites, covering the areas of Boka Kotorska Bay (namely, Kotor Bay and Tivat Bay) and the towns of Budva and Bar (Fig. 1; Table S1). In the Kotor Bay samples were collected at 5 locations (Kot1, Kot1a, Kot2, Kot3, Kot3a) near the town of Kotor, a tourist town with a marina, cruise port and a number of small ports. In the Tivat Bay, the samples were taken at the marina Porto Montenegro in the town of Tivat (Tiv1, Tiv2, Tiv3) and in front of the Bijela shipyard (TivB). In Budva (tourist town), samples were taken at 4 locations - in the marina (Bud1, Bud3, Bud4) and in the local port (Bud1a). In the town of Bar, one of the largest cargo, passenger and military ports on the Eastern Adriatic, samples were taken at 3 locations, including the cargo (Bar1), military port (Bar4) and the marina (Bar3). A brief description of each sampling site is given in Table S1 in the Supplementary Information. At sites labelled with "a" (which were a short distance from the site labelled with the same number) only mussels were collected. A total of 81 samples, including 14 samples of mussels and 67 samples of sediment obtained by cutting sediment cores (9 cores; 10-20 cm long, cut into 2 cm layers), were analysed for BuTs (i.e. MBT, DBT and TBT). In addition, bottom waters, i.e. water overlying the sediment (9 samples), were collected with a gravity corer to investigate the desorption of BuTs caused by sediment resuspension. The water overlying sediment in the gravity corer was transferred to a dark glass bottle after sediment particles had settled (approximately 15 min after sediment was taken out of the sea with the gravity corer), acidified to pH=2 with nitric acid (HNO_3) and stored in the dark at 4°C until analysis. Mussels (30 specimens per sampling site, with a shell length of 3 to 6 cm) were collected from the docks, rocky shores or boat strings. The shells were removed

and the soft tissue homogenised in a blender and later freeze-dried and stored at -20°C until analysis. Sediments were collected using a UWITEC gravity corer (Umwelt-und Wissenchaftstechnik, Austria) and frozen immediately after collection. The sediment cores were cut into 2 cm layers, freeze-dried, homogenised by milling and stored at -20 °C until analysis.

The analytical methods used for the analysis of BuTs (TBT, DBT and MBT) in seawater and mussels are described in Furdek et al. (2012), while that used for the analysis of sediments is described in Furdek et al. (2016). Briefly, BuTs were extracted from 300 ml of seawater, while simultaneous extraction and derivatisation were performed in a sodium acetate-acetic acid buffer (pH=4.8) by adding sodiumtetraethyl borate ($NaBEt_4$, 1% (w/v)) and hexane and mechanical shaking for 30 min at 350 rpm. Butyltins from sediments were extracted from 0.5-2 g of sample by acetic acid and ultrasound-assisted agitation (30 min, 55 Hz), while BuTs from mussel were extracted from 0.5-1 g of lyophilised tissue with 0.1 mol/L hydrochloric acid (HCl) in methanol using ultrasound-assisted agitation (30 min, 55 Hz). For both sediments and mussels, the derivatisation of compounds in the extract was performed in sodium acetate-acetic acid buffer (pH=4.8) with $NaBEt_4$ (1% (w/v)) and transferred into hexane by mechanical shaking at 350 rpm for 30 min. Analyses of BuTs in seawater were carried out on a gas chromatograph (GC Agilent 6890, Agilent Technology) coupled with inductively coupled plasma mass spectrometry (ICP-MS Agilent 7900, Agilent Technology), while detection of BuTs in mussels and sediments was performed using gas chromatograph (GC Varian CP 3800, Varian) with pulsed flame photometric detector (PFPD, Varian). Tripropyltin (TPrT) was used as an internal standard and quantification was performed by applying the standard addition calibration method. Quality control of the analytical procedure for the determination of BuTs in mussels and sediments was performed by analysing the certified reference materials (CRM) for mussels (CE 477, ERM, European Commission, Geel, Belgium) and marine sediment (PACS 2, NRC-CNRC, Canada). In addition, the accuracy of all three methods was further confirmed by successful participation in the proficiency tests. The detection limits for TBT, DBT and MBT were 0.1-0.5 ng(Sn)/L for seawater, 0.9-5.1 ng(Sn)/g for mussels and 0.9-6.1 ng(Sn)/g for sediment. Total Sn in sediments was analysed according to the method described in Furdek Turk et al. (2020) using high resolution inductively coupled plasma mass spectrometry (HR-ICPMS; Element 2, Thermo Finnigan). Sediments (0.1 g) were digested in a microwave oven (Multiwave 3000, Anton Paar) by two-step total digestion procedure HNO_3 (65% *s.p.*) + HCl (36% *s.p.*) + hydrofluoric acid (HF, 48% *s.p.*)), followed by boric acid

(H_3BO_3, 40 g/L). The quality control was performed by simultaneous measurements of certified reference materials for marine sediment (MESS-3, NRC-CNRC, Canada) for which satisfactory results were obtained.

The BuTs pollution of the water column was not detected directly, but indirectly by determining the accumulated BuTs in mussels. Indeed, mussels are a reliable biomonitor of TBT pollution as they are sessile and filter-feeding organisms, with a great ability to bioaccumulate TBT from water and a limited ability to metabolise it (Magi et al., 2008). The TBT concentration detected in mussels reflects the ambient TBT level during the last months (2-3 months; Salazar and Salazar, 1996) because during this period an equilibrium is reached between uptake from the water (i.e. accumulation), metabolism of TBT to DBT and MBT, and excretion of these compounds back into the water (Gomez-Ariza et al., 1999; Magi et al., 2008). Therefore, TBT levels measured in mussels are a reliable indicator of recent pollution in the water column over the last few months, in contrast to levels measured in seawater that only indicate the momentary level of pollution. The concentrations of BuTs (∑BuTs=MBT+DBT+TBT) in mussels varied from 19 to 402 ng(Sn)/g (Table 1, Fig. 2A); concentrations of DBT and TBT were of the same order of magnitude (10-173 ng(Sn)/g for DBT and 6.7-179 ng(Sn)/g for TBT), while MBT was the least abundant compound (<5-50 ng(Sn)/g). In all investigated marinas (Kot1a, Tiv1, Tiv2, Tiv3, Bud1 and Bar3), ∑BuTs were above 100 ng(Sn)/g, while the highest concentration (402 ng(Sn)/g) was found in mussels from the marina Bar3. At all locations, TBT and DBT account for 30-55% and 35-58% of the total BuTs, respectively (Fig. 2B). High concentrations and proportions of TBT in mussels reflect recent inputs of TBT, but may also be a consequence of the limited ability of mussels to metabolise TBT, as bivalves do not have an efficient detoxification system (Chandrinou et al., 2007; Diez et al., 2005; Lee, 1996). However, significant correlations between TBT and DBT ($r=0.912$, $p<0.05$, Spearman) suggest that DBT in mussel tissues may be the product of TBT metabolism, while lower but still significant correlation between TBT and MBT ($r=0.693$, $p<0.05$, Spearman) and DBT and MBT ($r=0.616$, $p<0.05$, Spearman) suggests that DBT could be eliminated from the organism before being degraded to MBT, or that it is metabolised more slowly compared to MBT (Furdek et al., 2012). In conclusion, the measured BuTs concentrations in mussels indicate that TBT pollution in seawater has been present at all investigated sites along the Montenegrin coast in recent months.

The spatial and depth distributions of BuTs in sediments were highly variable (Fig. 3A, Fig. 4, Table 1), with ranges encompassing 3-4 orders of magnitude (<9-20641 for BuTs; <1-14900

for TBT; <2-4279 for DBT; <6-3651 for MBT, expressed in ng(Sn)/g). The highest concentrations of BuTs were found in the sediments sampled in front of the Bijela shipyard (20641 ng(Sn)/g), while at all other sites maximum concentration did not exceed 2000 ng(Sn)/g (Table 1). TBT was the prevailing compound at all sites, accounting for up to 75% of all BuTs (Table 1). The depth profiles of BuTs in 9 sediment cores from different locations were highly variable, both in terms of the level of concentrations and the depth pattern (Fig. 4). Sediments in the city of Kotor (Kot1 and Kot2) may be considered as not polluted with TBT (Fig. 4); the concentrations of ∑BuTs were up to 35 ng(Sn)/g and were mainly in the form of TBT degradation products, i.e. DBT and MBT. In both sediment cores TBT was detected in only one layer (0-2 cm – Kot1 and 10-12 cm – Kot2). The sediment inside the marina in the town of Tivat (Tiv2) was heavily contaminated with BuTs throughout the core (up to 600 ng(Sn)/g), with a high proportion of TBT in each layer (30-40%). In contrast, in the sediment sampled outside the marina (Tiv3), BuTs concentrations were much lower, although the high proportion of TBT (up to 45%) was also detected in the deeper layers. The sediment sampled in front of the Bijela shipyard (TivB) was extremely contaminated; the BuTs concentrations in the upper 12 cm did not fall below 6500 ng(Sn)/g, while the maximum concentration of 20641 ng(Sn)/g was detected in the subsurface layer. In all layers, TBT was the prevailing compound and accounted for 50-75% of the total BuTs. The BuTs depth distributions in two sediment cores sampled within the same marina in the city of Budva (Bud1 and Bud3) were different, although these sites are only 100 m apart. While in the sediment core Bud1 only the surface sediment layers were slightly contaminated with BuTs, the sediments sampled closer to the service hoist (Bud3) were heavily contaminated to a depth of 12 cm, with BuTs concentrations up to 1180 ng(Sn)/g. In the city of Bar, the sediments of the marina (Bar3) and the military port (Bar4) were significantly contaminated with BuTs (concentrations of up to 1980 ng(Sn)/g), with the highest pollution observed in the deeper layers, where not only the concentrations were high, but also the percentage of undegraded TBT (up to 75%).

Based on the depth profiles obtained, several patterns of BuTs behaviour could be observed (Fig. 4). First, BuTs concentration profiles do not follow the same depth pattern; in some sediments BuTs decreased with depth, while in others concentrations were similar or vary throughout the depth of the core. Such different depth distributions may indicate different TBT inputs over time or different BuTs degradation efficiencies in different sediments. Since different BuTs depth distributions were observed in the sediments from marinas of similar size and similar intensity of marine traffic (such as Bud1, Bar3, Kot1 and Tiv2), the most likely

explanation is that the observed depth profiles are the result of different persistence of TBT in these sediments. It was demonstrated that sediments characteristics, namely grain size and organic matter, strongly affects the degradation process of TBT and its persistence in sediments (Furdek et al., 2016; Furdek Turk et al., 2020). Organic matter plays a crucial role as a TBT sorbent in sediments and, therefore, influences TBT degradation rate by determining the bioavailable fraction in porewater where TBT degradation occurs (Furdek at al., 2016). This means that, in sediments rich in organic matter, TBT will be less available to degradation and, consequently, more persistent over time (Furdek at al., 2016). Secondly, at most locations TBT degradation products, i.e. MBT and DBT, are the prevailing BuT compounds through the entire depth cores, which could indicate efficient degradation of TBT in these sediments. An exception is the sediments sampled in Bijela shipyard (TivB) and in two layers of cores Tiv2 and Bar4, where the percentage of TBT/∑BuTs was more than 50% (Fig. 4). Although the proportions of DBT and MBT prevail over TBT, their accumulation in the deeper layers was not observed in any sediment core. There are two explanations for this: i) DBT and MBT, being less hydrophobic compounds, are more easily desorbed from sediments than TBT (Poerschmann et al., 1997), and ii) the degradation of DBT and MBT to inorganic Sn is efficient in both oxic and anoxic sediments. Indeed, Furdek et al. (2016) demonstrated that the rate of DBT degradation in sediments is faster than the rate of its formation from TBT, which prevents accumulation of DBT in sediments, and that MBT, after desorption from sediment to anoxic porewater, is almost immediately degraded to inorganic Sn.

However, a reliable assessment of the long-term behaviour, persistence and historical input of TBT can only be made by simultaneously determining BuTs and total Sn in sediments (Furdek Turk et al., 2020). First, it should be confirmed that the inorganic Sn contamination originates mainly from TBT-antifouling paints and not from other possible sources such as municipal and industrial wastewater or landfill leachates. Namely, inorganic Sn is used in products such as food cans, welding equipment, electronics, and household products (Pougnet et al., 2014). Inputs of other organotin compounds such as mono- and disubstituted BuTs (often used as PVC stabilizers) and phenyltins (used as pesticides, while triphenyltin (TPhT) is also used as a biocide in antifouling paints) (Omae, 2003) could also lead to the formation of inorganic Sn. However, in Europe TPhT is used to a much lesser extent than TBT (Lagerstrom et al., 2017), which is confirmed by the data on TPhT contamination of the Croatian Adriatic coast, where only sporadic low levels of TPhT have been detected in mussels (Furdek et al., 2012) and sediments (Furdek et al., 2016).

