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Development of periphytic diatoms on different artificial substrates in the Eastern Adriatic Sea

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Abstract – The settling of diatoms as fouling organisms on a certain substrate is greatly influenced by substrate characteristics and the preferences of a diatom community and diatom species. A distinction among substrates can be made by analysing the specific abundance and composition of diatoms on different substrates. In this study, 11 different artificial substrates were exposed to a marine environment for a period of 30 days. Abundance and taxonomic composition of periphytic diatoms was determined on each of the substrates and on shoots of the marine seagrass *Posidonia oceanica*. The aim was to compare diatom community structure on different newly colonized surfaces. On all surfaces examined, periphytic diatoms were the pioneering organisms with differences in quantitative and qualitative composition on the different substrates. Taxonomic analysis of diatom communities on the substrates examined revealed 41 diatom taxa, with the dominant genera *Cylindrotheca, Amphora, Nitzschia, Cocconeis* and *Navicula*. Given that all the examined artificial substrates were solid materials, differences in the abundance and species composition of diatoms found between the materials point to the substrates' physical and chemical characteristics as a major influence on the final settling of diatoms. Knowledge from investigating the settlement of fouling organisms on anthropogenic substrates can have future use in management of waste materials that end up in the marine environment.

Key words: Adriatic Sea, anthropogenic materials, artificial substrates, diatoms, fouling, marine litter, periphyton

Introduction

Anthropogenic solid materials often end up in the marine environment and are commonly known as marine litter. In their exposure to sea water, those materials undergo chem-

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ical and biological conditioning known as the fouling process. Chemical conditioning produces a molecular film comprising compounds including glycoproteins, humic material, proteins, lipids, nucleic acids, polysaccharides, aromatic amino acids and/or unspecified macromolecules (Garg et al. 2009 and the references therein), whereas biological conditioning supersedes with the accumulation of prokaryotic and eukaryotic unicellular organisms (Railkin 2004) incorporated in the matrix of extracellular polymers (Donlan 2002). A newly formed conditioning matrix, or biofilm, is mostly composed of bacteria and diatoms. Other unicellular organisms, like flagellates, ciliates, yeasts and protozoans contribute less than 1% of the total cell count in the biofilm (Railkin 2004). The presence of bacteria and unicellular algae in the biofilm can enhance further colonization of the substrate by plants and animals (Totti et al. 2007 and the references therein). The initial colonizing biomasses, along with bacteria, are diatoms (Wetherbee et al. 1998), eukaryotic microalgae, which constitute the periphyton community on immersed substrates. Diatom adhesion is mediated by the physico-chemical properties of the substrate and the diatom cell properties. The surface charge of diatom cells is dependent on their cell wall. Although the cell wall is siliceous and therefore negatively charged, it is covered with an organic layer of polysaccharides, proteins and glycoproteins (Hecky et al. 1973, Staats et al. 1999), which allows cell surface potential to vary. Extracellular polymeric substances (EPS), secreted through numerous openings in the cell wall and the raphes is recognized as the extracellular adhesive which provides diatoms with the ability of permanent adhesion and motility on various substrates (Wang, et al. 1997, Wustman et al. 1997). In the investigation of the diatoms' cell surfaces and their ability of adhesion many techniques have been successfully used. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are traditional and the most commonly used techniques preformed on dried samples. Since diatoms form the bulk of initial colonizing biomass on immersed substrates, researchers have used artificial substrates to understand the preference of diatoms for a certain substrate (Rodrigues and Bicudo 2001, Danilov and Ekelund 2002, Tank and Dodds 2003, Townsend and Gell 2005). The first recorded study using an artificial substrate for periphyton settling was done by Hentschel in 1915. The advantage of newly introduced artificial substrates in the marine environment is the opportunity to monitor initial development and the succession of diatoms in the periphyton. Comparative research on nutrient concentration of periphyton on living substrates (macrophytes, shells), epilithion (Danilov and Ekelund 2002, Kahlert and Pettersson 2002) and the periphyton on inorganic (glass, plastic and rock) and organic substrates (wood, leaves) showed that living and organic substrates act as additional source of nutrients for attached communities (Danilov and Ekelund 2002, Tank and Dodds 2003). Some studies pertaining to the structural variations between artificial and natural substrates showed a significant difference between artificial and natural substrates (Townsend and Gell 2005) and others little (Rodrigues and Bicudo 2001) or no structural difference (Lane et al. 2003). Also, the development of periphyton on man-made structures in the marine environment has become a widespread issue and the literature about periphyton development has been rapidly growing since the 1980s (Bhosle et al. 1989, Railkin 2004, Yebra et al. 2004, Shultz et al. 2011).

