Science of the Total Environment 957 (2024) 177509

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effect of particles from wind turbine blades erosion on blue mussels *Mytilus edulis*

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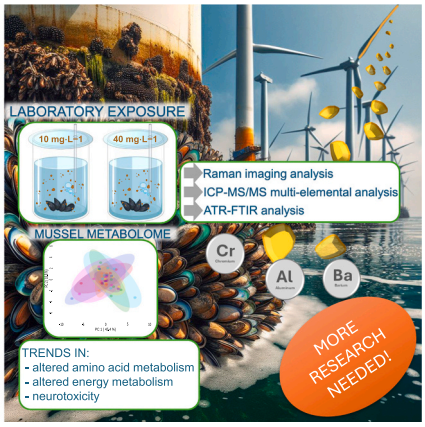
HIGHLIGHTS

- First controlled lab experiment to study biological effects of LEE particles
- Application of an innovative analytical toolbox for effect assessment
- Enrichment factors for metals and metalloids determined in *M. edulis* ranged between 0.93 and 6.1
- Metabolite investigation of the mussels' entire soft body tissue revealed only weak MP-induced changes

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Olga Pantos

Keywords:
Offshore wind farms
Pollution
Plastic
Bivalve
NMR spectroscopy
Metabolome

ABSTRACT

Offshore wind farms (OWFs) pose new anthropogenic pressures on the marine environment as the erosion of turbine blades release organic and inorganic substances with potential consequences for marine life. In the present study, possible effects of the released particles and their chemical constituents on the metabolic profile of the blue mussel, *Mytilus edulis*, were investigated, utilizing ^{1}H NMR spectroscopy. In the lab, mussels were exposed for 7 and 14 days to different concentrations (10 and 40 mg L^{-1}) of microplastic (MP) particles which were derived from cryo-milled rotor blade coatings and core materials (glass fiber polymer, GFP). Raman imaging techniques revealed that 30–40 % of the coating and GFP particles had MP sizes below 5 μm, with the majority (~98 %) being ≤50 μm. Despite the identified enrichment factors (EF) for metals and metalloids from the rotor blade materials, especially Ba, Cu, Cd, Cr and Ni with EFs between 0.93 and 6.1, untargeted metabolic

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https://doi.org/10.1016/j.scitotenv.2024.177509
Received 28 June 2024; Received in revised form 6 November 2024; Accepted 9 November 2024

ICP-MS/MS
Metals

profiling of the entire soft body tissues of *M. edulis* showed no significant metabolic disruption, regardless of the particle concentration. Observed trends in elevated concentrations of metabolites may indicate a possible short-term effect on mussels' neuroendocrine system and a possible long-term effect on energy metabolism. Experimental worst-case scenario of massive abrasion and the minimal response observed in *M. edulis* under the conditions tested suggest that erosion caused by wind turbine blades may pose little to no risk to bivalves at this stage. However, it is important to note that this study is only a preliminary step and further studies are needed to obtain a comprehensive overview of the issue before reaching a definite firm conclusion regarding the potential threat of OWFs abrasion to the marine environment, particularly considering the planned future extension of windpark construction in connection with the ongoing EU-wide energy transition.

Abbreviations

MP	microplastic
ICP-MS/MS	Inductively Coupled Plasma Tandem Mass Spectrometry
OWF	offshore wind farm
LEE	leading edge erosion
GFP	glass fiber polymer

1. Introduction

Renewable energy production systems such as offshore wind farms play an important role to achieve the goal of sustainable energy transition and climate neutrality by 2050. "The continuous rise and expansion of offshore wind power produced in offshore wind farms (OWFs) led to a European-wide production capacity of 30.3 GW by the end of 2022 with 8.1 GW contributed by Germany (WindEurope, 2023; Deutsche WindGuard, 2023)." However, OWFs pose new anthropogenic pressures to the marine environment. Along the multiple effects of OWFs on the environment (e.g. Christiansen et al., 2022; Degraer et al., 2020), the effect of emissions of polymer particles from turbine blades on marine life is poorly investigated (Kirchgeorg et al., 2018). Under harsh environmental conditions, turbine blades undergo rapid degradation and surface erosion over their leading edge, already after several years of wind turbine exploitation (Mishnaevsky et al., 2023). This degradation leads to the massive emission of particles to the environment from the turbine blade coatings and core materials. As highlighted in the special report of Asbjørn Solberg, Bård-Einar Rimereit and Jan Erik Weinbach from "THE TURBINE GROUP" in Norway, the leading edge errosion (LEE), together with pitting and delamination may cause the emission of approximately 62 kg particles per year per turbine. This approximate emission rate strongly depends on weather condition and the size of the turbines, thus with the prognosed increase of the turbine size and more frequent storm and hail events (due to the climate change), the emission rate will further increase (Solberg et al., 2021). Considering the approximate number of operating wind turbines in Germany in 2019 (31,000, both on- and offshore), and a maximum affected blade surface of approximately 10 m^2 and the coating thickness (up to 5 mm), the maximum material release can be estimated as 1395 t/y for all German wind turbines (with a specific material density of 1.2 t/m^3). The effects of the released microplastic (MP) particles and chemicals from the coatings and the blades of OWFs have been overlooked in recent ecotoxicity or environmental research so far, although there is rising concern toward marine paints as emerging pollutants (Turner, 2021; Ceia and Bessa, 2024; Hildebrandt et al., 2024; Murugan et al., 2023). Considering the ability of microparticles to adsorb and accumulate various contaminants, the effect can multiply drastically (Kinigopoulou et al., 2022; Rodrigues et al., 2019). Depending on the local energy regime, sediments in tidal flats, mussel banks and channels can act as a temporary sink for settling particles. However, they can also become re-suspended and further transported with tidal residual currents. Accordingly, the spatial distribution of particles is highly dependent on local dynamics that influence sedimentation, burial, re-suspension and re-surfacing.

Bivalves are key ecological species that play an important role in coastal ecosystems. They are not only the link between primary producers and main consumers in the marine food web but are also involved in bentho-pelagic coupling and act as ecosystem engineers (Vaughn and Hoellein, 2018). As active, sedentary filter-feeders, bivalve mollusks improve the water quality by removing particular organic matters and plankton but also pollutants such as heavy metals (Bates et al., 2021). They are well known for their ability to accumulate various contaminants in their tissues, and thus are widely used as bioindicators in monitoring aquatic pollution (Vijayavel et al., 2007). A significant number of studies have shown that MP has a negative effect on bivalves (summarized in Khanjani et al., 2023). As a result, bivalves have become one of the most studied organisms and bioindicators in microplastic research and monitoring programs. To summarize these studies, MP affects filter-feeders by multiple ways – clogging gills, digestive tracts and tissues, penetrating cells, and interfering with cellular processes via particle-associated chemicals, including organic and inorganic additives. This causes various physical-chemical effects on the organisms including altered immune response, inflammation, oxidative stress, DNA damage, reduced ability to absorb nutrients and feeding inhibition, reduced filtration activity, neurotransmission dysfunction, impairment of organ functions, reduced reproductive success and an increased formation of granulocytoma (Cappello et al., 2021; Khanjani et al., 2023; Li et al., 2022; Mkuye et al., 2022). Recent reviews highlight the concern about plastic pollution in general and summarize its impact on aquatic animals (e.g. Haegerbaeumer et al., 2019; Ward et al., 2019; Porter et al., 2023).

