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Chand, Rupa

DOI (link to publication from Publisher):
10.54337/aau745675701

Publication date:
2024

Document Version
Publisher's PDF, also known as Version of record

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BY
RUPA CHAND

PhD Thesis 2024

AALBORG UNIVERSITY
DENMARK

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PhD Thesis 2024

Submitted: June 2024

Main Supervisor: Professor Jes Vollertsen
Aalborg University

Co-supervisor: Associate Professor Asbjørn Haaning Nielsen
Aalborg University

Assessment: Associate Professor Søren Liedtke Thorndahl (chair)
Aalborg University, Denmark

Permanent Researcher Claudia Campanale
Istituto Superioredi Sanità, Italy

Professor Godecke-Tobias Blecken
Luleå University of Technology, Sweden

PhD Series: Faculty of Engineering and Science, Aalborg University

Department: Department of the Built Environment

ISSN: 2446-1636
ISBN: 978-87-94563-87-1

Published by:
Aalborg University Open Publishing
Kroghstræde 1-3
DK – 9220 Aalborg Øst
aauopen@aau.dk

RUPA CHAND

EDUCATION

Mar. 2019 - Jun. 2024 PhD Fellow at AAU, Denmark

Sep. 2015 - Jun. 2017 Masters in engineering in Water and Environment at AAU, Denmark

Dec. 2007 - Nov. 2009 Masters in environmental science at Tribhuvan University, Nepal

WORK EXPERIENCES

Jun. 2021 – Feb. 2024 **Research Assistant, AAU, Denmark**

- Managed the projects
- Coordinated with the project stakeholders
- Conducted and set up laboratory experiments
- Presentation of project outcomes

Nov. 2016 - Jun. 2017 **Research Assistant, AAU, Denmark**

- Worked on Pharmaceutical and biocide products
- Bench-scale batch experiments set up
- Micro-wave and reactor bottle sample extraction
- Solid-liquid separation
- HPLC-MS analysis
- Data analysis

Nov. 2011 - Aug. 2015 **Faculty Member and Coordinator, GoldenGate International College, Nepal**

- Delivered lectures
- Planned and prepared academic time plan for theoretical and field activities
- Coordinated and supervised students in research activities
- Monitored and evaluated overall academic activities

May 2012 - Jun. 2014 **Environmental Assessment Officer, Environmental Services Nepal (ESN), Nepal**

- Assessed environment impacts from road, hydropower, bridge and hotel construction.
- Organized and coordinated public consultation meeting
- Collected field data and analysed
- Monitored and evaluated the project progress
- Prepared report and presented outcomes

LIST OF PAPERS (INCLUDED IN THIS THESIS)

I. **Rupa Chand**, Lasse Abraham Rasmussen, Susanne Tumlin, Jes Vollertsen (2021). The occurrence and fate of microplastics in a mesophilic anaerobic digester receiving sewage sludge, grease, and fatty slurries. Science of the Total Environment, Volume 798. https://doi.org/10.1016/j.scitotenv.2021.149287

II. **Rupa Chand**, Komeil Kohansal, Saqib Toor, Thomas Helmer Pedersen, Jes Vollertsen (2022). Microplastic degradation through hydrothermal liquefaction of wastewater treatment sludge. Journal of Cleaner Production, Volume 335. https://doi.org/10.1016/j.jclepro.2022.130383

III. **Rupa Chand**, Lucian Iordachescu, Frida Bäckbom, Angelica Andreasson, Cecilia Bertholds, Emelie Pollack, Marziye Molazadeh, Claudia Lorenz, Asbjørn Haaning Nielsen, Jes Vollertsen (2024). Treating wastewater for microplastics to a level on par with nearby marine waters. Water research, volume 256, https://doi.org/10.1016/j.watres.2024.121647

IV. **Rupa Chand**, Ieva Putna-Nīmane, Elina Vecmane, Jeanette Lykkemark, Jytte Dencker, Asbjørn Haaning Nielsen, Jes Vollertsen, Fan Liu (2024). Snow dumping station–A considerable source of tyre wear, microplastics, and heavy metal pollution. Environment International, Volume 188. https://doi.org/10.1016/j.envint.2024.108782

OTHER SCIENTIFIC PAPERS (NOT INCLUDED IN THIS THESIS)

I. Nanna DR Klemmensen, **Rupa Chand**, María S Blanco, Jes Vollertsen (2024). Microplastic abundance in sludge-treated fields: Variance and estimated half-life. Science of the Total Environment, Volume 922. https://doi.org/10.1016/j.scitotenv.2024.171394

II. Komeil Kohansal, Saqib Toor, Kamaldeep Sharma, **Rupa Chand**, Lasse Rosendahl, Thomas Helmer Pedersen (2024). Hydrothermal liquefaction of pre-treated municipal solid waste (biopulp) with recirculation of concentrated aqueous phase. Biomass and Bioenergy, Volume 148. https://doi.org/10.1016/j.biombioe.2021.106032

ENGLISH SUMMARY

Microplastics (MPs) pollution has emerged as a pressing environmental issue, stemming from the pervasive use of plastic in various aspects of daily life. MPs found ubiquitously, present a new dimension to plastic pollution. These minuscule plastic particles, resulting from the breakdown of larger plastic items, infiltrate ecosystems and pose threats to living organisms. The potential for MPs to enter the food chain, ultimately reaching human consumption, underscores the urgency of addressing this issue. However, research on the sources, pathways, and impacts of MPs remains incomplete. Controversies persist regarding the health effects of MPs on humans and animals, necessitating further investigation. Understanding the distribution patterns of MPs in oceans and rivers, as well as their interaction with organisms, is crucial for developing effective mitigation strategies. However, the complex nature of environmental systems poses challenges in implementing legislative and technical solutions. Techniques for analyzing MP concentrations require advanced equipment and skilled manpower, adding to the complexity of the issue. Standardized protocols for sample collection and analysis are lacking, further complicating research efforts.

The study of MPs spans various fields, including wastewater treatment, agricultural systems, and marine ecosystems. Wastewater treatment plants (WWTPs) play a fundamental role in receiving and treating MPs from various sources before discharge into natural water bodies. The influx of MPs into WWTPs originates from household, commercial, industrial, and landfill sites, with significant contributions from cosmetics, personal care products, and textile washing. Industrial activities, including textile manufacturing and plastic production, also contribute to MP contamination of wastewater. Despite not being specifically designed to remove MPs, conventional WWTPs employ mechanical, biological, and chemical treatment methods that can effectively reduce MP concentrations in final effluents. The efficiency of MP removal in WWTPs depends on various factors, including treatment technologies, engineering design, and influent characteristics such as polymer types, density, and size. However, complete removal of MPs is challenging, and WWTPs have been argued to remain significant sources of MP discharge into aquatic environments. As urbanization and industrialization continue to increase wastewater volumes, the demand for efficient treatment facilities becomes more pressing to remove the pollutants from effluent to a significantly low level.

Research dedicated to analyzing MPs in WWTPs has been steadily increasing, resulting in numerous publications annually. However, majority of these research works reporting the presence of MPs in influent and effluent, offering only a general overview of treatment efficiency. Few studies have explored the effectiveness of different treatment stages within WWTPs in removing MPs, indicating a lack of comprehensive data on how individual processes influence MP removal. Furthermore, research on the fate of MPs within sludge digesters is lacking, along with a limited understanding of the impact of extreme thermal processes on MP breakdown in

sludge. Similarly, while mesophilic digestion is commonly used for sludge volume reduction and biogas generation, detailed studies on MP fate in mesophilic anaerobic digestion and including co-digestion process of grease and external fatty slurry are scarce.

Therefore, a thorough investigation was conducted to assess the efficacy of a cutting-edge WWTP in retaining MPs. This facility employs a comprehensive approach encompassing mechanical, biological, and chemical treatment methods, with rapid sand filtration for tertiary treatment. The overall efficiency of the system was determined to exceed 99.9% concerning both particle counts and mass. The mechanical component, encompassing pre-treatment and primary stages, exhibited a reduction of approximately 71% in both MP counts and mass. Subsequently, the biological treatment together with secondary settling, and rapid sand filtration effectively eliminated rest of the 29% MPs. Notably, MPs were successively reduced in size as they progressed through the various treatment stages.

Moreover, the PhD study assessed the MP balance over a mesophilic digester, taking in consideration both incoming and outgoing MPs. The corresponding mean mass reduction of the MPs was arbitrary and found approximately 10% to 30%. Similarly, the MP particle variation was approximately between 4% to 29%. The discrepancy observed in these studies may stem from various factors such as larger floatable polymer particles in the grease, and numerous small MPs in sewage sludge. Given the differing degradation rates of MPs observed in both studies, drawing conclusions regarding the effectiveness of mesophilic anaerobic digestion and complete elimination of MP particles proves challenging and most of them found resilient. There was no significant elimination of any specific polymer types, however, the MP composition varied in outgoing sludge compared to the incoming matrices.

This PhD also assessed the effectiveness of hydrothermal liquefaction (HTL) of sludge at 400°C, and 30 MPa in breaking down MP particles and elimination from the sewage sludge. This process resulted in a reduction of about 76% MPs count and 97% MPs mass and found the residual particles of very small fraction. The polymer composition underwent significant changes by HTL process, with polyurethane, polypropylene, and polyethylene being the prevalent polymers in the feedstock. Where, this study found elimination or disappearance of certain type of MP polymers and mostly polypropylene and polyethylene were predominant in the residual products. These findings suggest that excessive MPs in sludge from WWTPs can effectively be eliminated by HTL, resulting in significantly less polluted byproducts and residuals.

Furthermore, a spatial analysis was undertaken to assess the presence of MPs, tyre wear particles, and associated metal pollutants in freshly fallen snow collected from snow dumping sites. This investigation aimed to examine the potential for snowmelt to act as a source of contamination for water bodies, as well as a potential contributor of MPs to WWTPs in combined sewer systems. The extent of impact was

hypothesized to vary depending on the land use type, including commercial areas, residential areas, urban centres, and rural regions. The findings of the study revealed that snow can accumulate significant quantities of these pollutants, which, upon melting and subsequent release of meltwater into aquatic environments, could potentially affect receiving waters and contribute to an increased load of MPs in WWTPs.

Overall, this PhD has provided valuable insights into the behaviour of MPs within WWTPs and has offered in-depth understanding of the retention of MPs within sludge, grease, and fatty slurry undergoing mesophilic anaerobic digestion. Additionally, the findings have contributed scientific insights into the effects of hydrothermal liquefaction on MP presence within sludge. Furthermore, this study has made an additional contribution by enhancing our understanding of the potential pollution of nearby water bodies and WWTPs by MPs originated from melted snow.

DANSK RESUME

Mikroplastforurening (MP) er opstået som et presserende miljøproblem, stammende fra den udbredte anvendelse af plastik i forskellige aspekter af dagligdagen. MP, der findes overalt, præsenterer en ny dimension af plastforurening. Disse små plastikpartikler, der stammer fra nedbrydningen af større plastgenstande, infiltrerer økosystemer og udgør trusler mod levende organismer. Potentialet for, at MP kan komme ind i fødekæden og til sidst nå frem til menneskeligt forbrug, understreger vigtigheden af at håndtere dette problem. Imidlertid er forskning om kilderne, veje og virkningerne af MP ufuldstændig. Kontroverser består vedrørende de sundhedsmæssige virkninger af MP på mennesker og dyr, hvilket kræver yderligere undersøgelser. Forståelse af fordelingsmønstrene af MP i oceaner og floder samt deres interaktion med organismer er afgørende for udviklingen af effektive afbødelsesstrategier. Imidlertid udgør den komplekse natur af miljøsystemer udfordringer i implementeringen af lovgivningsmæssige og tekniske løsninger. Teknikker til analyse af MP-koncentrationer kræver avanceret udstyr og dygtigt personale, hvilket øger kompleksiteten af problemet. Standardiserede protokoller til prøveindsamling og analyse mangler, hvilket yderligere kompliserer forskningsindsatsen.

Studiet af MP spænder over forskellige områder, herunder spildevandsbehandling, landbrugssystemer og marine økosystemer. Spildevandsrenseanlæg (WWTP) spiller en afgørende rolle i modtagelse og behandling af MP fra forskellige kilder før udledning i naturlige vandlegemer. Tilstrømningen af MP til WWTP stammer fra husstande, kommercielle, industrielle og lossepladser, med betydelige bidrag fra kosmetik, personlige plejeprodukter og tekstilvask. Industrielle aktiviteter, herunder tekstilproduktion og plastproduktion, bidrager også til MP-forurening af spildevand. På trods af ikke at være specifikt designet til at fjerne MPs, anvender konventionelle WWTP mekaniske, kemiske og biologiske behandlingsmetoder, der effektivt kan reducere MP-koncentrationer i endeligt afløb. Effektiviteten af MP-fjernelse i WWTP afhænger af forskellige faktorer, herunder behandlingsteknologier, ingeniørmæssig udformning og influentegenskaber såsom polymertyper, densitet og størrelse. Imidlertid er fuldstændig fjernelse af MP udfordrende, og WWTP har været argumenteret som værende betydelige kilder til MP-udledning til akvatiske miljøer. Da urbanisering og industrialisering fortsætter med at øge spildevandsvolumenerne, bliver behovet for effektive renseanlæg mere presserende.

Forskning dedikeret til at analysere MP er støt stigende, hvilket resulterer i talrige publikationer årligt. Imidlertid fokuserer de fleste af disse studier på at rapportere tilstedeværelsen af MP i indløb og udløb og giver kun et generelt overblik over renseeffektiviteten. Få studier har udforsket effektiviteten af forskellige rensestadier inden for WWTP i fjernelse af MP, hvilket indikerer manglen på omfattende data om, hvordan individuelle processer påvirker MP-fjernelse. Desuden mangler der

forskning om MP skæbne inden for slamudrådning, sammen med en begrænset forståelse af virkningen af ekstreme termiske processer på MP-nedbrydning i slam. På samme måde, mens mesofil udrådning er almindeligt anvendt til reduktion af slamvolumen og biogasproduktion, er der få detaljerede undersøgelser om MP-skæbne i mesofil anaerob udrådning og inkludering af kombineret udrådning af fedt og ekstern fedt.

Derfor blev der udført en grundig undersøgelse for at vurdere effektiviteten af en topmoderne WWTP i at tilbageholde MP. Dette anlæg anvender en omfattende tilgang, der omfatter mekanisk, biologisk og kemiske behandlingsmetoder, med hurtig sandfiltrering til tertiær behandling. Den samlede effektivitet af systemet blev vurderet til at overstige 99,9% med hensyn til både partikelantal og masse. Den mekaniske komponent, der omfatter forbehandling og primære stadier, viste en reduktion på cirka 71% i både MP-antal og masse. Derefter fjernede den kombinerede biologiske behandling, sekundær sedimentation og endelig polering gennem hurtig sandfiltrering effektivt næsten alle de resterende 29%. Bemærkelsesværdigt blev MP successivt reduceret i størrelse, når de fortsatte gennem de forskellige behandlingsstadier.

Derudover vurderede Ph.d.-studiet MP-balancen over en mesofil rådnetank med henblik på både indkommende og udgående MP'er. Den tilsvarende gennemsnitlige masse reduktion af MP var tilfældig og blev fundet til cirka 10% til 30%. På samme måde var variationen i MP-partiklerne cirka mellem 4% og 29%. Forskelligheden observeret i disse studier kan skyldes forskellige faktorer såsom lettere polymerpartikler i fedt, og talrige små MP i spildevandsslam. Med de forskellige nedbrydningshastigheder af MPs, der blev observeret i begge studier, er det udfordrende at drage konklusioner vedrørende effektiviteten af mesofil anaerob udrådning og fuldstændig eliminering af MP-partikler, og de fleste af dem blev fundet resistente. Der var betydelig eliminering af bestemte polymer typer, men MP-sammensætningen var varierende i udgående slam sammenlignet med de indkommende matricer.

Dette Ph.d. undersøgte også effektiviteten af hydrotermisk behandling af slam under superkritiske vandbetingelser (400°C, 30 MPa) med henblik på at nedbryde MP-partikler og eliminering fra spildevandsslammet. Denne proces resulterede i en reduktion på cirka 76% i antallet af MPs og 97% i forhold til MP-massen og fandt restpartikler af en meget lille fraktion. Polymerkompositionen gennemgik betydelige ændringer som følge af HTL-processen, hvor polyurethan, polypropylen og polyethylen var de dominerende polymerer i råstoffet. Hvor denne undersøgelse fandt eliminering eller forsvinden af visse typer MP-polymerer, og mest polypropylen og polyethylen dominerede i de resterende produkter. Disse fund antyder, at HTL effektivt kan reducere MP i kraftigt forurenet slam fra spildevandsrenseanlæg, hvilket resulterer i betydeligt mindre forurenede biprodukter og rester.

Desuden blev der foretaget en analyse for at vurdere tilstedeværelsen af MP, dækpartikler og tilhørende metalforureninger i nyligt faldet sne, der blev indsamlet fra snedepoter. Denne undersøgelse havde til formål at undersøge potentialet for snesmeltning som en kilde til forurening af recipienter samt en potentiel bidragyder af MP til WWTP i kombinerede kloaksystemer. Omfanget af påvirkningen blev hypotetisk antaget at variere afhængigt af arealanvendelsen, herunder kommercielle områder, boligområder, bycentre og landlige områder. Resultaterne af undersøgelsen viste, at sne kunne akkumulere betydelige mængder af disse forurenende stoffer, som ved smeltning og efterfølgende frigivelse af smeltevand i vandmiljøer potentielt kunne påvirke modtagevand og bidrage til en øget belastning af MP i WWTP.

Denne ph.d. har givet værdifulde indsigter i MP adfærd inden for WWTP og har givet en dybdegående forståelse af fastholdelsen af MP'er i slam, fedt og fedtslam når det undergår mesofil anaerob udrådning. Derudover har resultaterne bidraget med videnskabelige indsigter i effekten af hydrotermisk behandling på MP-tilstedeværelse i slam. Derudover har denne undersøgelse bidraget yderligere ved at forbedre vores forståelse af potentiel forurening af nærliggende recipienter og WWTP af MP, der stammer fra smeltet sne.

PREFACE

This PhD thesis has been submitted for the assessment in partial fulfilment of the PhD degree. As part of the assessment, co-author statements have been made available to the assessment committee and are also available at the faculty.

This PhD study carried out in an extended period of March 2019 to June 2024 at the Department of the Built Environment, Aalborg University. The study also included a collaborative study with Latvian Institute of Aquatic Ecology (LIAE), Latvia. The joint study resulted in a manuscript (Paper -IV) which has been published in journal of environment international.

This PhD thesis is based on four published scientific paper (Paper-I, -II, -III, -IV). Contents of the papers are briefly discussed in the extended summary of the thesis. Additional details are given in the paper and manuscripts.

ACKNOWLEDGEMENTS

I would like to start my acknowledgement section with a short story of my own. I came to Denmark, a highly advanced and modern country to fulfill the gap in learning or achieving technical education that I could not fill in my home country. But the journey was not as easy as I was expecting. I had to stay away from my beautiful family and away from my little boy and caring husband. This was the hardest and most stressful period of my life. I found Professor Jes Vollertsen as a motivating supervisor and mentor. He has always showed his full interest in listening my problems and has suggested me with meaningful guidance. Today, I am grateful to express my heartfelt greetings to my supervisor for upbringing my talent, dedication, hard work and most important keeping me motivated to chase my destination by creating an easy and fruitful path. He has given my PhD journey a comfortable and safe landing. Without his all-time support, I would not be able to reach at this stage.

I would also like to say thank you to Asbjørn, my co-supervisor. I could easily access him when I need any guidance and help. Regardless of his busy schedule, he always showed his interest to listen carefully about my problems and ready to guide me. I did not need to explain a lot to him beforehand for getting his guidance.

I am thankful for Jytte and Henrik. I was always surprised seeing a great talent in Henrik and a constant energy in Jytte. Without them I feel like our research group is without guardians. They both are the best in managing the group by providing all the technical accessories in the lab and in the building. They are the core for making each of our projects successful by providing all the necessary equipment, labware, glassware, as well as keeping them safe. Not only the official work, but also the non-work-related motivations from them was worthy. Without any helps from Jytte, I don't think I would be able to place my things in a rightful manner, and with proper labelling. Her years long experience of managing the lab is a blessing to our research group.

I would like to give a special thanks to Lucian, my trainer/guru for the laboratory work and he taught me all the steps that I could use in the lab to accomplish my experimental projects. His way of taking the things effortlessly was wonderful. He is calm and passionate and taught me taking the failure of the experiments easily. His helping hands for driving me everywhere for collecting samples is unforgettable. He provided me with safe and secure zone, many times encouraged me when I got tired and corrected me with my mistakes not only in the lab, but equally while we were sharing office. I always felt grateful in his presence at the workplace and/or any other places. Thanks a lot Lucian for everything.

I also appreciate my close friend, more than a colleague, Shabnam. Her entry to the group brought up some sort of fresh air for me. Her trust upon me gave me certain kind of confidence. Her fearless talks and actions and power of thinking out of the

box was perfect with a lot of imperfections. I was always trying to do the things perfectly, but I learnt from her that chaos can even teach or improve us more. Her being with me always gives me a sense of sisterhood. I feel great on sharing our feelings. Her ways of motivating me or giving me compliments or simply showing me goodness is wonderful. Her positivity and appreciation toward me are priceless.

I want to express my appreciation to my another close, friendly, and fearless friend Lily. Her laughter is still in my ear. Her fearless and straight forward attitude taught me being honest and brave. Her helping hands beyond the workplace was great. Sharing an office with her kept me motivated to continue with the work. Thanks, Lily, for being there for me as well as trusting and motivating me.

Our working team (I prefer to call a working family) have so many wonderful people. All of them have equal role in shaping my working time here and I have gathered unforgettable memories for my whole life. Fan, who helped me with many things, not only professional field but also when I need emotional support. She showed me that if one door gets closed then another opens. Claudia, the coolest person with a lot of talent who tried her best to involve me in the group and offered her helping hands all the time. Alvise, the most energetic and experienced person in the group for microplastic research who always keeps updated and guides the group. Laura, the most determined person, and I admire her funny talks and laughter in the lab. Her presence gave me a motivation to continue with the work in the lab without being tired. Her trust and believes upon me made me confident. Jeanette, a strong and confident person who taught me a lot including Danish language. I always appreciate her efforts for arranging all the social gatherings and reviving the group. The same time I will always be grateful for her cross fingers for my goodness. Nanna, a cool, hardworking, and dedicated person. Herself being simply happy on my achievements was priceless. Luca, a factual person who was always honest and dedicated with his work. Offering help especially with the technical equipment was impressive. Thanks Luca, for being honest and always sharing your experiences. I am also thankful to very kind and hardworking couple Ashani and Gunalaan. Their presence in the group was simply beautiful and it gave me a kind of motivation to keep up my hope.

The same way I would like to thank Rasmus, Christoffer, and Janni for staying in touch with our research group and keeping the enjoyment memorable. I will always miss the morning coffee break and bread day.

I would equally like to thank student helpers, Alexandra, Camilla, Laura, Katrine, Maria, and Johanne. Their immense help in the lab made our lives little restful. They were all kind, helpful and most importantly were very talented.

Likewise, I would like to express my gratitude to old colleagues, Marta, Nikki, Kristina, Amelia, Lasse, and Fides. I would like to say thank you for pioneering the microplastics work in the department which took such a great space with many

projects and involved many people. Personally, I also have some time to learn from them.

Equally, our guest visitors are also thankful for their contributions in this research field. They came with different projects and allowed us to teach or learn from them.

I would also like to thank Ieva, and Elina from Latvian Institute of Aquatic Ecology, Latvia for external collaboration of my PhD work. Also, thanks to Sandra Sprukta for creating beautiful GIS maps. Ieva and Elina were wonderful colleagues and provided me all the ease for the success of the project.

Now time for thanking my sweet, and the sweetest family.

Through my heart, I owe my sincere thanks to my father (BABA) and mother (my sweet MUMMY) for their lifelong hard work and showing me (and my brother and sister) the true meaning and value of education. My father is a legendary person to me. Without his dream of making us educated, I don't think I would be here today. I am sure, my dear baba and mummy are very proud today of having me as their daughter, as always. Love you both. I want to thank my entire family for their kindness, love, support, and most important their trust upon me. Equally, I would like to thank you my parents-in-laws. They trusted on me and blessed me always to get success in my life. Unfortunately, my father-in-law is not here in the world today, but I am sure he is watching and blessing me.

Now, it's an opportunity to thank my darling dear family: my ever-supportive husband, Kapil, and my dear darling children Anugya (son) and Krisha (baby daughter). I feel like, I am the luckiest wife and the luckiest mother. Kapil, you are the gentlest man. No matter how far and how hard our lives knocked us out, you were and are there to hold all the pain. You sacrificed your dreams and started enjoying in mine. You are the most important steppingstone and a hidden treasure of my life. Without your support, I even cannot imagine what I will do. You are the strongest pillar of my life. Your simplicity and priority upon me, seeing the whole world through my eyes, enjoying each moment of my success, and making me a bold person is not lesser than any man on the planet can do for his wife. Our little son has grown up and have a good understanding of what I am working on. His way of accepting, trusting, and believing upon me gives another level of satisfaction. He made us proud, not one time but many times. Thank you, my boy, for being passionate about me and having your patience. Krisha and you make me to get up again and work further.

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CHAPTER 1. INTRODUCTION

Plastics, as polymeric substances derived from petrochemical compounds (Rios et al., 2007), have seen widespread use since the industrial revolution. Their unique characteristics, including being lightweight, resilient, inexpensive, durable, and versatile, have led to extensive consumption. Plastics have found applications in automotive, hardware, and plumbing industries, and have replaced many traditional materials like glass, metal, and natural substances in residential, commercial, and industrial settings. While plastics have brought numerous benefits to society, not all types are sustainable and pose unwanted health and environmental risks (North and Halden, 2013). Global plastic production has exceeded 300 million metric tons annually since 2014 and approached 390 million metric tons in 2021 (Statista 2023). Unfortunately, a significant portion of mismanaged and littered plastics ends up in the environment, with only a fraction being properly managed. Improper disposal methods, such as dumping in landfills or burning in open pits, contribute to land, water, and air pollution. Incinerating plastic waste releases carbon dioxide and other pollutants, exacerbating climate change. The accumulation of plastic waste is a pressing global issue, necessitating urgent action to preserve ecological balance.

Plastic waste poses a significant threat due to its increasing production and mishandling (Weinstein et al., 2016). Large quantities of plastics leak into marine ecosystems, adversely affecting marine life such as mammals, birds, and reptiles (Law, 2017). Marine organisms often ingest plastic debris, leading to prolonged and life-threatening impacts. Entanglement and ingestion are commonly reported interactions between wildlife and plastic debris, heightening public concern about plastic pollution. Researchers predict that if current trends continue, plastic waste in oceans will surpass the number of fish by 2050. Additionally, additives like antioxidants and stabilizers prolong the life of plastics, slowing down their decomposition rate to hundreds of years (Barnes et al., 2009; Chamas et al., 2020; Geyer et al., 2017; Weinstein et al., 2016). During degradation, plastics release harmful toxins into water and soil, posing significant risks to aquatic and terrestrial organisms.

Plastic is ubiquitous and undergoes degradation into smaller particles due to various physical, chemical, and biological factors. Particles smaller than 5 mm to 1 µm in size, regardless of their shape, are commonly classified as microplastics (MPs). MPs are divided into two categories based on their origin: Primary MPs are intentionally manufactured for specific purposes and are present in the environment in their original form. For instance, microbeads, typically ranging from 10 to 500 µm in size, are commonly used in rinse-off personal care products for cleansing. These microbead-containing products are applied to the body, washed off, and ultimately enter the wastewater stream (Mcdevitt et al., 2017). Conversely, items such as drinking water or soda bottles, plastic bags, fishing nets, textile fibers, tire wear, and personal protective equipment degrade into smaller pieces over time through natural

weathering processes upon entering the environment, forming secondary MPs (Akhbarizadeh et al., 2020). A single large plastic item can break down into hundreds to thousands of secondary MPs in various forms such as fibers, fragments, and films (Horton and Dixon, 2018). The degradation of plastic can also be facilitated by microbes and microorganisms. Furthermore, secondary MPs exhibit greater diversity in terms of size, shape, and polymer types compared to primary MPs.

Numerous research endeavours have concentrated on this matter, albeit with limited depth. Nonetheless, these minuscule plastic fractions have been documented extensively in nature, as evidenced by their presence in water, air, soil (Duis and Coors, 2016; Lusher et al., 2017), and even within animals and human bodies. The lightweight, small, finely textured, and vividly coloured MPs have also been identified in remote and pristine locales such as the summit of Mount Everest and the Antarctic region (Aves et al., 2022; Kelly et al., 2020; Napper et al., 2020). Furthermore, studies have highlighted clear evidence of detrimental impacts from MPs on the growth and reproductive capabilities of microalgae, bacteria, fungi, and other microorganisms (Cheng et al., 2023). Vianello et al. (2019) documented the presence of indoor airborne MPs and fibres, posing a significant health hazard due to their hydrophobic surfaces capable of readily adsorbing other pollutants. Moreover, impurities like additives, dyes, pigments, flame-retardants, and plasticizers found in MPs pose health risks upon entering the human body and organs. Consequently, scientists are delving into the presence of MPs in food and drinking water and their potential effects on human health (Cox et al., 2019; Kirstein et al., 2021). However, the risk assessment of MPs to human health is unresolved yet.

Furthermore, numerous studies have indicated that MPs possess the ability to adsorb organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), which upon ingestion by marine organisms could become bioavailable (Bowmer and Kershaw, 2010; Brennecke et al., 2016; Koelmans et al., 2014; Rios et al., 2007; Rochman et al., 2013). Similarly, other studies have also demonstrated the adsorption of heavy metals onto plastic surfaces (Holmes et al., 2012; Rochman et al., 2014). These organic pollutants and trace metals have the potential to spread to higher trophic levels through the food web, leading to bioaccumulation and becoming toxicants for organisms (Browne, 2015; Lusher et al., 2013; Rios et al., 2007; Setälä et al., 2014). Additionally, MPs could induce toxic effects through various mechanisms, primarily through direct effects caused by polymer constituents and additives, and secondarily through their size and shape, which may harm organisms (Liu et al., 2019; Sun et al., 2019). Consequently, there is an urgent need for comprehensive information on the fate and presence of MPs and their potential, whether partial or high-risk, to living organisms (Andrady, 2011; Hidalgo-Ruz et al., 2012). Likewise, the issue of MP pollution in marine and terrestrial ecosystems is raising significant concern among the scientific community, policymakers, and the public (Boerger et al., 2010; Brennecke et al., 2016; Claessens et al., 2013; De Stephanis et al., 2013; Graham and Thompson, 2009; Lazar and Gračan, 2011; Murray and Cowie, 2011).

Moreover, MPs are also characterized by their low density and small size, possing the ability to migrate across various environmental compartments, ranging from land to air and from rivers to oceans. Extensive research has unequivocally demonstrated the widespread presence of MPs in all environmental compartments, indicating their global dissemination driven by increasing inputs from both primary and secondary sources. Land-based sources, including direct littering and ineffective waste management practices such as landfill sites, waste disposal, and industrial spills, contribute significantly to the generation of MPs. This trend is expected to persist due to the escalating consumption of synthetic polymers and inadequate disposal and recycling infrastructure (Bretas Alvim et al., 2020). Consequently, there is a likelihood of substantial transformation of MPs from terrestrial to marine environments, with the potential for re-deposition on land during high tides and storm events (Horton and Dixon, 2018). Furthermore, wind and rainfall play pivotal roles in the transportation and deposition of MPs, facilitating their transfer from heavily polluted areas to remote and pristine regions (Klein and Fischer, 2019; Roblin et al., 2020). The shape, size, and polymer composition of MPs in the environment exhibit variability influenced by factors such as their sources, residence time, degradation, and transfer rates (Zhang et al., 2021). Studies have revealed considerable variations in the chemical composition, size, and shape of MP deposits in air, particularly across different locations such as urban, suburban, and remote areas (Allen et al., 2019; Cai et al., 2017; Ren et al., 2020). Notably, MPs have been detected not only on the surface of land, water, and the atmosphere but also within the deeper layers of soil. The presence of MPs in soil layers is influenced by soil permeability and the activities of soil-dwelling organisms, such as earthworms (Huerta Lwanga et al., 2017). Additionally, sewage sludge has been identified as a reservoir of MPs, with various countries employing disposal methods such as incineration, landfilling, or utilization as agricultural fertilizer (Duis and Coors, 2016). The application of sewage sludge to agricultural land can serve as a source of MPs for terrestrial environments (Hassan et al., 2023), subsequently mobilized and distributed by wind or transported via surface runoff to aquatic ecosystems (Duis and Coors, 2016).

1.1. SOURCE OF MICROPLASTICS IN WASTEWATER TREATMENT PLANTS

Wastewater treatment plants (WWTPs) receive a significant volume of MP particles daily from various sources including households, businesses, industries, and landfills. These plants serve as the primary receptors of MPs before the water is released into natural water bodies (Xu et al., 2021). Common personal care products like body lotions, soaps, scrubs, and toothpaste contain numerous MPs such as microbeads and exfoliants, which are washed down the drain during daily use (Carr et al., 2016). Additionally, textiles, particularly synthetic ones, have the potential to shed thousands of microfibers with each wash (Browne et al., 2011; Dris et al., 2016; Napper and Thompson, 2016). The discharge of these microfibers from textile manufacturing processes and washing machines contributes significantly to MP pollution in WWTPs

(Barnes et al., 2009; Carney Almroth et al., 2018; Napper and Thompson, 2016; Salvador Cesa et al., 2017). For example, a single garment wash can release over 1900 microfibers (Browne et al., 2011), and washing machines in Finland reported emitting millions of polyester and cotton microfibers annually (Sillanpää and Sainio, 2017). Furthermore, the release of microfibers is influenced by factors such as garment age, fabric type, washing techniques, and water temperature (Carney Almroth et al., 2018). Additionally, MPs from kitchenware contribute to the direct influx into wastewater. These continuous sources pose persistent challenges to wastewater treatment facilities.

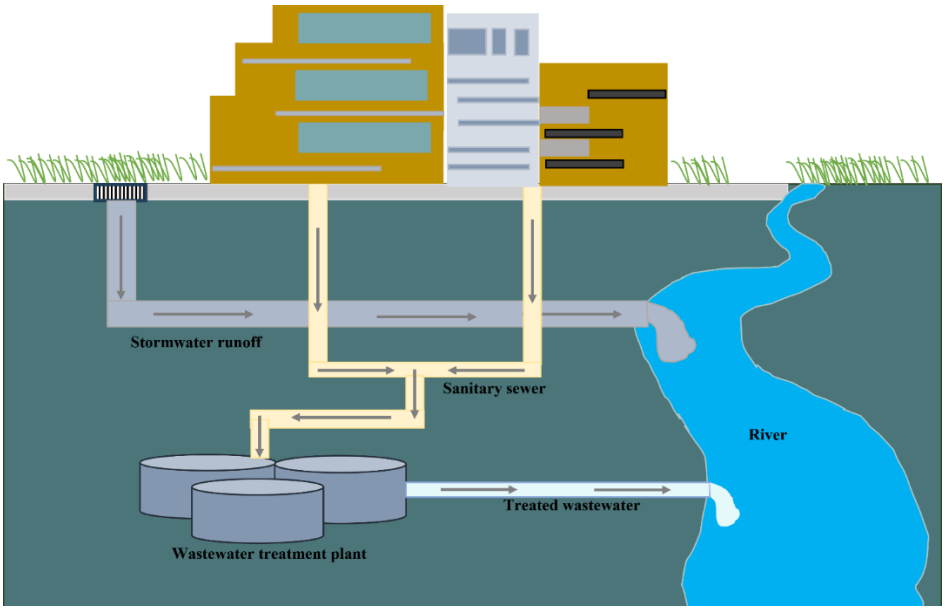

Figure 1: Transfer of land-based MPs to water resources.