Recent periphytic studies in the Adriatic Sea have focused on epiphytic diatoms of the northern Adriatic (Munda 2005) and the toxic bloom of benthic dinoflagellates *Ostreopsis* (Totti et al. 2007, Pfannkuchen et al. 2012), whilst in the middle Adriatic research is focused on the ecology and taxonomy of periphytic diatoms in the estuary of rivers, like the River Zrmanja (Burić et al. 2004, Caput et al. 2005).

In this research, we examined the initial colonization of diatoms in the periphytic community on a wide array of immersed artificial substrates with various physico-chemical properties. The abundance of diatoms on a newly formed biofilm has been studied, as has the diatom genera composition. The seagrass *Posidonia oceanica* in a nearby meadow was also sampled to compare the abundance and composition of the diatom community from artificial substrates with an already established community on a natural substrate. The aim of this study was to determine the difference in abundance and composition of diatom community on various artificial substrates and to discuss the difference in terms of diatom affinity to a specific artificial material. The relevance of investigating the affinity of diatoms as a major fouling community to a specific artificial material is to get an insight into the outcome of debris in the marine environment in order to systematically work towards alleviating this initially negative impact on the marine environment.

Materials and methods

Study area

The experiment was carried out in the Puntamika peninsula near Zadar, Croatia, coastal area of the Central Adriatic Sea (Fig. 1). The Adriatic Sea is an elongated basin, the northernmost part of the Mediterranean. The eastern coastal region is influenced by the ingoing current from the Ionian Sea, characterized by high salinity and low content of nutrients, and freshwater discharge from oligotrophic karstic rivers. The investigated station is situated in a region defined according to the physicochemical conditions as a region similar to the open waters of the middle Adriatic Sea (VIDJAK et al. 2012).

Artificial substrates were exposed to the marine environment at a depth of 12 m for the period of one month (March 7th to April 6th 2012). Eleven different materials were used as artificial substrates: asbestos, painted iron, wood, concrete, glass, plastic, unpainted iron, rubber, ceramics, stone and aluminium. Materials used as substrates were of variable shapes and sizes and were acquired from the local waste deposit site. Description of the materials is as follows: glass – transparent glass used as a window, asbestos – plates used as roofing material, ceramic – smooth, glazed ceramic indoor tiles, rubber – rubber inner tube of a bicycle tire, wood – processed, smoothed wooden bar, plastic – hard non-transparent plastic

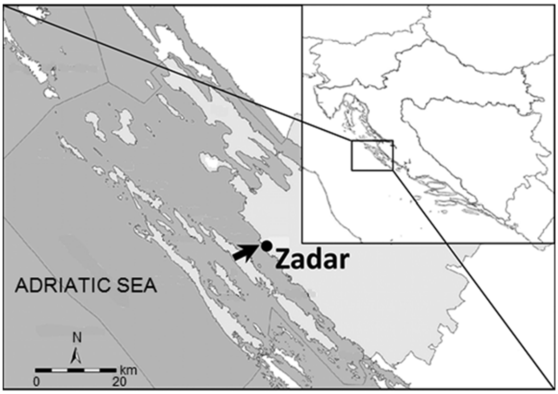

Fig.1. Study area at Puntamika, Zadar, Croatia.

plate, aluminium – flat plate, iron – flat plate, painted iron – painted flat plate, rock – karstic rock, concrete – building block. Two replicates of each substrate were used in case of eventual loss of one or more substrates. Substrates were submerged with the use of scuba diving to the depth of 12 m, which provided enough light for the development of periphyton, and protection from the influence of surge. Temperature of the sea while submerging was taking place was 11 °C, and 12 °C during the retrieval of the substrates. All substrates were placed on the seabed and arranged at 45º angle, leaning against a plastic pipe that followed the seabed at the location. The plastic pipe is an outlet pipe that has been there for a substantial amount of time. Plastic and rubber substrates were additionally anchored with materials (stone, glass jar) found at the location.