The effects of MP released from wind turbine blades due to LEE on marine filter-feeders are unknown and thus represent a significant knowledge gap in risk assessment studies related to the OWFs. In the field study by Wang et al. (2023) the authors describe an elevation of metabolic biomarkers indicating stress in bivalves, the Pacific oyster *Crassostrea gigas* and the blue mussel, *Mytilus edulis* from Rudong Offshore Wind Farm. However, it remains unknown whether this effect is related to OWF-MP or other factors such as general pollution. Therefore, a study under controlled laboratory conditions is required, especially with regard to the discussion on multi-use in offshore wind farms with mytilid mussel farming (Maar et al., 2023). Mytilid mussels are among the key species in coastal communities and are found in coastal waters around the world. By building byssus threads that hold the mussel to hard substratum, they can form large mussel beds making them competitive dominants on shorelines (Svane and Ompi, 1993; Buschbaum et al., 2009). Their shells are usually blue-black in color, and the animals can grow up to ~8 cm in size and reach up to 20 years of age (Theisen, 1973; Sukhotin et al., 2007). Mussels are of great commercial importance, and in 2019, the world exports of mussels exceeded 1 Mio tons (FAO, 2019).

In this work we aimed to investigate: 1. The possible effects of MP, which can be released from the turbine blades due to the LEE, on the metabolome of entire soft body tissues of blue mussel (*Mytilus edulis* Linnaeus, 1758) in a laboratory experiment; 2. the putative differential effects on the mussels' metabolome from the wind blades coating and

core material (glass fiber polymer) in comparison to the controls including exposure to natural particles (clay); 3. the possible release of metals and metalloids from the tested materials and a bioaccumulation of these elements in the exposed mussels. It was hypothesized that mussels could experience negative effects due to the physical properties and additives of different types of MP particles released from wind turbine blades, and that this effect would be detected by screening the metabolome of the entire soft body tissue (i.e. alteration of metabolite concentration involved in energy homeostasis, inflammation, osmolarity and stress response). It was further hypothesized that both types of material can release metals and metalloids, which are generally included in their formulation, and these inorganic additives could bioaccumulate in mussels potentially causing toxic effects. To check our hypotheses, cryo-milled samples of wind turbine blades, divided into coating and core materials were prepared and, in order to mimic a worst-case scenario, mussels were exposed to high concentrations of the particles produced. To differentiate the physical effect of MP (i.e. gill clogging) from the potential effect of the chemical additives, mussels were exposed to similar concentrations of natural particles (clay) alongside the synthetic particles (coatings and glass fiber polymer). Subsequently, the accumulation of metals and metalloids, as well as metabolite profiles of the soft body tissue of *M. edulis* were observed under these different conditions.

2. Methods

2.1. Collection and maintenance of blue mussel

Blue mussels (*Mytilus edulis*) were manually collected in October 2022 in the harbor on the Island of Heligoland, Germany (54.1745N, 7.8951E). The mussels were transported in a moist and cooled box and arrived at the Alfred Wegener Institute in Bremerhaven, Germany in <12 h. After arrival the mussels were kept in a 100-L recirculating seawater system at 15 °C and 33 PSU under direct illumination (12/12 h light-dark cycle). The mussels remained in the recirculating seawater system for the next 7 days for depuration and acclimation until the beginning of the exposure experiment. During this period, the mussels were not fed, in order to increase the uptake of the microparticles during the experiment (Korez Lupše *pers. obs.*). More details regarding mussel maintenance is given in S2.

2.2. Preparation of MP particles

MP particles were produced through cryo-milling. Initially, fragments up to 0.5 cm in size were obtained by manually scraping the surface of the test objects of turbine blades (provided by Fraunhofer Institute), which were in the form of a folded 24 cm × 11 cm plate using a paint scraper (50 mm stainless steel blade, BAHCO). The material samples were thus obtained from two fractions of the turbine material - coatings and glass fiber polymer (GFP). Larger fragments were additionally reduced in size using scissors. Next, the cryo-mill (Spex SamplePrep, Freezer Mill 6775) was cooled for 30 min by filling the tub of the cryo-mill with liquid nitrogen. Simultaneously, 1 g of the respective particles was weighed and placed into a stainless-steel vial, which was inserted into the grinding chamber of the cryo-mill. The grinding process commenced, consisting of a 15 min pre-cooling phase, followed by 4 cycles of alternating 2 min cooling and running periods, at a rate of 12 cycles per second. Once the grinding was complete, the microparticles were carefully extracted from the vial and placed onto a glass Petri dish. The process was repeated separately for the two synthetic particle types until a sufficient quantity of microparticles was obtained.

Clay (kaolin, aluminum silicate hydroxide, K7375, Sigma-Aldrich) with particle size of <20 μm was used as a control. Stock suspensions of clay, coating and GFP were prepared in ultrapure water at concentrations of 15 mg·mL^{-1}, respectively. The final microparticle concentrations in the experimental glass containers were 10 mg·L^{-1} and 40 mg·L^{-1} respectively, with a single type of microparticle in each glass container (Table 1). The material used, the sampling process and the resulting particles can be seen on the photographs in S1: Figs. 1, 2.

2.3. Exposure setup

One day prior to the exposure, the mussels (average length 2.77 ± 0.43 cm, width 1.43 ± 0.22 cm; mean ± SD, N = 140) were moved from a 100-L recirculating seawater system to 2-L glass containers. They were kept in a temperature-controlled room (15 °C) with a 12/12 h light-dark cycle. Each 2-L glass container held four mussels in 1 L seawater that were individually connected to an aeration system. The water was exchanged every other day. Microparticle type and concentration were adjusted accordingly. The seawater parameters and quality was continuously monitored, resulting in 10.29 ± 0.14 mg O_2 L^{-1} (mean ± SD, N = 11), 14.06 ± 0.14 °C (mean ± SD, N = 11) and 35.34 ± 0.07 salinity (mean ± SD, N = 11), 0.01 ± 0.00 mg nitrite L^{-1} (mean ± SD, N = 4), 0.17 ± 0.01 mg ammonium L^{-1} (mean ± SD, N = 4), and nitrate levels were below detection limit (N = 4).

