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„Impact of tire debris on the microbial community of the Mediterranean Sea"

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Contents

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Abstract

The accumulation of synthetic polymers in the ocean is a widely recognized problem and only recently the attention has been drawn towards tire debris as a major pollutant. As there are multiple commercial applications for end-of-life tires, the processed tire material, which is often small in size, is mainly transported into marine systems via freshwater runoff. Specific components of tire material are leaching into the water, likely enhanced by UV-irradiation. The consequences for marine microbial communities, such as in the Mediterranean Sea, remain, however, largely enigmatic. Here, we show that the exposure of tire particles to UV-radiation resulted in elevated leaching of dissolved organic carbon (DOC) while photo-oxidation of tire material did not occur. Furthermore, exposure of marine microbes to tire leachates led to higher bacterial growth and respiration as compared to the unamended controls, irrespective of whether the material was previously exposed to UV-radiation. Lastly, the exposure of marine microbes to tire particles over a period of several weeks resulted also in elevated bacterial abundance, hence growth. Thus, we conclude that natural marine microbial communities are able to utilize tire material originating from both, tire particles and leachate.

1. Introduction

When the plastic mass production started in the 1950s, the environmental impact of this new synthetic polymer was not critically scrutinized (Barnes *et al.,* 2009). What started as a great innovation led to one of the biggest pollution problems of our time. The widely perceived aesthetic problem of plastic litter emerged quickly into a major threat to marine wildlife (Barnes *et al.,* 2009). The amount of annually mismanaged plastic litter of a single country caused by the human population inhabiting the coastal areas ranges from 1.1 to 8.8 million metric tons (MT) (Jambeck *et al.,* 2015). Although there is a widespread recognition of the plastic littering problem, plastic production is still growing and even if it would be stopped immediately, the particles would persist in the environment for hundreds of years (Barnes *et al.,* 2009). Gradual fragmentation leads eventually to a moderate breakdown of the plastic debris into small pieces, coined microplastics (<5 mm) (Ryan *et al.,* 2009).

Synthetic polymers are generally termed plastics, including a wide spectrum of different sorts (Zheng *et al.,* 2005). One synthetic polymer-based material gaining increasing attention is the rubber derived from car tires (Wagner *et al.,* 2018; Knight *et al.,* 2020). For their production, a mixture of petroleum based synthetic rubber and natural rubber is used. To obtain specific properties, various components are added including sulfur (1-4%), zinc oxide (1%), black carbon (22-40%), silica and oil (Kole *et al.,* 2017). During driving, heat and shear processes between the road surface and tires result in the generation of tire wear particles of various sizes. Caused by the heat, the volatile content evaporates and releases particles of submicrometer size that will be eventually transported by water runoff and wind into rivers and ultimately reach the ocean (Kole *et al.,* 2017).

Tire wear and tear is however not the only source of tire derived microplastic. Each year around 1 billion end-of-life tires (ELT) are generated around the world (Wbcsd, 2015). However, with a recycling rate of 84% in Europe, there are many different applications for ELTs (Wbcsd, 2015). Discarded used tires can be recycled as a whole, grounded or devulcanized. Ground rubber applications include surface covers of playgrounds, establishment of athletic field turf and other landscaping applications (Bandyopadhyay *et al.,* 2008). Nevertheless, there is a specific concern using ground rubber directly since tire constituents will leach into the ambient surrounding. Several studies described potential

negative effects of tire material and tire leachate on marine organisms and human health (Llompart *et al.,* 2013; Rhodes *et al.,* 2012; Selbes *et al.,* 2015; Halsband *et al.,* 2020; Capolupo *et al.,* 2021). Halsband *et al.* (2020) identified various chemical compounds in car tire crumb rubber including polycyclic aromatic hydrocarbons, benzothiazole, phenolic compounds and different metals such as zinc, iron, or cobalt. Due to the toxic properties of some compounds, the exposure of tire leachates to marine copepods, for instance, caused death of the organisms (Halsband *et al.,* 2020). An analysis of tire leachate also revealed the presence of the plasticizer diethyl phthalate (Hennebert *et al.,* 2014). Plasticizers are commonly used to adjust the physical properties of plastic material and are not covalently bound to the material, thus making them subject to potential leaching into the ambient environment (Wright *et al.,* 2020). In a study by Nanthini & Sudesh (2016), the authors showed that bacteria of the strain *Streptomyces* sp. are able to degrade vulcanized, natural rubber car tires. Therefore, the environmental hazards of tires and the waste disposal problem of ELTs led to a general interest of the material's biodegradability (Nanthini & Sudesh, 2016).

When tire debris reaches the ocean, the material is not only exposed to seawater, but also to solar radiation. UV-radiation has the ability to change polymer properties during the process of photo-oxidative degradation (Singh & Sharma, 2008). In this process, solar radiation leads to the decomposition of the material by cleaving C-C bonds (Singh & Sharma, 2008). During this process, polymeric hydrocarbons are oxidized, resulting in low molecular weight products, carbon dioxide (CO_2) and carbon monoxide (CO) (Gewert *et al.,* 2015; Ranby & Lucki, 1980).

As tires are made up of various chemical compounds, the influence of UV-radiation might make specific carbon compounds available to biodegradation (Singh & Sharma, 2008; Tokiwa *et al.,* 2009). Besides photo-oxidation, physical disintegration and abiotic hydrolysis of polymers may lead to an enhanced biodegradation by increasing the surface to volume ratio of the particles (Palmisano & Pettigrew, 1992). This increase in surface area leads also to increased leachate concentrations in marine surface waters. Up to 23,600 MT of dissolved organic carbon (DOC) are estimated to be released into marine systems each year by marine plastic debris (Romera-Castillo *et al.,* 2018). The chemical compounds present in the plastic derived DOC in the ocean might be bioavailable and stimulate microbial carbon cycling. However, a study by Romera-Castillo *et al.* (2018) suggested that plastic leachate which was

previously exposed to artificial solar radiation has negative effects on microbial growth. Nevertheless, as previously described, tires can have a different chemical composition than the polymers used in these experiments and thus, the influence of solar radiation might not have the same effects on tire material.

As many other marine water bodies, the Mediterranean Sea is subject to plastic pollution. Due to its enclosed nature, it is especially vulnerable to plastic waste accumulation and has one of the highest plastic waste densities of regional seas (Barnes *et al.,* 2009). Rivers such as Po, Isonzo and Ebro transport plastic debris into the Mediterranean Sea, resulting in an estimated amount of 756 to 2969 tons of floating plastic debris in the Mediterranean Sea (Cózar *et al.,* 2015).

