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The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms

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A B S T R A C T

In studies of plastic ingestion by marine wildlife, visual separation of plastic particles from gastrointestinal tracts or their dietary content can be challenging. Earlier studies have used solutions to dissolve organic materials leaving synthetic particles unaffected. However, insufficient tests have been conducted to ensure that different categories of consumer products partly degraded in the environment and/or in gastrointestinal tracts were not affected. In this study 63 synthetic materials and 11 other dietary items and non-plastic marine debris were tested. Irrespective of shape or preceding environmental history, most polymers resisted potassium hydroxide (KOH) solution, with the exceptions of cellulose acetate from cigarette filters, some biodegradable plastics and a single polyethylene sheet. Exposure of hard diet components and other marine debris showed variable results. In conclusion, the results confirm that usage of KOH solutions can be a useful approach in general quantitative studies of plastic ingestion by marine wildlife.

1. Introduction

Marine pollution, and specifically plastic pollution, has gained major attention in the scientific world, as well as in the public perception. The United Nations Environment Programme (UNEP) has placed marine debris on the global agenda, and listed it as one of the major emerging environmental problems of this time (UNEP, 2011). By now at least 557 species of marine wildlife have been recorded to either ingest plastic or get entangled in marine debris (Kühn et al., 2015) including several species considered threatened by international standards (Gall & Thompson, 2015). Plastic ingestion by marine wildlife such as seabirds, marine mammals and turtles has received attention in many publications since the late 1960s (e.g. Kenyon & Kridler, 1969; Baltz & Morejohn, 1976; Ryan, 1987; Walker & Coe, 1990; Balazs, 1985; Duguy et al., 1980). Dedicated research on ingestion of plastic by fish however, only gained significant attention from 2010 onwards (e.g. Boerger et al., 2010; Foekema et al., 2013; Lusher et al., 2013; Rummel et al., 2016; see online Supplement Table 1). The increased attention for fish can be explained by potential toxicological impacts of chemicals associated with ingested plastics and the fact that these chemicals may be consumed by humans (Rochman et al., 2012, 2013). Some of these substances are additives integrated into the plastic during manufacture and processing to alter the characteristics of the product (Andrady & Neal, 2009). Other substances have been found to get adsorbed while plastic floats in seawater (Teuten et al., 2009; Mato et al., 2001). The uptake of chemical substances might have an effect not only on the organisms themselves, but also on predators higher up in the food chain, including ultimately human consumers (Tanabe et al., 2003; Tanaka et al., 2013; Van Cauwenberghe & Janssen, 2014; Rochman, 2015; Miranda & de Carvalho-Souza, 2016).

Seabirds, turtles and marine mammals can often only be sampled opportunistically in small numbers. However, in studies of smaller marine organisms dedicated sampling of a large number of individuals is possible (e.g. Dantas et al., 2012; Anastasopoulou et al., 2013; Foekema et al., 2013) and recommended by Galgani et al. (2013). This large sample size has an effect on the methodological approach, as individual dissection can be difficult and time consuming in smaller organisms. In particular when the frequency of occurrence of plastic is low and for reasons of financial efficiency it can be necessary to find methods to process larger bulks of samples even though details on individual occurrence are lost. Different methods to speed up these processes have been attempted. Recently, Dehaut et al. (2016) compared different protocols using chemical solutions intended to dissolve organic matter while retaining ingested plastics. Out of six potentially suitable

