

Trace element dynamics of biosolids-derived microbeads

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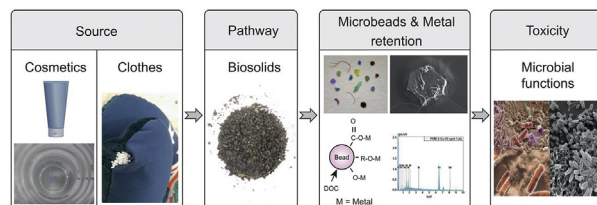
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HIGHLIGHTS

- Biosolids are a major source for microbeads in soil.
- A first-time study on microbial toxicity of biosolids-derived microbeads.
- Microbeads serve as a vector for transportation of trace elements in soil.
- Dissolved organic matter enhanced the adsorption of trace elements by microbeads.
- Microbeads decreased microbial respiration and dehydrogenase activity in soil.

GRAPHICAL ABSTRACT



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ABSTRACT

This study focused on quantifying and characterising microbeads in biosolids (i.e., treated sewage sludge), and in examining interactions of microbeads with trace elements when biosolids are added to soil. Under laboratory conditions, batch experiments were conducted to investigate the adsorption of Cu onto pure and surface modified microbeads suspended in soil. The ecotoxicity of microbead-metal complexes to soil microbial activities was also investigated by monitoring basal respiration and dehydrogenase activity. Concentrations of the microbeads were 352, 146, 324, and 174 particles kg⁻¹ biosolids for ≤50, 50–100, 100–250, 250–1000 μm size fractions, respectively. The Scanning Electron Microscope (SEM) images illustrated wrinkled and fractured surfaces due to degradation. The adsorption of dissolved organic matter onto microbeads was confirmed through FT-IR microscopy, while using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) the presence of trace metals including Cd (2.34 ng g⁻¹), Cu (180.64 ng g⁻¹), Ni (12.69 ng g⁻¹), Pb (1.17 ng g⁻¹), Sb (14.43 ng g⁻¹), and Zn (178.03 ng g⁻¹) was revealed. Surface modified microbeads were capable of adsorbing Cu compared to the pure microbeads, which may be attributed to the complexation of Cu with dissolved organic matter associated with the microbeads in the matrix. It was further revealed that the biosolids derived microbead-metal complexes

1. Introduction

Global production of plastics has exhibited an enormous increase to ~322 million metric tons in 2015, whereas in the 1950's it was only 1.5 million tons (Statista, 2017). Plastic products have been widely used worldwide due to their easy use, cost effectiveness, durability, plasticity, and transparency. They ultimately end up in the environment. Plastics found in the environment have been categorized into three categories based on size fractions of their diameters, i) macroplastics, > 5 mm (i.e., bottles, shopping bags), ii) large microplastic particles, 1–5 mm (i.e., plastic pellets, fragments), iii) small microplastic particles, < 1 mm (i.e., plastic fibers) (Wardrop et al., 2016). Microbeads, a type of primary microplastics, are plastic fragments or beads ranging in diameter from roughly 5 µm to 1 mm (Rochman et al., 2015). Table 1 summarizes the information on selected synthetic polymers made of microplastics, their origin, and characteristics.

Wastewater treatment plants (WWTPs) are a major source of microbeads that end up in the environment. Microbeads enter the WWTPs from several sources: domestic and industrial (i.e., sewage) [wastewater is the water that remains on the surface after sewage is precipitated-biosolids fall to the bottom and wastewater remains on the surface] surface runoff (i.e., stormwater), or from landfill leachate. During treatment and co-composting processes, large-size plastic debris is generally removed through screening, sieving, and hand picking. Although microbeads are effectively removed from final wastewaters in WWTPs, they are not effectively removed in treated sludge and eventually reach finished biosolids and related products (Rochman et al., 2015). Over one billion microplastic particles enter into biosolids on a daily basis at Los Angeles's WWTPs in the United States (Carr et al., 2016). Milling steps during co-composting of biosolids can form microbeads from large plastics. Lime stabilization in sewage sludge treatment shears

microplastics due to enhanced hydrolysis facilitated by an elevated pH, heat, and mechanical mixing, thereby influencing their morphology and sizes. A lower abundance of microplastics was observed in biosolids derived from anaerobic digestion compared to lime stabilization and thermal drying, highlighting the importance of generation process (Zubris and Richards, 2005; Mahon et al., 2016). Many countries utilise biosolids in agricultural, forest, and reclaimed lands, because they are rich in soil nutrients (e.g., nitrogen (N), phosphorus (P)) and organic matter (Shammas and Wang, 2008). To date, legislative authorities and policy makers have not focused their attention on disposal of microplastic-containing sources, including biosolids (Mahon et al., 2016). Therefore, microplastics enter into soils through land application of biosolids and, subsequently, into aquatic systems through runoff, thereby contaminating the environment.

Plastic products are remarkably persistent in the environment due to their resistivity to biodegradation (Yoshida et al., 2016). All forms of micro- and nano-scale plastics persist in nearly all terrestrial (i.e., soil) and aquatic (i.e., marine and freshwater) ecosystems, and they occur even in Polar Regions (Law et al., 2010; Rillig, 2012; Cole and Galloway, 2015; Amelineau et al., 2016). Because of their small size and persistence, they can be easily ingested and accumulated in bodies and tissues of many terrestrial and aquatic organisms including earthworms (Lwanga et al., 2016), birds (Holland et al., 2016; Zhao et al., 2016), freshwater invertebrates (Blarer and Burkhardt-Holm, 2016), fish (Lusher et al., 2017), and oysters (Vegter et al., 2014; Cole and Galloway, 2015). Because plastics are produced from petroleum, and many different organic compounds are added during the process of manufacturing them to improve their quality, microbeads contain organic contaminants such as petroleum hydrocarbons and polycyclic aromatic hydrocarbons (Horton et al., 2017). Hence, microplastics transport organic contaminants through adsorption, absorption, degradation,

Table 1
Summarized information on selected synthetic polymers made of microplastics, their origin, and characteristics.

