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Ecotoxicological Studies of Polyurethane Foams and Bio-Based Materials: Environmental Impacts and Applications

Ph.D. Dissertation

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"For God hath not given us the spirit of fear; but of power, and of love, and of a sound mind"

(2 Timothy 1:7)

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Supervisor's Recommendation for

Julie Mallouhi

for her doctoral dissertation entitled as

Ecotoxicological Studies of Polyurethane Foams and Bio-Based Materials: Environmental Impacts and Applications

It was a great honor to work with Julie Mallouhi over the past four years. During this time, We had the opportunity to observe her continuous progress in all aspects of research and soft skills.

Throughout these four years, Julie has contributed to 11 research articles, two of which were published in internationally recognized scientific journals (Q1). Additionally, she has participated in conferences with 11 oral and poster presentations.

During her doctoral studies, Julie developed toxicity testing protocols and demonstrated their applicability in assessing the toxic properties of polyurethane foams and carbon-based materials. Moreover, she prepared composite materials and successfully enhanced the mechanical and acoustic properties of polyurethane foams using carbon-based materials derived from natural resources.

Her ability to conduct research in diverse fields is further evidenced by her involvement in both biological sample analysis and material development studies during her PhD. Her commitment to continuous improvement has been an inspiration to others. Julie has a pleasant personality and works well both independently and in diverse cultural and team environments. She also spent some time at the University of Lodz in Poland, where she engaged in research across various fields.

All the findings presented in Julie's publications and doctoral dissertation are her own and serve as strong evidence of her independent scientific work.

Based on all these achievements, We are confident that Julie Mallouhi is fully capable of conducting independent research, and We strongly recommend that she be awarded a PhD degree.

Miskolc, 20.02.2025

Dr. Béla Fiser **Dr. Emma Szőri-Dorogházi**

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TABLE OF CONTENTS

ACKNOWLEDGMENT 1
LIST OF ABBREVIATIONS 7
1. Introduction 9
1.1 Basic chemistry of polyurethane 10
1.1.1 Isocyanate 11
1.1.2 Polyols 14
1.1.3 Additives 15
1.2 Carbonaceous material 15
1.3 Water treatment - removal of heavy metals from water 16
1.4 Ecotoxicity assessment tests 18
1.4.1 Model organisms used for toxicity tests 19
1.5 Objectives of this work 23
2. Materials and methods 24
2.1. Development of toxicity tests for polyurethane foams 24
2.1.1 Preparation of flexible polyurethane foam 24
2.1.2 Acute toxicity test using *Sinapis alba* (white mustard) seed 25
2.1.3 Liquid phase bacteria-based toxicity test using *Escherichia coli* model organism 28
2.2 Applicability of the developed toxicity tests to study carbonaceous materials 34
2.2.1 Preparation of the Activated carbon (AC) and Biochar (BC) 34
2.2.2 Metal content of the samples 36
2.2.3 Electro-kinetic (zeta) potential measurements 36
2.2.4 Fourier Transform Infrared Spectroscopy (FTIR) characterization and specific surface area analysis 36
2.2.5 Specific surface area measurements 36
2.2.6 Toxicity test of activated carbon and biochar 37
2.3. Potential applications of carbonaceous materials 39
2.3.1. Water treatment/ removal of heavy metals from water 39
1. *Effect of solution pH* 40
2. *Effect of the initial metal concentration and adsorption isotherm* 42
2.1. Adsorption isotherms 42
2.1.1. The Langmuir isotherm model 43
2.1.2. The Freundlich isotherm model 43
3. *Effect of AC/BC dose* 44

DOI: 10.14750/ME.2025.028

3. Results and Discussion 45
3.1 Development of toxicity tests procedure for polyurethane foams 45
3.1.1 Acute toxicity test 45
3.1.2 Bacteria-based toxicity test in the liquid phase 48
3.1.3 Verifying the sensitivity of solid PUF testing methods by using intentionally made Toxic foam…. 53
3.1.4 Conclusion 63
3.2 Applicability of the developed toxicity tests to study carbonaceous materials 65
3.2.1 Metal content measurement of carbonaceous materials 65
3.2.2 Electrokinetic (Zeta) potential measurements of AC/BC samples 66
3.2.3 Infrared spectroscopy characterization of the studied samples 67
3.2.4 Specific surface area measurements 68
3.2.5 Toxicity Test 69
3.2.6 Conclusion 73
3.3 Potential applications of carbonaceous materials 74
3.3.1 Water treatment/ removal of heavy metals from water 74
1. Influence of pH 74
2. Effect of initial cobalt concentration and adsorption isotherm investigation 77
3. Effect of adsorbent dosage 79
3.3.2 Conclusion 80
4. Summary 82
5. New scientific results 84
6. Scientific publications 85
References 88

DOI: 10.14750/ME.2025.028

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DOI: 10.14750/ME.2025.028

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DOI: 10.14750/ME.2025.028

Declaration

During the preparation of the thesis, I did not use the services of artificial intelligence, except for grammatical and stylistic corrections.

Julie Mallouhi

DOI: 10.14750/ME.2025.028

LIST OF FIGURES

Figure 1. Global consumption of polyurethane in 2022 9
Figure 2. Schematic representation of the chemical reaction of polyurethane formation 11
Figure 3. Reaction of isocyanate with water 12
Figure 4. Reaction of isocyanate with urethane..... 13
Figure 5. Reaction of isocyanate with urea 13
Figure 6. Schematic structures of polyols 14
Figure 7. Steps of acute toxicity measurement by using *Sinapis alba* seeds..... 26
Figure 8. *Sinapis alba* seed test: a) arrangement of the seeds in the plate at the start of the test, b) germinated seeds after incubation, and measurement of the length of the growing seed root (the numbers on the ruler represent cm)..... 27
Figure 9. Flowchart of the bacterial test. 29
Figure 10. Arrangement of the microplate for measuring the OD of the starter bacterial suspension. Sterile LB was used for blanking. For adequate OD measurement, overnight culture had to be diluted. Every time two columns with the same arrangement were used for measuring. 31
Figure 11. Steps for preparing a tenfold serial dilution of the OD600 = 0.15 bacterial suspension and spreading the LB agar plates with the 10^6 dilution to determine bacterial concentration. 32
Figure 12. Flasks with LB with or without polyurethane foam (PUF) samples inoculated with *E. coli* after overnight incubation 33
Figure 13. Bacteria-based toxicity tests in liquid phase: a) flasks LB with *E. coli* liquid medium, b) plates with *E. coli* grown in LB solid medium for colony counting. 33
Figure 14. Preparation of activated carbon and biochar..... 35
Figure 15. *Sinapis alba* seed test: (**a**) different types of AC/BC suspended in 20 mL of dilution water; (**b**) germinated seeds after incubation. 38
Figure 16. Schematic representation of the adsorption process..... 40
Figure 17. The workflow of the adsorption tests employed during the study 41
Figure 18. A) Flask with LB medium and *E. coli* (Reference), B) spreaded sample from the Reference suspension, C) LB medium with Non-autoclaved Control PUF, D) spreaded sample from C suspension, E) LB medium with Autoclaved Control PUF, F) spreaded sample from E suspension, G) LB medium with Autoclaved Control PUF inoculated with *E. coli* suspension, H) spreaded sample from G suspension. 48
Figure 19. Percentage of the average colony number for the tested samples (Control foam, NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, and NCO-1.2). The colony number of the Control plate is considered 100%. The lack of columns (the consequence of sterilization) is indicated by using red arrows. 50
Figure 20. Plates with *E. coli* grown on LB solid plates: a) Autoclaved Control PUF + *E. coli* sample, b) Autoclaved NCO-0.8 PUF + *E. coli*, where NCO-0.8 refers to the isocyanate index of the foam. .. 50
Figure 21. a) Difference in the look of inoculated LB medium before and after incubation of sample NCO-0.8 (Non-autoclaved NCO-0.8 PUF + *E. coli*), b) Colony-forming units on the plate with sample NCO-0.8 inoculated with *E. coli* (Non-autoclaved NCO-0.8 PUF+ *E. coli*). 52
Figure 22. Percentage of the average root length distribution depends on the toxicity of the tested polyurethane foam (PUF). PUF samples are Control, a new NCO-1.0, and Toxic foam. Used soaking times were 24, 48, or 72 h respectively. The percentage of average root length exposed to the Control is considered 100%. 55
Figure 23. Differences in the look of inoculated LB medium before and after incubation depend on the toxicity of the used foams. Non-Autoclaved Control PUF+*E. coli*: sterile LB medium with non-autoclaved Control PUF and inoculated with *E. coli*. Non-Autoclaved Toxic PUF+*E. coli*: sterile LB medium with non-autoclaved Toxic PUF and inoculated with *E. coli*. 57

DOI: 10.14750/ME.2025.028

Figure 24. Percentage of the average bacterial colony number in the case of the Control, new NCO-1.0, and Toxic foam samples. The lack of columns (the consequence of sterilization or the toxic behavior of the released chemical) is indicated by using red arrows. 58
Figure 25. Difference in colony number of *E. coli* depending on type and condition of PUF samples**.** 59
Figure 26. No growing of bacteria on the plates in the case of autoclaving the foams 60
Figure 27. Difference in the colony number obtained with the samples New NCO-1.0 foam (Autoclaved PUF + *M. luteus*) and Toxic (Autoclaved PUF + *M. luteus*) foam..... 61
Figure 28. a) Different morphologies of bacteria of the New NCO-1.0 foam (Non-Autoclaved PUF + *M. luteus*) LB agar plates b) Liquid suspension and a spread plate with the Toxic (Non-Autoclaved PUF + *M. luteus*) sample 62
Figure 29. Zeta potential distribution of the four different activated carbon/biochar samples..... 67
Figure 30. FTIR spectra of the four different activated carbon/biochar (COMAC, AC, and BC) samples. 68
Figure 31. Percentage of the length of the growing root of *Sinapis alba* (white mustard) seeds exposed to AC (activated carbon was prepared from *Sargassum sp.* by chemical activation), and BC **(**biochar prepared from *Sargassum sp.* by pyrolysis) compared to the growing roots exposed to the COMAC (commercial activated carbon). The red dotted line refers to the level of germination seeds for the samples in comparison to the COMAC..... 70
Figure 32. Bacteria-based toxicity tests: (**a**) flasks containing LB liquid medium with AC/BC samples and *E. coli*, (**b**) plates spread with liquid sample from the flask above for colony counting, and (**c**) a representative plate test of dry AC/BC samples. 72
Figure 33. Effect of the pH of the solution on the adsorption of Co^{2+} by 0.05 g of the four studied samples, COMAC, AC, and BC at 2 mmol/L initial concentration. 75
Figure 34. Adsorption isotherms fitted on the Co^{2+} adsorption data measured by using different adsorbents..... 78
Figure 35. Effect of adsorbent dosage on adsorption of Co^{2+} ions (pH= 6.8, initial concentration = 2 mmol/L, agitation rate = 210 rpm) 80

DOI: 10.14750/ME.2025.028

LIST OF TABLES

Table 1. International standard methods for toxicity tests using different organisms 22
Table 2. Formulation of the polyurethane foam 24
Table 3. Source of the carbonaceous materials 36
Table 4. The length of *Sinapis alba* roots in the case of all plates (Reference, Control, NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, and NCO-1.2, where NCO – isocyanate index) measured after 72 h incubation in dark at 20°C 45
Table 5. Percentage change of the average length of the growing root compared to the root length of the Control samples (NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, and NCO-1.2) applying different soaking times (24 h, 48 h, 72 h) 46
Table 6. Germination index (GI%) of the samples (Control, NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, and NCO-1.2) 47
Table 7. Colony forming unit (CFU) for all samples (Control, NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, NCO-1.2) as follows: Autoclaved foam, Autoclaved foam + *E. coli*, Non-autoclaved foam + *E. coli*. 49
Table 8. Percentage of colony forming units (CFUs) for all samples (Control, NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, NCO-1.2) in the following cases: Autoclaved foam, Autoclaved foam + *E. coli*, Non-autoclaved foam + *E. coli* compared to colony numbers of the Control plate. (-) refer to no colony-forming unit observed. 51
Table 9. Root lengths of the growing seeds in the presence of the soaking liquid applied for Control, new NCO-1.0 sample, and the Toxic foam with different soaking times of 24 h, 48 h, or 72 h. 54
Table 10. Percentage of the average length of the growing roots compared to the root length of the Control samples (the root length of the Control was considered as 100 % for each soaking period used. NCO-1.0 is a newly prepared foam with NCO = 1.0, and Toxic foam is a new NCO-1.0 foam made toxic after its production using a form separator 56
Table 11. Germination index (GI %) of the studied samples: Control, new NCO-1.0 sample, and the Toxic foam. 56
Table 12. Percentage of colony forming units (CFUs) for all samples NCO-1.0, and Toxic foam in the following cases: Autoclaved foam, Autoclaved foam + *E. coli*., Non-autoclaved foam + *E. coli*. compared to colony numbers of the Control plate. (-) refer to no colony-forming unit observed. 59
Table 13. Cell concentration (CFU/mL) for the samples: New NCO-1.0 foam (Autoclaved PUF + *M. luteus*) and Toxic (Autoclaved PUF + *M. luteus*) foams. 62
Table 14. Percentage of colony forming units (CFUs) for the samples: New NCO-1.0 foam (Autoclaved PUF + *M. luteus*) and Toxic (Autoclaved PUF + *M. luteus*) foams. 63
Table 15. Comparison of the metal content of activated carbon/biochar samples (COMAC, AC, and BC) determined by using ICP-OES. 66
Table 16. Electro-kinetic (zeta) potential of the activated carbon/biochar samples (COMAC, AC, and BC) 66
Table 17. Specific surface area of the samples. 68
Table 18. The average root lengths of the growing seeds without (e.g., Dilution water-1) and in contact with the studied samples (COMAC, AC, and BC). 69
Table 19. Percentage of the length of the growing root exposed to AC, and BC compared to those exposed to the COMAC sample 70
Table 20. Germination index GI of the samples (COMAC, AC, and BC) 71
Table 21. Colony forming units (CFU) for all samples (COMAC + *E. coli*, AC + *E. coli*, BC + *E. coli*). 73
Table 22. The adsorbed amount of Co^{2+} ions by the tested samples: COMAC, AC, and BC 76
Table 23. Adsorption isotherm model and the corresponding parameters for Co^{2+} removal by using the studied adsorbents 79

DOI: 10.14750/ME.2025.028

LIST OF ABBREVIATIONS

PU	Polyurethane
EMEA	Europe, Middle East, and Africa
NCO	Isocyanate
MDI	Methylene Diphenyl Diisocyanate
TDI	Toluene Diisocyanate
HMDI	Hydrogenated Methylene Diphenyl Diisocyanate
IPDI	Isophorone Diisocyanate
TDA	Toluenediamine
PUF	Polyurethane Foam
DABCO	1,4-diazabicyclo[2.2.2]octane
DBTDL	Dibutyltin dilaurate
CFCs	Chlorofluorocarbons
HFCs	Hydrogenated fluorocarbons
TPU	Thermoplastic polyurethane
HS	hard segments
SS	Soft segments
PUD	Waterborne polyurethane dispersions
PUB	Polyurethane binder
ISO	International Organization for Standardization
OECD	Organization for Economic Cooperation and Development
EPA	Environmental Protection Agency
E. coli	Escherichia coli
PVA	Polyvinyl Alcohol
SET	Sea urchin Embryo Test
PLA	Polylactic Acid
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PP	Polypropylene
MSZ	Magyar Szabvány (Hungarian Standard)
GI	Germination Index
CFU	Colony Forming Unit
LB	Lysogeny broth
OD	Optical Density
AC	Activated Carbon
BC	Biochar
COMAC	Commercial Activated Carbon
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometer
FTIR	Fourier Transform Infrared Spectroscopy
BET	Brunauer–Emmett–Teller Isotherm
ASTM	American Society for Testing and Materials

DOI: 10.14750/ME.2025.028

CFD	Compression Force Deflection
C_e	equilibrium metal concentration
C_i	initial metal concentration
K_L	Langmuir equilibrium constant
q_m	Maximum monolayer adsorption capacity
K_f	Freundlich constant
n	heterogeneity factor

DOI: 10.14750/ME.2025.028

1. Introduction

Toxic materials pose significant risks to the environment and human health, as they can originate from industrial, agricultural, and synthetic sources and they can be harmful due to their raw materials, degradation byproducts, or heavy metal contamination [1], [2]. This is particularly important when considering polymeric materials. Synthetic polymers are usually created through the polymerization of monomers derived from petrochemicals which are then combined with other additives such as plasticizers, stabilizers, *etc* [3]. The first synthetic polymer, Bakelite, was developed in the early 20th century, and mass production began to flourish in the 1950s [3], [4]. From 1950 to 2018, plastic consumption has increased approximately 180 times, then it reached an estimated global production of 400.3 million tons in 2022 [3]. Polyurethane (PU), a significant type of polymer with versatile properties was discovered by Otto Bayer in 1937 [5]. The major types and applications of PU include flexible and rigid foams, thermoplastic elastomers, adhesives, and surface coatings [6] (**Figure 1**). The production of PU continues to grow at a rapid pace throughout the world, and it is highly demanded in many applications [7], [8]. PUs are ranked as the sixth most used plastic globally, and millions of tons are produced worldwide [4], [9]. The global market value of PU is estimated at 78 billion USD in 2023, with an expected annual compound growth rate of 4.5% through 2030 [10]. However, only 9% of the material is recycled, 12% is burned, and the rest ends up in landfills or in the environment [1], [9]. About half of them have been estimated to accumulate in landfill sites worldwide, and others completely escape the collection system [4] (**Figure 1**).

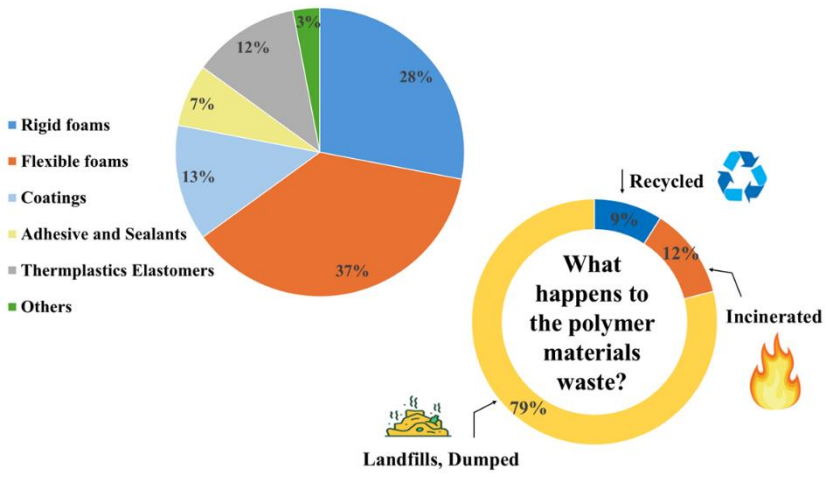

Figure 1. *Global consumption of polyurethane in 2022 [11]*

DOI: 10.14750/ME.2025.028

One of the most concerning issues is that this large amount of polyurethane waste accumulating in terrestrial and marine environments poses significant health and ecological risks for both humans and the environment [12], [13]. Due to the chemical stability and low bio-degradability, traditional landfilling not only consumes valuable land resources but also causes environmental pollution [14] because of long-term persistence in the environment, and the potentially toxic chemical content of PUs can be harmful [15], [16], [17]. To minimize the negative environmental effects of PUs, it is necessary to develop new polyurethane types that have a lower carbon footprint [18]. This can be achieved either by applying bio-based and nontoxic (*i.e.,* made from renewable resources rather than fossil fuels) or biodegradable (*i.e.,* the structure is willing to break down in the environment through physical, chemical, and biological processes) raw materials [19], [20]. Before testing the biodegradability of PU, their toxicity needs to be assessed first. Because if a material is toxic, it will kill microorganisms before they can initiate the degradation process. Studying the ecotoxicity of a pollutant can provide information about its biological accessibility and help to understand its behavior in the environment [21], [22].

Polyurethane is produced using a variety of chemical components, such as isocyanates, polyols, and additives, each of which might cause potential toxicological risks depending on their type, concentration, and exposure conditions. Understanding the properties of these individual chemicals is essential for assessing the overall environmental impact of polyurethane-based materials. The following is a list of chemicals used in the production of polyurethane, along with a description of their properties.

1.1 Basic chemistry of polyurethane

Polyurethanes (PUs) are polymers formed by the reaction between hydroxyl (OH) groups of a polyol and isocyanate (NCO) groups of an isocyanate, resulting in the formation of urethane linkages (-NH-COO-). This exothermic reaction produces urethane groups, giving the polymer its name [7], [18], [22], [23] (**Figure 2**). The urethane bond is relatively stable, but it can become toxic when it degrades and releases hazardous chemicals, particularly under certain conditions.

DOI: 10.14750/ME.2025.028

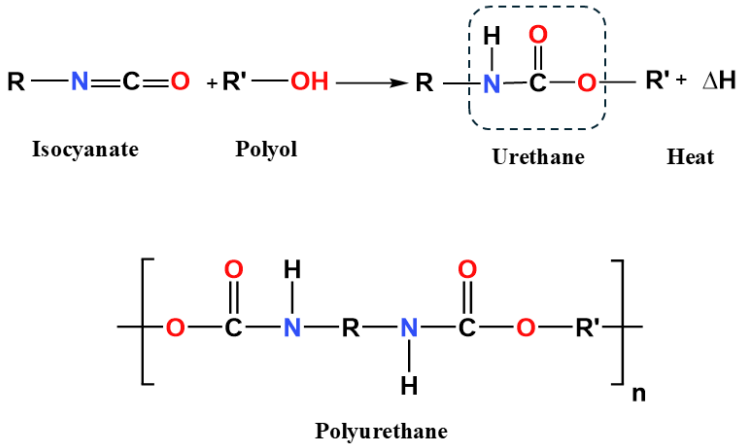

Figure 2. *Schematic representation of the chemical reaction of polyurethane formation* [23]

1.1.1 Isocyanate

Isocyanates are a highly reactive group of compounds defined by the functional group R−N=C=O, where R can be either an aliphatic or aromatic group [24]. The reactivity of isocyanates is determined by the positive partial charge of the carbon atom, making it vulnerable to nucleophilic attack by oxygen or nitrogen, and to electrophiles [23]. The most commonly used aromatic isocyanates are methylene diphenyl diisocyanate (MDI) and toluene diisocyanate (TDI), which together account for about 90% of total diisocyanate consumption [25]. Aliphatic isocyanates, such as isophorone diisocyanate (IPDI) and hexamethylene diisocyanate (HMDI), are also used, primarily in applications like coatings [9], [18]. However, these aliphatic molecules are less reactive and more costly than their aromatic counterparts. The fundamental reason for the difference in reactivity is the resonance stabilization of aromatic rings, which impacts the carbon atom in the NCO group [23].

Isocyanates exhibit high toxicity levels until they react with other chemicals during the PU synthesis process. However, polyurethane materials can degrade over time due to environmental factors such as heat, which may release toxic materials that can pose risks, especially if they leach into the environment. Therefore, while isocyanates are less hazardous once incorporated into the polyurethane structure, their potential toxicity remains a concern, especially during degradation or improper disposal [10].

Besides the reaction between polyols and isocyanates, several other reactions are also involved in polyurethane synthesis, including the reaction of isocyanates with alcohol, water, urethanes, ureas, and carboxylic acids [18].

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1.1.1.1 Reaction of isocyanate with alcohol

The reaction between isocyanates and alcohols is one of the most significant processes in polyurethane synthesis. It is an exothermic reaction that results in the formation of urethane bonds, where the nucleophilic oxygen of the alcohol group reacts with the partially positive carbon of the isocyanate group (**Figure 2**). This interaction facilitates the transfer of a proton from the hydroxyl group to the nitrogen of the isocyanate group, ultimately leading to the formation of the urethane bond [26], [27].

1.1.1.2 Reaction of isocyanate with water

The reaction between isocyanates and water produces carbon dioxide gas and urea **(Figure 3)**. This reaction is a convenient method for generating CO_2 to achieve the cellular structure of polyurethane foams [27]. The resulting amine quickly reacts with other isocyanate molecules to form a symmetrical disubstituted urea (**Figure 3**). Compared to alcohols, the reaction between isocyanates and water is more exothermic, releasing heat per mole of water at approximately 47 kcal/mol [27], [28]. In polyurethane foam production, water acts as an indirect chemical blowing agent, via its reaction with isocyanate it will lead to the formation of CO_2 which will initiate foam formation [29].

