

Impregnation of activated carbon with ionic liquids for simultaneous removal of micropollutants and antibiotic-resistant bacteria from wastewater

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ARTICLE INFO

Article history:

Received 4 September 2025

Received in revised form

26 November 2025

Accepted 15 December 2025

Available online 17 December 2025

Keywords:

Antibiotic resistance genes

Wastewater treatment

Ionic liquids

Impregnated activated carbon

Bacterial elimination

ABSTRACT

Antibiotic resistance genes (ARGs), often resulting from the misuse of antibiotics, have emerged as significant environmental pollutants. Their presence in wastewater poses challenges for conventional treatment methods, which fail to eliminate ARGs completely. This study investigates the use of ionic liquids (ILs) impregnated onto granular activated carbon (GAC) to enhance the removal of ARGs. Two ILs, TEDA ((N,N,N-triethyl-1-dodecylammonium bis(trifluoromethyl-sulfonyl)imide) and TEGO Dispers 662 C (commercial IL; an imidazolium-based surfactant), were used for impregnation and tested against bacteria *Bacillus subtilis* and *Aeromonas* sp., as well as bacteria present in real water samples from wastewater treatment plants (WWTP) and greywater. The IL-impregnated GAC demonstrated strong antimicrobial activity, particularly against ARG strains A3 and A4, with >99 % bacterial elimination. Notably, the adsorption capacity of GAC for most pharmaceuticals was not significantly reduced by impregnation with TEDA. Analytical and ecotoxicological tests (using *Vibrio fischeri*, *Sinapis alba*, and *Eisenia andrei*) confirmed that the ILs remained strongly bound to the GAC surface, reducing their environmental risk. The findings highlight the potential of IL-impregnated activated carbon as a selective antimicrobial agent for wastewater treatment, especially in addressing the spread of antibiotic resistance genes.

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1. Introduction

Despite the benefits of antibiotics, the widespread use of antibiotics has led to the emergence of resistant bacteria [1,2] and antibiotic resistance genes (ARGs) as parts of the bacteria that may have mutated due to the improper use of antibiotics. As a result, ARGs have become a new type of pollutant with a high risk to the environment and humanity. The sources of antibiotics in the environment can be divided into three branches – human

medicine, veterinary medicine, and agriculture [3–6]. From all these sources, antibiotic resistance genes enter the wastewater and subsequently wastewater treatment plants (WWTP), where they are transferred further into the environment as part of the effluent or in the sewage sludge. Wastewater treatment plants should therefore pay more attention to the removal of these bacteria [7,8].

Conventional wastewater treatment removes most bacteria, but a considerable portion of antibiotic resistance genes (ARGs) remains in the effluent [1,2]. Advanced disinfection technologies are therefore applied, with UV irradiation followed by chlorination being the most effective; the efficiency of chlorination alone can be reduced by ammoniacal nitrogen. Ozonation, commonly used for hospital wastewater, is generally less effective. Further reduction of microbes and ARGs can be achieved by combining ozonation with filtration (activated carbon or slow sand), as demonstrated by Lüddecke et al. (2015) [9]. Sludge stabilization

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Peer review under the responsibility of KeAi Communications Co., Ltd.

processes such as anaerobic digestion and (vermi)composting can also reduce microbial loads [10]. Ionic liquids represent a promising emerging option due to their strong antimicrobial activity and their ability to remove selected ARGs [1,7,11], with potential applications in eliminating antibiotic-resistant bacteria (ARB), disinfectant detergents, antiseptics, or dressing additives [1,12,13].

Ionic liquids (ILs) are liquid salts with melting points below water and negligible vapor pressure, making them non-volatile. Their use in green chemistry has expanded due to recyclability, non-flammability, high polarity, electrical conductivity and thermal stability, as well as their ability to dissolve a wide range of organic, inorganic and biological materials. Their properties can be tailored by combining different cations and anions [14–16]. ILs have been applied in areas such as nanocomposites [14], catalysis [15–17], biomolecules [18] and gas capture [19]. Many ionic liquids also exhibit antimicrobial activity [7,20,21] Fojtášková et al. (2020) [22] synthesized several ionic liquids and their precursors and evaluated their properties. Michalski, Sommer et al. (2023) [23] investigated antimicrobial and antiviral effects of morpholinium-based herbicidal ILs. Yagci et al. (2011) [24] prepared antimicrobial polyurethane films modified with ionic liquids, and Ślubik et al. (2023) [25] synthesized an antimicrobial IL based on a guanidine cation with thiocyanate or benzoate anions.

The ecotoxicity of ionic liquids varies widely, from low to very high toxicity [26]. Because of the vast number of possible cation–anion combinations, conventional ecotoxicity testing is difficult and costly [27]. Computational toxicology based on functional groups is therefore often used to relate chemical structure to biological effects and predict IL toxicity across species [28,29]. One approach to reducing their environmental impact while retaining antimicrobial activity is immobilizing ILs onto suitable substrates.

Although ionic liquid-impregnated materials have been widely investigated for gas capture and pharmaceutical adsorption, their use as selective antimicrobial agents targeting antibiotic resistance genes (ARGs) in wastewater remains largely unexplored. ILs have been immobilized on various substrates, such as chitosan [30], date pits [31] and mesoporous silica [32], and activated carbon is also frequently functionalized. Previous studies focused mainly on gas adsorption, including SO₂ [33], CO₂ [34,35], H₂S [36], H₂ [37,38], or on pharmaceutical and phenol removal [39,40].

However, no systematic study has evaluated the ability of IL-impregnated activated carbon to selectively remove ARGs from wastewater. This work therefore provides the first combined assessment of IL-modified activated carbon, its antimicrobial activity against ARG-bearing bacteria, and its potential ecotoxicity—introducing an innovative application that extends beyond previous gas- or pharmaceutical-oriented research.

The aim of this work was to impregnate granular activated carbon with two types of ionic liquids – the commercially available TEGO and the laboratory-synthesized TEDA (N,N,N-triethyl-1-dodecylammonium bis(trifluoromethyl-sulfonyl)imide). The next steps involved testing the materials' ability to suppress ARG-bearing wastewater bacteria, confirming that their pharmaceutical adsorption capacity was preserved, and examining any associated ecotoxic effects. Since the application of ionic liquid-impregnated activated carbon for ARGs has not been systematically studied, this research addresses a relevant gap in current knowledge.

2. Material and methods

2.1. Ionic liquids

Two ionic liquids (ILs) were used for the impregnation of

granular activated carbon (GAC): TEGO® Dispers 662C (Evonik, Germany), based on an imidazolium cation and methylsulfate anion, and TEDA (N,N,N-triethyl-1-dodecylammonium bis(trifluoromethylsulfonyl)imide). TEGO was selected as a representative of cost-effective ILs, while TEDA represents quaternary ammonium ILs (QAILs) known for their biocidal properties. TEDA was synthesized in two steps: (1) quaternization of triethylamine with 1-bromododecane, and (2) bromide anion exchange with the lithium salt of bis(trifluoromethylsulfonyl)imide, following the procedure described in Refs. [22,41].

2.2. Impregnation of activated carbon (AC)

Commercial GAC Hydrarffin 30 N (Donau Carbon, Germany; 8 × 30 mesh, 0.60–2.36 mm), produced from bituminous coal, was used. The carbon was repeatedly washed with hot and cold water and dried at 50 °C for 24 h to obtain pristine GAC.

For impregnation, IL was diluted with propan-2-ol (20 mL/g IL), and GAC was added (5 g GAC/g IL). The mixture was stirred for 24 h at 250 rpm, filtered, and dried at 50 °C to obtain impregnated activated carbon (iGAC).

The extent of impregnation was calculated as a ratio of the weight increase of iGAC (given as the difference between the weights of iGAC (m_{iGAC}) and GAC (m_{GAC})) and the initial weight of ionic liquid in mixture (m_{IL}):

$$[1]w_{IL} = 100 \cdot \frac{m_{iGAC} - m_{GAC}}{m_{IL}}$$

Dried iGAC was washed 10 times with an excess of water (250 mL) before the application to eliminate the effect of potential washout of the IL from the AC surface.