To investigate the source of Sn contamination and confirm that inorganic Sn originates mainly from the degradation of TBT, we examined the relationships between BuTs and inorganic Sn in the sediments by determining the total Sn depth profiles and calculating butyltin degradation index (BDI), following the approach presented in previous studies (Furdek Turk et al., 2020; Pougnet et al., 2014). Total Sn concentrations ranged from 3.8 to 46.5 µg/g (Fig. 4) and were higher than the background levels of Sn, obtained by the analysis of older uncontaminated sediments of the Boka Kotorska Bay (2.8 µg/g) and the coastal zone of Montenegro (1.7 µg/g) (Mikac et al., 2022). The degree of Sn contamination was assessed by enrichment factors ($EF=(Sn/Al)_{sample}/(Sn/Al)_{background}$), according to the scale proposed by Sutherland (2000): EF<2 indicates no or minimal, EF=2-5 moderate, EF=5-20 significant, EF=20-40 very high, and EF>40 extreme enrichment or contamination. The calculated EFs showed that all sediments studied were enriched in Sn; the sediments from Kotor Bay (Kot1, Kot2) and Tivat Bay (Tiv1, Tiv2, TivB) were significantly contaminated with Sn (EFs between 5 and 20), while the sediments from Budva (Bud1, Bud3) and Bar (Bar3, Bar4) had EFs between 2 and 12, indicating moderate contamination with Sn.

To investigate the source of Sn contamination, we examined the relationship between TBT and its degradation products (DBT, MBT and inorganic Sn) by calculating the BDI index, which is a commonly used tool to study TBT degradation efficiency. We calculated the BDI using two approaches: i) the commonly known approach, which considers only DBT and MBT as TBT degradation products; BDI = (MBT+DBT)/TBT (Garg et al., 2009), and ii) a modified approach proposed by Pougnet et al. (2014), which considers the inorganic Sn as the final product of total degradation; $BDI_{mod} = (Sn_{inorganic}+MBT+DBT)/TBT$, while the $Sn_{inorganic}$ could be calculated as $Sn_{total}-Sn_{background}-\sum BuTs$. A stronger correlation between TBT and BDI_{mod} ($r=0.961$; $p<0.01$, Spearman) than between TBT and BDI ($r=0.722$, $p<0.05$, Spearman) was observed when combining samples from all sediment cores. This indicates that most of the TBT has been completely degraded to inorganic Sn, while the anthropogenic fraction of inorganic Sn mainly originates from the degradation of TBT released from antifouling paints.

Considering the above conclusion that most of the inorganic Sn originates from TBT antifouling paints in the studied environments, we used BuTs and total Sn depth distributions to explain the historical input and behaviour of TBT in the investigated sediments and to clarify whether the observed BuTs depth distributions are the result of different TBT input over time or different TBT persistence in sediments. From the observation of the determined depth profiles (Fig. 4) the following conclusion are drawn. The total Sn depth profiles in the Kotor

Bay (Kot1 and Kot2) show high (EF=8-18) and continuous input of Sn over time, while the ∑BuTs accounts for less than 0.5% of the total Sn at all depths, indicating that the low TBT and BuTs levels are a consequence of an efficient TBT degradation rather than low TBT input over time. In the marinas of Budva (Bud1, Bud3) and Tivat (Tiv2, Tiv3) all cores are similarly contaminated with Sn (EF=3-10) (except for a few layers where EF is higher), indicating a comparable input of TBT over time. However, the percentage of $\sum BuTs/Sn_{total}$ in cores Tiv3 and Bud1 (<1%) is much lower than in cores Tiv2 and Bud3 (1-18%), indicating less efficient TBT degradation in the later cores. In the sediment cores from Bar (Bar3, Bar4) the total Sn input is generally lower (EF=2-7) compared to the other sites, but $\sum BuTs/Sn_{total}$ was high (4-20%), indicating high persistence of TBT in these sediments, especially in the sediments from marina (Bar3), where the highest level of BuTs in mussels were also found. In the military port (Bar4) the absence of BuTs in the surface sediments suggests that TBT has not been introduced recently; however, the elevated total Sn and the relatively high TBT levels in mussels indicate the opposite, namely that there was a recent input of TBT and its efficient degradation to inorganic Sn in the surface sediments. By contrast, the high level of TBT in the bottom layer of this core indicates poor degradation of TBT in older sediments. After all, the observed depth profiles demonstrated less efficient TBT degradation in cores Tiv2, Bud3 and Bar3 that in the other sediment cores analysed. Finally, the sediment core TivB is clearly different from all the other cores; not only BuTs and total Sn concentrations (EF=15-20) are much higher than in the other sediments, but also the proportions of TBT/∑BuTs and $\sum BuTs/ Sn_{total}$ are very high (50-75% and 20-65%, respectively). As this core was taken near the shipyard, these results could be explained by the presence of paint particles containing TBT in the form that is not available to degradation (Furdek Turk et al., 2020).

It should be emphasized that the proportion of $\sum BuTs/Snt_{otal}$ was generally not higher than 10% in any of the sediments studied (excluding the sediment from the shipyard), which may be surprising considering that the degradation of TBT in sediments is a slow process ($t_{1/2}$ = several years to decades; Omae, 2003). The same was found by Furdek Turk et al. (2020), who discussed that most of the TBT leached from ships is degraded to inorganic Sn already in the water column ($t_{1/2}$ = several days to weeks; Rodriguez-Gonzalez et al., 2013), so that only a small fraction of TBT (~10%) reaches the sediments. Therefore, a reliable assessment of TBT input in the time period covered by the sediment cores can only be performed by taking into account the total amount of Sn; otherwise, the total TBT input to the certain environment could be underestimated (at least by a factor of ten).

TBT pollution of sediments is not only a problem because it poses a threat to the benthic organisms living there; the big problem lies in the fact that contaminated sediments are a potential source of BuTs pollution for the water column. Although it has been experimentally proven many times that BuTs can be desorbed from sediments (Berg et al., 2001; Burton et al., 2004), this has been demonstrated only in a few field studies (Chen et al., 2022; Furdek Turk et al., 2020). However, knowledge about the extent to which contaminated sediments act as a source of BuT pollution is still incomplete. To investigate the behaviour of BuTs during the resuspension of disturbed sediments, we analysed the water overlying the sediment in the gravity corer. It was assumed that the penetration of the gravity corer into the sediment would cause resuspension of fine particles from the sediment surface, which could mimic, to some extent, the resuspension of surface sediments caused by waves or bioturbation. The BuTs concentrations in the water above the sediment (Table 1, Fig. 5A, B) showed a predominance of MBT (8.8-277 ng(Sn)/L) and DBT (0.2-130 ng(Sn)/L) over TBT (<0.1-160 ng(Sn)/L), which was found only at two sites (Tiv2 and TivB) where the surface sediments contained more than 50% of BuTs in the form of TBT (Fig. 3B). As these concentrations are much higher than those normally found in the water column and porewater (<10 ng(Sn)/L; Furdek et al., 2012; Furdek Turk et al., 2020), we assume that most of the detected BuTs are desorbed from the sediments into the overlying water. These data support the results of a similar study conducted with contaminated coastal sediments in Croatia (Furdek Turk al., 2020). Both studies showed that contaminated sediments are a potential source of BuTs to the water column, especially MBT and DBT as less hydrophobic and weakly adsorbed compounds that can be released from the sediments more easily than TBT. However, this study has shown that resuspension of sediments can also lead to the release of TBT, especially from contaminated sediments with high levels of TBT in the surface layers.

An assessment of the extent of BuTs contamination in the coastal environment of Montenegro was performed by comparing BuTs and TBT levels determined in different coastal areas of Europe during the last decade (Table 2). The comparison of literature data given in Table 2 showed that the Eastern Adriatic coast (Croatian and Montenegrin) is highly contaminated with BuT compounds. However, the level of TBT pollution strongly depends on the type of sampling locations (marina, port, beaches) and is related to the intensity of marine traffic (Briant et al., 2013; Suzdalev et al., 2015), and, therefore, this conclusion should be taken with caution as it may be influenced by different sampling strategies of the presented studies and prevalence of more contaminated sites along the Croatian coast. In the Water Framework

Directive (WFD; Directive 2000/60/EC, 2000) TBT is listed as one of the priority substances that should be monitored to assess the chemical status of a water body, but within this Directive TBT presence in the environment is regulated only by the prescribed maximum allowed concentration in seawater (the annual average should be less than 0.2 ng/L). However, several regional conventions, such as OSPAR (Convention for the Protection of the Marine Environment of the North-East Atlantic; OSPAR, 2004) and HELCOM Conventions (Convention on the Protection of the Marine Environment of the Baltic Sea Area; HELCOM, 2018), have established Environment Assessment Criteria (EAC) for the assessment and monitoring of the environmental status of regional waters, which could be applied for assessments based on the measurement of TBT in sediments and biota. OSPAR (OSPAR, 2004) has established EAC for TBT in mollusks; the lower EAC (EAC_{low} = 4.9 ng(Sn)/g) is defined as the threshold for protection of all marine species from chronic effects and the upper EAC (EAC_{high} = 72 ng(Sn)/g) is defined as the highest level at which no acute toxic effects are expected. The TBT concentrations quantified in mussel tissues were above the EAC_{low} at all sites, while they were above the EAC_{high} at site Bar3, indicating that chronic effects for marine organisms sensitive to TBT are to be expected at all locations. The assessment of TBT pollution based on the TBT concentrations quantified in sediments was carried out according to the Norwegian environmental quality classification system defined for management purposes (Bakke et al., 2010); Background: <0.4 ng(Sn)/g, Good: 0.4-2.1 ng(Sn)/g, Moderate: 2.1-8.3 ng(Sn)/g, Poor: 8.3-41.7 ng(Sn)/g, and Very Poor: >41.7 ng(Sn)/g. At seven (out of nine) sites investigated surface sediments are contaminated with TBT; Kot1 and Bud1 were moderately contaminated with TBT, while the environmental status of Tiv3 and Bud3, as well as Tiv2, TivB and Bar3, can be evaluated as poor and very poor, respectively, indicating that TBT is expected to have toxic effects on marine organisms and the occurrence of imposex in gastropods is very likely at these locations (Erdelez et al., 2017; Laranjeiro et al., 2018).

The presented results show that the investigated areas on the Montenegrin coast are polluted with TBT, which indicates the need for further monitoring of the coastal area. Apparently, TBT-based antifouling paints, despite of its ban, have been used within a few months of sampling (as shown by BuTs levels measured in mussels), but also over a longer period of time (as shown by BuTs levels measured in sediments). The main source of pollution could be small local and leisure boats that are not subject to any control of antifouling paint applied. Therefore, they may be illegally painted with TBT-containing paints, for which is shown that were still present on the global market in 2021 (Uc-Peraza et al., 2022) or can have old layers of TBT-

based paint that are not properly removed from the boat (Lagerström et al., 2017, 2019). Indeed, a recent study in the Nordic countries has demonstrated the presence of TBT in the subsurface paint layers of all investigated leisure boats (Lagerström et al., 2017, 2019) and shown that they are source of TBT pollution. The results have also shown that resuspension of contaminated sediments may cause pollution of the water column, especially at the Bijela shipyard (TivB) where remediation measures are strongly recommended. Recently, extreme contamination with ecotoxic metals (Mikac et al., 2022; Radomirović et al., 2021) and significantly altered microbial community have been demonstrated in sediments sampled near this shipyard (Jokanović et al., 2021).

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Figures caption

Figure 1. Sampling locations along the Montenegrin Adriatic coast.

Figure 2. Distribution of BuTs in mussel tissue: (a) MBT, DBT and TBT concentrations, and (b) percentage of each chemical species (BuT/∑BuTs).

Figure 3. Distribution of BuTs and total Sn in surface sediments: (a) MBT, DBT, TBT and Sn concentrations, and (b) percentage of each BuT species (BuT/∑BuTs) along with proportion of $\sum BuTs/Sn_{total}$. The absence of bars for BuTs means that they were not detected – concentrations <LOD.