Furthermore, microbeads and pellets produced by industrial processes, originally intended for various industrial applications, can unintentionally or deliberately find their way into sewer systems. While some industries have implemented wastewater treatment systems to mitigate the mixing of pollutants with municipal wastewater, however, this practice is not universal. Consequently, MPs originating from manufacturing industries can substantially elevate the concentration in wastewater influent. These industrial effluents may introduce distinct types of MPs, differing in size, shape, and polymer composition, compared to those found in municipal wastewater. Municipal wastewater, sourced from households, commercial establishments, and, in some cases, combined sewer inflows, represents a diverse array of origins (Figure 1). In addition to direct contributions from dry weather sources, there are indirect sources exacerbated by wet weather events, such as tire wear from vehicles and the fragmentation of plastic surfaces from unmanaged waste

on roads and streets (Järlskog et al., 2020; Mason et al., 2016). For example, microorganisms acting upon garbage can lead to the transfer of MP particles into leachate, subsequently reaching WWTPs via surface runoff. Moreover, there is the potential for atmospheric deposition of floating MPs emitted by plastic industries and vehicles, which can contaminate urban air (Liu et al., 2021). Reports indicate that urban air is heavily laden with MPs, suggesting some deposition into open chambers of WWTPs, such as biological activated sludge and sedimentation chambers. The common types of MPs reported in WWTPs are Polyethylene (PE), Polypropylene (PP), Polyester, Polyamide (PA), Polystyrene (PS), Polyurethane (PU), Polyvinylchloride (PVC), Acrylic, Cellulose acetate, Acrylonitrile Butadiene Styrene (ABS), Poly-vinyl acetate (PVac), Pan acrylic fibre (PAN), Alkyd, Aramid and Poly (vinyl stearate).

1.2. FATE OF MICROPLASTICS IN WASTEWATER TREATMENT PLANTS

WWTPs are not specifically engineered to tackle MPs (Acarer, 2023). Nevertheless, previous research has demonstrated a significant decrease in MP levels in the final effluent. On the other hand, WWTPs can both impede and serve as entry points for MPs into water bodies (Talvitie et al., 2017a). However, the conventional methods employed in WWTPs, combining mechanical, chemical, and biological treatments, have proven effective in capturing MPs, with retention rates ranging from 64% to 99% by particle count (Carr et al., 2016; Liu et al., 2021; Magnusson and Norén, 2014; Talvitie et al., 2017a) and up to 98% by particle mass (Simon et al., 2018). But, it should be noted that existing technologies cannot completely eliminate MPs from wastewater (Acarer, 2023). Despite the low concentration of MPs reported in final effluents, the substantial volume of wastewater treated and discharged means significant quantities of MPs are still released into nearby water bodies on a daily or annual basis (Li et al., 2018; Simon et al., 2018; Talvitie et al., 2017b, 2015; Xu et al., 2021; Ziajahromi et al., 2017). For instance, daily MP loads in effluents have been documented to range from 5×10^5 to 1.39×10^{10} (Dris et al., 2015; Lares et al., 2018; Michielssen et al., 2016; Ziajahromi et al., 2017), with concentrations varying from 0.01 to 2.97×10^2 items L^{-1} (Liu et al., 2021). Studies have also revealed elevated MP concentrations in surface water downstream of WWTPs after treated wastewater is mixed, indicating WWTP effluents as significant sources of land-based MPs in rivers, receiving inputs from industries producing MPs and domestic effluents containing MPs, such as those from cleansing and cosmetic products (Eerkes-Medrano et al., 2015; Kay et al., 2018; Zbyszewski et al., 2014).

MPs have the potential to be extracted during preliminary, primary, secondary, and tertiary treatment stages and stored in the sludge and enter the digester. Both small plastic particles and larger ones are capable of retention. The efficiency of MP removal in WWTPs likely depends on treatment unit configurations, engineering designs, and technologies (Kurt et al., 2022; Sun et al., 2019). However, various

factors can influence MP removal rates, including polymer types and densities, particle shapes and sizes, water flow rates, and MP flow rates. Advanced final-stage treatment technologies like rapid sand filtration, membrane bioreactors (MBRs), and disc filtration have exhibited higher efficacy in MP retention (Talvitie et al., 2017a; Xu et al., 2019). Conversely, WWTPs relying solely on primary and secondary treatments may exhibit lower MP retention capacities. Table 1 provides a summary of influent and effluent MP loads in different WWTPs across various countries with different treatment processes, where removal efficiencies were calculated based on their concentrations in influent and effluent. However, comparing data is challenging due to significant variations in studies employing different MP sampling and analysis techniques (Mintenig et al., 2017).

Table 1: Influent and effluent concentration and removal rate of MPs in WWTPs with different treatment processes in different countries

S.N.	Treatment processes	Location/Country	Influent load (items L^{-1})	Effluent load (items L^{-1})	Removal efficiency (%)	Reference
1	Primary, Secondary	Scotland	15.7	0.25	98.4	(Murphy et al., 2016)
2	Primary, Secondary	United States	133	5.9	95.6	(Michielssen et al., 2016)
3	Primary, Secondary	United States	1	0.00088	99.9	(Carr et al., 2016)
4	Primary, Secondary	Sweden	15.1	0.0082	99.9	(Magnusson and Norén, 2014)
5	Primary, Secondary (Biofilter)	France	293	35	88.1	(Dris et al., 2015)
6	Primary, Secondary	Denmark	2223 – 18285	19 – 447	99.3	(Simon et al., 2018)
7	Primary, Secondary	Finland	57.6	1	98.3	(Lares et al., 2018)
8	Primary, Secondary, Tertiary (Granular filter)	United States	91	2.6	97.2	(Michielssen et al., 2016)
9	Primary, Secondary, Tertiary (Biological aerated filter)	Finland	610	13.5	97.8	(Talvitie et al., 2017b)
10	Tertiary (Disc filter)	Denmark	29	3	89.7	(Simon et al., 2019)
11	Primary, Secondary, Tertiary (Disc filter)	Sweden	533	4	99.2	(Rasmussen et al., 2021)
12	Primary, Secondary	Canada	31.1	2.6	91.7	(Gies et al., 2018)
13	Primary, Secondary, Tertiary (Reverse osmosis)	Australia	1.6	0.2	87.5	(Ziajahromi et al., 2017)
14	Primary, Secondary, Tertiary (Denitrification/Ultrafiltration/Ultraviolet)	China	12.03	0.59	95	(Yang et al., 2019)

1.2.1. PRELIMINARY AND PRIMARY TREATMENT

Mechanical processes employed in wastewater treatment plants (WWTPs) are designed to eliminate coarse solids, sand, grit, oil, fat, and grease contaminants. Consequently, MPs can be efficiently removed through mechanical means such as screening/filtration, flotation, skimming, and sedimentation. The effectiveness of this stage depends on the aperture size of the screening material (Kurt et al., 2022) and the shape of the particles, with larger openings having a lower likelihood of capturing MPs, though some may still be retained within the filter cake. Fine screens, typically less than 5 mm in size, can capture significant MP particles, although long fibers and particles smaller than the pore openings may still pass through. While larger particles are easier to capture, smaller ones present challenges for removal. Moreover, the removal of MPs can be enhanced through grit and grease elimination. As fat, oil, and grease in wastewater are generally low-density and hydrophobic, they can readily adhere to MPs of similar characteristics, forming aggregates that are subsequently removed during skimming (Carr et al., 2016; Chand et al., 2021). Additionally, settling of dense MPs and those trapped in solid flocs during grit and sand removal can further improve MP removal efficiency.

In the primary treatment phase, the density of MPs plays a crucial role. Low-density MPs, such as polyethylene (HDPE or LDPE), polypropylene (PP), and polystyrene (PS) with densities less than 1 g cm^{-3}, can be eliminated through flotation, while high-density MPs like polyester (PEST) and nylon, with densities greater than 1 g cm^{-3}, are removed through sedimentation. Previous studies have demonstrated that preliminary and primary treatments are effective in removing large MPs, with removal rates ranging from 50% to 90% (Dris et al., 2015; Lares et al., 2018; Murphy et al., 2016; Talvitie et al., 2017a; Yang et al., 2019; Ziajahromi et al., 2017).The removal of larger MPs during pre-treatment stages also influences the size distribution of particles in subsequent treatment stages.

1.2.2. SECONDARY TREATMENT

During the secondary treatment phase, microorganisms such as bacteria, fungi, protozoa, and rotifers play a pivotal role in processing the incoming wastewater from the primary effluent. These microorganisms break down organic and biodegradable waste, as well as some inorganic waste, converting them into new bacterial mass and byproducts. The resulting bacterial flocs and byproducts are subsequently eliminated through settling and other physical methods. Meanwhile, MPs tend to aggregate with biological flocs and other suspended organic or particulate matter, effectively settling in the secondary clarifier (Wu et al., 2021). However, the instability of these flocs can lead to the redistribution of MPs, allowing them to escape into further processing stages (Carr et al., 2016). Additionally, the presence of MPs can detrimentally impact the treatment process, potentially reducing performance or increasing costs.

Secondary treatment, encompassing biological treatment and secondary sedimentation could further remove 0.2% to 14% MPs (Sun et al., 2019; Wu et al., 2021). Talvitie et al. (2017b) also noted a 7% to 20% decrease of microlitter through activated sludge treatment. Essentially, the duration of contact between MPs and biofouling can influence removal efficiency; longer contact times increase the likelihood of particle adsorption (Carr et al., 2016). Denser aggregates have a higher propensity to settle in the clarifier. Moreover, coagulation has demonstrated a positive impact on MP removal from WWTPs (Tang et al., 2022). Coagulants generate suitable suspended particulate matter to aggregate MPs, forming insoluble large flocs that aid in particle settlement alongside other solid fractions. Consequently, coagulation processes have achieved relatively high removal rates ranging from 47.1% to 81.6% ((Hidayaturrahman and Lee, 2019; Ruan et al., 2019; Zhou et al., 2021). However, a relatively low abundance of MPs, particularly fibers and the smallest, lightest fragments, may still pass through the secondary effluent. Nonetheless, the majority of small bead fragments are typically removed during the secondary treatment process.

1.2.3. TERTIARY TREATMENT

Tertiary treatment in WWTPs has been identified as a crucial step in further refining the removal of MPs and serving as the ultimate polisher in MP removal. Despite primary and secondary treatments accounting for 72% to 98% of MP removal (Leslie et al., 2017; Mason et al., 2016; Murphy et al., 2016; Talvitie et al., 2017a) a tertiary treatment remains valuable for enhancing WWTP efficiency. A reduction in MPs ranging from 0.2% to 2% relative to influent has been reported in effluent, depending on the technologies employed (Sun et al., 2019). Technologies utilized in tertiary treatment, such as disc filters (DF), rapid sand filtration (RSF), and dissolved air flotation (DAF), have demonstrated removal efficiencies as high as 98% (Simon et al., 2019; Talvitie et al., 2017a). Membrane technologies, including membrane bioreactors (MBRs), exhibit superior MP removal efficiency compared to other methods (Leslie et al., 2017; Talvitie et al., 2017a). However, the effectiveness of MBRs may diminish over time as this technology operates under high pressure and has not been specifically optimized for MP polishing (Leslie et al., 2017).

1.2.4. SEWAGE SLUDGE DIGESTER

The sludge contains a considerable amount of organic materials and nutrients, making it a valuable resource for recovery. One common method is anaerobic sludge digestion (AD), which has been widely used for methane gas production, resulting in a 30% to 50% reduction in organic load. AD operates at either mesophilic (35 °C to 40 °C) or thermophilic (50 °C to 55 °C) temperatures. In some WWTPs, additional organic materials such as skimmed grease and fatty slurry from restaurants and food factories are added to increase biogas production and manage waste (Chand et al., 2021). However, MPs are also concentrated in the sludge (Michielssen et al., 2016), primarily in grit, grease, and primary settling tanks. Although sludge treatment processes are not designed to stabilize MPs, they may undergo changes in size, mass, and number

due to shearing in the digester. Furthermore, research is underway on biofuel production through hydrothermal liquefaction (HTL), where biomass macromolecules break down under high temperature and pressure to produce bio-oil products (Toor et al., 2011). Sewage sludge is considered a promising resource for HTL process, which has been found to convert some common plastic waste (Iwaya et al., 2006; Madsen et al., 2016; Su et al., 2004; Yildirir et al., 2015; Zhao et al., 2018), leading to the expectation that it can also help to reduce MPs in the sludge.

CHAPTER 2. STUDY AIM AND OBJECTIVES

WWTPs are receiving a significant influx of MPs from diverse sources, with a considerable portion being captured and accumulated in sludge, grease, and other solid byproducts. Consequently, WWTPs have emerged as prominent focal points in the study of MPs. The volume of research dedicated to analyzing MPs has been steadily rising, resulting in hundreds of research publications each year. However, the majority of these studies are limited to reporting on the abundance of MPs in influent and effluent, often providing only a general overview of efficiency. Few studies have delved into investigating the efficacy of different treatment stages within WWTPs in removing MPs. This underscores the inadequacy of available data and information for comprehensively understanding how individual processes within WWTPs influence MP removal. Furthermore, there has been a notable dearth of research on the fate of MPs within sludge digesters. Additionally, the extent to which extreme thermal processes contribute to the breakdown or complete degradation of MPs in sludge remains largely unexplored. Similarly, while mesophilic digestion is a commonly practiced method for reducing sludge volume and generating biogas, detailed studies examining the fate of MPs in this process are scarce.

Furthermore, inconsistencies persist in MP analysis, with much of the existing research relying on manual sorting and less technologically advanced tools. Additionally, there is a lack of standardized protocols for extracting MPs from complex sample matrices, such as sludge, grease, fatty slurries from food industries, biocrude, and residual materials. These challenges have led to discrepancies in reported MP abundances, likely stemming from differences in analytical techniques (Chand et al., 2021). Visual and manual sorting of particles can introduce uncertainty, particularly with small particles below 500 µm. Therefore, the objective of this PhD study is to analyze MPs using an automated spectroscopic imaging approach, such as µ-FTIR for small particles (< 500 µm), to minimize human bias. Also, large particles (> 500 µm) were analyzed using ATR-FTIR. Additionally, this study aims to refine the procedure for extracting MPs from complex sample matrices.

To make this study a systematic investigation on MPs in WWTPs several research questions were formulated, and they were answered accordingly.

Research question 1*: What is the fate of MPs in a mesophilic anaerobic digester receiving WWTP sludge, grease, and fatty slurries from external sources?*

The research question was addressed by determining the quantities of MPs in both numbers and mass at the inlet and outlet of a mesophilic anaerobic digester. The digester's inlet received waste streams from various sources, including materials from grease traps, a mixture of primary and secondary sludge, and fatty slurries from grease

traps at external food industries such as restaurants and food factories/ice-cream factories. The outlet product comprised the discharged materials from the digester. Additionally, a mass balance of the MPs entering and exiting the anaerobic digester was established.

Research question 2*: Does hydrothermal liquefaction degrade or break down MPs in wastewater treatment sludge?*

HTL is a thermochemical method known to break down macromolecules and rearrange them into different compounds. Typically, this process operates at moderate temperatures (280–400°C) and high pressures (10–35 MPa). In terms of the research inquiry, the investigation centred on evaluating MPs in sludge subjected to the HTL process and the resulting residual products. The study aimed to determine if there were any significant effects on specific polymers, such as the disappearance or resilience of certain types of plastic following HTL treatment. Furthermore, the study conducted a comprehensive assessment of MP numbers and mass to establish a balance across the HTL reactor.

Research question 3*: What is the fate of MPs in WWTPs and how much of the MPs enter receiving waters from a modern state-of-the-art WWTP?*

This research inquiry was explored through an investigation into the presence of MPs within an advanced wastewater treatment facility employing mechanical processes for preliminary and primary treatment, biological treatment for secondary treatment, and sand filtration for tertiary treatment. The study examined the abundance of MPs at various stages of the wastewater treatment process as well as in the final effluent. Additionally, the quantities of MPs in the sludge entering and exiting an anaerobic mesophilic digester were quantified.

Research question 4: *Can snow melt contribute significantly to the influx of MPs, TWP, and metal pollutants to WWTPs and adjacent water bodies?*

This goal was achieved through an investigation into MPs in six distinct locations across Riga, Latvia, where freshly fallen snow was collected from designated snow dumping sites. The selection of these locations was based on varied land use practices, including business/commercial centers, pedestrian streets, residential areas, parking lots, rooftops in the city center, and remote areas within the national park. Advanced analytical techniques, such as μ-FTIR and Pyr-GCMS, were employed to analyze MPs and rubber particles from tire wear, respectively. Additionally, the concentration of metals was determined using Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP – AES). Subsequently, correlations between MPs, rubber (tire wear), and metal elements were established.

CHAPTER 3. METHODOLOGY

This PhD study was mainly focused on the fate of MPs entering wastewater treatment plants and physiological or morphological changes of MP in sludge digesters. Specifically, in mesophilic anaerobic digesters and hydrothermal liquefaction treatment. Furthermore, snowmelts from urban and remote regions were analyzed to investigate the possible source of MPs and tire wear from urban surface runoff to WWTPs. Based on the research objectives formulated in Section 2, the following sub-topics were formulated:

- Fate of MPs in sludge and slurries of a WWTP and external sources and their treatment in a mesophilic anaerobic digester.
- Fate of MPs in WWTP sludge treated by hydrothermal liquefaction.
- The occurrence and fate of MPs in a modern state-of-the-art WWTP with sand filtration.
- Occurrence of MPs in freshly fallen snow from different urban and remote areas.

An overview of how the study was carried out and its results is given in the following sections. Details are given in **Paper I – IV**.

3.1. THE OCCURRENCE AND FATE OF MICROPLASTICS IN CO-DIGESTION OF SLUDGE, GREASE, AND FATTY SLURRIES

The sludge digester in Ryaverket WWTP receives skimmed grease and sludge products from the treatment system as well as organic and fatty slurries from food industries, such as restaurants, bakeries, and other food processing industry (Figure 2). The WWTP grease samples were taken from the grease trap with metal buckets. Likewise, the sludge samples were grabbed from the inlet and outlet of the digester by means of metal buckets and stored in aluminum bottles. The fatty slurries were taken in aluminum bottles from the outlet hose of the transport truck. Further details, here among on the WWTP, skimmed grease and produced sewage sludge and received fatty slurries has been given in **Paper I**. The subsequent purification of samples, MP extraction and polymer identification were done following the protocol described in the paper and summarized later in this section.

Subsequently, the number and mass of the MPs per unit of dry weight of the waste streams entering and exiting a mesophilic anaerobic digester was estimated. Hence, an annual mass balance of MPs was calculated based on the annual waste received and discharged over the digester. The major contribution of MPs from the received streams and discharged to digested sludge was measured. Likewise, the reduction of MP mass and number over digester was calculated and presented in **Paper I**.

A somewhat similar study was done for Käppala WWTP. Three inlet and 3 outlet sludge samples from the mesophilic sludge digester were collected. The research and its results in terms of reduction of MP particles and mass over the digester was calculated and presented in **Paper III.**

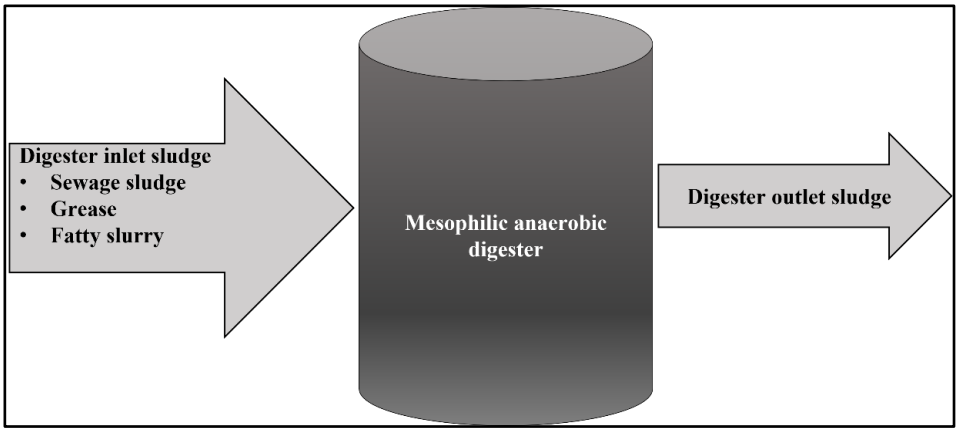

Figure 2: Schematic diagram for mesophilic anaerobic sludge digester.

Before analysis, the samples were uniformly mixed and sub-samples were taken depending on the solid content. For instance, for digester inlet approximately 100 g and for digester outlet 200 g of sub-sample was taken and processed in the lab for MP extraction.

The collected samples were treated using different chemical and physical processes to remove the organic contents, such as, cellulose fibers and proteinaceous materials and inorganic substances, by which the MPs were extracted. The major treatment steps included pre-oxidation, surfactant treatment, enzymatic treatments, oxidation and density separation. The particles were collected on stainless steel filters of 10 µm mesh size after each treatment step. Further, the large particles (>500 µm) and small particles (< 500 µm) were fractionated using a 500 µm mesh sized sieve. Manually selected large MPs mapped with a stereoscopic microscope (ZEISS, SteREO Discovery.V8). The images were taken with an Axiocam 105 color camera and max. 8x magnification. ZenCore software (Zen2Core SP1 from ZEISS) coupled to the stereoscopic microscope used to measure the particle size (Chand et al., 2022). Cary 630 FTIR (Agilent Technologies) with a diamond ATR was used to identify the polymer type (Figure 3 – 5). Particles between 500 and 10 µm were concentrated into 50% ethanol of HPLC grade and subsamples were analyzed by FTIR spectroscopy hyperspectral imaging, using Agilent Cary 620 FTIR microscope and Agilent 670 IR spectroscope (Chand et al., 2022, 2021). Three separate sub-samples were analyzed for each sample. The hyperspectral images were analyzed by the software siMPle, designed by Aalborg University and Alfred Wegener Institute, to identify MPs (Primpke et al., 2020). The outcome of the analysis was information on particle

numbers, polymer types, surface area, maximum and minimum Feret diameters, as well as an particle mass estimation.

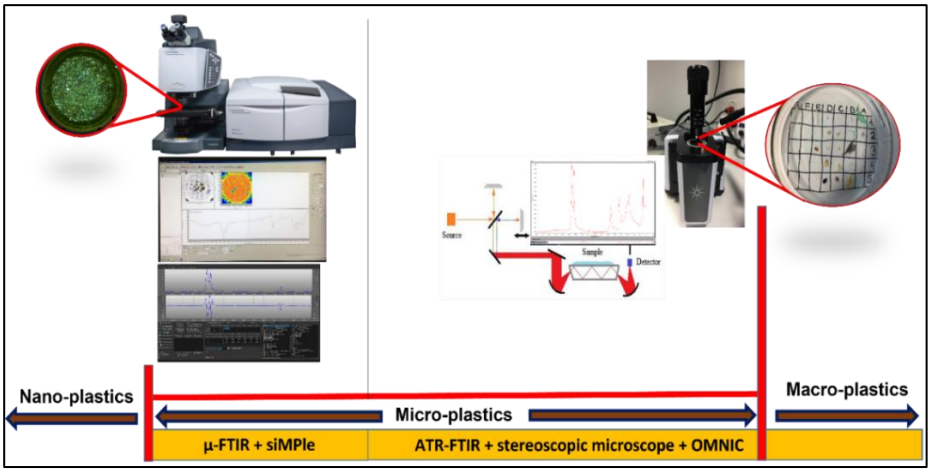

Figure 3: Used analytical techniques (Agilent Technologies) to analyze microplastics (10 – 5000 µm) in the present study.

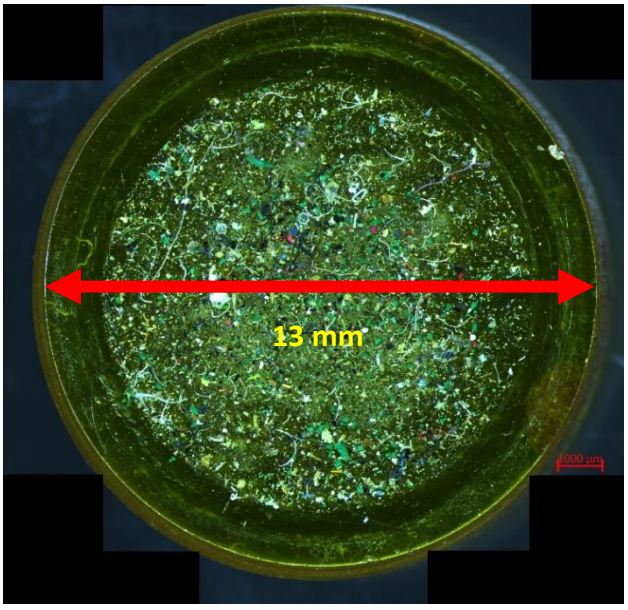

Figure 4: Microplastics (10 - 500 µm) analyzed with FPA-µ-FTIR.

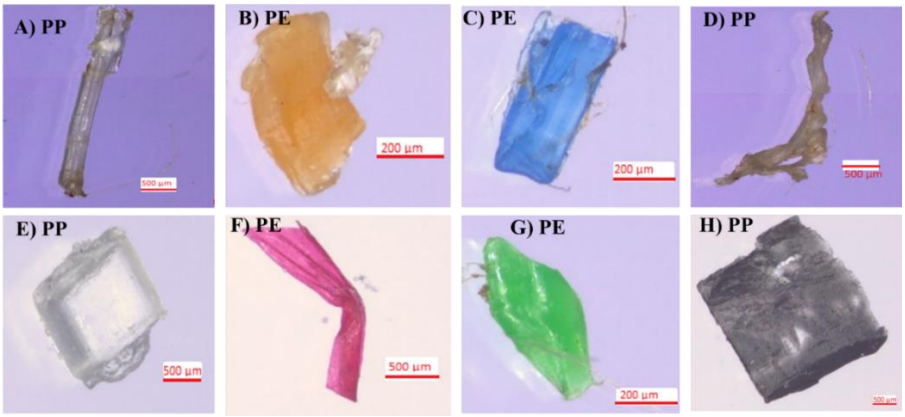

Figure 5: Microplastics (> 500 µm) analyzed with ATR-FTIR.

3.2. EFFECT OF HYDROTHERMAL LIQUEFACTION ON MICROPLASTICS IN SEWAGE SLUDGE

Partially dewatered sewage sludge was collected from Stistrup WWTP, Farsø, Denmark. The percentage of moisture and ash content (dry organic matter) was measured in the sewage sludge and water, industrial grade potassium carbonate (K_2CO_3) and sodium hydroxide (NaOH) were added before HTL processing. The prepared slurry was pressurized to 30 MPa and injected into two successively connected induction heaters at 400 °C. Then the slurry was transmitted to two 5 L serially connected heat resistant reactors. Further the effluent was discharged to concentric-tube heat exchanger and passes through high pressure filters. The as-received product was further separated into multi-phase products, such as aqueous, emulsion, solid residue, and useful bio-crude using lab-scale physio-chemical phase separation methods.

Duplicate samples of sewage sludge, slurry, as-received product, aqueous product, emulsion, distillate, concentrate, solid residue and bio-crude were collected (Figure 6). Standard protocols were developed to extract the MPs from these samples. The fate of MPs in a continuous HTL process was quantified, as was the impact of the HTL process on reduction and degradation of MPs. The residual MPs were further investigated in the residual products, such as solid and liquid residues. The details of the HTL project process, lab-scale experiments, MPs extraction, analysis of extracted MPs and obtained results presented in detail in **Paper II**.

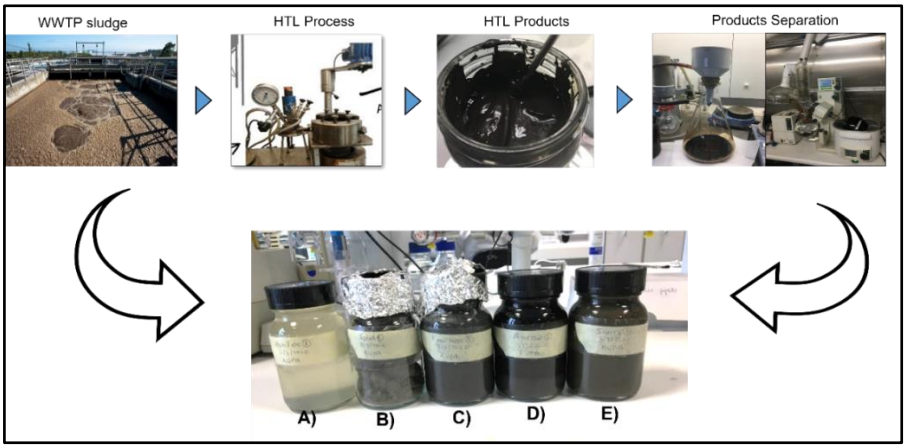

Figure 6: Sewage sludge treated with hydrothermal liquefaction (HTL) process and separation of the received products into different forms: A) Distillate water, B) Solid residue, C) Emulsion, D) As-received product from HTL chamber, and E) Slurry.

3.3. THE OCCURRENCE AND FATE OF MICROPLASTICS IN A STATE-OF-THE-ART WASTEWATER TREATMENT PLANT EQUIPED WITH SAND FILTRATION

The abundance of MPs in inlet and treated wastewaters of Käppala WWTP, an advanced wastewater treatment plant with sand filtration as tertiary treatment was assessed. This facility receives wastewater from over 500,000 inhabitants. Wastewater was collected at different treatment steps (Figure 7). The concentration and state of MPs in both liquid phase and solid phase was investigated, which was achieved by studying: A) How different treatment units retrain MPs, and B) How mesophilic sludge digester manage MPs.

The wastewaters from inlet and effluent from different treatment steps were collected flow proportionally. The outlet was sampled by filtering large water volumes using a custom-made filtration device.

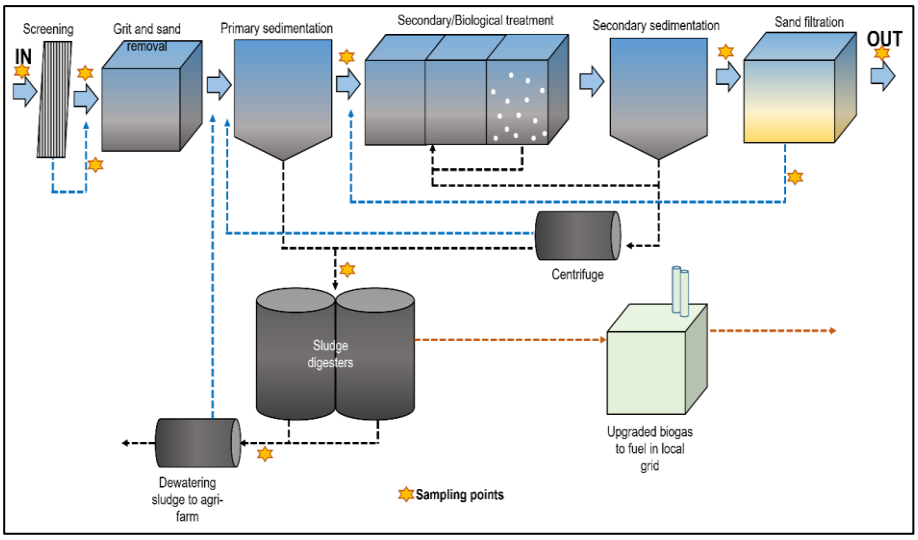

Figure 7: Flow diagram of Käppala WWTP and sampling points.

3.4. OCCURRENCE OF MICROPLASTICS, TWP, AND METALS IN FRESHLY FALLEN SNOW FROM URBAN AND REMOTE AREAS

Triplicate snow samples were collected from six sites in Riga; a central market (C1), an old town (C2), a parking lot (C3), and from a 50 m high rooftop of an academic science building (C4); one from a residential area (R1), and the last one from a national park (P1) 50 km far from the city (Figure 8). Besides, plastic canister was used to take sample for metal analysis. Snow samples were stored and left to melt at room temperature (22 – 23 °C). The melted snow volume was measured and then filtered through a 10 µm stainless-steel filter of Ø167 mm (Chand et al., 2024). MPs and tire wear particles (TWPs) were extracted via various treatments as described in Section 3.1. The large MPs were analyzed by ATR-FTIR spectroscopy while small MPs were analyzed by FPA-µFTIR spectroscopy, also as described in Section 3.1. TWPs were measured using pyrolysis gas chromatography mass spectrometry (Py-GCMS). The concentrations of selected metal elements (Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn) were quantified by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES). Further details are given in **Paper IV**.

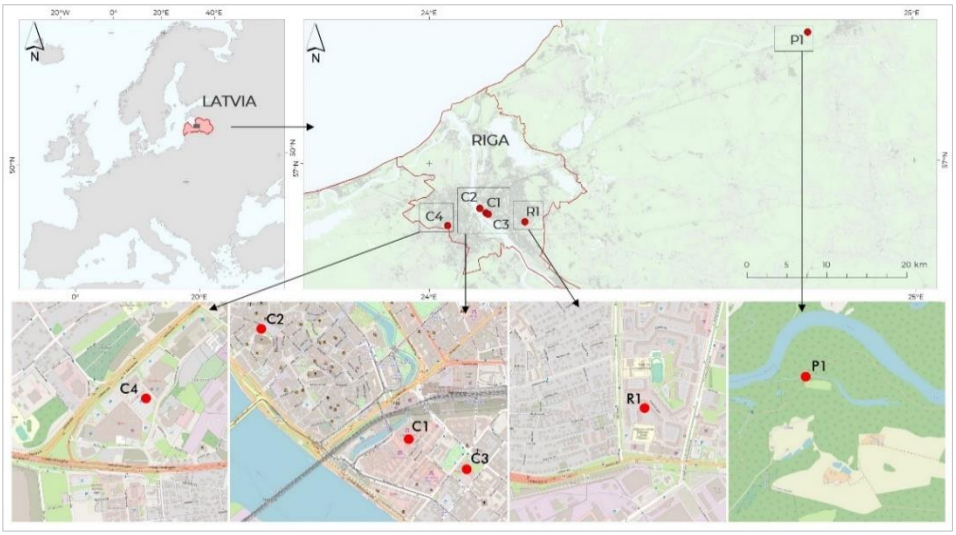

Figure 8: Sampling locations in Riga.

CHAPTER 4. RESEARCH OUTCOMES

The following sections present main outcomes of the PhD study following the structure presented in Chapter 3.

4.1. THE OCCURRENCE AND FATE OF MICROPLASTICS IN CO-DIGESTION OF SLUDGE, GREASE, AND FATTY SLURRIES

Paper I and **Paper III** assessed the overall status of MPs in the solid matrices produced at WWTPs and fatty slurries from food industries that underwent a mesophilic anaerobic digestion in a WWTP. In both papers, the concentration of MPs was rather similar in the inlet to the mesophilic digester. At Ryaverket WWTP, the MP particle count concentration in the inlet and outlet sludge was 4.08 (±0.25) × 10^6 counts kg^{-1} (dry weight) and 6.36 (±0.80) × 10^6 counts kg^{-1} (dry weight), respectively, while it was respectively 1.11 (±0.009) × 10^5 counts kg^{-1} (wet weight) and 1.16 (±0.0003) × 10^5 counts kg^{-1} (wet weight) at Käppala WWTP. The increasing concentration in terms of MP counts might have been due to disintegration of larger particles during the digestion. However, the digestion itself consumes organic matter, and the concentration per dry weight would hence increase correspondingly for a non-degradable pollutant. This could also contribute to explaining the observed increase in concentration.

An annual MP balance was assessed in **Paper I** and it showed a significant reduction rate. The MP removal rate was 29% by the MP counts while it was 33.3% by MP mass. In **Paper III** a daily assessment of MP counts in digester inlet and outlet sludge showed removal rates of approximately 4% by MP count and approximately 10% by MP mass. This difference between studies might be due to various aspects. In **Paper I** the source of MPs were sewage sludge and grease from a WWTP and fatty slurries from food industries while in **Paper III** only sewage sludge underwent mesophilic anaerobic digestion. The grease from the primary treatment stage contained more and larger MP particles. Along with the smaller MPs, a few larger floatable plastic particles were found in the grease, but not considered in the MP balance. However, seeing the variation on degradation rate of MPs in both studies, it is difficult to draw a conclusion on the effectivity of mesophilic anaerobic digestion to break down MPs retained in the sewage sludge.

