Characteristics of wastewater originating from dental facilities using predominantly mercury-free dental filling materials

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Abstract:

Dental materials are currently undergoing a revolution. Mercury use, including traditional amalgam (mercury-containing) material used in dental fillings, is now being widely regulated under the Minamata convention, and dental amalgam is currently being replaced by resin formulations in dentistry. These resin-based materials can be tuned to offer varying material properties by incorporation of a range of nano- and micro-particle based 'fillers' for different dental properties and applications. However, these innovations may have a concomitant effect on the waste streams associated with common dental applications, in particular the potential for higher concentrations of novel micro- and nanomaterials within wastewater streams, and a potential route for novel nanomaterials into the wider Environment. These new materials may also mean that wastewater filtering apparatus commonly deployed at present, such as amalgam separators, may be less efficient or insufficient to capture these new filler materials in dental

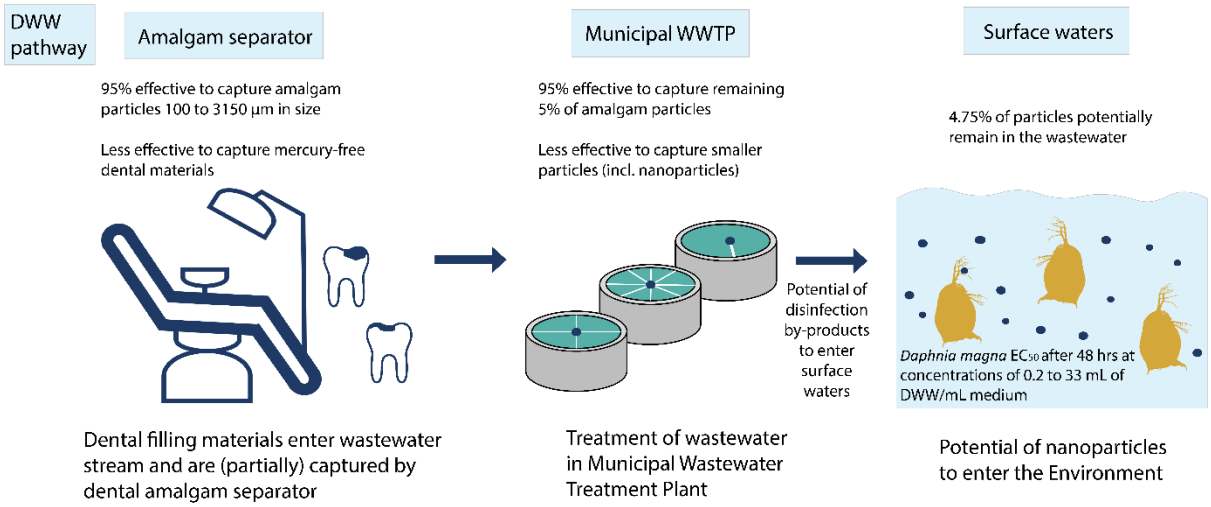
facility wastewater. In this work, we analyse dental wastewater streams from three dental facilities in Ireland with differing amalgam separators in place. The potential overall toxicity, particulate load and physicochemical properties are analysed. The overall risk posed by these new materials is also discussed.

Keywords: Dentistry, Minamata Convention, waste streams, nanomaterials, particulate matter

Highlights

- Dental wastewater streams are understudied.
- Current particle capture methods are outdated since introduction of novel materials.
- Potential for nanoparticle release in dental wastewater.
- Fillers present in dental materials are a potential pathway for metals to enter the wider Environment.

Graphical abstract



1. Introduction

The field of dentistry, and with it, the use of dental filling materials (DFMs), is currently evolving to adopt sustainability and environmental considerations into the clinical environment (Duane et al., 2019). Traditional Hg-based DFMs have long raised well-recognised environmental concerns, starting with the introduction of dental amalgam in the 1820s (Cataldi et al., 2017; Jamil et al., 2016; Mulligan et al., 2018). The Minamata Convention of 2013 has triggered the reduction of all mercury-containing products in use, including dental amalgam, when it entered into force in August 2017 (UNEP, 2017). Further environmental hazards were addressed with the introduction of Regulation (EU) 2017/852 by reducing the amount of Hg in use in all sectors, including dentistry, which contributes 20% of the global consumption of Hg (Regulation (EU) 2017/852, 2017; Tibau and Grube, 2019; UNEP, 2016). The Minamata convention, and the EU regulated phase-down of mercury (Hg)-based materials, have directly led to an increase in the use of Hg-free DFMs, more of which incorporate nano-fillers of various compositions (Radhi et al., 2021). These nano-fillers are incorporated to enhance desirable properties such as hardness, wear, colour, anti-bacterial properties and marginal integrity (Agha et al., 2017; Arenholt-Bindslev, 1992; Bonsor et al., 2013; Ibrahim et al., 2020; Kidd et al., 2011; Mulligan et al., 2018; Nicholson, 2007). This now means that current DFMs increasingly contain a range of novel materials and different constituents, and that updated information on potential risks posed to the environment and to human health by dental waste streams is required (Van Landuyt et al., 2011).

The main DFMs in use today are resin composites (RCs) and glass ionomer cements (GICs) (Table 1). RCs consist of a chemically active resin mixed with glass or ceramic fillers and a silane coupler for bonding, GICs consist of a polyacrylic acid mixed with glass fillers and water (Bonsor et al., 2013; Rohani and Nicholson, 2009; Sidhu, 2010). Two modified DFMs are also in use, which are polyacid modified resin composites (PMRCs) and resin modified glass

ionomers (RMGICs), which both utilise methacrylate resins in addition to glass and other fillers. Indeed, constituents of the current generation of DFMs include polyacids, heavy metals, metal oxides, plastics, glass and ceramic fragments, and a range of other small filler materials (Bonsor et al., 2013; Kidd et al., 2011). These filler materials may contain a range of engineered particle sizes, ranging from macro (10 – 100µm) to mid (1 – 10µm), mini (0.1 – 1µm), micro (0.01 – 0.1µm) and nano (0.005 – 0.01µm) sized scales (Table 1) (Mohamed Abdel-Hamid et al., 2008). Waste generated during application and removal of materials during dental applications may contain particles of different sizes.