Chemical compound	Chemical formula	Origin or source to environment	Characteristics	References
Low-density polyethylene (LDPE)	(C ₂ H ₄) _n	Squeeze bottles, toys, carrier bags, chemical tank linings, heavy duty sacks, general packaging, gas, and water pipes	Low density 0.91–0.94 g cm ⁻³ , non-biodegradable, most common plastics	Lambert et al., 2014; Lassen et al., 2015
High-density polyethylene (HDPE)	(C ₂ H ₄) _n	Chemical drums, toys, household and kitchenware, cable insulation, and carrier bags	Low density 0.92–0.99 g cm ⁻³ , non-biodegradable	Lambert et al., 2014, Lassen et al., 2015
Acrylic	Acrylate polymers: based with acrylic acid: CH ₂ =CHCOOH	Most used fibres in textiles: knitware, plastic flakes	High density 1.16 g cm ⁻³	Lassen et al., 2015
Polyethylene terephthalate (PET)	(C ₁₀ H ₈ O ₄) _n	Drink bottles, oven-ready meal trays, cable lining	High density 1.41 g cm ⁻³	Lambert et al., 2014, Lassen et al., 2015
Polypropylene (PP)	(C ₃ H ₆) _n	Food containers and microwavable meal trays	Low density 0.90–0.91 g cm ⁻³	Lambert et al., 2014, Lassen et al., 2015
Polystyrene (PS)	(C ₈ H ₈) _n	Food containers, stuffed animals, and protective packaging	High density 1.04–1.13 g cm ⁻³	Lassen et al., 2015
Polyvinyl chloride (PVC)	(C ₂ H ₃ Cl) _n	Water pipes, cable insulation, packaging, and healthcare applications	High density 1.39–1.43 g cm ⁻³ , non-biodegradable	Lassen et al., 2015

and leaching in the environment (Teuten et al., 2009). Polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbons, organochlorine pesticides, and bisphenol A are transported into marine systems via microbeads (Teuten et al., 2009).

Not only can microplastics transport organic contaminants, they also can transport inorganic contaminants such as trace elements. The high surface area and functionalized surfaces of the microbeads facilitate to adsorb trace elements. Virgin polystyrene beads and aged polyvinyl chloride fragments have shown a high affinity to trace elements; for example, copper (Cu) and zinc (Zn) leached from antifouling paints in sea waters (Brennecke et al., 2016). Aged plastics, which have been altered by attachment of organic matter and long-term pre-modification through photooxidation and attrition of charged materials, have been shown to enhance metal sorption (Turner and Holmes, 2015). Microplastics have shown interactions with metals in surface (i.e., depth up to 2 cm of the Beijiing River littoral zone) sediments (Wang et al., 2017). However, little information is available concerning the fate and transport, and effects of microplastics on microbial functions in terrestrial systems (i.e., soils); rather studies have focused attention on aquatic environments, especially marine ones. The complex nature of extracting microplastics from organo-mineral soil matrixes has been found to be a main reason for this scarcity (Rillig, 2012). Hence, the interactions of soil with microbeads (hereafter micro- and nano-plastics will be called “microbeads”) remain unclear. It is important to examine the interactions of soil with biosolids containing trace elements and microbeads. Therefore, the objectives of this study were (a) to quantify trace element contents in biosolids with microbeads, (b) to identify possible mechanisms for microbead-trace element associations in the soil environment, and (c) to study the effect of microbead-trace element associations on microbial activity in soils.

2. Materials and methodology

2.1. Biosolids, soil, and microbeads

Biosolids samples produced through aerobic and anaerobic digestion processes were collected from a wastewater treatment plant in Sydney, Australia. Surface soil samples (i.e., 0–15 cm depth) were collected from two agricultural lands in Grenfell, Sydney, New South Wales (33° 56' 31.5924" S and 148° 2' 15.72" E) and Ngapala, Eudunda, South Australia (34° 07' 06.85" S and 138° 99' 50.47" E). Sediment samples were collected from the Hunter river, Lorn, New South Wales (32° 43.579' S and 151° 33.304' E). Pure polyethylene microbeads (hereafter “PMB”), size ~ 100 µm (Microscrub®) were purchased from Micro Powders, Inc. Tarrytown, New York, USA. Additionally, microbeads were extracted from biosolids as well as commercial products i.e., a facial scrub (Positive Radiant®, Johnson & Johnson, New Brunswick, New Jersey, USA) and one neck pillow (Outbound™, TRS Trading Group Pty Ltd, Kensington, Victoria, Australia). The properties of the soils and biosolids are given in Table S1.

2.2. Extraction of microbead

The 2 mm diameter fraction of oven dried (36 °C) samples of the biosolids were used for extraction of microbeads. Steps described by Carr et al. (2016) were used to extract microbeads from the biosolids and commercial products. In brief, four main steps were performed in the extraction: density separation using distilled water and a saturated barium chloride (density ~ 3.0 g cm⁻³) solution; sieving; filtration; and visual sorting (Hidalgo-Ruz et al., 2012; Carr et al., 2016). A series of stainless-

steel screens (1000, 250, 125, and 53 µm) and filter papers (Whatman No.1, Qualitative, pore size 11 µm) were used in sieving and vacuum filtration, respectively. Microbeads extracted from facial scrubs were purified by exhaustively washing them with Milli-Q water and methanol using a glass vacuum filtration unit (Carr et al., 2016).

2.3. Characterization and quantification: microbeads and trace elements

Microbeads were identified based on several criteria: colour (transparent or their colour), size (≤ 50 , 50–100, 100–250, 250–1000 µm diameter), and shape (fiber, irregular, trigonal, pentagonal, spherical, cube). A stereomicroscope (OLYMPUS, SZ2-ILST, Tokyo, Japan) with sequentially gridded Petri dishes was used in segregation of lumps/particles and to identify particle shapes/sizes. Fourier transform infrared (FT-IR) microscopy (Agilent Cary 620 Series, Santa Clara, CA, USA), using a liquid nitrogen cooled MCT detector, was employed to identify the functional groups. Processed samples were scanned in a retro-reflection mode through single point scanning with a resolution of 4 cm⁻¹, and a total of 16 scans were co-added for spectral averaging. A field emission scanning electron microscope coupled with an EDS (energy dispersive spectroscopy) detector (FESEM) (Zeiss Sigma VP + Bruker EDS) was used to identify morphology and localized elemental composition. Fifty microbeads were extracted from biosolids and were digested in a microwave digester (MARS 6250/50, Matthews, NC, USA) with 10 mL of *aqua regia* for metal analysis. Trace metal analysis was carried out by using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Agilent 7900, G8403A, Tokyo, Japan), and a Total Organic Carbon (TOC) analyser (Shimadzu TOC-L CSH, Kyoto, Japan) was used to get TOC. All pH measurements were done by using a LAQUA PC1100 (Kyoto, Japan) instrument.

2.4. Quality control

Procedures to validate the methods were conducted as described by Carr et al. (2016). In brief, a 10 g sample of PMB was mixed with 50 g of soils. Then, the mixture was added to the sieving setup comprised of the 1000, 250, 125, and 53 µm screens; it was extracted to calculate the extraction efficiency. Extraction efficiency values were used as correction factors to calculate the concentrations of the microbeads. In each of the steps during sample preparation and analyses, cotton laboratory coats and nitrile gloves were used, and synthetic clothing was avoided to evade possible contamination by microbeads. In addition, at all times during experimental steps, samples were covered and the working surfaces were cleaned with alcohol prior to use. Accuracy of our analytical method for elemental analysis using ICP-MS was verified by measuring Standard Reference Materials (SRMs): NIST 1640-trace elements in natural water and NIST 1643e – trace elements in water, from the National Institute of Standard and Technology (NIST) which were described earlier (Rahman et al., 2015). Blanks and continuing calibration verification (CCV) were also included throughout the metal analysis.