A leachate of dried iGAC (before washing) was carried out in order to study the leachability of impregnated ILs to water. The weight of 5 g of iGAC was mixed with 50 mL of water and the concentration of leached IL was determined with the aid of UV–VIS spectroscopy. To assess the effectiveness of the impregnation process, the materials were comprehensively characterized using FTIR, SEM/EDS, BET, and UV–VIS analyses. The procedures and results are provided in the supplementary file (chapters S1.1–S1.3; Figs. S1–S4).

2.3. Isolation of resistant bacteria from wastewater and detection of ARGs

Resistant bacterial isolates from wastewater were used for antimicrobial activity testing as representatives of environmental bacteria. These isolates were obtained in a previous study [42], which provided a detailed description of their cultivation, identification, and the detection of antibiotic resistance genes.

Wastewater sampling was conducted at a WWTP (ÚČOV Ostrava, Czech Republic) in November 2020. The samples were plated on Tryptone Soya Agar (TSA, Sigma-Aldrich) supplemented with ampicillin (Sigma-Aldrich) at a concentration of 500 mg L^{−1} and incubated at 30 °C for 24 h. Genomic DNA was extracted from the resistant bacterial isolates, and genes conferring resistance to beta-lactams (*bla*TEM, *mec*A), carbapenems (*bla*NDM-1, *bla*KPC, *bla*OXA-48), tetracycline (*tet*W), and vancomycin (*van*A) were detected using PCR. In this study, ARGs detection was further expanded to include sulfonamide resistance genes (*sul*1), which were not analyzed in the previous publication. All primers and PCR conditions are listed in [Supplementary Table S2](#).

Resistant bacterial isolates confirmed to exhibit multidrug resistance were identified via Sanger sequencing of the 16S rRNA gene (SeqMe s. r.o., Dobříš, Czech Republic). The obtained

sequences were deposited in the NCBI GenBank under their respective accession numbers.

In this study, selected resistant bacterial isolates were subsequently used to evaluate the antimicrobial activity of impregnated activated carbon.

2.4. Liquid chromatography-mass spectrometry

A reliable LC-MS/MS method was developed and validated in our laboratory for the quantification of target analytes in model solutions, grey waters and wastewaters. A gradient liquid chromatograph Nexera X2 (Shimadzu, Kyoto, Japan) coupled with a QTRAP 6500+ mass spectrometer (AB Sciex, Framingham, MA, USA) was used for the analysis. A detailed description of the method is provided in the Supplementary file (chapter S1.4 and Table S3).

2.5. Adsorption tests

The batch adsorption tests with model solutions and real water samples were carried out to examine how the adsorption properties of GAC were affected by the ILs' impregnation. Four pharmaceuticals - paracetamol, ampicillin, ibuprofen, and sulfamethoxazole (all Sigma-Aldrich, MA, USA) - were selected to prepare the model test solutions. The weight of 10 mg of adsorbent (GAC or iGAC) was mixed with 25 mL of demineralized water in a 50 mL Erlenmeyer flask. The volume of 1.5 mL of solution was taken out and filtered to an Eppendorf tube. Then, the volume of 1.5 mL of the pharmaceutical solution (concentration 1 mg L⁻¹) was added to the flask. The procedure was repeated every 24 h. The WWTP influent and effluent (ÚCOV Ostrava, Czech Republic) and greywater (from bathrooms and laundry) from Hotel Zámek Valeč (Czech Republic) were used as real water samples. The weight of 150 mg of the adsorbent was mixed with 5 mL of WWTP sample in a 5 mL Eppendorf tube and the mixture was left at rest for 3 h. Then, the mixture was filtered through cellulose filters (regenerated cellulose membrane filter, 0.45 µm (Fisherbrand, NH, USA)). The greywater samples were treated with the adsorbents in the same way as the WWTP samples.

2.6. Antimicrobial activity tests

The antimicrobial activity of impregnated activated carbon (iGAC) was evaluated using four approaches: (i) a model solution of *Bacillus subtilis* in water, (ii) resistant *Aeromonas* sp. strains isolated from wastewater, (iii) wastewater from a municipal wastewater treatment plant (WWTP), and (iv) greywater from a hotel.

Bacteria were cultivated on Paddle tests (VWR, Czech Republic) with tryptone glucose extract agar containing TTC (2,3,5-triphenyltetrazolium chloride) or on 3M Petrifilm Aerobic Count plates (3M, USA). Colony growth was analyzed using ImageJ software.

For all tests, 1 g of iGAC was mixed with 1 mL of bacterial suspension or real water sample and kept in contact for 3 h. Subsequently, 200 µL of the mixture was diluted with 800 µL of distilled water and inoculated on the cultivation medium.

- (i) Model *B. subtilis* solution: Six replicates were tested with *B. subtilis* (Merck, Czech Republic) diluted 1:100. Plates were incubated at 30 °C for 24 h.
- (ii) Isolated *Aeromonas* sp. strains: Four strains (A1–A4) isolated from WWTP were tested in six replicates with bacterial suspensions diluted 1:1000. Incubation was performed at 28 °C for 24 h.

- (iii) WWTP wastewater: Samples of WWTP effluent (Ostrava, Czech Republic) were incubated at 28 °C for 48 h.
- (iv) Greywater: Greywater samples (Hotel Zámek Valeč, Czech Republic) were incubated at 28 °C for 24 h under identical conditions.

2.6.1. Antimicrobial test evaluation procedure

Bacterial colonies cultivated on agar were analyzed by image analysis using the software ImageJ. ImageJ is an open-source software developed by the National Institutes of Health (NIH), widely used in biology, microbiology, and other scientific fields [43]. Its main functions include both automatic and manual colony counting, measuring their diameter, area, shape, and color intensity. Each sample was photographed together with the scale. The core of the analysis was image conversion to binary (thresholding), which facilitated the detection of individual colonies against a contrasting background. The total area of colonies was recalculated to the scale and expressed in mm². In each experiment, a control sample (bacteria in water without activated carbon) was also placed on the tester, thus eliminating the influence of conditions, and the colony areas were expressed relative to this control in percentage. The evaluation of the antimicrobial tests using ImageJ software is presented in Supplementary in Fig. S5.

2.7. Ecotoxicity tests

As the iGAC is intended for wastewater treatment, aquatic tests performed with an aqueous leachate prepared from ten times-washed iGAC according to the European standard EN 12457-4:2002 (liquid to solid ratio 10 L kg⁻¹ dry weight) were selected from the battery of ecotests. A leachate from the pristine (non-impregnated) GAC was also tested using the same methods. The indicator organisms employed were *Vibrio fischeri*, *Sinapis alba*, and *Eisenia andrei*, representing bacteria, plant and animal species, respectively.

2.7.1. *Vibrio fischeri* bioluminescence inhibition

The bioluminescence inhibition test was performed following European Standard EN ISO 11348-3:2008. Freeze-dried marine bacteria *Vibrio fischeri* NRRL-B-11177 (Hach Lange GmbH, Germany) were reactivated and utilised for luminescence measurements of leachates, with the pH value adjusted to 7 and the salinity modified by the addition of 20 g L⁻¹ NaCl. Light emission of *V. fischeri* after exposure for 15 and 30 min was measured with LUMISTox 300 luminometer (Hach Lange GmbH, Germany) at 15 °C in duplicate. Due to the low inhibitory effect of the leachates, bioluminescence was determined only for undiluted leachates and leachates that had been diluted to half of their original concentration.

2.7.2. *Sinapis alba* root growth inhibition

The root growth inhibition test was performed using *Sinapis alba* (mustard) seeds. The test was conducted in 100 mm diameter Petri dishes lined with filter paper, 5 mL of aqueous leachate or dilution water (control), with 20 seeds placed in each dish. The test was conducted within a thermostatically controlled Lovibond ET 619-4/140 L cabinet (Tintometer GmbH, Germany), maintained at 20 °C in the absence of light for a period of 72 h. The assessment of root length was performed utilizing the ImageJ software [43]. The average root length (determined in triplicates for each sample) was related to the control, and the percentage of root growth inhibition was calculated.