Table 1. Details of the most commonly used Hg-free dental filling materials and particle size distribution of different filler sizes in use (Bonsor *et al.*, 2013; Mohamed Abdel-Hamid et al., 2008); Rohani and Nicholson, 2009; Sidhu, 2010).

Year of introduction	Hg-free dental filling material	Chemical composition				
1960s	Resin Composite	resin	+	silane coupler	+	filler (glass/ceramic)
1970s	Polyacid modified resin composite	methacrylate resin	+	methacrylate resin	+	reactive filler (glass)
1980s	Glass ionomer cement	polyacrylic acid	+	water	+	filler (glass)
1990s	Resin modified glass ionomer cement	Glass ionomer cement	+	resin	+	filler

Filler materials size category	Filler size
Macro	10 – 100µm
Mid	1 – 10µm
Mini	0.1 – 1µm
Micro	0.01 – 0.1µm
Nano	0.005 – 0.01µm

Dental wastewater (DWW) is a heterogeneous mixture of particles and liquids: with the particles resulting from dental amalgam, tooth constituents, soft tissue and bacteria and the liquids resulting from water, oral fluids, blood, saliva, plasma, daily/ routine cleaning solutions, in-line cleaners, surfactants and mouthwash fluids (Cailas et al., 2002). The processes of placing and removing dental fillings produce different waste streams, including wastewater, particulates and aerosols (Adegbembo et al., 2002; Cailas et al., 2002; Cataldi et al., 2017; Drummond et al., 2003; Jírová et al., 2019; Shraim et al., 2011). Current measures put in place to counteract release of particles into the environment from dental facilities have included various filter traps, and dental amalgam separation technologies designed to catch primarily Hg released from dental wastewater streams (Cailas et al., 2002; Vandeven and Mcginnis, 2005). The filter traps are divided into sink and spittoon filters, each capable of catching particles between 1000 and 2000 μm in size, and in line filters, capable of capturing particles between 2000 and 4000 μm in size (Table 2) (Vandeven and McGinnis, 2005). The different amalgam separation technologies in use in dental facilities (also known as amalgam separators, amalgam separator devices, MRUs (mercury recovery units) or mercury traps) are ISO 11143:2008 certified (Hylander et al., 2006; International Organisation for Standardisation, 2008).

The various ISO 11143:2008 certified separation technologies are required to be at least 95% effective in capturing a pre-determined particle size distribution consisting of a 30% mass fraction (3 g of the test slurry) of amalgam particles ≤100 μm in size, a 10% mass fraction (1 g of the test slurry) of amalgam particles between 100 μm and 500 μm in size, and a 60% mass fraction (6 g of the test slurry) with amalgam particles between 500 μm and 3,150 μm in size (Table 2) (International Organisation for Standardisation, 2008). Although amalgam separation devices are in theory at least 95% effective (based on mass fraction), efficiency testing in dental facilities has shown that particle removal varies from as low as 26.5% and up to 99.9% effective

(Drummond et al., 2003; Hylander et al., 2006; Vandeven and Mcginnis, 2005). Equally, efficiency testing of the various filter traps has shown them to be effective in capturing between 40 and 80% of the particles (Table 2) (Vandeven and Mcginnis, 2005). This capture efficiency is dependent on the setup and usage of the filters and amalgam separation device and it also showed that dissolved Hg fails to be trapped, causing significant Hg emissions from dentistry even under current best use.

Table 2. Particle size distribution and capture efficiency for dental chair filters and ISO 11143:2008 certified amalgam separators.

	Particle fraction	Particle size captured	Mass fraction	Test slurry proportion	capture efficiency in theory	capture efficiency in operation	References
sink and spittoon filters	3	1000 – 2000 µm	/	/	/	40 – 80%	(Vandeven and Mcginnis, 2005)
in line filters	3	2000 – 4000 µm	/	/			
amalgam separator	1	≤100 µm	30%	3 g	95%	26.5 – 99.9%	(Drummond et al., 2003; Hylander et al., 2006; International Organisation for Standardisation, 2008; Vandeven and Mcginnis, 2005)
	2	100 – 500 µm	10%	1 g			
	3	500 – 3,150 µm	60%	6 g			

With a concerted effort to reduce the use of Hg-based materials in dentistry, there is now a question as to how effective, or suitable, amalgam separation technologies are, when DFMs contain an increasing fraction of engineered nanocomposite materials and/or plasticisers such as Bisphenol A (Arenholt-Bindslev, 1992; Bonsor et al., 2013; Cataldi et al., 2017; Mulligan et al., 2018; Polydorou et al., 2020).

While infection prevention and control are largely regulated in dental facilities, and guidelines address the use of disinfectant for biofilm management, and the health hazards associated with DFMs (Dental Council of Ireland, 2015; Health and Safety Authority, 2013; HIQA, 2018; S.I. No. 533, 2018), the potential environmental aspects of novel Hg-free DFMs have not yet been addressed in detail.

