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Assessment of the potential environmental impacts arising from mercury-free dental restorative materials

Thesis presented by

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for the degree of

Master of Science

University College Cork

School of Biological, Earth and Environmental Science

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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

Signed

Hannah Binner, author.

Abstract

The field of dentistry and with it, the application of dental filling materials, is currently undergoing changes to adopt sustainability and environmental considerations into the clinical environment. This was largely triggered by the reduction of all products in use that contain Hg (mercury), including dental amalgam, through the Minamata Convention of 2013, which has in turn caused a rise in Hg-free dental filling materials that are now becoming increasingly nano-filled.

The focus of this study is on the particles released from Hg-free dental fillings. Knowledge gaps regarding the particle load and potential ecotoxicity of the particulate matter resulting from Hg-free materials exist. Moreover, the widely known environmental and human health impacts of Hg contained in dental amalgam have led to the widespread introduction of an amalgam capture device, the amalgam separator. Amalgam separators capture Hg and dental amalgam particles before wastewater discharge occurs. These amalgam separators are required to be installed in Ireland since the 1st of January 2019 in accordance with EU Directive 2017/852. The overarching objective of this thesis has been to assess whether existing amalgam separators are also effective in capturing particulate matter resulting from the use of Hg-free dental filling materials.

In order to meet this objective, this study has assessed the wastewater and amalgam separator capture efficiency in three dental practices in Cork, Ireland. Three dental practices were selected based on the type of amalgam separator in use and focus of service based on private or public practice and high, medium or low utilisation. Physical and chemical parameters of dental wastewater (DWW), including pH, temperature, conductivity, Total suspended solids (TSS) and Total Dissolved Solids (TDS) have been measured. Detailed analysis of particles found in these wastewater streams has been conducted using optical and scanning electron microscopy. The potential ecotoxicity of these waste streams has also undergone preliminary assessment by conducting standardised *Daphnia magna* immobilisation tests.

The results indicate that variation in the discharged DWWs exists, which is likely linked to the use of disinfection products, and has led to extreme observations of pH, conductivity, Total suspended solids and Total dissolved solids. Ecotoxicity results confirmed this and showed that the raw DWW caused an EC_{50} response at

concentrations between 0.1 to 6.69 % DWW/L medium. The particulate load in the three DPs was substantial. A high abundance of microparticles was identified and trends were consistent across the three DPs. It was therefore concluded that amalgam separators may not be sufficient in capturing the particulate matter released from Hg-free dental filling materials.

Further research is needed to identify the environmental fate of the particles that are released, particularly nanomaterials, as they have the potential to remain in the water after wastewater treatment has occurred.

List of abbreviations

Abbreviation	Explanation
Ama	Amalgam dental filling materials
DFMs	Dental filling materials
DIW	Deionised water
DMs	Dental materials
DP1	Dental practice 1
DP2	Dental practice 2
DP3	Dental practice 3
DPs	Dental practices (meaning DP1, DP2 and DP3)
DTT	Dental treatment type
DW	Distilled water
DWW	Dental wastewater
GIC	Glass ionomer cement dental filling materials
Hg	mercury, elemental
MSDS	Material Safety Data Sheets
RC	Resin composite dental filling materials
RM	Resin-modified dental filling materials
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
WW	Wastewater
WWTP	Wastewater treatment plant

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Chapter 1 Introduction

1.1 Dental filling materials and their regulation – historical context

Dental filling materials (DFMs) have raised environmental concerns for as long as they have been in use, which started with the introduction of dental amalgam in the 1820s and includes recently introduced novel materials such as nanocomposites (Arenholt-Bindslev, 1992; Bonsor *et al.*, 2013; Cataldi *et al.*, 2017; Mulligan *et al.*, 2017). Dental filling material constituents range from polyacids, heavy metals, metal oxides, plastics, glass and ceramic fragments to a range of other small filler materials (Kidd *et al.*, 2011; Bonsor *et al.*, 2013). While infection prevention and control are strictly regulated in dental practices (Dental Council of Ireland, 2015; HIQA, 2018), and guidelines address health hazards associated with DFMs, the potential environmental impacts arising from DFMs are not generally addressed. Potential environmental hazards resulting from the use of DFMs were mentioned for the first time in the 1950s, when the extensive contamination of watercourses by Hg from industry became apparent (UN Environment, 2017). These 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 amounts 20 % of the global consumption of Hg (Tibau and Grube, 2019; UNEP, 2016).

In order to understand the health and environmental risks posed by the waste generated by DFMs, it is important to understand why there is a need for DFMs in general and how they are used. The current study focuses on the waste streams resulting from the use of DFMs by dental practices (DPs). In cases where one or more teeth show signs of decay or damage, DFMs are used as an intervention to repair the damage on the affected tooth and to allow the patient to retain their teeth for longer (Kidd *et al.*, 2011; Yengopal *et al.*, 2016; Lynch *et al.*, 2018). The processes of placing and removing dental fillings produce different waste streams, including wastewater (Adegbembo *et al.*, 2002; Cataldi *et al.*, 2017; Drummond *et al.*, 2003; Cailas *et al.*, 2002; Jírová *et al.*, 2019; Shraim *et al.*, 2011). The wastewater contaminants can be categorised as physical, chemical, biological or radiological contaminants (US EPA, 2014b). Physical and chemical contaminants can be solid or dissolved materials that originate from the DFMs, while biological contaminants, such as saliva, blood and tissue, are a result of the dental treatment type (DTT) with patient contact (Bonsor *et al.*, 2013;

Dental Council of Ireland, 2015). Dental wastewater (DWW) is therefore often a heterogeneous mixture of particles and liquids: the particles resulting from dental amalgam, dentine, enamel, oral tissue, pulp and bacteria and the liquids resulting from water, oral fluids, blood, saliva, plasma, surfactants and mouthwash fluids (Cailas *et al.*, 2002).

It is also important to understand that there are two major groups of DFMs when assessing the potential environmental risks of DFMs: the traditional Hg-based dental fillings that have been in use since the 1820s (Jamil *et al.*, 2016) and the Hg-free dental filling materials (DFMs) that have been in use since the 1960s (Oral Health Foundation, 2003). Hg-based dental fillings consist of an alloy of mercury (Hg), silver (Ag), tin (Sn), copper (Cu) and zinc (Zn) (Bonsor *et al.*, 2013; FDA and Health, 2019; Oral Health Foundation, 2003). The aim of this study is to assess the potential environmental impacts of the Hg-free DFMs, of which there are four main types in use today (described in detail in *Section 1.2*).

Figure 1 shows a timeline of the most important milestones of dental material regulation. The Minamata convention was adopted by 128 signatories on the 10th of October 2013 and became legally binding for all parties on the 16th of August 2017 (Regulation (EU) 2017/852, 2017; UN Environment, 2017). Formal consent to the convention in Ireland occurred on the 18th of March 2019 (UN Environment, 2019). The convention sets out the controls and reductions for all areas in which Hg is used, released or emitted as well as mined, exported or imported, stored and disposed of (UN Environment, 2017). While this treaty applies to 128 signatories (or 110 parties) globally, more stringent regulation applies on a European level.

EU Regulation 2017/852 was passed on the 17th of May 2017 (Fig. 1) and applies to EU member states since the 1st of January 2018. As of the 1st of July 2018, this regulation restricts dental amalgam use on deciduous (milk) teeth, in children under the age of 15 and in pregnant or breastfeeding women, except “where strictly necessary” (Regulation (EU) 2017/852, 2017).

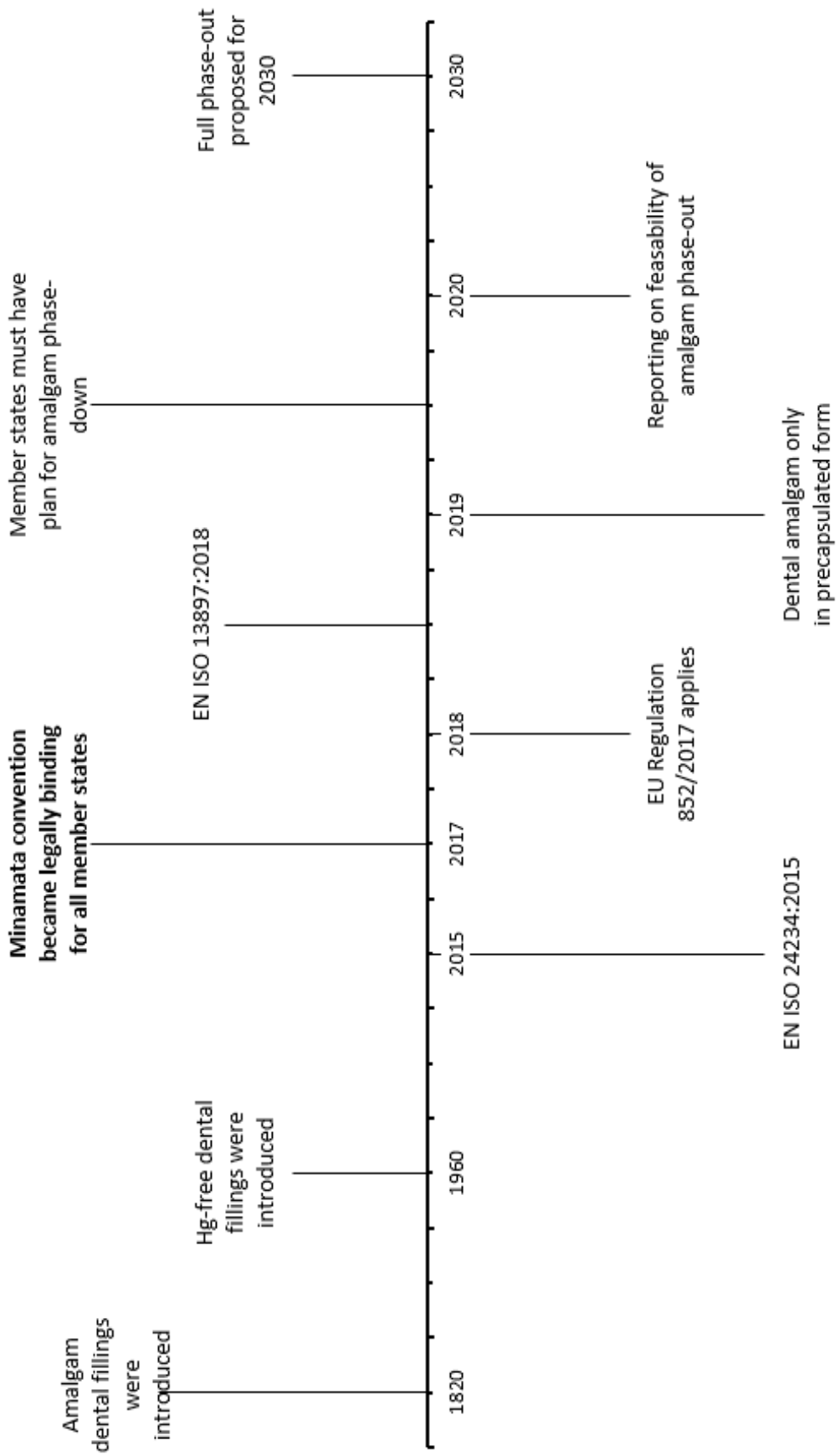


Figure 1: Timeline of introduction and regulation of dental materials, including Minamata convention and EU Regulation 852/2017 (Regulation (EU) 2017/852, 2017; UN Environment, 2017).

Effective from the 1st of January 2019, the restriction entails the prohibition of dental amalgam in bulk form and only permits its use in pre-dosed encapsulated form according to standards EN ISO 13897:2018 and EN ISO 24234:2015 (International Organisation for Standardisation, 2018; International Organisation for Standardisation, 2015). Hg-free dental filling materials are also produced in pre-dosed encapsulated form to promote single use and to avoid cross contamination (Fig. 2).



Figure 2: Current encountered Hg-free dental filling materials in pre-dosed encapsulated form as commonly used in current dental practices.

EU Regulation 2017/852 was enforced in Ireland in December 2018 under S.I. 533 of 2018 (Department of Health, 2019). As of 1st of July 2019, member states must have a plan in place for the phase-down to phase-out of dental amalgam and report on their feasibility (Regulation (EU) 2017/852, 2017). The National Plan for Ireland has indicated that it would be feasible to phase-out amalgam by 2030, except for clinical exemptions where the use is justified (Department of Health, 2019). The planned phase-out of dental amalgam will of course inevitably lead to a rise in use of Hg-free dental filling materials. It is therefore timely to review the potential environmental impacts of dental filling materials that are most likely to increase in use with the phase-down of dental amalgam (UN Environment, 2017).

1.2 Dental material constituents

Certain fundamental or key properties are required from a dental filling, which include that these fillings be non-toxic to the user, of high strength, longevity, easy to use and provide strong adhesion to a tooth surface (Bonsor *et al.*, 2013). In order to achieve this, several different components are mixed to form an amalgam or composite. Dental amalgam was used without competition since the 1820s (Jamil *et al.*, 2016), when it was introduced as a cheaper alternative to gold fillings. Environmental concerns were raised about Hg contained in amalgam fillings soon after their introduction, but only addressed after effects were observed due to Hg bioaccumulating in the environment and causing toxic effects to fish populations and, through the food chain or via direct exposure to Hg vapour, harmful effects to humans (FDA and Health, 2019; Oral Health Foundation, 2003).

Hg-free dental filling materials were introduced in the 1960s and then were largely polycarbonate-based, including BPA (Oral Health Foundation, 2003). These materials, which are often white in appearance, are made up of entirely different components than dental amalgam and were introduced with the aim of substituting Hg-based amalgam fillings where possible (Bonsor *et al.*, 2013; Kidd *et al.*, 2011). However, this process was complicated by their initial relative lack of strength, reduced durability and reduced ease of use when compared to dental amalgam (Bonsor *et al.*, 2013; Jamil *et al.*, 2016). These drawbacks were reduced in the 1990s with the introduction of fillers that were much smaller in size, which led to different compositions of dental fillings. As a result, Hg-free dental fillings were adjustable at design and production level to desired properties, such as high strength, low curing time and high durability to name but a few, which are continuously improved and informed by dental practitioners. Therefore, Hg-free dental fillings are produced for specific applications, such as for example a high filler-bearing material to provide high strength for the application in molars or a flowable material for the use in small cavities (Bonsor *et al.*, 2013). However, throughout the process of introducing different Hg-free dental materials, some dentists still considered Hg-free fillings a poor replacement for dental amalgam for certain applications, such as the restoration of cavities in the back or posterior teeth of the mouth (Roulet, 1997; Forss and Widström, 2001). Nevertheless, some countries, such as Sweden and Denmark, have drastically reduced all dental amalgam in use, while others continue to use amalgam for posterior

restorations (Arenholt-Bindslev, 1992; Cailas *et al.*, 2002; Swedish Chemicals Inspectorate, 2005).

However, in general, the use of Hg-free dental filling materials has greatly increased in the 21st century and this in turn has led to concerns regarding the potential health and environmental impacts of these materials (Mulligan *et al.*, 2017). These concerns are topical when set against current public concerns surrounding the release and detection of emerging contaminants such as microplastics and nanoparticulate wastes in the environment (Reijnders, 2009; Brar *et al.*, 2010; Froggett *et al.*, 2014).

1.3 The chemical and physical properties of Hg-free dental filling materials

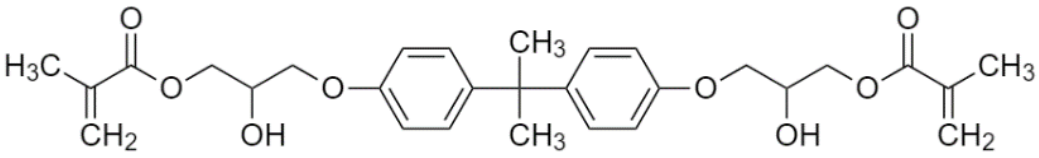
Currently there are four main classes of Hg-free dental filling materials in common use: 1) Resin composites (RC), 2) Polyacid modified resin composites (PMRC), 3) Glass ionomer cements (GIC) and 4) Resin modified glass ionomer cements (RMGIC). In order to understand potential wastes arising from these materials, it is important to understand how these materials are used, their common constituents and potential waste streams arising from their use. Therefore, all 4 classes are discussed in detail below.

1.3.1 Resin composites

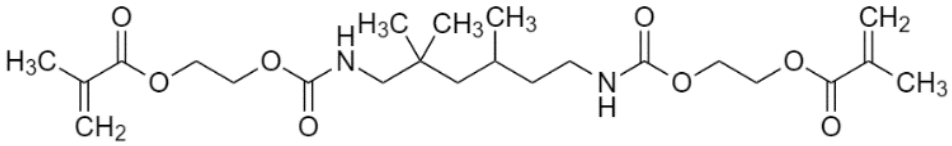
Resin composites are typically made from a chemically active resin, fillers and a silane coupler. The resin is generally a Bis-GMA (Bowen's resin/-dimethacrylate/bisphenol A-glycidyl methacrylate) or UDMA (urethane dimethacrylate oligomers) monomer with addition of other monomers such as mono- or di-methacrylates, MMA (methyl methacrylate), EDMA (ethylene glycol dimethacrylate) or TEGDMA (triethylene glycol dimethacrylate) (Kidd *et al.*, 2011). Their chemical structure can be seen in Figure 3.

This polymerisable resin medium is filled with a mixture of glass and quartz fillers, such as crystalline silica, silica glass (SiO_2), alumina glass (Al_2O_3) or a combination of glass and sodium fluoride (Bonsor *et al.*, 2013). These fillers are also composed of particles of different sizes to provide different properties in the final materials, which include so-called micro-filled composites that contain particles with a mean diameter of 0.04 μm that are used as thickening agents, macroparticles (5-100 μm) that are

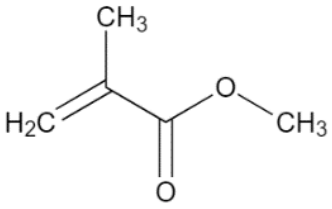
considered easier to polish, nanoparticles (20-70 nm) made up of discrete non-agglomerated and non-aggregated particles that provide high filler loading and high strength and hybrids of these three particle sizes, of which a trimodal distribution was found to be optimal to provide high strength and allow for easier smoothing and polishing (Bonsor *et al.*, 2013; Kidd *et al.*, 2011).



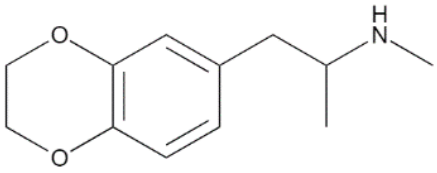
Bis-GMA



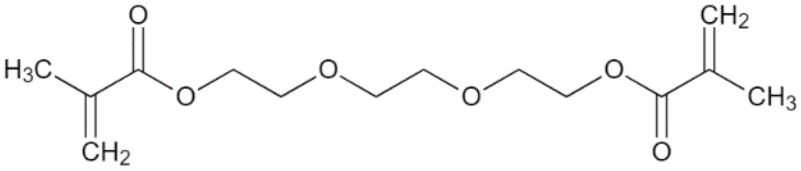
UDMA



MMA



EDMA



TEGDMA

Figure 3: Monomers used for the manufacturing of Resin Composite dental filling materials in dentistry.

More recently, resin composites have become increasingly nano-filled or densified in order to provide a dental filling with high strength (Schmalz *et al.*, 2018; Reijnders, 2009; Xia *et al.*, 2008). Ba (barium) or Sr (strontium) are added to resin composites when restoring posterior teeth to increase radiopacity and to allow for easier to polishing of the filling. Radiopacity serves the purpose of distinguishing the dental

filling material from tooth material after placement (Gul *et al.*, 2017). Synthetic compounds such as Oxybenzone (2-hydroxy-4-methoxybenzophenone) may be added to prevent discolouration and various organic oxides and organic compounds can be added to adjust the composite shade, which is in increasing demand for aesthetic reasons (Bonsor *et al.*, 2013).

1.3.2 Polyacid modified resin composites (compomers)

These materials were first available as commercial dental materials in the early 1990s and contain features of both the resin composites and of glass-ionomer cements (Meyer *et al.*, 1998; Nicholson, 2007; Milward *et al.*, 2011). Polyacid modified resin composites are also resin-based, but contain not only one resin, but two methacrylate resins that are mixed with fillers and a photo-activator to allow for setting via a polymerisation reaction (Bonsor *et al.*, 2013; Meyer *et al.*, 1998). The primary monomer that forms the resin matrix can be BisGMA (bis-glycidyl ether dimethacrylate) or UDMA (urethane dimethacrylate) (Meyer *et al.*, 1998). TCB resin (a reaction product of butane tetracarboxylic acid and hydroxyethyl methacrylate) is added to act as a bifunctional monomer that serves the purposes of cross-linking components during the primary reaction as well as providing a source of carboxyl groups during the secondary reaction with glass (Bonsor *et al.*, 2013; Kidd *et al.*, 2011; Milward *et al.*, 2011). Fluoro-alumino-silicate glasses with the addition of Li (lithium) or Sr (strontium) are added as a filler to impart strength, to release fluoride and to impart radiopacity (Nicholson, 2007; Bonsor *et al.*, 2013). Filler loading is between 42 and 67% and their size generally sub-8 μm. Camphor quinone (2,3-bornanedione) in combination with a tertiary amine serves the purpose of photo initiator in order to cure the filling via light-activated polymerization (Nicholson, 2007; Bonsor *et al.*, 2013). The major disadvantage of compomers is that these fillings do not adhere to the dentin and require bonding agents (Meyer *et al.*, 1998; Milward *et al.*, 2011).

1.3.3 Glass ionomer cements

These cements were invented in 1969 and are formed by mixing a polyacrylic acid with glass and water (Sun *et al.*, 2018; Bonsor *et al.*, 2013; Kiri and Boyd, 2015). The reaction between the polyacrylic acid and the basic glass powder leads to a

formation of a polysalt matrix in which the unreacted glass is encased (Bonsor *et al.*, 2013; Kiri and Boyd, 2015). Different glasses that are used in GICs are: alumina (aluminium oxide), silica (silicon dioxide), calcium fluoride, aluminium fluoride and sodium fluoride (Bonsor *et al.*, 2013). However, a novel aluminium-free GIC is being tested in order to produce a biocompatible material (Kiri and Boyd, 2015). Fluoro-alumino-silicate glass with added strontium or lithium are used to impart radiopacity to the material (Bonsor *et al.*, 2013; Nicholson, 2007). The properties of the final cement can be changed by, for example, increasing the glass proportion to provide a material with higher strength (Kiri and Boyd, 2015). The glass particles are up to 20 μm in size for cements used as restorative, while particles <5 μm are used for luting cements (Bonsor *et al.*, 2013).

1.3.4 Resin modified glass ionomer cements

These dental filling types are derived from glass ionomer cements with the addition of light-curing and resin components (van Dijken *et al.*, 2010; Agha *et al.*, 2017). The resin, usually HEMA (hydroxyethyl methacrylate), the polyacrylic acid and tartaric acid are used to graft together the different functional groups via acid-base and polymerization reactions (Bonsor *et al.*, 2013; Agha *et al.*, 2017). During this process, water prevents a reaction between the polyacid and the glass. Barium, strontium and alumina-silicate glass are added to improve strength and impart radiopacity of the filling. As a last step, polyacrylic acid is added to react with the glass to form a glass polyalkenoate cement and embed the unreacted salt in a polysalt matrix (Alhalawani *et al.*, 2015). Potassium persulphate and ascorbic acid are added to function as a redox catalyst system providing the methacrylate (dark) cure (Bonsor *et al.*, 2013).

To summarise, Figure 4 below shows the range of Hg-free dental fillings materials that are currently commercially available and summarises their chemical makeup.

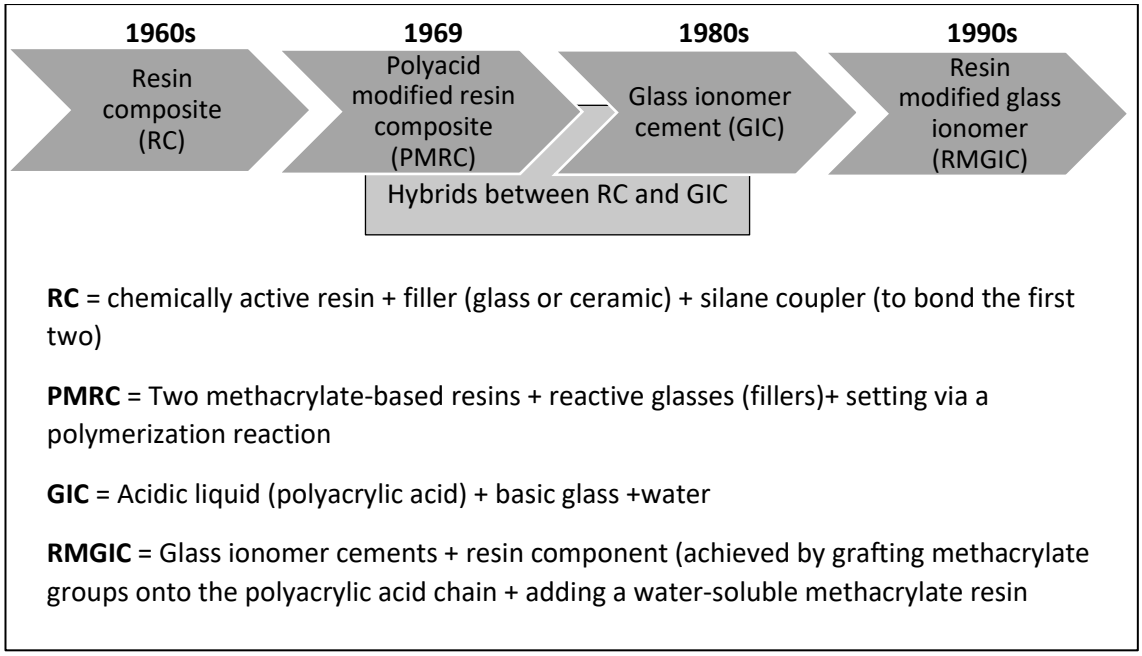


Figure 4: Range of the most commonly used Hg-free dental filling materials in order of year of introduction from the 1960s to the 1990s with information on their chemical composition (Sidhu, 2010; Rohani and Nicholson, 2009; Bonsor *et al.*, 2013).