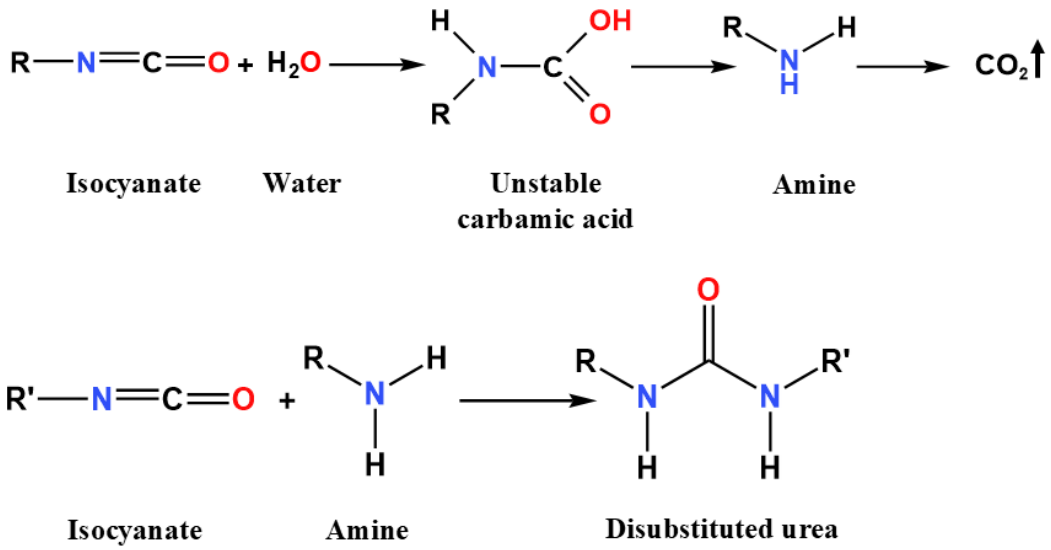

Figure 3. *Reaction of isocyanate with water*

DOI: 10.14750/ME.2025.028

1.1.1.3 Reaction of isocyanate with urethanes

Urethane groups are considered hydrogen-active compounds because of the hydrogen atom attached to the nitrogen. When an isocyanate reacts with the urethane group, it forms an allophanate (**Figure 4**). However, due to the electron-withdrawing effect of the carbonyl group, urethane is much less reactive than aminic -N-H groups. To promote allophanate formation, higher temperatures (> 110 ºC) are required [30]. It is also important to note that allophanate formation is a reversible reaction [26], [31], [32].

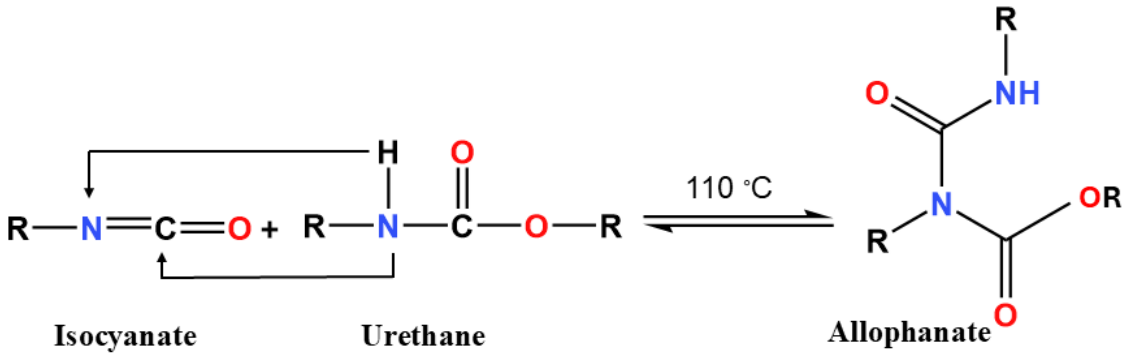

***Figure 4**. Reaction of isocyanate with urethane*

1.1.1.4 Reaction of isocyanate with urea

Similar to allophanate formation, the -N-H groups of urea can react with isocyanates to form biurets (**Figure 5**). Like the allophanate reaction, the reaction between urea and isocyanates is an equilibrium process that also requires elevated temperatures (above 110°C) [32]. The formation of allophanates and biurets is particularly common in polyurethane chemistry, especially when an excess of isocyanate is present [26].

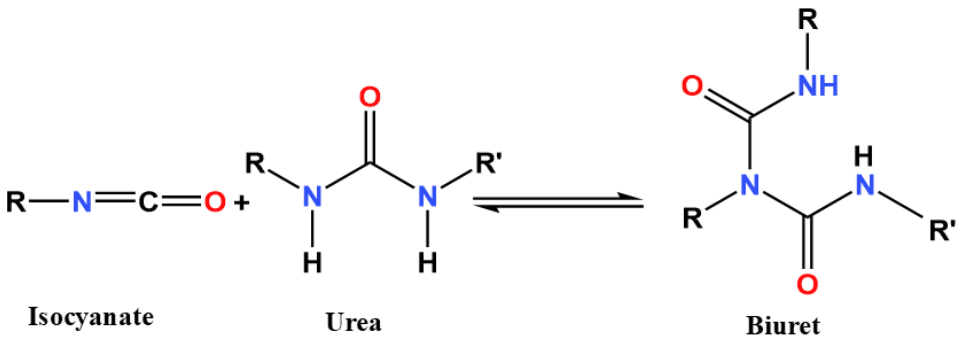

***Figure 5.** Reaction of isocyanate with urea*

DOI: 10.14750/ME.2025.028

1.1.2 Polyols

Polyols, typically containing two or more –OH groups, are the primary reaction partners of isocyanates used in polyurethane synthesis [33]. Various types of polyols can be prepared using different methods [23], [34]. Approximately 75% of all polyols are polyether polyols, while the remaining 25% consist of polyester polyols and other types [34]. Polyether polyols are produced through the reaction of an epoxide with a compound containing active hydrogen. They can also be synthesized via ring-opening polymerization of epoxy monomers. Another category, polyester polyols, is formed through the polycondensation of hydroxyl compounds with multifunctional carboxylic acids (**Figure 6**) [7].

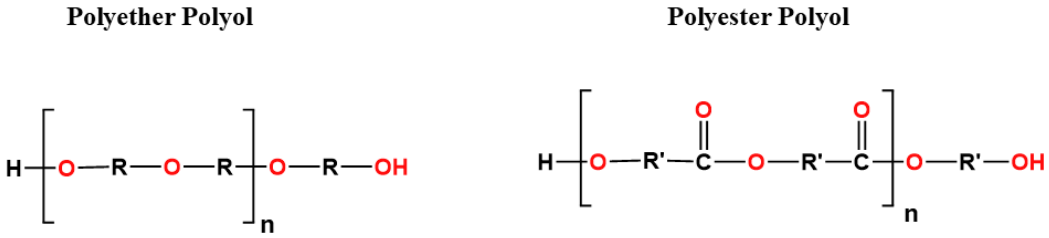

Figure 6. *Schematic structures of polyols*

Polyols can also be classified based on their end use, with their chemical composition and molecular weight determining the properties of the resulting polyurethanes. High molecular weight polyols (ranging from 2,000 to 10,000) are primarily used for synthesizing flexible polyurethanes, while low molecular weight polyols are used to produce rigid polyurethanes [7], [25].

Additionally, various properties of polyurethane foams (PUFs) can be adjusted by modifying the polyol functionality. For instance, increasing the functionality without changing the molecular weight results in a slight increase in foam hardness, and a small decrease in tensile strength, tear strength, and elongation. Conversely, increasing the equivalent weight of the polyol (molecular weight divided by functionality) while keeping the functionality constant enhances the tensile strength and elongation [18].

Polyether polyols are more resistant to hydrolysis but less stable against oxidation compared to polyester polyols [24]. As a result, polyether polyols are typically stabilized with antioxidants. On the other hand, polyester polyols tend to be more viscous and less resistant to hydrolysis. However, PUs derived from them exhibit superior physical properties compared to

DOI: 10.14750/ME.2025.028

those made from polyether polyols [22], [34]. Although, polyols are generally less toxic than other components such as isocyanates, certain polyols, especially those derived from petroleum-based sources, may contain residual toxic substances that can pose toxicity risks.

1.1.3 Additives

In addition to polyols and isocyanates, various additives are often needed during polyurethane production to control the reaction, modify processing conditions, and enhance the properties of the final product [32]. These additives include catalysts [35], chain extenders [36], cross-linkers [34], [37], surfactants [38], and blowing agents [34], [38]. While these additives play crucial roles in fine-tuning the properties of the material, they can also pose toxicity risks and cause skin irritation or respiratory issues with prolonged exposure [35]. Furthermore, some blowing agents, such as those releasing gases like nitrogen or carbon dioxide (CO_2), can be harmful when inhaled in high concentrations [38].

1.2 Carbonaceous material

In recent years, carbonaceous materials have gained significant attention alongside polymeric materials due to their exceptional properties, making them highly promising for applications in catalysis, separation science, environmental protection, energy storage, and water purification [14]. These materials can be derived from various carbon-rich sources, including biomass, organic waste, and synthetic polymers, through processes such as pyrolysis and carbonization [14], [39]. However, to develop more sustainable and environmentally friendly alternatives, extensive research is focused on producing and utilizing carbon materials from renewable natural resources. This approach aims to create cost-effective solutions with reduced reliance on non-renewable petroleum-based materials while maintaining or enhancing the functional performance of the resulting carbon-based materials [40], [41].

Carbonaceous materials are a class of porous substances, including carbon black, graphite, activated carbon, and biochar. These materials can be derived from a variety of natural and carbon-rich sources [40], [41]. Among these, activated carbon (AC) and biochar (BC) have emerged as particularly promising materials due to their large specific surface area, ability to be regenerated, desirable pore structure, strong chemical resistance, and the presence of various oxygen-containing functional groups on the surface [42], [43] making them suitable for a range of applications such as water treatment and agricultural enhancement [41], [44].

DOI: 10.14750/ME.2025.028

There is an increasing demand for AC and thus, its production has to be cheap and efficient. Activated carbon can be made from any carbonaceous substance, but the starting materials will define the cost and other properties of the final product. To achieve cost-effective production of AC, agricultural by-products that are easily available and abundant can be used as raw materials. Accordingly, wood, waste nut shells, cherry stones, tea waste, sugarcane bagasse, or fruit pits can be used as AC base material, and they are more cost-effective than the production of granular AC from non-renewable coal [40], [41].

The most important step in the production of activated carbon is activation. There are two methods to produce AC: physical activation and chemical activation, where the activation method depends on the starting material and whether powdered, granular, or low-density carbon is required [40]. Chemical activation is preferred over physical activation because this type is more economical, requires lower temperatures, produces a more uniform product, and takes less time [40].

Besides activated carbon, biochar is another type of versatile porous material primarily comprising carbon [45]. Biochar is produced by pyrolysis of biomass such as wood, grass, peanut straw, crop residues, or *Sargassum* sp. [46], [47], [48], [49]. Pyrolysis is a thermal decomposition process that involves the presence of little or no oxygen and occurs at temperatures ranging from 300 to 800°C [50].

These porous materials AC and BC with a large specific surface area have attracted much interest [44]. However, a significant challenge in the use of these carbonaceous materials is that toxic substances can be released from the materials matrix and affect living organisms [45]. Therefore, it is crucial to understand their environmental impact particularly when used in applications such as water purification and soil remediation [51].

1.3 Water treatment - removal of heavy metals from water

Water is one of the most crucial resources on Earth, responsible for the survival of all living organisms [42]. The accelerated rate of industrialization has significantly exacerbated the issue of water pollution [52], [53]. Heavy metals are toxic pollutants that enter surface and groundwater through various activities, including industrial processes, mining, and agriculture [54], [55]. Heavy metals provide a significant environmental risk due to their toxicity and availability. Although certain metals are essential in trace amounts, exceeding their tolerance levels can lead to various diseases, posing significant risks to both human and environmental

health [40], [42], [52]. The removal of hazardous heavy metal pollutants from water and wastewater is one of the most critical environmental challenges currently under investigation [56]. Various techniques, such as adsorption, chemical precipitation, electrochemical precipitation, chemical oxidation, ion exchange, and membrane filtration, are employed for this purpose which vary in their effectiveness and cost [56]. Among these, adsorption is considered one of the most effective methods due to its high removal efficiency, low cost, ease of operation, and the ability to regenerate adsorbents through an appropriate desorption process [57].

The adsorption process can be categorized into physical adsorption (physisorption) and chemical adsorption (chemisorption), based on the nature of the interaction [40]. Physisorption occurs through van der Waals forces, forming a multilayer of adsorbate, while chemisorption involves strong chemical bonds, such as ionic or covalent, resulting in a monolayer of adsorbate [40], [58] and leads to the formation of a monolayer of adsorbate on the adsorbent [59].

To understand and employ the adsorption processes, particularly in environmental remediation and wastewater treatment facilities, adsorption isotherms are crucial and can provide insights into the maximum adsorption capacity, which is a key factor in assessing the performance and effectiveness of adsorbents. Isotherm refers to the relationship between the equilibrium adsorbate concentrations in the liquid phase and the equilibrium adsorption amount in the solid phase at a certain temperature [58]. Several isotherm models exist for analyzing experimental adsorption equilibrium parameters such as Langmuir, Volmer, Brunauer-Emmett-Teller, Aranovich model, Freundlich, Redlich-Peterson, Temkin, Toth, and Sips.

Although several models also exist, the Langmuir and Freundlich isotherms are the most commonly used ones to describe the adsorption of metal ions, dyes, as well as other types of organic pollutants onto adsorbents [60], [61]. These two models are commonly employed in adsorption studies due to their applicability. Both models effectively describe the relationship between q_e (the quantity adsorbed at equilibrium, mg/g) and C_e (the concentration of adsorbate ions remaining in the bulk solution at equilibrium, mg/L) [62]. In general, experimental data is fitted to different adsorption isotherm models, and the best-fitting model is used to define equilibrium adsorption. After selecting an appropriate adsorption model, the model's adsorption parameters were obtained and utilized to characterize the properties of the process [63].

Key adsorption parameters such as pH, initial metal concentration, and adsorbent dose should also be considered as these are the most important parameters influencing the efficiency

and performance of the adsorption process [40]. Furthermore, electrostatic interactions between positively charged metal ions and negatively charged functional groups on the surface of adsorbents can play a significant role in enhancing the adsorption capacity [58].

The key criteria for an adsorption process are the adsorbent's selectivity, high adsorption capacity, and low cost [64]. Activated carbon and biochar have been shown to be effective adsorbents for the removal of a wide range of contaminants that exist in the aquatic environment, due to their high surface area and porosity, in addition to a variety of surface functional groups like carboxyl (-COOH), and hydroxyl (-OH), which play a role in adsorbing contaminants [56], [65], [66]. These functional groups attract heavy metal ions either through electrostatic attraction or surface metal complexation [42].

Despite activated carbon and biochar being highly efficient materials utilized in various applications, there are concerns regarding their possible toxicity [67]. Toxic materials can be released from these since heavy metals or other toxic compounds can be present in the original matrix utilized to prepare AC or BC [45]. Thus, since these materials can be used in various applications, such as adsorbing pollutants in water and soil amendment, it is important to know how they can affect organisms in the environment [51]. Similarly, while polyurethane is widely used in many applications, its toxicity remains a concern. PU products often end up in landfills once they are no longer useful and can release toxic compounds when degraded by humans or microbes. Therefore, the ecotoxicological assessment of PU foams and AC/BC is essential and will be discussed in detail in this dissertation.

1.4 Ecotoxicity assessment tests

Ecotoxicity tests assess the impact of environmental contamination on organisms by examining factors such as survival, growth, reproduction, and behavior where these tests help to determine whether contaminant concentrations are high enough to cause harmful effects on living organisms [68], [69], [70]. Ecotoxicology not only detects hazardous substances but also evaluates their biological effects on the environment. By integrating the synergistic and antagonistic interactions of all contaminants, ecotoxicology reveals the bioavailable fraction that cannot be fully assessed through chemical data alone. Therefore, bioassays serve as an essential complementary tool in the environmental risk assessment of bioremediation sites [70]. Most bioassay protocols currently in use are based on international standardization guidelines from organizations such as the ISO (International Organization for Standardization), OECD

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(Organization for Economic Cooperation and Development) [71], and U.S. EPA (United States Environmental Protection Agency) [68], [72].

1.4.1 Model organisms used for toxicity tests

Several bioassays are available for toxicity evaluation, utilizing organisms such as algae, fish, rats, and plant model organisms like *Sinapis alba* which are known for their sensitivity to various toxic substances. Additionally, invertebrates are also frequently utilized as test organisms in toxicity bioassays [73]. In this group *Daphnia magna* is considered the most commonly used to assess the acute and chronic toxicity of chemicals in aquatic environments [72], [74]. The usage of *Daphnia* offers several advantages, including high sensitivity and a short reproductive cycle. Key parameters measured in these tests are mortality and reproduction. The tests involve exposing the organisms to toxic substances under controlled conditions. Acute lethality tests with *Daphnia*, typically conducted over 21 days, are well-established and standardized [75].

Several commercial toxicity test kits and assays have been utilized to assess various biological responses, including 1) microbial activities, such as enzyme functions (*e.g.,* Toxi-Chromotest Kit) [76], 2) inhibition of respiration (*e.g.,* PolyTox®) [77], 3) reduction of the luminescence (*e.g.*, *Vibrio fischeri* – TOXcontrol) [78], 4) locomotor behavior analysis (*e.g.*, DaphTox II) and mortality (*e.g.*, Daphtoxkit F Magna).

1.4.1.1 Bacteria-based toxicity tests

The methods using the above-mentioned model organisms have several limitations, including being expensive, requiring specialized equipment and expertise to maintain the model organisms, and consuming a significant amount of time to obtain results. In comparison, bacteria offer a relatively quick and cost-efficient alternative [78], which belongs to the trophic level of decomposers, since they degrade dead organisms (plants and animals) [79] and can grow quickly in basic specified media [78], [80].

One of the most widely recognized bacterial tests for toxicity screening is the Ames assay, which utilizes *Salmonella typhimurium* [81]. In addition to that, luminescent bacteria are considered an effective indicator for toxic substances [75], [82]. When exposed to toxic substances, bacteria's bioluminescence reduces, resulting in lower light intensity which can be used to determine the level of toxicity in the environment. Due to this reason, they have been widely used in toxicology research [82], [83]. The Gram-negative luminescent bacteria, *Vibrio*

fischeri (also known as *Photobacterium phosphoreum* or *Aliivibrio fischeri*) [82] is a luminescence marine bacterium, which serves as a model organism in toxicity tests due to its high sensitivity to a wide range of toxic materials, cost-effective, ease to operate, and requires only 5–30 min for toxicity prediction [78].

Recently, Gram-negative *Escherichia coli* (*E. coli*) has been effectively used in toxicological investigations offering rapid and sensitive methods for assessing various environmental contaminants [84]. One of the most common ways to assess the toxicity of substances on *E. coli* is by monitoring its growth. Toxic compounds can inhibit cell division and replication, which can be quantified by measuring bacterial density over time [78], [84].

As mentioned previously, polymer-based materials have become a major environmental concern due to waste accumulating in terrestrial and marine environments. Therefore, toxicity tests are developed and conducted to evaluate the potential harmful effects of certain polymers on biological organisms or the environment. In previous studies, *Escherichia coli* has been used to test the toxicity of polystyrene particles of different sizes, and it was observed that the cell viability decreased as polystyrene concentrations increased, showing that plastic exposure inhibited cell growth [79]. In another study, the toxicity of leachates of PLA (polylactic acid), PHA (polyhydroxyalkanoates), PHBv (polyhydroxybutyrate-covalerate), and PP (polypropylene) on marine planktonic species were investigated. Bioassays using bacteria, microalgae, sea urchin embryos, mussel embryos, and copepod nauplii were conducted to assess the toxicity of leachates from those polymers. Results showed that PHBv leachates exhibited higher toxicity compared to other polymers with the microalgae *Rhodomonas salina* being the most sensitive species to the tested leachates. The toxicity was attributed to the presence of two specific compounds, 2,4,6-trichlorophenol and estragole, identified in the PHBv leachates. On the other hand, PP and PLA generally displayed minimal to no toxicity in the studied species [85].

Ecotoxicological evaluation of plastic at various stages of abiotic and biotic degradation is a challenging issue that should be carried out not only on conventional aquatic test species in water leachates but also on soil organisms [19].

1.4.1.2 Plant-based toxicity tests

Plants, as primary producers, play a crucial role in maintaining various ecosystem functions, such as soil stability, fertility, water availability, and the composition of soil microbial

communities. Due to their ecological significance, plants are commonly used in toxicity tests to evaluate the potential adverse effects of chemicals and materials on the environment.

Phytotoxicity refers to the adverse effects of chemical materials on plants. The phytotoxic symptoms can manifest in various parts of the plants, and assessment criteria commonly include measurements (*e.g.,* height, root length) as well as visual estimations, such as deformations (*e.g.,* curling, rolling, changes in size and shape of roots) and discoloration [86], [87]. Moreover, in ecotoxicological assessments, the germination test is considered a short-term evaluation aimed at identifying acute toxicity by focusing on the germination rate, root, and shoot length [70], [88]. Agricultural soils are prone to exposure to plastic which could affect the physiochemical properties of soil and harm the organisms in it [89]. Therefore certain species of plants are often used in the evaluation of phytotoxicity [90], which refers to the adverse effects of chemical and materials (fungicides, metals, or plastic waste) on plants. The *Sorghum saccharatum* (a monocotyledonous plant) and *Sinapis alba* (a dicotyledonous plant) are among the most commonly used model organisms due to their sensitivity and easily measurable growth parameters. Studies involving plastics like polyhydroxybutyrate, polylactic acid, and polypropylene have shown that *Sinapis alba* is more sensitive to plastic exposure compared to *Sorghum saccharatum*, indicating that these simple plants are excellent bioindicators for evaluating how plastics affect the early growth of plants [90].

Commercial tests are also available to facilitate plant-based toxicity assessments. One widely used bioassay is the Phytotoxkit, which is used to screen phytotoxicity using seeds from higher plants like *Sorghum saccharatum* (a monocot), *Lepidium sativum* (garden cress), and *Sinapis alba*. It measures the inhibition of seed germination and root growth after 3 days, in line with ISO Standard 18763 [91]. This commercial test is cost-effective, easy to use, and provides all the necessary materials to run the experiment in triplicate. The Phytotoxkit bioassay is particularly useful for assessing the toxicity of soils, sediments, wastewater, and various chemicals [72]. *Sinapis alba* is an ideal test species for evaluating the effects of contaminants on seed germination and root growth due to its thin, easily measurable roots, erect shoots, clear green leaves, and relatively large, round seeds, making it a reliable indicator in early plant development studies [88].

As detailed in this section, different toxicity tests employ different model organisms depending on the type of pollutant being assessed and whether it occurs in soil or water. Since

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many of these tests have been in use for a long time, several standardized methods and commercial tests are available to evaluate the negative impact of substances on the environment. The following table presents some standardized procedures that utilize the test organisms discussed in this section, demonstrating how important and complex the assessment of ecotoxicity is (**Table 1**):

Table 1. International standard methods for toxicity tests using different organisms

Method toxicity test	Organism	Comment	Reference
Water and degradational medium quality test: SOS-Chromotest	bacteria, *Escherichia coli* PQ37 strain	Detection of genotoxic activity and genotoxic materials in environmental water or sediment	[92]
Bacterial Microtox® bioassay ISO 11348-3-2007	bacteria, *Vibro fischeri*	Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri*	[93]
OECD 471: Bacterial Reverse Mutation Test (Ames Test)	bacteria such as *Salmonella typhimurium*	Evaluates whether a chemical can cause mutations in bacterial DNA	[71]
ISO 17126:2005	*Lactuca sativa L.*	Determination of the effects of pollutants on soil plant	[94]
ISO 22030:2005	*Brassica rapa* and *Avena sativa*	Chronic toxicity in higher plant	[95]
Water and soil toxicological test ISO Standard 18763	*Sorghum saccharatum, Lepidium sativum,* and *Sinapis alba*	Seed germination and early growth test with higher plants	[91]
ISO 11269-1:2012	*Lactuca sativa, Avena sativa,* and *Brassica napus*	Determine the effects of contaminated soils on root elongation	[96]
Microbiotest Thamnotoxkit F^{TM}	small scrimp *Thamnocephalus platyurus*	Toxicity screening of freshwater contaminants	[97]
Acute toxicity test ISO 6341:2012	planktonic crustacean *Daphnia magna Straus (Cladocera, Crustacea)*	Toxic chemical testing in water samples	[98]
Water quality test using ISO 8692:1989	*Scenedesmus subspicatus* and *Selenastrum capricornutum*	Fresh water algal growth inhibition test	[99]

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1.5 Objectives of this work

Building on the previously discussed importance of polyurethane foams and the significance of utilizing bio-based and non-toxic materials in various applications, the following specific objectives were established for this dissertation:

1- To develop toxicity testing protocols to assess the potential harmful effects of PU foams.
2- To assess the applicability of the developed toxicity tests for carbonaceous materials.
3- To explore potential applications of bio-based carbonaceous materials.

