Interactions of Trace Metals with Plastic Production Pellets in the Marine Environment

by

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Abstract

This study investigates the interactions between dissolved trace metals and plastic debris under controlled laboratory conditions by using polyethylene pellets as a model plastic particle. Specifically, the study compared virgin pellets sourced from a local moulding plant with those collected from local beaches and subjected to aging, attrition and deposition of extraneous material.

Pellets collected from the coastline of Southwest England were mainly polyethylene according to Fourier transform infra-red spectroscopy (FTIR), although occasional polypropylene pellets were present. Additionally, FTIR was used to identify the extent of degradation of pellets according to a photo-oxidation index derived from the relative magnitudes of specific absorbance peaks, and suggests pellet colour may give an indication of polymer degradation. Acid extractions of pellets collected from the coastline of Southwest England yielded metal concentrations ranging from low ng g^{-1} for metals such as Cd, Ni and Cr, to 7.7, 10.3 and 290 μg g^{-1} for Cu Pb and Zn, respectively, while Al, Fe and Mn were present on beached pellets at concentrations of up to 171, 314 and 308 μg g^{-1}, respectively. Metal concentrations exhibited a high degree of inter- and intra-site variability. Correlation of metal concentrations with the photo-oxidation index indicates that pellet age is not a reliable indicator of metal concentrations.

Batch experiments where metals (Cd, Co, Cr, Cu, Ni, Pb, Zn) were added to suspensions of pellets in seawater and estuarine water were performed in order to understand the extent and rates of trace metal adsorption to pellets. Langmuir modelling of adsorption isotherms in seawater indicated a range of maximum values for the accumulation of trace metals on beached pellets from 10 ng g^{-1} to 720 ng g^{-1} for Cd and Pb, respectively, in seawater spiked with 5 μg L^{-1} of each trace metal. Langmuir constants for these relationships ranged from 0.140 (Cd) to 2.67 (Ni) mL g^{-1}. Differences between trace metal affinities for pellets collected from the environment (beached) and those obtained directly from a plastics processing facility (virgin) were also identified. Adsorption maxima for virgin pellets ranged from < 1 ng g^{-1} (Cd) to 300 ng g^{-1} (Cr) with corresponding, respective Langmuir constants of 0.413 and 0.127 mL g^{-1}. Adsorption of trace metals to beached pellets exceeds that to virgin pellets owing to the development of viable surface sites by photo-oxidation, biofouling and deposition of fine sediment particles on the former during exposure to environmental conditions. Adsorption kinetics were modelled successfully using a pseudo first-order reversible model. Chemical response times derived from kinetic constants ranged from 1.5 – 12 h for beached pellets and 0.2 to 4.5 h for virgin pellets, and were generally lower in seawater than in river water.

Applying a similar batch approach to a system simulating an estuarine salinity gradient, the controlling effects of ionic strength and pH on the adsorption of trace metals to pellets were identified. Partitioning of Cd, Co and Ni exhibited inverse relationships with salinity, with partition coefficients (K_D) decreasing from values of the order 10^1 to 10^{-1} or 10^{-2} mL g^{-1} (for beached pellets) as salinity is increased from < 0.05 to 33. Chromium exhibited contrasting behaviour within the estuarine gradient, with partitioning increasing with salinity, while Pb appears to be independent of salinity within this system. Chemical modelling was used to explain the results in context of changes in trace metal speciation which occur through the estuarine gradient.

The bioaccessibility of metals on beached pellets to avian species was determined using an *in vitro* approach. Metal bioaccessibility in a simulated avian gastric environment comprising 10 g L^{-1} pepsin, 0.1 M NaCl and HCl at pH 2.8 indicated metals on pellets are labile (bioaccessibility ranges from < 10 % (Cr) to > 80 % (Mn)) and may be released readily within the digestive tract. Dissolution kinetics were modelled using the Noyes-Whitney equation, from which rate constants were determined in the range 3.1×10^{-4} (Cr) to 8.7×10^{-1} $\%^{-1}$ h^{-1} (Mn).

This study has shown, for the first time, that plastic pellets have the potential to accumulate and transport trace metals in the marine environment. Furthermore, metal accumulation on plastic pellets is controlled by estuarine master variables such that conditions which favour or limit adsorption can be defined. Pellets present a previously unreported vector for the transport of metals in the environment, and have the potential to convey metals to organisms upon ingestion. The findings of this thesis have implications for interactions between metals and plastics more generally in the marine environment.

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Nomenclature

ADC	Analog-to-digital converter
ALC	Above load coil
BET	Brunauer, Emmet & Teller
COSHH	Control of substances hazardous to health
CRM	Certified reference material
DIW	Deionised water
FTIR	Fourier transform infra-red spectroscopy
FTIR-PAS	Fourier transform infra-red photo-acoustic spectroscopy
HEPA	High efficiency particulate air filter
HDPE	High density polyethylene
HOC	Hydrophobic organic compound
ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
K_D	Partition coefficient, mL g^{-1}
LDPE	Low density polyethylene
LOD	Limit of detection
LM	Light microscopy
MDS	Multi-dimensional scaling
MQW	Milli-Q water
Nd	not detected
NP	Nonylphenol
PAH	Polycyclic aromatic hydrocarbon
PC	Polycarbonate
PCB	Polychlorinated biphenyl
PBDE	Polybrominated diphenyl ethers
PBET	Physiologically based extraction test
PE	Polyethylene
PET	Polyethylene terephthalate
POP	Persistent organic pollutant
PP	Polypropylene
PS	Polystyrene
PTFE	Teflon
PVC	Polyvinyl chloride
RF	Radio Frequency
RSD	Relative standard deviation
SEM	Scanning electron microscopy
SPM	Suspended particulate matter
SSA	Specific surface area
t_{resp}	System response time
UV	Ultraviolet
XRF	X-ray fluorescence analysis

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Author's declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

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Holmes, L.A., Turner, A., Thompson, R.C.T., 2011. Accumulation and transport of trace metals by plastic production pellets in the marine environment. 5th International Marine Debris Conference, Honolulu, Hawaii, USA, 20-25th March 2011.

Holmes, L.A., Turner, A., Thompson, R.C., 2010. The role of plastic production pellets in the accumulation and transport of trace metals in the marine environment. 2nd Annual BGC conference, Plymouth, UK, 17th December 2010.

Holmes, L.A., Turner, A., Thompson, R.C., 2009. The role of plastic production pellets in the accumulation and transport of trace metals in the marine environment. 6th Annual BEACh conference, Plymouth, UK 14th December 2009.

Chapter 1: Introduction

1.1 Introduction

Marine debris is a growing environmental concern, whose implications are not yet thoroughly understood despite its global importance. In recent years, the role of plastic as the primary component of marine debris has come to the attention of the scientific community, and the ubiquity and potential effects of plastic debris are now being disseminated to the wider public and government bodies (e.g. European Commission, 2011). This thesis addresses an aspect of research into plastic debris which has not yet been considered - interactions of trace metals with plastic in the marine environment.

The present chapter offers a summary of the relevant background on marine debris (Section 1.2), and introduces the common types of plastic debris, including plastic production pellets, providing rationale for their use as the basis of the experimental work presented in Chapters 3-6. Section 1.3 describes the known interactions between plastic debris and co-contaminants, whilst highlighting the lack of research into interactions between trace metals and plastics in the environment. The ingestion of plastic by organisms is described in Section 1.4, which also suggests why research into possible effects of ingestion must be carried out. Section 1.5 puts the subsequent research into context and identifies how it may contribute to current knowledge. Finally, the aims and objectives of this thesis are described in Section 1.6.

1.2 Background on plastic debris

1.2.1 People and plastics

Synthetic polymers are constructed by the process of linking monomers derived from hydrocarbons, and are commonly known under the general term “plastics” which gives an indication of some of their properties - plastics are capable of being moulded, blown into films or extruded in order to fulfil numerous applications (Andrady & Neal, 2009). Increasing demand since mass production of plastics began in earnest in the 1940s and 1950s has resulted in a continual growth in production (Fig. 1.1), with current estimates of 265 million tonnes in 2010 (Plastics Europe, 2011).

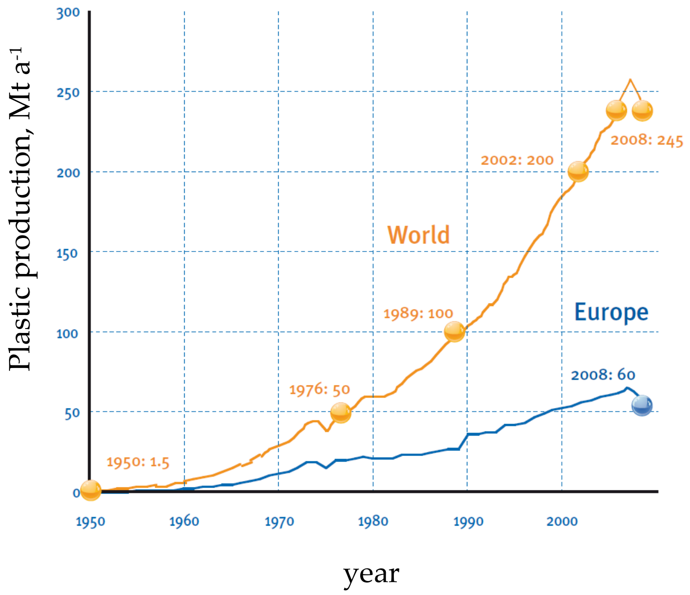

Figure 1.1: Trends in global plastic production over the period 1950 – 2008 (Plastics Europe, 2009).

Plastic production is continuing to increase at a rate of approximately 5 % per annum (Andrady & Neal, 2009), and now accounts for 8 % of oil consumption globally (Thompson *et al.*, 2009a). Owing to the desirable properties of plastics (low cost, low density and high durability) they have become pervasive globally yet it is the very properties which make plastics so desirable which results in environmental concern (Derraik, 2002).

Plastics have become globally important in a range of applications, bringing societal benefits such as waste minimisation within the food industry, where the use of durable packaging with low permeability to moisture and oxygen has extended shelf-lives for food (Andrady, 2011). Widespread use of plastics in the manufacture of lightweight components for vehicles has contributed to increased efficiency and reduced carbon emissions within the transport industry (Andrady & Neal, 2009). Furthermore, advances in hygiene have arisen from the ease of cleaning of plastic surfaces, which has led to their widespread use within hospitals and other environments sensitive to cleanliness. These three examples are just a small fraction of the innumerable applications for plastics (Andrady & Neal, 2009), but outline the versatility and range of properties which have led the demand for these materials to increase to the current level.

Nearly one third of plastics produced annually are used for consumer packaging (Andrady, 2011), constituting 10 % of municipal waste generated globally, which is largely deposited in landfills where it has the potential to persist for centuries (Barnes, *et al*., 2009). The remaining packaging will be either recycled or potentially handled irresponsibly, resulting in release to the environment. The diverse routes via which plastics may enter the environment give rise to challenges in estimating the magnitude of the issue, and make preventative measures difficult.

Reliable measurements of the proportion of marine debris comprised of plastic materials are not available. However, it is estimated that 60-80 % of marine debris is plastic (Gregory & Ryan 1997). Myriad sources, ranging from litter discarded on land, to accidental spillages during maritime shipping (Barnes *et al*., 2009), cause plastic debris to be a very difficult problem to manage, and with the level of plastic production continuing to increase, it is important for all possible impacts of plastic manufacture, use and disposal to be considered. While estimates of the global abundance of plastic debris are unavailable, the scale of the issue is exemplified by studies which indicate abundance on the shoreline may exceed 100 000 items m^{-2}

(Gregory, 1978), and over 3.5 million items km^{-2} in the surface waters of the North Pacific Ocean (Yamashita & Tanimura, 2007). A comprehensive review of plastic debris in general is beyond the scope of this chapter, but the reader is directed to Barnes *et al*. (2009), Derraik, (2002) and Ryan *et al*. (2009) for a broad perspective.

1.2.2 Macro-plastic debris

Marine debris is composed of materials including wood, glass and metal. However, the most abundant and persistent type of marine debris is plastic (Derraik, 2002). Macro-plastic is defined as a plastic item, fragment or strand whose greatest dimension is > 5 mm (Arthur *et al*., 2009). Debris in this size range impacts natural systems and ingestion by large predatory organisms including birds (Auman *et* al., 1997) turtles (Mrosovsky *et al*., 2009) and marine mammals (Laist, 1997), smothering of benthic habitats (Goldberg, 1997), and entanglement of fish, birds and mammals (Gregory, 2009) are well documented.

Furthermore, the increasing presence of buoyant substrates in the marine environment has the potential to alter assemblages of organisms which require buoyant material in order to reproduce, such as the pelagic insect *Halobates sericeus* (Goldstein et al., 2012) or inhabit, as noted for hydroids, bivalves, foraminifera and other sessile and motile organisms (Gregory, 2009). These changes in buoyant substrate availability may alter population dynamics for those species which rely on such sites through increasing abundance, as well as allowing attached organisms to more readily enter distant ecosystems, becoming invasive species (Barnes 2002; Gregory, 2009). In addition to these direct impacts on the marine environment, macroplastic will slowly degrade and break down physically, becoming one of the many sources of microplastic to the marine environment.

Degradation of plastics occurs in all environments, while the rate at which this process occurs will be controlled by several environmental factors. The primary means by which plastics are degraded involves photo-oxidation which initiates upon exposure to ultraviolet (UV) light, and is accelerated by thermal stress (Valadez-Gonzalez *et* al., 1999). As such, plastics in the marine environment will be more stable and persistent than those on land owing to the thermal insulation and UV attenuation afforded by immersion in the water column (Pegram & Andrady, 1989). Additionally, the development of biofilms on plastic debris will minimise the exposure of the plastic surface to UV potentially retarding the degradation process, while microbial activity may provide a means of biological degradation of the polymer (Artham *et al*., 2009). This has implications for the interactions between plastics and co-contaminants, as described in Section 1.3.

1.2.3 Micro-plastic debris

Microplastics are defined as plastic particles measuring < 5 mm in diameter (Arthur, *et al*., 2009) and may be introduced by industrial or domestic activities or can be generated by the degradation and fragmentation of macro-plastic as noted above. Fragmentation and degradation of the plastic debris currently in the environment is a major concern. Plastic debris has been recorded in all areas of the water column, from surface to the deepest oceans, as well as on coastlines globally (Ryan *et al*., 2009), rendering remediation a difficult or impossible task. Some schemes, such as Hawaii's Nets to Energy project are attempting to recover large items such as discarded fishing gear for use as an energy source (National Research Council, 2008), yet there remains an enormous stock of plastic in the environment which will, in time, be degraded into innumerable microplastic particles.

To examine domestic sources of microplastic, it must be considered that these pathways are typically passive - the polluter is not necessarily aware that they are contributing to the microplastic flux to the oceans. For example, microplastics have been observed in the effluent from domestic washing machines, where synthetic clothing has been shown to lose fibres which are flushed out with grey water discharges (Browne *et al*., 2011). Discharges of this type can contribute > 1900 individual fibres of microplastic to the environment with every wash, for a single item of clothing (Browne *et al*., 2011). Additionally, use of certain cosmetic products may result in inadvertent contributions of microplastic to the environment as plastics have begun to replace natural abrasives in cosmetics marketed as scrubs or exfoliators (Zitko & Hanlon, 1991; Fendall & Sewell, 2009). Plastics used in such applications are of a size which is not filtered out during the sewerage treatment process, resulting in discharge to the natural environment (Gregory, 1996).

Industrial practices are also known to cause microplastic particles to enter the marine environment owing to the accidental release of plastic raw materials such as powders or pellets, as well as the practices of shot blasting using plastic abrasives to clean machinery of paint and rust (Cole *et al*., 2011). Manufacture of plastic consumer items frequently relies on plastic production pellets, or nurdles, as a raw material, and it is these pellets, and their potential to act as a significant environmental contaminant which forms the basis for the work presented in the following chapters.

1.2.4 Plastic production pellets

Plastic production pellets have been described by Redford *et al.* (1997) as ovoid, cylindrical or spherical pieces of polymer, between 2 and 5 mm in diameter (Fig. 1.2), and it is in this form that raw plastics are transported to plastic processing facilities prior to forming or moulding into consumer items (Takada, 2006).

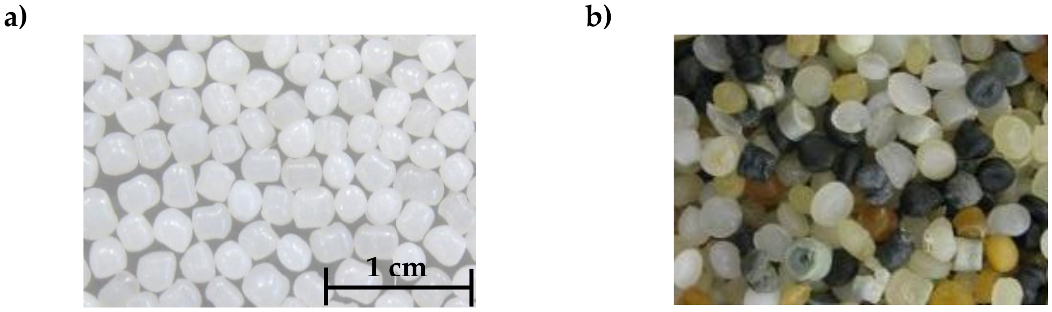

Figure 1.2: Plastic production pellets typical of those used within the plastics industry obtained (a) directly from the processing facility and (b) collected from the coastline of Southwest England.

Releases of pellets to the environment occur through careless handling and accidental spillage during transport and processing (Derraik, 2002). Following the polymerisation process, pellets are packed into sacks for transport to processing facilities, or directly pumped into cargo trains (Fig. 1.3). However, Redford *et al.* (1997) note that where packaging is used, it is often not robust, or is specifically designed for ease of rupture to facilitate emptying into hoppers.

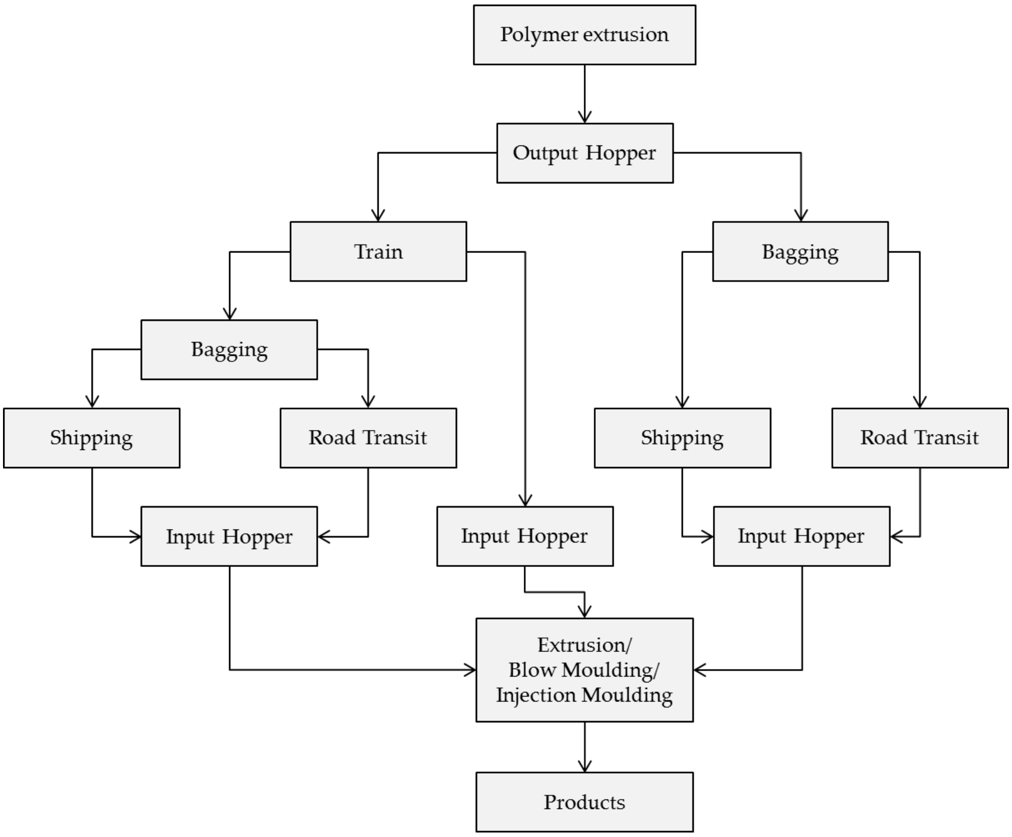

Figure 1.3: Stages in the transit of polymer pellets for production of consumer items. Releases of pellets may occur during transfers between stages of the process (adapted from Redford *et al*., 1997).

Additionally, during the packaging process, where pellets are put into bags, there is potential for release if the seal between the packaging and load valve is not complete. Poor connections between rail hopper cars and trucks constitute another means by which releases may occur, particularly during connection and disconnection of transfer valves, and spillages through these routes are well documented (Redford *et* al., 1997; Shiber & Barrales-Rienda, 1991).

Pellets are likely to be buoyant as the most abundant polymers, polyethylene and polypropylene, have specific gravities less than 1 (Table 1.1), therefore the majority of those released inland will be carried in surface water run-off, streams and rivers to the marine environment (Takada, 2006).

Table 1.1: Specific gravity of common polymers and natural waters (US EPA, 1992); and fraction of global plastic production (FGP) (Andrady, 2011).

Polymer	Minimum	Maximum	FGP
Polypropylene	0.890	0.910	24 %
Low density polyethylene	0.890	0.940	21 %
High density polyethylene	0.940	0.960	17 %
Polyvinyl chloride	1.380	1.410	19 %
Polycarbonate	1.200	1.200	< 6 %
Polystyrene	1.040	1.040	6 %
Polyethylene terephthalate	1.290	1.400	7 %
Typical river water	1.000		
Typical sea water	1.025		

Pellets may also enter the marine environment directly via drainage systems at coastal shipping sites (Wilber, 1987), or following cargo loss at sea due to storms. This has been demonstrated recently when several containers, containing a total of approximately 150 tonnes of pellets (Rochman, 2013), were swept from the deck of a cargo ship moored off the coast of Hong Kong in July 2012 during Typhoon Vicente, resulting in dense accumulations of pellets on the local coastline (Fig. 1.4), stimulating public and governmental clean-up efforts.

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Figure 1.4: Volunteers removing pellets from the coastline of Lamma Island, Hong Kong, August 2012 (Siu, 2012).

Methods to limit the discharge of plastic pellets to the environment include placing screens over drains to ensure pellets do not enter drainage or storm water channels, as well as ensuring any spillages are promptly recovered and disposed of responsibly (Redford *et al.*, 1997). These clean practices are outlined in voluntary initiatives such as Operation Clean Sweep, introduced in the early 1990s aimed at ensuring zero pellet loss to the environment (Ryan, 2008). However, there are currently no legal obligations to prevent pellet release.

While the focus of this work is plastic production pellets, plastic debris exists in many forms, each with their own properties and characteristic effects on the environment to which they are introduced. However, pellets constitute one of the most widely studied forms of plastic debris to date, and were one of the first types of plastic to be identified as an environmental contaminant (Carpenter & Smith, 1972). The prevalence of pellets in the literature stems not only from their abundance, but also their relatively uniform morphology which facilitates their use in controlled experiments and comparative assessments.

1.2.5 Environmental compartments for plastics

The environmental fate of plastics can, at a simplistic level, be determined from their densities as compared to natural waters (Table 1.1). The most common polymers are polypropylene (PP), and polyethylene (PE) (Andrady & Neal, 2009), and because of their wide variety of applications, PE, PP, polyvinyl chloride (PVC) and polystyrene (PS) dominate the market, representing 85% of all consumer plastics (McCrum *et al.*, 1997).

Debris composed of positively buoyant plastics such as PE and PP can be expected to be highly mobile, being transported by water currents and wind, until deposited in a location or system from which physical processes cannot move them. In the marine environment, mobility may be inhibited by stranding beyond the typical tidal range of a particular beach during a storm event; or by the slow process of biofouling and subsequent density gain, which causes the plastics to settle out of suspension. The low density of PE and PP has resulted in accumulations in the marine environment globally (Thompson *et al.*, 2004), and debris of this type is evident in remote locations far from industrial discharges or urban areas (Gregory, 1999; Hirai *et al.*, 2011; McDermid & McMullen, 2004; Nakashima *et al.*, 2012).

Negatively buoyant plastics, such as PVC and polyethylene terephthalate (PET) are likely to be immobile if introduced to the environment as a solid piece or fragment, readily settling out of suspension. However, the mobility of these plastics may be enhanced if the debris contains air pockets or is hollow, as seen, for example, in PVC fishing floats (Nakashima *et al.*, 2012) and PET drinks bottles (Nakashima *et al.*, 2011a).

Despite the various mechanisms by which plastics are transported, degraded, fragmented, fouled or deposited, what must be considered is that they are a very persistent material which has not been in existence long enough for reliable estimates of residence times in natural systems to have been determined. Therefore, it can be assumed that all of the plastic released to the natural environment until the present day is still in existence in one form or another (Thompson *et al.*, 2005). Sources, sinks and processes described throughout this introductory chapter are summarised in Figure 1.5.

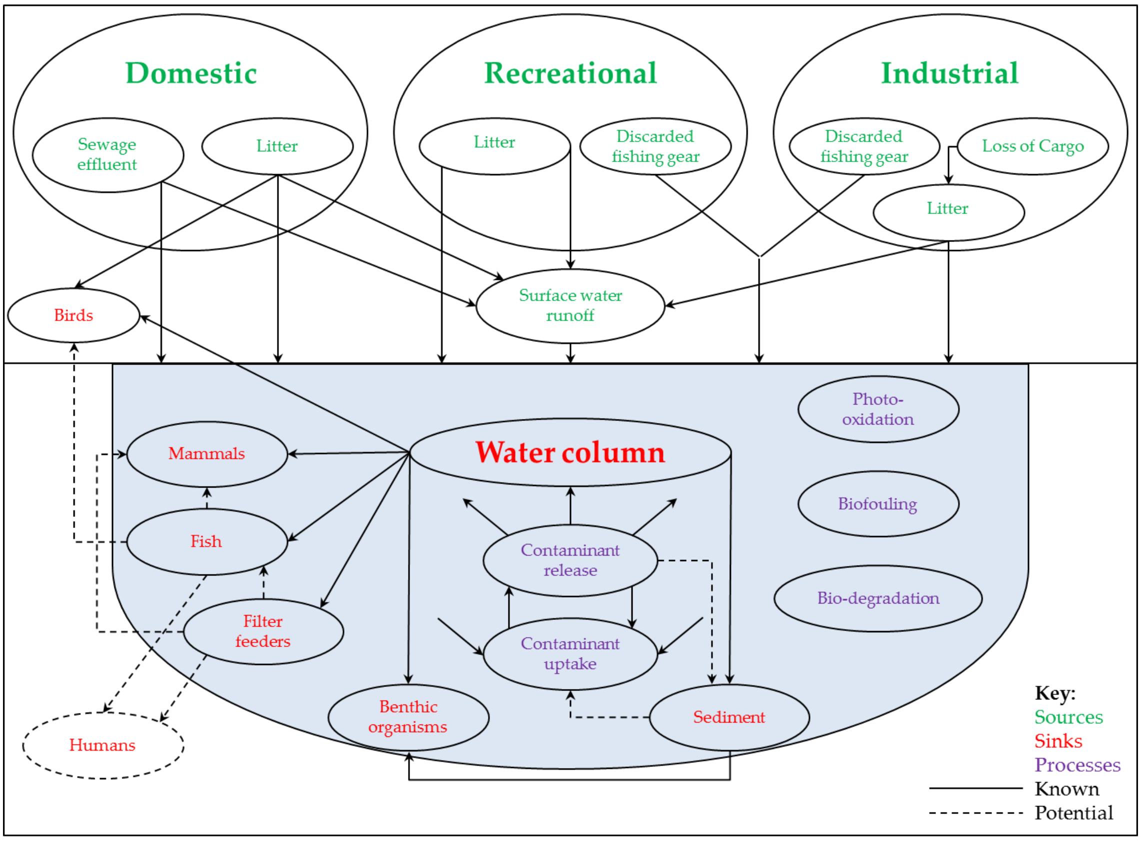

Figure 1.5: Known and potential sources, sinks and processes for plastic debris in the environment. Due to the persistence of plastic in the environment, most sinks must also be considered potential sources since re-suspension of sediment or decomposition of an organism following plastic ingestion will result in release of plastic back into the environment.

1.3 Plastics and co-contaminants

Since pellets were first identified in the natural environment in the 1970s (Carpenter & Smith, 1972; Gregory, 1977), it has been of concern that the plastics carry contaminants of toxicological importance, such as polychlorinated biphenyls (PCBs) (Carpenter *et al.*, 1972; Colton *et al.*, 1974). However, owing to the use of chemicals such as PCBs as plasticizers, it was not clear whether pellets were sequestering PCBs from the surrounding water or contributing them to the marine environment (Gregory, 1978). As interest in plastic pellets grew, investigations focused on the abundance and geographical distribution of plastic pellets (Dixon & Dixon, 1981; Morris, 1980), and it was discovered that the persistence and mobility of plastics had led to accumulations in remote locations and the ingestion of pellets by organisms, such as seabirds (Azzarello & Van Vleet, 1987; Harper & Fowler, 1987). Thus the focus shifted towards the abundance, location and ingestion of plastics, with few studies concerned with the co-contaminants adsorbed to and/or leaching from plastic debris.

Work was then carried out by Mato *et al.* (2001) in order to ascertain whether concentrations of PCBs on pellets could be attributed to adsorption from the environment, or were pre-existent in the pellets owing to the use of PCBs during their manufacture. This study confirmed that due to the hydrophobic nature of the pellets, hydrophobic pollutants such as PCBs accumulate on their surfaces (Mato *et al.*, 2001). During this study, samples of PP pellets were deployed at the sea surface within an industrial complex in Tokyo bay for a period of six days. In this time, PCB concentrations did not reach equilibrium with the surrounding waters, as suggested by their kinetic profiles. Nevertheless, the hydrophobic properties of pellets resulted in accumulations of PCBs on their surfaces comparable with those on natural sediment, despite the differences in specific surface area between natural sediment ($\sim 10^4$ cm^2 g^{-1}) and pellets ($< 10^2$ cm^2 g^{-1}) (Mato *et al.*, 2001).

Further work was carried out by Endo *et al*. (2005), who confirmed the interactions between PCBs and pellets, and Rios *et al.* (2007), who identified interactions between pellets and polycyclic aromatic hydrocarbons (PAHs). Through a series of kinetics experiments, Karapanagioti & Klontza (2008) showed that the adsorption of phenanthrene to pellets was altered by degradation of the polymer in the environment, with pellets increasing in adsorption capacity with increasing polymer weathering. The accumulation of hydrophobic contaminants by plastic pellets is of concern owing to the possibility of ingestion by organisms, discussed further in Section 1.4.

While even recent literature states that plastics may be sources or sinks for persistent organic pollutants (POPs) (Moore, 2008), what seems to have been overlooked thus far, is the potential for metals to accumulate on pellets when present in the environment. It is known that polymers may contain heavy metals as impurities and colourants, as well as organic plasticizers which can cause metal complexation or adsorption (Batley, 1989), but the interactions between dissolved metal concentrations and pellets is yet to be examined.

Metal loss to container surfaces has been noted by various authors in the context of environmental water sampling and subsequent storage (Batley, 1989; Pellenbarg & Church, 1978), and during experimental incubations (Fischer *et al.*, 2007; Giusti *et al.*, 1994; Li *et al.*, 2001). While these observations have not yet prompted studies on plastic debris in the marine environment, it can be seen that there is considerable potential for accumulations of metals by such materials.

Metals of major environmental concern today include cadmium, chromium, cobalt, copper, lead, manganese, mercury, nickel, silver and zinc (Bhattacharyya & Gupta, 2008; Turner & Millward, 2002). Adsorption of these contaminants to natural particulates is effective at very low concentrations (Bhattacharyya & Gupta, 2008), which suggests there may be potential for significant accumulation of such metals on surfaces of pellets, depending on the surface properties of the pellets and the speciation of the metals themselves.

The presence of any charged phases on pellet surfaces may facilitate the adsorption of trace metals, and the development of biofilms on hard substrates in the environment is well documented (Wahl, 1989). Therefore, pellets are likely to present a substrate on which biofilms may develop, with the consequence of providing a reactive surface for the adsorption of trace metals (Petrash *et al*., 2011). Biofilms develop over broad timescales, but initiate almost immediately upon immersion of a substrate in natural waters. Initially, biochemical conditioning and bacterial colonisation occur, with the deposition of proteins and extracellular polymeric substances (Munn, 2004). Once the initial stages of biofouling have occurred, there is further potential for the development of charged sites on the pellet surface. Suspended particulate matter (SPM) of biogenic and inorganic origins are known to become entrained in biofilms (Wesley & Satheesh, 2009), which will add to the surface rugosity of the pellet, as well as introducing localised regions of reactive sites capable of scavenging dissolved metals from the water column. Furthermore, Artham *et al*. (2009) have shown the effects of biofilm development on substrates to include increasing hydrophilicity of the substrate, which would favour immobilisation of trace metals due to the presence of polar groups on the surface. Additionally, biofilms are known to convey a net negative charge to surfaces in aqueous environments (Wahl, 1989).

Many metals are present in the aqueous environment as divalent cations, or are associated with organic or inorganic ligands which will govern the charge of the complex. Therefore negatively charged surface sites will be conducive to the adsorption of aqueous ions, while sites with zero or positive charges may facilitate adsorption of zerovalent or anionic metal species (Du Laing *et al*., 2009).

Complexing of dissolved trace metals by organic ligands stabilises the metals in solution and so can lead to movement throughout the water column (Chester, 2000). As trace metals are actively assimilated or passively adsorbed on to reactive sites of plankton or other biological particles, they ordinarily will be transferred out of surface layers (Chester, 2000). However, when adsorbed onto buoyant pellets, the cycling of these trace elements will be altered, affecting the vertical distribution.

At present, few studies have considered the presence of trace metal concentrations on plastics in the environment. However, recent work by Ashton *et al.* (2010) indicates that pellets may carry concentrations of mineral acid extractable metals ranging from < 2 ng g^{-1} (Cd) to 65 μg g^{-1} (Fe), approaching concentrations present in associated particulate matter. These results are surprising given that compared to natural sediment, the specific surface area of pellets is low and potentially limited in its abundance of reactive sites for the accumulation of metals. However, Nakashima *et al.* (2011b) used x-ray fluorescence analysis (XRF) to determine metal concentrations in marine macroplastic, noting mean concentrations of trace metals orders of magnitude greater than those reported by Ashton *et al.* (2010) which implies that metal concentrations associated with plastics in the environment may be highly variable.

While the surface coatings of pellets will have a major impact on sorptive behaviour, it must also be considered that conditions in the surrounding environment will dictate the behaviour of trace metals. Solution pH will impact sorption owing to hydrogen ions competing with metal ions for adsorption sites, while pH also determines the valence state of the ions and the oxide precipitation process (Quintelas *et al.*, 2009). Additionally, pellets transported through estuaries will be exposed to varying salinities, which will affect the partitioning of trace metals through the complexing and competitive effects of seawater ions on trace metals, affecting speciation, charge and hydrophobicity (Turner & Millward, 2002). At the present time there is a paucity of information on interactions between plastics and any co-contaminants within the estuarine environment.

1.4 Plastic ingestion

Organisms reported to ingest plastics include invertebrates (Graham & Thompson, 2009), fish (Boerger, *et al*., 2010; Colton *et al*., 1974), and turtles (Müller *et al*., 2011).

Plastic ingestion by seabirds has been well documented (Avery-Gomm *et al*., 2012; Colabuono *et al*., 2009; Furness, 1985; Robards *et al*., 1997; Ryan, 2008; van Franeker, 1985; van Franeker *et al*., 2011) and has provided data which suggests that the incidence of plastic ingestion is increasing in line with the production of plastics (Vlietstra & Parga, 2002) which raises concerns that the same could be occurring in other organisms. Data collected by Auman *et al.* (1997) state that 98% of 134 necropsied Laysan albatross chicks had plastic in their digestive tract, an increase from 74% in the 1966, which may reflect increasing concentrations of plastic in the surface waters of the north central Pacific Ocean. This is not an issue limited to one species of seabird, with 44% of all seabird species shown to ingest floating plastic while feeding on or near the surface (Rios *et al.*, 2007).

Ingestion of pellets by organisms may have varied effects. In terms of the physical impact of the plastic, some birds found to have plastic pellets in their gizzard were shown to not have fresh food in the proventriculus, suggesting suppressed feeding activity. Additionally, the pellets may begin to form a blockage preventing the movement of food through the digestive tract (Furness, 1985).

Perhaps more difficult to measure than the physical impacts of ingesting plastic – reduced apetite and altered foraging behaviour – are the possible chemical impacts. Plastics have been shown to accumulate hydrophobic contaminants such as PCBs (Mato *et al.*, 2001) which may become desorbed within the digestive system, making available significant concentrations of compounds known to impair reproductive efficiency of many birds (Barron *et al.*, 1995; Chen *et al.*, 2009). It is not only large vertebrates which are capable of ingesting pellets, as shown by Carpenter *et al.* (1972), who demonstrated selective feeding on PS pellets in 8 out of 14 fish species studied, and in recent research by Graham & Thompson (2009), where sea cucumbers were shown to ingest plastic fragments including PVC pellets 4 mm in size.

In a study of planktivorous fishes in the North Pacific Central Gyre, Boerger *et al*. (2010) observed that plastic ingestion was occurring in 35 % of mesopelagic fish caught, ranging in frequency from 1 to 83 pieces of plastic in fish up to 7 cm in length, and that the most abundant type of plastic ingested were fragments in the size range 1 – 2.8 mm. These low trophic level organisms have the potential to bio-accumulate toxic compounds, allowing bio-magnification within the ecosystem to which they belong (Graham & Thompson, 2009).

A broad spectrum of organisms from various trophic levels have therefore exhibited ingestion of plastic both under controlled conditions and in their natural environment (See Figure 1.6). This could potentially have impacts upon entire ecosystems as marine invertebrates often occupy a low trophic level, and may cause a biomagnification effect of any contaminants associated with pellets, as well as an accumulation of the pellets themselves.

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Figure 1.6: Clockwise from top left: Typical plastics found in the stomach of the Northern Fulmar (Ryan *et al.*, 2009); Plastic fragments found in a rainbow runner (Algalita Marine Research Institute, 2008); Albatross chick carcass decomposing to leave plastic stomach contents (Jordan, 2009); Green turtle stomach containing ingested plastic debris (Stahelin, 2012).

Top predators such as seabirds are likely to be exposed to high dietary levels of POPs from prey due to biomagnification, and the possibility of exacerbating this problem by directly aqcuiring POPs from plastics is of concern (Colabuono *et al.*, 2010). A positive correlation between mass of ingested plastic and PCB concentrations in Great Shearwaters, *Puffinus gravis* indicates this is of concern (Mato *et al.*, 2001).

While there has been some focus on this issue with regard to POPs there remains the possibility that plastics will also contribute significant metal loads to organisms ingesting them. It is therefore a priority to understand whether this is the case, particularly when some organisms have been shown to not only accidentally ingest, but also selectively feed on floating pellets (Mato *et al.*, 2001). As resin pellets have been shown to reflect regional differences in contaminant concentrations (Ogata *et al.*, 2009) it is of particular concern where plastics are found in the vicinity of industrial centres or areas of high population. It is highly likely that plastics ingested by organisms such as birds will release surface-bound metals due to the chemical and physical conditions typical of an avian gizzard.

1.5 Context of the present study

While research has been carried out to determine the interactions between plastics and co-contaminants, there are still large areas for which little work has been undertaken. It is the purpose of the present research to address some of these areas.

Despite experimental losses of trace metals being documented in plastic containers during controlled experiments and environmental sample collections, there has been very little research carried out on the propensity of plastic debris to interact with metals in the environment. This study aims to determine whether metals are interacting with plastics in the environment, and if so, identify what the drivers of these interactions are. Furthermore, it is the intention of this thesis to draw attention to the lack of studies concerned with the interactions between plastics and co-contaminants in estuarine systems. Finally, the question of whether trace metal concentrations associated with pellets be accessible to organisms upon ingestion will be investigated.