DWW effluent is currently not regarded as trade effluent, it does however require treatment in a municipal wastewater treatment plant (WWTP), which in Ireland generally includes secondary and tertiary treatment (Environmental Protection Agency, 2007; Irish Water, 2019). Standardised parameters to measure the WWTP effluent before discharge to surface waters include: the water temperature, pH, EPA Priority Pollutants, Suspended Solids, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) (EPA Ireland, 2021). Municipal WWTPs have been found to insufficiently remove small particles from the WW, leading to the formation of disinfection by-products, such as trihalomethanes (THMs), haloacetic acids (HAAs) and aldehydes (Bond et al., 2011; Jírová et al., 2019; Monarca et al., 2000; Park et al., 2016). Therefore, considering that novel materials are introduced into the amalgam separation units, which have been installed for the purpose of removing dental amalgam from the WW, and considering that WWTPs have been found to be equally insufficient in removing the full particle load that is currently discharged by human activity, current WWT technologies may need to be reviewed.

This study measures the particle load and potential ecotoxicity of the particulate matter arising from a shift to predominately Hg-free DFMs in Irish dental facilities. Internationally standardised and industry-approved testing is utilised to determine the ecotoxicity of the dental effluent by using the *Daphnia* sp. acute immobilisation test, which determines the immobilisation rate of the *Daphnia* sp. after 48 hrs of exposure to varying concentrations of the test substance (OECD, 2004). Furthermore, since amalgam separators are required to be

installed in Ireland since the 1st of January 2019 in accordance with EU Directive 2017/852, this study also assesses whether amalgam separators, designed with Hg-containing waste in mind, are also effective in capturing particulate matter resulting from the use of Hg-free DFMs.

2. Materials and methods

Dental wastewater (DWW) samples were collected from three dental facilities/practices (DPs) in Cork, Ireland, which included one private dental facility, one public dental hospital and one public dental school as part of the Cork University Dental School and Hospital (founded 1913, and part of University College Cork). The dental facilities were chosen based on ease of access and permissions granted for sampling, type of installed amalgam separators, and were largely deemed typical of local dental facilities in terms of volumes of patients treated, procedures undertaken and awareness and compliance with good dental practices and waste disposal. The characteristics of these DPs, including type and brand of amalgam separator, sampling period, daily volumes of WW discharged, predominant dental filling materials (DFMs) used, and disinfectant used are shown in Table 3. Each of the three dental facilities had between one and two clinical interventions involving Hg-free dental filling materials on each of the sampling days. The DWW sample collection was carried out by one individual (HB), adopting Health and Safety protocols published by the Health and Safety Authority (2013) and the Health Information and Quality Authority (HIQA) (2018), to allow for the safe extraction of DWW from the selected dental facilities. This included the use of a disinfection product, which was used to disinfect the dental tubing lines, as well as prevent potential hazards of Hazard Group 2 / Containment Level 2 and reduce the risk of contamination with blood borne viruses to the individual collecting and analysing the samples (Health and Safety Authority, 2013). In all three DPs, the DWW samples were collected once the DWW had passed through the dental

spittoon, dental chair tubing, amalgam separation unit, and before the wastewater entered the sewerage system (Fig. 1).

Table 3. Dental facility (DP) ID, amalgam separator (AS), sampling period, quantity of dental wastewater (DWW), typical dental filling material (DFM) and dental unit disinfectant used (Dis.) in this study.

DP ID	AS	Brand name of AS	No. of dental chairs connected to AS	Sampling period (no. of sampling days)	Maximum volume of DWW/day	Average number of patients per day	Total volume of DWW collected	Average L of DWW /patient	DFM	Dis.
DP1	Type 1 – centrifugation	Dürr Dental SE© CA1	1	Dec. 2018 – June 2019 (19)	Up to 11 L/day	1 – 2	143.8 L	~10 L	RC and GIC some Am	MD 555 cleaner, daily
DP2	Type 2 – sedimentation	Metasys ECO II	4 or 5	June – July 2019 (9)	Up to 4 L/day	6 – 7	18.1 L	~0.5 L	RC and GIC	Puli Jet Classic disinfectant, daily
DP3	Type 1 – centrifugation	Dürr Dental SE© CAS1	3	July 2019 (5)	Up to 6 L/day	5	7.8 L	~1.2 L	RC and GIC some Am	MD 555 cleaner, daily

* RC = Resin composite, GIC = Glass ionomer cement, Am = Amalgam.