To the best of our knowledge, the impact of tire leachates on natural marine microbial communities in the Mediterranean Sea has not been investigated yet. The aim of this study was to examine whether the exposure of shredded tire particles to UV-radiation results in photo-oxidation. Furthermore, we examined whether natural microbial communities are capable of using tire leachate as their sole carbon source, as previously described by Vukanti *et al.* (2009) for specific bacterial strains. Here, we hypothesized that leachate that was created under UV-radiation leads to higher bacterial growth due to increased concentrations of bioavailable DOC resulting in higher oxygen consumption. Also, we expected that under solar radiation, DOC leaching rates are higher than in the samples held in the dark. To study the direct influence of tire material on microbial communities, a long-term tire exposure experiment over 14 weeks was conducted to investigate the microbes' ability to utilize tire particles directly and investigate furthermore the biofilm formation on tire material.

2. Material and Methods

2.1. Sampling and preparation

Natural seawater (NSW) was collected in the Northern Adriatic Sea off Rovinj, Croatia and Piran, Slovenia. The water samples were aged in the dark to remove labile organic matter in polycarbonate containers and glass bottles that were previously rinsed with HCl (1M) and ultrapure water (18.2 MΩ·cm, Merck Millipore®, Germany). Prior to the experiments, NSW was filtered through 0.8 µm pore size to remove most of the non-bacterial cells.

All borosilicate glassware used in the following experiments was rinsed with ultrapure water and then combusted at 450°C for 6 h to remove all organics. Biological oxygen demand (BOD) bottles and BOD quartz glassware were rinsed thoroughly with HCl solution (1M) and ultrapure water. Artificial seawater (ASW) was prepared by adding to 1 L of ultrapure water 20.28 g NaCl, 9.18 g $MgCl_2*6H_2O$, 3.398 g Na_2SO_4, 1.286 g $CaCl_2*H_2O$, 0.564 g KCl, 0.1662 g $NaHCO_3$, 0.0826 g KBr, 0.022 g H_3BO_3, 0.021 g $SrCl_2*6H_2O$ and 0.0026 g NaF. The mixture was then autoclaved and stored at 4°C. Under sterile conditions, ASW was spiked with 1 µM NaH_2PO_4 and 10 µM NH_4Cl to ensure that the microbial communities were not limited by the availability of nitrogen and phosphorus.

Mixed non-sterile shredded tire material of 1-4 mm in size was provided by the Dept. of Geosciences, University of Vienna and stored in a 500 ml Schott flask at room temperature.

Each experiment, with the exception of the long-term experiment, was performed in duplicate, coined Experiment A and B.

Experimental setup

2.1.1. Photochemical oxygen demand

To determine the photochemical oxygen demand of tire material, the protocol of Reinthaler *et al.* (2006) for the determination of dissolved oxygen was followed. Calibrated BOD bottles of an approximate nominal volume of 120 cm^3 and Quartz BOD bottles were carefully filled under sterile conditions and room temperature. BOD bottles were filled in triplicate (Quartz bottles) and quadruplicate (borosilicate BOD bottles) with ASW + 2 g of shredded tires per 100 ml (ASW + tires) and ASW only (ASW) serving as a control. To assess the difference between the UV-radiation and dark treatment, the Quartz bottles comprising the samples 'ASW light' and 'ASW + tires light' were exposed to UV-radiation and borosilicate BOD bottles, comprising the samples 'ASW dark' and 'ASW + tires dark' were wrapped in aluminum foil. Artificial solar radiation was simulated over 13 d by 3 UVA-340 (QUV lamp, Q-lab) lamps and artificial photosynthetic active radiation (PAR) was supplied by two HQI-T Powerstar lamps (250 W, Osram) with a radiation intensity of 500 µmol m^{-2} s^{-1} for the 400-700 nm wavelength range. Prior to the incubation, the radiation intensity of the light tubes was tested using a Voltgraft UV-500 UV-measuring device with a range of 0.002 - 19.99 mW/cm^2. To determine the initial

O_2 concentration, one set of samples was fixed with Winkler reagents immediately (*T0*) and incubated in a flow-through water system together with the live samples (*T1*) while the water temperature was monitored. In Exp. A, the average temperature was (mean ± SD) 16.4 ± 1.2°C and in the Exp. B 18.2 ± 1.7°C. After an incubation period of 13 d, the live samples were fixed with the Winkler reagents. The O_2 concentrations at *T0* and *T1* were determined spectrophotometrically (Reinthaler *et al.,* 2006) at a wavelength of 466nm using a Shimadzu UV1800 spectrophotometer. Prior to the O_2 determination, the spectrophotometer was calibrated with standard additions of KIO_3. For this, BOD bottles were filled with ASW and with the Winkler chemicals I, II and III in reverse order. Subsequently, 50 µl, 100 µl, 200 µl, 400 µl and 1000 µl KIO_3 were added to five borosilicate BOD bottles and the absorbance measured (Reinthaler *et al.,* 2006).

2.1.2. Leachate production

Tire leachate was generated with tire material (2 g of tires per 100 ml) submerged in ASW (ASW + tires) and a control filled with ASW only (ASW) under sterile conditions. These experiments were performed in triplicate. For the UV-radiation exposed samples ('ASW light' and 'ASW + tires light') quartz bottles were used, for the samples kept in the dark ('ASW dark' and 'ASW + tires dark'), 500 ml Schott bottles were used and wrapped in aluminum foil. After an incubation period of 13 d, the leachate was collected by filtering each sample through a 0.2 µm polycarbonate filter. As the bottles for the leachate production were simultaneously incubated with the samples described in section **2.1.1. Photochemical oxygen demand**, the water temperature was the same as described in that section.

2.1.3. Bacterial respiration

To determine the biological oxygen demand of natural bacterial communities when exposed to tire leachate, samples from section **2.1.2. Leachate production** were filled in quadruplicate borosilicate BOD bottles together with 0.8 µm filtered natural seawater in a ratio of 1:10 (NSW : ASW). To determine bacterial respiration, the same approach as described in section **2.1.1.** was followed. Depending on whether the samples were previously exposed to UV-light or kept in the dark, the designations 'L' for light and 'D' for dark are used below. The bottles were kept in the dark in a water bath at 20°C. In Exp. A, the incubation time was 6 d and in Exp. B it was 1 d.

2.1.4. Microbial incubation with tire leachates

To reveal the ability of natural bacterial communities to utilize organic carbon derived from tire leachate, combusted borosilicate bottles were filled in triplicate with the four distinct samples generated in section **2.1.2.** and inoculated with 0.8 µm filtered natural seawater in a ratio of 1:10 (NSW : ASW). The samples termed 'ASW + NSW L', 'ASW + NSW + tires L', 'ASW + NSW D' and 'ASW + NSW + tires D' were shaken continuously at room temperature in the dark for several weeks. Samples (1.5 ml) were taken at different time intervals (12h - 24h) and fixed with glutaraldehyde (2% final concentration) for 10 min and subsequently stored at -80°C.