Owing to their relatively standard morphology, environmental abundance and recognition within the current literature with regard to interactions with co-contaminants (Heskett *et* al., 2012; Mato *et* al., 2001; Ogata *et* al., 2009), plastic production pellets have been selected as a model plastic particle for the experiments which follow. However, the results have implications for plastic debris on all scales, from macroplastics through to the smallest of microplastics. The accumulation of metals on plastics is currently not well documented, and plastics are still considered to be a newly emerging, and long-lasting contaminant of natural systems. Therefore the importance of understanding how plastic particles may alter metal cycling and bioaccessibility to all trophic levels remains to be seen.

With this in mind, the focus initially will be on the interactions between plastics and trace metals in the marine environment – the scenario where plastic debris has been most widely studied. Following a characterisation of plastic pellets in terms of their composition and metal concentrations, a batch approach will be used to investigate plastic-metal interactions under marine conditions. This is followed by an investigation replicating the estuarine environment to elucidate how these varied and complex conditions may control plastic-metal interactions. This constitutes the first study of any co-contaminants' interactions with plastic debris within an estuarine context. Finally, the bioaccessibility of metal concentrations present on pellets will be investigated with an *in vitro* investigation, mimicking the gastric conditions of avian species which has been widely shown to ingest plastics from the sea surface.

1.6 Aims and Objectives

The aims of this thesis are to elucidate interactions between plastic pellets and trace metals in the environment, and determine whether there is a possible threat to ecosystems owing to the presence of metals on plastics. Thus the objectives may be summarised as follows:

1) Characterise pellets to determine predominant polymer types.

2) Determine metal concentrations on pellets collected from various locations.

3) Identify the extent, rate and mechanisms of uptake from the environment to pellets under marine conditions using a batch approach.
4) Consider estuarine controls on metal uptake by pellets by synthesising an estuarine gradient for use in further batch experiments.
5) Quantify likely metal doses to organisms proven to ingest plastic by application of an *in vitro* model for simulating the avian digestive tract.

The chapters which follow begin with a brief introduction to the general methods and approaches employed during experimental work (Chapter 2). Chapter 3 summarises the characteristics of the plastic pellets collected from various sources and used subsequently in experimental work, addressing objectives 1 and 2. Chapters 4 & 5 outline interactions between trace metals and plastic production pellets under differing conditions in marine, estuarine and freshwater environments, according to the requirements of objectives 3 and 4. Chapter 6 examines the bioaccessibility of metals present on beached plastic pellets to birds, as determined using a physiologically based extraction test to fulfil objective 5. Finally, Chapter 7 offers a synthesis of the data presented in the preceding chapters and identifies future research directions.

Chapter 2:

General methods & approaches

2.1 Introduction

Plastic particles present as debris in the environment are a poorly studied medium compared to natural suspended particles, and for this reason it was necessary to identify suitable methods to characterise the surface properties and metal accumulations on plastic production pellets. It is the purpose of this chapter to outline the various analytical procedures and experimental approaches utilised in the experimental work which follows, and to give rationale for the methods and approaches employed throughout. Each experimental chapter includes a summary of methods with specific details of any alterations or additions to the standard procedures.

2.2 Materials, reagents and cleaning protocols

During all experimental work, attention was paid to maintaining high levels of cleanliness in order to minimise contamination within samples, standards and blanks, thereby generating reproducible results. Furthermore, all work was carried out within an ISO 9001:2008 accredited facility, assuring a high standard of monitoring and maintenance of equipment.

2.2.1 Reagents

Reagents used throughout all methodologies were of analytical grade or better. Specifically, nitric acid and hydrochloric acid used for acidification of samples and extraction procedures were of trace analysis grade. All reagents were supplied by Fisher Scientific (Loughborough, UK) or Sigma Aldrich (Cambridge, UK) and were used exclusively by the author to prevent mishandling by other workers. All reagent solutions prepared from salts were of analytical grade, and were dissolved in high purity (18.2 MΩ) water from a Millipore Milli-Q system (MQW). Prior to any work involving reagents, control of substances hazardous to health (COSHH) paperwork was completed so any personal protective equipment requirements and specific handling instructions were observed.

2.2.2 Materials and cleaning protocols

When conducting work with any aqueous solutions, there is potential for loss of analytes inherent in the methodology (Batley, 1999; Hoenig, 2001). These losses can be controlled by careful planning, minimisation of the number of unconditioned surfaces encountered by analytes, and selection of appropriate materials. While sample preservation and storage are covered in Section 2.6.3., it is appropriate to describe materials selected for experimental work alongside the standard cleaning protocols.

The selection of appropriate materials for bottles, flasks, and all other apparatus is vitally important when storing samples for any period of time, owing to the loss of analytes to various containers, as summarised for a selection of metals by Massee *et al*. (1981). Additionally, contamination can be minimised by omission of any equipment containing metal components, instead opting for all-plastic, or plastic coated materials. For sample storage and preparation, borosilicate glassware is inappropriate when conducting work on metals at low concentrations owing to its affinity for metal ions in solution, therefore all experimental work was carried out in plasticware. Polyethylene and Teflon (PTFE) were the predominant materials selected as they are known to have low affinity for metals (Batley & Gardner, 1977; Laxen & Harrison, 1981). Where PE or PTFE equipment was not available, polypropylene or polycarbonate (PC) equipment was used. When necessary, equipment was fabricated in order to eliminate the use of any metal components.

Prior to use, all equipment was carefully washed using a standard protocol to remove manufacturing residues, or, if equipment was being re-used, to remove contaminants or dust accumulated during storage. Thus, all items were rinsed thrice with deionised water (DIW), available to the laboratory on demand from a central reverse osmosis system. Items were subsequently soaked for > 24 h in a 2 % solution of Decon 90, a detergent with non-ionic and anionic surfactants which is widely used throughout analytical laboratories as a means to remove loosely adhered particles and organic material from surfaces; following removal from the 2 % Decon bath, apparatus was rinsed five times with DIW, and placed directly into a bath containing 1.2 M HCl, where it soaked for > 48 h. Equipment was removed from the HCl bath, rinsed five times with MQW, and placed in clean zip-lock polyethylene bags for transfer to a Bassaire S6 laminar flow cabinet to be dried for a period of 72 h. The laminar flow cabinet was fitted with a high efficiency particulate air (HEPA) filter to remove airborne particulates which may contaminate samples or apparatus. A moderate flow rate was maintained whenever the cabinet was in use, and when dormant, the working space was sealed and positive pressure maintained with a low flow rate to prevent accumulation of airborne particulates within the cabinet.

2.3 Sampling procedures

2.3.1 Water sampling and storage

Water samples were required for incubations, and were collected in bulk from two locations as required, observing clean sampling techniques to ensure they represented the environmental compartment from which they were collected (Kramer 1994). River water was collected in a 10 L high density polyethylene (HDPE) carboy from the River Plym (50.410°N 4.079°W), which is characterised by a granitic moorland catchment, and low concentrations of organic matter (Turner *et al*., 2004). The carboy used for sampling Plym river water was conditioned by rinsing numerous times *in situ* with river water before the final sample was retained for use in experiments. Gloves were worn throughout sampling procedures.

Seawater was collected from station L4 of the Western Channel Observatory (50.250°N 4.217°W) by staff from Plymouth Marine Laboratory aboard RV Plymouth Quest *via* an inboard sampling pump which was conditioned rapidly and continuously by constant circulation of water. Water collected from this location is representative of coastal seawater from the UK. Conditioning of the 10 L HDPE carboy used to collect seawater was achieved by triplicate rinsing before final sample collection.

All water samples were vacuum filtered immediately upon return to the laboratory by passing through a 0.45 μm cellulose nitrate filter (Whatman) housed in a polysulfone vacuum filter unit (Nalgene), and subsequently stored in 1 L HDPE bottles in the dark at 4 °C until use. In order to maintain flow rate, minimise pore size alterations and prevent excessive pressure within the filter unit, filters were changed regularly using plastic tweezers, and the filter unit was conditioned with approximately 100 mL of the sample each time filters were replaced.

2.3.2 Pellet collection and processing

Throughout experimental work, pellets are broadly defined as “virgin” or “beached”, and were obtained from a plastics processing facility or collected from the natural environment, respectively. About 1 kg of virgin polyethylene pellets (n = ~40,000) were obtained from Algram Plastics (Plymouth, UK), as they were required for observations of background levels of metals present immediately upon introduction of pellets to the natural environment, as well as adsorption experiments. Virgin pellets were collected in person from the processing facility, where they had been stored in a zip-lock bag. Upon return to the laboratory, virgin pellets were stored double-bagged in re-sealable bags in the dark until required for use.

Samples of beached plastic resin pellets were collected from field locations for adsorption and desorption experiments as required. Several beaches across the southwest of England were visited (Fig. 2.1), and sites with high concentrations of plastic pellets became the focus of sample collections owing to the speed at which large numbers of pellets could be collected from these sites. The four primary sites, namely Saltram, Ninney Rock, Sharrow Point and Watergate Bay, may be influenced by different sources of pellets and environmental factors affecting local metal concentrations. Saltram lies within the estuary of the River Plym, which drains granitic moorland and is influenced by historical china-clay and metalliferous mining activities. This site is in close proximity to Plymouth, a city with a population of approximately 260,000 in 2010 (Office for National Statistics, 2010), with local recreational boating and commercial shipping activities. Ninney Rock and Sharrow Point are located within the broader setting of Whitsand Bay, Cornwall. This site is well flushed by tides and local currents as it is exposed to the western English Channel. There is a legacy of dumping of dredged material from the military dockyard located on the River Tamar several miles offshore, an activity which has received attention for its potential to impact Whitsand Bay itself (CEFAS, 2005). Watergate Bay is located on the north Cornish coast, and exposed to the Atlantic Ocean. The closest urban centre is Newquay, with a population of 27,100 as of 2010 (Office for National Statistics, 2010).

Samples were collected using polypropylene tweezers to select individual pellets from the strandline of beaches, and stored in polycarbonate centrifuge tubes as a composite sample for transport to the laboratory. In the laboratory, samples were stripped of loosely adhered debris by sieving through a 1 mm plastic sieve, which retained pellets but allowed extraneous material to pass through. Subsequently, ultrasonication for five minutes in filtered seawater was used to remove firmly adhered particles. Pellets were finally dried under laminar flow and stored in sealed pots contained in re-sealable bags.

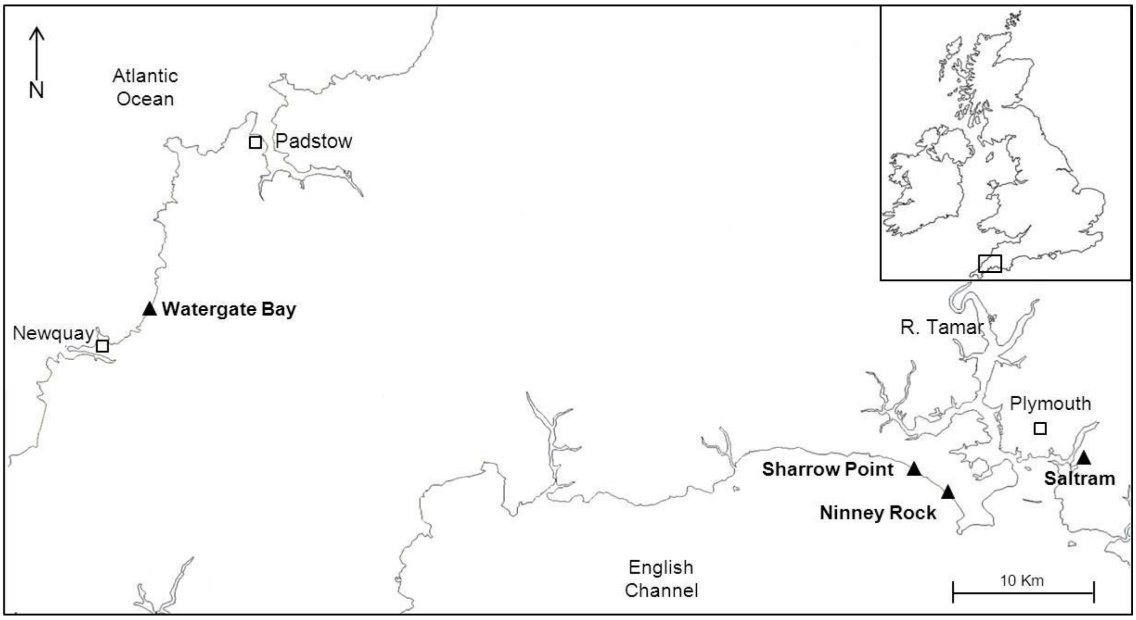

Figure 2.1: Pellet collection sites in southwest England. Sampling locations and nearby urban centres are indicated by ▲and □, respectively.

2.4 Metals selected for study

Metals were studied both *in situ* and in terms of their behaviour during adsorption experiments. Initially a broad suite of metals were selected for analysis, as preliminary work was used for scoping. Metals were selected on the basis that they represent either important geochemical phases (Al, Fe and Mn), or toxicologically important trace metals (Cd, Co, Cr, Cu, Ni, Pb and Zn). In the context of adsorption experiments, only trace metals were studied.

2.5 Analytical techniques

A range of analytical techniques was used throughout experimental work, to characterise physical properties of pellets, and to analyse for metal concentrations (Fig. 2.2). Whenever using equipment for which calibration was possible, including pipettes, balances and analytical instruments, this was performed immediately prior to use of the equipment, according to ISO 9001:2008 protocols.

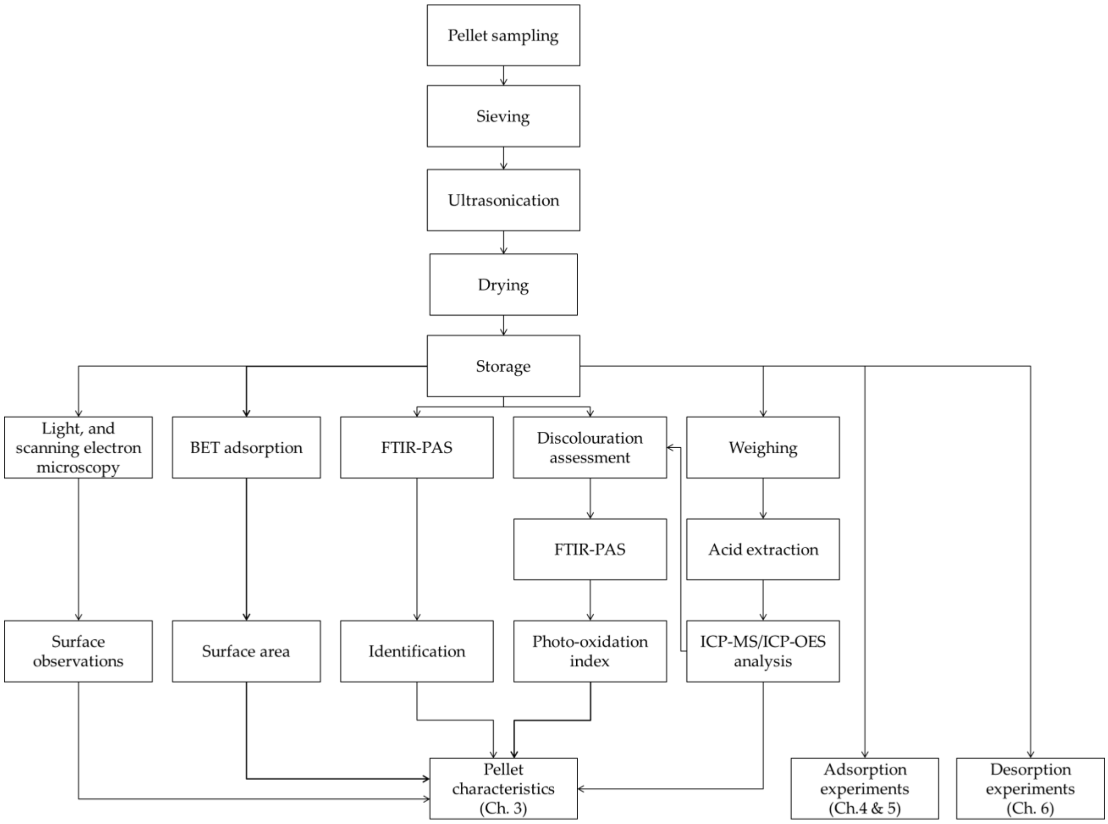

Figure 2.2: Summary flow chart of pellet handling and analytical techniques.

Pellets were analysed for surface area using the BET method of gas adsorption (Brunauer, *et al.*, 1938), while pellets were examined and photographed using both light microscopy and scanning electron microscopy (SEM). Polymer type was determined using Fourier transform infra-red photo-acoustic spectroscopy (FTIR-PAS) as has recently been strongly recommended by Hidalgo-Ruz *et al.* (2012). This technique also served to provide a means to determine the relative age of pellets (See Artham *et al.*, 2009), while metal concentrations were analysed using inductively coupled plasma-optical emission spectroscopy (ICP-OES) or inductively coupled plasma-mass spectrometry (ICP-MS).

2.5.1 Inductively coupled plasma – mass spectrometry

Inductively coupled plasma-mass spectrometry was used extensively for the analysis of trace metals in acid extractions using a Thermo X-Series II instrument (Thermo Elemental, Winsford, UK). Samples were introduced via a concentric glass nebuliser coupled with a conical spray chamber. Acquisition parameters and gas flow rates were constant throughout analyses (Table 2.1) with the exception of nebuliser gas flow, which was manipulated slightly to optimise the system for sensitivity and minimise interferences. Optimisation was carried out using a multi-element tune-up solution (Thermo Scientific) at the beginning of each data acquisition.

Table 2.1: Operating parameters for Thermo X-Series II ICP-MS.

Parameter	**Value**	**Gas**
RF power	1400 W	n/a
Coolant flow	13 L min^{-1}	Argon
Auxiliary flow	0.70 L min^{-1}	Argon
Nebuliser gas flow*	0.75 L min^{-1}	Argon
Collision cell gas flow	3.5 mL min^{-1}	7 % H_2 in He
Dwell time	10 ms	n/a
Sweeps	50	n/a
Replicates	3	n/a

RF: Radio frequency; *Typical value, optimised before each acquisition; n/a: not applicable

Calibration of this instrument was achieved using 5 calibration standards and a calibration blank, with ^{115}In and ^{193}Ir added to all standards, blanks and samples for internal standardisation to compensate for instrumental drift throughout sample runs. Frequent additional checks were carried out to ensure analytical accuracy by regular analysis of a multi-element solution prepared to a known concentration within the calibration range by an independent worker.

An essential requirement of ICP-MS analysis of environmental samples, or solutions with high concentrations of dissolved ions is that matrix effects are minimised. This is achieved by matrix-matching of all calibration standards and blanks to the samples which rely upon them. Matrix matching involves ensuring the bulk solution composition is the same in standards, samples and blanks for each analysis. For example, if samples were composed of diluted seawater, calibration standards and blanks are prepared in seawater of the same dilution. This is necessary to minimise analytical errors pertaining to mass interferences. Additionally, the use of a collision cell, where 7 % H_2 in helium gas is added, further reduces the likelihood of inaccuracies by splitting molecules which are likely to cause spectral interferences. Limits of detection for ICP-MS analysis were calculated from the standard deviation of the calibration blank standards

2.5.2 Inductively coupled plasma – optical emission spectroscopy

Analysis of metals Al, Fe and Mn was achieved by ICP-OES, owing to the greater level of accuracy and precision possible using this technique for these elements at the concentrations extracted from pellets in this investigation. Analysis was carried out using a Varian 725 ES (Mulgrave, Australia) fitted with a V-groove nebuliser coupled with a Sturman-Masters spray chamber. The same method of calibration and matrix-matching was used for ICP-OES as ICP-MS (Section 2.5.1), and similar checks were performed every 10 samples to ensure precision was maintained throughout analysis. Operating conditions for ICP-OES analysis are summarised in Table 2.2. Optimisation of ICP-OES, when necessary, was achieved by altering nebuliser gas flow rates between 0.52 and 0.72 L min^{-1}; or by increasing viewing height by 2-3 mm to improve signal to background ratios.

Table 2.2: Operating parameters for Varian 725 ES ICP-OES.

Parameter	Value	Gas
RF Power	1400 W	n/a
Plasma flow	15 L min^{-1}	Argon
Auxiliary flow	1.5 L min^{-1}	Argon
Nebuliser gas flow	0.68 L min^{-1}	Argon
Viewing height	8 mm ALC^	n/a
Dwell time	1 s	n/a
Replicates	3	n/a

RF: Radio frequency; ^ALC: above load coil

Limits of detection (LODs) were calculated as 3 standard deviations of blank measurements. Typical limits of detection for ICP-MS and ICP-OES analyses are presented in Table 2.3 for the suite of metals investigated. Values presented are from direct measurements of sample concentrations, rather than absolute values following calculations to take into account pre-concentration or dilution steps.

Table 2.3: Limits of detection for ICP-MS and ICP-OES analyses derived from 3 standard deviations of blank concentrations.

	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
ICP-MS LOD, µg L^{-1}	3.7	0.01	0.01	0.17	0.60	4.7	0.20	0.03	0.17	0.51
ICP-OES LOD, µg L^{-1}	19.8	8.25	15.0	6.77	7.71	13.9	1.01	80.1	37.3	9.90

2.6 Statistical analysis

A range of statistical tests were used to determine the significance of relationships and trends in the data. To compare sample mean values, ANOVA was carried out using Minitab 16. Where skewness in the data existed, the non-parametric Kruskal-Wallis test was used to compare median values. This test is a non-parametric alternative to ANOVA, and was carried out using the Statgraphics Centurion statistical package. The Kolmogorov-Smirnov test was performed using Minitab 16 to compare probability distributions – specifically pellet mass distributions. Where multivariate analysis was required to allow comparisons between sites for metal assemblages, multi-dimensional scaling and analysis of similarity (ANOSIM) was performed in Primer 6.

Chapter 3:

Pellet characteristics

3.1 Introduction

Prior to any investigation into the interactions between trace metals and plastic pellets, it was necessary to characterise pellets for pre-existent metal concentrations and surface properties. Additionally, it was desirable to identify suitable sources of pellets which could be used for further experiments. This was achieved using a number of techniques, which have been briefly described in Section 2.5.

It is the purpose of this chapter to describe the key characteristics of pellets used throughout experimental work. As such, the primary objectives were to determine pellet composition (polymer type), assess the efficacy of calculation of the relative age of pellets, describe surface characteristics, identify the geochemical composition of pellets (by analysing for metals: Al, Fe, Mn), obtain baseline trace metal concentrations for pellets, and to determine a suitable field location to source pellets for further experiments.

3.2 Materials & methods

Materials and reagents were selected according to protocols outlined in Section 2.2, and involved selection of plastic apparatus and analytical grade reagents. Prior to use, all apparatus was cleaned according to the method described in Section 2.2. Methods employed to characterise pellets are summarised in Figure 2.2, and described in detail in the following sections.

Statistical methods employed for the comparison of physical and chemical characteristics of pellets include ANOVA and Kolmogorov-Smirnov tests performed with Minitab 16 software. Non-parametric analyses were implemented using Kruskal-

Wallis tests in Statgraphics Centurion, while multivariate analysis was carried out in Primer 6.

3.2.1 Sample collection and treatment

Virgin and beached pellets for characterisation were collected according to Section 2.3. Briefly, virgin pellets were obtained from a plastic manufacturer, while several locations in southwest England were selected for beached pellet collection owing to the abundance of pellets at these sites (Fig. 2.1). Pellets were individually collected with polypropylene tweezers and stored in 50 mL polypropylene centrifuge tubes, while a qualitative assessment of pellet abundance was achieved by recording the time taken to fill one centrifuge tube with pellets on each beach. Concurrently, sediment was collected by scooping with a plastic spatula into centrifuge tubes for analysis of metal concentrations. In the laboratory, sediment was transferred to petri dishes and dried under laminar flow at room temperature. Pellet mass was determined for pellets from each site by weighing 30 individual pellets selected at random from the bulk samples using a Salter ER-182A balance.

3.2.2 Surface area analysis

In order to characterise specific surface area (SSA) of pellets, the BET gas adsorption method was employed, based on the method developed by Brunauer, Emmet and Teller (1938). This method uses the mass of N_2 gas adsorbed to surfaces to determine the porosity and surface area of a solid material. Gas adsorption experiments were performed on a Gemini 2360 surface area analyser (Micromeritics Instrument Corporation, USA), by analysing 1.11 g (n = 42) of pellets (Mettler Toledo XP 504 balance), which filled the sample chamber. Additionally, geometric SSA can be calculated by considering pellets as cylinders or spheres of measured dimensions. However, as the surface porosity of a particle will not be taken into account by this calculation, surface area will be underestimated.

3.2.3 Microscopy

To visually characterise pellet surfaces, a selection representative of the bulk sample of pellets was analysed by light microscopy (LM) and scanning electron microscopy (SEM). Light microscopy was carried out using an Allied Vision Technologies Pike F210C camera (Stemmer Imaging, Surrey, UK) coupled with a Qioptic Optem Zoom 70 XL lens (Göttingen, Germany), and operated using AVT Smartview software. To gain a greater level of detail in surface characteristic mapping, SEM was used. This was achieved by initially coating a dry pellet with a layer of carbon using an Emitech K 450X high vacuum carbon-coating unit (Ashford, UK), and subsequently photographing pellets using a JEOL JSM-6100 (Welwyn, UK) operated at 20 kV and at a working distance of 15 mm.

3.2.4 Polymer identification

Polymers were identified by Fourier transform infra-red photoacoustic spectroscopy (FTIR-PAS). Fourier transform infra-red spectroscopy (FTIR) is widely used for the determination of solid compositions by gaining an absorbance spectrum. For many samples, including plastics, standard methods of FTIR – transmission or reflection – are not feasible, owing to the sample transmitting insufficient light. Photoacoustic spectra are acquired by sensing absorption induced heating of the sample upon exposure to infra-red wavelengths of energy. The thermal expansion caused by this heating pressurises the surrounding gas environment, and is detected as an acoustic signal by a microphone (McClelland *et al.*, 2002). Spectra were acquired with a Bruker IFS 66 FTIR spectrometer coupled with a MTEC 300 photoacoustic cell which was purged with helium gas, and recorded as an average of 64 scans in the range 4000 to 400 cm^{-1} at a resolution of 8 cm^{-1}. Glassy carbon was used as a reference to give a background scan, and analogue-to-digital converter (ADC) counts were maintained between the range 11,000 and 16,000 using a manually controlled signal amplifier. Once spectra had been obtained, they were compared to a reference library of synthetic polymer spectra for identification, using Bruker OPUS spectroscopic software. Pellets were characterised for polymer type by obtaining individual spectra for 30 randomly selected pellets from each location, plus 30 virgin pellets, which were then compared with a standard polymer spectrum library.

3.2.5 Polymer degradation analysis

In addition to the identification of polymers, FTIR spectroscopy allows the relative age of individual pellets to be identified using a photo-oxidation index. Photo-oxidation caused by solar radiation is the principal means by which polymers are degraded in the environment (Andrady, 1990), and measurement and calculation of a photo-oxidation index has been used to determine the efficacy of polymer additives, such as $CaCO_3$, in retarding the aging process (Valadez-Gonzalez *et al.*, 1999; Yang *et* al., 2005), as well as comparing degradation of polymers under contrasting environmental conditions (Albertsson, *et al.*, 1987; Valadez-González & Veleva, 2004).

To determine the relative age of polyethylene pellets, a photo-oxidation index was calculated from carbonyl and methylene peaks (wavenumbers 1715 and 1465 cm^{-1}, respectively) by integrating peaks in the ranges 1729-1696 cm^{-1} and 1486-1448 cm^{-1}, respectively. The carbonyl index was calculated by dividing the carbonyl signal amplitude by that of the reference methylene peak. According to Artham *et al*. (2009) and Sudhakar *et al*. (2007), the methylene peak is unaltered by photo-oxidative degradation, while the inclusion of oxygen into carbonyl groups during photo-oxidation causes distinct peaks within the range outlined above, and can be compared with the methylene reference peak to obtain the index. For the purposes of aging assessments, five individual pellets from five groups, defined by visual degree of aging (yellowing), were analysed. The groups were categorised as virgin pellets (V), and four groups of beached pellets (white (W), yellow (Y), orange (O) and brown (Br)). Beached pellets were separated into these groups by matching with a reference pellet.

3.2.6 Metal extraction

Metals were extracted from pellet surfaces using a modified aqua regia extraction. Aqua regia was prepared by mixing 12 M HCl and 16 M HNO_3 in a ratio of 3:1, and subsequently diluting to 20 % strength, as documented by Ashton *et al*. (2010) as this preparation was capable of extracting metals with efficiency close to full strength aqua regia. Typical protocols for aqua regia extraction of soils involve a long (up to 16 hour) period of cold digestion followed by heated digestion for two hours (Sastre *et* al., 2002). This approach breaks down complex matrices and releases the associated metal concentrations. Owing to the relatively inert composition of pellets, it was not necessary to heat the digestions; however a longer period was used to allow metals to be removed from pellet surfaces. Individual pellets were added to a 7 mL HDPE vial, to which 2.5 mL of 20 % aqua regia was added. Vials were shaken at 150 rpm for 24 hours on a Stuart SS1 orbital shaker, with vials on their sides to maximise agitation. To ensure precision in extraction and analysis, the same bulk preparation of 20 % aqua regia was used throughout extractions, and for all blanks and calibrations.

Sediments and certified reference materials (CRMs) were digested using a standard procedure, based on the Laboratory of the Government Chemist (LGC) aqua regia soluble metals procedure. Thus, approximately 0.5 g of sediment were weighed directly into digestion tubes, to which 4 mL HCl and 1 mL HNO_3 were added. Tubes were placed in a cold Tecator block and left for pre-digestion for two hours. Following this period of cold digestion, an additional 0.5 mL HNO_3 was added to the digestion tubes and the contents were heated at 150 °C for two hours. Following digestion, the contents were allowed to cool, transferred to 25 mL glass volumetric flasks, and subsequently diluted 100-fold prior to analysis by ICP-MS.

3.2.7 Metal analysis

The metals selected for analysis and the rationale behind these selections have been introduced in Section 2.4. Metals were analysed using either ICP-MS or ICP-OES, techniques which are described in detail in Sections 2.5.1 and 2.5.2, respectively.

3.3 Results

3.3.1 Abundance and distribution

Plastic pellets were present on all beaches visited. Qualitative assessment of distributions suggests elevated abundance at the strandline at each location, commonly exceeding 100 pellets m^{-2}, while large areas exhibited complete absence of pellets. According to the time required to fill a 50 mL centrifuge tube at each site, it was determined that the abundance of pellets at Watergate Bay was the greatest, requiring < 0.5 h to collect 50 mL of pellets (approximately 750 by count), as opposed to > 1.5 hours required at Ninney Rock. At all locations pellets formed the most abundant form of debris by count.

Pellet morphology could be classified as cylindrical, ovoid or oblate spheroid, and were typically 3-5 mm in diameter and 1-3 mm in height. Pellet colour was predominantly white or off-white (discoloured), however, black pellets were abundant (29 %) at Sharrow Point and Ninney Rock. Pellets coloured yellow, purple and blue were also collected but represented only small proportions (1 %, 4 % and 7 %, respectively) of the total number of pellets collected.

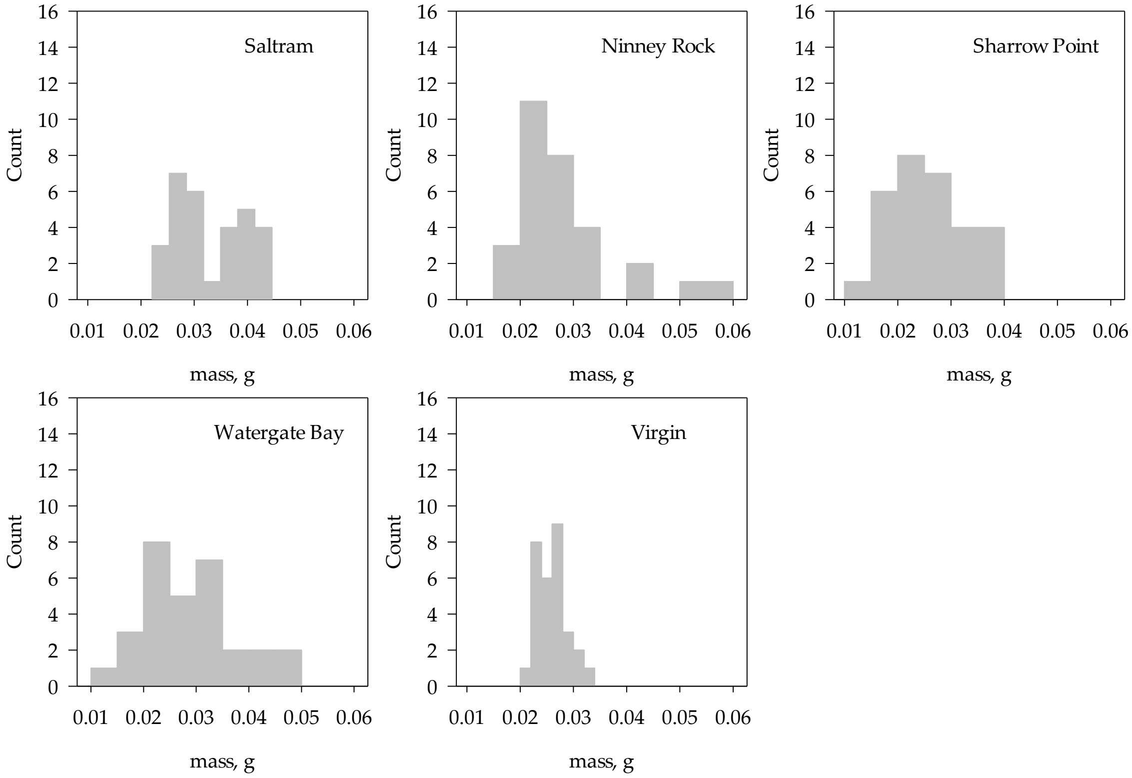

Figure 3.1: Mass distributions of 30 pellets from four field locations, and 30 virgin polyethylene pellets from Algram Plastics.

Pellet mass varied slightly between sites, exhibiting mean masses of between 24 and 29 mg, with differing levels of intra-site heterogeneity (relative standard deviation (RSD) values between 10 and 30 %). Mass distributions of virgin and beached pellets are shown in Figure 3.1, and it is evident that virgin pellets are more homogeneous, in terms of mass, than beached pellets. Comparisons of pellets from each field location with virgin pellets using Kolmogorov-Smirnov tests indicate mass distributions of pellets from each site, except Ninney Rock are significantly different to virgin pellets ($P < 0.05$). This can be attributed to the single source of virgin pellets, as compared to the many possible sources of beached pellets, as well as the effects of degradation and fouling of beached pellets during their time exposed to the marine environment.

3.3.2 Surface characteristics

Figure 3.2 displays a selection of light microscope images which provide a number of observations. Pellets exposed to the marine environment will vary in their surface appearance owing to the progressive degradation of the polymer, and accretion of additional biogenic and inorganic material upon their surfaces.

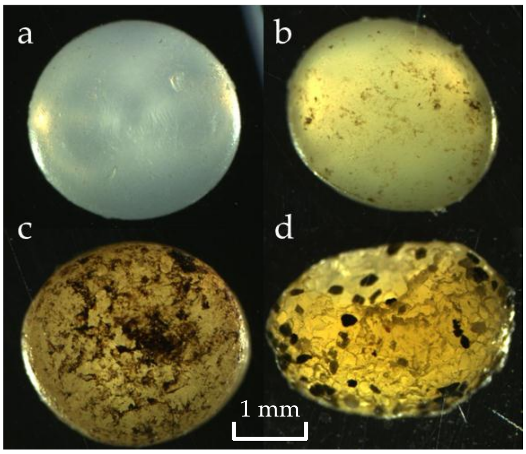

Figure 3.2: Composite light microscope image of beached pellets exhibiting different extents and types of fouling and degradation: (a) relatively pristine beached pellet, with minor surface striations; (b) pellet with minor biofouling present; (c) extensive biofouling; (d) deposits of inorganic material, biofouling and a moderate level of surface degradation, or “crazing”.

Scanning electron microscopy allows a greater level of detail to be examined for virgin and beached pellets. Examples of these two types are exhibited in Figure 3.3. The major conclusions to be drawn from images of this type are that virgin pellets have slight surface roughness and imperfections (Fig. 3.3a), while beached pellets are degraded, exhibiting fissures and striations, and accumulate grains of natural material (Fig 3.3b).

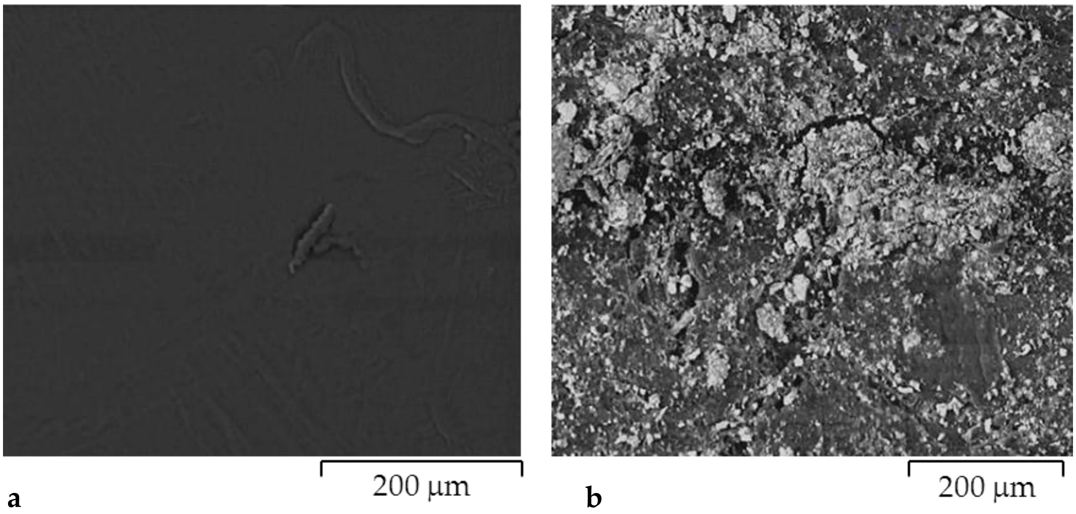

Figure 3.3: SEM images of the surface of a virgin pellet (a) and a beached pellet (b).

Surface area analysis using the BET method was inconclusive, as the pellets analysed had a surface area below the limits of detection for the selected method. Instead, the geometric surface area was calculated. The surface area of a cylinder is given by the formula: $A = 2 \pi r^2 + 2 \pi r h$, where r and h are radius and height of the cylinder, respectively. Thus, a pellet of 3 mm in diameter and 2 mm tall would have a surface area of 33 mm^2, and a mass of approximately 0.025 g, giving a specific surface area of 13.2 cm^2 g^{-1}.

3.3.3 Composition and aging

Determination of polymer type by FTIR-PAS confirmed that all virgin pellets were made of polyethylene, while beached pellets were predominantly polyethylene. A very low abundance of polypropylene pellets were present, representing < 1 % of the total number of pellets collected and analysed. Examples of spectra obtained from virgin and beached pellets are presented in Figure 3.4, with significant peaks, as determined by Bruker OPUS spectroscopy software, annotated with their corresponding wavenumber.