2.5. Experimental design

Soils collected from Eudunda, sediments, and biosolids were used to modify the PMB (purchased pure polyethylene microbeads) through sorption of dissolved organic matter (DOM) on their surfaces. The DOM sorbed microbeads were prepared by mixing 50 g of PMB with 500 g of biosolids, sediments, and soils in a 1 L borosilicate bottle and incubated for seven days under ~20% moisture

conditions. Afterwards, the extracted microbeads from biosolids (BSMB), sediment (SEMB), and soil (SMB) were dried overnight at 36 °C to remove the moisture. To examine the effect of microbead-trace element associations, 25 g of each type of the PMB, BSMB, SEMB, and SMB were sorbed in a 100 mg L⁻¹ Cu(II) solution (CuN₂O₆·3H₂O) and agitated overnight at 125 rpm in an orbital shaker (RATEK, OM 6, Boronia, Victoria, Australia). After shaking, the microbeads were separated using a vacuum filter; they then were oven dried overnight at 36 °C. These Cu(II) sorbed microbeads were named as PMB + Cu, BSMB + Cu, SEMB + Cu, and SMB + Cu. The soils collected from Grenfell were used in incubation experiments, which were done to examine the effects of microbeads on soil microbial respiration and enzyme activities.

2.5.1. Soil respiration study

The effect of microbead contamination on soil microbial activity was examined through soil respiration. Soils from Grenfell were separately mixed with PMB, BSMB, SEMB, SMB, PMB + Cu, BSMB + Cu, SEMB + Cu, and SMB + Cu. The soil and microbead mixing ratio was 1:0.064 w/w, and these mixtures were used to measure the release of CO₂ using respiration reagent bottles. For the incubation, 50 g of soil were mixed with 3.2 g of extracted microbeads; each mixture was placed in a 250 mL borosilicate screw capped reagent bottle containing 10 mL of 0.5 M NaOH as a CO₂ trap. Additional screw capped reagent bottles containing only the CO₂ traps and untreated soil, with moisture maintained at 20%, served as the controls. The screw capped reagent bottles then were incubated in the laboratory environment at 22 °C for fourteen days. At the end of the incubation, evolved CO₂ was quantified by analysing the trapped CO₂ as BaCO₃ using 1 M BaCl₂ and back-titrating unreacted NaOH using 0.5 M HCl.

2.5.2. Soil dehydrogenase activity analyses

The moist (~20% moisture) soil samples from the soil respiration study were stored at 4 °C until microbial enzyme analyses could be performed. Because the assay of dehydrogenase (DHA) activity has been considered to represent the overall microbial activity of soil microorganisms, and it is a reflection for biological oxidation of organic compounds in soil (Langer and Rinklebe, 2009), DHA activity was assessed following the method described in Klein et al. (1971). In brief, 1 g of moist soil was incubated for 24 h at 28 °C with a solution containing 0.2 mL of 3% (w/v) 2,3,5-triphenyltetrazolium chloride (TTC) and 0.5 mL 1% (w/v) glucose. The amount of triphenyl formazan formed was measured spectrophotometrically at 485 nm (Thermo Scientific, BIOMATE 3S, Waltham, MA, USA).

2.5.3. Soil organic matter-microbead-metal interaction

Batch isotherm experiments were carried out using four types of microbeads: PMB, BSMB, SEMB, and SMB. Five grams of each type of microbeads (PMB, BSMB, SEMB, SMB) were weighed into 250 mL borosilicate bottles before 225 mL of Milli-Q water was added. After a conditioning period of 24 h, 20 mL of slurry were abstracted per experiment and to that was added 10–500 µL (i.e., 10, 15, 20, 25, 50, 75, 100, 150, 200, 300, and 500 µL) from a 50 mg L⁻¹ Cu(II) solution. This resulted in initial Cu(II) concentrations in experiments ranging from 25–1250 ng L⁻¹; the mixtures were put in centrifuge tubes, which were shaken at 100 rpm overnight at 25 °C. To separate microbeads and slurry, the steps described in the “Extraction of microbeads” section were followed. After separating microbeads and slurry, the solutions (i.e., absence of microbeads) were used for metal analysis in the isotherm studies. The microbead solid portion was dried in an oven at 36 °C and characterized with FT-IR, and SEM-EDS. Control experiments were performed in the absence of DOM sorbed microbeads but with otherwise identical experimental

conditions.

2.5.3.1. Adsorption isotherms. Metal adsorptions were modeled with the Langmuir (Equation (1)) adsorption isotherm. The Langmuir adsorption isotherm is given by:

$$\Gamma_{ads} = \frac{k[M]\Gamma_{max}}{(1 + k[M])} \quad (1)$$

where, Γ_{ads} is the amount of adsorbate adsorbed per unit area of surface ($\mu\text{g g}^{-1}$); $[M]$, the equilibrium solution concentration ($\mu\text{g L}^{-1}$) of adsorbate; Γ_{max} , the maximum adsorption density ($\mu\text{g g}^{-1}$); and k , the equilibrium constant for the overall adsorption process (L g^{-1}). The Langmuir model assumes that all adsorption sites have equal affinity for the adsorbate and, therefore, only monolayer adsorption occurs (Shaheen et al., 2013).

2.6. Data analyses

Statistical analysis was performed using the IBM SPSS statistics version 24.0.0.1 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to determine the significance of differences ($P < 0.05$).

3. Results and discussion

3.1. Quantification and characterization of microbeads

3.1.1. Biosolids derived microbeads

The biosolids derived microbeads had varying physical characteristics (Table 2). The $\leq 50 \mu\text{m}$ particle size fraction was observed as the most counted size of microbeads, which had 352 particles per kg biosolids. The microbead size fractions of 50–100, 100–250, and 250–1000 were observed as having 146, 324, and 174 particles per kg biosolids, respectively. These microbeads had varying shapes such as irregular, fibreform, and spherical. The irregular and fibreform microbeads were the dominant shapes, which had 581 and 146 particles per kg biosolids, respectively. The frequently detected colours of these microbeads were white, blue and red. The green, yellow, orange, and magenta microbeads also were seen in biosolids, but in small numbers (Fig. 1a – b).

Table 2

Concentrations of microbeads in biosolids (particles per kg biosolids). Average values from two samples (i.e., $n = 2$) with standard deviations in parentheses.