The mussels were exposed for 7 and 14 days, respectively. A total of 140 mussels were randomly assigned to the respective particle types, concentrations, and incubation periods, resulting in an initial number of 10 replicates per treatment. Throughout experimental exposures, mussels were fed a commercial phytoplankton mixture (250.000 cells mL^{-1}, SA/DTs Premium Reef Blend live phytoplankton, Sustainable Aquatic's) after every water change. No mussels died during the exposure. After exposure, the specimens were rinsed with demineralized water to remove adhering particles from the body surface, wiped and sedated on ice. Measurements of shell length, width and total body mass (including the shell) were recorded. The mussel's entire soft body (0.39 ± 0.19 g, mean ± SD, N = 140) was put into a reaction tube, weighed and shock frozen in liquid nitrogen. The samples were stored at −80 °C until further analysis. The schematic representation of the entire experimental setup is shown in Fig. 1, photographs of the experimental setup can be found in S1: Fig. 3.

2.4. Particle size and ATR-FTIR analysis

The particle size distributions of the coating and GFP were determined by confocal microscopy using a WITEC Alpha300R Raman microscope. Particle images were collected in dark-field modes with a 20-fold magnification. Particle numbers and size distributions were determined by means of ParticleScout software (Oxford Instruments, UK). To achieve this, around 1 mg of each of the two synthetic microparticles were suspended in 100 mL Mili-Q ultrapure water, respectively. Subsequently, both resulting mixtures underwent immediate agitation for 2 min using a Vortex apparatus (GENIUS3, IKA) to proactively inhibit particle aggregation. Prior to filtration, the glass vials containing the samples (resultant suspensions) were manually shaken, and 1 mL of each sample was filtered on gold-coated polycarbonate membranes (d = 25 mm/0.8 μm mesh size, Jamil Orfali GIANT labs GmbH, Berlin, Germany) employing a MIMAS filtration device (Jamil Orfali GIANT labs GmbH, Berlin, Germany). Each sample underwent triplicate filtration. Following filtration, the filters were placed in a petri dish and subjected to overnight drying in a desiccator cabinet (Star-Vitrum Borosilicate glass, Sicco). The remaining two suspensions were sealed with glass caps

Table 1
Features of microparticles used in the exposure experiments.

Type	Origin	Size (μm)
Clay	Natural	<20[a]
Coating microparticles	Synthetic	≤50 (see results)
GFP microparticles	Synthetic	≤50 (see results)

GFP - glass fiber polymer.
[a] Can mechanically disintegrate into smaller microparticles.

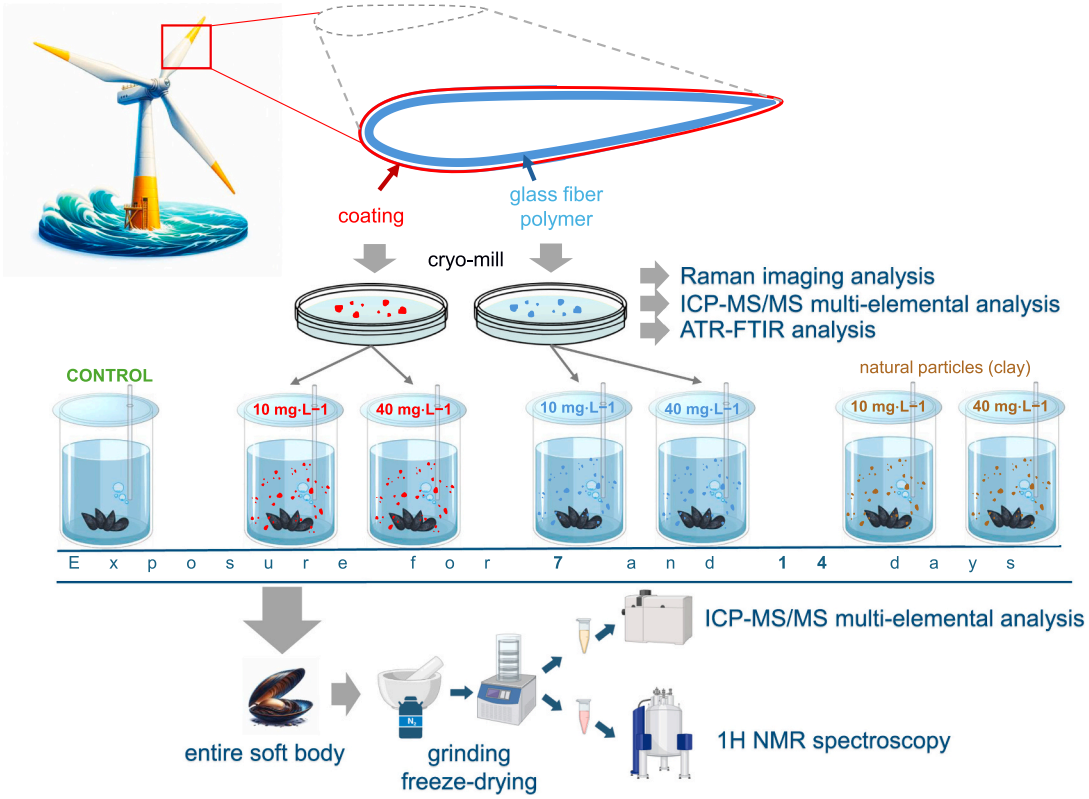

Fig. 1. A scheme of the experimental protocol and setup showing how *Mytilus edulis* was exposed to the different treatments and particle concentrations: control (no particles), clay (natural particles), coating and glass fiber polymer (GFP) (synthetic particles from wind turbine blades). Image is created using Microsoft PowerPoint™ and BioRender.com. An image of a wind turbine is AI-generated (Bing).

and stored at a temperature of 4 °C to mitigate the possibility of microbial contamination. The samples of cryo-milled coating and GFP materials were measured using attenuated total reflection (ATR)-FTIR-spectroscopy on a Bruker Tensor 27 System (Bruker Optics GmbH) with a diamond Platinum ATR-unit (Bruker Optics GmbH). The spectra were recorded in triplicate of each material in absorbance mode in the range from 4000 to 400 cm^{-1} using a resolution of 4 cm^{-1}, 32 co-added Scans, a Blackman-Harris 3-term apodization and zero filling factor of two using Bruker OPUS 7.5 (Bruker Optics GmbH). The spectra were compared against the Bruker ATR-polymer library (Bruker Optics GmbH) and the library of Primpke et al., 2018 using different data treatments (raw spectra, 1. derivative and 2. derivatives, vector-normalization) and correlation techniques (Bruker Standard methods and correlation factor).

2.5. Metal analysis of soft body tissue and exposure material

2.5.1. Acid digestion

Six replicates of the CRMs (Recommended masses: 50–100 mg (±10 % (1 *RSD*, $N = 6$)) were weighed into pre-cleaned 55 mL TFM (modified polytetrafluoroethylene (PTFE)) vials (MARS 6, CEM Corp., Kamp Lintfort, Germany). According to Zimmermann et al. (2020), digestions (40 samples per run) were conducted at 230 °C (ramp time: 20 min; hold time: 60 min; max. power: 950 W) in 5 mL HNO_3, 2 mL HCl and 1 mL HBF_4. Mussel tissue samples were freeze-dried and homogenized prior to MWAD (Gamma 1–16 LSC plus, Christ, Osterode, Germany). Depending on the available sample mass, 4 replicates (per treatment: $t = 7/14$ d; $m = 10/40$ mg; coating/fiberglass/clay particles and controls) of 47 ± 24 mg (1 *SD*, $n = 42$, Median = 45 mg) of the mussel tissue and windmill coating, windmill GFP as well as the clay material were weighed into pre-cleaned 55 mL TFM vials and subjected to MWAD as described afore.