Figure 4. Depth distributions of butyltins (MBT, DBT, TBT and ∑BuTs) and total Sn in sediment cores along with proportions of $\sum BuTs/Sn_{total}$. The absence of bars for BuTs means that they were not detected – concentrations <LOD.

Figure 5. Distribution of BuTs in water overlying sediment: (a) MBT, DBT and TBT concentrations, and (b) percentage of each BuT species (BuT/∑BuTs).

Table 1. Summarizing statistic for butyltins (MBT, DBT, TBT, ∑BuTs) concentrations and proportions of TBT/∑BuTs and ∑BuTs/Sn_{total} in different types of samples collected along the Montenegrin Adriatic coast.

	Concentration (ng(Sn)/g; ng(Sn)/L)				Proportion (%)	
	MBT	DBT	TBT	∑BuTs	TBT/∑BuTs	∑BuTs/Sn_{total}
			Mussels			
min	<5	10.4	6.7	19.7	28.2	-
max	50.7	173	179	402	55.5	-
average	15.1	43.0	40.6	98.6	39.1	-
median	11.1	30.6	25.9	67.8	39.3	-
			Sediment cores (all depths)			
min	<6	<2	<1	<9	-	0.01
max	3651	4279	14900	20641	75.4	62.0
average	248	288	829	1365	25.9	6.09
median	20.8	14.1	12.4	43.1	17.2	0.43
max*	650	445	1304	1979	74.8	26.5
average *	62	41	85	188	22.0	2.37
median*	11.1	3.9	0.5	31.2	11.1	0.10
			Water overlaying sediment			
min	8.8	0.2	<0.1	9.1	<0.2	-
max	277	130	160	566	28.2	-
average	91.3	52.4	24.4	168	6.0	-
median	38.2	58.9	0.3	80.4	0.4	-
max*	219	114	58	333	22.9	-
average *	68.1	42.7	7.5	118	3.2	-
median*	29.0	40.9	0.3	70.9	0.4	-

*without samples from Bijela shipyard

Table 2. Literature data on butyltins (BuTs=TBT+DBT+MBT) and tributyltin (TBT) concentration in seawater, sediment and biota from the coastal areas of Europe reported in the last decade.

Area and year of sampling	Seawater (ng(Sn)/L)	Sediment (ng(Sn)/g)	Biota (ng(Sn)/g)	Reference
Southeast Adriatic coast (Montenegro); 2015	-	BuTs: <9-2000 (20641)* TBT: <1-1300 (14900)*	BuTs: 20-400 TBT: 7-179	This study
East Adriatic coast (Croatia); 2010-2012	BuTs: 0.5-28 TBT: <0.2-15	BuTs: 3-1362 (66345)** TBT: 1-790 (32317)**	Mussels BuTs: <10-1676 TBT: <10-1045	Furdek et al. (2012); Furdek Turk et al. (2020)
East Adriatic coast (Croatia), 2015-2017	TBT: <0.2-6.3	TBT: <1-92	Gastropoda BuTs: 1.9-155 TBT: 0.5-59	Furdek Turk et al. (2019) ; Erdelez et al. (2017)
East Adriatic coast (Slovenia, Croatia, Montenegro, Albania); 2014	TBT: <2	BuTs: 7-731 TBT: 3-566	Mussels BuTs: 25-220 TBT: 20-170	Romanelli et al. (2019)
West Adriatic coast (Italy); 2014	TBT: <2	BuTs: 7-240 TBT: 2-186	Mussels BuTs: 25-100 TBT: 60-75	Romanelli et al. (2019)
North Adriatic coast (Italy, Slovenia); 2016	-	BuTs: 121-352 TBT: 53-174	-	Formalewicz et al. (2019)
North Sea (Sweden); 2014	-	BuTs: 10-500 TBT: 10-300	-	Egardt et al. (2017)
Baltic sea (Lithuania); 2010-2012	-	TBT: 1-5200	-	Suzdalev et al. (2015)
Baltic Sea (Poland); 2014-2015 (fish), 2018 (sediment)	-	BuTs: 5.7-3321 TBT: 3.7-1942	Fish muscle BuTs: nd-715 TBT: nd-503	Filipkowska et al. (2016); Filipkowska and Kowalewska (2019)
Baltic Sea (Poland); 2012-2013	-	BuTs: nd-22	Mussels BuTs: 41-164 TBT: 11-103	Filipkowska and Lubecki (2016)
Baltic Sea (Germany); 2015	-	BuTs: 100-1426 TBT: 2.5-380	-	Abraham et al. (2017)
Mediterranean (France); 2009, 2011, 2013	Porewater BuTs: 40-600 TBT: 0.1-70	BuTs: 8-16000 TBT: nd-10700	-	Briant et al. (2013, 2016)
Mediterranean and Atlantic coast (Portugal, Italy, Turkey); 2012	-	BuTs: 8-200 TBT: 3-75	Gastropoda BuTs: 30-720 TBT: 12-172	Anastasiu et al. (2016)
Mediterranean and Atlantic coast (Spain); 2015	BuTs: nd-20 TBT: nd-17	BuTs: nd-100 TBT: nd-75	-	Concha-Grana et al. (2021)
Barents Sea (Russia); 2019	-	BuTs: 28-83 TBT: 2-24	Bivalves BuTs: nd-50 TBT: nd-38	Metelkova et al. (2022)

*including samples from shipyard Bijela; ** including samples from shipyard and service hoists

Figure 1. Sampling locations along the Montenegrin Adriatic coast.

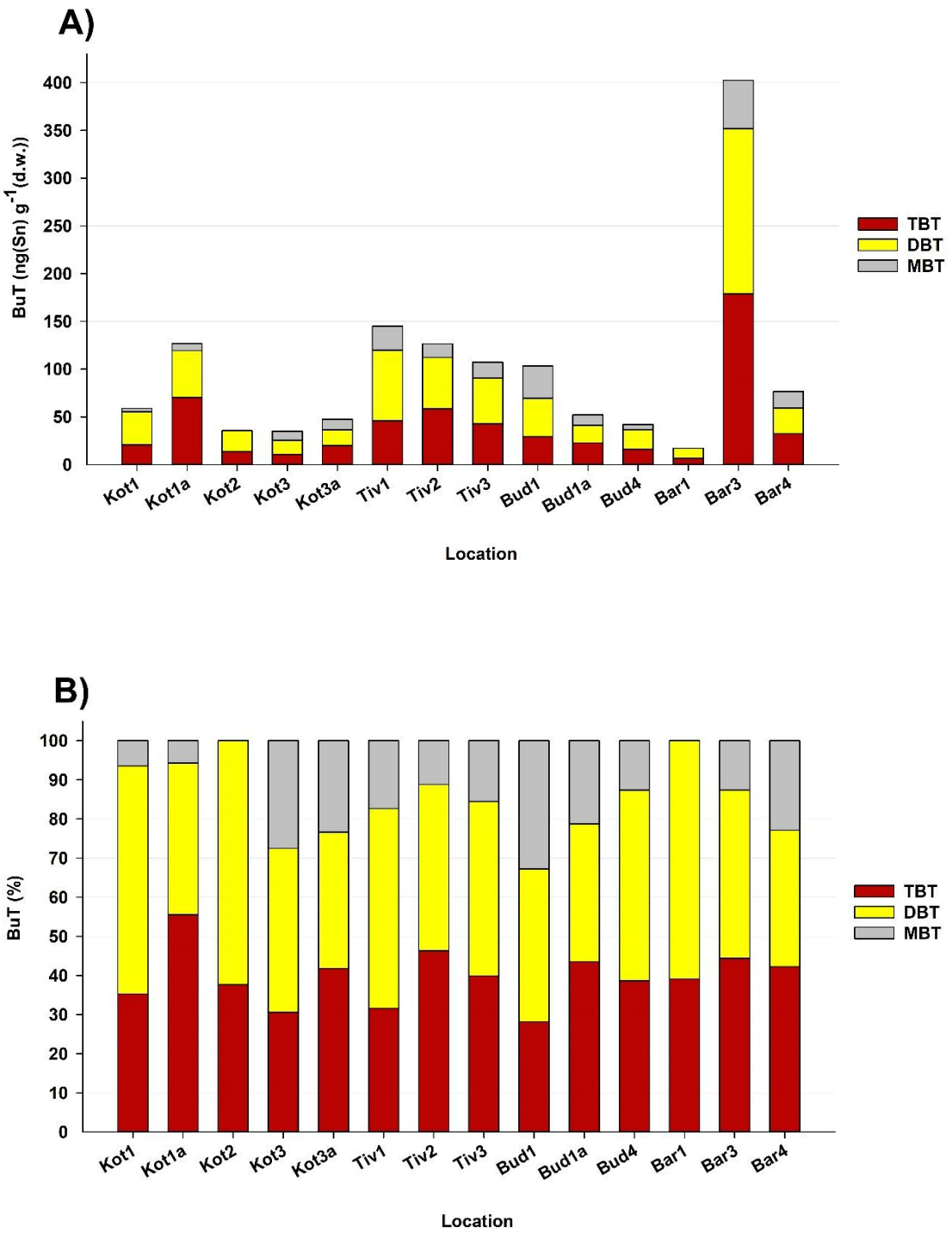

Figure 2. Distribution of BuTs mussel tissue: (**a**) MBT, DBT and TBT concentrations, and (**b**) percentage of each species (BuT/∑BuTs).

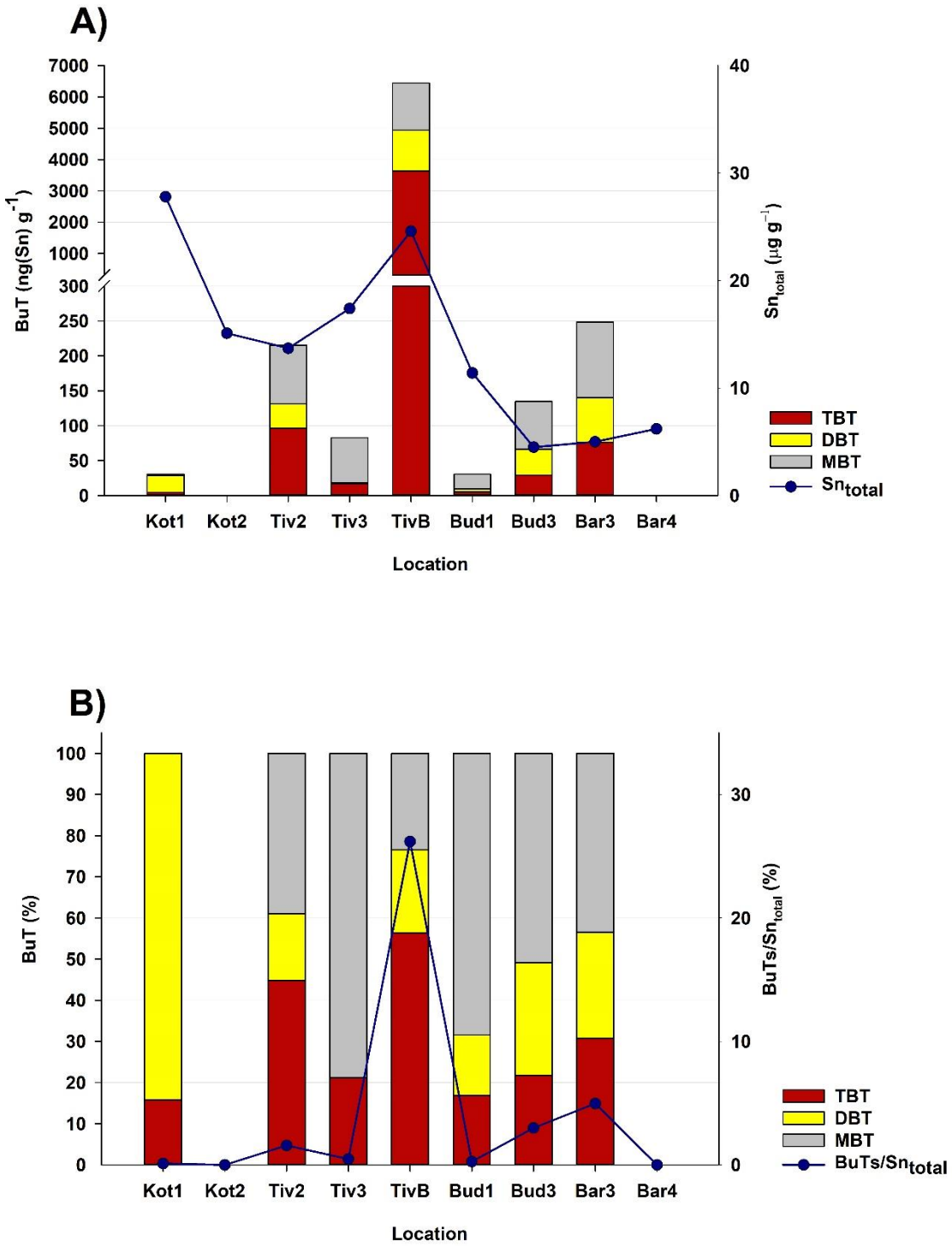

Figure 3. Distribution of BuTs and total Sn in surface sediments: (**a**) MBT, DBT, TBT and Sn concentrations, and (**b**) percentage of each BuT species (BuT/∑BuTs) along with proportion of $\sum BuTs/Sn_{total}$. The absence of bars for BuTs means that they were not detected – concentrations <LOD.