As can be seen from Figure 4, a broad range of materials is necessary in order to provide for the many desired properties that may be required from a dental filling. For example, from a dentistry practice perspective, low shrinkage during setting and improved hardness of the dental filling are necessary, which is achieved by adding fillers such as glasses, ceramics and silica (Agha *et al.*, 2017; Nicholson, 2007). However, dentists are also required to have a palette of white-coloured filling material available to them for different use cases (Kidd *et al.*, 2011; Bonsor *et al.*, 2013), which requires the addition of additional fillers and oxides. Additionally, while a filling *in situ* may be considered chemically inert, any particles resulting from the grinding and polishing of a dental filling may not be (Warwick *et al.*, 2019).

1.4 Amalgam separators and Hg-free based dental wastes

Amalgam separators are required to trap at least 95 % of dental amalgam particles in the DWW before the WW is released into the sewage system (Regulation (EU) 2017/852, 2017). The original purpose of an amalgam separator has been to retain amalgam particles from DWW by separating secretions, air and amalgam particles via sedimentation, filtration, centrifugation, ion exchange or a combination of these (Vandeven and Mcginnis, 2005) and to retain the amalgam particles in a collector vessel that allows proper disposal of these. An objective of the current study will be to determine if amalgam separators are also effective in removing particulate matter resulting from the use of Hg-free dental filling materials. Therefore, the most commonly used amalgam separation devices in Ireland were assessed for their efficacy to retain particles arising from the use of Hg-free materials. This section introduces amalgam separators that are currently in use in Ireland, and their main operational parameters, including their potential to trap particles resulting from the use of Hg-free dental filling materials.

1.4.1 Amalgam separators: Historical context

There was a long process of regulations leading up to the implementation of the amalgam separators that are in use today. Starting in 1984, EC-Council Directive of March 8, 1984 was introduced in order to introduce amalgam removal devices that were at least 95 % efficient in removing dental amalgam in order to tackle Hg pollution of waterways in Europe (Cailas *et al.*, 2002). This was followed by ISO standard 11143 in 1998, which was introduced to regulate and standardise these amalgam separators. Up until this point, the installation of amalgam separators had not been internationally regulated. Following this, at the Minamata convention of 2013, 128 signatories committed to the reduction of Hg in use. In the EU, this led to the passing of EU Regulation 2017/852 (Regulation (EU) 2017/852, 2017), which made it a requirement that as of 1st of January 2019, dental practices must be fitted with an amalgam separator that is at least 95 % effective in removing amalgam particles from the wastewater. In order to meet these legal requirements, dental practices are expected to newly fit or retrofit, as applicable, with amalgam separators. This was enforced in Ireland under S.I. 533 of 2018 and specified that as of the 1st of January 2021, all

amalgam separators in Ireland, whether retro-fitted or newly-fitted, must provide a 95 % retention level (Department of Health, 2019).

As part of the assessment of amalgam separation devices it is important to look at their ISO certification process and the particle sizes that are used during testing.

ISO testing for amalgam separators currently tests the removal efficiency with a test slurry of 1 L. This test slurry is prepared with a dispersing agent, such as sodium pyrophosphate ($Na_4P_2O_7$) and 100 g of dental amalgam divided into three mass fractions. The amalgam is prepared by amalgamating capsules of dental amalgam until a weight of 100 g is reached and allowing hardening of this for 30 days at 23 ±2 °C. The amalgam then undergoes grinding and is sieved to obtain the three mass fractions: a 30 % mass fraction (3 g of the test slurry) with particles equal to or less than 100 µm in size, a 10 % mass fraction (1 g of the test slurry) of particles between 100 and 500 µm in size and a 60 % mass fraction (6 g of the test slurry) with particles between 500 and 3,150 µm in size (International Organisation for Standardisation, 2008). This mass fraction was established from investigations in the United States, the Netherlands and Germany and is included in Annex D of ISO 11143:2008.

However, Cailis *et al.* (2002) found that 90% of the amalgam particle fraction in DWW is between 10 and 700 µm in size, while the remaining 10 % contain particles in suspension of size below 10 µm. These two findings explore two very different size distributions. It is widely recorded in the literature that amalgam separators are not completely effective at capturing particles of the lower mass fraction, which also includes dissolved Hg, and hence amalgam separators are generally between 95 and 98 % effective in removing amalgam particles (Batchu *et al.*, 2006; Vandeven and Mcginnis, 2005). This is an important consideration when testing these amalgam separators for their removal efficiency of Hg-free dental materials, as these materials may be much lighter in weight and, given by their design (explored in *Section 1.2*), are expected to produce more particles in the size fraction below 100 µm and potentially include nanoparticles.

Efficiency of the separators to retain amalgam particles is tested under full and empty container conditions (International Organisation for Standardisation, 2008). To do so, the amalgam separation unit is flushed with 1 µm filtered water that is three times (3x) the volume that the separation unit can hold before the certification test starts. If

particles are found to be present in the effluent, the system is flushed again. The aim is to fill the amalgam separator with water before the test is carried out. During testing, the 1 L of test slurry is added to the water flow rate of 0.5 L/min at a constant rate over 120 (± 10) s. The wastewater is collected after it has passed through the amalgam separation unit and analysed after it has been filtered through a series of pre-weighed filters in order to determine the collection efficiency of the tested unit (International Organisation for Standardisation, 2008). A warning system that indicates when a container should be replaced or malfunctions, is visually inspected during testing.

A second important consideration here is that although amalgam separators are in theory 95 % effective, they never receive all of the particle load that enters a dental chair (Batchu *et al.*, 2006; Cailas *et al.*, 2002; Vandeven and Mcginnis, 2005). This is due to chairside traps and vacuum pump filters. As the wastewater is suctioned through the dental vacuum lines, between 40 and 80 % of amalgam particles by weight can be trapped by chairside traps and vacuum pump filters (Lutchko and Gulka, 2004). For example, Vandeven and McGinnis (2005) recorded that 78 % of amalgam particles were trapped. Chairside traps remove particles down to 700 μm, while vacuum pump filters remove particles down to 840 μm (20 mesh screen, industry standard) or 420 μm (40 mesh screen, industry standard) (Lutchko and Gulka, 2004). Hence, between 20 to 60 % of amalgam particles remain to be caught by the amalgam separator with 95 % removal efficiency according to ISO 11143:2008. The remaining 5 % of amalgam particles are considered to be suspended in the liquid phase of the DWW and include dissolved Hg (Cailas *et al.*, 2002). Figure 5 below illustrates the particle trapping efficiencies of each step during the wastewater pathway as described.

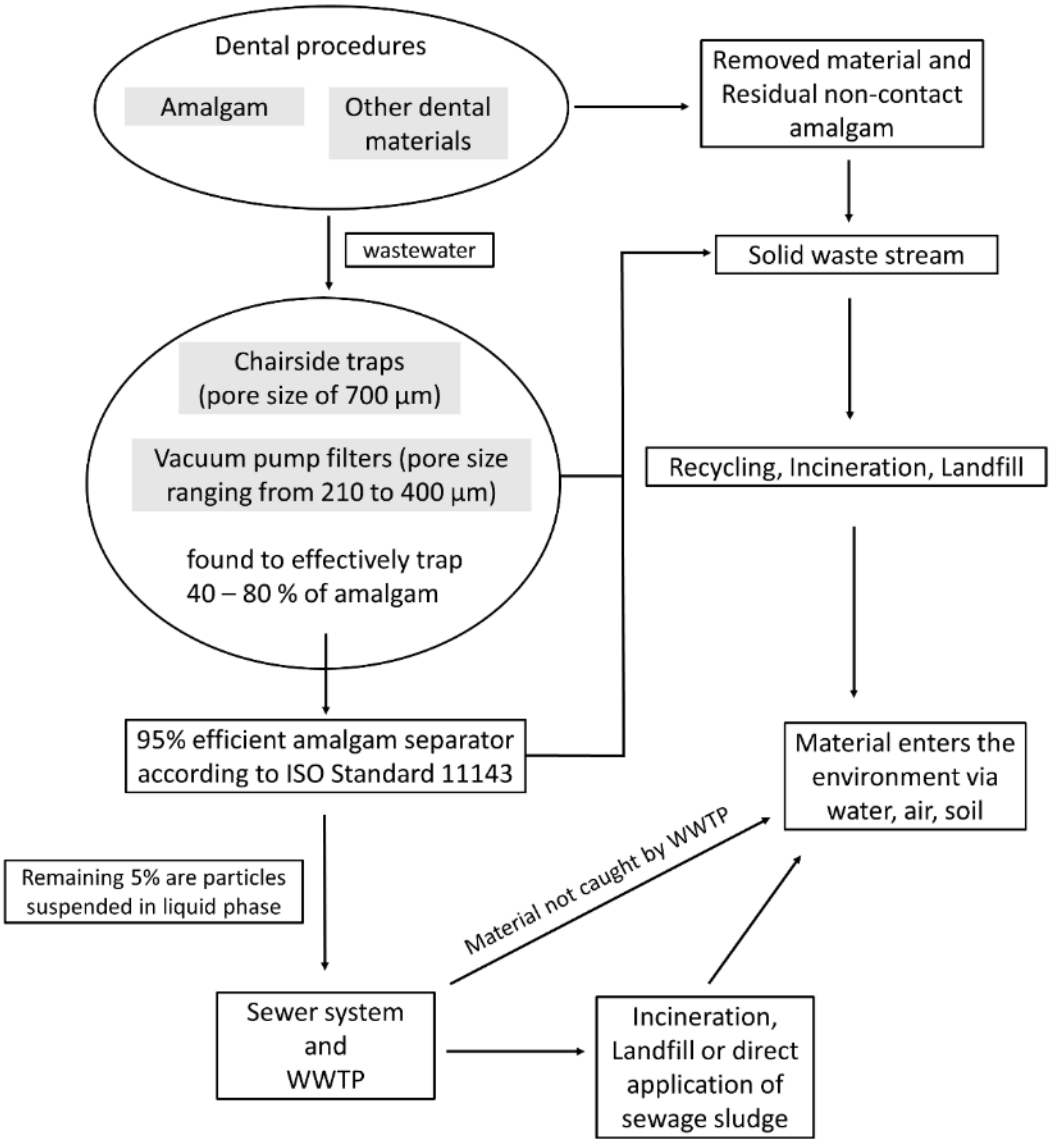


Figure 5: Schematic overview of the dental waste streams and the pathways by which dental waste materials may ultimately enter the environment (Batchu *et al.*, 2006; Cailas *et al.*, 2002; Vandeven and Mcginnis, 2005).

1.4.2 Amalgam separators in use in Ireland

Currently, there are five different commercially available amalgam separators in use in Ireland. These are the Dürr Dental CAS1 centrifugation amalgam separation unit, the Cattani Microsmart and the Turbo Smart sedimentation separators, the Metasys Type 2 ECO II sedimentation amalgam separator and the Amalsed sedimentation separator from Initial Medical (Kenny, R., Personal Correspondence, 2019). According to the ISO standards, amalgam separators are generally either classed as Type 1: centrifugation amalgam separation systems, Type 2: sedimentation

systems, as Type 3: filtration systems, while Type 1, 2 or 3 *in any combination* are classed as Type 4 amalgam separator systems (International Organisation for Standardisation, 2008).

The Dürr Dental CAS1 Combi Separator centrifugation amalgam separation system (Type 1 system) operates as follows. Wastewater flows from the spittoon to the centrifuge. During this flow, the separation unit is passed in which the aspirated secretions are separated from the aspirated air (Dürr Dental SE, 2019). All secretions that accumulate within the separation unit are continuously transported to the centrifuge, where amalgam (or other particulate matter) separation takes place. Once the centrifuge is switched off, the separated amalgam particles are rinsed into the amalgam collector vessel, which is located underneath the centrifuge and replaceable. A float sensor within the collector vessel checks the fill level and sends a signal to the display panel when it needs replacing.

Sedimentation amalgam separation systems are generally designed to operate as a system in which the flow is regulated and slowed down via obstruction or chambers and baffles to allow the sediment to settle (Lutchko and Gulka, 2004). Some units include mechanical filtration, which is usually a fine screen or porous material capable of trapping small particles. Some separators even include a third stage and utilise ion exchange to capture the dissolved Hg fraction in the wastewater by adsorbing the dissolved Hg onto the surface of a resin, which is currently not included in the ISO certification process but may offer future solutions for a higher efficiency rating than the current 95 % (Lutchko and Gulka, 2004). However, the study did not specify the amount of dissolved Hg that is expected to be released from dental amalgam. Moreover, the tested devices that utilised ion exchange to remove dissolved Hg have shown a variation in the overall removal efficiency between 95.2 % and 99.8 % (Lutchko and Gulka, 2004). The Cattani Micro Smart and the Turbo Smart sedimentation systems allow the fitting of a hydrocyclone amalgam retention system, which is advertised as being 98 % effective in removing dental amalgam (Cattanni, 2019). The Hydrocyclone operates as follows. Fluid from the suction passes through diffusion holes and is spiralled downwards through a cone. At the bottom of the cone, rotation is reversed and the fluids are required to backflow, at which point the particles fall into the collection container below and the remaining wastewater drains out via the outlet (Cattanni, 2016). The Metasys Type 2 ECO II sedimentation amalgam

separator also utilises sedimentation with the additional advantage of requiring no electricity during operation (Metasys, 2019). Because of this it does require, however, visual inspections every 4 weeks. The Amalsed sedimentation separator from Initial Medical is advertised to be 98% effective in removing dental amalgam (Initial, 2019).

One last consideration to be accounted for is the fact that amalgam separation systems are chosen based on their cost and their technical compatibility to the existing equipment in the dental practice, and not based on an environmental consideration which would take into account the loading and size distribution of the discharged materials and how that corresponds to the removal efficiency of a separator. This is due to the fact that installation of separators is limited, especially if retrofitted, to the existing system: if the installed suction unit is a wet vacuum system then the amalgam separation unit can only be installed at the vacuum pump inlet or at the vacuum pump discharge (Lutchko and Gulka, 2004). If a dry vacuum system is installed, then the amalgam separator can only be installed at the suction line before or in the air/water separator. This may change as new amalgam separation systems are released onto the market. Other considerations that are important when choosing the type and brand of an amalgam separator to be installed are where the layout of a clinic determines if amalgam separators can be installed at each dental chair unit or if a combi separator for up to 8 dental chairs is more suitable (Lutchko and Gulka, 2004).

1.5 Potential environmental impacts

Having highlighted the importance of preliminary wastewater treatment of DWW through amalgam separators, this section explores the known and potential environmental impacts of release of Hg-free dental filling materials into the environment via wastewater. It should be noted that the solid waste stream within dentistry is regulated and recycling implemented where possible (Arenholt-Bindslev, 1992; Drummond *et al.*, 2003; Mulligan *et al.*, 2017). Once the dental wastes have passed through any filtration, sedimentation or amalgam separation unit traps, they are suspended in copious amounts of water and discharged directly into the sewer. From the main sewerage system, the wastewater is generally passed on to a wastewater treatment plant (WWTP), which discharges the water into surface waters after wastewater treatment of various types has generally taken place. The WW treatment generally includes secondary or tertiary treatment, however, some particles can remain after these processes, which leads to the formation of disinfection by-products, such as trihalomethanes (THMs), haloacetic acids (HAAs) and aldehydes (Monarca *et al.*, 2000; Bond *et al.*, 2011; Park *et al.*, 2016; Jírová *et al.*, 2019).

It has further been established that WWTPs are not able to fully remove all nanoparticulates from healthcare wastewater, which includes TiO_2 and SiO_2 nanoparticles (Monarca *et al.*, 2000; Reijnders, 2009). This is of concern as both of these substances may be ecotoxic and in the case of TiO_2 nanoparticles have been found to interact with heavy metals to from ecotoxic substances (Reijnders, 2009; Rosenfeldt *et al.*, 2014). As a result of this, TiO_2 and SiO_2 nanoparticles have been found in the effluent and in the sewage sludge of WWTPs (Reijnders, 2009; Brar *et al.*, 2010; Rosenfeldt *et al.*, 2014). This sludge is applied directly to land or disposed of via landfill or incineration and hence enters the air, water and soil either directly or by depositing/settling back down onto land and water over time (Lutchko and Gulka, 2004; Ministry for the Environment New Zealand, 2008). Studies such as Mulligan (*et al*., 2017) have stated the need to fill knowledge gaps on the "nature, magnitude and effect" of potential environmental impacts from Hg-free dental filling materials.

Close examination of the material safety data sheets (MSDS) of some of the most commonly used Hg-free dental materials highlights why there may be concerns of these materials potentially bioaccumulating or persisting in the environment (Table 1).

However, manufacturers are currently not required to list all ingredients, especially those below 1% of the total product (Van Landuyt *et al.*, 2011).

Table 1: Summary of the Material safety data sheets (MSDSs) information on stated environmental hazards, risks and impacts of commonly used constituents of Hg-free dental filling materials.

Potential environmental hazard/risk/impact	**Commercial Product**	**CAS number**
Water hazard class 1: slightly hazardous for water. Do not allow product reach ground water, water course or sewage system.	Structur 2 SC Catalyst	
	Structur 2 SC Base	
	Fissurit F	7681-49-4
	ChemFil Rock Capsule	87-69-4
	Sedanol Liquid	97-53-0
	ViscoGel Liquid	64-17-5
	ViscoGel Lubricant	8012-95-1
	SDR	
	Ceram X	
	Phosphate cement liquid	
	Phosphate cement powder	
Water hazard class 2: hazardous to water, very toxic for fish and plankton in water bodies	Sedanol Powder	
Avoid release of product into the environment	3M Unitek Transbond XT Light Cure Adhesive	
	3M Unitek Transbond XT Primer	
	3M ESPE Filtek Supreme XTE Flowable Restorative	
Marine pollutant/ toxic to marine life	DENTSPLY calibra esthetic resin cement- base paste	65997-18-4
	Fleck's cement powder	1309-48-4
Persistence(water/soil): low, mobility: high, bioaccumulation potential: low	DENTSPLY calibra esthetic resin cement- base paste-4993-54	109-16-0
	DENTSPLY calibra esthetic resin cement- base paste	13463-67-7
Mobility: moderate, bioaccumulation potential: moderate	SNAP Provisional Crown and Bridge Resin - SNAP Monomer	97-86-9
Dangerous for environment according to directives 67/548/EEC & 99/45/EC, may cause long term adverse effects in the aquatic environment	Temp Bond Accelerator (tubes& dual syringes)	
	Temp Bond Base N.E.	1314-13-2
Avoid dispersal of spills (soil, waterways, drains, sewers) and contact relevant authorities if product has caused environmental pollution	SonicFill	
	XRV Herculite	

After cross-referencing the dental material constituents with the EPA Priority Pollutant List and the Water Framework Directive Monitoring Programme it was

identified that Ba (barium) and Zn (zinc) were listed as priority substances (US EPA, 2014a; Regan *et al.*, 2013; Environmental Protection Agency, 2006; European Parliament, 2000).

Due to the chemically complex nature of the materials in question, and due to there currently being no requirement to reveal the exact composition of materials in use, potential environmental impacts need to be assessed through careful study (Rodríguez-Farre *et al.*, 2016). While keeping in mind that each of the filling types has been developed for its unique set of applications and that these applications often require particles of a range of sizes, this study aims to give more information on any potential environmental impacts.

1.6 Aims and objectives of the thesis

As stated previously, the phase-out of dental amalgam is planned by 2030 and therefore it can be expected that Hg-free dental filling materials will dominate dental material usage in the future. However, while information on Hg exposure and release within the DWW stream is of course extensive (Fan *et al.*, 1997; Adegbembo *et al.*, 2002; Cailas *et al.*, 2002; Bose-O'Reilly *et al.*, 2010; Shraim *et al.*, 2011; Jamil *et al.*, 2016; Bjorklund *et al.*, 2017), the impact of constituent materials and wastewater arising from Hg-free dental materials are less studied and their environmental impacts resulting from their pollution load, particle size, shape and distribution and their ecotoxicity are unknown (Mulligan *et al.*, 2017). The aims of this thesis are to:

- Determine the efficacy of amalgam separators to trap materials resulting from the use of Hg-free dental filling materials
- Determine the particle size, shape and distribution of these particles that do not get caught by the amalgam separator
- Determine the pollution load within the DWW and carry out ecotoxicity testing to gain an understanding of the potential environmental impacts arising from the DWW stream.

The overall aim of this thesis has been to explore and partially address the existing knowledge gaps, where possible.

Chapter 2 General DWW sampling protocol

2.1 Introduction

The sampling and handling of the DWW presented a set of challenges due to the presence of both organic and inorganic contaminants, and possible risk posed by health and safety aspects of handling raw wastewater contaminated by human tissue (albeit copious disinfectant was added). Therefore, a general project sampling protocol for DWW was developed to allow sampling across a range of different clinics in a safe and reproducible manner. The details of this protocol are outlined below so that future studies may replicate the approach where necessary.

2.2 General project Health and Safety protocol

Given the nature of sampling of the wastewater outlet in dental practices, there were several hazards that needed to be addressed before any work could be carried out. When extracting samples from the point at which the wastewater pipes are exiting the dental chair and entering the sewer system, there is a risk of contamination from human saliva, tissue and blood resulting from different types of dental treatment that the patients receive, which is why samples containing saliva are classified as Hazard Group 2 /Containment Level 2 (Health and Safety Authority, 2013; University of Birmingham, 2014; HIQA, 2018). Particles and substances are released in dental practices whenever a dental filling is placed or removed. It is known that a range of polymers, ceramics, composites and glass are used in dental fillings (Kidd *et al.*, 2011; Bonsor *et al.*, 2013). For these reasons it was decided that samples would be disinfected, and special attention given to wear adequate Personal Protective Equipment (PPE).

As for the risk of contamination from human saliva, tissue or blood, there is a risk of contamination with blood borne viruses (BBVs) such as Hepatitis B, Hepatitis C or HIV (Human Immunodeficiency Virus), airborne viruses such as *Mycobacterium tuberculosis*, DNA viruses such as the Human Papillomavirus and viruses of the herpes group such as Cytomegalovirus and the Epstein Barr virus (University of Birmingham, 2014; HIQA, 2018). Considering the high risk of viral infection, it was required to wear PPE when collecting samples and when analysing samples in the laboratory at all times, which included a laboratory safety coat, safety glasses, gloves

and mouth protection (such as a surgical mask or face shield). As a precautionary method, all staff handling samples had the HBV vaccinations. Food and drink in rooms in which handling of samples took place was not permitted.

Sample collection was carried out using a closed and sealable container, made of inert HDPE (high-density polyethylene) material. The tubes, fittings and seals, for the wastewater inlet pipe coming from the dental chair unit, as well as for the overflow pipe going into the sewer, were installed by a qualified dental engineer. These tubes, fittings and seals were the same that are used in the dental chair unit and provided by Dürr Dental SE©, hence ensured a high-quality standard fitting. Once the samples were collected, the container and all its fittings were sealed in order to minimise release (Health and Safety Authority, 2013). Samples were stored in the refrigerator and in the dark/ under minimal light conditions.

As per Health and Safety Authority guidelines (2013), biohazard signs were posted on all samples and lab access restricted to pre-approved users. Furthermore, work benches were impervious to water and resistant to acids, alkalis, solvents and disinfectants (Health and Safety Authority, 2013). In case of a spill, the protocol stated that contaminated areas underwent disinfection and all spillages were removed using paper towels that were then placed into a healthcare risk waste bag. Once the area had been cleaned, it should be wiped with disinfectant once more and then washed with detergent and water to remove traces of the disinfectant. Hand hygiene should be performed afterwards. Protocols (included in the Appendix) were set to ensure the recording of sample collection and storage and the use of disinfectant were observed.

2.3 Sampling methodology

2.3.1 Selection of Dental Practices for sampling

DWW was collected from three dental practices that had different amalgam separation units of Type 1 and 2 installed. Sampling of the wastewater was achieved by installing a diversion into the waterline after amalgam separation had occurred, and before the wastewater was discharged into the sewer system.

The identities of dental practices have been anonymised as DP1, DP2 and DP3. The abbreviations for each practice and the abbreviations that were used for the most commonly used Hg-free dental filling materials during testing are shown in Table 2 below.

Table 2: Abbreviations used for the study on the potential environmental impacts of Hg-free dental filling materials in three dental practices.

Abbreviation	Abbreviation explained	Amalgam Separator Type	Details
DP1	Dental practice 1	Type 1	Teaching unit with amalgam separator attached to each dental chair
DP2	Dental practice 2	Type 2	Amalgam separator for collective WW of multiple dental chairs
DP3	Dental practice 3	Type 1	Amalgam separator for collective WW of three dental chairs
RC	Resin composite dental filling materials		*See Section 1.3.1 for details*
GIC	Glass ionomer cement dental filling materials		*See Section 1.3.3 for details*
RM	Resin-modified composite dental filling materials		*See Section 1.3.4 for details*
Ama	Amalgam dental filling materials		*See Section 1.3 for details*

DP1 was used as a base for piloting the sampling methodology and set-up and hence the highest number of samples were collected from this location. This dental practice used a Dürr Dental CA1 centrifugation amalgam separation unit (Type 1), fitted to a single dental chair. DP2 used a Metasys Type 2 ECO II amalgam separator (Type 2), fitted to collect the combined wastewater of four dental chairs, and DP3 used a Dürr

Dental CAS1 combi separator (Type 1), fitted to collect the combined wastewater of three dental chairs. Each of these separation units will be described in greater detail in section 2.3.3.

2.3.2 Accessing the DWW line

In order to collect a representative wastewater sample, the DWW samples were collected closest to the point of origin, just after filtration and amalgam separation had occurred. Sampling containers, 12.5 L and 1 to 2 L storage containers, were made of UN approved HDPE material and sourced from ibottles.co.uk. The tube and seal fittings that were attached to the sampling containers were the same that are used on the dental chair (dental tubes and amalgam separator seals) and sourced from Dürr Dental (Kettering, UK).

One key challenge was presented when installing the first sampling container at DP1. The container height needed to be below the level at which the amalgam separator was installed (ca. 15-18 cm above the ground) in order to continue the gravitational flow of the system. If that were not the case, a pump would need to be installed to establish a flow within the system. Secondly, the normal operation of the dental chair could not be interrupted, which is why all work on and around the sampling container (such as installing and collecting the container) was carried out outside of business hours and when no patients were treated in the clinic before 9 am and after 5pm. Moreover, when no samples were collected, the wastewater outlet pipe needed to be easily re-connected to discharge into the sewer system again, which was established by removing the extra piece of pipe that was connected to the wastewater outlet and sampling container (Fig. 6). Another important consideration during sample installation in DP1 was the aesthetics of the sampling container. Due to the container being located directly next to the dental chair it was inevitable that it may be noticed by patients, hence a semi-transparent container was chosen, which was expected to blend in with the dental surroundings, but still allow visual checks throughout the day to ensure that no wastewater was overflowing.