Furthermore, the quantification fibers were challenged by their variable shape and size. Fibers are furthermore strongly entangled with each other which made it difficult to separate them for analysis. Therefore, only a qualitative analysis was done by taking entangled fibers and analyze them as one by ATR-FTIR. This showed that the fibers were mainly of polyester, which indicated that their origin was textiles and probably came from laundering. The information about the fibers was not included in the paper to prevent misinterpretation of their quantity. But fibers, and their missing

quantification, might also to some degrees have impacted MPs concentrations in inlet and outlet sludge since fibers might have been broken down to smaller MPs during digestion. This can further have impacted the MP balance over the digester. The presence of few but large plastic particles not taken into account might have influenced the mass balance, as the larger MPs contribute the majority of the MP mass. The composition of MP polymers seemed unaffected by the mesophilic anaerobic digestion.

4.2. EFFECT OF HYDROTHERMAL LIQUEFACTION ON MICROPLASTICS IN SEWAGE SLUDGE

Paper II assessed the fate of MPs in sewage sludge that underwent hydrothermal liquefaction (HTL) where the sewage sludge was treated at supercritical water condition at high temperature (400 ºC) and pressure (30 MPa). The HTL process converts the sludge into bio-crude which can be further treated to produce biofuel. The MPs in the sludge were determined prior to the HTL treatment and after treatment in all produced solid and liquid phase. MPs in sewage sludge (before HTL treatment) was 8.10×10^5 counts kg^{-1} while the concentration in the as-received products (after HTL treatment) was 3.53×10^5 counts kg^{-1}. The MP mass was 9.64×10^7 ng kg^{-1} and 1.31×10^5 ng kg^{-1} in the feed sewage sludge and the as-received products, respectively. This study showed that the HTL process efficiently reduced MPs by 76% of MP numbers and 97% of MP mass. Furthermore, some polymer types disappeared completely after the treatment, where 18 polymer types were found in sewage sludge, while only 11 polymer types were found after the treatment. PU, PP, and PE were dominant polymer in sewage sludge where, PP and PE (polyolefins) were dominant in the residual products. Also, the MPs size was considerably lowered by the HTL treatment.

4.3. THE OCCURRENCE AND FATE OF MICROPLASTICS IN A STATE-OF-THE-ART WASTEWATER TREATMENT PLANT EQUIPED WITH SAND FILTRATION

In **Paper III**, a detail assessment of MPs at Käppala WWTP, Stockholm, Sweden was carried out. Here the role of treated wastewater on the MP pollution of receiving waters was addressed. The study demonstrated that Käppala WWTP achieved a high MP removal efficiency where the MP influent concentration decreased from 6.42×10^{10} counts day^{-1} and 8.99 kg day^{-1} to an effluent concentration of 1.04×10^7 counts day^{-1} and 0.00046 kg day^{-1}, that gives an overall efficiency of 99.98% and 99.99% for MP counts and mass, respectively. The MP concentration in the effluent were comparable to what a couple of other studies had reported for Danish/Swedish marine waters applying the same analytical techniques. The majority of the MPs in the raw sewage entering the WWTP were removed by the mechanical stages of the WWTP, the preliminary and primary stages. MPs were further reduced in the biological stages, and finally the tertiary treatment applying sand filtration polished

the effluent to levels comparable of those found in said marine waters. The sand filtration was especially efficient in removing the small and fine MPs.

4.4. OCCURRENCE OF MICROPLASTICS, TWP, AND METALS IN FRESHLY FALLEN SNOW FROM URBAN AND REMOTE AREAS

Paper IV assessed the potential of snow transferring MPs, TWP, and metals to nearby water resources and/or local WWTPs. The investigation was done in different locations based on land use such as a national park, a remote urban site, and highly crowed urban and residential centers. The results showed a significant spatial variation in the targeted pollutants. MPs were found at all locations and varied from 26 to 2549 counts L^{-1} corresponding to 19 – 573 µg L^{-1}. The mass of TWP ranged between 44 and 3026 µg L^{-1} and was not detected in two referenced locations. Fourteen metals were assessed and found to vary significantly between sites. Na had the highest concentration (449 – 819536 µg L^{-1}), while Cd was detected at the lowest concentrations (0.05 – 0.94 µgL^{-1}). Metal concentrations showed a weak to moderate correlation with the MPs number and mass, while they showed strong correlation with TWP. The results suggest that the melting of snow, if untreated, could be a significant source of MPs, TWP, and metals to water bodies. The presence of MPs in remote areas indicated that these were affected by atmospheric deposition of MPs.

CHAPTER 5. CONCLUSIONS

The PhD study targeted the fate of MPs in WWTPs and in sludge management. The latter specifically addressed mesophilic sludge digestion and HTL treatment of sludge, that is, treatment at high temperature and pressure. Melted snow was included as a possible source of MPs to WWTPs via wet weather runoff. This PhD study was designed to address four research questions, from which the following conclusions were drawn.

Research question 1*: What is the fate of MPs in a mesophilic anaerobic digester receiving WWTP sludge, grease, and fatty slurries from external sources?*

This work provided a complete overview of the MPs in different waste streams entering and exiting anaerobic digesters. This study was a first of its kind to quantify such details. **Paper I** and **Paper III** showed different reduction rates which can be explained by different aspects of the WWTPs. The study indicated that bigger particles might be fragmented into smaller ones during digestion, but that the digestion did not affect the total plastic mass significantly.

Research question 2*: Does hydrothermal liquefaction degrade or break down MPs in wastewater treatment sludge?*

Paper II found that HTL significantly reduces MPs in sludge. Some polymer types were completely degraded, while some were more resilient to the extreme conditions of HTL, especially PE and PP. The reduction of MP mass was larger than MP counts, likely because the HTL process caused larger MPs to break up. Most of the resilient MPs were found in the solid residue of the process and in its liquid waste, while the bio-crude was free from MPs. It could be concluded that HTL can be effective to reduce MPs in WWTP sludge.

Research question 3*: What is the fate of MPs in WWTPs and how much of the MPs enter receiving waters from a modern state-of-the-art WWTP?*

Paper III identified MPs in succeeding stages of an advanced WWTP and found very high treatment efficiency. The concentration in the final effluent was on par with MP concentrations in close by marine waters. Major removal of MPs was observed for the pre-treatment and primary treatment stage while the other treatment steps further removed MPs. The sand filter used to polish the water prior to discharge was able to bring down concentrations to very low levels, and also remove very fine MPs.

Research question 4: *Can snow melt contribute significantly to the influx of MPs, TWP, and metal pollutants to WWTPs and adjacent water bodies?*

Paper IV investigated the level of MPs, TWP, and metals in snow from snow dumping stations in Riga, and untouched snow from remote sites. Concentrations varied with human activity and land use. Remote sites held MPs, but not TWP. MP and TWP did not correlate, while TWP correlated to several of the metals, indicating that the sources of TWP and metals overlapped.

CHAPTER 6. MAIN CONTRIBUTIONS TO SCIENCE

WWTPs are essential for controlling the discharge of MPs and other pollutants to the aquatic environment. WWTPs have consequently been thoroughly studied by various researchers focusing mainly on the retention capacity of MPs. There are nevertheless significant knowledge gaps, some relating to the detailed waste streams inside a WWTP and some related to the subsequent sludge management. The same goes for MPs coming from wet weather runoff. This PhD study has contributed to filling some of these gaps.

MPs in sludge may become fragmented and degraded by anaerobic digestion. **Paper I** and **Paper III** present details on the fate of MPs during mesophilic digestion. The size and mass of the MPs, and their polymer composition of polymer types was investigated using µ-FTIR imaging spectroscopy allowing quantification of MP balances over the digesters. To the best knowledge of the author, these studies were a first in attempting to establish such balances. There is furthermore scarce data on MPs in WWTP grease and external organic materials such as fatty slurries, and the studies contributed to extending this database.

Paper II contributes to understanding the fate of MPs in sludge treated at extreme temperatures and pressure to create bio-crude. This study was a first to quantify MPs in such process and helped to understand which polymer types were affected by the process to which degree. Biomass conversion into biofuel could be a way to the future renewable energy. At the same time, the MPs in the sludge can be reduced significantly by this process. The process furthermore allowed persistent polymer types to be concentrated into a small volume of residue, which can easily be handled.

Paper III conducted a stepwise investigation on MPs in a WWTP with sand filtration as an advanced tertiary treatment process. This study provided knowledge about the effectiveness of the WWTP on retaining MPs in its different treatment steps. Major retention occurred at preliminary and primary stages, while the secondary and tertiary treatment processes significantly polished the water for MPs, particularly the small sized MPs. The study also showed that proper treatment can reduce the level of MP in effluents to levels found in many marine waters.

Paper IV investigated the MPs in snow from different snow dumping areas, both urban and remote places. This study showed that snowmelt can hold quite high MP concentrations, and that TWP and some metals correlated, indicating overlap in sources for the two pollutants.

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APPENDICES

Paper – I

The occurrence and fate of microplastics in a mesophilic anaerobic digester receiving sewage sludge, grease, and fatty slurries

Science of the Total Environment 798 (2021) 149287

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

The occurrence and fate of microplastics in a mesophilic anaerobic digester receiving sewage sludge, grease, and fatty slurries

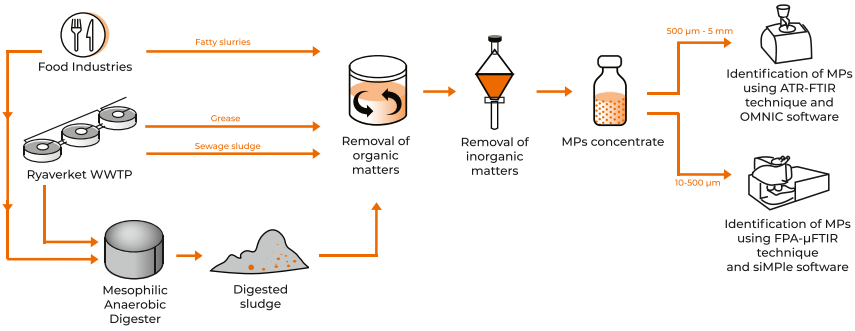
Rupa Chand [a,*], Lasse Abraham Rasmussen [a], Susanne Tumlin [b], Jes Vollertsen [a]

[a] Department of the Built Environment, Aalborg University, Thomas Manns Vej 23, 9220 Aalborg, Denmark
[b] Gryaab AB, Norra Fågelrovägen 3, 418 34 Gothenburg, Sweden

HIGHLIGHTS

- A full microplastics mass and particle number balance on a WWTP digester
- Microplastics quantified in sludge and grease down to 10 μm applying μFTIR imaging
- Mass balance on the digester: 0.025 g capita^{-1} day^{-1} in; 0.017 g capita^{-1} day^{-1} out
- Number balance on the digester: 4.14 × 10^5 N capita^{-1} day^{-1} in; 2.95 × 10^5 N capita^{-1} day^{-1} out
- Each single source, e.g. human excretion, must contribute less than 0.025 g capita^{-1} day^{-1}.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 April 2021
Received in revised form 19 July 2021
Accepted 22 July 2021
Available online xxxx

Editor: Kevin V. Thomas

Keywords:
MP
Wastewater treatment plant sludge
Digestion
Fatty slurries
Grease
Sludge

ABSTRACT

The mesophilic digester of a wastewater treatment plant serving 790,000 inhabitants was analyzed for incoming and outgoing microplastics (MPs). The annual MP load on the digester was 7326 kg y^{-1} and 1.20 × 10^{14} N y^{-1}, while the digested sludge contained 4885 kg y^{-1} and 0.85 × 10^{14} N y^{-1}. The corresponding mean reduction of approximately 30% was though within the variability of the analyzed samples, and size distributions and polymer composition before and after the digester could similarly neither confirm nor deny if MPs were lost in the digester. The load on the digester corresponded to a per capita load of 0.025 g capita^{-1} day^{-1} or 4.14 × 10^5 N capita^{-1} day^{-1}. In terms of MP numbers, the values were high compared to most previous studies, which most likely was due to differences in analytical methods, where the present study applied FPA-μFTIR hyperspectral imaging with automatized MP recognition in the size range 10–500 μm and ATR-FTIR on all particles in the range 500–5000 μm. The polymer composition was quite diverse, with 15 identified polymer types, of which polyethylene and polyester were the most common in the sludge, while the variation in composition was larger in the grease that came from the plant's grease trap and the fatty slurries which came from grease traps in the drainage area. The load on the digester was finally used to demonstrate how especially the determined mass loads can be used to set upper boundaries for specific sources, for example human excretion.

1. Introduction

Plastic particles smaller than 5 mm, so-called microplastics (MPs), have received much scientific and societal attention (Liu et al., 2019) as they are suspected to have detrimental impacts on the environment

* Corresponding author.
E-mail address: ruc@build.aau.dk (R. Chand).

https://doi.org/10.1016/j.scitotenv.2021.149287

(Bucci et al., 2020). MPs may be vectors for organic and inorganic micropollutants originally present or subsequently adsorbed to their surfaces. They may be vectors for pathogenic microorganisms (Kirstein et al., 2016), and they may cause harm due to their physical properties, for example by translocation (Alimba and Faggio, 2019). They enter the environment as intentionally produced particles or fragments of larger plastic items (Cole et al., 2011).

MPs are among other formed in urban areas and conveyed by urban wastewaters, where treatment plants (WWTPs) control their discharge to the aquatic environment (Browne et al., 2011; Talvitie et al., 2017a). WWTPs are quite efficient in retaining MPs of all sizes by concentrating them in solid waste streams such as sludge from its various treatment processes, fat and grease from its grease traps, and material collected at screens (Mintenig et al., 2017; Simon et al., 2018; Sun et al., 2019; Rasmussen et al., 2021). MP removal rates in advanced WWTPs, and consequently their retainment in WWTP waste streams, have been reported as high as 99.3% in terms of particle counts and 98.3% in terms of mass (Simon et al., 2018). Other researchers also found retainment rates significantly above 90%, e.g. Lares et al. (2018) who found an overall retainment of 98.3% in an activated sludge treatment plant, Talvitie et al. (2017b) who found retainment rates above 99% in a similar plant, and X. Xu et al. (2019) who found between 89 and 97% retainment in 11 WWTPs in China.

Advanced WWTPs generally comprise several treatment stages, where the first consists of mechanical means to remove gross solids, sand, grit, oil, and grease. This is commonly followed by a mechanical separation of part of the organic matter by a settling tank, creating primary sludge. At the next stage, the wastewater is frequently treated biologically through activated sludge or fixed film processes, creating secondary sludge (Tchobanoglous et al., 2011). Floatable fat, oil, and grease are typically removed in a skimming trough or an aerated grit chamber, where the latter combines sedimentation of grit, sand, and silt with the flotation of fat, oil, and grease. Fat, oil, and grease tend to be of low density and hydrophobic, as are many plastic types. MPs can hence readily form agglomerates with these sticky substances and be skimmed off during the grease removal (Carr et al., 2016). Where a WWTP has an anaerobic digester, this material is commonly fed here, as fats increase the overall methane production of the digester. Fat, oil, and grease received from external sources is also commonly fed to the digester (Luostarinen et al., 2009).

There are quite large differences in concentrations reported by the various studies, for example Raju et al. (2020), Murphy et al. (2016), Bayo et al. (2020), and Blair et al. (2019) detected MP concentrations orders of magnitude below what for example Simon et al. (2018) reported for raw wastewater, or for that matter, what Bergmann et al. (2019) reported for a different matrix, namely European snow. Similarly, MPs in WWTP sludge have been reported to range somewhere between some hundreds and some hundred thousands of MPs per kg of dry sludge (Gatidou et al., 2019; Sun et al., 2019), albeit data on this matrix is scarce.

The issue of inconsistency in results when attempting to quantify MPs in environmental matrices is well recognized. Already in the early days of microplastics research, the review of Hidalgo-Ruz et al. (2012) pointed towards issues causing poor comparability across studies. This has been a recurring theme since then, as for example revived for freshwater microplastics by Lu et al. (2021), who discussed that differences in sampling techniques, sample processing, quantification and identification, reporting units, as well as the degree of quality assurance/quality control significantly affected the obtained results. The authors concluded that these differences made it difficult to compare studies on freshwaters and freshwater sediments. In concrete terms, they reported that MP abundances in freshwaters varied by 8 orders of magnitude, of which a large part could be attributed to the above mentioned differences in experimental approaches.

The discrepancies in MP contents related to WWTPs is most likely due to differences in analytical techniques, where for example some studies applied Focal Plane Array (FPA)-Micro Fourier Transform Infrared spectroscopy (μFTIR) imaging, while others used visual sorting followed by Attenuated Total Reflectance (ATR)-FTIR quantification. The issue of visual sorting versus a full analysis is thoroughly discussed by J.L. Xu et al. (2019) who points out that visual sorting becomes highly uncertain below 100–500 μm in MP size, as small MPs tend to be overlooked. Automated spectroscopic imaging approaches based on μFTIR or μRaman, on the other hand, are able to detect a larger part of the small MPs and not biased by manual pre-sorting. They are hence able to find more MPs in a matrix and doing it with less human bias.

The present study has the objective to increase the knowledge on MPs in WWTP sludge, applying a FPA-μFTIR imaging approach for MPs of 10–500 μm and ATR-FTIR for 500–5000 μm. It attempts to quantify MPs in terms of particle number and mass in waste streams entering and exiting a mesophilic anaerobic digester and evaluate whether significant changes can be observed. The incoming waste streams are WWTP grease trap materials, fatty slurries from external grease traps (at restaurants and food factories referred as food industries), and mixed primary and secondary WWTP sludge. The exiting waste stream is sludge discharged from the digester.

2. Materials and method

2.1. Sampling and sample type

The studied WWTP, Ryaverket, is one of the largest in Sweden, collecting on average 4000 L s^{-1} of wastewater from seven municipalities and providing services to some 790,000 people. It applies mechanical, biological, and chemical treatment and produces some 440,000 tons y^{-1} of wet sewage sludge from its primary sedimentation and biological treatment stages, which is fed to its anaerobic digester. Around 1000 tons y^{-1} of wet grease is skimmed at the primary treatment and fed into the digester along with 14,000 tons y^{-1} of fatty slurries from external sources such as restaurants, bakeries, and food factories. The digester is fed continuously and run at mesophilic conditions (35 °C) with a 20 days retention time (Tumlin and Berholds, 2020). The fatty slurries samples were taken in 1 L aluminum bottles from the outlet hose of the transporting tanks. Grease samples were taken with the help of metal buckets from the grease trap and taken to the laboratory in 1 L aluminum bottles. Likewise, the sewage sludge and digested sludge samples were taken in 1 L aluminum bottles from the respective inlet and outlet hoses of the sludge digestion chamber. The three types of bio-solids fed to the digester, as well as the digested sludge, were analyzed to establish a MP-balance over the digester (Table 1).

2.2. Sample preparation

Approximately 800 g of wet grease and 800 g of wet sludge were dried at 50 °C, homogenized, and 2.5 g dry grease and 5 g dry sludge subsampled. Approximately 1 L of fatty slurries was homogenized and 0.4 L subsampled. All samples were analyzed for MPs in the range of 10–5000 μm. An additional 30 g of grease was processed to extract MPs >500 μm in order to develop a simple algorithm to estimate the particle thickness from a 2-dimensional visual particle image and its polymer type.

2.2.1. Extraction of MPs, 10–5000 μm

Grease and fatty slurries were incubated with 500 mL of sodium dodecyl sulfate solution (SDS, 5% w/vol) for 48 h at 50 °C, upon which 1 mL lipase (Lipozyme® TL 100 L, Strem Chemicals Inc.) was added. The solution was again incubated for 48 h at 50 °C. The particles in the sample mixture were filtered onto a 10 μm stainless steel filter and transferred to 400 mL Milli-Q, and 100 mL of 50% hydrogen peroxide (H_2O_2) added. Sludge was pre-oxidized by adding 100 mL of 50% H_2O_2 to a mixture of 5 g dry sludge and 400 mL Milli-Q. After filtration on the 10 μm steel

Table 1
Samples analyzed for MPs.

Type	Label	Sample collected period	Dry matter	Descriptions about the samples
Fatty slurries	Fatty slurry 1	June 2019	5.5%	Waste from four grease traps at restaurants
	Fatty slurry 2		2.4%	Waste from two grease traps at a bakery and a butter factory
	Fatty slurry 3		15.4%	Waste from two grease traps at an ice-cream factory
Grease	Grease 1	December 2018–January 2019	19.5%	Waste from the grease trap of the primary treatment stage
	Grease 2		17.6%	
	Grease 3		7.3%	
Sewage sludge	Sewage sludge 1	April 2019	6.5%	Thickened sludge produced from the primary and secondary treatment processes
	Sewage sludge 2		6.6%	
	Sewage sludge 3		6.8%	
Digested sludge	Digested sludge 1	April 2019	3.0%	Anaerobically digested sludge, mesophilic at 35 °C and 20 days residence
	Digested sludge 2		3.0%	time. Sludge collected before dewatering
	Digested sludge 3		3.0%	

filter, the particles were transferred to 500 mL of SDS solution and left for 48 h at 50 °C. The samples were kept gently stirred at all times.

Hereafter all samples followed the same procedure. The solutions were filtered on 10 μm steel filters and particles transferred by sonication into 300 mL of acetate buffer (pH 4.8). 500 μL of cellulase (Cellulase enzyme blend®, Sigma-Aldrich) and 500 μL cellulolytic enzyme mixture (Viscozyme®L, Sigma-Aldrich) was added and the solution incubated for 48 h at 50 °C. The solution was again filtered on 10 μm steel filters and particles transferred to 300 mL of tris buffer (pH 8.2). 500 μL protease (Protease from Bacillus sp. ®, Sigma-Aldrich) was added and the solution incubated for 48 h at 50 °C. The solution was again filtered on 10 μm steel filters and the collected particles subjected to a Fenton oxidation by adding 145 mL of 50% H_2O_2, 65 mL of 0.1 M NaOH, and 62 mL of 0.1 M $FeSO_4$, resulting in a pH between 2.5 and 3. The temperature was kept at 15–30 °C by keeping the reactors on ice. The process was followed closely until the temperature stabilized at room temperature. Further details can be found in Simon et al. (2018).

After the Fenton reaction, remaining particles were collected on a 10 μm steel filter and transferred to a 250 mL pear-shaped separation funnel containing a sodium polytungstate (SPT) solution of 1.8 g cm^{-3}. The sample mixture in the funnel was mixed by passing dry and filtered compressed air from the bottom for 30 min, left to separate for 24 h, upon which the floating particles were collected. The process was repeated 3 times to ensure high flotation efficiency. The collected particles were filtered over a 500 μm and a 10 μm steel mesh. The particles retained by the larger mesh were collected for analysis by ATR-FTIR. The particles on the 10 μm filter were collected into approximately 25 mL 50% ethanol, dried in an automated solvent evaporator (TurboVap® LV, Biotage), and finally re-suspended to a fixed volume of 5 mL 50% ethanol. As far as possible, the 10 μm steel filters were reused for same samples in order to minimize potential losses due to particles sticking on the filters.

2.2.2. Additional extraction of MPs >500 μm from grease

30 g dry grease was mixed into 500 mL of SDS solution, 2 mL of lipase (Lipozyme® TL 100 L, Strem Chemicals Inc.) gradually added, and incubated for 72 h at 50 °C. The suspension was filtered on a 500 μm steel mesh and the collected particles transferred to 400 mL Milli-Q to which 100 mL of 50% H_2O_2 was added. The mixture was kept for 24 h at room temperature, upon which the suspension again was filtered on a 500 μm steel mesh and particles transferred to 500 mL acetate buffer (pH 4.8). 1 mL cellulase (Cellulase enzyme blend®, Sigma-Aldrich) and 1 mL cellulolytic enzyme mixture (Viscozyme®L, Sigma-Aldrich) were added and the solution incubated for 48 h at 50 °C. The solution was again filtered on a 500 μm steel mesh, transferred to 500 mL of tris buffer (pH 8.2), 1 mL protease (Protease from Bacillus sp.®, Sigma-Aldrich) added, and incubated at the same temperature and time. Finally, the clean particles were dried at 50 °C and stored for further analysis.

2.3. MP identification

2.3.1. MPs of 10–500 μm

The particle concentrate was homogenized on a vortex mixer before taking a subsample with a disposable glass capillary pipette 50/100 μL. The subsample was deposited on a 13 × 2 mm zinc selenide window (Crystran, UK) held in a compression cell (Pike Technologies, USA), restricting the free opening to 10 mm in diameter. The window was dried at 50 °C and visually inspected under a microscope to judge the particle density. The deposition was repeated until the particle density was deemed suitable for further analysis, resulting in deposited volumes of 50–200 μL. For each sample, three such windows were prepared and analyzed.

The deposited particles were analyzed by μFTIR using an Agilent Cary 620 FTIR microscope equipped with a 128 × 128 pixel FPA (Mercury Cadmium Telluride detector) and coupled to an Agilent 670 IR spectroscope. The microscope used a 15× Cassegrain IR objective yielding a pixel size of 5.5 μm. 14 × 14 FPA-tiles were combined into one spectral image, corresponding to 3,211,264 individual IR spectra per image. To create the image, 30 scans per sample were co-added, while the background was acquired by 120 co-added scans. The spectral resolution was 8 cm^{-1}, covering the wavenumber range 3750–850 cm^{-1}. The acquired IR-spectra were analyzed using the software siMPle (previously MPhunter) which compares the raw spectra to a custom-made reference database, in this case containing 114 spectra of different materials (Liu et al., 2019; Primpke et al., 2020b). The output of the analysis was MP numbers, minimum and maximum Feret diameter of each particle, its polymer type, and mass estimated from the particle size as described by Simon et al. (2018).

2.3.2. MPs >500 μm

All potential plastic particles >500 μm were analyzed using a Cary 630 FTIR from Agilent Technologies equipped with a diamond ATR. The software OMNIC 8.2.0.387 (Thermo Fisher Scientific Inc., version 1) and its library were used to identify the material of the recorded IR spectra. Plastic particles were further imaged using a stereoscopic microscope (ZEISS, SteREO Discovery.V8) with Axiocam 105 color camera and max. 8× magnification. The software ZenCore (Zen2Core SP1 from ZEISS) was used to quantify the particle's area, minimum, and maximum Feret diameter. Randomly selected particles from the additionally extracted 30 g of grease were weighted (Mettler Toledo XSE205 DualRange) and the weight used to estimate the particle's third dimension (thickness).

2.4. Contamination and quality control

Contamination is an issue when analyzing MPs and steps were taken to reduce it. All tools and vessels were of glass or metal and rinsed at least thrice with particle-free Milli-Q water. Steel filters were muffled at 500 °C and stored in clean and closed Petri dishes till use. When a

filter was used multiple times for the same sample, it was stored in a closed Petri dish with Milli-Q water between uses. Liquid chemicals and solutions were filtered through 0.7 μm glass fiber filters before use. Filtration and sonication of filters were done inside a clean fume hood. Incubation of samples was done in a covered water bath. The wearing of natural fabric laboratory clothes was mandatory in the lab. A laminar flow cabinet was used for the final subsample deposition on a zinc selenide window and the window was covered by a clean glass beaker during drying. A continuous air-filtering device (Dustbox® Hochleistungsluftreininger) was installed in the room of the FTIR microscopes.

Some contamination was though unavoidable, and three blanks were hence processed: One L of filtered Milli-Q was treated with lipase and pre-oxidized with H_2O_2, upon which the processing followed the steps described for the 10–500 μm particles. A recovery test was not conducted as the main steps of the method were similar to Simon et al. (2018) who, working in the same lab with similar equipment, found recovery rates of 58-78%, indicating that the method used in the present study was likely to yield acceptable recovery rates.

3. Results and discussion

3.1. Blank sample analysis

A sum of 6053 particles were identified as MPs in the 12 samples of the study. In comparison, the blanks held a total of six MPs (Supplementary materials, Table S1). These six MPs were all of polyester (PEsT) and had an estimated total mass of 2399 ng. They were detected after scanning 33% of each of the 3 blanks (1650 μL per blank). No other plastic types were found in the blanks. This corresponds to an average MP contamination per processed sample of 18 PEsT particles with an estimated mass of 7269 ng. The contamination quantified by the blanks relates to the sample processing and not to the sample volume or mass analyzed. Hence this was the contamination per processed sample and not per processed volume or mass. Table 2 shows the corresponding level of contamination by MP number and mass in each analyzed sample when normalizing the contamination towards sample mass. The contamination was quite low, namely 0.05–3.88% by particle number and 0.10–4.07% by mass and the analyzed samples hence not corrected for blank values. In comparison, Liu et al. (2019) found eight MPs in three blanks scanning a similar-sized subsample. They found 3 PE, 2 polyamide (PA), 2 PEsT, and 1 acrylic particles, corresponding to a final blank concentration of 22.2 items and 942 ng of polymers per processed sample. Simon et al. (2018) reported contamination of 2110 N L^{-1} and 84 μg L^{-1} of raw wastewater, which was 16.3% of the particles and 28.4% of the mass in their sample. Talvitie et al. (2017b) achieved a microliter particle contamination of 0.4–0.8 N L^{-1}, which corresponded to 30% of the estimated microliter in effluent water. Murphy et al. (2016) found 25 MPs on a filter left in the laboratory to examine atmospheric contamination for the duration of the sample preparation. Lares et al. (2018) presented their field sampling and sample preparation contamination for wastewater and sludge as 4.4 and 0.1 MPs per sample, respectively, when addressing particles above some 250 μm.

3.2. Thickness ratio

For the smaller MPs detected using siMPle (MPs <500 μm), the volume and mass estimation was based on Simon et al. (2018), assuming an ellipsoidal shape together with a ratio of thickness to Feret minimum diameter of 0.60. It stands though to reason, that thickness ratio and shape depends on particle size and what is a suitable shape and size ratio for smaller MPs is not necessarily so for larger MPs (examples of MPs >500 μm illustrating this are in Supplementary materials, Fig. S1). The 30 g of grease analyzed to evaluate this yielded 162 MPs, for which visual inspection indicated the dominant shape to be flake-like. For calculation purposes, such shape was hence assumed, with the visible area being the flat part of the flake. The third dimension (thickness) could then be estimated from the equation: area × thickness × density = mass, where the mass had been measured by a scale. Based on a sub-sample of 19 randomly selected and weighted particles, a ratio between thickness and Feret minimum diameter of 0.18 was determined, while the ratio to the Feret maximum diameter was 0.11. To be consistent with the mass estimation approach of the small MPs, the Feret minimum diameter was then used to estimate masses of all MPs >500 μm in all analyzed samples. For the 162 MPs from the additionally analyzed grease samples, the calculated thickness ranged from 61 to 645 μm (Supplementary materials, Fig. S2).

3.3. MP abundance

The digested sludge held 20.5, 5.2, and 1.5 times more MPs than the fatty slurries, the grease, and the sewage sludge, respectively. The highest dry matter mean concentration and the standard error (SE) in terms of MP numbers (Table 2) was in digested sludge with 6.36 (±0.80) × 10^6 N kg^{-1}, followed by sewage sludge with 4.08 (±0.25) × 10^6 N kg^{-1}, grease with 1.22 (±0.78) × 10^6 N kg^{-1}, and fatty slurries with 3.11 (±1.70) × 10^5 N kg^{-1}. The difference between the means of the digested sludge and sewage sludge, as well as the difference between the means of grease and fatty slurries, were though not sufficient to reject the possibility that the difference was due to random sampling variability. In comparison, Edo et al. (2020) reported 1.33 × 10^5 N kg^{-1} in digested sludge from a Spanish WWTP and Lusher et al. (2017) found an average MP abundance of 6 × 10^3 N kg^{-1} in samples from Norwegian WWTPs (mix of raw and digested sludge types). Carr et al. (2016), Lares et al. (2018), Li et al. (2018), Magnusson and Norén (2014), Mahon et al. (2017), and Mintenig et al. (2017) reported MP concentrations in sludge between 4 × 10^2 and 1.7 × 10^5 N kg^{-1}. For materials from grease skimmers and grease traps there has so far been presented only a few

Table 2
MP number and mass concentrations with standard error (SE) and contamination calculated from blanks. All sludge and slurry masses are measured as dry matter.

Sample ID	Particle conc. [N kg^{-1}]	Mean particle conc. [N kg^{-1}]	Mass conc. [g kg^{-1}]	Mean mass conc. [g kg^{-1}]	Estimated particle contamination [N kg^{-1}]	Estimated mass contamination [g kg^{-1}]
Fatty slurry 1	5.99 × 10^5	3.11 (±1.70) × 10^5	0.07	0.045 (±0.02)	7.69 × 10^2	3.11 × 10^{-4}
Fatty slurry 2	3.23 × 10^5		0.065		1.65 × 10^3	6.65 × 10^{-4}
Fatty slurry 3	9.81 × 10^3		0.003		2.67 × 10^2	1.08 × 10^{-4}
Grease 1	6.65 × 10^5	1.22 (±0.78) × 10^6	1.10	3.23 (±2.06)	7.64 × 10^3	3.09 × 10^{-3}
Grease 2	2.44 × 10^5		1.22		9.37 × 10^3	3.79 × 10^{-3}
Grease 3	2.76 × 10^6		7.36		7.73 × 10^3	3.12 × 10^{-3}
Sewage sludge 1	4.59 × 10^6	4.08 (±0.25) × 10^6	0.29	0.23 (±0.03)	3.60 × 10^3	1.45 × 10^{-3}
Sewage sludge 2	3.84 × 10^6		0.18		3.60 × 10^3	1.45 × 10^{-3}
Sewage sludge 3	3.81 × 10^6		0.23		3.60 × 10^3	1.45 × 10^{-3}
Digested sludge 1	5.12 × 10^6	6.36 (±0.80) × 10^6	0.27	0.37 (±0.05)	3.43 × 10^3	1.38 × 10^{-3}
Digested sludge 2	6.08 × 10^6		0.37		3.60 × 10^3	1.45 × 10^{-3}
Digested sludge 3	7.86 × 10^6		0.46		3.60 × 10^3	1.45 × 10^{-3}

studies. Murphy et al. (2016) reported around 19 particles in 2.5 g of grease from the grease removing stage of a WWTP, which corresponds to 7.86×10^3 N kg^{-1}. The size range of the MPs was not reported. They furthermore found nearly ten-fold more particles in the grease than in undigested sludge of that WWTP. For a somewhat related matrix, namely skimmed materials from a primary tank, Carr et al. (2016) reported a MP concentration of 4–5 $\times$ 10^3 N kg^{-1} (wet basis). They found that these skimmings contained more MPs than the biosolids produced at the plant.

These previous studies reported between one and four orders of magnitude less MPs in sewage sludge and digested sludge than the present study found in these matrixes. A contributing factor to this difference is most likely the applied analytical approach, where the previous studies applied manual sorting approaches combined with single-particle identification techniques. As discussed thoroughly by J.L. Xu et al. (2019) and Primpke et al. (2020a) the automated chemical imaging approaches suggested by Löder et al. (2015), further developed by Primpke et al. (2017), and now applied for example in the present study, are able to reliably identify smaller and hence more MPs than visual sorting based methods. While the quantification bias due to different analytical approaches can be quite substantial, the concentration ratio between samples analyzed following the same analytical protocol should though be less affected. Hence the ratio of MP concentrations within a study is more likely to be comparable to that of another study, than are the actual numbers. This is for example reflected in most studies finding somewhat comparable MP removal rates at WWTPs, even though the analytical methods and consequently the absolute numbers are quite different (Bretas Alvim et al., 2020; Ngo et al., 2019).

Unfortunately there is no peer reviewed published data on WWTP sludge applying the same analytical method as in the present study. Only indirect comparison of the obtained data can hence be done. Considering 1 L of raw wastewater produces roughly 0.25 g of dry sludge in case of the studied WWTP, and that roughly all MPs ended up in the sludge, this leads to a rough MP estimation of 1320 N L^{-1}, which is less than what Simon et al. (2018) found in raw wastewater, namely 7216 N L^{-1}. A study conducted by Rasmussen et al. (2021), on the other hand, found 533 N L^{-1} in raw wastewater and 1.4×10^6 N kg^{-1} in sludge from the same WWTP which is lower compared to the present study. A study conducted by a different group on marine sediments affected by discharges of untreated wastewater found 0.2×10^6 N kg^{-1} in the most affected sediments, indicating that the wastewater must have held substantially amounts which otherwise would have ended up in the sludge of a WWTP (Haave et al., 2019).