2.7.3. *Eisenia andrei* acute toxicity

An acute toxicity test was performed with earthworms *Eisenia andrei* in accordance with OECD guideline No. 207 (1984) for testing of chemicals. In the paper contact toxicity test, individual worms were placed in vials lined with a 64 cm² square of filter paper moistened with 1 mL of undiluted aqueous leachate. The vials were then sealed with parafilm and placed in the thermostatically controlled cabinet set at 20 °C without light for 48 h. As the minimum requirement, ten replicates were used for each treatment. After the test, the mortality of the earthworms was assessed based on their response to a gentle mechanical stimulus.

2.8. Data processing and statistical analysis

Data were processed and visualized using OriginPro 2018b software (OriginLab Corporation, USA). Descriptive statistics (mean and standard deviation) were applied for statistical evaluation, and the datasets were compared using a *t*-test or one-way ANOVA at a 95 % significance level. The number of replicates is specified for each figure, and the statistical evaluation results are indicated using letters (a, b, c) above the boxes in boxplots.

3. Results and discussion

3.1. Characterization of impregnated activated carbon

The ratio of IL impregnated on the AC surface from the propan-2-ol solution was determined gravimetrically and related to the weight of GAC after drying at 50 °C. Based on the calculated ratios we can state, that TEDA was impregnated to a greater extent than TEGO (Table 1). Textural properties of washed GAC and iGACs are shown in Table S1. The specific surface as well as the pore volume were reduced more than twice due to the impregnation by both ILs which have indicated the pore plugging during impregnation.

The FTIR spectra of the original GAC and both impregnated GACs are shown in Fig. 1. The broad band at the 3200–3600 cm^{−1} region is assigned to OH-stretching vibrations. Small bands between 2800 and 3000 cm^{−1} indicate the C–H stretching of alkanes, whereas alkenes are represented by small bands at around 1600 cm^{−1}. Other small bands at around 1400 cm^{−1} are assigned to the C–H bending of alkanes.

Broad band is present at 1000–1200 cm^{−1}, representing aromatic CO groups [34,44,45]. Small bands at around 1100–1400 cm^{−1} appeared, corresponding to the S=O stretching of the sulphate or sulfonyl group which are present in TEGO or TEDA (Fig. S1), respectively [44,46]. In the case of GAC impregnated with TEDA (iGAC-TEDA), a band appeared at around 1100 cm^{−1}, indicating the presence of the fluoro group [34,44,45].

Table 2 represents the results of the SEM/EDS analysis of the initial GAC and impregnated GACs. It is apparent that the content of sulphur, which is present in both ionic liquids, has risen on the surfaces of both iGAC-TEGO and iGAC-TEDA. Furthermore, impregnation of GAC with ionic liquids has led to the increase of the content of fluorine which is also present in the TEDA structure. Based on the results, supported by the images shown in Fig. S2, we

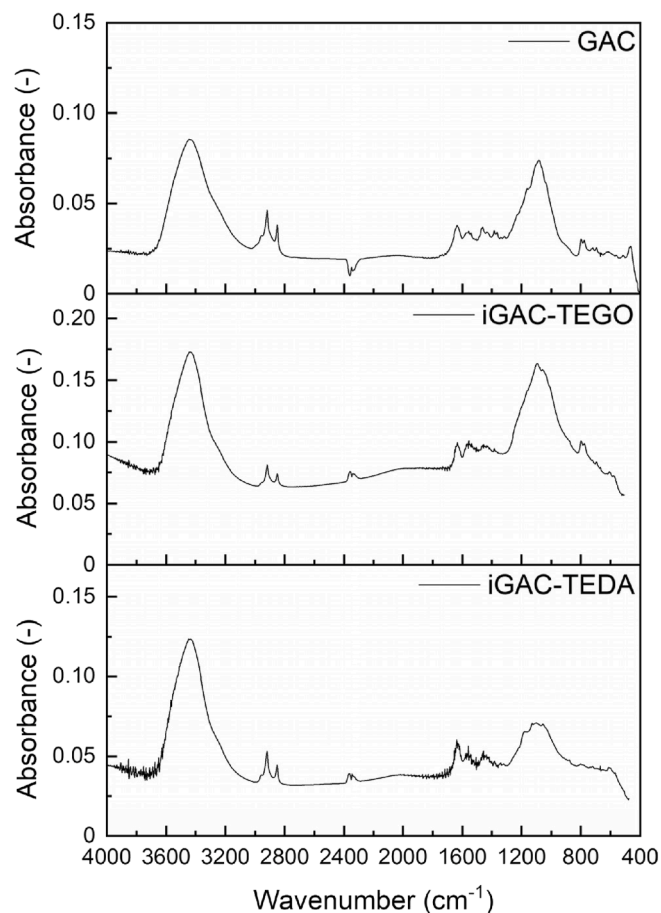


Fig. 1. FTIR spectra of initial GAC and both impregnated ACs.

Table 2

SEM/EDS analysis of the surfaces of GAC and impregnated activated carbons.

Sample	C (at. %)	O (at. %)	S (at. %)	F (at. %)
GAC	96.32	3.36	0.16	0.16
iGAC-TEGO	96.77	2.33	0.53	0.38
iGAC-TEDA	92.84	5.82	0.80	0.56

can conclude that fluorine was somehow present in the TEGO IL as well.

As shown in Fig. S3, it is possible to determinate the TEGO concentration in water with UV–VIS spectroscopy. Based on Table 1 and impregnation conditions we assume that the maximum amount of TEGO on the sample lied in hundreds mg. Results presented in Fig. S4 indicate that the amount of leached TEGO was negligible (less than 0.5 mg). This means that the maximum ratio of leached TEGO lied in hundredths of % related to the amount of TEGO bonded to the GAC surface.

Table 1

Textural parametres of washed GAC and iGACs and extent of impregnation of ionic liquids TEGO and TEDA.

	Specific surface (m ² g ^{−1})	Micropore volume (cm ³ g ^{−1})	Maximum pore volume (cm ³ g ^{−1})	Impregnation extent (wt. %)
GAC	850.06	0.250	0.353	–
iGAC-TEDA	327.20	0.106	0.133	71.51
iGAC-TEGO	380.23	0.068	0.155	85.72

3.2. Adsorption tests

3.2.1. Batch adsorption test with model solution

Impregnation of the GAC surface with TEDA had only a negligible impact on the adsorption of most of the contaminants used in this work (Fig. 2). Only in the case of paracetamol removal, a slight deterioration of adsorption capacity was observed (318 mg L^{-1} compared to 482 mg L^{-1} for pristine GAC). A similar effect has occurred in the case of sulfamethoxazole removal; nonetheless, this deterioration was observed only in the case of higher initial concentrations (5.315 mg L^{-1} compared to 5.816 mg L^{-1} for pristine GAC). Impregnation of GAC with TEGO had a similar effect on the adsorption of paracetamol, ibuprofen, and sulfamethoxazole compared to TEDA. Unlike the case of TEDA, impregnation with TEGO lead to a slight reduction of adsorption properties for ampicillin (830 mg L^{-1} compared to 980 mg L^{-1} for pristine GAC). Polymerized ionic liquid, used by Xu and An [39], led to the enhancement of ibuprofen removal from water, whilst the ionic liquid used in this study did not affect the adsorption capacity of GAC significantly. Enhancement of adsorption properties for pharmaceuticals (including ibuprofen, ampicillin, and sulfamethoxazole) due to the impregnation of the adsorbent surface (*Kigelia pinnata* biomass or montmorillonite, respectively) with 1-methyl-3-decahexyl imidazolium ionic liquid was also observed in two studies by Lawal and Moodley [47,48].