Concentration extraction	Particles per kg biosolids ^a		Total
	Water	BaCl ₂	
Particle diameter (μm)			
≤ 50	63 (27)	289 (149)	352
50–100	103 (60)	43 (33)	146
100–250	194 (163)	130 (80)	324
250–1000	138 (49)	36 (19)	174
Shape			
Irregular	308 (78)	273 (145)	581
Fibreform	63 (69)	83 (5)	146
Spherical	44 (21)	12 (7)	56
Pentagonal	16 (7)	40 (35)	56
Cube	12 (8)	43 (4)	55
Trigonal	0	4 (3)	4
Colour			
White	221 (159)	150 (25)	371
Blue	186 (139)	95 (29)	281
Red	111 (104)	119 (29)	230
Green	43 (18)	16 (14)	59
Yellow	8 (5)	4 (3)	12

^a Original average particle numbers from 253 g of biosolids converted to “Particles per kg biosolids”.

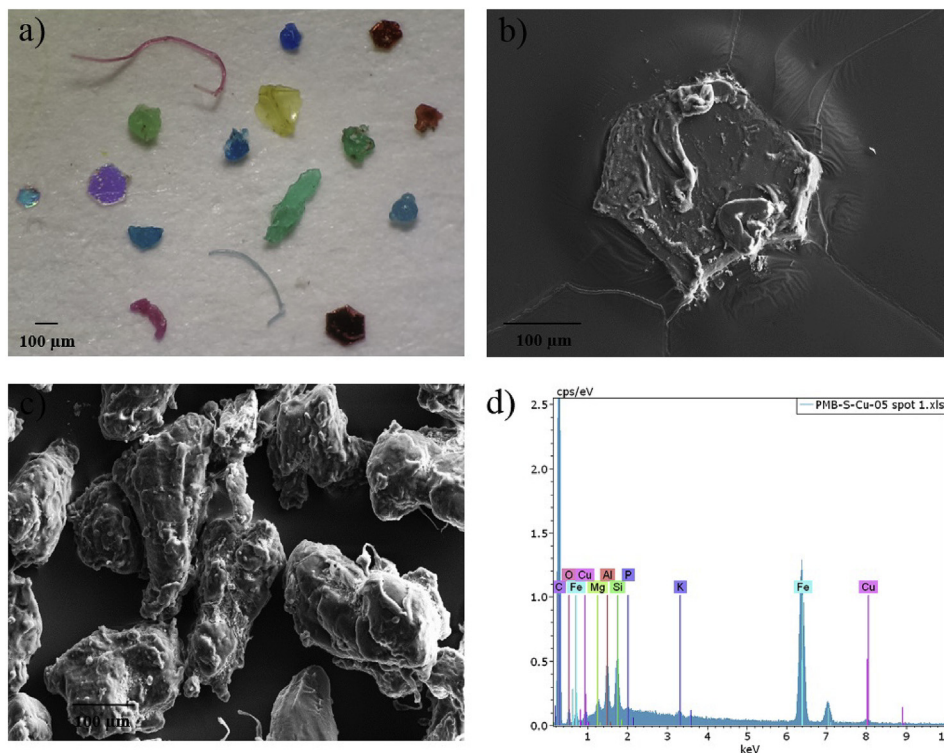


Fig. 1. Stereoscopic and scanning electron microscope images of microbeads. a) few of numerous microbeads found in biosolids under stereomicroscopy, b) a biosolids derived microbead showing wrinkled and fractured surfaces as patterns of its degradation, c) morphology of SMB (i.e., surface modified microbeads through soil organic matter sorption) after Cu(II) adsorption, and d) Energy dispersive spectroscopy showing evidences for Cu(II) adsorption to surfaces of SMB.

3.1.2. Polyethylene and surface modified microbeads

The purchased pure polyethylene microbeads (PMB) were shown in irregular shape, $\sim 100\text{--}150\ \mu\text{m}$ size and white colour (Figs. S1a–b). The microbeads extracted from the facial scrub were similar to the PMB (Fig. S1c), while the microbeads found in a neck pillow showed spherical shapes and $\leq 100\ \mu\text{m}$ size (Fig. S1d).

The FT-IR results of microbeads derived from biosolids, commercial products, and pure microbeads showed characteristic peaks, which were distinctive for each material; the peaks changed when the microbeads were exposed to dissolved organic matter. The microbeads in the biosolids were made of polyethylene terephthalate (PET), high density polyethylene (HDPE), polyethylene (PE), and polypropylene (PP) (Fig. 2). For example, well defined IR peaks at $\sim 2919\ \text{cm}^{-1}$ for the CH_2 asymmetric stretching, $\sim 2851\ \text{cm}^{-1}$ for the CH_2 symmetric stretching, $\sim 1460\ \text{cm}^{-1}$ for CH_3 symmetric deformation, and $\sim 1367\ \text{cm}^{-1}$ for wagging deformation were observed in some microbeads, thereby confirming the presence of PE microbeads (Fig. 2c). These microbeads were able to be extracted using water, indicating that the PE microbeads are less dense in nature during the extraction stage. These peak characteristics are consistent with previous FT-IR observations (Gulmine et al., 2002). When analyzing biosolids-derived microbeads under the stereomicroscope, pale yellow colour regions on the surfaces were noticed. Later, they were identified as mineral surfaces such as goethite (data not shown). For example, peaks $\sim 864\ \text{cm}^{-1}$ could be attributed to the Fe–OH in plane (ip) and out of-plane (oop) bending vibrations, from Fe-oxyhydroxide in goethite (Wijesekara et al., 2014). Similar observations in PE microbeads were found for the pure PMB and microbeads extracted from facial scrubs, suggesting that they are made from polyethylene. The microbeads derived from the neck pillow were characterized mainly as polystyrene microbeads (data not shown). They had peaks at $\sim 3024\ \text{cm}^{-1}$ for the CH asymmetric stretching of aromatic ring,

$\sim 2950\ \text{cm}^{-1}$ for the CH asymmetric aliphatic stretching, $\sim 1734\ \text{cm}^{-1}$ for the CO stretching, and $\sim 1600\ \text{cm}^{-1}$ corresponding to the C=C stretching of the aromatic ring, thereby confirming them as polystyrene beads (Babu and Dhamodharan, 2009).

The IR characteristics of the microbeads that contained iron minerals (i.e., biosolids-derived microbeads), as described above, were evident also for the surface modified microbeads (i.e., BSMB, SEMB, SMB). Compared to PMB, SEMB showed a strong peak at $\sim 1100\ \text{cm}^{-1}$ for the C–O stretching modes from primary and secondary alcohols, possibly associated with organic matter found in the sediments (Fig. S3) (Oh et al., 2005). In addition, the SEMB showed a weak peak at $\sim 1375\ \text{cm}^{-1}$ indicating the C–H and O–H deformation modes of alcohol and phenolic groups, which are present in decomposed cellulose material (Sewwandi et al., 2012). Compared with the surface modified microbeads, their Cu(II) sorbed microbeads did not show distinct IR peak changes (data not shown).