Sampling

After 30 days' exposure, scuba divers retrieved one replicate of each substrate. Upon retrieval, the substrates were enclosed, each in a separate plastic bag to minimize the effect of the phytoplankton to the substrates. Upon retrieval, a 36 cm^2 area of the mucous biofilm covering the surface of the substrates was scraped from both the top and bottom sides of each substrate with a scalpel and brush. Scraped surface of each substrate was rinsed with distilled water into marked, wide-mouthed sample plastic vials and preserved in a 4% formaldehyde solution. Two shoots of the seagrass *Posidonia oceanica* L. (Delile) from a nearby seagrass meadow were also sampled. The top parts of the shoots were cut, placed in a 0.5 L wide-mouthed sample container and preserved in 4% formaldehyde solution. In all, 22 samples were collected, 20 from the studied substrates and two shoots of *P. oceanica*. In two substrates, ceramics and concrete, sampling of the bottom side was not possible, due to roughness of the bottom surface of the materials and the inability to sample properly.

Cell abundance analysis

Fixed samples were stirred gently in plastic vials until they became homogeneous. Subsamples were taken with a pipette and poured into 10 cm^3 sedimentation chambers. Samples containing *Posidonia oceanica* shoots were shaken vigorously and the surfaces of the shoots gently rubbed to remove the remaining periphytic diatoms and allowed to detach from the shoot surfaces into the suspension. After 24 hours of sedimentation, samples were analyzed in sedimentation chambers following the standard Utermöhl (1958) method using an inverted microscope (ZEISS Axiovert 200, Zeiss GmbH). Due to the substantial amount of organic matter secreted by the cells and the small size of the cells, it was not possible to determine diatoms under the light microscope, with the exception of *Cylindrotheca closterium* (Ehrenberg) Reinmann. *C. closterium* cell abundance was counted separately, due to its high dominance in the samples and the specificity of the cells, which allowed easy determination.

Taxonomic analysis

For a more detailed taxonomic analysis using scanning electron microscopy (SEM), samples needed to be cleaned from cellular residue and organic matter. The cleaning process is based on the oxidation of organic matter with the method of strong acid oxidation. Samples were treated according to the von Stoch method (Hasle and Syvertsen 1997) using nitric acid (65%) and sulfuric acid (97%) in the quantity 1:1 and 3:1 in proportion to the sample volume respectively. The sample was stirred and heated to boiling point, which made the sample clear. After the sample was cooled, it was rinsed five times with distilled water to remove the acid. After acid cleaning of the samples, samples were prepared for examination under the SEM. A drop of the cleaned diatom material was mounted on aluminium stubs, air dried and gold coated with a sputter coater (S150A Sputter coater; Edwards Ltd., Crawley, UK). Observations were made with a Philips 515 SEM (FEI Co.). Diatoms were identified to the genus level according to the classification system of ROUND et al. (1990) using standard determination keys.

Results

Upon retrieval, after 30 days of exposure, a visible light brown macroscopic biofilm could be observed on all submerged substrates with uniform distribution. There were no apparent visual differences between the biofilms covering the substrates. Microscopic analysis showed that the biofilm consisted mostly of diatoms, with dinoflagellates, microscopic green algae (*Chlorophyta*) and brown algae *(Phaeophyta*) appearing sporadically. A marked difference in abundance of diatoms between different materials was recorded.

Abundance of diatoms on artificial substrates

The highest abundance of diatoms (6948 cells cm^{-2}) was recorded on asbestos. Diatom abundance on other materials is as follows from greatest to least: painted iron (4622 cells cm^{-2}), wood (4186 cells cm^{-2}), concrete (3666 cells cm^{-2}), *Posidonia oceanica* (2769 cells cm^{-2}), glass (2266 cells cm^{-2}), plastic (2261 cells cm^{-2}), unpainted iron (1180 cells cm^{-2}), rubber (1100 cells cm^{-2}), ceramics (839 cells cm^{-2}), stone (777 cells cm^{-2}) and the lowest recorded abundance was on aluminium (216 cells cm^{-2}) (Fig. 2). The mean abundance of diatom cells on studied substrates was 2569 cells cm^{-2}.