For the comparison of the adsorption efficiencies between individual pharmaceuticals, it is necessary to recalculate both the adsorbed amount (q) and initial concentration (c_0) to moles. The adsorption capacities on pristine GAC selected for the comparison

were $1.46 \mu\text{mol g}^{-1}$ ($220.64 \mu\text{g g}^{-1}$) at an initial concentration $0.61 \mu\text{mol L}^{-1}$ ($91.69 \mu\text{g L}^{-1}$) for paracetamol, $1.18 \mu\text{mol g}^{-1}$ ($243.52 \mu\text{g g}^{-1}$) at $0.56 \mu\text{mol L}^{-1}$ ($114.89 \mu\text{g L}^{-1}$) for ibuprofen, $1.14 \mu\text{mol g}^{-1}$ ($399.62 \mu\text{g g}^{-1}$) at $0.55 \mu\text{mol L}^{-1}$ ($191.27 \mu\text{g L}^{-1}$) for ampicillin, and $0.59 \mu\text{mol g}^{-1}$ ($148.5 \mu\text{g g}^{-1}$) at $0.65 \mu\text{mol L}^{-1}$ ($164.27 \mu\text{g L}^{-1}$) for sulfamethoxazole. Paracetamol, ibuprofen, and ampicillin seem to be adsorbed remarkably better than sulfamethoxazole, with paracetamol being slightly better attracted to the GAC surface than the latter two molecules. The adsorption efficiency can be affected by different factors such as charge of the molecule (related to its dissociation constant), functional groups, molecular size and shape, preferred adsorption mechanism, etc. Regarding the dissociation constants, we can consider that paracetamol is mainly uncharged at neutral pH, ibuprofen is present in an anionic form, and ampicillin is in a zwitterionic form. On the other hand, sulfamethoxazole has the highest negative charge due to the deprotonation of sulphonamide nitrogen and a pyridine nitrogen.

3.2.2. Batch adsorption experiments with WWTP samples

Batch adsorption experiments were used to compare individual materials (GAC and iGAC); therefore, the ratio of the amount of adsorbent and concentration and the volume of the adsorbate solution were chosen so that the concentrations after adsorption were measurable. The treatment of WWTP influent with any of the three materials has led to the decrease of the concentration of more or less all of the 24 micropollutants present in the sample (Fig. 3). The studied substances were of various nature – pharmaceuticals and their metabolites (metformin, paracetamol,

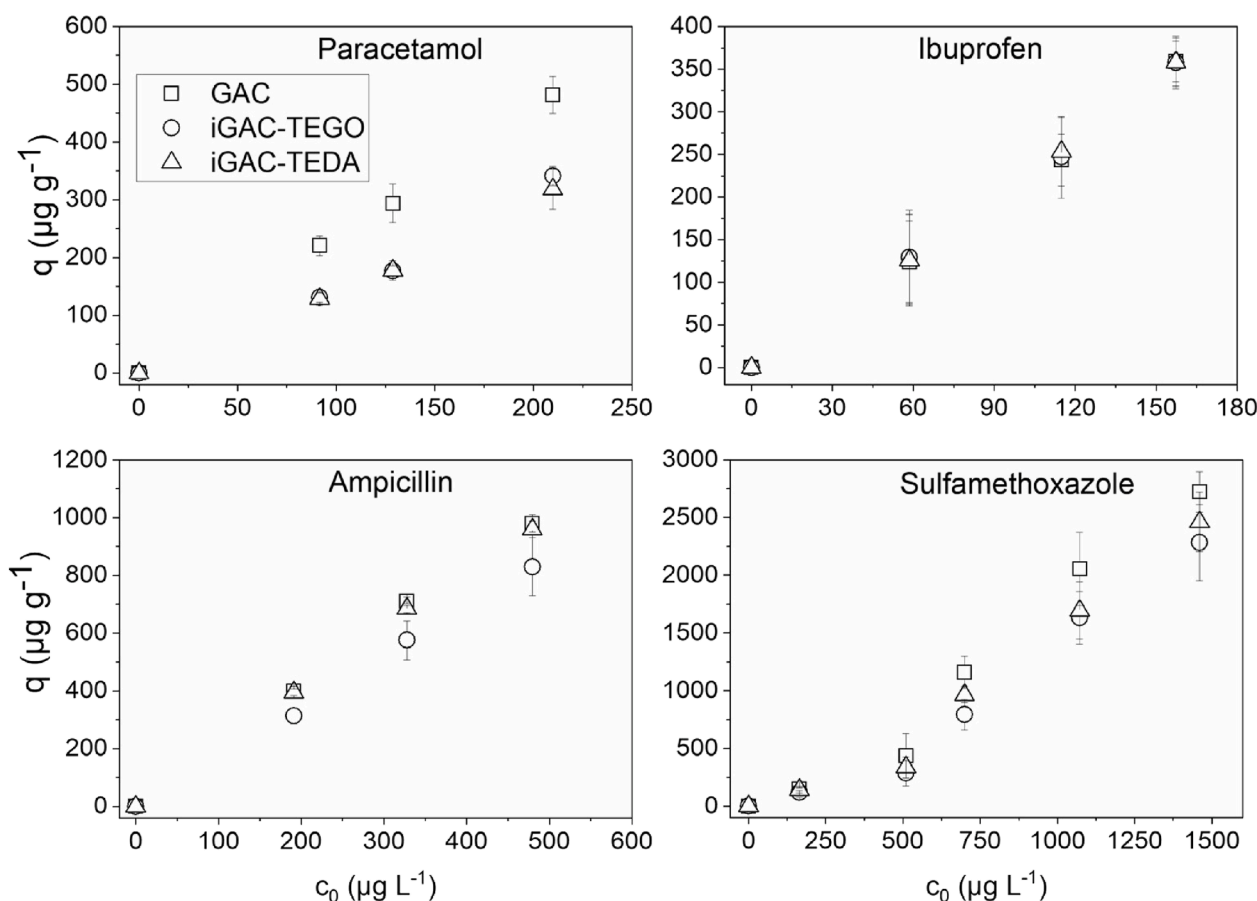


Fig. 2. Adsorption of single contaminants (paracetamol, ibuprofen, ampicillin, and sulfamethoxazole) from model solutions on pristine GAC and ionic liquid impregnated GACs. Adsorption tests were performed in three replicates, and the mean values $\pm$ standard deviations are presented.

paraxanthine, theophylline, lamotrigine, metoprolol, bisoprolol, telmisartan, irbesartan, losartan, candesartan, diclofenac, furosemide, ibuprofen), stimulants and their metabolites (nicotine, cotinine, caffeine, theobromine), industrial chemicals (1H-benzotriazole, 5,4-methylbenzotriazole), pesticides (DEET), and sweeteners (acesulfame K, saccharin, sucralose). The micropollutants with the highest concentrations were metformin, sucralose, caffeine, and 1H-benzotriazole with the initial concentrations over $40 \mu\text{g L}^{-1}$. The removal efficiency surpassed 50 % only for five studied contaminants: lamotrigine (84 %), bisoprolol (61 %), DEET (59 %), 1H-benzotriazole (58 %), and metoprolol (51 %). The adsorption of the studied pollutants was not significantly improved after the modification of the GAC surface with ILs. In the case of the modification with TEGO the adsorption was even decreased for 18 from 24 of the studied pollutants. The highest uptake improvement was observed in the case of ibuprofen (45 % compared to 23 % for pristine GAC). Unlike in the previous case, the surface modification with TEDA ionic liquid led to the slight enhancement of the adsorption of most analyzed contaminants. The most distinct improvements were observed in the case of saccharin (74 % compared to 43 % for pristine GAC), ibuprofen (53 % compared to 23 % for GAC), and caffeine (58 % compared to 37 % for pristine GAC).

