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**PVC does not influence cadmium uptake or effects in the mussel (*Mytilus edulis*)**

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Running title: Cadmium and microplastics in mussels
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## Abstract

Microplastics have become a global concern in recent years. In this study, we studied (i) whether the presence of polyvinyl chloride (PVC) microparticles may affect cadmium (Cd) uptake in mussel (*Mytilus edulis*); and (ii) the biological effects of PVC microparticles exposure alone or in combination with Cd. Significant Cd uptake in digestive gland was observed following Cd exposure. However, PVC did not significantly increase Cd uptake compared with Cd alone treatment. In terms of biological impacts, significantly lower neutral red retention (NRR) time and elevated expression of *Metallothionein isoform 20-IV (MT-20*) were observed in mussels exposed to Cd alone, or combined with microplastics, yet there was no significant difference between them. *Catalase* (*CAT*) expression only showed a significant increase in mussels exposed to Cd alone. This work provides an insight into the relationship on resulting biological impacts between these two contaminants.
Keywords: PVC microplastics; Cd; mussel; toxicity

## 1. Introduction

Microplastics (particles less than 5 mm) have become an issue of great scientific and social concern due to their wide presence and accumulation in many environmental matrices globally (Lusher et al. 2017). The blue mussel is commonly used as a sentinel organism for anthropogenic pollution (Beyer et al. 2017). Field studies have shown that microplastics are widespread in mussels around the world (Lusher et al. 2017; Li et al. 2016, 2018) with uptake, accumulation and toxicity characteristics well documented (Browne et al. 2008; Van Moos et al. 2012; Paul-Pont et al. 2016).

Interactions between metals and plastics have recently been studied in both laboratory and field survey approaches (Holmes et al. 2012; Brennecke et al. 2016; Munier and Bendell 2018). Akhbarizadeh et al. (2018) found linear relationships between microplastics and metals in the muscles of some fish species. Mixture of chromium (Cr) and microplastics caused significantly increased lipid peroxidation levels in the fish and this effect was absent under single Cr or microplastic exposures (Luís et al. 2015). It is well known that Cd is a highly toxic heavy metal with the properties of persistence and bioaccumulation. Cd has been impregnated in PVC products as a heat and UV stabilizer as well as adding color (Hansen et al. 2013). Although Cd in plastics has been banned, it is likely to be detected in products still in use and litter derived from older products (Turner 2016). Cd has also been detected in about 7% of ~900 beached microplastic samples from south west England (Ashton et al. 2010; Massos and Turner 2017).

PVC has as an important role in the manufacture of plastic products and has been one of the dominant microplastic types identified in coastal waters and beach sediments (Browne et al. 2010). It has also been widely detected in organisms, including mussels (Qu et al. 2018). Brennecke et al.

(2016) report that PVC has a higher adsorption for selected metals relative to polystyrene (PS). Also, PVC has been reported as the most abundant polymer recovered from intertidal sampling sites with high associated metal concentrations from 0.038-0.09 mg of Cd per gram of PVC (Rochman et al. 2014; Munier and Bendell 2018). Cd and PVC are thus a real combination in the environment. Their respective biological effects and health risks have also been extensively studied. However, little information is available about the extent to which their interaction changes their separate toxicity to organisms.

In this study, we examine whether the presence of PVC microparticles in seawater affects Cd uptake and toxicity, measured using a number of biomarker responses, in blue mussel (*M. edulis*). *Metallothionein isoform 20* (*MT-20)* has been selected as a specific biomarker of Cd-induced biological effects. *CAT* and *PK* were chosen to indicate oxidative stress and metabolism changes.

## 2. Methods and Materials

### 2.1. Model microplastic preparation

Virgin PVC powder (Sigma-Aldrich, Gillingham, U.K.) was ground with a mortar and pestle. Distilled water was added to the mortar and stirred with a glass rod. The mixture was successively filtered with a steel mesh with a pore size of 75 μm and a filter paper (1 μm). Afterwards, the PVC particles ranging from 1 to 75 μm on the filter paper were transferred to a centrifuge tube and dyed with 500 μg/mL Nile red solution in acetone for half an hour. The dye liquor was filtered on a filter paper (1 μm) and distilled water was added to remove remaining dye. The dyed particles were transferred to a small amount of distilled water in a glass bottle. Three replicates of 2 μl mixture were pipetted to a hemocytometer (Agar Scientific, Stansted, U.K.) and the number of red particles counted under an Olympus SZX10 Research High-Class Stereo microscope (Olympus Corporation, Japan). The solution was stored below 4 °C in the dark.

