DISSERTATIONES
TECHNOLOGIAE
UNIVERSITATIS
TARTUENSIS
93

ALINA ISMAGILOVA

Safety assessment of novel bio-based polymers and compounds used in low carbon technologies

DISSERTATIONES TECHNOLOGIAE UNIVERSITATIS TARTUENSIS

93

DISSERTATIONES TECHNOLOGIAE UNIVERSITATIS TARTUENSIS
93

ALINA ISMAGILOVA

Safety assessment of novel bio-based polymers and compounds used in low carbon technologies

UNIVERSITY OF TARTU
Press
1632

Institute of Technology, Faculty of Science and Technology, University of Tartu, Estonia

The dissertation was accepted for the commencement of the degree of Doctor of Philosophy in Engineering of Physical Engineering on 15.08.2025 by the Joint Council of the Doctoral Program of Engineering and Technology of the University of Tartu.

Supervisors: Lauri Vares, PhD
Associate Professor of Organic Chemistry
Institute of Technology, Faculty of Science and Technology
University of Tartu, Estonia

Veljo Kisand, PhD
Associate Professor of Molecular Ecology
Institute of Technology, Faculty of Science and Technology,
University of Tartu, Estonia,

Reviewer: Monika Mortimer, PhD
Senior Research Fellow
National Institute of Chemical Physics and Biophysics
Estonia

Opponent: Anita Jemec Kokalj, PhD
Associate professor
Biotechnical Faculty,
University of Ljubljana, Sloveenia

Commencement: Auditorium 121, Nooruse 1, Tartu, Estonia, at 13.15
on September 4th, 2025

Publication of this thesis is granted by the Institute of Technology, Faculty of Science and Technology, University of Tartu.

ISSN 2228-0855 (print)
ISBN 978-9916-27-970-0 (print)
ISSN 2806-2620 (pdf)
ISBN 978-9916-27-971-7 (pdf)

University of Tartu Press
www.tyk.ee

ABSTRACT

The worldwide rise in material and chemical usage, especially plastics, has raised significant concern over their long-term effects on the environment and human health. As a result, there is a growing interest in sustainable alternatives, leading to the development of innovative bio-based monomers and polymers from renewable resources. Although these options are intended to reduce reliance on fossil-derived plastics, their safety is not assured; some bio-based monomers, polymers, or their degradation products may still be toxic. This underscores the need for a thorough environmental evaluation of such alternatives.

This thesis investigates the environmental risks associated with bio-based materials, including isosorbide-based acrylates and methacrylates, PLA-derived acrylates, lignin-based methacrylates, and amines used in CO_2 capture and "switchable water" solvent systems. A series of aquatic ecotoxicological assays using bacteria (*Alivibrio fischeri*, *Escherichia coli*), vascular plants (*Spirodela polyrhiza*), and invertebrates (*Thamnocephalus platyurus*, *Daphnia magna*) revealed that some monomers, especially acrylates, exhibited moderate to high toxicity, while their corresponding polymers were mainly non-toxic. Cytotoxicity testing on human HeLa cells further indicated a significant reduction in toxicity upon polymerization. Ecotoxicological evaluation of amines used in CO_2 capturing technologies revealed that these compounds are relatively safe towards aqueous life, except for the more hydrophobic diamines, which should be carefully considered. Furthermore, biofilm analysis on various materials showed that bacterial colonization patterns depend on the type of material, with conventional plastics and coated paperboard supporting distinct microbial communities. These biofilms include taxa with known biodegradability, highlighting their ecological significance.

Overall, these findings emphasize that the chemical toxicity and microbial interactions of new bio-based materials need to be carefully assessed prior to their wider use. This comprehensive evaluation is essential to ensure that such materials not only lower carbon emissions but also safeguard environmental and human health.

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications. The articles are referenced in the text using Roman numerals:

I. **Ismagilova, A**.; Matt, L.; P. Jannasch, Kisand V.; Vares, L. Ecotoxicity of isosorbide acrylate and methacrylate monomers and corresponding polymers, *Green Chemistry*, 2023, 25, 1626–1634.

II. Palà, M.; **Ismagilova, A**.; Moreno, A.; Plaza, J.; Ronda, J. C.; Galià, M.; Vares L.; Lligadas, G. Thermoresponsive lactate amide acrylic polymers developed from PLA bags, *Polym Chem*, 2025, 16, 1692–1703.

III. Sedrik, R.; Bonjour, O.; de Souza, N. R. D.; **Ismagilova, A**.; Tamsalu, I.; Kisand, V.; Cherubini, F.; Jannasch P.; Vares, L. Aromatic Polymethacrylates from Lignin-Based Feedstock: Synthesis, Thermal Properties, Life-Cycle Assessment and Toxicity, *ChemSusChem*, 2025, 18, 1–7.

IV. **Ismagilova, A**.; Kisand, V.; Vares, L. Ecotoxicity risk assessment of amines used in 'switchable water' and CO_2-capturing processes, *Environ Sci Process Impacts*, 2025, 27, 974–980.

V. **Ismagilova, A**.; Shanskiy, M.; Vares, L.; Kisand, V. Bacterial biofilms on novel bio-based materials. (unfinished manuscript)

Author contribution:

Paper I: Performed all ecotoxicological tests, contributed to the monomers and polymers synthesis. Prepared the first draft of the manuscript and supporting information.

Paper II: Performed all ecotoxicological tests. Contributed to the preparation of the manuscript and supporting information.

Paper III: Performed cytotoxicity tests. Contributed to the preparation of the manuscript and supporting information.

Paper IV: Performed all ecotoxicological tests, contributed to the monomers and polymers synthesis. Prepared the first draft of the manuscript and supporting information.

Paper V: Performed all experiments and analysis. Prepared the first draft of the manuscript.

ABBREVIATIONS

A. fischeri	*Alivibrio fischeri*
AMP	2-amino-2-methyl-1-propanol
ANOVA	analysis of variance
ASV	amplicon sequence variants
BMA	4-hydroxybenzyl methacrylate
bio-PE	bio-based polyethylene
CAP	canonical analysis of principal coordinates
CCS	carbon capture and storage
CO_2	carbon dioxide
D. magna	*Daphnia magna*
DEA	diethanolamine
DEEA	diethylethanolamine
DMEA	dimethylethanolamine
EC_{50}	effective concentration for 50% of the population
E. coli	*Escherichia coli*
EPS	extracellular polymeric substances
ERA	Environmental Risk Assessment
IA	isosorbide 5-acrylate
IAA	isosorbide 5-acrylate 2-acetate
IC_{50}	half-maximal inhibitory concentration
IM	isosorbide 5-methacrylate
IMA	isosorbide 5-methacrylate 2-acetate
ISO	International Organization for Standardization
LAA	lactate amide acrylate
LCA	life-cycle assessment
LDH	lactate dehydrogenase
LEfSe	linear discriminant analysis of effect size
MDEA	methyldiethanolamine
MEA	monoethanolamine
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
PAA	poly(acrylic acid)
PBMA	poly(4-hydroxybenzyl methacrylate)
PC	polycarbonate
PE	polyethylene
PET	poly(ethylene terephthalate)
PI	poly(isosorbide 5-acrylate)
PIA	poly(isosorbide 5-acrylate)

PIAA	poly(isosorbide 5-acrylate 2-acetate)
PIMA	poly(isosorbide 5-methacrylate 2-acetate)
PIM	poly(isosorbide 5-methacrylate)
PLA	poly(lactic acid)
PLAA	poly(lactate amide acrylates)
PMMA	poly(methyl methacrylate)
PP	polypropylene
PS	polystyrene
PSMA	poly(syringyl methacrylate)
PUR	polyurethane
PVMA	poly(vanillin methacrylate)
PVC	polyvinyl chloride
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
OECD	Organization for Economic Co-operation and Development
SA-latex	styrene-acrylate latex
SMA	syringyl methacrylate
S. polyrhiza	*Spirodela polyrhiza*
SW	switchable water solvent system
T. platyurus	*Thamnocephalus platyurus*
TEA	triethanolamine
TMEDA	tetramethylethylenediamine
TMPDA	tetramethyl-1,3-propanediamine
UV	ultraviolet light
VMA	vanillin methacrylate

INTRODUCTION

The demand for materials and chemicals is growing every year, and simultaneously, concerns about their impact are also growing, as the increasing production and use lead to various detrimental climate and environmental effects. Currently, the chemical industry mainly uses raw materials derived from fossil fuels, which not only contribute to greenhouse gas emissions but also depend on non-renewable resources that are expected to become increasingly scarce in the future. Conventional plastic materials, such as polypropylene, polyethylene, and polystyrene, have excellent performance and economic advantages, but they pose significant environmental problems due to their persistence in ecosystems, low recycling efficiency, and contribution to global pollution. Because of this, there is a need to transition from traditional fossil-based production systems to more sustainable alternatives.

Green chemistry has emerged as a guiding framework for the development of more sustainable chemical processes and materials. The principles of green chemistry emphasize, e.g., the use of renewable feedstocks, the development of safer chemicals with reduced toxicity, increased energy efficiency, and the promotion of (bio)degradable end products. This approach has spurred research into bio-based polymers and materials derived from renewable sources such as agricultural crops and lignocellulosic biomass. These bio-based alternatives alleviate our dependence on fossil resources while potentially offering improved environmental profiles through carbon-neutral or carbon-negative life cycles. Life cycle analysis is used to estimate the influence of new materials on the environment, which also includes toxicological assessment.

The transition to bio-based materials also has challenges. The assumption that "bio-based equals green" cannot be taken as granted and requires careful examination through a comprehensive environmental risk assessment and toxicological evaluation. Recent studies have shown that several biomaterials can exhibit toxicity levels similar or even higher compared to their conventional counterparts, highlighting the critical importance of careful safety assessment throughout the life cycle of these materials. This includes not only the final polymer products, but also monomers and intermediates that may exhibit higher biological activity and may be released during production, use, or degradation.

In parallel with the development of bio-based materials, carbon capture and utilization technologies have emerged as additional strategies for environmental sustainability. Carbon capture and storage (CCS) technologies, including chemical absorption processes using amine-based solvents, offer promising approaches to reducing atmospheric CO_2 concentrations. In addition, new technologies such as "switchable water" processes provide energy-efficient alternatives for separating organic compounds from aqueous solutions, solving one of the major challenges in biomass-based chemical production.

The aquatic environment serves as an endpoint for many anthropogenic pollutants, making aquatic ecotoxicology an important area in the evaluation of new

materials and chemicals. Standard ecotoxicological tests using a variety of organisms at different trophic levels – from bacteria and algae to crustaceans and fish could provide important data for understanding the potential environmental impacts of new materials relatively quickly. In addition, cytotoxicity assessments give information about potential risks to human health, which is also important as bio-based materials find use in food packaging, consumer products, and biomedical devices. An additional consideration when assessing the environmental impact of both conventional and biomaterials is their interactions with microbial communities through the formation of biofilms on plastic surfaces. These biofilms can influence material degradation processes and serve as reservoirs for pathogens and antimicrobial resistance genes, adding another dimension to the environmental and health impacts of plastic materials in aquatic and terrestrial environments.

In this thesis, the primary focus was to evaluate the environmental risk of novel bio-based materials and compounds used in green technologies. Firstly, the effects of isosorbide-based acrylate and methacrylate monomers and their corresponding polymers were evaluated toward bacteria, vascular plants, and invertebrates. Also, the toxicity level of isosorbide-based industrially relevant latexes was determined using bacteria. Secondly, the toxicity level of lactate amide acrylic monomers and polymers developed from PLA bags was evaluated on several aquatic organisms. Furthermore, the cytotoxicity of lignin-derived aromatic methacrylate monomers and their corresponding polymers was established on an immortalized cell line, HeLa. Additionally, environmental risk assessment for amines used in CO_2-capturing and "switchable water" processes was evaluated on aquatic organisms. Finally, biofilms formed in aquatic and terrestrial environments on different conventional and bio-based polymers, and coated paperboards used as packaging materials were analyzed and compared. Overall, this work advances understanding of the environmental and ecotoxicological implications of emerging biobased materials, supporting the safer design and deployment of low carbon technologies. The findings can lead to the development of next-generation bio-based materials that minimize ecological harm and promote sustainability.

1. LITERATURE OVERVIEW

1.1 Sustainable chemical materials and processes

1.1.1 Green chemistry

Green chemistry is the approach to the chemical design of products and processes that aims to minimize the environmental impact.[1] The main principles of green chemistry include using renewable feedstock, designing safer chemicals with reduced toxicity, enhancing energy efficiency, and promoting (bio)degradability of end products (Fig. 1).[2] These principles guide academia and industry to find creative and innovative ways to reduce waste, save energy, and discover substitutes for hazardous substances.

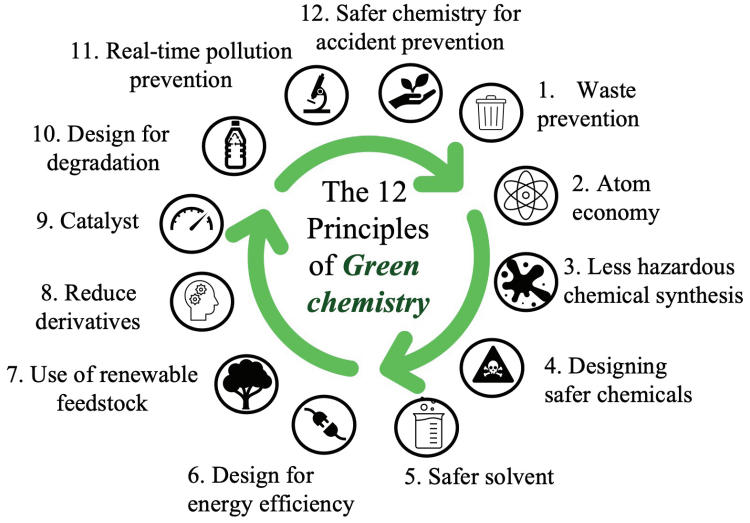


Fig. 1. The 12 principles of green chemistry (the figure is based on data published in ref. 3).

The need to transition from conventional methods and materials to more sustainable ones is a result of growing environmental concerns. Pollution threatens human health and the ecosystems, with rising carbon emissions from various human activities contributing to global environmental change.[4] Nowadays, most organic chemicals and materials are derived from fossil-based feedstock.[5] Fossil-based feedstock mainly consists of light petroleum fraction, but it can also be coal or natural gas. Most fossil fuels formed during the Carboniferous Period between 362–286 million years ago,[6] and are considered non-renewable, limiting their future availability. Moreover, our current extensive exploitation of fossil carbon is the main contributor to increasing carbon dioxide levels in the atmosphere.[7] While underground, the carbon is safely stored. However, when extracted and used as a fuel or a source for materials, most of this fossil carbon is eventually converted into additional CO_2 in the atmosphere.

Conventional plastic materials such as polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polyurethane (PUR), polystyrene (PS), are produced from fossil-based resources (Fig. 2). However, new plastics can also be produced from alternative sources – such as plastic waste, or carbon obtained through the carbon capture technologies. There are two main methods to recycle plastic waste: mechanical recycling, which consists of shredding and remolding, and chemical recycling, in which the plastic waste is broken down into its basic chemical components (monomers or other chemicals) and re-polymerized into new materials.[8] Over the past few decades, plastic production has increased exponentially due to its wide use in packaging, construction, healthcare, electronics, and transportation applications.[9] Plastic is a synthetic polymer material composed of a long chain of repeating units, called monomers, attached to each other.[10] Monomers are small molecules that combine with other molecules of the same or different type to form a polymer through chemical reactions.[11] Plastics also often contain various additives to improve performance and functionality. Common fossil-based monomers used in plastic production include, e.g., ethylene, propylene, vinyl chloride, and styrene.

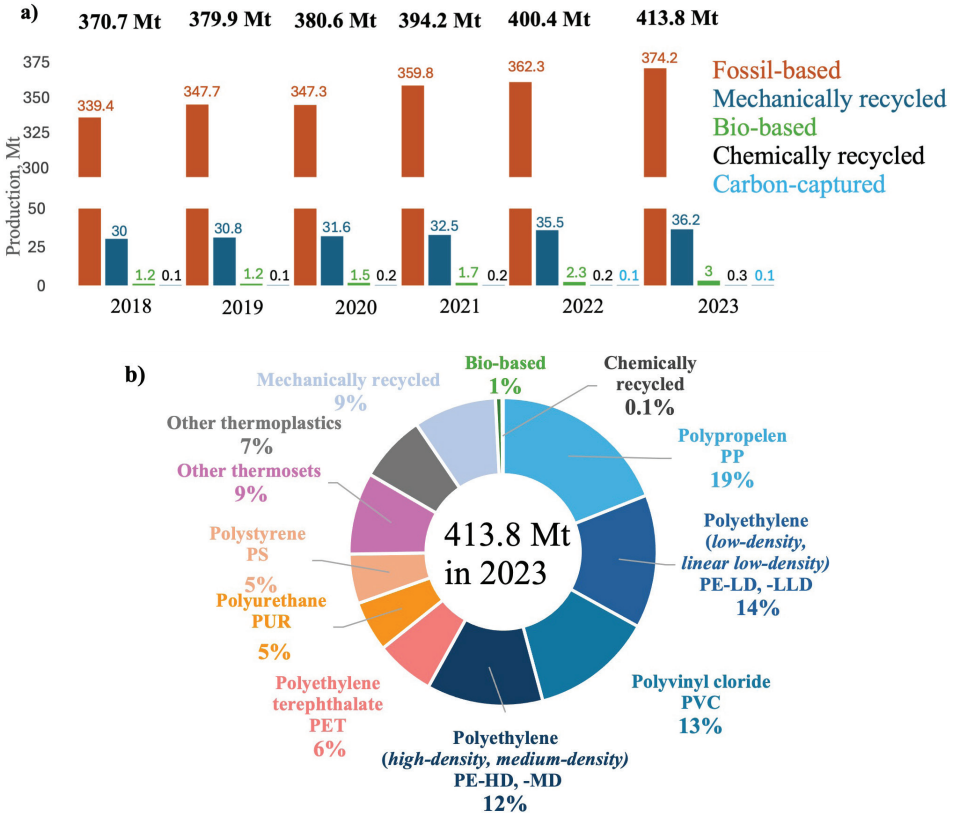


Fig. 2. a) The sources for plastic production from 2018 to 2023; b) World plastic production in 2023 (the figure is based on data published in ref. 14).

Plastics derived from petroleum-based monomers are lightweight, cheap, and easy to process.[12] Unfortunately, however, they have become a serious environmental threat due to poor recycling efficiency and their resistance to aging and degradation, which may lead to contamination after disposal.[13] Also, green chemistry principles suggest avoiding fossil feedstock and replacing it with renewable bio-based feedstock as a more sustainable alternative.

A renewable bio-based feedstock is a source obtained from recently living organisms, such as plants, algae, and microorganisms, which can be naturally and quickly replenished. If used responsibly, it is considered a sustainable alternative to fossil-based feedstock, supporting the transition to a circular economy and reducing environmental impact.[15] In contrast to fossil feedstock, which releases CO_2, bio-based feedstock absorbs CO_2 from the atmosphere via photosynthesis during growth. Even when bio-based materials are decomposed or burned, they release CO_2, but this CO_2 can be absorbed again by new plants, maintaining a balanced carbon cycle rather than adding new carbon to the atmosphere. Because of that, their use can be carbon neutral or even carbon negative, especially when combined with sustainable agricultural practices or carbon capture technologies.[16]

Bio-based feedstocks are generally divided into three generations based on source and sustainability.[17] First-generation feedstocks comprise traditional agricultural crops such as corn and sugar cane. Second-generation feedstocks include lignocellulose biomass, agricultural waste, and residues, offering a more sustainable alternative as non-food biomass. Third-generation feedstocks are derived from unconventional sources, such as algae and other microorganisms, which have the potential for higher productivity. Typically, the biomass is first converted into platform chemicals, small molecules that serve as key intermediates in the production of a wide range of materials, which can be further converted into different bio-based polymers or other products.[18]

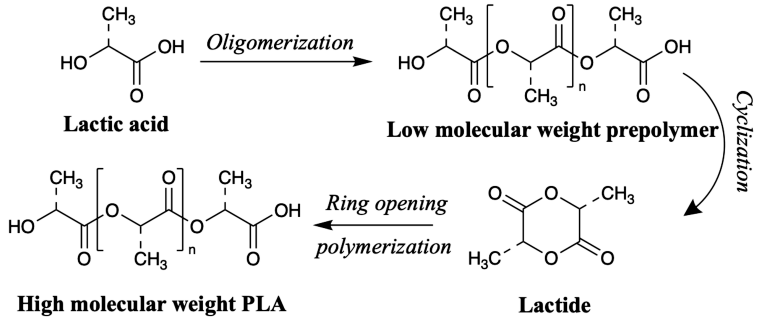
The environmental impact of different feedstocks, products, or processes can be evaluated and compared using life-cycle assessment (LCA). LCA is an analytical method for evaluating various effects of a product or process on the environment during its entire life cycle.[19] According to the principles of green chemistry, LCA allows for the quantification of how changes in chemical design, material sourcing, or processing can help to decrease emissions, reduce toxicity, and increase sustainability. Toxicological impacts (such as human health risks, ecotoxicity, and bioaccumulation) are a part of LCA analysis.[20] Because LCA results are numeric and standardized, they enable comparisons between different materials under the same conditions, giving an opportunity to develop more sustainable production systems.

1.1.2 Bio-based polymers

Bio-based polymers are materials fully or partially produced from bio-based feedstock. Currently, bio-based plastic takes up approximately 1% of global plastic production (Fig. 2).[21] However, it is expected that the production of bio-based

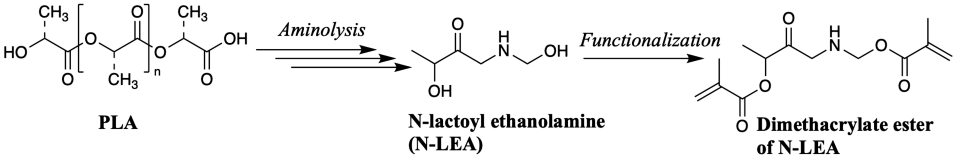
plastics will increase significantly in the coming years due to the growing demand for such materials.

Lactic acid, derived from biomass through carbohydrate fermentation or chemocatalytic processes, is a widely used platform chemical in producing bio-based materials.[22] The most common product derived from lactic acid is a poly-lactic acid (PLA) polymer.[23] High molecular weight PLA is generally obtained via polycondensation of lactic acid or ring-opening polymerization of lactide (Scheme 1).[24]



Scheme 1. Ring-opening polymerization of lactide to produce PLA.[22]

PLA has advantages such as excellent biocompatibility, easy processability, and sufficient mechanical properties, which make it suitable for a wide range of applications: single-use plastics, textiles, food packaging, and biomedical products.[23,25] However, there are problems such as brittleness, limited thermal stability, relatively low glass-transition temperature (T_g = 58 °C), poor recyclability, and the need for industrial composting conditions for degradation.[26] Many studies have investigated modifying PLA or changing its molecular structure to improve its properties.[27,28] For example, adding *Pinus sylvestris* (Scots pine) char to PLA composite increased its tensile strength by 98%, and the bending strength by 25%, compared to that of pure PLA.[29] The palm fiber-reinforced PLA composites with bran filler composite have T_g = 125 °C, which is significantly higher compared to regular PLA.[30] Also, industrial degradation of the PLA into CO_2 and H_2O occurs only under controlled environmental conditions such as temperature (usually around 58 °C), pH, and moisture content.[31] Chemical depolymerization can be a good solution in converting PLA into valuable chemicals using alcohols and amines.[32] For example, the reaction of PLA with ethanolamine followed by derivatization of the resulting lactate amide with methacrylic anhydride has been shown to be promising for producing photocurable resins for 3D printing (Scheme 2).[33] Alternatively, diamines have been explored to synthesize valuable diol derivatives that, when reacted with dicarboxylic acids, yield poly(esteramide) structures with tunable properties when reacting with dicarboxylic acids.[34]



Scheme 2. Synthesis of dimethacrylate ester from PLA for 3D printing.[33]

Isosorbide is another intensively investigated bio-based platform which is produced on a commercial scale. It is derived from sorbitol via double dehydration and is used as a building block to produce various polymers such as polyesters, polyethers, polycarbonates, polyterephthalates and polyurethanes (Fig. 3).[35–38] In all these polymers, isosorbide is part of the main polymer chain.

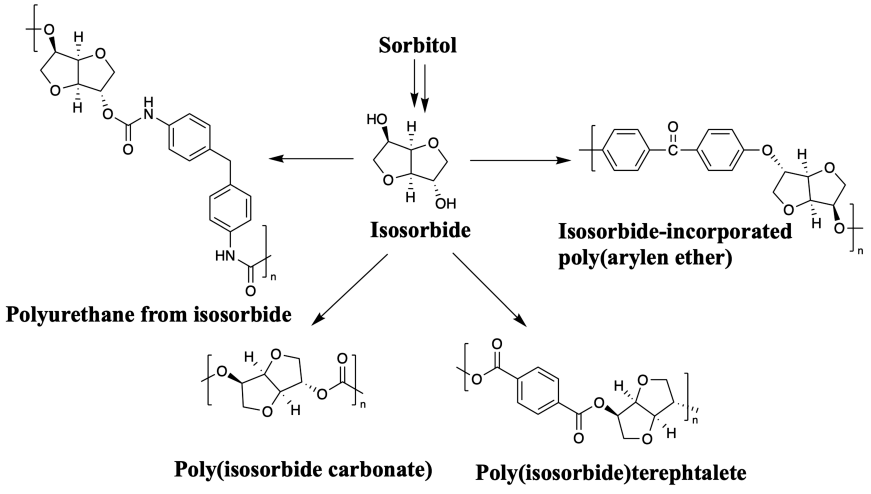
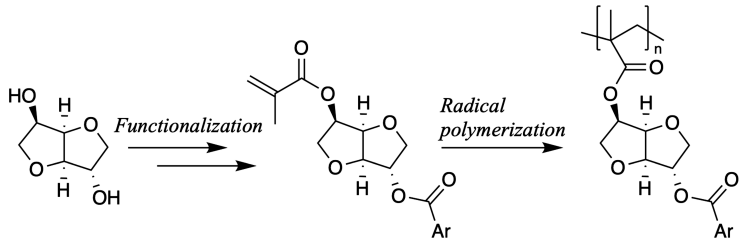


Fig. 3. Isosorbide-based polymers.[35–38]

For example, the UV and scratch-resistant isosorbide-containing polycarbonate DURABIO™ is offered by the Mitsubishi Chemical Corporation and used in the car industry, for sunglasses production, and other purposes.[39] However, studies by our and other groups have shown that various isosorbide-based monoacrylate and monomethacrylate monomers can also be prepared.[40–45] Such monomers can undergo radical homo- or co-polymerization and afford corresponding polymers where the isosorbide units form pendant side groups attached to all-carbon backbones (Scheme 3). Isosorbide-based polymethacrylates have good thermal properties, e.g., glass-transition temperatures (T_g) are up to 168 °C and thermal stabilities up to approximately 250 °C.[43] These thermoplastic isosorbide poly(meth)acrylates offer viable bio-based alternatives as a potential replacement of PMMA, PS, and other fossil-derived counterparts in coatings, adhesives, and engineering plastics.