Artificial substrates were placed at an angle of 45 ° on the sea bed, so the bottom sides of all the substrates, except concrete and ceramics, were sampled and abundance analyzed. The highest abundance on the bottom side of the substrates was on glass (2225 cells cm^{-2}), followed by wood (1550 cells cm^{-2}), rubber (1329 cells cm^{-2}), plastic (653 cells cm^{-2}), asbes-

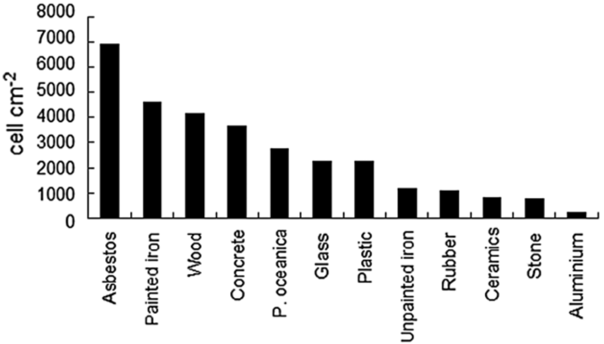

Fig. 2. Abundance of diatoms on top sides of examined substrates.

tos (466 cells cm^{-2}), unpainted iron (455 cells cm^{-2}), stone (360 cells cm^{-2}), aluminium (50 cells cm^{-2}) and the least was on painted iron (46 cells cm^{-2}) (Fig. 3). Mean abundance of diatom cells on bottom sides of studied substrates was 793 cells cm^{-2}.

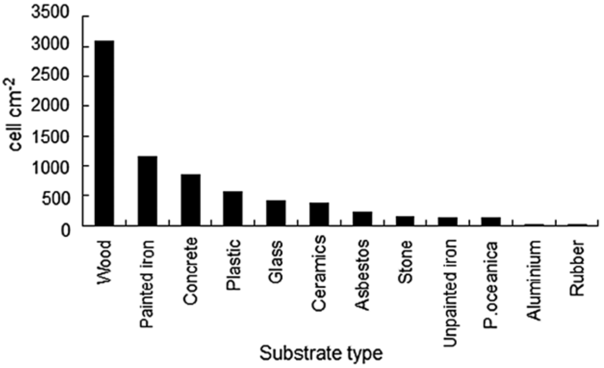

Fig. 3. *Cylindrotheca closterium* abundance on top sides of examined substrates.

After preliminary analysis of the samples using light microscopy, it was noted that the diatom *Cylindrotheca closterium* was the dominant species of diatoms present in all the samples. Therefore, in addition to the analysis of the abundance of all diatoms on investigated substrates, the abundance of the diatom *C. closterium* was analyzed independently. The highest abundance of *C. closterium* was recorded on wood (3091 cells cm^{-2}), then painted iron (1161 cells cm^{-2}), concrete (861 cells cm^{-2}), plastic (569 cells cm^{-2}), glass (424 cells cm^{-2}), ceramics (383 cells cm^{-2}), asbestos (238 cells cm^{-2}), stone (161 cells cm^{-2}), unpainted iron (140 cells cm^{-2}), *P. oceanica* (137 cells cm^{-2}), aluminium (16 cells cm^{-2}) and the lowest on rubber (11 cells cm^{-2}). The mean of *C. closterium* diatoms on the substrates studied was 599 cells cm^{-2}. Abundance of *C. closterium* on the bottom sides of the substrates was also analyzed. The highest was on glass (132 cells cm^{-2}), followed by wood (100 cells cm^{-2}), stone (60 cells cm^{-2}), plastic (50 cells cm^{-2}), rubber (17 cells cm^{-2}), asbestos (16 cells cm^{-2}), painted iron (9 cells cm^{-2}), unpainted iron (5 cells cm^{-2}) and none was recorded on aluminium. The mean abundance of diatoms *C. closterium* on the bottom sides of studied substrates was 43 cells cm^{-2}.