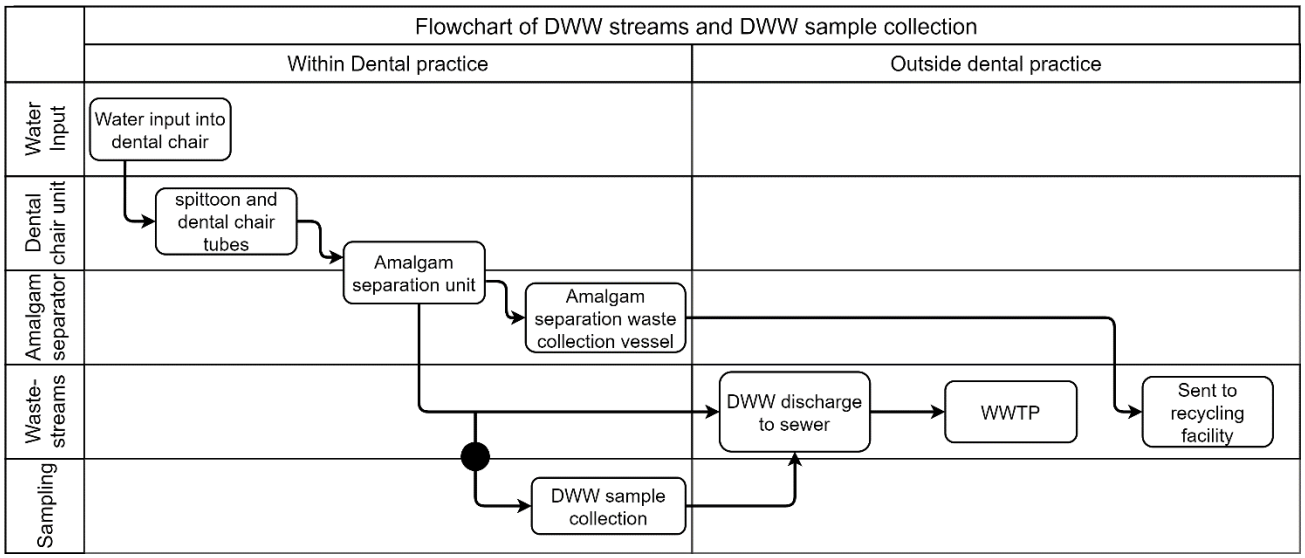


Fig. 1. Diagram of DWW collection progress (flowchart) for pathway followed in this study.

Samples were collected over a 24 h period in DP1, and over 36-48 h in DP2 and DP3. Once collected, the samples were transported to the laboratory and stored (4 ºC) and analysed within 36 h.

The laboratory analyses of the dental wastewater had three major components: 1) the analysis of physicochemical parameters, 2) inorganic particulate analysis and 3) ecotoxicity testing of DWW (Fig. 2).

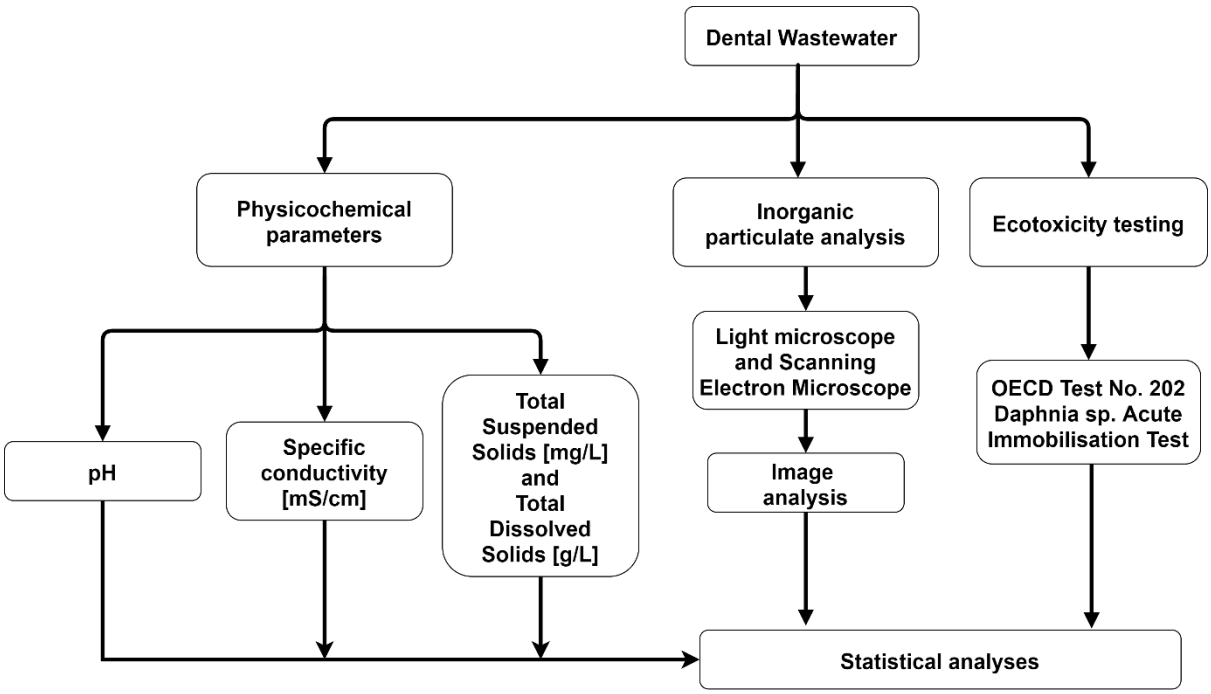


Fig. 2. Flowchart of materials and methods in this study of dental wastewater.

2.1 Physicochemical measurements of DWW

The physicochemical parameters measured were pH, specific conductivity, Total suspended solids (TSS) and Total dissolved solids (TDS). Specific conductivity and pH measurements were obtained by submerging calibrated probes into 200 mL of the DWW samples (at ambient temperature) and by recording the values once stabilised (WTW Handheld Conductivity meter

340i and OxyGuard Handy pH with software version 1.5). Regular blanks were obtained by submerging the probes in laboratory and dental tap water (pH 6 to 7) and by comparing the obtained measurements with reference values.

TSS and TDS measurements were carried out in triplicate according to standardised procedures, with no deviations from the standardised method (Fisher Scientific, 2007).

2.2 Inorganic Particulate Analysis in DWW samples

Particle detection, sizing and characterisation was carried out using the ALC Multispeed centrifuge (PK 121 series), the Leica DM 500 light microscope fitted with an ICC50 HD camera and the JEOL JSM-IT200 InTouchScope™ Scanning Electron Microscope (SEM).

Well mixed DWW in 1 L pre-weighed beakers (Fisher scientific, product code: 15409083) or polypropylene tubs (Fisher Scientific, product codes: 11358073, 11348073 and 11338073) covered with perforated lids, were placed in a drying oven at 60 °C for 72 to 96 hours to gently remove the excess liquid, until a volume below 0.5 L was reached.