Scheme 3. Synthesis and polymerization of isosorbide monomethylacrylate substituted with an aryl side group.[43]

Another widely available biobased raw material is lignin. It is a part of the lignocellulosic biomass. Lignin has a highly crosslinked polyphenolic structure, and after cellulose, it is the second most abundant biopolymer from wood.[46] It comprises three main phenylpropanoid units: hydroxyphenyl, guaiacyl, and syringyl (Fig. 4).[47]

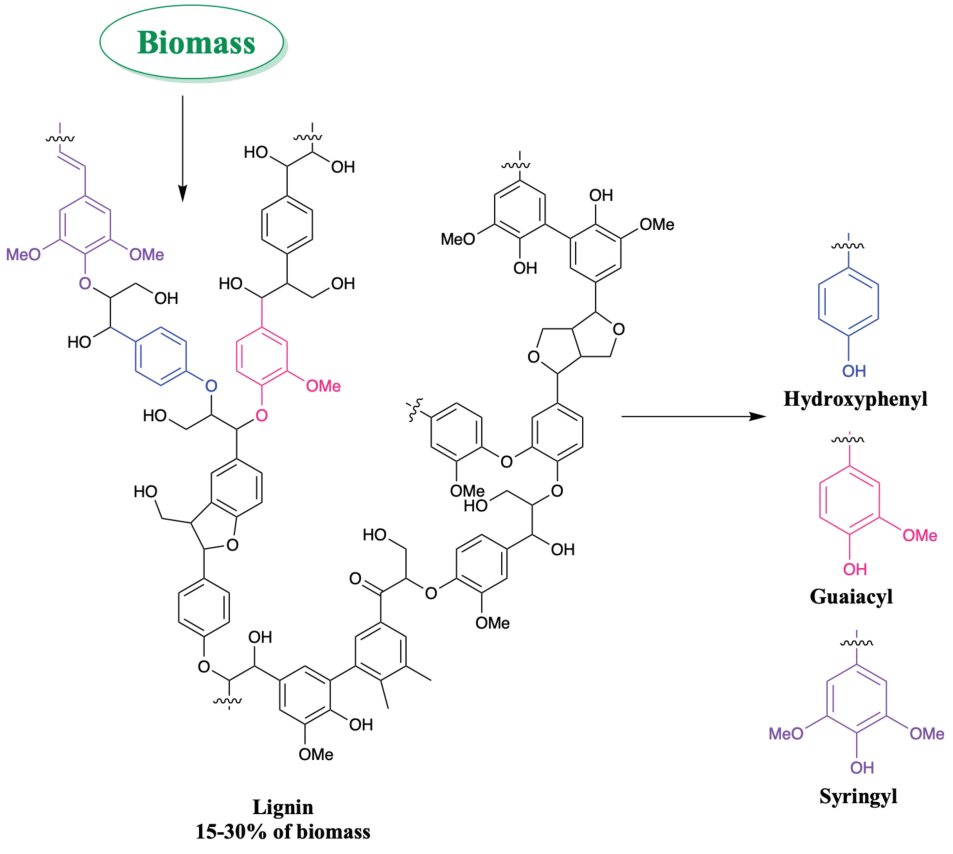
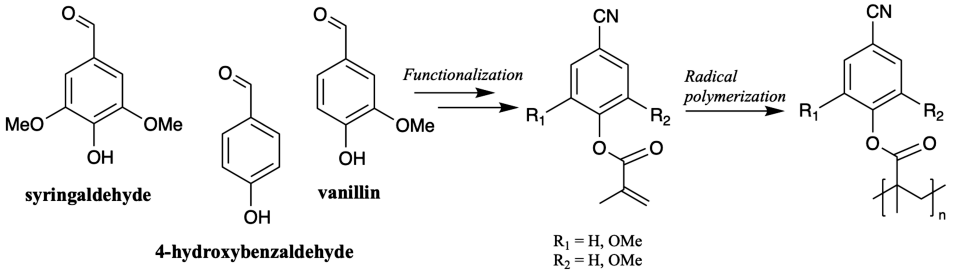


Fig. 4. Structural fragment of lignin and some main lignin units.[46]

Lignin presents both a challenge and an opportunity for valorization because of its heterogeneous and irregular structure. Traditionally, lignin has been considered a by-product of the paper and pulp industries and is often burned for energy as a low-value fuel.[48] However, due to its aromatic nature, lignin is particularly interesting as a renewable alternative to fossil-derived aromatic compounds.[49] Additionally, lignin can be used as an additive to well-known plastics. The blend of lignin with different polymers has been reported.[50–52] However, the different hydrophilic and hydrophobic properties of lignin and polymers can decrease the performance characteristics of the resulting composite materials. Polymeric lignin can be chemically modified through different functional group modifications or grafting with various monomers, which can make it a functional additive in plastic. For example, kraft lignin esterification with different-length fatty acids via acylation exhibited thermal stability and processability, indicating its potential as a component in polymer blends.[53] Depolymerization of lignin into low molecular weight monomers such as vanillin, guaiacol, syringaldehyde, and other phenolic derivatives can be another strategy to obtain bio-based building blocks (Fig. 4).[54,55] For example, nitrile-containing methacrylate monomers were prepared from 4-hydroxybenzaldehyde and syringaldehyde by direct nitration of the aldehydes, while vanillonitrile was obtained from a commercial source (Scheme 4). The polymethacrylates exhibited very high glass-transition temperatures, 150, 164, and 238 °C for 4-hydroxybenzonitrile, vanillonitrile, and syringonitrile derivatives, respectively, and were thermally stable up to more than 300 °C.[56]



Scheme 4. Synthesis and polymerization of nitrile methylacrylate monomers from vanillin, syringaldehyde, and 4-hydroxybenzaldehyde.[56]

Bio-based polymers are not only newly developed materials but can also be well-known, conventional plastics in which the fossil-derived feedstock has been replaced with renewable, bio-based sources. For instance, PE can be classified as bio-based polyethylene (bio-PE) if ethylene, the monomer used for PE production, is derived from bioethanol.[57]

The studies assessing the environmental impact of bio-based polymers mostly focus on the evaluation of the beginning and end of the product life cycle, considering such aspects as CO_2 emissions during production, feedstock renewability, and degradability. As a result, there is often limited data on potential toxic

effects on humans and other species. However, due to the increasing use of such materials, it is necessary to be aware of the potential risks to ensure overall safety and sustainability. For example, several bio-based plastics and plant-based materials (PLA, bio-PE, starch, cellulose) were evaluated for potential toxicity and compared to conventional plastics.[58] Six out of ten PLA samples, four out of ten bio-PE inhibited the bioluminescence of *Vibrio fischeri*, which indicates their toxicity toward bacteria. In the same study, six out of seven cellulose-based samples, four out of eight starch-based samples, four out of ten bio-PE samples, two out of ten PLA samples activated oxidative stress response, and one PLA sample demonstrated estrogenic activity *in vitro*. Comparisons between conventional plastics, bio-based plastics, and plant-based materials showed equally toxic levels. This study indicated that compared to conventional fossil-based plastics, bio-based materials will not necessarily have a lower effect on humans or the environment. Thus, the environmental risk assessment for all new bio-based materials is necessary.

1.1.3 CO_2 capture technology

Increasing carbon dioxide levels are one of the major contributors to global climate change. Its emissions primarily originate from various industrial activities, transportation, electricity and heat generation, and other activities where fossil fuels are burned. According to reports, in 2024, approximately 37.4 billion tons of CO_2 were released into the atmosphere, highlighting the urgent need for effective mitigation strategies.[59] Implementation of CO_2 carbon capture and storage (CCS) technologies is considered as one option to slow down the global environmental and climate changes. CCS is a process that was designed to capture CO_2 emissions from industrial facilities and power plants, preventing their release into the atmosphere.[16,60,61] This technology involves three main stages: capture, transport, and storage. The captured CO_2 could be stored in deep geological formations or reused for industrial applications, reducing its overall contribution to atmospheric greenhouse gas concentrations.

Several technologies have been developed for CCS to effectively separate CO_2 from gas streams before transportation and storage. One of the studied technologies is chemical absorption, which is one of the most explored methods for CO_2 removal.[62] In this process, a liquid amine-based sorbent is used to separate CO_2 from flue gas (Fig. 5). Gaseous CO_2 is absorbed into an aqueous amine solution, where CO_2 reacts with the amines to form carbamate or bicarbonates,[46,47] depending on the amine type (primary, secondary, or tertiary).[63,64] The CO_2 can be released upon heating, and the water and amines can be recovered and reused. It is a well-established process that offers operational convenience, rapid and high-capacity CO_2 absorption, and recyclability. Usually, monoethanolamine (MEA), diethanolamine, and potassium carbonate are used in this process.[65] Also, other alkanolamines have shown the possibility to absorb CO_2: methyldiethanolamine (MDEA), 2- amino-2-methyl-1-propanol (AMP), dimethylethanolamine (DMEA), diethylethanolamine (DEEA).[66–68]

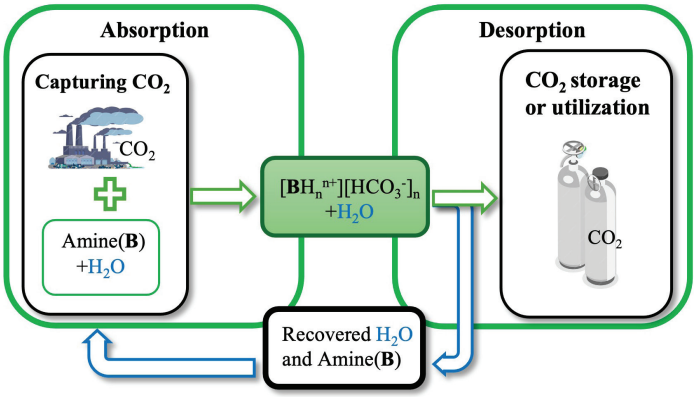


Fig. 5. Basic principles of amine-mediated CO_2-capturing process.

1.1.4 "Switchable water"

The development of new "green solvents" is crucial for advancing chemical processes that are economically competitive and environmentally sustainable compared to current fossil-based methods. Many biomass-based chemicals are water soluble and separating them from the water phase can cause serious difficulties.[69,70] The conventional separation techniques, such as distillation, have high energy requirements and can thus negate the environmental benefits of biomass conversion.[71] There are alternative methods that can make this process more economically attractive, but they have other disadvantages. For example, salting-out extraction produces large amounts of salt water, the purification of which is ecologically and economically expensive.[72,73]

Solvent-assisted "switchable water" (SW) process (Fig. 6) facilitates the removal of water-soluble organic compounds (e.g., hydrophilic alcohols, acetic acid) from the water.[74–77] In this process, an amine is added to a mixture of water and an organic compound, causing a reversible change in the polarity of the water, allowing the organic compound to be selectively extracted into a separate phase. Upon introducing CO_2 gas into the system, the amine reacts to form its bicarbonate salt, significantly impacting the solvent environment. The shift in ionic strength induces the precipitation of the organic product if it is a solid, or facilitates its separation in the form of an "organic-rich" liquid phase if it is a liquid. After separating the organic component, the aqueous phase can be decarbonated, and the removed CO_2 can be reused. Decarbonation reverses the reaction, converting the amine back to its neutral state. Finally, the amine can be recovered from the decarbonated water via reverse osmosis or by filtration and reused for another cycle.

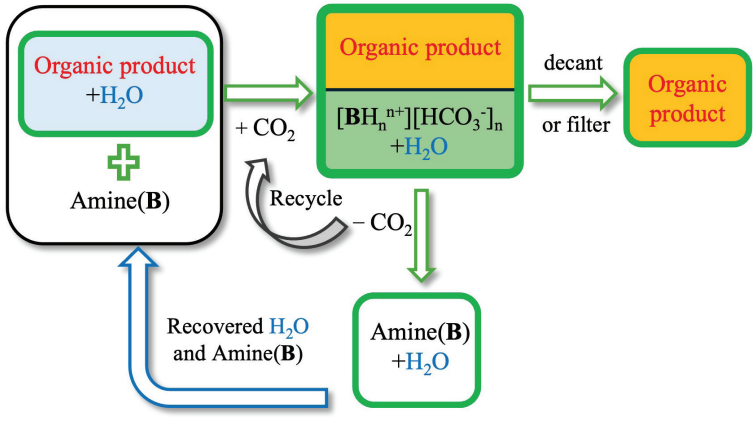


Fig. 6. Basic principles of the "Switchable water" process.

1.2 Environmental and human safety assessment of biomaterials and green technologies

1.2.1 Environmental risk assessment

Environmental risk assessment (ERA) is a method used to estimate and predict the likelihood of harmful effects on humans or ecosystems due to exposure to environmental stressors.[78] ERA involves three main phases: problem formulation, where stressors, exposure pathways, and affected ecological receptors are identified; analysis, which assesses exposure levels and toxicological effects; and risk characterization, which integrates data to estimate the likelihood and severity of ecological harm (Fig. 7).

Stressors can be classified as physical, biological, and chemical.[80] Physical stressors involve environmental changes that modify habitat conditions and disrupt ecosystem functions. These changes can be caused by natural processes, such as hurricanes or volcanic eruptions, or anthropogenic processes, such as deforestation, which leads to habitat fragmentation, soil erosion, and changes in local climate.[81] Biological stressors occur by introducing invasive species, pathogens, or genetic modifications that disrupt ecosystems. For example, an invasive species known as *Dreissena polymorpha* (zebra mussel) can significantly affect trophic interactions, displace native species, and influence water quality in freshwater ecosystems.[82] Additionally, fungal pathogens such as *Batrachochytrium dendrobatidis*, which cause the disease chytridiomycosis in amphibian populations, have caused a decline in biodiversity, particularly among species with restricted geographic distributions.[83] Chemical stressors arise from the introduction of synthetic or natural substances into the environment, often at concentrations exceeding the ecological tolerance thresholds. These stressors contribute to bioaccumulation, toxicity, and long-term ecosystem disruptions. Industrial pollutants, such as heavy metals, solvents, plasticizers, and byproducts from manufacturing processes, can harm human health, microbial communities, and aquatic

ecosystems. Plastics production often involves the use of toxic compounds such as vinyl chloride, ethylbenzene, phthalates, etc., which can leach into the environment and pose risks to both ecosystems and human health. For example, a widely used polymer building block, bisphenol A (BPA), has been linked to endocrine disruption, reproductive toxicity, and immune system dysfunction in both humans and wildlife.[84]

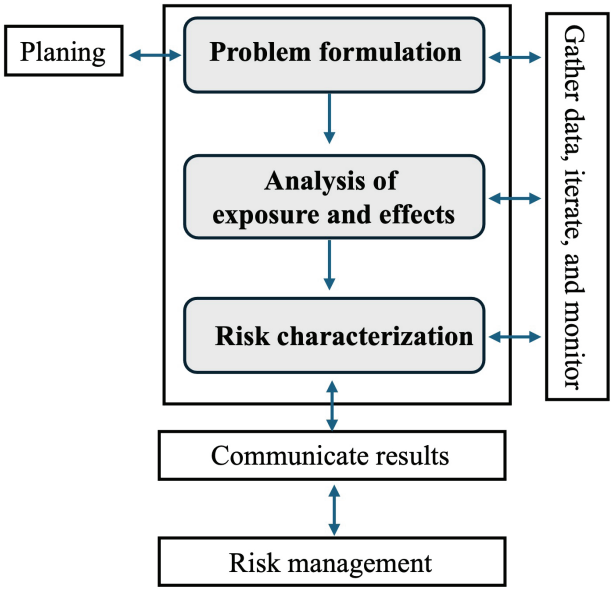


Fig. 7. General framework of environmental risk assessment (ERA) (the figure is based on data published in ref. 79).

Environmental risk assessment of chemicals can be divided into two main categories: human health risk assessment and ecological risk assessment. Human health risk assessment examines the potential effects of chemical exposure on human health. This evaluation considers toxicity, exposure pathways (inhalation, ingestion, and dermal contact), and particularly vulnerable populations.[85] On the other hand, ecological risk assessment focuses on the impact of chemicals on ecosystems, plants, animals, and microorganisms.[86] It is considered that the aquatic environment becomes the final point for most anthropogenic contaminants.

1.2.2 Aquatic ecotoxicology

Aquatic ecotoxicology is an important part of ERA. The aquatic environment is divided into freshwater ecosystems (rivers, lakes, and streams) and marine ecosystems (oceans and seas). Thus, freshwater and marine ecotoxicology assess how pollutants such as industrial chemicals, pesticides, pharmaceuticals, plastics and other contaminants affect aquatic species' health, survival, growth, reproduction, and behavior of aquatic organisms from bacteria to fish.[87]

In aquatic ecotoxicology, toxic effects are assessed using standardized tests that measure both acute (short-term, high dose) and chronic (long-term, low dose)

exposure.[88] Tests recommended for measuring ecotoxicity should be validated to ensure the repeatability and quality of results. This validation should follow standardized guidelines currently available through international organizations such as the Organization for Economic Co-operation and Development (OECD) or the International Organization for Standardization (ISO). Ecotoxicological bioassays are designed to assess the impact of chemicals on organisms at different trophic levels. Model organisms for testing include representatives of producers (e.g., autotrophic organisms such as algae and aquatic plants), consumers (e.g., invertebrates and vertebrates), and decomposers (microorganisms including bacteria). For example, common assays used in assessment include 48 h immobilization test of crustacean *Daphnia magna* (ISO 6341), 72 h algal growth inhibition test with *Raphidocelis subcapitata* (ISO 8692), and 30-min test with naturally luminescent bacterium *Alivibrio fischeri* that measures the decrease in light emission upon exposure to toxic substances (ISO 11348).[89–91] Ecotoxicity testing is also mandatory under regulatory frameworks to assess the environmental impact of chemicals. In the European Union, chemical safety is governed by the REACH regulation (Registration, Evaluation, Authorization and Restriction of Chemicals). For substances produced or imported onto the European market in quantities exceeding one ton per year, ecotoxicity data are required, including results from short-term (48 h) crustacean toxicity tests (the preferred species is *D. magna*; OECD, 2004)[92] and 72 h aquatic plant growth inhibition tests, usually algae (OECD, 2011).[93] When quantities exceed 10 tons per year, short-term (96 h) acute toxicity tests on fish are additionally required (OECD, 1992).[94]

Ecotoxicity testing of substances involves exposing test organisms to a series of different concentrations of the testing substance, as well as a negative control in parallel. After a certain exposure period specific to each test organism, the relevant endpoint, such as growth inhibition, mortality, or reproductive yield, is measured and compared with a negative control. The toxicity of the test substance is then quantified by determining the effective concentration (EC_{50}), which is the concentration at which 50% of the test population exhibits the stated adverse effect under specified conditions.

1.2.3 Aquatic ecotoxicity of bio-based monomers and polymers

Aquatic ecotoxicity of bio-based monomers and polymers is becoming an important topic as the use of renewable materials increases in various industries. However, these materials are often promoted as environmentally friendly alternatives to fossil-based materials. Before larger-scale industrial development, the ecotoxicity and other environmental aspects of these new materials should be carefully evaluated. It is important to evaluate not only the polymers but also the monomers, as they are more biologically active and reactive. Also, unreacted monomers may remain in the final polymer product and can leach out during use or degradation. In addition, monomers may be released into the environment during polymer synthesis or processing, making their assessment necessary to assess the full life cycle impact of the material.

Lactic acid is considered to have low aquatic ecotoxicity because it is biodegradable. It is naturally occurring in various biological systems, which decreases the possibility of adverse effects on the aquatic environment. One study evaluated the effects of lactic acid on aquatic organisms such as crustacean *Daphnia magna* (EC_{50} = 240 mg L^{-1}), alga *Selenastrum capricornutum* (EC_{50} > 2800 mg L^{-1}), and fish *Brachydanio rerio* (EC_{50} = 320 mg L^{-1}).[95] It was explained that the observed lactic acid toxicity was associated for *D. magna* and *B. rerio* with low pH values of the test substance solutions (4.1 and 3.5). Although this effect is not observed in algal media, because it is neutralized according to the relevant manual for this test. Another study tested lactic acid with the fish *Oreochromis mossambicus* (EC_{50} = 257 mg L^{-1}), the cladoceran *Moina micrura*, (EC_{50} = 329 mg L^{-1}) and the oligochaete worm *Branchiura sowerbyi* (EC_{50} = 50.8 mg L^{-1}).[96] They also found a decrease in pH in the tested medium. However, they considered that this parameter alone cannot explain the toxicity of the acid, since the high concentration of lactic acid, which provided the maximum decrease in media pH, had the least toxic effect on *Moina micrura* compared to the other acids tested in this study.

The data on isosorbide-based monomers in aquatic species are scarce. Isosorbide dimethyl ether, a potential aprotic solvent used in cosmetic and pharmaceutical products, was tested toward such aquatic species as algae and *D. magna* (EC_{50} >100 mg L^{-1}) by the Dutch National Institute for Public Health and the Environment.[97] Also, the compound was described as not readily biodegradable.

Whereas the toxicity of PS and several types of other conventional plastics, such as PE, PET, PU, and PVC, have been evaluated in bioassays, the data on bio-based monomers and polymers are scarce.[98] The direct acute ecotoxicity of the polymers is usually low due to low bioavailability (EC_{50} cannot be achieved, i.e. >1000 mg L^{-1} in standard assays). However, the introduction of various reactive functional groups needed for polymerization can affect the properties of the compound in different ways, and even minor changes in the chemical structure may have a large impact on its biological activity.[99]

Acrylate and methacrylate groups are important and widely used building blocks in polymer chemistry due to their high reactivity and versatility in forming polymers via free radical polymerization.[100] Derivatives with acrylate- and methacrylate functional groups have been reported to possess moderate to high toxicity towards algae and other organisms.[101–103] In general, acrylates have somewhat higher toxicity compared to corresponding methacrylates, and the toxicity decreases when the compounds become more lipophilic. For example, whereas acrylic acid has shown high toxicity towards algae, methyl methacrylate showed moderate toxicity in similar tests.[102] Also, acrylates exhibit higher toxicity than methacrylates due to their greater electrophilic reactivity, enabling Michael-type addition to cellular nucleophiles like glutathione and protein thiols. Methacrylates are less reactive and generally act through baseline narcosis unless metabolically activated.[104] Thus, due to potential toxicity-related issues with acrylates and methacrylates, any such new derivative of potential industrial use needs to be thoroughly assessed.

1.2.4 Amine's aquatic ecotoxicity

Studies on the ecotoxicity of amines used in the CO_2 capture and SW process have primarily focused only on a few alkanolamines, such as monoethanolamine (MEA), diethanolamine (DEA), and triethanolamine (TEA).[105–108] Only limited and divergent information is available, mainly for some decomposer, producer, and first-level consumer organisms. These amines have been evaluated previously toward single-celled organisms (*Entosiphon sulcatum* and *Chilomonas paramecium*), bacterium (*A. fischeri*), invertebrate (*D. magna*) and alga (*Skeletonema costatum*). Generally, the findings from these studies suggest a low toxicity level of alkanol amines towards most tested species.[109–111]

On the other hand, amines developed more recently, especially for the SW process, have not been evaluated. Moreover, the potentially harmful effects of amine emissions, such as the formation of nitrosamines and nitramines via photooxidation in the atmosphere, which could harm human health and the environment, have been observed.[112,113] The amine structure plays a critical role in the potential for the formation of these by-products. Studies have shown that secondary and tertiary amines have a higher propensity to form nitrosamines compared to primary amines.[114] Also, it was found that structural characteristics such as hydrophilicity and molecular configuration significantly affect toxicity.

Given these results, there is a clear need for comprehensive ecotoxicological assessments of both traditional and novel amines used in CO_2 capture technologies. Such assessments are necessary to fully understand their environmental impacts and develop mitigation strategies that minimize potential risks to aquatic ecosystems and human health.

1.2.5 Cytotoxicity

Cytotoxicity is an important toxicological assay to estimate disturbances in cellular homeostasis that may lead to cell death due to necrosis or apoptosis.[115] Cytotoxic effects may be caused by disturbances in basal cellular mechanisms and cell integrity, such as cell morphology, cell viability, cell growth, metabolic rate, and transcription of genes controlling basal functions. Compared to the ecotoxicity tests that uses organisms with various biological complexity, cytotoxicity testing focuses specifically on the cellular level and is typically conducted *in vitro* using mammalian cell lines. Mammalian cell-based assays provide important information at the cellular level, allowing for the early detection of compounds that may pose a risk to human health. Commonly used cell lines in cytotoxicity tests include immortalized cell lines such as HeLa (human cervical cancer cells), HepG2 (human hepatocellular carcinoma), Caco-2 (human colon adenocarcinoma), NIH/3T3 (mouse fibroblast), and CHO (Chinese hamster ovary).[116] These lines are usually chosen because of their ease of reproducibility and rapid growth, also, they are well characterized and widely used in research. The level of toxicity is usually evaluated by *in vitro* assays such as MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay or LDH (lactate dehydrogenase) release assay.[117,118]

The MTT assay is one of the most commonly used *in vitro* methods for assessing cytotoxicity by quantifying cell viability and metabolic activity. It is based on the principle that metabolically active cells can reduce the yellow tetrazolium salt of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan crystals by mitochondrial dehydrogenase enzymes. The amount of formazan produced is directly proportional to the number of viable cells because enzymatic reduction occurs only in living cells. Although the MTT assay is widely used due to its simplicity, reproducibility, and cost-effectiveness, it requires a solubilization step and can be affected by some test compounds. To overcome these limitations, related assays such as the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay, the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay, and the WST-1 (water-soluble tetrazolium salt-1) assay have been developed, offering soluble formazan products and simplified protocols.[119–121] In addition, fluorescent-based assays such as resazurin (commonly known as Alamar blue), calcein-AM (calcein acetoxymethyl ester), and annexin V-FITC/propidium iodide staining provide higher sensitivity and differentiate apoptotic and necrotic cells, although they are more expensive and require specialized equipment.[122–124] Despite these alternatives, the MTT assay still remains a reliable and affordable method for cytotoxicity screening.

The half-maximal inhibitory concentration (IC_{50}) is a key parameter obtained from the MTT assay and other methods that assess cell viability, metabolic activity, or cytotoxic endpoints. It indicates the concentration of the test substance that causes a 50% reduction in cell viability compared to untreated controls. IC_{50} is widely used to compare the potency and toxicity of different chemicals or pollutants. Lower IC_{50} values correspond to higher cytotoxicity, while higher values indicate less effect on cell viability. This makes the MTT assay a reliable and reproducible method for screening toxic effects and determining safe exposure levels in toxicological and other studies.

1.2.6 Lignin-derived monomers cytotoxicity

Evaluation of the human health safety of bio-based monomers and polymers is necessary to ensure their safety in their future use as packaging and consumer materials, as well as biomedical applications. Although these materials are often considered more environmentally friendly than their fossil-based analogs, their biological interactions, especially at the cellular level, still need to be assessed.

Vanillic acid and syringic acid are phenolic acids that can be produced from lignin by oxidation and used in bio-based polymer development.[125] Vanillic acid had no cytotoxic effect on Hepatoma Tissue Culture (HTC) cells (cancer cell line) over a range of concentrations (1–100 μM) or after different exposure times (24, 48, 72, and 96 h.) as measured by cell viability using the MTT assay.[126,127] In human lymphocytes, vanillic acid demonstrated protective effects by significantly reducing hydrogen peroxide (H_2O_2)-induced DNA damage.[127] At the same time, syringic acid exhibited cytotoxic effects on different cancer cell lines. For

instance, syringic acid is cytotoxic at varying concentrations (5–40 µg/mL) against gastric cancer cells with an IC_{50} of 30 µg mL^{-1}.[128] In another study, the cytotoxic effect of syringic acid against the human hepatoma cell line (HepG2) was assessed using the MTT assay.[129] At 100 µM concentration after 24 h. of syringic acid treatment, a significant inhibitory effect (> 80%) on the growth of HepG2 cells was observed compared to untreated control cells. It was concluded that syringic acid can induce cytotoxicity in HepG2 cells by apoptosis via the mitochondrial pathway. 4-hydroxybenzoic acid is another phenolic derivative of lignin that can be isolated by hydrolysis.[130] 4-hydroxybenzoic acid was tested against breast cancer cell lines with a range of concentrations (0–40 µg mL^{-1}) for 24 h using the MTT assay.[131] The results demonstrated that it induced apoptosis in the cancer cells, indicating its potential as a cytotoxic agent targeting breast cancer.

1.2.7 Biofilms on plastic

Biofilm is a complex community of microorganisms (such as bacteria and micro-eukaryotes) attached to an inert (e.g., plastics, glass, rocks) or living surface and embedded in a self-produced matrix of extracellular polymeric substances (EPS) (Fig. 8).[132] EPS is mainly composed of exopolysaccharides, lipids, proteins, nucleic acids, and other biomolecules produced by microorganisms during growth, reproduction, and lysis.[133] The EPS matrix helps the microbial community keep biofilm stability, adhere to surfaces, transport nutrients and waste, and protect from environmental threats.