Taxonomic analysis

Taxonomic analysis revealed 41 diatom genera (Tab. 1**)**, of which ten were identified as planktonic genera sporadically present on several substrates. The dominant diatom species recorded on all substrates were *Cylindrotheca closterium*, identified and counted with light microscopy, and diatom genera *Amphora,* identified with SEM. The iron substrate had the highest diversity (20 taxa), the same as the diversity recorded in natural periphytic assemblages on *Posidonia oceanica* (20 taxa). The lowest diatom diversity was recorded on plastic (4 taxa), concrete (4 taxa) and rubber (2 taxa) (Tab 1). Other diatom genera with frequent occurrence noted in the samples were *Nitzschia* (8 substrates + *P. oceanica*) (Fig. 4B), *Cocconeis* (7 substrates + *P. oceanica*) (Fig. 4C) and *Navicula* (7 substrates + *P. oceanica*) (Fig. 4D).

Tab. 1. Diatom diversity recorded on all examined substrates. Asterisk presents planktonic genera, *P.o.* – *Posidonia oceanica*, Ir. – iron, Gls. – glass, Al. – aluminium, Cer. – ceramics, P.Ir. – painted iron, As. – asbestos, St. – stone, W. – wood, Pls. – plastic, Crt. – concrete, Rub. – rubber.

Substrate Genus	*P.o.*	Ir.	Gls.	Al.	Cer.	P.Ir.	As.	St.	W.	Pls.	Crt.	Rub.
Amphora spp.	×	×	×	×	×	×	×	×	×	×	×	×
Coconeis spp.	×	×	×	×	×	×	×			×		
Nitzschia spp.	×	×	×		×	×	×	×	×			×
Navicula spp.	×	×	×	×		×		×	×	×		
Haslea spp.*		×				×			×		×	
Entomoneis spp.		×	×			×	×	×			×	
Fallacia spp.	×	×		×	×							
Lyrella spp.	×	×		×			×					
Licmophora spp.	×			×		×						
Striatella spp.	×	×	×									
Striatella spp.	×	×	×									
Grammathopora spp.	×	×						×				
Tabularia spp.	×			×				×				
Ardissonia spp.						×	×					
Campylodiscus spp.	×	×										
Campyloneis spp.	×			×								
Actinoptychus spp.	×	×										
Plagiogrammopsis spp.		×										
Dimeregramma spp.		×										
Microtabella spp.		×										
Paralia spp.		×								×		
Opeophora spp.		×	×									
Pleurosygma spp.*	×	×										
Climaconeis spp.			×		×							
Diploneis spp.		×										
Fragilaria spp.					×							
Thalassiosira spp.*					×							
Thalassionema spp.*											×	
Auricula spp.									×			
Parlibellus spp.									×			
Toxarium spp.	×											
Rophalodia spp.	×											
Planktioniella spp*.				×								
Astreromphalus spp.*					×							
Bactreiastrum spp.*					×							

Tab. 1. – continued

Substrate Genus	*P.o.*	Ir.	Gls.	Al.	Cer.	P.Ir.	As.	St.	W.	Pls.	Crt.	Rub.
Membraneis spp.*							×					
Chaetoceros spp.*					×							
Delphineis spp.*	×											
Mastogloia spp.	×											
Berkeleya spp.	×											
Psammodictyon spp.				×								
Proschkinia spp.			×									

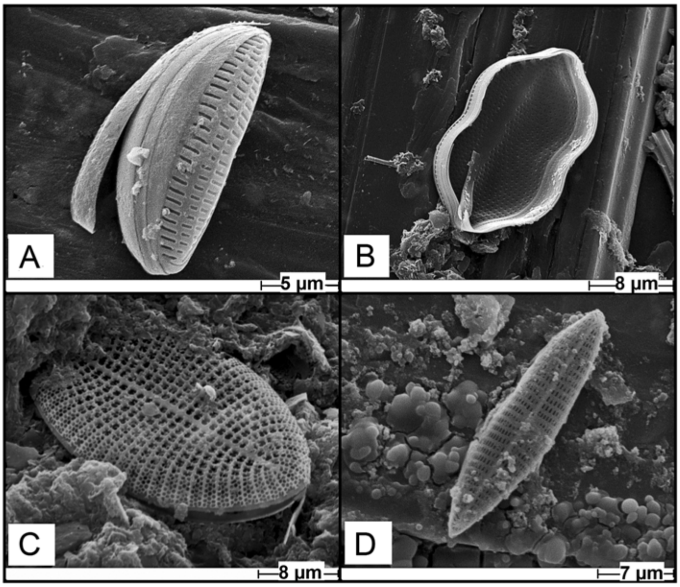

Fig. 4. Scanning electron microscope microphotographs of dominant genera of diatoms found on various substrates: A) *Amphora* sp. on glass substrate, B) *Nitzchia* sp. on ceramic tile substrate, C) *Cocconeis* sp. on aluminium substrate, and D) *Navicula* sp. on painted iron substrate.