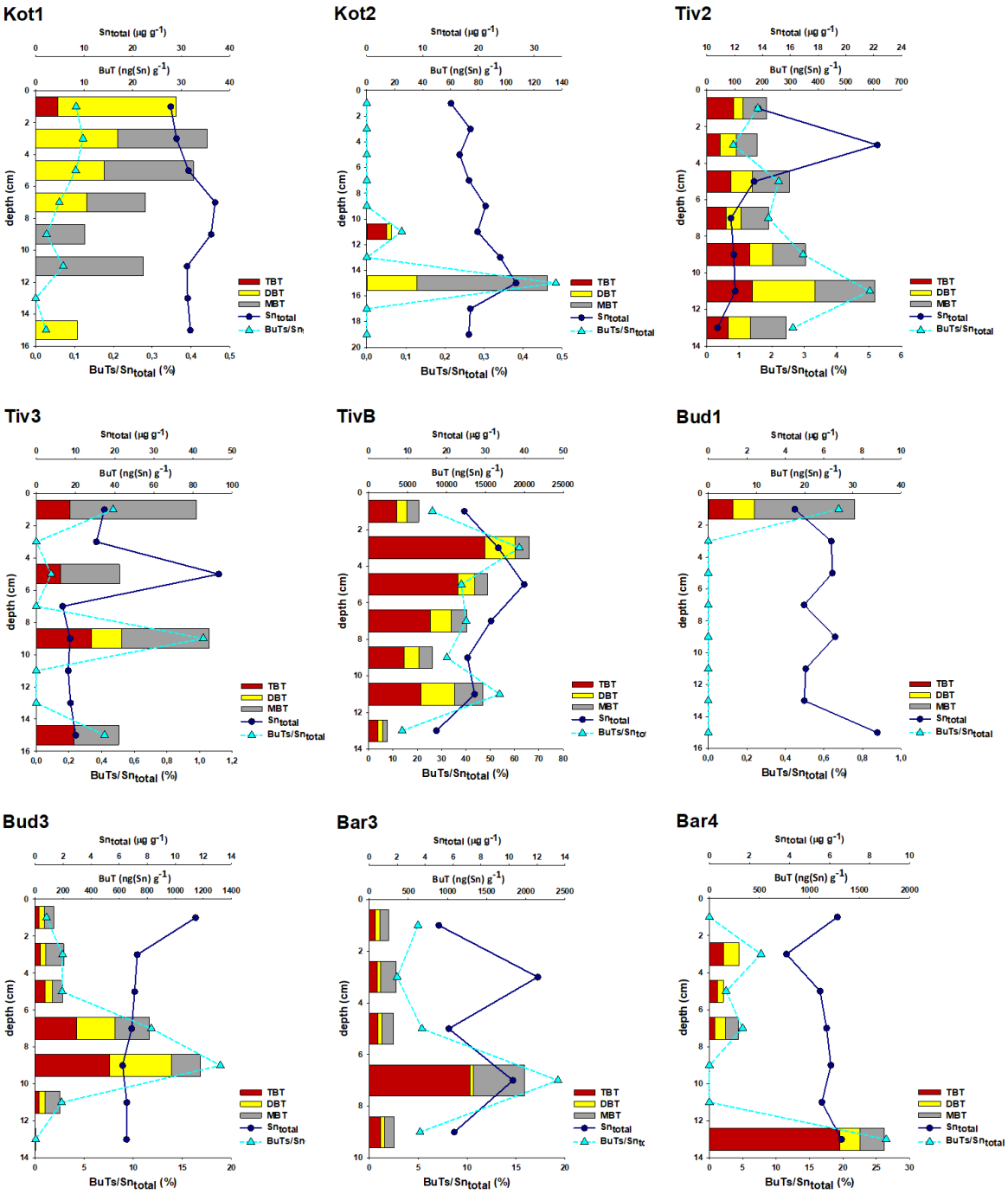

Figure 4. Depth distributions of butyltins (MBT, DBT, TBT and ∑BuTs) and total Sn in sediment cores along with proportions of $\sum BuTs/Sn_{total}$. The absence of bars for BuTs means that they were not detected – concentrations <LOD.

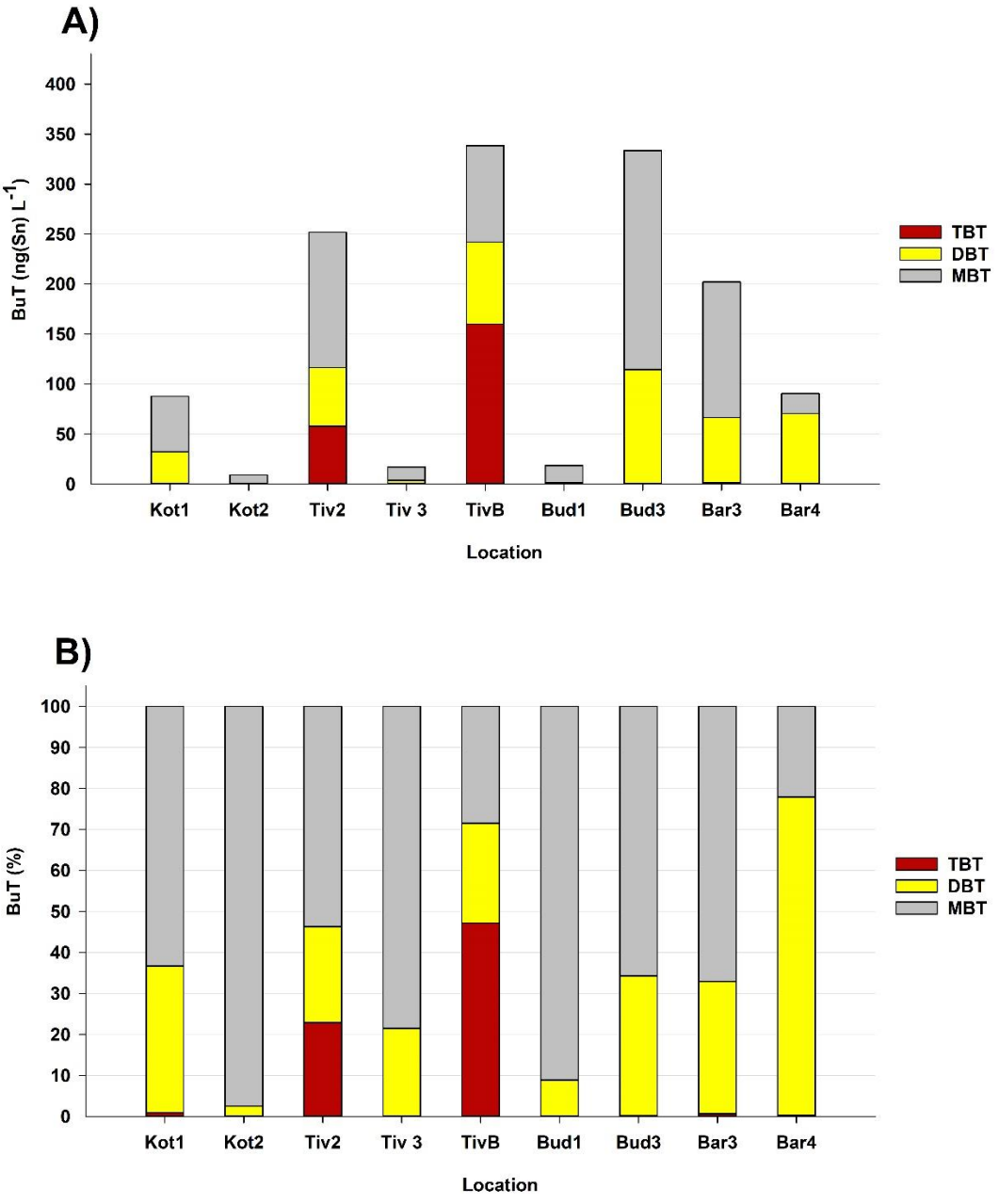

Figure 5. Distribution of BuTs in waters overlying sediment: (a) MBT, DBT and TBT concentrations, and (b) percentage of each BuT species (BuT/∑BuTs).

First assessment of butyltins (BuTs) contamination of the Montenegrin coast (Southeast Adriatic): Tributyltin (TBT) poses a threat to the marine ecosystem

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Abstract

This study presents the first assessment of butyltins (BuTs) pollution of the Montenegrin coast ~~(Southeast Adriatic)~~. The distribution of tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) was investigated in mussels, sediment~~s cores~~ and ~~in~~ water overlying sediment after the sediment resuspension. The results showed that the investigated sites (marinas, ports, shipyard~~s~~) are ~~significantly~~ contaminated with BuTs (19-402 ng(Sn)/g in mussels; 43-20641 ng(Sn)/g in sediments; 9-566 ng(Sn)/L in ~~water~~ overlying water~~ssediment~~) ~~despite the ban of TBT-based paints more than a decade ago~~. The measured TBT concentrations ~~were above the proposed ecotoxicological criteria at most locations,~~ indicat~~eing~~ that toxic effects on marine organisms are expected at most locations. The simultaneous analysis of BuTs and total Sn in sediment cores allowed the assessment of TBT historical input ~~and its long-term behaviour at the investigated sites. Finally~~, and it was demonstrated that resuspension of contaminated sediments leads to the release of all BuTs into the water column~~, especially MBT and DBT~~. This study shows that, despite the ban of TBT-based antifouling paints more than a decade ago, pollution of the marine environment with TBT is still a problem and regular monitoring remains essential.

Keywords: tributyltin (TBT), butyltins (BuTs), pollution, Montenegrin Adriatic coast, sediments, mussels

Tributyltin (TBT), one of the most toxic anthropogenic pollutants intentionally introduced into the aquatic environment, has been used as biocide in antifouling paints since the 1960s (Hoch, 2001). After its high toxicity to various aquatic non-target organisms was established, the use of TBT in antifouling paints was banned in many countries worldwide (Omae, 2003). ~~The regulation on the use of TBT based paints in Europe was enacted as early as 1989, but was fully implemented in 2008 (Furdek et al., 2012).~~ In 2001, the International Maritime Organization (IMO) established the AFS Convention (International Convention on the Control of Harmful Antifouling Systems on Ships) (IMO, 2008), calling for a global ban on TBT-based paints from 2003. In Europe, the ban was implemented in 2003 by Regulation (EC) No. 782/2003 and 2008 was set as the deadline for the complete ban of their use. Subsequently, a decrease in TBT levels has been observed in European coastal areas (Cacciatore et al., 2018; ~~Cuevas et al., 2014;~~ Guomundsdóttir et al., 2011; Harrison et al., 2020; Langston et al., 2015; Laranjeiro et al., 2018~~, Nicolaus and Barry, 2015~~), but TBT levels have still been above concentrations toxic to biota. In the last decade, high levels of TBT have been reported in European seas: Adriatic Sea (Erdelez et al., 2017; Formalewitz et al., 2019; Furdek Turk et al., 2020; Romanelli et al., 2019), North Sea (Eghart et al., 2017), Baltic Sea (Abraham et al., 2017; Filipkowska et al., 2016; Filipkowska and Kowalewska, 2019), Mediterranean and Atlantic coasts (Anastasiou et al., 2016; Concha-Grana et al., 2021; El Ayari et al., 2018) and in the Arctic (Kucklick and Ellisor, 2019; Metelkova et al., 2022). Recently observed TBT pollution was even worse in parts of the world where the use of TBT-based paints is not yet fully regulated (Bandara et al., 2021; Batista et al., 2016; Paz-Villarraga et al., 2015; Quintas et al., 2019). The above literature data clearly show that TBT pollution is still a major environmental problem that is far from being solved more than a decade after attempt for its global ban. However, the ban on the use of TBT never became "global" as the restrictions only applied to signatories of the IMO-AFS Convention (91 member states in 2021; Uc-Peraza et al., 2022), EU member states and countries with specific regulation in that sense. To make matters worse, TBT-based antifouling paints were still being manufactured in the Unites States and available on the market in 2021, even in some countries that are signatories of IMO-AFS Convention (Uc-Peraza et al., 2022).