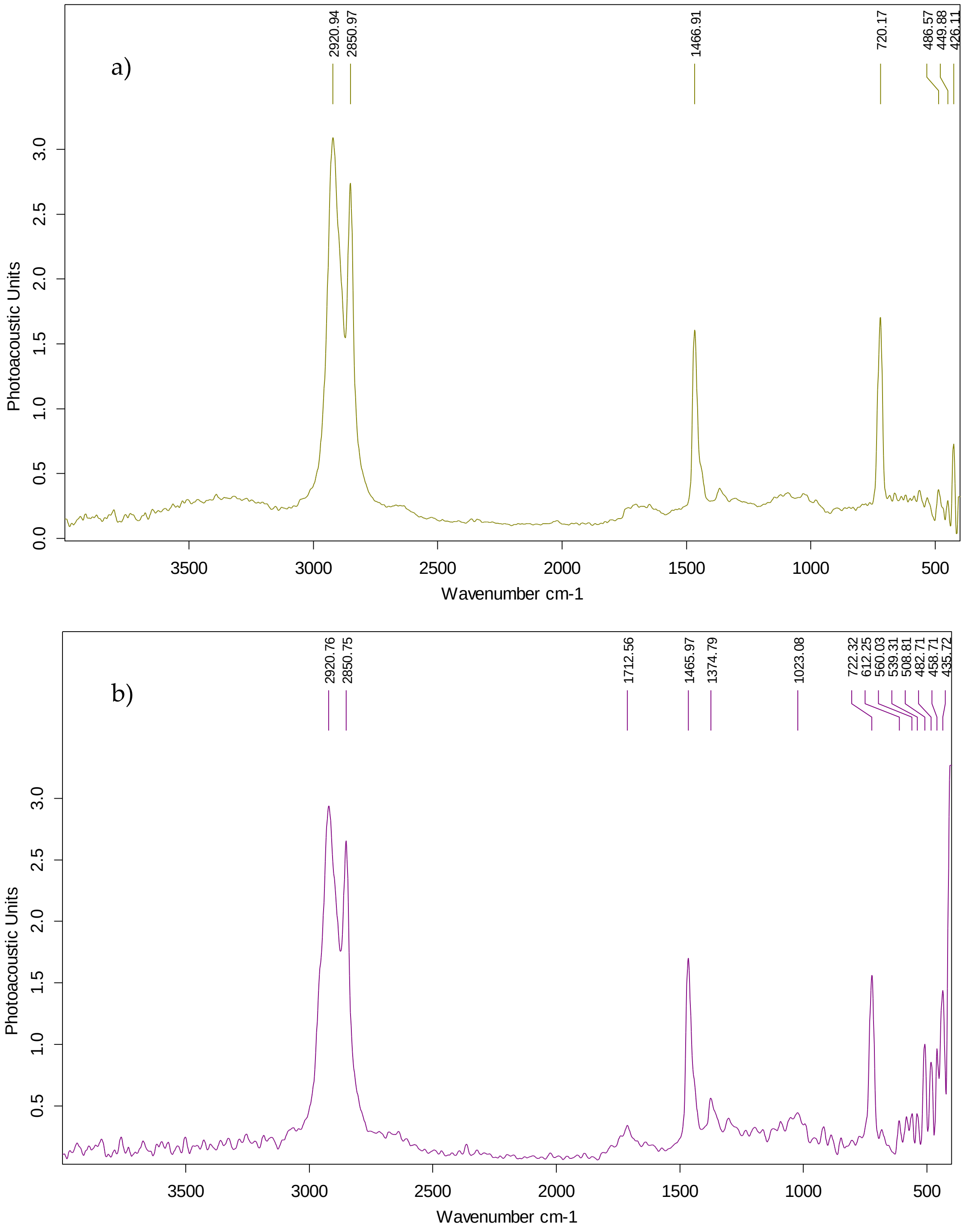

Figure 3.4: Example FTIR-PAS spectra for virgin (a) and beached (b) polyethylene pellets, with significant peaks annotated automatically by OPUS software. Thirty spectra were obtained for individual pellets from each field location and for virgin pellets..

Spectra obtained from pellets of polyethylene construction, when compared to a standard library of polymer types, exhibited the characteristic indicators - asymmetric and symmetric C-H stretching at 2921 and 2852 cm^{-1}, respectively, and methylene scissoring and rocking vibrations at 1460 cm^{-1} and 722 cm^{-1}, respectively.

From the wavenumbers annotated on Figure 3.4, it can be seen that the beached pellet from which this example originates has developed carbonyl functional groups, indicative of photo-oxidation (indicated by the presence of a peak at 1713 cm^{-1}). However, comparison to a reference peak is necessary in order to establish the extent of photo-oxidation. Using the photo-oxidation index calculation described in Section 3.2.5, the degree of degradation was determined and compared to pellet colour, as it has been stated that the degree of “yellowing” (Endo *et al.*, 2005) will be dependent on the degree of degradation. The carbonyl index for several categories of pellets is presented in Figure 3.5 and demonstrates how pellets are altered in appearance with the degree of photo-oxidation, an effect which seems to increase in the order: Virgin = White < Yellow < Orange < Brown. Analysis of photo-oxidation index data using ANOVA, however, identifies three significantly different groups (as annotated on Fig. 3.5). Thus discolouration yields only an approximate measure of the photo-oxidation of pellets.

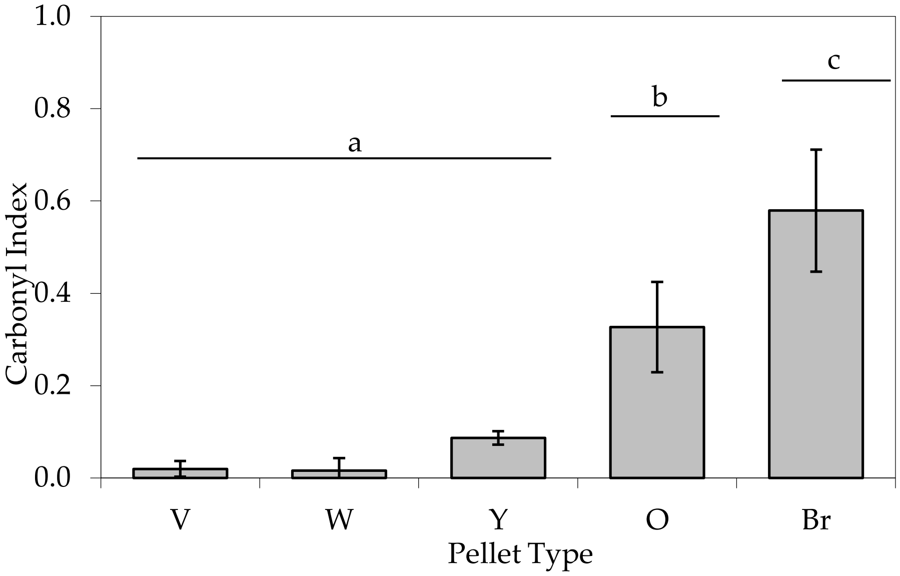

Figure 3.5: Carbonyl index for virgin pellets (V), and beached pellets divided into colour categories: white; yellow; orange, and brown (W; Y; O, and Br, respectively). Mean values for five replicates are presented ± 1 SD. Homogeneous groups, as determined by ANOVA, are annotated above each column.

3.3.4 Metal concentrations

Analysis of CRM highlights a reasonable degree of accuracy (Table 3.1), with all elements present at concentrations within the uncertainty of certified values. Furthermore, precision of sample processing and analysis is acceptable, with RSD values ranging from 2.6 % to 8.5 % (Al and Cu, respectively).

Table 3.1: Certified and measured aqua regia extractable metals in LGC6156 CRM (harbour sediment) determined using ICP-MS and ICP-OES (n=3).

	Metal concentration, µg g^{-1}									
	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Certified	19000 ±3700	2.9 ±0.5	28.3 ±2.8	111 ±15	2400 ±122	72000 ±5200	553 ±27	161 ±13	1685 ±140	3530 ±195
Measured	21400 ±550	3.0 ±0.813	34.9 ±2.41	117 ±6.54	2610 ±220	79800 ±3110	573 ±34.5	167 ±13.1	1830 ±87.6	3680 ±312

3.3.4.1 *Sediment metal concentrations*

Values for aqua regia extractable metals from sediment collected alongside pellets are presented in Table 3.2, and indicate inter-site variability, with Saltram presenting the lowest concentrations of metals. This is not an expected result owing to the alluvial inputs to this sampling site from the River Plym, however as sediments were analysed as whole sediment, differences in particle size distributions between sites which may account for some disparity between results. This assertion is supported by the comparatively low Al concentrations at Saltram since low Al content is consistent with low clay content and coarse mean grain size.

Table 3.2: Summary of concentrations of hydrogenous metals and trace metals in whole sediment sampled from four locations in south west England. SD represents 1 standard deviation of three individual samples from each location.

	Al μg g^{-1}	Cd μg g^{-1}	Co μg g^{-1}	Cr μg g^{-1}	Cu μg g^{-1}	Fe μg g^{-1}	Mn μg g^{-1}	Ni μg g^{-1}	Pb μg g^{-1}	Zn μg g^{-1}
Saltram										
Mean	1880	0.213_a	1.01	0.856	2.69	2690	57.6	nd	11.7	14.8
SD	111		0.365	0.138	0.551	440	3.43	nd	6.48	1.35
Ninney Rock										
Mean	8740	1.08	8.22	17.2	4.72	19300	423	34.2	78.9	44.3
SD	257	0.054	0.386	0.0301	0.774	640	22.3	0.240	6.29	1.80
Sharrow Point										
Mean	8510	0.925	7.89	16.4	4.81	18300	390	30.7	65.0	43.3
SD	428	0.139	0.438	0.968	0.326	810	18.0	0.745	7.95	2.14
Watergate Bay										
Mean	4970	1.34	6.61	11.1	9.89	14400	402	27.8	96.4	47.0
SD	60.8	0.091	0.326	0.145	1.48	745	13.3	1.55	5.79	1.58

SD: Standard deviation; nd: not detected; a : value from one replicate as other replicates < LOD

Additionally, sediment collected from Saltram was collected in the vicinity of the greatest concentration of plastic pellets, which was above the typical extent of tidal inundation, therefore the sediment collected may have been partially derived from weathered rock and organic matter as opposed to deposition of alluvium. Typical values for metal concentrations in the Plym more closely resemble those observed in the other sediment types, with Al and Fe concentrations of approximately 8,700 and 20,000 μg g^{-1}, respectively (Turner *et al*., 2010). Contrastingly, Turner (2010) observes Al, Fe and Mn concentrations in sediment from this location to be 6200, 8500 and 140 μg g^{-1} respectively, indicating a degree of variability is inherent in this system.

3.3.4.2 Plastic-associated metal concentrations

Acid extractions of metals from pellets collected from the coast of southwest England provide data which contribute to baseline observations of metals associated with plastic pellets (See Ashton, *et al*., 2010; Turner & Holmes, 2011). Concentrations of Al, Fe and Mn on pellets from the four locations described in Section 2.3 are presented in Figure 3.6, while trace metal concentrations are presented in Figure 3.7. Initial observations prior to statistical analysis suggest intra- and inter-site variability exist for all metals, with some underlying trends which may be explained by the characteristics of the environment from which pellets have been collected.

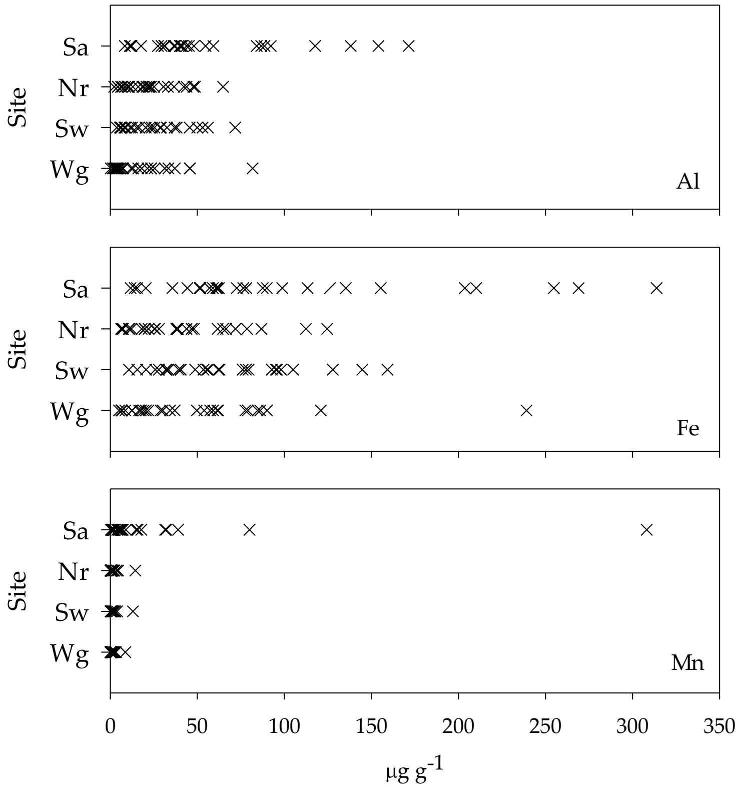

Figure 3.6: Aluminium, iron and manganese concentrations extracted from beached pellets collected from sites in southwest England (Sa: Saltram; Nr: Ninney Rock; Sw: Sharrow Point; Wg: Watergate Bay). Each point indicates a single pellet extraction.

Elevated levels of Fe, Al and Mn exist on pellets from Saltram, as compared to the other three sites, which generally present similar concentrations of these metals. As stated in Section 2.3, Saltram is situated within a tidal estuary and is therefore likely to be influenced by fluvial depositions which will alter the local metal concentrations. Additionally, the estuary is heavily influenced by urban and industrial land-use (Langston *et* al., 2003), including a landfill site and commercial shipping activities in close proximity to the sampling site. Pellets collected from Saltram may have been influenced by the greater metal concentrations typical of the intertidal zone of estuaries, rather than the lower metal concentrations found at the upper shoreline (Table 3.2), as they were presumably deposited from the water column.

Trace metal concentrations (Figure 3.7) exhibit similar degrees of intra-site variability to Fe, Al and Mn, but inter-site comparison does not reveal the same elevation at Saltram for all trace metals. For Co and Ni, elevation at Saltram exists, while elevation of Cd, Cr, Cu, Pb and Zn is apparent at Ninney Rock and Sharrow Point. Generally, pellets from Watergate Bay exhibit the lowest concentrations of all metals. This may be attributed to the absence of industrial and urban development in the vicinity of the site, a greater degree flushing of the coastline exposed to the Atlantic Ocean, the absence of major rivers in the immediate vicinity, or a combination of these factors. However, this contradicts the background metal concentration data obtained from sediments collected alongside pellets (Table 3.2), suggesting that pellets accumulate metals in a manner which allows them to differ from their surrounding environment. Additionally, metals in sediments are subject to grain size variability, therefore lower values for metals on sediment may be indicative of larger grain size, and vice versa.

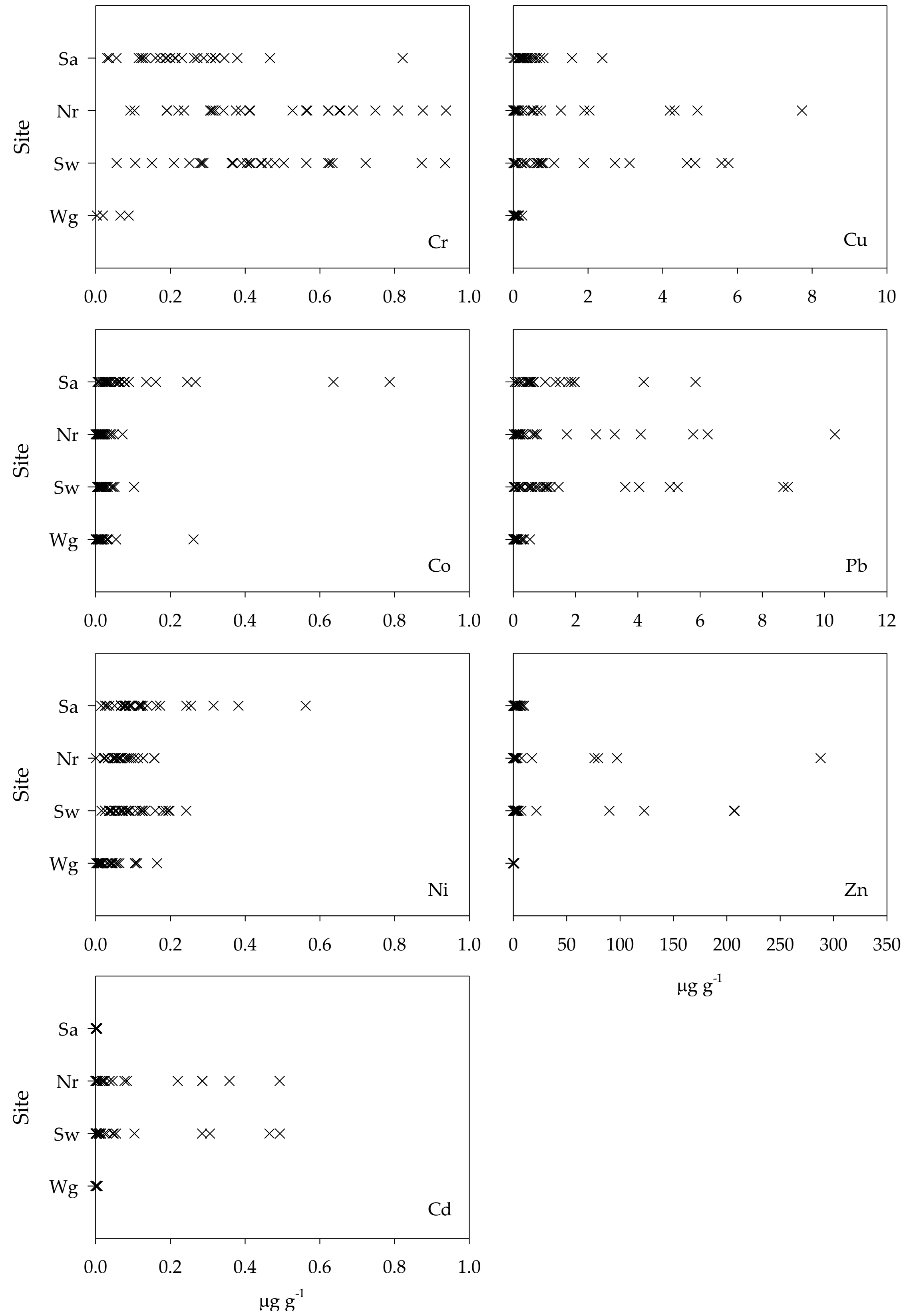

Figure 3.7: Metal concentrations on pellets from four sites: Saltram, Ninney Rock, Sharrow Point and Watergate Bay. Each point represents a single pellet extraction.

Table 3.3 summarises the concentrations of metals extracted from beached pellets. Metal concentrations on virgin pellets were below limits of detection, with the exception of Cu (mean Cu on virgin pellets: 20 ng g^{-1}) and are therefore omitted from Table 3.6. Owing to the high degree of intra-site variability, which can be examined by observing mean and standard deviation values, it is valuable to use the median value to interpret the typical metal concentrations extracted from pellets. Kruskal-Wallis tests were performed using the statistical package Statgraphics Centurion to identify inter-site differences. Statistical analysis shows that differences in metal distributions as illustrated in Figures 3.6 and 3.7 are not all significant. The greatest concentrations of Al, Fe and Mn, as well as Co and Ni are found on pellets from Saltram, Sharrow Point contains pellets with the greatest concentrations of Cu, Zn, and Pb, while Ninney Rock presents the location with pellets associated with the greatest concentrations of Cd.

Overall, there exists a large degree of variability between pellets for all trace metals, with some metal concentrations approaching values comparable with natural sediment, particularly if values are normalised for surface area. This is an unexpected finding, as the common assumption of plastics being relatively inert towards dissolved metal concentrations appears to no longer be valid once plastics have been exposed to the natural environment. Metal concentrations were correlated with the photo-oxidation index (Section 3.3.3) to determine whether the degree of ageing could be used as a predictor for metal concentrations present on pellet surfaces; however, no significant relationship existed between these two parameters for the pellets examined in this study.

Table 3.3: Summary of concentrations of hydrogenous metals and trace metals in pellets sampled from four locations in south west England (n = 30 in each case).

	Al	Fe	Mn	Cu	Zn	Pb	Cr	Co	Ni	Cd
	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	ng g^{-1}	ng g^{-1}	ng g^{-1}	ng g^{-1}
Saltram										
Mean	55.8	97.8	20.5	0.441	2.68	1.02	237	107	131	1.65
SD	42.7	79.1	56.8	0.470	2.40	1.24	159	177	117	1.19
Median	41.0	67.5	5.01	0.283	1.63	0.544	212	44.9	91.1	1.17
Minimum	8.33	11.7	0.35	0.012	0.051	0.052	nd	nd	14.9	0.208
Maximum	171	314	308	2.38	10.0	5.85	821	787	562	4.33
Ninney Rock										
Mean	22.3	41.9	1.51	1.11	21.0	1.34	751	17.7	69.8	76.7
SD	15.7	30.5	2.68	1.90	58.5	2.39	142	15.8	40.5	134
Median	20.8	38.3	0.760	0.265	0.818	0.269	413	14.7	62.8	14.9
Minimum	nd	6.21	0.075	nd	nd	0.0232	nd	nd	nd	nd
Maximum	64.8	125	14.4	7.73	288	10.3	7970	71.8	157	492
Sharrow Point										
Mean	23.0	64.6	1.51	1.32	23.3	1.64	430	20.7	95.1	74.4
SD	18.2	38.9	2.39	1.80	56.7	2.40	210	19.8	59.3	143
Median	18.4	55.8	0.669	0.652	1.99	0.74	408	13.9	81.3	9.61
Minimum	3.77	nd	0.193	nd	0.065	0.016	nd	4.43	15.5	nd
Maximum	71.8	159	13.0	5.76	207	8.82	935	103	242	493
Watergate Bay										
Mean	16.9	48.5	1.16	0.064	0.299	0.149	44.0	22.5	40.0	1.09
SD	18.4	48.2	1.62	0.0618	0.292	0.181	39.5	47.7	40.4	1.39
Median	7.43	34.4	0.712	0.047	0.196	0.109	42.5	13.8	29.3	0.523
Minimum	nd	nd	0.079	nd	nd	nd	nd	nd	nd	nd
Maximum	81.8	239	8.66	0.239	1.04	0.885	88.0	262	164	5.36

SD: Standard deviation derived from 30 individually extracted pellets from each location; nd: not detected.

Correlation of trace metals with Al, Fe and Mn across all samples (n = 120) indicates strong ($r > 0.7$) and significant relationships ($P < 0.001$) exist between Al, Fe, Mn and Co & Ni. While some relationships are strong, as is the case of Ni and Al, with a correlation coefficient of 0.9 ($P = < 0.001$), for other metals, relationships may be significant, yet very weak. For example the relationship between Fe and Pb has a coefficient of 0.358 (*P* = < 0.001). Similarly, Cu has a significant correlation with Al (*P* = 0.002), yet a correlation coefficient of 0.283 suggests a very weak correlation. These relationships may be driven or skewed by occasional very high metal concentrations. Zinc, Cd and Cr exhibit no correlation with Al, Fe or Mn at statistically significant levels. It is anticipated that trace metals will be associated with Al, Fe and Mn owing to their high surface area and known reactivity towards dissolved metals which facilitate adsorption. Where no correlation exists it may be due to heterogeneity in adsorption surfaces.

Multivariate analysis was used to identify whether differences exist between pellets from the four sites with regard to their metal assemblages. Multidimensional scaling (MDS) was performed in Primer 6 and the 2-dimensional graphical output of this test is presented in Figure 3.8

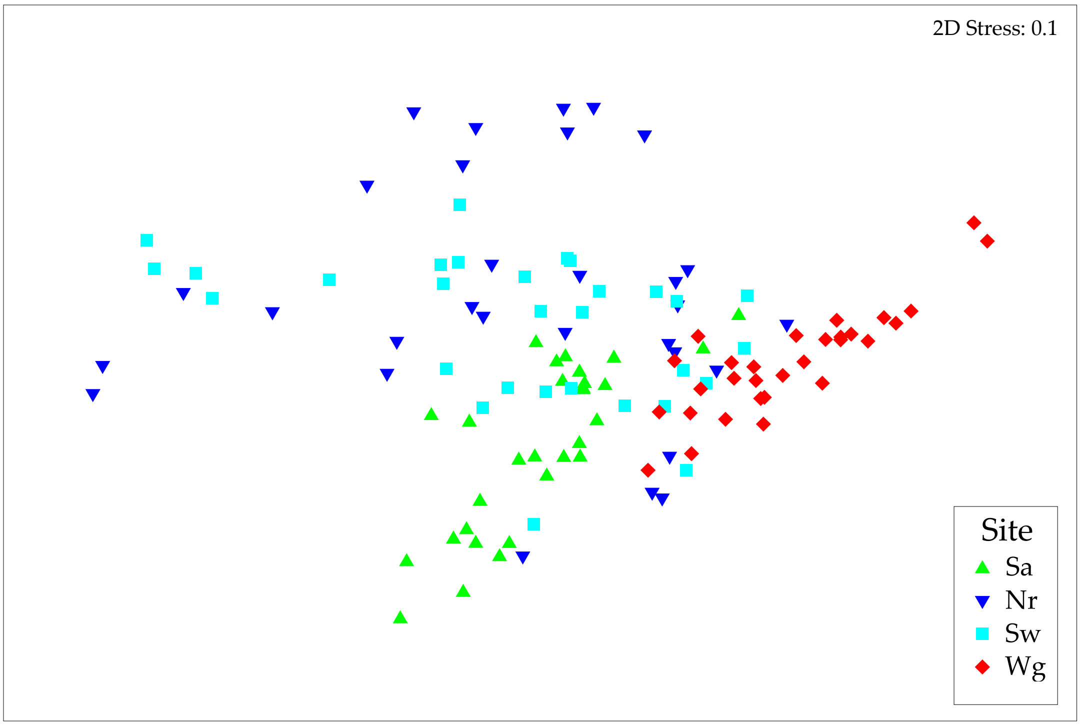

Figure 3.8: Multidimensional scaling plot to compare metal assemblages on pellets from the four sites.

The relationships shown in Figure 3.8 highlights the similarities between pellets from Nr and Sw, while Sa and Wg appear to form two discrete groups. To further analyse the relationships seen in Figure 3.8, analysis of similarity (ANOSIM) was employed to test whether metal assemblages on pellets from these sites were significantly different. This test confirms the similarity between Nr and Sw ($P = < 0.2$) while all other pairwise comparisons reflect no similarity ($P = < 0.001$). Results of pairwise comparisons are summarised in Table 3.4.

Table 3.4: Test statistic (R) and significance level (*P*) for pairwise ANOSIM comparisons of metal assemblages on pellets from four sites.

Pairwise comparison	R	*P*
Nr – Sw	0.012	> 0.2
Sa – Sw	0.230	< 0.001
Sa – Nr	0.241	< 0.001
Nr – Wg	0.430	< 0.001
Sw – Wg	0.479	< 0.001
Sa – Wg	0.689	< 0.001

Trace metal enrichment can be investigated by normalising to Al concentrations derived from sediment collected alongside pellets, to identify whether pellets are enriched with regard to any particular metals. The unitless enrichment factor (EF) for metals is calculated using the equation:

$$EF = ([Me]_P/[Al]_P) / ([Me]_s/[Al]_s) \quad \text{Eq. 3.1}$$

where $[Me]_P$ and $[Me]_s$ are metal concentrations (w/w) extracted from plastic pellets and sediment, respectively, and aluminium is used to normalise for particle size. Enrichment factors are summarised in Table 3.5.

Table 3.5: Metal enrichment (unitless) on pellets collected from SW England, normalised for Al using Eq. 3.1.

Location	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Saltram									
Mean	0.0791	1.22	12.6	5.78	1.20	7.96	3.73	2.92	6.75
SD	0.0989	1.18	11.9	2.98	0.247	12.8	1.11	2.54	4.83
Ninney Rock									
Mean	5.22	0.400	48.5	79.0	0.918	1.25	0.852	5.57	110
SD	8.52	0.591	171	111	0.399	2.53	1.05	6.94	261
Sharrow Point									
Mean	5.82	0.441	16.6	134	1.53	1.66	1.61	11.0	184
SD	11.6	0.206	16.6	193	1.15	1.76	1.35	14.1	486
Watergate Bay									
Mean	0.0964	0.420	0.324	3.55	1.31	1.19	0.467	0.641	2.29
SD	0.357	0.476	1.59	11.4	1.40	1.36	0.617	0.783	5.41

SD: Standard deviation

From the values presented in Table 3.5, it is clear that enrichment of several elements exists on plastic pellets. Variability is very high, as may be expected from the disparate metal concentrations presented in Table 3.3, particularly in the case of Cu and Zn. Such widely spread metal concentrations suggest the presence of a small number of pellets containing elevated metal concentrations alongside an abundance of pellets with lower metal concentrations. Following normalisation, it is possible to identify Watergate Bay as a site in which metals are not enriched on pellets to the same extent as in other locations.

3.4 Discussion

The various approaches aimed at characterising pellets collected from a number of sources have given a broad picture of the surface properties and metal concentrations likely to be encountered on pellets collected from beaches in the southwest of England, as well as those of virgin pellets. Pellets were most abundant at the high tide line of the beaches where they were observed, which is in accordance with McDermid & McMullen (2004) who found order of magnitude differences between the high tide line and berm areas of the beaches which they surveyed.

3.4.1 Physical properties

Mass distributions of beached pellets, with the exception of those collected from Ninney Rock, are heterogeneous when compared to virgin pellets, which may be attributed to the range of sources of beached pellets as opposed to the single virgin pellet supplier. Additionally, pellets in the environment may lose mass owing to photo-degradation and/or abrasion, or gain mass through the processes of biofouling and inorganic accretions on the pellet surface (Artham *et al*., 2009).

Analysis of FTIR spectra identifies virgin pellets as polyethylene, and while beached pellets were predominantly polyethylene, occasional polypropylene pellets were collected (< 1 % polypropylene, >99 % polyethylene). The aging of pellets, as determined by the carbonyl index, indicates that it is possible to compare pellets exhibiting differing degrees of yellowing on the basis of their levels of photo-degradation (Fig. 3.5). Inspection of microscope images (Figures 3.2 & 3.3) indicates that as well as chemical changes, described by the FTIR spectra, various physical changes occur during a pellet's time exposed to marine and coastal environments. These changes include increasing surface area through abrasion, and the adhesion of particulate matter onto the pellet surface.

3.4.2 Chemical properties

Heterogeneity in metal concentrations was one of the important preliminary findings of the work presented (See Figures 3.6 and 3.7), thus wherever possible, pellets used in further experiments will be grouped together in an effort to attain a representative value of metal concentrations and interactions with aqueous-phase metals.

Following the sample collection and characterisation procedure it was possible to identify a site (Watergate Bay, Cornwall) where pellet abundance was high, and concentrations of trace metals associated with those pellets was relatively low. This was an essential requirement of the adsorption experiments presented in Chapters 4 and 5. In addition to the identification of a suitable source of pellets for further experiments, the metal concentrations observed on beached pellets suggest that heterogeneity of certain metals, notably Cu and Zn, would cause precision or background correction issues. Therefore, these metals have been omitted from further consideration in adsorption experiments.

The high degree of variability and greater concentrations of the metals Al, Fe and Mn on pellets sourced from Saltram may be attributed to the presence of these pellets within an estuarine system. Pellets sourced from estuarine systems have not been analysed for associated metals or other chemicals prior to these observations and the relationships presented here raise important questions, particularly with regard to the interactions between dissolved chemicals and pellets under such conditions. Additionally, the proximity of Sharrow Point and Ninney Rock to the urban centre of Plymouth (Fig. 2.1) may be the cause of elevation of some metals, notably Cu and Zn.

This is particularly important when considering the historic dumping of dredged material from the River Tamar, a catchment which has a long history of metal mining.

Comparison of metal concentrations on pellets from southwest England to those from another region may indicate the likelihood of pellets representing local metal concentrations. Pellets from the Island of Malta have been analysed for Fe and Al concentrations (Turner & Holmes, 2011), and it is evident that the degree of intra-site variability is substantial whether considering pellets from SW England (e.g. RSD for Al = 70 % – 108 %), or those from Malta (RSD for Al = 6 % – 73 %). Additionally, mean Al, Fe and Cu values for pellets from SW England are generally 3-7 times greater than those reported for pellets collected on Malta. For example, to consider Sharrow Point, concentrations of Al, Fe and Cu, are 23.0 μg g^{-1}, 64.6 μg g^{-1} and 1.32 μg g^{-1}, respectively, while pellets from Malta exhibit metal concentrations up to 6.72 μg g^{-1}, 16.8 μg g^{-1} and 0.25 μg g^{-1} for Al, Fe and Cu, respectively. However, owing to discrepancies between sample sizes between the two studies, these values have not been compared statistically. Despite this limitation, the differences between the metal concentrations on pellets from the two locations may be explained either by a greater environmental exposure period for pellets collected from England, as a longer time in the environment could allow for metals to have accumulated on pellets, or by the local geology, assuming pellets represent local metal concentrations when collected from the natural environment. It is known that Cornish and Devonian geology is dominated by igneous and metamorphic rocks including several known to contain metalliferous deposits (Langston *et al*., 2003), whereas the geology of Malta is comprised of various types of limestone (Pedley *et al*., 1976), lacking in mineral deposits.

Mean concentrations of metals on pellets are orders of magnitude lower than those determined on sediments from their sampling locations. This is expected given the disparity between particle size distributions of plastics and natural sediment, with smaller, natural particulates presenting surface areas orders of magnitude greater than that of pellets. If it is considered that plastic pellets are largely formed of non-porous material, the metal concentrations on pellets are surprisingly high and do not follow the metal distributions found on sediment from the site from which they are collected. This suggests that plastics are exposed to a range of conditions which will influence their metal accumulations before being deposited within a system. Additionally, despite intra-site variability, values reported for pellets from Saltram correspond closely to values reported by Ashton *et al*. (2010) who studied pellets collected from a range of sites on the south Devon coastline.

It may be expected that the concentrations of metals on pellets will correlate with photo-oxidation indices for beached pellets, as the greater surface area of degraded plastics should facilitate metal adsorption, while the greater level of degradation would suggest a greater period of exposure to dissolved metals in the water column. However, when correlations of pellet age and metal concentrations were attempted, the correlations were insignificant, and may be explained by a number of factors. Firstly, when suspended in the water column, pellets are not as susceptible to heat build-up which initiates the photo-oxidation process (Andrady, 1990). Secondly, fouling of the polymers enhances the availability of adsorption sites on the pellet, and simultaneously shields it from light, retarding the degradation process. Finally, the adsorption of metals on the surface is unlikely to be facilitated during periods in which pellets are deposited on land, and it is during these periods that photo-oxidation rates are likely to be greatest, owing to the mechanisms outlined above.

3.5 Conclusions

Previous studies on the presence of chemical compounds on the surface of plastic pellets focus on hydrophobic organic pollutants which are known to associate with plastics, predominantly through mechanisms of hydrophobic adsorption. The results in this chapter are amongst the first values available for the concentrations of metals associated with plastic pellets which have been collected from the natural environment. Of the metals determined from pellets collected from southwest England, several are of toxicological interest. Detectable concentrations of Cd, Co, Cr, Cu, Ni, Pb, and Zn exist on pellets and merit further study. The following chapters investigate the accumulation and release of trace metals from polyethylene pellet surfaces using a batch approach in order to further investigate likely mechanisms of association of these metals with plastic debris in the environment.

Chapter 4:

Adsorption of trace metals to plastic resin pellets in the marine environment

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This chapter, alongside background metal concentration data from Chapter 3, forms a research article published in Environmental Pollution 160 (2012).

4.1 Introduction

Research concerning the interactions between dissolved chemicals and plastic debris under environmental scenarios has primarily focused on interactions between plastics and hydrophobic organic pollutants in the marine environment. The most obvious cause of this focus is that the marine environment was the first environmental compartment in which pellets were observed. Additionally, the oceans represent the ultimate destination for many items of uncontrolled manmade debris, whether it is released inland or at sea (Andrady, 2011). The mobility of plastics in the marine environment gives cause for concern because of the abundance and diversity of products reported in regions remote from any urbanisation (Shiber & Barralesrienda, 1991; Khordagui & Abuhilal, 1994; Derraik, 2002). In addition to their aesthetic impacts, plastics in the marine environment pose a direct threat to animals by accumulation, entrapment, entanglement, choking and suffocation (Ryan, 1987; Boren *et al*., 2006; Browne *et al*., 2008; Gregory, 2009). Indirectly, plastics can act as a source of organic contaminants to animals through the ingestion of material that is mistaken for food (Teuten *et al*., 2007). Contaminants include components of the plastics themselves, including plasticisers, and hydrophobic organic compounds, such as polychlorinated biphenyls and polycyclic aromatic hydrocarbons, that are sorbed onto the material from seawater (Mato *et al*., 2001; Endo *et al*., 2005; Rios *et al*., 2007).

Empirical studies of the interactions between organic contaminants and plastics in the marine environment have largely focussed on resin pellets, which are comprehensively described in Chapters 1 and 3. The literature to date describes pellets as being capable of accumulating POPs at concentrations several orders of magnitude greater than the surrounding water (Takada, 2006), with a high degree of variability between individual pellets. Until recently, interactions between metals and plastic resin pellets or any other polymeric debris had not been considered, presumably because polymers are generally considered to be relatively inert towards aqueous metal ions. However, loss of metal to plastic containers is a commonly reported problem during sample storage or in experiments involving spiking of metal standards (Giusti *et al*., 1994; Li *et al*., 2001; Cobelo-Garcia *et al*., 2007; Fischer *et al*., 2007). Therefore, it may be assumed that this phenomenon will occur in the environment, although currently this has not received any attention in the literature.

Analysis of composite samples of resin pellets collected from a variety of beaches in south west England revealed measurable trace metal concentrations (See Chapter 3) and, in some cases, metal enrichment relative to local sand after normalisation with respect to Al (Ashton *et al*., 2010). The precise mechanisms by which metals associate with pellets are unclear but likely involve adsorption of ions to the polymer and coatings thereon and adherence of small, metal-bearing mineral particles to the pellet surface. Moreover, the buoyancy of pellets ensures they are exposed to relatively high concentrations of metals and other contaminants in the sea surface microlayer (Wurl & Obbard, 2004). Trace metals associated with plastic pellets in the marine environment have the propensity to be transported considerable distances and are potentially available to animals and birds that mistake plastics for food (Robards *et al*., 1995; Tourinho *et al*., 2010).

The transfer of substances from a mobile phase to a solid phase is a universal phenomenon governing the mobility of these substances within a system (Limousin *et al*., 2007), which can be investigated using a number of techniques. Adsorption kinetic and isotherm experiments have a legacy of use within geochemistry and sedimentology, where they are widely applied to assess the adsorptive capacity of natural sediments, (Jannasch *et al*., 1988; Couceiro *et al*., 2007), and modified-natural or engineered substrate compositions used in remediation (Bhattacharyya & Gupta, 2008; Miretzky & Cirelli, 2010). The adsorption experiments presented here exhibit elements of classical experimental design, with slight modifications to allow the application of these methods to plastic pellets.

Adsorption kinetic investigations can help to identify mechanisms of association between dissolved analytes and solid phases, with rapid uptake kinetics indicative of surface-controlled mechanisms and slower rates suggesting diffusion of analytes into the solid matrix in a more recalcitrant form (Millward, 1995). Adsorption kinetic experiments are also a pre-requisite to any adsorption isotherm study, as isotherms must reach equilibrium for the isotherm to be considered. Within the context of the experiments presented, adsorption to PE pellets is observed over a period of one week, which is a typical period of time for studies of this type (Jannasch *et al*., 1988; Hatje *et al*., 2003). However, it must be considered that various kinetically controlled phenomena will occur over longer timescales on the order of years (Limousin *et al*., 2007), particularly when the primary mode of association of trace metals is controlled by the presence and continuous accretion of substrates such as Fe, Al and Mn precipitates and/or biofilms.

In the present study, the mechanisms and kinetics of trace metal uptake by plastic resin pellets are examined in metal-amended seawater under controlled laboratory conditions. Experiments use both new (virgin) pellets sourced from a plastics moulding facility and aged pellets collected from a local beach and whose chemical characteristics (e.g. polymer type, pre-existent metal content) had been previously established (Chapter 3). Results of the study are discussed in terms of the implications of plastic-metal interactions on trace metal behaviour and cycling in the marine environment.

4.2 Materials and methods

4.2.1 Materials and reagents

Reagents were of analytical grade or better, and all equipment was cleaned according to a standard protocol (see Section 2.2 for details).

4.2.2 Sample collection

Surface seawater of salinity 34 and pH 7.8 was collected in a 10 L high density polyethylene (HDPE) carboy from Station L4 in the English Channel as detailed in Chapter 2. In the laboratory, seawater was filtered through a 0.45 μm cellulose nitrate filter housed in a polysulfone vacuum filtration unit and stored at 4 °C and in the dark until required in the experiments.