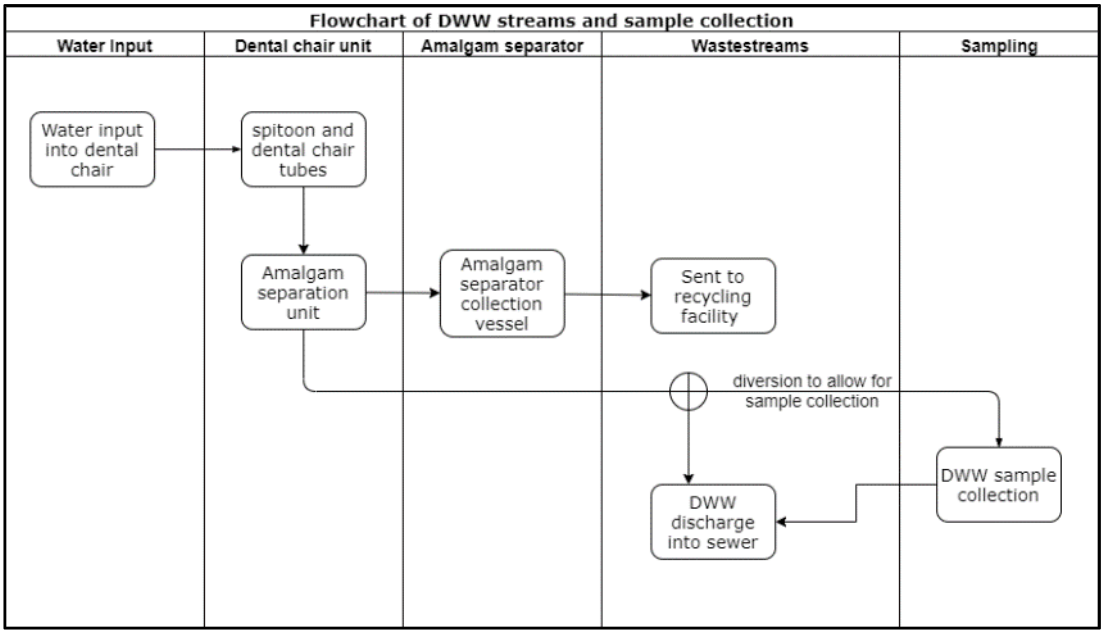


Figure 6: Flowchart of the DWW streams arising in dental practices and intersection of these to allow for sample collection of DWW in this study.

The sample installation in DP2 and DP3 required the same consideration as DP1 in terms of gravitational flow, but due to the amalgam separation units being located in an adjacent outbuilding, their aesthetics were only secondary.

The collected samples were a composite of all dental procedures that were carried out at the respective dental chairs on each sampling day.

2.3.3 Sampling approach

In the following, the sample set-up in each of the three dental practices is shown in order to highlight the different factors that may influence results. Samples in DP1 were collected over a period of 7 months between the months of December 2018 to June 2019. A total number of 21 samples with a total volume of 144 L were collected and analysed. Sample collection and installation took place at the start and end of each working day. Figure 7 below illustrates the set-up of the sampling containers at the dental chair unit wastewater outlet in DP1.

In detail, the water mains and materials used during the dental procedure enter the dental chair unit and are identified as components of the wastewater once they enter the system via the sink or spittoon. The wastewater then passes a set of filters

(labelled ① to ④) that trap particles that are 2mm or bigger in diameter and aim to protect the tubing system. There was a total of five filters within the dental chair unit (Fig. 7). Three of these were located in the waste water line going from the chair sink to the amalgam separator unit; the first (①) was located in the sink and designed to catch any larger particles (> 2 mm), the second filter(③) was located 15 cm away from the first filter and an in-line filter designed to prevent the clogging of the pipes by particles (> 4 mm). A third filter (2 mm mesh) was located further down the pipe (④), just before the wastewater line entered the amalgam separator (⑤) and its aim was also to prevent the clogging of pipes. The other 2 filters were located in the waste water line going from the dental suction unit to the amalgam separator unit; the first of these aimed to catch larger material (1x1 mm) and was located at the spittoon outlet (②), while the second filter (③) further down the waste water line aimed to prevent the clogging of pipes (4 mm max.) before the wastewater entered the amalgam separation unit (⑤), which in the case of DP1 was a CA1 centrifugation amalgam separation (Type 1) system from Dürr Dental SE©.

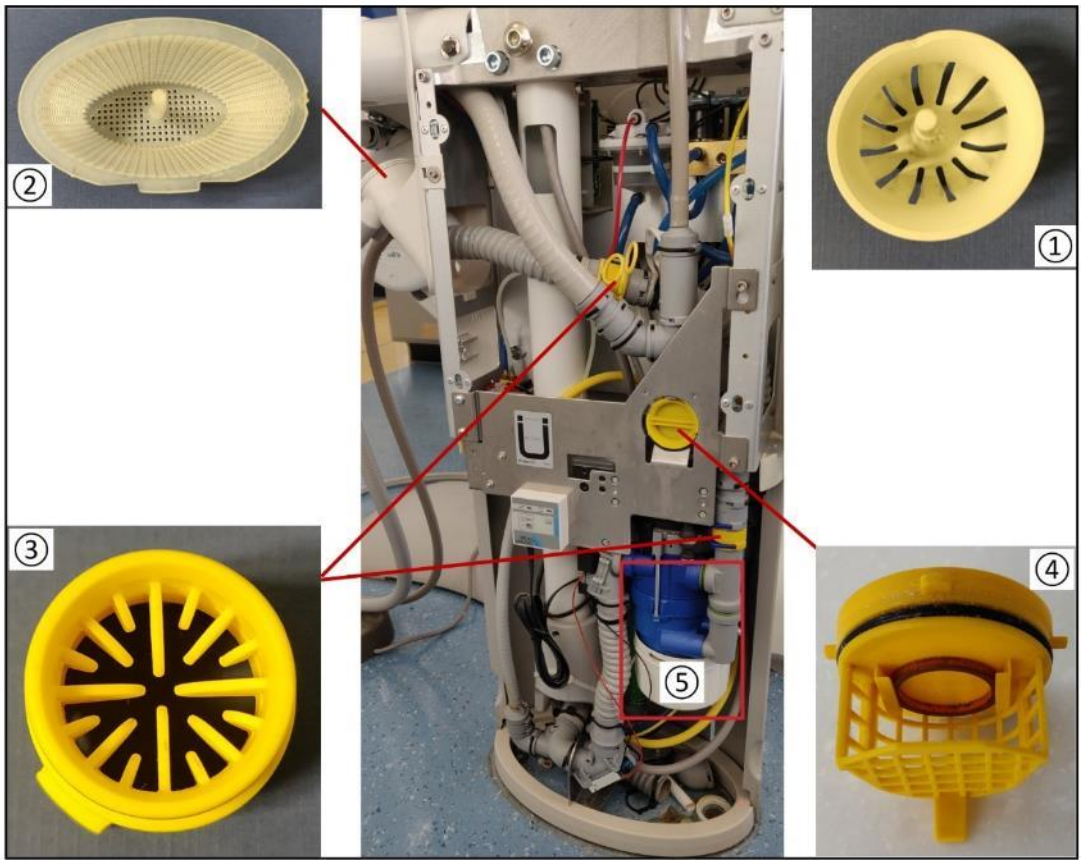


Figure 7: Schematic of dental chair unit, internal filters and amalgam separation unit in DP1 (1 = filter catching particles > 2 mm in size, 2 = 1x1 mm filter, 3 = filter catching particles > 4 mm in size, 4 = 2 mm mesh filter, 5= Dürr Dental CA1 amalgam separator).

Samples in DP2 were collected over a period of 2 months in June and July 2019. A total number of 6 individual samples with a total volume of 18 L were collected and analysed. DP2 had a combi sedimentation amalgam separation unit (Type 2) for the collected wastewater from several dental chairs, which is why in this clinic the amalgam separation unit was located outside of the main building in an adjacent outbuilding in which the wastewater line was intersected with the amalgam separation unit before the wastewater was discharged into the main sewerage system. Another deviation from DP1 was the sampling interval; in DP2 the samples were installed and collected at lunchtime on each sampling day and left between 1 to 3 days in order to collect a significant sampling volume of at least 2 L for analysis. The amalgam separation unit that was used here was the Metasys Type 2 ECO II sedimentation amalgam separator (Type 2).

DP3 was sampled in the month of July 2019. A total number of 2 samples with a total volume of 8 L were collected and analysed. Similar to DP2, the amalgam separation unit operated on the combined wastewater from three dental chairs within the dental practice. The amalgam separation unit was manufactured by Dürr Dental and similar to the unit installed in DP1, a centrifugation amalgam separator (Type 1), with the difference being that this model was a combination separator that collected the wastewater from several dental chairs rather than being installed directly at the dental chair.

These separators were ISO standardised and operated on the basic principles of "centrifugation, sedimentation, filtration or *any combination* of those" (International Organisation for Standardisation, 2008; Regulation (EU) 2017/852, 2017).

Data recording sheets were given to each dental practice which instructed the dental practitioners to record the type of dental treatment that was carried out, the dental materials that were used and the application of any line cleaner or disinfectant (data recording sheet are included in the Appendix).

Following collection, samples were transported to the laboratory and stored in the fridge at 4°C, if required, or processed immediately. In the final design of the study it was aimed to store the samples no more than 24 h (or at most 72 h on weekends but avoided where feasible) before processing in order to minimise bacterial growth and secondary reactions of the samples. Each sample had a unique sample identifier and

efforts were made to record the detailed dental procedures, that were carried out on each dental chair, on each sampling day. An aliquot of each sample was frozen once it reached the laboratory.

2.4 Summary

Three dental practices were selected for the collection of DWW for the analysis of (a) physical and chemical WW parameters (b) particle size and characterisation and (c) the assessment of the ecotoxic potential of the DWW streams. All samples were collected after they had passed through the installed amalgam separation unit. Wastewater was collected closest to the source of origin using inert HDPE containers. Protocols for sample collection, recording and to minimise hazards caused by the handling of these samples were developed.

Chapter 3: Physical and chemical parameters of DWW

3.1 Introduction

The discharge of wastewater in Ireland is regulated by the Wastewater Discharge (Authorisation) Legislations S.I. No. 684 (2007), the Protection of the Environment Act (2003), European Directive 2004/35/CE on environmental liability with regard to the prevention and remedying of environmental damage and Council Directive 91/271/EEC concerning urban wastewater treatment. The Environmental Protection Agency (EPA) Ireland issues wastewater discharge licences and certificates of authorisation based on set parameters such as pH, temperature, Dissolved oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS) and conductivity, all of which have permissible limits that the discharged wastewater has to fall under (Environmental Protection Agency, 2001; Council Directive 91/271/EEC, 1991; Protection of the Environment Act, 2003; Waste Water Discharge (Authorisation) Regulations, 2007; Directive 2004/35/CE, 2004). The EPA has issued a Wastewater discharge licence to Cork City under Licence register number D0033-01 (Environmental Protection Agency, 2009). There is currently no licence required for wastewater discharge from hospitals or dental practices, as these are not considered to form a trade effluent, but are treated as a domestic wastewater that discharges into the public sewers, which are then treated in the public wastewater treatment plant (WWTP) (Environmental Protection Agency, 2007; Irish Water, 2019). By assessing the physical and chemical parameters of the wastewater that was discharged by DP1, DP2 and DP3 (Dental Practice 1, 2 and 3), this chapter sets out to evaluate the wastewater load going into the public WWTP, while keeping the focus on the parameters that are relevant for the particle analysis that is conducted in this study.

3.2 Rationale

Water quality and pollution load with respect to particle load (suspended and dissolved) of the raw DWW that was discharged from three dental practices was identified through pH, conductivity, total suspended solids (TSS) and total dissolved solids (TDS) standardised testing. Hereby, pH, was obtained as an indicator of the nature of the wastewater that was sampled on a scale from acidic to basic. Specific

conductivity measurements were obtained as an indicator of dissolved ions within the water. Lastly, total suspended solids (TSS) and total dissolved solids (TDS) testing was carried out as indicators of water quality, namely TSS indicating the particle content of particles larger than 2 microns and TDS indicating the particle content below 2 microns (Fisher Scientific, 2007).

3.3 Materials and Methods

3.3.1. Set up and preparation

When cleaning glassware, standard practices for water sampling were observed, such as removing manufacturing residue before first using new equipment, decontaminating equipment immediately after use and rinsing with deionised water while still wet and before drying on a clean designated drying area (Wilde *et al.*, 2004). Glassware was cleaned with Lipsol® detergent (sourced from Fisher Scientific, product code: 12549965) and triple rinsed with deionised water. Deionised water was sourced from a Synergy® UV Water Purification system with a water resistivity at 25°C of 18.2 MΩ-cm. Regular sampling and equipment blanks were processed to ensure the cleaning protocol was adequate and to ensure the accuracy of the physical and chemical parameter results.

3.3.2. Process

The schematic below (Fig. 8) illustrates the sample analysis steps of the final sample design and indicates the processing time of each step. The total volume of each DDW sample (between 1.2 and 11 L, depending on the sampling day and clinic) was well-mixed and an aliquot of at least 400 mL was set aside for pH, conductivity, total suspended solids (TSS) and total dissolved solids (TDS) testing, which required up to 2 days to obtain results. An additional 0.1 to 0.5 L aliquot of the raw wastewater was placed in the freezer for ecotoxicity testing.

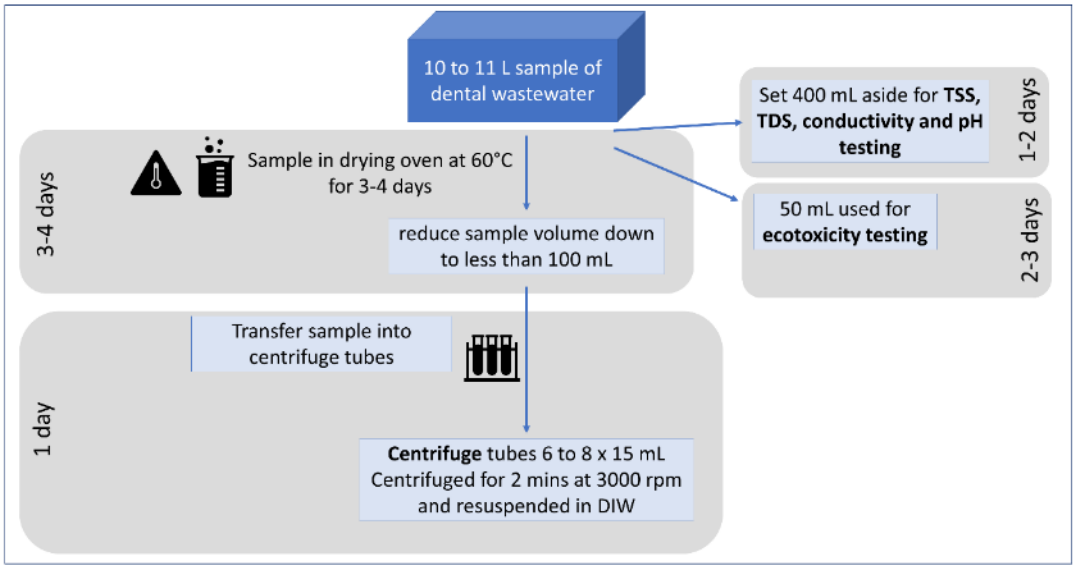


Figure 8: Study design schematic for the analysis of samples from dental wastewater analysis for Hg-free dental materials, indicating processing time.

Specific conductivity and pH measurements were obtained by submerging pre-calibrated probes into 200 mL of the sample and recording the values (WTW Handheld Conductivity meter 340i and OxyGuard Handy pH with software version 1.5). Calibration of the specific conductivity meter was conducted by using 0.01 M KCl to obtain a conductivity cell constant between 0.800 and 1.200 cm^{-1} and carried out weekly. The pH probe was calibrated using a 2-point calibration with pH 4 and pH 7 at 20°C (room temperature) weekly. Regular blanks were obtained by submerging the probes in tap water and by comparing the obtained measurements to reference values. TSS and TDS measurements were carried out in triplicate according to standardised procedures (Fisher Scientific, 2007) as follows: three pre-weighed and pre-washed 47 mm glass fibre filters (Whatman® glass microfiber filters, Grade GF/C sourced from Agar Scientific, product code: WHA9907047) were labelled and three 250 mL beakers were cleaned, labelled and weighed using a precision balance (Ohaus Explorer EP413 balance with 410 g x 0.001 g capacity and reported linearity of ± 0.002 g). To conduct the TSS test, each filter was placed on the TSS filtration apparatus, which consisted of a Buchner flask, rubber bung and Buchner funnel with a porous plate in which the filter paper was placed. The opening of the Buchner flask was connected to a vacuum pump via rubber tubing. A few drops of deionised water were added to the filter paper before the vacuum pump was turned on in order to create a vacuum. 100 mL of the sample were then passed through the apparatus and rinsed three times with 20 mL of deionised water. After three minutes the pump was turned off and the filter paper

placed in a clean, labelled Al weighing boat. The filtrate was placed into the pre-weighed 250 mL beakers for TDS analysis. This procedure was repeated in triplicate for each sample. Regular sample blanks and laboratory equipment blanks were processed, and sources of contamination identified and eliminated where needed. The TSS filter papers were then placed in the drying oven at 104 ± 1 °C for 1 h. Following that, the filter papers were placed in a desiccating chamber for at least 30 min to allow the filter papers to cool without collecting moisture. The filter papers were then weighed, and the weight difference recorded as shown in Eq. 1 below. The three 250 mL beakers were placed in the drying oven at 104 ± 1 °C for 24 h, followed by 180 °C for 2 h to remove organics and occluded water. The beakers were then placed in the desiccating chamber for 3 to 4 h to cool and the final weight was recorded, and the weight difference calculated as shown in Eq. 1:

$$[Eq.\,1] \quad TSS\ or\ TDS\ in\ mg/L = \frac{(weight\ after - weight\ before)\ x\ 1000}{mL\ sample}$$

The remaining well-mixed sample was transferred into 1 L pre-weighed beakers (Fisher scientific, product code: 15409083) or, as the methodology developed, polypropylene tubs (Fisher Scientific, product codes: 11358073, 11348073 and 11338073) that could hold between six to nine litres of liquid, and placed into a drying oven at 60°C in order to remove the excess water without potentially altering the chemistry of it under high heat; the samples were largely suspended with very little particles suspended in copious amounts of water. Generally, samples needed between three to four days in the drying oven at 60 °C in order to reduce the volume in each beaker or polypropylene tub down to between 0.1 and 1 L. Samples were checked on regularly and once sufficiently settled, the supernatant carefully decanted off while not disturbing the sediment, in order to speed up the process. The remaining sample volume of each beaker or polypropylene tub was then measured using a graduated cylinder and transferred into 15 mL centrifuge tubes (Fisher Scientific, product code: 11889649). The sample material in the tubes was carefully balanced and centrifuged at 3000 rpm for 2 min in an ALC Multispeed centrifuge (PK 121 series). The supernatant was then carefully removed, and the sample material resuspended in deionised water (DIW). At least eight 15 mL centrifuge tubes were processed in that way, per sample, and labelled according to further analysis and placed in the freezer.

3.4 Results

The pH and specific conductivity measurements were obtained from the wastewater of three dental practices (DP1, DP2 and DP3). The measurements are shown as boxplots according to sample size, which was n = 19 for DP1, n = 6 for DP2 and n = 2 for DP3 (Figs. 9 and 10). Fig. 9 shows that DP1 and DP3 had the overall lowest median pH values around a pH of 2 and DP2 recorded the highest median pH value of 9. The pH results of DP1 were within a range of 4.3 pH with a standard deviation of 1.2 pH units. Three outliers, at 4, 5 and 5.5, were detected and a mean of 2.5 (± 1.2) and a median of 2.2 pH were recorded. The pH results of DP2 showed a negative skew and a large range of 6.5 pH. The mean pH recorded was 7.0 (± 3.2) and the median 9.0. The pH measurements of DP3 showed the lowest variation within, due to the small sample size, and a mean and median pH of 1.9 was recorded.

The specific conductivity results in Fig. 10 show the same low variation for DP3 with a mean and median of 5.1 mS/cm and standard deviation of 0.1. DP1 measurements of conductivity showed the highest range with 18.5 mS/cm and a standard deviation of 4.9 mS/cm. The mean conductivity was 3.4 mS/cm and median conductivity was 4.4 mS/cm. Two large outliers, at 15 and at 19 mS/cm were recorded. The conductivity results of DP2 showed a positive skew with a mean conductivity of 2.5 mS/cm, a median conductivity of 1.2 mS/cm, a range of 5.8 mS/cm and a standard deviation of 2.7 mS/cm (Fig. 10).

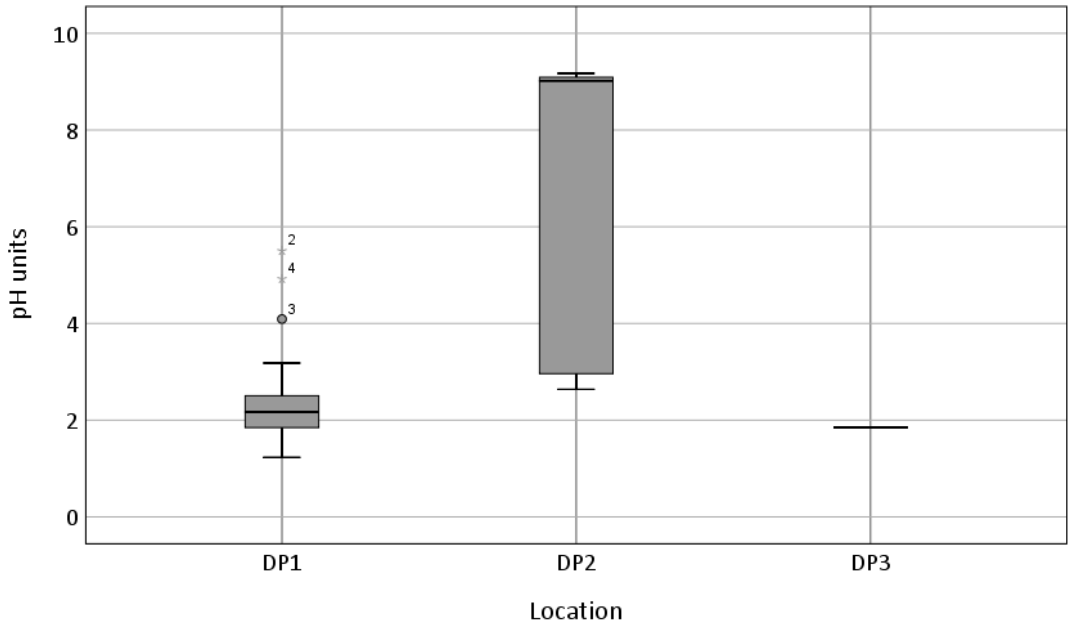


Figure 9: Boxplots of the pH measurements (on a scale from 0 to 14) from the wastewater of 3 dental practices (DP1 with n=19 samples, DP2 with n=6 samples, DP3 with n=2 samples).

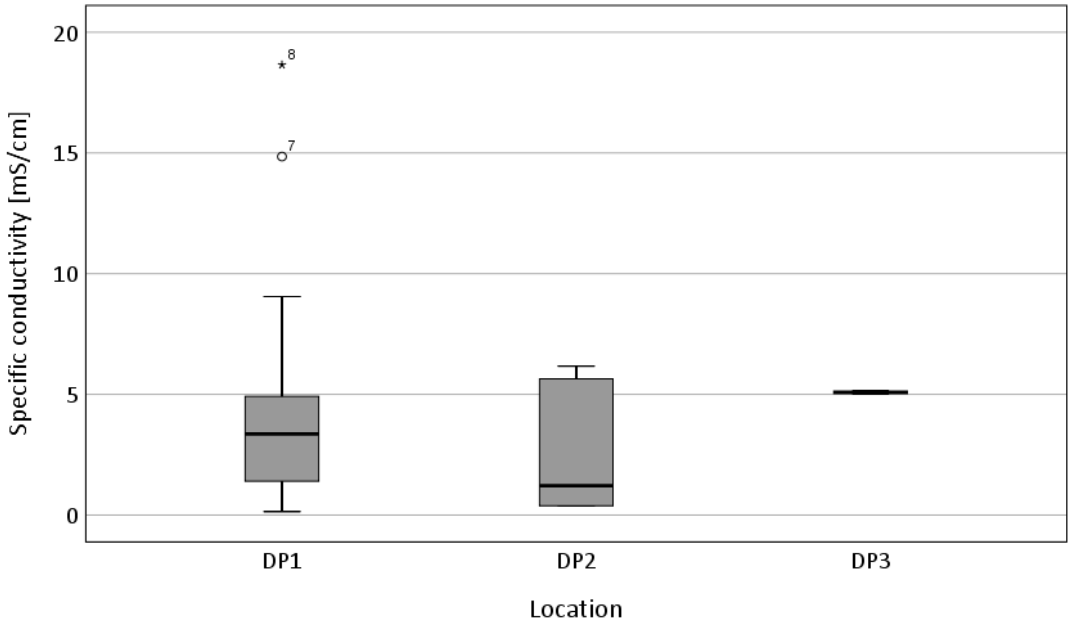
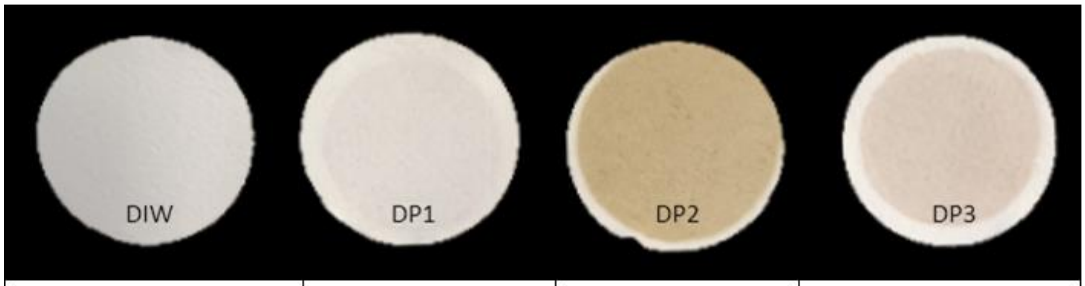


Figure 10: Boxplots of the specific conductivity measurements [in mS/cm] from the wastewater of 3 dental practices (DP1 with n=19 samples, DP2 with n=6 samples, DP3 with n=2 samples).