Once TBT has entered the marine environment, it undergoes various processes such as microbiological and photolytic degradation, bioaccumulation by biota (e.g. gastropods, bivalves, fish) and adsorption to suspended particles (Furdek et al., 2012, 2016). The degradation of TBT occurs both in sediments and in the water column, and consists of a

stepwise loss of butyl groups, via dibutyltin (DBT) and monobutyltin (MBT) to the inorganic Sn (Furdek et al., 2016, Furdek Turk et al., 2020). This process in the water column is quite rapid (half-lives of several days to weeks; Rodriguez-Gonzalez et al., 2013), and, therefore, analysis of seawater provides information on the current level of butyltins (BuTs) in the marine environment. On the other hand, BuTs concentrations accumulated in marine organisms, such as mussels, reflect an average pollution of a water column over a longer period of time of about several months (Furdek et al., 2012). Due to its hydrophobic characteristics, TBT has an affinity to adsorb onto the suspended particles in the water column, which settle in the sediments over time. Once in the sediment, TBT degrades slowly, with half-lives of several years to decades (Furdek et al., 2016; Omae, 2003); therefore, TBT content in sediments could provide information on TBT input over a long period of time, from several years to decades. Not only BuTs are highly persistent in sediments, but they can also be released back into bottom waters by diffusion or resuspension of sediments, making sediments a potential source of new pollution to the water column (Furdek Turk et al., 2020; Hoch and Schwesig, 2004; Ruiz et al., 2008). Nevertheless, the field evidence of desorption of BuTs from contaminated sediments are rare (Furdek Turk et al., 2020). Therefore, it is important to determine the status of BuTs pollution of coastal areas not only in the water column but also in sediments, and to assess the risk of BuTs desorption from the contaminated sediments.

The level of TBT pollution in the Adriatic Sea (the northernmost arm of the Mediterranean Sea) has been assessed in several studies, covering the coastal waters of Croatia (Erdelez et al., 2017; Furdek et al., 2012; Furdek Turk et al., 2020), Slovenia (Milivojevič Nemanič et al., 2009; Formalewicz et al., 2019) and Italy (Romanelli et al., 2019; Formalewicz et al., 2019; Berto et al., 2007). All these studies showed that TBT pollution is a significant environmental problem in coastal areas. Regular monitoring of the priority pollutants in Croatian coastal waters carried out under the Water Framework Directive (WFD; Directive~~EC~~ 2000/60/EC, 2000) also revealed that TBT levels in water are frequently above the prescribed maximum annual average concentration (0.2 ng/L) (Furdek Turk et al., 2019). However, the level of TBT pollution in the southern part of the Eastern Adriatic that belongs to Montenegro has never been investigated and there are no data on the status of BuTs in this area. There is only one data set for the port of Bar from 2014, when the total BuTs in sediments ranged from 18 to 33 ng(Sn)/g (Romanelli et al., 2019).

The Montenegrin coastline has a length of 294 km, of which 105 km belong to the large fjord-like Boka Kotorska Bay, which is naturally divided into several smaller bays (Kotor, Risan,

Tivat and Herceg Novi). The entire coast is under high anthropogenic pressure due to urbanisation and discharge of municipal wastewaters, maritime activities (marine traffic, marinas, shipyards), fishing and tourism (Joksimović et al., 2017, 2020). The studies on the distribution of ecotoxic metals in mussels, sediments and seagrass (Jokanović et al., 2021; Joksimović et al., 2011, 2020; Radmirović et al., 2021; Stanković et al., ~~2014~~2015) have shown that most of the investigated sites, especially shipyards and urban areas, are contaminated with metals due to significant anthropogenic pressure.

The aims of this work were: i) to assess the level of BuTs pollution along the Montenegrin coast by studying BuTs distribution in mussels and sediments; ii) to assess the extent of BuTs desorption from contaminated sediments into the water column caused by sediment resuspension~~,~~; and iii) to assess the historical input of BuTs along the Montenegrin Adriatic coast by studying the relationship between total Sn and BuTs in sediments.

Sampling locations included various areas along the Montenegrin coast which are affected by intensive shipping and industrial activities, such as marinas, shipyards and municipal and local ports. Samples of mussels *Mytilus galloprovincialis* (Lamarck, 1819) and sediments were collected in July 2015 from 16 sampling sites, covering the areas of Boka Kotorska Bay (namely, Kotor Bay and Tivat Bay) and the towns of Budva and Bar (Fig. 1; Table S1). In the Kotor Bay samples were collected at 5 locations (Kot1, Kot1a, Kot2, Kot3, Kot3a) near the town of Kotor, a tourist town with a marina, cruise port and a number of small ports. In the Tivat Bay, the samples were taken at the marina Porto Montenegro in the town of Tivat (Tiv1, Tiv2, Tiv3) and in front of the Bijela shipyard (TivB). In Budva (tourist town), samples were taken at 4 locations - in the marina (Bud1, Bud3, Bud4) and in the local port (Bud1a). In the town of Bar, one of the largest cargo, passenger and military ports on the Eastern Adriatic, samples were taken at 3 locations, including the cargo (Bar1), military port (Bar4) and the marina (Bar3). A brief description of each sampling site is given in Table S1 in the Supplementary Information. At sites labelled with "a" (which were a short distance from the site labelled with the same number) only mussels were collected. A total of 81 samples, including 14 samples of mussels and 67 samples of sediment obtained by cutting sediment cores (9 cores; 10-20 cm long, cut into 2 cm layers), were analysed for BuTs (i.e. MBT, DBT and TBT). In addition, bottom waters, i.e. water overlying the sediment (9 samples), were collected with a gravity corer to investigate the desorption of BuTs caused by sediment resuspension. The water overlying sediment in the gravity corer was transferred to a dark glass bottle after sediment particles had settled (approximately 15 min after sediment was taken out

of the sea with the gravity corer), acidified to pH=2 with nitric acid (HNO_3) and stored in the dark at 4°C until analysis. Mussels (30 specimens per sampling site, *with a shell length of 3 to 6 cm*) were collected from the docks, rocky shores or boat strings. The shells were removed and the soft tissue homogenised in a blender and later freeze-dried and stored at -20°C until analysis. Sediments were collected using a UWITEC gravity corer (Umwelt-und Wissenchaftstechnik, Austria) and frozen immediately after collection. The sediment cores were cut into 2 cm layers, freeze-dried, homogenised by milling and stored at -20 °C until analysis.

The analytical methods used for the analysis of BuTs (TBT, DBT and MBT) in seawater and mussels are described in Furdek et al. (2012), while that used for the analysis of sediments is described in Furdek et al. (2016). Briefly, BuTs were extracted from 300 ml of seawater, while simultaneous extraction and derivatisation were performed in a sodium acetate-acetic acid buffer (pH=4.8) by adding sodiumtetraethyl borate ($NaBEt_4$, (1% (w/v)) and hexane and mechanical shaking for 30 min at 350 rpm. Butyltins from sediments were extracted from 0.5-2 g of sample by acetic acid and ultrasound-assisted agitation (30 min, 55 Hz), while BuTs from mussel were extracted from 0.5-1 g of lyophilised tissue with 0.1 mol/L hydrochloric acid (HCl) in methanol using ultrasound-assisted agitation (30 min, 55 Hz). For both sediments and mussels, the derivatisation of compounds in the extract was performed in sodium acetate-acetic acid buffer (pH=4.8) with $NaBEt_4$ (1% (w/v)) and transferred into hexane by mechanical shaking at 350 rpm for 30 min. Analyses of BuTs in seawater were carried out on a gas chromatograph (GC, Agilent 6890, Agilent Technology) coupled with inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7900, Agilent Technology), while detection of BuTs in mussels and sediments was performed using gas chromatograph (GC, Varian CP 3800, Varian) with pulsed flame photometric detector (PFPD, Varian). Tripropyltin (TPrT) was used as an internal standard and quantification was performed by applying the standard addition calibration method. Quality control of the analytical procedure for the determination of BuTs in mussels and sediments was performed by analysing the certified reference materials (CRM) for mussels (CE 477, ERM, European Commission, Geel, Belgium) and marine sediment (PACS 2, ~~Ottawa~~NRC-CNRC, Canada). In addition, the accuracy of all three methods was further confirmed by successful participation in the proficiency tests. The detection limits for TBT, DBT and MBT were 0.1-0.5 ng(Sn)/L for seawater, 0.9-5.1 ng(Sn)/g for mussels and 0.9-6.1 ng(Sn)/g for sediment. Total Sn in sediments was analysed according to the method described in Furdek Turk et al. (2020) using high resolution inductively coupled plasma mass

spectrometry (HR-ICPMS; Element 2, Thermo Finnigan). Sediments (0.1 g) were digested in a microwave oven (Multiwave 3000, Anton Paar) by two-step total digestion procedure (HNO_3 (65% *s.p.*) + HCl (36% *s.p.*) + hydrofluoric acid (HF, ~~(~~48% *s.p.*)), followed by boric acid (H_3BO_3, ~~(~~40 g/L). The quality control was performed by simultaneous measurements of certified reference materials for marine sediment (MESS-3, NRC-CNRC, Canada) for which satisfactory results were obtained.

The BuTs pollution of the water column was not detected directly, but indirectly by determining the accumulated BuTs in mussels. Indeed, mussels are a reliable biomonitor of TBT pollution as they are sessile and filter-feeding organisms, with a great ability to bioaccumulate TBT from water and a limited ability to metabolise it (Magi et al., 2008). The TBT concentration detected in mussels reflects the ambient TBT level during the last months (2-3 months; Salazar and Salazar, 1996) because during this period an equilibrium is reached between uptake from the water (i.e. accumulation), metabolism of TBT to DBT and MBT, and excretion of these compounds back into the water (Gomez-Ariza et al., 1999; Magi et al., 2008). Therefore, TBT levels measured in mussels are a reliable indicator of recent pollution in the water column over the last few months, in contrast to levels measured in seawater that only indicate the momentary level of pollution. The concentrations of BuTs (∑BuTs=MBT+DBT+TBT) in mussels varied from 19 to 402 ng(Sn)/g (Table 1, Fig. ~~2a~~2A); concentrations of DBT and TBT were of the same order of magnitude~~similar~~ (10-173 ng(Sn)/g for DBT and 6.7-179 ng(Sn)/g for TBT), while MBT was the least abundant compound (<5-50 ng(Sn)/g). In all investigated marinas (Kot1a, Tiv1, Tiv2 and Tiv3, ~~and~~ Bud1 and Bar3), ∑BuTs were above 100 ng(Sn)/g, while the highest concentration (402 ng(Sn)/g) was found in mussels from the marina Bar3. At all locations, TBT and DBT account for 30-55% and 35-58% of the total BuTs, respectively (Fig. ~~2b~~2B). High concentrations and proportions of TBT in mussels reflect ~~a~~ recent inputs of TBT, but may also be a consequence of the limited ability of mussels to metabolise TBT, as bivalves do not have an efficient detoxification system (Lee, 1996; Chandrinou et al., 2007; Diez et al., 2005). However, significant correlations between TBT and DBT ($r=0.912$, $p<0.05$, Spearman) suggest that DBT in mussel tissues may be the product of TBT metabolism, while lower but still significant correlation between TBT and MBT ($r=0.693$, $p<0.05$, Spearman) and DBT and MBT ($r=0.616$, $p<0.05$, Spearman) suggests that DBT could be eliminated from the organism before being degraded to MBT, or that it is metabolised more slowly compared to MBT (Furdek et al., 2012). In conclusion, the measured

BuTs concentrations in mussels indicate that TBT pollution ~~with TBT~~ in seawater has been present at all investigated sites along the Montenegrin coast in recent months.