The dissolved organic carbon (DOC) contents were recorded as 43.21 , 63.32 , and $40.21\ \text{mg L}^{-1}$ for BSMB, SEMB, and SMB, respectively.

3.1.3. Trace elements of biosolids-derived microbeads

Table 3 shows the variation of metals in biosolids-derived microbeads. The concentrations of Cu, Zn, Mn, Sb, Mo, and Cd were observed to be 180.64 , 178.03 , 71.33 , 14.43 , 2.01 , and $2.34\ \text{ng g}^{-1}$, respectively. In addition, concentrations of trace elements such as Ag, As, Pb, and Se on biosolids-derived microbeads varied within the range of $<1\text{--}1.72\ \text{ng g}^{-1}$. The mineral elements such as Al, Ca, Fe, K and Mg had concentrations of $73,069$, 1240 , $91,502$, $77,170$, and $28,595\ \text{ng g}^{-1}$, respectively. Compared to a previous study (Turner and Holmes, 2015), most metal concentrations were lower in the present study. Nevertheless, the concentration of Cu in biosolids-derived microbeads was remarkably

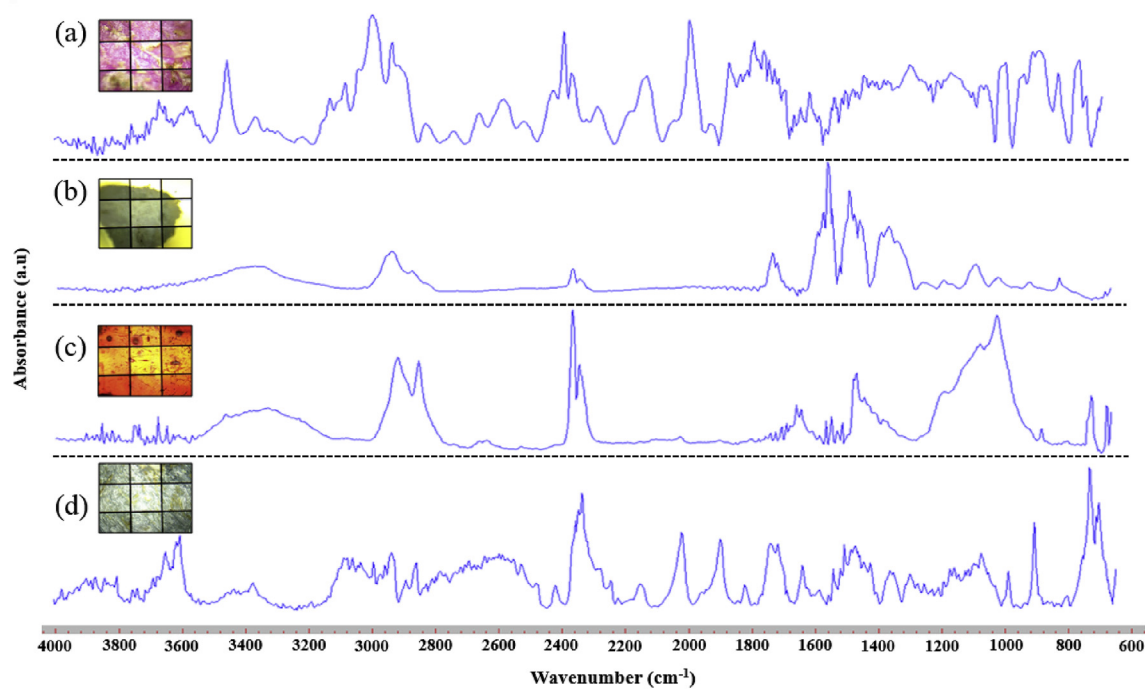


Fig. 2. FT-IR microscopy spectrums of microbeads found in biosolids. Microbeads made from a) polyethylene terephthalate (PET), b) high density polyethylene (HDPE), c) polyethylene (PE), and d) polypropylene (PP). The squares to the left represent the corresponding visible light maps of samples in $300 \times 250 \mu\text{m}$ panels.

Table 3

Metal concentrations on biosolids-derived microbeads (ng g^{-1}) and a comparison with a previous study. Average values from three samples (i.e., $n = 3$).

Metal	Concentration (ng g^{-1})	
	This study	Turner and Holmes 2015
Ag	1.21	<3
Al	73,069	7430
As	<1	–
Ca	1240	–
Cd	2.34	5
Co	ND	13.8
Cr	ND	42.5
Cu	180.64	47
Fe	91,502	34,400
Hg	NM	<3
K	77,170	–
Mg	28,595	–
Mn	71.33	712
Mo	2.01	–
Ni	12.69	29.3
Pb	1.17	109
Sb	14.43	–
Se	1.72	–
Zn	178.03	196

Note: ND and NM denote “not detected”, and “not measured”, respectively.

higher in our study, thereby indicating a possible threat of Cu transport in the environment. However, the biosolids used in our study contained a number of trace elements (Table S1). For example, the concentrations of Cu, Zn, and Mn in biosolids were observed 637.07 , 710.83 and $503.29 \text{ mg kg}^{-1}$, respectively. When microbeads interact with these trace metals in biosolids, they seem to retain a considerable amount of the metals, thereby transporting them in the environment. High concentrations of mineral metals such as Al, Fe, and Mg also were found in the biosolids-derived microbeads.

3.2. Microbeads on soil microbial respiration

Fig. 3 shows the effect of microbeads on microbial respiration measured two weeks after soil incubation. A decreased soil respiration was observed in the Grenfell soil mixed with microbeads (Fig. 3a). When compared with the average control (i.e., soil only) value of $230 \text{ mg CO}_2 \text{ kg soil}^{-1}$, the addition of PMB, BSMB, SEMB, and SMB resulted average of 204 , 187 , 165 , and $177 \text{ mg CO}_2 \text{ kg soil}^{-1}$, respectively, for the microbial respiration, thereby indicating significant ($p < 0.05$) effects (Table S2). Compared to the control, a larger effect was observed from the SEMB on soil respiration, indicating $\sim 28\%$ decrease over the control. In the case of Cu(II) treated microbeads, their influence on soil respiration was observed to be greater than the microbeads only with organic matter, possibly the toxicity occurred to microorganisms by high metal availability in soil (Usman et al., 2013). After fourteen days, the measured average CO_2 contents were 181 , 169 , 145 , and $162 \text{ mg CO}_2 \text{ kg soil}^{-1}$ for PMB + Cu, BSMB + Cu, SEMB + Cu, and SMB + Cu, respectively. SEMB + Cu had a $\sim 37\%$ decrease over the control, which was significant ($p < 0.05$). Its effect on microbial respiration was greater than the other treatments. Overall, the microbeads caused a decrease in soil respiration, thereby suggesting that degradation of soil organic matter had been affected by the addition of various microbeads. Nevertheless, it is important to mention that polyethylene can also be a source of carbon for microorganisms. For example, Kathiresan (2003) reported that, during a one-month period, *Pseudomonas* species and *Aspergillus glaucus* degraded 20.54 and 28.80% , respectively, of polythene present in soils under mangroves, which were rich with diverse microbial communities. When examining the surfaces of microplastics, McCormick et al. (2014) found a less diverse and significantly different taxonomic composition of bacterial assemblages in river water compared to those in water column and in suspended organic matter.