Discussion

Difference in quantitative and qualitative composition in the diatom community recorded on the substrates investigated impliess the preference of diatoms for specific substrates.

Quantitative values of diatom abundance were recorded on the top sides of all the substrates and shoots of the seagrass *Posidonia oceanica* from a nearby meadow. Materials of substrates used in this study vary in different physico-chemical properties, e.g. surface roughness, hydrophobicity, surface energy, solubility etc. With the substrates being placed at 45° angle, the top sides of the substrates had expectedly higher abundance of diatoms than the bottom side of the substrates due to the availability of the light diatoms use for photosynthesis. The interest in recording the diatom abundance on the bottom sides of substrates, where light is much more reduced, was in investigating whether any of the substrates acts as a nutrient source for the diatom community.

The highest abundance of diatoms in this study was recorded on the asbestos substrate. High abundance of diatoms on asbestos suggests that this substrate is a favorable habitat for diatoms. Asbestos is composed of silicate fibers, and as such, is chemically inert and resistant to biodegradation. Fibers could provide high protection from grazers and the space between the fibers can serve as a nutrient trap. Due to its porosity, it absorbs water and prolonged exposure of asbestos in water leads to slow, progressive rinsing of metal and silicate compounds. Fibers of the most commonly used chrysotile asbestos (asbestos fibers are divided into chrysotile and amphiboles) are coated with a brucite layer ($MgOH_2$) which dissolves relatively fast in water. The highly polar surface of chrysotile promotes adsorption of various organic and inorganic substances (SPEIL and LEINERVEBER 1969). As the brucite layer of chrysotile asbestos dissolves, magnesium ions (Mg^{2+}) are rinsed into the surrounding medium as $MgOH_2$ (CHISSICK 1985). High concentration of Mg^{2+}, as shown by (SONG and LEFF 2006) in a study with *Pseudomonas fluorescens* MIGULA 1895, can augment the production of exopolisaccharids (EPS) in bacteria, stabilize the structure of biofilms and facilitate further surface settlement (COSTERTON et al. 1995). Further investigation is needed to ascertain if asbestos used in this research releases Mg^{2+} to the surrounding water and what effect Mg^{2+} has on biofilm formation.

Painted iron had the second highest abundance of diatoms, after asbestos. The paint covering the surface of the iron makes the substrate's surface very smooth, so the high abundance of diatoms on such a smooth surface is unexpected. In their study on microbial colonization, CHARACKLIS et al. (1990) recorded an increase in the extent of microbial colonization with substratum surface roughness. Common physico-chemical properties of metal substrata that could influence the settlement of organisms are their high surface energy and hydrophilicity. High surface energy of metal surfaces could promote adhesion, but is soon after immersion reduced by the adsorption of organic particles (KINLOCH 1990; CAILLOU et al. 2008). Hydrophilicity of metal surfaces, as shown in several previous studies (PEDERSEN 1990; BECKER and WAHL 1991; BECKER 1996), makes adhesion of diatoms more difficult as opposed to adhesion on hydrophobic surfaces. The composition of the paint covering with its specific, yet undetermined, chemical characteristics could have facilitated the adhesion and settlement of the diatoms and been responsible for the high abundance recorded on coloured iron.

Abundance of diatoms on wood was similar to that on painted iron. The high abundance of the diatom *Cylindrotheca closterium* on wood substrate greatly contributed to the high overall abundance of diatoms on wood. Our study showed that wood has proven to be a very favorable substrate for the diatom *C. closterium.* Wood can pose as a source of nutrients, as suggested in a study by VADEBONCOEUR and LODGE (2000) which concluded that the availability of nitrogen and phosphorus in the water column can depend on the level of wood degradation, and ZHANG et al. (2013) proposed that wood is a potential source of nitrogen due to saprophyte domination. Furthermore, SCHOLZ and BOON (1993) showed that wood can be a substantial source of carbon for periphytic algae due to bacterial and fungal decomposition.

