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Pigments and plastic in limnetic ecosystems: A qualitative and quantitative study on microparticles of different size classes

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ABSTRACT

Recently, macroplastic (>5 mm) and especially microplastic (<5 mm) particles have been reported as emerging contaminants in marine and limnetic ecosystems. Their coloration is gained by the addition of pigments to the polymer blend which is the major component of the respective product. However, color is also a feature of paint and coatings whereby the pigment is the major component. Once abraded from a surface, paint particles may enter the environment via similar pathways as microplastic particles.

So far no detailed studies of microplastic particles (pigmented and non-pigmented) as well as paint particles have been performed focusing on very small microparticles (1–50 μm), in either marine or limnetic ecosystems. Using Raman microspectroscopy with a spatial resolution down to 1 μm, we report a remarkable increase in the occurrence of (pigmented) microplastic particles below 500 μm. Among those, most particles were found at a size of ~130 μm in a freshwater ecosystem (subalpine Lake Garda, Italy). Moreover, our qualitative and quantitative analyses revealed that the number of paint microparticles significantly increased below the size range of 50 μm due to their brittleness (the smallest detected paint particle had a size of 4 μm). Inductively coupled plasma mass spectrometry measurements showed that both colored particles found in nature as well as virgin particles contain a high variety of metals such as cadmium, lead and copper. These additives may elicit adverse effects in biota ingesting these microparticles, thus paints and associated compounds may act as formerly overlooked contaminants in freshwater ecosystems.

1. Introduction

Technical advances are often accompanied by the development of modern and highly innovative materials, chemicals and compounds. One recent change in material use is reflected in plastic production which has grown up to 311 million metric tons per year worldwide (PlasticsEurope, 2015), and has led to a change in the composition of our household and industrial waste. As a consequence, an increase of plastic litter has been reported in marine ecosystems (Thompson et al., 2009). Plastic waste is introduced into the sea as larger waste fragments (macroplastic particles, >5 mm), is then degraded by UV-radiation and subsequently fragmented through mechanical forces and biological processes into tiny "secondary" microplastic particles (<5 mm, Browne et al., 2007). In contrast pre-production pellets lost during transport or spilled at the production plants belong to "primary" sources (Hidalgo-Ruz et al., 2012). Small microplastic fibers released from synthetic clothing through mechanical processes in washing machines, in addition to "primary" microplastic particles, often used as abrasives in cosmetics or cleaning products, are introduced into the aquatic environment via sewage effluents (Browne et al., 2011; Fendall and Sewell, 2009; McCormick et al., 2014). As a consequence, not only marine habitats are affected. Just recently scientists discovered the existence of microplastic particles in limnetic ecosystems (reviewed in: Dris et al., 2015).

To achieve one appealing feature of plastic products – their

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coloration – the industry adds pigments to the polymer blends whereby the polymer usually remains the major component of the respective product (Freitag and Stoye, 2008). Though colorfulness is not exclusively a feature of plastic products. The utilization of pigments in colorful paintings and coatings is ubiquitous not only for wooden as well as metal items, but also lightweight products made of plastic, fiberglass or carbon fibers (Freitag and Stoye, 2008; Turner, 2010). In this case, the pigment is the major component and the polymer serves as a binder, such as an alkyd for industrial paints (e.g. in marine applications, Shtykova et al., 2006) or a (poly)acrylate/styrene (Song et al., 2014).

In the same manner as plastic particles, pigment coated surfaces are both mechanically weathered and abraded leading to the formation of so called paint particles. Heavy abrasion occurs while sandblasting buildings or in ship maintenance and repair facilities, in both cases generating dust as well as large fragments (Takahashi et al., 2012). Several studies have already reported on paint particles in terrestrial environments (Bogden and Louria, 1975; Sturges and Harrison, 1985). These paint particles can subsequently enter the aquatic ecosystem either directly or indirectly through wind and water run-off, likely with additional fragmentation occurring. Paint particles observed on rooftops in coastal locations were found to travel long distances via wind and water (Turner, 2010). These particles have also been discovered in the marine environment, including tidal inlet sediments along the English coast, the seabed next to marinas in Malta or on the sea surface (Norén and Naustvoll, 2011; Song et al., 2014; Takahashi et al., 2012; Turner, 2010).

Similar to the ubiquitous macro- and microplastic particles, microscale paint particles are prone to consumption by aquatic organisms from different trophic levels and feeding strategies. Ingestion may incur negative effects on the biota, for example through toxic or potentially harmful additives, mixed directly into the polymer blend (Cole et al., 2013; Eerkes-Medrano et al., 2015; Lithner et al., 2011; Wright et al., 2013). Another threat from particle ingestion arises from their ability to adsorb hazardous substances such as persisting organic pollutants (POPs) or toxic metals (Bakir et al., 2012; Holmes et al., 2012). Colored plastic particles containing pigments may supply an additional high burden of metals. Furthermore, other substances associated with paints and coatings may have adverse effects on organisms or the environment. These mixture of hazardous substances, either adsorbed or intentionally added, can be eventually released in the digestive tract of the organisms that swallowed the respective particle(s) (Browne et al., 2013). In addition, very small particles (1 μm–50 μm) pose a higher risk of being incorporated into the organism's tissue (Van Cauwenberghe et al., 2015). Despite the high potential ecological risk of tiny particles, few studies have been able to identify particles with sizes below 500 μm, even fewer yet below 50 μm, from environmental samples (reviewed in: Dris et al., 2015; Hidalgo-Ruz et al., 2012; Ivar do Sul and Costa, 2014; Rocha-Santos and Duarte, 2015; Van Cauwenberghe et al., 2015). The lack of an extensive qualitative and quantitative analysis of microparticles in the environment may arise from the limited number of reliable identification methods. Until now only two methods (micro-FTIR and Raman microspectroscopy, RM) can provide the identification of environmental microparticles with a spatial resolution down to ~20 μm and 1 μm, respectively (Enders et al., 2015; Imhof et al., 2013; Löder and Gerdts, 2015; Van Cauwenberghe et al., 2013).

In this study we confirm for the first time the existence of paint particles in a freshwater ecosystem based on samples from beach sediments of Lake Garda. To get further insights in the size distribution of plastic particles compared to paint particles from freshwater beach sediments we quantitatively and qualitatively assess the composition of microparticles including those below 50 μm by means of Raman microspectroscopy. As metals can adsorb to plastic particles or can be an inherent load, in a second step additional inductively coupled plasma mass spectrometry (ICP-MS) measurements reveal the elemental composition of sampled plastic particles (pigmented and non-pigmented) and provide a first time assessment of freshwater plastic particle metal contamination. A comparison with the metal burden of unused colored plastic particles may indicate possible sources.

In detail we aimed to analyze: (i) the concentration of non-pigmented and pigmented plastic particles in comparison to paint particles (ii) the size distribution of the particles (especially in the size range of very small microparticles (1–50 μm), (iii) the spatial distribution of plastic and paint particles and (iv) the elemental composition of a subsample of particles compared to unused plastic products.