### 2.2. Mussel collection and acclimatization

*M. edulis* were collected from Filey, U.K. (54.12600, 01.72101) at low tide during August 2017. Mussels were acclimatized in glass aquaria (at 14 °C) with aerated artificial seawater (Tropic Marin, 35‰ salinity, pH=7.8) for 7 d. Mussels were fed daily with phytoplankton suspension (PhytoGreen-M, Brightwell Aquatics, Elysburg, USA) during the acclimation phase.

### 2.3. Acute exposure experimental setup and sampling procedure

A total of 80 mussels were randomly distributed into 4 L glass tanks with full artificial seawater (5 mussels per tank). Four experimental conditions with 4 replicates were used: control, $CdCl_2$ (200 μg/L $Cd^{2+}$), PVC (20 particles/ml) and $CdCl_2$+PVC (200 μg/L $Cd^{2+}$ and 20 particles/ml PVC, with a total of 20 mussels per treatment. Concentrations higher than present environmental concentrations were set in order for an assessment of future risk and for comparison with other studies (Browne et al. 2008; Gonçalves et al. 2019). Cd and microplastics were mixed together and shaken repeatedly in a glass bottle 3 days before addition to the aquaria with aeration to keep particles in suspension. The concentrations of Cd at the start and end of 3 days' mixing process were tested and compared with control group only containing Cd to determine if Cd could be adsorbed by PVC particles (n=3). The exposure experiments were conducted at a constant temperature of 14 °C in the cold room and a 12 h light-dark illumination regime. Mussels were fed daily with phytoplankton suspension that was added to the tanks directly at a concentration of 13 million cells/L. All the exposure medium and chemicals was renewed in each tank on the fourth day. 500 ml medium was collected randomly from each treatment on the first day, fourth day (before and after media changed) and the seventh day of the exposure phase for chemical analysis. To this end, the 500 ml medium from each treatment group was collected from several aquaria and mixed. Mortalities were recorded (n=1 from the $CdCl_2$ exposure group). After the 7 d exposure period, mussels were removed from the aquaria and

haemolymph samples immediately taken from 10 mussels for each exposure group for lysosomal membrane stability (LMS) assay. The shell length was then recorded before dissection. The digestive gland was removed from individual mussels and cut into three pieces and processed as follows: one third was frozen at -80°C for later chemical analysis; one third was preserved in RNAlater Stabilisation Solution (Thermo Fisher Scientific, Loughborough, U.K.) for later molecular analysis; and the final third was fixed in 5% formalin for later histological analysis. Gills were removed and preserved in 5% formalin for histological analysis.

2.4. Chemical analysis to characterize Cd uptake

Concentrations of Cd in artificial seawater and mussel tissue were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 5300 DV, Perkin Elmer, Beaconsfield, U.K.). Mussel digestive glands from the same treatment group (n=20) were pooled and freeze-dried. These were ground to ensure homogeneity. Up to 0.1g dry tissue sample from each group was digested by adding 3 ml $HNO_3$ (Romil Ltd, Cambridge, U.K.) in a microwave digestion system (CEM, Milton Keynes U.K.) at 200ºC for 20 min and diluted with purified water. Cd was extracted from seawater by solid phase extraction (SPE) after complexation with 8-hydroxyquinoline (8-HQ). The samples were adjusted to pH 8 and passed through a C18 type cartridge. The retained complexes were eluted with nitric acid. Quantification was performed by the use of calibration standards. Results of Cd content in tissue were expressed as µg/g.d.w (dry weight) tissue weight.

2.5. Histological analysis to confirm uptake of microplastics

Fixed digestive glands and gills were washed with 0.01 M phosphate buffered saline (PBS), dehydrated with ethanol and cleared with Histoclear II (National Diagnostics, Fisher Scientific, U.K.), before being embedded in paraffin wax (VWR, Lutterworth, U.K.). 10 µm sections were cut on a microtome. After staining with Haematoxylin and eosin Y solution (Sigma-Aldrich), slides were observed through polarized light microscopy (GXMXPL3230, GT Vision Ltd., U.K.).