Virgin pellets were obtained from a local plastic processing facility, while beached pellets were collected as described previously from Watergate Bay, UK, following the identification of this site as one where pellets with comparatively low concentrations of metals occurred (Chapter 3). In the laboratory, pellets were processed as described in Chapter 2. Briefly, pellets were stripped of adherent material by ultrasonication for 5 minutes in native seawater, followed by drying under laminar flow, and stored in clean centrifuge tubes.

4.2.3 Adsorption experiments

Because of the relatively low metal concentrations in pellets from Watergate Bay (see Chapter 3), a large quantity of pellets (*n* ~ 2000) was collected from this location and used in the adsorption experiments along with virgin pellets obtained from the processing facility. For the kinetic experiments, undertaken in triplicate and including a metal-free control, 300 pellets were weighed and added to 750 ml of filtered seawater (pellet mass: seawater volume = 10 g L^{-1}) in 1 L Teflon (PTFE) bottles. After 24 hours, 5 μg L^{-1} of Cr, Co, Ni, Cu, Zn, Cd and Pb, prepared by serial dilution of 1000 mg L^{-1} plasma emission standards in MQW, was added. Concentrations greatly exceed aqueous metal concentrations in open seawater but are closer to values encountered in contaminated inshore waters (Jonas & Millward, 2010) and in the sea surface microlayer where pellets reside during transport (Wurl & Obbard, 2004). The contents were then orbitally agitated at 150 rpm for a period of 7 d at room temperature (20 ±1 °C), and subsamples of 50 ml and 20 pellets were abstracted using a plastic bulb pipette and 1 mm mesh, respectively, at time intervals of 0.25, 0.5, 2, 6, 24, 50, 120 and 168 hours. Water samples were transferred to polypropylene centrifuge tubes and acidified to pH < 2 with 50 μl of HNO_3. Pellets were transferred to 8 ml HDPE vials and 2.5 ml of 10% HCl added in order to extract adsorbed trace metals and minimise digestion of pre-existent metal present on pellet surfaces prior to adsorption experiments. Adsorption isotherms were undertaken in triplicate for a period of 48 h in a series of 60 ml PTFE bottles, each containing 20 pre-weighed pellets, 50 ml of filtered seawater and different concentrations of metals added in combination ranging from 0 to 20 μg L^{-1}, under otherwise identical conditions. On completion of the experiments, metal loss to the PTFE container surfaces was evaluated by rinsing empty bottles with 50 ml of 10 % HCl for 48 h under orbital agitation.

4.2.4 Metal analysis

Metals were analysed using ICP-MS, general procedures and operating conditions for which are given in Section 2.5. Instruments were calibrated using five matrix-matched, mixed standards and a blank; internal standardisation during ICP-MS analysis was achieved by the addition of 50 μg L^{-1} of ^{115}In and ^{193}Ir to all standards and samples. Seawater samples resulting from the adsorption experiments were diluted tenfold in MQW to reduce the concentration of dissolved ions, thereby minimising mass interferences and instrumental damage; acid digests of the corresponding pellets and acid rinses of the empty containers, to evaluate container adsorption, were also analysed for trace metals by ICP-MS.

4.3 Results

4.3.1 Pellet characteristics

As discussed in the previous chapter, pellets were predominantly white or off-white cylinders and ovoids, composed of polyethylene. Mean pellet mass was 26 mg, with an RSD of 23 %.

4.3.2 Trace metal adsorption

Since polymer (PTFE) bottles were employed during examination of the uptake of trace metals by plastic resin pellets, it was important to quantify the loss of analyte to the container surfaces during the incubations. Analysis of the acid rinsings of empty bottles at the end of the experiments showed that, on average, about 5% of Cr had adsorbed to the PTFE surfaces, and that < 1 % of other metals had been lost by this process.

Although trace metal loss to containers was small relative to percentages remaining in seawater or adsorbed to pellets, results below have been computed from direct measurements of the aqueous and pellet phases and do not rely on mass balance. Thus, kinetic results are expressed as percentage aqueous metal, calculated from aqueous concentration relative to aqueous plus pellet concentration (both on a w/v basis), while isothermic results are shown as pellet metal concentration on a w/w basis versus aqueous metal concentration.

4.3.3 Adsorption kinetics

Examples of the time-dependencies of metal uptake by virgin and beached pellets are shown in Figure 4.1. In most cases, profiles consisted of a period of rapid metal adsorption and subsequent approach to equilibrium or, in some cases, a more protracted period of slower adsorption, particularly in the case of Cr adsorption to beached pellets. Nickel adsorption to beached pellets over the time course appears to reach a maximum after 50 hours, with some remobilisation evident after this time. Owing to the high degree of variability in Cu and Zn concentrations mentioned in Chapter 3, Cu and Zn were omitted from adsorption studies as precise measurements were not possible. The extent of metal uptake was greater on beached pellets than on virgin pellets, presumably because of the changes to surface properties incurred while pellets are in the marine environment (described below).

Rates of interaction between metal, Me, and pellet surface, X, were modelled with a pseudo first-order reversible reaction (Turner et al., 2006):

$$\mathrm{Me} + \mathrm{X} \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} \mathrm{MeX} \qquad (4.1)$$

where MeX (w/v) represents metal adsorbed to the pellet surface and k_1 and k_{-1} are forward and reverse rate constants, respectively. The rate equation for this process is given as:

$$\frac{d[\mathrm{Me}]}{dt} = -k_1[\mathrm{Me}]_\mathrm{t} + k_{-1}[\mathrm{MeX}]_\mathrm{t} \qquad (4.2)$$

In each case, rate constants and an equilibrium constant ($k = k_1/k_{-1}$) were derived from the integrated form of equation 4.2 by assuming equilibrium concentrations were represented by the mean of the final two aqueous percentage measurements in the time-course ($[\mathrm{Me}]_\mathrm{e}$), to yield:

$$([\mathrm{Me}]_\mathrm{t} - [\mathrm{Me}]_\mathrm{e}) = ([\mathrm{Me}]_0 - [\mathrm{Me}]_\mathrm{e}) \exp[\,-(k_1 + k_{-1})\mathrm{t}] \qquad (4.3)$$

Under equilibrium conditions, $d[\mathrm{Me}]/dt = 0$; therefore it is possible to define an equilibrium constant, k, and rewrite equation 4.2 as:

$$k = \frac{k_1}{k_{-1}} = \frac{[\mathrm{Me}]_0 - [\mathrm{Me}]_\mathrm{e}}{[\mathrm{Me}]_\mathrm{e}} \qquad (4.4)$$

Rearranging equation 4.4 for k_{-1} and substituting this into equation 4.3 yields:

$$k_1 t = \frac{[\mathrm{Me}]_0 - [\mathrm{Me}]_\mathrm{e}}{[\mathrm{Me}]_0} \ln\left(\frac{[\mathrm{Me}]_0 - [\mathrm{Me}]_\mathrm{e}}{[\mathrm{Me}]_\mathrm{t} - [\mathrm{Me}]_\mathrm{e}}\right) \qquad (4.5)$$

Model fits to empirical data as calculated using equation 4.5 have been annotated on Figure 4.1, while rate constants are displayed in Table 4.1

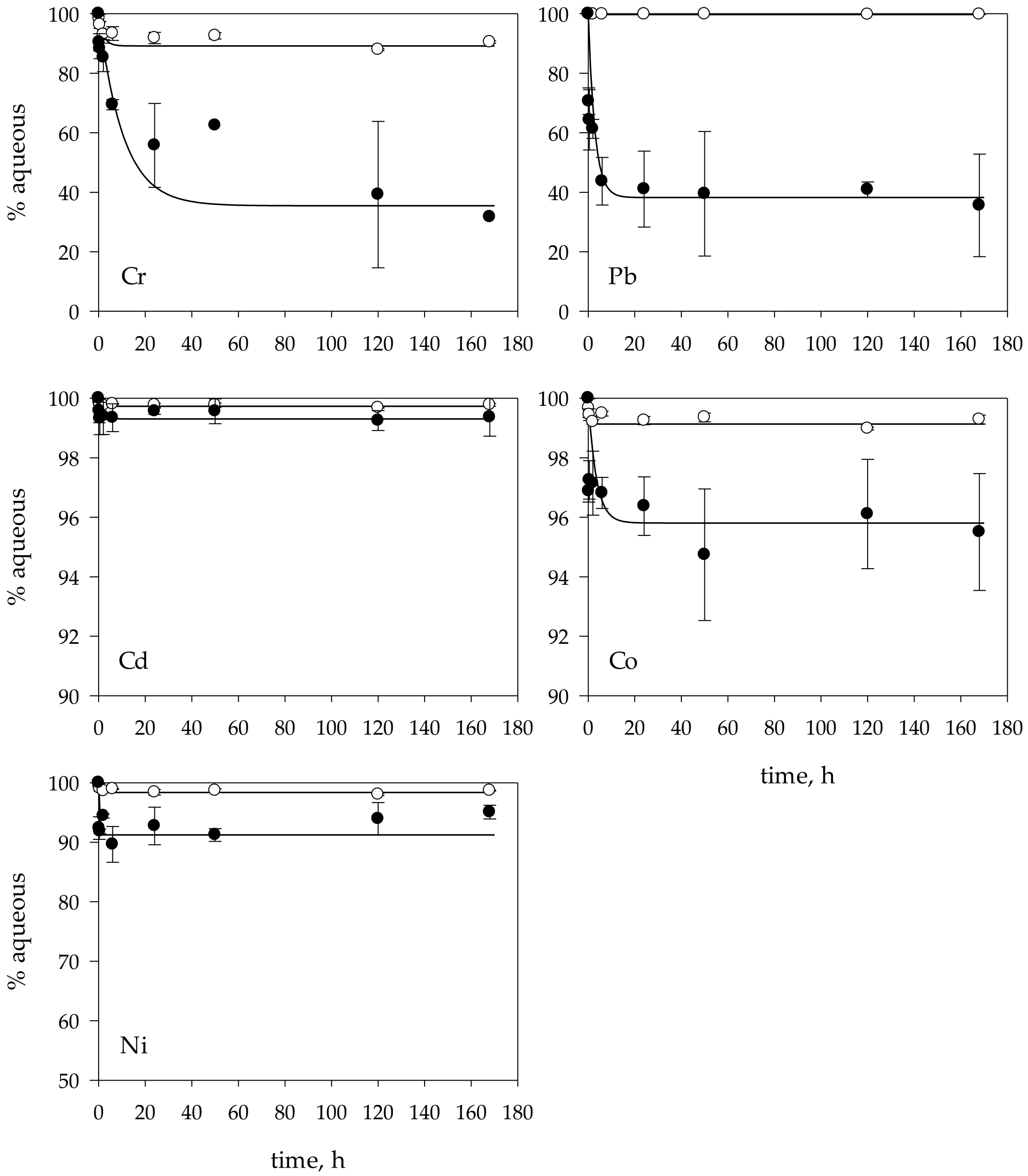

Figure 4.1: Time-dependent adsorption of Cr, Pb Co, Cd and Ni to virgin (o) and beached (●) polyethylene resin pellets. Error bars represent one standard deviation about the mean of three determinations and lines are fits to the data based on a pseudo-first-order model (equations 4.1 – 4.5), whose constants are given in Table 4.1.

Partition coefficients are widely used to compare the affinities of metals to different particulates, or to assess adsorption under varying conditions. K_D is defined:

$$K_D = \frac{[\mathrm{Me}]_p}{[\mathrm{Me}]_w} \quad (4.6)$$

where $[\mathrm{Me}]_P$ is the concentration of metal adsorbed to plastic pellets (w/w) and $[\mathrm{Me}]_W$ is the aqueous metal concentration (w/v). For the purposes of kinetics experiments, an equilibrium partition coefficient, $(K_D)_e$ (ml g^{-1}), was calculated from the ratio of w/w pellet metal concentration at equilibrium, $[\mathrm{MeX}]^*_e$, to aqueous metal concentration at equilibrium, $[\mathrm{Me}]_e$, and a system response time, defined as the time required for the reaction to reach 63% of the new equilibrium (Millward *et* al., 1992), was derived from the reciprocal of the sum of the forward and reverse rate constants:

$$T_{\mathrm{resp}} = \frac{1}{(k_1 + k_{-1})} \quad (4.7)$$

Constants defining all model fits with the exception of Zn, which was a contaminant of the control and whose pre-existent concentrations in beached pellets were relatively high and variable, are given in Table 4.1.

Table 4.1: Constants defining time-dependent profiles of trace metal adsorption to virgin and beached polyethylene pellets as fitted using a pseudo first-order model (equations 4.1 – 4.5).

	Virgin					**Beached**				
	k_1, h^{-1}	k_{-1}, h^{-1}	r^2	T_{resp}, h	$(K_D)_e$, ml g^{-1}	k_1, h^{-1}	k_{-1}, h^{-1}	r^2	T_{resp}, h	$(K_D)_e$, ml g^{-1}
Cr	0.055	0.45	0.974	1.97	10.1	0.061	0.025	0.598	11.6	221
Co	0.010	1.18	0.974	0.84	0.661	0.089	2.04	0.552	0.47	4.03
Ni	0.014	0.83	0.931	1.18	1.28	0.464	4.82	0.914	0.19	8.87
Cd	0.0002	0.01	0.649	105	0.204	0.002	0.265	0.126	3.75	0.318
Pb	0.002	0.67	0.986	1.49	0.225	0.210	0.130	0.912	2.94	149

Goodness of fit, represented by the regression coefficient defining the relationship between the integrated form of equation 4.2 and time, is better for trace metal uptake by the virgin pellets than by the beached pellets. This is because interactions between metal and the beached pellet surface did not always appear to attain equilibrium, an effect attributed to the greater heterogeneity and porosity of the coated and fouled surface of aged plastics. Assuming that equilibrium *is* attained, system response times for both pellet types are within about 100 h and, in many cases, are less than 1 h. With the exception of Cu, which may have been affected by interferences from pre-existent metal, equilibrium partition coefficients are considerably higher for beached pellets than for virgin pellets; with respect to Pb, the difference in partitioning between pellet types is almost two orders of magnitude.

4.3.4 Adsorption isotherms

Isotherms defining the adsorption of trace metals to virgin and beached pellets are shown in Figure 4.2 for the metals whose kinetic profiles were illustrated in Figure 4.1. The results presented in Figure 4.2 suggest order of magnitude differences between equilibrium concentrations of different metals on beached pellets within this experimental system. Chromium and Pb represent the trace metals with the greater affinity for beached plastic pellets, while Cd, Co and Ni display a lesser affinity for the plastic surface. This would be expected of trace metals for which affinities for the solid phase vary considerably. For all metals, virgin pellets present fewer viable sites for adsorption, which is reflected by the minimal associations of trace metals with virgin pellets through the concentration range.

Variations among replicates were greater for the beached pellets, presumably due to the greater heterogeneity of the plastic surface incurred by aging. Both Langmuir and Freundlich models were fitted to the isothermic data. The former assumes monolayer adsorption to a homogeneous surface and is given as follows:

$$[\mathrm{MeX}]^{*}_{e} = \frac{K_{L}[\mathrm{MeX}]_{\max}[\mathrm{Me}]_{e}}{1+K_{L}[\mathrm{Me}]_{e}} \qquad (4.8)$$

where $[\mathrm{MeX}]^{*}_{e}$ is the w/w concentration of metal adsorbed to the pellet surface, $[\mathrm{Me}]_{e}$ is the equilibrium concentration of metal in solution, $[\mathrm{MeX}]^{*}_{\max}$ is the adsorption capacity of the metal and K_L (ml g^{-1}) is the Langmuir isotherm constant.

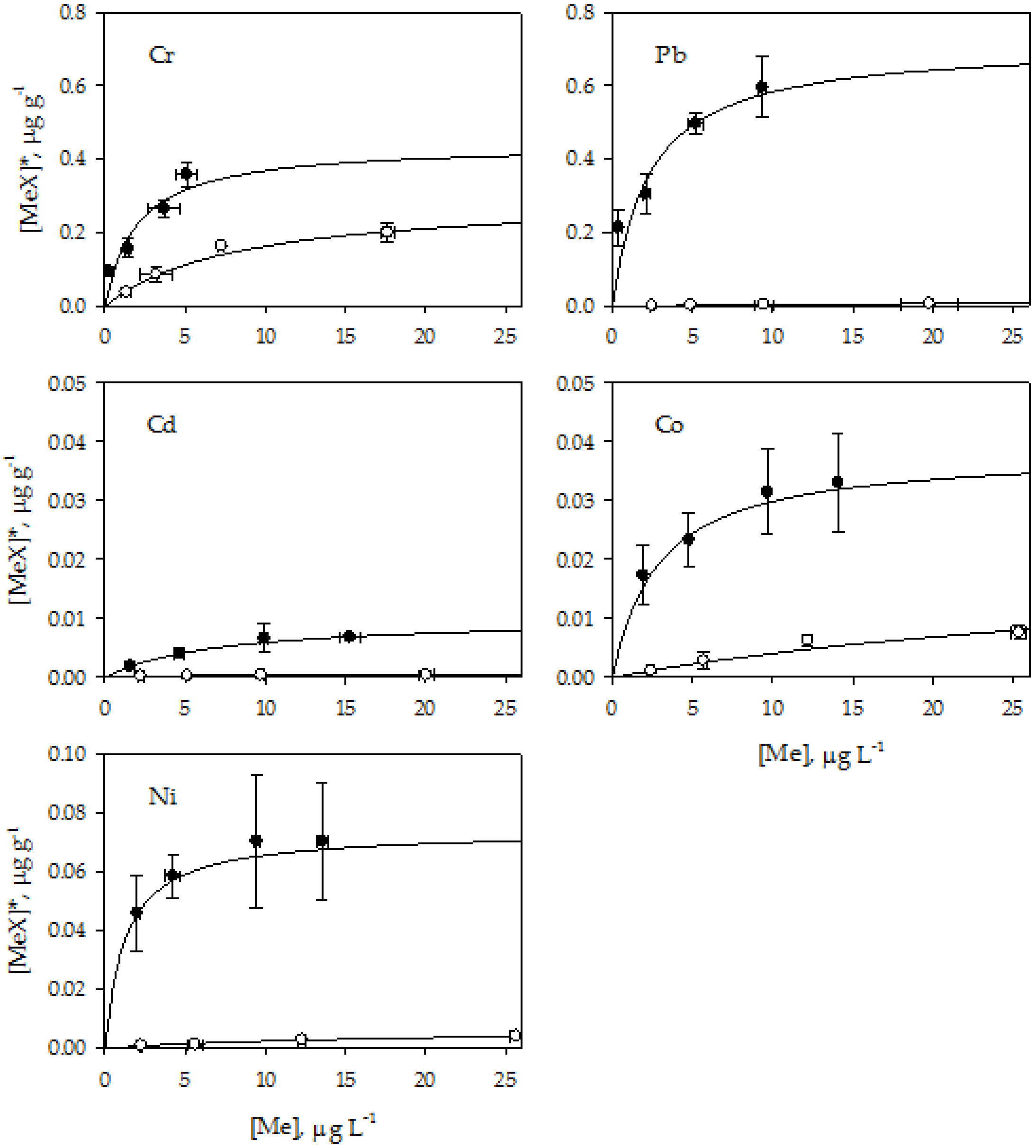

Figure 4.2: Isotherms defining the adsorption of Cr, Pb, Cd, Co, and Ni from seawater to virgin (o) and beached (●) polyethylene resin pellets. Error bars represent one standard deviation (n = 3), and lines represent Langmuir fits to the data according to equation 4 and whose constants are defined in Table 4.2.

The Freundlich equation assumes multilayer adsorption to heterogeneous surfaces and is given as follows:

$$[\mathrm{MeX}]^*_e = K_F[\mathrm{Me}]_e^{1/n} \tag{4.9}$$

where K_F, ($\mu g^{1-1/n} g^{-1}$) is the Freundlich constant and n is a measure of linearity. Values for the Langmuir and Freundlich constants, derived from linear regression analysis of $1/[\mathrm{MeX}]^*_e$ versus $1/[\mathrm{Me}]_e$, and non-linear regression of $[\mathrm{MeX}]^*_e$ versus $[\mathrm{Me}]_e$, respectively, are shown in Table 4.2 for trace metals that were unaffected by interferences from pre-existent metal or by experimental contamination.

Table 4.2. Constants defining trace metal adsorption to virgin and beached polyethylene pellets according to the Langmuir and Freundlich models (Equations 4.4 and 4.5, respectively). Note that interferences from pre-existent metal precluded fitting of beached Cu data and that Pb adsorption to virgin pellets was best defined by linear fitting (r^2 = 0.988; $(K_D)_e$ = 0.3 ml g^{-1}).

	Langmuir constants						**Freundlich constants**					
	Virgin			**Beached**			**Virgin**			**Beached**		
	$[\mathrm{MeX}]^*_{max}$ µg g^{-1}	K_L ml g^{-1}	r^2	$[\mathrm{MeX}]^*_{max}$ µg g^{-1}	K_L ml g^{-1}	r^2	$1/n$	K_F µg$^{1-1/n}$ g^{-1}	r^2	$1/n$	K_F µg$^{1-1/n}$ g^{-1}	r^2
Cr	0.297	0.127	0.96	0.441	0.503	0.78	0.624	0.038	0.83	0.570	0.131	0.79
Co	0.018	0.033	0.76	0.038	0.351	0.81	0.858	0.0006	0.84	0.344	0.014	0.61
Ni	0.008	0.047	0.80	0.070	2.67	0.82	0.671	0.0005	0.88	0.085	0.057	0.10
Cd	0.0004	0.413	0.98	0.010	0.140	0.77	0.287	0.0002	0.46	0.576	0.002	0.83
Pb	-	-	-	0.716	0.439	0.91	-	-	-	0.047	0.214	0.90

Despite the different assumptions of the two models and the different characteristics of the virgin and aged pellets, there are no clear or systematic differences in the goodness of fit (as r^2) to the datasets, either between the two models or between pellet type. (Note that Langmuir fits have been annotated for illustrative purposes in Figure 4.2.) Regardless of the model fits, and consistent with the kinetic results reported above, adsorption to beached pellets is greater than adsorption to virgin pellets, and adsorption capacities for beached pellets are greatest for Cr and Pb and lowest for Cd, Co and Ni.

4.4 Discussion

Despite the general assumption that plastics are relatively inert towards aqueous metals, virgin polyethylene pellets suspended in trace metal-amended seawater adsorb trace metals rapidly and in a Langmuir or Freundlich fashion with equilibrium partition coefficients (at [Me] = 5 μg L^{-1}) of up to about 12 ml g^{-1}. Presumably, metal adsorption proceeds through interactions between bivalent cations (e.g. Cu^{2+}, Cd^{2+}, Pb^{2+}) and oxyanions (e.g. $Cr_2O_4^{2-}$) with charged or polar regions of the plastic surface (effected by imperfections and the presence of charged contaminants and additives, for example), and via non-specific interactions between neutral metal-organic complexes and the hydrophobic surface of the bulk plastic medium. Aged, beached pellets accumulate trace metals to a significantly greater extent, with equilibrium partition coefficients ranging from about 4 ml g^{-1} (Co) to 220 ml g^{-1} (Cr). The reactivity of the surface of aged pellets is enhanced by both changes to the polymer itself and the presence of biofilms and chemical precipitates. With respect to the former, photo-oxidative weathering generates oxygen containing groups that increase the polarity of the polymer (Mato et al., 2001). Regarding the latter, accumulations of biofilms and hydrogeneous precipitates increase the charge, roughness, porosity and hydrophilicity of the surface (Artham et al., 2009).

Although these enclosed, batch experiments have revealed that interactions between trace metals and the surface of virgin or beached pellets are both considerable and relatively rapid, the equilibrium constants that have been derived are likely underestimates of environmental values. Thus, metal adsorption in situ is predicted to be a longer term process as chemical precipitates and biofilms continuously accrue at the plastic surface; trace metal accumulation is also likely to be enhanced by co-precipitation, a slow process not replicated in short-term experiments conducted without the addition of hydrogeneous metals. Overall, therefore, the relatively high concentrations of trace metals observed on beached pellets (see Chapter 3) may be attributed to these sorptive mechanisms acting on pellets that are exposed to aqueous metals in the sea surface microlayer for decadal timescales.

The association of trace metals with plastics is likely to be a more general phenomenon, both in terms of other aquatic environments and with respect to different types and sizes of plastic. Accordingly, plastics may be considered as a vehicle for transport of metals in aquatic systems that has not, thus far, been acknowledged. Metal transport with plastics is particulate by definition but, at least with regard to small pieces of litter, is quasi-conservative, enabling metals to be transported considerable distances while buoyant. Considering the abundance of inland sources of plastic debris, it is critical to examine the interactions between metals and plastics under freshwater and estuarine conditions – an environmental compartment currently under-represented in the field. The association of trace metals with plastics also has implications for the transfer of these contaminants into the foodchain. Thus, invertebrates, fish, birds and mammals that mistake plastics for food (Teuten et al., 2009) have the potential to mobilise metals in their acidic, enzyme-rich digestive systems. Consequently, metals may be either bioaccumulated or released back into seawater in a more soluble and biologically available form. Clearly, these and other potential impacts of plastic-metal interactions in the aquatic environment merit further study. With the noted presence of plastics in estuarine systems, and evidence for the scavenging of dissolved metals by plastics in marine systems, the first step is to understand metal uptake under the varied and complex conditions present within the estuarine environment. The following chapter begins to explore this through a series of controlled experiments which attempt to simulate the major physico-chemical gradients expected within an estuary.

Chapter 5:

Adsorption of trace metals to plastic resin pellets under estuarine and freshwater conditions

5.1 Introduction

Estuaries are complex and dynamic systems where biogeochemical cycling is governed by numerous physical, chemical and biological processes which change on various temporal and spatial scales (Millward, 1995). Within an estuarine system, the primary chemical controls over geochemical behaviour of trace metals are salinity, ionic strength and pH. Additionally, factors such as the concentration and composition of suspended particulate matter (SPM) are known to influence trace metal cycling (Money *et al.*, 2011). While marine waters have relatively consistent composition globally and are well buffered against changes in pH, rivers and estuaries have the potential for far greater variability of composition.

Inland spillage of plastic pellets, as well as tidal cycling, result in seaward and landward transport of plastic pellets within estuaries. Despite the abundance of plastic debris in riverine and estuarine systems (Browne, et al., 2011), there is currently a paucity of literature concerned with the chemical reactivity of plastic resin pellets under estuarine and freshwater conditions.

Owing to the ubiquity, persistence and mobility of synthetic polymers in the environment it is important to understand the trace metal accumulation properties of plastic pellets in freshwater and estuarine systems. The uptake of trace metals by plastics has been investigated under marine conditions (Holmes *et al*., 2012); however, owing to the aforementioned variability of composition, it is important to understand the consequences of releases of plastics into rivers and estuarine systems. In this chapter the interactions between trace metals and plastic pellets were investigated as functions of salinity and pH, and the behaviour of trace metals is interpreted in terms of speciation and their likely fate within estuarine systems.

5.2 Materials and methods

5.2.1 Materials and reagents

All equipment used for sample collection, storage, preparation and experimental work was washed and dried prior to use according to the standard protocol as described in Section 2.2. Riverine and marine end-member waters used in experimental incubations were collected, processed and stored according to the procedures outlined in Section 2.3. Aliquots were removed from the bulk sample and analysed for salinity with a YSI 85-10 FT salinity/O_2/temperature probe, and for pH using a calibrated Mettler Delta 340 pH meter.

5.2.2 Pellet sampling & characterisation

Beached pellets from Watergate Bay were selected for use in adsorption experiments owing to their relatively low background concentrations of metals (see Section 3.3), and consistent polyethylene composition. Virgin pellets were those supplied by Algram. Sample treatment was carried out following the method described previously (Section 2.3).

5.2.3 Adsorption experiments – salinity effects

Adsorption kinetics were studied over the period of one week in 0.45 µm filtered riverine and marine end members, while adsorption isotherms were prepared according to the method described in Section 4.3.4, substituting filtered seawater for filtered river water. This allows direct comparison between adsorption experiments conducted in river and marine end-members. Additionally, a salinity gradient was simulated by combining end members in varying proportions to obtain a range of salinities typically observed in estuaries.

Kinetic experiments were carried out by combining pellets with filtered water in triplicate, in 1 litre wide-neck PTFE bottles. Approximately 7.5 g of pellets were accurately weighed into each reaction vessel using a Salter ER-182A balance. These were conditioned in experimental waters whilst being agitated on an orbital shaker for 24 hours prior to addition of a mixed spike of trace metals. Trace metals were added as a mixed spike prepared from acidified calibration standards diluted with MQW to yield experimental concentrations of 5 μg L^{-1}. Due to the small volume of the spike addition, pH was not significantly altered upon addition of trace metals. A sub-sample of pellets (0.5 g) and two 25 mL aliquots of water were removed at designated time points (0.25, 0.5, 2, 6, 24, 50, 120, 168 hours) with a clean HDPE sieve and a low density polyethylene (LDPE) pipette, respectively. The initial 25 mL aliquot was discarded in order to condition the pipette, thereby minimising loss of dissolved metal analytes. Aliquots were acidified to $pH < 2$ with 25 μL concentrated HNO_3 and pellets were transferred to 7 mL HDPE vials, to be dried under laminar flow prior to extraction of adsorbed metals. This was achieved by addition of 2.5 mL 10 % HCl to the vials containing pellets, and shaking for 24 h with the vials on their sides to maximise contact between pellets and HCl. An aliquot of 2 mL was then removed for analysis by ICP-MS.

To simulate an estuarine gradient, 0.45 μm filtered end-member waters (salinities of 0.05 and 33 respectively) were combined in various proportions (Fig. 5.1) to obtain an estuarine gradient with a range of salinities (0.05, 1.7, 3.4, 5.0, 8.2, 16.4, 33) and a pH that ranged between 6.8 and 7.8. Prepared solutions were stored in the dark at 4 °C until use (< 14 days), whereupon they were allowed to equilibrate to room temperature (20 ± 1 °C).

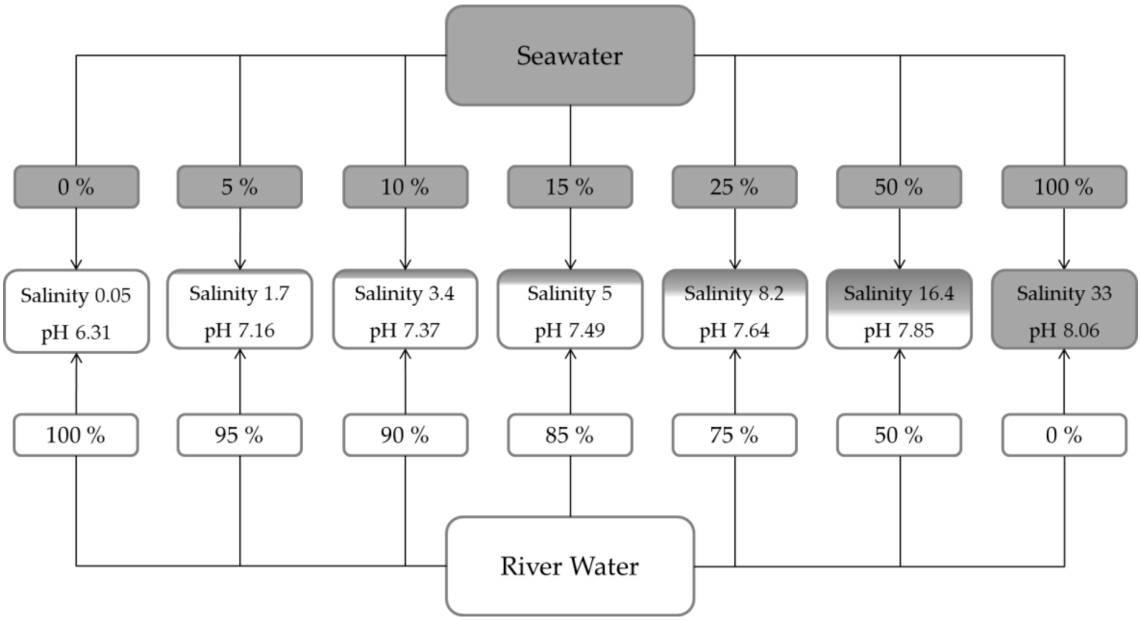

Figure 5.1: Proportions of filtered (0.45 μm) river and marine end members used to obtain a simulated estuarine gradient.

Triplicate 50 mL samples at each salinity point were added to individual 60 mL PTFE bottles and pellets were conditioned as above, followed by spiking with trace metals to 5 μg L^{-1}. Samples were then shaken for 24 hours to allow aqueous and adsorbed metal concentrations to reach equilibrium. Separation was achieved by removing 2.5 mL aliquots which were transferred to 7 mL HDPE vials and acidified to $pH < 2$ with HNO_3. The remaining water was discarded to leave behind the pellets, which were then transferred to vials and dried and extracted as above.

5.2.4 Adsorption as a function of pH

In order to identify the environmental scenarios most likely to promote adsorption of trace metals to plastic pellets, adsorption was studied as a function of pH to generate pH adsorption edges. This was achieved by controlling the pH of filtered river water by addition of 0.1 M NaOH (prepared from NaOH salts dissolved in MQW), or 0.1 M HNO_3 to pH values of approximately 0.5 unit intervals between 4 and 10.5, representative of all values likely in natural freshwaters. Seawater was not included in this study owing to its buffering capacity, which limits the range of pH found in natural waters to a narrow pH range of 7.8 – 8.2 (Pinet, 2006). Samples of each pH value were prepared by adding 60 mL filtered river water to 60 mL PTFE bottles, adding a predetermined volume of 0.1 M HNO_3 (< 230 μL) or NaOH (< 470 μL), and equilibrating overnight at 150 rpm on an orbital shaker before the addition of approximately 0.5 g pellets. These suspensions were equilibrated for pH for 24 h before addition of a multi-element (Cd, Co, Cr(VI), Ni, Pb) spike to a final concentration of 5 μg L^{-1}. Five millilitre aliquots were removed immediately before and after spiking, and analysed for pH using a Mettler Delta 340 pH meter, calibrated immediately prior to use. The remaining 50 mL preparations were then shaken for 24 h at 150 rpm before separation and sample treatment as above. A final pH measurement of each sample was also obtained and it is this value which is presented.

5.2.5 Metal analysis

Prior to analysis, water samples with seawater proportions > 10 % were diluted with MQW to salinities of 1.7 or 3.4 to minimise mass interferences and prevent instrumental damage. Trace metals Cd, Co, Cr, Ni and Pb in water samples and HCl extractions were analysed by ICP-MS according to the protocols outlined in Section 2.5. These metals were chosen owing to their adsorption to pellets in seawater, as confirmed in Chapter 4, and lack of contamination or background variability which cause difficulties in blank corrections. For example, concentrations of Cu and Zn adsorbed to pellets were indistinguishable from background variability resulting from pre-existent concentrations and/or procedural contamination (see Section 3.3). Preexisting metal concentrations on pellets were accounted for by blank correction by analysis of triplicate procedural blanks from which trace metal spikes were omitted.

5.2.6 Speciation modelling

Chemical speciation modelling was conducted using the Windermere Humic Aqueous Model, version VI (WHAM) in order to identify the likely metal species interacting with pellets within a simulated estuarine system. As with all geochemical models, WHAM is constrained by a number of assumptions, upon which speciation calculations are based.

Default formation constants contained within WHAM databases were used to model inorganic speciation of trace metals in experimental waters, using measured salinities of end-members to define the concentrations of major solutes, while estuarine values were calculated from conservative mixing. Typically, WHAM utilises the Extended Debye-Hückel equation for ionic strength corrections, however this equation is optimised for dilute (I < 0.1 M) solutions (Tipping *et al.*, 1998), suffering from inaccuracies above this value. Therefore the Davies equation was used to correct stability constants for ionic strength, owing to its applicability to greater ionic strengths. Literature values for dissolved organic carbon (DOC) in Plym River water (2 mg L^{-1}) and seawater (1.5 mg L^{-1}) were obtained from Turner *et al.*, (2004), and the widely accepted 4:1 ratio of fulvic acid (FA) to humic acid (HA) was employed to define FA and HA concentrations (Turner & Martino, 2006).

5.3 Results

5.3.1 Adsorption kinetics

Adsorption of Cd, Cr, Co, Ni and Pb to beached plastic pellets is expressed as a percentage calculated from aqueous and adsorbed concentrations, and is presented as a function of time in Figure 5.2. Data are presented as adsorbed percentages (calculated according to Section 4.3.2) owing to the requirements of the pseudo first order rate equation, detailed in Chapter 4, in order to obtain rate constants (presented in Table 5.1). While virgin pellet data are omitted from plots for clarity, in summary, adsorption to virgin pellets in river water reaches maximum values of 0.5, 0.2, 1.8, 0.5 and 3 % for Cd, Co, Cr, Ni and Pb respectively, while in seawater virgin pellets exhibit minimal adsorption, with values of < 1 % adsorbed, with the exception of Cr (9 %).

For all trace metals considered (Cd, Co, Cr, Ni, Pb) it is clear that the uptake from solution onto beached polyethylene pellet surfaces is rapid in both river water and seawater, reaching equilibrium within a few hours (Fig. 5.2), with little difference in time to equilibrium between freshwater and marine end-members (See Fig 5.1 for pH and salinity). However, the extent of uptake varied between metals, and for some metals, adsorption exhibited contrasting behaviour between riverine and marine end members.

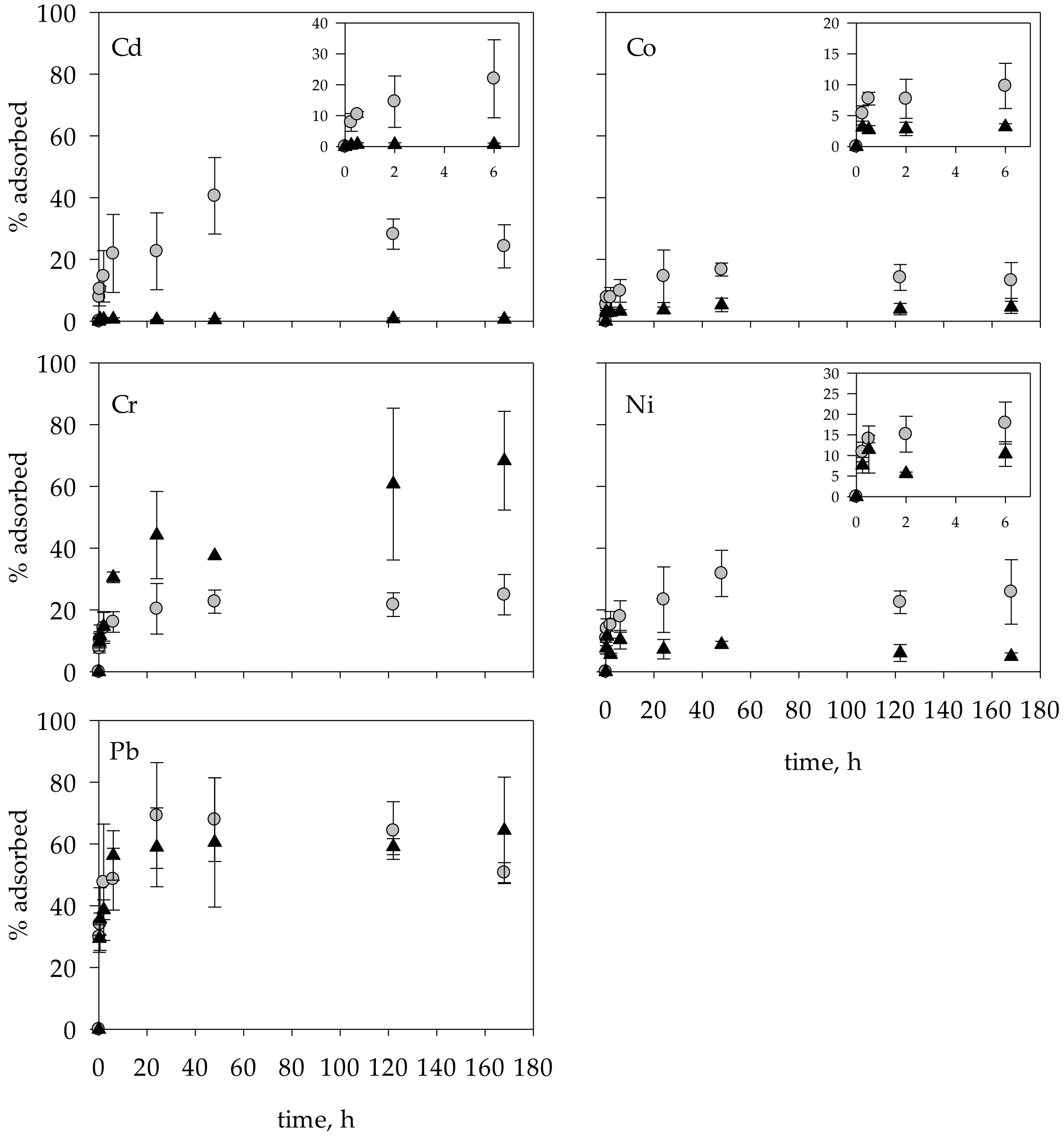

Figure 5.2: Adsorption kinetics of trace metals onto beached polyethylene pellets from freshwater (●) and seawater (▲) at 21 ±1 °C. Values are presented as mean values ± one standard deviation (*n* = 3). Inset figures show in detail uptake over the initial 6 hour period.