2. Materials & methods

2.1. Sediment collection

Beach sediment sampling was performed at Lake Garda, Italy. The lake is located at the eastern border of the subalpine region and its water body belongs to one of the key elements of the tourist economy (Salmaso and Mosello, 2010). Moreover Lake Garda is the largest freshwater supply in Italy. The water is used for domestic consumption, agricultural and industrial purposes such as the generation of hydroelectric power and it supports a small but productive fishery (Magni et al., 2008). We collected sediment samples at 5 different beaches along the coastline of Lake Garda, Italy. As the aim of this campaign was the establishment of a standardized sampling method we performed 3 replicates at each sample area within the littoral zone (at the waterline, in the flotsam and at the high water line). Sampling itself was performed using a 20 m transect in combination with 10 sediment cores (diameter 10 cm) to a depth of 5 cm with an interval of 2 m, resulting in a sample volume of approximately 4–6 L of each location. Each sample was stored in a wide neck polyethylene container of 6 L volume. Contamination through abrasion from the container was excluded, since no particles with the identical spectral pattern were detected by RM.

2.2. Particle extraction from Lake Garda beach sediments

The extraction of the particles was performed using the Munich Plastic Sediment Separator (MPSS, Imhof et al., 2012). As separation fluid a zinc chloride solution ($ZnCl_2$ technical grade, Th. Geyer, Germany) with a density of 1.6–1.7 kg/L was used. This technique enables us to extract all particles with a density lower than 1.6 kg/L with a recovery rate of 100% for macro- and large microplastic particles (>1 mm) and 95.5 ± 1.8% for small microparticles (<1 mm, Imhof et al., 2012). After introduction of the sample into the MPSS, large fragments (>1 cm) were singled out and thoroughly rinsed with zinc chloride solution in order to return potentially attached particles back into the MPSS. The sediment was stirred for 3 h, then the sediment had 12 h (overnight) to settle down. Similarly the less dense particles had enough time to rise to the surface. Depending on the amount of organic matter e.g. biofilm, algae, leaves, etc., the sample was subsequently (i) directly filtered on a quartz fiber filter (retention efficiency 98% at 2.2 μm, smaller particles will be retained with lower probabilities, Whatman QM-A, GE Healthcare Europe GmbH) and rinsed with hydrogen peroxide and distilled water; (ii) the samples were sieved using a 750 μm mesh into 250 ml centrifuge tubes (Eppendorf AG, Germany). Particles >750 μm were picked with tweezers and stored in petridishes until they were identified using RM. Centrifugation of the fraction with particles <750 μm with slow speed (~1500 rpm) facilitated the sedimentation of e.g. clay minerals. The supernatant was transferred in a glass jar and the centrifuge tube was carefully rinsed. With a large syringe we removed the zinc chloride solution and added hydrogen peroxide. To avoid precipitation (of e.g. $CaCO_3$) sulfuric acid (10%) was added dropwise. While having a clear solution we added more sulfuric acid until bubbles started rising resulting in peroxymonosulfuric acid, which oxidized organic residues. After stirring for approximately one week, the samples were filtered over a quartz fiber filter with a retention efficiency of 98% at 2.2 μm (Whatman QM-A, GE Healthcare Europe GmbH) and rinsed thoroughly with distilled water. As the quartz filter also will retain smaller particles with lower probabilities, we define the analyzed particles size range starting from 1 μm (since Raman microspectroscopic analysis was performed with a spatial resolution down to 1 μm, see below).

2.3. Identification by Raman microspectroscopy

All particles were identified by means of RM, which enables measurements of vibrational fingerprint spectra and hence the identification of polymer and pigment particles with spatial resolution down to 1 μm. The spectra were recorded in the laboratory of TUM-IWC using a Horiba LabRAM HR Raman microscope system (Horiba Scientific, Japan) with a He-Ne laser (632.8 nm, 40 mW, ca. 14 mW at the sample) and three objectives with 10- and 50-fold ($NA_{10} = 0.25$, $NA_{50} = 0.75$, Olympus, Germany) as well as 50-fold magnification with long working distance ($NA_{50LWD} = 0.50$, Olympus, Germany). A grating with 600 grooves/mm and a confocal slit width of 100 μm were used. The system was calibrated by zero-order correction of the grating and additionally on the 520.7 cm^{-1} peak of a silicon wafer. The spectra were recorded in the range of 50–4000 cm^{-1}. Macro- and large microplastic particles (>750 μm) were singled out and identified. All other microparticles were detected on the quartz fiber filter. As a fully automatized mapping of the entire filter would be very time consuming, we manually identified particles 500–750 μm on the entire filter surface and particles smaller than 500 μm on 5 spots on each filter with a surface of 4.185 mm^2 resulting in an analyzed surface of 20.92 mm^2 (1.6% or ~1/60 of the effective filter surface, diameter of 41 mm, 1320.25 mm^2, Fig. S3). Each spot was divided in 9 small squares with 700×700 μm^2. This resembles the field of view using a 10 × objective ($NA_{10} = 0.25$). In each field of view all particles were counted, analyzed and identified. For some particles "bleaching" (illumination with the laser before spectrum accumulation) was necessary to reduce the fluorescence background in Raman spectra.

The identification of plastic and paint particles was performed by comparison to commercial databases (SpecID, Thermo Scientific, USA under LabSpec 5, Horiba Scientific, Japan) and to Raman spectra of reference (colored) plastic materials collected in the TUM-IWC laboratory. However, we cannot completely exclude that the identification of polymers in some colored particles was hindered due to a high fluorescence background and therefore some particles may still count as microplastics. The detailed protocol for the differentiation of colored plastic particles and pure pigmented particles is available in the Supplementary Material.

2.4. Size measurements

After RM analysis the size of all particles identified as plastic particles (colored or uncolored) and paint particles was measured. Singled out particles were imaged using a camera or a digital image analyzing system (Leica MS5 equipped with an Altra 20 camera, Olympus, Germany and the software Cell^P, Olympus, Germany) and measured using ImageJ (U. S. National Institutes of Health, Bethesda, Maryland, USA). Particles on the filter were imaged using the microscope of the Raman system and measured with the RM Software LabSpec 5 (Horiba Scientific, Japan). The size is given as the largest distance between two spots for all fragmented particles, for all long and elongated particles (e.g. fibers) the diameter and the length of the particles is given.