2.1.5. Microbial incubation with shredded tire particles

To determine the ability of natural microbial communities to utilize carbon directly derived from shredded tire particles, a long-term incubation experiment was performed. Four different sets of treatments were established in combusted 500 ml borosilicate bottles. The bottles contained in triplicate ASW only (ASW), ASW and 2 g of shredded tire particles per 100 ml (ASW + tires), NSW and ASW in a ratio of 1:10 (NSW : ASW) (ASW + NSW) and NSW and ASW (in the same ratio) and shredded tire particles (ASW + NSW + tires). The bottles were shaken continuously for several weeks in the dark at room temperature. Sampling was conducted weekly in the same manner as described in **2.1.4. Microbial incubation with tire leachates**.

2.1.6. Bacterial abundance determination

To determine bacterial abundance, cells were stained with SYBR® Green fluorescent dye and the abundance was determined with a flow cytometer (Gasol & Del Giorgio, 2000) (BD Accuri® C6, BD Biosciences). Bacterial abundances were determined from the start of the experiment until the late stationary phase. Undiluted samples were used for determining bacterial abundance (250 µl sample, 2.5 µl SYBR® green) until the microbes reached a concentration of 1000 cells per µl. Once the concentration exceeded 1000 cells per µl, 5 µl of sample were mixed with 495 µl 1xTE buffer (1:100 dilution). Measurements on the flow cytometer were performed with medium flow velocity, limited to a running time of 3 min and a maximum of 10,000 events counted.

2.1.7. DOC (dissolved organic carbon) measurements

The dissolved organic carbon (DOC) concentration was determined in all experiments by collecting 50 ml of unfiltered samples at the initial and final phase (late stationary phase). The samples were transferred into acid washed (1 M HCl) and combusted 60 ml glass vials. From each sample, 25 ml were filtered through a 0.2 µm polycarbonate membrane using acid washed syringes into acid washed 30 ml glass vials to remove particles, while another sample was left unfiltered. The DOC samples were then stored unacidified at -20°C. Prior to analysis, CO_2 was removed by vigorous sparging with N_2 of high purity. Both the filtered and non-filtered DOC samples were used to determine the non-volatile fraction of DOC using a Shimadzu TOC-V organic carbon analyzer. DOC standards for the Shimadzu TOC-V were prepared as described elsewhere (Benner & Strom, 1993).

2.1.8. Statistical analyses

For data analysis and statistical tests, Microsoft® Excel, version 16.50 was used. All plots were created using SigmaPlot Version 14.5.

3. Results

3.1. Abiotic tire particle exposure experiment

3.1.1. DOC leaching from tire particles

Leaching of DOC was significantly higher (ANOVA; $p < 0.01$) in the light exposed treatment (ASW + tires light) (mean ± SD of the 3 technical replicates respectively of Exp. A and Exp. B: 2.62 ± 0.41 µmol g^{-1} d^{-1}) than in the dark treatment (ASW + tires dark) (1.33 ± 0.32 µmol g^{-1} d^{-1}) (Figure 1).

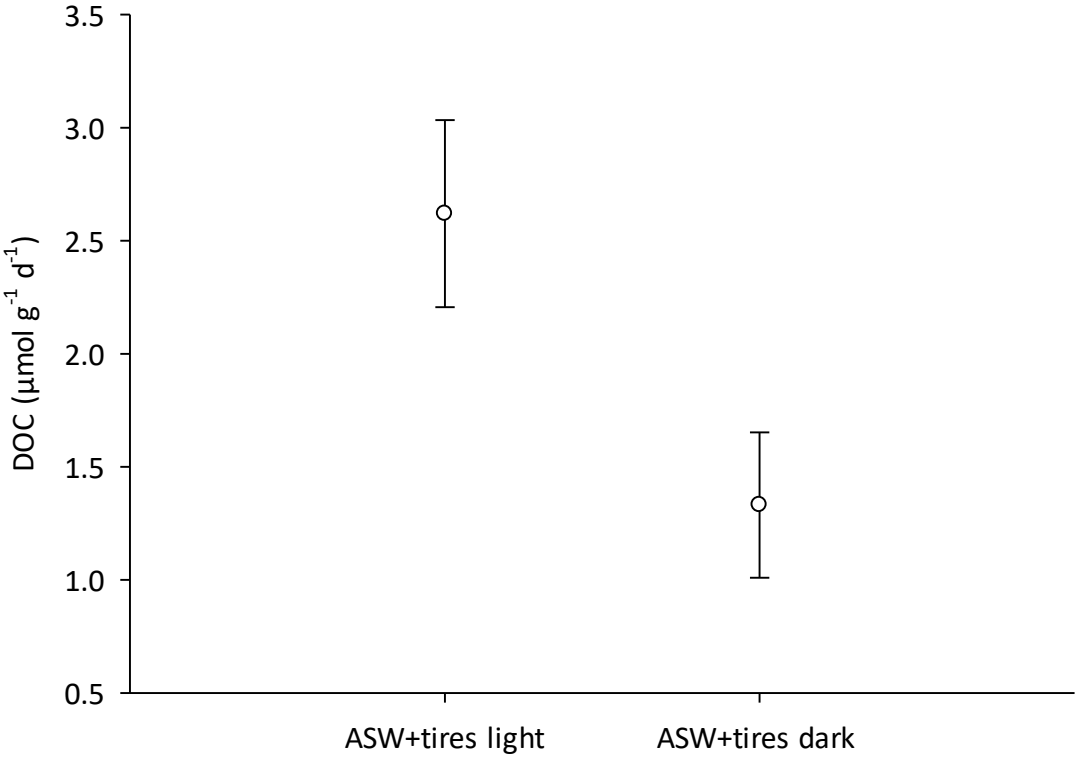

Figure 1: *Mean amount of leached DOC (µmol g^{-1} d^{-1}) in samples which were irradiated with artificial sunlight (ASW + tires light) and samples which were kept in the dark (ASW + tires dark).*

3.1.2. Photochemical oxygen demand

There was no significant (ANOVA; $p > 0.05$) difference between the light (0.79 ± 0.08 µmol g^{-1} d^{-1}) and the dark (0.73 ± 0.10 µmol g^{-1} d^{-1}) treatment (Figure 2). Hence, there was no photochemical oxygen demand measurable.