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fluids, potassium hydroxide (KOH) solutions proved to be the most effective for this purpose. A literature review on articles dedicated to plastic ingestion by marine organisms revealed that a 10% KOH solution has been used in six earlier studies, mainly analysing the ingestion of plastic in fish (Foekema et al., 2013; Rochman et al., 2015; Tahir & Rochman, 2015; Lusher et al. 2016; Tanaka & Takada, 2016; online Supplement Table 1), and also during the analysis of the complete gastrointestinal tract of a beached humpback whale (*Megaptera novaeangliae*; Besseling et al., 2015). As KOH is mainly sold as solid material it was assumed that the 10% solution used in these studies ranges from 1.5–1.8 Molar depending on purity of the solid material. Dehaut et al. (2016) recommend the use of KOH for future research after testing pristine polymer types, whereas plastics ingested by marine wildlife is mainly composed of plastic consumer wastes that have a) undergone various treatments during production processes b) have been subjected to variable periods of environmental degradation (Ter Halle et al., 2016), and c) may be affected by chemical or enzymatic processes in the gastrointestinal tract of the species studied. The pH in stomachs can vary depending on its fullness. It has been found that the pH in albatross stomachs can be as low as 1.5 (Gremillet et al., 2012) and 1.4 in fish (Lavell, 1989). Enzymatic degradation (Tokiwa et al., 2009) as well as temperature might also alter the characteristics of plastic in stomachs of organisms.

Thus, in addition to fresh industrial polymer types, the scope of the current study was extended to a range of consumer products and included environmentally degraded samples collected from beaches. Furthermore the potential impacts from digestive processes were evaluated by repeating KOH solution tests on a selection of samples that had been exposed to stomach fluids of northern fulmars (*Fulmarus glacialis*), acids, or both prior to the KOH solution treatment. A number of so-called biodegradable or compostable plastics was included in these KOH solution experiments. Circumstances at sea differ tremendously from degradation processes in the compost industry (Accinelli et al., 2012; Shah et al., 2013) and increasing proportions of biodegradable plastics may persist in the marine environment for long periods of time. Such plastics may already be or become a relevant issue in studies of plastic ingestion by marine wildlife.

2. Methods

2.1. Sample collection and preparation

For this study 74 different types of particles were examined, 63 of which belonged to different plastic types and shapes, and an additional 11 samples of other debris or natural hard dietary items. Of the 63 plastic particle types, six belonged to biodegradable plastics and 57 to conventional plastics. 16 particles were pristine industrial polymers, 36 were taken from fresh consumer products and 14 were collected from beaches (containing samples of both environmentally degraded industrial pellets and consumer waste). An overview of particles studied is given in Table 1 and full details are available in the online Supplement. All items have been categorized following Van Franeker et al. (2011) and OSPAR (2015) which distinguish industrial pellets, sheets, threads, foams, fragments, threads and 'other'. For the purpose of this study the category 'other' has been split into a category for rubber-like materials and one for other plastics. Plastic debris was collected from beaches in the north of the Netherlands, especially the island of Texel (53° 1′ N, 4° 8′ E) over a number of recent years. A majority of the 63 tested plastic samples (n = 48) had an identified polymer composition, either provided directly by the producer (supplied by Carat GmbH), read from product labels, or identified using FTIR-ATR (Fourier Transform Infrared-Attenuated Total Reflection; Shimadzu Prestige, No. of Scans: 10, Resolution: 4 (1/cm), Spectra: 4000–600 1/cm, Apodization: Happ-Genzel, Library ATR Polymer2, IRs Polymer 2, T-Polymer). The other 15 samples are of an unknown polymer type. The KOH solution resistance of 11 samples of items regularly encountered in marine organisms or on beaches was tested, including seaweed, squid beaks, polychaete beaks, sheep wool, seal whiskers, fish otoliths, bird feathers, manilla rope and a metal fish hook. Other debris includes lumps of beached paraffin and palm fat. Full details can be found in the online Supplement.

Table 1
Characterization of samples used in the KOH experiment (n = 74) by category, origin and (when applicable) polymer type. Classification of categories follows van Franeker et al. (2011).