In the case of WWTP effluent treatment (Fig. 3), only metformin and sucralose were present in significant amounts in the effluent; both of them were present in concentration levels higher than $50 \mu\text{g L}^{-1}$, while the rest of the contaminants were reduced under $5 \mu\text{g L}^{-1}$ during wastewater treatment. The removal efficiency >50 % was observed only in the case of DEET (60 %) and

losartan (59 %). The most distinct removal improvement was observed in the case of caffeine which went from negligible sorption to 60 % removal for iGAC-TEGO and 87 % for iGAC-TEDA, respectively. Impregnation of the GAC with TEDA led to the enhancement of the adsorption of multiple contaminants, such as lamotrigine (from 36 % to 71 %), furosemide (from 23 % to 54 %), losartan (from 59 % to 88 %), 1H-benzotriazole (from 49 % to 70 %), or metoprolol (from 36 % to 56 %). Ruhl et al., 2014 [49] used nine samples of powder activated carbon for the treatment of WWTP effluent, containing, among other diclofenac, metoprolol, 1H-benzotriazole, methyl benzotriazole, and acesulfame K. The initial concentration of diclofenac ($2.8 \mu\text{g L}^{-1}$) was very close to our work ($2.6 \mu\text{g L}^{-1}$), and the adsorption removals were in the range of 34–64 %. GAC used in our work removed 30 %, iGAC prepared with the use of TEGO and TEDA had removal efficiency of 25 and 32 %, respectively. For the other four contaminants, the initial concentrations were noticeably higher than in our work. The removal efficiencies were 42.9–89 % for 1H-benzotriazole (49–70 % in our work), 76–90 % for methyl benzotriazole (43–60 % in our work), and above 90 % for metoprolol (26–56 % in our work).

The impregnation of the GAC surface with ILs may have affected the possible adsorption mechanisms in several ways; lowered porosity and specific surface most likely led to the inhibition of the pore filling mechanism. On the other hand, both ILs are hydrophobic; therefore, we can consider that impregnation of GAC with ILs enhanced the hydrophobic interactions adsorption mechanism. Furthermore, impregnation with ILs affected the surface chemistry and most likely the surface charge of the GAC, which could have an impact on electrostatic interactions and hydrogen bond adsorption

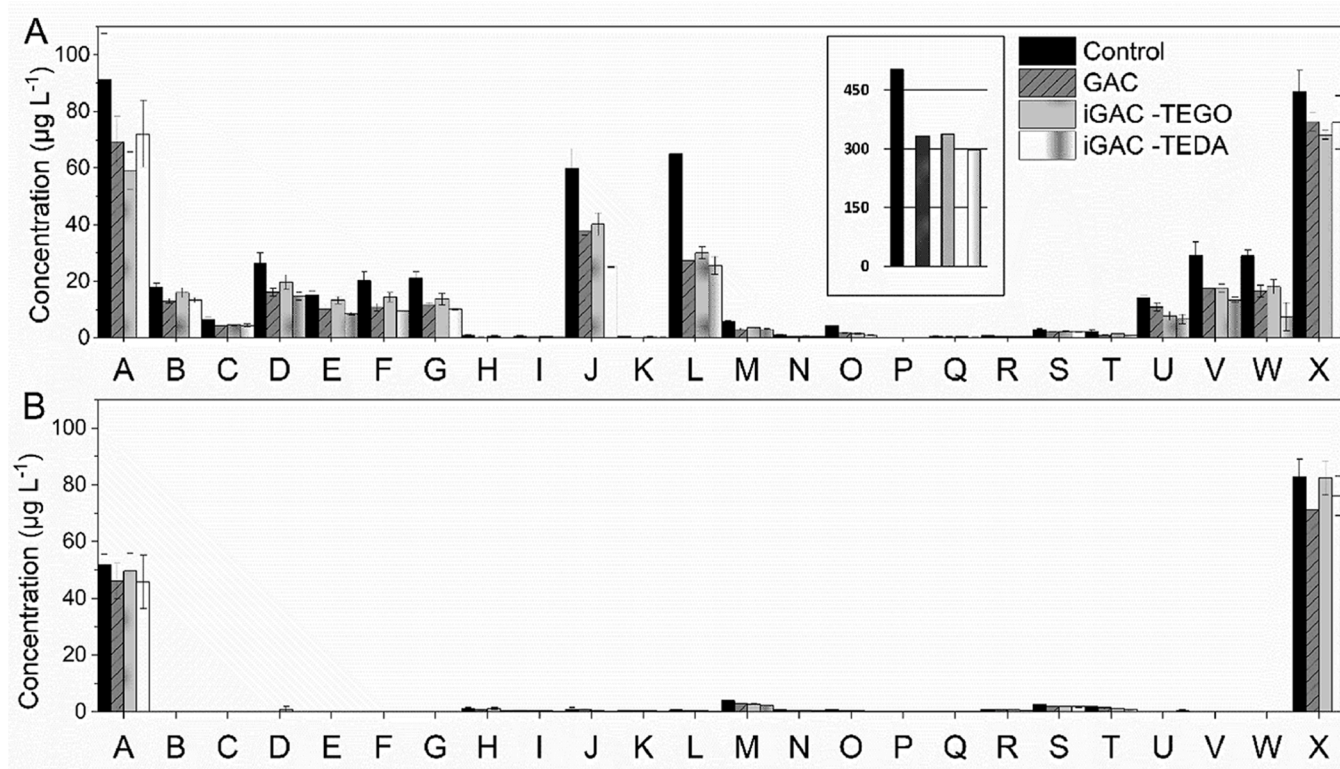


Fig. 3. WWTP influent (A) and effluent (B) treatment via adsorption on pristine GAC and ionic liquid impregnated GACs. The original wastewater sample is designated as the control. Insert in part A shows the total concentration of all micropollutants in the WWTP influent before (control) and after (GAC, iGAC-TEGO, iGAC-TEDA) treatment. Adsorption tests were performed in three replicates, and the mean values $\pm$ standard deviations are presented.

*A-Metformin, B-Nicotine, C-Cotinine, D-Theobromine, E-Paracetamol, F-Paraxanthine, G-Theophylline, H-Lamotrigine, I-Metoprolol, J-Caffeine, K-Bisoprolol, L-1H-Benzotriazole, M-Telmisartan, N-5/4 - Methyl-Benzotriazole, O-DEET, P-Irbesartan, Q-Losartan, R-Candesartan, S-Diclofenac, T-Furosemide, U-Ibuprofen, V-Acesulfame K, W-Saccharin, X-Sucralose.

mechanisms as well. The presence of another species in the real wastewater sample (including inorganic ions or diffused organic matter) also played an important role in affecting the adsorption process. All above mentioned aspects could explain why impregnation with TEDA leads to the general improvement of the removal of the studied contaminants, whilst the impregnation of TEGO had a rather opposite effect. It is important to consider the possible presence of various additives in TEGO IL, being a commercial product.

3.2.3. Batch adsorption experiments with greywater samples

In comparison with the WWTP samples, a reduced number of pollutants was selected to be examined in greywater samples – metformin, nicotine, theobromine, paracetamol, paraxanthine, theophylline, metoprolol, caffeine, 1H-benzotriazole, and DEET (Fig. 4). Metformin and caffeine were the most represented contaminants in greywater samples with the concentration range of 25–35 µg L⁻¹. The rest of the contaminants were present in concentrations lower than 5 µg L⁻¹. The adsorption efficiencies on GAC were generally lower than 50 %. The removal of micropollutants from greywater was not significantly improved by the impregnation of activated carbon. In the case of iGACs, a slight improvement in adsorption capacity was observed for paraxanthine and 1H-benzotriazole. Another slight improvement occurred in the case of the adsorption of theobromine and theophylline on AC impregnated with TEDA. Hernández-Leal et al., 2011 [50] reached 93 % removal of caffeine from greywater in a GAC column operated at low flow. Abd-ur-Rehman et al., 2022 [51] studied the removal of various common contaminants, including paracetamol and DEET, from synthetic greywater with the aid of various adsorbents. The removal efficiency on carbonaceous adsorbents was <20 % for paracetamol and approx. 10–60 % for DEET, the initial concentration of both substances was 5 µg L⁻¹ (See Fig. 4).

3.3. Characterization of resistant bacteria from wastewater

Four resistant bacterial strains (designated A1–A4) isolated from wastewater at a WWTP were selected to study the

antimicrobial activity of impregnated activated carbon (iGAC). All bacterial isolates were identified as *Aeromonas* sp. through 16S rRNA sequencing. The corresponding NCBI accession numbers are listed in Table 3.

Aeromonas sp. is a gram-negative bacterium commonly found in aquatic environments. It plays a critical role in the One Health concept, as it has been increasingly associated with the dissemination of ARGs in recent years, posing a significant public health concern [52–54].