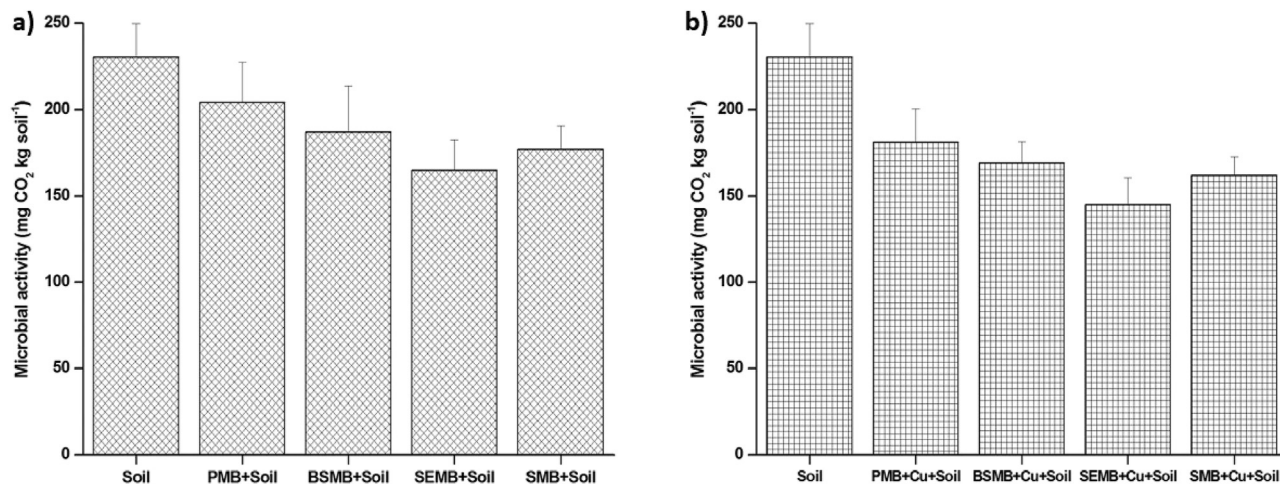


Figure 3. Effect of microbeads on microbial respiration in the soil from Grenfell. a) effect of pure (PMB) microbeads and microbeads extracted from biosolids; (BSMB), sediment (SEMB), and soil (SMB), and b) effect of copper treated microbeads: PMB + Cu, BSMB + Cu, SEMB + Cu and SMB + Cu on soil microbial respiration, (mean \pm standard deviation, $n = 3$).

3.3. Effect of microbeads on soil dehydrogenase activity

Microbeads had a negative effect on soil dehydrogenase activity (Fig. 4). When compared to the average DHA activity of $4.9 \mu\text{g TPF g}^{-1}\text{min}^{-1}$ for the control soil, the measured average values for PMB, BSMB, SEMB, and SMB were 4.43, 5.40, 3.54, and $3.02 \mu\text{g TPF g}^{-1}\text{min}^{-1}$, respectively. In the case of Cu(II) treated microbeads, the dehydrogenase activities for PMB + Cu, BSMB + Cu, SEMB + Cu, and SMB + Cu were 3.02, 2.64, 1.59, and $2.14 \mu\text{g TPF g}^{-1}\text{min}^{-1}$, respectively. Overall, DHA activity was less with the Cu-treated microbeads compared to microbeads without Cu. Further, the effect was significant ($p < 0.05$) for all the experiments. Ahmad et al. (2012) reported that a higher DHA activity in trace metal immobilised soils, thereby indicating the sensitivity of microbial activities to trace metal contamination. Because, DHA is an extracellular enzyme, it is assumed to be very sensitive to the environmental stress (Langer and Rinklebe, 2009; Paul and Clark, 1996). Therefore, the observed inhibition of DHA activity in our study could be attributed to the microbeads involved effects such as damaging the

microorganism's cells and their biopolymers (i.e., DNA), and limiting accessibility for their energy sources and nutrients. To our knowledge, there are no previous reports concerning the effect of microbeads on enzymatic activities in soil.

3.4. Interactions of dissolved organic matter-microbead-trace elements

In the isotherm study, the microbead concentration on a mass basis was $\sim 22.2 \text{ g L}^{-1}$. Using this solid solution, the maximum adsorption amount of Cu(II) for PMB, BSMB, SEMB, and SMB were 63.05, 105.79, 1319.63 and $85.07 \mu\text{g g}^{-1}$, respectively. Therefore, among the surface-modified microbeads, the SEMB and PMB showed the highest and lowest capacities, respectively, for Cu(II) adsorption. Nevertheless, the PMB, BSMB, and SMB showed approximately a similar trend in adsorption of Cu(II). In this experiment, Cu(II) adsorption data fitted well with a Langmuir isotherm model, suggesting a monolayer adsorption (Fig. 5). The Langmuir isotherm constants of k and Γ_{max} and correlation