2.6. LMS assay

LMS in haemocytes was analyzed using the NRR procedure of Martínez-Gómez et al. (2015) with slight modifications. Slides with haemolymph and neutral red solution were examined under a ZEISS AX10 Stereo microscope (ZEISS, Germany) with a digital camera at 15, 30, 60, 90, and 120 min incubation to determine and record the time at which 50% or a greater number of cells showed leakage of the dye from the lysosomal compartment into the cytosol. The mean NRR time represents 10 mussels per treatment.

2.7 *MT-20*, *CAT* and *PK* mRNA expression quantification from mussel digestive gland tissues

Transcriptional responses were measured in digestive glands for a metal-related stress, antioxidant defense and metabolic impact using *MT-20*, *CAT*, and *PK*. Primers were: *18S*, GTGCTCTTGACTGAGTGTCTCG, CGAGGTCCTATTCCATTATTCC; *EF1*, CACCACGAGTCTCTCCCAGA, GCTGTCACCACAGACCATTCC; *MT-20*, AATGGCTGGACCTTGTAACTGC, CTTTACATCCGGAACATCCGC; *CAT*, CACCAGGTGTCCTTCCTGTT, CTTCCGAGATGGCGTTGTAT; *PK*, GACTTGGAGCTGCCTTCAG, GGAATGCACAGAGGGTTCAT. For mRNA isolation, cDNA synthesis and real-time quantitative PCR, the same method we used before was applied (Chapman et al., 2017). Ten individuals from each exposure treatment were analyzed. Reference genes *EF1* and *18S* were selected for normalisation using the $2^{-\triangle Ct}$ method.

2.8. Statistical analyses

All statistical analyses were performed using SPSS. Any differences of NRR times, the gene expression and Cd concentration between control and different treatments was determined using One-Way ANOVA or non-parametric test according to the results of Kolmogorov-Smirnov test which

determine the normality. When the data were in a normal distribution, One-Way ANOVA with Dunnett's Test was performed. When the data was not normally distributed, a non-parametric test (Mann-Whitney U test) was performed. All the tests have a significance cut off value of $p$=0.05.

## 3. Results and Discussion

### 3.1. Concentration of Cd in seawater and mussel tissue

The size range of the mussels did not vary significantly between exposure treatment groups: control, 50.35±5.39 mm; $CdCl_2$, 49.37±3.04 mm; PVC, 51.35±5.38 mm; and $CdCl_2$+PVC, 49.45±4.21 mm. The Cd concentration in each exposure treatment at the four time points measured throughout the course of the experiment are shown in Fig.1a. All were close to the nominal concentrations (of 0 or 200 µg/L) except the sample from the $CdCl_2$+PVC treatment on the first day (Fig.1a). This aberrant Cd value was obtained at the outset of the exposure experiment and was attributed to poor mixing prior to sampling.

For mussel tissue, significant Cd uptake in digestive gland was observed in the $CdCl_2$ (130 µg/g.d.w) and $CdCl_2$+PVC (138 µg/g.d.w) treatment groups compared with the control and PVC alone treatment groups, after the seven-day exposure period (Fig.1b). These values are comparable with previous study reporting 210±54 µg/g.d.w Cd in digestive glands after twenty days at the same exposure concentration (Ciocan and Rotchell 2004). Cd exposure alone led to a similar concentration to the value obtained for Cd and PVC combined. Hence, the Cd uptake in the mussel digestive tissues was not significantly affected by PVC presence.