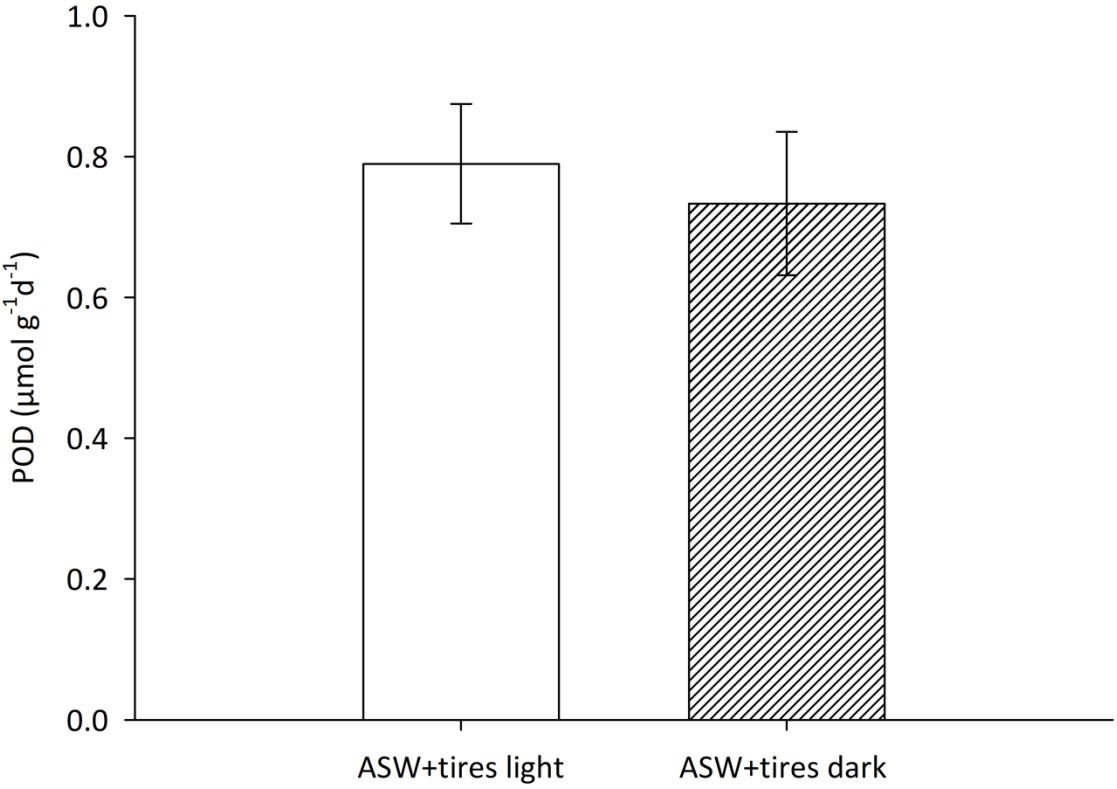

Figure 2: *Photochemical oxygen demand (POD) (µmol g^{-1} d^{-1}) in artificial seawater (ASW) containing tire particles under the exposure to UV-light (ASW + tires light) and under dark conditions (ASW + tires dark).*

3.2. Long-term biotic tire particle incubation experiment

3.2.1. Leached DOC from tire particles and NSW after 14 weeks of incubation

Although the DOC concentration was generally low, the DOC concentration was significantly higher (ANOVA, $p < 0.001$) in samples with tire particles compared to the controls containing only ASW (ASW) and ASW and NSW (ASW + NSW) (Figure 3). The samples containing ASW and tires (ASW + tires) exhibited the highest DOC concentration (0.28 ± 0.07 µmol g^{-1} d^{-1}) while the samples containing additionally NSW (ASW + NSW + tires), showed the second highest DOC concentration (0.24 ± 0.02 µmol g^{-1} d^{-1}). The DOC concentration of the control samples with ASW only (ASW) (0.05 ± 0.0 µmol g^{-1} d-1) and ASW and NSW (ASW + NSW) (0.03 ± 0.01 µmol g^{-1} d^{-1}) remained particularly low.

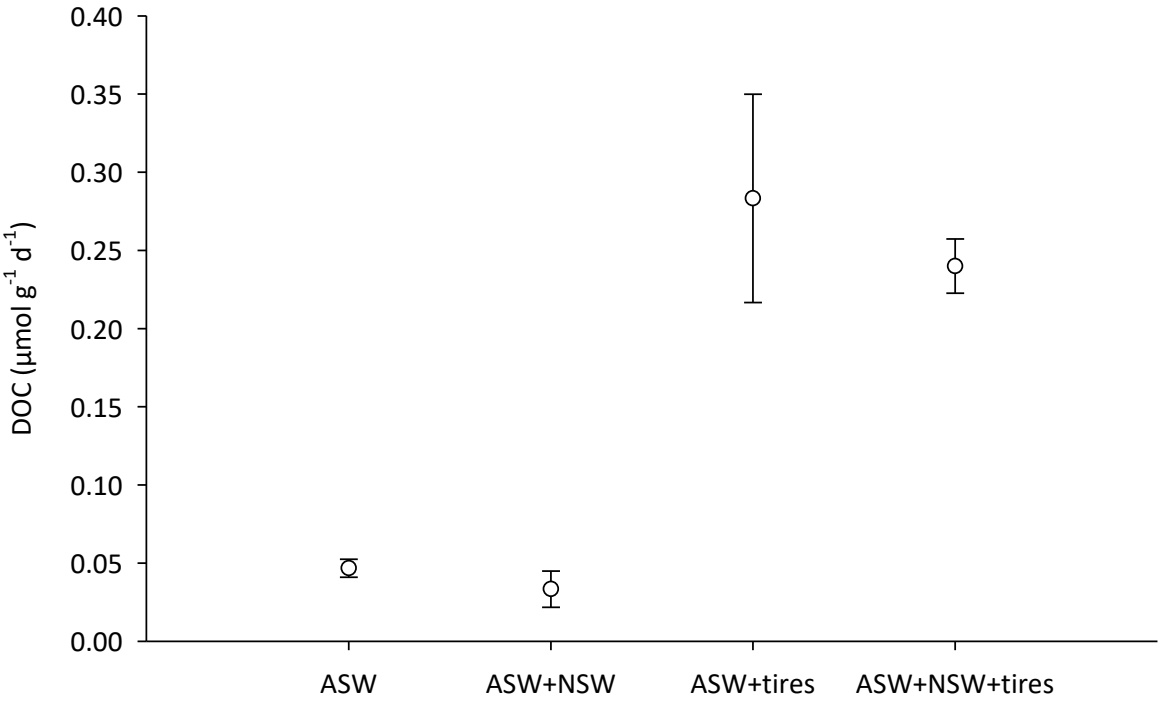

Figure 3: *Leached DOC (µmol g^{-1} d^{-1}) of the long-term tire particle exposure experiment. The samples contained ASW only (ASW), ASW and tire particles (ASW + tires), ASW and NSW (ASW + NSW) and ASW, NSW and tire particles (ASW + NSW + tires*).

3.2.2. Bacterial abundance in the long-term tire particle exposure experiment

To assess the impact of shredded tire particles on natural microbial communities, a long-term tire exposure experiment was conducted for 14 weeks. In comparison to the samples comprising ASW only (ASW), ASW and tire particles (ASW + tires) and ASW inoculated with natural microbial communities (ASW + NSW) as a control, the mixture of ASW, NSW and tire particles (ASW + NSW + tires) exhibited a strong exponential increase in bacterial abundance. Even after 14 weeks, stationary phase was not reached (Figure 4).