2.5. Prevalence of toxic metals—analyses of metal burden with ICP-MS

2.5.1. Metal burden of plastic particles from Lake Garda beach sediments

A subset of 11 of the plastic and pigment particles found during the first sampling campaign in 2011 (Imhof et al., 2013) were analyzed individually by ICP-MS to quantify the content of different elements. This subset included 2 paint particles, 1 white polystyrene particle (1.9 mm), 2 green polypropylene particles (size 3.0 & 14.11 mm), 4 polyethylene particles (green & transparent green, 15.5–24.4 mm). As control without pigments we included one polypropylene (size 6.3 mm) and one polyethylene particle (size 3.0 mm), both showing Raman signatures of only the polymer, indicating the absence of additional color pigments. For an overview about the analyzed particles please refer to Table S1 in the Supplementary Material.

2.5.2. Metal burden of unused plastic products

To compare the elemental content of already degraded plastic particles with almost fresh colorful plastic products we additionally analyzed unused examples of plastic products which are commonly found in the flotsam of lake ecosystems. These were colorful screw caps (N = 10) of plastic beverage bottles (red, green and blue), commercially available plastic bags (biodegradable or recycled) with color prints (N = 3) and colored packaging material (N = 1). The pigments and plastic types were previously characterized by means of RM. The screw caps were cut into small fragments using ceramic scissors. The plastic bags and packaging material were dissembled to small pieces to separate the different colors. For an overview about the analyzed particles please refer to Table S2 and Table S3 in the Supplementary Material.

2.5.3. Digestion and ICP-MS measurements

The samples were individually analyzed with a quadrupole ICP-MS (Elan 6100, Perkin Elmer, USA) to quantify the content of the different elements. All ICP-MS measurements samples (150–200 mg) were digested with 2 mL of hydrogen peroxide (30%, Carl Roth GmbH & Co. KG, Germany), 5 mL of nitric acid (65%, Suprapur, Merck KGaA, Germany) and 5 mL of sulfuric acid (95% Suprapur, Merck KGaA, Germany) by microwave-pressure decomposition. Resulting solutions were diluted for analysis. ICP-MS measurements of plastic bags and packaging material were replicated with three samples of each product with respect to the large surface area. These repeated measurements verified good reproducibility of ICP-MS analysis. Because of their small size the particles extracted from Lake Garda beach sediment were only measured once. The obtained values were corrected with acid blank values. All results are given in μg/g (μg element per g sample).

2.6. General precautions to prevent procedural contamination

During sampling all persons wore clothing made of cotton to prevent contamination with fibers from synthetic clothes. The used zinc chloride solution was reused and therefore filtered by a two-step filtration process using stainless steel candle filters of 20 μm (OES, 20 μm, 10“, MTS Filtertechnik, Germany) and subsequently by 1 μm candle filters (TAX, 1 μm, 10“, MTS Filtertechnik, Germany). All other fluids were filtered over 0.2 μm filter paper to prevent particulate contamination. All samples were always covered with aluminum foil to prevent aerial contamination.

3. Results

3.1. Identification of macro- and microparticles in Lake Garda beach sediment

In the samples from Lake Garda high numbers of (micro)particles were found and identified by Raman microspectroscopy (RM). Some of the particles could be identified as synthetic polymers (see Table S4 & S5 in the Supplementary Material). In addition for a large amount of particles we were able to identify pigments, however we were not able to distinguish the underlying polymer. The pigment containing fragments where synthetic polymers were not identified had a size distribution in the range of 5 μm–10 mm. Some of these colored particles had obviously mechanical and optical properties which were clearly different to typical plastic particles. In contrast to the polymer particles they were very brittle and had to be handled with additional care. Only two particles (1%) were larger than 5 mm. Both were very brittle and optically very similar to a paint fragment (one particle is shown in Fig. 1). Furthermore, RM analysis identified additional components in some of these particles which are related to paints and coatings (e.g. oleic acid, gypsum, Fig. 1).

We anticipate that these particles are paint particles which found their way into the aquatic environment as the use of paints and coatings is common in our everyday life. Therefore the abundance values of the microparticles found in Lake Garda beach samples is considered separately for plastic particles (polymer is the main component and belongs to the well-known polymers used for the so called plastic products e.g. polyethylene, polypropylene, polystyrene, polyamide, etc.) and paint particles (polymer as binder, high pigment content). This binder is often an alkyd in industrial paints, such as those used in marine applications (Shtykova et al., 2006), or a (poly)acrylate/styrene (Song et al., 2014). However, we were not able to clearly identify alkyds and (poly)acrylate/styrene at the low concentrations used in paint particles.

3.2. Size distribution of microparticles of (micro)plastic and (micro) paint particles

The size distributions of the microplastic particles and the micropaint particles found are very different (Fig. 2). The plastic particles represent almost 60% of the identified particles and contribute to all size classes. The contribution of the polymer types to the different size classes is visualized in Fig. 3. The paint particles have a clearly skewed size distribution towards a smaller size. Moreover both size distributions shows different maxima. The microplastic particles have a slight maximum between 50 and 500 μm. Among this size class most particles are found at ~130 μm. In contrast most paint particles were found in the size class <50 μm (Fig. 2).

3.3. Spatial distribution of (micro)paint and (micro)plastic particles

The mean abundance of plastic particles in Lake Garda beach sediment was 75 ± 134 particles/m^2. The abundance was very variable between the 5 sample areas which is resembled by a large standard deviation. On average similar amounts of paint particles were found but also here the spatial variability is high throughout the lake (58 ± 58 particles/m^2, some replicates did not contain paint particles). Plastic and paint particles were not equally distributed around Lake Garda. Spatial distribution of the particles is visualized in Fig. 4 for plastic and paint particles. Abundance of the entire size range is given for each sample area but the particles <50 μm are highlighted (Fig. 4). Detailed abundance values for plastic particles and paint particles of all size classes are listed in Table 1.

3.4. Pigment variability occurring in the plastic and paint particles found

3.4.1. Pigmented plastic particles

RM revealed that 5% of the found plastic particles with a size range between 60 μm and 27 mm do not only consist of polymer, but also of pigment(s). An overview about the identified polymers and pigments for the pigmented plastic particles in the different size classes is given in Fig. S4. The pigments identified in the plastic particle are given according to the definition of the International Color Index. They were namely: Pigment Blue 15 (33%, phthalocyanine blue), Pigment Green 7 (17%, phthalocyanine green) and Pigment White 6 (42%, Rutil/Anatase, TiO_2) as well as Pigment Yellow 83 in samples of Lake Garda. Additional pigments were identified during the analyses of the unused plastic products: Pigment Blue 29, Pigment Black 7 (carbon black), Pigment Red 170 and a commercial available dispersion mixture from BASF (Disperse green 85-0117) which was most probably a mixture of Pigment Green 7 and Pigment Blue 15. Some whitish, grey or brownish