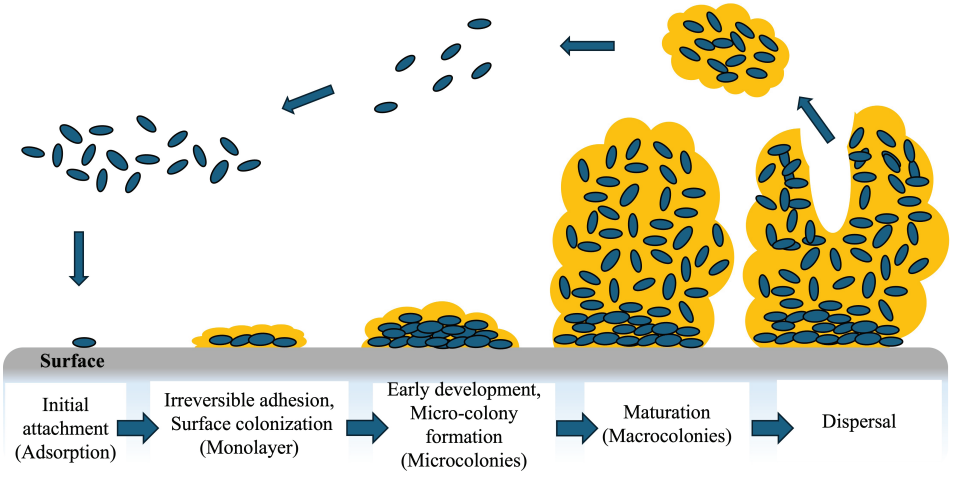


Fig. 8. Biofilm formation (adopted from ref. [134])

Biofilms easily accumulate on plastic materials present in the environment and have been demonstrated to be essential for microbial function and ecological processes in freshwater and terrestrial environments. Microorganisms can be one of the initial steps in the biodegradation of polymers.[135] This occurs under the action of enzymes generated by bacteria and is also dependent on the concentration of oxygen and biofilm density. Several known enzymes are involved in plastic degradation and can be classified as hydrolases and oxidoreductases.[136,137] Hydrolases, which include lipases, esterases, cutinases, and ureases, degrade hydrolyzable plastics such as PET, PUR, and PLA with important enzymes produced by *Candida antarctica* and *Aspergillus tubingensis*.[138–141] Ureases, a common soil enzyme important in the nitrogen cycle, have been identified as key contributors to PUR degradation.[142] Oxidoreductases, including laccases, peroxidases, and monooxygenases, oxidize polyolefins such as PE and PVC, with laccase from *Rhodococcus rubber C208* and manganese peroxidase from *Trichoderma harzianum* playing important roles.[143]

On the other hand, biofilms may operate as a place for harmful microbes, e.g., pathogens and antimicrobial-resistant bacteria (ARB), allowing them to persist and spread in the environment.[144–146] These microbial communities protect implanted bacteria from environmental stresses, antibiotics, and disinfectants, which improves their survival and resistance.[147] Studies have identified antimicrobial resistance genes ARGs, including sulfonamides (*sul1*, *sul2*), aminoglycoside (*strB*), β-lactams (*blaOXA*, *blaTEM*), macrolides (*ermB*), tetracyclines (*tetA*, *tetW*), and quinolones (*qnrS*), in biofilms on plastics such as PE, PP, PVC, and polyhydroxyalkanoates (PHA), often associated with bacterial genera such as *Pseudomonas*, *Bacillus*, *Mycobacterium*, and *Vibrio spp.*.[146,148,149] Pathogens such as *Aeromonas salmonicida*, *Aquabacterium*, and *Denitratisoma* were identified in the plastispheres, potentially posing threats to fish and human health.[150,151]

2. AIM OF THE STUDY

The current thesis aims to provide an environmental hazard assessment of novel bio-based monomers and their corresponding polymers, and amines that are used in the CO_2–capturing process and in the "switchable water" process. The specific aims of the study were as follows:

Paper I: Evaluate the ecotoxicity of isosorbide acrylate and methacrylate monomers, their corresponding polymers, and industrial isosorbide methacrylate-containing emulsion polymers

Paper II: Evaluate the ecotoxicity of lactate amide acrylic monomers and polymers developed from PLA bags

Paper III: Evaluate the cytotoxicity of lignin-derived methacrylate monomers and their corresponding polymers

Paper IV: Conduct ecotoxicity assessment of amines used in CO_2-capturing and "switchable water" processes

Paper V: Analyze and compare the structure and composition of biofilms formed in aquatic and terrestrial environments on different conventional and bio-based polymers, and coated paperboards used as packaging materials

3. METHODS AND EXPERIMENTAL DETAILS

Toxi-chromo testTM: bacterial chromo inhibition test using *Escherichia coli*

This analysis is based on the test compound's ability to inhibit the de novo synthesis of inducible β-galactosidase in a highly permeable mutant of *E. coli*.[152] Tests are carried out in 96-well plates in the kit's standard diluent. Mercury chloride [4–0.6 μg mL^{-1}] is used as a positive, highly toxic control. Samples with the bacterial mixture are incubated at 37 °C for 90 minutes. During the first incubation, the bacteria consume the sample components and attempt to induce the production and excretion of β-galactosidase. Next, a chromogen solution is added, lysing the cells and forming a blue color due to the presence of β-galactosidase. The color intensity, a measure of the toxicity, was recorded at 600 (±20) nm by a spectrophotometer.

WaterTOX^{TM} STD: bacterial luminescence inhibition test using *Alivibrio fischeri*

Toxicity of samples towards the bioluminescent marine bacterium *A. fischeri* was measured by comparing initial and final light emission after 15 min according to the ISO standard.[91] The toxic effect caused by a decrease in cellular metabolism is expressed as a decrease of the luminescence intensity. Tests were carried out at 15 °C in the kit's standard diluent. A series of dilutions were prepared for each sample according to the manufacturer's instructions.

Growth inhibition test using vascular plants *Spirodela polyrhiza*

This test is based on the measurement of growth retardation of the germinated dormant vegetative buds (turions) after 3 days of exposure to samples according to the ISP standard.[153] The tests were carried out on a 48-well plate containing a dilution series of tested samples at 25 °C in the plant growth chamber, using an illumination system enabling at least 6000 lux. A digital image of the multiwell plate was taken at the start of the test and after the incubation to determine the size of the vegetative buds (turions) before and after incubation by Image Analysis (Image J, National Institute of Mental Health, Bethesda, Maryland, USA, software for image processing and analysis). Next, by comparing the data obtained from the test plate, the growth inhibition of the duckweeds was calculated by subtracting the mean of the "initial" surface area of the first frond from the mean "final" surface area, in the control and at various concentrations of diluted samples. The 72 h EC_{50} concentration of the compound was obtained from the percentage of growth inhibition of the duckweed.

Crustacean toxicity screening test for freshwater using *Daphnia magna*

This test determines the lethal effects of toxicants on the *D. magna* after 48-h exposure. The 48-h immobilization test was performed in a multi-well test plate using the neonates of *D. magna* hatched from ephippia based on the ISO standard.[89] Ephippia hatching was initiated before the start of the toxicity test in a Petri dish with Standard Freshwater medium at 25 °C for 72-h, under continuous illumination (at least 6000 lux). Two hours before collecting the neonates for the test, they were pre-fed with *Spirulina* powder. The test incubation was carried out on a multiwell plate containing a dilution series of the tested samples in darkness for 48 hours. The number of immobilized (dead) organisms was counted after 48-h under a microscope (magnification 10–12×). The obtained data was used to determine the EC_{50} values.

Thamnotoxkit F: crustacean toxicity screening test for freshwater using *Thamnocephalus platyurus*

This test determines the lethal effects of toxicants on the *T. platyurus* after 24 h exposure. The 24 h immobilization test was performed in a multiwell test plate using the fairy shrimp *T. platyurus* hatched from cysts based on ISO standard.[154] Cyst hatching was initiated before the start of the toxicity test in a Petri dish with Standard Freshwater medium at 25 °C for 20–22 h, under continuous illumination (at least 3000 lux). The test incubation was carried out on a 24-well plate containing a dilution series of the monomers or polymers in the dark for 24 hours. The number of immobilized (dead) organisms was counted after 24 h under a microscope (magnification 10–12×).

Determination of EC_{50}

The level of toxicity of a substance was determined by establishing the effective concentration, EC_{50}. The EC_{50} values for monomers and polymers were defined as mg L^{-1} and the evaluation of toxicity followed the toxicological categories adopted by the European Commission.[155] According to this classification, the categories of aquatic toxicity are the following: very toxic: $EC_{50} < 1$ mg L^{-1}, toxic: $EC_{50} = 1–10$ mg L^{-1}, moderately toxic: $EC_{50} = 10–100$ mg L^{-1}, practically harmless: $EC_{50} = 100–1000$ mg L^{-1} and harmless compounds with $EC_{50} > 1000$ mg L^{-1} in this study.[156] The EC_{50} data were represented by mean values, and confidence intervals (95% CI) were calculated for each concentration.

All tested monomers and polymers were powders. They were dissolved in the tested medium directly or in a water/DMSO mixture (85:15 or 70:30, v/v). The potential background effect of DMSO on the test results was also evaluated and found to be negligible. All samples were tested in triplicate for each assay to ensure test reproducibility.

A comparison between tested substrates was analysed using one-way ANOVA, and a post-hoc pairwise comparison using Tukey test in R (version 3.6.0) using aov( ) and TukeyHSD( ) on EC_{50} data.

Cytotoxicity test

The cytotoxicity test of the tested materials was performed by using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (Sigma-Aldrich) assay. Human cervical carcinoma HeLa cells were plated to the 96-well plates at 5000 cells/well in 100 µL DMEM with 1% penicillin-streptomycin and 10% fetal bovine serum (FBS) and incubated at 37 ºC in 5% CO_2 and 100% relative humidity. After 24-h incubation to ensure adhesion, 100 µL of the tested material solution in DMEM was added for the cell cytotoxicity assay. Culture medium without additives was used as a negative control, and for the positive control, medium with 2% H_2O_2 was used. The samples were dissolved in DMSO at the concentration of 50 mg mL^{-1}. These sample solutions were diluted with the culture medium to concentrations of 20, 40, 100, and 200 µg mL^{-1} for monomers and 200, 500, 750, and 1000 µg mL^{-1} for polymers. After the cells were exposed to the monomers and polymers for 24 and 48 h respectively, the medium was removed. Next, 100 µL of fresh culture medium and 20 µL of MTT dissolved in phosphate-buffered saline (PBS) solution at a concentration of 5 mg mL^{-1} were added to each well. After 2 h of incubation, the medium was removed, and 200 µL of DMSO was added to each well to resuspend intracellularly stored MTT formazan. The optical density of formazan product was measured at 560 nm (OD_{560}). Relative cell viability was calculated using the following equation: relative cell viability (% of control) = (OD_{560} treated wells/OD_{560} control) × 100. All samples were tested 5 times to ensure test reproducibility. To determine the IC_{50} values of tested compounds, a dose-response curve of relative cell viability versus compound concentration was plotted. The IC_{50} was defined as the concentration that reduced MTT-formazan production by 50% compared to the untreated control. The IC_{50} values of materials were calculated using ED50plus v1.0 software.

Experimental setup for biofilm formation in river water

A river water biofilm experiment was conducted in the river Emajõgi, in Tartu, Estonia. In this study, materials were inserted separately into the cylindrical nets. All the samples were triplicate, and in total, we had 39 samples. Horizontally oriented nets were attached to the wooden frame in approximately 10 cm intervals and immersed 15 cm from the water surface. The frame had buoys and was attached to the wooden pier with a rope (Fig. 9). The long ropes gave the mounts the opportunity to slide vertically, and the exposure samples maintained the same position during the experiment. All samples were collected after 2 months of exposure together with 3 samples of river water. The river water samples were

taken from the water surface using Sterivex^{TM} presser filter with a pore size of 0.22 µm. The experiment was conducted from May 2021 to July 2021.

a) b)

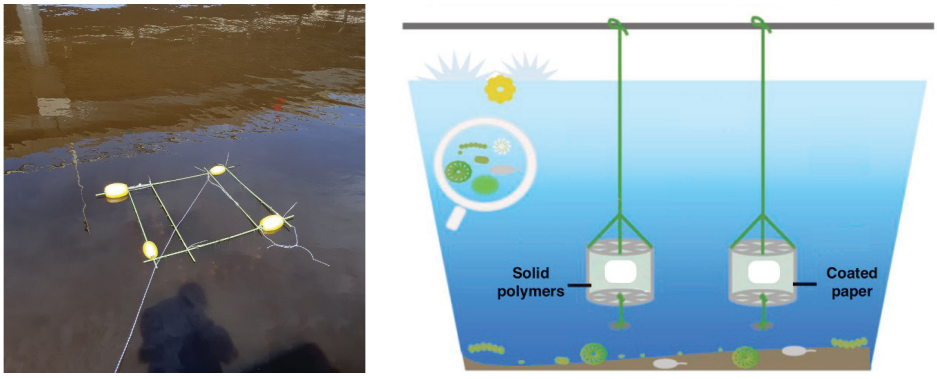


Fig. 9. a) Wooden frame with attached nets and buoys; b) scheme of attached nets.

Experimental setup for biofilm formation in soil

A soil exposure biofilm experiment was conducted in Rõhu test station, Estonian University of Life Sciences, Estonia, between July 2022 and April 2023. In this study, test materials were placed individually into nylon bags, which were then buried at a depth of approximately 15 cm in soil (Fig. 10). The experiment was carried out across four types of soil: compost 1 (com 1), compost 2 (com 2), mineralized soil (min), and untreated control soil (cont). Nylon bags were placed in four separate plots for each soil type. Half of the samples were collected after three months of exposure (first time point, 1tp), and the remaining samples were collected after nine months (second time point, 2tp). All materials were tested in triplicate. In total, 44 samples were collected at 1tp and 53 samples at 2tp.

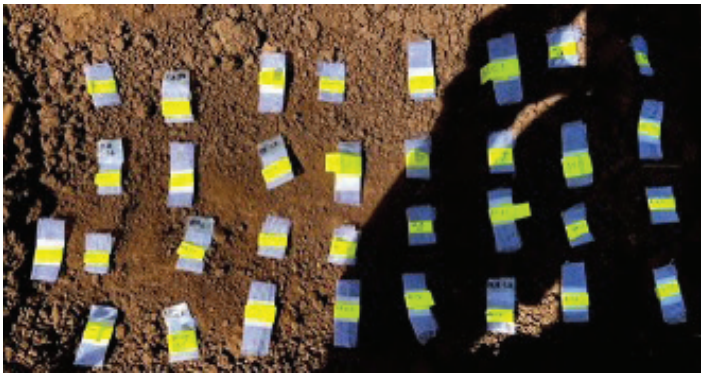


Fig. 10. Nylon bags with samples

Extraction of biofilm community DNA

The plastic and paperboard samples from river and soil biofilm formation experiments were stored at –84 °C until further processing. DNA extractions were carried out using DNeasy PowerWater Kit (Qiagen) according to the manufacturer's instructions. The amount and quality of the DNA were controlled with a NanoDrop™ UV–Vis spectrophotometer.

16S rDNA gene amplicon library preparation

The bacterial community was analyzed using a 2-step PCR amplicon library preparation and sequencing on an Illumina platform (MiSeq). First, amplicons were obtained in a 30 cycle PCR using the bacterial V3-V4 region of the bacterial 16S rRNA gene with universal primers Bakt_341F and Bakt_805R with Illumina TruSeq adapters added to these primers. In the second step, the amplicons were tagged using a combination of tails (P5/P7) including an 8 bp index sequence unique to each sample.

Bioinformatics and statistics

Sequencing data analysis was conducted using the QIIME2 bioinformatics pipeline.[157] Quality control, read pairing and chimera sequence removal were performed with the q2-dada2 plugin.[158] The 16S rRNA sequences were classified using the Greengenes2 release 2022.10 taxonomy with the q2-feature-classifier plugin.[159,160] Sequence alignment was done using the q2-alignment plugin before constructing a phylogeny with the FastTree method via the q2-phylogeny plugin.

General data processing and statistical analyses were performed in R. For alpha diversity analyses, the abundance table was assigned as an ASV table with taxa as rows, the taxonomy information was added as a taxonomy table, and the sample metadata was incorporated as a sample data object. Taxa identifiers were assigned to row names to ensure consistency across datasets. Alpha diversity indices, including observed richness and the Chao1 estimator, were calculated using the estimateR() function from the vegan package. Chao 1 plot was visualized with ggplot2. For beta diversity analyses, the phyloseq object was filtered to retain ASVs in at least one sample with a minimum abundance of 10 reads to focus on the most prevalent taxa. The data was then transformed using the centered log-ratio (CLR) transformation via the microbiome::transform function to mitigate the compositional nature of amplicon data. Redundancy Analysis (RDA) was performed using the vegan::rda function to explore the variation in community composition explained by environmental factors. The significance of RDA model terms was assessed using ANOVA with the anova.cca function. Ordination plots were generated using phyloseq::plot_ordination and visualized with ggplot2. For taxonomic composition analysis, the phyloseq object was taxonomically agglomerated at the Phylum, Class, Order, Family, and Genus levels using phyloseq::tax_glom. Relative abundance transformations were applied

to the phyloseq object using phyloseq::transform_sample_counts. Taxonomic summaries at theFamily levels were visualized using bubble plots created with ggplot2::geom_point, with point size representing relative abundance and color indicating materials type. Venn diagrams were generated using the MicEco::ps_venn function to visualize the LefSe and CAP taxa across sample types.

4. RESULTS AND DISCUSSIONS

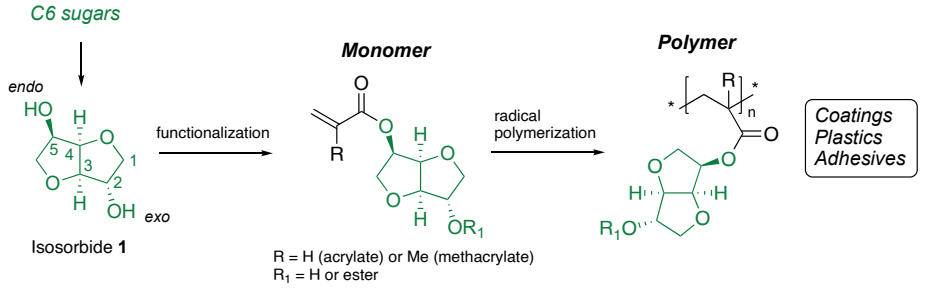
Even though bio-based materials are produced from bio-based feedstock, they may still pose a risk to the environment. The toxicity of the compounds is related to the structure, not origin. Therefore, thorough ecotoxicity assessment is essential to ensure that these materials align with sustainability goals. This thesis presents a series of studies evaluating the environmental and human health impacts of novel bio-based monomers, polymers and compounds used in low carbon technologies. First, the ecotoxicity study of isosorbide acrylate and methacrylate monomers and corresponding polymers was carried out. Secondly, the ecotoxicity of isosorbide methacrylate-containing emulsion polymers and industrially relevant SA latex (CHP BAR 1400) was investigated. Next, the ecotoxicity of lactate amide acrylic polymers developed from PLA bags was evaluated. Thirdly, the cytotoxicity of methacrylate lignin-based monomers and polymers was investigated because of their potential for use in a variety of fields, including biomedical ones, where the potential impact on humans requires assessment using mammalian cell lines. Fourthly, amines used in the CO_2 capturing and in the switchable water process were provided with ecotoxicological risk assessment. Across all studies, common methodologies such as standardized ecotoxicity tests and *in vitro* cytotoxicity assays were employed, allowing for a comparative and comprehensive evaluation of the different new bio-based materials. Finally, the biofilm formed on the different bio-based polymers and conventional polymers in the water and soil was investigated.

4.1 Ecotoxicity of isosorbide acrylate and methacrylate monomers and corresponding polymers (Paper I)

4.1.1 Development of isosorbide acrylate and methacrylate monomers, corresponding polymers and industrial based latex

The ecotoxicity of the isosorbide-based materials was evaluated toward several aquatic organisms with various biological complexities, including bacteria (*E. coli*, *A. fischeri*), vascular plants (*S. polyrhiza*), and invertebrates (*T. platyurus*).

Four isosorbide-based monomers were synthesized in our research group (Scheme 5), where the polymerization group is only attached to *endo* hydroxyl group: isosorbide 5-methacrylate (**IM**), isosorbide 5-methacrylate 2-acetate (**IMA**), isosorbide 5-acrylate (**IA**), and isosorbide 5-acrylate 2-acetate (**IAA**) (Fig. 11). The corresponding homopolymers were obtained by radical polymerization (**PIM**, **PIMA**, **PIA**, and **PIAA**) (Fig. 11). The polymers tested in this work have different average molecular weights (*Mn*), which varied from 4.8 to 69.3 kg mol^{-1}.



Scheme 5. Conversion of isosorbide into mono-(meth)acrylate monomers and subsequent radical polymerization.

Also, isosorbide methacrylate emulsion polymers **IMA-latex**, **IMP-latex**, and **IMB-latex** were generously provided by CH-Polymers (Finland). The polymerization process of isosorbide methacrylate emulsion polymers was analogous to the standard industrial production of styrene-acrylate (SA) latex (CHP BAR 1400), except 50% of the styrene monomer was replaced by isosorbide 5-methacrylates, either by the 2-acetate (**IMA**), 2-propionate (**IMP**), or 2-butyrate (**IMB**) derivative (Fig. 11). SA latex is widely used as a food-grade water and oil resistant coating in paper and cardboard food packaging (e.g., coffee cups, paper plates, pizzaboxes, etc.). All samples were synthesized using semi-continuous emulsion polymerization in laboratory-scale glass reactors.

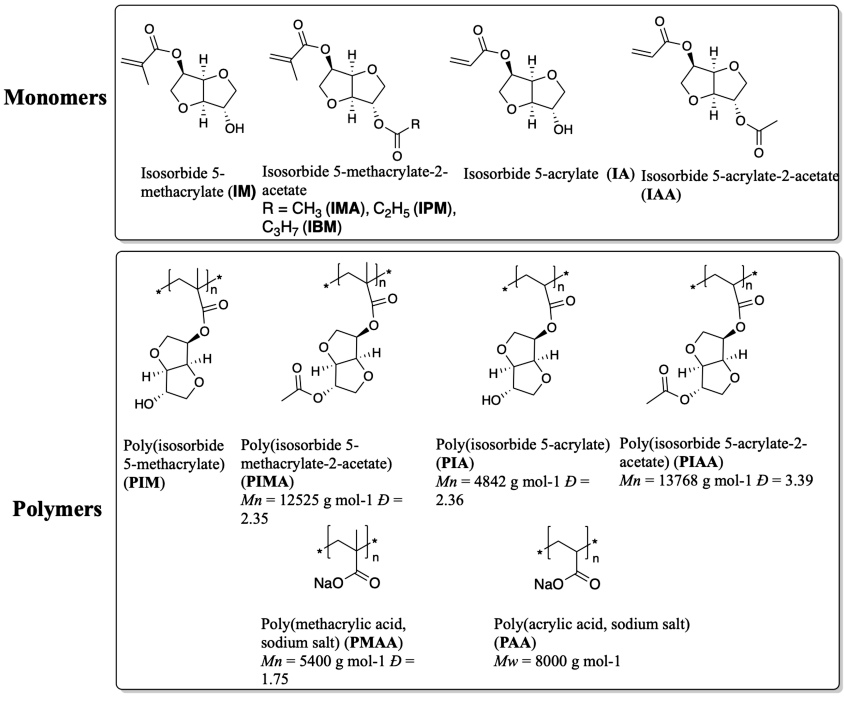


Fig. 11. The chemical structures, abbreviations, names, and molecular weights of isosorbide acrylate and methacrylate monomers and corresponding polymers.

Aqueous solutions of poly(methacrylic acid) (**PMAA**) and poly(acrylic acid, sodium salt) (**PAA**) were tested in this work as possible products of hydrolytic cleavage of the studied isosorbide-based polymers.

To conduct the aquatic toxicity tests, the solubility of the compounds in water was first determined. **IM**, **IA**, **PIA**, and the sodium salts of **PMAA** and **PAA** were determined to be fully soluble in water. **IMA**, **IAA**, **PIMA**, and **PIAA** were dissolved in a water/DMSO mixture (85/15, v/v), and **PIM** was dissolved in a water/DMSO mixture (70/30, v/v).

4.1.2 Toxicity evaluation of isosorbide acrylate and methacrylate monomers

The isosorbide methacrylate monomers **IM** and **IMA** exhibited low toxicity towards bacteria (*E. coli* and *A. fischeri*, Fig. 12a and b, respectively), vascular plant (*S. polyrhiza*, Fig. 12c), and invertebrate *(T. platyurus*, Fig. 12d) and can be considered as harmless. Except for one case where a certain effect of the acetate group in **IMA** on *S. polyrhiza* turions was found with the EC_{50} value 139 (95% confidence interval (CI): 115.8; 162.9) mg L^{-1}, which is below the threshold of the practically harmless range. In the same test, monomer **IM** without the acetate group has an EC_{50} value above 1000 mg L^{-1}. It is possible that the slightly higher toxicity of the acetate derivative **IMA** is potentially due to the release of acetic acid upon de-esterification, catalyzed by carboxylesterases present in the plants.[161]

In contrast, replacing the methacrylate group with the acrylate group significantly affected the toxicity level toward the tested organisms (Fig. 12). In the bacterial tests, two types of bacteria were used: *E. coli*, which evaluates toxicity based on inhibition of β-galactosidase synthesis, and *A. fischeri*, which measures toxicity through the reduction of bacterial bioluminescence. In the test with *E. coli* acrylate with free –OH group (**IA)** showed moderate toxicity with EC_{50} = 16 (95% CI: 8.8; 24) mg L^{-1}, compared to acrylate with the acetate group (**IAA**), which is practically harmless with EC_{50} = 124 (95% CI: 114; 158) mg L^{-1} (Fig. 12a). However, in the test with another bacteria, both acrylate monomers **IA** and **IAA** showed a practically harmless effect toward *A. fischeri* [EC_{50} = 456 (95% CI: 418; 511) mg L^{-1}, EC_{50} = 585 (95% CI: 420; 749) mg L^{-1}, Fig. 12b].

Both isosorbide acrylate monomers **IA** and **IAA** were toxic to *S. polyrhiza* with EC_{50} values 9 mg L^{-1} (95% CI: 5.8; 12.2) and 9.6 mg L^{-1} (95% CI: 6.8; 11.3) (Fig. 12c). In the test with invertebrates, **IA** was also toxic to *T. platyurus* [EC_{50} = 8.7 (95% CI: 6.5; 11.1) mg L^{-1}], while **IAA** was slightly less toxic and showed a moderately toxic effect [EC_{50} = 15.6 (95% CI: 8.46; 22.88) mg L^{-1}] (Fig. 12d).

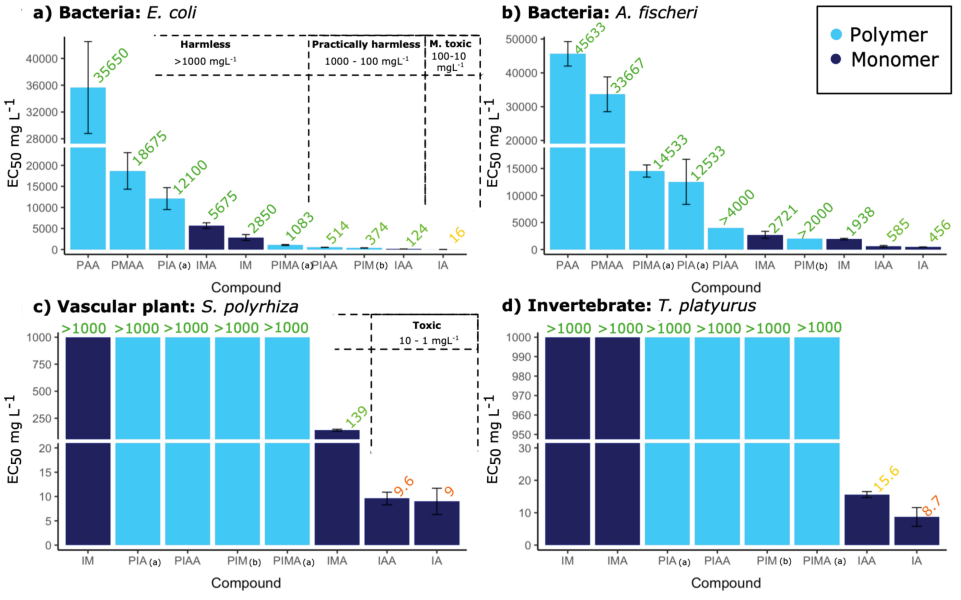


Fig. 12. Values of the mean effective concentration (EC_{50}, mg L^{-1}) of the tested monomers (light-blue columns) and polymers (dark-blue columns). The compounds are ordered according to the level of toxicity[156]: values in green represent the harmless (EC_{50} values > 1000 mg L^{-1}) and the practically harmless (EC_{50} values 100–1000 mg L^{-1}) compounds; values in yellow denote moderately toxic (EC_{50} values 10–100 mg L^{-1}) compounds and values in orange designate toxic compounds (EC_{50} values 1–10 mg L^{-1}).