The spatial and depth distributions of BuTs in sediments were highly variable (Fig. ~~3a~~3A, Fig. 4, Table 1), with ranges encompassing 3-4 orders of magnitude (<9-20641 for BuTs; <1-14900 for TBT; <2-4279 for DBT; <6-3651 for MBT, expressed in ng(Sn)/g). The highest concentrations of BuTs were found in the sediments sampled in front of the Bijela shipyard (20641 ng(Sn)/g), while at all other sites maximum concentration did not exceed 2000 ng(Sn)/g (Table 1). TBT was the prevailing compound at all sites, accounting for up to 75% of all BuTs (Table 1). The depth profiles of BuTs in 9 sediment cores from different locations were highly variable, both in terms of the level of concentrations and the depth pattern (Fig. 4). Sediments in the city of Kotor (Kot1 and Kot2) may be considered as not polluted with TBT (Fig. 4); the concentrations of ∑BuTs were up to 35 ng(Sn)/g and were mainly in the form of TBT degradation products, i.e. DBT and MBT. In both sediment cores TBT was detected in only one layer (0-2 cm – Kot1 and 10-12 cm – Kot2). ~~In marina in the town of Tivat~~, ~~the~~ The sediment inside the marina in the town of Tivat ~~at location~~ (Tiv2) ~~(inside the marina)~~ was heavily contaminated with BuTs throughout the core (up to 600 ng(Sn)/g), with a high proportion of TBT in each layer (30-40%). In contrast, in the sediment sampled outside the marina (Tiv3), BuTs concentrations were much lower, although the high proportion of TBT (up to 45%) was also detected in the deeper layers. The sediment sampled in front of the Bijela shipyard (TivB) was extremely contaminated; the BuTs concentrations in the upper 12 cm did not fall below 6500 ng(Sn)/g, while the maximum concentration of 20641 ng(Sn)/g was detected in the subsurface layer. In all layers, TBT was the prevailing compound and accounted for 50-75% of the total BuTs. The BuTs depth distributions in two sediment cores sampled within the same marina in the city of Budva (Bud1 and Bud3) were different, although these sites are only 100 m apart. While in the sediment core Bud1 only the surface sediment layers were slightly contaminated with BuTs, the sediments sampled closer to the service hoist (Bud3) were heavily contaminated to a depth of 12 cm, with BuTs concentrations up to 1180 ng(Sn)/g. In the city of Bar, the sediments of the marina (Bar3) and the military port (Bar4) were significantly contaminated with BuTs (concentrations of up to 1980 ng(Sn)/g), with the highest pollution observed in the deeper layers, where not only the concentrations were high, but also the percentage of undegraded TBT (up to 75%).

Based on the depth profiles obtained, several patterns of BuTs behaviour could be observed (Fig. 4). First, ~~the~~ BuTs concentration profiles do not follow the same depth pattern; in some

sediments BuTs decreased with depth, while in others ~~their~~ concentrations were similar or vary throughout the depth of the core. Such different depth distributions may indicate different TBT inputs over time or different BuTs degradation efficiencies in different sediments. Since different BuTs depth distributions were observed in the sediments from marinas of similar size and similar intensity of marine traffic (such as Bud1, Bar3, Kot1 and Tiv2), the most likely explanation is that the observed depth profiles are the result of different persistence of TBT in these sediments. It was demonstrated that sediments characteristics, namely grain size and organic matter, strongly affects the degradation process of TBT and its persistence in sediments (Furdek et al., 2016; Furdek Turk et al., 2020). Organic matter plays a crucial role as a TBT sorbent in sediments and, therefore, influences TBT degradation rate by determining the bioavailable fraction in porewater where TBT degradation occurs (Furdek at al., 2016). This means that, in sediments rich in organic matter, TBT will be less available to degradation and, consequently, more persistent over time (Furdek at al., 2016). Secondly, at most locations TBT degradation products, i.e. MBT and DBT, are the prevailing BuT compounds through the entire depth cores, which could indicate efficient degradation of TBT in these sediments. An exception is the sediments sampled in Bijela shipyard (TivB) and in two layers of cores Tiv2 and Bar4, where the percentage of TBT/∑BuTs was more than 50% (Fig. 4). Although the proportions of DBT and MBT prevail over TBT, their accumulation in the deeper layers was not observed in any sediment core. There are two explanations for this: i) DBT and MBT, being less hydrophobic compounds, are more easily desorbed from sediments than TBT (Poerschmann et al., 1997), and ii) the degradation of DBT and MBT to inorganic Sn is efficient in both oxic and anoxic sediments. Indeed, Furdek et al. (2016) demonstrated that the rate of DBT degradation in sediments is faster than the rate of its formation from TBT, which prevents accumulation of DBT in sediments, and that MBT, after desorption from sediment to anoxic porewater, is almost immediately degraded to inorganic Sn.

However, a reliable assessment of the long-term behaviour, persistence and historical input of TBT can only be made by simultaneously determining BuTs and total Sn in sediments (Furdek Turk et al., 2020). First, it should be confirmed that the inorganic Sn contamination originates mainly from TBT-antifouling paints and not from other possible sources such as municipal and industrial wastewater ~~(Pougnet et al., 2014).~~ or landfill leachates. Namely, inorganic Sn is used in products such as food cans, welding equipment, electronics, and household products (Pougnet et al., 2014). Inputs of other organotin compounds such as mono- and disubstituted BuTs (often used as PVC stabilizers) and phenyltins (used as pesticides, while triphenyltin

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(TPhT) is also used as a biocide in antifouling paints) (Omae, 2003) could also lead to the formation of inorganic Sn. However, in Europe TPhT is used to a much lesser extent than TBT (Lagerstrom et al., 2017), which is confirmed by the data on TPhT contamination of the Croatian Adriatic coast, where only sporadic low levels of TPhT have been detected in mussels (Furdek et al., 2012) and sediments (Furdek et al., 2016).

To investigate the source of Sn contamination and confirm that inorganic Sn originates mainly from the degradation of TBT~~this~~, we examined the relationships between BuTs and inorganic Sn in the sediments by determining the total Sn depth profiles and calculating butyltin degradation index (BDI), following the approach presented in previous studies (Furdek Turk et al., 2020; Pougnet et al., 2014). Total Sn concentrations ranged from 3.8 to 46.5 µg/g (Fig. 4) and were higher than the background levels of Sn, obtained by the analysis of older uncontaminated ~~recently determined for the~~ sediments of the Boka Kotorska Bay (2.8 µg/g) and the coastal zone of Montenegro (1.7 µg/g) (Mikac et al., 2022). ~~–~~The degree of Sn contamination was assessed by enrichment factors ($EF=(Sn/Al)_{sample}/(Sn/Al)_{background}$), according to the scale proposed by Sutherland (2000): EF<2 indicates no or minimal, EF=2-5 moderate, EF=5-20 significant, EF=20-40 very high, and EF>40 extreme enrichment or contamination. The calculated EFs showed that all sediments studied were enriched in Sn; the sediments from Kotor Bay (Kot1, Kot2) and Tivat Bay (Tiv1, Tiv2, TivB) were significantly contaminated with Sn (EFs between 5 and 20), while the sediments from Budva (Bud1, Bud3) and Bar (Bar3, Bar4) had EFs between 2 and 12, indicating moderate contamination with Sn.

To investigate the source of Sn contamination, we examined the relationship between TBT and its degradation products (DBT, MBT and inorganic Sn) by calculating the BDI index, which is a commonly used tool to study TBT degradation efficiency. We calculated the BDI using two approaches: i) the commonly known approach, which considers only DBT and MBT as TBT degradation products; BDI = (MBT+DBT)/TBT (Garg et al., 2009), and ii) a modified approach proposed by Pougnet et al. (2014), which considers the inorganic Sn as the final product of total degradation; $BDI_{mod} = (Sn_{inorganic}+MBT+DBT)/TBT$, while the $Sn_{inorganic}$ could be calculated as $Sn_{total}-Sn_{background}-\sum BuTs$. A stronger correlation between TBT and BDI_{mod} ($r=0.961$; $p<0.01$~~;~~, Spearman) than between TBT and BDI ($r=0.722$, $p<0.05$~~;~~, Spearman) was observed when combining samples from all sediment cores. This indicates that most of the TBT has been completely degraded to inorganic Sn, while the anthropogenic fraction of inorganic Sn mainly originates from the degradation of TBT released from antifouling paints.

Considering the above conclusion that most of the inorganic Sn originates from TBT antifouling paints in the studied environments, we used BuTs and total Sn depth distributions to explain the historical input and behaviour of TBT in the investigated sediments and to clarify whether the observed BuTs depth distributions are the result of different TBT input over time or different TBT persistence in sediments. From the observation of the determined depth profiles (Fig. 4) the following conclusion are drawn. The total Sn depth profiles in the Kotor Bay (Kot1 and Kot2) show high (EF=8-18) and continuous input of Sn over time, while the ∑BuTs accounts for less than 0.5% of the total Sn at all depths, indicating that the low TBT and BuTs levels are a consequence of an efficient TBT degradation rather than low TBT input over time. In the marinas of Budva (Bud1, Bud3) and Tivat (Tiv2, Tiv3) all cores are similarly contaminated with Sn (EF=3-10) (except for a few layers where EF is higher), indicating a comparable input of TBT over time. However, the percentage of $\sum BuTs/Sn_{total}$ in cores Tiv3 and Bud1 (<1%) is much lower than in cores Tiv2 and Bud3 (1-18%), indicating less efficient TBT degradation in the later cores. In the sediment cores from Bar (Bar3, Bar4) the total Sn input is generally lower (EF=2-7) compared to the other sites, but $\sum BuTs/Sn_{total}$ was high (4-20%), indicating high persistence of TBT in these sediments, especially in the sediments from marina (Bar3), where the highest level of BuTs in mussels were also found. In the military port (Bar4) the absence of BuTs in the surface sediments suggests that TBT has not been introduced recently; however, the elevated total Sn and the relatively high TBT levels in mussels indicate the opposite, namely that there was a recent input of TBT and its efficient degradation to inorganic Sn in the surface sediments. By contrast, the high level of TBT in the bottom layer of this core indicates poor degradation of TBT in older sediments. After all, the observed depth profiles demonstrated less efficient TBT degradation in cores Tiv2, Bud3 and Bar3 that in the other sediment cores analysed. Finally, the sediment core TivB is clearly different from all the other cores; not only BuTs and total Sn concentrations (EF=15-20) are much higher than in the other sediments, but also the proportions of TBT/∑BuTs and $\sum BuTs/ Sn_{total}$ are very high (50-75% and 20-65%, respectively). As this core was taken near the shipyard, these results could be explained by the presence of paint particles containing TBT in the form that is not available to degradation (Furdek Turk et al., 2020).

It should be emphasized that the proportion of $\sum BuTs/Snt_{otal}$ was generally not higher than 10% in any of the sediments studied (excluding the sediment from the shipyard), which may be surprising considering that the degradation of TBT in sediments is a slow process ($t_{1/2}$ = several years to decades; Omae, 2003). The same was found by Furdek Turk et al. (2020), who

discussed that most of the TBT leached from ships is degraded to inorganic Sn already in the water column ($t_{1/2}$ = several days to weeks; Rodriguez-Gonzalez et al., 2013), so that only a small fraction of TBT (~10%) reaches the sediments. Therefore, a reliable assessment of TBT input in the time period covered by the sediment cores can only be performed by taking into account the total amount of Sn; otherwise, the total TBT input to the certain environment could be underestimated (at least by a factor of ten).

TBT pollution of sediments is not only a problem because it poses a threat to the benthic organisms living there; the big problem lies in the fact that contaminated sediments are a potential source of BuTs pollution for the water column. Although it has been experimentally proven many times that BuTs can be desorbed from sediments (Berg et al., 2001; Burton et al., 2004), this has been demonstrated only in a few field studies (Furdek Turk et al., 2020; Chen et al., 2022). However, knowledge about the extent to which contaminated sediments act as a source of BuT pollution is still incomplete. To investigate the behaviour of BuTs during the resuspension of disturbed sediments, we analysed the water overlying the sediment in the gravity corer. It was assumed that the penetration of the gravity corer into the sediment would cause resuspension of fine particles from the sediment surface, which could mimic, to some extent, the resuspension of surface sediments caused by waves or bioturbation. The BuTs concentrations in the water above the sediment (Table 1, Fig. ~~5a~~5A,~~b~~B) showed a predominance of MBT (8.8-277 ng(Sn)/L) and DBT (0.2-130 ng(Sn)/L) over TBT (<0.1-160 ng(Sn)/L), which was found only at two sites (Tiv2 and TivB) where the surface sediments contained more than 50% of BuTs in the form of TBT (Fig. ~~3b~~3B). As these concentrations are much higher than those normally found in the water column and porewater (<10 ng(Sn)/L; Furdek et al., 2012; Furdek Turk et al., 2020), we assume that most of the detected BuTs are desorbed from the sediments into the overlying water. These data support the results of a similar study conducted with contaminated coastal sediments in Croatia (Furdek Turk al., 2020). Both studies showed that contaminated sediments are a potential source of BuTs to the water column, especially MBT and DBT as less hydrophobic and weakly adsorbed compounds that can be released from the sediments more easily than TBT. However, this study has shown that resuspension of sediments can also lead to the release of TBT, especially from contaminated sediments with high levels of TBT in the surface layers.