After digestion, the solution was transferred quantitatively to a pre-cleaned 50 mL DigiTUBEs (SCP Science, Quebec, Canada) and diluted to a final volume of 50 mL with ultrapure water. The diluted digests of SRM 2581 as well as the windmill coating and GFP were subjected to vacuum-filtration (0.45 µm PTFE filters; SCP Science) with a manifold to remove any remaining undigested particles, which may block the nebulizer of the used ICP-MS/MS system.

2.5.2. Multi-elemental analysis

The detailed description of reagent and standards is given in the S2. The instrument settings, measured isotopes and chosen measurement modes were very similar to our previous studies (Hildebrandt et al., 2020; Klein et al., 2021). However, O_2 was replaced by N_2O as reaction cell gas according to Klein et al. (2021). Multi-elemental analysis covering 46 elements in four different cell modes (no gas, He, N_2O and H_2) was performed using an ICP-MS/MS instrument (Agilent 8800, Agilent Technologies, Tokyo, Japan) coupled to an ESI SC-4 DX FAST autosampler (Elemental Scientific, Omaha, USA). The instrument was optimized for sensitivity and signal stability in a daily routine using a Li, Co, Y, Ce, Tl solution. Rh and Ir were used as internal normalization standards (Merck-Millipore) during analysis. An in-house quality control multi-elemental standard solution (Inorganic Ventures, Christiansburg, USA) was rigorously measured at least five times during each measurement batch to ensure a stable measurement and comparability within each measuring batch and in between batches. Additionally, wash blanks were measured between each sample replicate to monitor but also reduce potential carry-over effects.

2.5.3. Data evaluation and presentation

Processing of the multi-elemental raw data was conducted using MassHunter version 4.4 or higher (Agilent Technologies, Tokyo, Japan) in combination with a custom-written Excel© spreadsheet. The limits of detection (*LOD*s) and limits of quantification (*LOQ*s) were calculated

according to DIN 32645 (2008) and DIN ISO 11843-2 (2006). A *Kragten* spreadsheet approach (Kragten, 1994) was applied to determine combined uncertainties considering reproducibility, repeatability, and measurement precision for each sample. The significant number of digits of elemental mass fractions are given according to GUM and EURACHEM guidelines, whereby the uncertainty determines the significant number of digits to be presented with the value (EURACHEM/CITAC, 2012) (Klein et al., 2021; Reese et al., 2019; Ebeling et al., 2023).

In order to identify whether a metal has been enriched in the tissue or not, enrichment factors were calculated using the following equation:

$$EF = \left(\frac{\frac{[M]_{smpl}}{[P]_{smpl}}}{\frac{[M]_{control}}{[P]_{control}}} \right)$$

Here M always represents the mass fraction of the respective analytes while P refers to the mass fraction of the used reference element, Phosphorus. The lower scription indicates whether the mass fraction of a sample or the control group was used. For all calculations the mean mass fraction of control mussels only exposed to seawater ($n = 6$) were used as the control. Graphs were generated using Origin 2023 (Version 10.0.0.154).

2.6. Metabolic profiling of soft body tissue

Untargeted metabolic profiling based on ^{1}H NMR spectroscopy was performed on the entire soft body mass of the differently exposed *M. edulis* (for details see Götze et al., 2020). Briefly, after grinding the frozen tissue under liquid nitrogen, approximately 50 mg tissue was used for metabolite extraction via the methanol-chloroform-water method. Dried pellets containing the polar metabolites were resuspended in deuterized water (D_2O, final concentration 1 g mL^{-1} of the original sample weight) containing trimethylsilyl propionate as internal standard (TSP, 0.05 wt%, Sigma-Aldrich, St. Louis, USA).

Measurements were performed similar to Georgoulis et al. (2022) using an ultra-shielded vertical 9.4 T NMR spectrometer (Advance III HD 400 WB, Bruker-BioSpin GmbH, Germany) at 400.13 MHz with a 1.7 mm diameter triple tuned (^{1}H-^{13}C-^{15}N) probe, using a Carr–Purcell–Meiboom–Gill sequence (Bruker protocol cpmgpr1d, TOPSPIN 3.5, Bruker GmbH, Germany) with water suppression at room temperature and the following parameters: acquisition time (AQ) = 4.01 s, sweep width (SWH) = 8802 Hz (22 ppm), delay (D1) = 4 s, dummy scan (DS) = 4, and number of scans (NS) = 128. Spectra processing and analyses were done with Chenomx NMR Suite 8.4 software (Chenomx Inc., Edmonton, Canada) whereby the spectra were corrected for phase, shim and baseline and calibrated to the internal TSP signal (at 0.0 ppm, 2.3 mM). In total, signals of 44 compounds were annotated in the spectra and metabolites were identified using the internal database of Chenomx and an in-house library of ^{1}H NMR spectra from marine invertebrates.

2.6.1. Statistics and metabolic data evaluation for metabolic profiling

Statistical analyses of metabolite concentrations were performed using the MetaboAnalyst 5.0 web application. Treatment-dependent differences in normalized (log10 transformation) and Pareto scaled (in which each variable is divided by the square root of its standard deviation) concentrations were tested using univariate (ANOVA) and multivariate statistics (principal component analysis (PCA) and SAM (significant analysis of microarray-based on F-statistics), with Delta set so that FDR < 0.1). Partial least squares with discriminant analysis (PLS-DA) was found to be overfitted (checked by cross-validation and permutation analysis) and thus not shown. Clustering results are shown as dendrograms and heatmaps or visualization of treatment dependent shifts and changes in metabolite groups. PCAs and heatmaps were used to identify possible outliers as recommended by Xia and Wishart (2016). Although they highlight three possible outliers (samples ex40, ex41and ex42 in the control, coating and GFP-exposed group, respectively, see S1, Fig. 6), we have refrained from excluding these samples because the picture did not change when excluding them.

3. Results

3.1. Particle numbers and size distribution analysis

The quantity and size distributions of MP present in 1 μg of windmill coating and GFP material were directly determined through the Raman imaging techniques. The results revealed a mean of 1380 ± 140 (N/μg) of particles determined in coating material, while in the GFP material, a slightly higher mean particle count of 1423 ± 240 (N/μg) was observed (Fig. 2, A). Fig. 2, B illustrates the observed variation in MP size distribution within both synthetic particle samples revealing that a notable proportion of the coating (approximately 32.15 %) and GFP material (approximately 39.86 %) exhibited particle sizes smaller than 5 μm. Notably, a significant majority, around 97.76 % of particles of coating material and 97.18 % of particles of GFP material have a size ≤50 μm. Images of the Raman analyses can be seen in S1, Fig. 4.