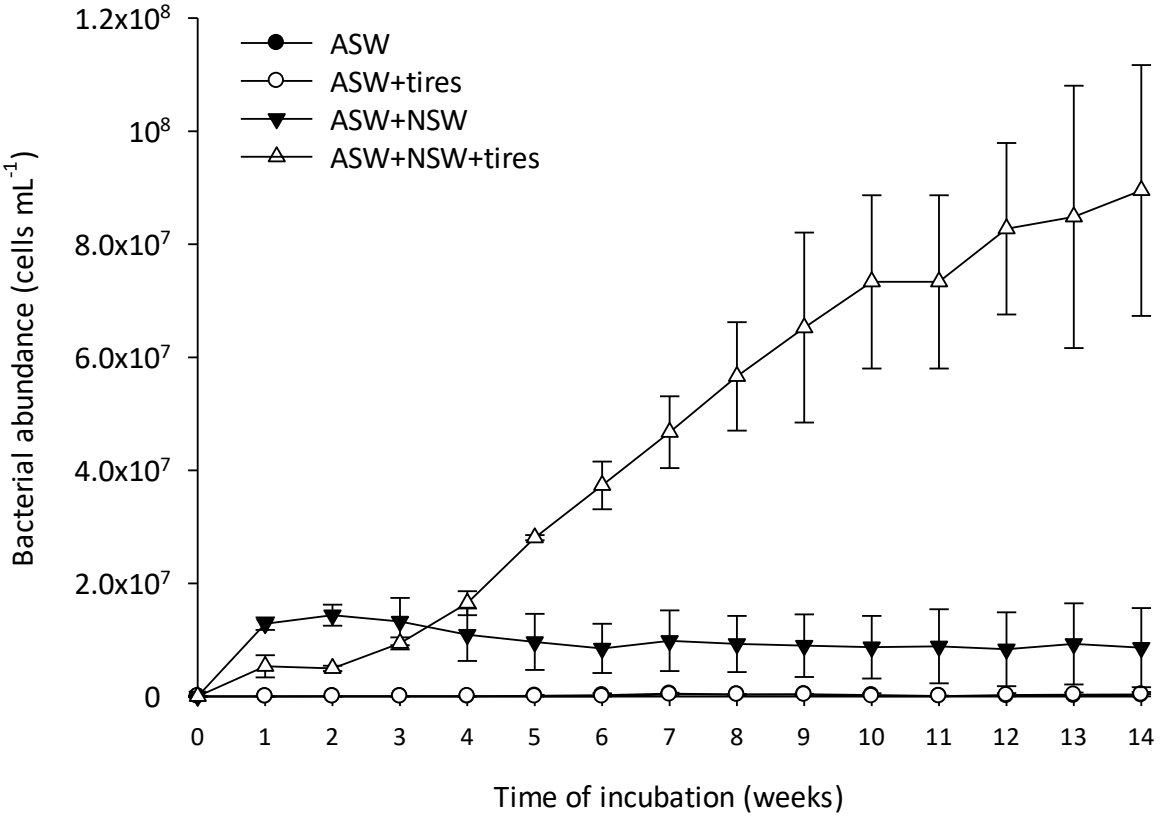

Figure 4: *Development of the bacterial abundance (cells ml^{-1}) in the treatments containing ASW only (ASW), ASW and tire particles (ASW + tires), ASW and NSW (ASW + NSW) and ASW, NSW and tire particles (ASW + NSW + tires) over 14 weeks.*

3.3. Bacterial utilization of tire leachate

3.3.1. DOC consumption by the bacterial community

The average DOC consumption rate in the treatment with irradiated tire leachate (ASW + NSW + tires L) was 2.38 ± 0.77 µmol l^{-1} d^{-1} (Figure 5). The average DOC consumption rate in the tire treatment held in the dark (ASW + NSW + tires D) was 2.39 ± 0.28 µmol l^{-1} d^{-1} and therefore, essentially identical (ANOVA; $p > 0.05$) to that in the UV-exposed treatment. The DOC consumption rates of the controls, containing ASW and NSW only remained low. However, the DOC consumption rate of the UV-exposed control (ASW + NSW L) was significantly higher (ANOVA; $p < 0.05$) (1.11 ± 0.28 µmol l^{-1} d^{-1}) than the dark control (ASW + NSW D) (0.71 ± 0.29 µmol l^{-1} d^{-1}).

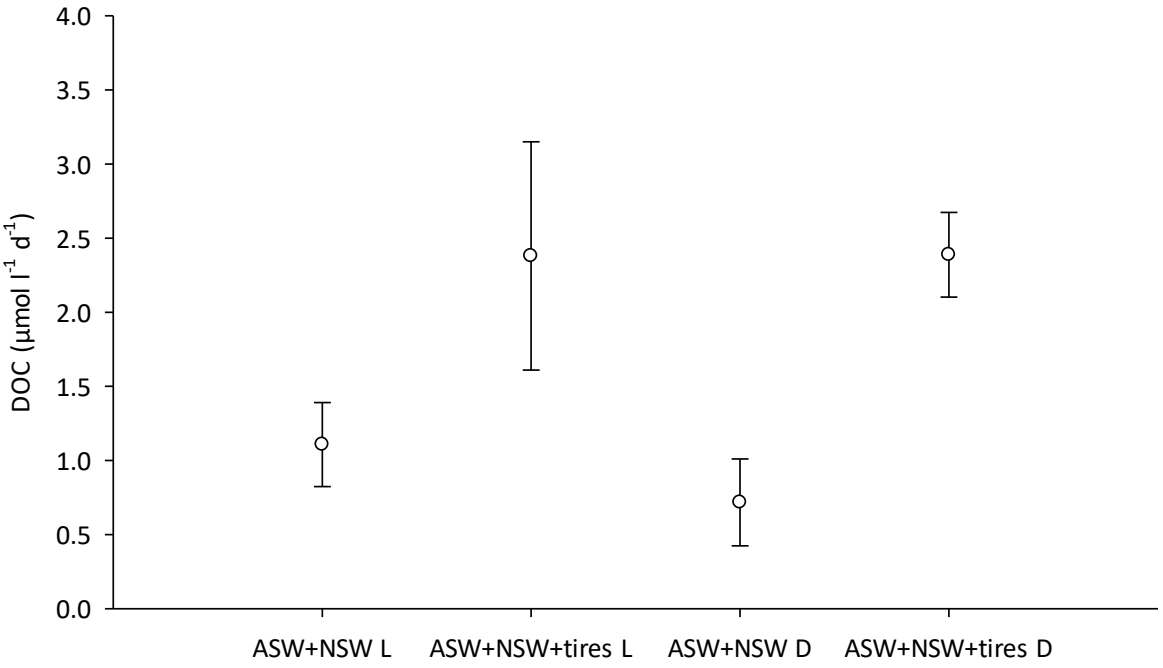

Figure 5: *DOC consumption by the bacterial community (µmol l^{-1} d^{-1}) previously irradiated with UV-light (ASW + NSW + tires L) and in the treatment held in the dark (ASW + NSW + tires D). As a control, the treatments with ASW and NSW (ASW+NSW L; ASW+NSW D) are also shown.*

3.3.2. Effect of tire leachate on the natural microbial community

Previously UV-exposed and non-exposed samples which were incubated for 13 d, containing either tire particles or ASW only, were subsequently filtered through 0.2 µm polycarbonate filters. The filtrate was then inoculated with 0.8 µm filtered NSW to add natural microbial communities to the samples and incubated in the dark on a shaker for 42 days. A higher bacterial abundance was recorded in tire leachate treatments (ASW + NSW + tires L; ASW + NSW + tires D) compared to the control samples (ASW + NSW L; ASW + NSW D) which did not contain tire material previously. No significant difference in bacterial abundance was obtained between the leachates from tire material previously exposed to UV-radiation or held in the dark (Figure 6).