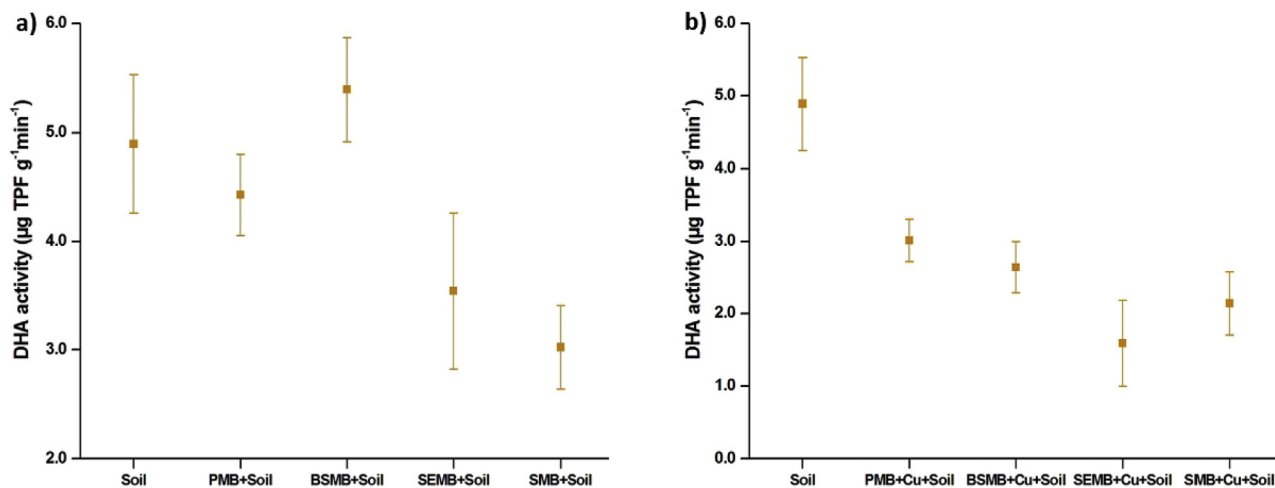


Fig. 4. Dehydrogenase (DHA) activity ($\mu\text{g TPF g}^{-1}\text{min}^{-1}$) change in soil over microbeads contamination (mean \pm standard deviation, $n = 3$). a) effect of pure (PMB), and extracted microbeads from biosolids; (BSMB), sediment (SEMB), and soil (SMB), and b) effect of copper treated microbeads as PMB + Cu, BSMB + Cu, SEMB + Cu and SMB + Cu on soil DHA activity.

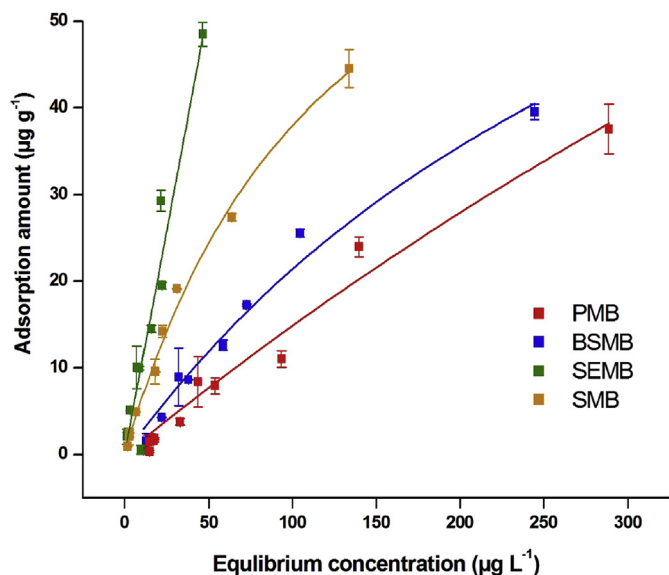


Fig. 5. Langmuir isotherms for Cu(II) by pure (PMB) microbeads and microbeads extracted from biosolids (BSMB), sediment (SEMB), and soil (SMB) using a solid solution of 22.2 g L^{-1} . Symbols represent experimental results and lines represent calculated results (mean \pm standard deviation, $n = 3$).

coefficients (R^2) for Cu(II) are presented in Table S3. The SEM-EDS analysis also confirmed the presence of Cu on surfaces of modified microbeads (Fig. 1c–d). These observations suggested that the microbeads need to be supported with organic matter and minerals to adsorb metals in high contents.

4. Conclusions

This study quantified the concentrations of microbeads in biosolids. It also examined the effect of pure polyethylene microbeads and surface-modified microbeads on metal retention and on specific microbial functions in soil. The results showed that the biosolids contained a substantial amount of microbeads including the polyethylene, polypropylene, and high density polyethylene beads. The biosolids-derived microbeads contained trace elements such as Cu, Zn, Mn, Sb, Mo, and Cd. Therefore, microbeads reaching biosolids during wastewater treatment are likely to serve as vectors for trace element contamination in soils. Soil microbial respiration and dehydrogenase activity were decreased when microbeads were mixed with soil. These effects were especially pronounced in the case of Cu-treated microbeads. Isotherm experiments confirmed that Cu retention was higher on surface-modified microbeads than on the pure polyethylene beads. We conclude that the microbeads affect specific microbial functions in soils. We provide evidence for microbeads enhancing metal retention, because of the organic matter associated with the microbeads. Further investigations are required to investigate the impact of contamination from trace metal sorbed-microbeads (e.g., as a result of application of biosolids) on specific microorganisms in soil.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.chemosphere.2018.01.166>.