4.1.3 Toxicity evaluation of isosorbide acrylate and methacrylate polymers

The ecotoxicity of corresponding isosorbide-based polymers **PIM**, **PIMA**, **PIA**, and **PIAA** showed that they exhibited harmless or practically harmless effects on all tested organisms (Fig.12a-d). The possible effect of polymers on the toxicity level can be caused by the leaching of unreacted monomers from polymer materials. Therefore, it is important to ensure that any residual monomer in polymers is removed. The toxicity observed for isosorbide-based acrylic monomers (**IA** and **IAA**) hasn't extended to the corresponding polymers (**PIA** and **PIAA**). Tests on vascular plants (*S. polyrhiza*) and invertebrates (*T. platyurus*) showed the polymers to be harmless, with EC_{50} values over 1000 mg L^{-1}, the highest concentration tested. There wasn't an obvious correlation found depending on the polymer backbone structure (polymethacrylate vs. polyacrylate) or isosorbide side chain (2-OH vs. 2-OAc) with the measured EC_{50} values.

The next step was to investigate the impact of different molecular weights (M_n) of the polymers **PIM**, **PIA**, and **PIMA** on their toxicity using the *E. coli* inhibition test (Table 1). All tested polymers showed a harmless (**PIA** and **PIMA**) or practically harmless (**PIM**) level of toxicity. It was found that the EC_{50} values

increase with increasing M_n. For instance, in the case with **PIMA**, where M_n increased roughly 5 times from 12.5 to 64.2 kg mol^{-1}, the EC_{50} increased from 1083 to 1895 mg L^{-1}. It can be considered that the effect is relatively small. This trend can be explained by the fact that with an increase in molecular weight, the bioavailability of the material decreases.[162]

Table 1. Ecotoxicology results of isosorbide-based polymers with different molecular weights in the bacterial chromo inhibition test with *E. coli*.

Entry	Polymer name	M_n, kg mol^{-1}	$Đ$	EC_{50}, mg L^{-1} (95% CI)
1	**PIM(a)**	36.1	2.03	319.5 (153; 486)
2	**PIM(b)**	69.3	1.3	374 (280; 468)
3	**PIA(a)**	4.8	2.36	12100 (5757; 18443)
4	**PIA(b)**	42.9	2.2	16410 (12026; 20794)
5	**PIA(c)**	55.1	1.8	18973 (15507; 22438)
6	**PIMA(a)**	12.5	2.35	1083 (800; 1364)
7	**PIMA(b)**	45.7	1.95	1215 (917; 1514)
8	**PIMA(c)**	64.2	1.9	1895 (1260; 2530)

In addition, the polyacrylic (**PAA**) or methacrylic acid (**PMAA**) sodium salts were evaluated in bacterial tests with *A. fischeri* and *E. coli*, and found these polymers to be harmless (Fig. 12a,b). These two salts can be formed during the possible degradation pathway of isosorbide-based polymethacrylates and acrylates due to hydrolytic cleavage of the ester bond between isosorbide and the polymer backbone.

4.1.4 Toxicity evaluation of isosorbide-based industrial latex

The industrially relevant isosorbide methacrylate-containing polymer dispersion samples **IMA-latex**, **IMP-latex**, and **IMB-latex**, and the standard styrene-acrylate-based latex (**SA-latex**), CHP BAR 1400, were evaluated using bacterial test with *A. fischeri*. This test was chosen because of its compatibility with the turbid dispersion of the tested materials. The results indicate that isosorbide-containing latexes have a considerably high EC_{50} value and have a harmless effect on the bacteria (Table 2, entries 1–3). However, for the commercial **SA-latex**, it was not possible to establish an EC_{50} value due to the lack of any effect on bacteria even at the highest concentration tested (Table 2, entry 4). The difference between the commercial sample and the isosorbide-based samples may be due to the presence of small amounts of unreacted isosorbide methacrylate monomers remaining in the emulsion. It was found that the length of the side chain of isosorbide monomers had a certain effect on the *A. fischeri*. As the side chain of isosorbide alkanoate was increased from acetate (C2) to propionate (C3) and butyrate (C4), the EC_{50} values increased, indicating a decrease in toxicity with decreasing monomer hydrophilicity. This trend is similar to the results for acrylic acid esters, where a similar decrease in aquatic toxicity was observed with increasing alkyl chain length.[163]

Table 2. Effect of industrially prepared latexes on *A. fischeri*.

Entry	Sample name	Sample description	Process type	WaterTOX™ *A. fischeri*	
				Toxicity[a]	EC_{50}, g L^{-1}[b] (95% CI)
1	**IMA-latex**	SA-latex, 50% of styrene replaced by IMA	lab synthesis	Harmless	158.2 (141.3; 175)
2	**IMP-latex**	SA-latex, 50% of styrene replaced by IMP	lab synthesis	Harmless	245.5 (157.2; 333.8)
3	**IMB-latex**	SA-latex, 50% of styrene replaced by IMB	lab synthesis	Harmless	451.5 (372.1; 575.8)
4	**CHP BAR 1400**	Conventional SA-latex binder	production grade	Harmless	>500[c]

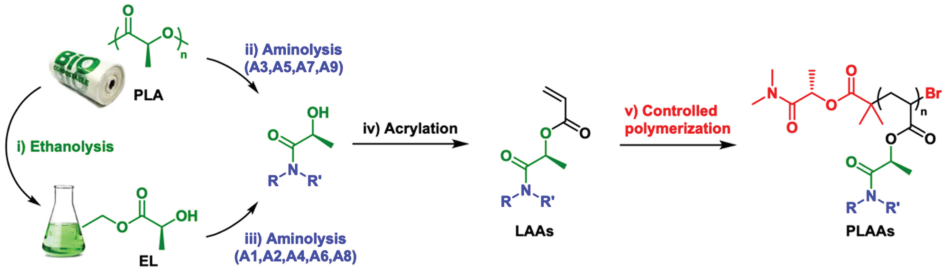
[a] H – harmless (EC_{50} values > 1000 mg L^{-1}). [b] Weight corresponds to the total solids of latex in 1 L of latex. [c] EC_{50} value could not be observed even at the undiluted samples (i.e., at maximum viable concentration).

4.2 Ecotoxicity of lactate amide acrylic polymers developed from PLA bags (Paper II)

4.2.1 Development of lactate amide acrylic polymers from PLA bags

Commercially available PLA bags were chemically depolymerized in two ways (Scheme 6). In the first case, PLA was depolymerized directly by aminolysis with the corresponding amines. In the second case, ethyl lactate (EL) was first obtained from PLA, and then aminolysis was carried out with the corresponding amines. As a result of depolymerization, lactate amides were obtained, which were subsequently converted into acrylic monomers (**LAAs**).

The corresponding poly(lactate amide acrylates) (**PLAAs)** were obtained using a Cu(II)Br_2/tris(2-dimethylamino)ethylamine (Me_6TREN)-mediated controlled radical polymerization under UV light.



Scheme 6. Synthesis of monomers via depolymerization of PLA by 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) catalyzed aminolysis or alcoholysis, followed by acrylation and subsequent controlled polymerization mediated by Cu(II)Br_2/Me_6TREN under UV-light

The resulting polymers exhibited thermoresponsive behavior, characterized by a lower critical solution temperature (LCST). The LCST could be modulated by altering the N-substituents on the lactate amide moiety. Polymers with more hydrophilic substituents displayed higher LCSTs, while those with hydrophobic groups had lower LCSTs. This tunability is essential for applications requiring specific temperature-responsive behaviors.

4.2.2 Toxicity evaluation of lactate amide acrylic monomers and polymers

The toxicity of a series of water-soluble lactate amide acrylic monomers and their corresponding polymers (Fig. 13) was evaluated toward aquatic organisms with different biological complexity: bacteria (*A. fischeri*), vascular plants (*S. polyrhiza*), and invertebrates (*T. platyurus*).

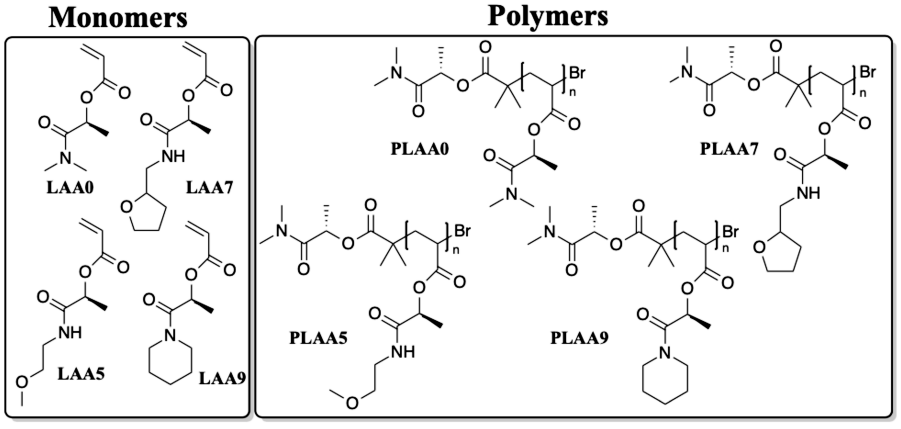


Fig. 13. The chemical structure and abbreviation of the tested lactate amide acrylic monomers and corresponding polymers.

Test results are summarized in Table 3 and indicate that tested monomers **LAA0**, **LAA5**, **LAA9** were harmless to *A. fischeri* with $EC_{50} > 8000$ mg L^{-1}. Only **LAA7** showed a practically harmless effect toward bacteria with $EC_{50} = 425$ (95% CI: 284; 566) mg L^{-1}. However, all tested monomers demonstrated moderately toxic effects toward *S. polyrhiza* [$EC_{50(LAA0)} = 17$ (95% CI: 15; 19) mg L^{-1}; $EC_{50(LAA5)} = 33$ (95% CI: 25; 40) mg L^{-1}; $EC_{50(LAA7)} = 16$ (95% CI: 12; 20) mg L^{-1}; $EC_{50(LAA9)} = 39$ (95% CI: 26; 49) mg L^{-1}]. Also, monomers exhibited toxic effects to *T. platyrus* [$EC_{50(LAA0)} = 6$ (95% CI: 4; 7) mg L^{-1}; $EC_{50(LAA5)} = 7$ (95% CI: 4; 9) mg L^{-1}; $EC_{50(LAA7)} = 5$ (95% CI: 5; 6) mg L^{-1}; $EC_{50(LAA9)} = 6$ (95% CI: 4; 8) mg L^{-1}]. The observed toxicity of the monomers is probably due to the acrylate group present in the monomers, because lactate esters (LE) and lactate amides (LA) such as ethyl lactate (EL) and dimethyl lactamide (DML) haven't shown any toxicity before. It is also correlated with the isosorbide acrylates, which also showed the same level of toxicity towards vascular plants and invertebrates. Acrylate groups – due to their reactive double bonds – can affect biological systems and metabolic processes, leading to toxic effects.[164,165] Notably, changes in the N-substituted side chains of the monomers did not result in significant differences in toxicity.

In contrast, the corresponding polymers (Fig. 12), despite being derived from these acrylate-based monomers, were classified as harmless or practically harmless among all tested organisms. This is consistent with the general classification

of acrylic polymers as non-toxic, although minor toxicity was sometimes associated with residual unreacted monomers or degradation by-products. Therefore, the observed low toxicity of the polymers reinforces the efficiency of the purification process and supports the conclusion that polymer backbone structure and side group variations had minimal impact on ecotoxicity, as reflected in the comparable EC_{50} values for all PLAAs tested.

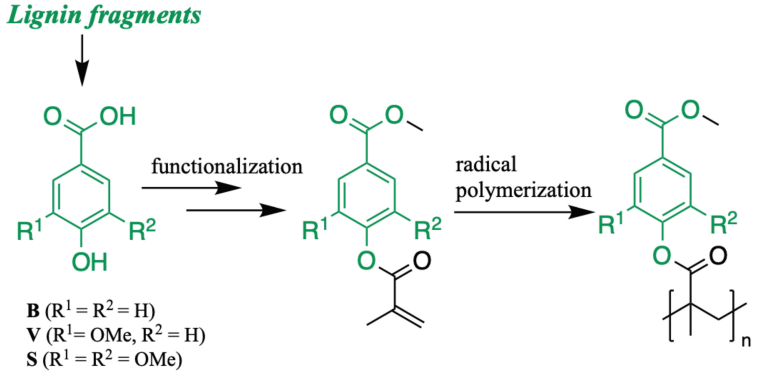
Table 3. Mean effective concentration (EC_{50}, mg L^{-1}) of the tested monomers and polymers on bacteria (*A. fischeri*), vascular plant (*S. polyrhiza*) and invertebrate (*T. platyurus*). Toxicity levels (Tox.) were stated as follows: H – harmless ($EC_{50} > 1000$ mg L^{-1}), PH – practically harmless ($100 < EC_{50} < 1000$ mg L^{-1}), MT – moderately toxic ($10 < EC_{50} < 100$ mg L^{-1}), and T – toxic ($1 < EC_{50} < 10$ mg L^{-1})

Entry	Sample name	*A. fischeri*		*S. polyrhiza*		*T. platyurus*	
		Tox.	EC_{50}, mg L^{-1} (CI 95%)	Tox.	EC_{50}, mg L^{-1} (CI 95%)	Tox.	EC_{50}, mg L^{-1} (CI 95%)
1	**LAA0**	H	>8 000	MT	17 (15; 19)	T	6 (4; 7)
2	**LAA5**	H	>8 000	MT	33 (25; 40)	T	7 (4; 9)
3	**LAA7**	PH	425 (284; 566)	MT	16 (12, 20)	T	5 (5, 6)
4	**LAA9**	H	>8 000	MT	39 (26; 49)	T	6 (4;8)
5	**PLAA0**	H	>20 000	H	>1000	H	>1000
6	**PLAA5**	H	14 638 (13696; 15579)	PH	533 (445; 619)	PH	394 (181; 606)
7	**PLAA7**	H	6677 (5995; 7358)	PH	325 (234; 415)	PH	263 (166; 360)
8	**PLAA9**	H	3915 (3764; 4066)	PH	440 (214; 667)	PH	252 (145; 359)

4.3 Cytotoxicity of aromatic polymethacrylates from lignin-based feedstock (Paper III)

4.3.1 Development of aromatic polymethacrylates from lignin-based feedstock

4-hydroxybenzoic acid, vanillic acid, and syringic acid are structurally related to lignin units and can be derived from lignin through oxidation processes. The methacrylate lignin-based monomers 4-hydroxybenzyl methacrylate (**BMA**), vanillin methacrylate (**VMA**), and syringyl methacrylate (**SMA**) were synthesized in two steps (Scheme 7). Corresponding lignin-based carboxylic acids were converted into methyl esters, followed by methacrylation of the phenolic hydroxyl group. The methacrylates obtained from the acylation with methacryloyl chloride in bio-based solvent 2-MeTHF have high yields and do not require chromatographic purification. The corresponding polymers (**PBMA**, **PVMA**, **PSMA**) were obtained by radical polymerization. The homopolymers exhibited high T_g values: **PBMA** at 106 °C, **PVMA** at 128 °C, and **PSMA** at 197 °C. These values are significantly higher than for **PMMA** (105 °C), indicating enhanced thermal performance. Rheological measurements demonstrated that the polymers could be processed in the melt, indicating their potential suitability as thermoplastic materials for a range of applications.



Scheme 7. Conversion of lignin-based carboxylic acids into methacrylate monomers and subsequent radical polymerization.

4.3.2 Cytotoxicity analysis of aromatic polymethacrylates from lignin-based feedstock

The possible cytotoxicity of monomers and polymers (Fig. 14) was studied on the HeLa (human cervical carcinoma) cell line. The viability of HeLa cells was measured using an MTT assay after 24-hour exposure to monomers and after 48-hour exposure to polymers. Based on the results, IC_{50} values for the chemicals were calculated. The higher the IC_{50} value, the lower the toxicity of the material to cells.

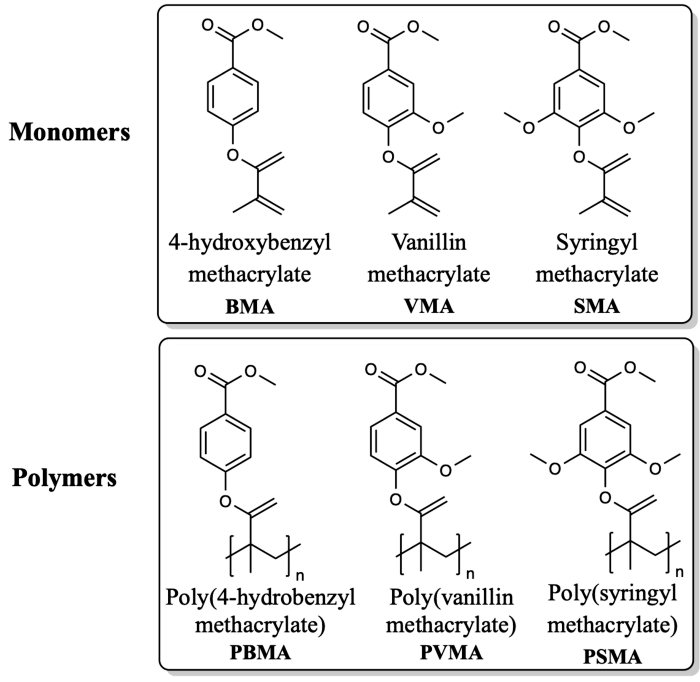


Fig. 14. The chemical structures and abbreviations of the tested lignin-based methacrylate monomers and corresponding polymers

The IC_{50} values for monomers were in the range from 42.8 to 95.1 μg mL^{-1} (Fig. 15). Those values are comparable to results obtained previously for other methacrylates.[166] All monomers showed a strong influence on cells compared to the corresponding polymers. The IC_{50} values for corresponding polymers were in the range from 577.6 to 962 μg mL^{-1} (Fig. 15). This difference between the polymer and the monomers occurs because the polymers do not contain the reactive methacrylate group, which may exhibit toxic effects on living organisms. At the same concentration of 200 μg mL^{-1} in the case of polymers, HeLa cells retained high viability after 48 hours, in the range from 86% to 100% (Fig.15 b) after 48 h of exposure to the polymers. In contrast, when exposed to the corresponding monomers for just 24 h, cell viability significantly decreased to 5–9% (Fig. 15a). At higher concentrations, all the polymers caused a reduction in cell viability (Fig. 15b). At 1000 μg mL^{-1}, 41–72% of cells remained viable after 48 hours of

treatment. These results are comparable to those observed for polymethylmethacrylate (**PMMA**) reference material. **PMMA** is a well-known polymer that is used as a bio-medical material and is considered to have very low cytotoxicity.[167,168]

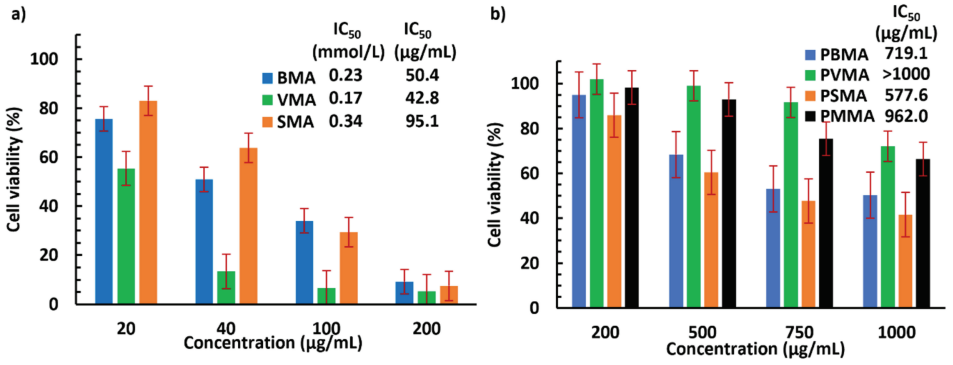


Fig. 15. HeLa cells viability after exposure to the methacrylate monomers during 24 h (a) and polymers during 48 h (b).

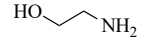
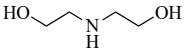
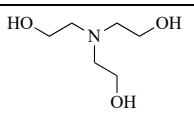
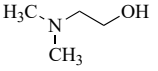
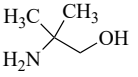
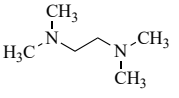
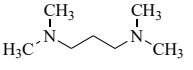
Therefore, **PBMA** and **PSMA** can be considered low-toxicity materials, while **PVMA** may be classified as non-toxic, as its IC_{50} value exceeds 1000 µg mL^{-1}.

4.4 Ecotoxicity of amines used in 'switchable water' and CO2-capturing processes (Paper IV)

4.4.1 Amines used in SW and CO_2-capturing processes

The ecotoxicity of structurally diverse amines utilized in emerging SW and CO_2 capture technologies was evaluated. The list of tested compounds (Table 4) includes primary monoethanolamine (**MEA**), secondary diethanolamine (**DEA**), tertiary triethanolamine (**TEA**), and dimethylethanolamine (**DMEA**), structurally hindered amino-2-methyl-1-propanol (**AMP**), and two diamines – tetramethylthylenediamine (**TMEDA**) and tetramethyl-1,3-propanediamine (**TMPDA**). Such amines are often used for SW and CC processes due to their ability to absorb gases efficiently. **MEA**, **DEA**, **TEA**, **AMP**, and **DMEA** are alkanolamines used in both processes.

Table 4. The list of tested compounds.

Entry	Name/ amine type	Formula	M_W (g mol^{-1})	Log K_{ow}*	Structure
1	Monoethanolamine (**MEA**) / Primary	C_2H_7NO	61.08	–1.6	
2	Diethanolamine (**DEA**) / Secondary	$C_4H_{11}NO_2$	105.14	–1.7	
3	Triethanolamine (**TEA**) / Tertiary	$C_6H_{15}NO_3$	149.19	–2.5	
4	Dimethylethanolamine (**DMEA**) / Tertiary	$C_4H_{11}NO$	89.14	–0.9	
5	Amino-2-methyl-1-propanol (**AMP**) / Hindered	$C_4H_{11}NO$	89.14	–0.7	
6	Tetramethylethylene diamine (**TMEDA**) / Diamine	$C_6H_{16}N_2$	116.24	–0.3	
7	Tetramethyl-1,3-propanediamine (**TMPDA**) /Diamine	$C_7H_{18}N_2$	130.23	0.2	

*Data taken from (Quantitative Structure-Activity Relationship) QSAR prediction analysis

4.4.2 Toxicity evaluation of amines used in SW and CO_2-capturing processes

The ecotoxicity of the tested amines was assessed on aquatic organisms with varying biological complexity: bacteria (*A. fischeri*), vascular plants (*S. polyrhiza*), and invertebrates (*D. magna*). The results of the measurements are visualized in Fig. 16. The tertiary amine **TEA** showed the lowest toxicity level across all tested organisms and can be rated as non-toxic (EC_{50} > 1000 mg L^{-1}, Fig. 16a–c). The secondary amine **DEA** and the primary amine **MEA** exhibited lower EC_{50} values compared to **TEA**. **DEA** and **MEA** and can be classified as practically harmless to *A. fischery* [$EC_{50(DEA)}$ = 468 (95% CI: 280; 656) mg L^{-1}; $EC_{50(MEA)}$ = 227 (95% CI: 150; 304) mg L^{-1}], *S. polyrhiza* [$EC_{50(DEA)}$ = 549 (95% CI: 479; 619) mg L^{-1}; $EC_{50(MEA)}$ = 358 (95% CI: 262; 454) mg L^{-1}] and *D. magna* [$EC_{50(DEA)}$ = 367 (95% CI: 288; 447) mg L$^{-1;}$ $EC_{50(MEA)}$ = 260 (95% CI: 203; 317) mg L^{-1}]. These results align with the previous study, where it was found that the toxicity decreased when alkyl substituents were added to the nitrogen group.[111,169] Also, alkylamines with shorter and less-branched alkyl chains exhibited higher toxicity than those with longer and more highly branched chains. [105]

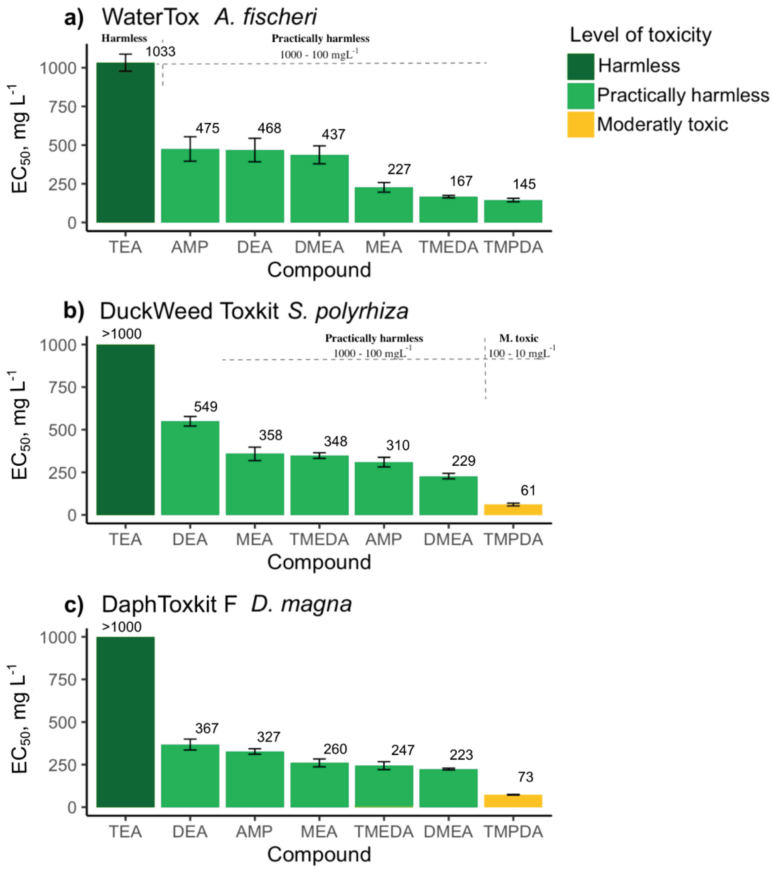


Fig. 16. Values of the tested compounds' mean effective concentrations (EC_{50}, mg L^{-1}) towards (a) *A. fischeri*, (b) *S. polyrhiza*, (c) *D. magna*. The compounds are ordered according to the level of toxicity: dark green represents the harmless (EC_{50} values > 1000 mg L^{-1}) compounds; light green represents the practically harmless (EC_{50} values 100–1000 mg L^{-1}) compounds; yellow denotes the moderately toxic (EC_{50} values of 10–100 mg L^{-1}) compounds.

DMEA is another tertiary alkanolamine, which has two ethanol groups replaced by methyl substituents. Compared to **TEA**, **DMEA** exhibited lower EC_{50} values and showed practically harmless level of toxicity to *S. polyrhiza* [EC_{50} = 229 (95% CI: 188; 269) mg L^{-1}], *D. magna* [EC_{50} = 223 (95% CI: 213; 234) mg L^{-1}] and *A. fischeri* [EC_{50} = 437 (95% CI: 293; 581) mg L^{-1}].