Sample aliquots were then centrifuged (3000 rpm, 2 min) in an ALC Multispeed centrifuge (PK 121 series) and the supernatant carefully decanted off. The sample material was resuspended in a mixture of 3 mL bleach (< 5% Sodium hypochlorite) and 11 mL de-ionised water (DIW) and refrigerated for 72 hours. Following this, the samples were centrifuged (2000 rpm, 2 min) and resuspended in DIW five to seven times to remove any excess chlorine salts. The resulting sample materials were then transferred to clean microscope slides with coverslips and images obtained using a Leica DM 500 light microscope fitted with an ICC50 HD camera (objective lenses x10, x40 and x100) and annotated with a scale bar in the LAS EZ software.

The remainder of the sample material was re-suspended in DIW and placed in the freezer at minus 18 °C for long-term sample storage.

2.3 Particle size and shape by light microscopy

Light microscope images were processed using ImageJ/Fiji (software version 1.46) (Rasband, 2018) for circularity, particle area and particle number. Results were exported from ImageJ/Fiji for further analysis and visualisation in a comma-separated value (.csv) file.

2.4 Particle size and shape by Scanning Electron Microscopy (SEM)

Sample material for analysis via SEM was prepared using the bleached material (Section 2.2) from the light microscopy preparation and by re-suspending this material in EtOH at increasing concentrations from 70:30 to 50:50 (DIW:EtOH) and centrifuging at 2000 rpm for 2 min. Sample material was mounted on 10 mm Al stubs with C tape and sputter coated with Au for 30 to 45 s initially but adjusted to up to 90 s as sample material showed signs of charging after short exposure to the electron beam. The JEOL JSM-IT200 InTouchScope™ SEM was used for analysis with general conditions at an accelerating voltage of 10 kV and a working distance of 10 mm.

2.5 Ecotoxicity testing of DWW

The *Daphnia magna* immobilisation test was carried out in line with the guidelines set out in OECD (Organisation for Economic Co-operation and Development) Test No. 202 (OECD, 2004). The growth medium used for *D. magna* culturing was the ADaM (Aachener *Daphnia* Medium) and batch cultures of the algae *Chlamydomonas reinhardtii* were grown in controlled laboratory conditions and fed to *D. magna* in line with their respective growth: 0.5 mg of C/day

for neonates 1 – 2 days old, 0.75 mg of C/day between days 3 – 7 and 1 mg of C/day for adults from day 8 onward. The testing was carried out in 48 h darkness periods. Test concentrations were established using range-finding tests. Only DWW samples containing mercury-free dental filling materials were used for ecotoxicity testing, any samples that contained dental amalgam (based on daily procedures recording in each dental facility) were excluded.

Immobilisation was recorded when neonates remained immobilised 15 s after agitation had occurred. Tests were valid when immobilisation of the control groups was < 10%. Using Graph Pad Prism (software version 8.2.1), the dose variable was log transformed using the natural logarithm (Ln) in order to normalise the data. The response variable was normalised in order to express the data on a common scale, with 0% and 100% representing the lowest and highest response. Nonlinear regression with curve fit was then carried out using the dose-response data, as part of which the EC_{50} was calculated, along with 95% confidence intervals (CI).

3. Results

Analysis of the physicochemical aspects of DWW from different dental facilities (Table 4), indicated that DP2 (n=6) had a wide range of pH values and DP1 (n=19) had the highest specific electrical conductivity range. However, DP2 had the highest mean specific conductivity and the highest mean TSS and TDS results. TSS and TDS results were restricted by the analytical balance to 4 significant figures.

The EC_{50} of *Daphnia magna* after 48 hrs (Table 4) was observed to be at a concentration of 0.2 (± 0.2) mL of DWW per L of medium in DP3 (n=2). The EC_{50} in DP2 (n=3) was met at a less diluted concentration of 0.6 (± 0.6) mL of DWW per L of medium. DP1 (n=7) required the least dilution to obtain the EC_{50} at a concentration of 32.9 (± 23) mL of DWW per L of medium.

Table 4. Results of specific conductivity, pH, triplicate Total Suspended Solids (TSS) and triplicate Total Dissolved Solids (TDS) measurements (±SD) from the wastewater of DP1 with n=19 individual wastewater samples, DP2 with n=6 samples, DP3 with n=2 samples (TSS and TDS carried out in triplicate and restricted by analytical balance to 4 sig. figures) and results of 48h *Daphnia magna* acute immobilisation testing according to OECD 202 standard methods using the wastewater from the wastewater of DP1 with n=7 samples, DP2 with n=3 samples, DP3 with n=2 samples, showing min, max and mean EC_{50} values in mL DWW/L medium (±SD).

		Min	**Max**	**Mean**	**± SD**
pH	**DP1 (n=19)**	1.23	5.50	n/a	n/a
	DP2 (n=6)	2.64	9.17	n/a	n/a
	DP3 (n=2)	1.85	1.85	n/a	n/a
Spec. cond. [mS/cm]	**DP1 (n=19)**	0.15	18.65	4.41	4.93
	DP2 (n=6)	0.38	6.17	2.50	2.72
	DP3 (n=2)	5.03	5.14	5.09	0.08
TSS [mg/L]	**DP1 (n=19)**	n.d.	56.67	19.85	2.55
	DP2 (n=6)	54.67	203.33	114.83	4.50
	DP3 (n=2)	121.00	144.00	132.50	2.82
TDS [g/L]	**DP1 (n=19)**	0.15	39.22	5.10	0.15
	DP2 (n=6)	4.92	7.22	6.13	0.25
	DP3 (n=2)	6.76	7.11	6.94	0.08
		min	**max**	**EC_{50}(48h)**	**± SD**
	DP1 (n=7)	0.20	133.80	32.93	23.00

OECD 202 Ecotoxicity testing (*D. magna*) [mL DWW/L medium]	**DP2 (n=3)**	0.20	0.80	0.60	0.60
	DP3 (n=2)	0.20	0.20	0.20	0.20

3.1 Analysis of inorganic particulates in DWW

The relative frequency [%] of each particle size bin width [Measurable 2-D particle area in μm^2] in the three DPs was observed to follow the same trends throughout (Fig. 3). For each DP, the particle data was divided into 5 bin widths: from 1.2 to 5 μm^2, from 5 to 10 μm^2, from 10 to 50 μm^2, from 50 to 100 μm^2 and from 100 to 500 μm^2. All particles contained in the respective bin width are then shown as a percentage with respect to the total number of particles found for that DP, e.g., X % = (100/total number of particles in DP) *number of particles in each bin width.