3.2. Multi-elemental analysis and ATR-FTIR analysis

The elemental mass fractions measured for the samples - different exposure concentrations, times spans - ranged from 18 μg kg^{-1} ± 7 μg kg^{-1} (U (k = 2) (Eu in windmill coating) to 62 g kg^{-1} ± 15 g kg^{-1} (Ca in windmill coating). The mussels on the other hand showed mass fractions ranging from 1.7 μg kg^{-1} ± 2.2 μg kg^{-1} (Eu) to 13 g kg^{-1} ± 24 g kg^{-1} (K). The corresponding high relative combined uncertainties of over 100 % for some elements can be assigned to the natural heterogeneity and variability of the mussel samples. For reasons of clarity, because of elevated mass fractions in the particles, significant enrichment factors and potential ecotoxicity, only the results for the selected elements Al, As, Ba, Cd, Cr, Cu, La, Mn, Ni, Pb, Sn, Ti, Th, V and Zn are considered here (S3, Table 1). Mass fractions of all measured elements in the raw materials (clay, windmill coating and GFP particles), and the mussel samples are provided in S2.

Especially, Ba, Cu, Cd, Cr, and Ni showed elevated mass fraction in the mussel samples exposed to coating and GFP particles compared to the natural clay matrix resulting in enrichment factors between 0.93 and 6.1. Because of these elevated elemental mass fractions during exposure, possible uptake effects in the mussels appear plausible.

In the case of Al, a moderate accumulation was observed in mussels exposed to clay and GFP particles at concentrations of 40 mg/L each, but it was found that this accumulation remained almost stable over both exposure times of 7 ($EF_{Clay} = 2.9$, $EF_{GFP} = 4.2$) and 14 days ($EF_{Clay} = 3.4$, $EF_{GFP} = 4.2$). For Ba similar results can be found here, the highest EF = 6, was found for the mussels exposed to coating material (40 mg/L, 7 days), whereas the longer exposed mussels showed an EF of 4.6. Cr also showed increased EFs for each exposure material over the different time periods. The highest accumulations for the GFP material were found at 40 mg/L for both 7 and 14 days of exposure. By contrast, the coating material only showed significant accumulations at the lower concentration of 10 mg/L, whereas the clay material showed minor enrichment along all tested parameters. Cr uptake, for instance, is significantly higher for the clay exposure compared to the coating particle exposure though the coating particles contain a significantly higher Cr mass fraction (Fig. 3, S3, Table 2). In contrast, Ba shows the highest EFs for the coating particles which is completely in line with the highest Ba loads. GFP exposure leads to higher Al enrichment than clay exposure even though GFP and clay particles contain similar mass amounts of Al.

Calculated EF varied between 0.41 and 6.0, showing minor to severe enrichments in the mussels. Here, it becomes evident that all elements except for Mn and Cu showed EF > 1 (for at least one of the three tested mussel samples, mean values are shown in S3, Table 1). This indicates

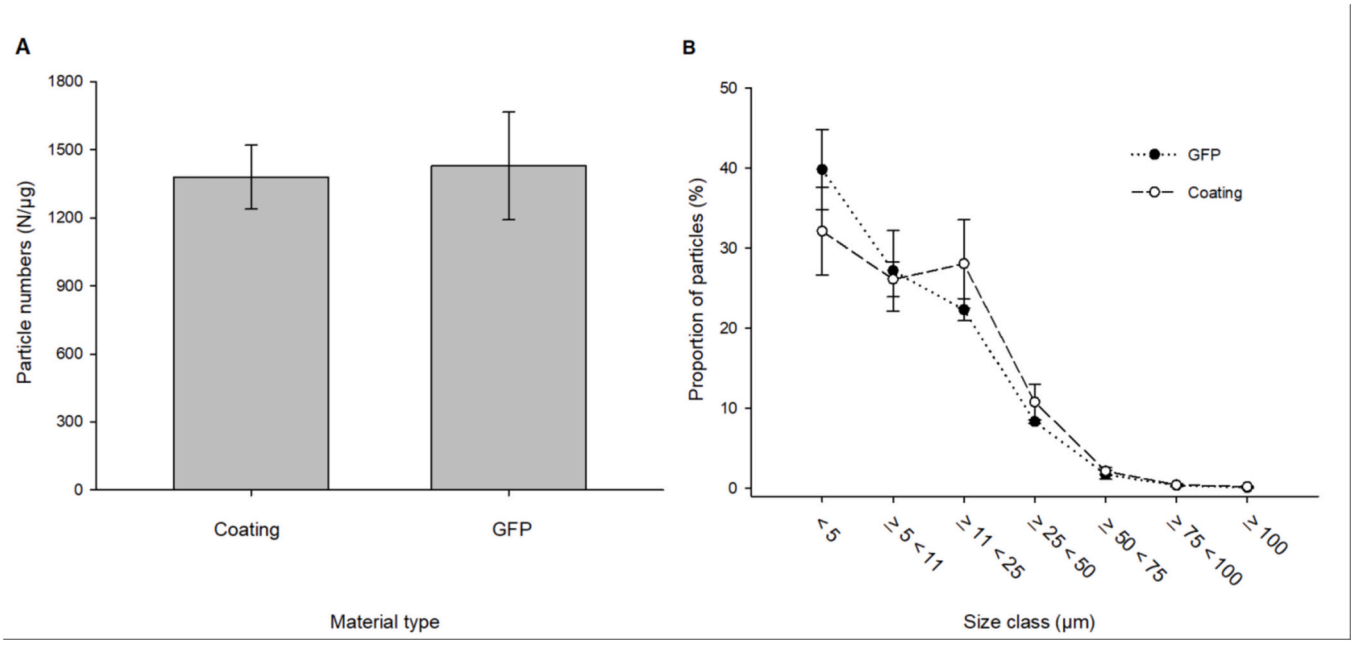

Fig. 2. Raman analysis of the number (A) and size distribution (B) of synthetic microparticles obtained from windmill coating and GFP material. Data are the means ± SD of technical triplicates.