AMP is a primary amine and structural isomer of DMEA with different arrangements of substituents. It has the same level of toxicity as **DMEA**, but the EC_{50} values for **AMP** were slightly higher than for **DMEA**: EC_{50} = 475 (95% CI: 279; 671) mg L^{-1} towards *A. fischeri*, EC_{50} = 310 (95% CI: 240; 380) mg L^{-1} towards *S. polyrhiza* and EC_{50} = 327 (95% CI: 287; 367) mg L^{-1} towards *D. magna*.

TMEDA and **TMPDA** are two diamines with different length of the carbon spacer between the two nitrogen atoms. **TMEDA** with an ethylene spacer showed a practically harmless effect toward *A. fischeri* [EC_{50} = 167 (95% CI: 149;

186) mg L^{-1}], *D. magna* [EC_{50} = 247 (95% CI: 192; 302) mg L^{-1}] and *S. polyrhiza* [EC_{50} = 348 (95% CI: 308; 388) mg L^{-1}]. However, **TMPDA** with propylene spacer was the only tested compound which showed moderately toxic level of toxicity to *D. magna* [EC_{50} = 73 (95% CI: 69; 77) mg L^{-1}] and *S. polyrhiza* [EC_{50} = 61 (95% CI: 41; 81) mg L^{-1}]. In the test with *A. fischeri*, **TMPDA** showed practically harmless effect with EC_{50} = 145 (95% CI: 117; 172) mg L^{-1}, but this is also the lowest value among all tested amines.

The relationship between the ecotoxicological results and the octanol-water partition coefficient ($logK_{ow}$) of the tested compounds (Table 4) was correlated. The $logK_{ow}$ parameter quantifies the hydrophobicity of a compound by measuring its partitioning between octanol (hydrophobic phase) and water (hydrophilic phase).[170] This parameter serves as a valuable predictor of environmental fate and potential environmental risk, since compounds with elevated $logK_{ow}$ values generally exhibit reduced solubility in water, increased bioaccumulation potential, and, as a consequence, increased toxicity to aquatic organisms.[171] A clear correlation was found between hydrophobicity and toxicological effects in all amine compounds tested. **TEA**, which had the lowest $logK_{ow}$ value (−2.5) among all tested substances, showed non-toxic effect to all organisms assessed, consistent with its highly hydrophilic nature. In contrast, the remaining alkanolamines with $logK_{ow}$ values ranging from –0.7 to –1.7 showed practically harmless effect of toxicity that generally increased with hydrophobicity. This relationship was most pronounced for the diamine **TMPDA**, which had the highest $logK_{ow}$ value (0.2) and consequently showed moderate toxicity to both *S. polyrhiza* and *D. magna.* The observed trend that the more hydrophilic compounds (**TEA**, **DEA**, **MEA**, **DMEA**, **AMP**, **TMEDA**) consistently showed lower toxicity compared to their more hydrophobic counterparts (**TMPDA**) confirms our previous ecotoxicological studies with isosorbide-based compounds.

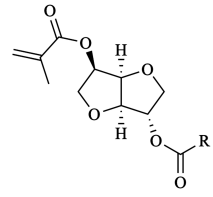
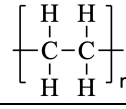
The mechanisms underlying amine-induced toxicity likely involve multiple pathways. One possible mechanism involves the induction of cellular oxidative stress through enhanced generation of reactive oxygen species (ROS), which can subsequently trigger lipid peroxidation cascades and ultimately lead to cytotoxicity.[172,173] Furthermore, the formation of reactive amine metabolites, including nitrosamines and imines, has been linked to genotoxic effects through direct DNA damage pathways.[113] These mechanistic insights, combined with the observed structure-activity relationships, provide a basis for predicting the environmental safety profiles of structurally related amine compounds.

4.5 Bacterial biofilm on bio-derived polymers, conventional plastics, and coated paperboards (Paper V)

4.5.1 Researched bio-derived polymers, conventional plastics, and coated paperboards

This study examines the interaction of freshwater bacteria and terrestrial bacteria with various bio-based and conventional polymer materials. Investigated polymer samples were incubated for 2 months in the river and in 4 types of soil for 3 months (1 tp) and 9 months (2 tp). Based on the physical appearance, the materials were divided into two categories: coated paperboards and plastics. Coated paperboard is a type of paperboard treated with one or more layers of polymer coating to improve its functional properties, often used for food packaging (e.g., coffee cups, juice cartons, etc.). The most common coating material is PE. However, the PE-coated paperboard packages (e.g., coffee cups) are practically not recyclable. Thus, in addition to the **PE**-coated paperboard (**PE-CP**), potentially recyclable paperboards with emulsion coatings based on three isosorbide methacrylates (**IMA-CP**, **IMP-CP**, **IMB-CP)**, and a corresponding uncoated paperboard (**UCP**) as a reference, were tested (**Table 5)**. These isosorbide-based industrial latex emulsions have been described and previously investigated for environmental toxicity in Paper I. A thin layer of emulsion covered the paperboard to achieve a bio-based and recyclable analog of regular coated paperboard.

Table 5. Overview of coated paperboard samples used in the study

Entry	Sample name	Sample description	Process type	Structure
1	**IMA-CP**	Coated paperboard with SA-latex, 50% of styrene replaced by **IMA**	Lab synthesis	 Isosorbide 5-methacrylate-2-acetate R = CH_3 (**IMA**), C_2H_5 (**IPM**), C_3H_7 (**IBM**)
2	**IMP-CP**	Coated paperboard with SA-latex, 50% of styrene replaced by **IMP**	Lab synthesis	
3	**IMB-CP**	Coated paperboard with SA-latex, 50% of styrene replaced by **IMB**	Lab synthesis	
4	**PE-CP**	Regular coffee cup paperboard coated with polyethylene	Production grade	
5	**UCP**	Uncoated paperboard	–	–

In the case of plastics, seven different types of polymers were chosen (Fig. 17). Polyurethane (**PU**(**1**–**4**)), polycarbonate (**PC-1**), and poly(thioether ester) (**TE-1**), based on rigid spirocyclic diols derived from citric acid, are high-performance materials from renewable feedstock. Poly(isosorbide 5-methacrylate-2-acetate) (**PIAMA**) film was chosen to evaluate whether the same isosorbide polymer behaves differently in different forms (i.e., as a coating or as a pure plastic film). All these polymers have been recently developed in our research group and can be generally characterized as high-performance plastics with potential applications in various fields of engineering.[40,174–176] Cellulose laurate (**C1**), cellulose stearate (**C2**), cellulose myristate (**C3**), and cellulose palmitate (**C4**) are polymers where cellulose hydroxyl groups are partially replaced with laurate, stearate, myristate, and palmitate.[177] All cellulose esters were a gift from Prof. Andres Krumme (TalTech) and these materials could be suitable as food packages. Granulated poly(lactic acid) (**PLA**) was turned into a film by using a standard solvent casting procedure. Polyethylene film (**PE**) is a common polymer for packaging and various other applications.

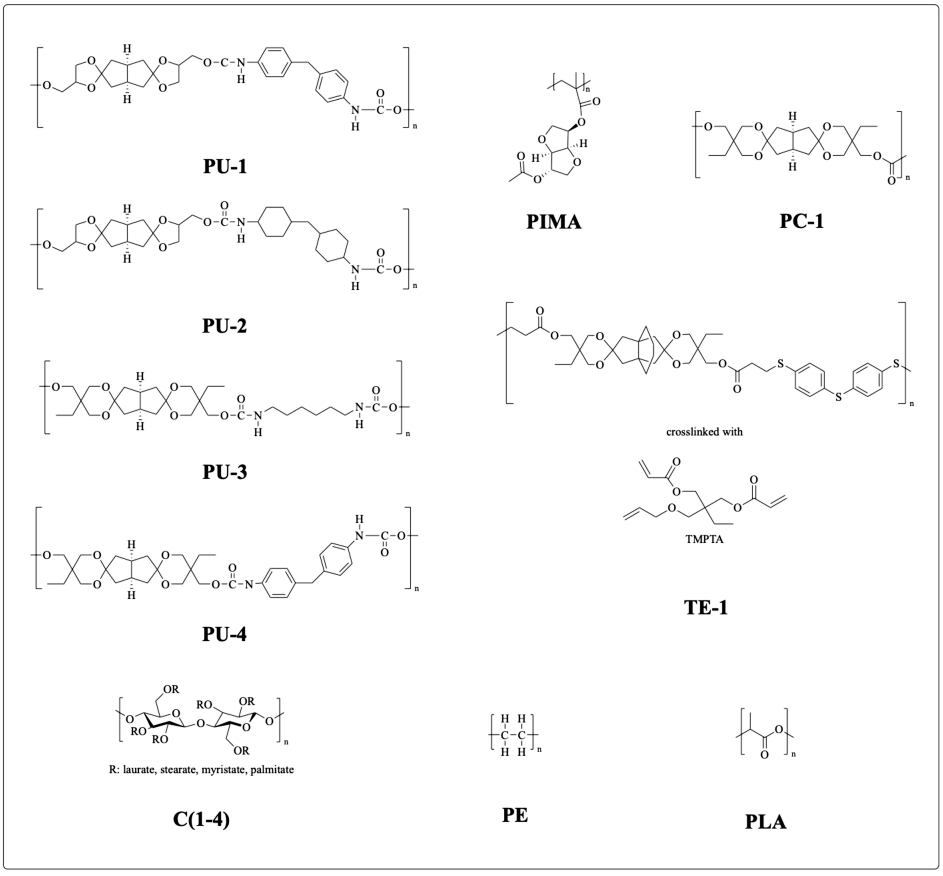


Fig. 17. Overview of chemical structures and abbreviations of the tested plastics.

4.5.2 Microbial richness and community composition of biofilms

A total of 149 841 amplicon sequence variant dates (ASVs) were obtained across all incubated samples from both aquatic and terrestrial environments. Rare taxa are ASVs with relative abundance < 0.01% across all samples, which present 99.2% of all ASVs, and their relative abundance is 57.7%. Only 64 ASVs were classified as abundant taxa (relative abundance > 0.1%), and their relative abundance is 12.8%.

To assess the diversity and structure of microbial communities on different materials, three standard ecological indices were analyzed: Chao1 taxonomic richness. Two-way ANOVA results indicated that this index was significantly influenced by the type of material ($p < 0.05$), suggesting that material composition plays a key role in shaping biofilm communities.

The Chao1 index (A), which estimates species richness, was substantially higher in materials incubated in a terrestrial environment compared to those incubated in an aquatic environment (Fig. 18). This pattern was consistent across most material types, suggesting that the terrestrial environment supports a more diverse microbial community, possibly due to its higher nutrient complexity, moisture retention, and microbial reservoir. Paperboard samples, bio-based polyurethane and cellulose-based polymers exhibited the highest richness values in soil. These materials may offer more accessible substrates or surface properties favorable for colonization and growth. In contrast, materials incubated in aquatic environments, such as bio-based polyurethane, polylactic acid, and uncoated paperboard samples, showed lower microbial richness. This reduced diversity may reflect the more selective pressures of the aquatic environment, such as limited nutrient availability and greater exposure to hydrodynamic forces.

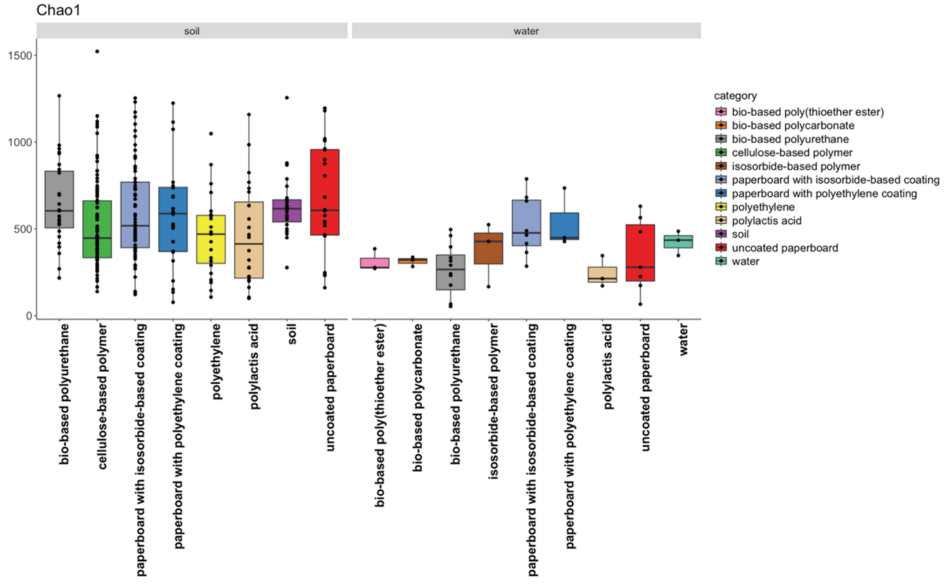


Fig. 18. Materials type significantly affected the Chao1 indices.

Overall, these patterns suggest that the type of material composition influences microbial colonization by affecting both the number of taxa and their relative distributions.

4.5.3 Distribution of bacterial community on the studied materials

Distance-based redundancy analysis (dbRDA) is a constrained ordination method used in microbial ecology to visualize how environmental variables influence community composition. This method was used to determine changes in microbial community composition depending on different chemical structures of materials and incubation environments (Fig. 19). The studied samples were grouped into ten categories depending on the chemical structure of the polymers or coatings on the paperboard. These groups include cellulose-based polymers (cellulose esters (**C**(**1–4**)), isosorbide-based polymer (**PIMA**), poly(thioether ester) (**TE-1**), polycarbonate (**PC-1**), polyurethane (**PU**(**1**–**4**)), polylactic acid (**PLA**), polyethylene (**PE**), paperboard with isosorbide-based coating (**IMA-CP**, **IMP-CP**, **IMB-CP**), paperboard with polyethylene coating (**PE-CP**), uncoated paperboard (**UCP**). There are also two control groups representing soil and water samples.

The x-axis (dbRDA1) explains 3% of the variation, while the y-axis (dbRDA2) explains 1.6%. Although these two axes explained only 4.6% of the total variance, they demonstrated that material composition is a major determinant of biofilm community structure. There is a clear separation between biofilms on paperboard and plastic materials incubated in soil. As we can see from Fig. 18, most of the plastic materials and control soil samples form a tight and overlapping cluster on the left side of the dbRDA1 axis. This may indicate that the microbial communities among these samples are not highly differentiated. While cellulose-based polymers also group near the other plastics, they are more widely distributed, particularly toward the upper region of the plot. This broader spread indicates a higher variability in microbial composition, suggesting that cellulose-based polymers support more diverse microbial communities compared to other polymer materials.

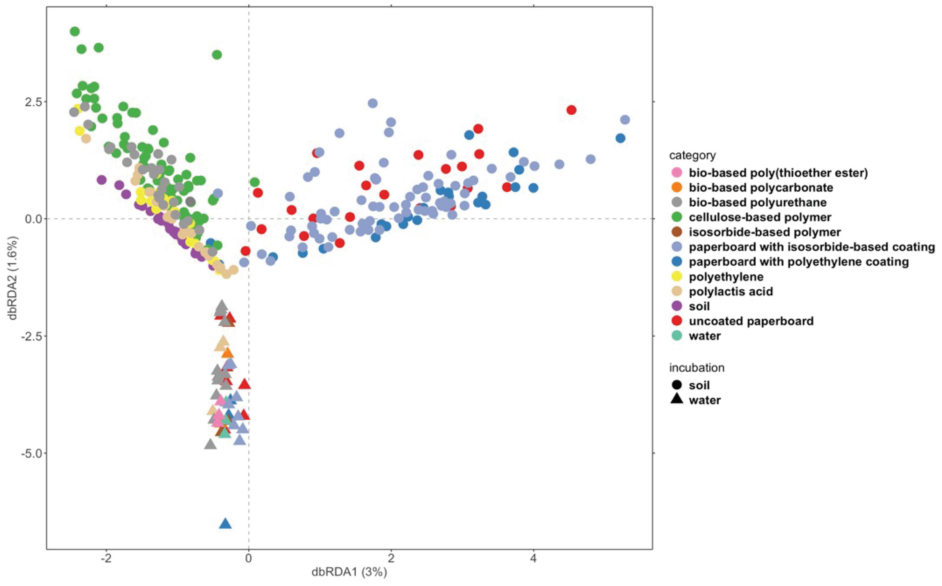


Fig. 19. Distance-based redundancy analysis (dbRDA).

Otherwise, paperboard materials are spread more widely along the right side of the dbRDA1 axis, showing greater variability in microbial communities compared to plastic samples. This clustering is likely due to the fact that cellulose-based cardboard backbone provides a stable substrate for microbial colonization, potentially favoring certain degradative organisms capable of using cellulose as a carbon source.

The incubation environment significantly influences community structure. Samples incubated in an aquatic environment (triangles) tend to cluster toward the lower half of dbRDA2, suggesting that water conditions limit microbial diversity or select for different taxa compared to soil. For example, even the same material (paperboards with isosorbide-based coating) shows different positioning depending on incubation.

4.5.4 Analysis of key bacterial families using different approaches

To better understand the distribution of microbial communities on the different types of materials tested, LEfSe (linear discriminant analysis of effect size) was applied at the family level of the taxonomic classification. LEfSe combines nonparametric statistical testing with linear discriminant analysis to identify microbial families that differ significantly in relative abundance between experimental groups. This method is particularly effective in identifying bacterial taxa that act as biomarkers.

In addition, we used CAPscale analysis (CAP), a restricted ordination method that is related to distance-based redundancy analysis (dbRDA). This ordination approach is useful for identifying which taxa have the greatest influence on shaping the overall community structure under the conditions tested.

After performing both analyses, we cross-compared the results to identify bacterial families that were significant in both LEfSe and CAP analyses (Fig. 20). A total of 71 families were found to be common to both methods. These are important because they are not only differentially abundant (as revealed by LEfSe) but also play a central role in shaping community differences (as revealed by CAP). Additionally, some families were uniquely detected by each method – 69 families by LEfSe and 20 by CAP, likely due to the differing statistical criteria used by the two approaches. This approach allowed a more complete interpretation of how the composition of the studied materials and the environmental context affect microbial community dynamics, especially at the family level.

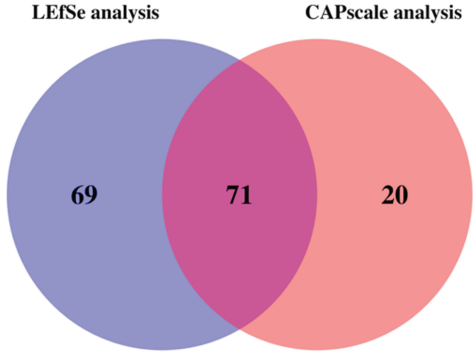


Fig. 20. LefSe and CAP circle diagram with numbers of shared bacterial families.

Common and unique bacterial families identified for LEfSe and CAP analyses were studied separately, depending on incubation type and time point. Specifically, comparisons were made for samples incubated in an aquatic environment, as well as for two different time points in the soil incubation.

4.5.5 Family-level taxonomic analysis of bacterial communities on materials incubated in an aquatic and terrestrial environment

Overall, 71 bacterial families were found to be shared between the two analyses in samples incubated across both environments. After excluding unclassified and potentially unknown taxa, 42 families remained. These were visualized in bubble diagrams to illustrate their relative abundance across different material types (Fig. A1, A3). Bio-based polyurethane polymers were incubated only for the second time point. In the discussion part, only samples included in the aquatic and the terrestrial second time point were analysed in detail. The bacterial families described in this part are illustrated in Fig. 21.

Among these, *Mycobacteriaceae* displayed consistently high abundance on all materials presented in these tests for both environments. This family includes acid-fast, lipid-rich bacteria commonly found in water and soil, known for their ability to form biofilms and degrade hydrophobic compounds and plastics – traits that support their dominance on polymer-based surfaces. Similarly, *Gaiellaceae*

is a family of Gram-positive, aerobic bacteria often found in soil and marine environments and known for their resilience in oligotrophic (nutrient-poor) conditions. Their ecological versatility likely enables them to thrive on various types of surfaces. *Tepidisphaeraceae*, a thermophilic, biofilm-forming group, was detected on most materials, except for **PC-1** in water. It also showed low abundance on **PE** sample in soil with compost 1, again highlighting how material composition may selectively shape microbial colonization patterns.

Pirelullacela appeared primarily on plastic materials but showed reduced or no abundance on paperboard with isosorbide-based coatings (**IMA-CP**, **IPA-CP**, **IBA-CP**) in water, control soil and in soil with compost 2. In soil with compost, 1 relative abundance for *Pirelullacela* for all samples was equal, while in mineralized soil, it was relatively the same for all samples, but wasn't present in **IMA-CP**. This suggests that specific surface chemistries and the type of environment may inhibit their colonization. Likewise, *Methylomonadaceae*, a family of methanotrophs often found in aquatic or moist environments, were present on the **IMA-CP** and **IPA-CP** at low abundance, on **PC-1** and **PIAMA** samples at higher abundance and absent from **PLA** and **PU-4** in the water incubated samples. *Methylomonadaceae* was detected on the terrestrial incubated samples at low abundance. These distribution patterns suggest a limited ability to adhere to or metabolize certain coated or polymer surfaces.

Halieaceae, bacteria often associated with hydrocarbon degradation, were detected exclusively on bio-based plastics (excluding **PLA**) and paperboard with **PE** coating, but were absent in the river water control at the water incubation test. In the soil test, it was found in practically all samples at low abundance; only paperboard samples with isosorbide coatings have higher abundance in mineralized soil and soil with compost 2. In contrast, *Geminicoccaceae* was found in higher abundance on hard-plastic samples, especially on cellulose-based polymers, but was absent practically in all paperboard samples under terrestrial incubation. Also, in the aquatic incubation test, this bacterium was present only in four samples (**IMA-CP**, **IPA-CP**, **PU-1**, **TE-1**, river water).

The **PU-4** sample showed a distinct bacterial community structure compared to other polyurethanes in water incubation test, possibly due to its smoother surface, which may influence microbial attachment. Notably, *Planococcaceae* – Gram-positive, spore-forming bacteria capable of degrading hydrocarbons and thriving in harsh conditions – were most abundant on **PU-4** and absent from the control, supporting their role in polymer-associated biodegradation. In addition, *Isosphaeraceae*, a family often found in wastewater and capable of forming complex biofilms, showed higher abundance on **PU-4** than on other polyurethane samples, suggesting that surface morphology or composition may favor their colonization. *Gemmatimonadaceae*, typically associated with oligotrophic environments and known for slow growth and potential photoheterotrophy, were present in low abundance on nearly all samples but absent from **PU-4.**

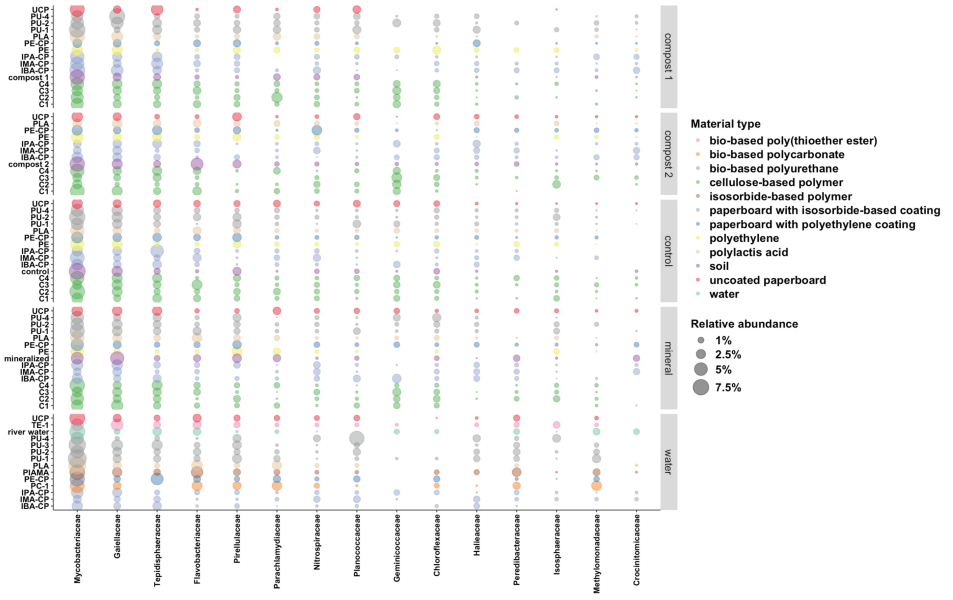


Fig. 21. Relative abundances of selected bacterial families on different materials across various soil types and water samples. Bacterial families displayed in the plot were identified based on LEfSe and CAP analyses. These families were selected for their statistically significant differences in abundance and strong ecological associations with specific materials and environments.

In the LEfSe analysis, 39 unique bacterial families were identified for the aquatic incubated materials. After filtering out unknown and unclassified taxa, 17 families remained for interpretation (Fig. A2). The bacterial families described in this part are illustrated in Fig. 22. Among these, *Pseudomonadaceae*, and *Steroidobacteraceae*, were consistently detected across all material types, though with varying relative abundances. These families are well-known for their metabolic versatility, biofilm formation, and ability to degrade complex organic compounds, which may contribute to their widespread colonization across diverse substrates.

Rubrobacteraceae was detected mainly on paperboard-based materials and generally in low abundance and in higher abundance in uncoated paperboard samples and bio-based polycarbonate **PC-1** under aquatic incubation. While under terrestrial conditions, it was present on practically all incubated samples, except **IPA-CP** and **IBA-CP** in the control soil. In contrast, *Turicibacteracea* was found mainly in hard-plastic samples incubated in water, but was absent in samples incubated in soil.

Cytophagaceae, a family involved in the degradation of complex polymers such as cellulose and chitin, was found in higher abundance on **PU-2** and **PU-4** under aquatic incubation, but it was present practically in all samples incubated in soil. This may indicate selective colonization, likely due to being influenced by the type of incubation.

Planctomycetaceae, *Micromonosporaceae*, and *Pedosphaeraceae* were detected in materials and generally in low abundance. These families are known for their roles in biofilm development, resistance to desiccation, and tolerance of low-nutrient environments, which could explain their association with porous, fibrous surfaces rather than dense plastics. *Fimbriimonadaceae* was detected on paperboard samples with coating but not on uncoated paperboard, and this family was also present on bio-based polyurethane samples in water incubated samples. In the soil, it was also found mainly on bio-based materials, in the uncoated paperboard, it was found in mineralized soil in low abundance, and in soil with compost 2. *Methylophilaceae*, a family of methylotrophic bacteria that utilize single-carbon compounds, appeared primarily on bio-based polyurethanes and the **TE-1** plastic, but among paperboard samples, it was only detected on one isosorbide-coated type and on the **PE-CP** under aquatic incubation. *Methylophilaceae* was present in low abundance in practically all samples incubated in soil. *Casimicrobiaceae* were found across materials, including **PE-CP**, **PLA**, **PC-1**, **PU-1**, and **IBA-CP**, but were absent from other samples and the river water.