With respect to the MP mass determined in the present study, it was the grease that held the highest concentrations, followed by digested sludge, sewage sludge, and fatty slurries. The difference between the means of the digested sludge and sewage sludge was though not sufficient to reject the possibility that the difference was due to random sample variability. The reason for the grease holding so much more mass than the other fractions relates to the particle size, where the grease held many large particles which contributed substantially to the MP mass concentration but little to the MP number concentration. This also illustrates the difficulties in comparing MP number and mass data, and the difficulties in estimating MP mass from particle numbers. The obtained MP mass data could unfortunately not be compared to scientific literature, as no such data has previously been published.

3.4. MP balance

The determined masses cover all waste streams entering and exiting the anaerobic digester. It was hence possible to establish a balance over the digester (Fig. 1). In the year of sampling, the digester received 1080 tons of fatty slurries, 148 tons of grease from the grease trap, and 29,166 tons of sewage sludge, all measured as dry matter. The same year, 13,342 tons of digested sludge dry matter was retrieved from it. The dry matter contents were deducted based on the water content of the analyzed samples (Table 1).

The total annual amount of MPs entering the digester was 7326 kg y^{-1} and 1.20×10^{14} N y^{-1} while 4885 kg y^{-1} and 0.85×10^{14} N y^{-1} exited it. Based on the 790,000 people served by Ryaverket, this corresponded to an annual per capita load of 9.3 g capita^{-1} y^{-1} and 1.5×10^8 N y^{-1} entering the digester and 6.2 g capita^{-1} y^{-1} and 1.1×10^8 N y^{-1} exiting it. Accordingly, the daily MP mass balance in the digester was 0.025 g capita^{-1} day^{-1} in and 0.017 g capita^{-1} day^{-1} out and MP number balance was 4.14×10^5 N capita^{-1} day^{-1} in and 2.95

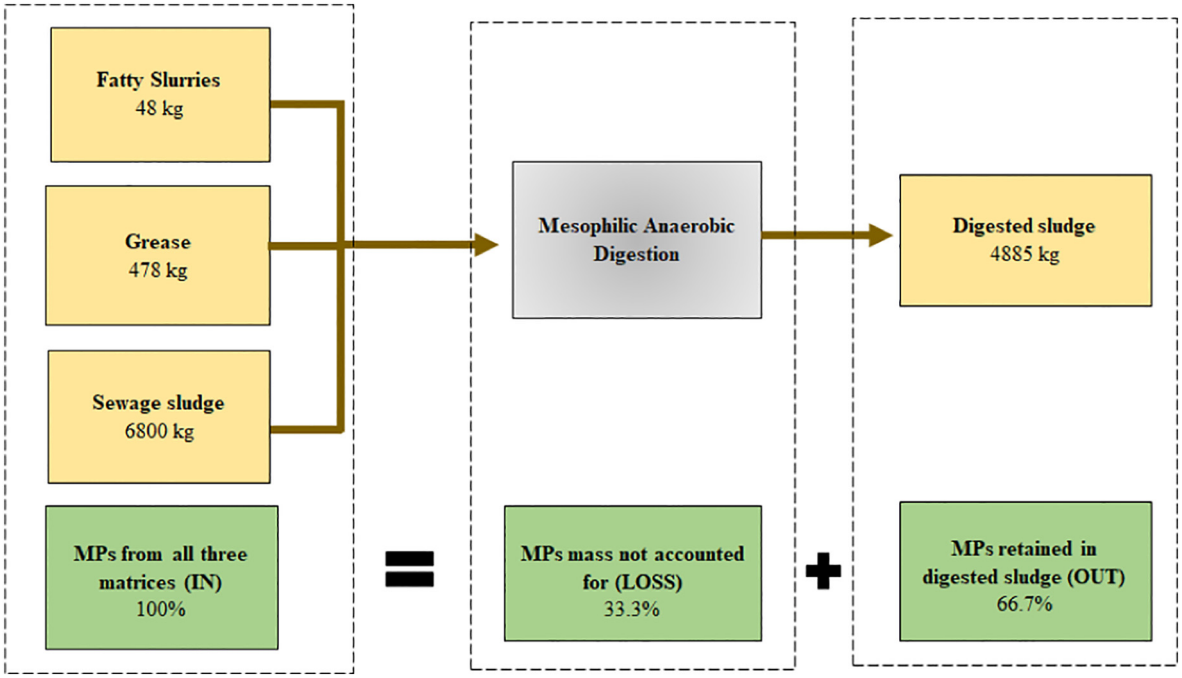


Fig. 1. Annual mass balance of MP over the digester (see also Supplementary materials, Tables S4 and S5).

$\times 10^5$ N capita^{-1} day^{-1} out. The fatty slurries and the grease together contributed 0.43% to the total number of MPs entering the digester, while they contributed 7.2% of the mass. The main part of the latter came from the grease trap, which contributed 6.5% of the MP mass entering the digester. In terms of removal rates, the digester reduced the number of MPs by 29% while it reduced the MP mass by 33.3%. While this at a first glance seems a substantial reduction, the differences were not statistically significant at a 95% confidence level.

Nevertheless, assuming that a reduction had actually taken place, such could have been caused by plastic degradation or depolymerization in the digester. No conclusive evidence has been published on whether or not such processes occur in mesophilic digesters, but some indication that this might happen has been presented by Mahon et al. (2017). Furthermore, Gómez and Michel (2013) performed a laboratory study of the mineralization of so-called biodegradable plastic materials during 50 days of mesophilic digestion. They found 20–25% degradation of some plastics of enhanced degradability while others only were degraded by 2%. Another potential reason for finding less MPs after the digestion could be fragmentation of the particles to sizes below the detection limit of the applied analytical approach (10 μm).

It might seem as if sludge from Ryaverket contained several orders of magnitude more MPs than reported for most other WWTPs. However, such conclusion would be a misinterpretation of the data as the difference in MP number is dominated by differences in analytical approaches and not by actual differences in MP concentrations. It is hence not possible to state that sludge from Ryaverket held more MPs than for example a Finish WWTP of a capacity of 10,000 m^3 day^{-1} for which Lares et al. (2018) found 1.7×10^{11} N y^{-1} in its sludge, or a Spanish WWTP of 45,000 m^3 day^{-1} capacity for which Edo et al. (2020) found 8×10^{11} N y^{-1} in its sludge. The same goes for an Italian WWTP for which Magni et al. (2019) reported 1.2×10^{12} N y^{-1} and a Canadian one, for which Gies et al. (2018) reported 1.64×10^{12} N y^{-1}. This issue of incomparability becomes quite obvious when comparing to the findings of Li et al. (2018) who investigated 28 Chinese WWTPs and estimated the MP content in the total amount of sludge produced in China to be 1.56×10^{14} N y^{-1}, i.e. about the same number as found in Ryaverket serving Sweden's second-largest city. It is obviously unreasonable to conclude that one single Swedish WWTP should contain a similar amount of MPs as all Chinese WWTPs put together.

The reason for differences in reported numbers in studies applying different quantification techniques is illustrated well by the MP size and mass distributions. Fig. 2 shows how particle sizes are distributed versus MP numbers in bins of 10 μm, and how smaller particles tend to be much more abundant in terms of number than the larger ones.

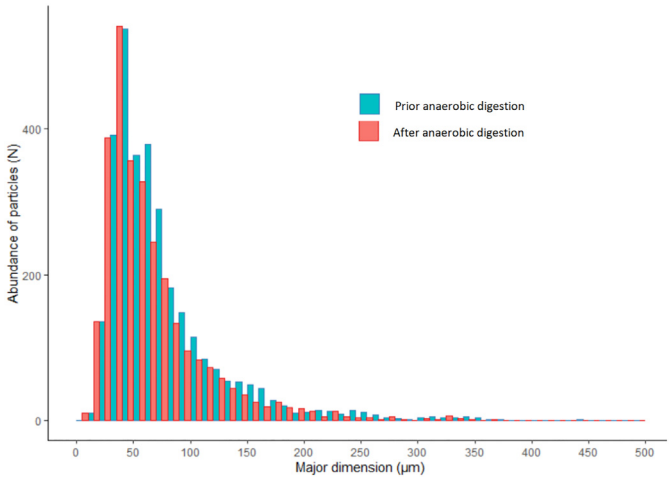


Fig. 2. Abundance of all MPs identified by μFTIR imaging (10–500 μm). "Prior anaerobic digestion" includes all identified MPs in fatty slurries, grease and sewage sludge and "After anaerobic digestion" includes MPs identified in digested sludge.

Hence a quantification method which allows precise quantification down to e.g. 500 μm will obviously find much fewer MPs than one which is able to quantify precisely down to a lower size.

This raises the question what the MP size quantification limit of the present study actually is. It is quite common in microplastics research that studies report their smallest detectable or detected particle size as the lower size limit of their study. One could call this the MP size detection limit. For the present study that would be 10 μm, defined by the filter size and the settings of the μFTIR imaging machine. One has to take care not to confuse such smallest detectable MP size with the size quantification limit, i.e. the size limit down to which a reliable and accurate quantification of the number of MPs in a sample can be made. When using spectroscopic techniques, being they based on manual sorting and single particle identification, or on imaging combined with automated recognition as in the present study, there will be an increasing number of false negatives with decreasing size. In other words, the smaller the MPs, the easier they are to overlook. While it stands to reason that the applied imaging approach recognizes significantly more small particles than manual sorting combined with single particle identification, it does not recognize them all. Part of this is due to small particles also being thin particles, and when analyzed in IR-transmission or transflection mode, the amount of absorbed light decreases with particle size. In other words, the signal to noise ratio tends to increase when particles become small. Another issue is that small MPs – all else being equal – have a higher risk of being affected by the sample purification and MP extraction. It is most likely a combination of these mechanisms that causes the particle sizes of the present study to decrease towards the MP size detection limit.

This can be illustrated by results of studies applying manual sorting followed by single particle identification. Magni et al. (2019) found for example that MPs were most abundant in the 100–500 μm range, while there were few particles in the 10–100 μm range. The authors recognized this issue, at least to some degree, stating that especially MPs of 10–30 μm are more difficult to identify using manual sorting. Another example is Kazour et al. (2019), who also found that particle numbers did not increase when sizes became small. On the other hand, studies applying automated imaging tend to find more smaller particles (Primpke et al., 2020a).

3.5. Removal over the digester

While an overall MP removal over the digester was not conclusive, as the observed approximately 30% reduction was not statistically significant at a 95% confidence level, more subtle changes could still be hidden behind the overall numbers. For example changes in particle size and polymer composition. The particle sizes of all analyzed samples (Fig. 3) did though not provide such confirmation, as the median size and size distribution of MPs in the sewage sludge and the digested sludge were quite similar. The variation between the triplicates was furthermore quite low, giving confidence in this comparison. The fatty slurries and the grease showed a higher variability between the samples, with some tendency towards somewhat larger sizes than the two sludge types. These two fractions did, however, contribute less than 10% to the total MP count in the sludge, and would hence not affect the overall MP size distribution much. Comparing the MP mass distributions yielded a quite similar picture (Supplementary materials, Fig. S3).

The polymer composition also seemed rather unaffected by the mesophilic digestion, albeit the variation between determinations was quite high, making it difficult to draw solid conclusions (Fig. 4). The variation between samples were most pronounced for the fatty slurries and the grease, where, for example PS in one grease sample accounted for 40% of the MP number and 45% of the mass, whereas it was below 1% in another. For one fatty slurry sample, PE accounted for 59% of the MP mass while it accounted for 4% in another. The triplicates of the sewage sludge and digested sludge showed less variation between samples, indicating that these waste streams were more homogeneous. The

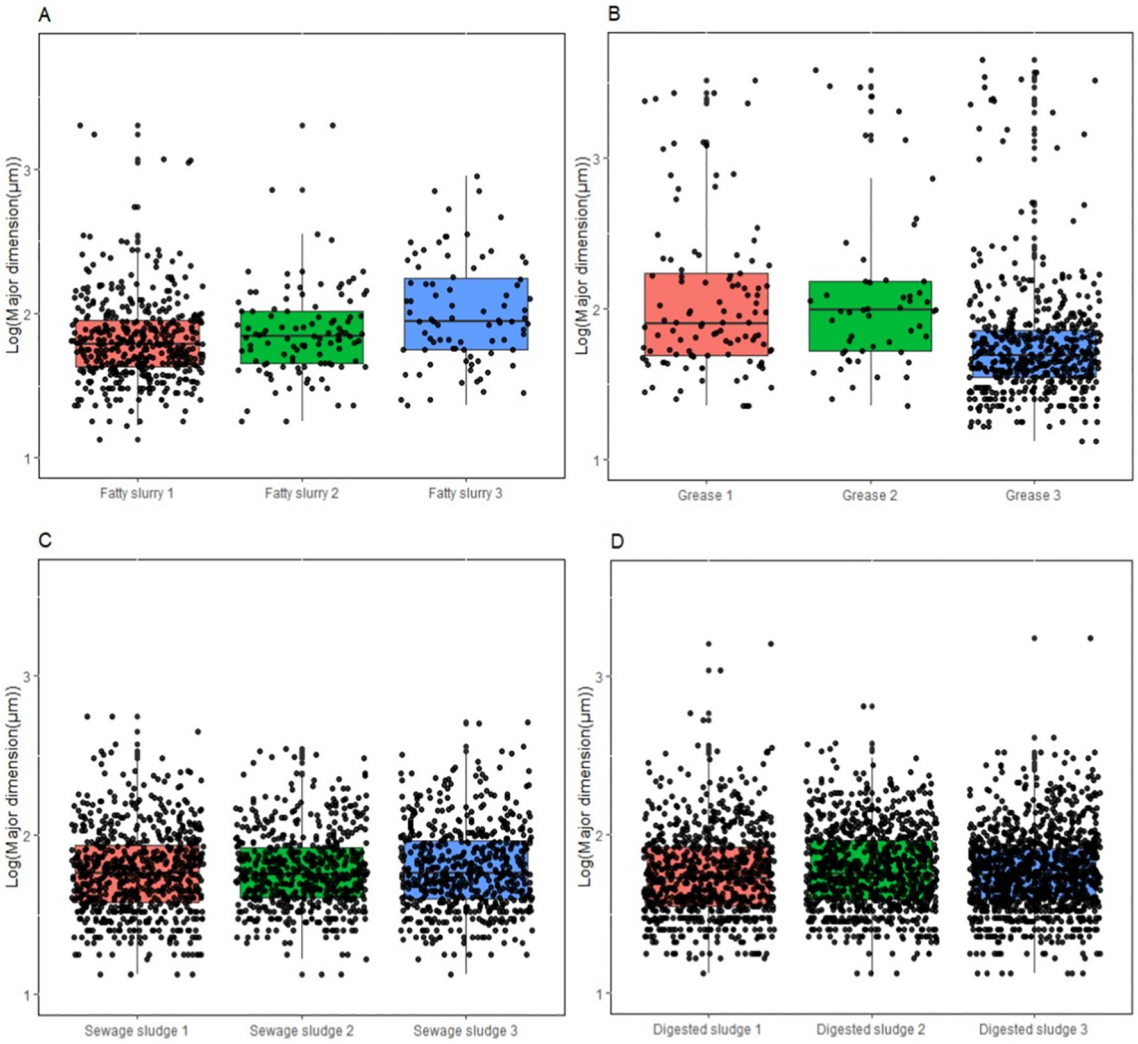


Fig. 3. Major dimension of MPs 10–5000 µm versus sample type. A: fatty slurries, B: grease, C: sewage sludge, and D: digested sludge (see also Supplementary materials, Table S3).

annual average loading on the digester, i.e. the weighted average of the mass flows entering it, and the mass flow exiting it (Fig. 4, the two columns to the right) shows that the difference in overall polymer composition was small. PE, the most abundant polymer, constituted 46% of the load and 50% of the discharge from the digester when measured as MP numbers. In terms of MP mass, it accounted for 51% of the load and 61% of the discharge. PEsT, the second most abundant polymer, accounted for 39% of the load and 26% of the discharge in terms of MP numbers, and 36% and 25% in terms of mass. Similarly with other polymer types. There was variation in average load and average discharge, where some polymers were more abundant after the digester, and some less. But there was no clear tendency that a certain type of polymer 'disappeared' in the digester. Taking the high (and difficult to quantify) uncertainty of the mass balance into account, it could not be concluded that the digester significantly affected MPs in general or specific polymer types. However, it can on the other hand not be excluded that the digester had an actual impact on the MPs, and that such hypothetical impact was hidden behind the random variability between samples.

3.6. Estimating source strengths

Wastewater is the main source of MPs entering the digester of Ryaverket. A modern WWTP like Ryaverket is highly efficient in treating for microplastics, and by far the largest part of MPs will end up in the sludge (Simon et al., 2018). It is hence fair to assume that the total load on the WWTP is close to the sum of the load on its digester. Assuming the average MP number and mass concentrations and 790,000 people served by the WWTP, this leads to a load per inhabitant of 9.3 g N y^{-1}, corresponding to 1.5×10^8 N y^{-1}. The main sources of these MPs are though only partly known. It is known that washing machines contribute MPs (Salvador Cesa et al., 2017), and other sources related to wear and tear of materials in households play a role. Industries will also contribute plastics, as will stormwater runoff when drainage systems are combined (Liu et al., 2019). Finally, human excretion is a source of MPs in wastewater (Schwabl et al., 2019). The latter study reported a median of 2.0 MPs per gram of human stool within a size range of 50–500 µm, but it did not report the MP mass. The MPs of Ryaverkets digester are the sum of MPs from such sources and can hence be used as a tool to backtrack MPs. Each single source must of course contribute less than the whole, which leads to source strengths between 0 and 0.025 g MP $capita^{-1}$ day^{-1}. Assuming purely for the sake of the argument that there are no other MP sources in the drainage area than human stool, this means human excretion in this drainage area cannot have exceeded $7 \times 0.025 = 0.175$ g MP $capita^{-1}$ $week^{-1}$. This is a factor 28 lower than what recently was up in the media, namely that 'an average person could be ingesting approximately 5 grams of plastic every week. The equivalent of one credit card' (Dalberg Advisors et al., 2019). Most likely the true value is substantially below the 0.175 g, as it seems unreasonable to assume that human stool is the only MP source

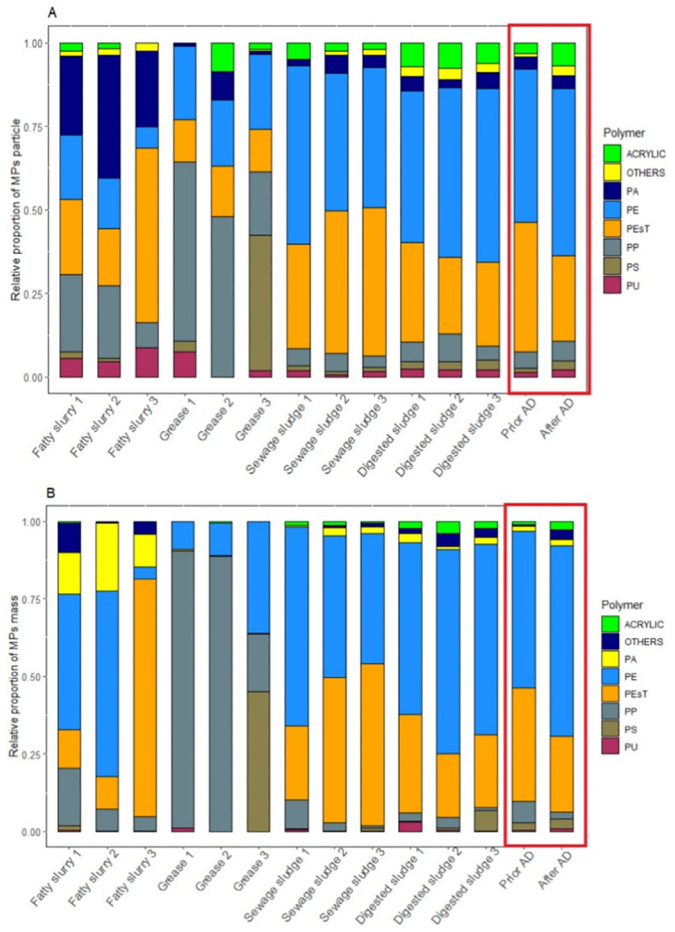


Fig. 4. Proportion of MP polymer types (10–5000 μm). A: MP numbers, B: MP mass. Polymer types labelled "OTHERS" cover PVC, PVAc, ABS, aramid, PAN, PVS and cellulose acetate. "Prior AD" refers to the MP load from fatty slurries, grease and sewage sludge and "After AD" refers to MP load in digested sludge. Details on number of particles identified are in Supplementary materials, Table S2.

in the drainage area. This example shows how studies on MPs in sewerage systems can be applied to qualify estimates on sources and also on human exposure.

4. Conclusion

The mesophilic digester of Ryaverket in Göteborg, Sweden, received an average MP load of 1.20×10^{14} N y^{-1} with an estimated mass of 7326 kg. The digester discharged 0.85×10^{14} N y^{-1} and 4885 kg y^{-1}, corresponding to an average reduction of 33% in terms of MP mass and 29% in terms of MP numbers. However, variation between replica meant that the observed reduction was not statistically significant. Close examination of particle size distribution and polymer composition showed that size and composition of MPs could neither confirm nor deny if they were affected by the digester.

The digester received material by three main streams: Sludge from primary settling and secondary treatment; grease, fat, and oil from the primary grease trap; and fatty slurries from external grease traps. The primary settling and secondary treatment sludge contributed the most MPs, namely 92.8% of the mass and 99.6% of the numbers. The grease trap accounted for 6.5% of the mass but only 0.2% of the numbers, meaning that material here from held larger MPs than the other matrices. The fatty slurries contributed 0.7% of the mass and 0.3% of the numbers.

The daily per capita load on the digester was 4.1×10^5 N capita^{-1} day^{-1} and 0.025 g capita^{-1} day^{-1}. These values, together with an assumption that the WWTP was efficient at retaining MPs, lead to the conclusion that no single MP source in the catchment, such as human stool and laundry machines, can exceed these values. Analyzing especially the mass-based data of wastewater treatment plant loads can hence be used to put other estimates, for example on human egestion, into perspective.

CRediT authorship contribution statement

Rupa Chand: Conceptualized the project, carried out the experiments, developed the methodology, analyzed data and wrote the original draft. **Lasse Abraham Rasmussen:** Assisted in conducting experiments and developing the methodology and, wrote, reviewed and edited the original draft. **Susanne Tumlin:** Conceptualized the project plan, coordinated the project work, contributed in sampling and sent to the lab for analysis, assisted in data interpretation, revised the work and reviewed the draft. **Jes Vollertsen:** Supervised the project work, writing, reviewing and editing. Equally, all authors contributed for the final manuscript.

Declaration of competing interest

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

Acknowledgements

This work was co-funded by Svenskt Vatten Utveckling, project number 19-106. We furthermore would like to thank Claudia Lorenz for proof-reading the manuscript and Safa Hadi, process engineer at Ryaverket, for his kind support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.149287.

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Paper – II

Microplastic degradation through hydrothermal liquefaction of wastewater treatment sludge

Journal of Cleaner Production 335 (2022) 130383

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Microplastics degradation through hydrothermal liquefaction of wastewater treatment sludge

Rupa Chand [a,*], Komeil Kohansal [b], Saqib Toor [b], Thomas Helmer Pedersen [b], Jes Vollertsen [a]

[a] Department of the Built Environment, Aalborg University, Thomas Manns Vej 23, 9220, Aalborg, Denmark
[b] Department of Energy Technology, Aalborg University, Pontoppidanstræde 111, 9220, Aalborg, Denmark

ARTICLE INFO

Handling Editor: Bin Chen

Keywords:
Microplastics
Hydrothermal liquefaction
Sewage sludge
Polymers
Bio-crude

ABSTRACT

Wastewater treatment plant sludge contains large quantities of microplastics (MPs), which is a problematic substance that impedes sustainability efforts, such as in land management. MPs are resilient to degradation, but extreme conditions, such as high temperature and pressure, can lead to residues that can be used as fertilizers on farmlands. Hydrothermal liquefaction (HTL) creates such conditions, converting sludge into valuable bio-crude. To this end, the current study examined the resilience of MPs in sewage sludge that were treated by continuous HTL operated at supercritical water conditions (400 °C, 30 MPa). MPs were extracted before and after the HTL process and quantified by Fourier Transformation Infrared Spectroscopy (FTIR). Particles of 10–500 μm were quantified using Focal Plane Array (FPA) based micro-FTIR (FPA-μ-FTIR) imaging combined with an automated analysis of the generated spectral image, while Attenuated Total Reflection (ATR)-FTIR was used for MPs >500 μm. The continuous HTL led to an MP reduction of approximately 76% in terms of MP number and 97% in terms of MP mass. The difference in reduction of the number of MPs versus their accumulated mass was the result of MPs being smaller after the HTL process. A total of 18 polymer types were detected in the sludge and slurry entering the continuous HTL while only 11 types were identified in the residual materials. No MPs were detected in the bio-crude, i.e. the most favorable product of the process. The polymer composition changed considerably as a result of the HTL process. Polyurethane, polypropylene, and polyethylene were the dominant polymers in the feedstock, while polypropylene and polyethylene were the most present in the residual products. The findings indicate that HTL can be efficient in reducing MPs in highly polluted sludge from wastewater treatment plants, leaving the byproducts and residuals significantly less polluted, hereby reducing the movement of MPs to the terrestrial environment. Thus the products are better suited for sustainability efforts than the raw material.

1. Introduction

As a major by-product stream of wastewater treatment plants, sewage sludge contains problematic pollutants, such as microplastics (MPs) that require further treatment to make the sludge eligible for environment-friendly disposal. The latter has recently gained much attention due to its accumulation in nature (Weinstein et al., 2016), adversely impacting ecosystems (Thompson et al., 2009) and human health (Prata et al., 2020). Although sludge is traditionally reused as fertilizer and soil improver, it may contain high levels of pollutants, such as MPs, that diminish its benefits to nature, leading to a non-sustainable disposure of the sludge. Therefore, future sustainable processes, such as hydrothermal liquefaction (HTL), must be considered when examining the resilience of MPs and its existence in residual products.

Scientists are working on resource recovery from sewage sludge, as it contains a high amount of organic and inorganic materials and nutrients. HTL is capable of treating the wet urban residue at relatively mild temperatures (280–400 °C) and high pressures (10–35 MPa). HTL is gaining popularity among researchers and policy-makers, as it provides a cost- and carbon-efficient way to convert complex feedstocks into sustainable chemicals and biofuels (Kohansal et al., 2021). As a thermochemical process, HTL offers an environment-friendly alternative to crude oil, or so-called 'bio-crude', that results from the thermal breakage and reforming of biomass macromolecules (Yang et al., 2019). This process is considered an up-and-coming technology for converting complex feedstock molecules into value-added products, for instance, polyethylene (PE) plastic waste (Su et al., 2004) and electronic waste (Zhao et al., 2019).

* Corresponding author.
E-mail address: ruc@build.aau.dk (R. Chand).

https://doi.org/10.1016/j.jclepro.2022.130383
Received 30 June 2021; Received in revised form 19 December 2021; Accepted 3 January 2022
Available online 5 January 2022

In recent decades, HTL technology has been extensively studied and used to tackle the ever-growing disposal challenges of sludge from WWTPs. Silva Thomsen et al. (2020) studied the effect of a pilot-scale HTL process conditions on the conversion of micropollutants (pharmaceuticals and biocides) in sewage sludge. They report that HTL effectively decreased the environmental risk of micropollutants being released to farmlands in the case of land fertilization. Some HTL studies targeted the depolymerization of common plastic wastes at various HTL conditions: these studies report the bio-crude derivatives of plastics, solid residues, and the recovery of some chemical components from plastic materials (Seshasayee and Savage, 2020). It has been reported that the component variation of the dark and viscous oil derivatives and chemical products is highly feedstock-dependent (Toor et al., 2011); moreover, there is an expectation that the HTL process may induce a certain degree of change to synthetic polymers as well as natural counterparts (e.g. cellulose and hemicellulose) in sewage sludge. However, the impact of the HTL process on the distribution and degradation of the large quantities of microplastics contained in sewage sludge has yet to be studied.

Sewage sludge is a waste product from wastewater treatment plants (WWTPs). It contains most of the MPs held in wastewater from households, institutions, industries, and urban surface runoff (Xu et al., 2021). Modern WWTPs implementing advanced treatment methods have been reported to retain as much as 99% of MPs from the wastewater stream. Lares et al. (2018), for instance, reported an MP retention capacity of 98.3% by a WWTP with a conventional activated sludge process and a pilot-scale advanced membrane bioreactor. Carr et al. (2016) reported a retainment efficiency of 99.9% by the studied WWTP. Talvitie et al. (2017) reported that the advanced final stage technologies in WWTPs can substantially reduce the MP discharge into nearby water resources.

The MPs are retained at various treatment steps and transferred into the sewage sludge (Sun et al., 2019). Mahon et al. (2017), for instance, found sewage sludge to contain from 4.2×10^3 to 15.4×10^3 N kg^{-1} dry matter (DM). The common trend of using sewage sludge as fertilizer on agricultural fields could therefore be a key source of MPs in the terrestrial environment (Edo et al., 2020). Despite these benefits, the application of sewage sludge on agricultural lands can be a double-edged sword. On the one hand, it is a source of sustainable fertilizer; on the other hand, it leads to environmental pollution. Therefore, alternative processes, such as HTL, are needed to preserve the beneficial outcomes while mitigating the negative consequences.

Quantifying MPs in complex matrices like sewage sludge and HTL products is challenging. A multitude of destructive and non-destructive analytical techniques have been tested and employed (Sun et al., 2019). Existing methods and approaches are in no way standardized, and concentrations obtained on the same or similar matrices vary by several orders of magnitude depending on which analytical approach is applied (Gatidou et al., 2019). The lack of standardization extends to other aspects of research, including sampling techniques, sample amount, and methods used to extract the MPs from samples. Analytical methods range from simple microscopy with visual identification of what counts as an MP to state-of-the-art methods, such as Focal Plane Array (FPA) based Micro Fourier Transformation Infrared Spectroscopy (FPA-μ-FTIR). The latter technique has been reported to yield reliable data regarding number, size, and composition (Löder et al., 2015). It does so at a low size resolution that also estimates MP mass (Simon et al., 2018). A customized polymer library with a large number of polymer spectra further aids in the identification of a recorded sample spectra (Mintenig et al., 2017).

The present study assesses the sustainability of an HTL process on sewage sludge with respect to MPs. It examines the resilience of MPs through the HTL process by quantifying their presence and distribution in feedstock and products. Further, the study addresses whether there is a significant impact on specific polymers, for instance, the disappearance of some plastic types after the HTL process. The effect on the number of MPs, their size and mass were analyzed. Finally, the study closes the balance over the HTL reactor based on the number and mass of the identified polymeric particles.

2. Material and methods

2.1. Continuous hydrothermal liquefaction (HTL) and separation of the products

Sewage sludge was obtained from Stistrup WWTP, Farsø, Denmark. The WWTP has a capacity of 20,000 Population Equivalents and operates as a one-step aerobic biological process for nitrification-denitrification, removal of organic matter, and simultaneous chemical precipitation of phosphorus. A pilot-scale continuous HTL plant was set up in close collaboration between the Department of Energy, Aalborg University, and Steeper Energy, to handle the wet organic wastes, converting them into a high-quality yield product or so-called bio-crude (Castello et al., 2018). In this project, the feeding material for the HTL process was partially dewatered sewage sludge, which was analyzed for MPs before and after the procedure. The sampled sludge contained 78.5 wt % of moisture and 5.5 wt % of ash content (dry basis). Dry matter (DM) content stands as an essential factor in the continuous processing of biomass. While processing, high DM can potentially cause slurry sedimentation and reactor clogging. In contrast, low DM suppresses the bio-crude yield – known as the most favorable product of the HTL process. To set the dry matter content of the slurry to the highly recommended range of 15–20 wt %, 7.6 kg of freshwater was added to the 50.6 kg of the partially dewatered sludge. Hence, the overall dry matter of the slurry was held at around 18 wt %. Shah et al. (2020) reported a significant increase in bio-crude yield as well as a reduction of solid residue while adding K_2CO_3 to the sewage sludge HTL reaction medium processing at supercritical conditions.

Moreover, using NaOH in low quantities can potentially increase the conversion of the biomass to the intended products (Posmanik et al., 2017). Therefore, to ease the biomass processability and increase the bio-crude production yield that consequently results in the reduction of solid residue, 2.5 wt % of potassium carbonate (K_2CO_3) and 0.5 wt % of sodium hydroxide (NaOH) were subsequently introduced to the slurry. Note that all of the chemicals used in the HTL plant were of industrial grade and as such, potentially containing MPs, reflecting the reality of a future full-scale installation.

For instance, the homogeneous alkali catalysts were stored in the HDPE vessels. Before being fed to the reactor, the slurry was heated to 60 °C in the mixing tank and subsequently pressurized to approximately 30 MPa through a high-pressure piston pump (Pedersen et al., 2016). The well-mixed and pressurized slurry was injected into two sequentially connected induction heaters to increase the slurry's temperature to 400 °C. The slurry was then transmitted to two 5 L serially connected reactors (operated at 390–420 °C) to maintain the residence time of the process. The produced effluent was discharged to a concentric-tube heat exchanger and subsequently passed through two high-pressure filters. Finally, prior to degassing, a series of capillary tubes were used to depressure the product stream. The depressurized products were stored without any phase separation. The single-phase emulsion was called 'as-received products'. A detailed description of the HTL process and phase separation of the as-received products is reported elsewhere (Jensen et al., 2017). No specific steps were taken to avoid MP contamination during the process, reflecting again the reality of a future full-scale system.

In contrast to many lignocellulosic biomasses that can be gravimetrically separated or simply broken down into the different product phases using a separation funnel, the products derived from the continuous HTL of sludge manifest as a single-phase emulsion. Initially, a certain amount of the products was centrifuged (Sigma 6–16 HS) at 3800 min^{-1} for 6 min at ambient temperature. The centrifuged effluent was termed 'aqueous phase' and stored for further treatment; the rest was termed 'emulsion'. The aqueous phase obtained from centrifugation

of the as-received product was further concentrated using a semi-pilot scale vacuum evaporator with a total volume of 10 L at 65 °C under a vacuum of 100 to 5 KPa. Due to foaming issues likely resulting from the light nitrogenous compounds dissolved in the aqueous phase, 2 mL of n-octanol was added to the evaporation medium as an anti-foaming agent.

Lab-scale physio-chemical phase separation was carried out by adding 100 g of methyl ethyl ketone (MEK) (VWR International) as a diluent agent to the partially dewatered emulsion. The mixture was continuously stirred using a magnetic stirrer at 1000 min^{-1} for 8 h. Thereafter, 100 g of 0.5 M citric acid was added to the mixture to facilitate emulsion breakage. The entire mixture was again centrifuged, and the liquid products were transferred to a decanter funnel, while the solid products that remained at the bottom of the vessel were washed with acetone and filtered through a 2 μm cellulose qualitative filter paper. The water phase and MEK-dissolved bio-crude were appropriately segregated in the decanter funnel. Furthermore, the MEK was evaporated using a lab-scale vacuum rotary evaporator (Büchi R210) at 60 °C for 30 min under a vacuum of 50 KPa. All procedures were carried out without the interference of any plastic material. The approach of liquid product separation is summarized in Fig. 1.

2.2. Sampling strategy

Samples were collected from various stages of the HTL process (Fig. 1, green boxes). All products were taken from the same batch experiment. The samples were collected in clean glass bottles and covered with aluminum foil to avoid plastic contamination. From each collected sample, duplicate sub-samples were taken to extract the MPs.