DOI: 10.14750/ME.2025.028

2. Materials and methods

2.1. Development of toxicity tests for polyurethane foams

Many studies have investigated the degradation of polymers (polyethylene, polystyrene, polyvinyl chloride, polypropylene, polyethylene terephthalate) [100], [101], [102] and toxicity tests were performed, as well as studies were carried out about how these materials affect different organisms, but limited results have been obtained regarding the impact of polyurethane foams. Therefore, in this study, we focus on investigating PU foams, considered as the most widely used type of polyurethane.

To examine the potentially toxic substances that can be formed during PU foam degradation and aging of the material, two different methods have been adopted and applied in my work. The toxicological assessment of different polyurethane foam samples was carried out using *Sinapis alba* (white mustard) seeds for liquid sample testing (*e.g.* dilution water used for soaking the PU foam) and bacteria-based toxicity test, where a piece of PU foam was soaked directly in the medium used for the growing of the bacteria.

2.1.1 Preparation of flexible polyurethane foam

The precursors for the production of flexible polyurethane foam samples were Ongronat TR4040 (methylene-diphenyl-diisocyanate isomer mixture, Wanhua-BorsodChem, Kazincbarcika, Hungary) and Ongropur FFP-303 (polyether type polyol premix, Wanhua-BorsodChem, Kazincbarcika, Hungary). Catalysts and additional additives like blowing agents and surfactants DABCO 33LV, Jeffcat ZF-22, Tegostab B4113, and Tegoamin DEOA 85 were mixed into a base polyol system (Alcupol F3831, Repsol, Madrid, Spain), to create the applied polyol (**Table 2**).

Table 2. *Formulation of the polyurethane foam*

Polyol premix (Ongropur FFP-303)	Ratio (pphp)*
Alcupol F3831	100
Water	3.6
Tegostab B4113	0.5
DABCO 33LV	0.15
Jeffcat ZF-22	0.1
Alcupol F3231	1
Tegoamin DEOA 85	0.5

*The values are in parts per hundred polyol (pphp)

DOI: 10.14750/ME.2025.028

NCO-index	Polyol premix Ongropur FFP-303 [% m/m]	Isocyanate Ongronat TR4040 [% m/m]
0.8	70.89	29.11
0.9	67.25	32.75
1.0	63.61	36.39
1.1	59.98	40.02
1.2	56.34	43.66

Five polyurethane foam samples were prepared by mixing the precursors, and the raw material ratios were determined in a way to achieve 0.8, 0.9, 1.0, 1.1, and 1.2 isocyanate indices (*e.g.* NCO-0.8). The isocyanate index is the ratio of the total number of NCO groups to the sum of the OH groups of the polyol premix which includes additives (*e.g.* water) within the reaction mixture. Previously, a Control sample (NCO-0.9) was also prepared, tested and it was found to be non-toxic. Thus, it was used as a reference to evaluate the toxicity of the newly prepared samples. The polyurethane samples were cut into small pieces approximately 1×1×1 cm^3, the mass range was 0.05 ± 0.01g and these PU foam cubes were used for the toxicity tests.

2.1.2 Acute toxicity test using *Sinapis alba* (white mustard) seed

Sinapis alba (commercially available seeds were used, and before usage, the germination potential was verified by using the (Magyar Szabvány) Hungarian Standard MSZ 6354-3 according to the Department of Environmental Protection, Nature Conservation, and Waste Management) [103] was chosen as a test organism to evaluate the toxicity of PU foam samples due to its agricultural significance and its sensitivity to changes in the environment [69]. The toxicity tests were carried out by using a special water-based liquid (dilution water). A solution of 1 L was prepared which contained 20 mM of $CaCl_2 \cdot 2H_2O$, 5.0 mM of $MgSO_4$, 7.5 mM of $NaHCO_3$, and 0.75 mM of KCl [98]. To test the PU samples, the foam cubes were soaked in 15 mL of the prepared special water-based solution in plastic centrifuge tubes for 24 h, 48 h, or 72 h, respectively (**Figure 7**).

Thereafter, the cubes were removed, and the remaining soaking liquid was used for the tests (5 mL/plate).

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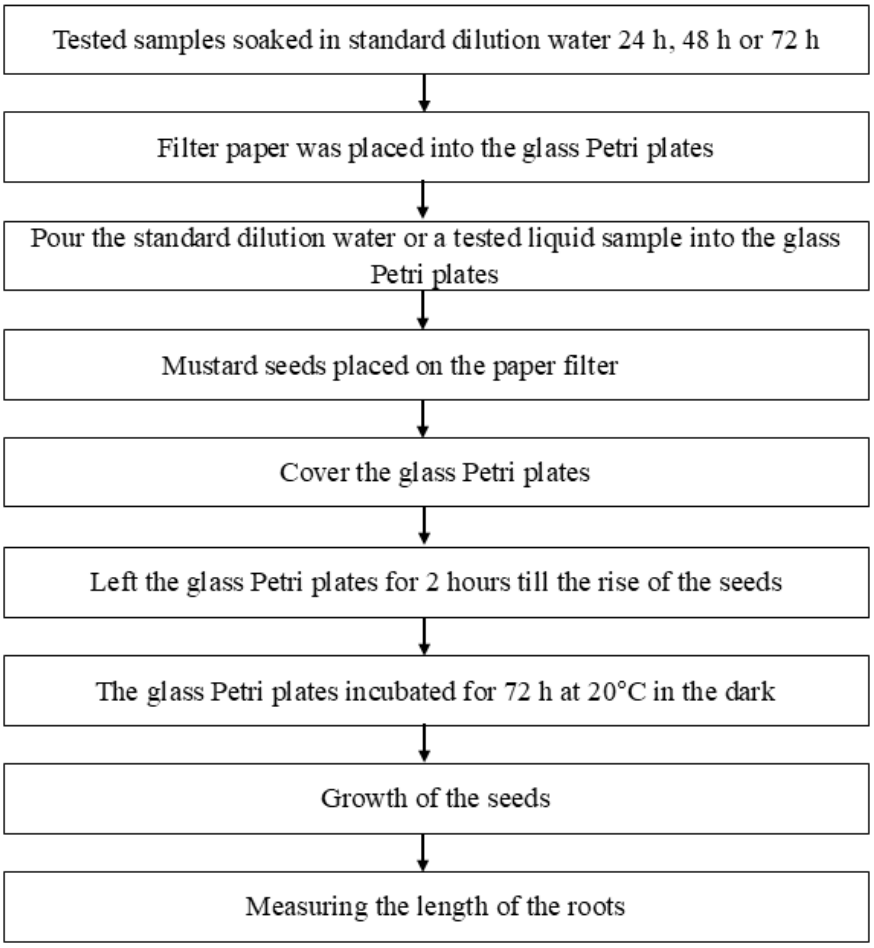

Figure 7. *Steps of acute toxicity measurement by using Sinapis alba seeds.*

In the meantime, for each sample, three glass Petri plates were prepared in the following way: circular shape filter paper was placed into the plates and heat sterilized together with the Petri dish at 100°C for 10 h. Then, 5 mL of the prepared water-based solution or 5 mL of the soaking liquid of the tested PU foams were put into Petri dishes using a 5 mL range automated pipette. After that, mustard seeds were distributed on the plates (30 seeds for each plate, (**Figure 8/a**). The plates were covered with a glass cover and left on the desk for approximately 2 h for a bit to the rise of the seeds and thus, to avoid their shift from their arranged position in the Petri dish. Then, the Petri dishes were placed in the incubator for 72 h in the dark at a temperature of 20°C. Thereafter the root length of the seeds was measured (**Figure 8/b**).

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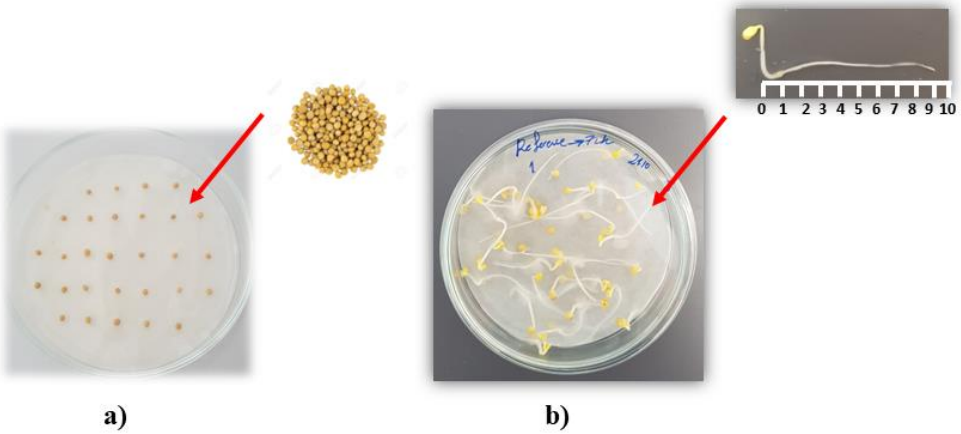

Figure 8. *Sinapis alba seed test: a) arrangement of the seeds in the plate at the start of the test, b) germinated seeds after incubation, and measurement of the length of the growing seed root (the numbers on the ruler represent cm).*

The average root length of the longest 25 seeds for each sample and the length of the roots of the Control sample were compared. Reference plates were made as well, for which only the pure prepared water-based solution was used, and the seeds were treated in the aforementioned way (as described for the plates with the soaking liquid of the PU foams).

Reference plates served to verify that the test was performed methodologically in a correct way and that the seeds were appropriate for germination. If the root length is shorter compared to what has been measured for the Control seeds, the tested liquid (the soaking dilution in which the foam cube was held) is considered toxic. The greater the difference, the more toxic is the tested PU foam which means that potentially toxic chemicals are released during the soaking of the foam.

For each plate, the germination index (GI) was calculated as the ratio of the average number of germinated seeds to the total number of seeds exposed to the test (in our test 30 seeds were placed on each plate). The ratio was multiplied by 100 [90] using the following formula:

$$GI\ \% = \frac{\text{Average number of germinated seeds in each PUF sample}}{\text{Total number of seed exposed to the test}} * 100 \qquad (1)$$

DOI: 10.14750/ME.2025.028

2.1.3 Liquid phase bacteria-based toxicity test using *Escherichia coli* model organism

Bacterial model organisms are commonly used in toxicity tests conducted in the liquid phase. In this case, the toxic effect of a chemical or material placed into the liquid is investigated by observing changes in bacterial viability.

To determine bacterial viability, conventional techniques such as plate counts and different dilutions of the initial bacterial suspension are utilized [82]. In this experiment, *Escherichia coli* DH5α strain (from the Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary) was used as a model organism because its growth is fast (*E. coli* divides every 20 min) [104]. The viability of bacterial cells is proportional to the number of colonies formed on the agar plate, as only viable cells can form colonies. Therefore, the concentration of living cells in a bacterial suspension is expressed in colony-forming units per milliliter (CFU/mL). To determine the toxicity of a substance, the bacterial cell concentration must be accurately measured and compared to the control sample. For this, the concentrated bacterial suspension usually needs to be diluted, as an excessive number of cells on the plate can cause colonies to merge, leading to inaccurate cell concentration determination, but at the same time, one has to take care to avoid over-dilution and thus, formation of too few cells or colonies on the plate. So, only plates with colonies between 30 and 300 should be taken into account [105], [106]. In the current study, a serial dilution was used to decrease the bacterial concentration to the level needed for the particular test which makes it easier to count the bacteria.

The steps of the liquid-phase toxicity test using a bacterial model organism are summarized in the flowchart shown in (**Figure 9**).

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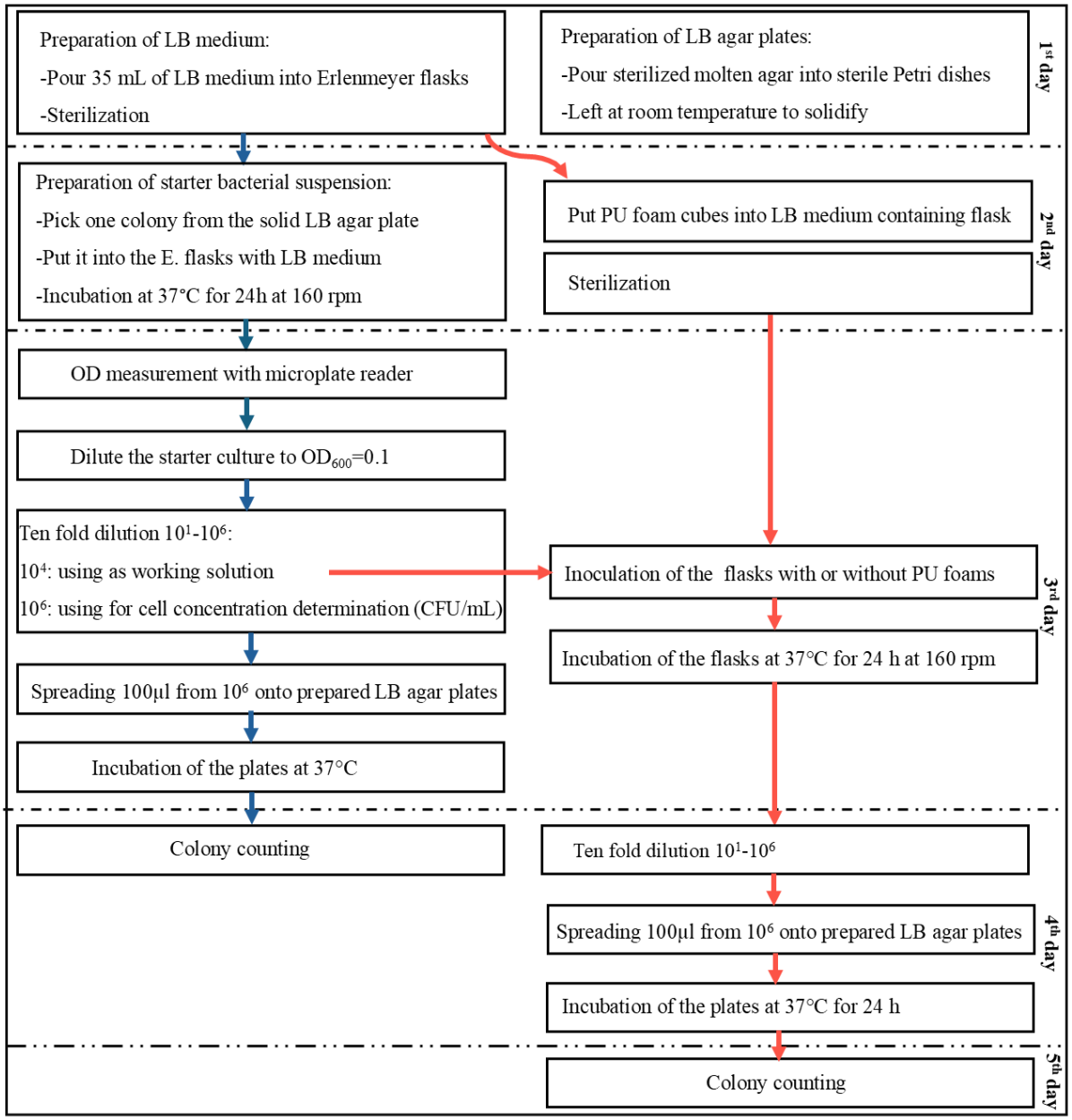

Figure 9. *Flowchart of the bacterial test.*

2.1.3.1. Description of the test procedure (Figure 9)

- *Sterilization of PU foams for bacterial tests*

As a first step, LB (*Lysogeny broth*) medium was prepared for the bacterial tests (10 g tryptone, 5 g yeast extract, 10 g NaCl, 1000 mL distilled water). Then, 35 mL LB was dosed into Erlenmeyer flasks. PU foams (selected for testing) were cut into 1×1×1 cm^3 cubes. One set of the PU foam cubes was put in flasks filled with LB medium, and was autoclaved together

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for sterilization of the foams and the medium (121°C, 15 psi for 45 min). This arrangement served not just for sterilization but to investigate whether high-temperature wet heat can promote the release of some chemical compounds from the foam (mimicking the aging process). The flasks were then left on the laboratory bench and cooled to room temperature. The other half of the flasks (with the other set of the foams) remained unsterilized.

- *Preparation of LB agar plates*

LB agar was made by adding 15 g agar to 1000 mL of LB medium and applied for the colony counting method as well as for maintaining the bacteria (model organism) for longer period of time. Petri plates were prepared by pouring the LB agar into the plates in the biological cabinet and leaving them to solidify overnight.

- *Preparing the starter culture*

Inoculation of the starter LB suspension was performed using a sterile inoculation loop and putting a single colony of *E. coli* into the LB medium. The starter was incubated in a shaker incubator overnight at 37 °C and 160 rpm.

- *Optical density measurement (OD_{600}) and inoculation of the flasks*

To measure the concentration of bacteria or other cells within a liquid sample, the OD_{600} measurement technique can be used [82], [107]. The OD_{600} technique measures the amount of light that passes through a bacterial solution at 600 nm. The amount of transmitted light can be used to determine the concentration of particles in a solution, since the amount of light absorbed by a sample is proportional to the concentration. Consequently, the greater the number of bacterial cells in the suspension, the more light they absorb or scatter. The OD_{600} was measured by a BMG Labtech Clariostar Microplate reader (**Figure 10**).

DOI: 10.14750/ME.2025.028

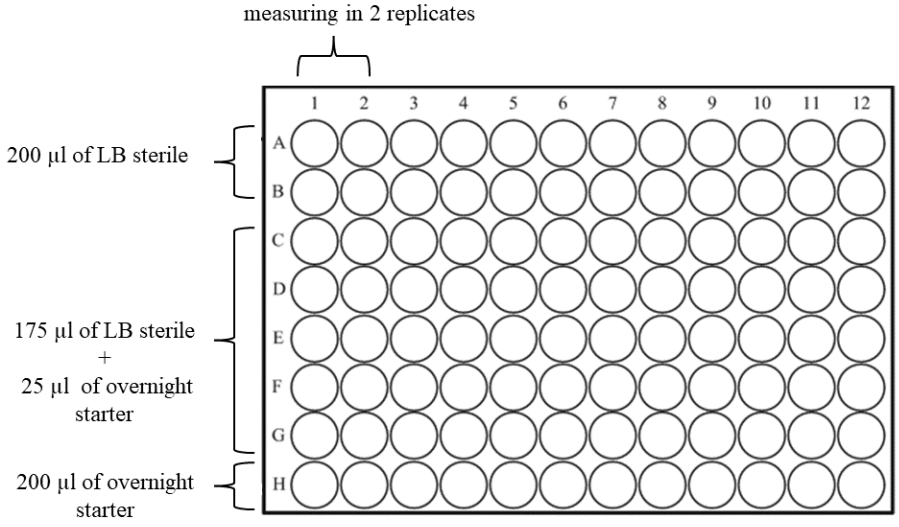

Figure 10. *Arrangement of the microplate for measuring the OD of the starter bacterial suspension. Sterile LB was used for blanking. For adequate OD measurement, overnight culture had to be diluted. Every time two columns with the same arrangement were used for measuring.*

According to the measured optical density of the overnight (OVN) starter culture, dilution was made with sterile LB medium to get bacterial suspension with OD_{600}=0.1 ± 0.05 optical density which was used for further work. By diluting to an initial OD of 0.1 ± 0.05, the reproducibility of the experiments was increased as the cell concentration of OVN starters can vary due to differences in incubation time (12–24 h) and the actual cell count in the inoculum. This dilution helped eliminate these variations. The success of this dilution was remeasured by spectrophotometer and after that ten-fold dilution series (10^1-10^6) was made from this bacterial suspension with OD_{600}=0.1 ± 0.05 (**Figure 11**). From the 10^4 dilutions (this dilution was chosen as a working bacterial solution) of the prepared dilution series, 30 µL was used to inoculate the flasks containing the PU foam to be tested. To determine the initial cell concentration, namely, to determine which CFU/mL corresponds to the working bacterial solution, 100 µL of the 10^6 dilutions was taken and spread on the top of five LB agar plates (**Figure 11**). After overnight incubation, the formed colonies were counted.

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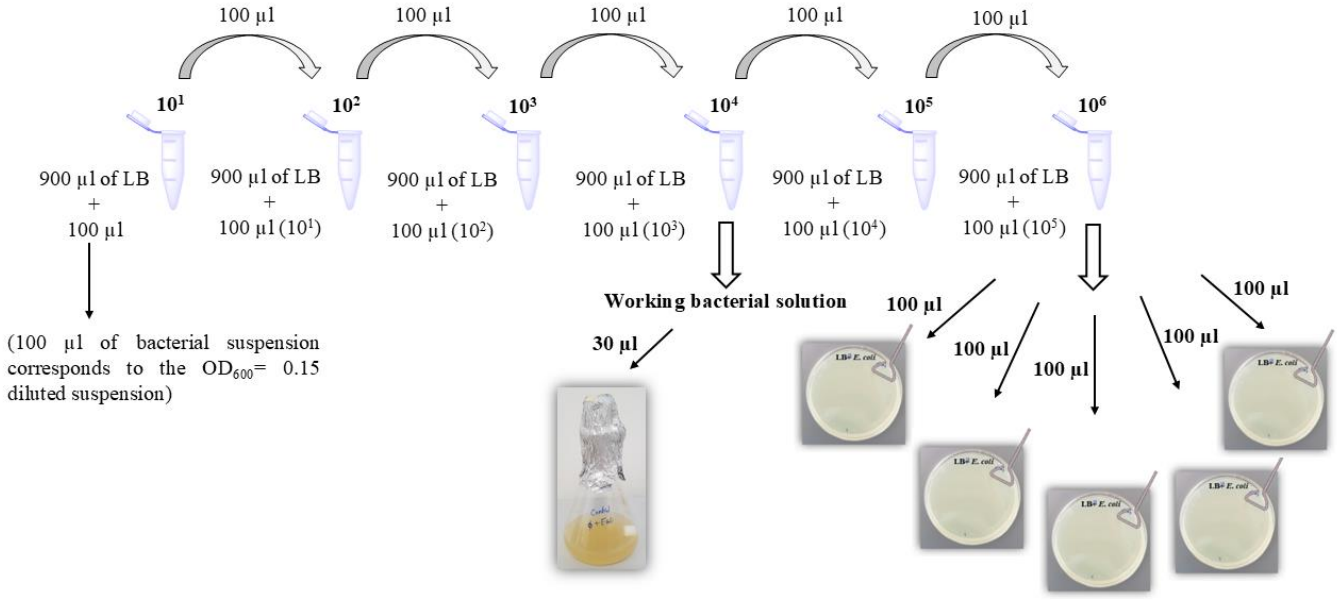

Figure 11*. Steps for preparing a tenfold serial dilution of the OD600 = 0.15 bacterial suspension and spreading the LB agar plates with the 10⁶ dilution to determine bacterial concentration.*

Flasks for the bacterial test were arranged as follows (**Figure 12**):

1. **LB + *E. coli*** - autoclaved LB medium, without PU foam; this flask serves as a Reference that the used media is appropriate for growing the bacteria as well as to demonstrate that the used working bacterial suspension contains viable cells.

2. **LB + Autoclaved PUF** - LB medium autoclaved together with the Control non-toxic PUF or the tested PUF samples.

3. **LB + Autoclaved PUF + *E. coli*** - LB medium autoclaved together with PUF (Control non-toxic PUF or the tested PUF samples) and inoculated with *E. coli* working bacterial suspension.

4. **LB + Non-autoclaved PUF + *E. coli*** - sterile LB medium with Non-autoclaved PUF and inoculated with *E. coli*.