The presence of ARGs *bla*TEM, *bla*NDM-1, *tet*W, and *sul*1 was confirmed in these bacterial isolates (Table 3). The individual bacterial isolates carried the following resistances: A1 - resistant to beta-lactams and tetracyclines, A2 - resistant to beta-lactams, tetracyclines and sulphonamides, A3 - resistant to beta-lactams, carbapenems, tetracyclines and sulphonamides, A4 - resistant to beta-lactams, tetracyclines and sulphonamides.

All isolates were tested positive for the *bla*TEM gene, one of the most widespread ARGs in the environment, often considered as an indicator of anthropogenic antibiotic resistance contamination [55]. The *sul*1 gene, commonly detected in WWTP effluents, is a key component of class 1 integrons and serves as an indicator of bacterial potential for horizontal gene transfer [56]. The *tet*W gene, associated with tetracycline resistance, is widely prevalent due to the extensive use of tetracyclines in veterinary medicine, making it

Table 3
Detection of ARGs in isolated bacterial strains from wastewater. Accession numbers of individual isolates come from the NCBI GeneBank database. The *bla*TEM gene encodes a TEM-type β-lactamase conferring resistance to penicillins and cephalosporins, the *bla*NDM-1 gene encodes the New Delhi metallo-β-lactamase responsible for carbapenem resistance, the *tet*W gene encodes a ribosomal protection protein conferring resistance to tetracyclines, and the *sul*1 gene encodes a resistant variant of dihydropteroate synthase responsible for sulfonamide resistance.

Isolate	<i>bla</i> TEM	<i>bla</i> NDM-1	<i>tet</i> W	<i>sul</i> 1	Accession Number
A1	+		+		OL832099
A2	+		+	+	OL832103
A3	+	+	+	+	OL832106
A4	+		+	+	OL832108

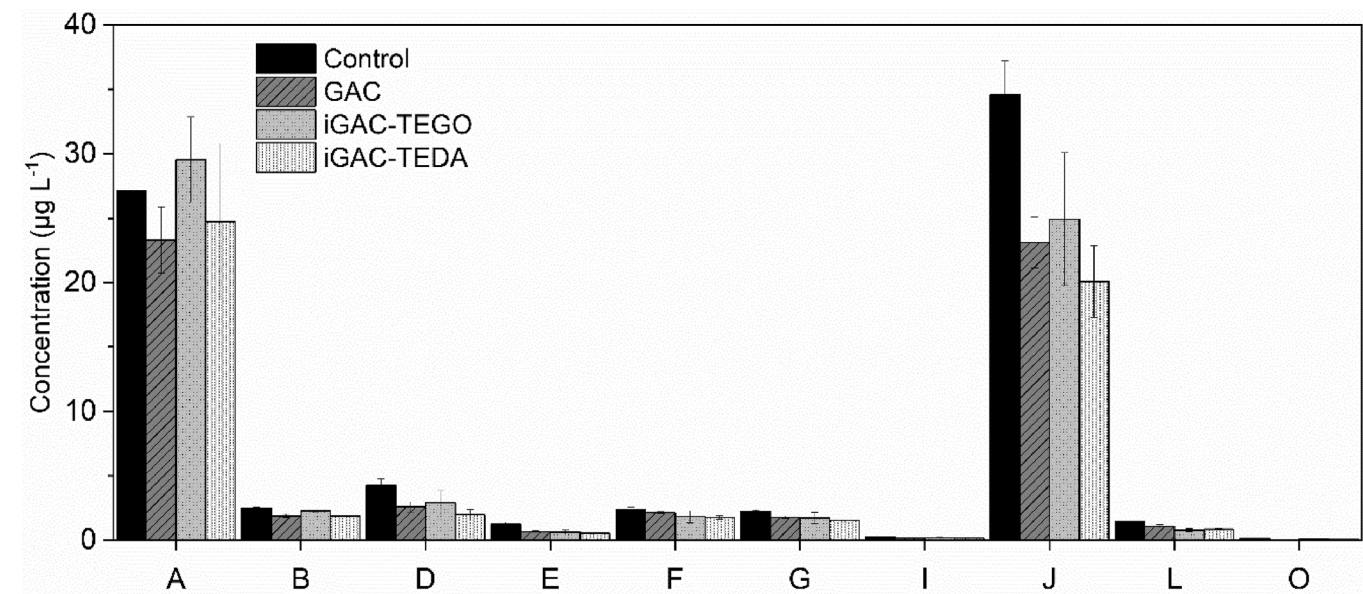


Fig. 4. Greywater with the use of initial GAC and impregnated GACs. The original greywater sample is designated as the control. Adsorption tests were performed in three replicates, and the mean values ± standard deviations are presented.
*A-Metformin, B-Nicotine, D-Theobromine, E-Paracetamol, F-Paraxanthine, G-Theophylline, I-Metoprolol, J- Caffeine, L- 1H-Benzotriazole, O-DEET.

a marker of veterinary antibiotic contamination [57]. Carbapenems are last-resort antibiotics, and the presence and spread of *bla*NDM-1 in the environment represent a serious threat to the effective treatment of bacterial infections [58].

3.4. Antibacterial activity of impregnated activated carbon

3.4.1. *Bacillus subtilis* – model organism

Tests were performed on the model organism *Bacillus subtilis*. As you can see in Fig. 5, the results confirmed the high efficacy of granular activated carbon impregnation with ionic liquids TEDA and TEGO against *Bacillus subtilis*. The average colony size for untreated GAC was 21.7 %, for iGAC-TEDA 13.4 %, and for iGAC-TEGO 21.2 %, with the control set at 100 %. Statistically significant differences were observed between the control and all tested groups (GAC, iGAC-TEDA, iGAC-TEGO), as well as between most tested pairs, except the GAC–iGAC-TEGO pair, where no significant difference was detected. This suggests that the use of ionic liquids, particularly TEDA, enhances the antimicrobial effectiveness of GAC.

3.4.2. *Aeromonas* sp. isolated from WWTP

Antimicrobial activity of iGAC was statistically more successful against *Aeromonas* sp. With antibiotic resistance genes (bacterial strains labelled A1–A4), see Fig. 6. Although impregnation did not lead to a statistically significant improvement in the A1 strain, there was a statistically significant improvement in the A2 strain for iGAC-TEGO, and in the A3 and A4 strains for both impregnated materials, with >99 % elimination of bacteria.

These results were achieved at a significance level $p < 0.05$ (t -test), confirming the high efficacy of granular activated carbon impregnation with ionic liquids TEDA and TEGO against bacteria carrying antibiotic resistance genes.

For strain A1, the average relative colony size for GAC impregnated with TEDA was 15.52 % and 17.35 %, while the control value was 100 % – however, these differences were not statistically significant at the 0.05 significance level (t -test). For strain A2, the relative colony size of iGAC-TEGO decreased to 2.31 %, representing a statistically significant decrease compared to the control (100 %) and pristine GAC (65 %). The most pronounced effect was observed for strains A3 and A4, where the relative colony size after impregnation with both ionic liquids decreased almost to zero. The relative colony size of A3 for iGAC-TEDA was 0.13 % and iGAC-TEGO

0.06 %, which represents statistically significant results, both against the control (100 %) and pure GAC (69 %). For the A4 gene, the relative colony size of iGAC-TEDA was 0 % and iGAC-TEGO 0.87 %, which confirms the high efficiency of this method in eliminating bacteria with antibiotic resistance genes.

3.4.3. Wastewater and greywater samples

For real wastewater samples (Fig. 7), both ILs contributed to the inhibition of bacterial growth compared to the control and GAC.

In the real water sample, the relative colony area for iGAC-TEDA decreased to 17.82 % and for iGAC-TEGO to 17.98 % compared to the control (100 %). For iGAC-TEDA, there was a statistically significant decrease also compared to GAC (49 %). For the grey water sample, a statistically significant inhibition at the 0.05 significance level was recorded for both impregnated materials iGAC-TEDA (44 %) and iGAC-TEGO (57 %) compared to the control (100 %) and also for iGAC-TEDA compared to GAC (75 %).