There was no obvious change of Cd concentration after mixing with microplastics for three days compared with control ($p < 0.05$), which indicated a poor adsorption ability of Cd to PVC in this study. This could be explained by the short time for mixture in preparation period. In addition, adsorption ability of virgin microplastics used in our study tend to be weaker than beached particles under weathering, which have a suitable, charged surface for the adsorption of various metal ions (Holmes et al. 2012). There are also environmental conditions (e.g. pH, temperature, ionic strength, dissolved organic matter) that may affect the adsorption performance of metals (Eom et al., 2019; Yang et al., 2019; Godoy et al., 2019). For example, Copper (Cu) adsorption by polymethyl methacrylate has been shown to significantly increase initially and then decrease with the increasing pH from 4 to 7, while Cd adsorption by polypropylene has been shown to be unaffected by pH change (Yang et al., 2019; Eom et al., 2019). Therefore, the influence of environmental conditions also varies with various metals and microplastics. All of these factors should be taken into consideration and characterized in the experiment. More chronic exposures and varying concentrations and environmental conditions are needed in the future to elucidate the combined effects of microplastics and other pollutants.

### 3.2. Uptake of microplastics in mussel tissue

Histological analyses of treated mussels revealed the presence of PVC particles in gills (Fig.1c) and digestive glands (Fig.1d) and gills in the PVC and $CdCl_2$+PVC treatment groups. In line with mussel filter feeding strategies, the microplastics appear to be trapped in mucus within the gills, and transported into the digestive system (Von Moos et al. 2012). Uptake and accumulation of PVC in mussel tissue has been observed previously in a related species, *Perna perna*, using a size range 0.1-1 µm and a longer exposure of 90 days (Santana et al. 2018). Similarly, other microplastics, PS and PE, of a similar size range (2 - 80 µm) have also been frequently reported as taken up by mussels (including *Mytilus* spp.), showing distribution within the gut lumen, gills and hemolymph (Browne et al. 2008; Von Moos et al. 2012; Avio et al. 2015; Paul-Pont et al. 2016; Magni et al. 2018). It is worth mentioning that no control to test for leakage of Nile Red from the microplastics was used in this study, which would help rule out artefact presence.

### 3.3. LMS assay

Photos of a healthy mussel granulocyte cell (Fig.2a), a stressed cell showing loss of neutral red in the cytosol (Fig.2b) and a dead cell (Fig.2c) were taken. Significantly lower NRR time was

observed in mussels exposed to Cd alone or in combination with PVC when compared to the control group ($p < 0.05$, Fig.2d). In contrast, no significant destabilization of LMS was observed in the mussels exposed to PVC particles alone or between the Cd and Cd+PVC exposure treatments. These results suggest that the Cd component within the exposure has an impact, as opposed to the PVC element. LMS in mussels is considered a sensitive, simple and low-cost biomarker of stress (Martínez-Gómez et al. 2015) and its correlation with oxyradical scavenging capacity, physiological scope for growth, and genotoxicity in mussels are well established (Moore et al. 2004). As such, LMS represents an integrated pathophysiological indicator of whole organism health status (Martínez-Gómez et al. 2015), that is routinely used as a robust biomarker of metal toxicity and bioaccumulation (Domouhtsidou et al. 2004; Liu et al. 2018). The Cd impact on LMS observed is thus predictable. In contrast, however, several studies have reported a significant destabilization of LMS in mussels exposed to microplastics alone (Von Moos et al. 2012; Avio et al. 2015), yet this was not observed following exposure to PVC particles in our study. This could be explained by the use of different polymer type, size range, and/or exposure concentration.

3.4 qPCR analysis of *MT-20*, *CAT* and *PK* mRNA expression

The expression level of three target gene in the digestive gland of mussels was measured. *MT-20* showed a significant effect of Cd in both Cd alone and Cd +PVC exposure groups relative to the control treatment ($p < 0.05$, Fig.2e). The elevated expression of *MT-20* in mussels following both exposures involving Cd is consistent with previous published studies whereby Cd salts induce *MT* expression in mussel tissues (Ciocan and Rotchell 2004: Scudiero et al. 2014). PVC alone, or in combination with Cd, had no significant difference from the control, or Cd+PVC, exposure treatments respectively ($p < 0.05$, Fig.2e). This indicates that, under these conditions, there is no evidence that PVC bound Cd increases the biological impact, measured as *MT-20* expression, compared with aqueous Cd exposure alone. Again, this is in contrast to previous studies whereby microplastics (PE and PS) have been reported to act as a vector for chemical contaminants leading to increased contaminant exposure levels and impacts, although none use the same metal and PVC combination (Avio et al. 2015).