A high abundance of diatoms was also recorded on a concrete substrate. Concrete was indicated to be an excellent substrate and habitat for the settling of periphytic organisms in

»Guidelines for marine artificial reef materials« by Lukens and Selberg (2004). It is known that macroalgae can use some elements from the concrete (calcium, aluminium, iron and potassium) that they need for metabolism (Berton 2004).The high roughness of concrete can trap detritus in the surface cracks which can serve as an additional source of nutrients (Taniguchi and Tokeshi 2004), as well as a protection from grazers (Bergey and Weaver 2004).

Diatom abundance on Posidonia oceanica was similar to the mean abundance recorded on studied artificial substrates, but the diversity of diatoms was the same as the highest diversity recorded on the unpainted iron substrate. Seagrass is a natural substrate for periphyton and the diatom community on shoots of seagrass was already a settled, more stable community. Macrophytes generally serve as a nutrient source for attached periphyton community (Cattaneo and Amireault 1992). Also, epiphytes on shoots of seagrass have better availability to light, as well as to nutrients from the water column and the substrate (Hutchinson 1975). Additionally, macrophytes as an elastic substrate can sustain wave energy and reduce turbulent effects on the biofilm surface, which could promote biofilm formation (Pfannkuchen et al. 2012).

Glass and plastic substrates showed very similar diatom abundance, around the mean value. Glass and plastic panels have been widely used as artificial substrates for the settlement of diatoms in both marine and fresh water environments by many researchers (Cooksey et al. 1984, Wahl and Mark 1999, Caput et al. 2005, Nayar et al. 2005, Webster and Negri 2006, Reisser et al. 2014). Although both materials are inert in the marine environment, glass and plastic have different physico-chemical properties. Glass is a high-energy hydrophilic surface, while plastic is a low-energy hydrophobic surface, and as reported by many studies, diatoms adhere more successfully to hydrophobic surfaces (Fletcher 1988, Becker and Wahl 1991, Becker 1996). The shared property of both substrates is the smoothness of the substrate, which could be the cause of the similar diatom abundance.

The unpainted iron substrate showed low diatom abundance, but the highest diatom diversity. The hydrophilicity of iron makes the adhesion of diatoms on metal surfaces difficult. This is also attributed to the low surface energy caused by organic layer, which is effective in preventing adhesion (Townsin and Anderson 2009) as mentioned before. Metals in sea-water form hydroxides biologically unavailable for uptake by algae (Lewandowska and Kosakowska 2004). Most studies of diatoms, and biofilm in general, on metal surfaces have investigated diatom biofilms on hydrodynamic drag on vessels (Bohlander 1991, Shultz et al. 2011) and the biofilm development on stainless steel surfaces (Schneider 1996, Landoulsi et al. 2011).

Diatom abundance on a rubber substrate was as low as that on unpainted iron. Although rubber is inert in the marine environment, some research has focused on leaching of heavy metals and organic compounds from rubber to surrounding water. Heavy-metal leaching in seawater could be effective in preventing the colonization of the substrate by fouling organisms, as reported by Jelić-Mrčelić (2006) in tests with heavy-metal leaching antifouling (AF) coatings. Zinc, as a major toxicant that rubber leaches into the water environment, was identified by Collins et al. (1995) and Gualtieri et al. (2005), but according to their study, the amount of zinc being released in that way does not have any significant effect on most marine organisms. The settling of fouling communities on rubber substrates should be studied in more detail, since rubber is one of the most common materials used for artificial reefs. Also, the disposal of rubber tires has become a widespread issue as large amounts of car tires are being disposed of in the marine environment (personal observation), especially for implementing artificial reefs and often without any prior investigation of the influence of tires on the marine environment and their suitability for the settling of marine fouling organisms.

The extremely smooth surface of the glazed ceramic tile could be the reason for low diatom abundance. The results of MURDOCK and DODDS (2007) in their study with benthic microalgae showed higher abundance of algae on unglazed than on glazed ceramic tiles. Studies by CHARACKLIS et al. (1990), investigating the adhesion of bacteria on surfaces of different roughness, and WOODS and FLETCHER, (1991), investigating adhesion of diatoms on rough and smooth surfaces, came to the same conclusion – that the adhesion for both bacteria and diatoms was more successful on rougher surfaces because of the bigger surface for attachment in comparison with smooth surfaces.