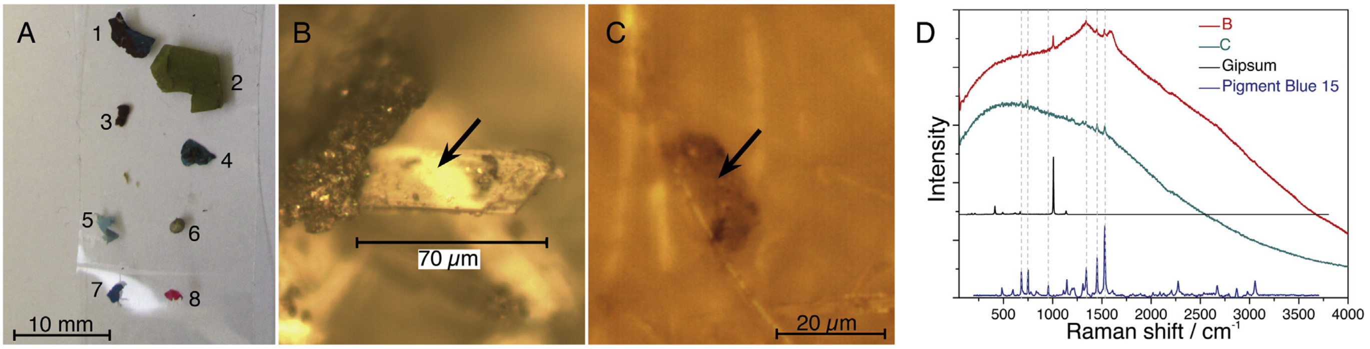

Fig. 1. Particles from Lake Garda beach sediments (A) Examples of extracted plastic and paint particles. (A1) is an example for relatively large particles were solely the Pigments Red 48 & Blue 15 were identified. The optical image shows a texture similar to paint fragments. (A2) is a green polyethylene particle with Pigment Blue 15 and Pigment Green 7. (A3) is a dark brown polystyrene particle without identified pigments. (A4 – A8) are paint particles with different pigments (e.g. Pigment Blue 15, Pigment Red 170 and Pigment White 6). (B & C) Microscopic images of two paint particles. (D) Raman spectra of the paint particle (B & C) and the reference spectra of Pigment Blue 15 as well as Gypsum.

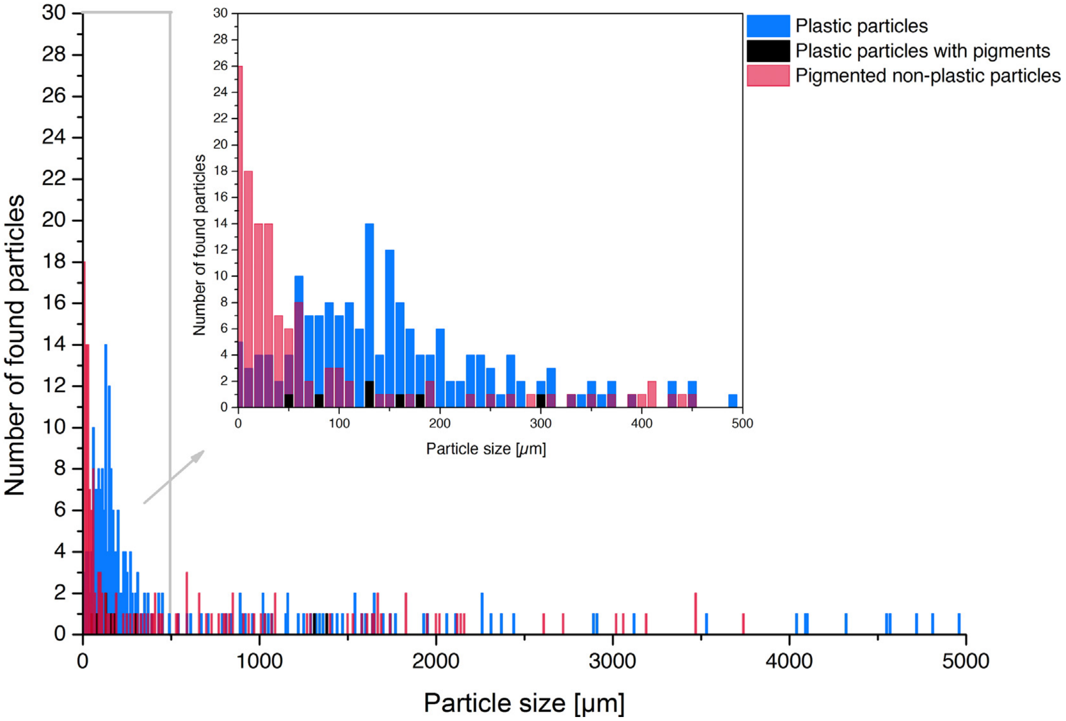

Fig. 2. Size distribution of the particles from Lake Garda beach sediment. The amount of paint particles increased with a decrease in size. This is highly pronounced in the size class of 1 µm–50 µm. Maximum for paint particles is visible between 1 and 50 µm and for plastic particles the maximum in the size class 50–500 µm was at ~130 µm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

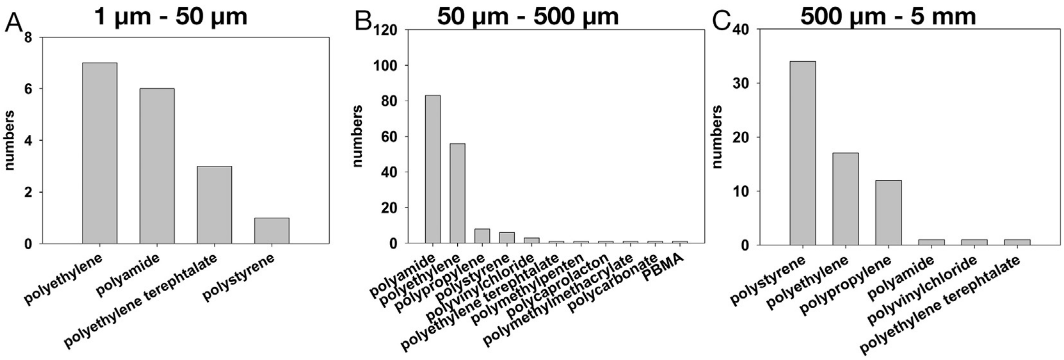

Fig. 3. Plastic types found for non-pigmented and pigmented plastic particles according to the size classes 1 µm–50 µm, 50 µm–500 µm and 500 µm–5 mm. Polyamide is mainly found for smaller particles, whereas polystyrene is only present at larger particles.

appearing particles did not contain color pigments. Their color here might have originated from degradation processes, organic material or dirt. The main polymers containing pigments were polyethylene (33%), polystyrene (33%), polypropylene (25%) and polyamide particles (8%).