*CAT* expression was elevated in all the treatment group compared with the control while there is only significant difference in mussels exposed to Cd alone ($p < 0.05$, Fig.2f). Interestingly, combining Cd and PVC did not add to the significant increase compared with the control and Cd single exposure. Previous studies have reported a negatively correlated relationship between *CAT* activity and microplastics after 6 days, as well as Cd in the environment (Avio et al. 2015; Paul-Pont et al. 2016). Our results are in line with Magni et al. (2018), showing increased *CAT* expression/enzyme activity as a result of microplastic (PS or PE) exposure, also over 6 days. In addition, a biphasic response of *CAT* has been previously reported in caged *M. galloprovincialis*, with an initial decrease over the 7 to 14 d time point followed by an increase in activity, over the remaining 28 d timescale (Serafim et al. 2011).

For *PK* expression, no significant differences between the exposure treatments relative to the control treatment were observed (Fig.2g). Previous studies have shown Cd-induced (using 50 μM/L) *PK* activity in mussel digestive gland tissues (Dailianis and Kaloyianni 2004). A significant increase of *PK* expression in gills of mussel caused by micro-PS (2, 6 μm) has also been observed, yet no similar induction in digestive gland tissue (Paul-Pont et al. 2016), suggesting an organ-specific response to microplastics.

This study shows that virgin PVC particles and Cd do not cause an additive detrimental effect on mussel health compared to Cd alone, as measured using selected biomarker responses of stress under the exposure regime adopted. These findings contribute to the knowledge about the relationship between microplastics with other pollutants, and resulting biological impacts. However, there are still some limitations due to the single concentration, environmental conditions and exposure time. Future studies should investigate defined types of plastic polymer, their weathered/virgin state, and their interaction with various chemical contaminant classes under different exposure conditions, before

conclusions can be made regarding their role as vectors affecting the health of aquatic organisms.

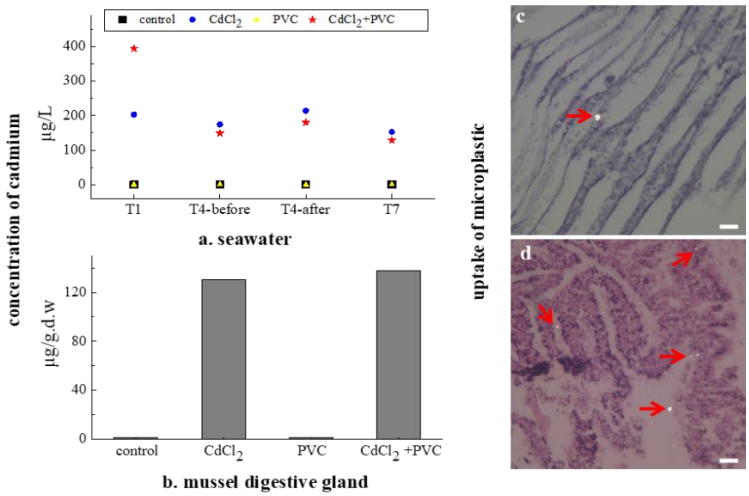


**Fig. 1** Uptake of Cd and microplastics. Concentration of Cd in seawater (a) and digestive glands of mussels (b) was analyzed. There is one replicate (including 20 pooled samples) for each group. T1, T4 and T7 are day 1, 4 and 7 of exposure respectively. T4-before and T4-after mean the time before and after the water change. Photos show haematoxylin and eosin Y stained tissue section of gills (c) and digestive glands (d) under polarized microscopy. The red arrows indicate microplastics. Scale bar =50 μm