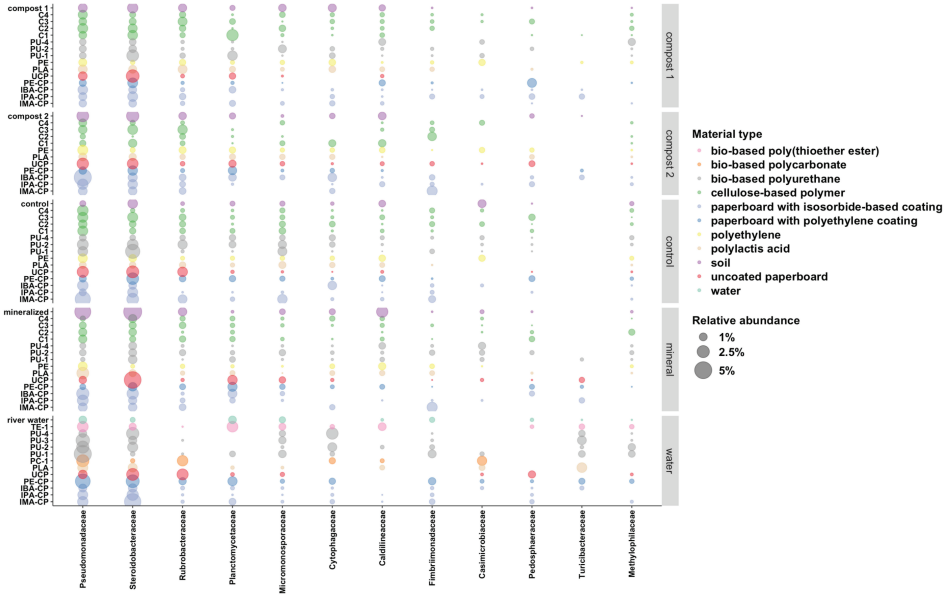


Fig. 22. The relative abundances of bacterial families on different materials in various soil types and water. Bacterial families displayed in the plot were identified based on LEfSe analysis.

The CAP analysis initially identified 20 unique bacterial families, but after filtering out unclassified or unknown taxa, only 6 remained (Fig. 23). Among them, *Micrococcaceae*, *Polyangiaceae* and *Sphingobacteriaceae* were consistently present across all material types, though with varying relative abundances. These families are commonly found in soil and aquatic environments and are known for their roles in organic matter degradation, biofilm formation, and environmental

adaptability – traits that may explain their widespread presence across different substrates.

Anoxybacillaceae, a thermotolerant, spore-forming family capable of surviving in fluctuating conditions, was detected on two paperboard with isosorbide coating samples **IMA-CP** and **IPA-CP,** and in the river water control sample under aquatic incubation. While in the samples under terrestrial incubation, it was detected in all paperboards with isosorbide coating samples in mineralized soil and soil with compost 2 in relatively high abundance, in soil with compost 1 in low abundance, and it was absent in the control soil. In contrast to aquatic incubation, in the terrestrial environment, the *Anoxybacillaceae* were also present in a paperboard sample with polyethylene coating in all soil types, also, in uncoated paperboard except soil with compost 1. *Anoxybacillaceae* was also present in all cellulose-based polymers in the control soil and in one **C3** cellulose-based polymer in soil with compost 1 but was absent in mineralized soil and soil with compost 2. Also, it was absent in all soil samples (compost 1, compost 2, control, and mineralized).

Desulfitobacteriaceae, a family involved in reductive dechlorination and anaerobic degradation of complex compounds, was found on two paperboard materials, **IMA-CP** and **IBA-CP,** on **TE-1** and **PIAMA**, in aquatic incubated samples. It was also detected in all paperboard samples with isosorbide coating in mineralized soil and soil with compost 1. In control soil was found only in **IBA-CP** sample, while in soil with compost 2 in **IMA-CP** and **IBA-CP**. Also, *Desulfitobacteriaceae* was detected in other materials incubated in a terrestrial environment, such as uncoated paperboard samples (mineralized soil and soil with compost 1 and 2), **PU-4**(control soil and soil with compost 1), **PU-1** (soil with compost 1), **PLA** (mineralized soil), **C2** (control soil and soil with compost 2), **C3** (soil with compost 2). All these materials are bio-based, only in control soil and in soil with compost, 1 *Desulfitobacteriaceae* was found in paperboard with polyethylene coating, which may suggest a colonization pattern possibly driven by the bio-based composition of materials. *Propionibacteriaceae* was detected at relatively low abundance on only a few materials incubated in an aquatic environment: the isosorbide-coated paperboard **IPA-CP** and bio-based polyurethane **PU-1,** in **PLA** sample abundance was higher. *Propionibacteriaceae* was found in all paperboard samples with isosorbide and polyethylene coatings, in cellulose-based sample **C2**, **PLA**, and uncoated paperboard. This family includes anaerobic or aerotolerant bacteria often associated with skin or fermentation environments, and their limited presence may reflect specific nutrient or surface preferences. Both *Propionibacteriaceae* and *Desulfitobacteriaceae* were absent from the river water control and soil control samples, indicating that their presence may be associated with surface colonization rather than passive environmental background.

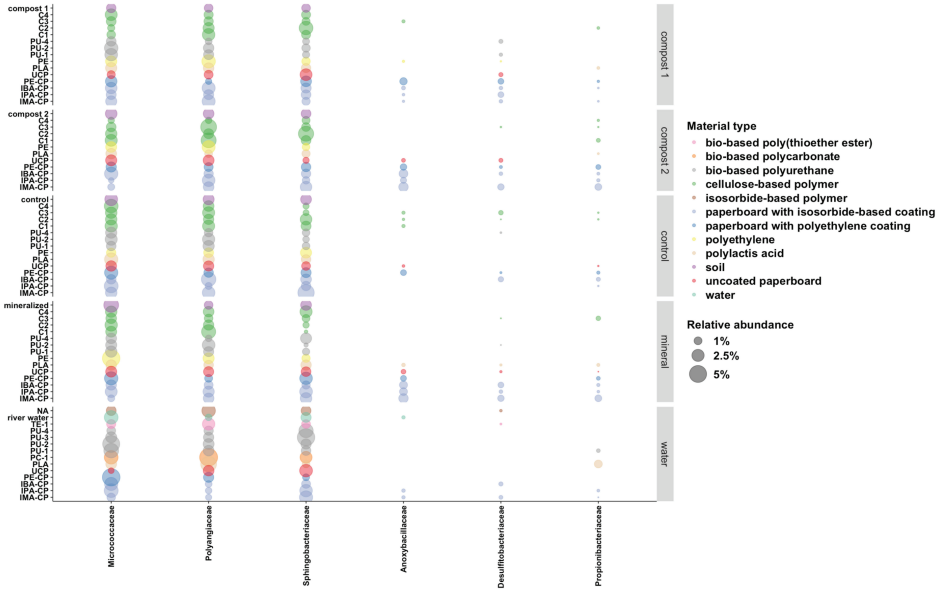


Fig. 23. The relative abundances of bacterial families on different materials in various soil types and water. Bacterial families displayed in the plot were identified based on CAP analysis.

SUMMARY

The global increase in the use of materials and chemicals has raised serious concerns about their long-term environmental and health impacts. Conventional fossil-based polymers are widely used due to their durability and low cost, but their persistence, low recycling rates, and accumulation in ecosystems contribute significantly to pollution. In response, bio-based materials derived from renewable feedstocks have attracted attention as more sustainable alternatives. However, their safety cannot be guaranteed as some bio-based monomers and degradation products exhibit significant toxicity, demanding careful environmental assessment.

In this study, several bio-based materials were assessed for their ecotoxicological, microbiological impacts, and cytotoxicity. These include acrylates and methacrylates derived from isosorbide, lactate amide acrylates from depolymerized PLA, lignin-based methacrylate polymers, and amines used in CO_2 capture and switchable solvent systems. The focus was on assessing the toxicity of monomers versus polymers and understanding how structural factors such as hydrophobicity, molecular weight, and solubility affect biological interactions.

Testing of isosorbide-based monomers showed that acrylate derivatives (**IA**, **IAA**) were more toxic than methacrylate derivatives (**IM**, **IMA**), especially to aquatic plants (*Spirodela polyrhiza*) and invertebrates (*Thamnocephalus platyurus*). This is likely due to the higher reactivity of acrylates. Their corresponding polymers (**PIA**, **PIAA**, **PIM**, **PIMA**) had significantly lower toxicity, often showing EC_{50} values >1000 mg L^{-1}, thus being classified as harmless. Increasing molecular weight further reduced toxicity, likely due to reduced bioavailability and limited cellular uptake. Industrially relevant latexes created using isosorbide monomers (**IMA-latex**, **IMP-latex**, **IMB-latex**) also showed low toxicity under standard conditions. The minor effects observed may have been due to residual monomer or surfactant content, but overall, these materials demonstrated safe profiles for packaging or coating applications.

Lactate amide acrylic monomers derived from PLA (**LAA0**, **LAA5**, **LAA7**, **LAA9**) showed moderate to high toxicity levels towards aquatic species, especially *S. polyrhiza* and *T. platyurus*. However, after polymerization (**PLAA0**–**PLAA9**), the toxicity of these materials decreased to practically harmless and harmless levels. These results highlight the critical role of polymerization in reducing the environmental impact of acrylate compounds and emphasize the importance of minimizing monomer residue levels in consumer products.

Lignin-derived methacrylate monomers (**BMA**, **VMA**, **SMA**) were evaluated using HeLa cells. The monomers demonstrated moderate cytotoxicity with IC_{50} values ranging from 42 to 95 μg mL^{-1}. In contrast, their corresponding polymers (**PBMA**, **PVMA**, **PSMA**) demonstrated much lower cytotoxicity with IC_{50} values greater than 500 μg mL^{-1}, and cell viability remained high at application-relevant concentrations. These results support the potential of lignin-derived methacrylates as safe and effective alternatives to conventional plastics such as **PMMA**.

Amines used in CO_2 capture and switchable water processes were also evaluated. The compounds tested included monoethanolamine **MEA**, **DEA**, **TEA**, **DMEA**, **AMP**, and diamines such as **TMEDA** and **TMPDA**. **TEA** was found to be the least toxic among all species tested, including bacteria, plants, and invertebrates. In contrast, **TMPDA**, which is more hydrophobic ($\log K_{ow} = 0.2$), showed moderate toxicity. A clear trend links increased hydrophobicity with increased toxicity, likely due to greater membrane permeability and bioaccumulation potential. These results highlight the importance of physicochemical properties in the early stage of amine screening for green applications.

In addition to toxicity, this study investigated how material properties affect microbial colonization through biofilm formation. A total of 149,841 ASVs were identified from materials incubated in aquatic and terrestrial environments, with rare taxa accounting for most of the microbial diversity. Families such as *Mycobacteriaceae*, *Gaiellaceae*, and *Tepidisphaeraceae* were widely distributed, while others, such as *Pirellulaceae*, *Halieaceae*, and *Methylomonadaceae*, exhibited material- and environment-specific patterns. Another families, such as *Desulfitobacteriaceae* and *Propionibacteriaceae,* were enriched on paperboards samples but absent from others, indicating active surface colonization. These results highlight the influence of polymer chemistry, surface structure, and environmental conditions on biofilm community formation.

SUMMARY IN ESTONIAN

Uute biopõhiste polümeeride ning madala süsinikuheitega tehnoloogiates kasutatavate kemikaalide ohutuse hindamine

Materjalide ja kemikaalide kasutuse suurenemine on tekitanud maailmas tõsiseid muresid nende pikaajalise keskkonna- ja tervisemõju osas. Fossiilset päritolu polümeerid on tänapäeval laialdaselt kasutusel oma vastupidavuse ja madala hinna tõttu, kuid nende püsivus, madalad taaskasutusmäärad ning kuhjumine ökosüsteemidesse põhjustavad olulist reostust. Sellele vastukaaluks on taastuvast toorainest saadavad biopõhised materjalid äratanud tähelepanu kui jätkusuut-likumad alternatiivid. Siiski ei saa nende ohutust garanteerida vastavate uuringuteta, kuna biopõhised monomeerid, polümeerid ja nende laguproduktid võivad osutuda samuti toksilisteks, mistõttu on vajalik põhjalik keskkonnamõju hindamine.

Käesolevas uuringus hinnati mitmeid biopõhiseid polümeere ning nende lähteaineid (s.o. monomeere) ökoloogilise toksilisuse, mikrobioloogilise mõju ja tsütotoksilisuse osas. Uuritud materjalideks olid isosorbiidist saadud polüakrülaadid ja -metakrülaadid, depolümeriseeritud PLA-st saadud laktaatamiidakrülaadid, ligniinipõhised metakrülaatpolümeerid ning CO_2 sidumisel kasutatavad amiinid. Fookuses oli monomeeride ja polümeeride toksilisuse võrdlemine ning struktuursete tegurite – nagu hüdrofoobsus, molekulmass ja lahustuvus – mõju bioloogilistele koostoimetele.

Isosorbiidipõhiste monomeeride testimine näitas, et akrülaadid (**IA**, **IAA**) olid toksilisemad kui metakrülaadid (**IM**, **IMA**), seda eriti veetaimedele (*Spirodela polyrhiza*) ja selgrootutele (*Thamnocephalus platyurus*). See võib olla tingitud akrülaatide suuremast reaktsioonivõimest. Nende vastavad polümeerid (**PIA**, **PIAA**, **PIM**, **PIMA**) olid oluliselt vähem toksilised, sageli EC_{50} väärtustega > 1000 mg L^{-1}, mistõttu liigitati need ohututeks. Molekulmassi suurenemine vähendas samuti toksilisust, tõenäoliselt tänu vähenenud biosaadavusele ja piiratud rakku sisenemisele. Isosorbiidipõhised tööstuslikud lateksid (**IMA-lateks**, **IMP-lateks**, **IMB-lateks**) näitasid samuti madalat toksilisust standard-tingimustes. Vähesed täheldatud mõjud võisid olla seotud jääkmonomeeri sisal-dusega, kuid üldiselt võib neid materjale pidada ohutuks.

PLA-st saadud laktaatamiidakrülaatmonomeerid (**LAA0**, **LAA5**, **LAA7**, **LAA9**) osutusid mõõdukalt kuni tugevalt toksilisteks veeorganismide *S. polyrhiza* ja *T. platyurus*. Kuid pärast polümerisatsiooni (**PLAA0**–**PLAA9**) vähenes nende toksilisus tasemele, mida loetakse praktiliselt ohutuks või ohutuks. Need tule-mused näitavad, et keskkonnamõju minimeerimiseks on oluline vältida mono-meerijääkide sisaldust lõpptootes.

Ligniinist saadud metakrülaatmonomeere (**BMA**, **VMA**, **SMA**) hinnati HeLa rakkudel. Monomeerid näitasid mõõdukat tsütotoksilisust IC_{50} väärtustega vahe-mikus 42–95 μg mL^{-1}. Seevastu nende vastavad polümeerid (**PBMA, PVMA, PSMA**) olid oluliselt vähem toksilised, IC50 väärtustega üle 500 μg mL^{-1}, ning rakuelujõulisus jäi kõrgeks ka suurtel kontsentratsioonidel. Need tulemused

toetavad ligniinipõhiste metakrülaatide potentsiaali ohutute ja tõhusate alternatiividena traditsioonilistele plastidele nagu **PMMA** ja polüstüreen.

Samuti hinnati amiinide toksilisust, mida kasutatakse näiteks arendatavates CO_2 sidumise tehnoloogiates. Uuritavate ainete hulgas olid monoetanoolamiin (**MEA**), dietanoolamiin (**DEA**), trietanoolamiin (**TEA**), dimetüületanoolamiin (**DMEA**), 2-amino-2-metüül-1-propanool (**AMP**) ning diamiiinid nagu tetrametüüleetaamindiamin (**TMEDA**) ja trimetüleentetrapropaan-1,4-diamin (**TMPDA**). **TEA** osutus kõige vähem toksiliseks nii bakterite, taimede kui ka selgrootute suhtes. Vastupidiselt sellele näitas **TMPDA**, mis on hüdrofoobsem ($\log K_{ow} = 0,2$), mõõdukat toksilisust. Täheldati selget seost hüdrofoobsuse suurenemise ja toksilisuse kasvu vahel, mis tõenäoliselt on seotud parema membraaniläbitavuse ja bioakumulatsioonipotentsiaaliga. Need tulemused näitavad amiinide keskonnamõju hindamise tähtsust enne nende laialdast tööstuslikku kasutuselevõttu.

Lisaks uuriti kuidas materjali omadused mõjutavad mikroobide koloniseerimist biokile moodustumise kaudu. Vee- ja maismaakeskkonnas inkubeeritud materjalidest identifitseeriti kokku 149 841 ASV-d, kusjuures haruldased taksonid moodustasid suurema osa mikroobide mitmekesisusest. Perekonnad nagu *Mycobacteriaceae*, *Gaiellaceae* ja *Tepidisphaeraceae* olid laialt levinud, samas kui teised, näiteks *Pirellulaceae, Halieaceae* ja *Methylomonadaceae*, näitasid materjali- ja keskkonnaspetsiifilisi mustreid. Teatud perekonnad, näiteks *Desulfitobacteriaceae* ja *Propionibacteriaceae*, olid rikkalikult esindatud pinnakatte-proovidel, kuid teistel puudusid, mis viitab aktiivsele pinnakoloniseerimisele. Need tulemused demonstreerivad polümeeride struktuuri ja keskkonnatingimuste mõju biokile koosluse moodustumisele.

APPENDIX

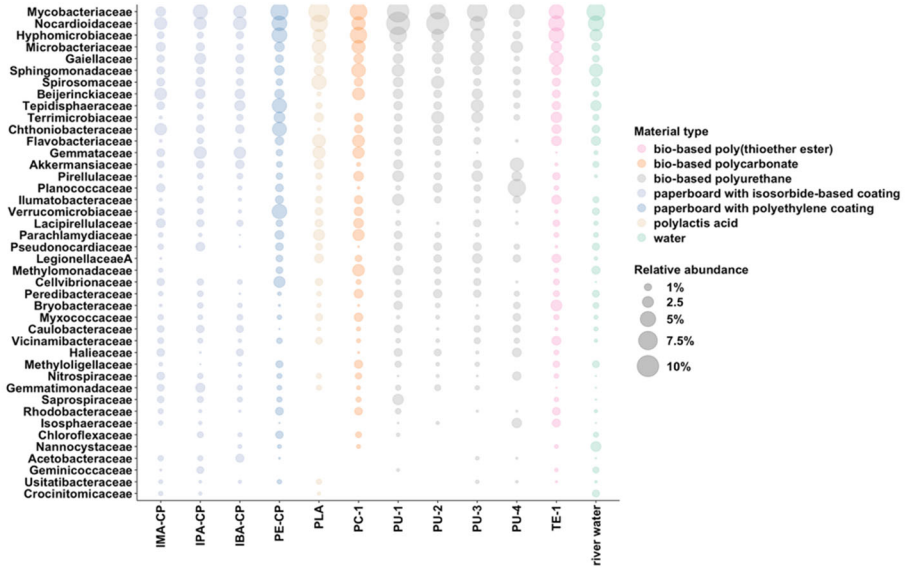


Fig. A1. The relative abundances of bacterial families on different materials in water. The bacterial families presented were identified as common based at family level on LefSe and CAPscale analysis.

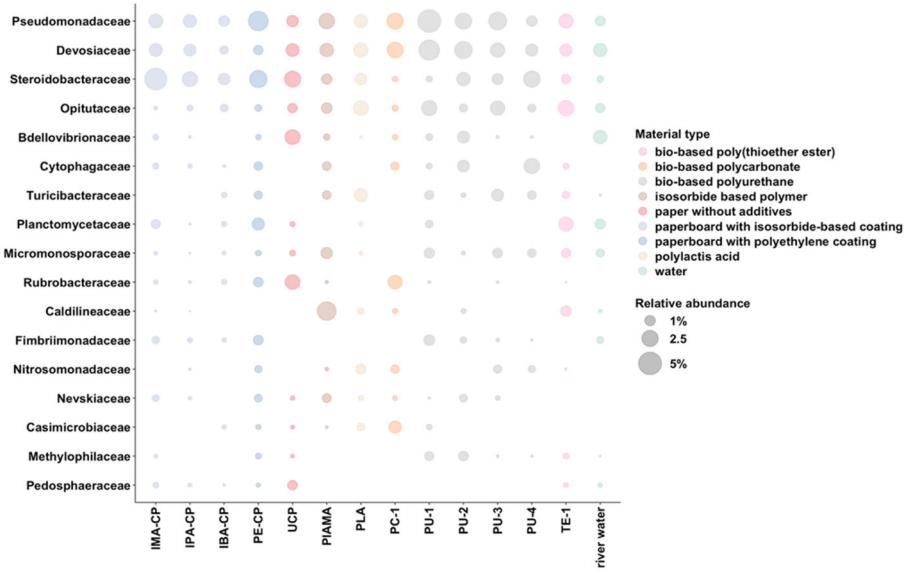


Fig. A2. The relative abundances of bacterial families on different materials in water. The unique bacteria at the family level for LefSe analysis.

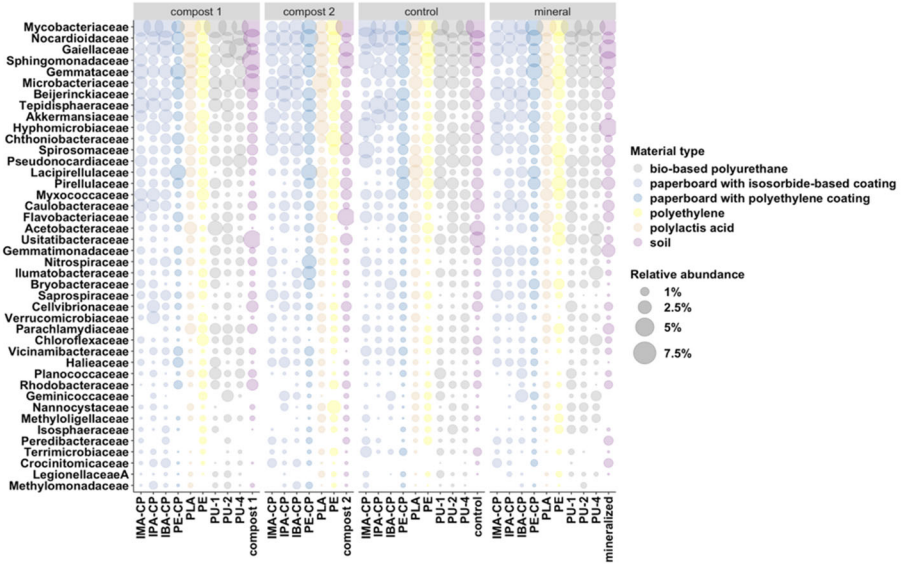


Fig. A3. The relative abundances of bacterial families on different materials in different soil for 2 time point. The bacterial families presented were identified as common based at family level on LefSe and CAPscale analysis.

REFERENCES

1 P. Anastas and N. Eghbali, Green Chemistry: Principles and Practice, *Chem Soc Rev*, 2010, **39**, 301–312.

2 M. Amoneit, D. Weckowska, S. Spahr, O. Wagner, M. Adeli, I. Mai and R. Haag, Green chemistry and responsible research and innovation: Moving beyond the 12 principles, *J Clean Prod*, 2024, **484**, 144011.

3 S. D. Jain, A. Awasthi and A. K. Gupta, Green Chemistry: A Sustainable Path to Environmental Responsibility and Innovation, *Asian J Res Pharm Sci*, 2024, 51–55.

4 D. Shindell and C. J. Smith, Climate and air-quality benefits of a realistic phase-out of fossil fuels, *Nature*, 2019, **573**, 408–411.

5 P. G. Levi and J. M. Cullen, Mapping Global Flows of Chemicals: From Fossil Fuel Feedstocks to Chemical Products, *Environ Sci Technol*, 2018, **52**, 1725–1734.

6 M. Aretz, H. G. Herbig, X. D. Wang, F. M. Gradstein, F. P. Agterberg and J. G. Ogg, The Carboniferous Period, *Geologic Time Scale 2020*, 2020, 811–874.

7 Dhakal, S., J.C. Minx, F.L. Toth, A. Abdel-Aziz, M.J. Figueroa Meza, K. Hubacek, I.G.C. Jonckheere, Yong-Gun Kim, G.F. Nemet, S. Pachauri, X.C. Tan, T. Wiedmann, 2022: *Emissions Trends and Drivers. In IPCC, 2022: Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [P. R. Shukla, J. Skea, R. Slade, A. Al Khourdajie, R. van Diemen, D. McCollum, M. Pathak, S. Some, P. Vyas, R. Fradera, M. Belkacemi, A. Hasija, G. Lisboa, S. Luz, J. Malley, (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA.

8 K. Ragaert, L. Delva and K. Van Geem, Mechanical and chemical recycling of solid plastic waste, *Waste Manag*, 2017, **69**, 24–58.

9 OECD (2022), *Global Plastics Outlook: Economic Drivers, Environmental Impacts and Policy Options*, OECD Publishing, Paris.

10 R. C. Thompson, S. H. Swan, C. J. Moore and F. S. Vom Saal, Our plastic age, *Phil Trans R Soc B-Biol Sci*, 2009, **364**, 1973–1976.

11 A. Rudin and P. Choi, Introductory Concepts and Definitions, *J Pol Sci Eng*, 2013, 1–62.

12 R. Geyer, J. R. Jambeck and K. L. Law, Production, use, and fate of all plastics ever made, *Sci Adv*, 2017, **3**, e1700782.

13 P. G. C. Nayanathara Thathsarani Pilapitiya and A. S. Ratnayake, The world of plastic waste: A review, *Clean Mater*, 2024, **11**, 100220.

14 Plastics Europe, Plastics – the Facts 2024: An Analysis of European Plastic Production. Demand and Waste Data. Plastics Europe, 2024, https://plasticseurope.org/knowledge-hub/plastics-the-fast-facts-2024/ (accessed 10.08.2025).

15 G. Lopez, D. Keiner, M. Fasihi, T. Koiranen and C. Breyer, From fossil to green chemicals: sustainable pathways and new carbon feedstocks for the global chemical industry, *Energy Environ Sci*, 2023, **16**, 2879–2909.

16 D. Y. C. Leung, G. Caramanna and M. M. Maroto-Valer, An overview of current status of carbon dioxide capture and storage technologies, *Renew Sustain Energy Rev*, 2014, **39**, 426–443.

17 C. Wellenreuther, A. Wolf, Innovative feedstocks in biodegradable bio-based plastics: A literature review, *HWWI Research paper*, 2020, **194**, 1–40.

18 Z. Terzopoulou and D. N. Bikiaris, Biobased plastics for the transition to a circular economy, *Mater Lett*, 2024, **362**, 136174.
19 International Organization for Standardization, *Environmental management – Life cycle assessment – Requirements and guidelines – ISO 14044*, 2006, vol. 2006.
20 S. Sala, F. Biganzoli, E. S. Mengual and E. Saouter, Toxicity impacts in the environmental footprint method: calculation principles, *Int J Life Cycle Assess*, 2022, **27**, 587–602.
21 The Directorate-General for Environment, *EUBP Statement on the EU Policy Framework on Biobased, Biodegradable and Compostable Plastics*, 2022, https://environment.ec.europa.eu/publications/communication-eu-policy-framework-biobased-biodegradable-and-compostable-plastics_en (accessed 10.08.2025).
22 M. Dusselier, P. Van Wouwe, A. Dewaele, E. Makshina and B. F. Sels, Lactic acid as a platform chemical in the biobased economy: The role of chemocatalysis, *Energy Environ Sci*, 2013, **6**, 1415–1442.
23 N. G. Khouri, J. O. Bahú, C. Blanco-Llamero, P. Severino, V. O. C. Concha and E. B. Souto, Polylactic acid (PLA): Properties, synthesis, and biomedical applications – A review of the literature, *J Mol Struct*, 2024, **1309**, 138243.
24 E. Castro-Aguirre, F. Iñiguez-Franco, H. Samsudin, X. Fang and R. Auras, Poly(lactic acid) – Mass production, processing, industrial applications, and end of life, *Adv Drug Deliv Rev*, 2016, **107**, 333–366.
25 T. A. Swetha, A. Bora, K. Mohanrasu, P. Balaji, R. Raja, K. Ponnuchamy, G. Muthusamy and A. Arun, A comprehensive review on polylactic acid (PLA) – Synthesis, processing and application in food packaging, *Int J Biol Macromol*, 2023, **236**, 123715.
26 S. Farah, D. G. Anderson and R. Langer, Physical and mechanical properties of PLA, and their functions in widespread applications – A comprehensive review, *Adv Drug Deliv Rev*, 2016, **107**, 367–392.
27 L. Zhang, Z. Ye and P. Hou, Biodegradable PLA/TPU blends with improved mechanical properties, *Mater Lett*, 2025, **387**, 138205.
28 M. Puthumana, P. Santhana Gopala Krishnan and S. K. Nayak, Chemical modifications of PLA through copolymerization, *Int J Polym Anal Charact*, 2020, **25**(8), 634–648.
29 M. Wei, Q. Li, F. Chen and Y. Zhang, Improving mechanical properties of PLA with pinus sylvestris char: Exploring thermal stability, interfacial strengthening mechanisms, and applications in 3D printing, *Sustain Chem Pharm*, 2024, **42**, 101759.
30 T. Raja, Y. Devrajan, S. Ali and N. L, Comprehensive analysis of thermal properties and stability in palm fiber-reinforced PLA composites with bran filler for sustainable lightweight materials, *Case Stud Therm Eng*, 2025, **69**, 106003.
31 S. M. Emadian, T. T. Onay and B. Demirel, Biodegradation of bioplastics in natural environments, *Waste Manag*, 2017, **59**, 526–536.
32 A. J. Spicer, A. Brandolese and A. P. Dove, Selective and Sequential Catalytic Chemical Depolymerization and Upcycling of Mixed Plastics, *ACS Macro Lett*, 2024, **13**, 189–194.
33 L. Shao, Y. C. Chang, C. Hao, M. E. Fei, B. Zhao, B. J. Bliss and J. Zhang, A chemical approach for the future of PLA upcycling: from plastic wastes to new 3D printing materials, *Green Chem*, 2022, **24**, 8716–8724.
34 S. Liu, L. Hu, J. Liu, Z. Zhang, H. Suo and Y. Qin, Zinc Catalyst for Chemical Upcycling of PLA Wastes: Novel Industrial Monomer Resource toward Poly(ester-amide), *Macromol*, 2024, **57**, 4662–4669.