Low abundance on stone substrata was surprising given that stone is a natural substrate for the periphyton, and has a rough surface texture. Periphyton on stone substrates can acquire nutrients from the surrounding water column or microbial regeneration inside the periphyton matrix (STEVENSON and GLOVER 1993).

Aluminium substrates had the lowest diatom abundance of all the substrates investigated. The very smooth surface of aluminium can make adhesion of diatoms difficult and facilitate detachment of cells from the surface. Also, it is possible that aluminium and aluminium oxide could have a toxic effect on periphytic organisms. Several studies showed that aluminium dissolved in sea-water inhibits the growth of some planktonic species by reducing the amount of available dissolved phosphorus (DICKSON 1978, DRISCOLL 1985) or with direct toxic effects (FOLSOM et al. 1986).

The interest in recording the abundance of bottom sides of the substrates where light is much more reduced was in investigating whether any of the substrates acts as a nutrient source for the diatom community. The bottom side of the glass substrate had the highest abundance of diatoms. The transparency of the glass plate used in this study made photosynthesis available to the diatom community on the bottom side of the substrate. High diatom abundance on the bottom side of wood substrate could be attributed to wood being a nutrient source for the periphyton community, as mentioned before. Rubber substrate showed high abundance of diatoms and was the only substrate that showed higher abundance of diatoms on the bottom side of the substrate. The reason for this could be in the method the rubber substrate was anchored to the sea bed. The rubber used in this research was a black bicycleinner tube anchored around a glass jar found on the location, so the bottom side of the rubber was in direct contact with the glass jar from which the already established diatom community could migrate to the rubber substrate. Plastic, asbestos, unpainted iron and stone showed similar low abundances, that can be explained by insufficient light availability. Aluminium and painted iron had extremely low abundances, which could be attributed to the antimicrobial effects of metals (JAIN 1990), as well as to the low light intensity on the bottom side of the substrates.

Abundance of the diatom *Cylindrotheca closterium*, due to its specificity and high abundances in the preliminary examination of substrates, was recorded for the top and bottom sides of all substrates. By far the highest abundance was on wood substrate, followed by painted iron, concrete, plastic, glass, ceramics, asbestos, *P. oceanica*, unpainted iron, stone, and the the lowest was on aluminium and rubber. The abundance of the bottom side followed the same trend, with the exception of the glass substrate, which showed the highest abundance of *C. closterium* due to its transparency and the availability of light. It can be concluded that the wood substrate is the most favorable substratum for the diatom *C. closterium*.

This study recorded *Cylindrotheca closterium* and the genera *Navicula, Nitzschia, Cocconeis* and *Amphora* as dominating diatoms in the periphyton community on the artificial substrates studied. It has been shown that pennate diatoms dominate diatom communities in biofilms (PATIL and ANIL 2005, and included citations) with frequently identified pennate genera *Navicula, Nitzschia, Cocconeis, Amphora* (RAILKIN 2004), as was recorded in this study. The majority of studies showed the dominance of pennate genera *Amphora, Navicula, Nitzchia* (KHATOON et al. 2007) and *Cylindrotheca* (MOLINO et al. 2009; DOBRETSOV and THOMASON 2011; BRIAND et al. 2012) on different artificial substrates as well as on fouling release coated substrates (CASSÉ and SWAIN 2006; ZARGIEL et al. 2011). The recorded genera of diatoms in this study are therefore typical periphytic diatoms in biofilms.

In conclusion, this study recorded a qualitative and quantitative difference of diatom abundance in periphyton community among different artificial substrates. It was proven that the settlement of diatom community on a specific substrate is dependent on light availability, surface roughness and properties. We hypothesize that some substrates, like wood and concrete, serve as a nutrient source to the periphyton community. Hydrophobicity of the substrates was not shown to have a major influence on diatom settlement.

As mentioned in the discussion, there have been many efforts at finding favorable artificial reef materials that promote the biodiversity of marine organisms. Development of periphyton is the first step towards the settlement of higher organisms, and, provided with a suitable substrate, the biodiversity could be enhanced. Thus, this initially negative impact that the disposal of litter has on the marine environment can be alleviated with proper management and further research.

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