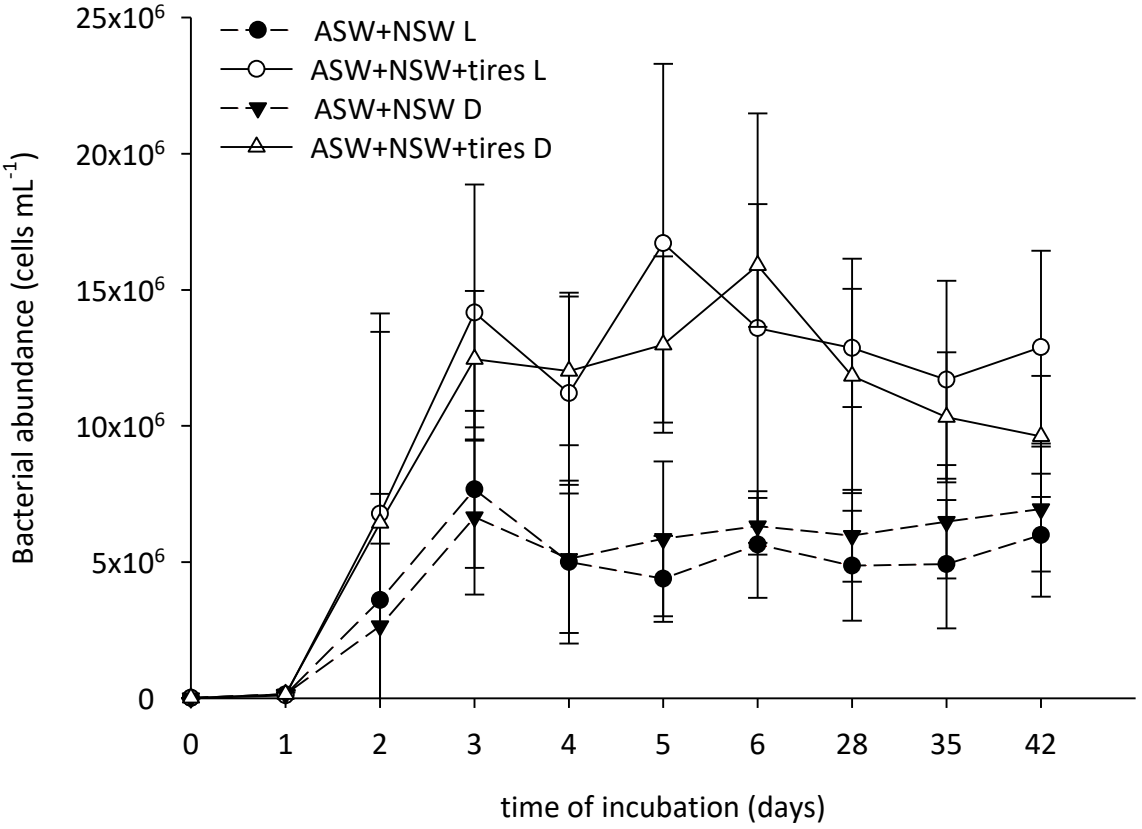

Figure 6: Effect of leachate obtained from previously UV-radiation exposed tire material (ASW + NSW + tires L) and from tire material held in the dark (ASW + NSW + tires D) on the growth of a natural microbial community. Samples with ASW and NSW served as a control and were also either exposed to UV-radiation (ASW + NSW L) or kept in the dark (ASW + NSW D).

3.3.3. Microbial respiration of tire leachate

Tire leachate of UV-irradiated tire material (ASW + NSW + tires L) resulted in a similar oxygen consumption rate (8.18 ± 1.93 µmol l^{-1} d^{-1}) as the leachate generated in dark treatment (ASW + NSW + tires D) (7.85 ± 2.60 µmol l^{-1} d^{-1}) (ANOVA; $p > 0.5$). The average oxygen consumption rate in the ASW control was 0.36 ± 0.29 µmol l^{-1} d^{-1} in the light (ASW + NSW L) and 4.63 ± 2.83 µmol l^{-1} d^{-1} in the dark treatment (ASW + NSW D) (Figure 7).