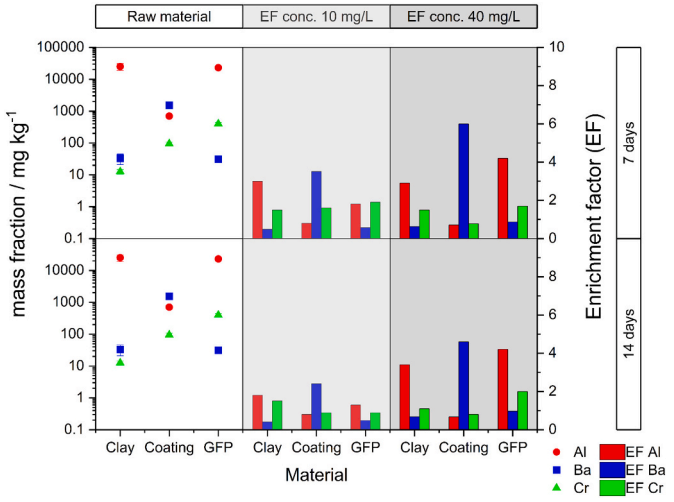

Fig. 3. Mass fractions of selected elements Al, Ba and Cr and corresponding enrichment factors (EF) in *M. edulis* after different exposure times (7 vs. 14 days) and concentrations (10 vs. 40 mg/L) dependent on exposure material (natural (clay) and synthetic blade coatings and core (glass fiber polymer, GFP)) Data are the means ± SD of technical triplicates. EFs are the average of 3 mussels.

possible intake of the particles or their additives (S3, Table 1). Most striking EFs were determined for Al, Ba and Cr (EF > 2) indicating moderate to severe elemental intake.

A comparison of the ATR-FTIR data with different polymer databases did not yield any hit or indicator for a similar material present in the database. The IR spectra (S1, Fig. 5) mainly showed the signals (peaks between 1730 and 1641 cm^{-1}) of the urethane groups of the coatings and in both cases various signals of the epoxy binders in the GFP and coating (peaks around 1610–1580 cm^{-1}, 1508 cm^{-1} and 828 to 700 cm^{-1}) based on acrylic compounds. Further, the coatings contain alkyd-chains (peaks at 2930 and 2860, 1455 and 1370 cm^{-1}) but these could not be further identified.

3.3. Metabolic profiling of soft body tissue

The analyses of the metabolic profiles obtained in the entire soft body tissue of *M. edulis* did not reveal any statistically significant treatment-induced changes, irrespective of particle concentration (confirmed by ANOVA and SAM). The focus of the following analysis is on the data set obtained from mussels exposed to the high load of MP (40 mg/L). Principal Component Analysis (PCA, Fig. 4, A) and Hierarchical Clustering (S1, Fig. 6) did not reveal any clear separation of treatment groups.

Despite no statistical significance, variability and average intensity of many metabolites was higher in mussels exposed to all types of studied MP in comparison to seawater controls (Fig. 4, B). Specifically, it was visible for the metabolic profiles obtained in mussels exposed to clay and GFP particles for 7 days and coating particles for 14 days. The highest fold-change was observed for sn-glycero-3-phosphocholine, lactate, tryptophane, inosine, alanine and 4-aminobutyrate (Fig. 4, B). Besides, a trend toward an increase in succinate concentration was detected in particle-treated mussels after 14 days of exposure, irrespective of material (Fig. 4, C).

4. Discussion

This is the first study investigating the possible effect of MP emitted from the wind turbine blades of OWFs on bivalves under controlled laboratory conditions. The worst-case scenario of massive abrasion of OWF materials which can occur under the conditions of LEE, was tested. Irregularly shaped particles in a size range similar to synthetic microparticles were produced from the coating and GFP-core material of the wind blade.

4.1. Chemical analytics of windmill particles composition and uptake of elements by mussels

Topcoats used for OWFs typically contain a polymer matrix with binders, pigments, fillers and extenders, along with different solvents, and additives (Mishnaevsky Jr et al., 2017). In this study, matte weathering-resistant polyurethane topcoats found on wind turbine rotor blades were tested. According to the material safety data sheet, the