Our results demonstrate the potential of granular activated carbon impregnated with ionic liquids to inhibit bacterial growth in various waters, including ARG strains isolated from WWTP. In the model organism *Bacillus subtilis*, no statistically significant improvement in antimicrobial activity was observed compared to non-impregnated GAC. It has been published several times, that ILs can exhibit antimicrobial activity against *B. subtilis*, with efficacy influenced by factors such as alkyl chain length, cation type, and anion composition [59–61]. However, direct studies on the antimicrobial effects of immobilized ILs on *B. subtilis* are limited.

In contrast, a notable antimicrobial effect was observed in *Aeromonas* strains isolated from WWTP. In some cases, GAC impregnation with ILs led to more than 99 % reduction in bacterial viability. It is therefore obvious that ionic liquids can have different effects on different microorganisms, as has been previously documented in the literature [62,63].

Similar effects were observed in real wastewater and greywater samples. It was shown that the significant inhibition of bacterial growth of GAC impregnated with TEDA and TEGO occurred not only under laboratory conditions but also in complex matrices. While ILs have strong potential as antimicrobial agents, as shown in sludge-based tests [64–66], their use for direct bacterial suppression in actual wastewater treatment processes is rare [67].

Compared to other advanced oxidation or membrane-based methods, adsorption offers advantages such as lower energy requirements and potential reusability of the material [68]. On the other hand, although ILs offer promising results, there are challenges related to their toxicity, biodegradability, and economic feasibility that need to be addressed for their broader practical application [69]. Therefore, further long-term testing under real operational conditions is required, along with an evaluation of the environmental impact of ionic liquid use.

3.5. Ecotoxicity of ionic liquids, granulated activated carbon and impregnated activated carbon

Ionic liquids are frequently classified as environmentally friendly green solvents. Nevertheless, when (eco)toxicity is the focal point, numerous ionic liquids pose an environmental threat [70]. The commercially available IL TEGO Dispers 662 C used in this study is considered a water pollutant based on the information provided in the product safety data sheet. It is not allowed to enter soil, waterways or wastewater canal. This assertion is supported with ecotoxicity data, ranging from EC50 0.065 mg L⁻¹ for *Daphnia magna* to EC50 564 mg L⁻¹ for activated sludge bacteria (see Supplementary material, Table S4). For the IL TEDA, EC50 of 0.51 and 0.45 mg L⁻¹ was observed for *Vibrio fischeri* luminescence inhibition after 15- and 30-min incubation, respectively [22]. Concerning these

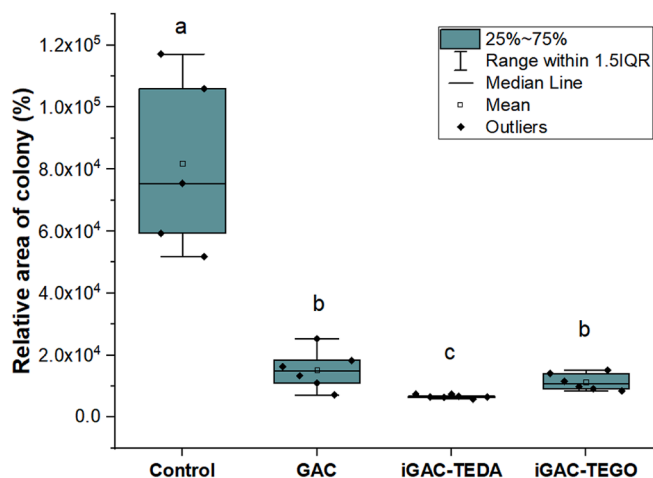


Fig. 5. Results of *Bacillus subtilis* antimicrobial tests. The mean value of the control sample area was set to 100 %. The tests were performed in six replicates. A one-way ANOVA was used for the statistical evaluation at a 95 % significance level. Different letters (a, b, c) indicate statistically significant differences between the datasets.

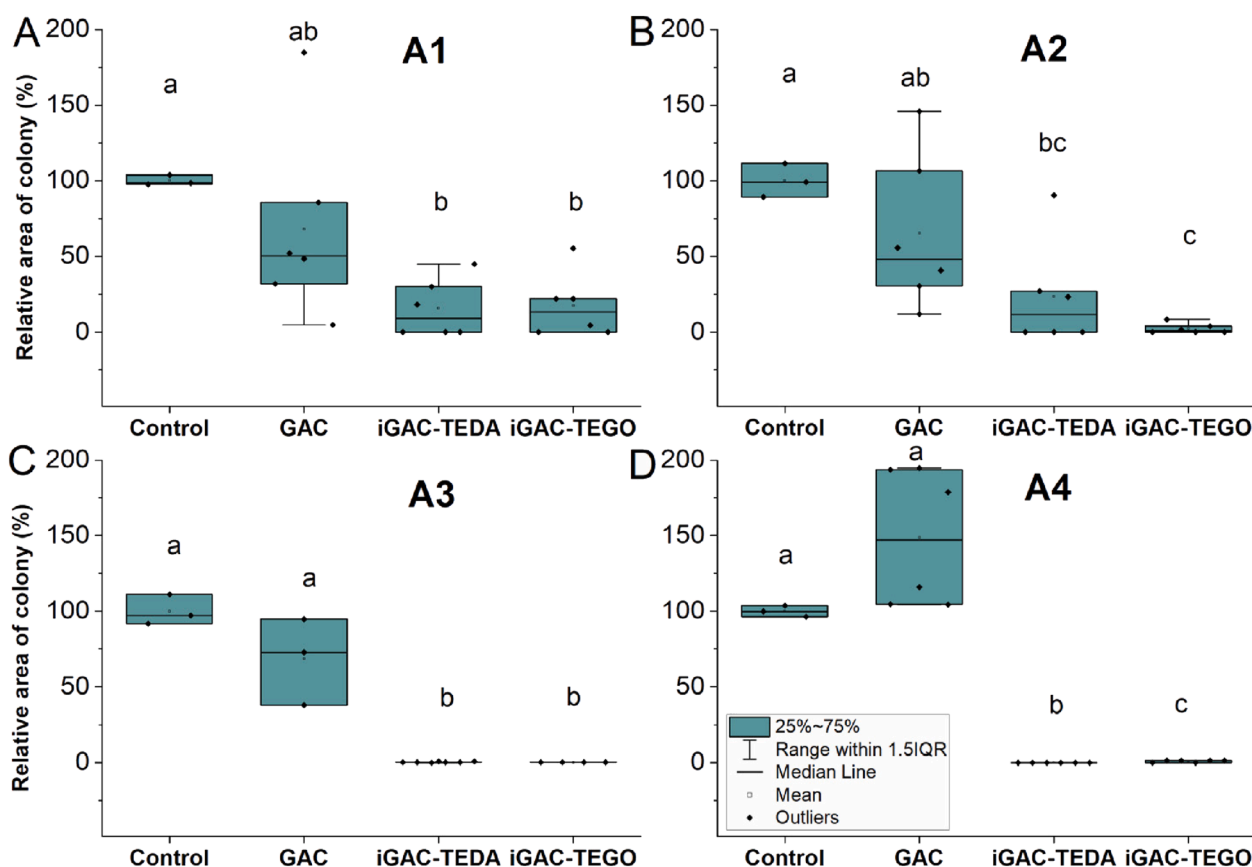


Fig. 6. Results of ARGs antimicrobial tests. The mean value of the control sample area was set to 100 %. **A1** - resistant to beta-lactams and tetracyclines, **A2** - resistant to beta-lactams, tetracyclines and sulfonamides, **A3** - resistant to beta-lactams, carbapenems, tetracyclines and sulfonamides, **A4** - resistant to beta-lactams, tetracyclines and sulfonamides. The tests were performed in six replicates. A one-way ANOVA was used for the statistical evaluation at a 95 % significance level. Different letters (a, b, c) indicate statistically significant differences between the datasets.