Total suspended solids (TSS) and Total dissolved solids (TDS) measurements were obtained in triplicate per sample from each of the three DPs. Figure 11 below shows some of the filter papers after TSS filtration, which highlights the different particulate matter load observed in each of the DPs.



Average of triplicate TSS results per DP	19.9 mg/L (n = 19)	114.8 mg/L (n = 6)	132.5 mg/L (n = 2)

Figure 11: Filter papers after TSS filtration of a DIW control and the dental wastewater arising from three dental practices (DP1, DP2 and DP3).

The mean average of each triplicate sample was obtained. These sample averages were then used to compute boxplots, showing the distribution of the measurements of DP1 with n = 19, DP2 with n = 6 and DP3 with n = 2. Figure 12 shows that the TSS measurements of the three DPs varied greatly between DPs and within DP1 and DP2. Measurements of TSS in DP1 showed a range of 57 mg/L and a standard deviation of 15.8 mg/L. TSS values in DP1 were overall lowest and a mean recorded at 19.9 mg/L and a median at 16.3 mg/L. DP2 showed the highest range in TSS results with results ranging between 148.6 mg/L and a standard deviation of 69.0 mg/L. The mean TSS for DP2 was 114.8 mg/L and the median was 88.0 mg/L. Mean and median TSS results from DP3 were the overall highest with 132.5 mg/L, a range of 23.0 mg/L and a standard deviation of 16.3 mg/L.

Fig. 13 shows the TDS results of the three DPs. DP1 shows the highest variation within with a range of 39.2 g/L and a standard deviation of 9.0 g/L. The mean TDS was 3.8 and the median TDS was 0.7 g/L. Two large outliers at 10.5 and 39 g/L were identified. DP2 had a mean and median of 5.9 g/L, a range of 2.3 and a standard deviation of 0.9 g/L. DP3 has a mean and median of 6.9 g/L, a range of 0.4 and standard deviation of 0.2 g/L.

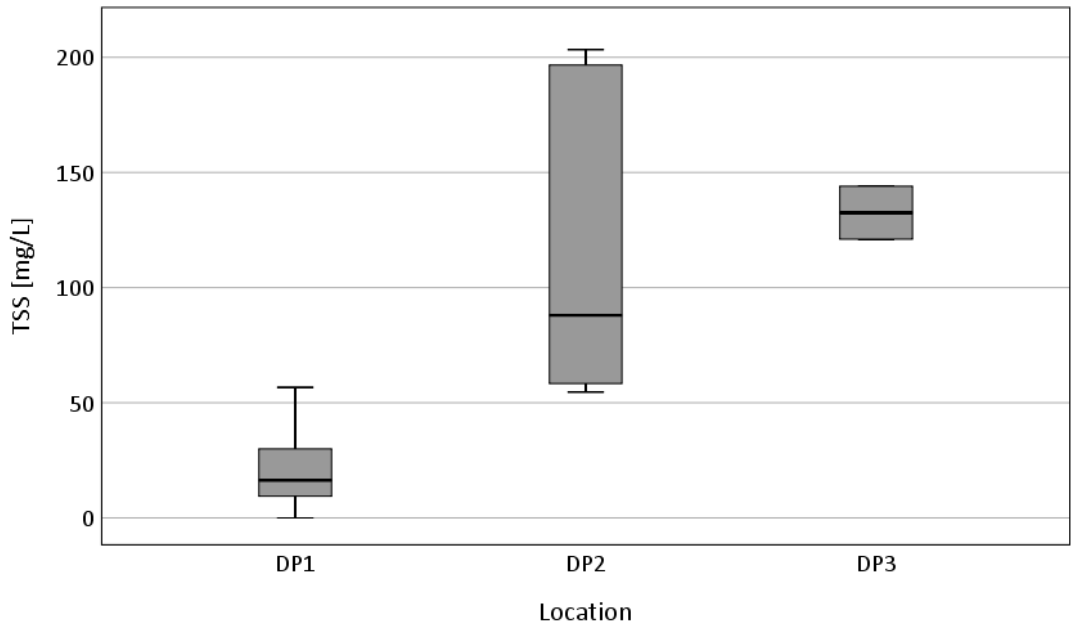


Figure 12: Boxplots of Total suspended solids (TSS) measurements [in mg/L], carried out in triplicate, from the wastewater of 3 dental practices (DP1 with n=19 samples, DP2 with n=6 samples, DP3 with n=2 samples).

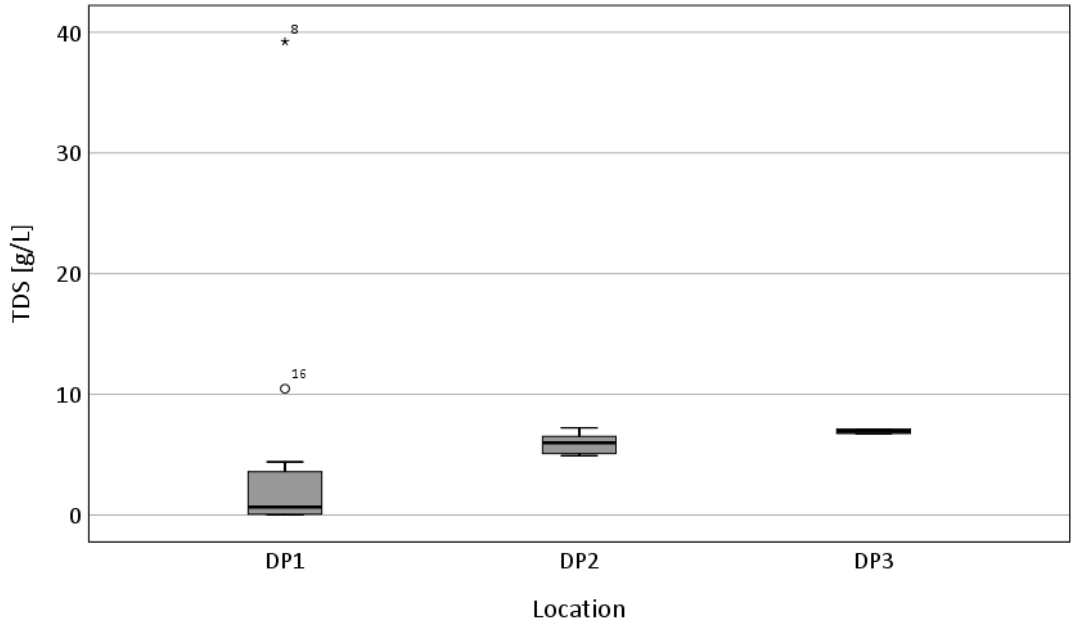


Figure 13: Boxplots of Total dissolved solids (TDS) measurements [in g/L], carried out in triplicate, from the wastewater of 3 dental practices (DP1 with n=19 samples, DP2 with n=6 samples, DP3 with n=2 samples).

The results of the measurements that were obtained from the three DPs are given in Table 3 below (more detailed results can be found in the Appendix in Table 10). The data from DP 1 was split into four groups, which were identified based on the data recorded by the dental practitioners, which included dental procedures and materials. The four major groups of materials that were identified were: the use of Resin Composite (RC) materials, Resin-modified (RM) composites, Glass ionomers (GIC) and use of amalgam (Ama) material. Days on which no detail on the treatment type was given were classed as 'other'.

Table 3 below shows the results of pH, specific conductivity, TSS and TDS measurements that were obtained from a total of 19 samples from DP 1, 6 samples from DP2 and 2 samples from DP3. TSS and TDS results were restricted by the number of significant figures on the analytical balance (max. capacity of 410 g x 0.001 g and reported linearity of ± 0.002 g), which is why TSS results especially show round numbers with similar error bars in cases where the triplicate filter paper measurements differed by less than 0.001 g.

Within the DP1 results, the RC group (n = 5) showed the overall lowest conductivity measurements between 0.15 and 5.16 mS/cm. The GIC (n=4) group recorded the

highest conductivity values between 3.04 and 18.65 mS/cm. The pH was overall lowest in the GIC group, ranging from 1.23 to 2.17, and highest in the RC group, ranging from 2.02 to 5.5. The RM, Ama and 'Other' groups fell between these extreme values and conductivity trends were observed to increase from RC < Other < RC+GIC < Ama < RM << GIC, pH trends were observed to decrease from RC > Other > RC+GIC > RM > Ama > GIC, TSS trends increased from Ama < Other < RM < RC < RC+GIC < GIC and TDs trends increased from RC < Other < Ama < RM < GIC (Table 3).

TSS and TDS results reflected the results obtained from the conductivity measurements. TSS was highest within the GIC group and recorded the three highest TSS values of 37, 47 and 57 (± 6) mg/L and the highest TDS values with up to 39 (± 1) g/L. The RC group followed with the second highest TSS values up to 39 (± 2) mg/L. TDS was lowest for the RC group overall. The RM group (n=1) produced another high TSS load with 17 (± 6) mg/L and high TDS with 4 (± 0.5) g/L. The Ama group produced a varied TSS load between 0 to 16 (± 1) mg/L and a TDS load between 1.6 to 4.4 (0.3) g/L. Highlighted in **bold** in Table 3 are those values that are considered above permissible limits if such wastewater were to be discharged directly into the environment according to the Environment Protection Act (2003) and EU Council Directive 91/271/EEC.

Concurrent with results in Figs. 9 – 13, the detailed results of DP2 and DP3 in Table 3 highlight the overall significantly higher conductivity, TSS and TDS results of DP3 and the lower pH values recorded. However, single outliers could be found in DP2 with conductivity measurements as high as 6.2 mS/cm and as low as 0.4 mS/cm. The pH was overall highest in DP2. TSS and TDS measurements have both in trend increased from DP1 < DP2 < DP3, however single measurements in DP2 were found to exceed those of DP3.

Table 3: Specific conductivity, pH, triplicate Total Suspended Solids (TSS) and triplicate Total Dissolved Solids (TDS) measurements from the wastewater of DP1 with n=19 samples, DP2 with n=6 samples, DP3 with n=2 samples and TW blanks. DP1 identifiers as follows: RC = Resin composite, GIC = Glass ionomer cement, RM = Resin-modified composite, Ama = Amalgam. TSS and TDS carried out in triplicate and restricted by analytical balance to 4 sig. figures).

		Spec. Cond. [mS/cm]	pH	Mean TSS [mg/L] (n=3)	TSS SD [mg/L]	Mean TDS [g/L] (n=3)	TDS SD [g/L]
DP1	RC	5.16	**2.02**	30.00	± 0.01	**3.82**	**± 0.22**
		0.17	5.50	10.00	± 0.01	0.19	± 0.01
		0.15	4.09	10.00	± 0.01	0.15	± 0.01
		0.15	4.92	**39.00**	**± 1.73**	0.16	± 0.01
		3.87	**2.30**	8.89	± 1.92		
	RC + GIC	3.68	**2.09**	20.00	± 0.01		
	GIC	**14.85**	**1.23**	**56.67**	**± 5.77**		
		18.65	**1.31**	**46.67**	**± 5.77**	**39.22**	**± 0.68**
		3.04	**1.71**	**36.67**	**± 5.77**	**1.24**	**± 0.07**
		3.36	**2.17**	3.33	± 5.77		
	RM	4.77	**2.05**	16.67	± 5.77	**4.07**	**± 0.39**
	Ama	1.98	**2.26**	16.33	± 0.58	**1.63**	**± 0.04**
		4.45	**1.43**			**3.36**	**± 0.12**
		5.06	**1.45**	3.33	± 5.77	**4.41**	**± 0.26**
	Other	1.94	**2.39**	16.67	± 5.77		
		9.05	**1.98**	30.00	± 0.01	**10.47**	**± 0.27**
		0.86	**2.37**	16.00	± 2.00	0.66	± 0.03
		2.10	**2.62**	10.33	± 0.58	**1.54**	**± 0.03**
		0.49	**3.18**	6.67	± 1.15	0.43	± 0.01
	TW blanks	0.18	6.60	0.00	± 0.00	0.03	± 0.05
		0.16	5.81	0.00	± 0.00	0.08	± 0.05
		0.15	7.00				
DP2		5.64	2.96	196.67	± 5.77		
		2.04	2.64	203.33	± 5.77	7.22	± 0.18
		6.17	9.17	111.00	± 9.17	6.52	± 0.17
		0.38	9.03	54.67	± 3.06	6.40	± 0.56
		0.38	9.00	58.33	± 1.53	5.58	± 0.24
		0.40	9.09	65.00	± 1.73	4.92	± 0.09
DP3		5.03	1.85	144.00	± 2.65	6.76	± 0.07
		5.14	1.85	121.00	± 3.00	7.11	± 0.10

3.5 Discussion

The results of the physical and chemical parameters of the wastewater from three dental practices have given clues about the extent of the wastewater load that was discharged from the dental practices. The pH results were unexpectedly low as the average pH values of DP 1 and DP 3 with 2.5 (± 0.3) and 1.9 (± 0.1) were at a level considered toxic to fish populations (below 10^{-3} mol/L H^{+}) and significantly more acidic than those found in urban wastewaters (Monarca *et al.*, 2000). Only DP 2 with a pH of 7.0 (± 1.3) was within what would be permissible limits for wastewater discharge from a treatment plant, which is a pH between 5 and 9 (Protection of the Environment Act, 2003; Environmental Protection Agency, 2001). The processing of tap water blanks from DP 1, which was taken from the same water stream that entered the dental chair, showed that the tap water had a pH in the range of 5.81 to 7, which highlights that the change in pH is directly linked to the procedures that were carried out at that dental practice, which includes the use of a line cleaner disinfectant (discussed below) at the start and end of each working day. The processing of regular laboratory tap water blanks recorded pH values within the normal range (pH 7 ± 1) and the probe was calibrated with a 2 point calibration (Platikanov *et al.*, 2017).

The specific conductivity results shown above were considered to be elevated. Conductivity is often reported in µS/cm, but due to the high values that were recorded in the three DPs, the scale was adjusted to show conductivity in mS/cm. Comparing the obtained conductivity measurements with the US EPA (2012) monitoring guidelines shows that the wastewater of the three DPs was above the conductivity range that is generally found in freshwater, which is up to 1.5 mS/cm, but below the range found in industrial wastewaters, which is up to 10 mS/cm (US EPA, 2012). Laboratory tap water blanks and the tap water blank from DP 1 fell within the normal range that is found for tap water (up to 2.5 mS/cm)(Platikanov *et al.*, 2017).

As per Infection Prevention and Control Protocol (IPCP) (Dental Council of Ireland, 2015), a minimum of 20 mL (1 % concentration) of MD 555 special suction unit cleaner was flushed through the dental chair systems in DPs 1 and 3 at the start and end of each working day. The active ingredients of the MD 555 cleaner that are listed in the MSDS are 20 – 25 % citric acid monohydrate ($C_6H_8O_7.H_2O$) and 15 – 20 % phosphoric acid (H_3PO_4) (Dürr Dental SE, 2015). DP 2 used the Puli Jet Classic disinfectant product at a concentration of 5 %. The Puli Jet product ingredients that

are listed in the MSDS are 0.95 % C_6H_5OH (phenol) , 0.38 % Na_2SiO_3 (sodium metasilicate) and 0.012 % $C_3Cl_2N_3NaO_3$ (sodium dichloroisocyanurate dihydrate) (Cattani, 2016). Therefore, the measured pH and conductivity values are likely due to the application of these line cleaner and disinfectant products in the three DPs. The acidic content of the MD 555 cleaning product explains the low pH values and high conductivity of the wastewater in DPs 1 and 3.
However, during testing it was observed that both cleaning/disinfecting products are not used according to the recommended dosage level. In the case of the MD 555 cleaner, the manufacturer recommends a dosage of 100 mL (5 %), but only 20 ml (1 %) were used. Moreover, the MD 555 product provides only half of the routine cleaning recommendations. This cleaner is used to clean the suction unit, but in order to disinfect the unit the use of MD 555 in combination with Orotol® plus is recommended (Dürr Dental SE, 2015). As for the Puli jet Classic product it was also observed that concentrations below the recommended 5 % were used to disinfect the dental chair unit. Therefore, an observation of other dental practices and their use of the disinfection and dental line cleaning products would perhaps determine whether using less disinfection is common practice or whether the selected dental practices were an anomaly and hence underestimated the chemical load of the wastewater.

TSS and TDS measurements were obtained to give clues on the particle load within the DWWs. Permissible TSS discharge limits from urban WWTPs to surface waters are set out in the Environment Protection Act (2003) and in the European Directive 91/271/EEC to be 35 mg/L. Therefore, DP 2 and DP 3 were releasing TSS to sewer that were over three times larger than the permissible discharge limit of an urban WWTP. Only DP 1 was below these limits, on average. There are currently no National or EU Directives for permissible TDS discharge limits (Environmental Protection Agency, 2001), however, the typical reported levels of TDS in urban wastewater are 1000 mg/L (Environmental Protection Agency, 1997). Therefore, all three DPs exceed these values by five to six times. The highest TSS and TDS values of DP1 were found as a result of the use of GICs.

The number of samples that were collected from each DP differed significantly from n=19 in DP1, n=6 in DP2 to n=2 in DP3. This variability was due to time constraints of the study and led to the highest number of samples being collected in DP1 as the

method development took place in DP1. Moreover, the daily WW volumes that were discharged from each DP ranged from one to 4 L/day in DP2 and from 2 to 6 L/day in DP3, while DP1 discharged around 10 L on most days. While this on the one hand impacted on the overall WW concentration or dilution of the samples, on the other hand it also impacted on the precision of the results. Ideally, the same number of samples would have been collected in each DP to allow for better comparison of the data. The effects of sample size on the accuracy of the results in environmental studies is important in order to avoid type 1 (false positive) and type 2 (false negative) errors that could lead to an incorrect environmental impact assessment (Fairweather, 1991; Wilson Van Voorhis and Morgan, 2007).

The 2 samples that were collected in DP3 provided only 2 data points for pH, specific conductivity and TDS, which were observed to be close together, which in turn can lead to a false sense of precision of the data. In the case of the TSS results, the data points were further apart, which indicated low accuracy and does not allow for definitive conclusions. The same principles apply to the results obtained from DP2 with a sample size of 6. In both DP2 and DP3, the mean and median results were very close together or identical, which in a large sample size indicates normality but at such a low sample size indicates that more data points are needed. In the case of DP1, 19 samples were obtained and the results showed a much larger standard deviation and range, in part due to the high variability in dental treatments that were carried out, which also highlights that the results in DP 2 and DP3 could potentially have shown a much higher variability if more samples had been collected.

It is recommended, that should any follow-up studies be carried out, to obtain at least 30 samples in each DP and, additionally, to divide the sampling days by the different dental treatments and to obtain at least 30 samples from each DP based on treatment type to decrease the chances of systematic errors and to allow for further statistical analyses (Springate, 2012; Wilson Van Voorhis and Morgan, 2007).

TSS as well as BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand) are standard parameters used for effluent testing (Hernando *et al.*, 2005). The collection of BOD and COD measurements was considered for this study, however, as a study by Hernando *et al.* (2005) highlighted, these measurements may not be a good indicator of pollution load and should be coupled with standardised ecotoxicity analysis. Ecotoxicity testing was conducted in Chapter 5 of this thesis. For

near real-time application of effluent monitoring, BOD and COD offer fast results. It was, however, suggested that a combined approach could provide the high accuracy that *D. magna* is able to detect even at very low concentrations (in ng/L), while also offering near real-time results to provide comprehensive quality control (Hernando *et al.*, 2005).

3.6 Conclusions

The physical and chemical parameter results have shown that treatment of the DWW by a WWTP is necessary and any accidental spill into the environment of these untreated wastewater streams could have potential environmental impacts due to the low pH (as low as 1.3 at times) and the high particle load (up to 200 mg/L TSS and 39 g/L TDS). Those values that were above discharge limits are highlighted in bold in Table 3 above. The physicochemical parameters indicated a high suspended particle load and presence of ions, especially resulting from the use of glass ionomer cements (GIC). Chapter 4 reports more detailed results on the particle size analysis and characterisation of these particles.

Chapter 4 Particulate matter load, size and characterisation in DWW streams

4.1 Introduction

Particulate matter resulting from the use of dental filling materials (both amalgam and Hg-free), makes up one of the main possible pollutants within DWW. Information on the particle size arising from dental drilling and polishing of Hg-free materials in clinical use may inform the behaviour and environmental fate of these materials. Previous work in this area has focused primarily on Hg particulate waste streams resulting from the use of dental amalgam (Lutchko and Gulka, 2004; Shraim *et al.*, 2011; Vandeven and Mcginnis, 2005; Fan *et al.*, 1997; Jamil *et al.*, 2016; Tibau and Grube, 2019). In the case of Hg from dental amalgam, it has been found that the particle and pollution load entering the sewer and treatment plants is substantial and poses an environmental risk in case of accidental environmental exposure (Adegbembo *et al.*, 2002). Another study found that healthcare facilities produced different levels of toxic wastewater based on 5 toxicity levels (1 – non-toxic, 2 – low toxic, 3 – toxic, 4 – very toxic, 5 – extremely toxic), all of which discharged wastewater directly into the urban sewer system and hence leaving the (sometimes substantial) pollution load to be treated by a single cycle of WW treatment in a WWTP, which may not be equipped to catch persistent pollutants such as micropollutants and nanoparticles (Jírová *et al.*, 2019).

The airborne exposure of Hg wastes in dental practices is well studied (Warwick *et al.*, 2019) and airborne particles are known to be generated by high-speed dental drills that are used during dental filling placement or removal. Moreover, the heat generated by the dental drill has the potential to vaporise the Hg component in dental amalgam. Such particulate matter and nanomaterial wastes are known to cause respiratory illness and amount to a substantial load of airborne particulate matter (Murr and Garza, 2009; Warwick *et al.*, 2019). In the case of Hg-free dental filling materials it was found that the airborne exposure amounted to a mean of 135 $\mu g/m^3$ over a 30 min period, while the highest proportion of particulate matter generated by the high-speed dental drill was caught by the dental suction pump and hence entered the wastewater (Warwick *et al.*, 2019). The particulate matter that entered the suction was flushed through the dental chair tubing and amalgam separator before it was discharged as wastewater. In

the case of dental amalgam, the amalgam separator is designed to catch 95 % (based on mass fraction) of the particulates (International Organisation for Standardisation, 2008; Regulation (EU) 2017/852, 2017). They were, however, not designed for Hg-free dental filling materials, which contain nanofillers and microparticles that are unlikely to be trapped by the amalgam separator due to their size.

A review of 54 nanomaterial release studies has shown that, of the particles that are frequently released, most remain bound to the composite matrix, while only a small proportion of discrete nanoparticles was released (Froggett *et al.,* 2014). Both the microparticle and the nanomaterial fraction eventually end up in the WWTP, which may not be equipped to catch these (Brar *et al.*, 2010). It is reported that nanomaterials entering WWTPs may not remain dispersed during the WWTP process and therefore behave very different to particles that are utilised as part of the WWTP process, which may impact on the treatment efficiency and require an adaptation of nanoparticle-removal processes into the current WWTP processes in the future (Brar *et al.*, 2010). One possible result of this is that these nanomaterials end up in the sewage sludge and enter the environment when this sewage sludge is incinerated, brought to landfill or applied to agricultural land (Brar *et al.*, 2010).

Considering previous findings and the role that particulate matter within DWW plays, this chapter details an assessment of particulate load, particle size distribution and shape characterisation resulting from the use of Hg-free dental filling materials.

4.2 Materials and Methods

Samples of DWW were collected as per the methods described in sections 2.3 and 3.3. The process for the particle detection, sizing and characterisation included the use of 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). Figure 14 below shows the processes involved in the study design covered in Chapters 3 and 4.

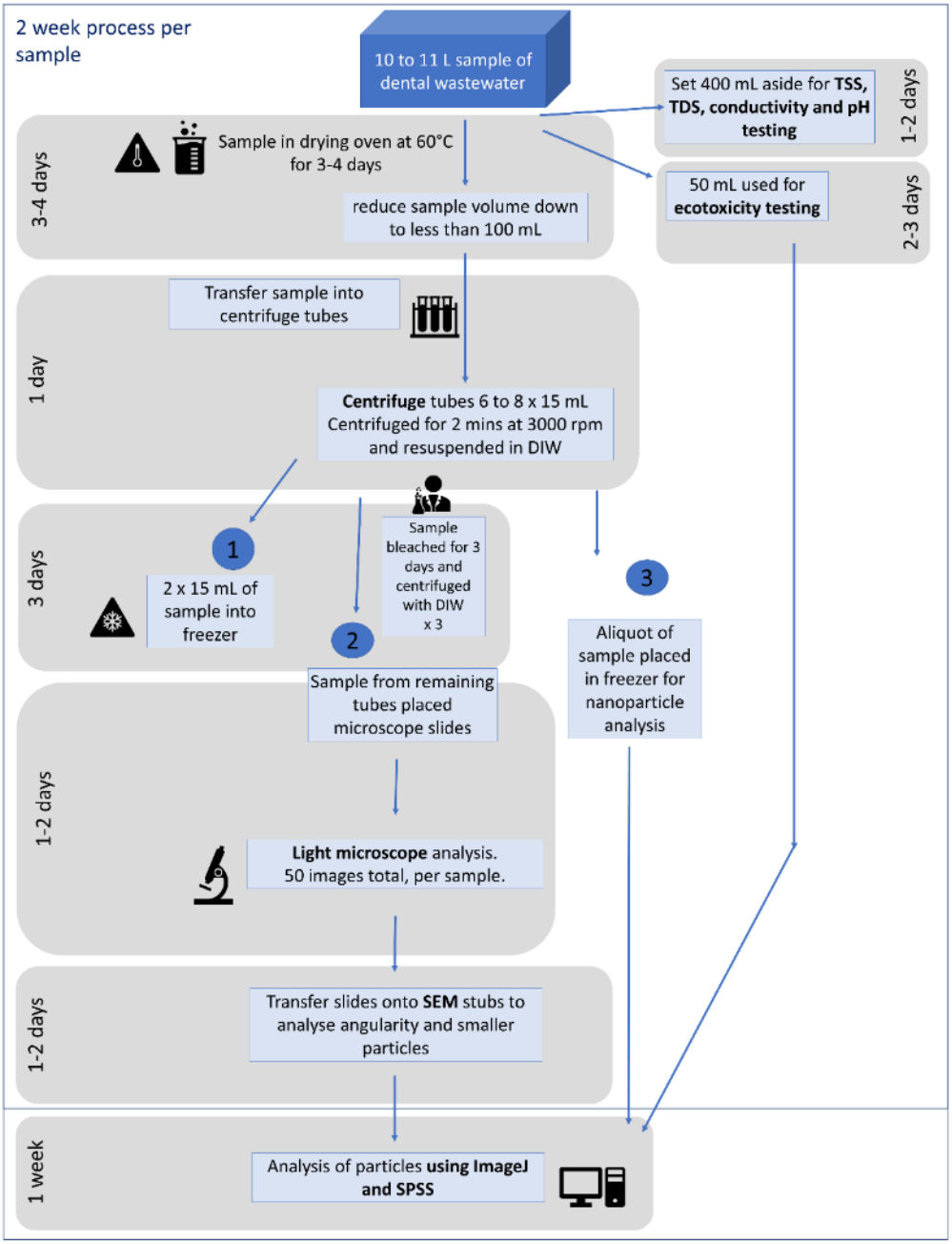


Figure 14: Study design schematic for the processing of samples from dental wastewater analysis for Hg-free dental filling materials.