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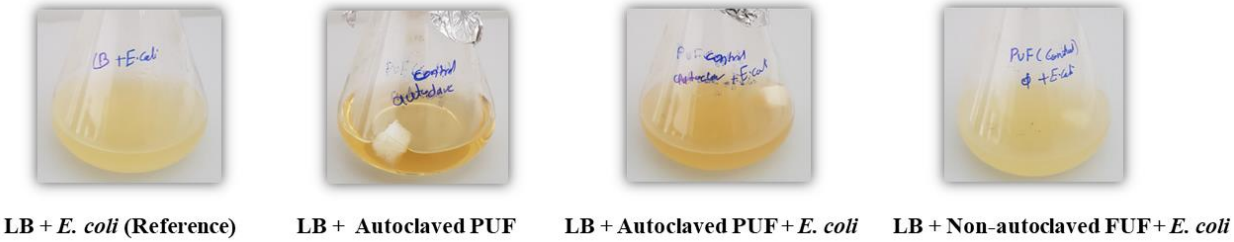


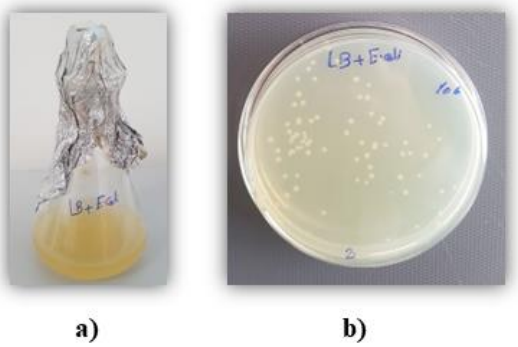
LB + *E. coli* (Reference) **LB + Autoclaved PUF** **LB + Autoclaved PUF + *E. coli*** **LB + Non-autoclaved FUF + *E. coli***

Figure 12. *Flasks with LB with or without polyurethane foam (PUF) samples inoculated with E. coli after overnight incubation*

The flasks were incubated overnight at 37°C in a shaker incubator (160 rpm).

After the incubation of the prepared flasks, the overnight cultures were diluted with a sterile LB medium by applying a 10^1-10^6 dilution for all flasks and spread 100 µL from the 10^6 on the top of the LB agar plates in triplicates (it was known from my preliminary experiments that a 10^6 dilution of OVN-grown suspensions would give a calculable number of colonies). After that, all the plates were put in the incubator overnight at 37°C and the colony number was counted for each plate (**Figure 13**) using the following formula:

$$\text{CFU/mL} = \frac{number\ of\ colonies \times dilution\ factor \times 10^3}{10^2} \quad (2)$$



a) **b)**

Figure 13. *Bacteria-based toxicity tests in liquid phase: a) flasks LB with E. coli liquid medium, b) plates with E. coli grown in LB solid medium for colony counting.*

DOI: 10.14750/ME.2025.028

2.2 Applicability of the developed toxicity tests to study carbonaceous materials

In Guadeloupe (Caribbean), it is a big problem that brown macroalga called *Sargassum* sp. grows in huge quantities on the shores [2], [108], [109], [110]. This is quite problematic because ammonia and hydrogen sulfide gases can be released during the macroalga's decomposition, and thus, the overgrowth of *Sargassum* sp. is causing several concerns, including health problems [109]. Additionally, because it has an indirect impact on tourism, it has negative economic effects as well. Thus, by using the *Sargassum* sp. as raw materials for AC and BC production, the negative effects of the overgrowth of the macroalga can be turned into an advantage and valuable products can be achieved [108], [109].

In this work, the carbonaceous material (activated carbon (AC) and Biochar (BC) samples) were prepared from *Sargassum* sp. and characterized by employing various techniques. The potential adverse effects of the AC and BC samples were also assessed by examining their toxicity using *Sinapis alba* (white mustard) seeds and a bacteria-based toxicity test by using *Escherichia coli* as a model organism and the AC and BC were compared to commercially available activated carbon sample.

2.2.1 Preparation of the Activated carbon (AC) and Biochar (BC)

Activated carbon and biochar are both porous carbonaceous materials derived from *Sargassum* sp., but they differ significantly in their production processes and pore structures. The preparation method is as follows:

The fresh *Sargassum* sp. *(Sargassum natans and Sargassum fluitans)* was collected in Guadeloupe at La Datcha beach [109]. Initially, the samples were sun-dried for 7 days to achieve dehydration, followed by placement in an oven set to 120 ºC for at least 24 h. The dry samples were grinded in a mixer grinder and then sieved between 0.4 to 1.5 mm serving as the chosen material for producing activated carbon and biochar. The activated carbon was prepared by chemical activation and biochar by the pyrolysis of the *Sargassum* sp. [111].

2.2.1.1. Activated carbon prepared by chemical activation

The *Sargassum* sp. was impregnated with 85% H_3PO_4 in a mass ratio of 3:1 (85% H_3PO_4 /Sargassum) for 15 h (as the concentration of phosphoric acid increases, the porosity and surface area of the material significantly improve) [66]. After impregnation, the sample was heated in

DOI: 10.14750/ME.2025.028

a tubular furnace at 5°C/min until it reached a temperature of 600°C. After two hours at the required temperature, the samples were naturally cooled. Throughout the process, a nitrogen flow of 80 mL/min was introduced into the furnace. Phosphoric acid (H_3PO_4, 85 wt %), was provided by Sigma-Aldrich (France) (**Figure 14**) and acts as a drying agent which can increase the pores in activated carbon [56].

2.2.1.2. Biochar sample preparation

Biochar was produced through the pyrolysis of crushed *Sargassum* sp. in an advanced microwave oven (PYRO) at 1200 W (~650 ºC) for 15 min under an inert nitrogen atmosphere. The resultant materials underwent washing initially with hydrochloric acid (5 M) followed by washing with deionized water until achieving a pH close to 7, and then they were dried (**Figure 14**).

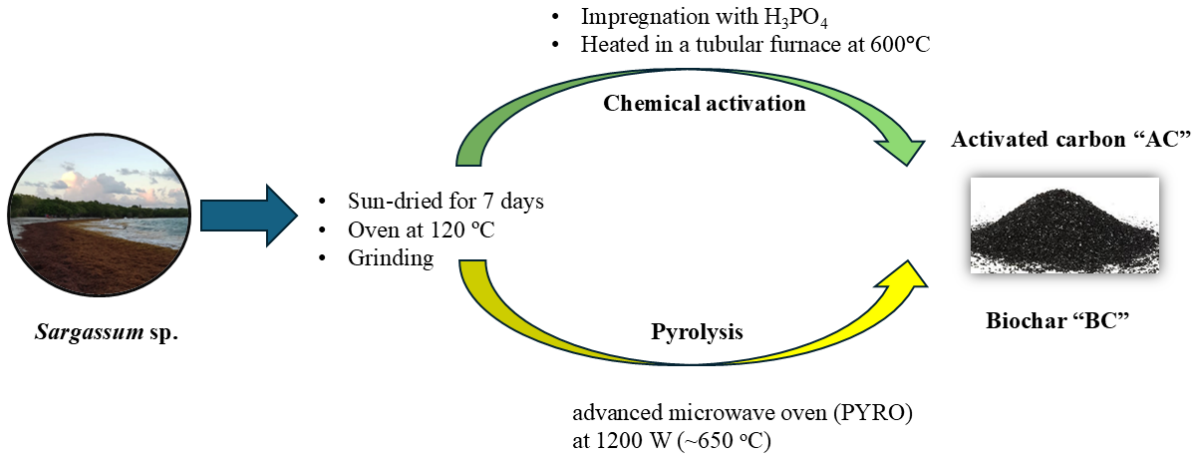


Figure 14. *Preparation of activated carbon and biochar*

In this work, different samples were tested and compared (**Table 3**): COMAC (commercial activated carbon, Sigma-Aldrich (France)), activated carbon (AC) prepared from *Sargassum* sp. by chemical activation with phosphoric acid, and biochar prepared from *Sargassum* sp. (elaborated using microwaves). The samples were identified as COMAC, AC, and BC, respectively.

DOI: 10.14750/ME.2025.028

Table 3. *Source of the carbonaceous materials*

Sample	Source
COMAC	Commercial activated carbon
AC	Prepared from *Sargassum* sp. chemical activation
BC	Prepared from *Sargassum* sp. by pyrolysis process

2.2.2 Metal content of the samples

The metal content of the samples (COMAC, AC, and BC) was measured by using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Varian 720 ES, Lab-EX, (Budapest, Hungary)) for 9 different metals (Ca, Cd, Co, Cu, Fr, Mg, Ni, Pb, and Zn), and the analysis was carried out by microwave-assisted digestion using nitric acid (HNO_3).

2.2.3 Electro-kinetic (zeta) potential measurements

Zeta potential can be used to quantify the magnitude of the charge on the surface of a particle in a liquid. It is an important parameter in determining the stability of colloidal suspensions, as well as the interaction of particles with each other and with the surrounding medium [112]. To compare the surface charge of studied activated carbon and biochar samples, zeta potential measurements were carried out on 0.05 g of powdered AC or BC suspended in 50 mL of distilled water.

2.2.4 Fourier Transform Infrared Spectroscopy (FTIR) characterization and specific surface area analysis

The functional groups on the surface of the studied activated carbon and biochar were determined by using a Bruker Optik GmbH Vertex 70 FTIR spectrometer (Ettlingen, Germany). All samples were investigated in potassium bromide pellets (0.05 g AC or BC in 0.16 g KBr).

2.2.5 Specific surface area measurements

The specific surface area was measured using nitrogen (N_2) adsorption studies. A Sorptomatic 1990 series analyzer was employed to evaluate the N_2 adsorption capacity at 77 K. Before the analysis, the samples were degassed at 250°C for 17 h to remove any residual moisture. The specific surface area was calculated by applying a linear fit to the Brunauer-Emmett-Teller (BET) equation within the relative pressure range (P/P_0) of 0.103 to 0.225 [111].

DOI: 10.14750/ME.2025.028

2.2.6 Toxicity test of activated carbon and biochar

To estimate the ecotoxicological effect of activated carbon and biochar on various organisms, two acute toxicity tests have been employed. For the seed tests, commercially available *Sinapis alba* (white mustard) seeds were used, which germination potential was verified previously. For the bacteria-based test, *Escherichia coli DH5α* was employed as a model organism (the same as in the toxicity test of PUFs).

2.2.6.1. Using Sinapis alba seed (mustard seed) for an acute toxicity test

The incorporation of activated carbon and biochar into soil has been shown to improve various physical, chemical, and biological properties, enhancing soil fertility, and soil amendment and ultimately promoting plant growth and productivity. Additionally, they have a good effect on soil enzymatic activity, making the environment more suitable for beneficial microbes and improving plant health [113]. However, *Sargassum* is reported to be able to bioaccumulate heavy metals and metalloids, particularly arsenic, which pose potential hazards to the environment when plants and animals are exposed to their leachates. Thus it was necessary to evaluate the toxicity of the AC and BC made from them.

In this work, mustard seeds were exposed to the three different types of activated carbon and biochar: COMAC, AC, and BC.

Each type of AC or BC (0.02 g) was suspended in 20 mL of a special water-based solution, which had the same composition as the dilution water used in the toxicity test of PUFs (section 2.1.2) [98]. After that, 5 mL of the solution containing the studied samples were put into Petri dishes, and mustard seeds were distributed on the plates. Then, the plates were placed inside an incubator for 3 days in the dark at 20°C . Thereafter the root length of the seeds was measured to assess the effects of the different activated carbon and biochar samples on the organism (**Figure 15**).

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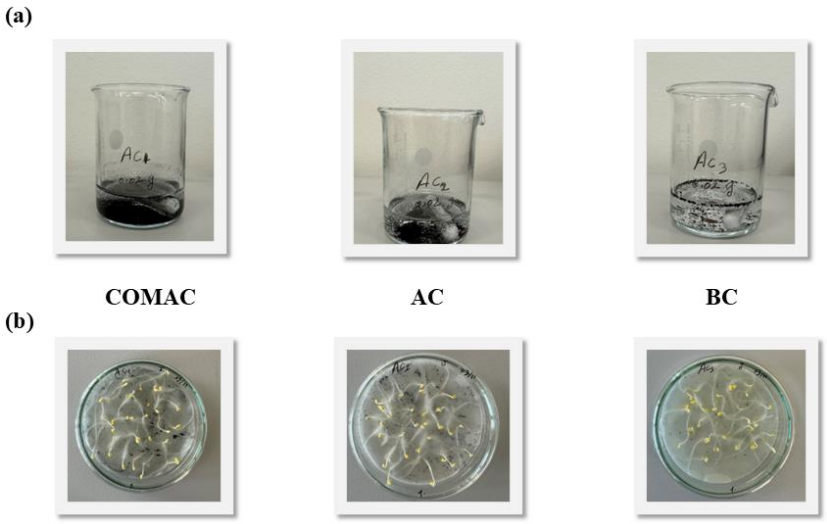

Figure 15. *Sinapis alba seed test: (**a**) different types of AC/BC suspended in 20 mL of dilution water; (**b**) germinated seeds after incubation.*

2.2.6.2. Bacterial test using Escherichia coli as a model organism

The preparation of the bacterial test is the same as explained in section 2.1.3.1. regarding the LB liquid medium, agar plates, and starter bacterial suspension. In this experiment, the prepared bacterial suspension was used for two types of bacterial tests:

- Flask experiment

As previously mentioned, the starter bacterial suspension was diluted, and a dilution series from 10^1 to 10^6 was prepared. The 10^4 dilution was selected as the working bacterial solution, as stated earlier, and was used to inoculate the flasks containing the tested AC and BC samples. 30 µL of the working solution (as inoculum) was added to each flask containing 0.02 g for each sample type. All activated carbon and BC samples were sterilized at 120°C in a drying cabinet for 2 h before use in bacterial tests. The inoculated flasks with the tested AC and BC samples were incubated at 37°C in a shaker incubator (160 rpm) for 24 h, after which observation was made to determine whether the liquid in the flask had turned turbid (indicating normal bacterial growth) or remains clear (indicating toxicity).

The LB + *E. coli* flask (autoclaved LB medium, without any of the tested samples) was prepared to prove the presence of live cells in the utilized working bacterial solution and to show that the medium used is suitable for bacterial growth.

DOI: 10.14750/ME.2025.028

After the incubation of the suspension, the prepared flasks were diluted with a sterile LB medium by applying a 10^1–10^6 dilution for all flasks. As mentioned before, a serial dilution was used to obtain a bacterial suspension with the appropriate concentration for accurate bacterial count determination. Accordingly, 100 µL from the 10^6 dilution of every flask (LB + *E. coli,* LB + COMAC + *E. coli,* LB + AC + *E. coli,* LB + BC + *E. coli*) was spread on the top of the LB agar (3 plates for each tested sample) and after another 12–16 h incubation of the plates, the colony count was determined.

- Plate test

The dry AC and BC samples (not their suspensions) were tested as well. For this, firstly the bacterial working suspension (with the OD_{600}=0.1 ± 0.05) was spread evenly over the surface of the agar plates. It was important to use the appropriate volume of the suspension so that it could dry quickly and evenly, forming a thin layer on the top of the plates. Due to the higher bacterial concentration in the suspension, the bacteria grew as a continuous layer across the plate instead of forming separate colonies, as seen in previous experiments.

If the tested particles were toxic, a clear zone (transparent ring) would appear where the sample was placed. The size of this clear (inhibition) zone indicates the level of toxicity. In the clear area, bacterial growth is inhibited, revealing the original yellowish color of the agar plate. In contrast, in areas without toxicity, bacteria grow and merge to form a smooth, buttery-colored layer.

After evenly spreading bacterial suspension on the plates, 0.02 g of different types of samples (COMAC, AC, and BC) was placed on the top of the plates. Following that, all the plates were placed in an incubator overnight at 37°C, and the potential formation of a zone of inhibition was tested.

2.3. Potential applications of carbonaceous materials

2.3.1. Water treatment/ removal of heavy metals from water

One of the main objectives of this study is to test the applicability of carbonaceous materials like activated carbon, and biochar derived from *Sargassum* sp., and other commercial AC samples as water treatment agents in heavy metal adsorption.

DOI: 10.14750/ME.2025.028

The adsorption process involves the accumulation of substances (adsorbates) on the surface of a material (adsorbent) at the interface between a solid and a surrounding medium (**Figure 16**) [58], [114].

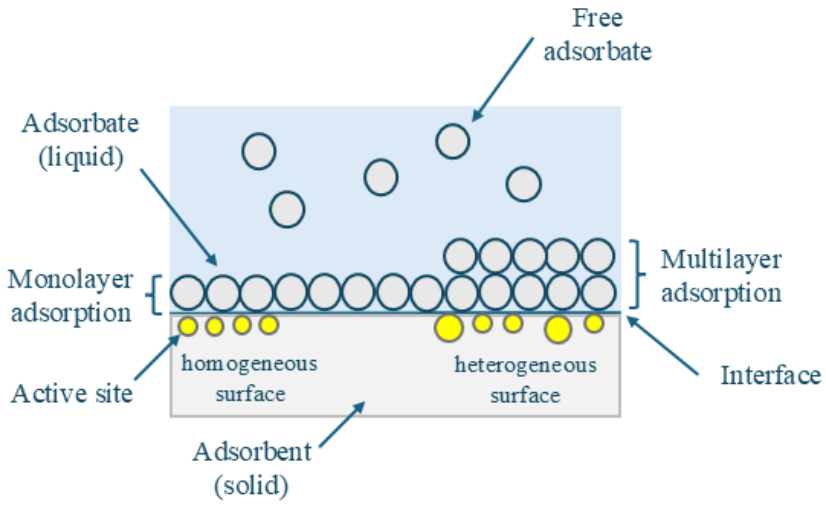

Figure 16. *Schematic representation of the adsorption process*

2.3.1.1. Adsorption Studies

Various parameters were studied to determine the optimal conditions in the adsorption test such as pH, initial concentration of the solution, and the amount of the dosage. The adsorption isotherm was evaluated as well.

1. ***Effect of solution pH***

The pH of the aqueous solution is an important parameter in ion adsorption [115]. During this process, the binding of metal ions to the adsorbent is often influenced by factors such as the functional groups, surface charges, degree of ionization, and solubility of the adsorbent [116], [117], and electrostatic interactions between metal ions and adsorbent surface groups which are affected by pH. To enhance adsorption, the pH of the solution must be adjusted so that the metal ions and adsorbent surface groups are of opposite charge. This will increase adsorption by promoting strong electrostatic interactions [118].

Given the significant role of pH in adsorption, it is essential to determine the point of zero charge (pHpzc) before conducting adsorption experiments, as pHpzc defines the linear range of pH sensitivity. This property provides insights into the surface polarity, which gives information on its adsorption capacity for ionic species. To determine the pHpzc of all carbonaceous samples, 0.1 g of carbonaceous material was added to 20 mL of a 0.1 M NaCl solution with

DOI: 10.14750/ME.2025.028

initial pH values ranging from 2 to 13, adjusted using 0.5 M NaOH or HCl. Then, the samples were agitated at 150 rpm for 24 hours at 25°C. The initial and final pH values were then determined to calculate the pHpzc. The total basic and acidic surface of the samples was determined using the Boehm titration method. For this, 500 mg of carbonaceous samples were added to 50 mL of NaOH 0.05M or HCl 0.05 M solution to determine the concentration of basic and acidic groups, respectively. The sealed glass bottles were placed in a shaker and agitated for 48 hours. The sample was then filtered with a 0.45 µm syringe filter to eliminate the AC. Subsequently, 10 mL of each filtrate sample was titrated with HCl or NaOH (0.05 M). All titrations were performed in triplicate, and the average values were provided [111].

For the adsorption test, the stock solution was prepared by dissolving the reagent, 1.455 g of cobalt(II) nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$) in volumetric flasks and diluted to 250 mL with distilled water with an initial concentration of 20 mmol/L. Then, 5 mL of stock solution was pipetted and diluted in a 50 mL flask with distilled water, where the pH values were adjusted to the range ~ 2.0 - 8.0 by 1 M HCl and 1 M NaOH and the initial concentration of the solution 2 mmol/L in 50 mL flask. The experiments were carried out using 9 Erlenmeyer flasks each containing 0.05 g of carbonaceous material in 50 mL of diluted cobalt solution. After that, these flasks were shaken in a horizontal shaker for 1 h at 210 rpm at room temperature. After shaking, the solutions were filtered by using a syringe filter (0.45µm-PVDF filter). The final concentration of metal ions was measured by Varian 720 ES Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (**Figure 17**).

Figure 17*. The workflow of the adsorption tests employed during the study*

The amount of metal ions adsorbed onto the carbonaceous materials (AC or BC) was calculated using the following equation:

$$q_e = \frac{(C_i - C_e) * V}{m} \qquad (3)$$

where q_e is the amount of metal adsorbed by the adsorbent (mg/g), C_i is the initial metal concentration (mg/L), C_e is the equilibrium metal concentration after filtration (mg/L), V is the volume of the initial solution (L), and m is the weight of carbonaceous material (g).

The percentage removal of metal ions was calculated using the following formula:

$$Removal\ \% = \frac{C_i - C_e}{C_i} * 100 \qquad (4)$$

2. *Effect of the initial metal concentration and adsorption isotherm*

The adsorption capacity of the adsorbents is affected by the metal concentration. The initial concentration of metal ions can impact the efficiency of metal removal by affecting the availability of specific surface functional groups and their ability to bind metal ions effectively [117].

To assess the effect of initial ion concentration on equilibrium, various concentrations were tested in a range of 0.04 – 2 mmol/L. Based on the results regarding pH effects, the optimal pH was selected and applied to the different concentrations of the solution, and 0.05 g of AC or BC was added to 50 mL Erlenmeyer flasks in 9 cases, and all the flasks were shaken at 210 rpm for 1 h. After filtration, the final metal ion concentrations were measured using ICP-OES.

2.1. Adsorption isotherms

The adsorption isotherm is essential for understanding the interactions between solution and adsorbents, making it crucial for optimizing the efficiency and effectiveness of adsorbent materials [59]. It is important to note that in adsorption isotherms, the amount of solute adsorbed per unit mass of the adsorbent is expressed as a function of the equilibrium solute concentration, not the initial concentration. Therefore, the model equation can only be used when the equilibrium concentration of the solute is known [116]. The linear regression method has been commonly used to estimate model parameters. However, linearizing adsorption models can alter the independent and dependent variables, potentially introducing propagated errors. To obtain more accurate parameter calculations, nonlinear regression is recommended, as it avoids the drawbacks associated with linearization. This method has been concluded to be a more reliable and powerful tool for parameter estimation [119].

Two different models were used to fit the adsorption isotherms:

DOI: 10.14750/ME.2025.028

2.1.1. The Langmuir isotherm model

The Langmuir isotherm model considers the monolayer formation process (*e.g.* chemical adsorption), where the monolayer adsorption capacity per unit mass of the adsorbent is determined along with the Langmuir constant, which reflects the solute's affinity for the adsorbent. According to this model, molecules are adsorbed onto a finite number of distinct active sites that are homogeneously distributed across the adsorbent's surface [58]. These active sites possess an equal affinity for the adsorption of a monomolecular layer, and there is no interaction (no mobility) among the adsorbed molecules [119], [120].

The Langmuir isotherm model is expressed by the following non-linear equation:

$$q_e = \frac{q_m \ K_L \ C_e}{1+K_L \ C_e} \qquad (5)$$

Where q_e is the equilibrium adsorption amount of heavy metal ions adsorb onto the unit mass (mg/g), K_L is the Langmuir equilibrium constant is related to the energy of adsorption, which quantitatively reflects the affinity between the adsorbent and the adsorbate (L/mg), C_e is the equilibrium ions concentration (mg/L), and q_m is the monolayer adsorption capacity (mg/g).

2.1.2. The Freundlich isotherm model

The Freundlich isotherm model has been regarded as an empirical equation that describes another multilayer adsorption process, characterized by a gradual increase in the amount of solute adsorbed per unit mass of the adsorbent [58], [63], [116] and thus, the adsorption occurs on heterogeneous surfaces, where interactions occur between the adsorbed molecules [58].