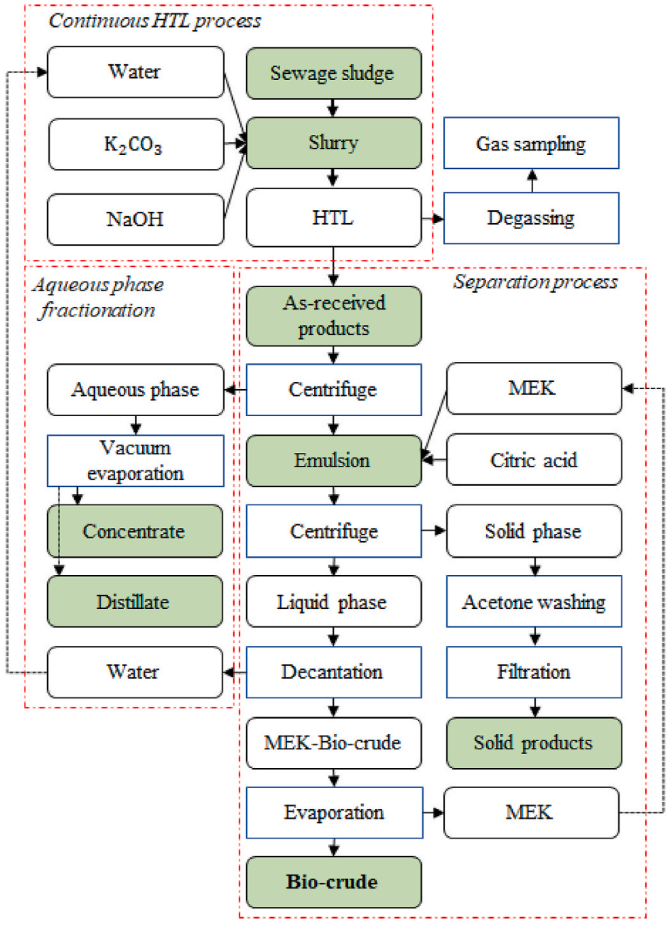

Fig. 1. An overview of the continuous hydrothermal liquefaction (HTL) process, product separation and aqueous phase fractionation.

2.3. Extraction of MPs

2.3.1. Sewage sludge and slurry

About 500 g of sewage sludge was homogenized, and 20.08 g of duplicate sub-samples equivalent to 5 g dry matter (each) were taken. Likewise, 23.8 g of duplicate sub-samples were taken from 500 g of homogenized slurry. The samples were pre-oxidized in 1 L beakers by gradually mixing 200 mL of 50% hydrogen peroxide (H_2O_2) and 250 mL of Milli-Q into the sample. The sample was left for 48 h and then filtered through a 10 μm stainless steel filter. The particles from the filter were detached by ultra-sonication into 500 mL sodium dodecyl sulfate solution (SDS, 5% w/vol). The sample was incubated for 48 h at 50 °C by continuously mixing with a glass-coated magnet. The filtered particles were transferred into 300 mL of acetate buffer (pH 4.8), then 500 μL of cellulase (Cellulase enzyme blend®, Sigma-Aldrich) and 500 μL of cellulolytic enzyme mixture (Viscozyme®L, Sigma-Aldrich) were added. The mixture was incubated at 50 °C for 48 h. The filtered particles were transferred to 300 mL of tris buffer (pH 8.2) where 500 μL of protease solution (Protease from Bacillus sp.®, Sigma-Aldrich) was added and incubated at 50 °C for 48 h. The solution was filtered and the particles were transferred into 200 mL of Milli-Q water. A Fenton oxidation was conducted by adding 145 mL of 50% H_2O_2, 65 mL of 0.1M NaOH, and 62 mL of 0.1M $FeSO_4$ while maintaining a temperature of 15–30 °C. After this step, the larger MPs (major dimension >500 μm) were separated from the smaller MPs (major dimension between 500 and 10 μm) using a 500 μm mesh sized sieve and a 10 μm stainless steel filter. The larger particles were removed from the sieve by backflushing with particle-free water, which were then stored in an aluminum tray. The water was evaporated in an oven at 50–60 °C. The cleaned and dried particles were stored for later analysis. The smaller particles were transferred into a 250 mL pear-shaped separation funnel containing a sodium polytungstate (SPT) solution of density 1.7 g cm^{-3}. The particles were mixed using compressed air bubbling from the bottom of the funnel for 30 min. The mixture was left to settle for 24 h. Three-fourth of the bottom part was removed: the process was repeated up to 3 times. The top floating particles were filtered using a 10 μm steel filter and washed with Milli-Q water to remove SPT. The filtered particles were removed using an ultra-sonicating bath and transferred into ultra-pure HPLC quality 50% ethanol. The particles were moved to 10 mL vials, and the ethanol evaporated in an evaporation bath (TurboVap® LV, Biotage) at 50 °C. Finally, the particles were suspended into 5 mL of ultra-pure HPLC quality 50% ethanol.

2.3.2. Continuous HTL products

Approximately 500 g of as-received product, 500 g of emulsion, 500 g of solid residue, and 200 g of bio-crude were taken as samples and homogenized. Duplicate sub-samples of each product (55 g of as-received product, 10 g emulsion, 10 g solid residue, 10 g bio-crude) were taken. The samples were initially treated with SDS solution and incubated for 24 h at 50 °C. Afterward, 1 mL of lipase (Lipozyme® TL 100 L, Strem Chemicals Inc.) was added to the sample and incubated for 48 h at 50 °C. The particles were filtered and transferred to 200 mL of Milli-Q water. A Fenton reaction was carried out following the aforementioned procedure. Particles were separated using a 500 μm sieve and a 10 μm steel filter. Density separation was carried out for the small MPs as described for sludge and slurry. The cleaned particles from the supernatant were transferred to 50% ethanol, resulting in a total volume of approximately 25 mL, which was later evaporated. The particles from the as-received product and emulsion were suspended in 15 mL of 50% ethanol; solid residue and bio-crude were suspended in 10 mL of 50% ethanol.

Approximately 500 g of concentrate and 2 L of distillate were collected and homogenized. Duplicate sub-samples were taken (13 g concentrate and 1 L distillate for each), filtered through a 10 μm steel filter, and flushed with Milli-Q water and 50% ethanol. The particles from the filter were diluted in 1 L of Milli-Q water and filtered through a

500 μm sieve and a 10 μm steel filter. The small MPs were transferred to 25 mL of 50% ethanol, which was later evaporated. Similarly, the small MPs from the concentrate were transferred to 10 mL of 50% ethanol, and those from the distillate samples were transferred to 5 mL of 50% ethanol.

2.4. Analytic techniques for MPs with ATR-FTIR and FPA-μFTIR

The extracted particles >500 μm were handpicked and imaged using a stereomicroscope (ZEISS, SteREO Discovery.V8) equipped with an Axiocam 105 color camera with a maximum magnification of 8 × . ZenCore (Zen2Core SP1 from ZEISS) software coupled to the microscope was used to measure particle dimensions, including area, minimum, and maximum Feret diameter. The particles' IR spectra were obtained with an Attenuated Total Reflection: ATR-FTIR (Agilent Cary 630 FTIR with a diamond ATR). Upon obtaining the background, the sample spectrum of the particle was recorded by 64 co-added scans in the spectral range of 650–4000 cm^{-1}. OMNIC software (Thermo Fisher Scientific Inc., 8.2.0.387 version 1) and its library were used to identify the material of the recorded IR spectra.

Particles between 10 and 500 μm were analyzed using FPA-μFTIR: Agilent Cary 620 FTIR microscope equipped with a 128 × 128 pixel FPA (Mercury Cadmium Telluride detector) and coupled to an Agilent 670 IR spectroscope. The particles suspended in 50% ethanol were homogenized with a vortex, and sample aliquots were taken with a glass pipette of 25 μL, 50/100 μL, and 200 μL. The sub-sample was deposited on a 13 × 2 mm zinc selenide window (Crystran, UK) held in a compression cell (Pike Technologies, USA), leaving a 10 mm diameter free area. The windows were left to dry for some hours on a heating plate at 50 °C. If needed, the process was repeated until the window was adequately covered by particles. The volume deposited on the window for each scanning of sludge and slurry was 100 μL. The deposited volumes for as-received products were 25 μL, concentrate 100–200 μL, solid residue 200 μL, distillate 600 μL, and bio-crude 50 μL. Three windows were scanned for each sample. Details of the technology are described in Simon et al. (2018), Vianello et al. (2019), and Kirstein et al. (2021). The background was acquired by 120 co-added scans, while the sample was obtained by 30 co-added scans at the wavenumber range 3750–850 cm^{-1}. The scan was done in transmission mode with a 15 × Cassegrain IR objective, producing a pixel resolution of 5.5 μm.

The resulting chemical images were analyzed with siMPle, which is an automated software program that provides particle dimensions, area, volume, and mass estimates of MPs (Primpke et al., 2020). To this end, the MPs were mapped by matching each pixel of the scan to a custom-built reference database, containing more than 110 spectra of different materials by a Pearson's correlation (Liu et al., 2019). Only particles consisting of at least two pixels were included as MPs.

2.5. Quality control and analysis of contamination

To remove traces of organic and synthetic contaminants, all glassware and the stainless steel filters were muffled at 500 °C before use. Filters were sonicated for 5 min in Milli-Q water to remove ash content and subsequently thoroughly flushed with particle-free water. The filter used for the first treatment was used throughout the sample preparation and was preserved in a clean Petri dish with Milli-Q water. All equipment was rinsed three times before use. Chemicals were filtered through 0.7 μm glass fiber filters prior to use. The sample preparation was performed inside a clean fume hood. Researchers wore cotton clothes (such as top and lab coat) throughout sample preparation and analysis. The deposition of samples for analysis on μFTIR was done inside a clean laminar flow bench, and windows were covered with a muffled beaker. The air in the FTIR and microscope lab room was continuously filtered with a Dustbox® (Hochleistungsluftreininger, Germany) housed with a HEPA filter (H14, 7.5 m^2) with over 99.99% filtration efficiency for particles <0.2 μm (Tan et al., 2021).

Contamination cannot be fully avoided despite the many protocols followed during sample preparation. Duplicate blank samples were prepared in parallel to sample preparation to quantify the possibility of contamination. For each blank sample, 1 L of Milli-Q water was filtered through a 10 μm stainless steel filter and sonicated in Milli-Q water; the final volume was adjusted to 250 mL after removing the filters. The blank control samples were processed and analyzed following the processing steps for sludge and slurry samples, i.e. the matrices requiring the most treatment prior to analysis.

2.6. Statistical analysis

R (version 3.5.2) was used for univariate analysis of datasets, and data visualization was done with the add-on ggplot2 package. The normality of the data was tested by a Shapiro-Wilk's test. Particle size and mass was verified with non-parametric tests, Wilcoxon rank-sum test between the sample matrices, and for prior and after HTL process at a significance level of 0.05.

3. Results and discussion

3.1. Continuous HTL products distribution

A sample (1.164 kg) of as-received products was extracted from the HTL storing tank. Centrifugation separated 1.020 kg of the aqueous phase without the intervention of chemicals. As expected, more aqueous phase mass was recovered than the input water content of the slurry 79 wt% (936 g) in the slurry, which is due to the dissolved polar organics in the aqueous phase. Hence, the aqueous phase yield was calculated to be 34 wt%. On the other hand, bio-crude and solid residue yielded around 25 wt% and 33 wt%, respectively. Approximately 92 wt% of the organics present in the as-received products got separated into the three mentioned phases, revealing the efficiency of the separation procedure.

3.2. MPs quantification

MPs were found in all matrices, but not detected in the bio-crude, with contents varying up to four orders of magnitude measured both as number and mass (Fig. 2). During sample preparation, minor contamination was observed compared to the abundance in the samples and the datasets, and thus not corrected for blank values. A detailed description of MP contamination in procedural blanks is given in supplementary materials in the section "MPs in procedural blanks" and in Table S3. In terms of MP numbers, the solid residue held as much as 5.7 × 10^4 times higher concentration than the distillate. The MP mass concentration in sludge was 0.096 g kg^{-1}, with ever-decreasing values in the other matrices (Fig. 2). The MP concentration in the slurry was significantly reduced compared to the concentration in the sludge, which was partly due to the latter being diluted by water and catalyst while preparing for HTL. Some variation is also due to uncertainties in sampling the various matrices. Finally, some chemical degradation and stress cracking of the polymers may have resulted because of the added K_2CO_3 and NaOH. Gohla et al. (2020) observed that many MP polymers found in beach sediments were resistant to K_2CO_3 treatment with the exception of polycarbonate (PC). Teófilo et al. (2010) found that NaOH led to significant surface damage and reduced mechanical properties of poly(ethylene terephthalate) (PET). Similar work by Baah and Baah (2002) revealed a weight loss of polypropylene (PP) upon immersion into a NaOH solution. In the present study, poly(oxymethylene) (POM) and epoxy were not detected in the slurry, which could also be due to degradation or dissolution by the added K_2CO_3 and NaOH.

The MP mass in the sewage sludge was reduced significantly by the continuous HTL process, showing that the process was efficient in degrading the plastics in the sludge. A closer look at the MP balance (Fig. 3) shows that approximately 68% of the reduction in terms of MP numbers and 60% in terms of MP mass occurred between the sludge and

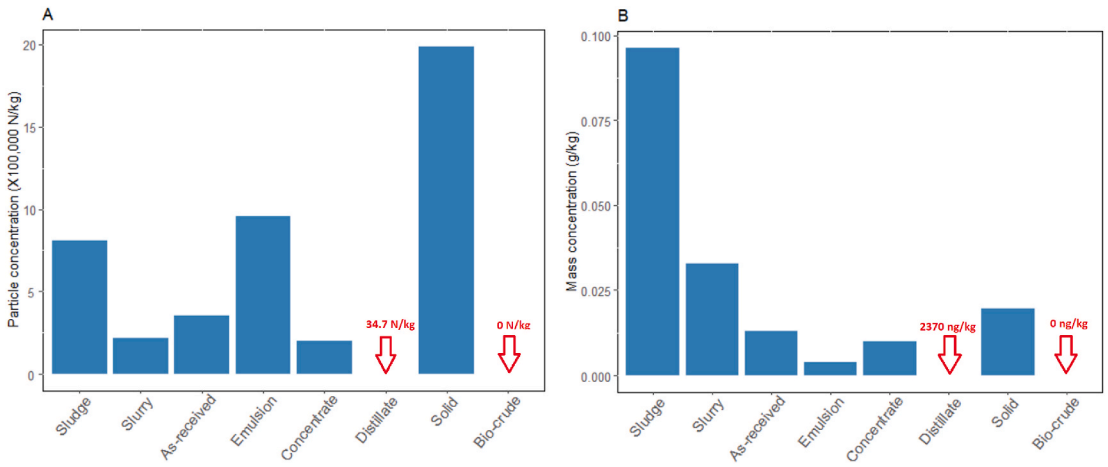

Fig. 2. The concentration of MPs in analyzed matrices: A) particle concentration and B) estimated mass concentration. The concentration is per wet weight of the sample matrices except for the matrix 'Solid'. Polymer distributions are provided in the supplementary materials Table S4 and Table S5.

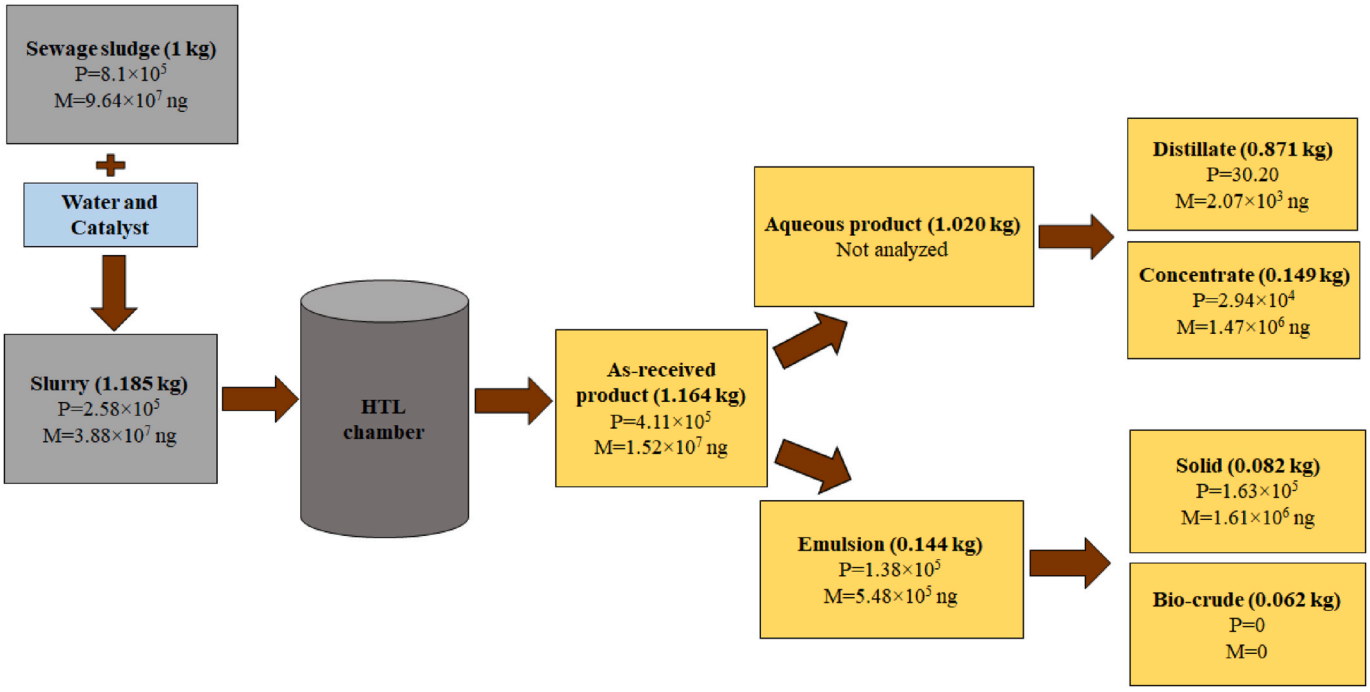

Fig. 3. MP balance over the continuous HTL process. P = particle numbers (N) and M = mass (as nanogram (ng). Details of the identified MP polymers in a unit mass of feeding material (1 kg sewage sludge) over the continuous HTL process by number and mass are provided in supplementary materials Table S6 and Table S7.

the slurry. The as-received product held more MPs than the slurry but less MP mass, indicating that particles might have fragmented in the HTL chamber. On the other hand, the sum of emulsion and aqueous product held significantly less MP mass and somewhat fewer MPs than the as-received products. The MP mass, and to a lesser degree, the particle numbers, increased in the sum of the final products. Some discrepancies are likely related to analytical uncertainties and subsampling uncertainties caused by inhomogeneity in the sampled matrices. Nevertheless, it is clear that the continuous HTL process resulted in a significant reduction of the number and mass of MPs in the raw product, the sewage sludge.

The present study is the first to quantify MPs in a continuous HTL process. There, the results cannot be compared to existing research; furthermore, more studies are needed to verify the effects of the process and the analysis used to quantify MP number and MP mass. However, there are several studies that have examined the raw product, the sewage sludge, in relation to particle numbers, including one investigation that analyzed quantified particle mass (Chand et al., 2021). The latter study quantified MPs in dry sewage sludge and digested sludge from a Swedish WWTP and found 4×10^6 N kg^{-1} and 6.4×10^6 N kg^{-1} MP in terms of particle concentration and 0.23 g kg^{-1} and 0.37 g kg^{-1} MP in terms of mass concentration, respectively. Li et al. (2018) analyzed 79 sewage sludge samples from 28 different WWTPs in China. They identified MP concentrations from 1.6×10^3 to 56.4×10^3 N kg^{-1} of dry sludge. Lares et al. (2018) investigated a WWTP in Finland and found 2.3×10^3 N kg^{-1}, 1.7×10^5 N kg^{-1}, and 2.7×10^3 N kg^{-1} in dry activated sludge, digested sludge, and sludge from a pilot membrane bioreactor (MBR), respectively. Xu et al. (2020) reported MPs from 2.9×10^3 to 5.3×10^3 N kg^{-1} in dry sludge from a WWTP in Beijing, China. Edo et al. (2020) discovered 1.8×10^5 N kg^{-1} in mixed sludge and 1.65×10^5 N kg^{-1} in heat-dried sludge from a WWTP in Madrid, Spain. It is difficult to compare the findings of said past studies with each other, as

well as with the present study, as different analytical approaches were applied. The present study, which applied FPA-μFTIR imaging with automated MP recognition, was capable of quantifying MPs down to a size of approximately 10 μm. Consequently, the present study was able to identify many more MPs than in studies that manually analyzed individual particles where the size limit is at least one order of magnitude above that reported in this paper.

3.3. Identification and composition MPs

A total of 5599 MPs were identified in the analyzed samples with an estimated total mass of 1.09×10^6 ng. The abundance of MPs identified in the sample matrices are provided in Table S1. The mean abundance values for identified polymers and the standard deviation from the sample matrices are presented in Table S2. The identified polymers were of 18 types: PP, PE, PEsT, PU, cellulose acetate (CA), polyvinyl chloride (PVC), polystyrene (PS), PA, pan acrylic (PAN), POM, PC, polyvinyl acetate (PVaC), acrylonitrile butadiene styrene (ABS), alkyd, acrylic, epoxy, phenoxy resin, and poly(ethyle acrylate). The most diverse polymeric composition was found in the sewage sludge where 17 polymer types were identified. 15 types were identified in the slurry, 3 in the as-received products, 4 in the emulsion, 3 in the concentrate, 8 in the distillate, and 8 in the solid residue.

Fig. 4 shows the polymer composition based on MP number and mass concentration. The polymer composition between samples was markedly heterogeneous with the exception of sewage sludge and slurry, which were comparable in composition. In terms of MP numbers, PP was the dominant polymer in the samples after the HTL process reactor with the exception of distillate. However, it must be noted that the total concentration for the distillate was very low (Figs. 2 and 3), and uncertainty on the polymer composition was therefore correspondingly high. In terms of mass, PP was the dominant polymer in emulsion and solid phases. Viewing the two polyolefins (PP and PE) under one showed that these were the most resilient to degradation in the HTL reactor. Some polymer types, namely CA, POM, PVaC, PC, alkyd, phenoxy resin, poly(ethyle acrylate) were not detected in as-received and residual products, indicating a considerable or even total degradation in the HTL reactor.

In addition to the overall picture outlined above, there were some discrepancies in the measured data, such as only 3 polymer types were identified in the as-received products while up to 8 were identified in the refined products. The discrepancies in the particle numbers and mass quantified in the balance over the reactor are apparently similar (Fig. 3). The true causes are difficult to establish conclusively, but can be partly attributed to sample complexity, particularly for as-received and emulsion samples, which were very difficult to purify and concentrate for analysis. The main reason for this was that they held charred particles, which were challenging to separate from the sample as they frequently entangle and float on the surface of the SPT solution used in the floatation step. Agglomerated particles were broken by using air bubbles passed through the bottom of the funnel, a process which was repeated 3–5 times. This allowed for a proper extraction of MPs, yet some MPs have most likely been lost in this process. Residual charred particles might also have covered MPs while scanning with the μFTIR, which would have led them to be overlooked in the analysis, but the first issue is judged the greater of the two. The other products after the HTL reactor contained less charred materials, and the extraction method was found much more efficient with fewer steps for purifying the particles.

It is finally worth repeating Simon et al.'s (2018) observation that the identification of polymer chemical composition of MPs is not always straightforward, though μFTIR is a powerful tool and routinely used in MP research. Since MPs are exceptionally heterogeneous in size, shape, and structure, such qualities can contribute to some baseline drift and noise, which interferes with the evaluation of the obtained spectra. The

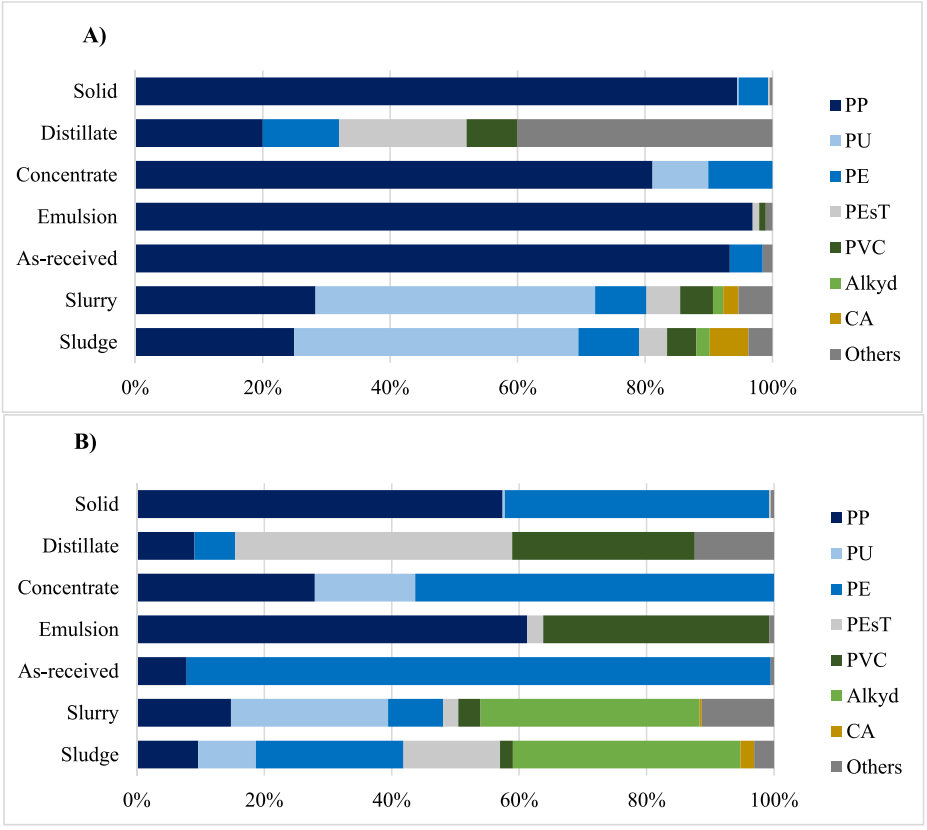

Fig. 4. Distribution of the identified polymers based on: A) particle concentration and B) estimated mass concentration. 'Others' indicates polymer types: acrylic, PS, PA, PAN, POM, epoxy, PC, PVaC, ABS, phenoxy resin and poly (ethyl acrylate).

HTL process might have affected such characteristics and accordingly introduced unknown bias into the analysis.

3.4. Effect of the HTL process on MPs size and mass

The size (major dimension) and the corresponding mass of the MPs were also investigated, revealing a change in the size and corresponding mass of the particles before and after the HTL process. In the sludge, approximately 83% of the particles were below 100 μm, whereas in residual products (aqueous phase and solid residue), above 96% were below 100 μm. The corresponding mass showed that approximately 84% and 96% of the identified particles were below 50 ng in sludge and residual products, respectively. A Shapiro-Wilk test showed that the datasets were non-normally distributed for all analyzed matrices. A Wilcoxon rank-sum tests revealed that the sizes of the identified particles were significantly different when comparing the categories before the HTL process (sludge and slurry) to those after the HTL process (as-received, emulsion, concentrate, distillate, and solid). The tests were performed at a 0.05 significance level, and p-values were found to be small ($<2.2 \times 10^{-16}$). Similar low p-values were observed for the mass of the identified particles that showed a significant difference in the mass datasets. However, Wilcoxon rank-sum tests showed non-significant differences for particle size and mass between sludge and slurry with p-values of 0.14 and 0.18, respectively. The same test also showed a non-significant difference between particle size and mass of the as-received and emulsion with p-values of 0.25 and 0.23, respectively. Fig. 5 illustrates the distribution pattern of the major dimension of the identified particles. In sludge and slurry, i.e., the sample matrices before the HTL process, the particle sizes ranged between 11 μm and 2343 μm; while in the products, i.e., the sample matrices after the HTL process, the sizes were between 11 μm and 515 μm. Likewise, Fig. 6 illustrates the distribution pattern of the estimated mass of the identified particles. In sample matrices before the HTL process, the estimated particle masses varied between 0.16 ng and 148145 ng, while MPs after the HTL process were between 0.13 ng and 2981 ng. For further details, see supplementary materials Table S8.

3.5. Resistant MPs polymers over HTL process

About 76% of the number of MPs and 97% of MP mass was removed by the HTL process. Furthermore, the HTL products had a less rich polymer composition, where some polymer types were completely absent (Table S1). The most resilient polymers seemed to be the polyolefins PE and PP, but also some PU made it through the liquefaction (Fig. 7 and Fig. S1). The solid residue held the largest amount of residual MPs and accounted for approximately 85% by number and 52% by mass of all MPs after HTL.

Some studies have raised concerns about the ability of HTL to remove MPs from sludge. Prestigiacomo et al. (2021) reported partial degradation of MPs, arguing that MPs in sludge could also challenge the performance of the HTL process. While the number of studies addressing aspects of MPs in HTL of sludge is limited, several studies have assessed residual products of plastic materials treated at sub/supercritical water conditions, either individually or in combination with organic biomass materials. A co-processing of different polymers, such as PE, PP, PET and nylon with pistachio hull (biomass) was carried out by Hongthong et al. (2020). The study showed that PE and PP were highly resistant toward the specified thermochemical operating conditions. On the other hand, nylon (polyamides) and PET showed significant degradation of 84% and 53%, respectively. Raikova et al. (2019) investigated co-liquefaction of microalgae with commonly used plastics such as PE, PP, and nylon. They reported that 3.7–18% of these were converted to bio-crude, depending on loading rates. However, the remaining was either converted to solid residue (char) or remained as unreacted plastics in the solid phase. Zhao et al. (2019) found an effect of temperature on four types of electronic plastic waste. This included PC and PA, which were susceptible to degradation at lower temperatures and yielded removal rates of 82% and 74% at 250 °C, respectively. Simultaneously, high impact polystyrene (HIPS) and acrylonitrile butadiene styrene (ABS) were degraded by 88.4% and 98.4% at 350 °C, respectively. Jin et al. (2020) identified

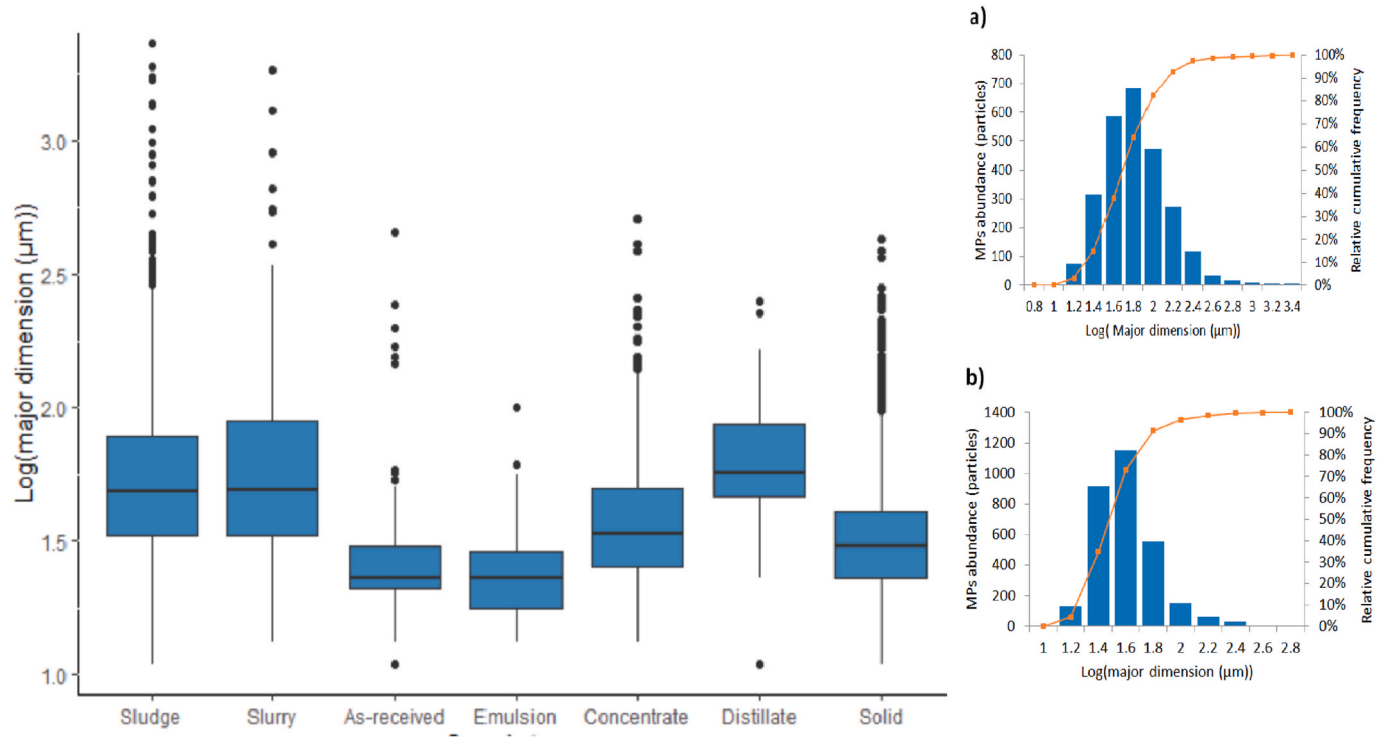

Fig. 5. Box plot for major dimension (as log of the major dimension (μm)) of identified MPs (a) size distribution and the relative cumulative frequency of the identified particles before HTL (in sludge and slurry) and (b) after HTL (in as-received, emulsion, concentrate, distillate, and solid residues).

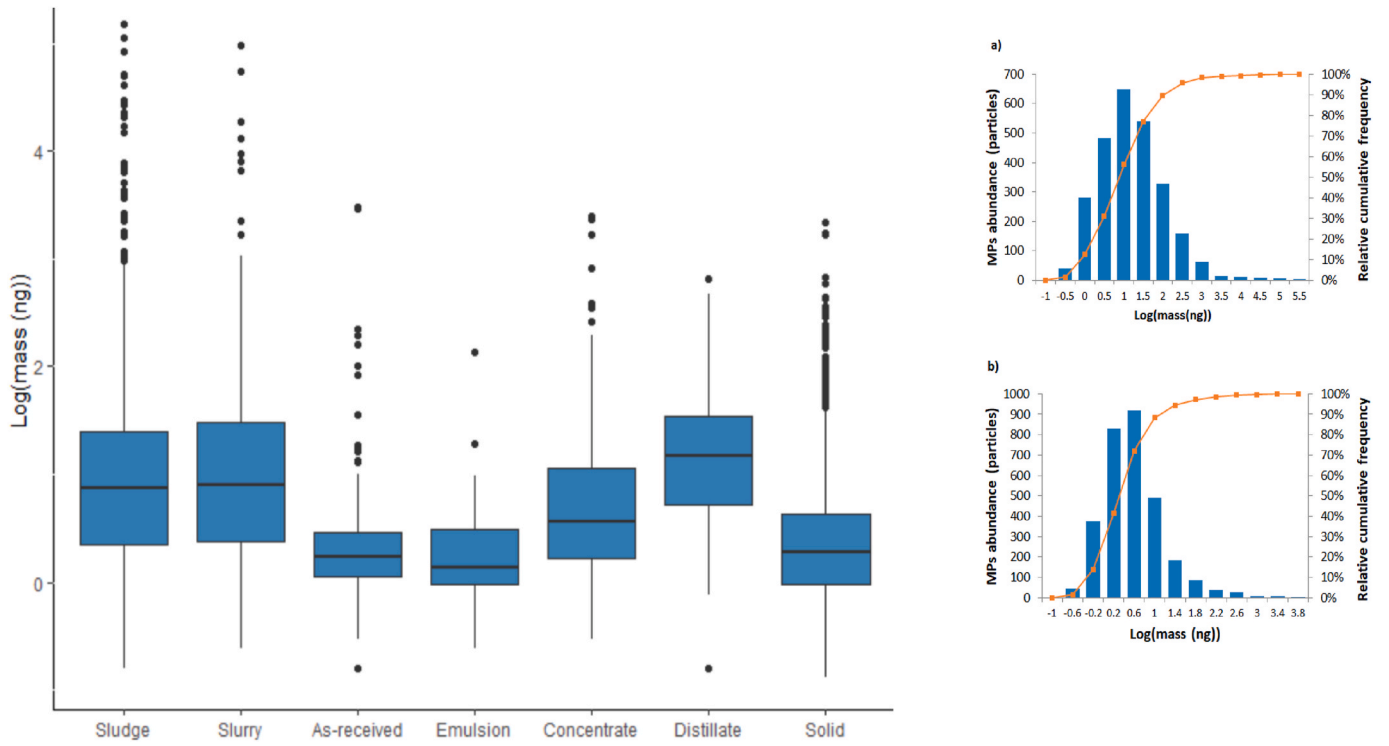

Fig. 6. Box plot for mass (as log of the mass (ng)) of identified MPs (a) mass distribution and the relative cumulative frequency of the identified particles before HTL (in sludge and slurry) and (b) after HTL (in as-received, emulsion, concentrate, distillate and, solid residues).