3.4.2. *Paint particles*

Using RM, we identified the same pigments as in the plastic particles (named according to the definition of the International Color Index): Pigment Blue 15, Pigment Green 7 and Pigment White 6 (Rutil/Anatase). In addition the following pigments were found: Pigment Black 7 (soot, carbon black), Pigment Blue 16, 17, 27, 29, 60, Pigment Brown 7, Pigment Orange 5, 34, Pigment Red 3, 19, 48, 102, 112, 170, 275, 276, Pigment Violet 23, 310, Reactive Yellow 3, Pigment Yellow 1, 7, 42, 154, 210 and the lead containing Pigment Yellow 34 (Chrome Yellow $PbCrO_4$). The most common pigments were Pigment Blue 15 (40%), Pigment Green 7 (17%), Pigment White 6 (Rutil/Anatase, TiO_2, 15%), Reactive Yellow (7%), Pigment Yellow 42 (7%), Pigment Red 112 (4%), Pigment Black 7 (soot, carbon black, 2%). In addition Pigment Blue 15 was found to be the pigment most often used in all size classes. This was highly pronounced in the size class of very small microparticles (1 µm–50 µm), herein ~53% of the particles contained Pigment Blue 15. For an overview about the found frequency of the pigments in the paint particles please refer to Fig. S5.

3.5. *Metal burden of particles from Lake Garda beach sediment and unused plastic products*

An overview about the found elements from the particles from Lake Garda beach sediments is given in Fig. S6 and Table S1. Due to the use of zinc chloride in the extraction of the particles from the beach sediments we excluded zinc from the analyses of the particles from Lake Garda in respect to the possible adsorption of zinc to

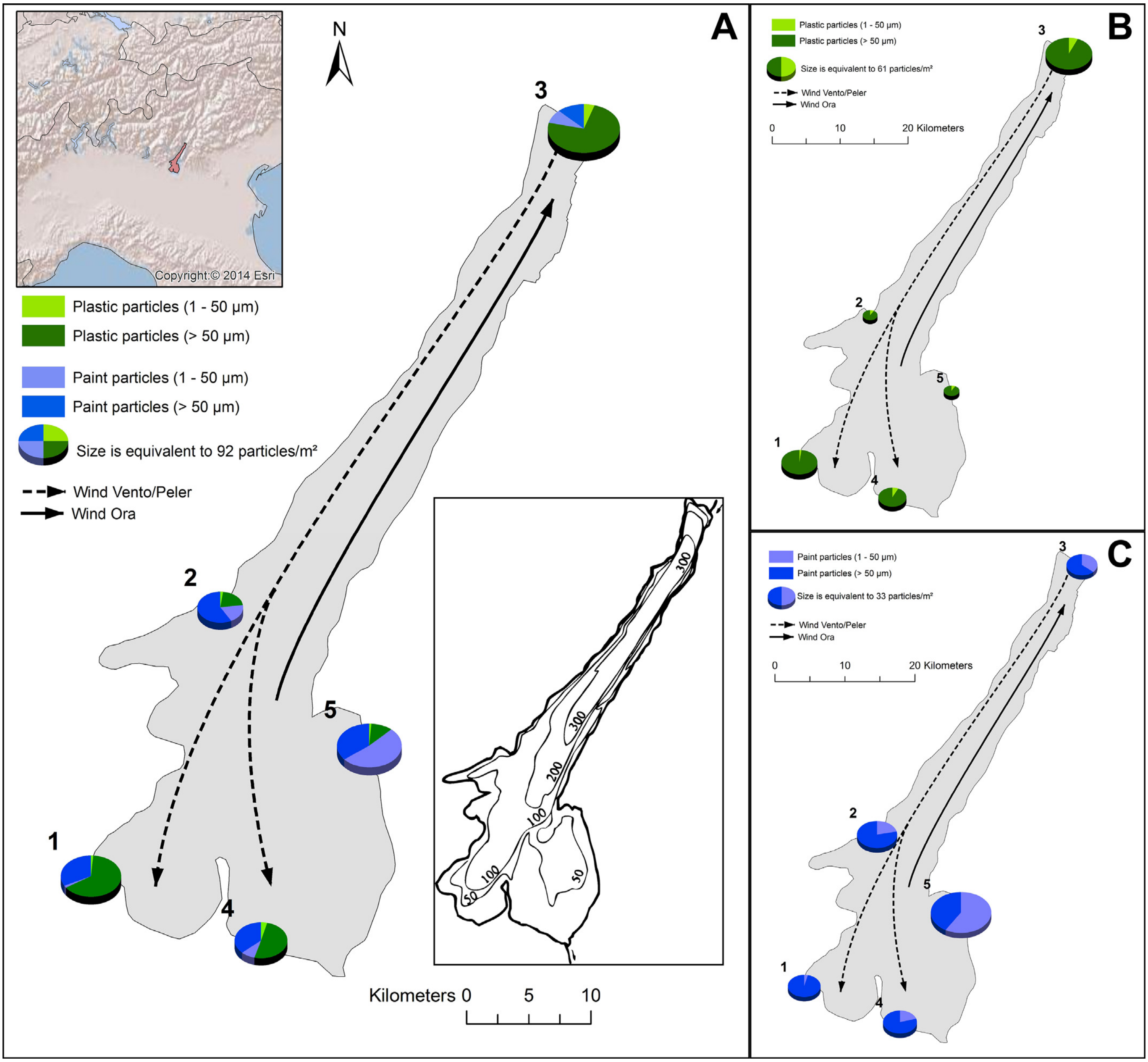

Fig. 4. Spatial distribution of plastic and paint particles from Lake Garda beach sediments. Abundance values are given for the entire size range but the particles between 1 and 50 µm are highlighted. The size of the circles resembles the particle numbers. (A) Map of Lake Garda with the spatial distribution of plastic particles. The inserted small maps show the localization of Lake Garda in northern Italy in red and the depth lines of the Lake. The small maps on the right side show the spatial distribution of plastic (B) and paint particles (C) individually. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Detailed abundance values of the plastic particles and the paint particles for the entire lake and for each sample area. The abundance is given for each size class separately and as total abundance of all size classes. In addition the highest abundance values are given for each size class separately and as total abundance.

	Plastic particles [particles/m^2]					Paint particles [particles/m^2]				
	All size classes	1–50 µm	50–500 µm	500 µm–5 mm	>5 mm	All size classes	1–50 µm	50–500 µm	500 µm –5 mm	>5 mm
Total	75 ± 134	5 ± 12	46 ± 122	20 ± 31	5 ± 6	58 ± 58	23 ± 38	23 ± 26	10 ± 18	2 ± 3
1	90 ± 78	2 ± 3	29 ± 34	54 ± 59	5 ± 9	44 ± 55	2 ± 3	20 ± 31	20 ± 27	2 ± 3
2	19 ± 28	2 ± 3	9 ± 11	9 ± 15	0 ± 0	63 ± 79	14 ± 19	27 ± 28	22 ± 34	0 ± 0
3	142 ± 295	11 ± 22	127 ± 216	2 ± 5	2 ± 3	26 ± 22	14 ± 17	11 ± 8	0 ± 0	0 ± 0
4	58 ± 15	5 ± 9	7 ± 8	36 ± 22	10 ± 9	48 ± 21	10 ± 10	20 ± 18	12 ± 12	5 ± 5
5	20 ± 9	2 ± 3	3 ± 6	9 ± 6	7 ± 6	129 ± 73	78 ± 70	46 ± 44	3 ± 6	2 ± 3
Max.	561	51	505	117	15	179	153	97	61	10

the particles. The elemental composition of the unused plastic products (colorful screw caps and packaging material) as a comparison for the inherent metal burden is shown in Fig. S7, Table S2 and Table S3.