4.2.1 DWW sample preparation for particle analysis

Continuing from the sample methodology in Chapter 3, where the sample volume was reduced by evaporating sample liquid off the surface using a drying oven at 60°C for 3 to 5 days and carefully decanted off before the sample was transferred into 15 mL centrifuge tubes (Fisher Scientific, product code: 11889649), the centrifuge tubes were then placed in the centrifuge and spun at 3,000 rpm for 2 minutes. The supernatant was carefully removed, and the centrifuge tubes filled with 12 mL of deionised water and 3 mL of bleach (< 5 % Sodium Hypochlorite) in order to remove biological material from the sample material.

Figure 15 below shows the testing that was undertaken to remove biological material from the sample material on untreated sample material, material treated with 10 % HCl (2.7 M), material treated with 10 % H_2O_2 (3.0 M) and material treated with 1, 2 or 5 mL of thin bleach (< 3 % sodium hypochlorite). The sample material still showed some biological material but confirmed that bleach sufficed in removing the biological material significantly. This was advantageous since HCl and H_2O_2 may have led to sample material becoming dissolved. In the final methodology, 3 mL of bleach (< 5 % Sodium Hypochlorite) were used on the DWW samples.

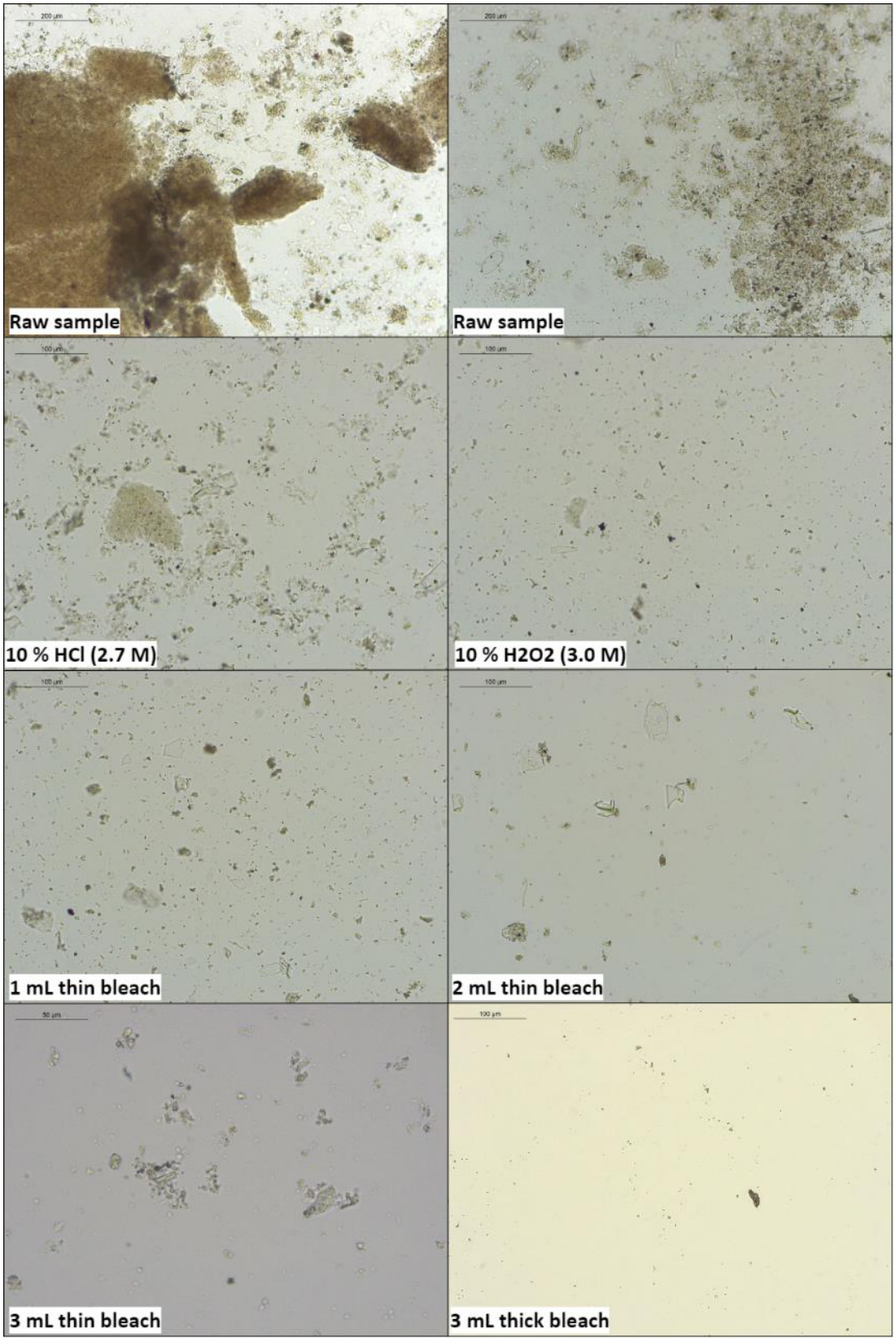


Figure 15: Treatment of DWW sample material to remove biological material with HCl, H_2O_2 and bleach, in comparison.

The centrifuge tubes were kept in the fridge at 4°C for 3 days and inverted daily to allow the bleach to work. Following the 3-day bleaching, the centrifuge tubes were carefully balanced with deionised water and centrifuged at 2,000 rpm for 2 minutes and the supernatant taken off carefully. A process of re-suspending the sample in deionised water, balancing the tubes and centrifuging at 2,000 rpm for 2 minutes was then repeated at least 3 times, until the chlorine salts and bleach remains were sufficiently removed. Once the sample was considered sufficiently "clean" of chlorine salts, the supernatant was carefully removed one last time and a small drop (10 – 20 µL) of sample material pipetted onto microscope slides that were cleaned with acetone wipes and covered with a clean cover slip. Microscope slides and cover slips were sourced from Fisher Scientific, product codes 11562203 and 12363128. The images below (Fig. 16 a) show particles in the centrifuge tube after bleaching, re-suspending and centrifuging a sample from DP 1 in deionised water 3 times. Some particles are clearly visible. The sample was then transferred onto a microscope slide and images of the same particles obtained (Fig. 16 b-c).

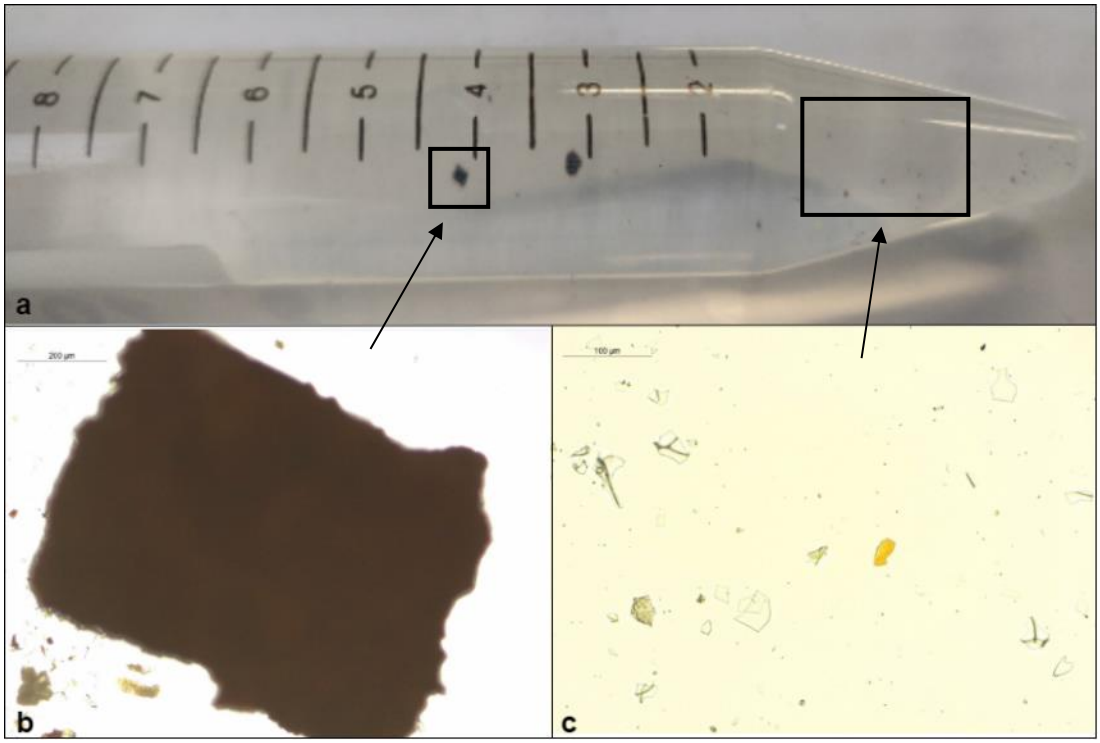


Figure 16: Dental wastewater sample material clearly visible to the naked eye (a) and under a light microscope (b) at a scale of 200 µm (c) and 100 µm.

4.2.2. Light microscopy analysis

Using the Leica DM 500 light microscope fitted with an ICC50 HD camera, images of each sample were obtained with three different objective lenses (x10, x40 and x100) and annotated with a scale bar in the LAS EZ software. In order to provide results of a representative sample, the initial goal was to obtain 50 light microscope images per sample, which was later adjusted to provide representative images for each dental filling material that was used in each dental practice, which were 50 images per dental filling material each and resulted in more images obtained from the more frequently used materials.

Blank slides were processed that were prepared in the same way that sample slides were prepared, which included the use of a drop of deionised water and the cleaning of the slide and cover slip with acetone wipes. These images were used to establish a baseline count of particles detected by the light microscope using the three different objective lenses and to account for room contamination and dust particles on the microscope lenses.

4.2.3 Scanning electron microscopy analysis

Sample material for analysis with the Scanning Electron microscope was prepared using the bleached material from the light microscopy preparation and by re-suspending this material in EtOH at increasing concentrations from 70:30 to 50:50 (Deionised water:Ethanol) and centrifuging those tubes at 2,000 rpm for 2 minutes. Sample material was mounted on Al stubs with C tape and sputter coated with Au for 30 – 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 at an accelerating voltage of 10 kV and a working distance of 10 mm.

4.2.4. Image analysis

The light microscope images were processed using ImageJ/Fiji (software version 1.46) (Rasband, 2018) in order to obtain the particle size and circularity. The semi-automated particle analysis from digital images with ImageJ/Fiji required calibration. Calibration was carried out by using images available from the ImageJ

website that allowed for comparison of the results and the methodology was established through these exercises and the ImageJ tutorials. Images needed to be of high quality and include a scale bar to be able to use the ‘Set Measurement scale’ function. This allowed later results to be shown in the set scale, which for the purposes of this study was chosen to be microns, rather than pixels. To set a scale, the line tool was selected, and a line drawn to cover the scale bar. By selecting ‘Analyse’ and ‘Set Scale’ the scale was set in units of µm. Once the scale was set, a box was drawn over the scale bar and the commands ‘Edit’, ‘Clear’ and then ‘Edit’ and ‘Invert’ were selected. Doing so removed the scale bar from the image and therefore eliminated errors of the scale bar being analysed like a particle. Next, the image was converted to an 8-bit image in order to reduce the memory requirements of the image (Rasband, 2018). To do so, the options ‘Image’, ‘Type’ and ‘8-bit’ were selected in the software. In the next step the option of turning the background black was eliminated by selecting ‘Process’, ‘Binary’, ‘Options’ and deselecting ‘Black Background’. This was important groundwork for the next step in which the image was converted to a black and white image by selecting ‘Process’, ‘Binary’ and ‘Make Binary’. After that the steps ‘Process’, ‘Binary’ and ‘Fill Holes’ and ‘Process’, ‘Binary’ and ‘Watershed’ were carried out in order to separate clustered particles. In the next step the measurements for particle analysis were selected in the ‘Set Measurement’ tool under the ‘Analyse’ tab. The measurements selected for this study were: area, standard deviation, min. & max. gray value, bounding rectangle, shape descriptors, area fraction, mean gray value, centroid, perimeter, feret's diameter and median. Decimal places were set to 3. The last processing step was to select ‘Analyse particle’ on the ‘Analyse’ tab and to set the size (μm^2) to 0-Infinity, the circularity from 0.00 to 1.00, select ‘Show: Outlines’ and select ‘Display results’, ‘Summarise’, ‘Record starts’ and click ‘OK’.

To speed up the process, Macros were written for each of the image size classes (50, 100 and 200 µm).

For each light microscope image that was processed in this way, a drawing of the image was produced, which showed the outline of each particle that was detected (see Fig. 17 below for samples of these) as well as a summary for the entire image including the total count of particles per image, their total area, % area, average size (mean) and other selected parameters. Detailed results were also provided for each of the particles

within each image which included the area [in µm^2] of each particle within the image and the circularity index from 0.0 to 1 (1 indicating a perfect circle and 0 indicating an elongated shape) of each particle. The ImageJ user guide specifies that the circularity index was calculated based on $4\pi * \frac{[Area]}{[Perimeter]^2}$, and values outside of the range between 0.0 and 1.0 were ignored (Rasband, 2018). The circularity index was chosen as it is considered to represent the 2D index of true sphericity based on particle area (Cruz-Matías *et al.*, 2019). The results were exported from ImageJ/Fiji in a comma-separated value (.csv) file.

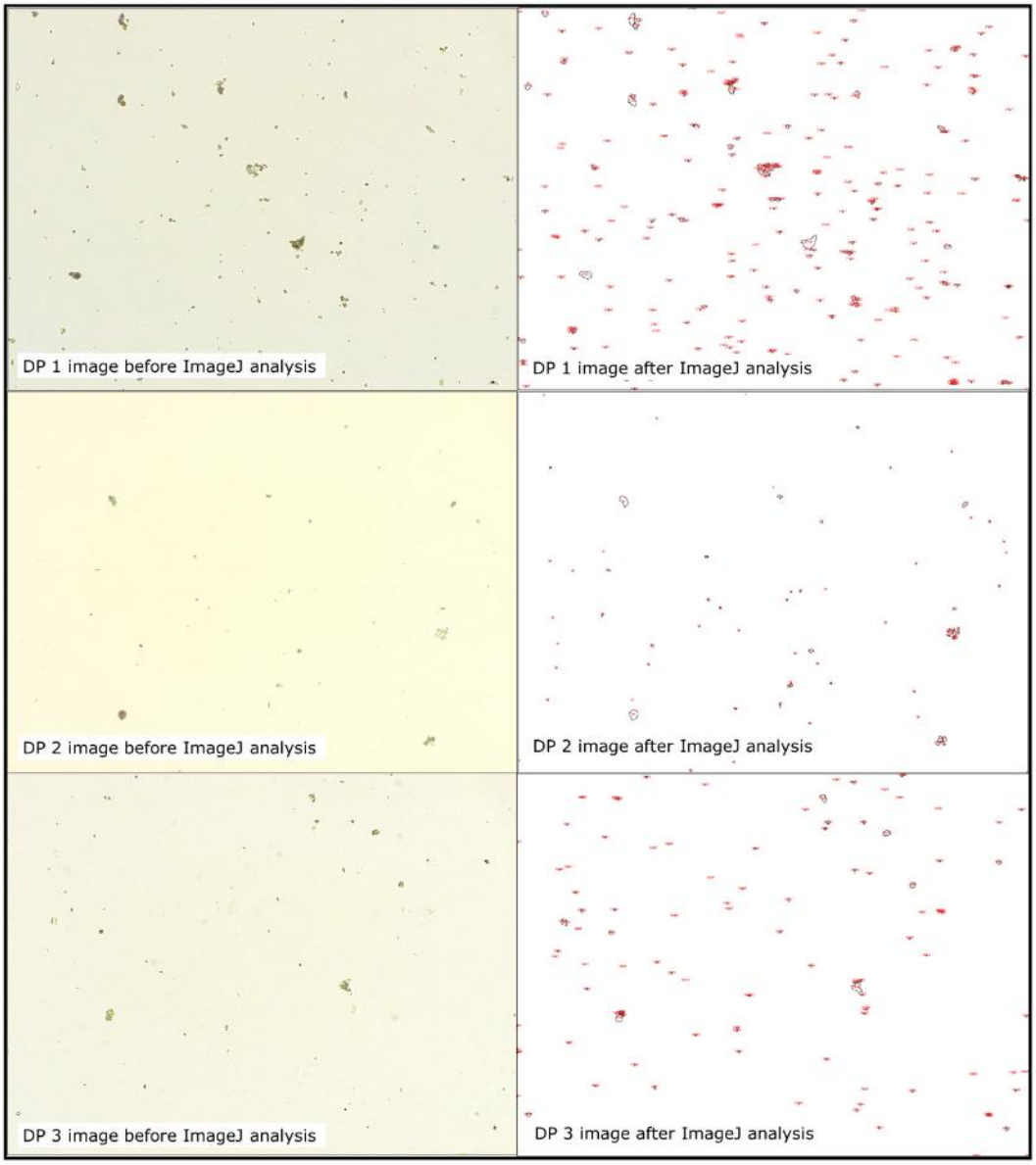


Figure 17: Light microscope images obtained from sample material of DP 1, DP 2 and DP 3 and processed using ImageJ particle analysis.

Erroneous images were excluded from the analysis. These images included a large number of particles on the edges of the image that did not correspond to particles. Moreover, some images showed clusters of particles that were not present in the original images and therefore these images were also excluded from the analysis.

4.2.5 Efficacy testing of amalgam separator

Known volumes of 4 commonly used Hg-free dental filling materials were fed through the dental chair. The four materials were (1) Ceram X manufactured by Dentsply Sirona, (2) SDR flow^{+} manufactured by Dentsply Sirona, (3) Filtek manufactured by 3M and (4) Herculite XRV manufactured by Kerr Dental (Fig.18). The materials were light-cured before use using a UV-lamp. Prior to testing the dental chair pipes and filters were cleaned, and the amalgam separator collection vessel was changed. The dental filling material was weighed before and after testing and the difference recorded.



Figure 18: Four commonly used Hg-free dental filling materials after light-curing: (1) Ceram X manufactured by Dentsply Sirona, (2) SDR flow^{+} manufactured by Dentsply Sirona, (3) Filtek manufactured by 3M and (4) Herculite XRV manufactured by Kerr Dental.

A qualified dental practitioner drilled the material with both the high and low speed drill and polished the materials with 4 different polishing disks of different grades. Any loose material was caught with a wet suction system, the test caps (shown in Fig. 18) were flushed with water and the resultant wastewater flushed down the spittoon at the end of the procedure. The objective here was to replicate a clinical scenario as closely as possible by using the same equipment and wastewater pathways. The sample passed through the wet suction, spittoon, dental chair filters and the amalgam separator until discharge as wastewater occurred. The wastewater was caught by the same HDPE collection vessel that was used for GWW analysis to allow for particles

analysis. After collection, the sample was processed as per procedure outlined in sections 3.3.2, 4.2.1 and 4.2.2 using the drying oven, centrifugation, light microscopy and image processing with ImageJ/Fiji.

4.2.6 Statistical analysis

The particle results were analysed using MS Excel and the statistical software package SPSS (IBM, software version 25). The results for this section are presented in the order from particle load analysis to particle size distributions and particle characterisation.

4.3 Results

4.3.1 Baseline data for particle size and characterisation

Optical microscopy is considered to be applicable to particles in the range between 0.8 – 150 µm (Wills and Finch, 2016). A size of 0.8 µm would include particles with an area size of 0.64 μm^2 (0.8 x 0.8 µm), while a size of 150 µm would include particles with an area size of 22500 μm^2 (150 x 150 µm).

Baseline data for particle size (in μm^2) of 19 images obtained from blank slides using the light microscope (objective lenses x10, x40 and x100) and processed using ImageJ/Fiji is shown in Figure 19. A declining trend in frequency with an increase in particle size based on μm^2 could be observed. Of the 515 particles that were detected in the 19 images, 57 % of the particles were below 0.64 μm^2 in size. However, high frequencies of particles (above 10) were detected up to 1.2 μm^2 particle area (equivalent to 1.1 x 1.1 µm). Particles up to 1.2 μm^2 made up 66 % of the particles that were detected. The remaining 34 % of values ranged from 1.2 to 704 μm^2 in size and included mostly frequencies of 1 and only few frequencies between 2 and 8. Therefore, 1.2 μm^2 was chosen as the cut-off for baseline data and all particles below that particle area size were excluded from the analysis of the DWW. Analysis of the circularity index of these particles showed that 78 % had a circularity index between 0.9 and 1.0.

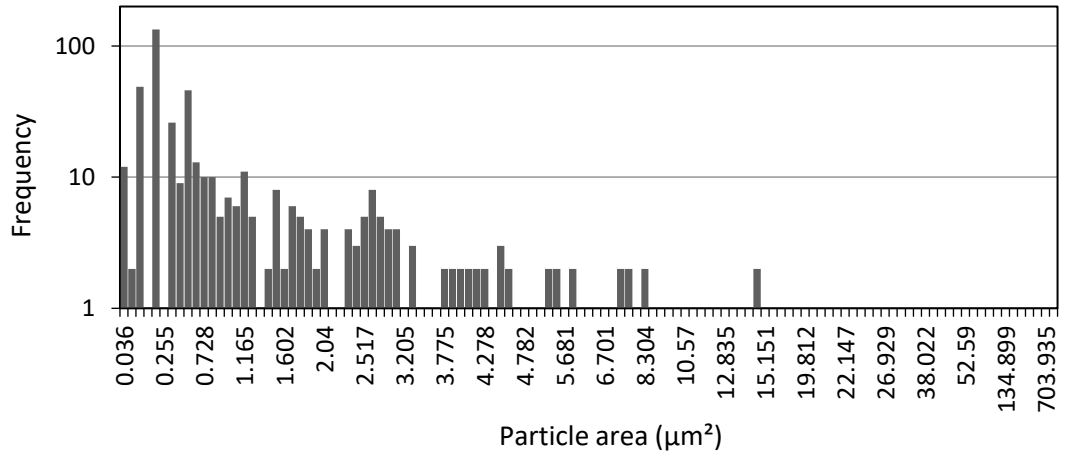


Figure 19: Baseline data of particle frequency plotted against particle area [μm^2] of 19 images taken from blank microscope slides with the light microscope objective lenses x10, x40 and x100.

As part of the particle characterisation, the raw Hg-free dental filling materials were imaged using the Hitachi TM 1000 SEM at an accelerating voltage of 10 kV (Fig. 20). This SEM required no sputter coating of the materials.

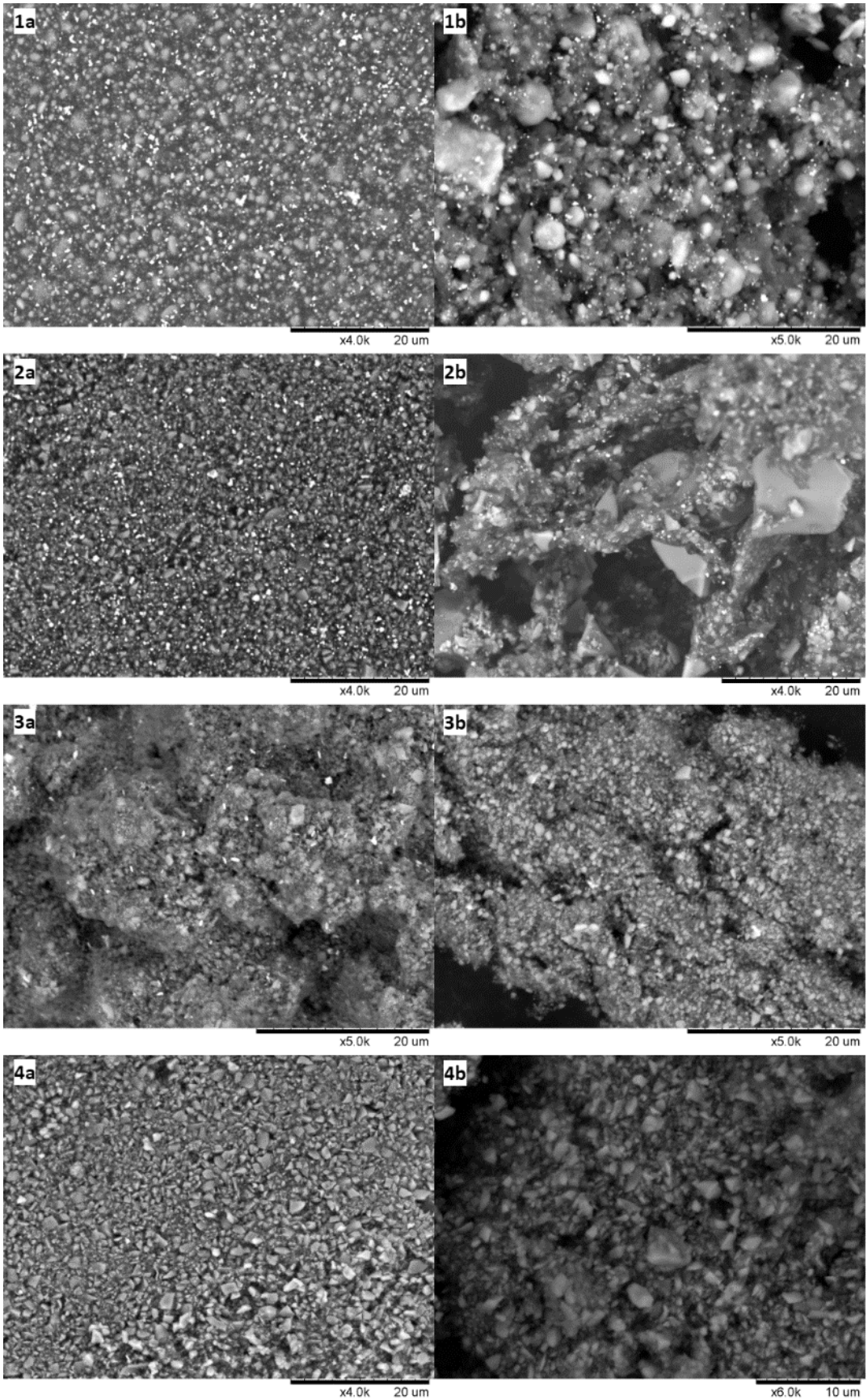


Figure 20: Cured Hg-free dental filling materials imaged with a Scanning Electron Microscope (SEM). Bulk material (a) and scrapings of the bulk materials (b) of Filtek (1), SDR flow^{+} (2), Ceram X (3) and XRV Herculite (4) dental filling materials.