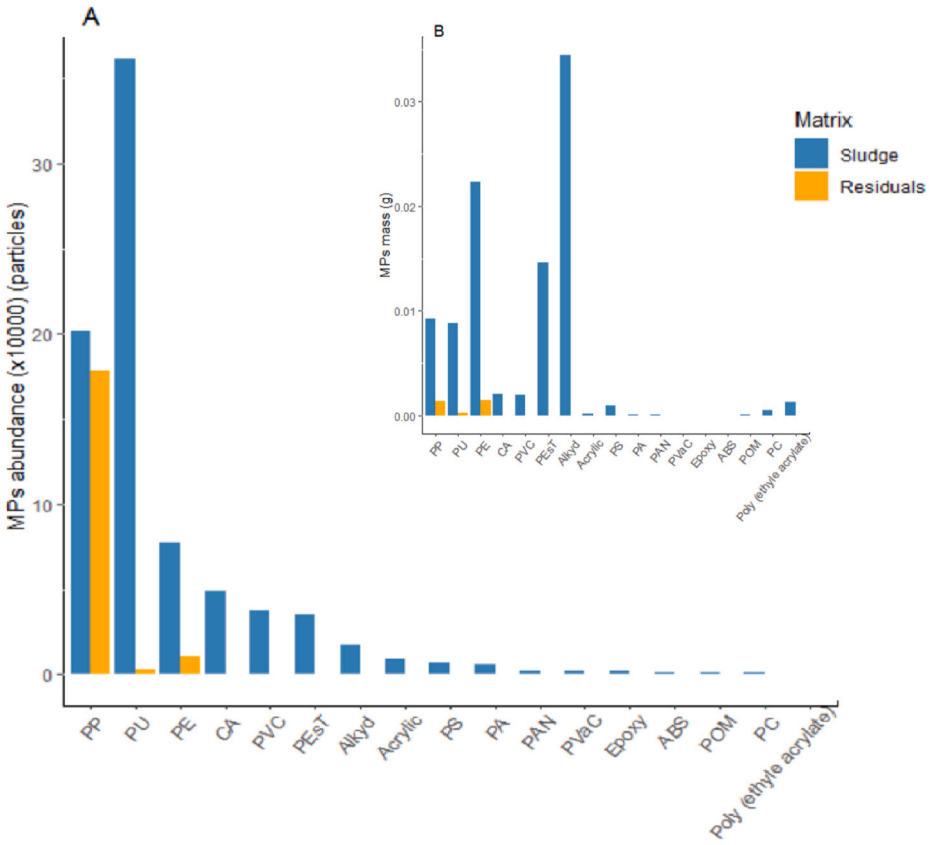

Fig. 7. MPs load reduction over HTL process A) by number and B) by mass.

PC as a temperature-sensitive plastic, which could be depolymerized at temperatures between 350 °C and 450 °C. Pedersen and Conti, (2017) found the solid residue from a HTL process between 1 wt% to 68.5 wt% for styrene-butadiene (SB), poly (vinyl alcohol) (PVA), poly (p-phenylene oxide) (PPO), poly(oxymethylene) (POM), PET, poly (butylene terephthalate) (PBT); while PC, Poly (lactic acid) (PLA), and Poly(methyl methacrylate) (PMMA) were non-detectable in the residue. They presented no information on the plastics, which seemed to be less reactive in their study (PP, PE, and PU). dos Passos et al. (2020) stated that due to the absence of heteroatoms and reactive sites in polyolefins, such as PE (HDPE and LDPE), PP, and PS, these plastic types are highly resistant to HTL processing and remained in their solid residue at over 90%.

The results of the present study, which shows that the HTL process can significantly reduce MPs in sewage sludge, demonstrates that the reported process has the potential to mitigate the MP pollution issue of this material when applying it as fertilizer on agricultural fields. The remaining amount of MPs in residual products would pose a lower environmental risk compared to the original product: sewage sludge. However, there is still room for improvement, and it is essential to further explore the viability of MPs during the HTL process in order to optimize the process.

4. Conclusion

Continuous hydrothermal liquefaction of sewage sludge is an innovative field of study that generally targets the production of bio-crude. This study showed that it also has the potential to significantly reduce microplastics in sludge, adding to its sustainable use for fertilization and soil improvement. It was discovered that the HTL process reduced the amount of MPs significantly, namely by 76% as particle numbers and 97% as plastic mass. Furthermore, it was seen that some polymer types were more resistant than others, but none were unaffected by the process. In addition, the HTL process was found to decrease the particle size of the more resistant polymers, such as the PP, PE, and PU. The HTL process is thus able to not only convert sludge to bio-crude, but also mitigate the issue of the sludge being polluted by MPs, creating residual products with significantly less MP than the original product. This clearly shows that the application of the HTL process significantly reduces the movement of MPs from the sewage sludge into the terrestrial environment. The present study is the first of its kind yet still opens up many unanswered questions. Some questions relate to the analytics involved in the quantification of MPs in the rather difficult matrices of HTL products. Others questions relate to how the HTL process can be optimized to remove as much MP as possible without compromising its main objective: the production of bio-crude. Overall, however, the picture is clear: The HTL process is highly effective in producing cleaner bio-crude and solid product from the highly microplastic-polluted sludge. The process could contribute significantly to mitigate MP pollution, and enhance sustainable reuse of waste products.

CRediT authorship contribution statement

Rupa Chand: Conceptualization, carried out the experiments, Methodology, Formal analysis, Writing – original draft. **Komeil Kohansal:** Formal analysis, Writing – review & editing, Writing – original draft, Assisted in conducting sampling and sent to the lab for analysis and, contributed in the original. **Saqib Toor:** Writing – original draft, Coordinated the project work, revised the work and reviewed the. **Thomas Helmer Pedersen:** Conceptualization, the project plan, coordinated the project work, revised the work and reviewed the, Writing – original draft. **Jes Vollertsen:** Supervision, Writing – review & editing, Equally, all authors contributed for the final manuscript.

Declaration of competing interest

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

Acknowledgement

We would like to acknowledge the funding support received from the Danish Energy Authority (EUDP) and the European Union's Horizon 2020 research innovation program under grant no. 818413 (NextGenRoadFuel).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2022.130383.

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Paper – III

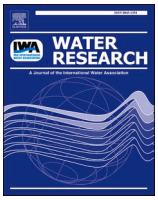
Treating wastewater for microplastics to a level on par with nearby marine waters

Water Research 256 (2024) 121647

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres



Treating wastewater for microplastics to a level on par with nearby marine waters

Rupa Chand [a,*], Lucian Iordachescu [a], Frida Bäckbom [b], Angelica Andreasson [b], Cecilia Bertholds [b], Emelie Pollack [b], Marziye Molazadeh [a], Claudia Lorenz [a,c], Asbjørn Haaning Nielsen [a], Jes Vollertsen [a]

[a] Department of the Built Environment, Aalborg University, Thomas Manns Vej 23, Aalborg 9200, Denmark
[b] Käppala, Södra Kungsvägen 315, Lidingö 18163, Sweden
[c] Department of Science and Environment, Roskilde University, Roskilde 4000, Denmark

ARTICLE INFO

Keywords:
Microplastics
Wastewater treatment plant
Treatment efficiency
Rapid sand filter
Digester inlet
Digester outlet

ABSTRACT

Retention of microplastics (MPs) at the third largest wastewater treatment plant (WWTP) in Sweden was investigated. The plant is one of the most modern and advanced of its kind, with rapid sand filter for tertiary treatment in combination with mechanical, biological, and chemical treatment. It achieved a significantly high treatment efficiency, which brought the MP concentration in its discharge on par with concentrations measured in marine waters of the same region. This novel data shows that properly designed modern WWTPs can reduce the MP content of sewage down to background levels measured in the receiving aquatic environment. Opposite to current understanding of the retention of MP by WWTPs, a modern and well-designed WWTP does not have to be a significant point source for MP. MPs were quantified at all major treatment steps, including digester inlet and outlet sludge. MPs sized 10–500 μm were analyzed by a focal plane array based micro-Fourier transform infrared (FPA-μFTIR) microscopy, a hyperspectral imaging technique, while MPs above 500 μm were analyzed by Attenuated Total Reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy. Mass was estimated from the hyperspectral images for MPs <500 μm and from microscope images >500 μm. The overall treatment efficiency was in terms of MP counts 99.98 %, with a daily input of 6.42×10^{10} and output of 1.04×10^{7} particles. The mass removal efficiency was 99.99 %. The mechanical part of the treatment, the pre-treatment, and primary stages, reduced both the MP counts and mass by approximately 71 %. The combined biological treatment, secondary settling, and final polishing with rapid sand filtration removed nearly all the remaining 29 %. MPs became successively smaller as they passed the different treatment steps. The digester inlet received 1.04×10^{11} MPs daily, while it discharged 9.96×10^{10} MPs, causing a small but not significant decrease in MP counts, with a corresponding MP mass reduction of 9.56 %.

1. Introduction

Plastic is a versatile material which enjoys widespread use. Compared to natural materials, plastics are durable, low cost, light weight, and rather strong (Ammala et al., 2011). Plastics take many sizes and shapes, and when manufactured in the size range 1–5000 μm, they are termed primary microplastics (MPs). Some of these are manufactured as additives to cosmetics, personal care products, industrial scrubbers, and cleansers, and some as plastic powders for molding (Talvitie et al., 2017a). MPs are also formed in the environment as breakdown products of larger plastic items (Thompson, 2005), these are termed secondary MPs (Andrady, 2011; Mason et al., 2016; Weinstein et al., 2016). The degradation usually occurs due to harsh mechanical, chemical, and biological processes (Mason et al., 2016; Singh and Sharma, 2008; Sussarellu et al., 2016). The continuous disintegration of larger items lead to accumulation of MPs, for example in marine sediments (Simon-Sánchez et al., 2022). Due to the ubiquity of plastics and their persistency, MPs are present in all environments, such as soil, sediment, water, and air (Duis and Coors, 2016; Gasperi et al., 2018; Simon-Sánchez et al., 2022). The list continues, and MPs have for example been reported in tap water and bottled water (Kirstein et al., 2021; Koelmans et al., 2019), and have been identified in marine

* Corresponding author.
E-mail address: ruc@build.aau.dk (R. Chand).

https://doi.org/10.1016/j.watres.2024.121647
Received 30 October 2023; Received in revised form 10 March 2024; Accepted 18 April 2024
Available online 19 April 2024

organisms (Galloway, 2015).

Wastewater is no exception when it comes to MPs, and various sources releasing MPs into it, such as domestic discharge of textile fibers, cleansing beads from personal care products, industrial plastics, and stormwater runoff have been identified (Carr et al., 2016; Iyare et al., 2020; Long et al., 2019). However, wastewater is commonly treated before discharge, and the retention of MPs by wastewater treatment plants (WWTP) has been the focus of many a study (Gies et al., 2018; Lares et al., 2018; Talvitie et al., 2017a). Since the beginning of MP research, there has been significant concern from various stakeholders on the ability of WWTPs to retain MPs, leading to a significant body of research on this topic. Most studies treated WWTPs as black boxes, focusing on particle concentration in the inlet versus the outlet, largely ignoring the huge variation in treatment technologies applied by different plants. Depending on WWTP layout and operation, treatment efficiencies have often been found to be high, commonly at or above 95–99 % (Simon et al., 2018; Talvitie et al., 2017b). The retained MPs are either concentrated in the sludge (Michielssen et al., 2016; Xu et al., 2019) or skimmed off with the grit and grease (Chand et al., 2021; Rasmussen et al., 2021). The larger-sized MPs have been reported residing in grit, grease, and primary sludge, while smaller MPs seem to have a higher probability to reach the outlet (Lusher et al., 2017; Murphy et al., 2016; Ren et al., 2020). Sun et al. (2019) reported that 35–59 % of MPs were removed by pre-treatment and 50–98 % by primary treatment, while Iyare et al. (2020) found that tertiary treatment could further remove 5–20 %. Other studies showed that pre-treatment could remove even more. Ziajahromi et al. (2021) found for example a retention of 79 % by the screening and grit removal. Where the MPs go will depend on the WWTP layout as well as the physical characteristics of the MPs, such as size, shape, density, and chemical characteristics. Moreover, different polymers may follow different hydraulic transport patterns (Molazadeh et al., 2023a) which further might affect their fate in the primary and secondary settling tanks.

Most MP ends up in the sludge, which at larger WWTPs often is further treated in anaerobic digesters (Jiang et al., 2022). The MPs in sludge have been found rather resistant to mesophilic anaerobic digestion, with the exception of some possible fragmentation (Chand et al., 2021; Jiang et al., 2022). An effective degradation of MPs in digester inlet sludge might only be possible at extreme conditions, for instance, the high temperatures of hydrothermal liquefaction (Chand et al., 2022). Fragmentation of large-sized plastics (>5 mm) that can enter the digester via primary sludge and grease (Rasmussen et al., 2021), might also contribute to MPs in its outlet sludge. Some macroplastics and larger MPs probably disintegrated into smaller MPs during anaerobic digestion, which increases the MP numbers. At the same time, the polymer composition by MP counts might be altered, which could affect their fate in the digester (Askham et al., 2022). Where sludge is used as fertilizer, this might increase the risk of MP soil pollution (Iyare et al., 2020; Raju et al., 2018; Wei et al., 2022).

Despite their high efficiency towards MPs removal, WWTPs have been reported to impact the aquatic environment (Carr et al., 2016; Gies et al., 2018; Mintenig et al., 2017). This has been evidenced for example by elevated MP-levels in receiving waters downstream of WWTPs (Molazadeh et al., 2023b; Sönmez et al., 2023). Schmidt et al. (2020) for instance found 4–4.5 $\times$ 10^5 MP m^{-3} in the effluent of 79 German WWTPs. Similarly, Ren et al. (2020) reported a daily discharge of 8.7 $\times$ 10^8 MPs from a Chinese WWTP treating combined municipal sewage and stormwater. Many studies on MP retainment at WWTPs overlook that WWTPs differ widely in the technology they apply. Some are limited to simple screening for gross solids before discharge, others add a gravimetric settling step. More advanced WWTPs make use of biological and chemical treatment processes, ranging from simple treatment in high-loaded activated sludge reactors to complex biological nutrient removal treatment trains. A few added a polishing step of the so-treated water (Funck et al., 2021; Kılıç et al., 2023). They are all called WWTPs, however, their efficiency to retain MPs will depend on the details of the treatment train they apply.

The objective of the present work is to further develop the understanding of how individual treatment steps affect MP retainment within a WWTP, including its anaerobic digester. The study investigates the MPs in successive treatment stages and internal flows at Käppala WWTP in Lidingö, Sweden, as well as the fate of MPs in its mesophilic anaerobic digester.

2. Materials and methods

2.1. Description of the WWTP and sample collection

Käppala WWTP is one of the three largest wastewater treatment facilities in Sweden, receiving sewage from eleven municipalities in the greater Stockholm area. It serves a population over 525,000 and is considered one of the most effective plants in Sweden with quite low nutrients and organic pollutants discharge, partly due to its tertiary treatment based on rapid sand filtration. Approximately 50.5 million m^3 of sewage is being treated annually and the treated water discharges to the Baltic Sea. The sewage sludge (digester inlet) is being treated in two mesophilic anaerobic digesters. The produced sludge (digester outlet) is used as fertilizer on arable land.

Wastewater was collected flow proportionally over 24 h applying the samplers used for routine measurements. Seven consecutive samples were mixed into a weekly sample. Three such weekly samples were collected per sampling point and transported to Aalborg University for MP analysis. The samples were collected at: Influent (inlet before preliminary treatment); Rinse water (water from the washing of screen debris); Screening effluent (inlet after screening and before grit and fat removal); Primary effluent (wastewater after primary sedimentation); Secondary effluent (wastewater after secondary sedimentation); and Backwash water (from the tertiary sand filters). All wastewater samples were collected in aluminium bottles, stored at 4 °C, and trasported to the laboratory.

Three final effluent samples were collected by pumped filtration using a plastic-free filtration device (Universal Filtering Object, UFO) holding 167 mm diameter stainless steel filters. First the water passed a 300 μm pre-screen upon which it was distributed onto two 10 μm screens (Rasmussen et al., 2021). More than 1000 L treated water was filtered per sample. Upon filtration, all three filters were treated together as one sample. Sludge was collected from the inlet and the outlet of the digesters as grab samples. Approx. 1 kg wet weight of each type was collected and stored in metal buckets. The sampling was conducted over a time span of one year (September 2020 to October 2021). The operation of and load on the WWTP was unchanged during this period. An overview of the plant and sampling spots is presented in the graphical abstract.

The collected sample volume depended upon the expected MP content in the matrix, as the goal was to identify a sufficient number of MPs to quantify the content herein. The higher the expected concentration, the smaller the volume, and samples like influent wastewater were hence sampled at much lower volumes than final effluent. Of the collected samples, a subsample of 3 L of influent, screening effluent, primary effluent, and secondary effluent was taken into work to extract MPs, while 2–3 L of backwash water and 1 L of rinse water was taken into work. For sludge, the sample was homogenized by manual mixing, upon which 100 g wet weight of digester inlet sludge and 200 g wet weight of digester outlet sludge was taken into analysis.

2.2. MPs extraction

2.2.1. Removal of natural organic materials

All samples required thorough treatment to remove cellulose, proteins, and other organic materials prior to analysis. For this purpose, all samples except effluent water were first pre-oxidized by adding 50 % hydrogen peroxide (H_2O_2). The amount of H_2O_2 needed depended on

the organic content of the samples. Pre-oxidation took 72–120 h and was considered completed when no more bubbles were seen upon adding H_2O_2. The undigested particles were filtered on a stainless-steel filter of 10 μm mesh and Ø47 mm, upon which the particles were transferred to 250–500 mL of sodium dodecyl sulfate solution (SDS, 5% w/vol). For the effluent samples, the particles collected on the Ø167 mm filters were detached by ultrasonication and transferred into 500 mL of SDS, 5% w/vol solution where they were incubated for 48 h at 50 °C and constantly mixed. All further filtration was done on 10 μm stainless steel filters and particles were detached by ultrasonication.

All samples were then treated enzymatically in two steps. The particles were first transferred to 300 mL of tris buffer (pH 8.2) and 500 μL protease (Protease from Bacillus sp. ®, Sigma-Aldrich) added. The solution was incubated for 48 h at 50 °C. Hereafter the liquid was filtered, and the collected particles transferred into 300 mL of acetate buffer (pH 4.8) to which 500 μL of cellulase (Cellulase enzyme blend®, Sigma-Aldrich) and 500 μL cellulolytic enzyme mixture (Viscozyme®L, Sigma-Aldrich) were added and the solution incubated for 48 h at 50 °C. The liquid was filtered, and particles transferred to 200 mL of milli-Q water. A catalytic oxidation (Fenton) was carried out by adding 145 mL of 50 % H_2O_2, 65 mL of 0.1 M NaOH, and 62 mL of 0.1 M $FeSO_4$. The NaOH was needed to adjust the pH to 2.5–3.0 throughout the process. The temperature was maintained between 15 and 30 °C by keeping the samples in an ice bath.

After Fenton oxidation, the liquid was filtered on a 500 μm stainless steel sieve followed by a 10 μm stainless steel filter. The particles collected on the 500 μm sieve were dried at 50 °C for 7 days and each particle analyzed chemically to identify its material (Section 2.3).

2.2.2. Removal of inorganic materials

The particles on the 10 μm filter were transferred into 250 mL of sodium polytungstate (SPT) solution of 1.78 g cm^{-3} density. The liquid was transferred to a pear-shaped separating funnel, mixed by compressed air from the bottom for 30 min, and left to settle for 24 h. The settled heavy materials were discharged, and the top floating materials were collected and transferred into approximately 25 mL of 50 % ethanol. The ethanol was evaporated, and the particles collected in a 10 mL vial. A final volume of 5 mL of 50 % ethanol was added, making the extract ready for chemical analysis (Section 2.3).

2.3. FTIR (ATR and FPA-μ-FTIR) analysis

Particles >500 μm were manually sorted and imaged with a stereoscopic microscope (ZEISS, SteREO Discovery.V8) with an Axiocam 105 color camera and max. 8x magnification. The dimension of each particle was measured by the software ZenCore (Zen2Core SP1 from ZEISS) coupled to the stereoscopic microscope. The material of each particle was identified by a Cary 630 FTIR (Fourier transform infrared, Agilent Technologies) equipped with a diamond ATR (Attenuated Total Reflectance) accessory. The obtained spectrum was interpreted by the software OMNIC 8.2.0.387 (Thermo Fisher Scientific Inc., version 1) applying a commercial library.

Particles between 500 and 10 μm were analyzed by hyperspectral imaging applying FTIR microspectroscopy (μFTIR). After a thorough mixing of the sample concentrate, a subsample was taken with a disposable glass pipette and deposited on a 13 × 2 mm zinc selenide (ZnSe) window (Crystran, UK) and the window dried on a heating plate at 50 °C. This process was repeated till the window was sufficiently covered for the hyperspectral imaging. The window was analyzed in full by μFTIR using an Agilent Cary 620 FTIR microscope equipped with a 128 × 128-pixel FPA (Focal Plane Array) with Mercury Cadmium Telluride detector and coupled to an Agilent 670 IR spectroscope. Three separate windows were analyzed for each sample. Not all the 5 mL of concentrate could be deposited on the 3 windows. How much depended on the particle content in the concentrate. The full hyperspectral image consisted of 14 × 14 FPA-tiles covering the whole area of the Ø10 mm window. For further details see Chand et al. (2021) and Simon et al. (2018). MPs were identified and characterized by an automated approach using the software siMPle combined with a dedicated reference database (Liu et al., 2019; Primpke et al., 2020b). Besides information on particle number and polymer types, the analysis yielded shape parameters such as particle area, maximum and minimum Feret diameters, and particle mass estimation (Simon et al., 2018).

2.4. Contamination control and recovery test

Proper precautions to avoid contamination during sample preparation and analysis were undertaken as described in Chand et al. (2022a, 2021). Contamination was assessed by three laboratory blanks where 1 L of filtered Milli-Q was treated following the same treatment processes as for the samples. A field air blank was also collected during effluent sampling by keeping a clean glass Petri-dish open near the sampling location. The particles from the Petri-dish were transferred into a vial and concentrated into 5 mL HPLC ethanol 50 % and approx. one third of it was analyzed by μFTIR.

The recovery efficiency was assessed by spiking with known MP particles. Spherical polystyrene (PS) beads (microparticles GmbH, Berlin, Germany) of mean diameter 106 μm and a clearly distinguishable red color were used alongside fragments of polyvinyl chloride (PVC), polyethylene (PE), and polypropylene (PP). These fragments were produced by cry-milling common plastic objects, such as PVC from a PVC pipe, PE from a water bottle, and PP from a plastic sheet, and were obtained in the size range of 20 – 200 μm, 40 – 80 μm, and 20 – 200 μm, respectively. Three different sets of particles were made for the recovery, always mixing the spherical PS beads with one of the fragmented polymer types: i) PS and PVC; ii) PS and PE; and iii) PS and PP. The particles were counted by fluid imaging with a FlowCam 8400 (Yokogawa Fluid Imaging Technologies) at 4x magnification (

Y information Figure S1). The counted particles were then spiked into 250 mL of 5 % SDS. The FlowCam analysis also yielded the size of the added beads and fragments stated above. The spiked particles were then treated following the same sample treatment protocol as the effluent samples and the extracted spiked particles were again counted by the FlowCam. The treatment efficiency was calculated as the proportion of recovered particles to the total spiked particles (Table 2).

2.5. Data analysis

Hyperspectral images acquired by FPA-μFTIR were analyzed by siMPle, an automated software that compares the FTIR-spectrum of each pixel with a library tailored to MP analysis and containing more than 450 spectra (Primpke et al., 2020; Simon-Sánchez et al., 2022). The IR spectra from ATR-FTIR were analyzed by OMNIC (Thermo Fisher Scientific Inc., 8.2.0.387 version 1) for chemical identification. Fibers were distinguished from fragments by their length to width ratio being >3 (Vianello et al., 2019). A Shapiro-Wilk normality test was performed on the distribution of the determined MP major dimensions and estimated masses. Likewise, a pair-wise Wilcoxon rank sum test was conducted to test the significance of data among different sampling spots. In all cases, the significance level was set to 0.05. Data analysis and visualization was performed using R studio (4.2.2) and Microsoft Excel.

3. Results and discussion

3.1. Background contamination and recovery

Almost one third (32 %) of the concentrate from the three laboratory blanks was analyzed by scanning 3 windows of each blank, and 5 MPs were detected: 2 polyester, 1 PVC, 1 PP, and 1 PS. The total estimated mass was 692.5 ng. The contamination per processed blank sample hence was 5.2 MP per sample (721.4 ng per sample). Polyester contributed 40 % of the MP-counts while PS contributed the most mass

(57 %). Comparing the per sample blank concentration to that of the samples, the digester outlet sludge was relatively speaking the least affected by contamination (0.02 %), while the most affected was secondary effluent (7.52 %). The relative contamination measured as mass was lowest in rinse water (0.001 %), and highest in secondary effluent (29.17 %). For the field blank collected during effluent sampling, 2 MPs were found: 1 PVC and 1 polyester. The PVC particle was the largest and contributed most to the estimated mass (83 %). Further details are in supplementary information Table S1 and Table S2.

The data were not blank corrected as the level of contamination was low compared to the concentration in most samples. There are furthermore several fundamental issues when blank-correcting MP data. If, for example, a blank holds an MP of a material not found in the related sample, a blank correction is meaningless as negative concentrations cannot exist. A similar issue arises when assessing MP size distributions. Blank correction might also lead to meaningless results when mass is estimated, e.g., having a positive number of MPs but at the same time having a negative mass. Blank correction can hence bias the data instead of improving it. Many studies took the same approach for similar reasons, for example Lares et al. (2018), Liu et al. (2019), Ren et al. (2020), and Simon et al. (2018). Instead, these studies reported background contamination to qualify how trustworthy the data was.

MPs may be lost in the extraction process, and assessing recovery rates during extraction is an essential part of quality assurance. A common way to assess recovery is by spiking with a known amount of MPs and recovering them after extraction. Three sets of recovery tests were prepared with PS beads (106 μm) together with fragments (20–200 μm) of PVC, PE, and PP (Section 2.4), yielding an overall recovery above 68 % (Table 1). PP, PVC, and PE fragments were recovered above 70 % while PS beads were recovered at about 61 %. In comparision, Simon et al. (2018) recovered 77.7 ± 11.6 % PS beads of 100 μm and 57.6 ± 25.1 % HDPE fragments of 80–150 μm following a similar protocol of MP extraction. Long et al. (2019) obtained recovery rates of 99.0 % and 89.3 % of 1000 μm and 90 μm PS, respectively, where the extraction was done by wet oxidation without enzymatic treatment. Various other researchers have presented recovery results applying spiked material (Ruggero et al., 2020). However, it is difficult to be sure that the recovery of artificially spiked material will reflect the true recovery of MPs naturally present in complex environmental samples as some few polymer types and standard sized particles can never fully reflect the variety of MPs present in such samples. It is on the other hand we used four different polymer types with two different shapes and rather small sized MPs which makes this recovery test fairly representative compared to other examples.

Table 1
Composite samples over the course of week, and sample amount.

Sample Matrices	Daily collected sample volume	Total volume/mass collected per sample
Influent	0.5 – 1 L	5 L (flow proportionally collected weekly sample)
Screening effluent	0.5 – 1 L	5 L (flow proportionally collected weekly sample)
Rinse water (from rinsing screen debris)	1 L	5 L (combined spot sample)
Primary effluent	0.5 – 1 L	5 L (flow proportionally collected weekly sample)
Secondary effluent	0.5 – 1 L	5 L (flow proportionally collected weekly sample)
Backwash effluent (from sand filters)	0.5 – 1 L	5 L (combined grab sample/spot sample)
Effluent	–	1042.7 L, 1269.9 L, and 1274 L (spot sample via collecting particles on filter)
Digester inlet sludge	–	1 kg (grab sample/spot sample)
Digester outlet sludge	–	1 kg (grab sample/spot sample)

Table 2
Recovery of MP particles.

Recovery sample sets	Spiked particles		Recovered particles			Overall average recovery
i) PS and PVC	PS = 1188	PVC = 1234	PS = 741 (62.37 %)	PS_{avg} = 61.45 ± 0.91 %	PVC = 922 (74.71 %)	68.16 ± 7.29 %
ii) PS and PE	PS = 450	PE = 397	PS = 271 (60.22 %)		PE = 278 (70.02 %)	
iii) PS and PP	PS = 364	PP = 213	PS = 225 (61.81 %)		PP = 170 (79.81 %)	

3.2. MP in the waters of the WWTP

A total of 3315 MPs were identified in the water samples (raw wastewater and various effluents) based on scanning on average 29 % of the 5 mL of microplastic concentrate from the sample preparation (supplementary information Table S8). Here of, 822 were in samples from the sand filter backwash, 766 in rinse water, 540 in influent, 513 in screening effluent, 375 in primary effluent, 170 in final effluent, and 129 in secondary effluent (Table S3 gives further details). The highest MP counts, and mass concentration were in the rinse water, while effluent held the lowest MP concentration (Table 3). For details see supplementary information Table S4 and Table S5.

Table 3
MP particle and mass concentration in various sample matrices. Sd stands for standard deviation.

Samples	Particle conc. [counts L^{-1} ±Sd]		Mass conc. [ng L^{-1} ±Sd]	
	MP < 500 μm	MP > 500μm	MP < 500 μm	MP > 500μm
Influent	$4.52 \times 10^2 \pm 2.24 \times 10^2$	$3.07 \times 10^{\circ} \pm 2.95 \times 10^{\circ}$	$4.00 \times 10^4 \pm 2.28 \times 10^4$	$2.37 \times 10^4 \pm 3.15 \times 10^4$
Screening effluent	$3.91 \times 10^2 \pm 1.08 \times 10^2$	$9.56 \times 10^{\circ} \pm 9.66 \times 10^{\circ}$	$4.08 \times 10^4 \pm 2.50 \times 10^3$	$2.21 \times 10^5 \pm 1.08 \times 10^5$
Rinse water (from screened debris)	$5.78 \times 10^3 \pm 2.33 \times 10^3$	$6.23 \times 10^1 \pm 7.02 \times 10^{\circ}$	$8.04 \times 10^5 \pm 4.41 \times 10^5$	$4.28 \times 10^7 \pm 3.76 \times 10^7$
Primary effluent	$1.14 \times 10^2 \pm 2.89 \times 10^1$	$8.64 \times 10^{-1} \pm 4.78 \times 10^{-1}$	$1.27 \times 10^4 \pm 7.40 \times 10^3$	$3.37 \times 10^3 \pm 1.36 \times 10^3$
Secondary effluent	$2.82 \times 10^1 \pm 5.22 \times 10^{\circ}$	$2.14 \times 10^{-1} \pm 3.70 \times 10^{-1}$	$1.39 \times 10^3 \pm 7.89 \times 10^2$	$2.86 \times 10^1 \pm 4.95 \times 10^1$
Back wash (from sand filters)	$5.84 \times 10^2 \pm 4.81 \times 10^2$	$5.05 \times 10^{\circ} \pm 7.48 \times 10^{\circ}$	$3.81 \times 10^4 \pm 3.24 \times 10^4$	$5.69 \times 10^4 \pm 4.95 \times 10^4$
Effluent	$8.64 \times 10^{-2} \pm 2.18 \times 10^{-2}$	Not detected	$3.80 \times 00 \pm 7.24 \times 10^{-1}$	Not detected

Samples	Particle conc. [counts (kg wet weight)$^{-1}$ ±Sd]		Mass conc. [ng (kg wet weight)$^{-1}$ ±Sd]	
	MP<500 μm	MP>500μm	MP<500 μm	MP>500μm
Digester inlet sludge	$1.10 \times 10^5 \pm 8.21 \times 10^3$	$5.67 \times 10^2 \pm 5.00 \times 10^2$	$7.32 \times 10^6 \pm 1.60 \times 10^6$	$3.66 \times 10^6 \pm 2.86 \times 10^6$
Digester outlet sludge	$1.16 \times 10^5 \pm 2.86 \times 10^4$	$5.11 \times 10^2 \pm 3.35 \times 10^2$	$8.61 \times 10^6 \pm 3.19 \times 10^6$	$2.28 \times 10^6 \pm 2.25 \times 10^6$

MP levels decreased continuously between influent, primary effluent, secondary effluent, and final effluent (Table 3), with the caveat that the internal stream of rinse water held the highest concentrations of the water samples. The MPs likely were trapped on the bar screen (opening: 3 mm) and its filter cake. During washing of the screenings, the MPs smaller than the bar screen opening were detached and carried on with the rinse water. Thus, the input of rinse water before the grit chamber represents an internal load which increased the MP concentration in its inflow. The rinse water MPs were larger than those of other sampled matrices (Fig. 3).

Comparing to other studies can be challenging as wastewater treatment technologies differ, as do catchment characteristics (Mason et al., 2016). Further complicating comparison is that studies have used different sampling approaches, sample preparation and MP isolation protocols, and analytical instruments (Y. Y. Liu et al., 2023). They have consequently achieved different size quantification limits (Liu et al., 2021; Simon et al., 2018; Talvitie et al., 2017a). Reported numbers on influent and effluent consequently vary substantially. For instance, Üstün et al. (2022) reported 135.3 ± 28 counts L^{-1} in influent and 8.5 ± 4.7 counts L^{-1} in effluent of a Turkish WWTP. Liu et al. (2021) reported 0.28–3.14 × 10^4 counts L^{-1} in the influent and 0.01–2.97 × 10^2 counts L^{-1} in the effluent of 38 WWTPs of 11 countries. Magni et al. (2019) reported effluent concentrations of 0.90 counts L^{-1}, Gies et al. (2018) found 0.53 counts L^{-1}, and Talvitie et al. (2017b) found 0.5–2.5 counts L^{-1}. Mason et al. (2016) reported an average of 0.05 counts L^{-1} in the effluent from WWTPs in the USA. Taking the last reference as an example, Mason et al. (2016) applied visual identification of MPs, and it is reasonable to assume that such analytical approach will yield lower concentrations than the μFTIR analysis of the present study.

Käppala WWTP achieved a high MP removal efficiency. It decreased an MP influent load of 6.42 × 10^{10} counts day^{-1} and 8.99 kg day^{-1} to an effluent load of 1.04 × 10^7 counts day^{-1} and 0.00046 kg day^{-1}, yielding an overall efficiency of 99.98 % and 99.99 % for respectively MP counts (Fig. 1A and Fig. 1B) and mass (Fig. 1C and Fig. 1D). The mechanical part of the treatment, the preliminary, and the primary stages, reduced most of MPs by approximately 71 %, which corresponds well with the 35–59 % and 57–98 % reported by respectively Michielssen et al. (2016) and Xu et al. (2021) for these stages. The efficiency of the combined biological treatment, secondary settling, and final polishing with sand filtration removed nearly all the remaining 29 % not retained by the previous stages. The individual treatment process all achieved good individual efficiencies, were especially that of the sand filtration was very high, namely 99 % (Fig. 2).