Category	Nr. of samples (Conventional/ biodegradable)	Pristine/ consumer/ beach	Nr. of samples with known polymer basis [a]
Pellets	20 (18/2)	16/0/4	16
Sheet	14 (11/3)	0/12/2	9
Thread	4 (4/0)	0/3/1	3
Foam	7 (7/0)	0/4/3	6
Fragment	8 (7/1)	0/8/0	7
Rubber	8 (8/0)	0/6/2	6
Other synthetic	2 (2/0)	0/2/0	1
Other debris[b]	3	0/1/2	na
Natural[c]	8	na	na

[a] Identified polymers used in this study: ABS = acrylonitrile butadiene styrene, CA = cellulose acetate, EVA = ethylene-vinyl acetate, HD-PE = high density polyethylene, GPPS = general purpose polystyrene, LD-PE = low density polyethylene, NBR = nitrile butadiene rubber, PA = polyamide, PBT = polybutylene terephthalate, PC = polycarbonate, PE = polyethylene, PET = polyethylene terephthalate PP = polypropylene, PS = polystyrene, PUR = polyurethane, PVC = polyvinyl chloride, SAN = styrene acrylonitrile resin and different biodegradable bioplastics: Bio4Pack, Cradonyl, Mater-Bi, PHB = Polyhydroxybutyrate, PLA = polylactic acid, Wenterra.
[b] Including: palm fat, paraffin, metal fish hook.
[c] Including: seaweed, squid beaks, polychaete beaks, sheep wool, seal whisker, fish otoliths, bird feather, manilla rope.

2.2. Sample processing

Larger objects were cut to particles sized approximately 5 mm diameter as industrial plastic pellets and some natural objects had such size already. With a few exceptions (e.g. natural substances, a fish hook, a soft airgun bullet), 10 particles were combined to one sample and were then weighed to the nearest 0.0001 g (Sartorius 1702MP8). The 63 samples weighed on average 0.29 g, sd ± 0.67 g (maximum weight 4.49 g). They were put in glass jars and submerged in circa 20 ml of a 1 Molar KOH solution (Fisher Chemicals) at 18–21 °C (hereafter referred to as room temperature) for two days. Samples were then rinsed under tap water on a 0.3 mm sieve, dried at room temperature for six days and weighed again.

Earlier publications using 10% KOH solutions (depending on purity 1.5–1.8 M concentration) and earlier trials (see online Supplement) had shown full dissolution of fish tissue. Therefore in the current study, the lower 1 M concentration was used in order to further reduce any potential effect on plastics. Drying the samples at room temperature was the chosen method to keep the procedure as simple as possible in other situations where no incubators are available. Furthermore this approach corresponds to the method used during the standard fulmar research (Van Franeker et al., 2011).

Tests exposing sprat (*Sprattus sprattus*) to a KOH solution of 1 M at room temperature for two days, showed that samples of stomachs and intestines (ca. 1 g), and even a whole sprat (10.5 cm, 8.9 g), dissolved completely (except for the otoliths), with remains passing through a 0.3 mm sieve without problems. Therefore he tests with individually known plastics using 1 M solution for two days exposure were executed.

2.3. Tests in gastrointestinal fluids

In a second experiment 22 samples were subjected to a simulated gastrointestinal environment prior to the KOH treatment (see Table 2). The treatment in KOH followed the same procedures as mentioned

Table 2
Characteristics of samples (n = 22) exposed to stomach fluid and/or acids (HCl) prior to the KOH experiment. None of the samples belonging to the categories "other synthetics" and "other debris" were tested. For details on polymer types and natural substances see Table 1.

Category	Nr. of samples (Conventional/ biodegradable)	Pristine/ consumer/ beach	Nr. of identified polymer types
Pellets	9 (9/0)	8/1/0	8
Sheet	1 (1/0)	0/1/0	0
Foam	4 (4/0)	0/3/1	3
Fragment	1 (0/1)	0/1/0	1
Rubber	3 (3/0)	0/2/1	1
Natural	2	na	na

Table 3
Summarized results of tests in which individually known synthetic substances were exposed to KOH solution of 1 M for two days at room temperature. Tabulated are mass changes in percentages of samples after KOH treatment and 6 days of drying time at room temperature. None of the changes in synthetics, rubbers and biodegradable plastics were significant.