Lead was detected in one paint particle from Lake Garda beach sediment (LG03, Pigment Green 7) with a concentration of 227.02 μg/g and in one polyethylene particle from Lake Garda with Pigment Green 7 (LG09, 219.70 μg/g). Increased concentrations of lead were also detected in two plastic bags samples from unused plastic products (Table S2). The lead containing polyethylene particle from Lake Garda (LG09) was the only particle with cadmium (23.64 μg/g) among all analyzed samples. Copper is a constitutional component of many blue and green pigments like phthalocyanine pigments (e.g. Pigment Green 7, Pigment Blue 15), which were found in a high percentage in the samples from Lake Garda. Elevated copper concentrations were found in the analyzed paint particle with Pigment Blue 15 of Lake Garda (LG04, 706.48 μg/g) as well in almost all unused plastic products containing Pigment Green 7 or Pigment Blue 15 with concentrations up to 373.71 ± 6.98 μg/g. No copper was detected in both plastic particles (LG10 and LG11) with no incorporated pigments. This further supports the assumption that the increased copper levels originated from the phthalocyanine pigments, although rather low concentrations were detected in the paint particle with Pigment Green 7 from Lake Garda (LG03, below detection limit) and the plastic particles containing Pigment Green 7 from Lake Garda (LG01, LG02, LG03, LG06, LG09, copper concentrations between 5.32 μg/g and 36.35 μg/g).

Titanium was detected in almost all paint and plastic particles from Lake Garda but with highest concentrations in two paint particles from Lake Garda (LG03 & LG04) but also in the polystyrene particle with the TiO_2 containing Pigment White 6 from Lake Garda (LG05). Like titanium, barium is used in pigment and polymer production as an extender and filler (Freitag and Stoye, 2008). In concordance it was found in most samples from Lake Garda (in one sample with a concentration of 4 mg/g) and in almost all unused plastic products (up to 402.25 ± 8.14 μg/g). Nickel was found in rather low levels (0.1–10 μg) in all unused plastic products apart from one screw cap (571.08 μg/g) and in some particles from Lake Garda (0.76–89.75 μg/g). Tin was found in one paint particle from Lake Garda (LG04, Pigment Blue 15) in a concentration of 975.25 μg/g. The tin concentrations in the unused plastic products samples were also rather low. Zinc was found with concentrations from 0.72 to 315.86 μg/g in the unused plastic products. The levels of cobalt and chromium were rather low (0.1–10 μg) for the unused plastic products, though for some particles from Lake Garda the concentrations were much higher (Co: 63.36–76.22, Cr: 4.06–456.25 μg/g).

4. Discussion

Here we provide a first time report on the existence of paint particles in a subalpine lake. This may be surprising since the discharge of micro-particulate waste, such as microplastics, into freshwater and marine ecosystems has already been widely described (Browne et al., 2011; Wagner et al., 2014). We distinguish between plastic particles comprised of well-known polymers (e.g. polyethylene, polypropylene, polystyrene, polyamide, etc.) containing coloration pigments, and paint particles having a high pigment content and a polymeric binder in lower amounts.

4.1. Size distribution of microparticles: plastic and paint particles

Both (micro)plastic and (micro)paint particles were found in Lake Garda beach sediments. However, plastic particles were on average larger (ranging from 50 to 500 μm) than paint particles. Only 7% of the plastic particles occurred in the size class <50 μm. These were mainly rigid polymers such as polyethylene, polyamide and polyethylene terephthalate. Above the 500 μm size, predominantly low density polymers such as polystyrene (both expanded and not), polyethylene and polypropylene were found. This follows a common pattern observed in both freshwater and marine beach sediments (reviewed in: Dris et al., 2015; Hidalgo-Ruz et al., 2012; Van Cauwenberghe et al., 2015). In contrast to studies which already assessed microplastic contamination in lake shore sediments (Faure et al., 2013; Imhof et al., 2013) high amounts of high density polymers and low amounts of low density polymers (e.g. expanded polystyrene) were detected in the size class below 500 μm. This may arise from an improved methodology to identify microplastics from environmental samples.

Paint particles contributed mostly to the smaller size classes. Only a small percentage of all paint particles were larger than 500 μm. This is clearly depicted when comparing the size distribution to that of microplastic particles, with the micropaint particle distribution curve being strongly skewed to smaller sizes and reaching a high number for very small micropaint particles (1 μm–50 μm). Similarly, the abundance of paint particles in surface samples taken in the Korean sea increased with decreasing size (Song et al., 2014).

In contrast to rare reports of very small paint particles, the discharge of larger paint chips into the marine environments has already been documented in the area of shipyards and boat maintenance facilities (Takahashi et al., 2012). Likewise, paint particles originating from refurbishment were discussed as a source of pollution for the marine and terrestrial environment, but not for freshwater (Bogden and Louria, 1975; Sturges and Harrison, 1985; Takahashi et al., 2012; Turner, 2010). Reports of paint particles in smaller size classes may be rare due to either sampled particles being ignored (e.g. Fischer et al., 2015; Lattin et al., 2004) or the lack of appropriate identification methods. Another reason for non-detection may lie in the fast disintegration of paint particles into even tinier pieces, as suggested by the observed sensitivity of extracted particles to mechanical stress.

Thus far most studies concerning microplastic in the marine as well as limnetic environments only assessed particles >1 mm or >300 μm due to technical constraints in sampling, particle handling, sample processing and necessary identification methods (Andrady, 2015; Dris et al., 2015; Hidalgo-Ruz et al., 2012; Van Cauwenberghe et al., 2015). Only a limited number of available identification methods, such as Raman microspectroscopy and micro FTIR, allow the identification of particles at the micron size. FTIR enables a faster screening of larger surfaces using a focal plane array (FPA) detector, however this method is diffraction limited to a minimum particle size of ~20 μm (Löder et al., 2015; Löder and Gerdts, 2015). The number of available studies decreases with the decreasing size of identifiable particles. Only a few studies were able to identify particles with sizes below 500 μm and even fewer below 50 μm (e.g. Enders et al., 2015; Imhof et al., 2013; Nor and Obbard, 2014; Norén, 2007; Van Cauwenberghe et al., 2013). Fully quantitative studies mainly sampled surface waters using a manta trawl with a mesh size of 300 μm, meaning that a significant number of particles smaller than this are lost (Enders et al., 2015; Song et al., 2014). Until now no published study has quantitatively assessed particles smaller than 50 μm from freshwater sediments and only a few studies are available for marine (beach) sediment samples (reviewed in: Dris et al., 2015; Hidalgo-Ruz et al., 2012; Van Cauwenberghe et al., 2015). To overcome these difficulties and to quantitatively assess all particles down to 1 μm, we applied density separation using the ‘MPSS’ (Imhof et al., 2012) followed by Raman microspectroscopy (RM) on singled out particles >750 μm and particles <750 μm on quartz fiber filters, which allows for a reliable characterization of microparticles (e.g. underlying polymers, pigments, etc.) with a smallest size of 1 μm. The feasibility of RM for characterization of plastic type and pigments has already been demonstrated (Imhof et al., 2013; Lenz et al., 2015; Remy et al., 2015; Signer and Weiler, 1932; Van Cauwenberghe et al., 2013; Van Cauwenberghe and Janssen, 2014).