Consistently for each DP the highest number of particles were between 1.2 to 5 μm^2 in size (52 to 65% of particles). Between 17 and 21% of the particles were between 5 and 10 μm^2 in size, and 13 to 22% of the particles were found to be between 10 to 50 μm^2 in size. Only very few particles (3% or less) were found to be larger in size, between 50 to 500 μm^2.

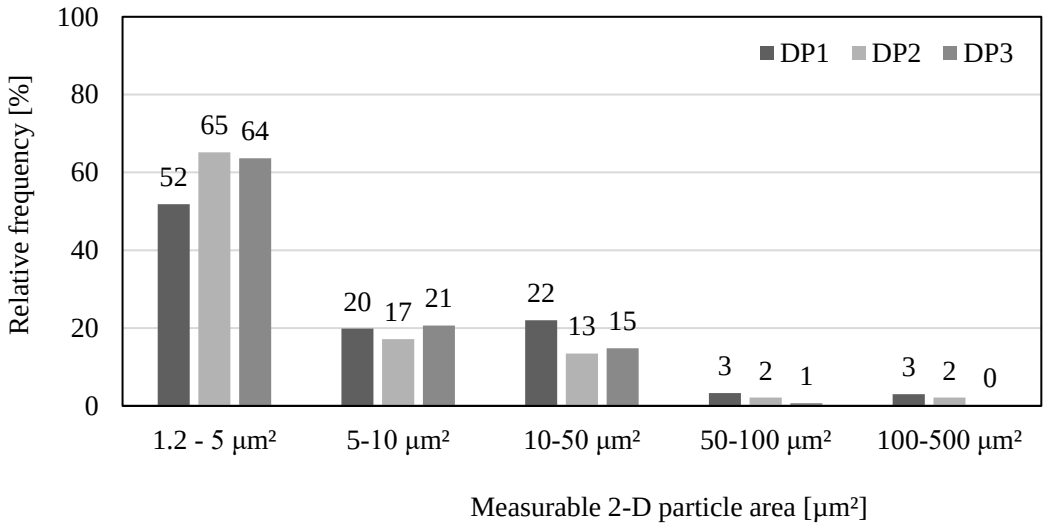


Fig. 3. Size distribution shown as relative frequency [%] of particles between particle area of 1.2 and 500 μm^2 in the wastewater from three dental facilities: DP1 (n=19), DP2 (n=6) and DP3 (n=2).

The particle shape was analysed by using a combined LM and SEM approach. The cured DFMs (bulk material and scrapings of the bulk) were observed under the LM and SEM on clean microscope slides and SEM stubs before and after contact with dental patients (Fig. 4). The circularity index of these particles under the LM consistently indicated that most of the particles (around 70%) were of elliptic (circ. index of 0.7) to round (circ. index of 1.0) shape. These particles were interpreted to be mini- to micro-sized filler materials (< 1 µm in size) that are used in the Hg-free DFMs. The remaining 30% of particles with lower circularity index were linked to dental materials resulting from the use of dental drilling and polishing and the SEM measured these to be of different shape and size than those identified as fillers (Fig. 4 e-h).

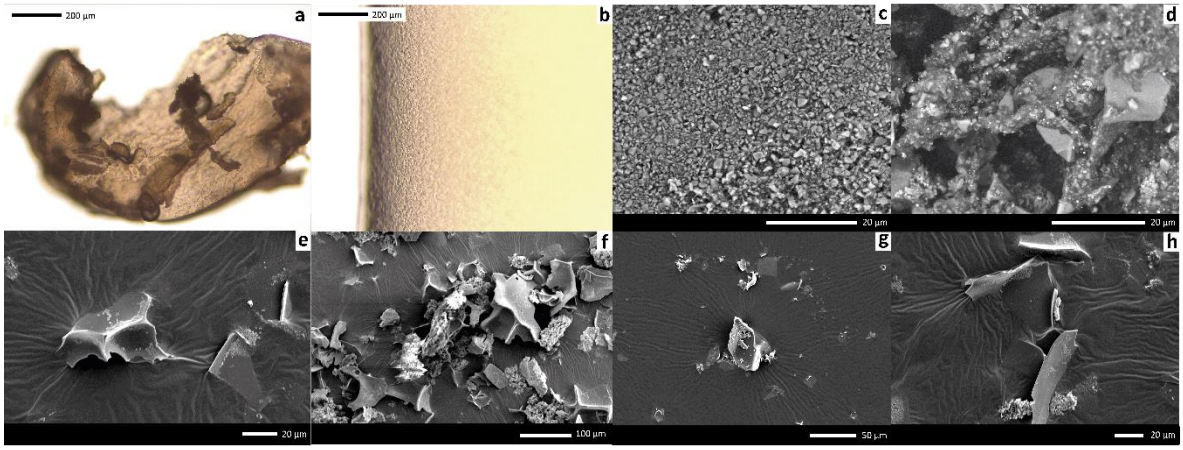


Fig. 4. Cured Hg-free dental filling materials (DFMs) imaged using (a-b) Light Microscopy (LM) and (c-h) Scanning Electron Microscopy (SEM), showing materials before use on dental patients (a-d), with (a) scalpel scraping of cured Hg-free DFM under LM, (b) bulk of cured Hg-free DFM under LM, (c) bulk of cured Hg-free DFM under SEM, (d) scalpel scraping of cured Hg-free DFM under SEM and (e-h) fragments of cured Hg-free DFM under SEM found in dental wastewater samples after use in dental facilities under typical use conditions.