References

- Ahmad, M., Hashimoto, Y., Moon, D.H., Lee, S.S., Ok, Y.S., 2012. Immobilization of lead in a Korean military shooting range soil using eggshell waste: an integrated mechanistic approach. *J. Hazard. Mater.* 209–210, 392–401.
- Amelineau, F., Bonnet, D., Heitz, O., Mortreux, V., Harding, A.M.A., Karnovsky, N., Walkusz, W., Fort, J., Grémillet, D., 2016. Microplastic pollution in the Greenland Sea: background levels and selective contamination of planktivorous diving seabirds. *Environ. Pollut.* 219, 1131–1139.
- Babu, K., Dharmodharan, R., 2009. Synthesis of polymer grafted magnetite nanoparticle with the highest grafting density via controlled radical polymerization. *Nanoscale Res. Lett.* 4, 1090–1102.
- Blarer, P., Burkhardt-Holm, P., 2016. Microplastics affect assimilation efficiency in the freshwater amphipod *Gammarus fossarum*. *Environ. Sci. Pollut. Res.* 23, 23522–23532.
- Brennecke, D., Duarte, B., Paiva, F., Caçador, I., Canning-Clode, J., 2016. Microplastics as vector for heavy metal contamination from the marine environment. *Estuar. Coast. Shelf Sci.* 178, 189–195.
- Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. *Water Res.* 91, 174–182.
- Cole, M., Galloway, T.S., 2015. Ingestion of nanoplastics and microplastics by pacific oyster larvae. *Environ. Sci. Technol.* 49, 14625–14632.
- Gulmine, J.V., Janissek, P.R., Heise, H.M., Akcelrud, L., 2002. Polyethylene characterization by FTIR. *Polym. Test.* 21, 557–563.
- Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environ. Sci. Technol.* 46, 3060–3075.
- Holland, E.R., Mallory, M.L., Shutler, D., 2016. Plastics and other anthropogenic debris in freshwater birds from Canada. *Sci. Total Environ.* 571, 251–258.
- Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total Environ.* 586, 127–141.
- Kathiresan, K., 2003. Polythene and plastic-degrading microbes in an Indian mangrove soil. *Rev. Biol. Trop.* 51, 629–633.
- Klein, D.A., Loh, T.C., Goulding, R.L., 1971. A rapid procedure to evaluate the dehydrogenase activity of soils low in organic matter. *Soil Biol. Biochem.* 3, 385–387.
- Lambert, S., Sinclair, C., Boxall, A., 2014. Occurrence, degradation, and effect of polymer-based materials in the environment. *Rev. Environ. Contam. Toxicol.* 227, 1–53.
- Langer, U., Rinklebe, J., 2009. Lipid biomarkers for assessment of microbial communities in floodplain soils of the Elbe River (Germany). *Wetlands* 29, 353–362.
- Lassen, C., Hansen, S.F., Magnusson, K., Hartmann, N.B., Rehne Jensen, P., Nielsen, T.G., Brinch, A., 2015. Microplastics: Occurrence, Effects and Sources of Releases to the Environment in Denmark. Danish Environmental Protection Agency. http://orbit.dtu.dk/files/118180844/Lassen_et_al_2015.pdf. (Accessed May 2017).
- Law, K.L., Morét-Ferguson, S., Maximenko, N.A., Proskurowski, G., Peacock, E.E., Hafner, J., Reddy, C.M., 2010. Plastic accumulation in the North Atlantic subtropical gyre. *Science* 329, 1185–1188.
- Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Anal. Methods* 9, 1346–1360.
- Lwanga, E.H., Gertsen, H., Gooren, H., Peters, P., Salanki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2016. Microplastics in the terrestrial ecosystem: implications for *Lumbricus terrestris* (Oligochaeta, Lumbricidae). *Environ. Sci. Technol.* 50, 2685–2691.
- Mahon, A.M., O'Connell, B., Healy, M.G., O'Connor, I., Officer, R., Nash, R., Morrison, L., 2016. Microplastics in sewage sludge: effects of treatment. *Environ. Sci. Technol.* 51, 810–818.
- McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an abundant and distinct microbial habitat in an urban River. *Environ. Sci. Technol.* 48, 11863–11871.
- Oh, S.Y., Yoo, D.I., Shin, Y., Seo, G., 2005. FTIR analysis of cellulose treated with sodium hydroxide and carbon dioxide. *Carbohydr. Res.* 340, 417–428.
- Paul, E.A., Clark, F.E., 1996. *Soil Microbiology and Biochemistry*, third ed. Academic Press, San Diego, London, Boston, New York, Sydney, Tokyo, Toronto.
- Rahman, M.M., Dong, Z., Naidu, R., 2015. Concentrations of arsenic and other

- elements in groundwater of Bangladesh and West Bengal, India: potential cancer risk. *Chemosphere* 139, 54–64.
- Rillig, M.C., 2012. Microplastic in terrestrial ecosystems and the soil? *Environ. Sci. Technol.* 46, 6453–6454.
- Rochman, C.M., Kross, S.M., Armstrong, J.B., Bogan, M.T., Darling, E.S., Green, S.J., Smyth, A.R., Verissimo, D., 2015. Scientific evidence supports a ban on microbeads. *Environ. Sci. Technol.* 49, 10759–10761.
- Sewwandi, B.G.N., Vithanage, M., Wijesekara, S.S.R.M.D.H.R., Rajapaksha, A.U., Jayarathna, D.G.L.M., Mowjood, M.I.M., 2012. Characterization of aqueous Pb(II) and Cd(II) biosorption on native and chemically modified *Alstonia macrophylla* saw dust. *Biorem. J.* 16, 113–124.
- Shaheen, S.M., Tsadilas, C.D., Rinklebe, J., 2013. A review of the distribution coefficients of trace elements in soils: influence of sorption system, element characteristics, and soil colloidal properties. *Adv. Colloid Interface Sci.* 201–202, 43–56.
- Shammas, N.K., Wang, L.K., 2008. Transport and pumping of sewage sludge and biosolids. In: Wang, L.K., Shammas, N.K., Hung, Y.T. (Eds.), *Biosolids Engineering and Management*. Humana Press, New Jersey, USA, pp. 1–64.
- Statista, 2017. *Global Plastic Production from 1950 to 2015*. <https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/>. (Accessed August 2017).
- Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 2027–2045.
- Turner, A., Holmes, L.A., 2015. Adsorption of trace metals by microplastic pellets in fresh water. *Environ. Chem.* 12, 600–610.
- Usman, A.R.A., Almaroai, Y.A., Ahmad, M., Vithanage, M., Ok, Y.S., 2013. Toxicity of synthetic chelators and metal availability in poultry manure amended Cd, Pb and as contaminated agricultural soil. *J. Hazard. Mater.* 262, 1022–1030.
- Vegter, A.C., Barletta, M., Beck, C., Borrero, J., Burton, H., Campbell, M.L., Costa, M.F., Eriksen, M., Eriksson, C., Estrades, A., Gilardi, K.V.K., Hardesty, B.D., Ivar do Sul, J.A., Lavers, J.L., Lazar, B., Lebreton, L., Nichols, W.J., Ribic, C.A., Ryan, P.G., Schuyler, Q.A., Smith, S.D.A., Takada, H., Townsend, K.A., Wabnitz, C.C.C., Wilcox, C., Young, L.C., Hamann, M., 2014. Global research priorities to mitigate plastic pollution impacts on marine wildlife. *Endanger. Species Res.* 25, 225–247.
- Wang, J., Peng, J., Tan, Z., Gao, Y., Zhan, Z., Chen, Q., Cai, L., 2017. Microplastics in the surface sediments from the Beijiang River littoral zone: composition, abundance, surface textures and interaction with heavy metals. *Chemosphere* 171, 248–258.
- Wardrop, P., Shimeta, J., Nuggeoda, D., Morrison, P.D., Miranda, A., Tang, M., Clarke, B.O., 2016. Chemical pollutants sorbed to ingested microbeads from personal care products accumulate in fish. *Environ. Sci. Technol.* 50, 4037–4044.
- Wijesekara, S.S.R.M.D.H.R., Basnayake, B.F.A., Vithanage, M., 2014. Organic coated nano-particulate zero valent iron for remediation of chemical oxygen demand (COD) and dissolved metals from tropic landfill leachate. *Environ. Sci. Pollut. Res.* 21, 7075–7087.
- Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y., Oda, K., 2016. A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science* 351, 1196–1199.
- Zhao, S., Zhu, L., Li, D., 2016. Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: not only plastics but also natural fibers. *Sci. Total Environ.* 550, 1110–1115.
- Zubris, K.A.V., Richards, B.K., 2005. Synthetic fibers as an indicator of land application of sludge. *Environ. Pollut.* 138, 201–211.