4.2. Spatial distribution of (micro)paint and (micro)plastic particles

Plastic particle concentration at the southern shore from 2011 (8.3 macroplastic particles/m^2 and 108 ± 55 microplastic particles/m^2, Imhof et al., 2013) and 2012 (5 ± 9 macroplastic particles/m^2 and 43 ± 56 microplastic particles/m^2) are quite similar, despite the changed sampling approach. In contrast to a random grid sampling (2011) a combined sampling method with transects and sediment cores was performed in 2012. Another recurring pattern is the relative increase in particles at the north shore, although the overall numbers are not as high as in 2011. This high particle abundance might be due to the lake topography with steep rocky shores in the northern basin, and strong winds blowing from south to north, resulting in a strong surface circulation and forming a counter clockwise rotating eddy at the northern tip of Lake Garda.

The second most abundant location of particles was near Bardolino and Garda (sampling area 5, Fig. 4), where paint particles made up the majority of all particles and comprised 60% of particles below 50 μm. This area is known to be a trapping zone for pollutants despite the changing wind conditions (Lovato and Pecenik, 2012).

Interestingly the spatial distribution of plastic and paint particles is clearly different among the five sample areas around Lake Garda, which might be due to different physical properties of the particles. In the trapping zone of Lake Garda, next to sampling area 5 (Bardolino and Garda) and 2 (Maderno), the majority of particles originated from paints, whereas in the sample areas in the north (3, Torbole) and in the south west (1, Desenzano del Garda) the majority originated from plastic. In the southeastern sample area (4) similar amounts of paint and plastic particles were found. We anticipate that the stability of the plastic particles and their partially low density facilitates long distance transport across the lake. In contrast, the paint particles disintegrate quickly into very small paint particles and settle down faster. A higher percentage of very small paint particles (1 μm–50 μm) were found throughout all sample areas in comparison to very small microplastic particles. Similar as for the paint particles, we expected dense plastic particles to quickly settle. This is supported by the polyamide particles (density 1.1–1.2 kg/L), which comprised the majority of the dense polymers at Lake Garda. Herein 90% of the polyamide particles were found in sample area 2 and most of them (92%) were between 50 and 500 μm in size. Similarly to the paint particles this might indicate local sources as the origin for the dense plastic particles rather than sources requiring long distance transport over the lake.

4.3. Biological implications of pigmented (micro)particles

The expanding prevalence of plastic and paint particles in sizes which are prone to an increased bioavailability in nature (Eerkes-Medrano et al., 2015; Enders et al., 2015; Van Cauwenberghe et al., 2015) combined with laboratory observations of the uptake by organisms (Besseling et al., 2014; Cole et al., 2013; Imhof et al., 2013; Setälä et al., 2014) indicates that particulate debris is inevitably entering the food web. In the marine system, some studies have shown that paint flakes are ingested by seabirds (Ryan, 1989; Sileo and Fefer, 1987; Turner and Hambling, 2012), and artificial cellulose fibers colored by industrial dyes were recently proved to be ingested by seagrass associated macrofauna (Remy et al., 2015). As a consequence to this, a variety of harmful effects can be caused by different compounds used in the production of a colored polymer, paint or coating.

Our findings show that plastic particles, occurring in a subalpine lake contain pigments which were directly mixed into the polymer blend during production. This is in concordance with recent studies, which found plastic particles with pigments also in the deep sea (Van Cauwenberghe et al., 2013), as well as in the tissue of bivalves from the North Sea and the Atlantic Ocean (Van Cauwenberghe and Janssen, 2014). Pigments used in paints, coatings or to colorize plastic products often have metals as constituent (e.g. arsenic yellow, antimony orange, cadmium red, chrome yellow, chromoxide green, molybden orange, white lead, Becker et al., 2010, Murphy, 2001, Wäger et al., 2012). Due to their toxicity, the use of metals such as lead, mercury and cadmium in pigments has been banned, especially in food contact material (European Communities, 2007) or children's toys (European Committee for Standardisation). Most of these substances have been substituted with pigments of lower toxicity, but especially for the red and yellow pigments based on cadmium or chrome, it has proven difficult and expensive to find suitable substitutes (Jansen and Letschert, 2000). In addition, older microplastic particles, persisting in the environment since the beginning of the plastic boom can contain toxic and currently banned pigments.

4.4. Metal burden of particles from Lake Garda beach sediment und unused plastic products

Many of the analyzed paint and plastic particles samples from Lake Garda as well as the unused plastic products were found to contain significant metal concentrations.

In following that copper is a constituent of many blue and green phthalocyanine pigments (e.g. Pigment Green 7, Pigment Blue 15), elevated copper concentrations were detected in many blue and green paint as well as plastic particles originating from Lake Garda. The same was true for the unused plastic products. Barium and titanium are used as either extenders and fillers in pigment and polymer production (Freitag and Stoye, 2008) or as pigments themselves (e.g. TiO_2 containing Pigment White 6). Both were found at high levels in the samples.

Uncommon metals in plastic and paint production include nickel, tin, cobalt and chromium. All were found at mostly low concentrations in the samples, apart from some from Lake Garda particles showing high concentrations. Zinc was only identified in samples of unused plastic products but at concentrations up to 315.86 μg/g. For the Lake Garda particles detected zinc concentrations were omitted due to possible adsorption of zinc during the separation process.

4.4.1. Origin of metals from inherent load or adsorption?

Although lead was only found in a few particles and cadmium only in one particle, concentrations found were much higher compared to macroplastic sampled at Ookushi Beach, Nagasaki, Japan (13.5 ± 8.4 μg/g lead, Nakashima et al., 2012) and preproduction pellets found along the English coast (lead concentrations below 1.08 μg/g, cadmium concentrations up to 10 ng/g, Ashton et al., 2010). The same holds true for copper, which was detected by Ashton et al. (2010) with concentrations below 1 μg/g. However, metal concentrations of the unused plastic products were equal to the high elemental concentrations of plastic particles isolated from Lake Garda, especially for lead and copper. Additionally, the analysis of two plastic particles where no pigments were detected by RM showed lower metal values.