The overall removal efficiency was comparable to data reported for Ryaverket (MP count efficiency 99.25 % and mass efficiency 99.60 %), another Swedish WWTP, which also applies tertiary treatment (disc filters) (Rasmussen et al., 2021). It was also comparable to Viikinmaki WWTP in Finland, which uses biologically active filters as tertiary treatment (overall retention >99 %) (Talvitie et al., 2017b). Despite very high removal rates, a complete MP elimination cannot be achieved (Yaseen et al., 2022), and the daily discharge of MPs through WWTPs is a product of the treated water volume and the effluent concentration

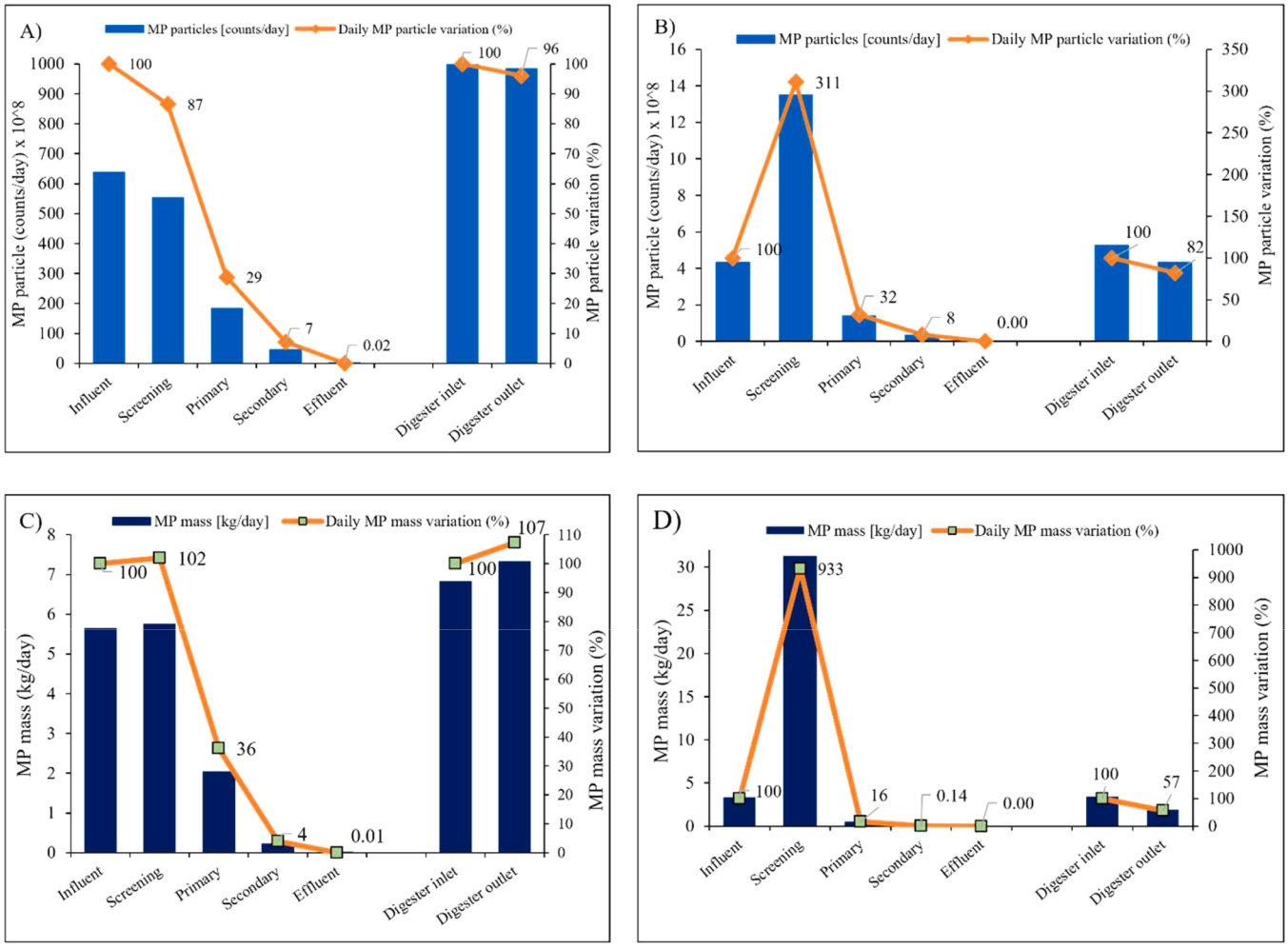
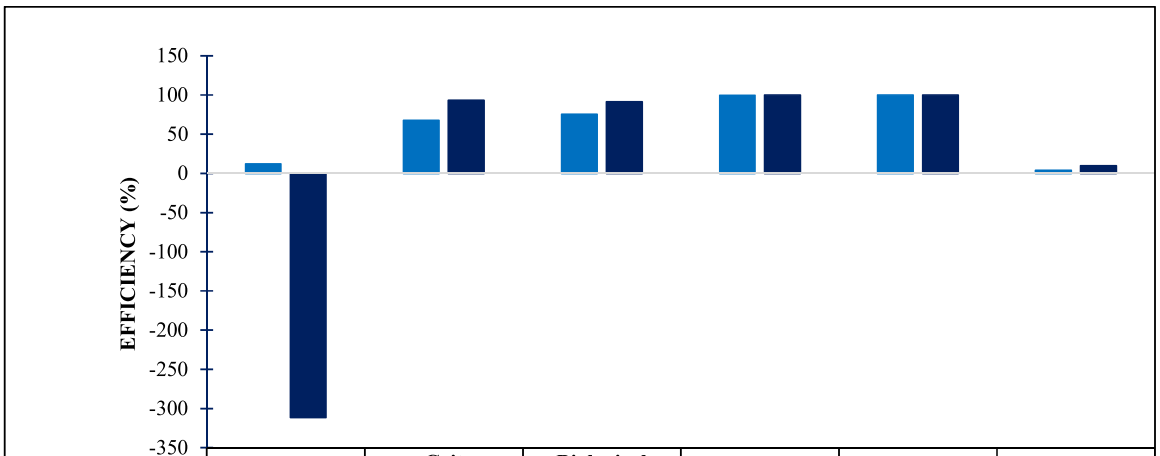


Fig. 1. Estimated daily variation of MPs in various treatment points based on flow rate of wastewater; A) particle variation for MPs < 500 μm, B) particle variation for MPs> 500 μm, C) mass variation for MPs < 500 μm, and D) mass variation for MPs > 500 μm.



	Pre-treatment	Grit chamber+Primary sedimentation	Biological treatment + Secondary sedimentation	Sand filtration	Overall	Daily variation in anaerobic digester
MP particle(%)	11.92	67.34	75.25	99.77	99.98	3.95
MP mass(%)	-311.43	93.02	91.14	99.80	99.99	9.56

Fig. 2. Overall MP removal efficiency at different treatment stages.

(Ziajahromi et al., 2017). In the present study, the effluent held very low concentrations (86.4 × 10–3 counts L^{-1}), but due to the large volume this still means that 1.04 × 107 counts day^{-1} were discharged to the nearby receiving waters. With the caveat that data are not really comparable, Magni et al. (2019) and Xu et al. (2019) found quite comparable numbers released into receiving waters, namely 1.6 × 108 and 3.63 × 108 MP counts day^{-1}, respectively.

Another way of evaluating the environmental impact of wastewater effluent is by comparing it to concentrations in the aquatic environment. Käppala effluent was on par with levels in the nearby marine waters between Sweden and Denmark where Y. Liu et al. (2023) and Gunaalan et al. (2023) found 17–28.6 × 10^{-3} counts L^{-1} and 11–87 × 10^{-3} counts L^{-1}, respectively. Both studies were done in the same laboratory as the present one and applied the same sampling tools, analytical protocols, and equipment. While the absolute numbers of an analytical pipeline always can be discussed, the fact that the same approach was used gives confidence in the comparability of concentrations between these studies.

3.3. Sludge digestion over mesophilic anaerobic digester

The MPs removed from the wastewater after the bar screens will largely be retained in the WWTP's sludge, which then undergoes anaerobic digestion. Here 3126 MPs were found of which 1007 were in digester inlet sludge and 2119 in digested sludge (supplementary information Table S3). The MP particle and mass concentrations in the inlet and outlet were rather similar (Table 3). The purpose of a digester is to convert sludge into methane and carbon dioxide gas, and a third or more of the sludge organic matter can be expected to be converted to gas (Appels et al., 2008; Zhang et al., 2018). To be able to compare inlet and outlet, the concentrations were hence stated per wet weight and not per dry weight of sludge. In comparison, Ryaverket WWTP, also in Sweden, showed a reduction of 29 % MP counts and 33.3 % mass (Chand et al., 2021).

While no statistically significant change in MP concentration over the digester was seen in the present study. A potential decrease could be caused by degradation of the polymers and by fragmentation below the size quantification limit of the analytical method, which was seen in earlier studies that have observed morphological changes on MP surfaces during anaerobic digestion (Akbay et al., 2022; Mahon et al., 2017). A study by Chand et al. (2021) reported a higher MP count concentration in digester outlet sludge, pointing in the same direction. However, detailed studies on anaerobic digesters are scarce, which makes it challenging to compare results. This is further complicated by different studies using different MP extraction protocols, analytical techniques, and data presentation. For example regarding the latter, Mahon et al. (2017) reported for seven Irish WWTPs an MP abundance in dry weight of sludge ranging from 4.2 × 10^3 to 1.5 × 10^5 counts kg^{-1} while Yuan et al. (2022) presented results from five Chinese WWTPs ranging from 0.02 × 10^3 to 5.81 × 10^3 counts kg^{-1} based on wet weight.

The amount of MP entering the treatment plant and the amount entering the digesters did not agree well, as about 38 % more MP by counts and 12 % more by mass entered the digesters than the treatment plant. Most likely this difference was caused by sampling uncertainty. Sludge samples were taken as grab samples during working hours, while wastewater was collected flow proportionally over 24 h. As MP concentrations vary over the day and between days, this means that the time window sampled was not the same. Furthermore, while the sludge retention time in the primary settlers is counted in hours, the sludge retention time in the biological processes is in the order of a month. Both these waste streams become mixed before the inlet to the digester, hence representing by themselves different time windows of influent. These different time scales of sources contributing to the digester inlet sludge and the WWTP inlet illustrates that comparison between MP load on the WWTP and the digesters must be treated carefully.

3.4. MP size and mass

Of the 6441 identified MPs from wastewater and effluents and sludge (supplementary information Table S3), 6326 had a major dimension <500 μm while 115 had a major dimension >500 μm. Of the latter, 44 were in rinse water, 18 in digester outlet, 9 in digester inlet, 17 in screening effluent, 15 in backwash, 6 in primary effluent, 5 in influent, 1 in secondary effluent and no large MPs were found in final effluent. Of the 6441 MPs, 1388 (22 %) had a length to width ratio >3 and could hence be characterized as fibers. The largest MP was 4775 μm and the smallest was 11 μm (corresponding to the lower size limit of detection). The median size of all particles was 56.9 μm with first and third quartiles of 37.6 and 95.6 μm, respectively (for detail of each sample type see supplementary information Figure S3). The corresponding MP mass of the identified particles varied by 9 orders of magnitude, between 0.14 ng and 1.11 × 10^7 ng with a median of 14 ng (first and third quartiles: 4 ng

and 49 ng, respectively. For the detail of each sample type see supplementary information Figure S4). A Shapiro-Wilk normality test showed that the MP major dimensions and estimated masses were non-normally distributed ($p < 2.2 \times 10^{-16}$).

The major dimension of the MPs from different treatment steps were compared by a pair-wise Wilcoxon rank sum test, showing a significant difference ($p < 0.05$) between the mean size of influent and screen effluent, between rinse water and primary effluent, and between influent and effluent water (Fig. 3A). The major dimensions of digester inlet and outlet sludge were also significantly different. The MP masses also showed a significant difference between influent and rinse water and between screening effluent and primary effluent. Likewise, the particle mass between digester inlet and outlet sludge was significantly different (Fig. 3B). The analysis furthermore showed that the presence of a few large MPs influenced the mass variation much, for instance, less than 2 % large MPs (>500 μm) contributed more than 99.5 % of all mass.

Overall, particle sizes decreased through the treatment plant (Fig. 3), leading to a complete retainment of the very large particles > 500 μm. Size, shape, and mass of the MPs play a significant role in their removal. Especially fibers have gained much attention in microplastics research and is also one of the shapes more readily defined (F. F. Liu et al., 2023). Fibers have by several studies been reported to dominate in wastewater and retained to a higher degree in sewage sludge, while other studies could not confirm this (Harley-Nyang et al., 2023). In the current study, there were less fibers in the effluent from the rapid sand filter for tertiary treatment compared to the influent to the treatment plant (10 % in the

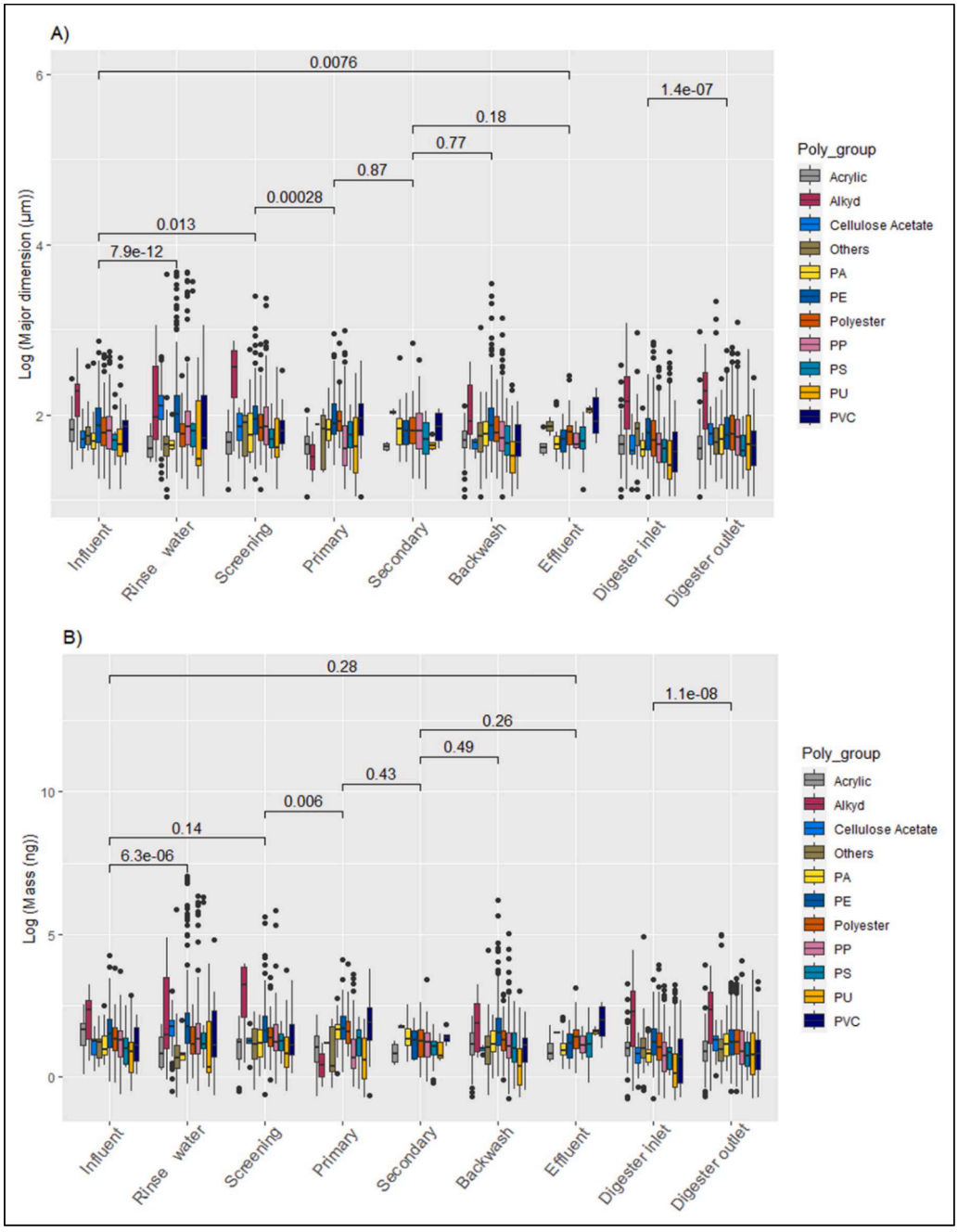


Fig. 3. Box plot for identified MP polymer types and the comparison (significance level of $P = 0.05$) between analyzed sample matrices at various treatment steps; A) major dimension of the identified particles, and B) estimated mass of the identified particles.'Others' indicates polymer types: aramid, acrylonitrile butadiene styrene (ABS), epoxy, polyvinyl acetate (PVaC), pan acrylic (PAN), poly(oxymethylene) (POM), vinyl copolymer, poly(ethyl acrylate), poly butylene terephthalate, poly (lauryl acrylate), and polyvinyl acetate (PVA).

outlet versus 20 % in the inlet) (supplementary information Table S7 and Figure S4). The inlet to the sand filters, on the other hand, held more fibers (26 %) than the inlet to the treatment plant. This implies that the tertiary treatment retained fibers better than less elongated MPs, while the other treatment step did not show such preference.

Large and buoyant particles of low density can remain at the surface and be removed by skimming in the primary treatment steps (Alavian Petroody et al., 2020; Bilgin et al., 2020). Larger and dense MPs can settle in the grit chamber and the primary sedimentation tank (Iyare et al., 2020; Ngo et al., 2019). Small particles, on the other hand, can pass the primary and secondary treatment. Talvitie et al. (2017a) found for example that approximately 80 % of the MPs in secondary effluent were 20–100 μm while 100–300 μm MPs were almost absent.

In the current study, 82 % of the MPs in the secondary effluent were smaller than 100 μm, which increased to 90.4 % in the final effluent. The tertiary treatment step hence not only reduced the MP concentration but also preferentially removed the larger particles which can also be seen in the data on the mass estimate (Table 3). Mintenig et al. (2017) similarly found few large MPs (>500 μm) in final effluent after the post-filtration, and Carr et al. (2016) showed that a tertiary treatment system with sand

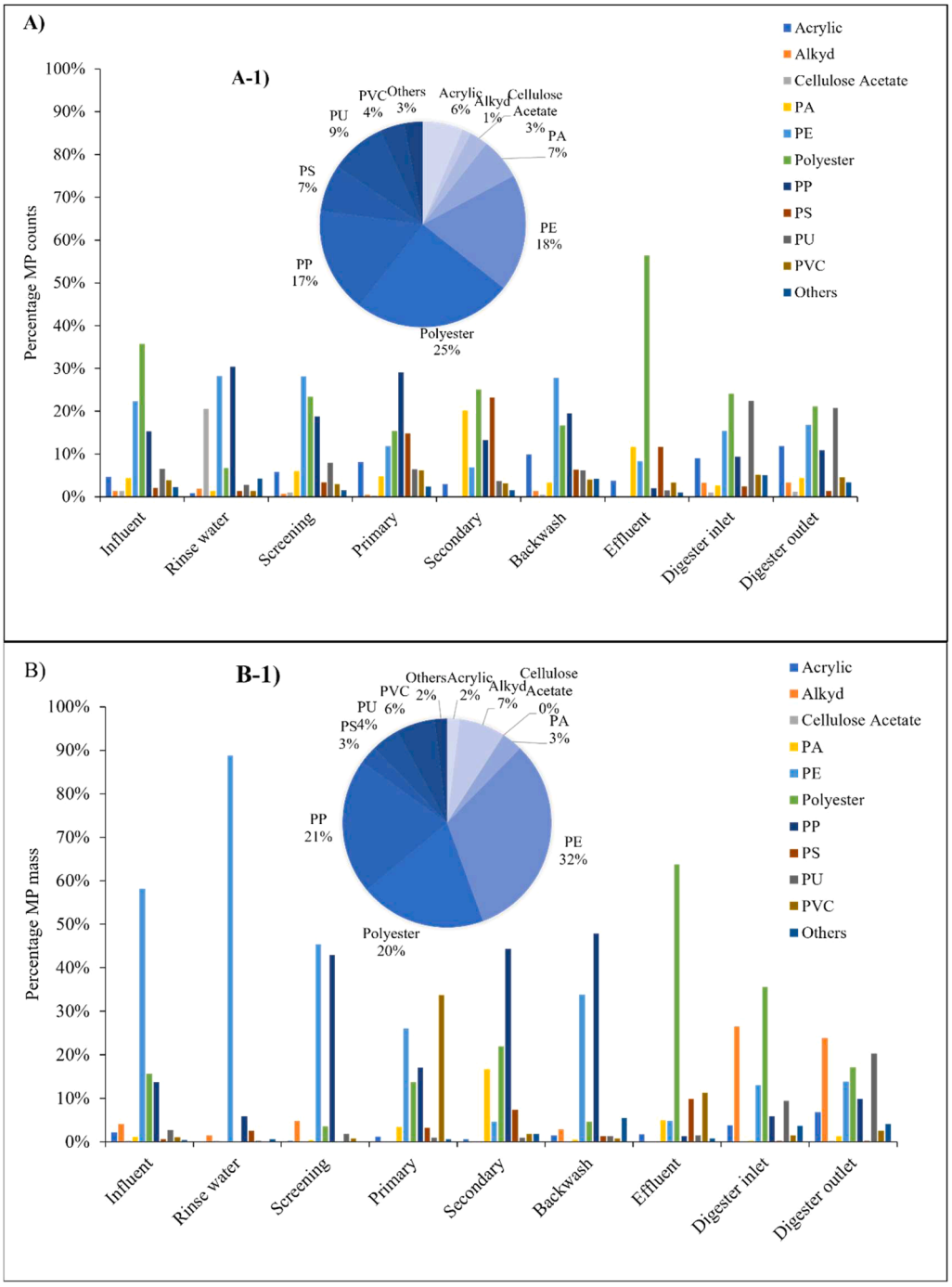


Fig. 4. Distribution of MPs among different polymer types as percentage of total; A) particle counts where A-A-1) shows the overall polymer distribution by particle counts, and B) particle mass where B-B-1) shows the overall mass distribution of the estimated mass from all sample matrices. 'Others' indicates polymer types: aramid, acrylonitrile butadiene styrene (ABS), epoxy, polyvinyl acetate (PVaC), pan acrylic (PAN), poly(oxymethylene) (POM), vinyl copolymer, poly(ethyl acrylate), poly butylene terephthalate, poly(lauryl acrylate), and polyvinyl acetate (PVA).

filtration removed most MPs >45 μm and that MPs <20 μm dominated the final effluent. Likewise, Lares et al. (2018) reported MPs between 0.5 and 1 mm as a major particle size in the final effluent. However, these studies address simpler treatment plants without tertiary polishing. This indicates that the treatment technique is important for which particle sizes are found in the effluent (Wu et al., 2021).

3.5. Polymeric composition

A total of 21 synthetic polymer types were identified (Fig. 4): acrylic, alkyd, aramid, acrylonitrile butadiene styrene (ABS), cellulose acetate (CA), epoxy, polyamide (PA), PE, PP, polyester (PEsT), PVC, polyvinyl acetate (PVaC), polyurethane (PU), PS, pan acrylic (PAN), poly(oxymethylene) (POM), vinyl copolymer, poly(ethyl acrylate), poly(butylene terephthalate), poly(lauryl acrylate), and polyvinyl acetate (PVA)). Polyester, PE, PP, PS, PU, PA, acrylic, alkyd, PVC were the major types of polymers, which have also commonly been found in wastewater treatment systems (Azizi et al., 2022; Sun et al., 2019). These polymers accounted for 80–95 % in the water samples. The rest of the polymers, grouped as "Others", contributed less than 1–5 %. The polymer composition was not systematically affected by the individual treatment steps, and neither was the size or mass of MP. Like for the water samples, there was little variation between digester inlet and outlet sludge. No systematic removal of specific polymer types could hence be identified, i.e., none of the treatment steps preferentially removed specific polymer types.

4. Conclusion

Käppala WWTP with its advanced treatment processes and tertiary polishing step by sand filtration technique was highly efficient in retaining MPs. It achieved an overall reduction of 99.98 % measured by MP counts and 99.99 % by MP mass, bringing the discharged water to a level on par with which was reported for marine environments in its vicinity. All steps contributed to the removal, with the final tertiary step, the sand filter, achieving percentually the highest efficiency. The MPs became continuously smaller as the water passed the mechanical treatment, the biological processes, and finally the sand filtration. The polymer composition, on the other hand, was not affected, indicating that none of the steps had a preference towards specific polymer types. Käppala WWTPs digesters similarly did not affect the polymer composition, that is, no polymer types were preferentially removed. Neither did it significantly affect the MP concentration measured as counts or mass. Overall, the study led to a novel realization, namely that a properly designed, advanced and modern WWTP can reduce MP down to background levels in the receiving aquatic environment. Such modern, advanced, and well-designed WWTP is hence not a significant point source for MP.

CRediT authorship contribution statement

Rupa Chand: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Lucian Iordachescu:** Writing – review & editing. **Frida Bäckbom:** Writing – review & editing. **Angelica Andreasson:** Writing – review & editing. **Cecilia Bertholds:** Writing – review & editing. **Emelie Pollack:** Writing – review & editing. **Marziye Molazadeh:** Writing – review & editing. **Claudia Lorenz:** Methodology, Writing – review & editing. **Asbjørn Haaning Nielsen:** Writing – review & editing. **Jes Vollertsen:** Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interest.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.121647.

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Paper – IV

Snow dumping station–A considerable source of tyre wear, microplastics, and heavy metal pollution

Environment International 188 (2024) 108782

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Full length article

Snow dumping station – A considerable source of tyre wear, microplastics, and heavy metal pollution

Rupa Chand [a], Ieva Putna-Nīmane [b], Elina Vecmane [b], Jeanette Lykkemark [a], Jytte Dencker [a], Asbjørn Haaning Nielsen [a], Jes Vollertsen [a], Fan Liu [a,*]

[a] Department of the Built Environment, Aalborg University, Thomas Manns Vej 23, 9200 Aalborg, Denmark
[b] Latvian Institute of Aquatic Ecology, Voleru str. 4, LV-1007 Riga, Latvia

ARTICLE INFO

Handling Editor: Marti Nadal

Keywords:
Microplastic
Tyre wear particles
Heavy metals
Snow dumping station
Atmospheric pollutants

ABSTRACT

Snow dumping stations can be a hotspots for pollutants to water resources. However, little is known about the amount of microplastics including tyre wear particles transported this way. This study investigated microplastics and metals in snow from four snow dumping stations in Riga, Latvia, a remote site (Gauja National Park), and a roof top in Riga. Microplastics other than tyre wear particles were identified with Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) (>500 μm) and focal plane array based micro-Fourier Transform Infrared (FPA-μFTIR) imaging (10–500 μm), tyre wear particles by Pyrolysis Gas Chromatography-Mass Spectroscopy (Py-GC–MS), and total metals by Inductively Coupled Plasma with Optical Emission Spectroscopy (ICP-OES). Microplastics detected by FTIR were quantified by particle counts and their mass estimated, while tyre wear particles were quantified by mass. The concentrations varied substantially, with the highest levels in the urban areas. Microplastic concentrations measured by FTIR ranged between 26 and 2549 counts L^{-1} of melted snow with a corresponding estimated mass of 19–573 μg/L. Tyre wear particles were not detected at the two reference sites, while other sites held 44–3026 μg/L. Metal concentrations varied several orders of magnitude with for example sodium in the range 0.45–819.54 mg/L and cadmium in the range 0.05–0.94 μg/L. Correlating microplastic measured by FTIR to metal content showed a weak to moderate correlation. Tyre wear particles, however, correlated strongly to many of the metals. The study showed that snow can hold considerable amounts of these pollutants, which upon melting and release of the meltwater to the aquatic environment could impact receiving waters.

1. Introduction

Plastic use has increased substantially (Li and Zhang, 2021) due to its distinctive benefits over other materials, and the production has exceeded most other man-made materials, for instance, concrete, cement, and steel (Geyer et al., 2017; Ivleva et al., 2017; Li et al., 2018). The applications range from packaging materials (Chen et al., 2021) to highly engineered plastic components, for example, in aircrafts (Fette et al., 2015). However, inappropriate waste management has caused plastic to be a persistent pollutant, threating ecosystems, organisms, and even human beings (Galloway, 2015; Li and Zhang, 2021). Once plastic litter enters the natural environment, it starts to degrade and fragment into smaller pieces (Browne et al., 2011; Ragusa et al., 2021). The degradation of plastic can facilitate the release of additives, which then impact the nearby surroundings and organisms (Teuten et al., 2009). The additives associated to plastics (Chen et al., 2022) can interact chemically and may disrupt the endocrine system of living organisms (Ivleva et al., 2017; Teuten et al., 2009). In the case of small plastic fragments, they can easily disperse and be transported to remote locations far away from the source (K. Liu et al., 2019; Üstün et al., 2022).

The general consensus is that synthetic polymers between 1 and 5000 μm in their longest dimension are called microplastics (Hartmann et al., 2019). Some microplastics, commonly called primary microplastics, are produced to this size for various commercial purposes such as cosmetics. Other microplastics, commonly called secondary microplastics, are formed by fragmentation of large plastic debris due to physical and chemical processes. Microplastics have been investigated in all environmental compartments (Dalu et al., 2021; Ivleva et al., 2017; Lusher et al., 2017; Zhang et al., 2020) to understand the environmental and ecological risk associated to them. Examples are marine

* Corresponding author.
E-mail address: fl@build.aau.dk (F. Liu).

https://doi.org/10.1016/j.envint.2024.108782
Received 4 March 2024; Received in revised form 18 May 2024; Accepted 27 May 2024
Available online 28 May 2024

environments (Alimba and Faggio, 2019; Alimi et al., 2018; Liu et al., 2022; M. (Shabnam) Molazadeh et al., 2023), freshwater environments (Alimi et al., 2018; Eerkes-Medrano et al., 2015; Koelmans et al., 2019; Rasmussen et al., 2024; Molazadeh et al., 2024), and technical systems such as wastewater treatment plants (Browne et al., 2011; Cheung and Fok, 2017; Kazour et al., 2019). Other compartments have received less focus, here among the cryosphere including freshly fallen snow (Bergmann et al., 2019; Zhang et al., 2020; 2022). One microplastic type has been studied significantly less than others, namely particles of tyre wear (TWP). It has, though, received increasing attention over the later years. Most studies report that it tends to end up in soil (Giechaskiel et al., 2024), even though some is also found in remote areas (Goßmann et al., 2023). The reason for the comparatively few studies is that the most common analytical techniques, FTIR and Raman spectroscopy, struggle to identify TWP due to the content of carbon black (Järlskog et al., 2022; 2020). Here thermal decomposition, mainly pyrolysis GC–MS, is used instead. TWP contains many toxic additives and has been reported to cause detrimental effects in the environment (Wik and Dave, 2009), for example on aquatic organisms, such as, algae, crustaceans, and fish (Marwood et al., 2011).

Microplastics can be transported in the atmosphere over long distances and have been found in pristine terrestrial and oceanic environments, as well as high altitude locations (Wagner et al., 2018; Zhang et al., 2021). According to X. Wang et al. (2020), microplastics can be conveyed over distances as far as 1000 km. They can also be transferred from the ocean surface to the atmosphere (Allen et al., 2020; Goßmann et al., 2023). Atmospheric conditions, such as wind direction, wind speed, and precipitation could have a significant impact on the transport of these micropollutants (Allen et al., 2019; Roblin et al., 2020). The inherent properties of microplastics such as small mass, small size, and irregular shape could enhance the transport and distribution pattern (Li et al., 2018), and atmospheric transport and deposition hereof has gained increasing interest (Cai et al., 2017; Ding et al., 2021; Dris et al., 2015). Different levels of microplastic contamination have been reported at rural versus urban sites when analysing wet deposition (rainfall and snowfall) versus dry deposition (Klein and Fischer, 2019; Wright et al., 2020). Precipitation is important for conveying airborne particles to terrestrial and aquatic surfaces, and are likely also able to convey microplastics, and microplastic concentrations have been found to show moderate to strong correlation with both rainfall and snowfall events and intensities (Allen et al., 2019). Furthermore, significant amounts of microplastic fibres and particles have been identified in rainwater and snow (Abbasi et al., 2022; Bergmann et al., 2019; Dris et al., 2016; Roblin et al., 2020).

Microplastics interact with other pollutants such as metals and organic micropollutants (Sun et al., 2019; Tamis et al., 2021). Many of these are persistent (Li et al., 2022; Q. Wang et al., 2020) and can be detrimental to organisms and humans (Jaishankar et al., 2014). During aging in the environment, the adsorption capacity of microplastics can increase due to an increase in specific surface area and oxygen-containing functional groups (Liu et al., 2021; Wang et al., 2019). The oxygen-containing functional groups of microplastics are more prone to react actively with charged heavy metals and other pollutants. Likewise, other chemical and physical factors such as pH, temperature, dissolved organic matter and electrostatic interaction also have significant impact on the adsorption capacity of microplastics (Guo et al., 2020; Li et al., 2022).

In regions with prolonged cold temperature, snow can be subjected to atmospherically transported microplastics. Such snow is accumulated and afterwards moved to snow dumps till it melts when the weather gets warmer. We hypothesize that such dumps could be hotspots for microplastics and other pollutants, affecting the waters to which the snowmelt discharges. The pollutant level could depend on the land use, i.e. commercial areas, residential areas, urban centres, and rural areas. The objective of the present work is to evaluate this hypothesis by analysing microplastics in snow from snow dumps in the urban centre and residential areas of Riga, Latvia, and compare it to snow from sites less affected by human activity, in this case, a National Park. This study aims to understand the contamination level based on land use practices and human activities. It applied μFTIR imaging to investigate microplastics in terms of counts, size, and material types, and estimated their mass from the hyperspectral image. As TWPs cannot be identified this way, it used pyrolysis GC–MS for this microplastic fraction and used ICP-OES to quantify metals.

2. Materials and method

2.1. Study sites and snow sampling

Riga, the capital of Latvia, is covered by snow a quarter of the year. To support the city traffic, the urban snow is collected in the city and dumped at specific dump sites. Snow was collected from five sites in the city in December 2021, four of which were in the inner city: A central market (C1); a square in the old town with controlled car access (C2); a 65 m high rooftop of an academic science building (C3); and a parking lot of a shopping mall (C4). One residential site was chosen in a yard with limited car access (R1); and one site was chosen outside of the city national park, the Gauja National Park (P1) 50 km from Riga (Fig. 1, Table 1). Snow was taken to approximately the same thickness from all sites, which was 15–20 cm from the surface. Triplicates were taken from each site in 20 L metal buckets with a metal shovel, both pre-cleaned with distilled ultrapure water. In addition, a separate sample was taken in a plastic canister for metal analysis.

2.2. Sample preparation

2.2.1. Microplastic extraction

Upon arrival at the lab, the snow samples were kept at room temperature (22–23 °C) until melted, measured for volume, and filtered through a 10 μm stainless-steel filter (Ø167 mm) (Table 1). The cut-off size (10 μm) was restricted by the limit of detection (LOD) of the analytical method for the common polymers (section 2.3.1.2). This cutoff was also applied to TWP, even though the analytical method used for its detection (Pyrolysis Gas Chromatography-Mass Spectrometry, Py-GC–MS) in principle has no lower particle size detection limit, with the attempt to avoid sample splitting during extraction. Triplicate samples were collected and treated separately for each sampling site. Microplastics were isolated via a multi-step treatment (Rasmussen L.A. et al., 2023; Chand et al., 2021), in short: Density separation ($ZnCl_2$, 1.78 g cm^{-3}), enzymatic digestion (protease and cellulase, Sigma-Aldrich), catalysed oxidation (Fenton reaction), and a final density separation. The details of the procedure are in the supplementary information (section S 1.1). The extracts were then fractionated into two size classes: 10–500 μm, and ≥ 500 μm. The small size fraction was concentrated in a 10 mL glass vial with a known amount of 50 % ethanol (HPLC grade), whilst the large fraction was dried at 50 °C and stored in an aluminium tray until analysis. Field blanks (section 2.4) were collected in 5 mL 50 % ethanol.

2.3. Analysis of pollutants

2.3.1. Microplastics

The microplastic mass estimated by Focal Plane Array based Micro-Fourier Transform Infrared imaging (FPA-μFTIR) is not necessarily comparable to the mass determined by Py-GC–MS (Primpke et al., 2020b; Kirstein et al., 2020).. Furthermore, particle counts could only be obtained by FPA-μFTIR. To distinguish the two groups of microplastics, the abbreviation MP is used for microplastics determined by ATR-FTIR and FPA-μFTIR, while TWP is used for tyre wear microplastics.