The two main observations were: (1) most particles were small in size (1.2 to 5 μm^2) and few large particles (50 to 500 μm^2) remained in the DWW, and (2) particle angularity overall was low during LM observation, but showed higher angularity under the SEM.

4. Discussion

As pointed out by Bayne *et al.*, (2019) dental filling materials (DFMs) have undergone a revolution rather than an evolution over the past century, and this revolution continues apace with the recent introduction of a wide range of novel Hg-(mercury)-free DFMs that contain a diverse range of engineered nanomaterials. A note of caution should now be sounded around dental wastewater as a potential route of entry for engineered nanomaterials and other pollutants into the environment.

The most common points of entry for contaminants from WW into the environment are through untreated discharge or through the re-use of biosolids arising from wastewater treatment for example.

This study addresses a knowledge gap pertaining to wastewater streams associated with the use of these Hg-free DFMs in the clinical dental environment. All facilities had various filter traps (spittoon, sink and in-line filters), as well as an amalgam separator of either Type 1 or Type 2 fitted to minimise the presence of amalgam in the DWW. Despite detecting minimal amalgam during sampling, suggestive of separators being efficient for dental amalgam (ISO, 2008), a high contribution from Hg-free DFMs and particulate matter overall was detected.

In terms of physicochemical measurements from the wastewater streams, DP1 and DP3 showed extremes of pH (pH 1.23 and pH 1.8, respectively) and high specific conductivity measurements were recorded in all three DPs (mean between 2.5 ± 2.7 and 5.1 ± 0.1 mS/cm). As expected, a relationship between the acidity (pH) and the high amount of suspended solids (TDS) was evident, as well as a link to the use of line cleaners and disinfection products, by referring to the Material Safety Data Sheet (MSDS) of the cleaning products. Exclusion of the disinfection products for DWW testing was not possible, due to adherence to the health and safety protocols necessary for sampling (see justification in Methods section 2). Measurement

of total suspended solids (TSS) indicated that particulates were detectable in wastewater streams from all three DPs, with DP2 and DP3 having the highest overall particulate loadings (between 114 ± 69.0 mg/L to 132.5 ± 16.3 mg/L TSS on average and 5.9 to 6.9 g/L on average). While the occurrence of enamel and other particulate material cannot be excluded, within DP1, the highest particle load was linked to sampling days on which GICs (glass ionomer cements), RCs (resin composites) and RMGICs (resin-modified glass ionomer cements) were used heavily.

Ecotoxicity results identified the wastewater of DP3 to have the highest potential to cause an environmental impact due to the EC_{50} (the effective concentration at which 50% of the *D. magna* become immobilised) of 0.2 mL DWW/L of medium, however, these volumes will become further diluted as the DWW is released to the sewerage system. Overall, the EC_{50} ranged from 0.2 to 32.9 mL DWW/L medium in the three DPs. This highly variable toxicity can very likely be attributed to high concentrations of disinfection products in the analysed dental wastewater, which could not be excluded from the sampling protocol due to health and safety measures in place for sampling. A similar study found the EC_{50} of the effluent to be as low as 2.3 and up to 335 µg/L (Park et al., 2016), which is below the levels found in this study.

Analysis of inorganic particulate size distribution curves confirmed that RCs were associated with higher particulate loadings at small particle sizes (2.5 to 5 µm²) and GICs and RMGICs products with larger particle sizes (12 and 50 µm²). Size distributions overall were found to be similar between the three DPs that were tested and to follow a trend of highest abundance (52 – 65%) of particles in the size between 1.2 and 5 µm², and lower frequencies (17 – 21%) as the particles increased to 5 and 10 µm² in size, and (13 – 22%) as the particles increased to 10 and 50 µm² in size. Angularity (sharp corners and edges) of these relatively larger particles was found to be low, with only few small particles showing high angularity. This is of significance in the dental environment in so far as particles of high angularity are more likely to become

trapped in the dental tubing and filters. In terms of wastewater treatment, angular particles, compared to smooth particles, take longer time to settle during the sedimentation stage of the WWTP. The size between 50 and 500 µm² was rarely detected with the analysis methods utilised in this study (below 6%). This is likely due to the high efficacy of the in-line filters and the amalgam separators to remove large particles (refer to Table 2).There is a challenge in interpreting particle size results that are taken from a three-dimensional object, but only measured in two dimensions: length and width. Table 5 below highlights the size difference as a particle that exists in 3D [µm²] is measured in 2D [µm]; a particle measured in 2D can have significantly smaller and/or larger dimensions in 3D. Particles that were found in the DWW samples that correspond to a size between 1.2 and 100 µm² have a corresponding 3D size as low as 1 µm and up to a maximum of 100 µm. According to the ISO testing of amalgam separators, all of these particles are associated with particle fraction 1, which makes up 30% of the mass fraction testing for ISO certification. The particle size of 500 µm² corresponds to a 3D particle size between 1 and 500 µm and therefore falls into particle fraction 2 of the ISO certification, which makes up 10% of the mass fraction test. Considering that 97 – 100% of particles released from DWW in this study were associated with particle fraction 1 (with a corresponding particle size between 1.2 – 100 µm², or 1 – 100 µm), it can be concluded that the amalgam separator does not sufficiently capture these smaller sized particles.