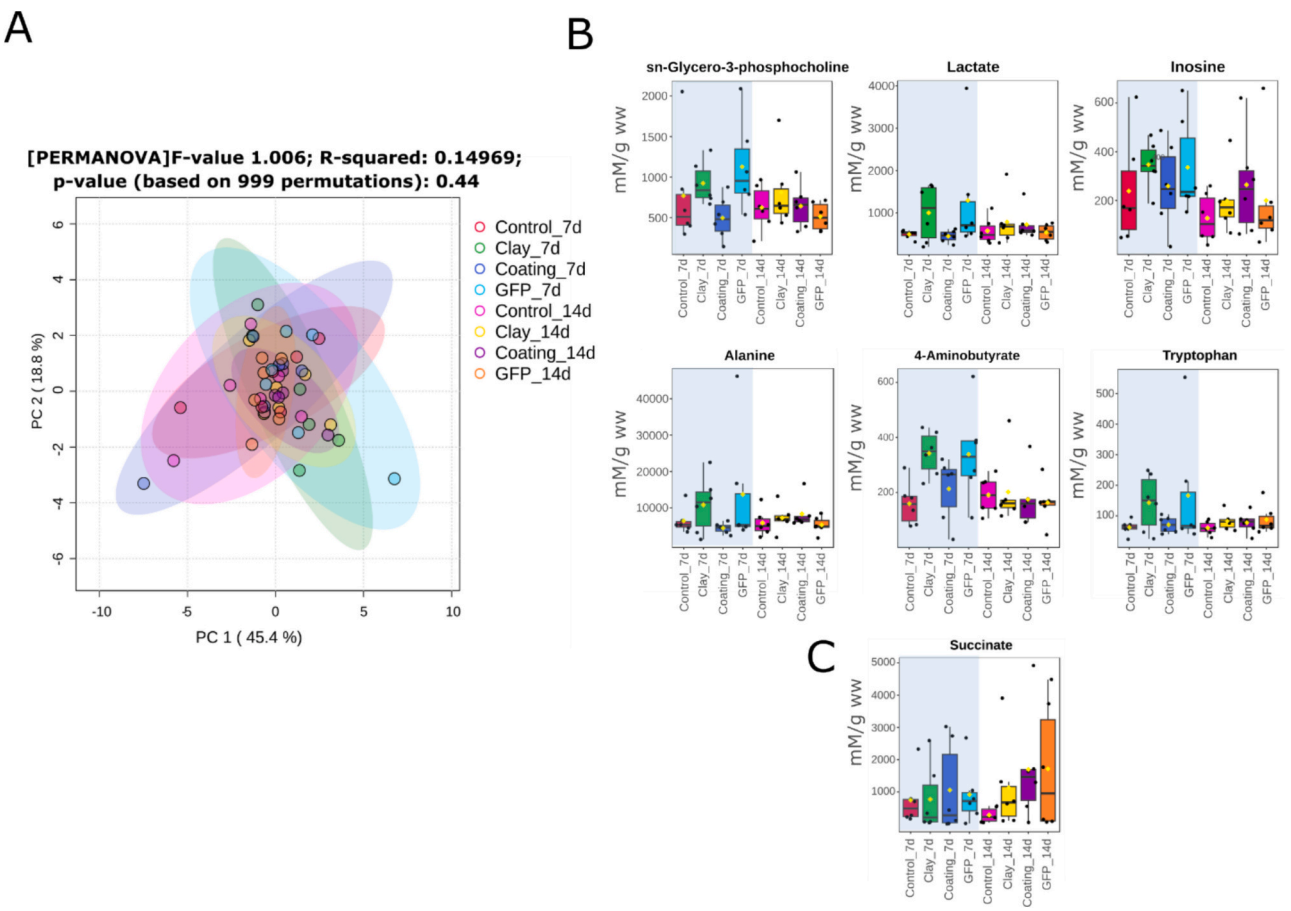

Fig. 4. Metabolic profiles of the entire soft body tissue of *M. edulis* exposed for 7 and 14 days to the high load (40 mg/L) of different microparticles (Control = seawater controls, no MP; Clay = seawater enriched with natural particles, Coating = seawater enriched with synthetic coating MP, GFP = seawater enriched with synthetic glass fiber polymer (GFP) MP). A) Principal Components Analysis Score Plot with dots representing individual animals, $N = 6$ per treatment. B) Selected metabolites with high variation and average intensity at 7- vs. 14-day exposure. C) Trend of increased succinate concentration in mussels exposed to all types of MP compared to seawater controls (7-day exposure data are highlighted in blue).

repair-kit for this coating includes n-butyl acetate, mica, titanium dioxide, silicon dioxide (amorphous), 1 bis (1,2,2,6,6-pentamethyl-4-piperidyl sebacate, xylene and 3-aminopropyltriethoxysilane. The information about studied GFP material was not available, however, these materials typically represent glass fiber-reinforced epoxy resins. ATR-FTIR analysis confirmed the presence of polyurethane and alkyd-chains in coating material (signals of urethane groups) and epoxy binders in both materials. Epoxy resin-based coatings are known to release toxic compounds (like bisphenol A and 4-tert-butylphenol), causing estrogenic effects and toxicity to various organisms (Bell et al., 2021; Vermeirssen et al., 2017) into aquatic environments. Ecotoxicity of polyurethane products is not well studied; however, it is proven that polyurethane can leach various toxic substances into water, posing environmental risks (Corapi et al., 2023; Lithner et al., 2009). Glass fibers, used to produce GFP for the windmill blades, are mainly composed of silicon dioxide and aluminum oxide with other oxides present in small quantities to increase the strength (Dathu and Hariharan, 2020). This can explain the high mass fraction (23,000 ± 1200 mg kg^{-1}) of Al followed by the relatively high mass fractions of Cr (400 ± 40 mg kg^{-1}) and Ni (177 ± 13 mg kg^{-1}) found in the GFP material. As expected, the clay particles (aluminum silicate hydroxide) were also enriched with Al (Mass fraction 25,000 ± 6000 mg kg^{-1}). High mass fraction of Al in the coating material can be attributed to mica, one of the components of this material. Similar assumption refers to the relatively high mass fraction of Ti that can be attributed to titanium dioxide. The highest mass fraction in the coating material was demonstrated for Ba (1530 ± 180 mg kg^{-1}). Even though the barium sulfate was not included in the material safety data sheet, the material is commonly used in the windmill blade coatings as a filler and pigment (Benin et al., 2022). Moderately high mass fractions were found for Cr (95 ± 12 mg kg^{-1}) in coating materials. This can be attributed to the impurities in the material composition.

Mussels are well known for their ability to accumulate metals from food and surrounding water, that is why they are commonly used in monitoring programs like Mussel Watch (Goldberg et al., 1978; Rainbow and Phillips, 1993; Stankovic and Jovic, 2012). In this study, enrichment factors (EF) for metals and metalloids were identified in natural and synthetic particles-exposed mussels and results demonstrate obvious uptake by the mussels of the elements with high mass fractions. Most striking EFs were determined for Al, Ba and Cr (EF > 2), indicating moderate to severe elemental intake. Aluminum can be toxic for bivalves at high concentrations, causing genotoxic effects, oxidative stress and affecting reproduction (Mao et al., 2011). The observed mass fractions of Al in the soft body of mussels, after exposure to clay (172.33 ± 49.70 mg kg^{-1}, S1) and to GFP (208.33 ± 108.03 mg kg^{-1}, S1), fell within the range detected in mussel samples collected from various regions (51–1237 mg kg^{-1}, Mao et al., 2011; Beiras et al., 2003). The EF of Al in mussels after exposure to natural clay and the GFP material were relatively similar, which suggests that these synthetic microparticles cannot be considered as a potential source of aluminum contamination for mussels. Existing data regarding the ecotoxicity of barium indicate little/no acute ecotoxicity, that can be attributed to the properties of Ba as a competitive antagonist for potassium channels and the ability of Ba to substitute calcium (Payne et al., 2011; Verbruggen et al., 2020). As

barium sulfate is hardly soluble in water (the solubility in seawater has been reported to be in the range of 37–52 μg L^{-1} (Chow and Goldberg, 1960a, 1960b), observed high EFs of Ba may indicate a predominant uptake of particles, rather than of the leached element from the media. Chromium is considered to be moderately toxic for aquatic invertebrates, in particular for marine bivalves (Ciacci et al., 2012). The mass fraction of Cr in mussels from the control condition found in this study was 5.15 ± 0.60 mg kg^{-1} and exposure to GFP material caused an increase in the mass fraction up to 11.06 ± 9.55 mg kg^{-1} in GFP-exposed mussels. This is in the range of field samples from different regions (i.e. Cr concentration in biota from southwest Atlantic Ocean varied in the range of 0.2 to 14.2 mg kg^{-1}, Trevizani et al., 2023). Although little/no effect was observed on the metabolome of the mussels` entire soft body tissue, the relatively high EF of Cr suggests that the use of certain materials may cause chronic toxicity for mussels.

4.2. Effect of windmill particles on mussel's metabolome

The study and comparison of metabolomic profiles are useful tools for characterizing the health status and metabolic disorders in marine organisms incl. bivalves (see review by Capello, 2020). In bivalves that were sampled in- and outside of offshore wind farms (OWFs), Wang et al. (2023) found significant changes in gill metabolites such as increased levels of epinephrine, sulphaniline, and inosine 5′-monophosphate and lower levels of L-carnitine in animals from OWF areas. The field study suggests that the physiology of bivalves is affected by OWFs, as the animals showed an inflammatory response and appeared to have increased energy requirements and oxidation/detoxification capacities (Wang et al., 2023).