Category	n	Average change	± sd	Minimum change	Maximum change
Synthetic pellets	18	0%	±1%	0%	3%
Synthetic sheets	11	4%	±23%	−7%	75%
Synthetic threads	4	16%	±25%	0%	51%
Synthetic foams	7	44%	±43%	9%	119%
Synthetic fragments	7	0%	±0%	0%	1%
Other synthetics	2	−16%	±22%	−31%	0%
Rubbers	8	2%	±2%	0%	6%
Biodegradable plastics	6	−7%	±42%	−100%	14%
Natural Materials	8	−31%	±60%	−100%	38%
Non natural materials	3	0%	±1%	−1%	1%
Animal tissues	4	−54%	±50%	−100%	0%

above (2 days, 1 M KOH). After weighing, the subsamples were put in stomach fluids of northern fulmars, or in undiluted hydrochloric acid (HCl, 1 N standard solution, Acros Organics) or in a mixture of these for one day. Stomach fluids originated from northern fulmars hunted for human consumption on the Faroe Islands and were stored at −20 °C until usage. For the chemical composition of fulmar stomach fluids see: Wang et al. (2007). From literature the pH value in stomachs of live seabirds was known to be around 1.5 (Gremillet et al., 2012). However, when measured in the lab the pH of the stomach fluid was found to be around 4. To correct for this change, acid was added artificially, as stomach fluid apparently changes its pH value after death or during storage. To be able to differentiate between possible effects caused by either stomach fluid or acid or both samples were treated with these substances separately and in combination. After treatments, the samples were rinsed as described for the KOH tests above. After an initial drying period of 3 days, parts of the samples still felt wet and had excess weight. For practical reasons the samples then had to be stored for four weeks in plastic bags. Following storage samples were dried for an additional arbitrary period of five days before weighing for a second time.

2.4. Statistical analysis

Statistical analysis were conducted for mass differences before and after KOH treatments and were performed using Genstat 18th Edition (Payne et al., 2015). Potential usage of *t*-test for paired samples was evaluated by the Shapiro-Wilk test for Normality, which showed that only data for industrial pellets were sufficiently close to normality to allow usage of the *t*-test. As a non-parametric alternative, the Mann-Whitney *U* test (equal to Wilcoxson rank-sum) was used for the tests. Significance level was taken as $p < 0.05$.

3. Results

Among the traditional plastics, most items with relatively smooth hard surfaces, (the industrial pellets and fragments) were completely unaffected with 0% average mass change, and at most 3% change in an individual type (Table 3). Results for pellets by *t*-test paired samples showed differences before and after KOH treatment to be non-significant ($n = 18$, $p = 0.076$). The Mann-Whitney *U* tests revealed no statistical differences in any of the categories of synthetics, rubbers or biodegradables (Categories and sample sizes shown in Table 3).

The categories contain a broad range of different polymers that remain unaffected from exposure to 1 M KOH solution, and this is valid also for particles showing environmental degradation (e.g. discoloured yellowish pellets collected from the beach) and also in tests simulating potential gastrointestinal degradation of the materials (Table 4). Full results are available in the online Supplement.

Other categories of synthetic materials showed much more variable results, although mostly not in the sense of mass loss. Some types of sheet- and threadlike materials, especially foams, retained considerable

Table 4
Details of standard KOH solution impacts on industrial plastic pellets, pristine and from beaches, including simulated impacts from digestion processes in seabird stomachs (stomach fluids SF; acid environment (HCL) or their combination). Shown are percentages of change in mass of plastic after treatments.