Table 5: Conversion of particle size from 3D [µm²] to 2D [µm] dimensions, with minimum, maximum and average/angular dimensions, and indicated particle fraction according to ISO 11143:2008.

Particle size [µm²] (2D)	Circular particle dimensions (3D)	Minimum and maximum particle dimensions (3D)	Particle fraction
1.2	1.1 x 1.1	1 x 1.2	1
5	2.24 x 2.24	1 x 5	1
10	3.17 x 3.17	1 x 10	1
50	7.1 x 7.1	1 x 50	1
100	10 x 10	1 x 100	1
500	22.35 x 22.35	1 x 500	2

The tested Type 1 and Type 2 amalgam separators did not show any significant difference in capture efficiency of Hg-free DFMs, but both fell short in catching the high particle load that was released from the DPs, especially smaller particles. Even under ideal testing conditions, the retention level of amalgam separators is a at maximum at 95% and under regular operation it is even lower. However, this study only carried out testing in three dental facilities and more research is needed to show if similar findings are observed in other dental facilities. Current research shows that Bisphenol A, which is a primary component of dental composites, has been observed to leach into wastewater, but may be removed using activated carbon filtration (Polydorou et al., 2020). Our results suggest that, under current requirements, a substantial amount of the inorganic particulate matter below 50 μm^2 in size, that is released from Hg-free DFMs, does not get trapped by the amalgam separator but remains suspended in the WW to be released into the sewerage system and undergo treatment in a municipal WWTP.

Therefore, if future regulations required an increased capture efficiency of Hg-free DFMs, then a need to redesign a capture system to account increasing use of these new materials will arise. Currently, treatment by a WWTP ensures the treatment of the dental wastewaters and removal of 95% of the particulate matter before release into the environment. Research is emerging on municipal WWTPs potentially falling short in capturing the particulate matter, including dissolved and nano-particulates, released from healthcare facilities (Brar et al., 2010; Jírová et al., 2019; Reijnders, 2009; Rosenfeldt et al., 2014). Furthermore, trihalomethanes (THMs), halo acetic acids (HAAs) and aldehydes are of concern as research on disinfection by-products is emerging (Bond et al., 2011; Jírová et al., 2019; Monarca et al., 2000; Park et al., 2016). The incorporation of double treatment was suggested in the literature (Jírová et al., 2019), which would require a change in infrastructure by installing a WWTP at the healthcare facility before WW treatment in the municipal WWTP takes place, with the aim of further reducing the remaining 4.75% fraction of particles currently remaining in the WW after treatment in a

municipal WWTP has been completed. Future research is needed to assess if major changes as such are necessary or if current amalgam separation devices can be adjusted to be made efficient in capturing the Hg-free dental filling particulates that are released.

This study identified that a detailed analysis of nano-particulates arising in dental wastewater is needed. The prospect is that dentistry is moving to increasingly incorporate nanomaterials and technology and under current assessment, WWTPs fall short in removing some of these materials (Emmanuel et al., 2015; Padovani et al., 2015; Schmalz et al., 2018; Suresh et al., 2014). While analysis of SEM micrographs highlighted the presence of smaller particles within samples analysed, detailed results based on further analyses of shape and composition for example are needed to be able to make predictions on the environmental fate of these nanoparticles (Froggett et al., 2014; Reijnders, 2009). More data is needed to determine the composition of the released debris, which may not be discrete particles and behave very differently to other particles due to the increase in surface area that nanoparticles present (Froggett et al., 2014).

The recent developments since the Minamata convention of 2013 have shown that in this short time-frame, the field of dentistry must incorporate sustainability and environmental awareness into decision-making processes (Duane et al., 2019a). This study has highlighted some of the environmental considerations that need to be made as future DFMs further incorporate exotic nanomaterials in increasing amounts yet continue to rely on technology for particulate capture and wastewater treatment facilities designed with other materials in mind.

5. Conclusions

The Minamata convention has brought about the continued phase down of dental amalgam use and an increase in amalgam separator technology. Implementation of this has meant, that there is an increased use of material alternatives to dental amalgam, and these alternatives increasingly contain micro- and nanoparticle-based filler materials. Here, a knowledge gap was examined as to whether pre-existing amalgam separators are likely to trap these new materials, and what the physiochemical characteristics of dental wastewater prior to discharge to a municipal sewer may be. We have found that dental wastewater from three dental facilities in Ireland that utilise alternatives to dental amalgam contain a high proportion of microscale particles, but that larger particulate matter is effectively separated. Results also indicate that the physicochemical characteristics of dental wastewater is highly variable both within and between facilities, and reaches extremes of pH, contains many dissolved substances, and has a relatively potent toxicological profile when tested against *Daphnia magna*. The prevalence of microscale particles and smaller-sized materials in the dental wastewater tested, along with the toxicological and variable physicochemical characteristics indicate a potential and relatively widespread source of engineered nanomaterials into the environment. The findings of this study need to be addressed in a broader context and microparticle testing needs to be carried out at other dental facilities to establish if the results are similar to those of the three dental facilities that were investigated in this study. Furthermore, continued scrutiny of the ongoing suitability of amalgam separation technologies to effectively separate a broader range of nanomaterials, as they are introduced, should be conducted.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

HB: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – Original Draft, Writing – Review & Editing, Visualization

NK: Study design, Writing – Review & Editing

MH: Conceptualization, Funding acquisition, Study design, Resources, Supervision, Writing – Review & Editing

TS: Conceptualization, Methodology, Funding acquisition, Study design, Resources, Supervision, Writing – Original Draft, Writing – Review & Editing, Visualization

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