Contrary to our expectations and literature data, present study revealed no clear metabolomic disturbances in mussels exposed to MP-particles from wind turbines. However, some insignificant trends may indicate a possible short-term effect on mussels' neuroendocrine system (shown by neuronal-related metabolites sn-glycero-3-phosphocholine and 4-aminobutyrate), amino acid metabolism (tryptophane, alanine) and energy metabolism (lactate, succinate). These results support already existing data on bivalves exposed to different synthetic microparticles that were carried out using NMR spectrometry. I.e. Cappello et al. (2021) found that a short-term (72 h) exposure of *Mytilus galloprovincialis* to polystyrene microparticles (50 particles mL^{-1}) resulted in disturbed amino acid metabolic processes, energy metabolism and osmoregulatory processes in the digestive glands of the mussels. The study revealed increased levels of amino acids such as alanine, isoleucine, leucine, valine and tyrosine, of metabolites involved in the energy metabolism (lactate, glycogen and glucose) and in osmolytes and antioxidants such as betaine, homarine and glutathione. In a follow up study, MP-induced alterations in energy-related metabolites, osmolytes, amino acids, succinate, and neurotransmitters (e.g., acetylcholine) were also found in the gill tissue of this mussel species (De Marco et al., 2023). In line, gill metabolism of the Pacific oyster *Crassostrea gigas* was affected by MP exposure (25 μg L^{-1} for 12 days) whereas no MP-induced metabolic alterations were determined in the digestive gland (Paul et al., 2024). Particularly levels of metabolites related to amino acid and energy metabolism and osmoregulation such as threonine, glutamate, glucose, hypotaurine and betaine were elevated in gills of MP-exposed oysters. Methods based on liquid chromatography–mass spectrometry (LC-MS) also revealed changes in metabolites that are related to the metabolism of amino acids in mussels exposed to synthetic microparticles. Huang et al. (2021) demonstrated the alteration of phenylalanine metabolism in the mussel *Mytilus coruscus*, exposed for 7 and 14 days to four concentrations (0, 10, 10^4, 10^6 particles/L) of polystyrene microplastics. In the Asian clam *Corbicula flumine*, synthetic microparticles of unknown composition (640 mg L^{-1}) caused changes in metabolites that are related to purine, phenylalanine, tyrosine, and tryptophan biosynthesis, D-amino acid metabolism, and aminoacyl-tRNA biosynthesis (Zhang et al., 2023). Alterations in the amino acid metabolism were found in the Pacific oyster *C. gigas*, exposed to polyethylene and polyethylene terephthalate microparticles at concentrations of 10 and 1000 μg L^{-1} for 21 days (Teng et al., 2021). Considering that the main body of literature data including those mentioned refer to metabolic changes at the tissue level, with responses varying by tissue type, it appears that potential tissue-specific effects may have been masked by the present analysis of the whole soft tissue. In the worst-case scenario on which the present study was based, the impact was expected to be so strong that possible metabolomic responses would be detectable when examining the entire soft tissue. In a planned follow-up study on the effects of erosion particles from wind turbine blades, the possibility of masked tissue-specific effects will therefore be investigated in more detail.

It is also important to consider that the results of the present study may be related to particle sorting and rapid excretion through the digestive system. Mussels, like other bivalves, have the ability to sort particles based on their properties such as size, shape, nutritional properties and chemical surface composition and can also reject specific particles (Rosa et al., 2013; Baroja et al., 2021). The particles produced in this study fall within the size range of natural food particles for mussels (1 to 40 μm, Beecham, 2008). However, other factors such as surface properties or chemical composition may serve as sorting factors. In addition to sorting during the filtration process, bivalves are able of further sort of the particles after ingestion and rapidly excrete them as pseudofeces (Ward et al., 2019; Zhao et al., 2018). Results of the present study partially support this assumption, as potential disturbances in the metabolome were primarily observed after 7 days and disappeared after 14 days of exposure, suggesting an increased egestion of particles following an additional exposure week. Considering that Huang et al. (2021) observed an increasing MP accumulation with increasing exposure time in the digestive tract of mussels exposed to 2 μm MP particles, further studies are useful to clarify this aspect.

As no differentiation was done between different tissue types (digestive tract, gills) in the present study, no precise localization of the particles within the organism can thus be determined. For further investigation into the localization of the particles, utilizing a spatially resolved technique such as laser ablation ICP-MS of cross-sections could be beneficial. Furthermore, to decipher the discussed possibility of a masked tissue-specific effect at the metabolite level in our present study (see above), tissue-specific investigations will be included in a planned follow-up study to gain a deeper understanding of the potential variations of the MP-induced response in different tissues of bivalves.

5. Conclusion

Offshore wind farms (OWFs) have the potential to have a positive impact on climate change as the use of wind energy can replace conventional fossil fuels, thereby reducing CO_2 emissions and contributing to the fight against climate change. However, it is crucial to consider the potential environmental consequences of OWFs, specifically the erosion of rotor blades, which can result in unintended side effects such as enhanced plastic pollution of the oceans and effects on the marine ecosystem. In the present study, an experimental worst-case scenario of massive abrasion was investigated, in which mussels *Mytilus edulis* were exposed to a high MP load (40 mg L^{-1}) for up to 14 days. Mussels exposed to blade coatings and core (GFP) particles showed moderate to severe intake of metals, in particular barium and chromium with high enrichment factors of >2. Some trends of elevated metabolite concentrations may indicate a possible short-term effect on mussels' neuroendocrine system (sn-glycero-3-phosphocholine and 4-aminobutyrate), and amino acid metabolism (tryptophane, alanine) as well as a possible long-term effect on energy metabolism (lactate, succinate). According to the principles of One Health (Lebov et al., 2017), the presented results indicate a potential threat to the ecosystem functioning within the wind park area (as blue mussels are the ecological engineers in the epibenthic ecosystem), as well as a possible impact on the human health (due to the commercial use of blue mussels for human consumption). While these

results are promising, the current study is far from providing a comprehensive and reliable understanding of the potential OWFs risks in the marine environment. To achieve this, short- and long-term studies with different turbine blade materials and an integrative approach that examines parameters at various biological levels and life stages are necessary, preferably on an international scale.

Ethical statement

All procedures complied with the current laws of the country in which they were performed. An ethical approval by the German Council for Animal Care was not required for this study on bivalves.

CRediT authorship contribution statement

Daria Bedulina: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Špela Korez Lupše:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lars Hildebrandt:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Yaqing Duan:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis. **Ole Klein:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis. **Sebastian Primpke:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis. **Christian Bock:** Writing – review & editing, Validation, Methodology, Investigation, Funding acquisition, Conceptualization. **Stefan Krause:** Writing – review & editing, Resources, Conceptualization. **Steffen Czichon:** Writing – review & editing, Resources, Conceptualization. **Daniel Pröfrock:** Writing – review & editing, Validation, Methodology, Formal analysis, Conceptualization. **Gunnar Gerdts:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Gisela Lannig:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

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.

Acknowledgement

We thank our AWI colleagues A. Tillmann, F. Feliz Moraleda, and A. Karamyar for their great technical support in NMR analyses and animal care. We thank the Biological station of Helgoland (AWI), in particular U. Kieb for support in animal supply. The authors acknowledge funding of the project P-LEACH (https://www.ufz.de/P-LEACH) from the Helmholtz Association, Innovation Pool of the Research Field Earth and Environment in support of the Research Program "Changing Earth – Sustaining our Future".

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.177509.

Data availability

The generated datasets for this study will be made available after publication in the data repository of the AWI, https://www.pangaea.de/.

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