The nonlinear form of the Freundlich model is given by the following equations [111]:

$$q_e = K_f \ C_e^{1/n} \qquad (6)$$

Where q_e is the amount of heavy metal ions adsorbs onto the unit mass (mg/g), C_e is the equilibrium concentration of adsorbate in the solution (mg/L), K_f is the Freundlich constant, serving as the adsorption or distribution coefficient (it indicates the quantity of metal ions adsorbed per unit of equilibrium concentration on the adsorbent), n is the heterogeneity factor (constant factor) that represents the deviation from the linearity of adsorption and provides insight into the favorability of the adsorption process. The value of 1/n ranges between 0 and 1. If the value n >1 (1/n<1) adsorption is considered favorable, suggesting a strong interaction

DOI: 10.14750/ME.2025.028

between the adsorbent and adsorbate. If n=1, the adsorption is linear implying uniform adsorption sites, while if n<1 (1/n>1) adsorption is less favorable indicating that the solvent may have a higher affinity for the adsorbent surface than the adsorbate does [116].

3. *Effect of AC/BC dose*

Adsorbent dosage is also a critical parameter in adsorption, as it determines the amount of metals adsorbed. Since adsorption requires active sites on the adsorbent, a higher dosage of adsorbent increases the number of active sites, making it more effective in removing metals. However, increasing the adsorbent dose even further can reduce the adsorption capacity because more active sites remain unoccupied throughout the process [121].

In this experiment, the effect of adsorbent dose on the uptake of Co^{2+} was investigated using different amounts of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 g of the adsorbents. The experiments were carried out by adjusting the pH to the optimal value of 6.8, initial Co^{2+} concentration of 2 mmol/L, and shaking time for 1 h at 210 rpm.

DOI: 10.14750/ME.2025.028

3. Results and Discussion

3.1 Development of toxicity tests procedure for polyurethane foams

3.1.1 Acute toxicity test

The *Sinapis alba* test is valid if 90% of the seeds grow on the reference plate, and the tested sample is not toxic if the roots are nearly reached the same length as on the Control [88]. During the seed test, the root lengths for all samples have been measured and compared (**Table 4** and **Table 5**). To analyze the results of each soaking period, the average root lengths achieved on the Control plate were taken as 100%. The percentage of average root length of the other plates was compared to this.

The root length of *Sinapis alba* for the Control plate fell within a similar range as observed in the reference plates, where the seeds were germinated using only the standard water-based solution. However, in the case of the tested foams where the soaking solution of the foams was used for germination, as the soaking time of the foams increased the length of the root slightly decreased. This indicates that the toxicity is higher when the tested foams are soaked for a longer period (*i.e.* 72 h).

Table 4. *The length of Sinapis alba roots in the case of all plates (Reference, Control, NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, and NCO-1.2, where NCO – isocyanate index) measured after 72 h incubation in dark at 20°C.*

	Soaking Time		
	24 h	**48 h**	**72 h**
Sample	**Length of the root (cm)**		
Reference_1	3.3	3.6	2.8
Reference_2	3.9	3.6	3.9
Reference_3	3.4	3.5	4.0
Average Reference	3.5±0.3	3.5±0.05	3.5±0.6
Control_1	5.0	4.5	3.9
Control_2	5.0	4.3	4.6
Control_3	4.7	4.6	3.9
Average Control	4.9±0.1	4.4±0.1	4.1±0.4
NCO-0.8_1	4.9	4.2	3.5
NCO-0.8_2	5.0	4.6	4.0
NCO-0.8_3	4.7	4.1	4.1
Average NCO-0.8	4.8±0.1	4.3±0.2	3.8±0.32
NCO-0.9_1	4.9	4.2	4.0
NCO-0.9_2	5.3	4.8	4.2
NCO-0.9_3	5.0	4.5	4.3
Average NCO-0.9	5.0±0.2	4.5±0.3	4.1±0.1

DOI: 10.14750/ME.2025.028

NCO-1.0_1	5.3	3.7	4.3
NCO-1.0_2	5.0	4.3	3.9
NCO-1.0_3	5.2	4.7	4.1
Average NCO-1.0	5.1±0.1	4.2±0.5	4.1±0.2
NCO-1.1_1	5.5	4.9	3.8
NCO-1.1_2	5.3	4.3	4.1
NCO-1.1_3	4.9	4.9	3.8
Average NCO-1.1	5.2±0.3	4.7±0.34	3.9±0.1
NCO-1.2_1	5.1	4.7	3.6
NCO-1.2_2	5.3	4.7	4.1
NCO-1.2_3	4.8	4.6	3.6
Average NCO-1.2	5.0±0.2	4.6±0.05	3.7±0.2

In this series of experiments, it was also seen, that applying 24 h or 48 h for foam soaking was possibly too short for the release of toxic compounds from the foams, or the released amount was not enough to trigger a toxic effect. From all the data the highest sensitivity of *Sinapis alba* was observed for the sample with the highest isocyanate index (NCO-1.2), where the root growth was reduced in relation to the Control foam (4.1 cm for the Control, 3.7 cm for NCO-1.2, respectively, with reduction percentage 9.8%) (**Table 5**) applying 72 h of soaking. It was also noticed that the largest difference between the 24 h and 72 h soaking time was found for the NCO-1.2 foam, with a difference of approximately 11.8% (**Table 5**).

Table 5. *Percentage change of the average length of the growing root compared to the root length of the Control samples (NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, and NCO-1.2) applying different soaking times (24 h, 48 h, 72 h).*

Soaking time	**Percentage (%) Control 100%**				
	NCO-0.8	**NCO-0.9**	**NCO-1.0**	**NCO-1.1**	**NCO-1.2**
24 h	97.9±3.1%	102±4.2%	104±3.1%	106±6.2%	102±5.1%
48 h	97.7±6.0%	102.2±6.8%	95.4±11.4%	106.8±7.8%	104.5±1.3%
72 h	92.68±7.8%	100±3.7%	100±4.8%	95.1 ±4.2%	90.2±7.0%

For the Acute toxicity test, the germination index was calculated according to equation (1) (see Material and Method). The germination index (GI%) values differed based on soaking times and the composition of the samples. At 24 h, GI% ranged from 91.0% to 93.3% for all samples. After 48 h, the GI% ranged from 83.3% to 94.3%. While at 72 h, the GI% for all samples varied between 85.3% and 92.0% (**Table 6**) where the lower germination rate was typically observed with PUF sample NCO-1.2.

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***Table 6**. Germination index (GI%) of the samples (Control, NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, and NCO-1.2)*

	Soaking time		
	24 h	**48 h**	**72 h**
Samples		**GI %**	
Reference	92.0	93.3	87.6
Control	91.0	88.6	92.0
NCO-0.8	92.0	83.3	87.6
NCO-0.9	93.3	91.0	91.0
NCO-1.0	91.0	94.3	91.0
NCO-1.1	93.3	90.0	90.0
NCO-1.2	93.3	91.0	85.3

Therefore, it can be concluded that the tested foam compositions did not affect noticeably seed germination, but the root length slightly varied depending on the chemical composition and concentration of the substances present in the soaking solution of the polymers. Similar findings were presented formerly when polylactide (PLA), polyhydroxybutyrate (PHB), and petroleum-derived non-biodegradable polypropylene (PP) were used in germination tests [90]. When the impact of different types of plastics on root growth was compared, it was observed that PHB and PLA had a more significant effect compared to PP, and all these polymers cause a reduction in the root length at higher concentrations of polymer particles used in soil [90]. This reduction may be attributed to the greater biodegradability of PLA and PHB compared to PP, and as they degrade, they release smaller particles or chemicals into the soil which may have toxic effects.

The primary reason for the differences in Acute toxicity test results may be the different compositions of the PUFs tested. Of the two main components used in the formulation of polyurethanes (polyol mix and isocyanate), the isocyanate component may have a greater influence on the toxicity of the product due to the reactive nature of the isocyanates. This is also because the components of the polyol mix (catalysts, blowing agents, surfactants, and flame retardants) individually appear in smaller percentages [122]. Therefore, the isocyanate index can significantly affect the toxicity of the final product, and the main toxic effect is likely induced by the presence of more isocyanate (*e.g.,* NCO-1.2).

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3.1.2 Bacteria-based toxicity test in the liquid phase

Bacterial toxicity tests were also performed to determine the effect of the prepared flexible polyurethane foam samples on microorganisms (NCO-0.8, 0.9, 1.0, 1.1, and 1.2) and compared with the Control foam sample. This sample (Control foam with NCO-0.9 isocyanate index) was tested previously and considered non-toxic (**Figure 18**).

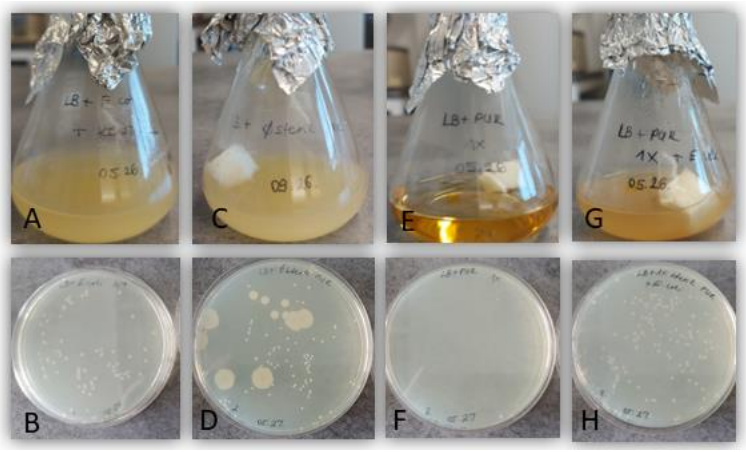

Figure 18. *A) Flask with LB medium and E. coli (Reference), B) spreaded sample from the Reference suspension, C) LB medium with Non-autoclaved Control PUF, D) spreaded sample from C suspension, E) LB medium with Autoclaved Control PUF, F) spreaded sample from E suspension, G) LB medium with Autoclaved Control PUF inoculated with E. coli suspension, H) spreaded sample from G suspension.*

All the samples (NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, and NCO-1.2) were compared with the Control foam. As shown in **Figure 18/D** if the Control foam was not autoclaved, at least three different cell morphologies were seen on the plate, which confirms that the Non-autoclaved foam was not toxic. Also, if the Control foam has been autoclaved (and made sterile) (**Figure 18/E**) and then, inoculated with *E. coli*, the colony number of the plates where nearly the same as the Reference where $(1.08\pm0.25)\times10^9$ CFU/mL for the Reference plate (**Figure 18/B**) and $(1.17\pm0.21)\times10^9$ CFU/mL for the Control foam (**Figure 18/H**), respectively. This indicates that no detectable toxic material is released from the Control foam during sterilization. The percentage of colony number of Control samples in comparison to the colony number of the studied foams with different isocyanate indices (NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, and NCO-1.2) was calculated (**Figure 19**).

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When the samples were autoclaved, no colonies were observed on the plates because by autoclaving, the PUF became sterile (**Figure 19**, **Table 7**). In case when the foam samples were autoclaved and inoculum of *E. coli* was added, the colony number of the Control plate and the Reference plate was comparable (**Table 7**, Reference (LB + *E. coli*) and the Control (Autoclaved foam + *E. coli*)) presuming that only non-toxic materials were released from the Control foam into the bacterial growing media during heat treatment (autoclaving) of the foam. However, as the NCO index increases, the number of NCO groups exceeds that of OH groups, leading to a greater influence of isocyanate on the toxicity of PUF. This is because PU foams can undergo thermal degradation under heat sterilization conditions (autoclaving at 121°C) [123]. Consequently, foams with higher isocyanate indices may release a greater proportion of chemical components during sterilization, potentially resulting in increased toxicity in the model organism and a reduction in cell concentration (**Table 7**).

Table 7*. Colony forming unit (CFU) for all samples (Control, NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, NCO-1.2) as follows: Autoclaved foam, Autoclaved foam + E. coli, Non-autoclaved foam + E. coli.*

		CFU/mL	
Sample	**Autoclaved foams**	**Autoclaved foam + *E. coli***	**Non-autoclaved foam+ *E. coli***
Control	0.0	9.6×10^8	6.3×10^8
NCO-0.8	0.0	13.1×10^8	7.7×10^8
NCO-0.9	0.0	5.9×10^8	6.4×10^8
NCO-1.0	0.0	6.7×10^8	6.5×10^8
NCO-1.1	0.0	5.0×10^8	4.1×10^8
NCO-1.2	0.0	4.9×10^8	6.0×10^8
		CFU/mL	
Reference1 (LB+*E. coli*)		9.5×10^8	
Reference 2 (LB+*E. coli*)		6.5×10^8	
Reference 3 (LB+*E. coli*)		5.4×10^8	

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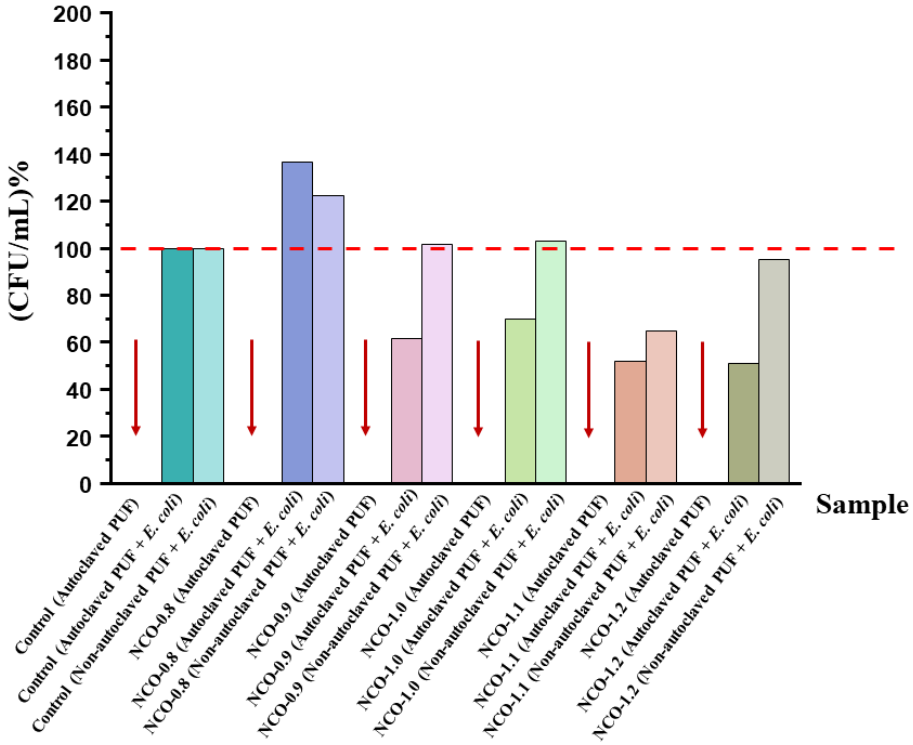

Figure 19. *Percentage of the average colony number for the tested samples (Control foam, NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, and NCO-1.2). The colony number of the Control plate is considered 100%. The lack of columns (the consequence of sterilization) is indicated by using red arrows.*

Formation of the colonies can occur exclusively in the case when there is no toxic material released from the tested foam during the sterilization process. Similarly, comparable bacterial concentrations were observed for both NCO-0.8 PUF and Control when autoclaved foam was examined with *E. coli* inoculum. This suggests that the samples with this isocyanate index did not inhibit the tested bacterial strain (**Figure 20**).

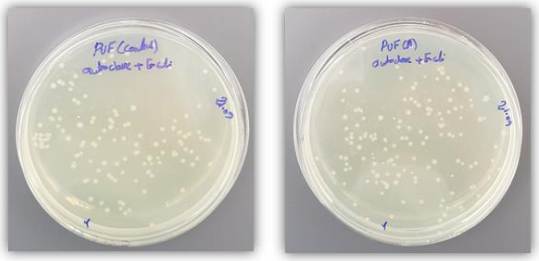

Figure 20. *Plates with E. coli grown on LB solid plates: a) Autoclaved Control PUF + E. coli sample, b) Autoclaved NCO-0.8 PUF + E. coli, where NCO-0.8 refers to the isocyanate index of the foam.*

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For NCO-0.9 and NCO-1.0 foams, a slightly reduced cell concentration was determined, but samples with NCO-1.1 and NCO-1.2 had the lowest colony numbers compared to other samples with the same condition (see in the column named Autoclaved foam + *E. coli* in **Table 8**). The bacterial concentration for the NCO-1.1 and NCO-1.2 was 5.0×10^8 and 4.9×10^8 CFU/mL respectively, which means 48 % and 49 % lower colony numbers than that of the Control sample, respectively (**Table 7** and **Table 8**).

Table 8. *Percentage of colony forming units (CFUs) for all samples (Control, NCO-0.8, NCO-0.9, NCO-1.0, NCO-1.1, NCO-1.2) in the following cases: Autoclaved foam, Autoclaved foam + E. coli, Non-autoclaved foam + E. coli compared to colony numbers of the Control plate. (-) refer to no colony-forming unit observed.*

	Percentage %		
Sample	**Autoclaved foams**	**Autoclaved foam+ *E. coli***	**Non-autoclaved foam+ *E. coli***
Control	-	100%	100%
NCO-0.8	-	136.4%	122.2%
NCO-0.9	-	61.4%	101.5%
NCO-1.0	-	69.7%	103.1%
NCO-1.1	-	52%	65%
NCO-1.2	-	51%	95.2%

The Non-autoclaved PUFs were also tested. Since the PUF itself is not sterile and when it is not toxic, microorganisms from the surface of the foam cube can grow in the used medium, so the liquid will turn turbid (**Figure 21/a**). However, primarily it is not known whether the tested foam is toxic or not, a flask with Non-autoclaved foam was also inoculated with *E. coli*. It turned out that Non-autoclaved Control and tested PUF samples (NCO-0.8 - 1.2) are non-toxic since bacteria could grow and numerous bacterial colonies with different morphology were counted on the plates belonging to these samples (as shown in a representative plate of NCO-0.8 sample, **Figure 21/b**).

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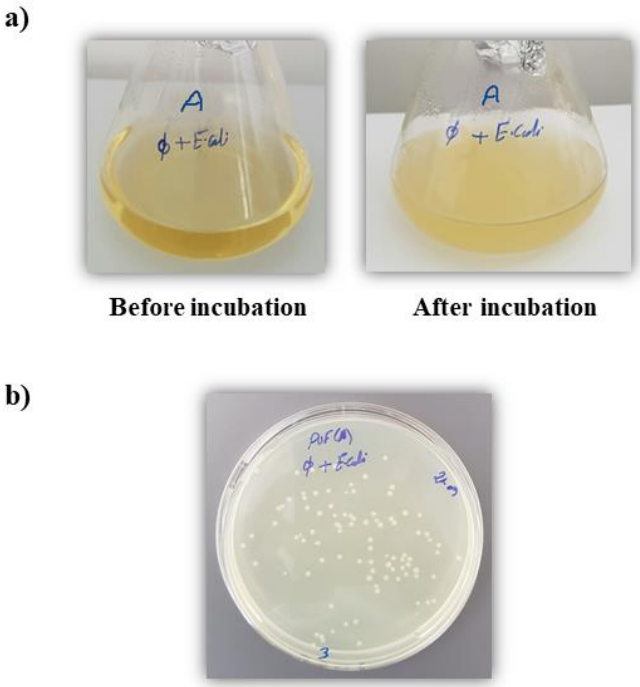

***Figure 21**. a) Difference in the look of inoculated LB medium before and after incubation of sample NCO-0.8 (Non-autoclaved NCO-0.8 PUF + E. coli), b) Colony-forming units on the plate with sample NCO-0.8 inoculated with E. coli (Non-autoclaved NCO-0.8 PUF+ E. coli).*

The bacterial concentration of the samples NCO-0.9, NCO-1.0, and NCO-1.2 was nearly the same as that of the Control sample ~6.3×10^8 CFU/mL, but the reduction in the colony number was obvious in the case of NCO-1.1 which is 35% lower than Control sample (**Figure 20**, **Table 7** and **Table 8**).

These results are in good agreement with other findings, where *E. coli* bacterium was also used in toxicology studies. As it was observed exposure of *E. coli* to polystyrene (PS) could allow bacteria to grow but the viability of *E. coli* cells decreased with increasing concentrations of PS [79].

In another study, the cytotoxic effect of open-cell polyurethane foams with different isocyanate indices was investigated on the keratinocyte cell line (HaCaTas). This study showed that the isocyanate index strongly influences the chemical structure of polyurethane foams, which is also reflected in cytotoxicity. These results provide important information for the design of polyurethane foams when used *e.g.* as composite matrices in biomedical applications [124].

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All our results were verified using a Reference (reference plates spread from the flask containing only sterile LB medium and the appropriate amount of *E. coli* overnight starter suspension) to have evidence that the applied bacterial suspension includes viable cells and that the LB agar plates are appropriate for growing the bacteria.

3.1.3 Verifying the sensitivity of solid PUF testing methods by using intentionally made Toxic foam

3.1.3.1. Seed test

Moderate toxicity of the PUF samples was demonstrated with previous experiments, and the toxic effect depended on the time used for soaking the foams and on the isocyanate index as well. To verify that the applied method is adequate for the demonstration of the toxic effect of the solid foams, if any, a Toxic sample was used, and the previously presented seed test was carried out. In this case, a Control foam (the same as was used in previous tests), a newly prepared NCO-1.0 foam, and an intentionally toxic foam (named Toxic foam) were used. The Toxic foam was made by soaking the NCO-1.0 foam into a commonly used form separator (GORAPUR LK 8779-61 E, consisting of hydrocarbons, C_9-C_{10}, n-alkanes, isoalkanes, cyclics, < 2% aromatics) applied in flexible polyurethane production. This Toxic foam served to validate the sensitivity of the adapted tests for the polyurethane foam.

Based on the information sheet of the used foam separator it releases agents harmful to aquatic life (flammable liquid and vapor, may cause an allergic skin reaction, drowsiness, or dizziness) with long-lasting effects as well. After soaking the foam cubes in this toxic material, excess liquid was squeezed from the foam cubes, and for several minutes they were left on the laboratory desk and dried. The average root length of the seeds grown in the presence of the solution prepared by soaking Control foam, a new NCO-1.0 foam, and the Toxic foam in water-based liquid (with the same composition as used previously in this work) was calculated (**Table 9**).

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Table 9. *Root lengths of the growing seeds in the presence of the soaking liquid applied for Control, new NCO-1.0 sample, and the Toxic foam with different soaking times of 24 h, 48 h, or 72 h.*

	Soaking Time		
	24 h	**48 h**	**72 h**
Sample	**Length of the root (cm)**		
Reference_1	3.7	3.8	4.0
Reference_2	3.2	3.0	4.1
Reference_3	3.7	3.3	4.3
Average Reference	3.5±0.2	3.3±0.4	4.1±0.1
Control_1	4.1	3.6	4.0
Control_2	4.2	3.6	4.1
Control_3	3.9	4.1	4.1
Average Control	4.0±0.1	3.7±0.2	4.0±0.05
NCO-1.0_1	4.5	4.3	3.5
NCO-1.0_2	4.0	3.5	3.6
NCO-1.0_3	3.9	3.7	3.7
Average NCO-1.0	4.1±0.3	3.8±0.4	3.6±0.1
Toxic_1	2.3	2.8	3.1
Toxic_2	2.9	2.8	3.2
Toxic_3	2.1	2.9	3.2
Average Toxic	2.4±0.4	2.8±0.05	3.1±0.05

The root length of the seeds when soaked into liquid from the Control and new NCO-1.0 foams was in the same range when a 24 or 48 h soaking period was used. The root length was between ~3.7 and 4.1 cm, while in the case of the Toxic sample, the length of the root decreased significantly. The reduction was the most obvious in the case of the 24 h soaking time, since in comparison to the Control the average root length was just around 60% (The percentage of average root length exposed to the Control is considered 100%) (**Figure 22**, **Table 10**).