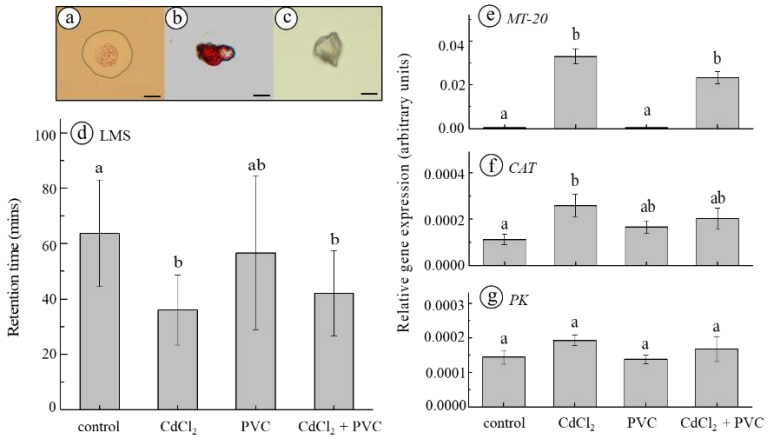


**Fig. 2** Biological effects of microplastics and Cd. Photos of a healthy mussel granulocyte cell (a), a stressed cell showing loss of neutral red in the cytosol (b) and a dead cell (c) were taken. Scale bar =200 μm. Lysosomal membrane stability (LMS) in haemolymph of mussels was analyzed (d). Relative mRNA expression of *MT-20* (e), *CAT* (f) and *PK* (g) was also included. Different letters (a/b) indicate significant difference. n=10, $p<0.05$. The vertical bars mean the standard deviations

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## References

Akhbarizadeh R, Moore F, Keshavarzi B (2018) Investigating a probable relationship between microplastics and potentially toxic elements in fish muscles from northeast of Persian Gulf.

Environ Pollut 232: 154-163
Ashton K, Holmes L, Turner A (2010) Association of metals with plastic production pellets in the marine environment. Mar Pollut Bull 60: 2050-2055
Avio CG, Gorbi S, Milan M, Benedetti M, Fattorini D, d'Errico G, Pauletto M, Bargelloni L, Regoli F (2015) Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ Pollut 198: 211-222
Beyer J, Green NW, Brooks S, Allan IJ, Ruus A, Gomes T, Bråte ILN, Schøyen M (2017) Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: A review. Mar Environ Res 130: 338-365
Brennecke D, Duarte B, Paiva F, Caçador I, Canning-Clode J (2016) Microplastics as vector for heavy metal contamination from the marine environment. Estuar Coast Shelf Sci 178: 189-195
Browne MA, Dissanayake A, Galloway TS, Lowe DM, Thompson RC (2008) Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). Environ Sci Technol 42: 5026-5031
Browne MA, Galloway TS, Thompson RC (2010) Spatial patterns of plastic debris along estuarine shorelines. Environ Sci Technol 44: 3404-3409
Chapman EC, O'Dell AR, Meligi NM, Parsons DR, Rotchell JM (2017) Seasonal expression patterns of clock-associated genes in the blue mussel *Mytilus edulis*. Chronobiol Int 34(9): 1300-1314
Ciocan CM, Rotchell JM (2004) Cadmium induction of metallothionein isoforms in juvenile and adult mussel (*Mytilus edulis*). Environ Sci Technol 38: 1073-1078
Dailianis S, Kaloyianni M (2004) Cadmium induces both pyruvate kinase and Na+/H+ exchanger activity through protein kinase C mediated signal transduction, in isolated digestive gland cells of *Mytilus galloprovincialis* (L.). J Exp Biol 207: 1665-1674
Domouhtsidou GP, Dailianis S, Kaloyianni M, Dimitriadis VK (2004) Lysosomal membrane stability and metallothionein content in *Mytilus galloprovincialis* (L.), as biomarkers: combination with trace metal concentrations. Mar Pollut Bull 48: 572-586
Eom JH, Park JH, Kim SH, Kim YJ, Ryu SK, Seo DC (2019) Evaluation of Cd Adsorption Characteristic by Microplastic Polypropylene in Aqueous Solution. Korean J Environ Agric 38(2): 83-88
Godoy V, Blázquez G, Calero M, Quesada L, Martín-Lara MA (2019) The potential of microplastics as carriers of metals. Environ Pollut 255: 113363
Gonçalves C, Martins M, Sobral P, Costa PM, Costa MH (2019) An assessment of the ability to ingest and excrete microplastics by filter-feeders: A case study with the Mediterranean mussel. Environ Pollut 245: 600-606
Hansen E, Nilsson NH, Lithner D, Lassen C (2013) Hazardous Substances in Plastic Materials. COWI and the Danish Technological Institute on behalf of The Norwegian Climate and Pollution Agency, Oslo. pp 150
Holmes LA, Turner A, Thompson RC (2012) Adsorption of trace metals to plastic resin pellets in the marine environment. Environ Pollut 160: 42-48
Li J, Qu X, Su L, Zhang W, Yang D, Kolandhasamy P, Li D, Shi, H (2016) Microplastics in mussels along the coastal waters of China. Environ Pollut 214: 177-184
Li J, Green CG, Reynolds A, Shi H, Rotchell JM (2018) Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. Environ Pollut 241: 35-44
Liu Q, Wang F, Meng F, Jiang L, Li G, Zhou R (2018) Assessment of metal contamination in estuarine surface sediments from Dongying City, China: Use of a modified ecological risk index. Mar