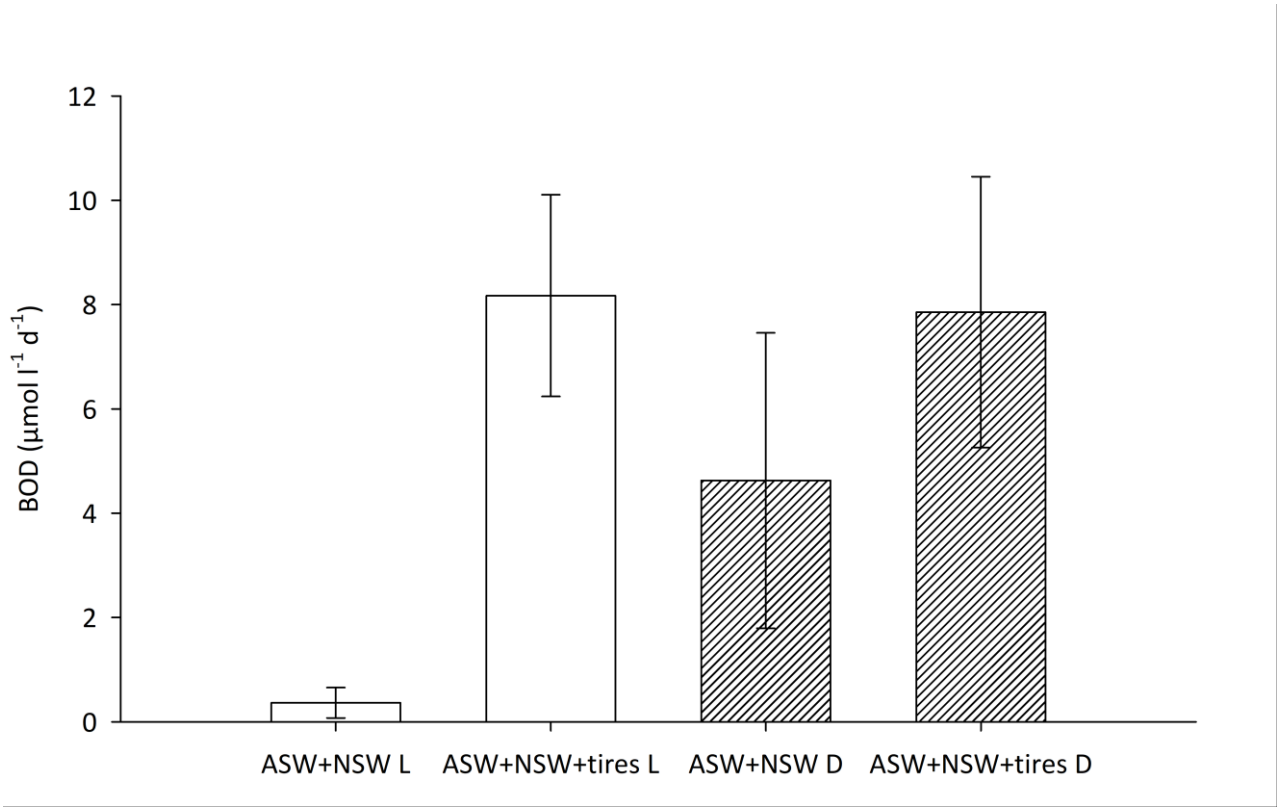

Figure 7: Biological oxygen demand (BOD) (µmol l^{-1} d^{-1}) of a natural microbial community using tire leachate generated under UV-exposed (ASW + NSW + tires L) and dark (ASW + NSW + tires D) conditions. Samples with ASW and NSW (ASW + NSW L; ASW + NSW D) served as a control.

4. Discussion

As tire debris is known to be a contributor to plastic pollution in the sea (Knight *et al.,* 2020), the aim of this study was to investigate the consequences of tire debris for the marine microbial community. We found that the presence of tire particles or leachate results in a decreased oxygen content and an increased DOC leaching (Figures 1, 2, 3, 5, 7). The leachates and tire particles are stimulating microbial activity as indicated by higher bacterial abundance compared to controls without tire particles or leachates added. Furthermore, it does not influence the microbial activity to a significant extent whether the tires were exposed to UV-radiation or not (Figures 5, 6, 7). However, the exposure of tire particles to UV-light without the addition of NSW resulted in higher DOC leaching rates compared to the samples shielded from artificial solar radiation.

Exposure of tire particles to UV-radiation provoked about twice as high DOC leaching rates than in the dark (Figure 1). Similar results were obtained by Zhu *et al.* (2020) in a study from

the North Pacific Gyre with synthetic polymers reporting 10 times higher leaching rates than under dark conditions.

The high oxygen consumption of the dark control (Figure 2) is most likely caused by a handling problem of the samples and therefore, an artifact. As tire material is hydrophobic, a layer of air might have formed around the tire particles when submerged in the water. However, as there was overall no difference between the light and dark treatment, we conclude that photochemical oxidation of tire material is insignificant. Nevertheless, a visible coloration of the UV-light exposed tire samples at the end of the incubation period was noticeable. The color change of the tire material is known to be an indication of photodegradation processes as shown by a similar study conducted by Gewert *et al.* (2018).

The DOC concentrations in the long-term experiment were rather low compared to the DOC values obtained in the abiotic incubation experiments. While both the abiotic and biotic experiments were incubated for around 13 d (Figures 1, 5), the long-term experiment was running for 14 weeks (Figure 3). Romera-Castillo *et al.* (2018) conducted a similar experiment with different polymers and reported that in the first 200 h of incubation around 45% of the DOC that leached was progressively lost. As their samples were also kept in the dark and without microbes, the loss of DOC can neither be due photo-oxidation nor caused by microbial utilization. The authors suggested therefore that DOC might be lost by volatile leachates and sorption onto the tire material.

The exposure of a natural microbial community to tire particles over a period of 14 weeks, resulting in a strong increase of bacterial abundance (Figure 4), is consistent with a similar experiment performed by Vukanti *et al.* (2009) using, however, a single bacterial strain known to occur naturally at buried tire shreds. They found that only a part of the bacterial strain was able to utilize tire material. In comparison to our study, Vukanti *et al.* (2009) determined the microbial cells attached to the tire material only while we determined the bacterial abundance in the ambient seawater. Essentially on any type of solid surface that reaches the sea a microbial biofilm will develop, for plastics coined plastisphere (Wright *et al.,* 2020). As there was no additional source of organic carbon supplied in our treatments, it indicates that microbes are able to utilize organic carbon derived from tire material. As tire rubber is composed of a variety of components (Kole *et al.,* 2017), some microbes of the consortium

might be able to metabolize tire material while other microbes might not be able to utilize it. Consequently, microbial taxa incapable of degrading leachate material may benefit from metabolites of other bacterial taxa.

Similar to the DOC leaching rate and the bacterial abundance, the biological oxygen consumption was highest in the tire treatments (Figure 7) indicating biodegradation of tire material. Due to the absence of a difference between the UV-light exposed and the dark treatment containing tire leachate, the extent of biodegradation was not affected by the previous exposure of the tire material to UV-light.

Although tire material is made up of a variety of toxic and non-toxic chemicals, our results do not support the assumption that the leached components have a general negative impact on marine microbes. Hartwell *et al.* (2000) state that tire leachate is generally more hazardous to freshwater than to marine systems.

5. Conclusion

Based on our results we conclude that UV-radiation of tire material did not cause photo-oxidation, but it did enhance leaching rates of dissolved organic carbon. Also, a stimulation of microbial respiration on leached material was observed. Thus, tire material stimulates microbial activity in the sea.

Zusammenfassung

Die zunehmende Akkumulation synthetischer Polymere in marinen Systemen ist ein allgegenwärtiges Problem. Neben den klassischen Polymertypen ist nun auch vermehrt Reifenmaterial als Umweltschadstoff in das Bewusstsein der Menschen getreten. Unbeabsichtigter Transport in marine Systeme durch Wind oder Wasser ist besonders bei Reifenpellets durch ihre geringe Größe begünstigt. Der hohe Anteil kohlenstoffhaltiger Verbindungen von Reifen erlaubt die Annahme, dass der Einfluss von Meerwasser und UV-Strahlung das Herauslösen dieser Verbindungen begünstigt. Die daraus resultierenden Konsequenzen für mikrobielle Gemeinschaften mariner Systeme wie dem Mittelmeer sind jedoch wenig bekannt. In dieser Studie konnte gezeigt werden, dass das Bestrahlen von Reifenmaterial mit UV-Licht zu einem verstärkten Herauslösen von gelöstem organischem Kohlenstoff (DOC) führt wobei jedoch die photochemische Aktivität durch UV-Licht nicht gesteigert wurde. Von Reifen in Lösung gehende organische Verbindungen stimulieren außerdem bakterielles Wachstum. Dies zeigt, dass trotz der diversen in Reifen vorhandenen, vermeintlich schädlichen Chemikalien, marine Mikroben befähigt sind, Kohlenstoff aus dem Reifenabrieb als alternative Kohlenstoffquelle zu nutzen und somit den Reifenabrieb zumindest teilweise abbauen können.