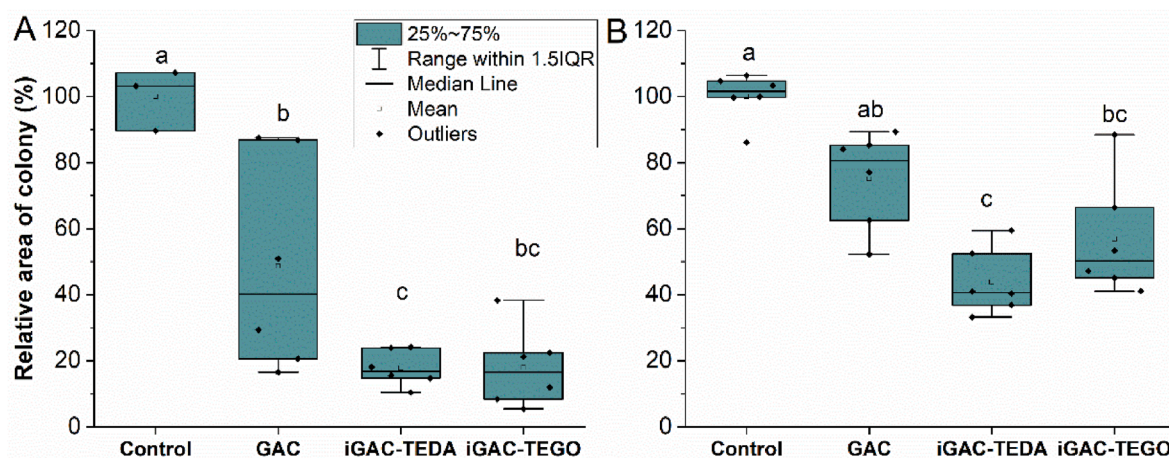


Fig. 7. Antimicrobial test of A – wastewater (WWTP Ostrava, effluent) and B – greywater samples. The mean value of the control sample area was set to 100 %. The tests were performed in six replicates. A one-way ANOVA was used for the statistical evaluation at a 95 % significance level. Different letters (a, b, c) indicate statistically significant differences between the datasets.

values, TEDA can be classified as highly toxic, according to the already used hazard ranking by Passino and Smith [71].

The findings of our study demonstrate that the aforementioned toxic ILs have the potential to bind firmly to the surface of GAC, thereby negating their environmental hazard, despite the fact the ILs are present in iGAC in high concentrations (up to 17 wt%). The

results of the ecotoxicity tests (see Table 4) confirmed that the aqueous leachate from iGAC did not exert a significant ecotoxic effect on any of the indicator organisms. *V. fischeri* bioluminescence was only slightly inhibited for iGAC-TEGO leachate, with the inhibition value not exceeding 10 % after 30 min incubation. Raw data on luminescence intensity can be found in Table S5 *S. alba* root

Table 4
Results of ecotoxicity tests conducted with indicator organisms *V. fischeri*, *S. alba*, *E. andrei*. (mean value ± standard deviation; 2 replicates for *V. fischeri*, 3 replicates (3x20 seeds) for *S. alba*, 10 replicates for *E. andrei*) Negative values indicate stimulation of the monitored parameters (luminescence, root growth).

Sample	<i>V. fischeri</i> luminescence inhibition (%)		<i>S. alba</i> root growth inhibition (%)	<i>E. andrei</i> mortality (%)
	15 min	30 min		
GAC	1.60 ± 6.09	1.70 ± 4.42	−3.79 ± 5.09	0
iGAC-TEGO	14.41 ± 1.24	9.81 ± 0.82	−10.71 ± 9.14	0
iGAC-TEDA	8.73 ± 0.83	1.01 ± 0.05	12.10 ± 6.42	0

growth was slightly inhibited in case of the iGAC-TEDA leachate (12.10 % inhibition compared to control). Conversely, a slight growth stimulation was observed for the iGAC-TEGO leachate. Considering the values of standard deviations, it can be stated that the average root length is comparable to the control determination for all samples (see Fig. 8). Acute toxicity test conducted with earthworms *E. andrei* exhibited no mortality in any of the determinations.

Given the negligible inhibitory (and zero mortality) effects, no ecotoxicity assessment of the dilution series was conducted. Consequently, no median effective nor lethal concentration (EC50, LC50) could be ascertained for any of the indicator organisms employed. The ILs were likely to be firmly attached to the surface of the AC, which resulted in the aqueous leachate from the ten-times-washed iGAC being considered non-toxic.

4. Conclusions

Commercial granular activated carbon was impregnated with two ionic liquids (ILs), TEGO® Dispers 662C and N,N,N-triethyl-1-dodecylammonium bis(trifluoromethyl-sulfonyl)imide (TEDA). TEGO was selected as a representative of cost-effective ILs, while TEDA represented a group of quaternary ammonium ionic liquids known for their biocidal properties. The impregnation of GAC by ILs did not significantly decrease its sorption efficiency for micropollutants in wastewater from WWTP and greywater. Both ionic liquids showed a statistically significant improvement in effective inhibition of viable strains of *Aeromonas* sp. Carrying ARGs, which had been isolated from the wastewater of WWTP. In conclusion, the attachment of ionic liquids to a suitable carrier represents a promising strategy to limit the environmental spread of antibiotic resistance.

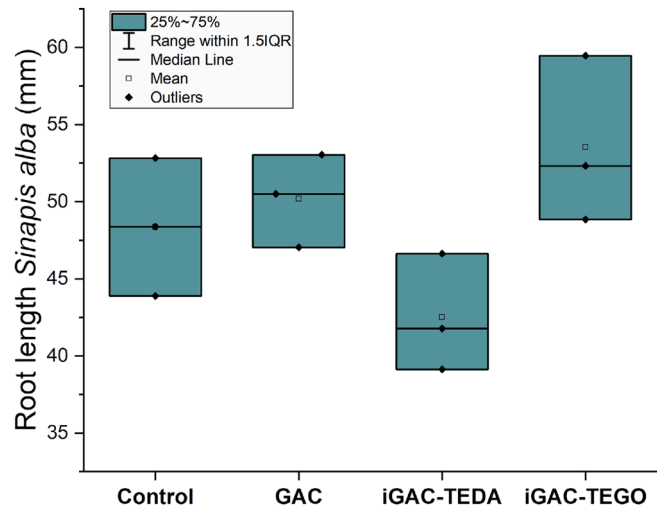


Fig. 8. Root length of *S. alba* after 72 h of germination, each treatment was performed in triplicate.

CRediT authorship contribution statement

Lucie Řepečká: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Jan Bednárek:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Tereza Stachurová:** Writing – original draft, Methodology, Investigation. **Anna Gavlová:** Writing – original draft, Methodology, Investigation. **Kateřina Smutná:** Writing – original draft, Methodology, Investigation. **Jana Vařtýl:** Writing – review & editing, Methodology, Investigation. **Marek Šváb:** Writing – review & editing, Methodology, Investigation. **Martina Vráblová:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization.

Funding

The work was supported by the OP JAK project “INOVO!!!”, No. CZ.02.01.01/00/23_021/0008588 supported by the Ministry of Education, Youth and Sports of the Czech Republic and co-financed by the European Union. This work was financially supported by the European Union under the REFRESH Research Excellence For Region Sustainability and High-tech Industries project No. CZ.10.03.01/00/22_003/0000048 via the Operational Programme Just Transition. Experimental results were accomplished by using Large Research Infrastructure ENREGAT supported by the Ministry of Education, Youth and Sports of the Czech Republic under project No. LM2023056.

Data is available online via the ZENODO repository <https://doi.org/10.5281/zenodo.17311234>.

A preprint has been submitted to Research Square. Title: Modification of activated carbon using ionic liquids for concurrent elimination of micropollutants and antibiotic-resistant bacteria from wastewater. DOI: <https://doi.org/10.21203/rs.3.rs-8206670/v1>.

Declaration of competing interest

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

Acknowledgements

The authors thank Kamil Maciej Górecki for performing FTIR analyses, Jana Strakošová for performing Elemental analyses and Jaroslav Lang for his support and assistance during the experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.emcon.2025.100615>.

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