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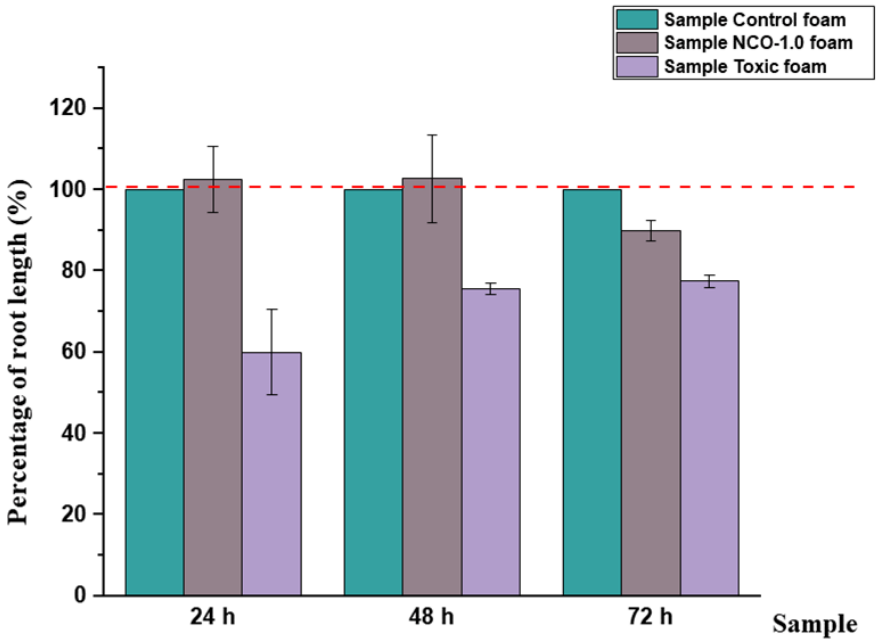

Figure 22. *Percentage of the average root length distribution depends on the toxicity of the tested polyurethane foam (PUF). PUF samples are Control, a new NCO-1.0, and Toxic foam. Used soaking times were 24, 48, or 72 h respectively. The percentage of average root length exposed to the Control is considered 100%.*

It was also observed that the difference in the root length (measured for the roots from the Control and the Toxic foam's plate) decreased as the soaking time increased. The root length difference between the Control and the Toxic foam from 40% (using 24 h soaking time) decreased to 22.5% (after 72 h soaking period) (**Figure 22**, **Table 10**). The reason behind this phenomenon can be the presence of volatile materials in the used form separator liquid (hydrocarbons, C_9-C_{10}, n-alkanes, isoalkanes, cyclics, < 2% aromatic). Therefore, as the incubation time was longer, the quantity of the toxic compound in the foam (and consequently in the soaking liquid) decreased, presumably due to the evaporation of the volatile toxic compounds.

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Table 10. *Percentage of the average length of the growing roots compared to the root length of the Control samples (the root length of the Control was considered as 100 % for each soaking period used. NCO-1.0 is a newly prepared foam with NCO = 1.0, and Toxic foam is a new NCO-1.0 foam made toxic after its production using a form separator*

Soaking time	Percentage (%)	
	Control 100%	
	NCO-1.0	Toxic foam
24 h	102.5±8.0%	60.0±10.4%
48 h	102.7±10.8%	75.6±1.5%
72 h	90.0±2.5%	77.5±1.4%

The germination index (GI%) was also determined for these tests for all samples and at different soaking times. The GI% ranged from 85.3% to 92% at 24 h, from 85.3% to 93.3% at 48 h, and from 87.6% to 96.6% at 72 h (**Table 11**), which means that the tests were evaluable, and the results obtained with the Toxic foam were relevant.

Table 11*. Germination index (GI %) of the studied samples: Control, new NCO-1.0 sample, and the Toxic foam.*

	Soaking time		
	24 h	48 h	72 h
Samples		GI %	
Reference	88.6	87.6	90.0
Control	88.6	93.3	87.6
NCO-1.0	92.0	91.0	94.3
Toxic foam	85.3	85.3	96.6

By performing experiments with the aforementioned setup, the sensitivity of the mustard seed model organism was shown. With this test, adapted to the toxicity evaluation of PUFs, it was not only possible to demonstrate the toxic behavior of a tested material but also its potential volatile properties (**Figure 22**). It can be concluded that this test is sufficiently sensitive to detect the toxicity of solid PUFs, more specifically the toxicity of chemical compounds used for polyurethane preparation, or any chemicals released from the foams, if present.

3.1.3.2. Bacteria-based test

Similarly, to the mustard seed test, the sensitivity of the bacterial test was also verified. The changes in the LB medium are visible when there is bacterial growth (**Figure 23**, Non-autoclaved Control PUF + *E. coli* before and after incubation) since due to bacterial division the used liquid medium becomes turbid. As explained earlier, the Non-autoclaved foams are

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not sterile, and microorganisms existing on the surface of the foam can easily divide, causing the initially transparent LB medium to become opaque when incubated at 37°C in a basic bacterial medium. In the case of the Toxic Non-autoclaved foam, there was no change in the color and appearance of the used medium before and after incubation (**Figure 23**, Non-autoclaved Toxic PUF + *E. coli*). Moreover, there was no turbidity observed even if a certain amount of *E. coli* was added to the Non-autoclaved Toxic foam. This means, that the chemical used for making the sample toxic releases toxic compounds into the liquid medium, which in turn kills all the microorganisms used for the test (including those naturally occurring on the surface of the foam, and the extra added *E. coli* bacterium as well).

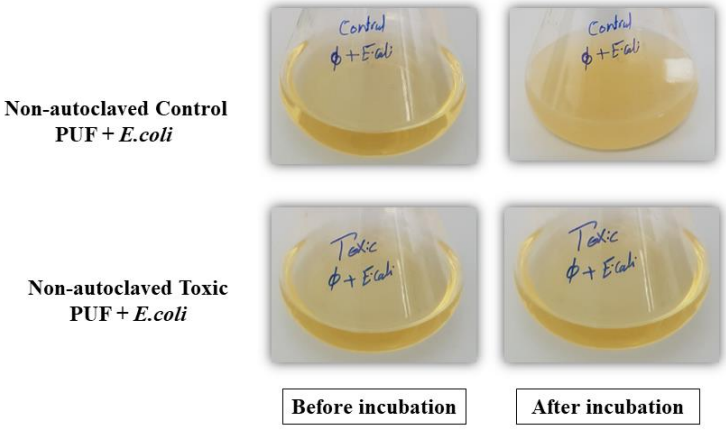

***Figure 23**. Differences in the look of inoculated LB medium before and after incubation depend on the toxicity of the used foams. Non-Autoclaved Control PUF+E. coli: sterile LB medium with non-autoclaved Control PUF and inoculated with E. coli. Non-Autoclaved Toxic PUF+E. coli: sterile LB medium with non-autoclaved Toxic PUF and inoculated with E. coli.*

In the case of using the autoclave sterilization process, after incubation of the flasks, no bacterial growth was observed, resulting in the LB medium remaining transparent regardless of whether Toxic or another foam was used. This was confirmed by spreading the required amount of these incubated liquids onto LB agar plates, where no colonies were formed (**Figure 24** and **Figure 25**).

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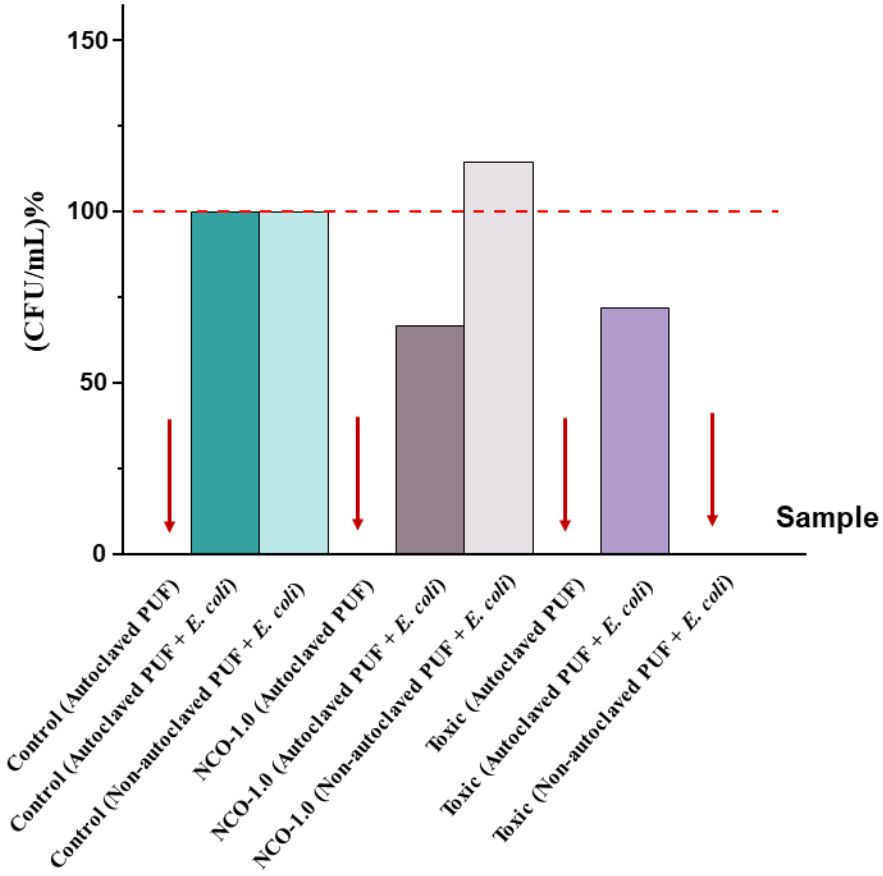

Figure 24. *Percentage of the average bacterial colony number in the case of the Control, new NCO-1.0, and Toxic foam samples. The lack of columns (the consequence of sterilization or the toxic behavior of the released chemical) is indicated by using red arrows.*

When Control foam (Autoclaved) was put into a flask inoculated with *E. coli* bacterial suspension, it did not affect the growth of the bacteria, since the colony number of the Reference flask (see reference conditions in the Materials and Methods section) and that of the Control flask were almost the same in a range 7.5×10^8 CFU/mL. The percentage of the CFU/mL in the case of autoclaved and inoculated new NCO-1.0 foam was 66.6% compared to the Control (**Figure 24** and **Table 12**), which was in agreement with our previous observations (**Table 12**, **Table 12**). While the Toxic foam enabled bacterial growth only when its heat sterilization was performed before the inoculation (**Figure 24**).

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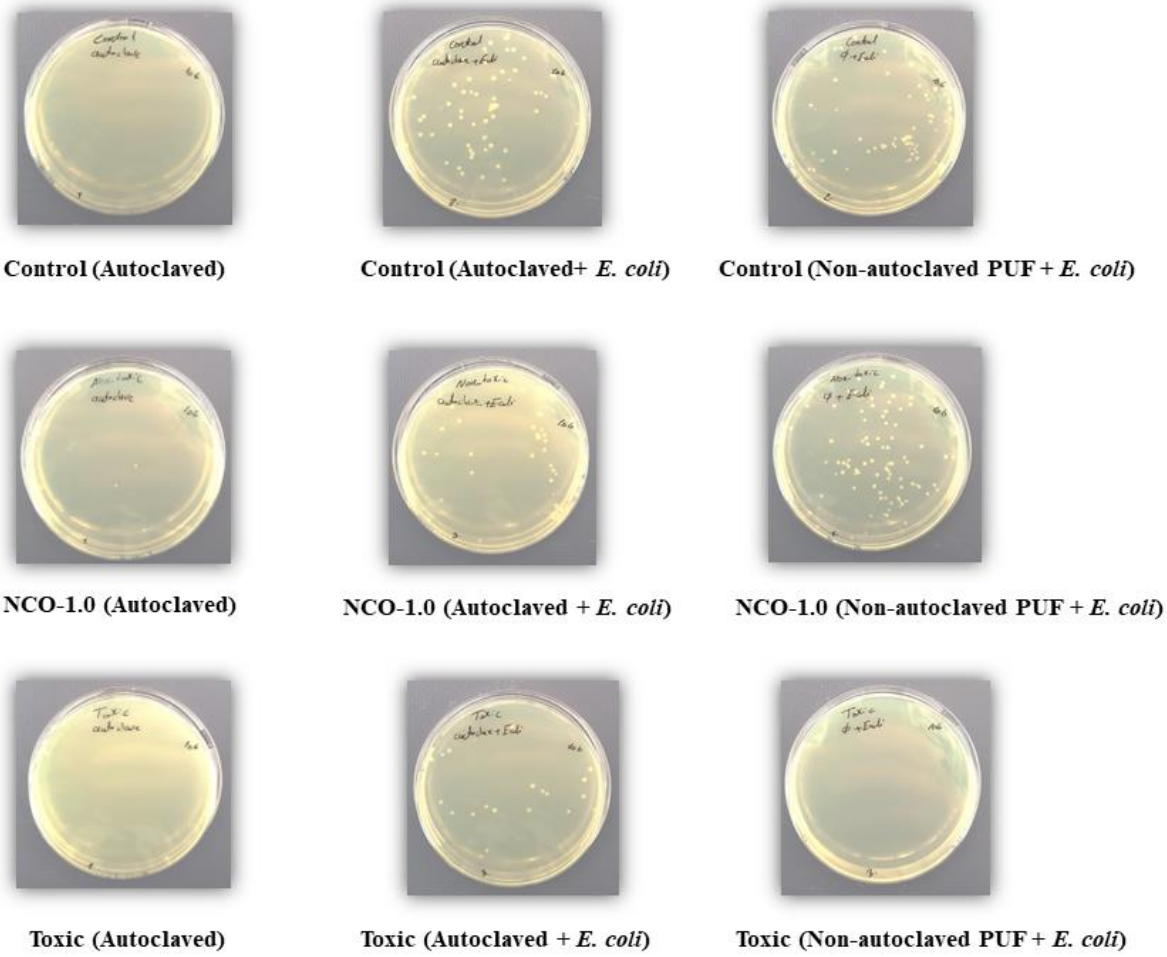

Figure 25. *Difference in colony number of E. coli depending on type and condition of PUF samples.*

Table 12*. Percentage of colony forming units (CFUs) for all samples NCO-1.0, and Toxic foam in the following cases: Autoclaved foam, Autoclaved foam + E. coli., Non-autoclaved foam + E. coli. compared to colony numbers of the Control plate. (-) refer to no colony-forming unit observed.*

	Percentage %		
Sample	**Autoclaved foams**	**Autoclaved foam+ *E. coli***	**Non-autoclaved foam+ *E. coli***
Control	-	100	100
NCO-1.0	-	66.6	114.5
Toxic	-	72.0	0.0

Thus, it can be concluded that the developed and applied *E. coli*-based test procedure is also sensitive enough to detect the toxicity of polyurethane foam samples. When evaluating the

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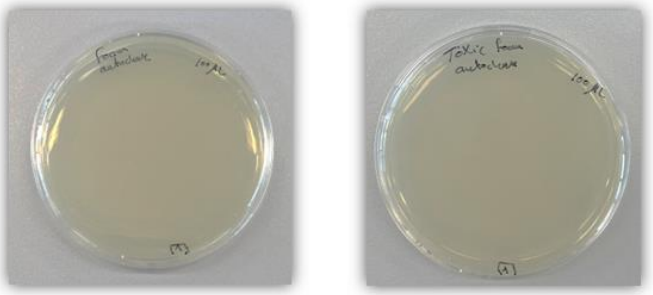
effectiveness of the *E. coli* inhibition test, it was found that this method is not only sufficiently sensitive but also rapid, providing results within 24 h, which is a significant advantage compared to other tests [82], which highlights the benefits of bacterial-based tests.

In addition to *Escherichia coli* bacteria, the Gram-positive bacterium, *Micrococcus luteus* (*M. luteus*, an available Gram-positive type microorganism in our laboratory) has been used to validate the sensitivity of the toxicity test of polyurethane foams. For the bacterial test, the same procedure and steps were applied as previously, while the new NCO-1.0 foam compared to the Toxic PUF which is the same type of foam (NCO-1.0) but was intentionally made toxic. The Toxic foam was prepared in the same way as described previously by soaking the foam into a form separator generally applied in flexible polyurethane production. Tested PUFs in bacterial suspension were incubated at 30°C for 48 h (since these are the optimal growing conditions for our *Micrococcus luteus* laboratory model organism).

The results showed that when new NCO-1.0 foam and Toxic PU foam cubes were immersed in the appropriate amount of LB broth and autoclaved (121°C, 15 psi for 45 minutes), then samples from the liquid were spread onto LB agar plates, no bacterial growth was observed on these plates. Thus, confirming that the foams were sterile (**Figure 26**).



New NCO-1.0 foam (Autoclaved PUF) **Toxic (Autoclaved PUF)**

Figure 26*. No growing of bacteria on the plates in the case of autoclaving the foams*

When the samples were autoclaved and then incubated with *M. luteus*, the cell concentration of the bacterial solutions was determined, and the resulting cell concentration values were compared. The average cell concentration for the new NCO-1.0 foam (Autoclaved PUF + *M. luteus*) sample was determined to be 9.8×10^7 CFU/mL (**Table 13**), while 1.5×10^7 CFU/mL was obtained for the Toxic (Autoclaved PUF + *M. luteus*) foam. This means an 84.7% reduction in

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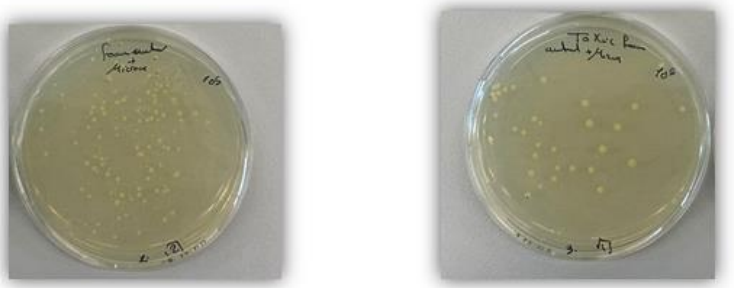
cell concentration (**Figure 27**). These results suggested that our Gram-positive model organism (*Micrococcus luteus*) was sensitive enough to be used in toxicity assays and that it was more sensitive to the toxic compound used than *E. coli* (84.7 % vs. 28% reduction).



New NCO-1.0 foam (Autoclaved PUF + *M. luteus*

Toxic (Autoclaved PUF + *M. luteus*)

Figure 27. *Difference in the colony number obtained with the samples New NCO-1.0 foam (Autoclaved PUF + M. luteus) and Toxic (Autoclaved PUF + M. luteus) foam*

In the case of new NCO-1.0 foam (Non-Autoclaved PUF + *M. luteus*), which was not sterile and had microorganisms remaining on the foam surface, a large number of bacterial colonies of different morphologies appeared on the plates (**Figure 28/a**). As seen in the figure, an uncountable number of colonies developed on the new NCO-1.0 plates, making it impossible to determine the exact CFU/mL concentration. In contrast, for the Toxic foam (Non-Autoclaved PUF + *M. luteus*), when *M. luteus* was added, no turbidity was observed in the flask, and no bacteria grew on the plates. This indicates that the chemical used to make the foam toxic was potent enough to kill all the microorganisms (**Figure 28/b**).

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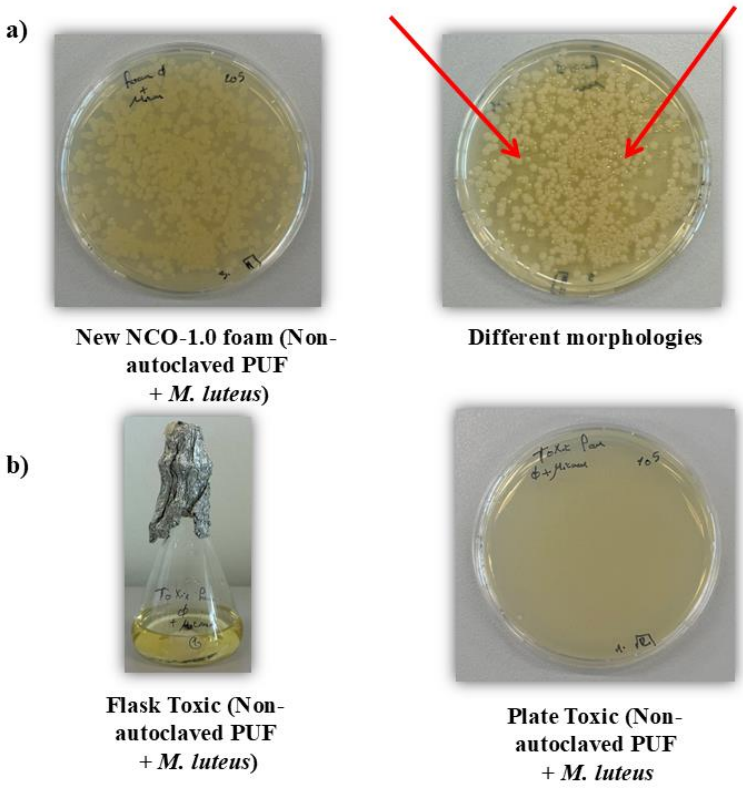

Figure 28. *a) Different morphologies of bacteria of the New NCO-1.0 foam (Non-Autoclaved PUF + M. luteus) LB agar plates b) Liquid suspension and a spread plate with the Toxic (Non-Autoclaved PUF + M. luteus) sample*

Table 13*. Cell concentration (CFU/mL) for the samples: New NCO-1.0 foam (Autoclaved PUF + M. luteus) and Toxic (Autoclaved PUF + M. luteus) foams.*

	CFU/mL		
Sample	**Autoclaved PUF**	**Autoclaved PUF + *M. luteus***	**Non-autoclaved PUF + *M. luteus***
New NCO-1.0 foam	0.0	9.8×10^{7}	Uncountable
Toxic foam	0.0	1.5×10^{7}	0.0

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Table 14. *Percentage of colony forming units (CFUs) for the samples: New NCO-1.0 foam (Autoclaved PUF + M. luteus) and Toxic (Autoclaved PUF + M. luteus) foams.*

	Percentage %		
Sample	**Autoclaved PUF**	**Autoclaved PUF + *M. luteus***	**Non-autoclaved PUF + *M. luteus***
New NCO-1.0 foam	-	100%	Uncountable
Toxic foam	-	15.3%	0.0

All in all, the adapted bacterial test proved to be sensitive to detect the toxicity of PUF using any bacteria, whether *E. coli* (as a representative of Gram-negative bacteria) or *Micrococcus luteus* (as a representative of Gram-positive bacteria). When *E. coli* is used in the test procedure, results are obtained faster (only 24 h is needed), whereas for *Micrococcus luteus*, the examination takes longer, requiring a minimum of 48 h for each incubation.

3.1.4 Conclusion

Five PU foam samples were prepared with different NCO indices (NCO-0.8, 0.9, 1.0, 1.1, and 1.2) and together with the Control sample (a previously tested non-toxic foam sample) were applied to develop toxicity tests, while intentionally prepared Toxic foam has been used to verify the accuracy of the developed testing procedures. Three test organisms were successfully applied, *Sinapis alba* (white mustard) seeds, *Escherichia coli* (non-pathogenic), and *Micrococcus luteus* bacterial model organisms, and toxicity tests were adapted for the examination of PUF-derived substances. Regarding *Sinapis alba* test, the prepared samples (NCO-0.8 to NCO-1.2) did not express toxicity after 24 h soaking period used since there was no relevant decrease in the root length compared to the Control. However, by comparing the root length of the samples with different NCO indices to that of the Control foam, a slight decrease can be observed when foams were soaked for 72 h. The most obvious decrease was noticed in the case of NCO-1.2 where the root length reduced by 9.8% compared to the Control sample. In the case of using an intentionally made Toxic foam, the average root length was lower than the Control for all soaking times used which means that the test was sensitive enough to detect toxicity.

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In the bacterial test, it was observed that the NCO-0.8 sample in both cases (Autoclaved PUF +*E. coli* and Non-autoclaved PUF +*E. coli*), as well as the Non-autoclaved PUF (NCO-0.9, NCO-1.0 and NCO-1.2) +*E. coli* were non-toxic as bacteria could grow on the plates. In the case of the Autoclaved PUF (NCO-0.9, NCO-1.0, and NCO-1.2) +*E. coli*, the cell concentration was reduced to about half of the cell concentration of the Control sample. However, the sample NCO-1.1 had the lowest colony number in both conditions. The opposite was observed for the Toxic sample since no bacteria could grow when the Non-autoclaved Toxic PUF +*E. coli* condition was tested. So, the bacterial test is also sensitive enough to detect toxic compounds and their volatile behavior, if any. Moreover, testing PUF toxicity with other bacteria (e.g. *M. luteus*) confirmed its effectiveness in detecting PUF toxicity.

DOI: 10.14750/ME.2025.028

3.2 Applicability of the developed toxicity tests to study carbonaceous materials

To validate the applicability of the developed toxicity tests, they were applied to carbonaceous materials like activated carbon and biochar. Before that, all samples were thoroughly characterized using various techniques to examine their structure and functionalities, as outlined in this section.

3.2.1 Metal content measurement of carbonaceous materials

The metal content of activated carbon and biochar was determined to identify the levels of heavy metals in these samples, which could pose environmental or health risks and contribute to the potential toxicity of these materials and any composites made from them.