35 R. Marín, A. Alla, A. Martínez De Ilarduya and S. Muñoz-Guerra, Carbohydrate-based polyurethanes: A comparative study of polymers made from isosorbide and 1,4-butanediol, *J Appl Polym Sci*, 2012, **123**, 986–994.
36 W. Fang, F. Xu, Y. Zhang, H. Wang, Z. Zhang, Z. Yang, W. Wang, H. He and Y. Luo, Acylamido-based anion-functionalized ionic liquids for efficient synthesis of poly(isosorbide carbonate), *Catal Sci Technol*, 2021, **12**, 1756–1765.
37 D. Kim, I. C. Kim, Y. N. Kyun and S. Myung, Novel bio-based polymer membranes fabricated from isosorbide-incorporated poly(arylene ether)s for water treatment, *Eur Polym J*, 2020, **136**, 109931.
38 M. Rose and R. Palkovits, Isosorbide as a renewable platform chemical for versatile applications-quo vadis?, *ChemSusChem*, 2012, **5**, 167–176.
39 Mitsubishi Chemical Corporation, https://www.m-chemical.co.jp/en/products/departments/mcc/pc/product/1201026_9368.html (accessed 10.08.2025).
40 L. Matt, J. Parve, O. Parve, T. Pehk, T. H. Pham, I. Liblikas, L. Vares and P. Jannasch, Enzymatic Synthesis and Polymerization of Isosorbide-Based Monomethacrylates for High-T_g Plastics, *ACS Sustain Chem Eng*, 2018, **6**, 17382–17390.
41 S. Laanesoo, O. Bonjour, J. Parve, O. Parve, L. Matt, L. Vares and P. Jannasch, Poly(alkanoyl isosorbide methacrylate)s: From Amorphous to Semicrystalline and Liquid Crystalline Biobased Materials, *Biomacromol*, 2021, **22**, 640–648.
42 L. Matt, I. Liblikas, O. Bonjour, P. Jannasch and L. Vares, Synthesis and anionic polymerization of isosorbide mono-epoxides for linear biobased polyethers, *Polym Chem*, 2021, **12**, 5937–5941.
43 S. Laanesoo, O. Bonjour, R. Sedrik, I. Tamsalu, P. Jannasch and L. Vares, Combining isosorbide and lignin-related benzoic acids for high-Tg polymethacrylates, *Eur Polym J*, 2024, **202**, 211–228.
44 F. Nonque, A. Sahut, N. Jacquel, R. Saint-Loup, P. Woisel and J. Potier, Isosorbide monoacrylate: A sustainable monomer for the production of fully bio-based polyacrylates and thermosets, *Polym Chem*, 2020, **11**, 6903–6909.
45 F. Nonque, A. Benlahoues, J. Audourenc, A. Sahut, R. Saint-Loup, P. Woisel and J. Potier, Study on polymerization of bio-based isosorbide monomethacrylate for the formation of low-Tg and high-Tg sustainable polymers, *Eur Polym J*, 2021, **160**, 110799.
46 S. Ez-Zahraoui, F. Z. Semlali Aouragh Hassani, M. El Achaby, A. el kacem Qaiss and R. Bouhfid, Natural fiber reinforcements: classification, extraction, treatment, and properties, *Multiscale Textile Preforms and Structures for Natural Fiber Composites*, *Woodhead Publishing*, ed. M. Midani, T. Hamouda, A. H. Hassanin, A. M. Seyam, *Woodhead Pub.*, 2023, 3–29.
47 D. M. Neiva, J. Rencoret, G. Marques, A. Gutiérrez, J. Gominho, H. Pereira and J. C. del Río, Lignin from Tree Barks: Chemical Structure and Valorization, *ChemSusChem*, 2020, **13**, 4537–4547.
48 L. A. Zevallos Torres, A. Lorenci Woiciechowski, V. O. de Andrade Tanobe, S. G. Karp, L. C. Guimarães Lorenci, C. Faulds and C. R. Soccol, Lignin as a potential source of high-added value compounds: A review, *J Clean Prod*, 2020, **263**, 121499.
49 C. Libretti, L. Santos Correa and M. A. R. Meier, From waste to resource: advancements in sustainable lignin modification, *Green chem*, 2024, **26**, 4358–4386.
50 J. Domínguez-Robles, E. Larrañeta, M. L. Fong, N. K. Martin, N. J. Irwin, P. Mutjé, Q. Tarrés and M. Delgado-Aguilar, Lignin/poly(butylene succinate) composites with antioxidant and antibacterial properties for potential biomedical applications, *Int J Biol Macromol*, 2020, **145**, 92–99.

51 X. Chen, Z. Li, L. Zhang, H. Wang, C. Qiu, X. Fan and S. Sun, Preparation of a novel lignin-based film with high solid content and its physicochemical characteristics, *Ind Crops Prod*, 2021, **164**, 113396.

52 S. Sen, S. Patil and D. S. Argyropoulos, Thermal properties of lignin in copolymers, blends, and composites: a review, *Green Chem*, 2015, **17**, 4862–4887.

53 K. A. Y. Koivu, H. Sadeghifar, P. A. Nousiainen, D. S. Argyropoulos and J. Sipilä, Effect of Fatty Acid Esterification on the Thermal Properties of Softwood Kraft Lignin, *ACS Sustain Chem Eng*, 2016, **4**, 5238–5247.

54 N. Shirke, V. Jamdar. Review on lignin-derived syringaldehyde-based polymers and its applications, preprint, *Green Mater*, 2024.

55 A. L. Holmberg, K. H. Reno, N. A. Nguyen, R. P. Wool and T. H. Epps, Syringyl Methacrylate, a Hardwood Lignin-Based Monomer for High-T_g Polymeric Materials, *ACS Macro Lett*, 2016, **5**, 574–578.

56 O. Bonjour, H. Nederstedt, M. V. Arcos-Hernandez, S. Laanesoo, L. Vares and P. Jannasch, Lignin-Inspired Polymers with High Glass Transition Temperature and Solvent Resistance from 4-Hydroxybenzonitrile, Vanillonitrile, and Syringonitrile Methacrylates, *ACS Sustain Chem Eng*, 2021, **9**, 16874–16880.

57 X. Y. D. Soo, J. K. Muiruri, W. Y. Wu, J. C. C. Yeo, S. Wang, N. Tomczak, W. Thitsartarn, B. H. Tan, P. Wang, F. Wei, A. Suwardi, J. Xu, X. J. Loh, Q. Yan and Q. Zhu, Bio-Polyethylene and Polyethylene Biocomposites: An Alternative toward a Sustainable Future, *Macromol Rapid Commun*, 2024, **45**, 2400064

58 L. Zimmermann, A. Dombrowski, C. Völker and M. Wagner, Are bioplastics and plant-based materials safer than conventional plastics? In vitro toxicity and chemical composition, *Environ Int*, 2020, **145**, 106066.

59 Global Carbon Budget 2024, https://essd.copernicus.org/articles/17/965/2025/essd-17-965-2025.html (accessed 10.08.2025).

60 J. Gibbins and H. Chalmers, Carbon capture and storage, *Energy Policy*, 2008, **36**, 4317–4322.

61 A. S. Bhown, B. C. Freeman, Analysis and status of post-combustion carbon dioxide capture technologies, *Environ Sci Technol*, 2011, **45**, 8624–8632.

62 A. G. Romero-lGarcía, N. Ramírez-Corona, E. Sánchez-Ramírez, H. Alcocer-García, C. De Blasio and J. G. Segovia-Hernández, Sustainability assessment in the CO_2 capture process: Multi-objective optimization, *Chem Eng Process – Process Intensif*, 2022, **182**, 109207.

63 B. Aghel, S. Janati, S. Wongwises and M. S. Shadloo, Review on CO_2 capture by blended amine solutions, *Int J Greenh Gas Con*, 2022, **119**, 103715.

64 F. Meng, Y. Meng, T. Ju, S. Han, L. Lin and J. Jiang, Research progress of aqueous amine solution for CO_2 capture: A review, *Renew Sustain Energy Rev*, 2022, **168**, 112902.

65 A. Gautam and M. K. Mondal, Review of recent trends and various techniques for CO_2 capture: Special emphasis on biphasic amine solvents, *Fuel*, 2023, **334**, 126616.

66 C. Nwaoha, C. Saiwan, P. Tontiwachwuthikul, T. Supap, W. Rongwong, R. Idem, M. J. AL-Marri and A. Benamor, Carbon dioxide (CO_2) capture: Absorption-desorption capabilities of 2-amino-2-methyl-1-propanol (AMP), piperazine (PZ) and monoethanolamine (MEA) tri-solvent blends, *J Nat Gas Sci Eng*, 2016, **33**, 742–750.

67 K. Maneeintr, R. O. Idem, P. Tontiwachwuthikul and A. G. H. Wee, Synthesis, solubilities, and cyclic capacities of amino alcohols for CO_2 capture from flue gas streams, *Energy Procedia*, 2009, **1**, 1327–1334.

68 G. Li, X. Shen, X. Jiao, F. Xie, J. Hua, H. Lin, F. Yan, H. Wu and Z. Zhang, Novel tri-solvent amines absorption for flue gas CO_2 capture: Efficient absorption and regeneration with low energy consumption, *Chem Eng J*, 2024, **493**, 152699.
69 Catalyst Separation, Recovery and Recycling: Chemistry and Process Design, ed. by D. Cole-Hamilton and R. Tooze, *Springer: Dordrect*, 2006, 250 pp.
70 J. Chang, High value chemicals and materials production based on biomass components separation, *Renewable Resources for Biorefineries,* ed. C. Lin, R. Luque, *RCS*, 2014, 146–175.
71 F. Ausfelder, A. Bazzanella, H. VanBracki, R. Wilde, C. Beckmann, R. Mills, E. Rightor, C. Tam, N. Trudeau and P. Botschek, *Technology Roadmap Energy and GHG Reductions in the*, 2013.
72 A. M. Hyde, S. L. Zultanski, J. H. Waldman, Y. L. Zhong, M. Shevlin and F. Peng, General Principles and Strategies for Salting-Out Informed by the Hofmeister Series, *Org Process Res Dev*, 2017, **21**, 1355–1370.
73 P. K. Grover and R. L. Ryall, Critical appraisal of salting-out and its implications for chemical and biological sciences, *Chem Rev*, 2005, **105**, 1–10.
74 M. Sanger, D. Barker and P. G. Jessop, Simultaneous switching of two different CO_2-switchable amines in the same solution, *Phys Chem Chem Phys*, 2024, **26**,11406–11413.
75 S. M. Mercer, T. Robert, D. V. Dixon, C. S. Chen, Z. Ghoshouni, J. R. Harjani, S. Jahangiri, G. H. Peslherbe and P. G. Jessop, Design, synthesis, and solution behaviour of small polyamines as switchable water additives, *Green Chem*, 2012, **14**, 832–839.
76 I. T. Cunha, H. Yang and P. G. Jessop, High pressure switchable water: an alternative method for separating organic products from water, *Green Chem*, 2021, **23**, 3996–4007.
77 I. T. Cunha, H. Yang and P. G. Jessop, High pressure switchable water: an alternative method for separating organic products from water, *Green Chem*, 2021, **23**, 3996–4007.
78 E. K. Okeukwu, O. C. Okeke, M. O. Irefin, H. I. Ezeala and C. C. Amadi, Environmental Impact Assessment and Environmental Risk Assessment: Review of Concepts, Steps and Significance, *IIARD Int J Geogr Environ Manag*, 2023, **9**, 25–51.
79 D. U.S. Environmental Protection Agency Washington, Guidelines for Ecological Risk Assessment, *Environ Toxicol Chem*, 1998, **11**, 1663–1672.
80 S. K. Stefanis, A. G. Livingston and E. N. Pistikopoulos, Environmental Risk Assessment of Chemical Plants, *Adv Saf Reliab*, 1997, 639–648.
81 M. R. Adam, T. El-Badawy, M. S. Shamsudin, S. K. Hubadillah, M. R. Jamalludin, M. H. A. Aziz and A. M. Nasir, Deforestation influence on climate change, *Greenhouse Gases Formation and Challenges*, ed. M. R. Rahimpour, M. A. Makarem, M. Meshksar, *Elsevier*, 2024, 169–183.
82 L. Evariste, E. David, P. L. Cloutier, P. Brousseau, M. Auffret, M. Desrosiers, P. E. Groleau, M. Fournier and S. Betoulle, Field biomonitoring using the zebra mussel Dreissena polymorpha and the quagga mussel Dreissena bugensis following immunotoxic reponses. Is there a need to separate the two species?, *Environ Pollut*, 2018, **238**, 706–716.
83 P. Nie and J. Feng, Global niche and range shifts of Batrachochytrium dendrobatidis, a highly virulent amphibian-killing fungus, *Fungal Biol*, 2022, **126**, 809–816.
84 Y. Ma, H. Liu, J. Wu, L. Yuan, Y. Wang, X. Du, R. Wang, P. W. Marwa, P. Petlulu, X. Chen and H. Zhang, The adverse health effects of bisphenol A and related toxicity mechanisms, *Environ Res*, 2019, **176**, 108575.

85 EPA, Human Health Risk Assessment, https://www.epa.gov/risk/human-health-risk-assessment (accessed 10.08.2025).
86 EPA, Ecological Risk Assessment, https://www.epa.gov/risk/ecological-risk-assessment (accessed 10.08.2025).
87 T. I. Moiseenko, Aquatic ecotoxicology: Theoretical principles and practical application, *Water Res*, 2008, **35**, 530–541.
88 EPA, Overview of Freshwater and Marine Toxicity Tests: A Technical Tool for ecological risk Assessment, 2004, 154.
89 International Organization for Standardization, *ISO 6341:2012. Water quality – Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) – Acute toxicity test*, 2012.
90 International Organization for Standardization, ISO 8692:2012 Water quality – Fresh water algal growth inhibition test with unicellular green algae, *International Standard*, 2012, 16–18.
91 International Organization for Standardization, *ISO 11348-3:2007 – Water quality – Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test)*, 2007.
92 OECD, *Daphnia sp. Acute Immobilisation Test*, 2004.
93 OECD, *Freshwater Alga and Cyanobacteria, Growth Inhibition Test*, 2011.
94 OECD, *Fish, Acute Toxicity Test*, 1992.
95 C. T. Bowmer, R. N. Hooftman, A. O. Hanstveit, P. W. M. Venderbosch and N. Van Der Hoeven, The ecotoxicity and the biodegradability of lactic acid, alkyl lactate esters and lactate salts, *Chemosphere*, 1998, **37**, 1317–1333.
96 N. C. Saha, F. Bhunia and A. Kaviraj, Comparative toxicity of three organic acids to freshwater organisms and their impact on aquatic ecosystems, *Hum Ecol Risk Assess*, 2006, **12**, 192–202.
97 S. L. ; D. Z. ; R. E. ; J. N. Waaijers-van der Loop, Toxicity screening of potential bio-based Polar Aprotic Solvents (PAS), *Methodology*, 2018, **5**, 1.
98 L. Zimmermann, G. Dierkes, T. A. Ternes, C. Völker, M. Wagner, Benchmarking the in Vitro Toxicity and Chemical Composition of Plastic Consumer Products, *Environ Sci Technol*, 2019, **53**, 11467–11477.
99 J. M. van Rossum, The Relation Between Chemical Structure and Biological Activity, *J Pharm Pharmacol*, 1963, **15**, 285–316.
100 T. Pirman, M. Ocepek and B. Likozar, Radical Polymerization of Acrylates, Methacrylates, and Styrene: Biobased Approaches, Mechanism, Kinetics, Secondary Reactions, and Modeling, *Ind Eng Chem Res*, 2021, **60**, 9347–9367.
101 C. A. Staples, S. R. Murphy, J. E. McLaughlin, H. W. Leung, T. C. Cascieri and C. H. Farr, Determination of selected fate and aquatic toxicity characteristics of acrylic acid and a series of acrylic esters, *Chemosphere*, 2000, **40**, 29–38.
102 H. Greim, J. Ahlers, R. Bias, B. Broecker, H. Hollander, H. P. Gelbke, S. Jacobi, H. J. Klimisch, I. Mangelsdorf, W. Mayr, N. Schön, G. Stropp, P. Stahnecker, R. Vogel, C. Weber, K. Ziegler-Skylakakis and E. Bayer, Assessment of structurally related chemicals: Toxicity and ecotoxicity of acrylic acid and acrylic acid alkyl esters (acrylates), methacrylic acid and methacrylic acid alkyl esters (methacrylates), *Chemosphere*, 1995, **31**, 2637–2659.
103 L. E. Sverdrup, T. Källqvist, A. E. Kelley, C. S. Fürst and S. B. Hagen, Comparative toxicity of acrylic acid to marine and freshwater microalgae and the significance for environmental effects assessments, *Chemosphere*, 2001, **45**, 653–658.

104 U. Blaschke, K. Eismann, A. Böhme, A. Paschke and G. Schüürmann, Structural alerts for the excess toxicity of acrylates, methacrylates, and propiolates derived from their short-term and long-term bacterial toxicity, *Chem Res Toxicol*, 2012, **25**, 170–180.
105 J. A. Finlay and M. E. Callow, The potential of alkyl amines as antifouling biocides I: Toxicity and structure activity relationships, *Biofouling*, 1996, **9**, 257–268.
106 L. Garnick, C. Bates, A. Massarsky, P. Spencer, P. Sura, A. D. Monnot and A. Maier, Developmental and reproductive toxicity hazard characterization of 2-amino-2-methyl-1-propanol (AMP), *J of Appl Toxicol*, 2024, **44**, 316–332.
107 B. Peric, J. Sierra, E. Martí, R. Cruañas, M. A. Garau, J. Arning, U. Bottin-Weber and S. Stolte, (Eco)toxicity and biodegradability of selected protic and aprotic ionic liquids, *J Hazard Mater*, 2013, **261**, 99–105.
108 G. Libralato, A. V. Ghirardini and F. Avezzù, Evaporation and air-stripping to assess and reduce ethanolamines toxicity in oily wastewater, *J Hazard Mater*, 2008, **153**, 928–936.
109 I. Eide-Haugmo, O. G. Brakstad, K. A. Hoff, K. R. Sørheim, E. F. da Silva and H. F. Svendsen, Environmental impact of amines, *Energy Procedia*, 2009, **1**, 1297–1304.
110 A. E. Poste, M. Grung and R. F. Wright, Amines and amine-related compounds in surface waters: A review of sources, concentrations and aquatic toxicity, *Sci Total Environ*, 2014, **481**, 274–279.
111 J. W. Davis and C. L. Carpenter, Environmental assessment of the alkanolamines., *Rev Environ Contam Toxicol*, 1997, **149**, 87–137.
112 F. Vega, A. Sanna, B. Navarrete, M. M. Maroto-Valer and V. J. Cortés, Degradation of amine-based solvents in CO2 capture process by chemical absorption, *Greenh Gases Sci Technol*, 2014, **4**, 707–733.
113 H. Bartsch and R. Montesano, Relevance of nitrosamines to human cancer, *Carcinogenesis*, 1984, **5**, 1381–1393.
114 N. Dai, W. A. Mitch, Influence of Amine Structural Characteristics on N – Nitrosamine Formation Potential Relevant to Postcombustion CO_2 Capture Systems, *Environ Sci Technol*, 2023, **47**, 13175–13183.
115 G. Eisenbrand, B. Pool-Zobel, V. Baker, M. Balls, B. J. Blaauboer, A. Boobis, A. Carere, S. Kevekordes, J. C. Lhuguenot, R. Pieters and J. Kleiner, Methods of in vitro toxicology, *Food Chem Toxicol*, 2002, **40**, 193–236.
116 J. Carter, Matt; Shieh, in *Guide to Research Techniques in Neuroscience*, 2010, pp. 281–296.
117 T. Mosmaa, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J Immunol Methods*, 1983, 55–63.
118 T. Decker and M. L. Lohmann-Matthes, A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity, *J Immunol Methods*, 1988, **115**, 61–69.
119 K. Berg, L. Zhai, M. Chen, A. Kharazmi and T. C. Owen, The use of a water-soluble formazan complex to quantitate the cell number and mitochondrial function of Leishmania major promastigotes, *Parasitol Res*, 1994, **80**, 235–239.
120 K. D. Paull, R. H. Shoemaker, M. R. Boyd, J. L. Parsons, P. A. Risbood, W. A. Barbera, M. N. Sharma, D. C. Baker, E. Hand, D. A. Scudiero, A. Monks, M. C. Alley and M. Grote, The synthesis of XTT: A new tetrazolium reagent that is bioreducible to a water-soluble formazan, *J Heterocycl Chem*, 1988, **25**, 911–914.

121 Y. Sasaki, M. Kakisaka, N. Chutiwitoonchai, S. Tajima, H. Hikono, T. Saito and Y. Aida, Identification of a novel multiple kinase inhibitor with potent antiviral activity against influenza virus by reducing viral polymerase activity, *Biochem Biophys Res Commun*, 2014, **450**, 49–54.
122 O. John, W. Ian, O. Terry and P. Fran§ois, Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity, *Eur J Biochem*, 2000, **267**, 5421–5426.
123 B. Jonsson, G. Liminga, K. Csoka, H. Fridborg, S. Dhar, P. Nygren and R. Larsson, Cytotoxic activity of calcein acetoxymethyl ester (calcein/AM) on primary cultures of human haematological and solid tumours, *Eur J Cancer*, 1996, **32**, 883–887.
124 I. Vermes, C. Haanen, H. Steffens-Nakken and C. Reutellingsperger, A novel assay for apoptosis Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V, *J Immunol Methods*, 1995, **184**, 39–51.
125 M. Nasrollahzadeh, Z. Nezafat, N. Shafiei, Lignin chemistry and valorization, *Biopolymer-Based Metal Nanoparticle Chemistry for Sustainable Applications: Volume 1: Classification, Properties and Synthesis*, 2021, 145–183.
126 I. V. Almeida, F. M. L. Cavalcante, V. E. P. Vicentini, Different responses of vanillic acid, a phenolic compound, in HTC cells: Cytotoxicity, antiproliferative activity, and protection from DNA-induced damage, *Genet Mol Res*, 2016, **15**, gmr15049388.
127 S. D. Ray, M. Krmic, A. Hussain, C. Marvilli, R. Fabian, A. Niha, M. Danai, Z. Smith, A. Jalshgari, N. Malik, A. Alhariri and R. Maldonado, Toxicity of natural products, *Encyclopedia of Toxicology, Fourth Edition: Volume 1–9*, 2023, **9**, V9-257-V9-282.
128 J. Pei, P. Velu, M. Zareian, Z. Feng and A. Vijayalakshmi, Effects of Syringic Acid on Apoptosis, Inflammation, and AKT/mTOR Signaling Pathway in Gastric Cancer Cells, *Front Nutr*, 2021, **8**, 788929.
129 S. Gheena and D. Ezhilarasan, Syringic acid triggers reactive oxygen species–mediated cytotoxicity in HepG2 cells, *Hum Exp Toxicol*, 2019, **38**, 694–702.
130 H. Rasmussen, K. H. Mogensen, M. D. Jeppesen, H. R. Sørensen and A. S. Meyer, 4-Hydroxybenzoic acid from hydrothermal pretreatment of oil palm empty fruit bunches – Its origin and influence on biomass conversion, *Biomass Bioenergy*, 2016, **93**, 209–216.
131 X. N. Wang, K. Y. Wang, X. S. Zhang, C. Yang and X. Y. Li, 4-Hydroxybenzoic acid (4-HBA) enhances the sensitivity of human breast cancer cells to adriamycin as a specific HDAC6 inhibitor by promoting HIPK2/p53 pathway, *Biochem Biophys Res Commun*, 2018, **504**, 812–819.
132 L. Karygianni, Z. Ren, H. Koo and T. Thurnheer, Biofilm Matrixome: Extracellular Components in Structured Microbial Communities, *Trends Microbiol*, 2020, **28**, 668–681.
133 H. C. Flemming and J. Wingender, The biofilm matrix, *Nat Rev Microbiol*, 2010, **8**, 623–633.
134 H. Y. Liu, E. L. Prentice and M. A. Webber, Mechanisms of antimicrobial resistance in biofilms, *npj Antimicrob Resist*, 2024, **2**, 1–10.
135 E. Ventura, A. Marín, J. Gámez-Pérez and L. Cabedo, Recent advances in the relationships between biofilms and microplastics in natural environments, *World J Microbiol Biotechnol*, 2024, **40**, 1–16.

136 A. Richert and G. B. Dąbrowska, Enzymatic degradation and biofilm formation during biodegradation of polylactide and polycaprolactone polymers in various environments, *Int J Biol Macromol*, 2021, **176**, 226–232.
137 Y. Han, R. Wang, D. Wang and Y. Luan, Enzymatic degradation of synthetic plastics by hydrolases/oxidoreductases, *Int Biodeterior Biodegradation*, 2024, **189**, 105746.
138 S. H. Lee, I. Y. Kim and W. S. Song, Biodegradation of polylactic acid (PLA) fibers using different enzymes, *Macromol Res*, 2014, **22**, 657–663.
139 S. Khan, S. Nadir, Z. U. Shah, A. A. Shah, S. C. Karunarathna, J. Xu, A. Khan, S. Munir and F. Hasan, Biodegradation of polyester polyurethane by Aspergillus tubingensis, *Environmental Pollution*, 2017, **225**, 469–480.
140 R. Gautam, A. S. Bassi and E. K. Yanful, Candida rugosa lipase-catalyzed polyurethane degradation in aqueous medium, *Biotechnol Lett*, 2007, **29**, 1081–1086.
141 M. Osman, S. M. Satti, A. Luqman, F. Hasan, Z. Shah and A. A. Shah, Degradation of Polyester Polyurethane by Aspergillus sp. Strain S45 Isolated from Soil, *J Polym Environ*, 2018, **26**, 301–310.
142 S. Loharch and Ł. Berlicki, Rational Development of Bacterial Ureases Inhibitors, *Chemical Record*, 2022, **22**, e202200026.
143 L. Qiu, X. Yin, T. Liu, H. Zhang, G. Chen and S. Wu, Biodegradation of bis(2-hydroxyethyl) terephthalate by a newly isolated Enterobacter sp. HY1 and characterization of its esterase properties, *J Basic Microbiol*, 2020, **60**, 699–711.
144 K. Li, L. Xu, X. Bai, G. Zhang, M. Zhang and Y. Huang, Potential environmental risks of field bio/non-degradable microplastic from mulching residues in farmland: Evidence from metagenomic analysis of plastisphere, *J Hazard Mater*, 2024, **465**, 133428.
145 L. Wang, Z. Luo, Z. Zhen, Y. Yan, C. Yan, X. Ma, L. Sun, M. Wang, X. Zhou and A. Hu, Bacterial community colonization on tire microplastics in typical urban water environments and associated impacting factors, *Environ Pollut*, 2020, **265**, 114922.
146 X. pan Guo, X. li Sun, Y. ru Chen, L. Hou, M. Liu and Y. Yang, Antibiotic resistance genes in biofilms on plastic wastes in an estuarine environment, *Sci Total Environ*, 2020, **745**, 140916.
147 F. Bydalek, G. Webster, R. Barden, A. J. Weightman, B. Kasprzyk-Hordern and J. Wenk, Microplastic biofilm, associated pathogen and antimicrobial resistance dynamics through a wastewater treatment process incorporating a constructed wetland, *Water Res*, 2023, **235**, 119936.
148 Y. Sun, N. Cao, C. Duan, Q. Wang, C. Ding and J. Wang, Selection of antibiotic resistance genes on biodegradable and non-biodegradable microplastics, *J Hazard Mater*, 2021, **409**, 124979.
149 E. M. Stevenson, A. Buckling, M. Cole, P. K. Lindeque and A. K. Murray, Selection for antimicrobial resistance in the plastisphere, *Science of the Total Environment*, 2024, **908**, 168234.
150 M. K. Viršek, M. N. Lovšin, Š. Koren, A. Kržan and M. Peterlin, Microplastics as a vector for the transport of the bacterial fish pathogen species Aeromonas salmonicida, *Mar Pollut Bull*, 2017, **125**, 301–309.
151 K. Kaur, S. Reddy, P. Barathe, U. Oak, V. Shriram, S. S. Kharat, M. Govarthanan and V. Kumar, Microplastic-associated pathogens and antimicrobial resistance in environment, *Chemosphere*, 2022, **291**, 133005.
152 K. K. Kwan, Direct toxicity assessment of solid phase samples using the toxi-chromotest kit, *Environ Toxicol Water Qual*, 1993, **8**, 223–230.