Although these facts indicate that the metallic element burden of the particles from Lake Garda originate from an inherent load, adsorption of metal ions during the residence time of the particles in the lake ecosystem cannot clearly be excluded (Boucher et al., 2016; Cobelo-Garcia et al., 2007; Fischer et al., 2007; Giusti et al., 1994; Holmes et al., 2014; Rochman et al., 2014; Weijuan et al., 2001). Some particles from Lake Garda contained high concentrations of nickel, cobalt and tin whereas others did not. In freshwater, the adsorption of trace metals might be further increased for beached particles with organic material on the surface as compared to virgin particles (Turner and Holmes, 2015). However, the relatively low metal concentrations found in Lake Garda water, sediments and organisms (Camusso et al., 2001; Chiaudani and Premazzi, 1990) indicate that the majority of metals detected on the sampled particles originate from an inherent load (e.g. additives, pigments) and were not adsorbed from the environment (For detailed information about the metal concentrations of Lake Garda please refer to the Supplementary Material). Future experimental studies considering the adsorption of metals to freshwater plastic particles would be necessary to clarify this, as recently performed for marine plastic particles (e.g. Ashton et al., 2010; Rochman et al., 2014) and freshwater pellets (Turner and Holmes, 2015). Toxic metals transported with either (micro)plastic or (micro)paint particles may pose a risk to the environment (Nakashima et al., 2012), regardless of whether they were added intentionally or as an impurity during production or were adsorbed to the surface.

4.4.2. *Toxicity due to inherent or adsorbed metals*

Most of the detected metals are declared as priority pollutants by the US EPA (Mahmood et al., 2012). Many of them are known to be toxic or harmful to aquatic organisms (Gade et al., 2012; Lithner et al., 2011; Muenzinger and Monicelli, 1991). Lead and cadmium are especially toxic for aquatic organisms (Mahmood et al., 2012), however both were only rarely detected. Copper has a no observed effect concentration (21 day, NOEC) based on reproduction of *Daphnia magna* ranging from 29.4 to 228 μg/L (De Schamphelaere and Janssen, 2004) which is clearly exceeded by the particles from Lake Garda and the unused plastic products. Zinc was only analyzed in the unused plastic products but concentrations were found to be occasionally quite high (up to 80.69 ± 2.02 μg/g). Especially for aquatic organisms like freshwater algae and *Daphnia magna*, zinc is both highly toxic as a single compound (EC50 for *D. magna*: 70 μg/L) and can cause significant reduction in *D. magna* body length as well as brood size when in combination with paint mixes (Gade et al., 2012; Lithner et al., 2011; Muenzinger and Monicelli, 1991). Whereas titanium used as a filler substance is of low toxicity, barium is toxic at high concentrations and leads to reproductive impairments in *D. magna* (Biesinger and Christensen, 1972; Lira et al., 2011). In the analyzed samples, barium was found at concentration levels up to 4 mg/g which is a magnitude higher than the other metal concentration levels.

Uncommon metals in the plastic and paint production such as nickel, tin, cobalt and chromium were found in low concentrations. Nickel can induce changes on body size and Life-History parameters of *D. magna* and therefore has also to be considered as harmful for aquatic organisms (Muenzinger and Monicelli, 1991). Tin is primarily of low toxicity, although some organotin compounds are highly toxic (e.g. TBT, Hoch, 2001). Cobalt and chromium are both known to cause allergenic reactions or skin irritations (Forte et al., 2009).

4.5. *Other potentially toxic substances used by the paint industry*

The most abundant pigments in Lake Garda were Pigment Blue 15 and Pigment Green 7, and both are based on phthalocyanine with copper as a constituent. They are declared as moderate or low toxic due to the covalently bound copper (OECD). Nevertheless produced phthalocyanin blue is known to be contaminated with traces of suspected carcinogenic or teratogenic compounds (e.g. PCB, Hu and Hornbuckle, 2009). Numerous components are used in industrial paint production which fulfill certain functions during production, liquid paint application, and later as a solid coating (Freitag and Stoye, 2008). Common examples are: plasticizers (e.g. phthalates), paint additives (e.g. catalysts, antiskimming, antifloating, antiflooding, matting, neutralizing and thicking agents), preservatives (e.g. biocides and fungicides), flame retardants (e.g. polybrominated biphenyls) and corrosion inhibitors (like oxidizing salts such as chromates organic amines, organic salts). Consequently, paint particle toxicity may arise from a broad variety of sources beyond simply from metals.

5. Conclusions

Enabled by recent technical advancements, the scientific focus is shifting from larger particulate waste to the monitoring of small particles down to the micron scale. This has led to increasing identification of previously ignored particulate micropollutants in aquatic environments.

In this study we performed a detailed quantitative and qualitative analysis of microplastic particles (both pigmented and non-pigmented) as well as paint particles. We gave especial attention to very small microparticles (1 μm–50 μm) and provide the first evidence of paint particles in a freshwater ecosystem. A higher amount of pigmented particles was detected in smaller size classes and found to be highest for very small microparticles (1 μm–50 μm). In contrast, microplastic particles tended to be larger, with most found to be between 50 and 500 μm (the maximum in this size class was around 130 μm). Furthermore spatial distributions of plastic and paint particles over Lake Garda was quite different.

The continuous discharge of particulate debris into freshwater ecosystems is potentially hazardous to the biota. This is compounded by the accelerated break down of larger plastic and paint particles to tiny microparticles and eventually nanoparticles, which can pose a high risk to aquatic organisms (Bouwmeester et al., 2015; Lambert et al., 2014; Lambert and Wagner, 2016; Mattsson et al., 2015). Until now studies concerning plastic debris in the environment mostly focused on the adsorption of persistent organic pollutants (Mato et al., 2001; Teuten et al., 2009) and toxic metals to the polymers (Holmes et al., 2014; Rochman et al., 2014; Turner and Holmes, 2015). Our work reveals that paint particles act as contaminants transporting toxic elements into lakes and streams. We additionally found that metals detected in both Lake Garda plastic particles and unused plastic products may originate from an inherent load. This load contributes to the chemical mixture plastic particles can transport into non-contaminated areas or transfer to aquatic organisms.

Proven ingestion by several pelagic and benthic invertebrate taxa, as well as subsequent bioaccumulation with all associated harmful consequences shows the need to include other anthropogenic particulate debris in future monitoring approaches. Moreover, it underpins the necessity to implement standardized/harmonized surveillance guidelines as now planned for macro- and microplastic particles in the marine system (e.g., Marine Strategy Framework Directive–indicator 10.1.3). The need is especially pertinent for very small microparticles (1 μm–50 μm). Such efforts would help to identify potential accumulation hotspots, high risk products and groups of organisms more vulnerable to the impacts of particulate anthropogenic debris and its associated chemicals.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.03.015.

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