The concentrations of various metals in the activated carbon/biochar samples from a natural source from *Sargasumm* sp. and one commercial activated carbon COMAC were analyzed using ICP-OES measurements, and the results were compared in terms of m/m%. The predominant metal observed in the AC/BC samples was calcium (Ca), with concentrations of 0.135, and 0.951m/m% for COMAC, and AC, respectively. Calcium was also present in BC but at a lower concentration compared to magnesium (Mg), which showed the highest concentration in the biochar sample with a value of 1.246 m/m%. Two heavy metals, lead (Pb) and cadmium (Cd) exhibited the lowest concentration, with values < 0.001% for all samples (**Table 15**). Additionally, other metals such as Co, Cu, Fe, Ni, and Zn were also present in all samples but only at low concentrations. The higher presence of calcium in activated carbon produced by using phosphoric acid chemical activation and the presence of magnesium in biochar prepared by pyrolysis primarily arise from the inherent calcium and magnesium content in the precursor material. During the activation process, calcium interacts with phosphoric acid to form stable, non-volatile compounds retained within the activated carbon structure [125]. Biochar was produced through the pyrolysis of crushed *Sargassum* sp. at 600°C, and residual volatile compounds began to volatilize at temperatures above 800°C, explaining a higher concentration of thermally stable magnesium components in the biochar.

DOI: 10.14750/ME.2025.028

***Table 15**. Comparison of the metal content of activated carbon/biochar samples (COMAC, AC, and BC) determined by using ICP-OES.*

Sample	Concentration of Metals in *m*/*m*%								
	Ca	**Cd**	**Co**	**Cu**	**Fe**	**Mg**	**Ni**	**Pb**	**Zn**
COMAC	0.135	<0.001	<0.001	<0.001	0.047	0.068	0.0011	<0.001	0.0064
AC	0.951	<0.001	<0.001	0.0016	0.048	0.097	0.0015	<0.001	0.0146
BC	0.969	<0.001	<0.001	<0.001	0.019	1.246	0.002	<0.001	0.0043

3.2.2 Electrokinetic (Zeta) potential measurements of AC/BC samples

The electrokinetic properties of the AC/BC samples differ due to the various activation processes, which create oxygenated functional groups on the surface [126]. The negative charge of the AC/BC particles can be attributed to the deprotonation of functional groups (e.g., carboxyl) [127], [128].

***Table 16.** Electro-kinetic (zeta) potential of the activated carbon/biochar samples (COMAC, AC, and BC)*

Sample	Zeta potential (mV)
COMAC	-13.21
AC	-20.07
BC	-22.80

The lowest zeta potential was associated with BC and AC samples, with a value of −22.80 and −20.07 mV, respectively (**Figure 29**,**Table 16**). This indicates that the BC particles have a strong negative surface charge, which will help repel each other, keeping the particles well-dispersed and preventing aggregation by causing electrostatic repulsion between the particles. This property is crucial for maintaining uniform dispersion, which enhances the effectiveness of AC or BC in applications such as water purification where uniform distribution is needed for optimal performance.

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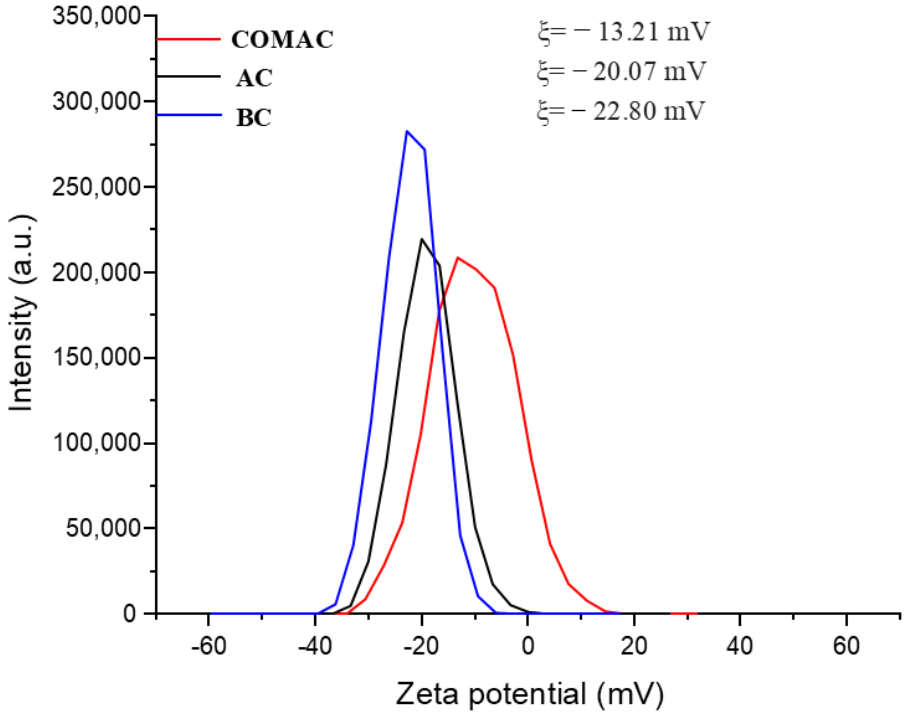

Figure 29. *Zeta potential distribution of the four different activated carbon/biochar samples.*

3.2.3 Infrared spectroscopy characterization of the studied samples

To identify the functional groups in the AC/BC samples, FTIR was used [115]. All studied samples have the same tendency, but the highest intensities were determined in the case of COMAC. The spectra of all samples contained peaks at 650 cm^{-1} attributed to aromatic C–H bending [129]. The peaks within the range of 1126–1236 cm^{-1} correspond to C-O stretching in alcohols and phenols [115], [129]. The band observed at about 1566 cm^{-1} may be associated with aromatic and C=O (carbonyl) stretching vibration [129]. The CO_2 infrared standard peak (2349 cm^{-1}) splits into two peaks (2330 cm^{-1} and 2360 cm^{-1}) caused by the asymmetric and symmetric stretching vibrations of CO_2 (peak at 2349 cm^{-1} is attributed to O=C=O) [130]. Very small peaks are found from 2842–2908 cm^{-1}, which are associated with the symmetric and asymmetric stretching vibration of aliphatic and aromatic C-H [115], [128]. The functional group represented by the bands about 3400 cm^{-1} is related to the stretching vibration of the surface hydroxyl group -OH [131]. The stretching vibration band of hydroxyl group –OH could be observed at 3851 cm^{-1} [128] (**Figure 30**).

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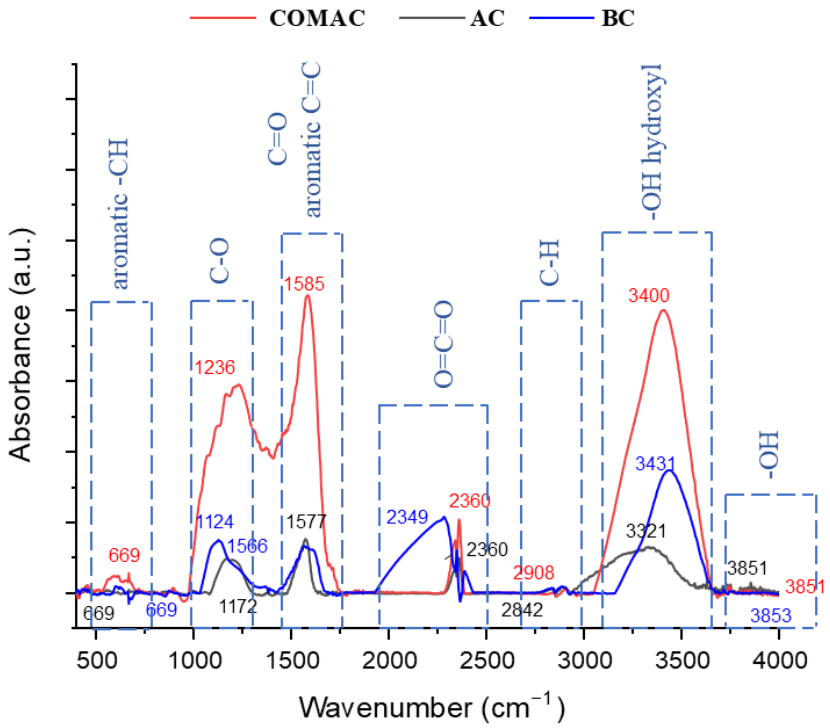

***Figure 30**. FTIR spectra of the four different activated carbon/biochar (COMAC, AC, and BC) samples.*

3.2.4 Specific surface area measurements

Surface area measurement is applied to activated carbon and biochar, where a larger surface area indicates a more porous structure, enhancing the materials' effectiveness in applications such as adsorbing the contaminants from water and soil.

The specific surface area of the samples was measured using the Brunauer–Emmett–Teller (BET) method with nitrogen adsorption at 77 K (**Table 17**). By comparing all the samples, AC samples showed the largest surface area of 1695 m^2/g, indicating a highly porous structure. The COMAC sample had a slight reduction in BET surface area, being 1120 m^2/g but still had high porosity. In contrast, biochar showed the lowest value of 854 m^2/g.

***Table 17**. Specific surface area of the samples.*

Sample	BET surface area (m^2/g)
COMAC	1120
AC	1695
BC	854

DOI: 10.14750/ME.2025.028

3.2.5 Toxicity Test

3.2.5.1 Acute toxicity test

Since *Sinapis alba* has shown sufficient sensitivity in toxicity tests, the developed procedure was also applied to assess the toxicity of AC and BC samples.

The root lengths and percentages of the average of the root length for all samples have been measured (**Table 18** and **Table 19**) and compared to the COMAC sample. The root length of *Sinapis alba* seeds for the AC plates was within a similar range to those of the COMAC plates. The average root length was 4.9 cm with percentages of 102.08%, compared to COMAC (**Table 18** and **Table 19**).

Table 18. *The average root lengths of the growing seeds without (e.g., Dilution water-1) and in contact with the studied samples (COMAC, AC, and BC).*

Sample	Root Length (cm)
Dilution water-1	3.9
Dilution water-2	3.6
Dilution water-3	3.3
Average Dilution water	3.6 ± 0.3
COMAC-1	5.0
COMAC-2	4.4
COMAC-3	5.0
Average COMAC	4.8 ± 0.3
AC-1	5.0
AC-2	4.9
AC-3	4.9
Average AC	4.9 ± 0.1
BC-1	4.2
BC-2	4.7
BC-3	3.7
Average BC	4.2 ± 0.5

The results indicate that root length slightly decreased with biochar compared to COMAC, reaching approximately 87.50%, representing a ~12.5% reduction (**Table 19**). The potential impact of biochar on plant germination varies significantly based on feedstock composition and pyrolysis temperature [53]. However, regardless of whether commercial activated carbon (COMAC), AC, or BC was used, root length remained comparable to that of seeds germinated in simple dilution water, confirming that the tested activated carbons are not toxic.

DOI: 10.14750/ME.2025.028

Table 19. *Percentage of the length of the growing root exposed to AC, and BC compared to those exposed to the COMAC sample*

Sample	Percentage (%)
COMAC	100.0
AC	102.08±2.04
BC	87.50±11.9

Among all tested samples, the highest root length percentage for mustard seeds was observed with AC, which increased the average by 102.08%, surpassing BC (**Figure 31**, **Table 18** and **Table 19**).

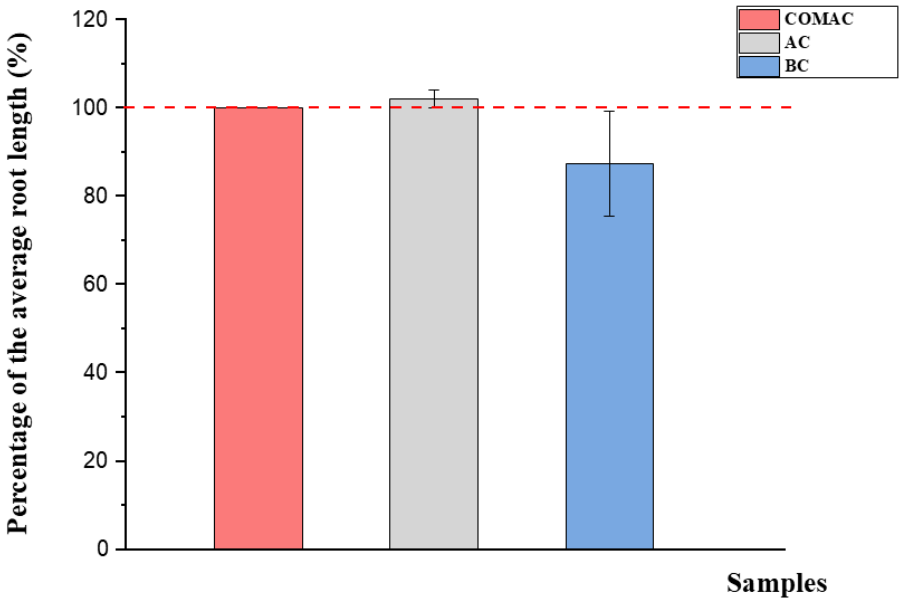

Figure 31. *Percentage of the length of the growing root of Sinapis alba (white mustard) seeds exposed to AC (activated carbon was prepared from Sargassum sp. by chemical activation), and BC (biochar prepared from Sargassum sp. by pyrolysis) compared to the growing roots exposed to the COMAC (commercial activated carbon). The red dotted line refers to the level of germination seeds for the samples in comparison to the COMAC.*

While all samples contributed to mustard seed growth, the average root length varied, likely due to factors such as particle size and material composition. Activated carbon and biochar can enhance seed germination and growth due to their metal content and the partial bioavailability of carbon within their porous structure [53]. Generally, the metal content of activated carbon

DOI: 10.14750/ME.2025.028

from COMAC, AC, and BC does not negatively affect mustard seed growth; instead, it has a beneficial impact, particularly when compared to seeds germinated in dilution water alone, where the average root length was 3.6 ± 0.3 cm (**Table 18**).

The germination index (GI%) was also determined (**Table 20**) for these tests for all samples (COMAC, AC, and BC) according to equation (1). Comparing the GI% for all the carbonaceous materials it was observed the BC sample has the highest germination index of 92% despite having a shorter root length than other samples. COMAC and AC had similar GI values 87% and 86.6%, respectively.

Table 20*. Germination index GI of the samples (COMAC, AC, and BC)*

Samples	GI%
COMAC	87.0
AC	86.6
BC	92.0

3.2.5.2 Bacterial test for activated carbon and biochar

Toxicity tests using *E. coli* were also performed to make sure the samples were safe and suitable for a wide range of applications such as adsorbing pollutants in water [67], soil amendment, and as filler in composite materials [132].

The sterilization process in this test was applied as previously described (at 120°C for 2 hours). As stated earlier, bacterial colony formation occurs only if no toxic substances are released from the tested samples that could inhibit bacterial growth (**Figure 32**).

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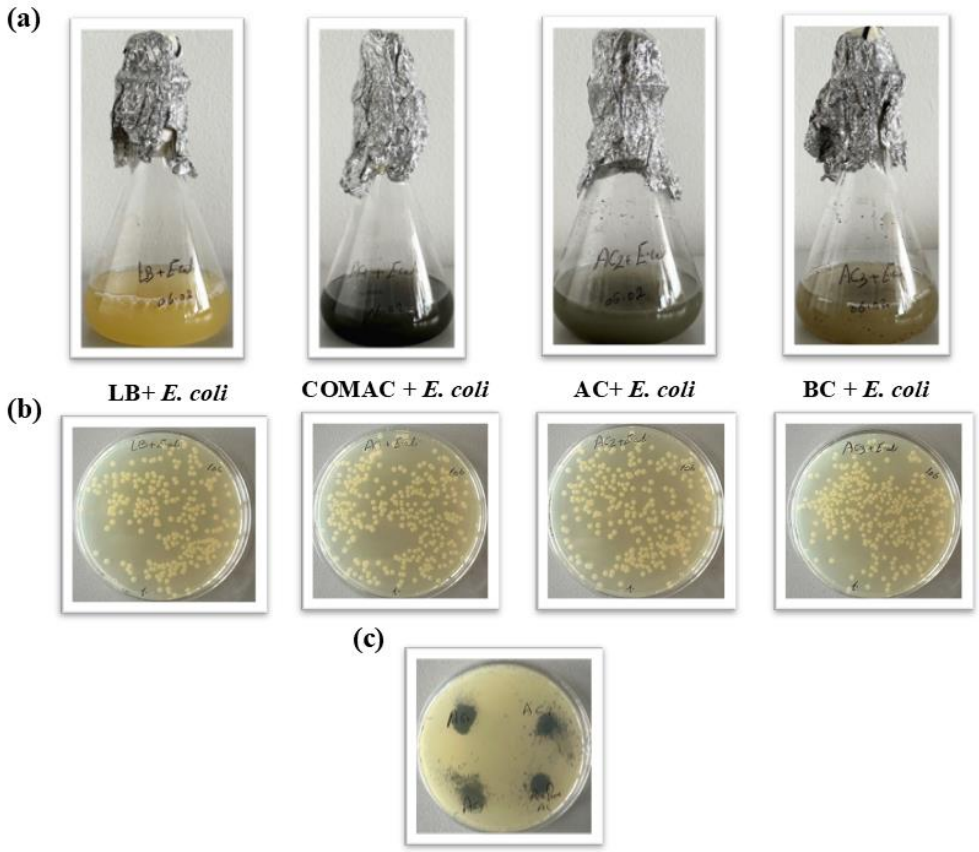

Figure 32. *Bacteria-based toxicity tests: (**a**) flasks containing LB liquid medium with AC/BC samples and E. coli, (**b**) plates spread with liquid sample from the flask above for colony counting, and (**c**) a representative plate test of dry AC/BC samples.*

When all AC- or BC-containing flasks (filled with LB medium) were sterilized and then inoculated with *E. coli*, the change in bacterial concentration under the given condition was detected by spreading an appropriate amount of liquid sample on LB agar plates and by counting the number of colonies formed on the plates. It was found that in case of both the COMAC + *E. coli* plate and plates made from the flasks with various AC or BC samples coincubated with *E. coli*, the colony counts fell within a similar range of ~ 3.1×10^9 CFU/mL, showing a similarity in the growth and viability of bacteria. This observation assumes that only non-toxic materials were released from the various AC or BC samples into the bacterial growth medium (**Table 21**).

DOI: 10.14750/ME.2025.028

Table 21. *Colony forming units (CFU) for all samples (COMAC + E. coli, AC + E. coli, BC + E. coli).*

Sample	CFU/mL
LB + *E. coli*	2.7×10^9
COMAC + *E. coli*	3.1×10^9
AC + *E. coli*	3.2×10^9
BC + *E. coli*	2.9×10^9

The potential toxicity of tested samples was assessed using another technique (inhibition zone test) as explained previously. This involved spreading 100 µL of a concentrated *E. coli* bacterial suspension onto an agar plate and adding a certain amount of dry particles from the tested samples. In this method, a clear zone (the zone of inhibition) around the material placed on the plate indicates toxicity, meaning the tested material has inhibited or killed the bacteria in that area. If the material is not toxic, the bacteria will grow normally around the tested samples, and no clear zone will be visible. As no clear zone was noticed around the samples, the tested materials were determined as not toxic (**Figure 32/C**).

This result aligns with the previous test using *Sinapis alba* seeds where these materials were found to be non-toxic as well. Therefore it could be established that AC and BC can be applied in various applications, including water purification, removal of heavy metals from water, or soil amendment.

3.2.6 Conclusion

Three different samples of activated carbon and biochar (COMAC, AC, and BC) have been studied. All samples were characterized using various techniques to understand their structure and functionalities. The metal content of the samples was characterized by using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Despite *Sargassum sp*. originated from the sea and possibly being contaminated with heavy metals, the results showed that the metal content of the AC and BC from *Sargassum sp*. did not contain excessive heavy metals, indicating the materials are not hazardous. FTIR spectra revealed that all the samples have the same functional groups, and carbonyl and hydroxyl groups were identified on the surface of the AC/BC samples. Toxicity tests were also carried out to determine the environmental impact of the prepared activated carbon and biochar samples. Using *Sinapis alba* seeds as model organisms the test results confirm that the analyzed activated carbons, including the commercial activated carbon (COMAC), and the *Sargassum sp.* based AC, or BC, do not exhibit toxicity,

DOI: 10.14750/ME.2025.028

as root length remained comparable to that of seeds germinated in simple dilution water. In addition, the toxicity of the samples was also determined by using *Escherichia coli* as a model organism, where there was no observed decrease in bacterial viability in the presence of the studied samples. Thus, it was concluded that all investigated carbonaceous materials are non-toxic and that it is safe to employ them in various applications such as the removal of heavy metals from water.

3.3 Potential applications of carbonaceous materials

3.3.1 Water treatment/ removal of heavy metals from water

Since the carbonaceous materials were found to be non-toxic, there is a possibility of using them as adsorbent materials. This section describes the adsorption performance of activated carbon, and biochar samples derived from *Sargassum* sp. compared to commercial activated carbon COMAC, where the adsorption test was carried out at different parameters.

3.3.1.1. Adsorption studies

1. Influence of pH

The pH at the point of zero charge (pHpzc) was determined for the COMAC, AC, and BC samples. Estimating the pHpzc is crucial, as it influences the adsorption behavior of the materials. When the pH of the solution is lower than the pHpzc, the surface of the porous materials becomes a bit more positively charged, and thus, the anion adsorption is favored. Conversely, if the solution pH is higher than the pHpzc, the surface of the samples acquires a bit more negative charge, enhancing the attraction of cations adsorption. The pHpzc of the COMAC, AC, and BC were 6.98, 3.40, and 6.50, respectively.

Given the influence of pH on adsorption mechanisms, it is essential to assess how surface charge variations affect metal ion removal. At low pH, certain functional groups may become protonated which reduces their ability to provide negatively charged active sites, thereby preventing the formation of complexes between Co(II) ions and the other functional groups present on activated carbon or biochar surfaces [120]. The Co(II) removal at strong acidic pH values was found to be small in a range of 5.7-13.2 mg/g (5.48%-12.62%) (**Figure 33**, **Table 22**). However, the adsorbed amount of cobalt ions increases consistently with increasing pH values and this is noticed clearly in the case of a sample of activated carbon derived from *Sargassum* sp. which has a better uptake capacity compared to the biochar sample at different

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pH. It was found that pH=6.8 is the optimum value, in which the Co(II) removal was the highest for sample AC with an adsorbed amount of 26.5 mg/g and removal efficiency of 25.25% which is higher than the adsorbed amount by COMAC and biochar BC with removal efficiency 10.37% and 7.41%, respectively at the same pH.

The observed results align with the pHpzc values of the materials. Since AC has a low pHpzc of 3.40, its surface remains negatively charged at pH 6.8, favoring Co(II) cation adsorption leading to higher adsorption. On the other hand, COMAC and BC, with higher pHpzc values of 6.98 and 6.50, respectively, exhibit less favorable surface charges for Co(II) removal for cation attraction, resulting in lower adsorption capacities.

The adsorption of this metal at pH>6.8 is related to the formation of metal hydroxide species such as soluble $Co(OH)^+$ and/or the formation of insoluble precipitate of $Co(OH)_2$ [120]. In this case, the metal ions are removed from the solution due to the formation of $Co(OH)_2$ and not due to the adsorption of free Co(II) ions. Thus, the pH has to be kept at pH=6.8 to avoid precipitation.