Synthetic pellets			Treatment weight change			
Description	Origin	Polymer type	KOH solution	SF + KOH	HCl + KOH	SF + HCl + KOH
Black pellets, possibly recycled	Beach		−0.1%			
White pellets	Pristine	LD-PE	0.0%	0.6%	0.2%	0.4%
White pellets	Pristine	PP	0.0%	0.4%	−0.6%	0.1%
White pellets	Pristine	PBT	0.0%			
White pellets	Beach		0.0%			
Transparent pellets	Pristine	EVA VA19%	0.0%			
Purple pellets	Pristine	PET	0.1%	0.4%	7.0%	−2.9%
Green pellets	Pristine	PE-LLD recycled	0.1%			
Transparent pellets	Pristine	SAN	0.1%			
White pellets	Pristine	PA66	0.2%	1.9%	1.4%	1.7%
Transparent pellets	Pristine	GPPS	0.2%			
Discoloured (yellowish) pellets	Beach		0.2%			
Black biobead (sewage usage?)	Beach		0.2%	0.2%	−0.1%	0.1%
Transparent pellets	Pristine	PC	0.3%	0.1%	0.1%	0.3%
Transparent pellets	Pristine	PA6	1.0%	1.4%	1.4%	1.6%
White pellets	Pristine	ABS	1.1%			
White pellets	Pristine	HD-PE	1.6%	0.5%	0.0%	0.6%
Orange pellets	Pristine	HD-PE	2.8%			
Total	n = 18		0.4%	0.6%	1.0%	0.2%

moisture under the test conditions, and became heavier, apparently given insufficient time to fully dry.

In a few samples, however, substantial loss of mass was observed. One sheet-like plastic of low density polyethylene (LD-PE) lost 7% of original mass. Cigarette filter material (CA cellulose acetate) lost 31% of its mass, and among the biodegradable plastic types, a sample from a bag made from PLA (polylactic acid) dissolved completely, while pellets from a material known as Cradonyl lost 33% of their original mass.

Non-plastic debris that was tested showed resistance to KOH solution. In this short experiment, lack of change in a fish hook was expected, but remarkably, the fairly soft materials of beach collected lumps of paraffin and palm fat did not suffer weight loss from exposure to the KOH solution.

Among non-plastic materials found in, for example, bird stomachs, many substances were dissolved by the KOH solution (Polychaete jaws, sheep wool, seal whiskers, bird feathers) but others were largely retained, two notable examples being fish otoliths and squid beaks. The difference between squid beaks and polychaete worm jaws is remarkable as both are assumed to be built from the same natural polymer, keratin. Table 3 summarizes the results by category of material. Full details are given in the online Supplement.

Balloon rubber, likely largely consisting of natural latex, did not show a decrease in weight, but rather, exceeded its original mass after processing. It was also seen that the exposure of balloon rubber to the stomach fluid of northern fulmars triggers a change in material characteristics. The rubbery structure was lost and the material became fluid and sticky. These results correspond to the personal observations of the authors when finding balloon remains or similar substances in the stomachs of northern fulmars: although not evident inside the stomach, once removed and laid out to dry, many of these items become a mucoid mud rather than a dried particle.

Foamed materials in particular when exposed to fulmar stomach fluids, sometimes showed an excessive increase in weight (see online supplement) indicating that the oily substances in these stomach fluids had settled in the airy spaces and were not removed by simple rinsing in cold water and subsequent drying. Results for industrial pellets (Table 4) indicate, however, that the conclusion is justified that exposure to stomach fluids or HCL does not make plastic polymers more prone to dissolving in KOH solution.

4. Discussion

When promoting a new method it is important to make sure that it works under various circumstances. Exposing stomachs and their contents to KOH solutions is a newly proposed method to efficiently study plastic ingestion in situations where individual visual separation of plastics from gastrointestinal tracts is too difficult or too labour intensive. However, the assumption that indeed organic components are dissolved and all synthetic materials are retained needs proper testing. Such testing should include not only virgin polymer types, but must consider a wide variety of synthetic consumer products, also taking into account the potential impacts from material degradation in the environment or in the gastrointestinal tracts of species studied.