6. References

Bandyopadhyay, S., Agrawal, S. L., Ameta, R., Dasgupta, S., Mukhopadhyay, R., Deuri, A. S., Ameta, Suresh C. & Ameta, Rakshit. (2008). **An overview of rubber recycling**. Progress in rubber, plastics and recycling technology, Vol.24 (2), p.73-112

Barnes, D. K. A., Galgani, F., Thompson, R. C. & Barlaz, M. (2009). **Accumulation and fragmentation of plastic debris in global environments**. Philosophical transactions. Biological sciences, Vol.364 (1526), p.1985-1998

Benner, R. & Strom, M. (1993). **A critical evaluation of the analytical blank associated with DOC measurements by high-temperature catalytic oxidation.** Marine chemistry, Vol. 41 (1-3), p. 153-160

Capolupo, M., Gunaalan, K., Booth, A. M., Sørensen, L., Valbonesi, P. & Fabbri, E. (2021). **The sub-lethal impact of plastic and tire rubber leachates on the Mediterranean mussel Mytilus galloprovincialis**. Environmental pollution (1987), Vol.283, p.117081

Cózar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J. I., Ubeda, B., Gálvez, J. Á., Irigoien, X. & Duarte, C. M. (2015). **Plastic Accumulation in the Mediterranean Sea.** PloS one, Vol.10 (4), p.e0121762

Gasol, J. M. & Del Giorgio, P. A. (2000). **Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities.** Scientia marina, Vol.64 (2), p.197-224

Gewert, B., Plassmann, M., Sandblom, O. & MacLeod, M. (2018). **Identification of Chain Scission Products Released to Water by Plastic Exposed to Ultraviolet Light.** Environmental Science & Technology Letters, Vol.5 (5), p.272-276

Gewert, B., Plassmann, M. M. & MacLeod, M. (2015). **Pathways for degradation of plastic polymers floating in the marine environment.** Environmental Science: Processes & Impacts, Vol.17 (9), p.1513-1521

Halsband, C., Sørensen, L., Booth, A. M. & Herzke, D. (2020). **Car Tire Crumb Rubber: Does Leaching Produce a Toxic Chemical Cocktail in Coastal Marine Systems?** Frontiers in environmental science, Vol.8

Hartwell, S. I., Jordahl, D. M. & Dawson, C. E. O. (2000). **The effect of salinity on tire leachate toxicity**. Water, air, and soil pollution, Vol.121 (1), p.119-131

Hennebert, P., Lambert, S., Fouillen, F. & Charrasse, B. (2014). **Assessing the environmental impact of shredded tires as embankment fill material**. Canadian geotechnical journal, Vol.51 (5), p.469-478

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R. & Law, K. L. (2015). **Plastic waste inputs from land into the ocean**. Science (American Association for the Advancement of Science), Vol.347 (6223), p.768-771

Knight, L. J., Parker-Jurd, F. N. F., Al-Sid-Cheikh, M. & Thompson, R. C. (2020). **Tyre wear particles: an abundant yet widely unreported microplastic?** Environmental science and pollution research international, Vol.27 (15), p.18345-18354

Kole, P. J., Löhr, A. J., Van Belleghem, F. G. A. J. & Ragas, A. M. J. (2017). **Wear and Tear of Tyres: A Stealthy Source of Microplastics in the Environment**. International journal of environmental research and public health, Vol.14 (10), p.1265

Llompart, M., Sanchez-Prado, L., Lamas, J. P., Garcia-Jares, C., Roca, E. & Dagnac, T. (2013). **Hazardous organic chemicals in rubber recycled tire playgrounds and pavers**. Chemosphere, Vol.90 (2), p.423-431

Nanthini, J. & Sudesh, K. (2016). **Biodegradation of Natural Rubber and Natural Rubber Products by *Streptomyces* sp. Strain CFMR 7.** Journal of polymers and the environment, Vol.25 (3), p.606-616

Palmisano, A. C. & Pettigrew, C. A. (1992). **Biodegradability of plastics**. Bioscience, Vol.42 (9), p.680-685

Ranby, B. & Lucki, J. (1980). **New aspects of photodegradation and photo-oxidation of polystyrene**. Pure and applied chemistry, Vol.52 (2), p.295-303

Reinthaler, T., Bakker, K., Manuels, R., van Ooijen, J. & Herndl, G. J. (2006). **Fully automated spectrophotometric approach to determine oxygen concentrations in seawater via continuous-flow analysis**. Limnology and oceanography: methods, Vol.4 (10), p.358-366.

Rhodes, E. P., Ren, Z. & Mays, D. C. (2012). **Zinc Leaching from Tire Crumb Rubber.** Environmental science & technology, Vol.46 (23), p.12856-12863

Romera-Castillo, C., Pinto, M., Langer, T. M., Álvarez-Salgado, X. A. & Herndl, G. J. (2018). **Dissolved organic carbon leaching from plastics stimulates microbial activity in the ocean**. Nature communications, Vol.9 (1), p.1430

Ryan, P. G., Moore, C. J., van Franeker, J. A. & Moloney, C. L. (2009). **Monitoring the abundance of plastic debris in the marine environment.** Philosophical transactions of the royal society B. Biological sciences, Vol.364 (1526), p.1999-2012

Selbes, M., Yilmaz, O., Khan, A. A. & Karanfil, T. (2015). **Leaching of DOC, DN, and inorganic constituents from scrap tires**. Chemosphere, Vol.139, p.617-623

Singh, B. & Sharma, N. (2008). **Mechanistic implications of plastic degradation.** Polymer degradation and stability, Vol.93 (3), p.561-584

Tokiwa, Y., Calabia, B. P., Ugwu, C. U. & Aiba, S. (2009). **Biodegradability of Plastics**. International journal of molecular sciences, Vol.10 (9), p.3722-3742

Vukanti, R., Crissman, M., Leff, L. G. & Leff, A. A. (2009). **Bacterial communities of tyre monofill sites: growth on tyre shreds and leachate.** Journal of applied microbiology, Vol.106 (6), p.1957-1966

Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T. & Reemtsma, T. (2018). **Tire wear particles in the aquatic environment - A review on generation, analysis, occurrence, fate and effects**. Water research, Vol.139, p.83-100

Wbcsd. (2015). **Tire industry project 10-year progress report**. World Business Council for Sustainable Development

Wright, R. J., Erni-Cassola, G., Zadjelovic, V., Latva, M. & Christie-Oleza, J. A. (2020). **Marine Plastic Debris: A New Surface for Microbial Colonization**. Environmental Science & Technology, Vol.54 (19), p.11657-11672

Zheng, Y., Yanful, E. K. & Bassi, A. S. (2005). **A Review of Plastic Waste Biodegradation**. Critical reviews in biotechnology, Vol.25 (4), p.243-250

Zhu, L., Zhao, S., Bittar, T. B., Stubbins, A. & Li, D. (2020). **Photochemical dissolution of buoyant microplastics to dissolved organic carbon: Rates and microbial impacts**. Journal of hazardous materials, Vol.383, p.121065-121065