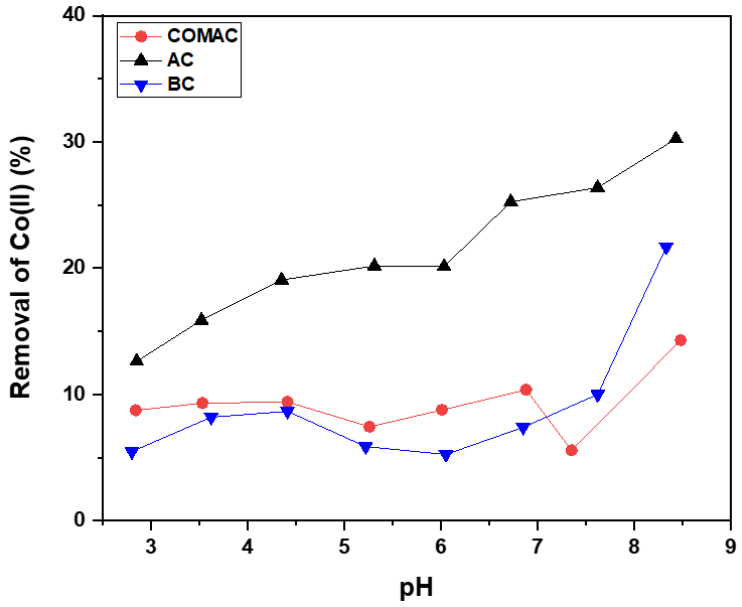

Figure 33. *Effect of the pH of the solution on the adsorption of Co^{2+} by 0.05 g of the four studied samples, COMAC, AC, and BC at 2 mmol/L initial concentration.*

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Table 22. *The adsorbed amount of Co^{2+} ions by the tested samples: COMAC, AC, and BC*

	Adsorbed amount mg/g		
pH	**COMAC**	**AC**	**BC**
2.8	9.1	13.2	5.7
3.6	9.7	16.6	8.6
4.4	9.8	20.0	9.1
5.2	7.8	21.1	6.1
6.0	9.2	21.1	5.5
6.8	10.8	26.5	7.7
7.6	5.8	27.6	10.5
8.4	15.0	31.7	22.7

It can be concluded that activated carbon and biochar samples display varying capacities for adsorbing metal ions. Previous measurements indicate that the surface zeta potential of all samples ranges between -22.80 mV and -13.21 mV, indicating a negative charge on the surface of the COMAC, AC, and BC particles. Consequently, these materials probably adsorbed metal ions by electrostatic interaction between positively charged metal ions and the negatively charged adsorbents. This electrostatic interaction likely contributes to the enhanced adsorption of metal ions as pH increases. Additionally, all samples contain surface functional groups such as -COOH and -OH, which can interact with metal ions to form surface complexes on both activated carbon and biochar. By increasing pH, more of these functional groups will be deprotonated, thereby these negatively charged groups will further enhance the possibility of complex formation with metal ions [58], [120], [133], [134]

Similar results were presented previously in some experiments, activated carbon derived from pyrolyzed potato peels was utilized to remove cobalt ions [120]. Chemical activation was achieved by treating the material with phosphoric acid at temperatures of 400°C, 600°C, and 800°C for 2 hours. The study revealed that as the pH increased from 2 to 5, the percentage of removal also increased across all temperatures. At pH=6, the highest cobalt uptake was recorded, reaching 85.7% by employing the AC prepared at 400°C and 92% by using samples prepared at 600°C, while the adsorption capacities were 373 mg/g and 405 mg/g, respectively [120].

DOI: 10.14750/ME.2025.028

2. Effect of initial cobalt concentration and adsorption isotherm investigation

The removal efficiency percentage of metal ions is directly affected by their initial concentration in the solution [59]. At low concentrations, metals are adsorbed onto specific sites. As the concentration increases, all adsorption sites become occupied, and the adsorbent surface may become covered by the initially adsorbed metal ions [135]. The higher initial metal ion concentrations provide a strong driving force between the liquid and solid phases [116]. The results showed that the adsorption capacity increased with increasing initial concentration for all the adsorbents. This is attributed to the high initial concentration increases the driving force for the mass transfer of metal ions onto the adsorbent's surfaces and more efficient utilization of the active sites on the adsorbent thereby enhancing the efficiency of the adsorption process [59], [136]. Based on the results the optimal metal concentration was ~2 mmol/L.

The study of concentration effects plays a crucial role in improving the accuracy of isotherm results. The adsorption isotherms provide essential data for understanding the adsorption behavior [137]. The two models of Langmuir and Freundlich were fitted to the experimental adsorption isotherms data of Co^{2+} onto the adsorbent surface at pH 6.8 and different initial concentrations and the obtained results are shown in (**Figure 34** and **Table 23**).

The Langmuir isotherm, which assumes monolayer adsorption on a surface, provided insights into the maximum adsorption capacities of tested adsorbents. The maximum monolayer adsorption capacities estimated by the Langmuir isotherm were 468.97, 361.23, and 334.36 mg/g for AC, COMAC, and BC respectively. Among these, the sample AC derived from *Sargassum* sp. exhibited better capacity and greater potential to adsorb cobalt ions at saturation than biochar and commercial AC, where high q_m and a steep initial isotherm slope indicate strong favorability (as the initial concentration increases, the available sites may become saturated, and adsorption reaching a plateau) as shown in (**Figure 34**). Additionally, the Langmuir constant K_L which reflects the affinity between the adsorbent and the cobalt ions was also higher for AC with values of 0.9 (L/mg). These higher values suggest a stronger interaction between cobalt ions and the adsorbent surfaces, indicating that these adsorbents may be more efficient in removing cobalt ions from the solution. The Langmuir isotherm applied to the data with a correlation coefficient (R^2) of ~ 0.9 for all the samples indicating that Co^{2+} adsorption likely occurred on a homogeneous surface through monolayer adsorption, with no interaction between the adsorbed molecules [138], [139].

DOI: 10.14750/ME.2025.028

The Freundlich isotherm was also applied which describes the multilayer adsorption on a heterogeneous surface primarily driven by physisorption. The R^2 values for BC are close to 1 (**Table 23**), indicating favorable adsorption and suggesting that cobalt ions are preferentially adsorbed onto the surfaces of the applied materials. The Freundlich model shows a better fit compared to the Langmuir model, though no plateau is observed, indicating incomplete surface coverage due to insufficient metal ion concentration. All in all, it can be concluded that multilayer adsorption does not occur, instead, aggregation of BC may occur which would seemingly tilt the preference towards the Freundlich model. However, high K_F and n values were observed for AC, indicating a high binding capacity and strong affinity between these adsorbents and Co^{2+} ions [140].

All in all, the significantly high surface areas of AC (1695 m^2/g) contribute directly to their high adsorption capacities, as higher surface areas provide more active sites for adsorbate interactions. This increased availability of adsorption sites allows these materials to capture and retain more cobalt ions than samples with lower surface areas COMAC (1120 m^2/g) and BC (854 m^2/g).

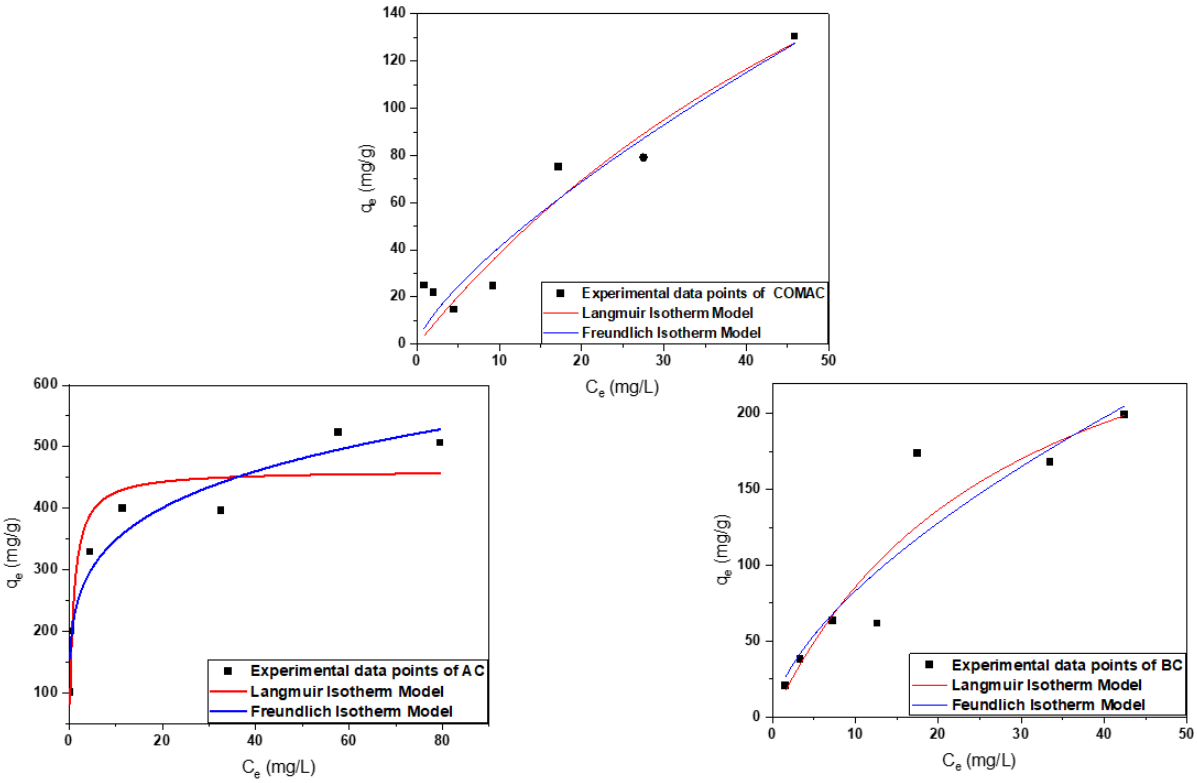

Figure 34. *Adsorption isotherms fitted on the Co^{2+} adsorption data measured by using different adsorbents*

DOI: 10.14750/ME.2025.028

Table 23. *Adsorption isotherm model and the corresponding parameters for Co^{2+} removal by using the studied adsorbents*

Adsorbent	Model						
	Langmuir model			Freundlich model			
	q_m (mg/g)	K_L (L/mg)	R^2	K_F (mg/g)	n	1/n	R^2
COMAC	361.23±262.26	0.01±0.01	0.90	7.45±3.61	1.35±0.25	0.74	0.91
AC	468.97±30.58	0.9±0.37	0.91	192.53±29.1	4.24±0.76	0.23	0.90
BC	334.36±111.11	0.03±0.02	0.90	21.30±0.17	1.66±0.006	0.60	0.99

3. Effect of adsorbent dosage

The influence of adsorbent dosage on the adsorption of metal ions was examined by varying dosages from 0.05 to 0.8 g while initial cobalt concentration was held constant at 2 mmol/L at room temperature and pH kept at ~ 6.8 (**Figure 35**). It was observed that the removal efficiency (percentage of metal ions removed from the solution) increased with an increase in adsorbent dosage. This is attributed to the greater overall surface area provided by the higher amount of adsorbent, which increases the number of available binding sites for adsorption [114], [140], [141].

The results showed that the AC sample was the most effective adsorbent for removing Co^{2+} ions compared to COMAC and BC at all dosages. This superior performance can be attributed to their higher surface area, which provides more active sites for adsorption. The removal efficiency of AC exhibited an increase in removal efficiency, from 28% to 94.94% as the dosage increased.

Several studies observed this trend previously, where the adsorbent from *Sargassum* sp. was used to adsorb four metal ions, Pb, Cu, Zn, and Mg from a synthetic multi-solute system and real stormwater runoff and it was observed that the metals removal efficiency increased with increasing adsorbent amount whereas metals uptake capacity decreased at higher adsorbent dosages [140].

In contrast, biochar showed significantly lower efficiency for Co^{2+} ion removal than AC (**Figure 35**). For COMAC, even at higher dosages, the removal efficiency remained limited, reaching only 28%.

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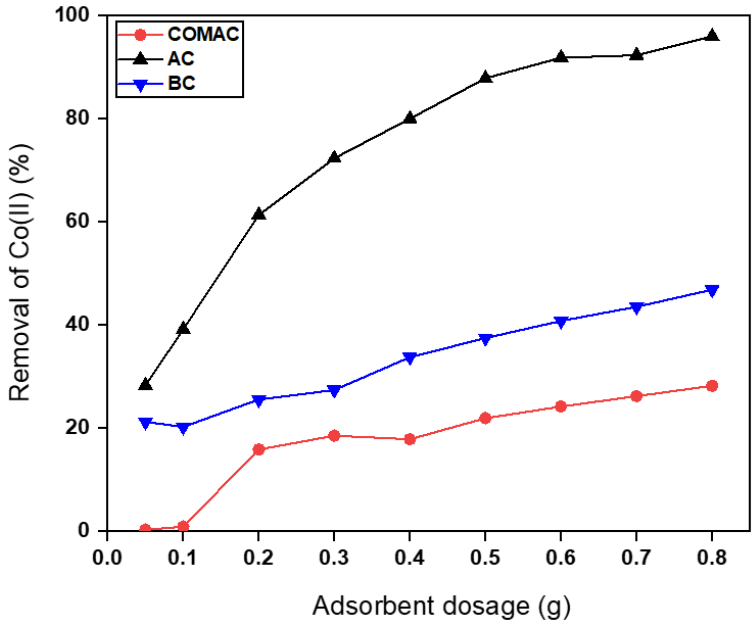

Figure 35. *Effect of adsorbent dosage on adsorption of Co^{2+} ions (pH= 6.8, initial concentration = 2 mmol/L, agitation rate = 210 rpm)*

The optimal dosage for COMAC would be approximately 0.2 g, as increasing the dosage beyond this point does not yield a significant increase in removal efficiency. For AC and BC samples, the optimal adsorbent dose would be chosen based on maximum removal efficiency. In such cases, adding more adsorbent can further enhance the efficiency of the removal of metal ions as can be seen in the case of AC and BC samples where the removal efficiency increases steadily with dosage. The differences in adsorption capacities among the various adsorbent dosages could be attributed to the type of surface functional groups involved in the adsorption of metal ions from the solution [137].

Overall, AC demonstrated its suitability for high-efficiency cobalt removal, whereas BC, despite its lower performance, could still be considered for applications.

3.3.2 Conclusion

AC and BC samples, prepared from *Sargassum* sp. waste, were investigated as materials potentially applicable in heavy metal removal and compared with commercial activated carbon sample to assess their applicability. The tests were carried out by varying different parameters, including pH, initial concentration, and amount of the adsorbents. The adsorption ability of Co^{2+} ions by the AC/BC samples was tested at an initial concentration of 2 mmol/L and 0.05 g

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at various pH values. As the pH increases, the deprotonation of the metal binding sites increases the negative charge on the surfaces of activated carbon and biochar, which enhances adsorption. However, precipitation could occur above a certain pH making the optimal pH ~ 6.8. The pH at the point of zero charge pHpzc was determined as well and it was found AC has a low pHpzc of 3.40, so its surface remains negatively charged at pH 6.8, which favors the adsorption of Co(II) cations and leads to higher adsorption than other samples.

The results also showed that the adsorption capacity increased with increasing initial concentration for all the adsorbents reaching the optimum at ~2 mmol/L. The Langmuir and Freundlich isotherm models were applied to Co^{2+} adsorption data as well and both fit the experimental data. Langmuir's maximum adsorption capacities were 468.97, 361.23, and 334.36 mg/g for AC, COMAC, and BC, respectively where the AC sample showed superior adsorption capacities than other samples indicating that it may be more efficient in removing cobalt ions from the solution with adsorption occurring on a homogeneous surface through monolayer adsorption. The K_f adsorption capacity in Freundlich isotherm was higher in AC with 192.53 mg/g compared to other samples.

The high surface areas of AC (1695 m^2/g) significantly enhance the adsorption efficiency by providing abundant active sites. Applying different amounts of the adsorbent with constant pH = 6.8 and an initial metal concentration of 2 mmol/L for all the samples was also carried out. The results showed that AC was the most effective adsorbent for removing Co^{2+} ions compared to COMAC and BC at all dosages with a removal efficiency of up to 94.4%.

DOI: 10.14750/ME.2025.028

4. Summary

Polyurethanes (PUs) are a special class of polymeric materials that differ significantly from most other types of plastics in many aspects and are widely used in convenience products. However, they often end up in landfills, releasing toxic compounds when degraded by environmental or human factors. Therefore, the ecotoxicological assessment of PU foams is essential. In this doctoral dissertation, toxicity test protocols were developed by using three different organisms, *Sinapis alba* seeds, *Escherichia coli*, and *Micrococcus luteus* bacterial model organisms. Five PU foam samples with different NCO indices (NCO-0.8, 0.9, 1.0, 1.1, and 1.2) were prepared and compared to the Control sample, while intentionally prepared Toxic foam was also used to verify the accuracy of the developed testing procedures. Regarding the *Sinapis alba* test, the highest NCO index (NCO-1.2) significantly reduced root length by 9.8% compared to the Control sample. In the bacterial test, it was observed that the samples containing NCO-1.1 and NCO-1.2 had lower colony numbers (5.0×10^8 and 4.9×10^8 CFU/mL, respectively) in comparison to the Control plate (9.6×10^8 CFU/mL). Additionally, testing PUF toxicity with other bacteria, such as *M. luteus*, further validated its effectiveness in detecting PUF toxicity. All in all, two toxicity tests were successfully adapted for PU foams, and both are applicable in the ecotoxicological assessment of the samples. The toxicity tests were also applied to carbonaceous materials derived from natural sources, including activated carbon (AC) and biochar (BC) from *Sargassum* sp., and compared with commercial AC samples. The results confirmed the protocols' reliability in detecting toxicity in various materials. Bio-based AC and BC from *Sargassum* sp., and commercial activated carbon COMAC being non-toxic, show great potential for soil remediation and water purification applications. Therefore these non-toxic bio-based carbonaceous materials were used as adsorbents to remove cobalt ions from water. Adsorption tests were conducted to assess the capacity of activated carbon and biochar to remove cobalt ions from water. The results showed that the adsorption capacity was highly dependent on pH where the optimal pH was ~ 6.8 for all the samples, while above that precipitation could occur and hydroxy precipitates can form during this process. Furthermore, the results showed that the AC derived from *Sargassum* sp. has the highest adsorption capacity due to the high surface area which offers more active sites for interactions with adsorbates, and increases the availability of adsorption sites. This enables materials with larger surface areas to capture and retain more cobalt ions compared to those with smaller surface areas.

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It can be concluded that this doctoral dissertation, provides a comprehensive understanding of the toxicity of polyurethane foam and examines the applications of bio-based materials in water purification.

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5. New scientific results

Based on the experimental research conducted during my Ph.D. studies on the ecotoxicity of polyurethane foams and the role of bio-based materials in applications like the removal of heavy metals from water, the following results have been identified as new scientific findings:

1st Thesis

Two toxicity test protocols were developed in which two different types of model organisms were successfully applied, *Sinapis alba* (white mustard) seeds and non-pathogenic bacteria (Gram-negative *Escherichia coli,* or Gram-positive *Micrococcus luteus*). The protocols were successfully adapted and validated for the examination of polyurethane foam samples and it was found both are applicable in the ecotoxicological assessment of polyurethane foams.

2nd Thesis

Applicability of the developed toxicity tests to study carbonaceous materials derived from natural sources, specifically activated carbon (AC) and biochar (BC) synthesized from *Sargassum* sp., and COMAC commercial activated carbon was evaluated. It was found that the developed toxicity test protocols were not only effective for assessing polyurethane foam samples but could also be employed to detect the toxicity of other materials, expanding the applications of the methods.

3rd Thesis

The toxicity of bio-based AC and BC which are derived from *Sargassum* sp. was determined and it was found that both are non-toxic materials and thus, they offer promising potential for use in many industrial applications as an adsorbent to remove heavy metals from water.

4th Thesis

The applicability of carbonaceous materials (AC and BC) derived from *Sargassum* sp. as water treatment agents for heavy metal adsorption has been investigated and compared to COMAC, specifically, for the removal of cobalt ions from water. The results demonstrated that all carbonaceous materials could adsorb cobalt ions in varying capacities, with AC from *Sargassum* sp. showing the highest adsorption capacity making it a promising material for environmental remediation.

6. Scientific publications

Publications related to the subject of the dissertation

1. **Julie Mallouhi**, Enikő Hornyák-Mester, Miklós Varga, Béla Viskolcz, Béla Fiser, Emma Szőri-Dorogházi, ″Development of toxicity tests for Polyurethane foams″, *Heliyon,* 2024, 10, doi:10.1016/j.heliyon.2024.e38440. (**Q1**)
2. **Julie Mallouhi**, Miklós Varga, Emőke Sikora, Kitty Gráczer, Olivér Bánhidi, Sarra Gaspard, Francesca Goudou, Béla Viskolcz, Emma Szőri-Dorogházi, and Béla Fiser, ″Activated Carbon and Biochar Derived from *Sargassum* sp. Applied in Polyurethane-Based Materials Development″, *Polymers*, 2024, 16 (2914), doi: 10.3390/polym16202914. (**Q1**)

Further Publications

1. **Julie Mallouhi**, Hadeer Q. Waleed, Emma Szőri-Dorogházi, Michael Owen, Béla Fiser, ″Molecular dynamics study of the closed conformations of Candida rugosa lipase 2 (CRL),″ *Symposium on Polyurethane Innovation - SPI 2022, Conference Publications*, 2022, 203, 82-90.
2. **Julie Mallouhi**, Béla Viskolcz, Emma Szőri-Dorogházi, Béla Fiser, ″Bond Dissociation Enthalpy as a Tool to Study Urethane Degradation″, *Materials sciences and Engineering,* 2023, 47, 100-108.
3. **Julie Mallouhi**, Emma Szőri-Dorogházi, Béla Fiser, ″Toxicity Tests Developed for Polyurethanes″, *Symposium on Polyurethane Innovation - SPI 2022, Conference Publications,* 203, 44-52.
4. **Julie Mallouhi**, Emma Szőri-Dorogházi, Béla Fiser, ″Biodegradation of Polyurethanes-A mini-review″, *Doktorandusz Almanach,* 2022, 1, 141-147.
5. **Julie Mallouhi**, Béla Fiser, Emma Szőri-Dorogházi, ″Developing Protocols to Evaluate the Toxicity Test of Polyurethane-based Materials″, *Doktorandusz Almanach,* 2023, 1, 111-116.
6. **Julie Mallouhi**, Béla Fiser, Emma Szőri-Dorogházi, ″Activated Carbon: A Promising Adsorbent for Heavy Metal Removal from Water″, *Symposium on Polyurethane Innovation - SPI 2023, Conference Publications,* 230, 57-62.
7. **Julie Mallouhi**, Béla Fiser, Emma Szőri-Dorogházi. ″ Introduction of the Applications of Activated Carbon″, *Doktorandusz Almanach,* 2024, 1, 216-220.

DOI: 10.14750/ME.2025.028

8. **Julie Mallouhi**, Béla Fiser, Emma Szőri-Dorogházi. ″ Ecotoxicity assessment tests-mini review″, *Doktorandusz Almanach,* 2024, 1, 177-181
9. **Julie Mallouhi**, Emőke Sikora, Kitty Gráczer, Olivér Bánhidi, Sarra Gaspard, Francesca Goudou, Béla Viskolcz, Emma Szőri-Dorogházi, and Béla Fiser, ″Adsorption of Nickel(II) ions by activated carbon and biochar″, *Symposium on Polymers Innovation,* 2024, 45-51.

Presentations and posters

1. 25th Spring Wind Conference
   Poster presentation/Pécs, Hungary, 2022.06-08.05.
   Computational study of urethane degradation.
2. 10th Visegrad Symposium on Biomolecular Interactions
   Poster presentation/Nove Hrady, Czech Republic, 2022.06.16.
   Polyurethane degradation: A computational study
3. Symposium on Polyurethane Innovation SPI 2022
   Oral presentation/Miskolc, Hungary, 2022.08.26.
   Toxicity Tests Developed for Polyurethane
4. Hungarian Science Day
   Oral presentation/Miskolc, Hungary, 2022.11.09.
   Development of Toxicity Testing Protocols for Polyurethane-based Materials
5. 26th Spring Wind Conference
   Oral presentation/Miskolc, Hungary, 2023.05-07.05.
   Toxicity assessment of Polyurethane Foams
6. 11th Visegrad Symposium on Biomolecular Interactions
   Poster presentation/Szidonia Castle, Hungary, 2023.20-23.06.
   Ecotoxicological Assessment of Polyurethane Foams: Impacts on Mustard Seeds and Bacterial Model Organisms
7. Symposium on Polyurethane Innovation SPI 2023
   Oral presentation/Miskolc, Hungary, 2023.08.24.
   Toxicity tests of polyurethane foams using different organisms
8. 27th Spring Wind Conference
   Poster presentation/Budapest, Hungary, 2024.03-04.05
   Characterization and Using Activated Carbon for Diverse Applications

DOI: 10.14750/ME.2025.028

9. 11th Doctoral Symposium on Chemistry
   Poster presentation/Lodz, Poland, 2024.16-17.05.
   Characterization and application of activated carbon
10. 12th Visegrad Symposium on Biomolecular Interactions
    Poster presentation/Piešany, Slovakia, 2024.23-25.05.
    Evaluation of *Sargassum*-Derived Activated Carbon and Biochar: Ecotoxicity, and Heavy Metal Adsorption Performance
11. Polish-Hungarian Symposium on Chemistry and Materials Science
    Oral presentation/Miskolc, Hungary, 2024.01.10
    Removal of cobalt ions from the aqueous solution by activated carbon and biochar materials derived from *Sargassum sp.*

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