Figure 20 shows these materials at a scale of 10 - 20 μm shortly after light-curing has taken place in bulk form (a) and after they were scraped with a scalpel (b). Four widely used Hg-free dental filling materials were used, which included (1) Filtek manufactured by 3M (1), (2) SDR flow^{+} manufactured by Dentsply Sirona, (3) Ceram X manufactured by Dentsply Sirona and (4) Herculite XRV manufactured by Kerr Dental. The bright coloured materials that are visible on images 1a to 2b are likely glass or quartz fillers. The images also show the range of particle sizes that are used for the different filling types: from larger angular fragments (20 μm) in image 2b to smaller and more abundant particles visible in images 1b and 3b (less than 1 μm).

4.3.2 Particle load of DP1, DP2 and DP3

A total of 724 light microscope images were processed using ImageJ. Of these, 574 images were obtained from samples from DP1 (with n = 19 samples), 62 images from samples from DP 2 (with n = 6 samples) and 88 images from samples from DP 3 (with n = 2 samples).

Particles with an area ranging from ≥ 1.2 to ≤ 150 μm^2 in size were analysed due to the data cut-off determined in Section 4.3.1. Due to the cut-off, 41 % of the particles were excluded from the analysis of the DWW of DP1, 52 % from DP2 and 37 % from DP3 (Table 4).

The particle area that was recorded for particles ranging from ≥ 1.2 to ≤ 150 μm^2 in size was found to be highest in DP1 with a mean particle area (± standard deviation) of 11.4 ± 19.4 μm^2 and median particle area (± standard error) of 4.5 ± 0.07 μm^2 (Table 4). DP2 showed the second highest mean particle area with 8.2 ± 16.2 μm^2 and lowest median particle area with 3.2 ± 0.2 μm^2. DP3 showed the lowest mean particle area with 6.4 ± 9.0 μm^2 and second highest median particle are with 3.5 ± 0.06 μm^2.

The circularity index (1 indicating a perfect circle and 0 indicating an elongated shape) of the three DP was obtained. The mean circularity index (± standard deviation) was recorded to be 0.74 ± 0.2 for DP1, 0.72 ± 0.3 for DP2 and 0.80 ± 0.2 for DP3 (Table 4).

Table 4: Statistics of particle count, particle size and circularity from wastewater generated by 3 dental clinics examined in this study.

	DP 1	DP 2	DP 3
Total number of images used for analysis (FOV 18)	574	62	88
Data points excluded due to cut-off [%]	41	52	37
Mean particle area (± SD) [μm^2]	11.4 (± 19.4)	8.2 (± 16.2)	6.4 (± 9.0)
Median particle area (± SE) [μm^2]	4.5 (± 0.07)	3.2 (± 0.2)	3.5 (± 0.06)
Mean circularity index (± SD)	0.74 (± 0.2)	0.72 (± 0.3)	0.80 (± 0.2)

The results from DP1 were explored in greater detail as the highest number of DWW samples were collected from this site, which led to a higher number of light microscope images being processed and therefore greater detail of information was available from this clinic which also allowed the analysis of the data based on four

treatment groups. The treatment groups were RC (Resin Composite), RM (Resin-modified glass ionomer cement), GIC (Glass ionomer cement) and Ama (Amalgam), which allowed comparison of particle sizes per treatment group.

In DP1, a total of 574 images were used for particle analysis. Of these, 215 images were obtained from the RC treatment group, 207 from the GIC treatment group, 51 from the RM treatment group and 101 images from the Ama treatment group (Table 5).

The mean and median particle area that was recorded for particles ranging from ≥ 1.2 to ≤ 150 μm^2 in size for the four treatment groups showed that on average, particles from the GIC group were largest with a mean particle area of 13.1 ± 21.6 μm^2 and median particle area of 5.2 ± 0.1 μm^2. The RM group was second highest with a mean particle area of 12.7 ± 21.4 μm^2 and median particle area of 4.8 ± 0.2 μm^2. The Ama group followed with a mean particle area of 10.6 ± 17.9 μm^2 and median particle area of 4.5 ± 0.2 μm^2. The RC group showed the overall smallest particle area with a mean particle area of 9.2 ± 15.9 μm^2 and median particle area of 3.9 ± 0.1 μm^2.

The mean circularity index of the 4 treatment groups ranged from 0.66 ± 0.7 in the RM group to 0.79 ± 0.9 in the Ama group.

Table 5: Statistics of average particle size and circularity of the wastewater from DP 1 obtained with light microscopy and processed with ImageJ (RC = Resin composite, GIC = Glass ionomer cement, RM = Resin-modified composite, Ama = Amalgam).

	RC	GIC	RM	Ama
Total number of images used for analysis (FOV 18)	215	207	51	101
Mean particle area (± SD) [µm²]	9.2 (± 15.9)	13.1 (± 21.6)	12.7 (± 21.4)	10.6 (± 17.9)
Median particle area (± SE) [µm²]	3.9 (± 0.1)	5.2 (± 0.1)	4.8 (± 0.2)	4.5 (± 0.2)
Mean circularity index (± SD)	0.72 (± 0.8)	0.75 (± 0.8)	0.66 (± 0.7)	0.79 (± 0.9)

4.3.3 Particle size and characterisation of DP1, DP2 and DP3

The size distributions of particles from ≥ 1.2 to ≤ 150 µm^2 in size from the three dental practices were observed to decrease in frequency with increasing particle size. Consistently for each DP, the largest volume of particles was in the size category between 1.2 to 5 µm². Figure 21 shows these trends as size distributions based on percentage of each particle size class. The size class 1.2 – 5 µm² included the highest percentages of particles for all three dental practices with 53 % of particles in DP1, 66 % of particles in DP2 and 64 % of particles in DP3 falling into that category (Fig. 21). Between 17 to 21 % of the particles were in the size class 5 – 10 µm², and between 14 to 22 % of the particles were in the size class between 10 – 50 µm². Percentages below 3 % were recorded for particles sized 50 – 100 µm² and percentages below 1 % for particles sized 100 – 150 µm² (Fig. 21).

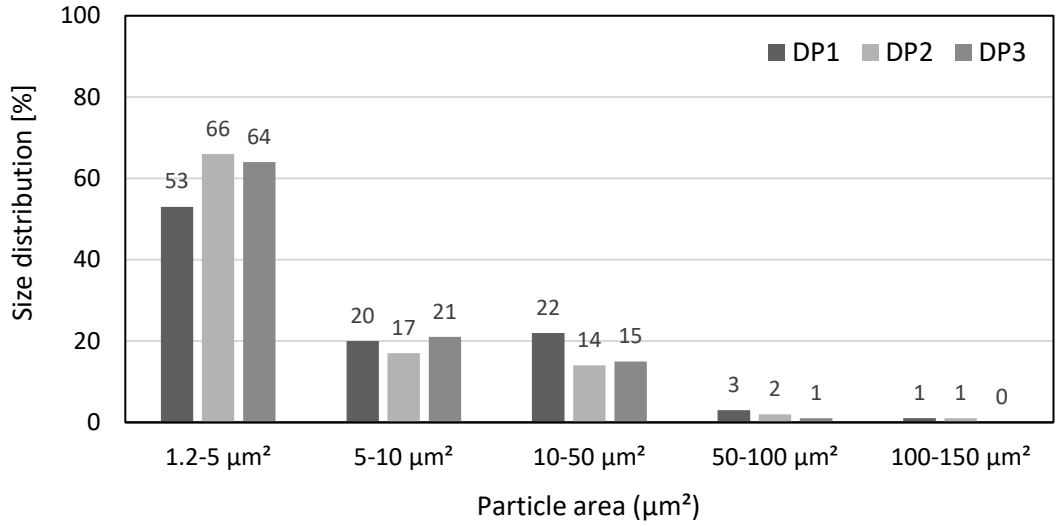


Figure 21: Size distribution [%] of particles between particle area of 1.2 and 150 µm^2 in the wastewater from three dental practices: DP1 (n=19), DP2 (n=6) and DP3 (n=2).

On a more detailed level, size distribution curves based on particle area (µm²) showing the abundance of particles as total particle count and as relative percentages were computed for particles in the range from ≥ 1.2 to ≤ 150 µm^2 in size (Fig. 22). The declining trend in abundance as particle area size increases that was observed in Fig. 21 can be explored in more detail in Fig. 22.

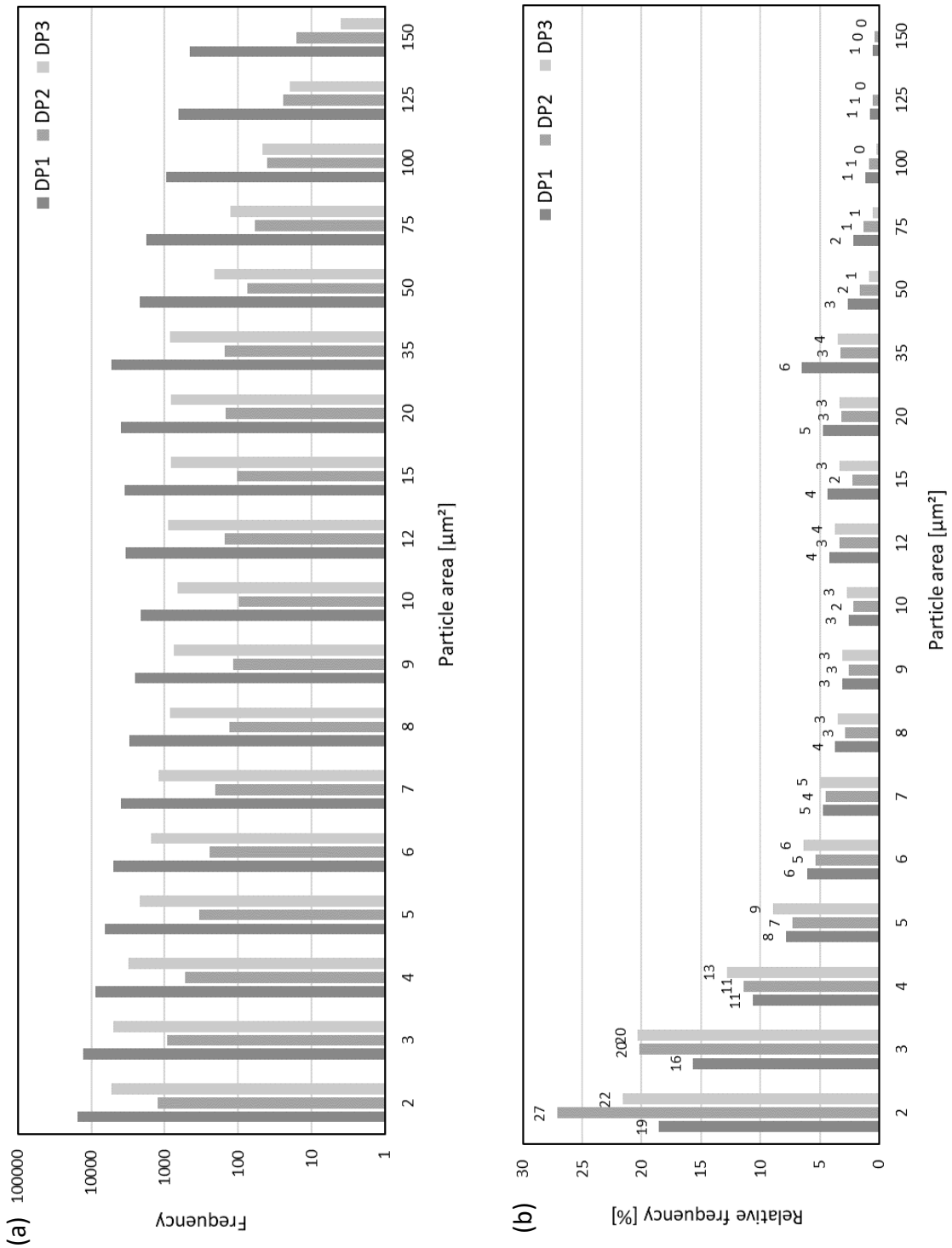


Figure 22: Histograms of particle sizes based on particle area [µm²] showing (a) frequency as count and (b) frequency as relative percentage [%] on a scale from 1.2 to 150 µm² released from the wastewater of three dental practices: DP1 (n=19), DP2 (n=6) and DP3 (n=2).

The highest abundance of particles, which was consistent for each bin width, was captured in DP1 (n=19) (Fig. 22a). DP3 (n=2) showed the second-highest abundance of particles and DP2 (n=6) the lowest abundance. These size distributions were computed as the relative percentage of each bin width of the histogram and compared in Fig. 22b. The relative percentages were calculated per DP, meaning that a percentage of 20 % shows, that 20 % of the particles of the corresponding DP were of that size category.

As a relative percentage, DP2 showed the highest abundance of particles sized between 1.2 and 2 µm² with 27%, whereas DP2 recorded 22 % and DP1 recorded 19 % of the particles to be within this size range. In the size range between 2 and 3 µm², DPs 2 and 3 both recorded 20 % and DP1 recorded 16 % of the particles to be within this size range. As the size range increased to 4, 5 or more µm², the three DPs relative percentages of particle abundance could be observed to become increasingly similar until in the 8 to 9 µm² size range, all three DPs recorded 3 %. As particle area increased further, the relative percentages of particles in DP2 and DP3 became increasingly less until at 150 µm² no more particles of that size category were observed. For DP1, however, abundance increased as particle area increased to record up to 6 % relative percentage in the size range between 20 and 35 µm² but declined again as particles neared 150 µm² in size.

All three DPs showed an overall trend of particle abundance decreasing as particle area size decreased, which was observed in Figs. 21 and 22. However, the particles in the size range from 1.2 to 4 µm² in DP1 showed a unique trend, which was not observed in DPs 2 and 3. Fig. 23 shows that the particles in DP1 had a high abundance of particles (count above 1,000) for very specific particle sizes such as 1.26, 1.31, 1.46, 1.51, 1.60, 1.75, 1.76, 1.90, 2.01, 2.04, 2.19, 2.27, 2.33, 2.48, 2.52, 2.77 and 3.02 µm², but low abundance (below 10) for particle sizes in between these. This trend ebbs off as the particle area of the particles increases.

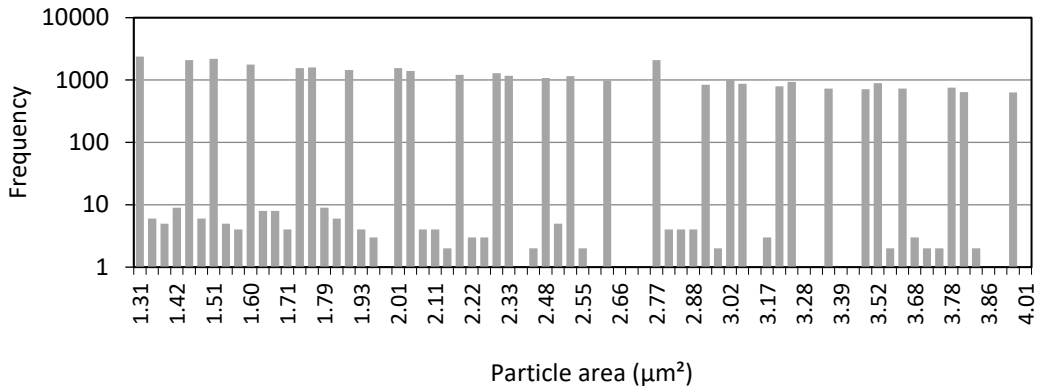


Figure 23: Histograms of particle sizes based on particle area [µm²] on a scale from 1.2 to 4 µm² released from the wastewater of DP1 (n=19).

The DP1 results were again divided by treatment groups (RC, RM, GIC and Ama), to allow for a more detailed analysis. Figure 24 below shows the particle size distributions on a scale from 1.2 to 150 µm² for the four treatment groups.

Fig. 24a shows that within DP1, the RC and the GIC treatment groups released the highest frequency of particles overall and the RM treatment group the overall lowest. Moreover, the RC and GIC group show a bimodal trend of high abundance of particles sized between 1.2 and 4 µm² and those sized between 12 and 50 µm².

Observing these trends as relative percentages for each treatment group shows that the RC group has the overall highest abundance of particles sized between 1.2 and 2 µm² with 22 % of the particles of the RC group in this size range. The RM, GIC and Ama group follow closely with 17 – 18 %. The bimodal trends observed in the particle count can also observed in the relative percentages, however, the bimodal behaviour is most prevalent for the RM and GIC group and the second peak is located in the size range from 20 to 35 µm².

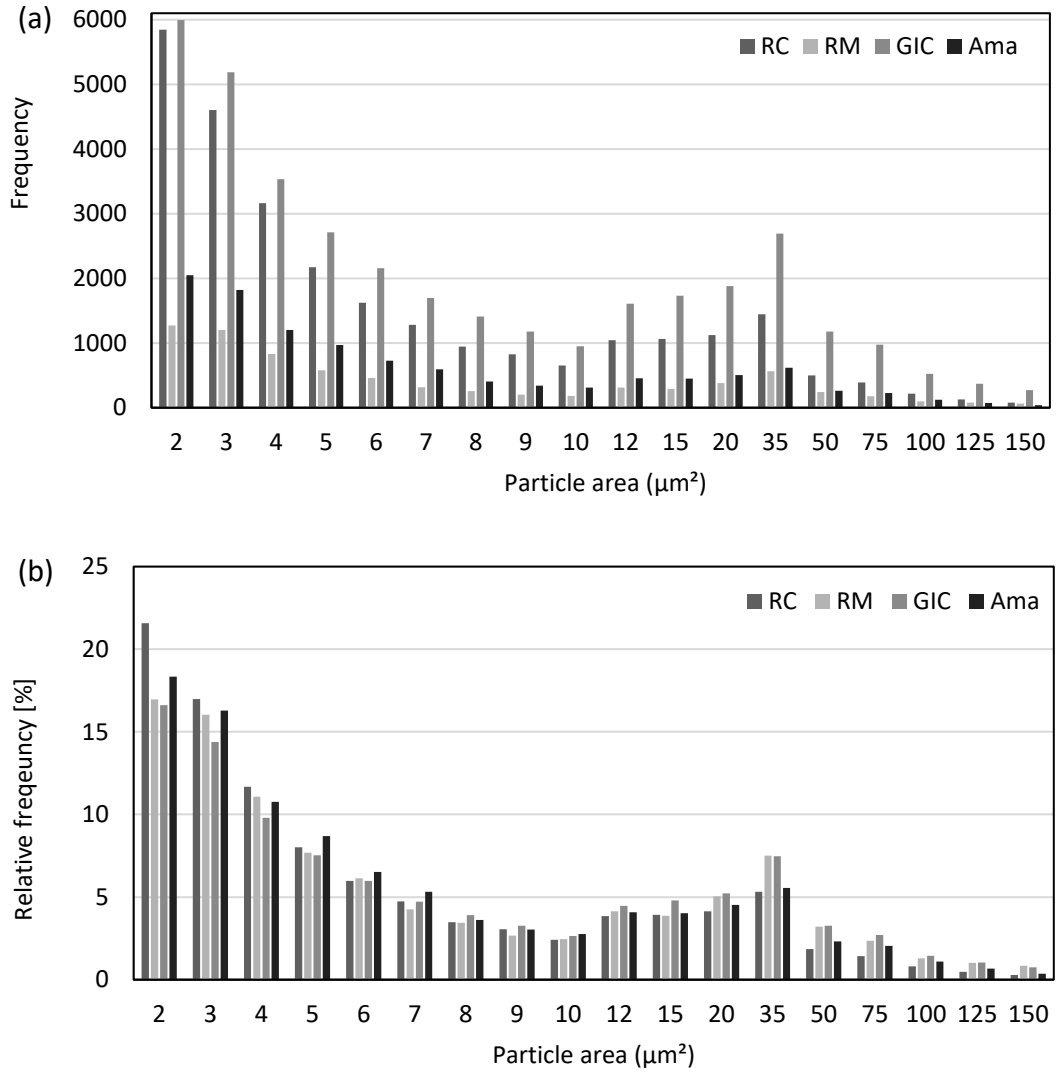


Figure 24: Histograms of particle sizes based on particle area [µm²] released from the wastewater of DP1 (n=19), divided by treatment group RC = Resin composite, RM = Resin modified glass ionomer cement, GIC = Glass ionomer cement and Ama = Amalgam, showing (a) frequency as count and (b) frequency as relative % on a scale from 1.2 to 150 µm².

The circularity index of the three DPs is given as a frequency table (count and %) in Table 6. Particles with a circularity index between 0.91 and 1.0 were most abundant across all three DPs in both count and relative percentage. A brief exploration of correlation plots showed that a weak relationship between high circularity index and small particle size exists, and that circularity decreases as particle size increases. Table 6 highlights that around 70 % of the particles released from DP 1, 2 and 3 have a circularity index of 0.7 or higher.

Table 6: Frequencies of circularity index [in count and %] of particles released via the wastewater of three dental practices: DP1 (n=19), DP2 (n=6) and DP3 (n=2).

Circularity index	Frequency [count]			Relative frequency [%]		
	DP1	DP2	DP3	DP1	DP2	DP3
0.1	717	27	30	1	1	0
0.2	1674	113	61	2	2	0
0.3	2975	278	386	4	6	2
0.4	4519	321	884	6	7	4
0.5	5902	342	1429	7	7	6
0.6	7157	405	1938	9	9	8
0.7	8241	393	2421	10	9	10
0.8	9878	449	2890	12	10	12
0.9	11706	598	3834	14	13	16
1.0	29053	1684	10452	36	37	43

4.3.3 Particle characterisation using the Scanning Electron Microscope (SEM)

Figure 25 shows sample material from DP1 that was imaged using the JEOL JSM-IT200 InTouchScope™ SEM. These materials have been either placed in or removed from a patients' tooth and been flushed through the dental chair system and have passed through the amalgam separation unit before being caught by the wastewater collection container. These particles have likely undergone drilling, grinding and polishing procedures that were carried out by the dentist when placing or removing these fillings. Figure 25 shows that these particles have ragged ends and sharp corners and different particle sizes can be observed ranging from 1 to 150 μm in size.

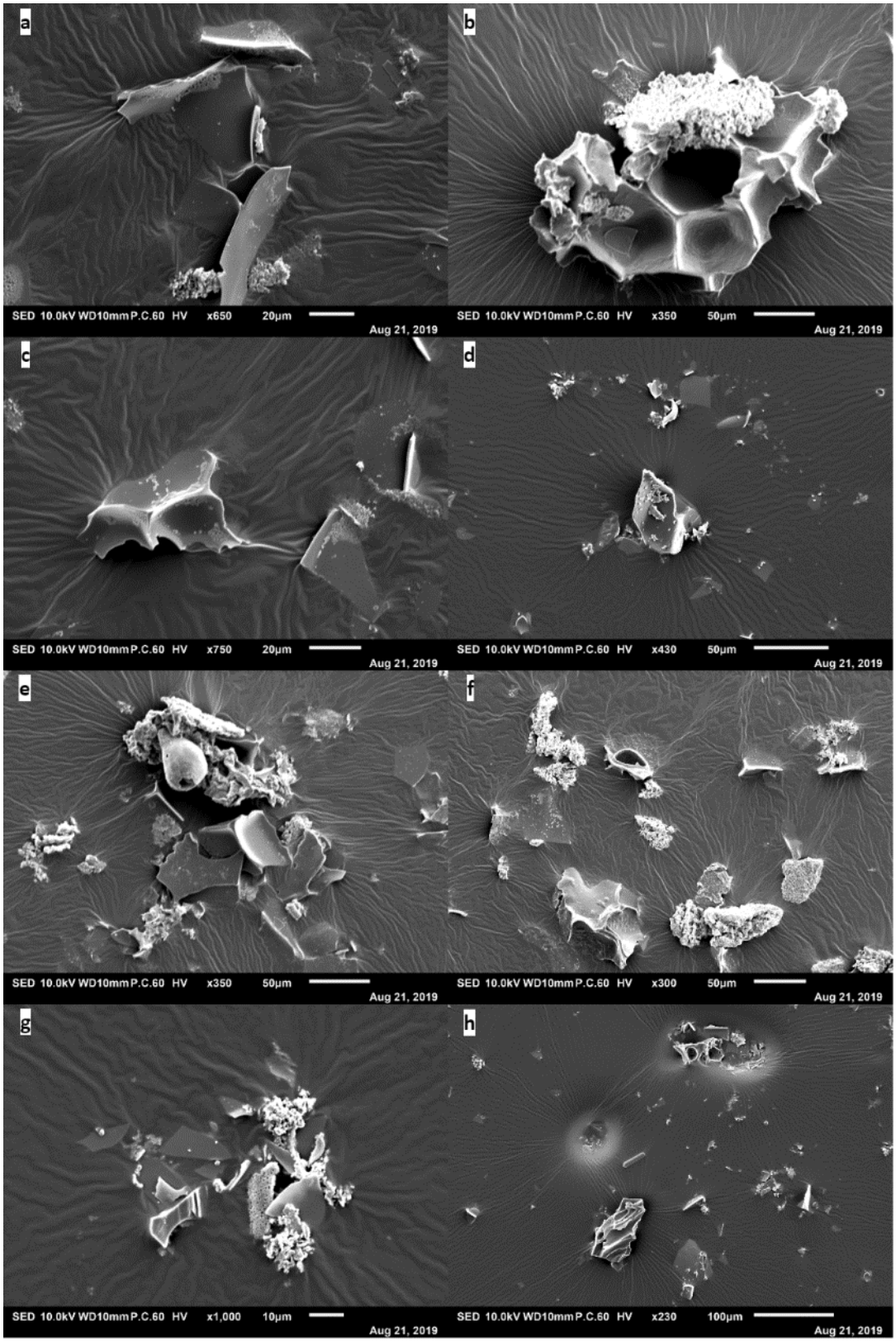


Figure 25: SEM images of particles found in the dental wastewater streams from DP1 after sample processing, imaged using the JEOL JSM-IT200 InTouchScope™ SEM.