During this study, plastics of different categories, polymer types and origins as well as natural substances and items were tested and exposed to a KOH solution in a concentration which easily dissolved the tissues of a small fish species (*Sprattus sprattus*, solution of 1 Molar KOH concentration, applied during 2 days at room temperature). The results show that a wide range of polymer types, in different shapes and forms, and partly subjected to earlier environmental degradation were not affected by this KOH solution treatment. As was reported by Dehaut et al. (2016) one of the few synthetic substances that was dissolved in KOH solution was cellulose acetate (CA), in this study represented by a cigarette filter. Cigarette filters are often found in high numbers on tourist beaches (Ocean Conservancy, 2016). Besides additives added to the polymer during production processes, the toxic compounds of cigarettes are attached to the filters and have been proved to be toxic to fish (Slaughter et al., 2011). However, in plastic samples from the marine environment, cigarette filters seem infrequent. Once soaked, the material will probably disintegrate and loose fibrous elements (Puls et al., 2011) and will not be easily detected in the water or in animal stomachs or intestines. In long term studies of plastic ingestion in northern fulmars in the North Sea (Van Franeker et al., 2011) there are only a few records of ingested cigarette filters. Results for the Netherlands suggest maybe 3 such cases in over a 1000 stomachs analysed.

Remarkably, among several samples representing polyethylene (PE) one bag labelled as being made of LD-PE showed some degradation, with 7% mass loss in the experiment. The reason for this contradictory case is not clear, but could indicate degradation or production issues.

Two out of six biodegradable plastics showed substantial degradation following KOH treatment: fragments from a bag made from polylactic acid (PLA) disappeared completely, and granules of a material called Cradonyl lost one third of their original mass. In 2014, bio-based and biodegradable plastics together represented <1% of the global plastic production (EUBP, 2016). Even though the degradation of bioplastics in the ocean is very slow (Accinelli et al., 2012; O'Brine & Thompson, 2010), this suggest that only a small share of the marine debris currently consists of this material. In the near future, the proportion of such polymers will likely remain low, even though the production is expected to quadruple by 2019 (EUBP, 2016). However, the effects of this development should be monitored, as on the longer term this might have consequences on the composition of plastics found in the ocean and subsequently in marine organisms.

Treatment of samples with seabird stomach fluids and acids to simulate digestive processes did not change the results of the KOH tests. The results of this study indicate that the chosen KOH solution concentration (1 M, two days at room temperature) can be safely applied in a quantitative study to estimate levels of plastic ingestion by small fish. Proportions of ingested plastics that are lost through KOH treatment will be relatively small. The tests, however, also showed that drying times needed to get rid of moisture in foamed plastics were substantial, and that such problems were aggravated by fatty stomach fluids. This implies that when a study species ingests a substantial proportion of foamed materials, additional procedures for cleaning and drying the isolated plastics should be considered and tested. Standard protocols often advise to quantify plastic ingestion by marine wildlife in terms of plastic mass (Ryan et al., 2009; Van Franeker et al., 2011; OSPAR, 2015; Provencher et al., 2016). Components of the dissolved KOH could also contribute to the increased weight.

An initial trial with samples containing a mix of plastics collected on beaches (see online supplement) suggests that the conclusions are similarly valid with increased concentrations of 10% KOH solutions. Results by Dehaut et al. (2016) suggest that such a concentration can also be applied safely at a temperature of 50 °C. On the other hand, a preliminary test on digestion of seabird intestines under our standard exposure of 1 M KOH for 2 days, showed them to be immune to that concentration even if duration was prolonged. Depending on the tissue to be dissolved, concentration and duration may need to be increased to achieve disintegration of the organic material. The fulmar intestines tested in these studies were found to dissolve completely in three days in 5 M KOH solution at 60 °C. Resistance of plastics to such other conditions will vary and needs additional testing. When studies of plastic ingestion are combined with research of the diet, care should be taken with KOH applications. Important diet items such as fish otoliths and squid beaks appear to be resistant, but some other similar components disappeared. Therefore it is recommended to test typical prey items occurring in the species studied to make sure they are resistant to the KOH dissolution procedure.

Overall, the results of this study confirm in detail that the KOH approach to separate plastics from organic matter is a valuable and practical method in studies of plastic ingestion by marine wildlife.

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Appendix A. Supplementary data

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