For adsorption experiments in river water, Pb reaches a maximum adsorption to beached pellets of 69 %, while Cd, Co, Cr, and Ni show lesser affinities for pellets, with adsorbed fractions reaching maximum values of 40, 16, 25, and 32 %, respectively. In seawater experiments, adsorbed, Cd, Co, Cr, Ni and Pb reach maximum values of

0.8, 5.2, 64, 10 and 65%, respectively. Despite differences in the extent of uptake onto pellets, the time to reach equilibrium was rapid, and remained fairly consistent between freshwater and seawater for each metal. Values of k_1 and k_{-1} which are the forward and reverse rate constants for trace metal uptake calculated using the pseudo first order model described in Chapter 4 (Eqs. 4.1-4.5), are presented in Table 5.1

Table 5.1: Forward and reverse rate constants for adsorption to beached and virgin pellets exposed to 5 µg L^{-1} trace metals in seawater (Sw) and river water (Rw).

	Beached pellets						Virgin pellets					
	Rw			Sw			Rw			Sw		
	k_1, h^{-1}	k_{-1}, h^{-1}	r^2	k_1, h^{-1}	k_{-1}, h^{-1}	r^2	k_1, h^{-1}	k_{-1}, h^{-1}	r^2	k_1, h^{-1}	k_{-1}, h^{-1}	r^2
Cd	0.085	0.224	0.64	0.002	0.265	0.13	0.010	1.46	0.48	0.003	12.2	0.90
Co	0.041	0.256	0.46	0.089	2.04	0.55	0.002	1.08	0.88	0.010	1.18	0.97
Cr	0.099	0.370	0.83	0.061	0.025	0.60	0.002	0.130	0.72	0.055	0.453	0.97
Ni	0.087	0.271	0.93	0.464	4.82	0.91	0.002	0.424	0.85	0.014	0.834	0.93
Pb	0.369	0.217	0.60	0.210	0.130	0.91	0.036	0.255	0.64	0.002	0.668	0.99

Values of k_1 and k_{-1} indicate adsorption is reversible, with the magnitudes of k_{-1} exceeding k_1 in most instances. Notably, however, adsorption of Cr to beached and virgin pellets in seawater is less reversible than in river water. Lead adsorption to beached pellets exhibits a lesser degree of reversibility than other metals, with values of k_1 slightly exceeding those for k_{-1}. Lead adsorption to virgin pellets, however, exhibits the same pattern as other metals, with k_{-1} exceeding k_1. Where k_{-1} in seawater is greater than k_1 in river water, it can be inferred that there is potential for adsorbed metal concentrations to be released where increasing salinity is encountered. The opposite may be true where k_{-1} in river water exceeds k_1 in seawater, as in the case of Cr on beached and virgin pellets, suggesting mobilisation of Cr may occur if pellets are exposed to decreasingly saline conditions.

The chemical response time, T_{resp}, has been used to compare between metals and identify whether adsorption kinetics are altered by variations in salinity. The derivation of T_{resp} is described in full in Section 4.3. Briefly, T_{resp} is calculated from the reciprocal of the sum of forward and reverse rate constants, and gives an indication of the time required for analytes in an experimental system to reach 63 % of their equilibrium values. The values of T_{resp}, summarized in Table 5.2 illustrate the differences between time to equilibrium for trace metal adsorption to beached and virgin pellets in river and marine end members. For Cd, Co and Pb, T_{resp} in river water exceeds that in seawater for virgin and beached pellets. The same can be said for Ni only for beached pellets, since T_{resp} for virgin pellets is comparable in seawater and river water. For Cr the greater T_{resp} values are seen in seawater for beached pellets and river water for virgin pellets.

Table 5.2: Mean (*n* = 3) chemical response times (T_{resp}) for trace metal interactions with virgin and beached pellets in river water (^{rw}) and seawater (^{sw}).

	Beached pellets		Virgin pellets	
Element	T_{resp}^{rw}, h	T_{resp}^{sw}, h	T_{resp}^{rw}, h	T_{resp}^{sw}, h
Cd	3.23	1.63	0.681	0.167
Co	3.38	0.843	0.928	0.843
Cr	2.13	11.6	4.46	1.97
Ni	2.80	1.18	2.35	2.96
Pb	1.71	1.49	3.44	2.96

5.3.2 Adsorption isotherms

Adsorption isotherms determined in river water are presented in Figure 5.3 alongside observations determined in seawater (reported in Section 4.3.4) to allow direct comparison. Adsorption of Cd, Co, Cr, Ni and Pb to beached and virgin pellets in river water were modelled with Langmuir and Freundlich equations (Equations 4.4 and 4.5

respectively). Values for Langmuir and Freundlich constants are presented in Table 5.3, while results of Langmuir modelling are annotated on Figure. 5.3.

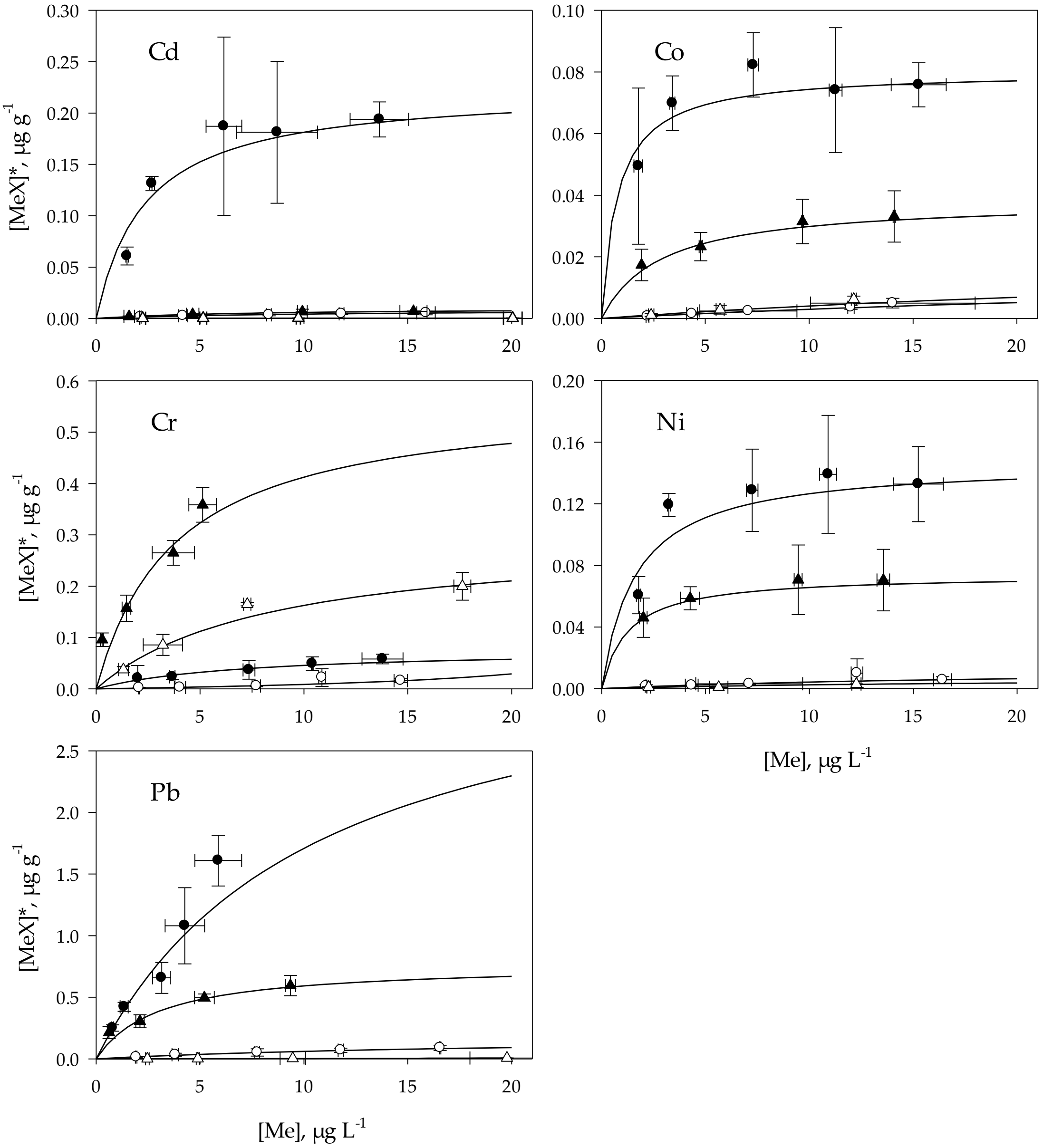

Figure 5.3: Comparison of adsorption isotherms for virgin and beached pellets (open and filled symbols, respectively) in river water and seawater (circles and triangles, respectively). Data points indicate the mean of three replicate incubations, with error bars denoting one standard deviation. Lines represent Langmuir model fits to data. Langmuir and Freundlich constants are presented in Table 5.3.

Figure 5.3 shows that adsorption to beached pellets exceeds that to virgin pellets in river water, consistent with comparisons between virgin and beached pellets in Chapter 4. Furthermore, adsorption isotherms presented in Figure 5.3 highlight differences between the extent of adsorption in river water and seawater, in agreement with Figure 5.2. Adsorption of Cr is greater in seawater than in river water, while adsorption of Cd, Co, Ni and Pb in river water exceeds that in seawater. For Pb, this is in disagreement with Fig. 5.2, which suggests that adsorption to beached pellets in river water and seawater should be similar.

The constants presented in Table 5.3 indicate that generally, virgin pellets exhibit better fits to the Freundlich model, while beached pellets are better explained by Langmuir adsorption. The maximum concentrations predicted to adsorb to beached pellets suggest that Pb will accumulate to concentrations over one order of magnitude greater than Cd, Co, Cr or Ni.

Table 5.3. Constants defining trace metal adsorption to virgin and beached polyethylene pellets in river water according to the Langmuir and Freundlich models (Equations 4.8 and 4.9, respectively).

	Langmuir constants						Freundlich constants					
	Virgin			**Beached**			**Virgin**			**Beached**		
	$[MeX]^*_{max}$ µg g^{-1}	K_L ml g^{-1}	r^2	$[MeX]^*_{max}$ µg g^{-1}	K_L ml g^{-1}	r^2	$1/n$	K_F $µg^{1-1/n}$ g^{-1}	r^2	$1/n$	K_F $µg^{1-1/n}$ g^{-1}	r^2
Cd	0.009	0.091	0.60	0.224	0.427	0.73	0.615	0.0007	0.97	0.471	0.062	0.84
Co	0.019	0.018	0.11	0.080	1.293	0.87	0.950	0.0004	0.85	0.205	0.048	0.22
Cr	-	-	-	0.093	0.106	0.88	1.23	0.0006	0.81	0.380	0.018	0.34
Ni	0.013	0.046	0.16	0.147	0.617	0.85	0.812	0.0007	0.57	0.319	0.063	0.52
Pb	0.191	0.050	0.84	2.74	0.124	0.78	0.773	0.0107	0.78	0.820	0.316	0.85

5.3.3 Adsorption in an estuarine gradient

To examine changes in trace metal affinity for pellets through the salinity gradient, partition coefficients (K_D) were calculated as they are widely used to compare the affinity of trace metals for particulates across a range of different conditions. Values are plotted as a function of salinity for virgin and beached pellets in Figure 5.4, where it can be seen that partitioning to beached exceeds that to virgin pellets in all instances.

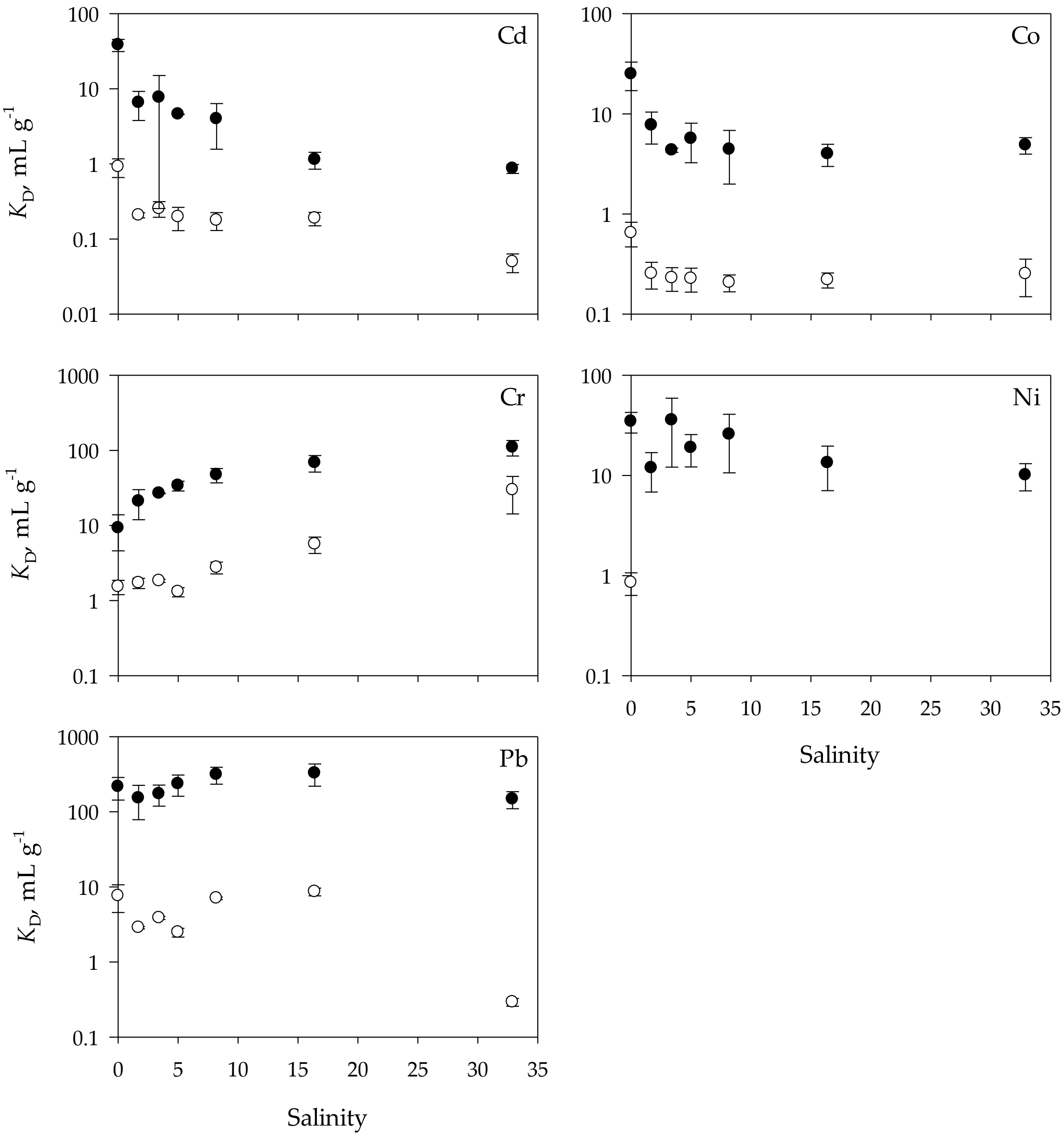

Figure 5.4: Partition coefficients, K_D (mL g^{-1}), for Cd, Co, Cr, Ni and Pb associations with beached (●) and virgin (o) polyethylene pellets as a function of salinity in waters prepared from combined river and marine end-members. Symbols denote mean values ± one standard deviation (n = 3).

Adsorption to virgin pellets

As discussed in Chapter 4, virgin plastic pellets exhibit a lesser affinity for trace metals than do beached pellets. This is evident for all metals considered, and may be attributed to the development of charged sites and accretion of reactive inorganic and organic coatings on the pellet's surface as it is aged in the environment.

While exhibiting lower K_D values than beached pellets, virgin pellets are shown to adsorb trace metals through the salinity range typical of an estuarine system. Partitioning of Cr increases alongside salinity, with K_D values rising from 1.53 to 29.7 mL g^{-1} between riverine and marine end-members.

Cadmium adsorption to virgin pellets is highly sensitive to increasing salinity, with an exponential decrease in K_D, from 0.92 – 0.20 mL g^{-1} evident between salinities of < 0.05 and 5 as shown in Figure 5.4, continuing to decrease to 0.05 mL g^{-1} at salinity 33. Again, this indicates the low affinity of Cd to virgin pellets throughout the salinity range when compared with natural sediment, which is on the order of 10^4 mL g^{-1} (Tang, *et al*., 2002). Cobalt partitioning to virgin pellets exhibits a trend similar to Cd within the low salinity range, with an exponential decrease in partitioning from 0.65 – 0.23 mL g^{-1} between salinities of < 0.05 and 5. Further increases in salinity appear to have minimal effect on Co partitioning, with K_D stabilising between 0.21 and 0.25 mL g^{-1}. Nickel adsorption to virgin pellets was below detection limits at all salinities, with the exception of riverine end member, where K_D is 0.85 mL g^{-1}, indicating a low affinity for virgin pellets even in river water. The partitioning of Pb exhibits no clear systematic relationship with salinity. However, the marine end-member appears to retard Pb adsorption to virgin pellets, with K_D decreasing by more than one order of magnitude between brackish (2.5 – 8.6 mL g^{-1}) and marine end-member waters (0.3 mL g^{-1}). These values indicate a low affinity of Pb to virgin plastics, considering K_D values for partitioning of Pb to natural sediments is of the order 10^5 mL g^{-1} (Tang *et al*., 2002).

5.3.3.1 *Adsorption to beached pellets*

To consider adsorption to beached pellets, Cr exhibits a similar trend as reported for virgin pellets, with a concomitant increase in K_D and salinity – an approximately linear partitioning-salinity relationship (Fig 5.3). However, K_D values for adsorption to beached pellets are approximately one order of magnitude greater than those for virgin pellets. Both Cd and Co exhibit consistent behaviour between virgin and beached pellets with regard to changes in partitioning through the salinity gradient, Pb partitioning to virgin and beached pellets does not exhibit this trend.

As observed for virgin pellets, Cd adsorption to beached pellets exhibits a high degree of sensitivity, with an exponential decrease in K_D with increasing salinity. The maximum K_D value for Cd, 38.4 mL g^{-1}, drops to 6.5 mL g^{-1} between the riverine end-member and a preparation of experimental water of salinity 1.7, reaching a minimum K_D of 0.87 mL g^{-1} in the marine end-member. This compares with a marine end-member K_D of 0.05 mL g^{-1} for virgin pellets.

Cobalt adsorption to beached pellets exhibits a similar trend to that of Cd, with K_D decreasing exponentially from 25 to 4.87 mL g^{-1} between river and marine end-members – remaining over one order of magnitude greater than K_D for virgin pellets (≈ 0.23 mL g^{-1}). Nickel is less sensitive to changing salinity than are Cd or Co, with a less pronounced decrease in K_D with increasing salinity. However, there is an apparent decrease in partitioning in the upper salinity range, dropping from a river end-member K_D of 35 mL g^{-1} to 10 mL g^{-1} in the marine end-member.

Despite a degree of variability, the affinity of Pb for beached pellets does not appear to be affected by variations in salinity. This effect has been observed in the context of natural sediments by Schafer *et al.* (2009) and Gambrell *et al.* (1991), where salinity did not significantly affect Pb mobility. However, this contrasts with Pb partitioning to virgin pellets, for which there was an order of magnitude difference in partitioning between intermediate salinity and seawater. This difference between virgin and beached pellets may be due to the weakly bound Pb being more readily out-competed for surface sites on virgin pellets.

For the two metals exhibiting an exponential decrease in K_D with increasing salinity (S), the empirical partitioning-salinity relationship can be defined as follows (Turner & Millward, 1994):

$$\ln K_D = b[\ln(S+1)] + \ln K_D^0 \qquad \text{Eq. 5.1}$$

where S is salinity; and K_D^0 and b are data fitted constants derived from a plot of $\ln K_D$ versus $\ln(S+1)$. This equation suggests that that partitioning-salinity relationships will be similar in a wide range of estuaries, since changes in partitioning will be primarily controlled by the magnitude of the constants b, the sensitivity of K_D to salinity; and K_D^0, the partitioning in water with a salinity of zero (Turner, 1996). Values for b and K_D^0 are presented in Table 5.4.

Table 5.4: Empirical constants defining the K_D – salinity relationships of Cd and Co (Eq. 5.1).

Pellet type	Metal	K_D^0, mL g^{-1}	b	r^2	P
Beached	Cd	31.6	-1.08	0.94	0.0003
	Co	14.2	-0.438	0.62	0.035
Virgin	Cd	1.03	-0.657	0.80	0.006
	Co	0.339	-0.232	0.46	0.093

Values of K_D^0 indicate the hypothetical partitioning in water of salinity 0; in this instance the values are below empirical mean K_D values in river water. Discrepancies between calculated K_D^0 values (Table 5.4) and empirical data presented in Figure 5.4

may be attributed to deviation from the non-linear regression used to define the constants. From the magnitudes of b in Table 5.5 it is clear that adsorption of Cd to beached pellets is more sensitive to changing salinity than is Co (constants for b of -1.08 and -0.438, respectively). These relationships between partitioning and salinity are shown to be significant, with Cd and Co exhibiting P values of < 0.001 and < 0.05, respectively. For virgin pellets, the r^2 value indicates that Co adsorption exhibits a poor fit to the power law which typically defines adsorption through the simulated salinity gradient ($r^2 = 0.46$, $P > 0.05$). Cadmium adsorption to virgin pellets, however, exhibits a statistically significant fit ($r^2 = 0.8$; $P < 0.01$) to this equation.

5.3.4 Metal speciation modelling

Using WHAM calculations as a tool to visualise changes in speciation which are developed through an estuarine gradient, it is possible to identify the predominant mechanisms of adsorption to the plastic particle surface. Speciation data were calculated using input parameters consistent with the estuarine gradient used in incubations. Figure 5.5 presents speciation data as a function of salinity for Cd, Co, Ni and Pb, for which complexation constants are available within the WHAM master database. Chromium is omitted from speciation calculations on the basis that Cr speciation in oxic waters is dominated by oxyanions. Within this experiment, Cr is assumed to be present as Cr(VI) owing to the addition of Cr(VI) and oxygenated conditions of the incubations. Turner *et al*. (1981) state that Cr(VI) speciation in freshwater at pH 6 is $HCrO_4^-$ (74 %) and CrO_4^{2-} (26 %), while in seawater of pH 8.2, CrO_4^{2-} (71 %) and $NaCrO_4^-$ (28 %) are the dominant species.

By plotting metal speciation as a function of salinity, changes in the composition of the aqueous medium are shown to alter chemical behaviour within the experimental system used for incubations. Cadmium is evidently dominant as Cd^{2+} at very low salinities, with considerable chloro-complexation occurring as salinity exceeds 5, where > 90 % Cd is present as $CdCl^+$ and $CdCl_2$. Additionally, it is apparent that organic complexation plays a major role in Cd speciation in river water. However, this declines steeply with salinity, with < 1 % Cd associated with organic ligands at a salinity of 5. This corresponds with Turner *et al*. (2008a) who modelled Cd speciation in the Beaulieu estuary using measurements of hydrophobic Cd to define organic complexation.

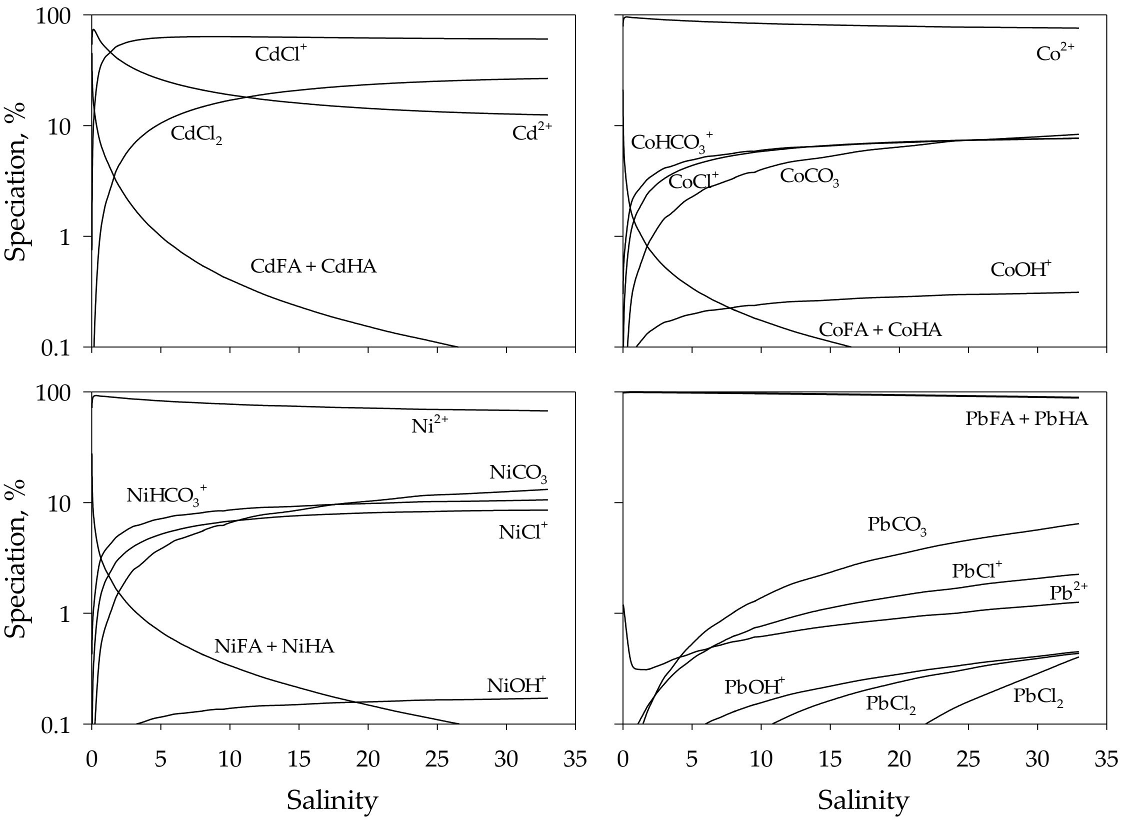

Figure 5.5: Aqueous speciation of dissolved Cd, Co, Pb and Ni modelled using WHAM, at trace metal concentrations of 5 μg L^{-1}. The component denoted (Me)FA +(Me)HA refers to the sum of metal associated with colloidal fulvic and humic acids.

Cobalt speciation is dominated by the free ion throughout the estuarine gradient, decreasing from approximately 90 % in river water to 70 % in sea water. Cobalt is increasingly complexed by carbonate and chloride ions through the estuarine gradient, with the most pronounced changes occurring between river water and waters with salinity of 5. Above a salinity of 5 the relative significance of these complexes is approximately constant. Organically complexed Co represents 10 % of total Co in river water, decreasing through the salinity gradient to < 0.1 % at a salinity of 17.

Nickel exhibits speciation behaviour very similar to Co, however, the importance of different inorganic complexes varies between the two metals. Despite differences in relative importance of different ligands, the sum of carbonate and chloride species of Ni is comparable to that of Co, at approximately 30 % above a salinity of 10, in agreement with Turner & Martino (2006), who modelled Ni speciation in the Tweed estuary. Complexation of Ni with FA and HA decreases as salinity increases (Fig. 5.5), falling from > 20 % in river water, to 0.1 % at a salinity of 26.

According to model calculations, Pb speciation is dominated by organic complexation through the estuarine gradient, decreasing from 99 % in river water to 82 % in seawater. This effect has been demonstrated in the salinity range 15 – 35 by Turner (2008b), where complexation by spiked humics exceeded 65 % through the salinity gradient. As salinity approaches that of the marine end member, $PbCO_3$ increases gradually to a maximum of 8 %, while Pb^{2+} is present at < 1 % throughout the salinity range.

To further develop the mechanistic understanding of trace metal adsorption to pellets it is possible to compare speciation directly with partitioning behaviour. It is reasonable to assume that a relationship should exist between the partition coefficient and the relative abundance of a metal species which is interacting with the sorbent surface. To take Cd as an example, there is an approximately linear relationship between K_D and abundance of Cd^{2+}, expressed as the free ion activity of Cd^{2+}, suggesting the association of Cd^{2+} with the pellet surface (Fig. 5.6). This supports the notion that free ionic Cd^{2+} is the species of Cd interacting with plastic pellets.

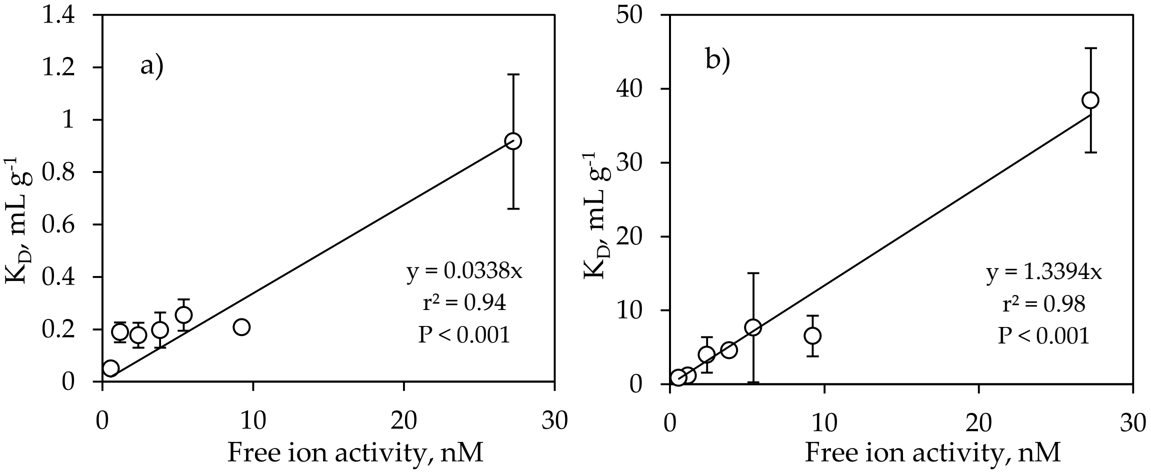

Figure 5.6: Partition coefficients defining Cd adsorption to plastic pellets at a range of salinities versus relative abundance of Cd^{2+} (as % of total Cd), for virgin (a) and beached (b) pellets. Concentrations of the free ion were calculated using WHAM 6. Error bars represent one standard deviation about the mean of three replicates.

If adsorption of Cd were entirely reliant on the free ion activity of Cd^{2+}, then it would be expected that this relationship between K_D and the free ion activity of Cd^{2+} would be linear, with no intercept. The linear plots expressed in Figure 5.6 suggest interactions between Cd^{2+} and pellets are important, with r^2 values of 0.95 ($P < 0.01$) and 0.98 ($P < 0.01$) for plots for virgin and beached pellets, respectively. Cobalt also exhibits this relationship, although respective r^2 values of 0.70 and 0.79 for virgin and beached pellets indicate that relationship between K_D and free ion activity is weaker in these instances. For other elements, there was no clear relationship observed between calculated speciation and partitioning.

For other metals there is not such a clear relationship between the proportion of the metals present as divalent metal cations and partitioning, which suggests there may be other processes governing trace metal-pellet interactions not being taken into account by speciation calculations. Specifically, changes to particle surfaces effected by changes in salinity could cause changes in partitioning behaviour. Additionally, as the model outputs are is based on literature values for DOC and default parameters defining organic matter, it is likely that it only affords an estimate of the interactions in the experimental system.

5.3.5 Influence of pH on trace metal adsorption

To determine the environments likely to lead to metal associations with pellets, and further investigate the mechanisms of adsorption of trace metals to plastic pellets, adsorption was investigated as a function of pH. Figure 5.7 presents trace metal partitioning to pellets as a function of equilibrium pH in filtered river water, widely described as pH adsorption edges.

Adsorption of Cd, Co and Ni to beached pellets exhibits sigmoid curves typical of transition metals (Hatje *et al*., 2003). However this may not be immediately evident owing to the log scale used in Figure 5.7 to present virgin and beached pellets in a comparable format. From these observations, plastic-bound Cd, Co and Ni speciation can be identified as cationic, if it is assumed that the surface charge on the plastics is negative, as shown for PE pellets by Fotopoulou & Karapanagioti (2012). The widely recognised increasing affinity of cations for SPM with increasing pH (Phillips *et al*.,

2004) suggests it is the free aqueous ion which is predominantly associated with plastic pellets within this experiment. The observed enhanced metal uptake with increasing pH can be attributed to increasing affinity for deprotonated sites, as suggested for natural particulate surfaces by Warren & Haack, (2001).

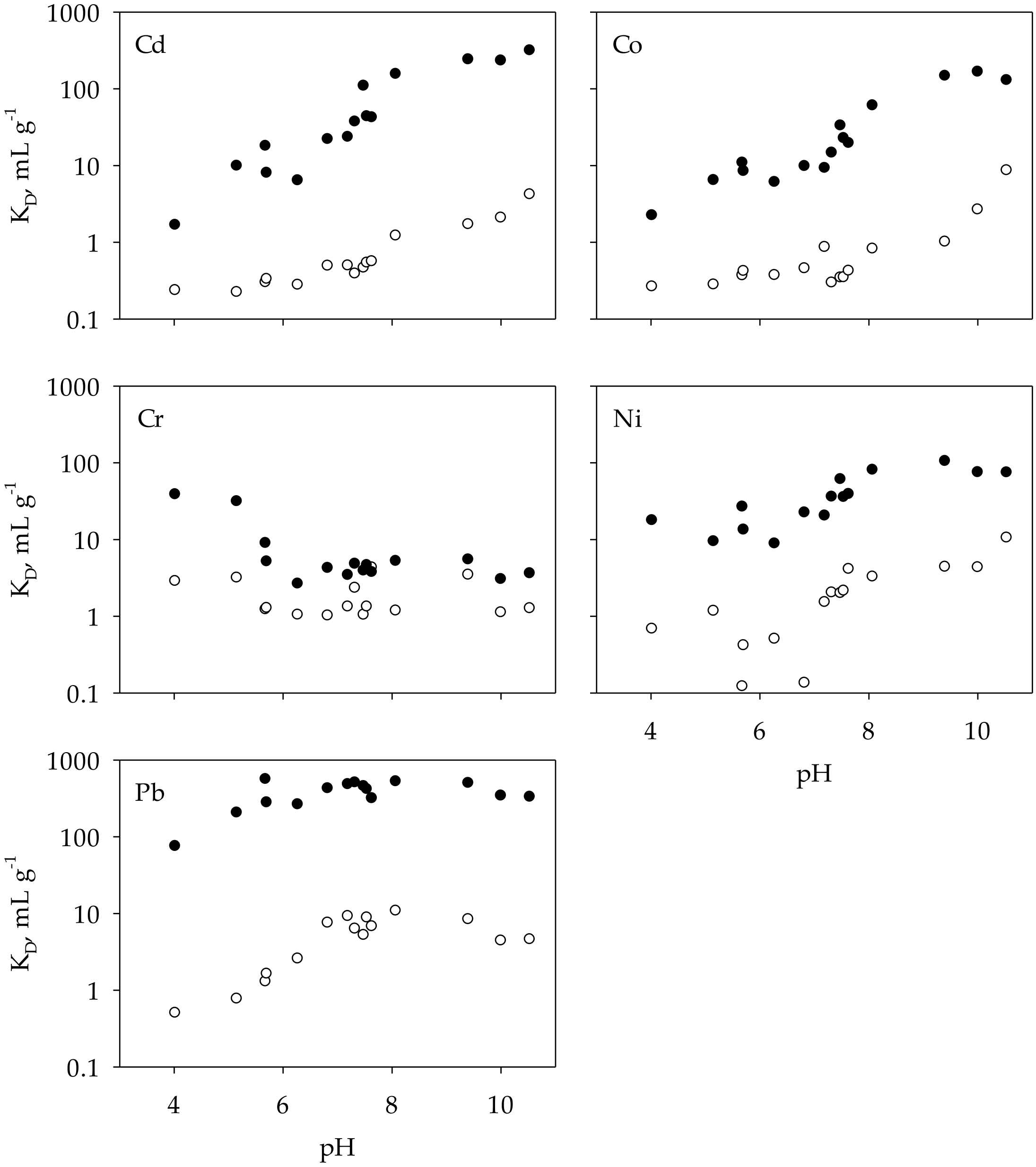

Figure 5.7: Adsorption edges for pH. Beached (●) and virgin (o) polyethylene resin pellets exposed to trace metals Cd, Co, Cr, Ni and Pb in filtered river water for 24 h.

Adsorption to beached pellets increased over a range of 2 pH units between pH 6 and 8 for Cd and Ni, and between 7 and 9 for Co. While virgin pellets exhibit minimal adsorption for all metals, there is a slight increase in uptake at the upper range of pH (Fig 5.7). Chromium exhibits a sigmoidal curve for adsorption to beached pellets, decreasing in affinity as pH increases, predominantly between pH 5 and 6, indicative of anionic speciation. Adsorption of Cr to virgin pellets shows no clear pH dependency.

Affinity of Pb for pellets is greatest of the metals in this experiment. For example, partitioning to beached pellets increases from a K_D of 75 mL g^{-1} at pH 4 to reach K_D values over 450 mL g^{-1} as pH exceeds 6.5, however the slope for Pb suggests the pH range examined was not wide enough to encompass the entire adsorption edge, with the steepest section of the curve being terminated below pH 4. Lead adsorption to virgin pellets increases slightly in the mid-range of pH values, but is minimal when compared to the extent of adsorption to beached pellets, reaching a maximum K_D of 10.8 mL g^{-1} at pH 8.

5.4 Discussion

While trace metal adsorption from natural waters has been widely studied with regard to natural particulates, very little research has considered associations of trace metals with synthetic polymers, which are now ubiquitous in the environment (Thompson *et al*., 2004). Furthermore, the complex conditions which define estuarine systems are yet to be considered for any chemical adsorption studies focusing on plastic debris, despite extensive research into the associations of POPs with plastics, particularly resin pellets, in the marine environment (Mato *et al*., 2001; Endo *et al*., 2005; Teuten *et al*., 2009).

Throughout the present study, the changing chemical conditions within an estuarine system have been shown to alter the characteristics of trace metal adsorption to polyethylene pellets considerably, while differences between virgin and beached pellets, noted in Chapter 4, remain present.

5.4.1 Particulate surface properties

It is widely accepted that for natural particulates there is a strong functional relationship between K_D and salinity (Turner, 1996; Turner & Millward, 2002; Hartnett *et al*., 2006). This may be due to the presence of dissolved chloride or carbonate anions which mobilise trace cations by forming soluble complexes (Du Laing *et al*., 2009), and competition for adsorption sites from dissolved cations such as Ca^{2+} or Mg^{2+} which are abundant in seawater (Hatje et al., 2003; Phillips *et al*., 2004). Additionally, the changes to surface properties which result from changing estuarine conditions have consequences for the affinity of dissolved metals for particle surfaces.