153 International Organization for Standardization, *ISO 20079:2005 – Water quality – Determination of toxic effect of water constituents and waste water to duckweed (Lemna minor) – Duckweed growth inhibition test*, 2005.
154 International Organization for Standardization, *ISO 14380:2011 – Water quality – Determination of the acute toxicity to Thamnocephalus platyurus*, 2011.
155 EU classification categories for hazardous to the aquatic environment, https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:083:0001:0053:en:PDF (accessed 10.08.2025).
156 *EU Environmental Hazards*, 2011, https://unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev01/English/04e_part4.pdf (accessed 10.08.2025).
157 E. Bolyen, J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. Bin Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y. X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight and J. G. Caporaso, Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2, *Nat Biotechnol*, 2019, **37**, 852–857.
158 B. J. Callahan, P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson and S. P. Holmes, DADA2: High-resolution sample inference from Illumina amplicon data, *Nat Methods*, 2016, **13**, 581–583.
159 N. A. Bokulich, B. D. Kaehler, J. R. Rideout, M. Dillon, E. Bolyen, R. Knight, G. A. Huttley and J. Gregory Caporaso, Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin, *Microbiome*, 2018, **6**.
160 D. McDonald, Y. Jiang, M. Balaban, K. Cantrell, Q. Zhu, A. Gonzalez, J. T. Morton, G. Nicolaou, D. H. Parks, S. M. Karst, M. Albertsen, P. Hugenholtz, T. DeSantis, S. J. Song, A. Bartko, A. S. Havulinna, P. Jousilahti, S. Cheng, M. Inouye, T. Niiranen, M. Jain, V. Salomaa, L. Lahti, S. Mirarab and R. Knight, Greengenes2 unifies microbial data in a single reference tree, *Nat Biotechnol*, 2024, **42**, 715–718.
161 M. C. Gershater and R. Edwards, Regulating biological activity in plants with carboxylesterases, *Plant Science*, 2007, **173**, 579–588.
162 K. Duis, T. Junker and A. Coors, Environmental fate and effects of water-soluble synthetic organic polymers used in cosmetic products, *Environ Sci Eur*, 2021, **33**, 1–20.

163 L. Zimmermann, G. Dierkes, T. A. Ternes, C. Völker and M. Wagner, Benchmarking the *in Vitro* Toxicity and Chemical Composition of Plastic Consumer Products, *Environ Sci Technol*, 2019, **53**, 9b02293.
164 M. Suh, D. Proctor, G. Chappell, J. Rager, C. Thompson, S. Borghoff, L. Finch, R. Ellis-Hutchings and K. Wiench, A review of the genotoxic, mutagenic, and carcinogenic potentials of several lower acrylates, *Toxicology*, 2018, **402–403**, 50–67.
165 T. J. McCarthy and G. Witz, Structure-activity relationships in the hydrolysis of acrylate and methacrylate esters by carboxylesterase in vitro, *Toxicology*, 1997, **116**, 153–158.
166 E. Yoshii, Cytotoxic effects of acrylates and methacrylates: Relationships of monomer structures and cytotoxicity, *J Biomed Mater Res*, 1997, **37**, 517–524.
167 G. Mahadevan and S. Valiyaveettil, Understanding the interactions of poly(methyl methacrylate) and poly(vinyl chloride) nanoparticles with BHK-21 cell line, *Sci Rep*, 2021, **11**, 2089.
168 S. A. Salim, E. A. Kamoun, S. Evans, T. H. Taha, E. M. El-Fakharany, M. M. Elmazar, A. F. Abdel-Aziz, R. H. Abou-Saleh and T. A. Salaheldin, Novel oxygen-generation from electrospun nanofibrous scaffolds with anticancer properties: Synthesis of PMMA-conjugate PVP-H2O2nanofibers, characterization, and: In vitro bioevaluation tests, *RSC Adv*, 2021, **11**, 19978–19991.
169 G. Libralato, A. Volpi Ghirardini and F. Avezzù, Seawater ecotoxicity of monoethanolamine, diethanolamine and triethanolamine, *J Hazard Mater*, 2010, **176**, 535–539.
170 S. Amézqueta, X. Subirats, E. Fuguet, M. Roses and C. Rafols, Octanol-water partition constant, *Liq Extr*, 2019, 183–208.
171 E. Popek, Environmental Chemical Pollutants, *Sampl Anal Environ Chem Pollut*, 2018, 13–69.
172 S. Awasthi and P. J. Boor, Lipid peroxidation and oxidative stress during acute allylamine-induced cardiovascular toxicity, *J Vasc Res*, 1994, **31**, 33–41.
173 Y. Feng, S. Chen, Y. Zhao, D. Wu and G. Li, Heterocyclic aromatic amines induce Neuro-2a cells cytotoxicity through oxidative stress-mediated mitochondria-dependent apoptotic signals, *Food Chem Toxicol*, 2022, **168**, 113376.
174 I. Liblikas, O. Bonjour, N. Savest, A. Krumme, P. Jannasch and L. Vares, Bis-spirocyclic diol monomers and polyurethanes derived from citric acid: Synthesis, properties, electrospinnability, and evaluation of chemical recyclability, *Chem Engin J*, 2025, **515**, 163525.
175 O. Bonjour, I. Liblikas, T. Pehk, T. Khai-Nghi, K. Rissanen, L. Vares, P. Jannasch and P. Jannasch, Rigid biobased polycarbonates with good processability based on a spirocyclic diol derived from citric acid, *Green Chemistry*, 2020, **22**, 3940–3951.
176 R. Sedrik, O. Bonjour, S. Laanesoo, I. Liblikas, T. Pehk, P. Jannasch and L. Vares, Chemically Recyclable Poly(β-thioether ester)s Based on Rigid Spirocyclic Ketal Diols Derived from Citric Acid, *Biomacromolecules*, 2022, **23**, 2685–2696.
177 E. Tarasova, N. Savale, L. Trifonova, I. Krasnou, I. Reile, M. Kudrjašova, A. Mere, T. Kaljuvee, V. Mikli, R. Sedrik and A. Krumme, Effect of green co-solvents on properties and synthesis of cellulose esters in superbase ionic liquid, *Cellulose*, 2024, **31**, 4911–4927.

ACKNOWLEDGEMENT

First of all, I would like to express my deepest gratitude to my supervisors, Veljo Kisand and Lauri Vares, for their valuable guidance, support and encouragement throughout my PhD work. Their expertise, constructive feedback and patience have been crucial in shaping this research and helping me grow. I am also sincerely grateful to my group members: Rauno, Livia, Siim, Amina, Ilme and Peeter. Your advice has always been helpful to me. I would also like to thank all my co-authors for making it possible to conduct research on various materials.

To my family, thank you for your unconditional love, support, and belief in me. I especially want to thank my brother, Radmir, for his constant motivation and for always being available during my most difficult moments. Your support has helped me overcome many challenges and made this journey possible. To my very first friends in Tartu, Katarina and Alexandra, thank you for being a company to me during the beginning and beyond. Our meetings sometimes ended unpredictably and made life even more fun. Veronika, Anna, Masha, and Kate, thank you for our group chat for being a source of laughter, perspective, comfort throughout this process, and most of all, a place to spill a great tea.

Most of all, I am immensely grateful to my husband, Mihhail, for his constant support, patience, understanding, and love.

PUBLICATIONS

CURRICULUM VITAE

Name: Alina Ismagilova
Date of birth: 14th August 1996
Citizenship: Russian
Contact: Institute of Technology, University of Tartu
Nooruse 1, 50411, Tartu
E-mail: alina.ismagilova@ut.ee

Education:

2021–…	University of Tartu, PhD studies in engineering and technology
2018–2020	Saint-Peterburg State Forest Technical University, MSc in energy and resource saving processes in chemical technology
2014–2018	Saint-Peterburg State Forest Technical University, BSc in chemical technology
2003–2014	Gymnasium №1, Salavat

Language skills:

Russian	native language
English	excellent in speech and writing
Estonian	elementary in speech and writing

Professional employment:

2020–…	University of Tartu, Institute of Technology, Junior Researcher Fellow
2019–2020	Saint-Peterburg State Forest Technical University, Laboratory Technician at the Department of Chemistry

Scientific publication:

Ismagilova, A.; Zakusilo, D.; Osetrova, L.; Vasilyev, A. Reaction of 5-phenyl-penta-2,4-dienoic acid with benzene in trifluoromethanesulfonic acid, *Russian Chemical Bulletin*, 2020, 69, 1928–1932.

Kochurin, M.; **Ismagilova, A**.; Zakusilo, D.; Khoroshilova, O.; Boyarskaya, I; Vasilyev, A. Reactions of linear conjugated dienone structures with arenes under superelectrophilic activation conditions. An experimental and theoretical study of intermediate multicentered electrophilic species, *New Journal of Chemistry*, 2022, 46, 12041–12053.

Ismagilova, A.; Matt, L.; P. Jannasch, Kisand V.; Vares, L. Ecotoxicity of isosorbide acrylate and methacrylate monomers and corresponding polymers, *Green Chemistry*, 2023, 25, 1626–1634.

Sedrik, R.; Bonjour, O.; de Souza, N. R. D.; **Ismagilova, A**.; Tamsalu, I.; Kisand, V.; Cherubini, F.; Jannasch P.; Vares, L. Aromatic Polymethacrylates from Lignin-Based Feedstock: Synthesis, Thermal Properties, Life-Cycle Assessment and Toxicity, *ChemSusChem*, 2025, 18, 1–7.

Palà, M.; **Ismagilova, A**.; Moreno, A.; Plaza, J.; Ronda, J. C.; Galià, M.; Vares L.; Lligadas, G. Thermoresponsive lactate amide acrylic polymers developed from PLA bags, *Polym Chem*, 2025, 16, 1692–1703.

Ismagilova, A.; Kisand, V.; Vares, L. Ecotoxicity risk assessment of amines used in 'switchable water' and CO2-capturing processes, *Environ Sci Process Impacts*, 2025, 27, 974–980.

ELULOOKIRJELDUS

Nimi: Alina Ismagilova
Sünniaeg: 14. august 1996
Kodakondsus: Venemaa
Kontakt: Institute of Technology, University of Tartu
Nooruse 1, 50411, Tartu
E-mail: alina.ismagilova@ut.ee

Haridus:

2021–…	Tartu Ülikool, doktorantuur (tehnika ja tehnoloogia)
2018–2020	Peterburi Riiklik Metsatehnika Ülikool, magistriõpe (energia- ja ressursisäästuprotsessid keemiatehnikas)
2014–	Peterburi Riiklik Metsatehnika Ülikool, bakalaureuseõpe (keemia tehnoloogia)
2003–2014	Gümnaasium №1, Salavat

Keeleoskus:

vene keel	emakeel
inglise keel	väga hea kõnes ja kirjas
eesti keel	algtase kõnes ja kirjas

Teenistuskäik:

2020–…	Tartu Ülikool, Tehnoloogiainstituut, nooremteadur
2019–2020	Peterburi Riiklik Metsatehnika Ülikool, keemia osakonna laboritehnik

Teaduspublikatsioonid:

Ismagilova, A.; Zakusilo, D.; Osetrova, L.; Vasilyev, A. Reaction of 5-phenylpenta-2,4-dienoic acid with benzene in trifluoromethanesulfonic acid, *Russian Chemical Bulletin*, 2020, 69, 1928–1932.

Kochurin, M.; **Ismagilova, A**.; Zakusilo, D.; Khoroshilova, O.; Boyarskaya, I; Vasilyev, A. Reactions of linear conjugated dienone structures with arenes under superelectrophilic activation conditions. An experimental and theoretical study of intermediate multicentered electrophilic species, *New Journal of Chemistry*, 2022, 46, 12041–12053.

Ismagilova, A.; Matt, L.; P. Jannasch, Kisand V.; Vares, L. Ecotoxicity of isosorbide acrylate and methacrylate monomers and corresponding polymers, *Green Chemistry*, 2023, 25, 1626–1634.

Sedrik, R.; Bonjour, O.; de Souza, N. R. D.; **Ismagilova, A**.; Tamsalu, I.; Kisand, V.; Cherubini, F.; Jannasch P.; Vares, L. Aromatic Polymethacrylates from Lignin-Based Feedstock: Synthesis, Thermal Properties, Life-Cycle Assessment and Toxicity, *ChemSusChem*, 2025, 18, 1–7.

Palà, M.; **Ismagilova, A**.; Moreno, A.; Plaza, J.; Ronda, J. C.; Galià, M.; Vares L.; Lligadas, G. Thermoresponsive lactate amide acrylic polymers developed from PLA bags, *Polym Chem*, 2025, 16, 1692–1703.

Ismagilova, A.; Kisand, V.; Vares, L. Ecotoxicity risk assessment of amines used in 'switchable water' and CO2-capturing processes, *Environ Sci Process Impacts*, 2025, 27, 974–980.

DISSERTATIONES TECHNOLOGIAE UNIVERSITATIS TARTUENSIS

1. **Imre Mäger**. Characterization of cell-penetrating peptides: Assessment of cellular internalization kinetics, mechanisms and bioactivity. Tartu 2011, 132 p.
2. **Taavi Lehto**. Delivery of nucleic acids by cell-penetrating peptides: application in modulation of gene expression. Tartu 2011, 155 p.
3. **Hannes Luidalepp**. Studies on the antibiotic susceptibility of *Escherichia coli*. Tartu 2012, 111 p.
4. **Vahur Zadin**. Modelling the 3D-microbattery. Tartu 2012, 149 p.
5. **Janno Torop**. Carbide-derived carbon-based electromechanical actuators. Tartu 2012, 113 p.
6. **Julia Suhorutšenko**. Cell-penetrating peptides: cytotoxicity, immunogenicity and application for tumor targeting. Tartu 2012, 139 p.
7. **Viktoryia Shyp**. G nucleotide regulation of translational GTPases and the stringent response factor RelA. Tartu 2012, 105 p.
8. **Mardo Kõivomägi**. Studies on the substrate specificity and multisite phosphorylation mechanisms of cyclin-dependent kinase Cdk1 in *Saccharomyces cerevisiae*. Tartu, 2013, 157 p.
9. **Liis Karo-Astover**. Studies on the Semliki Forest virus replicase protein nsP1. Tartu, 2013, 113 p.
10. **Piret Arukuusk**. NickFects–novel cell-penetrating peptides. Design and uptake mechanism. Tartu, 2013, 124 p.
11. **Piret Villo**. Synthesis of acetogenin analogues. Asymmetric transfer hydrogenation coupled with dynamic kinetic resolution of α-amido-β-keto esters. Tartu, 2013, 151 p.
12. **Villu Kasari**. Bacterial toxin-antitoxin systems: transcriptional cross-activation and characterization of a novel *mqsRA* system. Tartu, 2013, 108 p.
13. **Margus Varjak**. Functional analysis of viral and host components of alphavirus replicase complexes. Tartu, 2013, 151 p.
14. **Liane Viru**. Development and analysis of novel alphavirus-based multifunctional gene therapy and expression systems. Tartu, 2013, 113 p.
15. **Kent Langel**. Cell-penetrating peptide mechanism studies: from peptides to cargo delivery. Tartu, 2014, 115 p.
16. **Rauno Temmer**. Electrochemistry and novel applications of chemically synthesized conductive polymer electrodes. Tartu, 2014, 206 p.
17. **Indrek Must**. Ionic and capacitive electroactive laminates with carbonaceous electrodes as sensors and energy harvesters. Tartu, 2014, 133 p.
18. **Veiko Voolaid**. Aquatic environment: primary reservoir, link, or sink of antibiotic resistance? Tartu, 2014, 79 p.
19. **Kristiina Laanemets**. The role of SLAC1 anion channel and its upstream regulators in stomatal opening and closure of *Arabidopsis thaliana.* Tartu, 2015, 115 p.

20. **Kalle Pärn**. Studies on inducible alphavirus-based antitumour strategy mediated by site-specific delivery with activatable cell-penetrating peptides. Tartu, 2015, 139 p.
21. **Anastasia Selyutina**. When biologist meets chemist: a search for HIV-1 inhibitors. Tartu, 2015, 172 p.
22. **Sirle Saul**. Towards understanding the neurovirulence of Semliki Forest virus. Tartu, 2015, 136 p.
23. **Marit Orav**. Study of the initial amplification of the human papillomavirus genome. Tartu, 2015, 132 p.
24. **Tormi Reinson**. Studies on the Genome Replication of Human Papillomaviruses. Tartu, 2016, 110 p.
25. **Mart Ustav Jr**. Molecular Studies of HPV-18 Genome Segregation and Stable Replication. Tartu, 2016, 152 p.
26. **Margit Mutso**. Different Approaches to Counteracting Hepatitis C Virus and Chikungunya Virus Infections. Tartu, 2016, 184 p.
27. **Jelizaveta Geimanen**. Study of the Papillomavirus Genome Replication and Segregation. Tartu, 2016, 168 p.
28. **Mart Toots**. Novel Means to Target Human Papillomavirus Infection. Tartu, 2016, 173 p.
29. **Kadi-Liis Veiman**. Development of cell-penetrating peptides for gene delivery: from transfection in cell cultures to induction of gene expression *in vivo*. Tartu, 2016, 136 p.
30. **Ly Pärnaste**. How, why, what and where: Mechanisms behind CPP/cargo nanocomplexes. Tartu, 2016, 147 p.
31. **Age Utt**. Role of alphavirus replicase in viral RNA synthesis, virus-induced cytotoxicity and recognition of viral infections in host cells. Tartu, 2016, 183 p.
32. **Veiko Vunder**. Modeling and characterization of back-relaxation of ionic electroactive polymer actuators. Tartu, 2016, 154 p.
33. **Piia Kivipõld**. Studies on the Role of Papillomavirus E2 Proteins in Virus DNA Replication. Tartu, 2016, 118 p.
34. **Liina Jakobson**. The roles of abscisic acid, CO_2, and the cuticle in the regulation of plant transpiration. Tartu, 2017, 162 p.
35. **Helen Isok-Paas**. Viral-host interactions in the life cycle of human papillomaviruses. Tartu, 2017, 158 p.
36. **Hanna Hõrak**. Identification of key regulators of stomatal CO_2 signalling via O_3-sensitivity. Tartu, 2017, 260 p.
37. **Jekaterina Jevtuševskaja**. Application of isothermal amplification methods for detection of *Chlamydia trachomatis* directly from biological samples. Tartu, 2017, 96 p.
38. **Ülar Allas**. Ribosome-targeting antibiotics and mechanisms of antibiotic resistance. Tartu, 2017, 152 p.
39. **Anton Paier**. Ribosome Degradation in Living Bacteria. Tartu, 2017, 108 p.
40. **Vallo Varik**. Stringent Response in Bacterial Growth and Survival. Tartu, 2017, 101 p.

41. **Pavel Kudrin**. In search for the inhibitors of *Escherichia coli* stringent response factor RelA. Tartu, 2017, 138 p.
42. **Liisi Henno**. Study of the human papillomavirus genome replication and oligomer generation. Tartu, 2017, 144 p.
43. **Katrin Krõlov**. Nucleic acid amplification from crude clinical samples exemplified by *Chlamydia trachomatis* detection in urine. Tartu, 2018, 118 p.
44. **Eve Sankovski**. Studies on papillomavirus transcription and regulatory protein E2. Tartu, 2018, 113 p.
45. **Morteza Daneshmand**. Realistic 3D Virtual Fitting Room. Tartu, 2018, 233 p.
46. **Fatemeh Noroozi**. Multimodal Emotion Recognition Based Human-Robot Interaction Enhancement. Tartu, 2018, 113 p.
47. **Krista Freimann**. Design of peptide-based vector for nucleic acid delivery in vivo. Tartu, 2018, 103 p.
48. **Rainis Venta**. Studies on signal processing by multisite phosphorylation pathways of the *S. cerevisiae* cyclin-dependent kinase inhibitor Sic1. Tartu, 2018, 155 p.
49. **Inga Põldsalu**. Soft actuators with ink-jet printed electrodes. Tartu, 2018, 85 p.
50. **Kadri Künnapuu**. Modification of the cell-penetrating peptide PepFect14 for targeted tumor gene delivery and reduced toxicity. Tartu, 2018, 114 p.
51. **Toomas Mets**. RNA fragmentation by MazF and MqsR toxins of *Escherichia coli.* Tartu, 2019, 119 p.
52. **Kadri Tõldsepp**. The role of mitogen-activated protein kinases MPK4 and MPK12 in CO_2-induced stomatal movements. Tartu, 2019, 259 p.
53. **Pirko Jalakas**. Unravelling signalling pathways contributing to stomatal conductance and responsiveness. Tartu, 2019, 120 p.
54. **S. Sunjai Nakshatharan**. Electromechanical modelling and control of ionic electroactive polymer actuators. Tartu, 2019, 165 p.
55. **Eva-Maria Tombak**. Molecular studies of the initial amplification of the oncogenic human papillomavirus and closely related nonhuman primate papillomavirus genomes. Tartu, 2019, 150 p.
56. **Meeri Visnapuu**. Design and physico-chemical characterization of metal-containing nanoparticles for antimicrobial coatings. Tartu, 2019, 138 p.
57. **Jelena Beljantseva**. Small fine-tuners of the bacterial stringent response – a glimpse into the working principles of Small Alarmone Synthetases. Tartu, 2020, 104 p.
58. **Egon Urgard**. Potential therapeutic approaches for modulation of inflammatory response pathways. Tartu, 2020, 120 p.
59. **Sofia Raquel Alves Oliveira**. HPLC analysis of bacterial alarmone nucleotide (p)ppGpp and its toxic analogue ppApp. Tartu, 2020, 122 p.
60. **Mihkel Örd**. Ordering the phosphorylation of cyclin-dependent kinase Cdk1 substrates in the cell cycle. Tartu, 2021, 228 p.
61. **Fred Elhi**. Biocompatible ionic electromechanically active polymer actuator based on biopolymers and non-toxic ionic liquids. Tartu, 2021, 140 p.

62. **Liisi Talas**. Reconstructing paleo-diversity, dynamics and response of eukaryotes to environmental change over the Late-Glacial and Holocene period in lake Lielais Svētiņu using sedaDNA. Tartu, 2021, 118 p.
63. **Livia Matt**. Novel isosorbide-based polymers. Tartu, 2021, 118 p.
64. **Koit Aasumets**. The dynamics of human mitochondrial nucleoids within the mitochondrial network. Tartu, 2021, 104 p.
65. **Faiza Summer**. Development and optimization of flow electrode capacitor technology. Tartu, 2022, 109 p.
66. **Olavi Reinsalu**. Cancer-testis antigen MAGE-A4 is incorporated into extracellular vesiclesand is exposed to the surface. Tartu, 2022, 130 p.
67. **Tetiana Brodiazhenko**. RelA-SpoT Homolog enzymes as effectors of Toxin-Antitoxin systems. Tartu, 2022, 132 p.
68. **Georg-Marten Lanno**. Development of novel antibacterial drug delivery systems as wound scaffolds using electrospinning technology. Tartu, 2022, 175 p.
69. **Liubov Cherkashchenko**. New insights into alphaviral nsP2 functions. Tartu, 2023, 171 p.
70. **Kristina Kiisholts**. Peptide-based drug carriers and preclinical nanomedicine applications for endometriosis treatment. Tartu, 2023, 138 p.
71. **Kai Rausalu**. Alphaviral nsP2 protease: From requirements for functionality to inhibition. Tartu, 2023, 175 p.
72. **Laura Sandra Lello**. Unraveling the intricate nature of the alphavirus RNA replicase. Tartu, 2023, 219 p.
73. **Houman Masnavi**. Visibility Aware Navigation. Tartu, 2023, 180 p.
74. **Kadir Aktas**. Cosmic Ray Tomography based Object Reconstruction and Recognition. Tartu, 2023, 104 p.
75. **Egils Avots**. Brain abnormality detection using statistical analysis of individual structural connectivity networks and EEG signals. Tartu, 2023, 223 p.
76. **Sainan Wang**. Structure-guided insights into the functions of CHIKV nsP2. Tartu, 2024, 154 p.
77. **Anneli Samel**. Unveiling the characteristics of cancer-testis antigen MAGEA10. Tartu, 2024, 136 p.
78. **Ikechukwu Ofodile**. Fault tolerant attitude control for nanosatellites: ESTCube-2 case. Tartu, 2024, 130 p.
79. **Olena Zamora**. Impacts of plant hormones on controlling stomatal conductance. Tartu, 2024, 166 p.
80. **Mariliis Hinnu**. *In vitro* methods for studying the mechanisms of ribosome-targeting antibiotics. Tartu, 2024, 143 p.
81. **Chung-Yueh Yeh**. Characterization of MPK and HT1 kinases in CO_2-induced stomatal movements. Tartu, 2024, 118 p.
82. **Iman Dadras**. Low power neural network-based control and actuation solutions for insect-scale robots. Tartu, 2024, 149 p.
83. **Fatemeh Rastgar**. Towards reliable real-time trajectory optimization. Tartu, 2024, 158 p.

84. **Maria Maloverjan**. Optimizing cell-penetrating peptide-based nanoparticles for delivery of nucleic acid therapeutics. Tartu, 2024, 172 p.
85. **Joonas Merisalu**. Resistive switching in memristor structures with multilayer dielectrics. Tartu, 2024, 149 p.
86. **Siim Laanesoo**. Novel high-performance biomass-based polymers. Tartu, 2024, 117 p.
87. **Henri Ingelman**. Systems-level characterisation and improvement of *Clostridium autoethanogenum* metabolism. Tartu, 2024, 164 p.
88. **Mailis Laht**. Using the One Health approach for mapping the spread of antibiotic resistant bacteria in Estonia. Tartu, 2024, 188 p.
89. **Ingrid Rebane**. Structure-property relationships of moldable silicone foams. Tartu, 2024, 164 p.
90. **Robert Valner**. Design of TeMoto, a software framework for dependable, adaptive, and collaborative autonomous robots. Tartu, 2024, 182 p.
91. **Kristiina Kurg**. Exploring the potential of a liquid biopsy approach for melanoma diagnostics and the role of extracellular vesicles in atherosclerosis development. Tartu, 2025, 201 p.
92. **Rauno Sedrik**. Synthesis and investigation of polymers from different cyclic bio-based monomers. Tartu, 2025, 155 p.