4.3.4 Efficacy testing of the amalgam separator

When testing the efficacy of the amalgam separator to trap materials from Hg-free dental filling materials, 1.00 g of Ceram X, 0.78 g of SDR flow^{+}, 0.92 g of Filtek and 0.88 g of XRV Herculite was removed from the cured dental filling material through drilling and polishing and fed through the dental chair system. A total of 5L of wastewater was collected during this test. Figure 26 shows the particle curve of material that was detected using the light microscope from 8 images based on particle area (μm²) and frequency of occurrence of each particle size. A total of 1,128 particles were detected of which 44 % of the particles were between 1.2 and 5 μm² in size, a further 18 % were between 5 and 10 μm² in size, 33 % were between 10 and 50 μm² in size and 5 % were above 50 μm² in size. Analysis of the circularity index showed that particles of the efficacy test had a mean and median circularity index of 0.68.

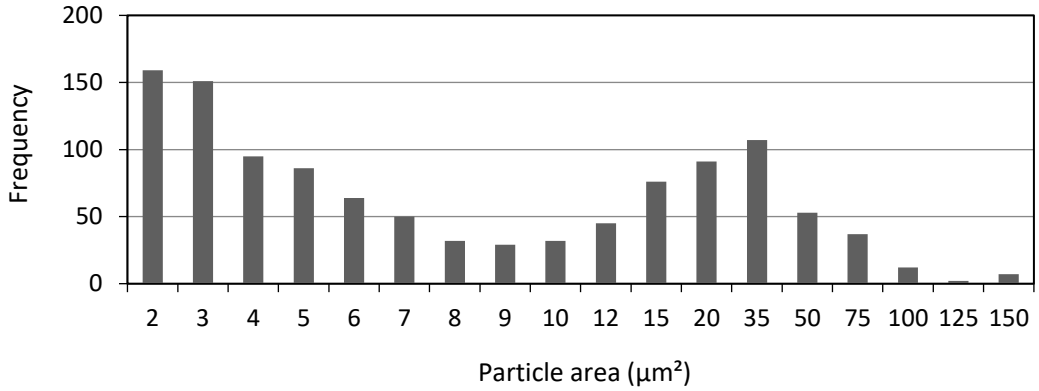


Figure 26: Particle size distribution of particles detected during efficacy testing of the amalgam separator using 4 commonly used Hg-free dental filling materials: Ceram X, SDR flow^{+}, Filtek and XRV Herculite.

4.4 Discussion

The particulate matter resulting from the use of Hg-free dental filling materials within the DWW of three dental practices was assessed in terms of particle load, size and characterisation. The results have shown that consistently for each DP, a high number of small particles with high circularity index were discharged as part of the wastewater. Moreover, as particle size increased, the frequency of occurrence decreased, concurrent with a decrease in circularity. Findings like these were expected due to amalgam separators being utilised to largely capture particles above 500 µm in size. Figure 27 shows the size distributions of the test slurry that is used to conduct the ISO standard 11143:2008 testing of amalgam separators to ensure 95 % efficiency to trap amalgam particles contained in the test slurry. The test slurry was explored in detail in *Section 1.3* and included an account of the mass fractions of each particle size (30 % mass fraction with particles equal to or less than 100 µm in size, a 10 % mass fraction with particles between 100 and 500 µm in size and a 60 % mass fraction with particles between 500 and 3,150 µm in size). Figure 27 shows these mass fractions in comparison with the relative size distribution % of each of the particle size classes obtained from the results from the three DPs as a DP average.

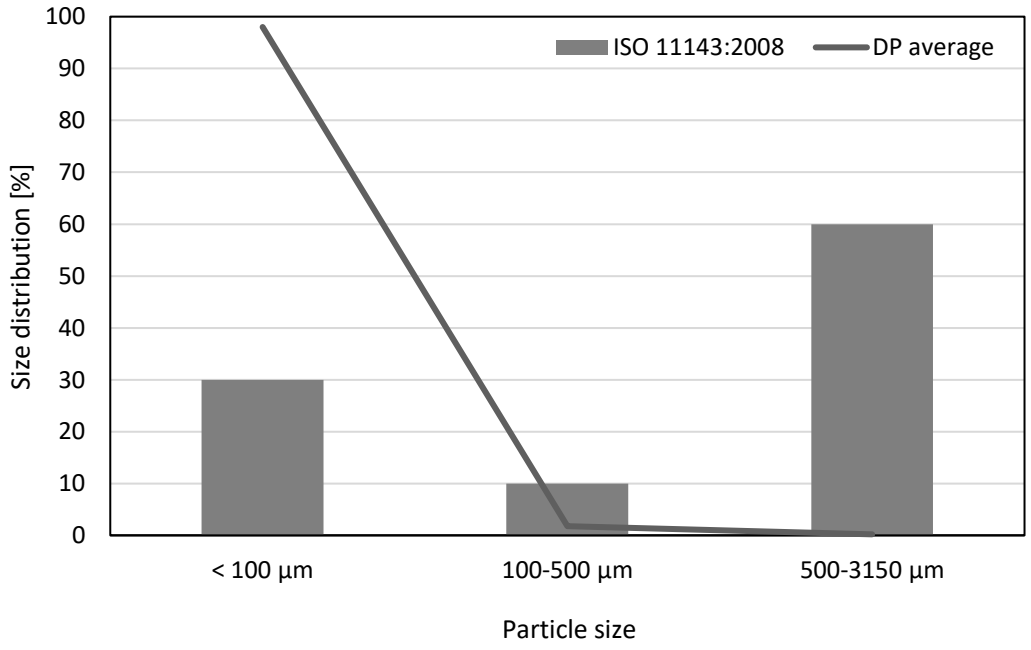


Figure 27: Particle size distribution comparison of DP average size distribution [%] compared to the ISO 11143:2008 test slurry particle size distribution.

The comparison illustrates that the amalgam separators are ISO certified under the assumption that the highest number of particles are larger than 500 µm. The particle results have shown that particles from Hg-free dental filling materials that remain in the wastewater after amalgam separation has taken place are largely (at least 97 %) below 100 µm² in size (equivalent to particles sized 10 x 10 µm), which, under ISO testing only makes up 30 % of the mass fraction. Other studies have also found that the particle fraction of dental amalgam consists to 90 % of particles in the size between 10 and 700 µm (Cailas *et al.*, 2002), which differs significantly to the size distribution that is used for the current ISO certification process.

Another important finding was that while on average, DP1 had the highest mean particle area with 11.4 (± 19.4) µm² in size (Table 4), as a relative percentage, DP1 showed a similar size distribution as DP2 and DP3 (Fig. 22). The same was found for overall abundance of particles: even though DP1 showed an overall higher count, as a relative percentage, DP1, 2 and 3 showed the same trend of high abundance of particles sized between 1.2 and 2 µm² in size, with DP2 showing the highest abundance (27 %), and a declining trend in abundance as particle area increased (Figs. 21 and 22).

Within DP1 it was found that the largest particles, on average, were released as a result of the use of glass ionomer cements and resin-modified glass ionomer cements, which were 13.1 (± 21.6) µm² and 12.7 (± 21.4) µm². In terms of amalgam particle capture, Table 5 has shown that after amalgam use, particles with a mean size of 10.6 (± 17.9) µm were captured and an abundance of up to 2,000 particles in the size range between 1.2 and 2 µm² were recorded in Fig. 24. This count was lower than the other treatment groups, but it was found to be higher than expected due to the amalgam capture rate of 95 % by the amalgam separator.

The circularity index results given in Table 6 showed that around 70 % of the particles had a circularity index between 0.7 and 1.0, which corresponds to an elliptical to round shape. Particles of that shape likely correspond to fillers as seen in Fig. 20, while the low abundance of elongated/angular shapes likely corresponds to material broken off during dental procedures with the dental drill and polishing disks. The SEM images in Fig. 25 showed some of these angular particles.

When testing the amalgam separator capture efficiency in DP1 without patient contact, on a clean dental chair, similar results to those in the real-life application in DPs 1, 2 and 3 were obtained. However, the relative percentage for particles between 10 and 50 μm² was 10 – 15 % higher and between 2.1 – 5 μm² was 10 – 15 % lower than those observed in the three DPs. From revising sampling protocols, this was likely due to utilising high-speed drilling of material during testing at a much higher rate than the polishing disks. Nonetheless, this confirms that the amalgam separator did not trap these particles that resulted from the use of Hg-free dental filling materials. Moreover, the mean circularity index of these particles was found to be 0.68, which was in line with the circularity index observed during sampling in the three DPs.

Limitations of this study were given by the sample design. Due to the non-uniform nature of particles, results are often shown as a distribution plotting particle quantity versus particle size (Hogg, 2008). In an ideal scenario, sampling size would have been based on a 95 or 99.9 % CI (Confidence Interval) with a sample size of $n > 30$ per location (Allen, 1997). However, due to time limitations of this study, this requirement could not be met which is why it is recommended to repeat this study on a larger scale and in other locations to gain a better understanding of the particle load that is discharge by the dental practices. Moreover, there are limitations when reducing sampling variation caused from the use of many different dental materials in dental practices. Therefore, an improved study design would be to designate each sampling day with only one type of dental material used per dental chair. This would allow isolating of samples from one another and hence more detailed analysis of the components and particle sizes associated with each material. However, as was the case with this study, there is always a potential of material from previous procedures that is lodged in the dental chair tubes and filters to become loose and therefore cause sampling error (Arenholt-Bindslev, 1998; Vandeven and Mcginnis, 2005). In this study it was chosen to conduct a study that was reproducible in different dental practice scenarios and potential errors were identified and kept small, with the aim of the results to be of high accuracy and high precision. Further testing would need to confirm the accuracy of the data as the actual particle size resulting from the use of Hg-free dental filling materials in the wastewater was previously unknown but was attempted to be assessed in this study for the first time.

The particle detection, sizing and characterisation presented a set of challenges. Common methods used are sieving, sedimentation, microscopy and the use of automatic particle analysis instruments. All of these were considered in detail.

Sieving of the samples was considered unsuitable for this study due to the potential of damaging or wearing down of and therefore altering of the size and shape of particles in the process. Moreover, issues of reproducibility as well as effects of irregularity due to particles not being perfectly round are well known disadvantages of sieving and other particle sizing methods (Hogg, 2008). Sedimentation and settling rates were also considered as these offer reproducible results. However, the materials that were analysed in this study have not been previously tested and important information such as the particle density and their solubility were unknown (Hogg, 2008). The material in this study was found to settle out to some extent in the course of a few hours but some fractions also partly remained suspended after several days as well as remained floating on the surface of the wastewater.

The Coulter counter was tested as a method of automatic particle sizing. Sheldon and Parsons (1967) have described the use of the Coulter counter in marine application to be profound due to offering particle size results of samples that are not only at low concentrations but also stretch across a wide size range, which commonly makes analysis of these samples difficult. Their published manual describes the adjustments that were made in a model B Coulter counter in order to obtain the sizing results for marine samples (Sheldon and Parsons, 1967). It was assumed that these adjustments may aid this study in detecting a particle size distribution of particles in the wastewater resulting from the use of Hg-free dental materials because the samples, like the marine samples described, were expected to contain low concentrations of particles overall and similarly were expected to include a wide range of particle sizes from the micron to sub-micron range. The Coulter counter model used during this study was the Z2 model, manufactured by Beckman, fitted with a 100 µm aperture. During operation, the particle volume (sphere diameter) is determined by the machine through a change in resistance as the particles pass through an aperture that is suspended in electrolyte solution that contains the sample. It was expected that with the fitted 100 µm aperture a particle range between 2 µm and 60 µm could be detected as the practical working limit is restricted to within 2 to 60 % of the aperture diameter (Beckman Coulter Inc, 2002). The size range of the Coulter counter is restricted by the aperture and adjusted

through the gain and current settings. The first set of tests were carried out between the size ranges of 3.5 and 8 (3.432 and 7.985) µm under a gain of 256 and a current of 0.250. Count values were obtained by the Coulter counter for every increment and given as the mean value of each particle size in a range between 3.5 and 8 µm. Baseline testing was carried out using 20 mL of Isotone II Diluent, manufactured by Beckman, with the aim to keep the baseline count below 50. During baseline testing it was found that baseline counts did not persistently show values below 50 and at times reached over 1'000 when using the Isotone blank. Generally, the Coulter counter is considered to give results that represent the true volume of the particles at a wide range, which is, however, restricted by the sample: porous, very small or too large particles can all cause issues. Moreover, light microscope images showed biological growth in the samples. Therefore, it was identified that the Coulter counter is not suitable for the analysis of particles in DWW.

The Malvern Mastersizer 3000 was identified as an alternative particle sizer to the Coulter counter. Commonly used for sediment samples, the Malvern Mastersizer is expected to give results on the particle size in the micron range. This particle sizer conducts baseline testing as part of the start-up procedure of the machine and software and therefore eliminated the baseline issues that were encountered with the Coulter counter. However, when analyses on the DWW were conducted, results showed a higher occurrence of larger particles and only few smaller particles, which is likely due to aggregates forming. From light microscopy images this was known to be an incorrect representation of the data. Additionally, cross-contamination from sediment samples that were regularly processed in the Malvern could not be excluded. Therefore, it was concluded that the Malvern is also not a suitable means for analysis of the particles within DWW.

The final methodology used light microscope and scanning electron microscope images to determine the particle size. Microscopy is considered more accurate due to several factors. For one, particle size is measured on an absolute scale (from zero to infinity) and agglomerated particles are visible and can be distinguished using specialised software. Through image analysis in Image J multiple parameters can be inferred from these images. When utilising a light microscope for image acquisition, the diffractive index plays an important role. As the light interacts with the specimen, some light is diffracted (one-half wavelength out of phase) which causes destructive

interference with the direct light at the objective rear focal plane (Rottenfusser *et al.*, 2018). This effect is magnified by the eye lens resulting in the image that is received by the retina and image acquisition sensor to show a spectrum of grayscale values, which combine to form a representative image of the original specimen (Rottenfusser *et al.*, 2018). According to Ernst Abbe, the developer of optical instruments in the 19th century, the image is more accurately represented if at least 2 orders of light and 1 order of diffraction are captured by the objective; higher numeral aperture objectives capture more diffracted light (Rottenfusser *et al.*, 2018). This is important when analysing particles, as it allows for them to be distinguishable from one another and for higher resolution (Rottenfusser *et al.*, 2018).

The use of image acquisition and analysis through image processing software has long been established and is commonly utilised for the detection and characterisation of (nano-) particles (Defante *et al.*, 2018; Orts-Gil *et al.*, 2011; Pyrz and Buttrey, 2008). There are a number of errors that apply to particle size analysis such as: limitations of the instrument and errors due to procedure, the operator or the sampling design (Allen, 1997) as well as issues with data acquisition, presentation and interpretation (Hogg, 2008). One limitation is the depth of focus being restricted by the magnification (e.g. a magnification of 100 is restricted to ~10 µm). Moreover, the diffraction effects can cause blurring of the edges and make results below 3 µm less reliable. When acquiring a 2D image of a 3D object, a certain measure of error is expected due to the nature of the procedure. Image processing programmes, such as ImageJ, utilise code to find the most accurate representation of each particle. The code used to analyse particles in ImageJ is contained in a 2D Region of Interest (ShapeRoi.java) file (Tigaret, 2012; Rasband, 2018) that constructs the shape based on the basic shapes of rectangle, oval, line and polygon flattened at 0.1 [in units of calibration, either pixel or µm] and the size based on the shape approximated by straight lines (vertices) or curve segments that were arranged around each particle (Tigaret, 2012). These curve segments have a maximum tolerance of 1.0e-3 (Tigaret, 2012). Another factor is that biological material has the potential to falsify the results because the ImageJ software does not distinguish between particles of biological or non-biological nature. In order to obtain clear light microscope and SEM images, biological material needed to be removed from the sample material using bleach.

In order to detect particles in the sub-micron and the nano-size range the Scanning Electron Microscope was used. Rather than light, this type of microscope uses electrons at high acceleration to obtain images of a sample. Limitations of SEM imaging are that the vacuum chamber is quite small and can therefore only analyse small samples that require specialised sample preparation. Therefore, SEM images were used on a small scale, in order to assess particle in the sub-micron and nano-size range and to aid the characterisation of these smaller sized particles, but not for quantification purposes.

The light and scanning electron microscope images that were obtained for this study were calibrated and attention was given to validate these results. However, the study could have benefitted from additional particle size analysis to validate and confirm the data. While sieving and automated particle sizing were considered for this study, in testing they were found unsuitable. However, future studies could highly benefit from utilising particle sizing techniques such as those employed by pharmaceutical science, for example, which uses particle sizing such as laser diffraction or FBRM (Focused beam reflectance measurement) for the manufacture of powders and granule particles in the micron size-range and often requires real-time results (Kumar *et al.*, 2013). For nanoparticle analysis, NTA (nanoparticle tracking analysis), a type of light scattering of the sample in liquid suspension, has shown to offer accurate and reproducible results, if the protocol is adhered to (Hole *et al.*, 2013). These methods often incur high costs but offer high performance and would greatly aid the validation of the results, as well as strengthen the conclusions drawn.

4.5 Conclusions

The particle results have shown that a substantial particle load passed through the amalgam separation units of Type 1 and 2 in the three tested dental practices. This particle load was shown to be highest as a result of the use of glass ionomer cements and resin-modified glass ionomer cements with a mean particle size of 13.1 (± 21.6) μm^2 and 12.7 (± 21.4) μm^2. Particle size distributions confirmed that most of these particles were small (below 5 μm^2). Characterisation with image processing software has further shown that these particles are largely within a circularity index between 0.7 and 1.0, and likely results from the fillers contained in the dental materials while visual analysis with SEM images has shown that some of these particles, while

somewhat round, have sharp edges, which likely result from the use the dental drill and polishing disks and therefore present particles released during dental procedures.

Assessment of the efficacy of amalgam separation units to remove Hg-free dental filling materials has concluded that these are not equipped to capture the large quantities of particles that are released, which is due to dental amalgam producing much larger particles, but Hg-free materials producing largely particles below 100 µm in size, for which amalgam separators are only set out to catch 30 % of the particle load.

This study also concluded that further testing is needed to assess the accuracy of these results in different dental clinics, which potentially use different Hg-free dental filling materials.

Chapter 5 Ecotoxicity testing of DWW

5.1 Introduction

The previous chapters have assessed the physical and chemical parameters, including particle load of DWW streams arising from Hg-free dental filling materials. These results have highlighted the need to conduct ecotoxicity testing of these wastewaters in order to further assess if potential environmental impacts exist. Previous and current assessments of DWW streams mainly focused on dental amalgam as a source of contamination (Arenholt-Bindslev, 1998; Bjorklund *et al.*, 2017; Cailas *et al.*, 2002; Fan *et al.*, 1997). Disinfectants, line cleaners and materials trapped by the filters and screens within the dental chair form part of the additional biohazardous materials waste streams (Valli and Anand, 2015).

Ecotoxicity testing is used to assess the risk to the environment of a chemical or compound that is released into the environment (Walker, 2008). One such test is the acute toxicity test, which is carried out to gain an understanding of the dose-response of chemicals, waters and wastewaters (International Organisation for Standardisation, 2012). The test organisms are, in relation to freshwaters, preferably small, sensitive freshwater species with short life cycles and high reproductive output (Dehghani *et al.*, 2019). The crustacean, *Daphnia magna* Straus, a water flea, is one such species and has been used for ecotoxicity testing for many years (International Organisation for Standardisation, 2012). There are two relevant documents that outline the standardised testing guidelines for Daphnia acute immobilisation testing: OECD guideline 202 and ISO standard 6341:2012.

The ISO standard includes more stringent guidelines compared to the OECD guideline, with respect to defining the growth medium to be used, a maximum specified time between sample collection and testing and the requirement that a sensitivity test using $K_2Cr_2O_7$ be carried out within one month of the test period (International Organisation for Standardisation, 2012). After reviewing the OECD 202 guideline and the ISO 6341:2012 standard, it was decided to use the OECD 202 guideline for the purposes of this study, due to the use of a different growth medium, which was only permitted for the OECD 202 guideline.

5.2 Materials and Methods

5.2.1 *Daphnia* Culturing

In line with the OECD protocol and to carry out testing, a single female of *Daphnia magna* Strauss was isolated to ensure that the daphnids were all from the same stock. This single female was captured from a small pond on the grounds of Trinity College, Dublin, Ireland in May 2019 using a plankton net. The first 15 young daphnids (neonates) were used to form the first culture. Growth of the neonates was monitored to ensure the growth of a healthy stock that produces the first brood on day 7, the second brood on day 9, the third brood on days 12 to 14, the fourth brood on day 17 and the fifth brood on day 21 (Heckmann and Connon, 2007). Subsequent cultures were produced from age-synchronised adults from the 3rd to 5th brood as recommended (Heckmann and Connon, 2007). Neonates of the 2nd, 3rd, 4th and 5th brood progeny of subsequent *Daphnia* cultures were used for the acute immobilisation tests in order to minimise clonal variations (OECD, 2014; Heckmann and Connon, 2007). Neonates from the 1st to 5th brood were used for range-finding testing. Range-finding tests were carried out in order to determine the test concentrations by exposing five daphnids per vessel to the test substance at widely spaced concentrations for 24 or 48 hrs until data on 100 % immobilisation of the highest concentration and no observable effect in the lowest concentration could be obtained (OECD, 2014). The cultures were kept in 2 L inert plastic buckets that were filled to the 500 mL mark with growth medium (as specified below).

D. magna requires very specific growth conditions for ecotoxicity testing. These growth conditions include the growth medium, the food supply, light and temperature conditions, which are detailed in the following.

5.2.1.1 Daphnia growth medium

D. magna were grown in controlled conditions using the artificial *Daphnia* medium ADaM (Aachener *Daphnia* Medium) (Klüttgen *et al.*, 1994). The medium was made up of sea salts and three stock solutions (detailed below) that were added to distilled water. Distilled water was produced by a Laboratory Glass Water Distiller with a distillate resistivity in the range of 0.25 - 0.30 MΩ-cm. The sea salts and stock solution reagents were sourced from Sigma Aldrich (product codes S9883, 31307,

S5761 and 325473). Sea salts were added at a concentration of 0.333 g/L. Stock solution A was prepared at a concentration of 117.6 g/L $CaCl_2$ x 2 H_2O, stock solution B at a concentration of 25.2 g/L $NaHCO_3$ and stock solution C at a concentration of 0.07 g/L SeO_2. For every 10 L of distilled water, 3.33 g of sea salts, 23 mL of stock solution A, 22 mL of stock solution B and 1 mL of stock solution C were added.

The ADaM growth medium was replaced incrementally at 20 to 30 % every 2-3 days to reduce stress caused by rapid changes in growth conditions. The culturing containers were cleaned weekly using Lipsol® detergent (sourced from Fisher Scientific, product code: 12549965) and triple rinsed with distilled water.

5.2.1.2 Daphnia food supply

Batch cultures of algae (*Chlamydomonas reinhardtii*) were grown and used to feed the daphnids. These algae cultures were grown in a laboratory set-up using chemostats that contained seven stock solutions (Ebert, 2012). Ebert (2012) developed this medium to grow the green algae *Scenedesmus*, but it was found to also successfully grow *Chlamydomonas reinhardtii*. The algae growth medium was made up using the seven stock solutions and concentrations listed in Table 7, of which all compounds were sourced from Sigma Aldrich and Honeywell.

The stock solutions were added in the order A to G (Table 7) to 20 L of distilled water. This medium reservoir was then placed in the autoclave for six hours, cooled overnight and connected to a pump that operated between 3 to 6 rpm. From the medium reservoir the medium was pumped to the chemostats after passing through 0.2 µm filters. Also connected to the chemostats were air pumps that operated 24h/day. Effluent tubing within the chemostat led to a collecting receptacle into which flowed the algae that were to be used for feeding.

The C (carbon) concentration in mg/L was detected from each algal batch culture using a spectrophotometer (Shimadzu UV mini- 1240). To do so, between 1 and 2 L of algal batch culture (exact volumes depended on the amount of food produced by the chemostats) was centrifuged at 3000 to 4000 rpm for 3 min in 50 mL centrifuge tubes in an Eppendorf 5810 centrifuge every few days. Of each tube, the supernatant (algae medium) was discarded and the algae “cake” was added to 500 mL of ADaM medium.

Table 7: Concentrations of stock solutions used for the growth of batch cultures of algae (*Chlamydomonas reinhardtii*) for the feeding of *Daphnia magna* Strauss (Ebert, 2012).

Stock solution	Chemical compound	Concentration [g/L]
A	$CaCl_2$ x 2 H_2O	36.8
B	$MgSO_4$ x 7 H_2O	37.0
C	$NaHCO_3$	12.6
D	K_2HPO_4 x 3 H_2O	11.4
E	$NaNO_3$	85.0
F	Na_2SiO_3 x 5 H_2O	21.2
G	NaEDTA	4.36
	$FeCl_3$ x 6 H_2O	3.15
	$CuSO_4$ x 5 H_2O	0.010
	$ZnSO_4$ x 7 H_2O	0.022
	$CoCl_2$ x 6 H_2O	0.010
	$MnCl_2$ x 4 H_2O	0.180
	Na_2MoO_4 x 2 H_2O	0.006
	H_3BO_3	1.00

The algal food medium was then analysed for C content in the spectrometer by analysing a well-mixed aliquot. The spectrometer was calibrated with an ADaM blank at a wavelength of 800 λm and the absorbance was recorded for the algal food medium. Based on the absorbance, the amount of algal food needed to feed the daphnids 1 mg of C/day was calculated according to the following three formulas:

From initial testing that established a stable C concentration of different dilutions of food at 10, 20, 40, 50, 60, 80, 100 and 200 % the factors 174.2 and 1.4 were found to reflect a stable food concentration in mg/L. Hence, after using the ADaM blank, the spectrometer results were added to the equation below.

$$[\text{Eq. 1}] \quad \text{Concentration of food} \left[\frac{\text{mg C}}{\text{L}}\right] = 174.2 * Spectrometer\ result + 1.4$$

The Food concentration in mg/L was then added to Eq.2 in which the 500 mL of ADaM medium used for dilution was used and the Total C [mg] in the food solution calculated.

$$[\text{Eq. 2}] \quad \text{Total C [mg]} = \frac{Concentration\ of\ food\ \left[\frac{mg\ C}{L}\right] * 500\ mL\ ADaM\ medium}{1000}$$

A third equation calculated the amount of food needed in mL of food solution to feed the adult daphnids 1 mg of C per day by multiplying the C concentration needed per day with the amount of ADaM used for dilution of the food and then dividing it by the total C concentration of the solution.

$$[Eq. 3] \quad Amount\ of\ food\ per\ day\ [mL] = \frac{1\ \frac{mg\ C}{day}\ *\ 500\ mL\ ADaM\ medium}{Total\ C\ [mg]}$$

Algae food was started at 0.5 mg of C/day for neonates 1-2 days old and increased to 0.75 mg of C/day as the neonates matured on day 3 to 7. Once adulthood was reached at day 8, the food was increased to 1 mg of C/day. When no testing was carried out over the weekend, food supply was increased by 50 % to 1.5 mg of C. Monitoring of the food supply ensured that the daphnids had a continuous population growth as stated within OECD guideline 202.