Most particulates have a net negative charge when immersed in natural waters (Newton & Liss 1987), but become decreasingly electronegative as the salinity of the system increases (Hunter & Liss, 1982). Zachara *et al*. (1987) observe that Ca^{2+} adsorption reduces the magnitude of the net negative charge, electrostatically encouraging anion retention. Therefore, the increasing affinity of Cr for plastic surfaces with salinity may be attributed to anionic forms of chromium (CrO_4^{2-} , $HCrO_4^-$) adsorbing to decreasingly electronegative sites on particle surfaces, as illustrated in Figure 5.8.

In addition to the acute changes to particulate surfaces caused by changing ionic strength of the medium with tidal cycling, more long-term changes are known to progressively alter a plastic surface exposed to sunlight in the aqueous environment. This concept was proposed in Section 4.4 and supported by evidence of photo-oxidation in plastics in studies by Sudhakar *et al*. (2007) and Artham *et al*. (2009), and specifically by the photo-oxidation of plastic resin pellets as shown by Turner & Holmes (2011).

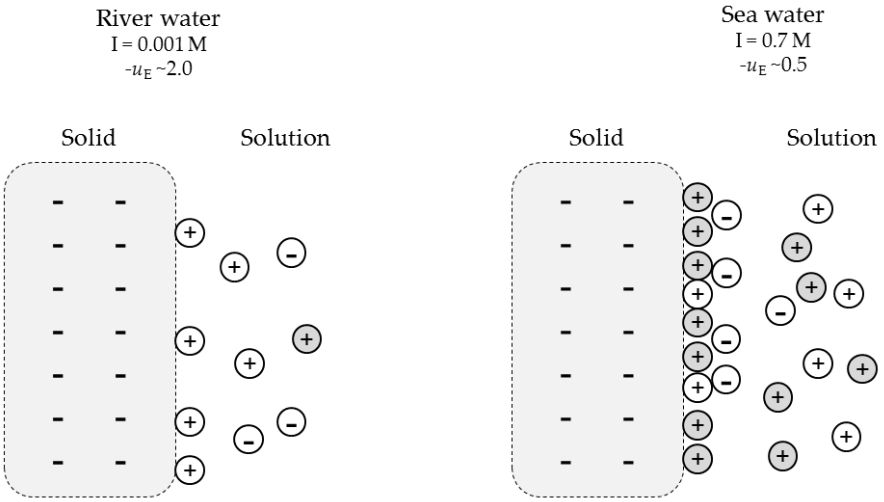

Figure 5.8: The effect of increasing ionic strength (*I*) on suspended particle surfaces. *I* represents the approximate ionic strength of river water and seawater, and *u*E is the surface charge (10^{-8} m^2 s^{-1} V^{-1}). Trace metals (white circles) bound to surface organic matter or hydroxyl sites can be displaced by seawater cations (filled circles), reducing the magnitude of the negative charge *u*E, minimising repulsive forces between the particle surface and anionic metal species (adapted from Turner & Millward, 2002).

At all salinities, and for all metals studied, partitioning to beached pellets exceeds that of virgin pellets, which reinforces this concept of changing surface properties which are developed as plastics are aged in the natural environment. From this it can be conceived that persistent plastics will become more reactive towards dissolved metals as they are weathered, increasing in surface area as well as in the number of viable sites through processes of biofouling and accretion of inorganic material. Virgin pellets therefore enter the environment as a relatively inert material and through the processes mentioned above, will develop a surface similar to a natural sediment particle over time.

The surface charge of beached pellets has been identified as negative above pH 6.1 by Fotoupoulou & Karapanagioti (2012), which supports observations shown in Figure 5.7. This suggests associations of trace metals predicted to be cationic in river water (Fig. 5.5), with pellet surfaces are enhanced by deprotonation of surface sites as pH increases.

5.4.2 Mechanisms of adsorption

Chemical speciation modelling has facilitated the interpretation of the mechanisms driving changes in partitioning behaviour through the estuarine salinity gradient as shown in Figure 5.4. Changes in speciation over the salinity gradient as calculated using WHAM are shown in Figure 5.5 and have been combined with K_D values to interpret associations of chemical species with suspended particles – in this instance, plastic pellets (Fig. 5.6). These various comparisons and calculations are intended to elucidate the mechanisms by which trace metals are being accumulated on plastic pellets. The relationship between free ion activity and partitioning of Cd is quite clear, with the chloro-complexation driving the change in partitioning through the salinity gradient; however this relationship does not explain the behaviour of the other metals investigated.

To consider the partitioning-salinity relationship for Co, the reduction in K_D with increasing salinity is substantial (Fig. 5.4), however, modelled Co speciation indicates that complexation with dissolved anions is likely to be less extensive than Cd, with > 75 % Co remaining in solution as Co^{2+}. This decrease in K_D despite the presence of Co^{2+} through the salinity gradient suggests that the predominant mechanism behind the limited affinity of Co for pellets with increasing salinity is competitive adsorption by dissolved seawater cations. This may also be the case for Ni, for which the partitioning in river water is greater than in seawater, despite the presence of Ni^{2+} in marine end-member waters (67 % of total Ni).

Lead adsorption is shown to be largely independent of salinity (Figs. 5.2 & 5.4), as has been observed for marsh sediment (> 0.45 μm) by Gambrell *et al*. (1991), and only slightly affected by changing pH (Fig. 5.7). Speciation analysis indicates that Pb has a high affinity for dissolved organic carbon in this system, which may be driving the adsorption of Pb to pellets at all salinities. Lead is also known to be highly reactive towards particle surfaces (Tang *et al*., 2002), with a high affinity for Fe oxy-hydroxides (Lion *et al*., 1982). Therefore, adsorption of Pb will be altered in the natural environment by changes in the concentration of dissolved organic matter, and oxides of Al, Fe and Mn which will continue to develop over increasing exposure times in natural systems. Owing to the consistent K_D over the salinity range, it is likely that plastics may contribute a long-range transport medium for Pb as it will not be removed by changing water chemistry.

Chromium displays contrasting behaviour to that of Cd, Co and Ni, exhibiting increased partitioning to beached and virgin polyethylene pellets with increasing salinity (Fig. 5.4). Chromium (VI) is present in oxygenated environments predominantly as dissolved oxyanions which have been shown to exist in this experimental system by the pH sorption edge indicative of anionic speciation (Fig. 5.7), where the greater adsorption in the low pH range indicates the presence of anionic Cr species. This corresponds with the increasing affinity of Cr for pellet surfaces with increasing salinity. Enhanced adsorption of Cr in seawater when compared to freshwater has been reported by Hatje *et al*, (2003), where it was attributed to the pH dependence of Cr adsorption to estuarine SPM (< 63 μm). Conversely, while pH increases alongside salinity within this investigation, the effect described above is not supported by the adsorption edge for Cr, where adsorption is greatest between pH of 4 and 6 (Fig. 5.7), dropping significantly at greater pH. Therefore changes in the particle surface due to salinity (as described in Figure 5.8) present a more important driver of Cr partitioning than pH within these experiments.

5.4.3 Environmental Implications

While empirical and modelled data represent one specific system, many of the observations are likely to be more generally applicable. The present work has shown rates of adsorption to occur rapidly through the full range of conditions typical of a tidal estuary, and this must be borne in mind when considering the likely fate of plastic-associated trace metals within an estuarine system. If it is assumed that pellets remain in the water column, they present a mobile particle carried by surface water, and are likely to be retained within an estuary for a time approximately equal to the flushing time of that system (Turner *et al*., 1994). Timescales for this process exhibit inter- and intra-estuary variability as a result of differences in estuary size, tidal regimes and seasonal variations in river discharge (Morris, 1990). Whole estuary flushing times, or freshwater turnover times, are known to range from one tidal cycle in the smallest, to more than one year in the largest systems (Morris, 1990). Greater flushing times allow for chemical reactions to propagate over a longer period, yet the rapid adsorption kinetics exhibited by pellets suggest it is likely that equilibrium will be reached in all but the smallest of estuaries.

Trace metals will be either accumulated and/or released from the pellet surface as it is transported through an estuary. The spatial scale of this phenomenon will be a function of the sensitivity of each metal's K_D to salinity, the type of estuary (i.e. salt wedge/well mixed, etc.), and the length of the estuary, owing to the different spatial scales over which salinity can vary as illustrated in Figure 5.9.

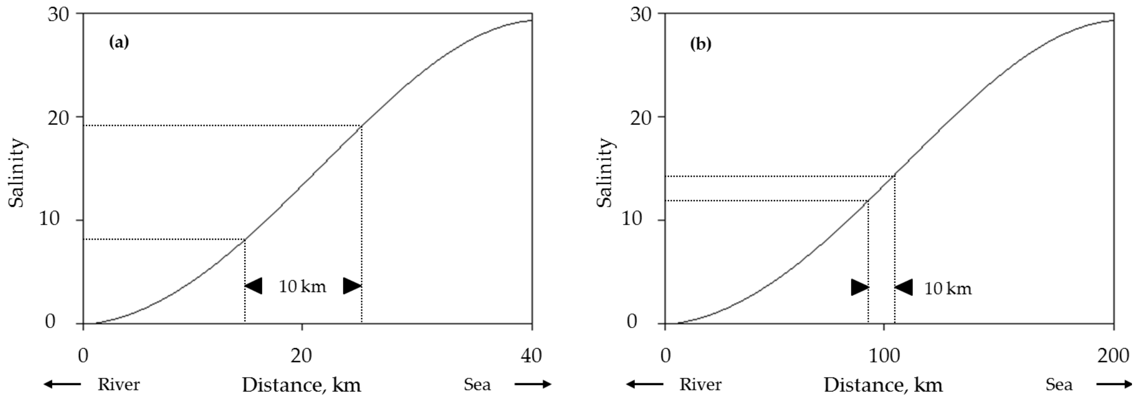

Figure 5.9: Variations in salinity encountered by a suspended during a 10 km axial transit through hypothetical small (a) and large (b) estuaries (adapted from Morris, 1990).

Owing to their steep salinity gradients (Fig. 5.9a), small size and rapid flushing times, smaller estuaries do not present systems in which long range transport of those trace metals associated with pellets, whose K_D decreases with increasing salinity will occur. However, the less steep salinity gradients (Fig. 5.9b) and greater freshwater residence times of larger systems may allow longer range transport of trace metals bound to plastics.

The importance of differences between partitioning in riverine- and marine-end members has connotations for transport of metals which are associated with plastic particles. Low K_D values for Cd, Co and Ni in seawater suggest they may accumulate on plastics above the tidal limit of a system. As they are transported seaward through the estuary, they are likely to release any trace metal concentrations which exist in exchangeable phases, owing to the combined effects of competition by seawater cations and complexation with seawater anions. This has been confirmed by the relative magnitudes of k_1 and k_{-1} shown in Table 5.1, which indicates reversibility of the trace metal concentrations adsorbed to the plastic surface. Contrastingly, Cr will be accumulated more readily under more saline conditions, which indicates that transport and release of Cr by plastic pellets may be more important as plastics are transported up-estuary. This observation is supported by the relative magnitudes of k_1 and k_{-1} presented in Table 5.1 which indicate reversibility of adsorption as Cr is transported on a pellet surface from seawater to river water conditions.

Assessment of Pb transport by plastic particles within estuarine systems is difficult to interpret, as there appears to be few environmental factors which would facilitate release of Pb from pellet surfaces, except perhaps in the event of significant input of dissolved organic material to the system. This suggests that long-range transport of Pb associated with buoyant plastic particles is possible.

While the waters of the river Plym had a slightly acidic pH of 6.8, other systems will present different conditions, and it is valuable to understand which systems are most likely to promote adsorption of metals to plastics. The adsorption edges presented in Figure 5.7 indicate that Cr will be more readily accumulated on plastic surfaces in acidic, rather than basic environments, while basic conditions favour Cd, Co and Ni adsorption to plastics, and Pb is relatively pH independent through the typical range expected in river water. Therefore contaminated river waters draining basic catchments present the most favourable conditions for the uptake of Cd, Co and Ni, while acidic river catchments will promote Cr adsorption. As salinity increases, the increasing abundance of dissolved ions takes precedence over pH as the main driver of trace metal affinity for plastics, and concentrations may then be released as the particles are transported through the estuarine gradient. In addition to changes in pH and salinity of the system, other changes to the composition of the medium may cause changes to trace metal affinities to plastics. Notably, the concentration and composition of dissolved organic matter may influence trace metal speciation and associations with particulate matter.

Rapid uptake kinetics may cause plastics passing through highly contaminated areas to rapidly concentrate trace metals, and subsequently be transported into less impacted areas locally, or ingested by organisms from trophic levels ranging from benthic invertebrates (Graham & Thompson, 2009) to predators including seabirds (Furness, 1985; van Franeker, 2011), potentially conveying bioaccessible metals to the organism (Ryan *et* al., 1988; Teuten *et al*., 2009; Colabuono *et al*,. 2010). Longer-term trends in trace metal cycling may be controlled changes to plastic particles caused by exposure to natural conditions, with plastics increasing in surface area and availability of sites for adsorption.

Typically, trace metals bound to natural particulates would be removed from the water column by processes of flocculation and settling (Chiffoleau *et al*., 1994), while trace metals associated with buoyant plastics will be mobile over greater distances. This may be of minor importance when plastic concentrations are low. However with the progressive degradation and fragmentation of plastic debris in the natural environment, and continued addition of microplastics to freshwater and marine systems, the future potential for plastics to alter trace metal cycling must be considered.

The K_D values presented for adsorption of metals to plastic pellets are several orders of magnitude lower than those described in the literature for natural SPM (See Turner & Millward, 2002; Tang *et* al., 2002; Takata *et* al., 2010), suggesting that pellets are unlikely to concentrate metals to levels exceeding those found in sediments. However, the specific surface area of natural sediment is several orders of magnitude greater than plastic pellets, which may explain these differences. Furthermore, the increasing specific surface area and enhanced chemical reactivity of progressively degrading plastic particles will bring K_D values for plastics closer to those presented by natural particulates. This is an important point as it has been shown in Chapter 3 that virgin plastics are being degraded by photo-oxidation, and are accumulating concentrations of metals including Fe and Mn, oxyhydroxides of which are known to have high geochemical reactivity. It must be considered when evaluating the potential impacts of plastics within natural systems that changes to the properties of plastic debris are occurring, and that these changes may alter the interactions between plastics and natural systems which have been noted above.

5.5 Conclusions

Plastic resin pellets have been considered an important anthropogenic contaminant of marine systems for decades, with recent work focusing on their chemical properties within marine systems. Here for the first time it has been shown that synthetic polymers can behave similarly to natural particulates for some metals, *vis-à-vis* adsorption characteristics under conditions of changing salinity and pH. The estuarine environment is important for the cycling of trace elements, and with increasing inputs of plastics into such systems it is likely that trace metal cycling will be altered. This effect may be particularly important where microplastic inputs to estuarine systems make significant contributions to the particulate flux. The work presented here demonstrates the complex interactions between trace metals and the surfaces of plastic pellets in an estuarine system. These observations will stimulate further research on the interactions between plastics and a range of other pollutants under freshwater and estuarine conditions, for which there is currently a significant gap in knowledge.

Chapter 6:

In vitro determination of metal bioaccessibility from resin pellets to avian species

6.1 Introduction

As described in the preceding chapters, plastic debris is becoming increasingly pervasive in natural systems with many obvious consequences, such as entanglement of organisms, smothering of habitats and ingestion - impacts known to affect over 660 species (Convention on Biological Diversity, 2012). The number of species with records of plastic ingestion exceeds 190 according to the most recent review (Convention on Biological Diversity, 2012) but is increasing as more research is conducted. Plastic ingestion is evident at all trophic levels, including invertebrates, fish, turtles, mammals, and seabirds (Boerger *et* al., 2010; Gregory, 2009; Laist, 1997; Thompson *et* al., 2004). Consequences of ingestion include choking, internal injuries, and false sensation of satiation, which may lead to malnourishment (Duke, 1986a; Robards *et al*., 1995; Ryan, 2008). In addition to these physical impacts upon organisms following the ingestion of plastic debris, there is potential for co-contaminants - chemicals which are bound to the surface of plastic debris - to be released upon ingestion with possible toxicological effects (Teuten *et al*., 2009).

Several workers have focussed on the presence and/or accumulation of POPs on plastic particles in the marine environment (Endo *et al*., 2005; Heskett *et al*., 2012; Hirai *et al*., 2011; Mato *et al*; 2001, Ogata *et al*., 2009, Teuten *et al*., 2007), while the work presented in the preceding chapters has examined the interactions of trace metals with plastic pellets, giving evidence for rapid uptake under a range of environmental scenarios. It is therefore important to investigate whether ingested plastics provide a direct route for exposure of potentially toxic concentrations of chemicals to organisms known to ingest plastic debris. This is important for organisms in higher trophic levels, as they face impacts directly through ingestion of plastic, and indirectly through the ingestion of contaminated prey (van Franeker *et al*., 2011).

Ingestion of plastic by seabirds occurs on a global scale and is known to affect 119 species of seabirds (Convention on Biological Diversity, 2012), with some species exhibiting this behaviour in up to 98 % of individuals (n = 329; van Franeker *et al*., 2011). Thus developing knowledge of potential impacts of plastic ingestion is vital as continued exposure of organisms to physical and chemical stressors may have effects which have not yet been considered. The need for research into the bioaccessibility of co-contaminants carried by plastic has been a recurrent conclusion in studies of plastic ingestion, as stated in policy documents (European Commission, 2011b) and most recently in the scientific literature by Tanaka *et al.* (2013).

While bioavailability, or the fraction of a pollutant absorbed through the gastro-intestinal tract by crossing a physiological membrane (Semple & Doick, 2004), is important, the measurement of bioavailability requires *in vivo* investigations which can be difficult both economically and ethically (Denys 2008), depending on the organism used. Instead, this study intends to estimate bioaccessibility of pollutants from plastic pellets using *in vitro* protocols. Bioaccessibility is defined as the fraction of pollutant extracted from the contaminated matrix by digestive fluids (Hamel *et al*., 1998), and does not directly relate to the quantity of the extracted chemical which may be assimilated by the organism.

The amount of metal absorbed by an organism will be dependent on the form of metal associated with the ingested solid and the physical constraints of metal mobilisation caused by properties of the solid phase itself. Therefore the determination of the bioaccessibility of a metal may indicate the levels available for absorption following ingestion and is important in quantifying the risk to organisms of the presence of plastic in the environment.

6.1.1 Avian gastric conditions

The process of digestion in the avian digestive system takes place in a number of stages. Firstly, the arrival of food in the proventriculus stimulates the secretion of digestive juices (Fig. 6.1a) composed of HCl and pepsin to begin chemical breakdown of material (Duke, 1986b). Secondly, ingested material, now combined with digestive fluid, is transferred from the proventriculus to the gizzard - a muscular organ which mechanically breaks down material. Muscular contractions in the gizzard and proventriculus break down and propel food within the digestive tract (Fig. 6.1b). Thirdly, sufficiently macerated material is transported towards the duodenum for assimilation or excretion, while any food which has not yet been broken down is retained in the gizzard and transported back towards the proventriculus (Fig. 6.1c). Further addition of pepsin and HCl within the proventriculus continues the chemical breakdown, and material is propelled towards the gizzard (Fig. 6.1d) for further mechanical breakdown (Klasing, 1998).

This reflux process is repeated until all food has been broken down sufficiently for transfer to the duodenum while some indigestible matter, including squid beaks, fish otoliths and plastics (van Franeker & Meijboom, 2006), may remain in the gizzard. The relative importance of the proventriculus and gizzard are dependent on diet. In carnivorous birds, whose diet is composed primarily of meat or fish, the proventriculus is large while the gizzard is thin-walled and weak. Conversely, in granivorous species, which feed mainly on seeds, the gizzard is very muscular (Klasing, 1998).

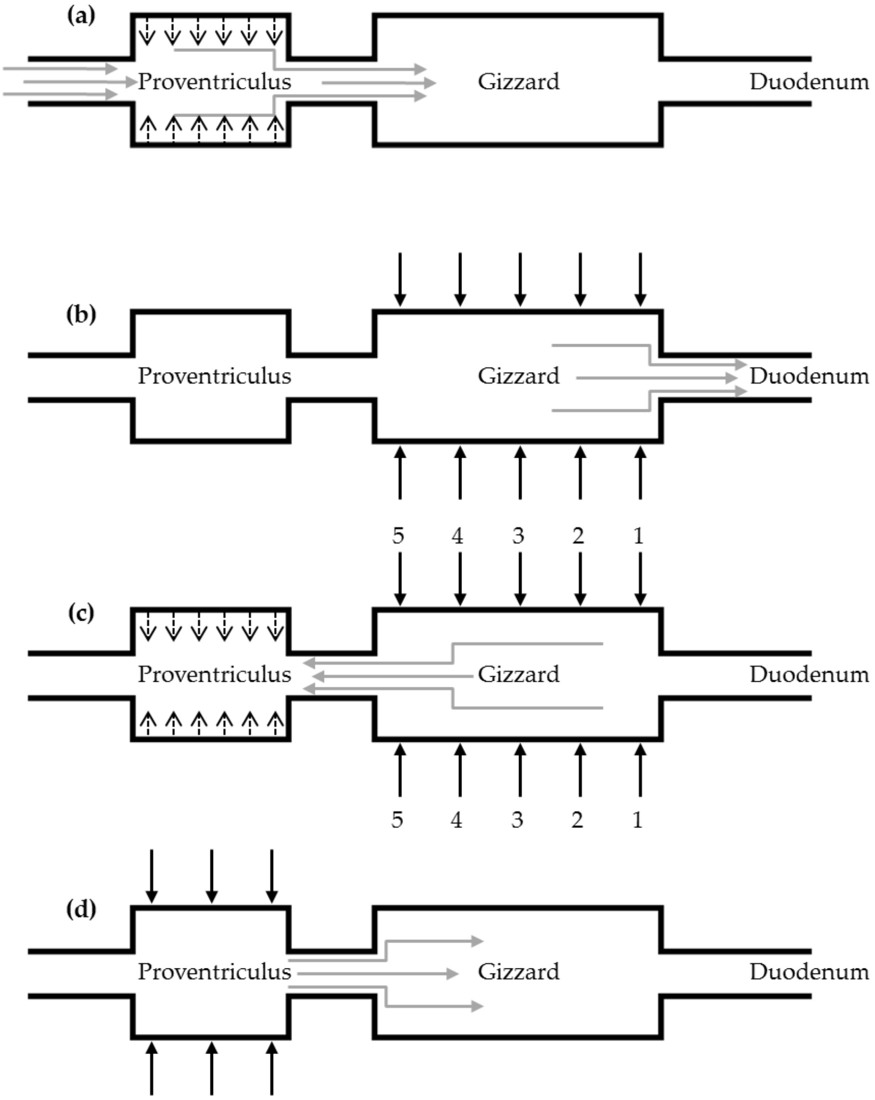

Figure 6.1: A schematic of the initial stages of the avian digestive system. Solid and dotted black arrows indicate muscular contractions and addition of digestive fluid respectively, while grey arrows indicate the path of ingested material. Ingesta entering the proventriculus (a) stimulate secretion of gastric fluid composed primarily of HCl and pepsin. The muscular gizzard then mechanically breaks down solid material (b), allowing the transit of dissolved matter through to the duodenum while solid material is retained within the gizzard. Sequential contractions (c), indicated above by notation 1-5, expel undigested food back into the proventriculus for further addition of HCl and pepsin. Contractions in the proventriculus return food to the gizzard (d) for repetition of steps b-d.

While the digestive tracts of bird species are optimised for the digestion of food specific to dietary habits, the physical and chemical conditions of the digestive system may facilitate the release of compounds from inorganic or anthropogenic items upon ingestion.

It is the purpose of this study to estimate the bioaccessibility of metals released from ingested plastic within the avian digestive system. The primary objectives were to critically evaluate similar studies from the literature, facilitating the optimisation of methods to simulate the avian gizzard, with a particular focus on the Northern Fulmar, *Fulmarus glacialis*, a species for which plastic ingestion is well documented (Avery-Gomm *et al*., 2012; van Franeker 1985), and to examine the kinetics and extent of metal release within this environment. Of particular interest was the rate of release of bioaccessible metals, specifically, whether it is important that plastics are retained within the digestive tract for extended periods, greater than typical retention times for food (Laist *et al*., 1987). Furthermore this study will address the current lack of protocols for the measurement of bioaccessibility of plastic co-contaminants to seabirds and provide the first measurements of this type, since neither metals nor POPs have been assessed for bioaccessibility under simulated avian gastric conditions.

6.2 Materials and methods

All plastic and glassware used was thoroughly cleaned and dried using the standard protocol defined in Section 2.2.

6.2.1 Materials and reagents

Wherever possible, plastic apparatus was used, as has been the case throughout experimental work to minimise adsorptive losses to container surfaces. The major exception in this instance was the use of glass volumetric flasks for the preparation of synthetic digestive fluid, which preceded all stages in which metal concentrations may be affected by adsorptive losses to containers.

6.2.2 Sample collection and processing

Pellets were collected using the standard method outlined in Section 2.3. However, in contrast to adsorption experiments, it was desirable to obtain pellets from a site likely to present a sample of pellets with relatively high concentrations of metals, to ensure detectable concentrations of analytes. Therefore pellets were collected from Ninney Rock (Fig. 2.1) owing to the prior determination of this site as a location containing pellets with moderately high metal concentrations (see Section 3.3). While pellets have been handled as a composite sample, representing pellet populations for adsorption experiments, it is important to understand whether particular pellet types pose a greater threat to organisms than others. Therefore pellets were divided into type according to morphology and colour for metal bioaccessibility experiments. Polymer types were identified using FTIR using the approach described in Section 3.2.4.

6.2.3 Physiologically based extraction test conditions

Physiologically based extraction tests (PBETs) have been used extensively to in order to identify the bioaccessibility of chemical components of ingested material, particularly contaminated food and incidentally ingested soil (Hamel *et al*., 1999; Intawongse & Dean, 2006; Ruby *et al*., 1996). Typically the PBET is optimised for the human digestive system, however there are several papers regarding bioaccessibility in bird species. To investigate the bioaccessibility of metals associated with plastic pellets, PBETs have been conducted following a method similar to those used by Kimball & Munir (1971), Furman *et al.*, 2006 and Martinez-Haro *et al.*, (2009). However, slight alterations were made in order to more closely represent the likely conditions found in the gizzard of Procellariiform seabirds such as *F. glacialis*.

Modifications to the prescribed method were deemed necessary in order to more accurately replicate the digestive environment present in marine avian species, particularly Procellariiformes, for which plastic ingestion is well documented in the North Sea (van Franeker *et al.*, 2011), South Atlantic (Colabuono *et al.*, 2009) and North Pacific (Avery-Gomm *et al.*, 2012). The major parameters which define the avian gastric environment and are typically controlled in PBET studies are temperature, pH and the concentrations of NaCl and pepsin. A summary of conditions used to assess bioaccessibility to avian species is presented in Table 6.1 alongside conditions used in the present study.

Table 6.1: Parameters used in previous avian PBET studies, and conditions used in the present study.

Study	**Reaction vessel**	**[NaCl]**	**pH**	**[pepsin], g L^{-1}**	**Temp. °C**	**Duration**
Kimball & Munir, 1971	Not described	1 N	2.0	10, 20, 40	42	1-2 weeks
Levengood & Skowron, 2001	Beaker	1 N	2.0	10	42	1 hour
Furman *et al.*, 2006	PP centrifuge tube, 50 mL	1 M	2.6	10	42	1 hour
Martinez-Haro *et al.*, 2009	PP centrifuge tube, 50 mL	1 N	2.0	10	42	3 hours
This study	PP centrifuge tube, 50 mL	0.1 M	2.8	10	40	1 week

The temperature used for incubations in previous studies was 42 °C (Table 6.1), a temperature that may be relevant for waterfowl such as ducks or geese, but is inappropriate for marine birds, which have body temperatures ranging between 38 and 42 °C (Warham, 1996). For this reason, 40 °C was chosen as the incubation temperature in the present study. The pH of gastric secretions has been measured to range from 2.0 – 3.5 (Klasing, 1998) and may be controlled to some extent by the pH and composition of ingested material. For the present study, therefore, a target pH of 2.8 was selected as it represents the mid-point of known values.

Marine birds spend large proportions of their lives at sea, relying on seawater to drink. As a result of this, they have well developed nasal salt glands which secrete hypersaline solutions to remove excess salts from ingested water. Consequently, it is unlikely that the gizzard content of seabirds will have a NaCl concentration greater than that of seawater, yet previous studies use a NaCl concentration of 1 M as the bulk medium of digestive fluid. Thus the present PBET is conducted in a NaCl concentration of 0.1 M to reflect the lowest concentrations of NaCl likely in the avian digestive tract, and to minimise instrumental damage and analytical interferences.

Lacking data on gizzard concentrations of pepsin derived from dissection of *F. glacialis* specimens, concentrations were based on previous work by Levengood & Skowron, (2001) Furman *et al*. (2006) and Martinez-Haro *et al*. (2009), all of which used concentrations of 10 g L^{-1} pepsin for the gastric secretions. Piscivorous and carnivorous birds are known to have the highest concentrations of pepsin of all avian species (Klasing, 1998), hence this considerable concentration of pepsin is a realistic estimate.

The final parameter which needed to be addressed was that of retention time of ingested material in the gizzard. In previous studies, typical gizzard retention times were between 1 and 3 hours, with the exception of work by Kimball & Munir (1971). Piscivores have mean retention times ranging from 6 - 12 hours, with food items remaining in the proventriculus and gizzard for approximately 50 % of this time (Klasing, 1998). However, ingested plastic may remain in the digestive tract for greater periods, with some authors suggesting retention times on the order of weeks to months (Avery-Gomm *et al*., 2012), or in excess of one year (Laist, 1987; Ryan & Jackson, 1987), during which time bioaccessible co-contaminants may be released. Therefore, desorption kinetics were chosen as a necessary observation, to determine whether ingested plastics will be stripped of bioaccessible metal concentrations within one typical digestive cycle (4 h) or whether it is important that plastics may be retained for longer periods.

While the avian digestive tract is composed of various stages, each with a different role in the digestion of food, plastic has only been recorded within the gizzard and proventriculus, but not within the duodenum in studies which dissected the entire digestive tract of specimens (Furness, 1985; Robards *et* al., 1995). Therefore this work focuses on the mobilisation of metals from plastic under the conditions found in the gizzard. Metal concentrations associated with plastics are unlikely to reach the duodenum until mobilised, in which case the chemical conditions specific to the duodenum will govern metal mobility.

6.2.4 Incubation and sampling

Synthetic gastric fluid was prepared as a large batch for immediate use in a 1 litre volumetric flask. The bulk solution was prepared by adding 5.844 g NaCl and 10 g pepsin (Sigma Aldrich, UK) to the volumetric flask and filling up to the mark to obtain a 0.1 M NaCl solution containing 10 g L^{-1} pepsin. Subsequently, HCl was added to amend pH to 2.43, which had been determined from prior investigations to yield an equilibrium pH between 2.76 and 2.81. This pH was chosen as it lies within the pH range of gastric secretions, (typically pH 2.0 - 3.5), which may be altered by the composition of ingested material (Klasing 1998).

Forty millilitres of gastric fluid were added to each centrifuge tube which formed the reaction vessel for the PBET extraction, and equilibrated for pH and temperature for three hours prior to the addition of plastic. Samples of 20 pellets were randomly selected from six categories of pellets based on colour and morphology (See Table 6.2 for details). Pellets were weighed and added to the centrifuge tubes which contained the pH and temperature equilibrated synthetic gastric fluid. Initial abundances of 20 pellets (approximately 0.5 g) were used in order to obtain a sufficiently large sample throughout the sampling regime to compensate for potential variability between individual pellets, thereby obtaining results more representative of the population. Furthermore, quantities of plastic ingested by *F. glacialis* are variable, but some studies have reported mean plastic abundances of 0.6 g per individual (van Franeker *et al*., 2005).

Samples were then shaken for a period of one week in a Tecam SB16 shaking water bath while sample temperature was maintained at 40 °C with a Grant Instruments combined immersion heater & pump. Subsamples were taken at predetermined time points throughout the experiment by removing two 1 mL aliquots (1 mL removed and discarded to condition the pipette and 1 mL to be stored for analysis) and a single pellet, in order to maintain constant solid-solution ratios. Sample aliquots were immediately diluted five-fold with 2 % HNO_3, to a total dissolved solids concentration of 0.2 %. This allowed dilution and acidification to be achieved in one step which was completed under laminar flow. Pellets were rinsed briefly with a few mL of MQW to remove any gastric solution from the pellet surface, and pellets were stored as a single sample in a 7 mL vial, dried under laminar flow and extracted for remaining metals in order to obtain a mass balance.

6.2.5 Complete extraction

Metal concentrations remaining on pellets after *in vitro* incubations were extracted with aqua regia (prepared as described in Section 3.2)in order to obtain a measurement of total metal concentration on pellets, and calculate the fraction of metals which exist in a bioaccessible form on the different pellet types. This offers an approximate value for the total metal concentrations, but will not be an accurate measurement of total extractable metal owing to the different periods pellets were exposed to the simulated gastric secretions prior to total extraction. This approach was used due to the often disparate concentrations of metals on pellets. Analysis of a separate batch of pellets to obtain data on total metal concentrations would yield metal concentrations which were not representative of the conditions within the incubations used in the present study. Despite slight inaccuracies caused by this method, it is still possible to observe the kinetics of desorption within the digestive tract, and draw conclusions regarding the likely fate of plastic-bound metals following ingestion by marine birds.

6.2.6 Metal analysis and data handling

Metals were analysed using ICP-MS and ICP-OES according to the standard protocol adopted throughout previous chapters including matrix-matched calibration standards prepared with synthetic digestive fluids. The major consideration during analysis of these samples however, was the concentration of dissolved solids. Due to the concentration of pepsin in the gizzard solution, it was necessary to dilute samples tenfold, which resulted in many elements approaching analytical limits of detection. Statistical methods employed included ANOVA and correlation analysis using Minitab 16 software.

6.3 Results

6.3.1 Sample characteristics & composition

Pellets varied slightly in mass between different categories, with black disc pellets forming the group with the lowest mean mass. White ridged cylinders were the pellet type with the greatest mass and greatest variability between replicate samples. However, all pellet samples were representative of the typical mass and number of plastic items found in the digestive tract of collected *F. glacialis* specimens (van Franeker *et al*., 2005). Despite differences in morphology and colour, all pellets used in the *in vitro* bioaccessibility investigation were composed of polyethylene as determined by FTIR analysis. Pellets divided into groups are hereafter described as white disc (WD), black disc (BD), White ridged cylinder (WRC), white smooth cylinder (WSC), black ridged cylinder (BRC), orange (degraded) (OR).

Table 6.2: Mean mass of plastic pellet samples by pellet type (defined above). Values are presented as mean values arising from three replicates of samples which were each comprised of 20 randomly selected pellets.

Pellet type	WD	BD	WRC	WSC	BRC	OR
Mean mass	0.5754	0.4044	0.6224	0.6186	0.5276	0.5843
RSD, %	0.982	7.61	11.1	1.21	2.67	1.40
Polymer	PE	PE	PE	PE	PE	PE

Total metal concentrations were calculated using mass balance, and these values may be compared with values determined as part of the pellet characterisation investigations in Chapter 3. Total metal concentrations are presented in Table 6.3, and despite a degree of heterogeneity, are comparable with values presented in Chapter 3, indicating consistency with previously determined metal concentrations. Concentrations of most metals vary between pellet types, though there is no pellet type which consistently exhibits enrichment of all metals. For some metals, including Al, Cr, and Fe, pellet types do not exhibit significant ($P < 0.05$) differences in concentrations according to ANOVA analysis.

Table 6.3: Total metal concentrations extractable from plastic pellets used in PBET incubations, grouped by pellet type. Values are given as mean values for three replicate extractions of 20 pellets of each type, ± 1 S.D.

	WD	BD	WRC	WSC	BRC	OR
Ag, ng g^{-1}	24.3 ± 5.14	53.5 ± 13.1	58.3 ± 12.1	20.4 ± 4.53	79.9 ± 9.07	29.3 ± 7.27
Al, µg g^{-1}	18.9 ± 2.61	17.5 ± 1.02	22.7 ± 3.88	13.5 ± 3.69	20.2 ± 4.00	15.6 ± 2.40
Cd, ng g^{-1}	8.20 ± 0.505	11.0 ± 1.00	7.12 ± 1.49	7.19 ± 1.19	10.7 ± 0.393	8.19 ± 0.715
Co, ng g^{-1}	308 ± 9.94	743 ± 76.6	590 ± 223	287 ± 79.7	500 ± 183	293 ± 37.4
Cr, ng g^{-1}	108 ± 81.1	123 ± 61.5	88.8 ± 65.7	79.3 ± 45.8	95.0 ± 60.0	82.4 ± 25.7
Cu, ng g^{-1}	145 ± 2.32	210 ± 33.9	106 ± 25.9	117 ± 28.2	160 ± 29.0	136 ± 5.77
Fe, µg g^{-1}	28.1 ± 3.06	29.3 ± 1.96	29.7 ± 9.98	23.2 ± 6.33	25.8 ± 2.88	31.5 ± 12.0
Mn, µg g^{-1}	34.8 ± 10.87	57.6 ± 1.51	71.0 ± 18.3	34.7 ± 10.6	53.0 ± 20.4	33.9 ± 4.82
Ni, ng g^{-1}	35.6 ± 7.87	62.3 ± 22.4	57.0 ± 40.0	28.8 ± 10.1	46.7 ± 15.5	22.1 ± 37.4
Pb, ng g^{-1}	117 ± 3.04	140 ± 27.2	192 ± 29.3	108 ± 52.0	229 ± 89.9	130 ± 63.2
Zn, µg g^{-1}	3.30 ± 1.33	5.01 ± 0.775	6.92 ± 0.453	2.56 ± 0.470	7.45 ± 1.12	3.73 ± 0.953

6.3.2 Bioaccessible metal

Quasi-equilibrium bioaccessibility, $(BA)_e$ (%), was calculated according to Equation 6.1:

$$(BA)_e = (C_{BA})_e / C_e \times 100\ \% \qquad \text{Eq. 6.1}$$

using (w/w) total metal concentrations (C_e) as shown in Table 6.3, and bioaccessible metal concentrations (w/w) determined in the gizzard phase at the end of the one-week incubation, $(C_{BA})_e$. Bioaccessibility of metals are presented in Table 6.4.

From these values it is evident that all metals investigated during the present study are bioaccessible within the avian digestive system. Bioaccessibility of metals range from < 8 % (Cr on OR pellets) to > 80 % (Pb on OR pellets), with many trace metals exhibiting considerable bioaccessibility, > 30 % over the incubation period.

Table 6.4: Bioaccessibility of metals (%) extracted from plastic pellets over a 168 hour period calculated using Eq. 6.1. Values are presented as mean values of three replicates, ± 1 S.D. nd = not detected.

	WD	BD	WRC	WSC	BRC	OR
Ag	62.6 ± 16.8	61.4 ± 45.9	50.2 ± 27.6	46.2 ± 19.6	28.5 ± 9.20	nd
Al	65.0 ± 9.85	43.1 ± 4.60	41.6 ± 3.56	62.4 ± 6.98	47.0 ± 8.72	56.4 ± 8.94
Cd	nd	1.59 ± 21.2	nd	nd	25.5 ± 37.1	nd
Co	49.7 ± 9.83	20.0 ± 6.96	34.3 ± 8.75	29.6 ± 11.8	46.4 ± 14.8	50.6 ± 29.9
Cr	38.9 ± 26.8	20.06 ± 1.14	46.8 ± 27.5	33.8 ± 18.8	10.3 ± 10.2	7.60 ± 16.1
Cu	52.4 ± 15.5	29.0 ± 14.7	29.0 ± 13.6	42.5 ± 9.86	20.0 ± 5.94	27.9 ± 7.22
Fe	78.5 ± 8.07	72.1 ± 7.69	60.9 ± 8.88	76.7 ± 8.24	60.0 ± 12.9	57.6 ± 3.45
Mn	83.3 ± 2.67	70.8 ± 7.87	64.9 ± 16.5	75.4 ± 4.65	82.4 ± 4.46	82.1 ± 3.22
Ni	51.9 ± 8.39	41.3 ± 29.3	28.5 ± 7.10	42.1 ± 29.0	38.12 ± 5.20	39.5 ± 22.1
Pb	79.5 ± 10.3	48.4 ± 10.4	65.5 ± 6.95	56.4 ± 13.6	55.5 ± 22.2	66.5 ± 13.9
Zn	76.5 ± 13.4	65.5 ± 22.3	45.4 ± 11.3	56.9 ± 32.5	74.7 ± 11.5	82.7 ± 11.0

Continued release of metals from plastics is a possibility considering the replenishment of gastric fluid, not represented here, coupled with the aforementioned retention times for plastics within the digestive tract. With plastics known to be retained for periods greater than 168 hours, further desorption may be expected. However, kinetic observations are necessary to determine the importance of extended residence times for plastics in the digestive tract.