Pollut Bull 126: 293-303
Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(T) (-Delta Delta C) method. Methods 25: 402-408
Luís LG, Ferreira P, Fonte E, Oliveira M, Guilhermino L (2015) Does the presence of microplastics influence the acute toxicity of chromium (VI) to early juveniles of the common goby (*Pomatoschistus microps*)? A study with juveniles from two wild estuarine populations. Aquat Toxicol 164: 163-174
Lusher A, Bråte ILN, Hurley R, Iversen K, Olsen M (2017) Testing of methodology for measuring microplastics in blue mussels (*Mytilus* spp) and sediments, and recommendations for future monitoring of microplastics (R & D-project). Norsk institutt for vannforskning
Magni S, Gagné F, André C, Della Torre C, Auclair J, Hanana H, Parenti CC, Bonasoro F, Binelli A (2018) Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel *Dreissena polymorpha* (Mollusca: Bivalvia). Sci Total Environ 631: 778-788
Martínez-Gómez C, Bignell J, Lowe D (2015) Lysosomal membrane stability in mussels. ICES TIMES No. 56, pp41
Massos A, Turner A (2017) Cadmium, lead and bromine in beached microplastics. Environ Pollut 227: 139-145
Moore MN, Depledge MH, Readman JW, Leonard DP (2004) An integrated biomarker-based strategy for ecotoxicological evaluation of risk in environmental management. Mutat Res-Fund Mol M 552: 247-268
Munier B, Bendell LI (2018) Macro and micro plastics sorb and desorb metals and act as a point source of trace metals to coastal ecosystems. PloS One 13: e0191759
Paul-Pont I, Lacroix C, Fernández CG, Hégaret H, Lambert C, Le Goïc N, Frère L, Cassone AL, Sussarellu R, Fabioux C, Guyomarch J (2016) Exposure of marine mussels *Mytilus* spp. to polystyrene microplastics: Toxicity and influence on fluoranthene bioaccumulation. Environ Pollut 216: 724-737
Qu X, Su L, Li H, Liang M, Shi H (2018) Assessing the relationship between the abundance and properties of microplastics in water and in mussels. Sci Total Environ 621: 679-686
Rochman CM, Hentschel BT, Teh SJ (2014) Long-term sorption of metals is similar among plastic types: implications for plastic debris in aquatic environments. PloS One 9: e85433
Santana MF, Moreira FT, Pereira CD, Abessa DM, Turra A (2018) Continuous exposure to microplastics does not cause physiological effects in the cultivated mussel *Perna perna*. Arch Environ Contam Toxicol 74: 594-604
Scudiero R, Cretì P, Trinchella F, Esposito MG (2014) Evaluation of cadmium, lead and metallothionein contents in the tissues of mussels (*Mytilus galloprovincialis*) from the Campania coast (Italy): levels and seasonal trends. C R Biol 337: 451-458
Serafim A, Lopes B, Cravo A, Gomes T, Sousa V, Bebianno MJ (2011) A multi-biomarker approach in cross-transplanted mussels *Mytilus galloprovincialis*. Ecotoxicol 20: 1959-1974
Turner A (2016) Heavy metals, metalloids and other hazardous elements in marine plastic litter. Mar Pollut Bull 111: 136-142
Von Moos N, Burkhardt-Holm P, Köhler A (2012) Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ Sci Technol 46: 11327-11335
Yang J, Cang L, Sun Q, Dong G, Ata-Ul-Karim ST, Zhou D (2019) Effects of soil environmental

factors and UV aging on $Cu^{2+}$ adsorption on microplastics. Environ Sci Pollut Res 26: 23027-23036.