5.2.1.3 Light and temperature conditions

Halogen lights (Philips Master TL-D Reflex 36W/840) with a luminous flux of 3350 Lm and a peak in the red and green wavelengths (at 550 and 630 λ nm) were left on in a temperature-controlled (CT) room for 24 hours every day to maintain growth of the algal food. For testing, the test vessels were covered in order to carry out the tests in complete darkness. The temperature was kept stable at 22 ± 1°C.

5.2.2 OECD Standard testing

Acute 48 h immobilisation tests using *D. magna* were carried out in line with OECD guideline 202 for testing of chemicals in order to obtain EC_{50} values for the DWW from DP1, DP2 and DP3 (OECD, 2014). The EC_{50} is defined as the concentration of the test substance (DWW) that results in an acute immobilisation response in *D. magna* halfway between the baseline and the maximum concentration after an exposure duration of 48h.

5.2.2.1 Range-finding tests

Several range-finding tests were carried out before the acute immobilisation tests were conducted. The aim of the range-finding tests was to determine the highest test concentration, which should result in 100 % immobilisation, as well as the lowest test concentration, which should result in no observable effect (OECD, 2014). It was necessary to carry out separate range-finding tests for the wastewater resulting from each clinic due to their different particle load, which was reflected in the TSS and TDS measurements. The test concentrations and immobilisation results of the different range-finding tests are presented in the results section in Table 8.

5.2.2.2 Acute immobilisation testing

The immobilisation of young daphnids (from the 2^{nd}, 3^{rd}, 4^{th} or 5^{th} brood progeny and aged less than 24 hrs) in a range of concentrations was recorded after 48 hours. For each concentration at least 20 animals were used. These 20 animals were split into four groups of 5 animals each. The same procedure was carried out with the control, which consisted of four vessels with 5 animals each. A minimum of 2 mL of test solution per animal was used, which resulted in a minimum of 10 mL of test solution being used per vessel containing 5 daphnids. The test concentrations used were those specified through range-finding testing shown in Table 8. Tests were carried out over a period of 48 hours and in complete darkness. Neonates were transferred from the culture containers into test containers using a plastic pipette and special attention was given to transfer no food or growth medium from the cultures. Temperature, pH and dissolved oxygen (DO) concentrations were measured in the control group and the highest concentration test group before and after tests were

carried out to ensure the temperature was stable at 22 ± 1°C and to record any pH or DO fluctuations.

Temperature and pH measurements were obtained by submerging the conductivity and pH probes into the sample (WTW Handheld Conductivity meter 340i and OxyGuard Handy pH with software version 1.5). Calibration details of both probes were detailed in Section 3.3.2. The DO probe had an internal calibration (OxyGuard Handy Polaris 2).

Immobilisation of the neonates was recorded by gently agitating the test vessels and recording any neonates that remained immobilised 15 s after agitation had occurred. Tests were considered valid when immobilisation in the test groups was < 10 %.

EC_{50} values were estimated from the dose-response curves and calculated using Graph Pad Prism (software version 8.2.1). For the calculations in the Prism software, 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).

5.3 Results

The initial range-finding test with DWW from DP1 was carried out with concentrations ranging from 4 to 50 % DWW/L of medium (Table 8). This range was observed to be too high to result in the required immobilisation information for the DWW from DP1, which is why a second range-finding test was carried out with much lower concentrations ranging from 0.01 to 4 % DWW/L of medium (Table 8). Testing with this range was repeated and found to achieve the required data of 100 % immobilisation of the highest test concentration and no observable effect in the lowest test concentration.

Range-finding tests for DP2 showed that concentrations as low as 0.04 % DWW/L of medium resulted in a 100% immobilisation response. However, to keep testing consistent, the same range of test concentrations ranging from 0.01 to 4 % DWW/L of medium was used for ecotoxicity testing of DP2 and DP3 using *D. magna*.

Table 8: Concentrations [% DWW/L medium] used for (24h/48h) range-finding tests for *D. magna* acute immobilisation testing of DWW showing initial range-finding test concentrations from 4 – 50 % DWW/L medium and the adjusted concentrations from 0.01 – 4 % DWW/L medium, resulting in no/low effect at the lowest test concentration and 100 % immobilisation at the highest test concentration.

Initial conc. [% DWW/L medium]	% immob.	Adj. conc. [% DWW/L medium]	% immobilisation			
	DP1 sample 1 (48h)		DP1 sample 1 (48h)	DP1 sample 3 (24h)	DP1 sample 4 (48h)	DP2 sample 1 (48h)
0	0	0	0	0	0	0
4	100	0.01	0	0	0	0
8	100	0.02	0	60	0	0
12.5	100	0.04	0	80	0	100
25	100	0.1	0	100	0	100
50	100	0.2	0	100	0	100
		0.5	0	100	60	100
		1	0	100	0	100
		2	100	100	100	100
		4	100	100	100	100

The results of the acute immobilisation testing using *Daphnia magna* Strauss, after 48h exposure to different concentrations of DWW from three dental practices are shown in Table 9 below. The immobilisation % of each sample is given, as well as the estimated and calculated EC_{50} values and 95 % confidence intervals, if available.

The immobilisation % shows that for DP1 the immobilisation of 100 % of *D. magna* ranged from a concentration of 1 to 4 % DWW/L of medium, for DP2 it ranged from 0.1 to 0.2 % DWW/L of medium and for DP3 it ranged from 0.04 to 0.1 % DWW/L of medium (Table 9). Three of the samples from DP1 did not reach an 100 % immobilisation endpoint.

Estimated and calculated EC_{50} values were found to match closely for most cases (Table 9). Calculated EC_{50} values were obtained through processing in Graph Pad Prism (software version 8.2.1), which highlighted data as inconclusive when a best fit that included the values of all parameters could not be achieved and hence 95 % confidence intervals were very wide or unavailable. The EC_{50} values for DP1 ranged from as low as 0.01 to as high as 6.69 % DWW/L of medium. EC_{50} values for DP2 ranged from 0.01 to 0.04 % DWW/L of medium and DP3 showed an EC_{50} value of 0.01 % DWW/L of medium.

Table 9: Results of 48h *Daphnia magna* acute immobilisation testing according to OECD 202 standard methods using the wastewater from three dental practices (DP1, DP2 and DP3) showing % immobilisation results, calculated and estimated EC_{50} values and 95% Confidence Intervals (CI). (Data with an * were flagged by the software as inconclusive).

Conc. [% DWW/L medium]	% immobilisation											
	DP1 sample 1	DP1 sample 2	DP1 sample 3	DP1 sample 4	DP1 sample 5	DP1 sample 6	DP1 sample 7	DP2 sample 1	DP2 sample 2	DP2 sample 3	DP3 sample 1	DP3 sample 2
0	0	0	0	0	0	0	0	0	0	0	0	0
0.01	0	0	0	5	0	0	0	10	0	0	50	55
0.02	5	0	0	0	0	0	0	10	0	0	N/A	75
0.04	5	0	0	0	0	0	5	60	0	75	100	90
0.1	15	0	0	0	0	20	10	100	65	85	100	100
0.2	0	15	0	0	0	30	0	100	100	100	N/A	100
0.5	35	85	20	0	0	10	N/A	100	100	100	100	100
1	85	100	100	0	10	0	100	N/A	100	100	100	100
2	0	100	100	0	20	40	100	N/A	100	100	N/A	N/A
4	0	100	100	N/A	10	100	100	N/A	100	100	100	N/A
Estimated EC_{50} (48h)	N/A	0.028	0.55	0.02	0.96	2.08	0.55	0.04	0.09	0.04	0.01	0.01
Calculated EC_{50} (48h)	N/A	0.28	0.42	0.01*	6.69	2.04*	0.44	0.04*	0.01*	0.04	0.01	0.01
95 % CI	N/A	0.26-0.31	N/A	very wide	N/A	1.98-17.86	N/A	N/A	N/A	0.03-N/A	0.007-0.01	0.002-0.02

5.4 Discussion

When interpreting the EC_{50} resulting from *Daphnia* testing, considerations regarding their sensitivity response and the reliability of the results need to be made. It is recorded in the literature that the amount of food affects brood size (Enserink *et al.*, 1990; Smolders *et al.*, 2005). Both studies identified that higher food supply increased brood size and lower food supply produced fewer but larger neonates. Moreover, it was observed that in the case of high food supply with higher brood size and smaller neonates, the population that is produced is less tolerant to stresses from toxicity testing. Smolders *et al*. (2005) noted that it is likely that the increased stress of high productivity produces more sensitive individuals, while reproduction under low food supply emphasizes survival. The purpose of toxicity testing in this study was to give a true account of the toxicity of the DWW, which is why the culturing conditions were adapted to neither overfeed nor underfeed the *Daphnia* cultures.

Another factor impacting the reliability of the results was observed adult mortality of *Daphnia*. The OECD guideline 202 states that, in case of adult mortality, the results obtained from their neonates during testing must be excluded. In this study, a high adult mortality was observed in the 2^{nd} and 3^{rd} culture, which is why only the 1^{st} culture was used for the ecotoxicity tests. After revising all aspects of the culturing conditions, the medium renewal was adapted from the suggested full change in medium every 7 days to a change in medium by 20-30 % every 3 to 4 days. However, the neonates were not impacted by the mortality observed in the adults, even though neonates are more sensitive to a change in growth conditions. Therefore, the adult mortality was unlikely to be attributed to a change in temperature, medium, food or contamination of the culturing vessels. Nevertheless, the culturing conditions were adapted to a more gradual change in medium and observed to reduce adult mortality gradually until healthy cultures were established after two weeks of reproduction of *D. magna*.

Keeping in mind the criteria for reliability of the data when interpreting the EC_{50} results, the EC_{50} results indicate that the wastewater in DP3 is more ecotoxic than that of DP1. However, an important consideration to be made is whether the wastewater of DP1 was simply more diluted. From sampling protocols, it was clear that DP1 discharged higher volumes of wastewater per day (up to 11 L/day with 1 – 2 patients treated per day) compared to DP2 (up to 4 L/day with 6 – 7 patients treated per day) and DP3 (up to 6 L/day with 5 patients treated per day) but that fewer patients were

treated in DP1. Hence, it is known that the wastewater in DP1 was more diluted, whilst the wastewaters of DP2 and DP3 were more concentrated. This is important when assessing the ecotoxicity of these practices as it shows that different dental practices can vary greatly.

Compared to a study conducted on textile and dye effluents (Verma, 2008), the EC_{50} of the DWW were significantly lower. But when compared to a study on the effluent of WWTPs that use disinfection products, levels as low as 2.3 and up to 335 µg/L were found to be ecotoxic (Park *et al.*, 2016), which were below those detected in this study. A third study that assessed the uptake of heavy metals in the presence of TiO_2 nanoparticles found that their toxicity to *Daphnia* was reduced in the presence of the nanoparticles (Rosenfeldt *et al.*, 2014). This link between ecotoxicity and presence of nanoparticles is of high interest as research on the release of nanoparticles from WWTPs is gaining interest (Reijnders, 2009; Brar *et al.*, 2010; Rosenfeldt *et al.*, 2014). It was found that a single cycle of wastewater treatment may only be 90 % effective in removing particulate matter and hence it was suggested that treatment of the wastewater should be undertaken twice, once locally at the healthcare facility and a second time at the municipal WWTP (Jírová *et al.*, 2019). Particles remaining in the water during the disinfection stage of the wastewater treatment process were found to form toxic by-products as the organic matter reacts with the disinfection products (such as chlorine, sodium hypochlorite, chlorine dioxide, ultraviolet radiation or ozonation) such as trihalomethanes (THMs), which are harmful to the aquatic environment and via the food chain to humans, haloacetic acids (HAAs) and aldehydes (formaldehydes and acetaldehydes) (Monarca *et al.*, 2000; Bond *et al.*, 2011; Park *et al.*, 2016; Jírová *et al.*, 2019). ClO_2 and O_3 were found to produce the biggest environmental impact (Monarca *et al.*, 2000). Besides "double cleaning" of the wastewater in two separate WWTPs, it was found that removing the precursors of these harmful by-products via biofiltration for example is possible (Bond *et al.*, 2011). However, such approaches are wastewater-specific due the pollution load and origin and disinfection application, but necessary to reduce environmental and human health risks (Bond *et al.*, 2011).

5.5 Conclusions

The wastewater of DP3 was found to be the most ecotoxic (EC_{50} of 0.01 % DWW/L of medium) and that of DP1 to be the least ecotoxic (EC_{50} between 0.01 and 6.69 % DWW/L of medium), which was attributed to the wastewater of DP1 being more diluted and that of DP3 being more concentrated. Toxicity effects by means of *D. magna* acute immobilisation tests was largely attributed to the presence of disinfection products in the wastewater. Further research is needed to isolate the particles content and test for the ecotoxicity of nanoparticles in particular. The variability within the DWWs was shown and highlights the need for review of protocols and guidelines for dental wastewater treatment.

Chapter 6 Summary

6.1 Overall conclusions and recommendations

This thesis set out to address some of the knowledge gaps pertaining to Hg-free dental filling materials, which are used by dental practitioners globally, and may cause environmental impacts. As such, the pollution load of the discharged DWW was assessed through conducting pH, conductivity, Total suspended solids and Total dissolved solids measurements and by assessing the particle load, size distributions and characterisation, as well as the ecotoxicity, of DWW that was released from three dental practices in the vicinity of Cork City, Ireland. Furthermore, the efficacy of amalgam separation devices, which are utilised to capture at least 95 % of the particles released from dental amalgam, to also capture particles released from the use of Hg-free dental filling materials were assessed. Between the three DPs, two Type 1 (centrifugation) and one Type 2 (sedimentation) amalgam separation unit were assessed.

The DWW sample collection was carried out in a repeatable manner and under revision of Health and Safety protocols to allow for the safe extraction of DWW from the selected dental practices. The DWW was intersected at the point in the wastewater line after which amalgam separation had occurred and before release to the sewerage system.

Based on the physical and chemical parameters that were recorded from the wastewater streams, DP1 and DP3 showed extremes of pH (mean pH of 2.5 ± 1.2 and 1.9 ± 0.1) and high conductivity measurements were recorded in all three DPs (mean between 2.5 ± 2.7 and 5.1 ± 0.1 mS/cm). A link of these results to the use of line cleaners and disinfection products was made. The TSS and TDS however, indicated that a substantial particle load was released from the three DPs, with DP2 and DP3 releasing the highest particle loads (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). Within DP1, the particle load was linked to the use of GIC (glass ionomer cements) and RC (resin composites). The analysis of particle size distribution curves confirmed that higher particle loads were associated with RC at small particle sizes (2.5 to 5 μm^2) and GIC and RM products at larger particle sizes (12 and 50 μm^2). Size distributions overall were found to be similar between the three DPs that were tested and to follow a trend of highest abundance (53

– 66 %) of particles in the size between 1.2 and 5 µm² and lower frequencies (14 – 22 %) as the particles increased to 10 and 50 µm² in size. The size between 50 and 150 µm² only showed very low frequencies of particles (below 3 %). The circularity index of these particles showed that consistently between the DPs, the majority of the particles (around 70 %) were of elliptic (circ. index of 0.7) to round (circ. index of 1.0) shape. These particles were linked to filler material that are used in the Hg-free dental filling materials. The remaining 30 % of particles with lower circularity index were linked to materials resulting from the use of dental drilling and polishing and the SEM identified these to be of different shape and size than those identified as fillers. The tested Type 1 and Type 2 amalgam separators did not show any significant difference in capture efficiency of Hg-free dental filling materials, but both fell short in catching the high particle load that was released from the use of Hg-free dental filling materials in the three DPs.

Ecotoxicity results identified the wastewater of DP3 to have the highest potential to cause an environmental impact due to the EC_{50} of 0.01 % DWW/L of medium. Overall, the EC_{50} ranged from 0.1 to 6.69 % DWW/L medium in the three DPs. This toxicity was attributed to the presence of disinfection products in the wastewater.

These results imply that under current requirements, the particle load released from Hg-free dental filling materials 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. If future regulations required the capture efficiency of Hg-free dental filling materials also, then a need to design a capture system for these material does arise. Currently, treatment by a WWTP ensures the treatment of the dental wastewaters before release into the environment. However, any accidental release or spill of these wastewaters into the environment is expected to cause harm to fish populations, given by the physicochemical parameters that were obtained from the three DPs. Furthermore, research is emerging on municipal WWTPs potentially falling short in capturing the particulate matter, including nano particulates, released from healthcare facilities (Jírová *et al.*, 2019; Reijnders, 2009; Brar *et al.*, 2010; Rosenfeldt *et al.*, 2014).

This study identified that the detailed analysis of nanoparticles 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 (Suresh *et al.*, 2014; Emmanuel *et al.*, 2015; Schmalz *et al.*, 2018; Padovani *et al.*, 2015). While the SEM images picked up some of the smaller particles, detailed results based on shape and composition are needed to be able to make predictions on the environmental fate of these nanoparticles (Reijnders, 2009; Froggett *et al.*, 2014). The release of nanoparticles from nanocomposites is increasingly made aware and 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 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. 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.

The recent developments since the Minamata convention of 2013 have shown that in this short time-frame, the field of dentistry has been increasingly incorporating sustainability and environmental awareness into decision-making processes (Duane *et al.*, 2019). This study has highlighted some of the environmental considerations that need to be made as future dental materials progress to incorporate nanomaterials in increasing amounts but remain to be discharged into existing wastewater treatment facilities that may soon be out-dated.

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Appendix

List of Publications

Oral presentation

Binner, H., Kamali, N., Harding, M., Sullivan, T. (2019) Assessment of the environmental impacts arising from mercury-free dental restorative materials. *Environ 2019, 'Engagement for Climate Action'*. April 15th – 17th, 2019. Carlow, Ireland.

Poster presentations

Binner, H., Sullivan, T., Kamali, N., Hayes, M., Harding, M. (2019) Assessment of the environmental impacts arising from mercury-free dental restorative materials. *Irish Division of IADR 35th Annual Scientific Meeting*. October 10th – 11th, 2019. Cork, Ireland.

Binner, H., Kamali, N., Harding, M., Sullivan, T. (2018) Assessment of the environmental and health impacts arising from mercury-free dental restorative materials. *EPA and HSE Environment, Health and Wellbeing Conference, 'Knowledge and Data'*. November 7th, 2018. Dublin, Ireland.

Protocol 1: Recording of dental procedures that were carried out on each sampling day, by the dental practitioner.

Attention to Dental Staff

A research project is currently carried out at this dental chair unit, which results in the chair unit being slightly modified. It is however operational as usual.

Could you please indicate below the type of dental treatment that was carried out with each patient using a short description or the dental code (we are particularly interested in non-mercury fillings being used).

Date	Type of treatment	Code	Filling type used

Protocol 2: Recording of the use of Orotol (disinfectant) by the dental practitioner on each sampling day.

Dental staff: Please sign when **Orotol** has been used on dental chair (at the start and end of each shift)

	26.11.18	27.11.18	28.11.18	29.11.18	30.11.18		03.12.18	04.12.18	05.12.18	06.12.18	07.12.18
Start of shift											
End of shift											

Protocol 3: Recording of sample collection, hand-over and storage information.

Protocol for sample collection of wastewater arising from dental chairs			
Sample number:			
Sample started:	Date:	Time:	
Sample collected:	Name:	Date:	Time:
Sample handed over:	Name:	Date:	Time:
Sample storage (tick):	Refrigerator:	Other: _____	
Sample analysis:	Notes:		

Table 10: Detailed results obtained for specific conductivity, pH, triplicate Total Suspended Solids (TSS) and triplicate Total Dissolved Solids (TDS) measurements from the wastewater of DP1 with n=19 samples, DP2 with n=6 samples, DP3 with n=2 samples and TW blanks. (TSS and TDS carried out in triplicate and restricted by analytical balance to 4 sig. figures).

	Dental Unit	Sample number	Date of sample collection	Total Volume (L)	Cond. [mS/cm]	pH	TSS 1 [mg/L]	TSS 2 [mg/L]	TSS 3 [mg/L]
DP1	Unit 4	1	04.12.17	11.00	-	-	-	-	-
DP1	Unit 4	2	04.02.18	11.30	-	-	-	-	-
DP1	Unit 4	3	12.03.19	11.00	3.87	2.30	6.67	10.00	10.00
DP1	Unit 4	4	27.03.19	10.74	4.45	1.43	0.00	0.00	0.00
DP1	Unit 4	5	29.04.19	5.80	5.06	1.45	0.00	0.00	10.00
DP1	Unit 4	6	09.05.19	6.83	3.36	2.17	0.00	10.00	0.00
DP1	Unit 4	7	13.05.19	3.21	5.16	2.02	30.00	30.00	30.00
DP1	Unit 4	8	11.06.19	5.54	0.15	4.09	10.00	10.00	10.00
DP1	Unit 4	9	13.06.19	7.50	0.86	2.37	14.00	16.00	18.00
DP1	Unit 4	10	14.06.19	11.00	0.15	4.92	38.00	38.00	41.00
DP1	Unit 4	11	17.06.19	10.20	1.98	2.26	16.00	16.00	17.00
DP1	Unit 4	12	18.06.19	9.00	2.10	2.62	10.00	11.00	10.00
DP1	Unit 3	1	14.03.19	11.00	3.68	2.09	20.00	20.00	20.00
DP1	Unit 3	2	25.03.19	1.20	14.85	1.23	50.00	60.00	60.00
DP1	Unit 3	3	26.03.19	1.08	18.65	1.31	50.00	50.00	40.00
DP1	Unit 3	4	04.04.19	10.50	3.04	1.71	40.00	40.00	30.00
DP1	Unit 3	5	15.05.19	9.60	1.94	2.39	20.00	20.00	10.00
DP1	Unit 3	6	21.05.19	5.08	4.77	2.05	10.00	20.00	20.00
DP1	Unit 3	7	04.06.19	9.00	0.17	5.50	10.00	10.00	10.00
DP1	Unit 3	8	05.06.19	9.80	9.05	1.98	30.00	30.00	30.00
DP1	Unit 3	9	21.06.19	2.80	0.49	3.18	6.00	6.00	8.00
DP1	Unit 3	TW blank	26.03.19	1.00	0.18	6.60	0.00	0.00	0.00
DP1	Unit 4	TW blank	04.04.19	1.00	0.16	5.81	0.00	0.00	0.00
DP2		1	05.06/06.06.19	3.60	5.64	2.96	200.00	190.00	200.00
DP2		2	11.06/12.06.19	3.45	2.04	2.64	200.00	200.00	210.00
DP2		3	17.06/18.06.19	2.10	6.17	9.17	103.00	109.00	121.00
DP2		4	03.07.19	3.70	0.38	9.03	58.00	52.00	54.00
DP2		5	11.07.19	4.20	0.38	9.00	60.00	58.00	57.00
DP2		6	16.07.19	1.00	0.40	9.09	66.00	63.00	66.00
DP3		1	24.07/25.07	5.90	5.03	1.85	141.00	146.00	145.00
DP3		2	25.07/26.07	1.90	5.14	1.85	118.00	121.00	124.00

Table 10 (continued)

	TSS average [mg/L]	TSS error	TDS 1 [g/L]	TDS 2 [g/L]	TDS 3 [g/L]	TDS average [g/L]	TDS error [g/L]	Comments
DP1	-	-	-	-	-	-	-	*method development
DP1	-	-	-	-	-	-	-	*method development
DP1	8.89	1.92	-	-	-	-	-	TDS analysis was added later into protocol
DP1	0.00	0.00	3.22	3.43	3.42	3.36	0.12	
DP1	3.33	5.77	4.27	4.24	4.71	4.41	0.26	
DP1	3.33	5.77	-	-	-	-	-	no TDS result because sample got contaminated in oven
DP1	30.00	0.00	3.58	4.01	3.87	3.82	0.22	
DP1	10.00	0.00	0.16	0.15	0.14	0.15	0.01	
DP1	16.00	2.00	0.63	0.69	0.65	0.66	0.03	
DP1	39.00	1.73	0.15	0.17	0.15	0.16	0.01	
DP1	16.33	0.58	1.59	1.64	1.66	1.63	0.04	
DP1	10.33	0.58	1.51	1.56	1.56	1.54	0.03	
DP1	20.00	0.00	-	-	-	-	-	TDS analysis was added later into protocol
DP1	56.67	5.77	-	-	-	-	-	TDS analysis was added later into protocol
DP1	46.67	5.77	38.53	39.24	39.89	39.22	0.68	
DP1	36.67	5.77	1.20	1.19	1.32	1.24	0.07	
DP1	16.67	5.77	-	-	-	-	-	no TDS result because load too high to filter
DP1	16.67	5.77	3.73	4.50	3.98	4.07	0.39	
DP1	10.00	0.00	0.19	0.19	0.19	0.19	0.00	
DP1	30.00	0.00	10.16	10.65	10.60	10.47	0.27	
DP1	6.67	1.15	0.44	0.43	0.43	0.43	0.00	
DP1	0.00	0.00	0.07	0.00	0.04	0.03	0.05	
DP1	0.00	0.00	0.11	0.02	0.11	0.08	0.05	
DP2	196.67	5.77	-	-	-	-	-	no TDS result because load too high to filter
DP2	203.33	5.77	7.06	7.20	7.41	7.22	0.18	
DP2	111.00	9.17	6.41	6.71	6.43	6.52	0.17	
DP2	54.67	3.06	7.01	5.91	6.28	6.40	0.56	
DP2	58.33	1.53	5.33	5.81	5.59	5.58	0.24	
DP2	65.00	1.73	4.97	4.99	4.82	4.92	0.09	
DP3	144.00	2.65	6.70	6.83	6.75	6.76	0.07	
DP3	121.00	3.00	7.03	7.22	7.09	7.11	0.10	