For clarity of presentation, Al, Fe and Mn have been separated from trace metal data as they are important geochemical phases which may control the mobility of other elements, and at environmentally relevant levels are essential nutrients rather than posing a toxicological threat to organisms. Bioaccessible Al, Fe and Mn are presented in Figure 6.2.

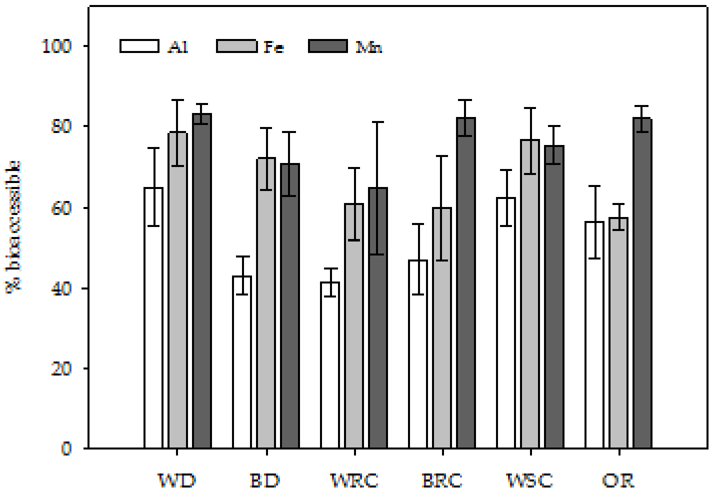

Figure 6.2: Equilibrium bioaccessibility of Al, Fe and Mn in PBET incubations (t = 168 h). Error bars indicate one standard deviation about the mean of three replicates.

All pellet types contain bioaccessible concentrations of Al, Fe and Mn, and despite a degree of variability, bioaccessible fractions of Al are shown to differ between pellet classifications (ANOVA, $P < 0.008$). However, homogeneous groups overlap and Figure 6.2 is indicative of where these differences and similarities may exist. Bioaccessible Al is greater than expected which may be due to underestimations of total Al concentrations. Bioaccessible Fe exhibits slight, but significant ($P = 0.04$) differences between pellet types, with two overlapping homogeneous groups, as evident in Fig. 6.2, while bioaccessible Mn was shown not to vary between pellets of different types, with a P-value of 0.11. While the bioaccessible fractions of Al, Fe and Mn are shown not to vary systematically between pellet types, the relationship between Al, Fe and Mn within each pellet type remains consistent, decreasing in bioaccessibility in the order Mn ≈ Fe > Al. Figure 6.3 exhibits the equilibrium bioaccessibility of trace metals released from pellets within the simulated gizzard for the metals described in Table 6.4.

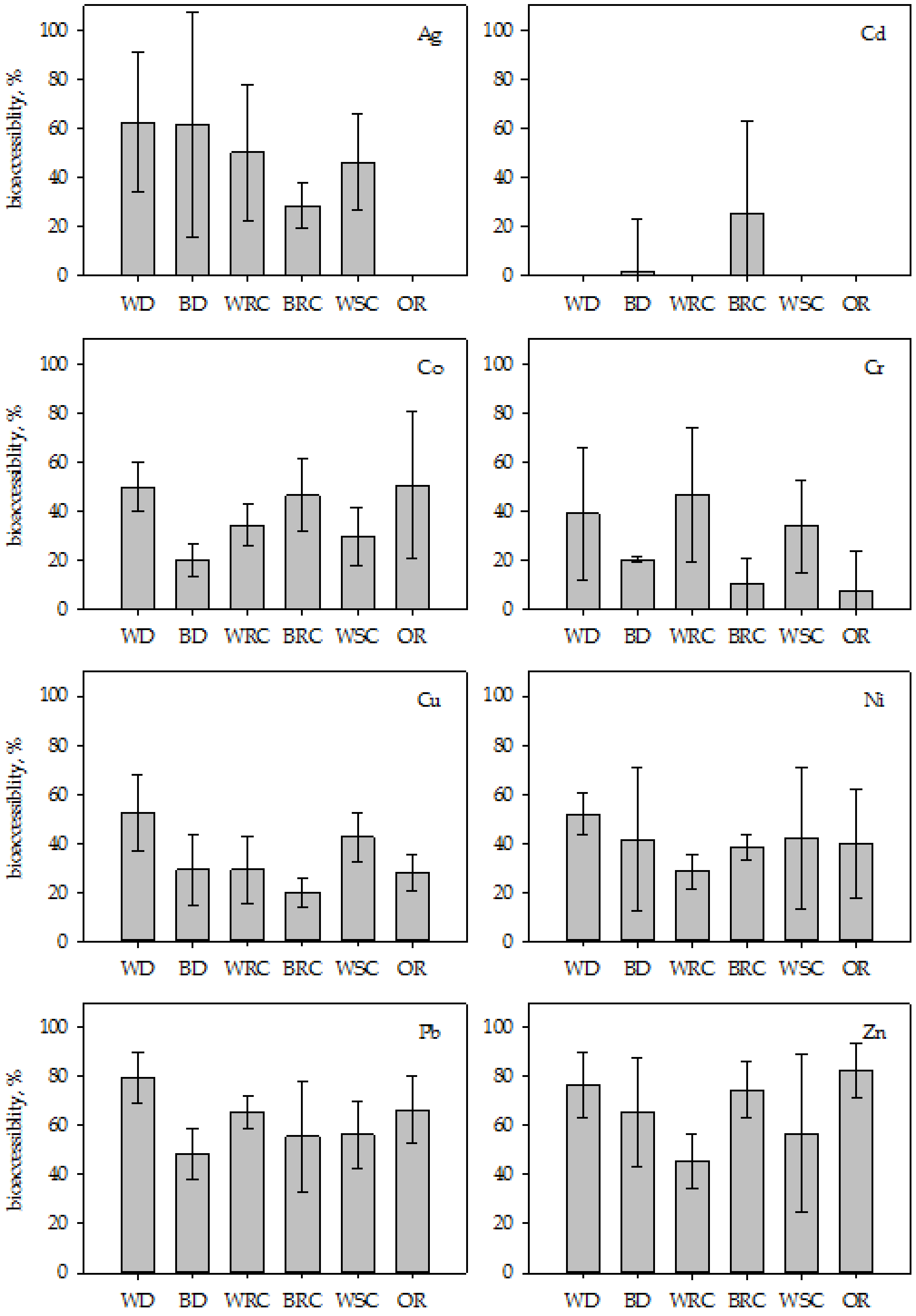

Figure 6.3: Mean (± 1 SD; *n*=3) bioaccessibility of metals from different pellet types (*t* = 168 h).

The results displayed in Figure 6.3 indicate that no single group of pellets consistently contains a greater or lesser bioaccessible fraction of metals than any other, while it does indicate that differences may exist between types of pellets.

Statistical analysis (ANOVA) confirms that differences in bioaccessible metal fractions between pellet types are not significant, with *P*-values exceeding 0.05 in all cases. Cadmium is the one exception to this, as it was not detected on any pellets except black discs and black ridged cylinders. This can be attributed to the collection of pellets which were deposited on beaches following a period of exposure to seawater, and as shown in Chapter 5, Cd does not have a high affinity for pellets in seawater. Overall mean values ($t = 168$ h) indicate bioaccessibility of metals decreases in the order Mn, Zn > Fe, Pb > Al > Cr > Co, Ni > Ag > Cu > Cd.

6.3.1 Relationships between metals

Metal concentrations from all pellet types were pooled to determine whether relationships existed between different metals for either total, or bioaccessible ($t = 168$ h) concentrations. Values, expressed as μg g^{-1}, were correlated using Minitab 16 and indicate there are few relationships between mobilised metal concentrations ($P < 0.05$). Relationships are summarised in Table 6.5, and suggest that the associations of metals with pellets are varied and may be interdependent in some instances. Notably, there exist a number of significant relationships between total metal concentrations which are not evident among bioaccessible concentrations. This suggests that the mechanisms and/or extent of release under gastric conditions differ from those which control metal extraction using acid digestion alone. Furthermore, it illustrates the complexity of metal release within the digestive system, and indicates that the relative abundance of a metal cannot be used to predict bioaccessible concentrations.

Table 6.5: Correlation matrix for total and bioaccessible (t = 168 h) metal concentrations extracted from polyethylene pellets. The letters T and B indicate significant ($P < 0.05$; $n = 18$) relationships between metals for total or bioaccessible concentrations, respectively.

	Ag	**Al**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**
Al										
Cd	T,B									
Co		T, B	T							
Cr										
Cu	T	B	T	T						
Fe		T, B		T, B						
Mn		T		B		T	T			
Ni		T			T	T, B				
Pb	T, B	T, B	T	T, B		T	T	T, B	T	
Zn			T, B*	T						

*$n = 8$

6.3.2 Kinetics of metal mobilisation

For clarity and continuity with preceding chapters, kinetic profiles are presented only for the trace metals for which adsorption kinetics and isotherms have been presented in Chapters 4 and 5, in addition to Al, Fe and Mn, which may have important controlling effects on the mobilisation of trace metals. Cadmium is omitted in this case, as very few data for Cd were above detectable limits.

While the data presented in Table 6.4 and Figures 6.2 & 6.3 indicate minimal differences in equilibrium metal bioaccessibility between pellet types, it cannot be assumed that the kinetics of mobilisation will also be consistent across different pellet types. Therefore it is valuable to determine rates of metal mobilisation over time.

The mobilisation of metals from pellets can be interpreted either as a concentration of metal released into the gizzard phase on a mass to volume basis, or as a percentage of the total metal. Observations expressed as the former indicate a degree of variability between each replicate, which is anticipated owing to the heterogeneity in metal concentrations reported in Chapter 3, though minimised by using many pellets within each replicate. Figure 6.4 shows the time-dependent mobilisations of metal from pellets. Replicates are presented individually as mean values may obscure trends within the data. All pellet types carry bioaccessible concentrations of metals, with biphasic mobilisation profiles evident in all instances. This indicates a rapid initial release of metal from the sites in immediate contact with the solution, followed by a more protracted mobilisation of more recalcitrant metal deposits. Time to equilibrium generally varies from less than 6 h to more than 24 h, while for some metals equilibrium is not attained in all instances.

Aluminium mobilisation is rapid initially (Fig. 6.4a), slowing as equilibrium is approached after the first few hours. However, there exists a degree of variability between pellet types, with the mobilisation of Al from some pellet types - specifically White Ridged Cylinders and Orange (degraded) pellets - exhibiting a slower approach to equilibrium. Furthermore, white smooth cylinders and black discs do not reach equilibrium in the studied time period. Iron mobilisation in the simulated gizzard generally follows a biphasic trend. However following the initial rapid desorption phase, equilibrium is only attained in black ridged cylinders, white ridged cylinders and some replicates of orange pellets. Maximum Fe concentrations in the gizzard phase range from 120 to 330 μg L^{-1} (Fig. 6.4b). Replicate samples show variability for some metals, notably Mn (Fig. 6.4c), with white ridged cylinders, black ridged cylinders and orange pellets exhibiting disparate concentrations of Mn. Nevertheless, equilibrium is attained following incubation periods of 24 h in all instances.

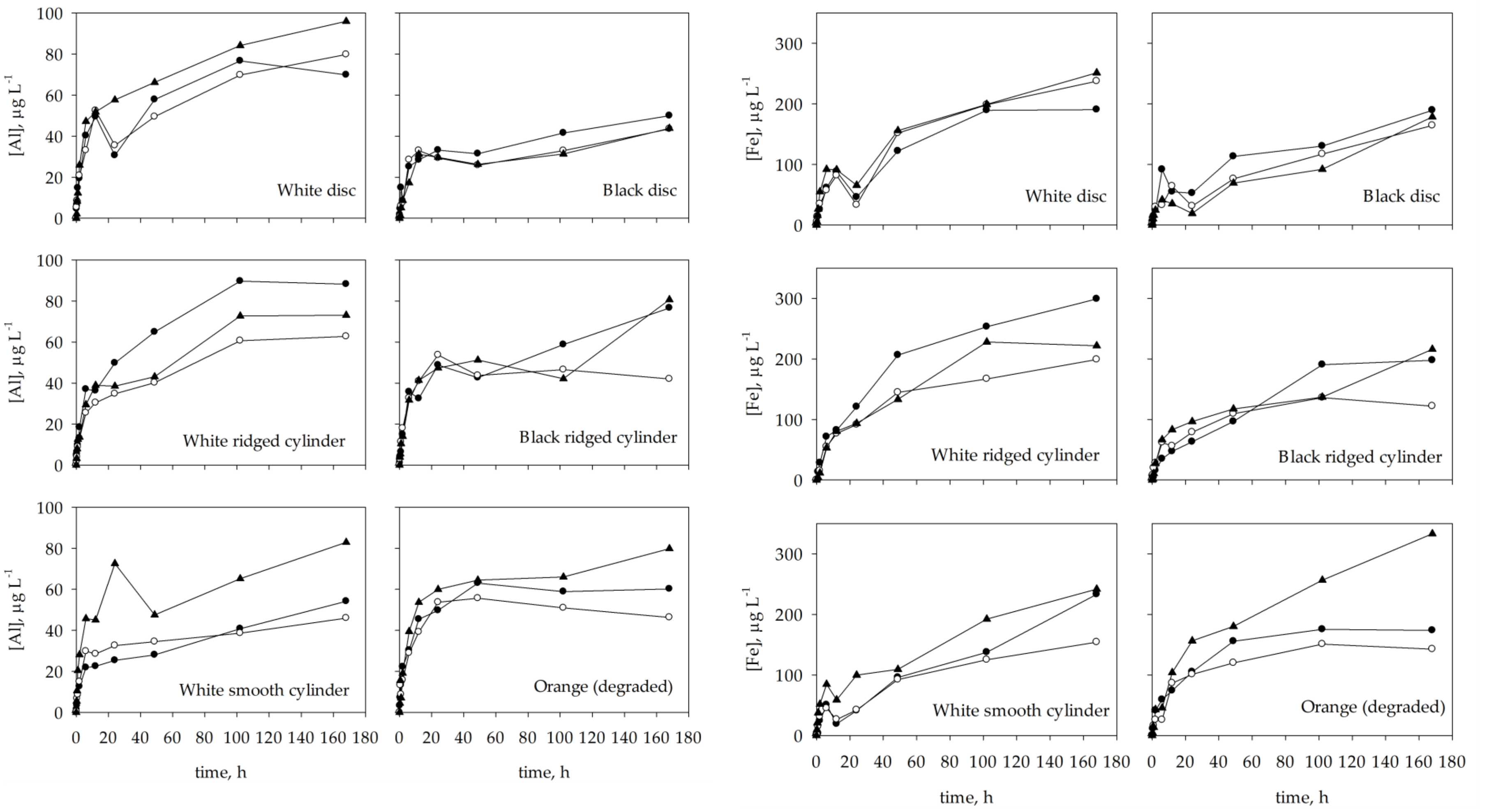

Figure 6.4a: Time dependent release of Al and Fe in a simulated avian gizzard over a period of one week. Plots are derived from individual replicates of each pellet type.

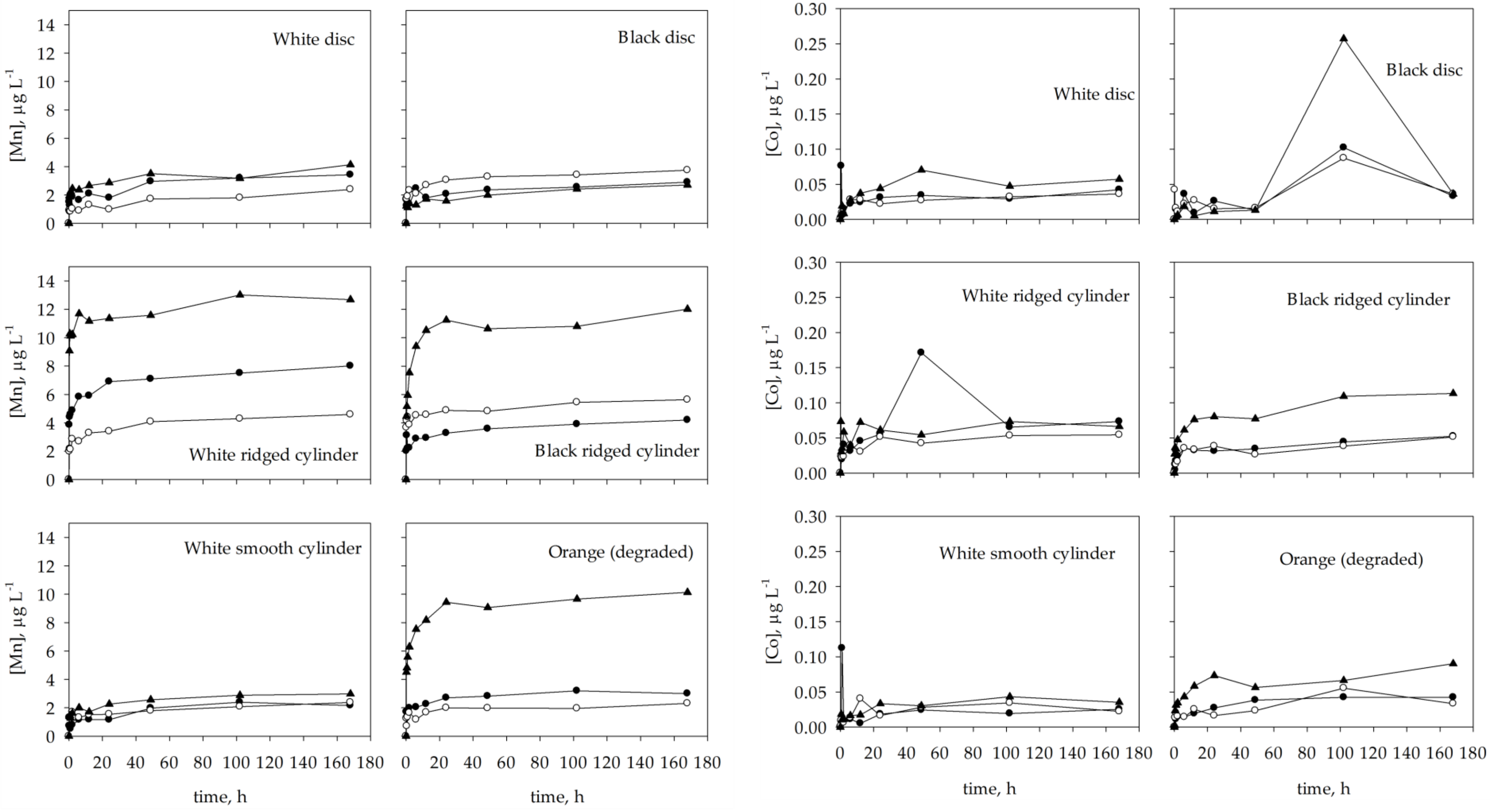

Figure 6.4b: Time dependent release of Mn and Co in a simulated avian gizzard over a period of one week. Plots are derived from individual replicates of each pellet type.

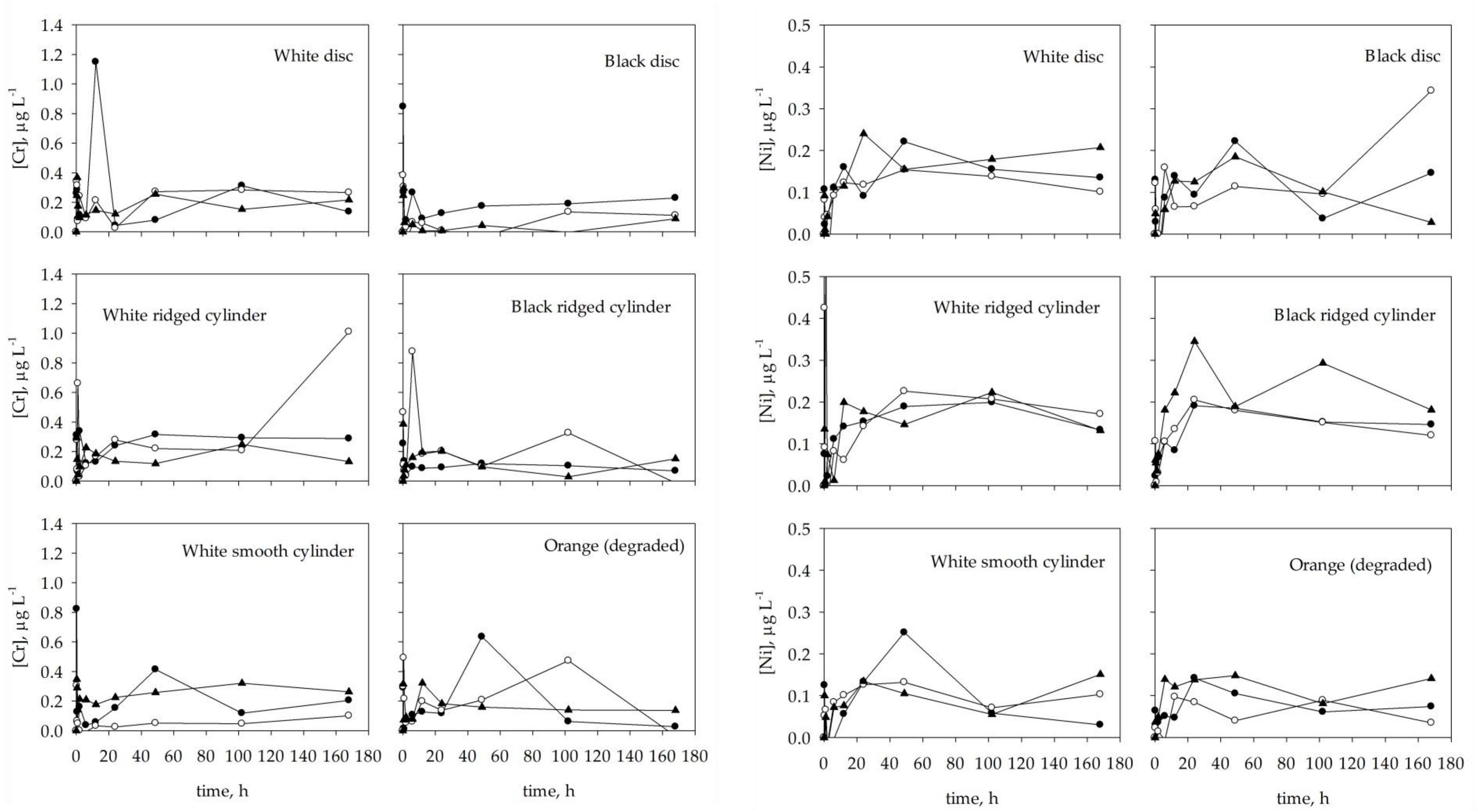

Figure 6.4c: Time dependent release of Cr and Ni in a simulated avian gizzard over a period of one week. Plots are derived from individual replicates of each pellet type.

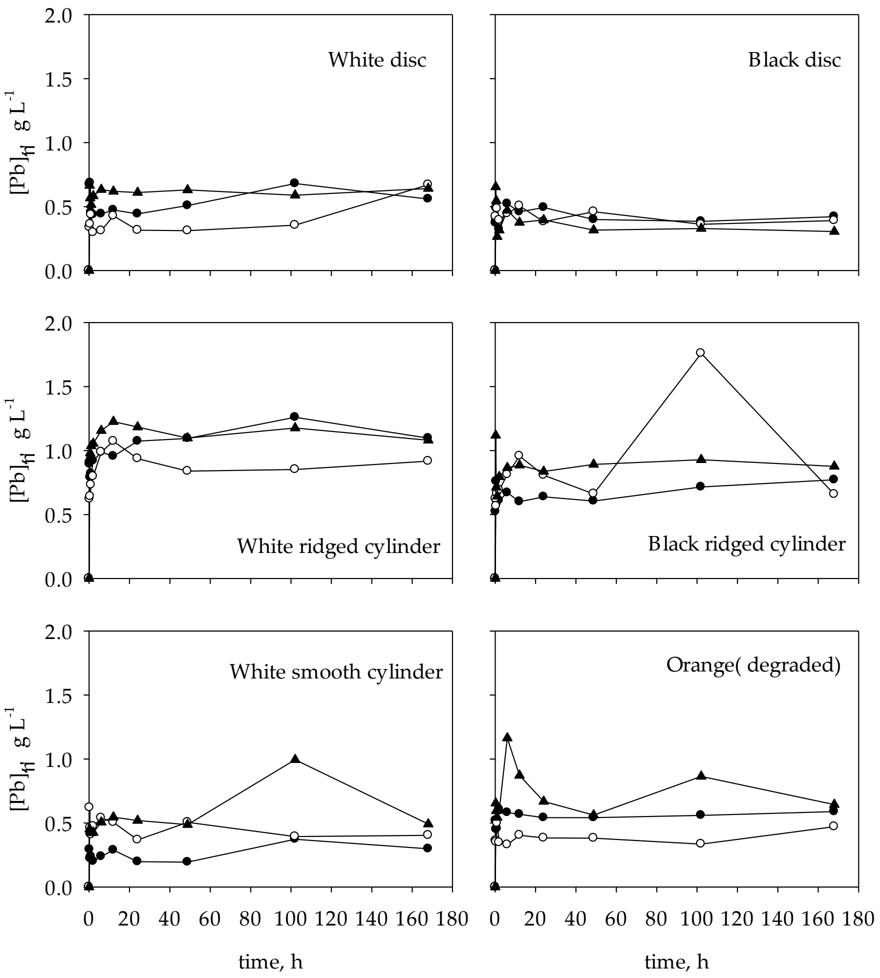

Figure 6.4d: Time dependent release of Pb in a simulated avian gizzard over a period of one week. Plots are derived from individual replicates of each pellet type.

Trace metal mobilisation from pellets in the simulated avian gizzard generally exhibits less distinct kinetics than those of Al, Fe and Mn. This may be partly due to the challenges of analysis of low concentrations in a complex medium, which cause some samples to be close to or below detectable limits. Additionally the complex associations of trace metals with the various organic and inorganic phases present on pellet surfaces may govern kinetics, resulting in considerable variability between replicate samples.

Release of trace metals appears to progress more rapidly than that of Al, Fe or Mn, with considerable proportions of the equilibrium concentrations released by the initial sampling time of 0.25 h. This rapid release is indicative of loosely bound trace metals which do not diffuse from within the plastic matrix, but are likely to be present on the outer surface of pellets. Labile metals are likely to be associated either with charged sites on the polymer surface, or its accumulated coatings.

Total metal concentrations (C_e) are not entirely indicative of the bioaccessible concentrations, $(C_{BA})_e$, mobilised during PBET incubations. The bioaccessibility of each metal is reliant on several factors which may limit or enhance its mobilisation, as suggested by the correlation matrix in Table 6.5, and seen in Figure 6.4. Therefore, total concentrations of metals on plastics do not afford an accurate indication of likely metal doses to organisms. Knowledge of the bioaccessibility of each metal is required, in addition to total metal concentrations in order to predict likely doses.

6.3.3 Kinetic modelling

Mobilisation kinetics were modelled using a modified form of the Noyes-Whitney equation, which represents a pseudo first-order dissolution process driven by the concentration gradient between particulate and dissolved concentrations (Turner & Price, 2008). *In vitro* analysis leads to a decrease in the magnitude of this gradient, therefore the decrease in dissolution rate may be determined by this increase in dissolved concentrations. Thus *in vivo* bioaccessibility may be greater than those described by *in vitro* simulations, owing to the transfer of solutes into the systemic circulation upon dissolution.

Kinetic models for mobilisation were calculated using values of bioaccessibility (BA, %), derived according to Equation 6.1 at each point in the time series. Modelling the mobilisation kinetics in this way allows for comparison between different metals in addition to inter-sample comparisons for each metal. Dissolution kinetics were modelled for bioaccessible percentages of total metal, BA, vs. time, t, with a modified form of the modified Noyes-Whitney equation (Ruby *et al*., 1992):

$$1/((BA)_e\text{-}BA) = 1/(BA)_e+kt \qquad \text{Eq. 6.2}$$

where $(BA)_e$ is a quasi-equilibrium bioaccessible fraction and k is a combined (forward and reverse) rate constant derived from the gradient of plots of $1/((BA)_e\text{-}BA)\text{-}1/(BA)_e$ versus time. The Noyes-Whitney equation is typically used to describe dissolution kinetics for pharmaceuticals in the gastric environment (Dokoumetzidis & Macheras, 2006). As such it is applicable to the processes which are present in the avian digestive tract. Despite occasionally disparate concentrations of metals released from replicate samples (Fig. 6.4), the bioaccessible fractions exhibit more consistency. Therefore mean values have been used to model mobilisation kinetics, and are presented in Figure 6.5.

Kinetic profiles suggest a biphasic release of metal from the surface of the pellets, initially releasing metal rapidly, followed by a period where metal is immobilised or the labile fraction has been completely removed. Despite the degree of variability which remains between replicates in some cases, general desorption trends can be observed in Figure 6.5, and reflect the rapid instantaneous desorption evident in Figure 6.4, and allow the direct comparison of release rates between pellet types and metals.

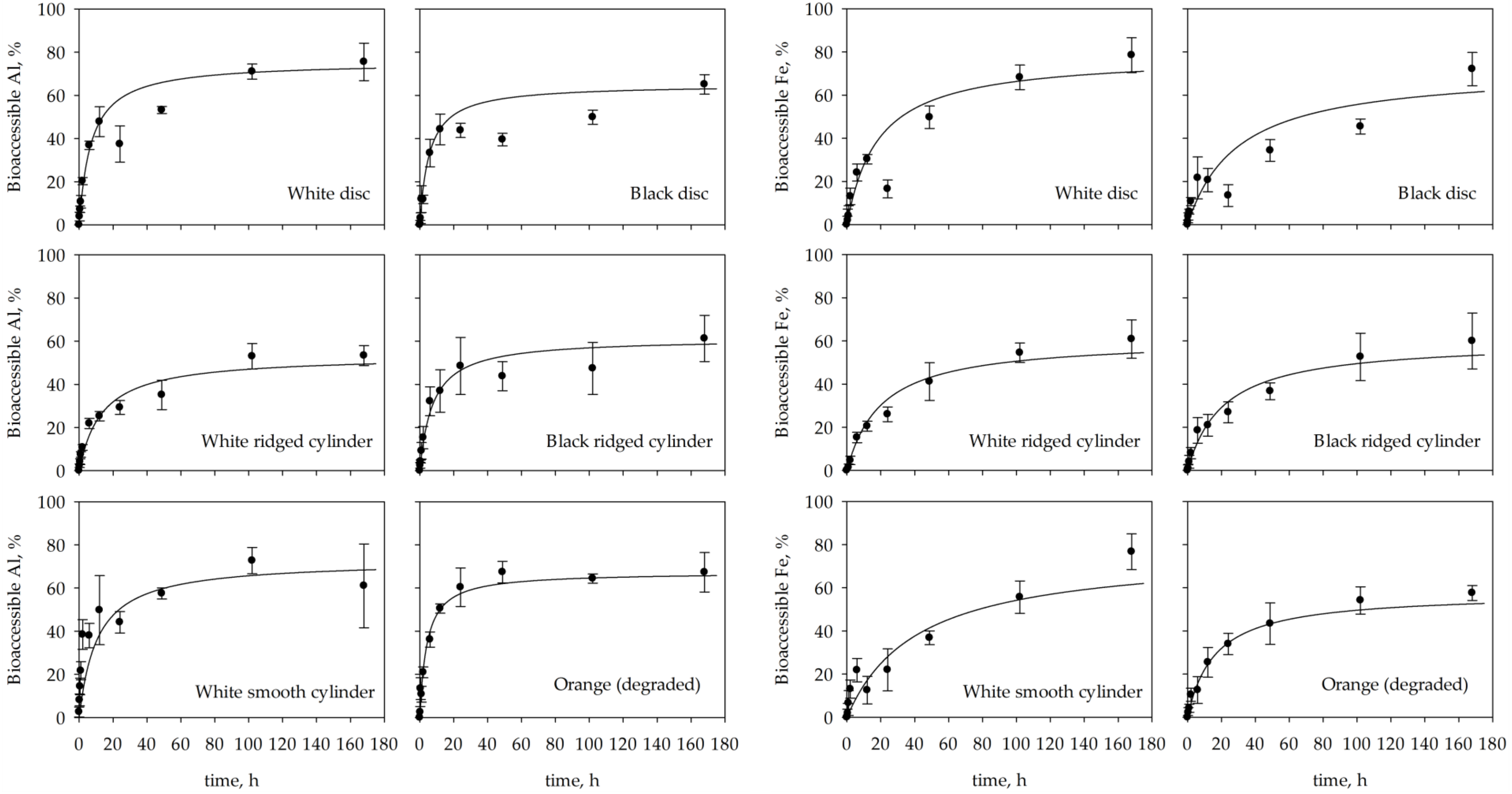

Figure 6.5a: Mobilisation kinetics of Al and Fe in a simulated avian gizzard over a one-week incubation at 40 °C. Data points represent mean empirical values (n = 3) while error bars indicate one standard deviation about the mean. Lines describe Noyes-Whitney model fits to the data, while values for model fits and rate constants are presented in Table 6.6.

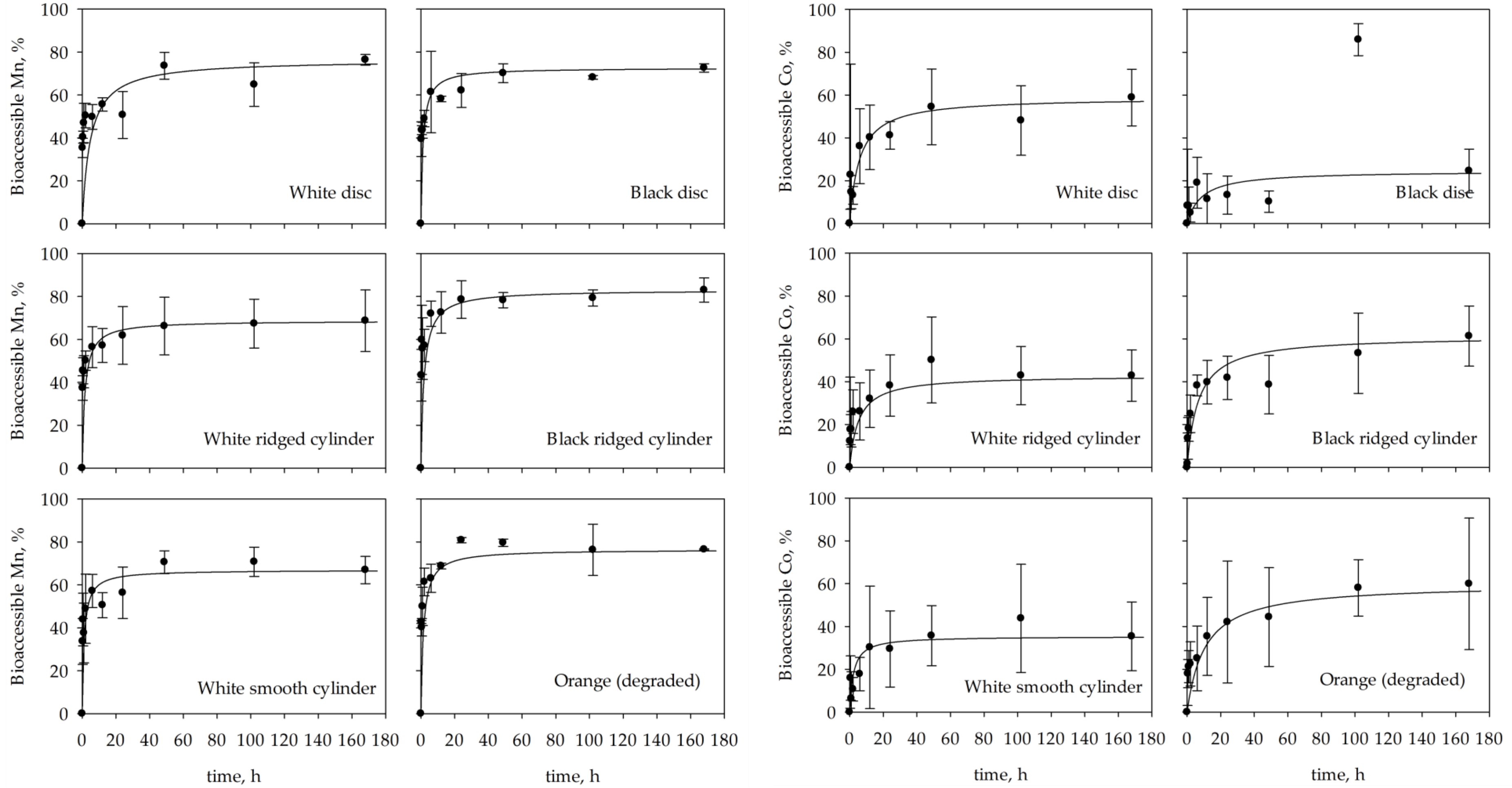

Figure 6.5b: Mobilisation kinetics of Mn and Co in a simulated avian gizzard over a one-week incubation at 40 °C. Data points represent mean empirical values (n = 3) while error bars indicate one standard deviation about the mean. Lines describe Noyes-Whitney model fits to the data, while values for model fits and rate constants are presented in Table 6.6.

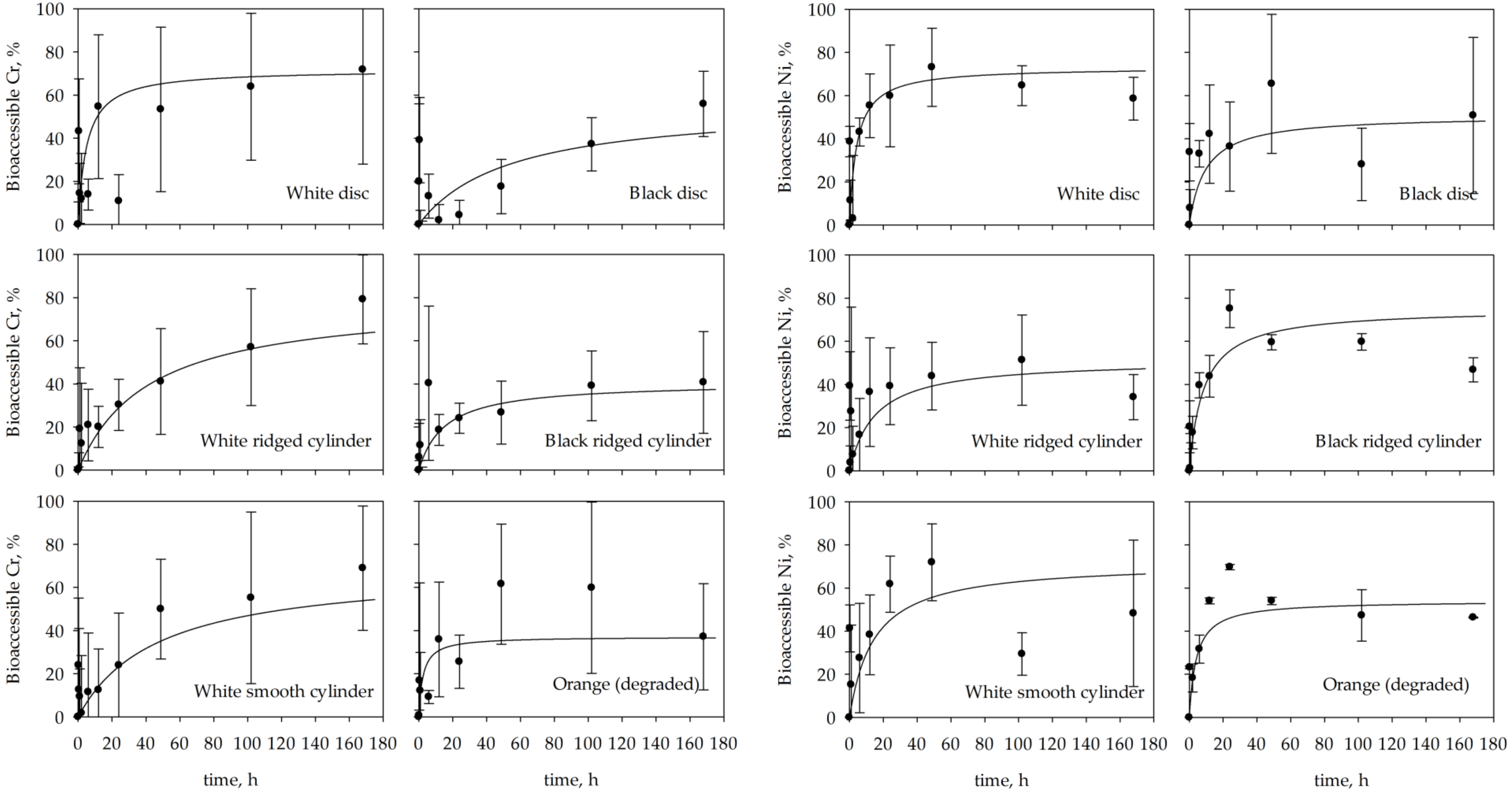

Figure 6.5c: Mobilisation kinetics of Cr and Ni in a simulated avian gizzard over a one-week incubation at 40 °C. Data points represent mean empirical values (n = 3) while error bars indicate one standard deviation about the mean. Lines describe Noyes-Whitney model fits to the data, while values for model fits and rate constants are presented in Table 6.6.