An assessment of the extent of BuTs contamination in the coastal environment of Montenegro was performed by comparing BuTs and TBT levels determined in different coastal areas of Europe during the last decade (Table 2). The comparison of literature data given in Table 2

showed that the Eastern Adriatic coast (Croatian and Montenegrin) is highly contaminated with BuT compounds. However, the level of TBT pollution strongly depends on the type of sampling locations (marina, port, beaches) and is related to the intensity of marine traffic (Brian~~t~~ et al., 2013; Suzdalev et al., 2015), and, therefore, this conclusion should be taken with caution as it may be influenced by different sampling strategies of the presented studies and prevalence of more contaminated sites along the Croatian coast. In the Water Framework Directive (WFD; Directive 2000/60/EC, 2000) TBT is listed as one of the priority substances that should be monitored to assess the chemical status of a water body, but within this Directive TBT presence in the environment is regulated only by the prescribed maximum allowed concentration in seawater (the annual average should be less than 0.2 ng/L). However, several regional convections, such as ~~(i.e.~~ OSPAR (Convention for the Protection of the Marine Environment of the North-East Atlantic; OSPAR, 2004) and~~,~~ HELCOM Conventions (Convention on the Protection of the Marine Environment of the Baltic Sea Area; HELCOM, 2018), ~~Barcelona Convection)~~ have established Environment Assessment Criteria (EAC) for the assessment and monitoring of the environmental status of regional waters, which could be applied for assessments based on the measurement of TBT in sediments and biota. OSPAR (OSPAR, 2004) has established EAC for TBT in mollusks; the lower EAC (EAC_{low} = 4.9 ng(Sn)/g) is defined as the threshold for protection of all marine species from chronic effects and the upper EAC (EAC_{high} = 72 ng(Sn)/g) is defined as the highest level at which no acute toxic effects are expected. The ~~measured~~ TBT concentrations quantified in mussel~~s~~ tissue were above the EAC_{low} at all sites, while they were above the EAC_{high} at site Bar3, indicating that chronic effects for marine organisms sensitive to TBT are to be expected at all locations. The assessment of TBT pollution based on the TBT concentrations ~~measured~~ quantified in ~~the~~ sediments was carried out according to the Norwegian environmental quality classification system defined for management purposes (Bakke et al., 2010); Background: <0.4 ng(Sn)/g, Good: 0.4-2.1 ng(Sn)/g, Moderate: 2.1-8.3 ng(Sn)/g, Poor: 8.3-41.7 ng(Sn)/g, and Very Poor: >41.7 ng(Sn)/g. At seven (out of nine) sites investigated surface sediments are contaminated with TBT; Kot1 and Bud1 were moderately contaminated with TBT, while the environmental status of Tiv3 and Bud3, as well as Tiv2, TivB and Bar3, can be evaluated as poor and very poor, respectively, indicating that TBT is expected to have toxic effects on marine organisms and the occurrence of imposex in gastropods is very likely at these locations (Erdelez et al., 2017; Laranjeiro et al., 2018).

The presented results show that the investigated areas on the Montenegrin coast are polluted with TBT, which indicates the need for further monitoring of the coastal area. Apparently, TBT-based antifouling paints, despite of its ban, have been used within a few months of sampling (as shown by BuTs levels measured in mussels), but also over a longer period of time (as shown by BuTs levels measured in sediments). The main source of pollution could be small local and leisure boats that are not subject to any control of antifouling paint applied. Therefore, they may be illegally painted with TBT-containing paints, for which is shown that were still present on the global market in 2021 (Uc-Peraza et al., 2022) or can have old layers of TBT-based paint that are not properly removed from the boat (Lagerstrom et al., 2017, 2019). Indeed, a recent study in the Nordic countries has demonstrated the presence of TBT in the subsurface paint layers of all investigated leisure boats (Lagerström et al., 2017, 2019) and shown that they are source of TBT pollution. The results have also shown that resuspension of contaminated sediments may cause pollution of the water column, especially at the Bijela shipyard (TivB) where remediation measures are strongly recommended. Recently, extreme contamination with ecotoxic metals (Mikac et al., 2022; Radomirović et al., 2021) and significantly altered microbial community have been demonstrated in sediments sampled near this shipyard (Jokanović et al., 2021).

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Figures caption

Figure 1. S~~The s~~ampling locations along the Montenegrin Adriatic coast.

Figure 2. D~~The d~~istribution of BuTs in mussel~~s~~ tissue: (a) MBT, DBT and TBT concentrations, and (b) percentage of each chemical species (BuT/∑BuTs).

Figure 3. D~~The d~~istribution of BuTs and total Sn in surface sediments: (a) MBT, DBT, TBT and Sn concentrations, and (b) percentage of each BuT species (BuT/∑BuTs) along with proportion of ∑BuTs/Sn_{total}. ~~(~~The absence of bars for BuTs means that they were not detected – ~~the~~ concentrations ~~are~~ <LOD.~~)~~

Figure 4. D~~The d~~epth distributions of butyltins (MBT, DBT, TBT and ∑BuTs) and total Sn in sediment cores along with proportions of ∑BuTs/Sn_{total}. ~~(~~The absence of bars for BuTs means that they were not detected – ~~the~~ concentrations ~~are~~ <LOD.~~)~~

Figure 5. D~~The d~~istribution of BuTs in water~~s~~ overlying sediment: (a) MBT, DBT and TBT concentrations, and (b) percentage of each BuT species (BuT/∑BuTs).

Table 1. Summarizing statistic for butyltins (MBT, DBT, TBT, ∑BuTs) concentrations and proportions of TBT/∑BuTs and ∑BuTs/Sn_{total} in different types of samples collected along the Montenegrin Adriatic coast.

	Concentration (ng(Sn)/g; ng(Sn)/L)				Proportion (%)	
	MBT	DBT	TBT	∑BuTs	TBT/∑BuTs	∑BuTs/Sn_{total}
			Mussels			
min	<5	10.4	6.7	19.7	28.2	-
max	50.7	173	179	402	55.5	-
average	15.1	43.0	40.6	98.6	39.1	-
median	11.1	30.6	25.9	67.8	39.3	-
			Sediment cores (all depths)			
min	<6	<2	<1	<9	-	0.01
max	3651	4279	14900	20641	75.4	62.0
average	248	288	829	1365	25.9	6.09
median	20.8	14.1	12.4	43.1	17.2	0.43
max*	650	445	1304	1979	74.8	26.5
average *	62	41	85	188	22.0	2.37
median*	11.1	3.9	0.5	31.2	11.1	0.10
			Water overlaying sediment			
min	8.8	0.2	<0.1	9.1	<0.2	-
max	277	130	160	566	28.2	-
average	91.3	52.4	24.4	168	6.0	-
median	38.2	58.9	0.3	80.4	0.4	-
max*	219	114	58	333	22.9	-
average *	68.1	42.7	7.5	118	3.2	-
median*	29.0	40.9	0.3	70.9	0.4	-

*without samples from Bijela shipyard

Table 2. Literature data on butyltins (BuTs=TBT+DBT+MBT) and tributyltin (TBT) concentration in seawater, sediment and biota from the coastal areas of Europe reported in the last decade.

Area and year of sampling	Seawater (ng(Sn)/L)	Sediment (ng(Sn)/g)	Biota (ng(Sn)/g)	Reference
Southeast Adriatic coast (Montenegro); 2015	-	BuTs: <9-2000 (20641)* TBT: <1-1300 (14900)*	BuTs: 20-400 TBT: 7-179	This study
East Adriatic coast (Croatia); 2010-2012	BuTs: 0.5-28 TBT: <0.2-15	BuTs: 3-1362 (66345)** TBT: 1-790 (32317)**	Mussels BuTs: <10-1676 TBT: <10-1045	Furdek et al. (2012); Furdek Turk et al. (2020)
East Adriatic coast (Croatia), 2015-2017	TBT: <0.2-6.3	TBT: <1-92	Gastropoda BuTs: 1.9-155 TBT: 0.5-59	Furdek Turk et al. (2019) ; Erdelez et al. (2017)
East Adriatic coast (Slovenia, Croatia, Montenegro, Albania); 2014	TBT: <2	BuTs: 7-731 TBT: 3-566	Mussels BuTs: 25-220 TBT: 20-170	Romanelli et al. (2019)
West Adriatic coast (Italy); 2014	TBT: <2	BuTs: 7-240 TBT: 2-186	Mussels BuTs: 25-100 TBT: 60-75	Romanelli et al. (2019)
North Adriatic coast (Italy, Slovenia); 2016	-	BuTs: 121-352 TBT: 53-174	-	Formalewicz et al. (2019)
North Sea (Sweden); 2014	-	BuTs: 10-500 TBT: 10-300	-	Egardt et al. (2017)
Baltic sea (Lithuania); 2010-2012	-	TBT: 1-5200	-	Suzdalev et al. (2015)
Baltic Sea (Poland); 2014-2015 (fish), 2018 (sediment)	-	BuTs: 5.7-3321 TBT: 3.7-1942	Fish muscle BuTs: nd-715 TBT: nd-503	Filipkowska et al. (2016); Filipkowska and Kowalewska (2019)
Baltic Sea (Poland); 2012-2013	-	BuTs: nd-22	Mussels BuTs: 41-164 TBT: 11-103	Filipkowska and Lubecki (2016)
Baltic Sea (Germany); 2015	-	BuTs: 100-1426 TBT: 2.5-380	-	Abraham et al. (2017)
Mediterranean (France); 2009, 2011, 2013	Porewater BuTs: 40-600 TBT: 0.1-70	BuTs: 8-16000 TBT: nd-10700	-	Briant et al. (2013, 2016)
Mediterranean and Atlantic coast (Portugal, Italy, Turkey); 2012	-	BuTs: 8-200 TBT: 3-75	Gastropoda BuTs: 30-720 TBT: 12-172	Anastasiu et al. (2016)
Mediterranean and Atlantic coast (Spain); 2015	BuTs: nd-20 TBT: nd-17	BuTs: nd-100 TBT: nd-75	-	Concha-Grana et al. (2021)
Barents Sea (Russia); 2019	-	BuTs: 28-83 TBT: 2-24	Bivalves BuTs: nd-50 TBT: nd-38	Metelkova et al. (2022)

*including samples from shipyard Bijela; ** including samples from shipyard and service hoists

Figure 1. ~~The~~ Ssampling locations along the Montenegrin Adriatic coast.