2.3.1.1. Large fraction (≥500 μm). For the large size fraction, particles

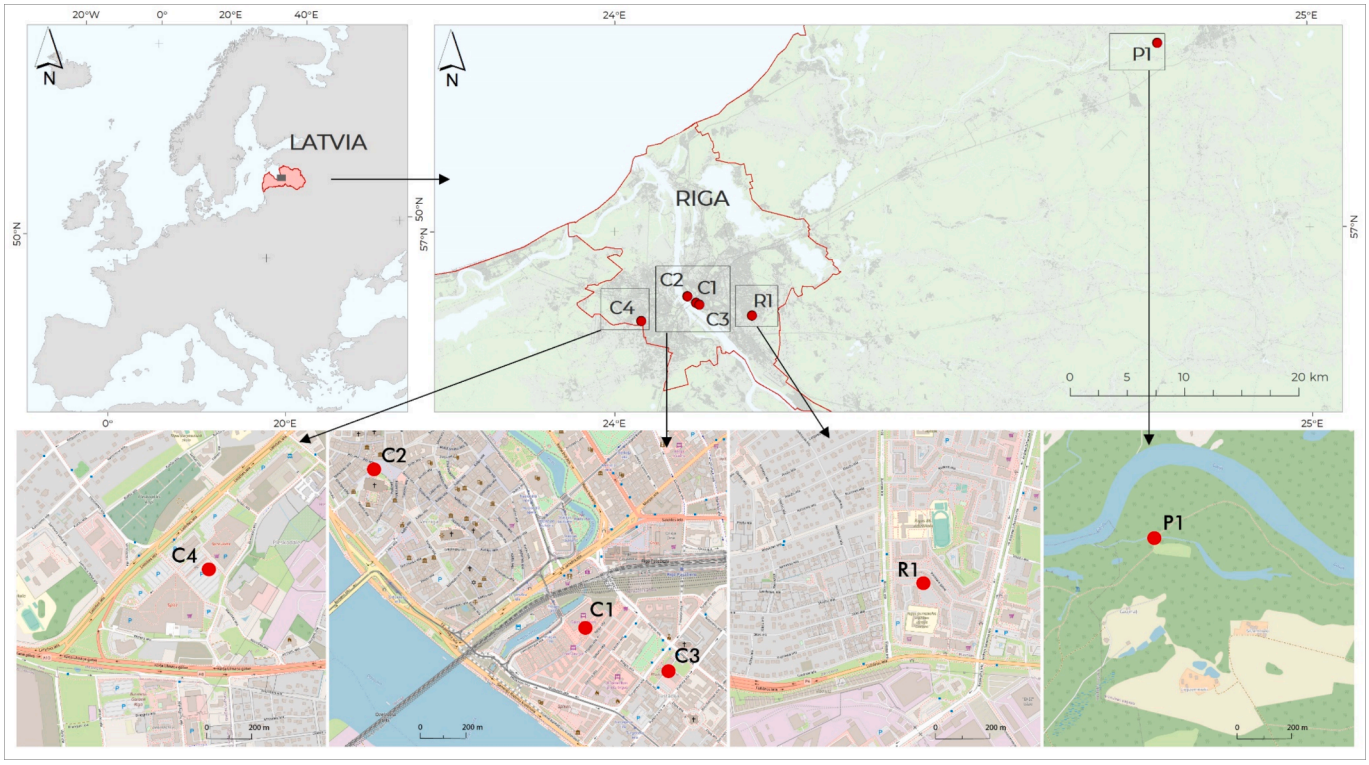

Fig. 1. Sampling locations. C1: Riga central market, C2: Old town centre (Doma Church square), C3: Latvian academy of science rooftop (65 m high), C4: Shopping mall "Spice home" parking lot, R1: Residential area (Plavnieki), and P1: Gauja National Park (appr. 50 km away from Riga city centre).

Table 1
Detail about the sampling sites, longitudinal and latitudinal position, and weather parameters and total volume of sampled snow.

Sampling sites	Land use	Longitude (E)	Latitude (N)	Humidity (%)	Wind speed (m/s) and direction	Temperature during sampling (°C)	Volume (L) of the melted snow of triplicate samples
C1	Central market and core business centre of Riga	24.11713°	56.94447°	55	1.3 Southeast	−7.1	i) 8.7 ii) 9.3 iii) 8 average: 8.7 total: 26
C2	Square in the old town with visitors throughout the year and controlled car access	24.10480°	56.94954°	32.9	2.3 South	−6.5	i) 9.1 ii)7.6 iii) 8.4 average: 8.4 total:25.1
C3	65 m high rooftop of the Latvian Academy of Science building	24.12207°	56.94298°	58.4	4.8 Southeast	−10.1	i) 6 ii) 6.1 iii) 5.8 average: 6.0 total:17.9
C4	Parking lot of a mall (Spice home) with 2000 parking spaces	24.03856°	56.93029°	51.6	1.0 South	−7.0	i) 11.5 ii) 10.9 iii) 11.7 average:11.4 total: 34.1
R1	Yard in a residential area with limited car access	24.19748°	56.93437°	52.8	1.5 South	−8.5	i) 7.7 ii) 7.9 iii) 7.9 average: 7.8 total:
P1	Gauja National Park, appr. 50 km from the city centre of Riga	24.78398°	57.14593°	57.7	0.0 South	−2.2	i) 7.1 ii) 7.3 iii) 7.1 average: 7.2 total:

were firstly characterised under a stereomicroscope (ZEISS, SteREO Discovery.V8) by measuring the surface area, circularity, major and minor dimension (software Zen2Core SP1), then analysed by Attenuated Total Reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy for chemical composition (Agilent Cary 630 FTIR with a diamond ATR) Chand et al. (2022).

2.3.1.2. Small fraction (10–500 μm). For the small size fraction (10–500 μm), an aliquot was deposited from the concentrates onto a zinc selenide window after thoroughly vertexing the concentrates as described in (Chand et al., (2022,2021) and Simon-Sánchez et al. (2022). The window had an effective area of Ø10 mm and a thickness of 2 mm. For each sample, three windows were prepared by sequentially depositing aliquots to a minimum of 100 μl of 10 mL to 1 mL of 5 mL of concentrate, thus, to ensure a sufficiently populated window for analysis. Windows were dried at 50 °C between aliquoting. The deposited amount depended upon the particle content of the samples. The identification of MPs was performed by FPA-μFTIR (Agilent Cary 620 FTIR microscope equipped with a 128-pixel FPA and coupled with a Cary 670 IR spectroscope), resulting in a pixel resolution of 5.5 μm. Details of the instrumental settings are found in Simon et al. (2018), Vianello et al. (2019) and Chand et al. (2022).

2.3.1.3. TWP. The content of TWPs was measured by Py-GC–MS by subsampling 25–1000 μL from the concentrates using a glass capillary, then depositing it to a sample cup and drying it on a heating plate at 50 °C. In this way, TWP shared the same cutoff size with MPs, which was 10 μm. The Py-GC–MS system comprised a micro furnace pyrolyzer EGA Py-3030D (FrontierLab, Japan), an autoshot sampler AS-1020E (FrontierLab, Japan), a Thermo Scientific TRACE 1310 GC, and an ISQ™ single quadrupole GC–MS with helium as the carrier gas. The analysis employed a solution of 0.2 g/L deuterated anthracene (A-d10) in n-hexane as an internal standard. The Py-GC–MS method involved pyrolysis at 600 °C with an interface temperature of 280 °C. The GC was operated with helium gas as the carrier (1 mL min^{-1}) in split mode 30:1. The temperature program ranged from 40 °C (2 min) to 300 °C (5 min), with a temperature gradient of 10.5 °C min^{-1}. The MS was operated in EI positive mode (70 eV, *m/z* range 35–500), while the transfer line and ion source were maintained at 250 °C and 200 °C, respectively (Molazadeh et al., 2023; Rasmussen et al., 2024). The TWP concentration was assessed by examining extracted ion 54 chromatograms for 4-vinylcyclohexene, a well-known indicator of styrene butadiene rubber and butadiene rubber (Goßmann et al., 2021). The same marker was also used by More et al., 2023 and Mun et al., 2023. The quantification process involved utilizing an external calibration curve composed of a standard tire mixture obtained from Genan, Denmark. This company specializes in recycling Danish and Swedish tires. Genan acquires approximately 85 % of all used tires in Denmark, ensuring that their product for calibration is representative of the types of tires commonly in use in the region where the samples were from. Moreover, their tire mixture is homogenized during the manufacturing, contributing to the consistency of the calibration process. This choice of calibration standard not only reflects the diversity of tire compositions but also accounts for variations in tire makeup.

2.3.2. Metals

For each sampling site, 50 mL melted snow was mixed with 1 mL nitric acid (SupraPur quality, SCP Science, Quebec, Canada) and analysed for aluminium (Al), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb), and zinc (Zn) by Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP-AES). The choice of these metals was based on their wide occurrence in various environmental matrixes, including fresh snows (Veysseyre et al., 2001). The spectrometer was a Thermo iCap 6300 Duo (Thermo Fischer Scientific, Waltham, MA, USA) operated in axial view mode. Concentrations of each element were calculated as total metal, i.e., the sum of their various forms, for instance, ions, organic bound, and precipitated metals. The sample was introduced by a concentric glass nebulizer attached to a glass cyclonic spray chamber with a rate of 2 mL min^{-1}. The RF source was set at 1150 W and the plasma and auxiliary gas flows were 2 L min^{-1} and 12 L min^{-1}, respectively. The nebulizer gas flow was pressure controlled at 0.2 MPa. A series of matrix matched multi-element standards, ranging from 10–1000 mg/L were used for calibration. Each element was quantified by selecting one to five wavelengths. 1000 mg/L yttrium solution was added to each sample (0.2 mL min^{-1}) as the internal standards (reference), which was reflected on the wavelength of 360.073 nm. Over-range samples were diluted from 10 to 1000 times using a matrix matched solution (type I ultra-pure water (ELGA LabWater, High Wycombe, UK) and nitric acid) until the signal was within the range of the calibration standards.

2.4. Quality control

Measures were taken during sampling and in the lab to minimize contamination. Only non-plastic tools were used during sampling, and only cotton lab coats were allowed in the lab. All sample processing was done in a clean fume hood or a laminar flow bench (TelstarAV-100). The air in the lab was continuously filtered with a Dustbox® (Hochleistungsluftreininger, Germany) holding a HEPA filter (H14, 7.5 m^2). All reagents were filtered through 0.7 μm GF filters (Whatman). All glass wares and filters were muffled at 500 °C, or thoroughly cleaned with filtered demineralized water.

One field blank was collected for each sampling site by opening a clean petri dish (Ø167 mm) when the sampling started and closing it when it ended. The particles collected in the petri dish were concentrated into 5 mL of 50 % ethanol (HPLC grade), upon which approx. 20 % of the concentrate was analysed by FPA-μFTIR.

2.5. Data analysis

The IR spectra from ATR-FTIR were analysed by OMNIC (Thermo Fisher Scientific Inc., 8.2.0.387 version 1) for chemical identification. IR images from FPA-μFTIR were analysed by siMPle (previously called MPhunter, F. Liu et al., 2019). It compares the IR-spectrum of each pixel with a custom-made library, containing more than 450 spectra of natural and synthetic materials. siMPle provides particle dimensions, 2D projected areas, estimated volume, and estimated mass (Primpke et al., 2020; F. Liu et al., 2019). The major dimension of the particle was reported as the particle size and only particles consisting of at least two pixels were considered as MPs. Data analysis and visualization was performed using R (4.2.2) with package ggplot2. To assess whether there were significant differences in the size and mass of MPs among the sites, Kruskal-Wallis test was applied. One-way ANOVA test was performed to assess whether polymer composition varied significantly between sites. The significant level was in all cases set to 0.05. The location map was produced using ArcMap 10.3.

3. Results and discussion

3.1. Contaminations

For the analysed aliquots, 12 MPs belonging to seven polymer types were detected in five field blanks: five MP particles in C3, three in R1, two in C2, and one in each C4 and C1. No MPs were found in P1. The corresponding total mass of MPs in the blanks was 2692 ng, 1527 ng, 409 ng, 253 ng and 3 ng, respectively. Polyester (PES) and polyvinylchloride (PVC) were the major polymers, each contributing 25 % to the particle counts, followed by PAN acrylic with 17 % contribution. The remaining four types were polyamide (PA), polyacrylamide (PAA), polyurethane (PU), and epoxy-phenoxy resin, each contributing 8 %. Particles found in the blanks could originate from activities during the sampling, where MPs were released to the air then deposited onto the petri dish. The contamination level is site-specific; thus, it is reasonable to interpret the results as counts $site^{-1}$, or mass $site^{-1}$. Taking particle counts as an example, this corresponds to the highest pollution level as 25 counts $site^{-1}$ (C3), followed by 15 (R1), 10 (C2), 5 counts $site^{-1}$ (C1 and C4), and not detectable at P1.

3.2. MP and TWP abundance

3.2.1. MPs

MPs were found in all snow samples. The highest concentration was found in the central market (C1), both in terms of particle counts 2549 (±836) counts L^{-1} and estimated mass 573 (±318) μg L^{-1}, while the lowest was in the national park (P1) with 26 (±23) counts L^{-1} and 19 (±13) μg L^{-1}, closely followed by the rooftop (C3, 88 (±30) counts L^{-1}, 43 (±11) μg L^{-1}) (Fig. 2, Table S 1 and Table S2). Compared with the MP content in field blanks for each specific site, MP concentrations in snow samples were much higher, hence the results were not corrected for contamination. Within the urban environment, Rasmus et al. (2023) found that the stormwater runoff contained 0.3–1200 counts L^{-1} MPs using the same analytical method and cutoff size. This is slightly lower than the MP level found in the snow at the dumping sites, which makes the snow dumping sites a more important hotspot for MP pollution than stormwater runoff.

The high abundance of MPs in snow in populated areas (C1, C2, and C4, Fig. 2A and B) seems reasonable as these sites are close to microplastic sources. Some of the microplastics in the snow dumps may have been caught while the snow was falling, while others might have been caught after it was deposited (Zhao et al., 2015). The snow from the residential area (R1) held lower concentrations than the sites in the dense city (C1, C2, and C4), which may be due to a lower intensity of anthropogenic activity in the residential area. All in all, these results support previous observations that human activities and population density may influence the occurrence of microplastics (Bergmann et al., 2019; Chernykh et al., 2022; Dris et al., 2016).

MPs have also been reported in urban air by analysing of wet and dry deposition (Abbasi et al., 2017; Qiu et al., 2020), but only few studies have reported them in snow. Differences in sampling and analytical methods do however make comparation across studies problematic.

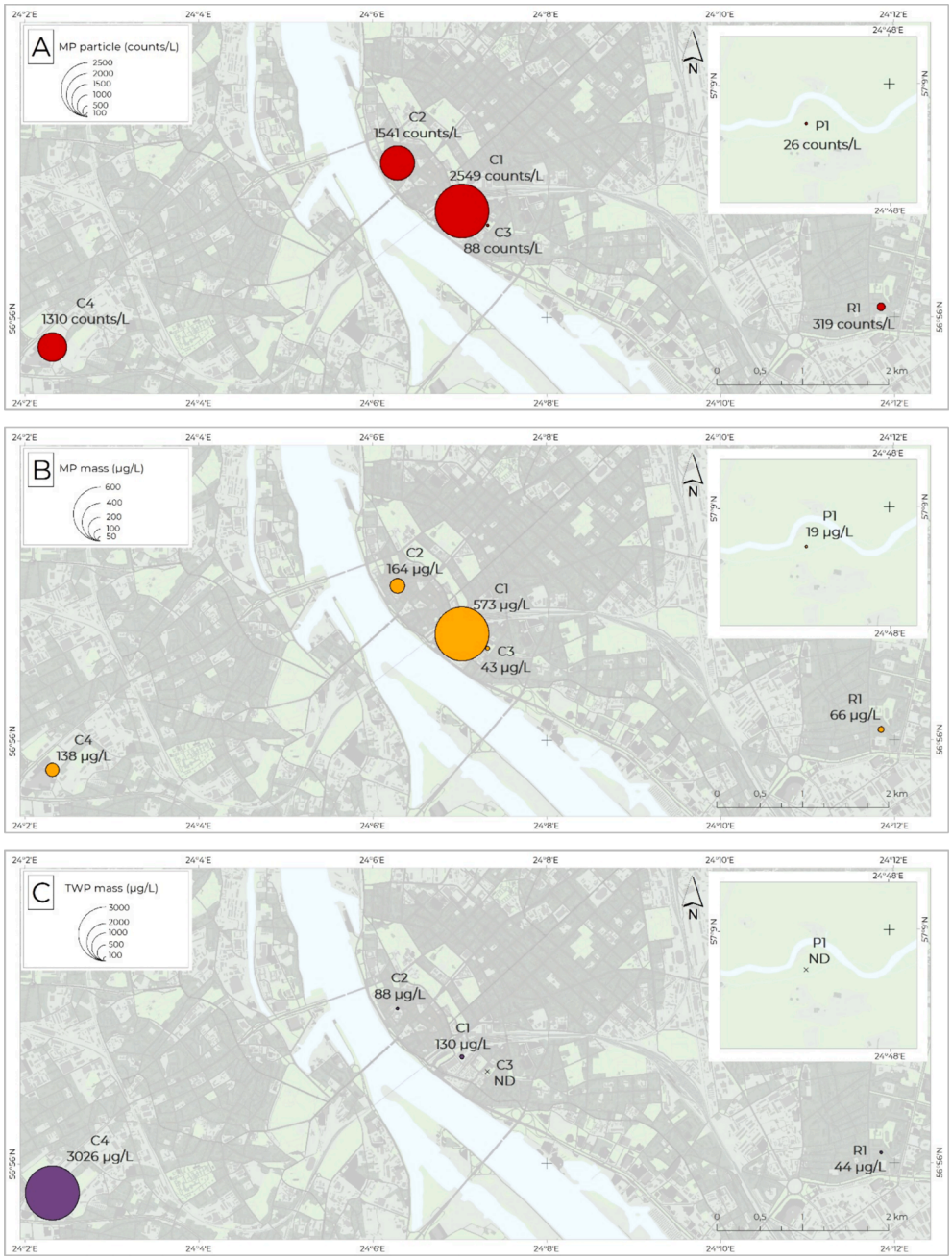

Fig. 2. Concentration: A) MP particle counts, B) MP mass, and C) TWP mass at the sampling locations. ND*: Not detected.

Malygina et al. (2020), for instance, investigated snow from Western Siberia employing visual identification coupled with fluorescent staining for MP identification, restricting the low cut-off size to 100 μm. They did not report concentrations, only that they found both particles and fibres. However, even if they had reported concentrations, such values cannot readily be compared to the current study, which applied a quite different instrumental approach and a lower size cut-off of 11 μm. Applying a similar hyperspectral imaging approach as the current study, and a similar size detection limit, Bergmann et al. (2019) found that snow from the Swiss Alps held significantly lower counts (0 to 14.4 × 10^3 counts L^{-1}) than urban sites from northern Europe (0.19 × 10^3 to 154 × 10^3 counts L^{-1}). The concentration difference between remote and urbanized regions was also notable in the current study, as MPs concentrations in snow from populated areas (C1, C2, C4, and R1) on average was 53 times higher than that of untouched snow in the Gaujas National Park (P1), which evidenced that MPs were less subjected to atmospheric transport.

MPs were also detected in snow from the 65 m high rooftop (C3). It held more than the snow in the national park (P1), but less than the snow on the city ground surfaces. As this site is publicly accessible for tourists, it is not possible to differentiate whether the identified MPs came from the visitors, for instance from synthetic clothing, or were transported to the snow via the atmosphere. The snow at P1 was, however, untouched by direct human activity, indicating that MPs were transported via the atmosphere to this remote area (Baztan et al., 2014; Kelly et al., 2020; Napper et al., 2020).

3.2.2. TWPs

TWPs were detected at four sampling sites (C1, C2, C4, and R1) but not in the snow from the national park (P1) and the rooftop (C3). In the four sites, the concentration ranged from 44 to 3026 μgL^{-1} (Fig. 2, Table S4). The highest abundance was found in snow from the parking lot (C4), while the lowest was in the residential area (R1). A study by Rødland et al. (2022) investigated TWP in snow along the roads in Norway, and reported concentrations 2–4 times higher than the most polluted site of the current study. TWP may be subject to atmospheric transport, as the deposited particles can be resuspended and brought up by airflow (Kole et al., 2017; Sun et al., 2022). However, in this study, TWP was absent in the snows from the rooftop and the national park. The reason could be that wind-driven transport of TWP was less pronounced than wind-driven transport of other microplastics. Another explanation could be that the concentrations were below the detection limit of the Py-GC–MS. All other sites than C4, the parking lot, held lower TWP mass than MP mass. This statement must be interpreted with caution, as the mass by FTIR is an estimation only, made by assuming that the imaged particle can be described by its equivalent ellipsoid, where the third dimension (thickness) equals 0.6 times its second dimension (width) (Simon et al., 2018), whilst the Py-GC–MS yields a direct and quantitative measurement. Hence, the two values are not necessarily comparable.

3.3. Polymer composition, size, and mass

3.3.1. Polymer composition

Twenty polymer types were identified by FTIR in the snows from the six sites. Polyester (PES), polypropylene (PP), and polyethylene (PE) were the most prevalent polymers in the samples. Some polymers were detected at a low frequency (1 % in terms of particle counts), and were lumped into a group termed 'Others' (Fig. 3A). Polymer composition though varied between sites, but with no statistical significance. Nevertheless, PES dominated at all sites except C4, both in terms of particle counts and estimated mass, and at R1 in terms of particle mass (Fig. 3B). Instead, most of MPs identified at C4 were PP, which dominated both as particle counts and mass. At R1, PP was the most abundant polymer in terms of particle mass, probably caused by the PP particles being comparably large.

PES was also a dominant polymer (56 %) in snow from Mount Everest (Napper et al., 2020). Likewise, Aves et al. (2022) found that 41 % of polymers identified in their 19 snow samples were polyethylene terephthalate (PET), which is a common thermoplastic resin of PES. As such PES is one of the polymers in high demand, and has been used extensively for textile filaments (Carr, 2017). This polymer can be shredded from the synthetic clothes and other textiles and end up in the snow. Other common polymers like PA, PE, PS, PU, and PVC were also observed in the snow but at lower frequency. With respect to mass composition, PES constituted over 90 % in both C3 and P1 (Fig. 3B), while PP dominated in R1 and C4 with 70 % and 54 %, respectively. There is little data on MPs in the Riga region, but MPs in adjacent marine areas, for instance the Baltic Sea, North Sea, Kattegat, and southern North Sea, showed a more or less similar trend with a high proportion of PE, PP, PES, and PS (Kirstein et al., 2016; Liu et al., 2022; Lorenz et al., 2019).

3.3.2. Size and mass and characteristics of identified polymers

A total 18,289 MP particles were identified in the size range 11–4427 μm when scanning all the snow samples by μFTIR and the larger particles by ATR-FTIR. The vast majority (17977 counts, 98.3 %) were small MPs (<500 μm) and only 312 particles (1.7 %) were above 500 μm. Considering the major dimensions of the MPs, the snow samples from different sites were statistically significant regarding particle size

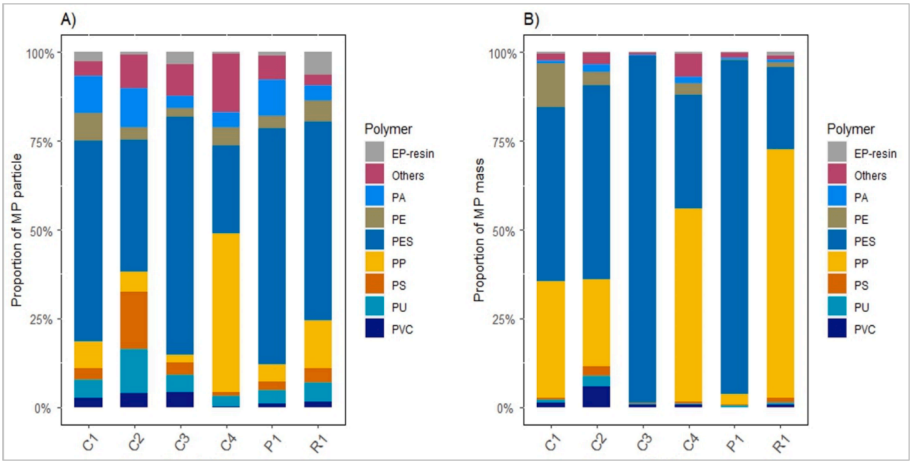

Fig. 3. Distribution of MPs based on: A) particle count and B) estimated mass. Others: ABS, AF Paint, Acrylic Paint, Acrylic, Alkyd, CE, EVA, PAM, PAN, PHB, VCC, and PC.

and mass (Wilcoxon rank sum test P-value < 0.05, Figure S 1A). Likewise, the estimated masses among the sampled snows were also significantly different (Wilcoxon rank sum test P-value < 0.05, Figure S 1B). The mass of individual MPs varied by 8 orders of magnitude, with the smallest particle having an estimated mass of 0.1 ng while the heaviest one weighted 1969 μg. The summary of the measured major dimension and estimated mass is provided in Table S 3 and figure S 3. The small MPs dominated the MP number concentration while the mass was governed by a few large particles, a finding which corresponds to e.g. that of Liu et al. (2022).

The identified MPs were further characterized as fibres and fragments based on their aspect ratio being above or below 3, that is, fibres have a ratio of major to minor dimension above 3 and fragments one below 3. In total, 16,611 (~91 %) of the detected MPs were fragments, while the remaining 1678 (~9%) were fibres. The ranking in terms of fibre content was P1 > C3 > C1 > R1 > C2 > C4 (Figure S 2). This study found a lower proportion of fibres than many other microplastic studies, which might be attributed to the detection of a large number of small MPs when using μFTIR with a low size detection limit combined with autodetection of particles using a dedicated software. For instance, 72 % of all MPs had a major dimension below 100 μm, while this is roughly the lower size limit that can be achieved when manually pre-sorting particles and applying visual detection.

3.4. Metals

The highest metal concentrations were found in snow from C4, except for Na and Pb which were high at C2 and C1, respectively. The high content of Na was likely from road de-icing salts, whilst Pb might be a legacy pollutant from both exhaust and non-exhaust traffic (Ye et al., 2022). The lowest concentrations were in snow from P1, except for Ca, which was lower in snow from C3. Metal concentrations tended to be high in densely populated and urbanized areas (Table 2 and Table S 5). However, the concentrations varied also between urbanised sites. The snow metal content was above that of several previous studies on snow from remote areas (Hur et al., 2007; Planchon et al., 2001), which probably was due to human activity at the studied sites (Hur et al., 2007; Li et al., 2022; Sakai et al., 1988; Sharma, 2014). For instance can Cd, Cr, Ni, Cu, and Zn come from traffic related activities close to the sampling sites (Pacyna et al., 2007). The metals in snow from P1 must, however, have come via long-range transport or originate from the local soils (Slukovskii et al., 2020; Valavanidis et al., 2011).

3.5. Correlation of MPs, TWP, and metals

There was a moderate positive corelation between MP counts and metals (Pearson's coefficient 0.4–0.67) (Fig. 4), while the estimated MP mass was only weakly to moderately correlated to metals (Pearson's coefficient 0.027–0.51). This is in contrast to findings of some laboratory studies reporting a positive correlation between MP concentrations and adsorbed metals (Q. Wang et al., 2020) and to a study on MPs in soil which showed a negative corelation between metals and MPs (Du et al., 2023). The latter could have been due to low organic content in their soil, as organic matter may increase adsorption of metals.

Except for Na, TWP and metal masses were on the other hand well correlated (0.70–0.98). Part of this might be caused by metals associated to tyres and traffic in general (Adamiec et al., 2016), which was particularly true for Mg, Ca, Co, Cu, Ni, and Fe with the Pearson coefficient all above 0.8 (Fig. 4). A study by Adachi and Tainosho (2004) stated that heavy metals in tire dust are not only attributed to TWPs, but also to road and traffic materials, for instance, brake linings and road paint, together enriching the diversity of metals released to the environment. Likewise, most metals were well correlated among themselves (Fig. 4). The exception was again Na, which generally was poorly corelated to other metals, a finding which agrees with a previous study on seasonal patterns of metals in snow (Hur et al., 2007). Whether there was an interaction between TWP, and metals is though not a given, as the co-occurrence of TWP and many metals might simply be due to similarity in sources.

MP counts and mass were, however, not correlated to TWP (Pearson's coefficient 0.2 and 0.0099, respectively). Considering the fact that metals correlated strongly with TWP, but only weakly to moderately with MPs, it is reasonable to assume that TWP and metals were likely to have originated from similar sources, which were distinct from those of MP. Since it is evident that the primary origin of TWP is traffic, which is also an important source for many metals (Rasmussen et al., 2023), it can be derived that MP were more associated with other types of human activities, a conclusion which is in line with findings by Bergmann et al. (2019) and Chernykh et al. (2022).

3.6. Fate of snowmelt

Upon melting, accumulated snow can convey microplastics and metals to surface waters. Riga city is situated on the Daugava River mouth on the south coast of the Gulf of Riga, Baltic Sea. Part of the

Table 2
Metals in the collected snow.

Sampling sites	Concentration [mg/L]				Concentration [μg/L]									
	Na	K	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Al	Pb
C1	80.4 (±28.5)	13.8 (±4.2)	12.7 (±7.6)	47.3 (±24.7)	24.0 (±15.5)	441 (±272)	8,910 (±6,145)	3 (±2.9)	14 (±8)	60 (±18)	525 (±60)	0.7 (±0.1)	3,350 (±2,129)	39 (±9)
C2	819. (±97.0)	8.8 (±1.7)	8.1 (±0.3)	36.9 (±3.3)	11 (±1.3)	177 (±14)	4,240 (±527)	2 (±0.3)	6 (±1)	58 (±9)	222 (±56)	0.3 (±0.5)	1,890 (±158)	21 (±3)
C3	1.1 (±0.4)	0.1 (±0.03)	0.1 (±0.04)	4.3 (±0.6)	1 (±0.6)	9 (±4)	105 (±40)	NQ	NQ	20 (±9)	94 (±71)	0.1 (±0.02)	50 (±10)	6 (±2)
C4	434.5 (±409.8)	37.7 (±30.2)	39.9 (±16.0)	132.5 (±55.5)	36 (±16.3)	622 (±246)	18,750 (±8,730)	11 (±5.5)	30 (±14)	130 (±50)	640 (±265)	0.9 (±0.4)	5,920 (±2,668)	33 (±15)
R1	33.7 (±53.5)	3.3 (±2.9)	5.4 (±6.4)	21.3 (±16.7)	6 (±6.8)	131 (±162)	2,190 (±3,084)	3 (±00)	4 (±5)	23 (±13)	154 (±182)	0.4 (±0.4)	1,460 (±1,757)	18 (±25)
P1	0.4 (±0.4)	0.05 (±0.01)	0.02 (±0.006)	5.9 (±2.9)	NQ	NQ	4 (0.3)	NQ	NQ	1 (±0.2)	1 (±00)	NQ	18 (±5)	1 (±0.1)

Ranked abundance of the specified metallic elements.
C1: Na > Ca > K > Mg > Fe > Al > Zn > Mn > Cu > Pb > Cr > Ni > Co > Cd.
C2: Na > Ca > K > Mg > Fe > Al > Zn > Mn > Cu > Pb > Cr > Ni > Co > Cd.
C3: Ca > Na > K > Mg > Fe > Zn > Al > Cu > Mn > Pb > Cr > Ni > Cd.
C4: Na > Ca > Mg > K > Fe > Al > Zn > Mn > Cu > Cr > Pb > Ni > Co > Cd.
R1: Na > Ca > Mg > K > Fe > Al > Zn > Mn > Cu > Pb > Cr > Ni > Co > Cd.
P1: Ca > Na > K > Mg > Al > Fe > Pb > Zn > Cu > Mn > Cr > Ni > Cd.
NQ: Non quantifiable, i.e., below the method quantification limit.

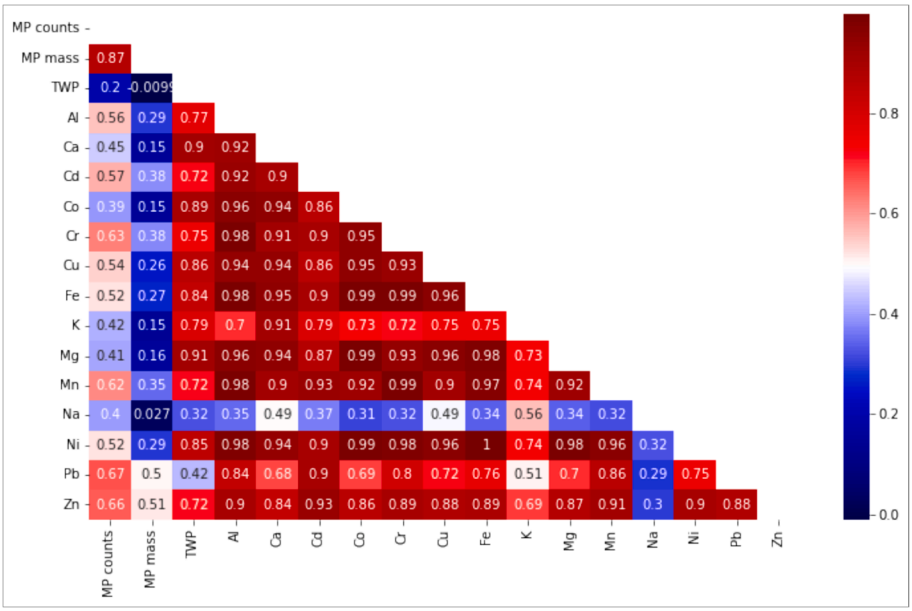

Fig. 4. Heat map of the correlation (R-coefficient) between MP counts, MP mass, TWP, and metals. The abundance of TWP was presented as mass.

snowmelt is collected by the city's combined drainage system and treated at its wastewater treatment plant, which discharges into the Gulf of Riga, and part goes directly into the Daugava via different watercourses. Snowmelts in Riga occur over quite short periods of time, leading to quite high pollutant loads being discharged during such events (Sakai et al., 1988). The microplastics and metals in the runoff may adversely affect the aquatic environment. A high Zn concentration, for instance, would negatively affect fish and invertebrates (Hagen and Langeland, 1973). Runoff from areas with dense traffic, for instance, highways, have been tested toxic towards various species (Kayhanian et al., 2008; McIntyre et al., 2015). Similarly, the marine fauna could be detrimentally affected by ingesting microplastics (Anderson et al., 2016), and additives in the microplastics, e.g., pigments, dyes, and plasticizers, might affect the organisms (Digka et al., 2018). Together, the findings of this study show that meltwater from urban snow should undergo treatment before being discharged to the aquatic environment.

4. Conclusions

Snow from snow dumping stations in Riga held significant concentrations of microplastics and metals compared to snow from an urban rooftop and a rural site in a national park. The latter were however also quite high compared to close by marine waters, as it held orders of magnitude more microplastics then previously reported for those. The content of tyre wear particles in the snow correlated strongly with most metals, while other types of microplastics showed little or no correlation. Consequently, tyre wear particle content did not correlate with the content of other microplastics. The snow dumps in the urban centre had higher concentrations of microplastics and metals than that of a residential area and those of a rooftop, indicating that urban activity influenced the pollution content of the snow. The quite high pollution level of the urban snow dumps proved that such dumping sites can be hotspots for microplastic and other pollution. If not treated prior discharge, it could impact receiving water quality. Urban snow melt should hence be treated prior to discharge to the environment, for instance, by sand filtration or sedimentation in a pond.

CRediT authorship contribution statement

Rupa Chand: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Ieva Putna-Nīmane:** Writing – review & editing, Resources, Project administration, Investigation. **Elina Vecmane:** Writing – review & editing, Investigation. **Jeanette Lykkemark:** Writing – review & editing, Methodology, Data curation. **Jytte Dencker:** Writing – review & editing, Methodology, Data curation. **Asbjørn Haaning Nielsen:** Writing – review & editing, Methodology, Data curation. **Jes Vollertsen:** Writing – review & editing, Supervision, Funding acquisition. **Fan Liu:** Writing – review & editing, Methodology.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgements

This project was supported by EU INTERREG Baltic Sea Region project "Initiatives to remove microplastics before they enter the sea - FanPLESStic-sea" (project number #RO92). We furthermore would like to thank Sandra Sprukta, Research assistant at LIAE for her kind support in creating GIS maps in the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2024.108782.

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