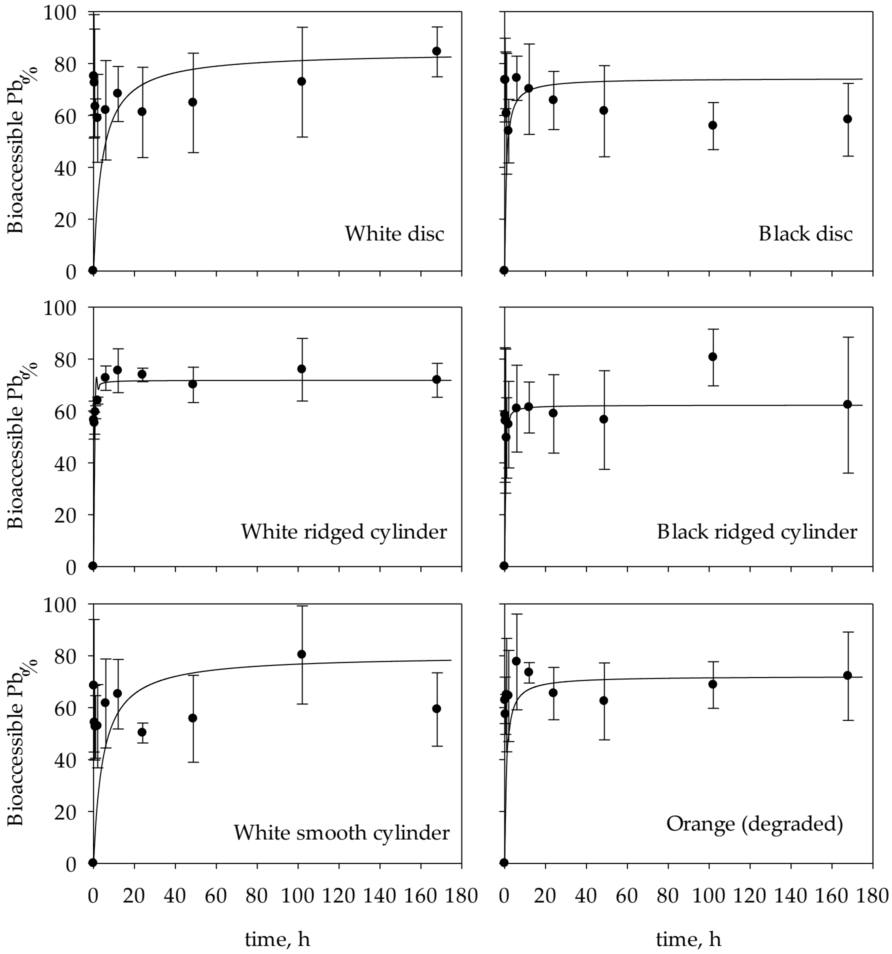

Figure 6.5d: Mobilisation kinetics of Pb in a simulated avian gizzard over a one-week incubation at 40 °C. Data points represent mean empirical values (n = 3) while error bars indicate one standard deviation about the mean. Lines describe Noyes-Whitney model fits to the data, while values for model fits and rate constants are presented in Table 6.6

Rate constants and model fits are presented in Table 6.6 for the data shown in Figure 6.5. Desorption kinetics exhibit two-stage (biphasic) behaviour for all metals and pellet types, however the relative importance of each of the two stages (rapid initial release/slow protracted release) varies. To consider Al, desorption reaches equilibrium within 24 hours, and while orange pellets exhibit a smooth transition from the rapid initial stage to equilibrium, other pellet types exhibit a more distinct change in rate after only a few hours. This is not, however, reflected by the rate constant which is comparable for all pellet types with regard to Al mobilisation, ranging between 1.3 $x10^{-3}$ and 3.6 $x10^{-3}$ $\%^{-1}$ h^{-1}. This contrasts with Fe mobilisation which is comparably slow, returning rate constants of 1.1 $x10^{-3}$ and 7.9 $x10^{-4}$ $\%^{-1}$ h^{-1}, reaching equilibrium in > 24 hours.

Despite the disparities in absolute values for Mn (Fig. 6.4b), the bioaccessible fractions of Mn are consistent within each pellet type (see Fig. 6.5 b), ranging from 65 to 83 % at equilibrium, as reported in Table 6.4. To compare desorption kinetics, *K* values for Mn range between 8.7 $x10^{-1}$ and 9.2 $x10^{-3}$ $\%^{-1}$ h^{-1}, indicating desorption rates vary between pellet types, yet are more rapid than desorption of Al and Fe for all pellet types. Cobalt also approaches equilibrium rapidly, within 12 hours, and while rate constants suggest all pellet types are consistent, with *K* values ranging from 1.3 – 4.7 $x10^{-3}$ $\%^{-1}$ h^{-1}. Cobalt exhibits a good fit to the Noyes-Whitney equation, with r^2 ranging from 0.84 to 0.91 ($P < 0.02$ to < 0.004). Chromium exhibits a poor fit to the Noyes-Whitney equation, with r^2 values < 0.8 for all pellet types, and *P*-values ranging from 0.02 to 0.6. This may be attributed to the anionic speciation of Cr described in Chapter 5, whereby low pH may enhance the affinity of Cr for plastic surfaces. Nickel mobilisation is rapid, with some pellet types – specifically white disc and black ridged cylinders pellet types - exhibiting desorption kinetics which follow the Noyes-Whitney equation ($r^2 > 0.8$, $P < 0.02$), and with K values of 3.24 $x10^{-3}$ and 1.6 $x10^{-3}$ $\%^{-1}$ h^{-1}, respectively, are comparable to the desorption rates expressed by Co.

Table 6.6: Rate constants and model fits for Noyes-Whitney equation (Eq. 6.2) used to model mobilisation kinetics for metals released from polyethylene pellets in a simulated avian gizzard.

Pellet type	WD	BD	WRC	BRC	WSC	OR
Al						
K (%$^{-1}$ h^{-1})	1.88 x 10^{-3}	2.77 x10^{-3}	1.40 x10^{-3}	2.14 x10^{-3}	1.29 x10^{-3}	3.60 x10^{-3}
r^2	0.997	0.995	0.939	0.955	0.801	0.982
P	3.6 x10^{-6}	8.93 x10^{-6}	1.4 x10^{-3}	7.6 x10^{-4}	1.6 x10^{-2}	1.2 x10^{-4}
Fe						
K (%$^{-1}$ h^{-1})	6.95 x10^{-4}	4.71 x10^{-4}	5.65 x10^{-4}	7.88 x10^{-4}	3.14 x10^{-4}	1.08 x10^{-3}
r^2	0.943	0.774	0.925	0.905	0.959	0.953
P	1.2 x10^{-3}	2.1 x10^{-2}	8.5 x10^{-4}	3.5 x10^{-3}	4.7 x10^{-3}	8.5 x10^{-4}
Mn						
K (%$^{-1}$ h^{-1})	2.81 x10^{-3}	1.10 x10^{-2}	9.18 x10^{-3}	6.55 x10^{-3}	1.31 x10^{-2}	8.66 x10^{-1}
r^2	0.773	0.594	0.856	0.822	0.940	0.940
P	2.1 x10^{-2}	7.3 x10^{-2}	8.8 x10^{-3}	1.1 x10^{-2}	3.1 x10^{-3}	1.4 x10^{-3}
Co						
K (%$^{-1}$ h^{-1})	2.99 x10^{-3}	4.73 x10^{-3}	4.51 x10^{-3}	2.48 x10^{-3}	1.28 x10^{-2}	1.58 x10^{-3}
r^2	0.891	0.891	0.905	0.866	0.864	0.842
P	4.6 x10^{-3}	1.6 x10^{-2}	3.5 x10^{-3}	7.0 x10^{-3}	7.3 x10^{-3}	9.9 x10^{-3}
Cr						
K (%$^{-1}$ h^{-1})	2.83 x10^{-3}	3.34 x10^{-4}	3.31 x10^{-4}	1.60 x10^{-3}	3.09 x10^{-4}	1.09 x10^{-2}
r^2	0.563	0.151	0.443	0.655	0.733	0.788
P	8.5 x10^{-2}	6.1 x10^{-1}	1.5 x10^{-1}	9.7 x10^{-2}	3.5 x10^{-1}	1.8 x10^{-2}
Ni						
K (%$^{-1}$ h^{-1})	3.24 x10^{-3}	2.08 x10^{-3}	1.30 x10^{-3}	1.57 x10^{-3}	9.75 x10^{-4}	4.46 x10^{-3}
r^2	0.809	0.575	0.054	0.859	0.247	0.791
P	1.5 x10^{-2}	8.0 x10^{-2}	6.5 x10^{-1}	7.8 x1^{-3}	3.8 x10^{-1}	1.8 x10^{-2}
Pb						
K (%$^{-1}$ h^{-1})	2.83 x10^{-3}	1.67 x10^{-2}	1.99 x10^{-1}	8.41 x10^{-2}	2.72 x10^{-3}	1.54 x10^{-2}
r^2	0.708	0.171	0.865	0.917	0.961	0.983
P	1.6 x10^{-1}	1.2 x10^{-1}	7.2 x10^{-3}	2.6 x10^{-3}	3.3 x10^{-3}	9.1 x10^{-4}

Lead desorption from pellets is not well described by the Noyes-Whitney equation owing to extensive instantaneous desorption which brings the initial measurement (0.25 h) of Pb, in most cases, to a value approximately equivalent to the equilibrium values used for modelling, within experimental error. This indicates that almost all Pb is desorbed within the initial exposure period (< 2 h).

In some instances - for example Cr mobilisation from BD and WRC pellets - modelled values poorly represent empirical data; predominantly where instantaneous desorption is followed by immobilisation and subsequently increased mobilisation according to the typical release profile described by the Noyes-Whitney model. However, generally model fits are significant ($P < 0.05$) for most metals and pellet types, suggesting the suitability of the Noyes-Whitney model for describing the release of metals from pellets in the simulated digestive tract.

6.4 Discussion

Application of an *in vitro* approach for the determination of metal bioaccessibility from plastic pellets has indicated that trace metals are readily mobilised from plastic pellet surfaces in the gastric environment, and that Al, Fe and Mn are also mobilised, albeit more slowly. There exists little evidence that a particular pellet type would pose a greater risk to an organism upon ingestion, as the variability between replicates of each pellet type often exceed differences between pellet types for each metal. Analysis of equilibrium gizzard-phase concentrations of metals using ANOVA has indicated that generally, homogeneous groups overlap (Figures 6.2 & 6.3). However, with pellets only representing one form of plastic debris in the environment, it should be considered that other forms of plastic debris may carry greater total concentrations of metal (Nakashima *et al.*, 2011b), with the potential to convey greater concentrations to organisms if bioaccessibility was consistent between types of plastic debris. Determination of the relative importance of other types of plastic would require extensive further investigations, which may be crucial in our understanding of the issue, given that recent measurements of some metals have exhibited concentrations over 700 times greater than those reported here for Pb (Nakashima *et al*., 2012).

6.4.1 Mobilisation mechanisms

All pellet types exhibit a biphasic release of metals whereby a rapid rate of metal desorption is evident during the initial exposure to the simulated gizzard. This period extends from < 2 h for Pb, to > 24 h, as exhibited by Fe. The rapid release of metals suggests labile phases are being mobilised immediately upon immersion in the gastric environment. Following this initial period of metal mobilisation, a more protracted increase to equilibrium is observed. This protracted desorption is the result of further chemical processes involving slow kinetic reactions, and the release of more energetically bound phases from the polymer surface. Metal associations with pellet surfaces are reliant on system pH, as seen in Chapter 5. The low pH (2.8) of the simulated gastric environment will mobilise metals due to competition between adsorbed cations and protons.

The bioaccessibility of metals from pellets can be compared with literature values for metal bioaccessibility from natural geosolids such as sediment, soils and dust. If metal desorption from plastics was reliant on dissolution of adhered sediment particles, it would be anticipated that the bioaccessibility of trace metals from pellets would be similar to that of sediment. The bioaccessibility of some metals suggests that associations of metals with plastics involves processes of precipitation and adsorption rather than being determined by attached sediment particles, as the bioaccessibility of Al, for example, from sediments and soils is minimal, on the order of 1 - 4 % total Al

(Shock *et al*., 2007), relying on the dissolution of aluminosilicate minerals. Contrastingly, Al bioaccessibility from pellets is in the range 42 – 65 %, suggesting more labile associations. The other major elements, Fe and Mn, also exhibit bioaccessibility from pellets (58 -79 % and 65 – 83 %, respectively) greater than would be expected from sediment, with some studies indicating bioaccessibility for Fe and Mn to be 1.3 – 12 % and 31 – 65 %, respectively (Turner & Ip, 2007).

Some trace metals also exhibit bioaccessibility in excess of what would be anticipated from a natural geosolid. This is the case for Cr and Ni, for which bioaccessibility from pellets are in the range 7.6 – 47 % and 29 – 52 %, respectively, while Hamel *et al*. (1998) report values for Cr and Ni bioaccessibility from soil of 0.2 – 4.2 and 11 – 14 %, respectively. Furthermore, even lower values, less than 1 %, are reported for Cr and Ni bioaccessibility from estuarine sediment by Turner & Hambling, (2012). This serves to highlight the variability in bioaccessibility for metals associated with different solids used in PBET experiments. Lead exhibits intermediate bioaccessibility from soil, as shown by Ruby *et al*. (1996) where Pb bioaccessibility was in the range 22 – 26 %. Contrastingly, bioaccessibility of Pb from pellets in this study lies in the range 56 – 80 %, which corresponds more closely to the value of 55 - 99 %, reported by Turner & Simmonds, (2006) for household dust.

Other metals are known to present a range of bioaccessibilities from soils, dust and sediments in the gastric environment. Copper bioaccessibility has been reported as 31 % for soil (Barsby *et al*., 2012), comparable with values presented in Table 6.4 which indicate a range of 20 – 52 % for Cu bioaccessibility from pellets, while Turner & Simmonds (2006) report greater Cu bioaccessibility (> 80 %). Cobalt bioaccessibility from pellets ranges from 30 – 50 %, which is comparable with mean Co bioaccessibility

(34 %) from soil from a mining region studied by Ettler *et al*. (2012). The same study reports Zn bioaccessibility of 45 %, which corresponds with the lower bound of Zn bioaccessibility from pellets (45 – 83 %). Thus generally, pellets represent the upper limits of bioaccessibility of trace metals when compared with values which have been determined from soil, and for some elements, particularly major elements Al, Fe and Mn, the bioaccessibility from pellets greatly exceeds that of soils and sediments.

6.4.2 Implications for organisms' health

The key observation from the *in vitro* determination of metal bioaccessibility is that the kinetics of metal mobilisation from polyethylene pellets within the gizzard environment can exceed the residence times for natural food items, which are typically passed through to the duodenum in less than 3 hours (Avery-Gomm *et al*., 2012). Consequently, since plastics are retained within the gizzard for periods exceeding residence times of natural ingested material, it must be considered that bioaccessible metals will continue to be released from plastic over greater timescales than would be expected for material which would typically pass through to the duodenum. Conversely, if plastics were passed within the same timescale as natural foodstuffs, then it is likely that the metal loadings accessible to the organism would be less.

Metal speciation, in addition to total concentration is of importance when considering toxicological perspectives or the absorption through the epithelium of metals associated with plastics. However, these are likely to be controlled by the specific conditions and dissolved constituents within the gizzard and duodenum. As such, accurate speciation is not available for complex matrices which exist in the digestive tract, and toxicological effects would require *in vivo* studies to determine any effects. Precipitation of inorganic compounds including hydroxides and carbonates and re-adsorption of metal cations to deprotonated surfaces proceed simultaneously in the intestine as may be expected of the avian duodenum where pH increases to 5.2 - 7.2, and bicarbonate ions, bile salts and pancreatin are abundant (Martinez-Haro *et al*., 2009). This reduced accessibility in the intestine is observed in other studies (Ruby *et al*., 1996; Turner & Ip, 2007), with metal being precipitated or re-adsorbed to surfaces within the intestine.

6.4.2.1 Metal dose from ingested plastic

Despite the constraints of *in vitro* analysis, it does facilitate an intake calculation which identifies the likely metal load conveyed to a model organism by ingestion of plastic, and the possible implications of these metal doses. The intake of metal from plastics (I_P) can be calculated using the formula:

$$I_P = W_P \cdot C \cdot BA \qquad \text{Eq. 6.3}$$

where W_P is the mass of ingested plastic, and C and BA are the plastic-bound metal concentration (w/w) and the bioaccessibility (%) of the metal, respectively. Values of I_P have been determined from the maximum concentrations for each metal determined during the present work, recorded in Table 6.3, and bioaccessible fractions after 168 h are taken from Table 6.4. While it is stated in Section 6.2.4 that mean abundances of ingested plastic in *F. glacialis* have been recorded at 0.6 g, it is worth considering that this is highly variable, and some individuals exhibit far greater accumulations of plastic – with a maximum of 20.6 g recorded in one individual (van Franeker *et al*., 2005). Therefore the mean mass of ingested plastic, 0.6 g, is used in intake calculations for I_p^{mean}, while I_p^{max} represents the 20.6 g maximum recorded value. Values for the intake of metals from plastic derived from Equation 6.3 are presented in Table 6.7.

Table 6.7: Bioaccessible metal intake calculated using Eq. 6.3, for mean (0.6 g) and maximum (20.6 g) plastic mass reported in the gizzard of *F. glacialis* (van Franeker *et al*., 2005).

	Al	Co	Cr	Fe	Mn	Ni	Pb
I_p^{mean}, µg	8.85	0.226	0.0345	14.8	35.5	0.0194	0.109
I_p^{max}, µg	304	7.74	1.19	509	1220	0.666	3.75

Greater metal doses than those calculated above, and reported in Table 6.7 may be derived from plastics containing elevated levels of metals, such as those reported by Nakashima *et al,* (2012). This is suggested by the relatively consistent bioaccessible fractions derived from pellets despite disparities in metal concentrations within replicate samples. It may be assumed therefore that ingestion of highly contaminated plastic could present a pathway for the transfer of considerable concentrations of metals to birds upon ingestion. In the present work, pellets were collected from the coastal environment following an undetermined period of time exposed to seawater conditions where metal concentrations are expected to be relatively low, while plastics in contaminated environments, such as industrialised estuaries, are likely to accumulate greater concentrations of metals.

Furthermore, the metal doses stated in Table 6.7 represent values determined from plastic abundances within the gizzard at a given time point – upon mortality of dissected specimens – which may not represent the true quantity of plastic ingested by an organism. Plastic may be slowly broken down in the gizzard over time and excreted, regurgitated, or transferred from adult birds to their young, though these processes are largely species-dependent.

6.4.2.2 *Toxicological considerations*

Based on toxicological data for chronic effects on avian species, (ATSDR, 2004; ATSDR, 2005; Edens *et* al., 1976; Sample *et al*., 1996) which offer lowest observed adverse effect levels for metals (LOAELs), it is possible to estimate the daily intake of plastic necessary to present a toxicologically important dose of metals. Equation 6.4 indicates how the LOAEL (µg kg^{-1} d^{-1}) normalised to 750 g (the mean mass of *F. glacialis* specimens (Nuttall, 2005)), and the bioaccessible metal concentration, C_{BA} (µg g^{-1}), can be used to calculate the mass of plastic required on a daily basis for adverse effects to be possible in terms of metal toxicity(M_{adv}).

$$M_{adv} = \mathrm{LOAEL}/C_{BA} \qquad \text{Eq. 6.4}$$

The mass of ingested plastic necessary to cause harm to these birds, according to Equation 6.4 ranges from 6.1 g d^{-1} for Cr (if all Cr is assumed to be present in hexavalent form), to 26.5 kg d^{-1} for Ni. Lead toxicity would begin to occur only if the bird was to ingest 3.7 kg of plastic, while Cd and Co toxicity would require daily intakes of 24.5 g d^{-1} and 10.1 g d^{-1}, respectively. Given that the maximum quantity of plastic found in a specimen of this species was 20.6 g, it is therefore highly unlikely that the metal concentrations present on plastics serve to cause chronic sub lethal toxicological effects to the individual, particularly not at levels which would not have already caused substantial physical harm to the bird. These calculations, however, make no assumptions of possible cumulative or synergistic effects arising from metal concentrations within an organism, as it is difficult to quantify these effects.

Additionally, some elements, e.g. Ni, are known carcinogens which may not exhibit immediate toxic effects.

6.4.2.3 Dietary intake of metals

To further put the bioaccessibility of metals from plastics into context, comparisons can be drawn from the dietary intake of major and trace metals from pelagic fish. Medeiros *et al*. (2012) and Saei-Dahkordi & Fallah, (2011) studied fish from Brazilian and Iranian markets, respectively. Results from these studies are comparable and indicate that for pelagic fish, major and trace metal concentrations may be similar in geographically distant areas. Comparisons of metals determined in fish (Medeiros *et al*., 2012), and pellets (this study) have been summarised below.

To compare the mean values documented in Medeiros *et* al. (2012) with mean values reported for pellets in Table 6.3, Cd, Pb and Zn are present on pellets (0.007 – 0.011, 0.108 – 0.229, and 2.56 – 7.45 μg g^{-1}, respectively) at concentrations comparable to pelagic fish (0.001 – 0.09, 0.1 - 0.3 and 2.7 – 9.3 μg g^{-1}, respectively). This is not the case for all reported metals; concentrations of Co, Fe and Mn on pellets (0.290 – 0.590, 23 – 36 and 34 – 71 mg g^{-1}, respectively) greatly exceed Co (0.007 – 0.02 μg g^{-1}), Fe (1.6 – 7.5 μg g^{-1}) and Mn (0.3 – 1.7 μg g^{-1}) in fish. However, some metals are less concentrated on pellets than in fish. For example Al in pellets ranges from 14 – 23 μg g^{-1}, compared with 49.1 – 394 μg g^{-1} in fish, while Cu concentrations in pellets (0.106 – 0.210 μg g^{-1}) are over an order of magnitude lower than in fish (1.2 – 2.9 μg g^{-1}).

These comparisons put ingestion of plastic into context when considering the likely contribution of metal to avian species through dietary intake. However, comparisons only take into account metal concentrations determined on pellets from one location. It must be considered that metal concentrations vary between pellets and sites, as shown in Chapter 3. Furthermore, values reported for Pb by Nakashima *et al*. (2012) are orders of magnitude greater than those presented in this thesis.

6.4.3 Evaluation of the PBET approach

Presently, there are several studies which identify presence of POPs in birds shown to have ingested plastic, yet information on the bioaccessibility of POPs from plastics is not yet known. Quantification of the likely bioaccessibility of POPs from ingested plastic could provide an estimate of the toxicological risk to organisms. Tanaka *et al*. (2013) report evidence for transfer of polybrominated diphenyl ethers (PBDEs) from ingested plastic to the adipose tissue of short-tailed shearwaters (*Puffinus tenuirostris*), though the authors state the need for studies which focus on release rates of chemicals in digestive fluids. Thus while the present study suggests that the risk of harm caused by bioaccessible concentrations of metals from plastic pellets is minimal, it should provide a baseline method for the development of desorption kinetic profiles which can be applied to the whole range of plastic co-contaminants.

According to the observations of this study, the concentrations of metals released from pellets suggest the likelihood of a toxicological effect on seabirds is low. However, it does indicate that metals on plastics are bioaccessible. Thus while acute toxicological effects may not be observed as a consequence of metal release from plastic, any anthropogenic contributions of synthetic materials to the natural environment contribute to a new, direct pathway for avian species to ingest metals, and potentially other co-contaminants which may have chronic effects on individuals over longer timescales, for example by reducing the physical fitness.

While *in vitro* determination of bioaccessibility is a useful measure from which conclusions may be drawn, it is important to consider, when discussing the mechanisms of metal mobilisation, that the bioaccessibility of a metal is dependent not only on the conditions of incubation – temperature, pH, enzyme activity, concentrations of inorganic and organic complexing agents – but also on the nature of associations of metals with biogenic and mineralogical phases deposited on the plastic surface, as well as with the polymer surface itself. This consideration is a well-known aspect of bioaccessibility studies (Hamel *et al*., 1998). Furthermore, it is possible that continued secretion of digestive fluid from the proventriculus may facilitate further leaching of metals from plastics, as the concentration gradient between particulate and dissolved concentrations will be increased.

6.5 Conclusions

Metals on the surface of plastic pellets have demonstrated bioaccessibility within a simulated avian digestive tract comprising pepsin, NaCl and HCl. Bioaccessibility of metals from pellets grouped according to morphology and colour ranged from 7.6 % (Cr) to 83 % (Zn), while overall mean values decrease in the order Mn, Zn > Fe, Pb > Al > Cr > Co, Ni > Ag > Cu > Cd. Comparisons of pellet groups did not indicate a type of pellet for which bioaccessibility was consistently greater. Dissolution kinetics were successfully modelled (with the exception of Cr for some pellet types) using the Noyes-Whitney equation, rate constants for which indicate dissolution of metals varies between pellet types, presumably due to differences in reactivity of pellet surfaces. Furthermore, rates of metal release are dependent on the metal in question, with times to equilibrium ranging from < 2 h (Pb) to over 24 h (Al).

On the basis of the data currently available and presented above, it is unlikely that the metals associated with plastics present a toxicological risk to avian species. However, several questions are raised by the confirmation that co-contaminants present on the plastic surface are bioaccessible. Firstly, additional investigations should attempt to determine whether polymer type affects the bioaccessibility of metals. Secondly, work should be carried out to identify whether POPs present on the surfaces of plastics are bioaccessible. Furthermore, given the likelihood that plastics which carry greater concentrations of metals may convey greater doses to organisms, broadening the scope of this study to include other forms of plastic debris, and other organism models from different trophic levels is essential to elucidate potential ecosystem effects.

Chapter 7:

Conclusions & future perspectives

7.1 Introduction

This study has shown that metals are present on plastic production pellets collected from the intertidal zone, and is one of the first studies to identify the accumulation of metals on plastics in the environment. Additionally, it has been demonstrated for the first time that trace metals (Cd, Co, Cr, Ni, Pb) will be removed from the water column by adsorption to PE pellets, a process which is enhanced by the polymer's exposure to environmental degradation. By examining this phenomenon under a range of conditions it has been possible to identify scenarios which may promote the adsorption of trace metals to PE debris, with the potential for transport in the environment. Furthermore, the work presented within this thesis indicates that metals on PE pellets are bioaccessible within the avian gastric environment, and may constitute an emerging pathway for metals to be assimilated by organisms.

7.2 Environmental implications

The initial objectives proposed at the beginning of this thesis were centred on the quantification of trace metals on pellets, and the application of a batch approach to identify trace metal uptake by PE pellets under different conditions. Acid extractions of pellets collected from four field sites indicates that metals are present on the surfaces of beached pellets, while inter-site dissimilarities in metal assemblages suggest that contaminant burdens on pellets most probably result from localised contaminant concentrations in the environment.

Adsorption experiments have shown that trace metals are accumulated on the surface of PE pellets, and that the extent and rate of adsorption will be determined not only by the trace metal in question, but also by the properties of the sorbent surface.

Furthermore the physico-chemical properties of the aqueous medium have been demonstrated to exert major controls over the uptake of trace metals to pellets. While this study makes use of PE pellets to address the specific research questions outlined at the outset, the results have implications which extend beyond interactions of this model particle with the environment, and may represent characteristic interactions of PE debris with dissolved trace metals. The wider implications of the findings of this thesis will be discussed in the following subsections.

7.2.1 Changes to plastics upon environmental exposure

Comparisons between virgin and beached pellets in Chapters 3-5 have shown that changes which occur to pellets upon environmental exposure will cause virgin plastic surfaces to become more favourable for trace metal adsorption. The physical and chemical degradation described in Chapter 3 will increase specific surface area of plastic pellets, while accumulation of biofilms and oxyhydroxides of Fe and Mn will confer reactive sites to pellets.

The presence of Fe and Mn on beached pellets has been confirmed in Chapters 3 and 6, in contrast with virgin pellets for which concentrations of Fe and Mn were below limits of detection. This suggests that over time the surface of plastics will become more analogous to that of sediment, forming a mobile particle which may accumulate and transport trace metals in the water column. The surface of plastic pellets in natural waters above pH 7 is likely to be negative, particularly when concentrations of Fe and Mn oxyhydroxides are present, due to the deprotonation of these phases. The negative charge of beached pellet surfaces has been confirmed by Fotopoulou & Karapanagioti (2012) who determined the point of zero charge for beached pellets to be pH 6.1. This is in agreement with the adsorption of trace metals to beached pellets in pH-controlled river water presented in Chapter 5. Under these conditions adsorption of Cd, Co and Ni was enhanced as pH increased above 6, while the opposite was observed for Cr. Furthermore, virgin pellets were shown to behave similarly under conditions of controlled pH. This is not an expected finding since Fotopoulou & Karapanagioti (2012) report virgin polyethylene to present a neutral surface. However, the surface charge observed in Chapter 5 for virgin pellets can be explained by the use of natural river waters and consequent immediate biofouling (Wahl, 1989) of virgin plastics in incubations.

7.2.2 Adsorption mechanisms

From the observations presented throughout this thesis, it is possible to summarise conditions whereby certain trace metals (Cd, Co, Cr, Ni, Pb) will be accumulated on, and transported by plastic debris. The data pertaining to trace metal uptake, presented in Chapters 4 and 5 illustrate that in addition to the effects of aging and degradation of the plastic itself, environmental factors will have considerable controls over the interactions between plastics and metals.

The major controlling variable in the coastal zone is likely to be salinity, owing to its effects on trace metal speciation and the surface charge of substrates. Kinetic modelling has also provided constants which indicate desorption is likely from pellets as they are exposed to increasingly (for cations) or decreasingly (in the case of anions) saline conditions. The experimental and modelling approaches used to understand the interactions between trace metals and pellets have elucidated differential behaviour between the elements studied, with Cd, Co, Ni exhibiting decreasing affinities for plastic as salinity increases and Cr exhibiting the reverse trend. For cationic species this has been attributed to competitive and complexing effects of seawater cations and anions, respectively. Adsorption of Cr has been attributed to its anionic speciation, and increases with salinity due to the decreasing electronegative surface charge of particles as ionic strength increases. The adsorption of Pb is independent of salinity within the context of this study, predominantly complexing with dissolved organic matter according to speciation modelling using WHAM. However, Pb is known to be highly reactive toward particulates (Tang *et al*., 2002), particularly Fe and Mn oxides.

Despite the constraining effects of increasing salinity on Cd, Co and Ni, there remains potential for the accumulation of these metals on plastics in the environment, as indicated by their presence on pellets collected from the coastline and analysed for pre-existent metal concentrations in Chapter 3. Currently estuarine and freshwater systems are under-represented in the field of contaminant uptake to plastics. However, the approach used in Chapter 5 has facilitated a greater understanding of the mechanisms of trace metal accumulation on polyethylene, and identified scenarios in which certain trace metals are likely to be accumulated by polyethylene particles. Using a similar approach for persistent organic pollutants (POPs) could identify potential priority environments for the control of plastic debris.

While some of the interactions between trace metals and polyethylene are comparable with those which are known to occur with natural suspended particulate matter (SPM), the consequences of metal associations with natural SPM and buoyant plastic particles differ. Natural SPM tends to reduce the mobility and accessibility of trace metals in the environment, sequestering them from the water column and settling out in sediments (Teuten *et al*., 2009). This is not the case for buoyant plastic particulates which are likely to be mobile over greater spatial and temporal scales than natural SPM.

The data modelling techniques used to interpret and describe the interactions of trace metals with sorbent surfaces, as well as associations with other dissolved constituents have been pivotal in the interpretation of results. However, understanding the processes involved in metal interactions with pellets relies on knowledge of chemical kinetics in light of residence times for different particles in the system in question. The interactions between pellets and trace metals progress at rates high enough to compensate for their short residence times in estuarine systems compared to natural particulates, therefore chemical equilibria are likely to be achieved within these systems. However, the batch approach used to determine adsorption of trace metals to plastics does not take into account changes which take place over longer timescales. Changes to the surface of plastics will occur as a result of photo-oxidation, biofouling, accumulation of hydrogenous metals, and the entrainment of natural SPM, as discussed throughout this thesis. These changes will convey a progressively more reactive surface to plastics in the water column.

Continued growth in the abundance of plastic debris will ensure that the potential for trace metal cycling to be altered will become increasingly important over time. Furthermore, as plastic debris changes in terms of its affinity for trace metals during its residence time in the water column, and is progressively degraded into smaller particles, the scope for interactions between plastic particles and dissolved metals will become greater.

7.2.3 Bioaccessibility to Procellariiform birds

The final objective of this study was to examine the bioaccessibility of metals associated with plastic pellets to avian species using an *in vitro* approach. Addressing this objective has shown trace metals to exhibit bioaccessibility from plastic, in some cases (e.g. Cr, Ni) exceeding bioaccessibility of metals from natural soils or sediments. The high degree of bioaccessibility of elements such as Cr and Ni from pellets suggests that considering plastic particles to be analogous to natural sediments is not appropriate despite the fact that they are coated in hydrogenous phases, as sediment.

While the results themselves are of interest, one of the key implications of this study is that an *in vitro* approach to bioaccessibility of contaminants carried by plastic can provide essential data on the magnitude of the impacts of plastic ingestion. This is a valuable contribution in the field, as at present links between plastic ingestion and contaminant loadings in avian species have been investigated by very few studies (Teuten *et al*., 2009). None of these studies utilise an *in vitro* approach to the question, and therefore are unable to unequivocally state that contaminants in organisms are derived from plastic. This thesis employs an approach which is novel to the study of chemical transfer from plastics to avian species and should support future work to determine bioaccessibility of non-metal contaminants to birds, and further work on the bioaccessibility of metals from other types of plastic debris.

7.3 Future directions

Plastics are becoming more widely recognised among the public and policy-makers for their environmental presence, and their potential role in uptake and transport of co-contaminants in natural systems. This is due to the work carried out in academic institutions globally, and their efforts in effective communication. Despite this, there remain considerable hurdles to be overcome in our understanding of the magnitude of the issue. This thesis has applied a number of techniques to determine the characteristics and trace metal interactions of plastic pellets, and forms a model approach on which future research into the behaviour of plastic debris in the environment can be developed. Based on the principles applied throughout this thesis, the following research could provide further insights into the fate and potential consequences of plastic debris in the environment.

7.3.1 Interactions between persistent organic pollutants and plastics under estuarine conditions

Chapter 5 highlighted the complex and varied interactions between metals and plastics in a simulated estuarine environment, and is the first study to focus on the uptake of contaminants to plastics under such conditions. While POPs have been proven to adsorb to plastics in seawater (Teuten *et al*., 2007), what remains to be seen is whether POPs are affected by changes which occur in an estuarine gradient.

An experimental approach based on Chapter 5, with regard to the preparation of an estuarine gradient could elucidate the partitioning behaviour of POPs under these conditions. However, the approach to sample handling, extraction and analysis would need to be optimised for POPs. The use of glass apparatus would be a necessity owing to leaching of interfering compounds such as phthalates which can co-elute with analytes of interest during analysis. Furthermore, POPs are readily lost to plastic surfaces during storage due to their affinity for hydrophobic surfaces (Teuten *et al*., 2009).

Following Teuten *et al.* (2007), glass reaction vessels could be charged with POPs dissolved in a solvent which is evaporated to dryness prior to the addition of plastic particles and estuarine waters. Extraction and analysis of POPs from pellets would require use of solvents such as hexane (Ogata *et al*., 2009), followed by multiple purification and fractionation steps using silica gel columns and selective elution for the separation of analytes of interest (Mato *et al*., 2001). Analysis of aqueous POP concentrations would require a technique such as solvent extraction, (e.g. liquid/liquid extraction) with dichloromethane (Endo *et al*., 2005), followed by purification and analysis according to the same process as pellet extractions. Depending on the degree of sample preparation, analysis could be conducted using gas chromatography-mass spectrometry (GC-MS), or preferably, comprehensive two-dimensional gas chromatography coupled to a time of flight mass spectrometer (GC*GC-TOFMS) (Focant *et al*., 2004).

7.3.2 *In vitro* bioaccessibility of persistent organic pollutants

The work presented in Chapter 6 has shown that concentrations of metals bound to the surface of plastics are bioaccessible within the avian gastric system. Owing to the low concentrations of metals present on the selected plastic particles, it was concluded that the risk to birds of metal doses delivered in this manner was low. Many studies have identified POPs to be present on plastic pellets at concentrations far exceeding those in the surrounding water column (Mato *et al*., 2001, Rios *et al*., 2007), and some studies have attempted to link plastic ingestion with POP concentrations in tissue samples and eggs of birds (Ryan *et al*., 1988). However, finding a link between plastic ingestion and POPs in tissue is analytically challenging due to the dietary intake of POPs by birds contributing to variable baselines. *In vivo* exposure has, however, shown transfer of PCBs from plastics to streaked shearwater (*Calonectris leucomelas*) chicks (Teuten *et al*., 2009).

To support these studies, and suggest the likely dose of POPs derived from ingested plastic, an *in vitro* method similar to the approach used in Chapter 6 could be used to determine the bioaccessibility of POPs to avian species. Modifications to this method would be necessary given the constraints of the handling and analysis of POPs. These have largely been addressed in Section 7.3.1, identifying the need for glass apparatus and solvent extraction for analysis of POPs by GC-MS or GC*GC-TOFMS. Application of this *in vitro* approach would avoid the complications and constraints of *in vivo* studies, whilst addressing the necessity of further research focusing on whether, and how, transfer of POPs to marine organisms occurs, as stated recently by Colabuono *et al*. (2010).

7.3.3 Interactions between metals and other polymer types and morphologies

The focus of this thesis has been on interactions between trace metals and polyethylene pellets, which are environmentally abundant and represent one of the most common polymer types in production. However, interactions between metals and other polymer types must be studied. Teuten *et al*. (2007) found that polyethylene had a greater affinity for phenanthrene than polypropylene and PVC, and evidence exists for differential affinities of dissolved trace metals for different polymer types during sample storage (Cobelo-Garcia *et al*., 2007). Batley (1999), states that polyethylene and Teflon both have low affinities for trace metals, which would suggest that other polymer types may interact more readily with trace metals in the marine environment.

Aside from polyethylene, which has been widely reported, records exist for the presence of many polymers in the environment, including PVC and polystyrene (Hidalgo-Ruz *et al*., 2012), acrylic, polyamide (nylon), polyester and polypropylene (Thompson *et al*., 2004). Study of the interactions between metals and polymers other than polyethylene should focus on the most commercially important, therefore most widely used polymers - polypropylene, PVC and polystyrene. Application of similar methods and approaches to those presented in Chapters 4 and 5 would allow direct comparison of interactions between trace metals and other polymer types with the interactions presented in this thesis.

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