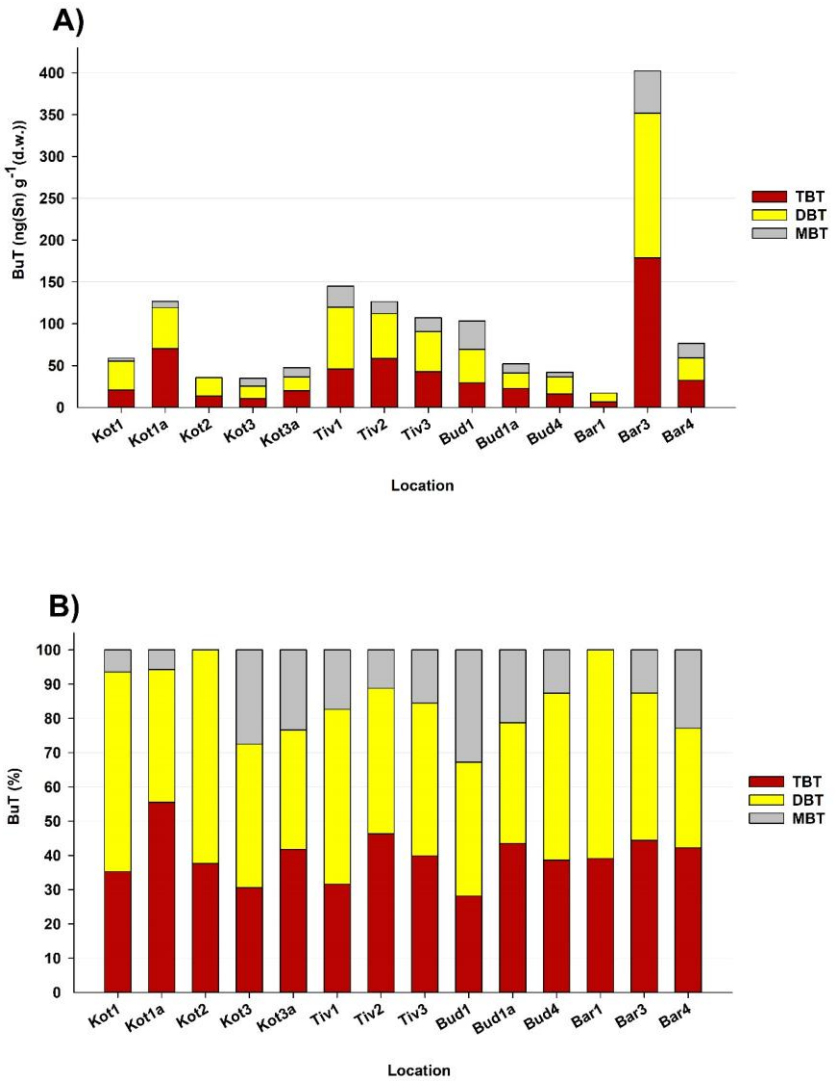

Figure 2. ~~The d~~Distribution of BuTs in ~~mussels~~mussel tissues: (**a**) MBT, DBT and TBT concentrations, and (**b**) percentage of each chemical species (BuT/∑BuTs).

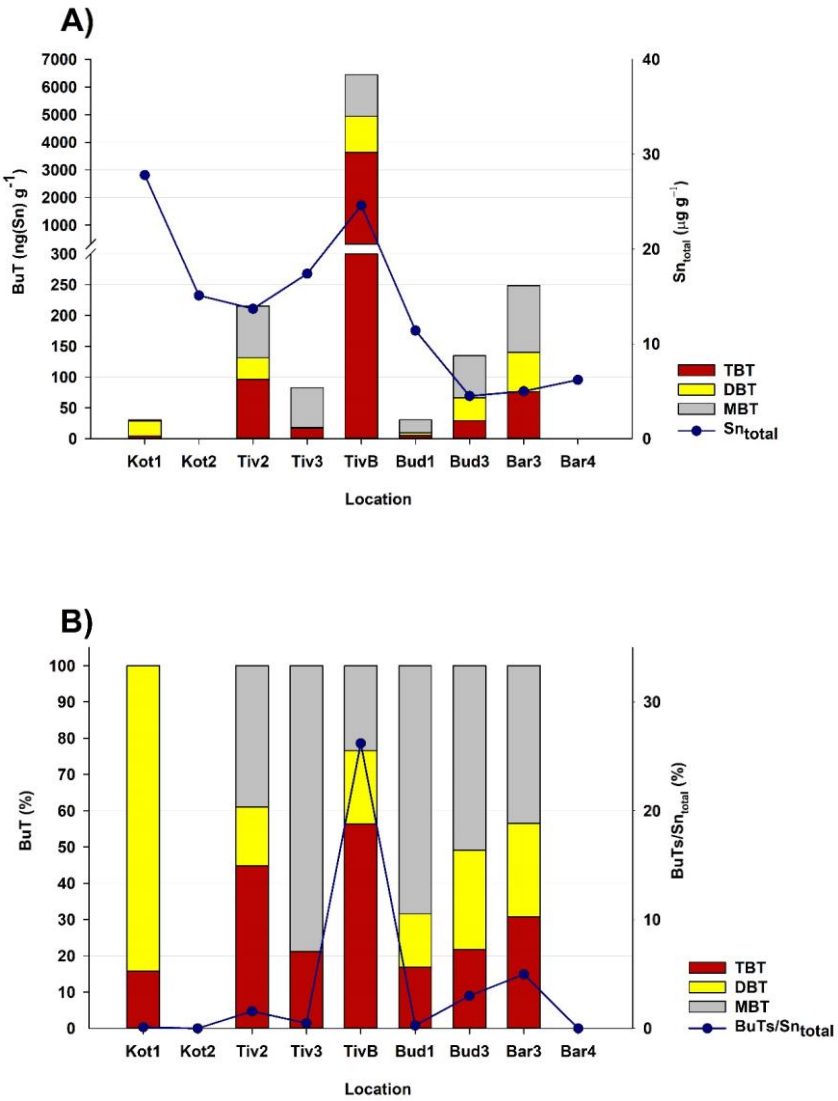

Figure 3. ~~The d~~Distribution of BuTs and total Sn in surface sediments: (**a**) MBT, DBT, TBT and Sn concentrations, and (**b**) percentage of each BuT species (BuT/∑BuTs) along with proportion of ∑BuTs/Sn_{total}. ~~(~~The absence of bars for BuTs means that they were not detected – ~~the c~~concentrations ~~are~~ <LOD.~~)~~

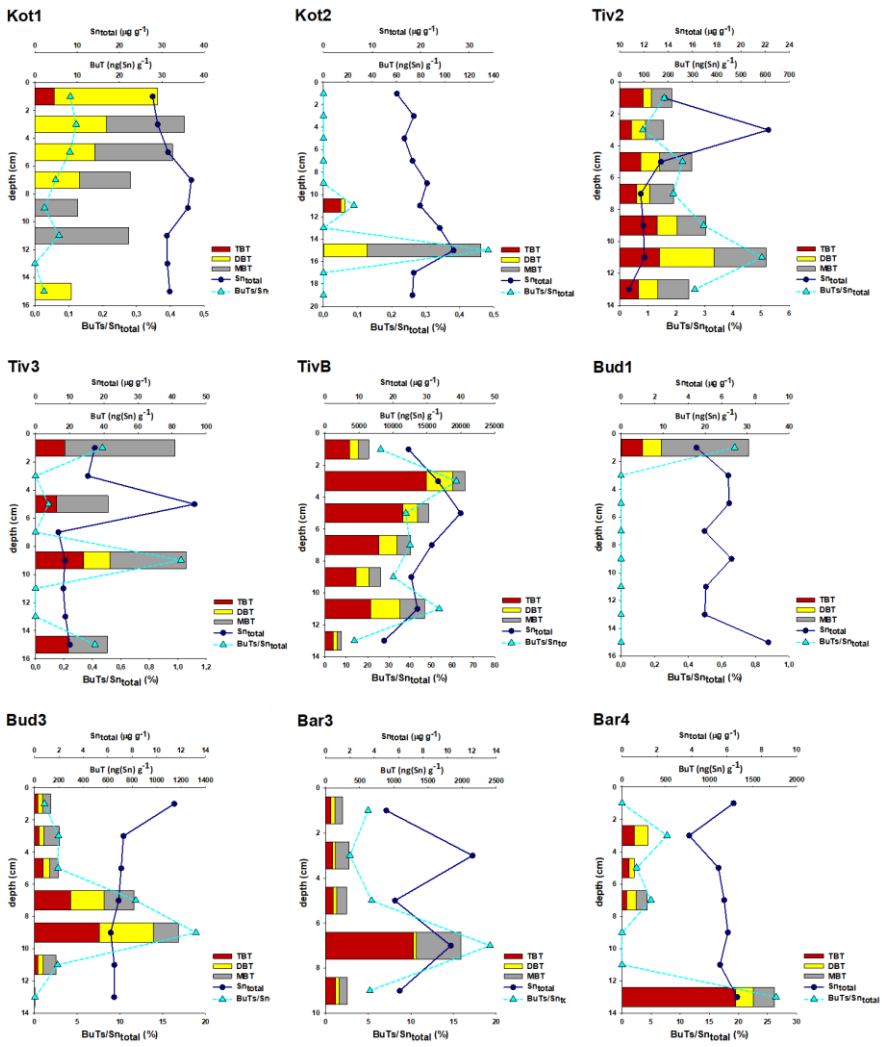

Figure 4. ~~The d~~Depth distributions of butyltins (MBT, DBT, TBT and ∑BuTs) and total Sn in sediment cores along with proportions of ∑BuTs/Sn_{total}. ~~(~~The absence of bars for BuTs means that they were not detected – C~~the c~~oncentrations ~~are~~ <LOD.~~)~~

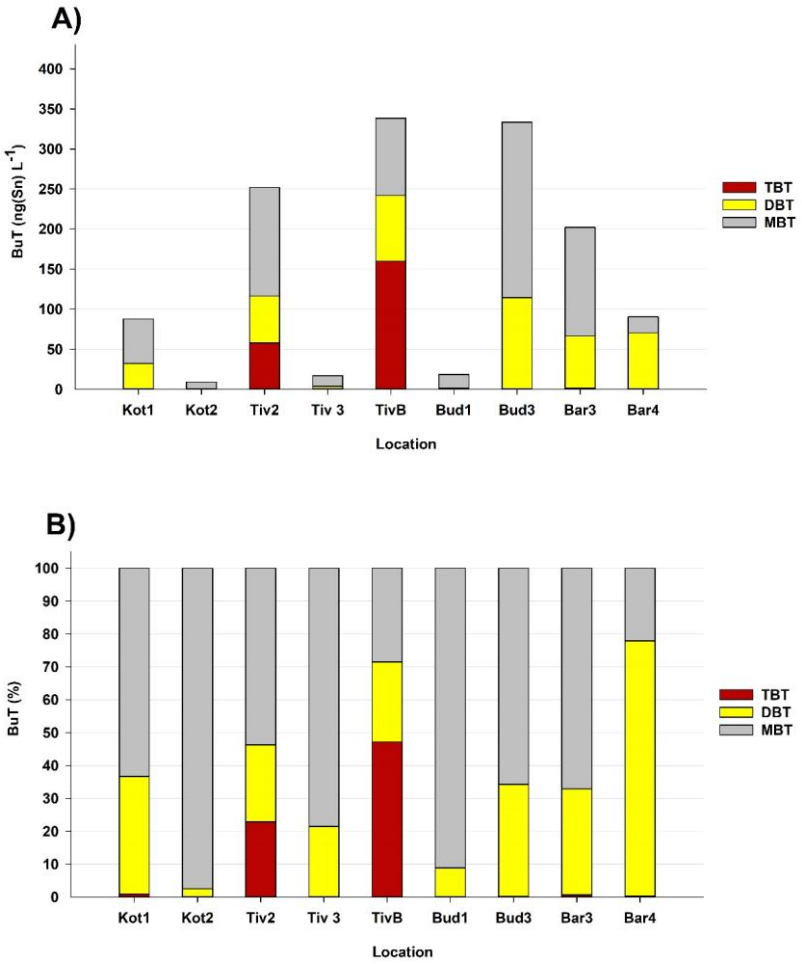

Figure 5. ~~The d~~Distribution of BuTs in water~~s~~ overlying sediment: (a) MBT, DBT and TBT concentrations, and (b) percentage of each BuT species (BuT/∑BuTs).

Table S1. The description of sampling locations along the Montenegrin coast.

Location	Type pf samples	Description of location	Water depth (m)
		Kotor Bay	
Kot1	sediment, mussels	marina in Kotor	7
Kot1a	mussels	marina in Kotor	3
Kot2	sediment, mussels	cruise port in Kotor	15
Kot3	mussels	small local port (Dobrota)	5
Kot3a	mussels	small local port (Dobrota)	3
		Tivat Bay	
Tiv1	mussels	marina in Tivat	8
Tiv2	sediment, mussels	marina in Tivat	6
Tiv3	sediment, mussels	outside the marina in Tivat	21
TivB	sediment	shipyard	24
		City of Budva	
Bud1	sediment, mussels	marina	4
Bud1a	mussels	local port	3
Bud3	sediment	marina (near the hoist)	5
Bud4	mussels	marina	4
		City of Bar	
Bar1	mussels	cargo port	15
Bar3	sediment, mussels	marina	6
Bar4	sediment, mussels	military port	7

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Credit Author Statement

Nevenka Mikac: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Martina Furdek Turk**: Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Visualization, Supervision. **Dragana Petrović**: Investigation. **Miljan Bigović**: Formal analysis, Investigation. **Sladjana Krivokapić**: Investigation, Funding acquisition.