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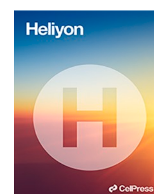

Research article

Industrial ecotoxicology in focus: The unexplored environmental impacts of pilot-scale advanced filtration in Sc recovery

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ABSTRACT

The demand within the European Union (EU) for the crucial raw material Scandium (Sc), coupled with the lack of sufficient recovery strategies, has gravitated research into exploiting alternative secondary sources. Utilizing residues from ore-production processes has proven to be a successful attempt for advanced Sc recovery. Despite the emergence of new technologies for Sc recovery from such residues, the potential environmental impacts of byproducts and technology wastes are often disregarded. Our study aimed to assess the environmental efficiency of a pilot-scale Sc recovery technology that relies solely on filtration. We employed a problem-specific ecotoxicity toolkit based on the approach of Direct Toxicity Assessment (DTA). The results of DTA provide an indication of the scale of the adverse effect of (contaminated) samples without the necessity of translating the results into chemical concentration. Standardized test methods (*Aliivibrio fischeri* bioluminescence inhibition, *Daphnia magna* lethality and *Sinapis alba* root and shoot elongation inhibition) were applied, supplemented by a bioconcentration assessment with the *D. magna* bioaccumulation test method to gain insight on the bioaccumulation potential of different metals in the case of all samples from the filtration technology. Comprehensive genotoxicity evaluations were also implemented using three distinct test methods (Ames test, Ames MPF test, SOS Chromotest). We conducted a comparative direct toxicity assessment to anticipate the potential environmental impacts of residues generated at each filtration step on the aquatic ecosystem. Our findings indicate that the environmental impact of the generated intermediate and final residues was alleviated by the consecutive filtration steps employed. The pilot-scale application of the Sc recovery technology achieved a high and statistically significant reduction in toxicity according to each test organism during the filtration processes. Specifically, toxicity decreased by 73 %, 86 % and 87 % according to the *Aliivibrio fischeri* bioluminescence inhibition assay, the *Sinapis alba* shoot elongation inhibition test, and the *Daphnia magna* lethality test, respectively. The toolbox of industrial ecotoxicology is recommended to predict the environmental performance of metal recovery technologies related to potential ecosystem effects.

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1. Introduction

For more than two decades, green chemistry has been standing at the forefront of efforts to revolutionize the chemical and product development landscape by prioritizing reduced toxicity and environmental harm [1–3]. While the safe-by-design approach seeks to minimize the environmental impact of chemicals, it does not guarantee that alternative chemicals will be inherently less ecotoxicologically harmful [4], therefore the complexity of ecological interactions and the varied pathways through which chemicals can impact ecosystems necessitate a more nuanced understanding [5,6]. Green toxicology is a necessary tool in assessing the risk of alternative chemicals to the environment and the eco-friendliness of potential green technologies by considering factors such as bioaccumulation, persistence, and potential harm to ecosystems, rather than just acute toxicity. As the field matures, there is a growing potential for increased emphasis on comprehensive environmental risk assessments (ERA) of input and output materials, as well as waste streams of chemical technologies [4,7]. This evolution signifies a move towards a more holistic and sustainable approach to technology development within the realm of green technology.

Adopting green toxicology principles may vary among industries and organizations, with some being more attuned to the importance of comprehensive ERA than others [7]. Conducting thorough ecotoxicity testing of all input materials, output materials, and waste streams, while essential for a holistic evaluation, can be resource-intensive and time-consuming, posing challenges in the early stages of technology development focused on proof of concept and feasibility studies [2].

Complex waste samples often contain a myriad of compounds with varying chemical properties [8]. Obtaining detailed data on the concentration and behavior of all constituents can be challenging. Incomplete or inaccurate data can compromise the reliability of the model used, which may struggle to capture the interactions and synergistic or antagonistic effects among these diverse substances leading to underestimation or overestimation of ecotoxicity [9]. Real ecosystems are dynamic and subject to constant changes. Computer models may not fully account for the dynamic nature of environmental systems, leading to inaccuracies in predicting the fate and effects of complex waste samples [10,11].

Direct toxicity assessment (DTA) describes the aggregated effects of complex waste samples [12–19], therefore DTA-based ecotoxicity results should be prioritized over single chemical compound-based effective concentration (EC_X) values from scientific literature in life cycle impact assessments (LCIAs). As LCIAs aim to provide a comprehensive assessment of the environmental impact of a product or process throughout its life cycle, DTA results align with this objective by offering a more holistic view of the aggregated effects of complex waste samples, contributing to a thorough LCIA. However, the current challenge of integrating DTA results into LCIA tools lies in the following limitations: ecotoxicity data is typically available only for individual compounds, not complex mixtures of real-world waste streams and the fact, that predominantly LCIA relies on literature data from databases. Consequently, there's a gap in translating DTA data – even if they are available - to the language of LCIA, hindering the accurate assessment of accurate environmental impacts within LCIA frameworks [11]. Current LCIA software integrates ecotoxicity information for individual compounds, resulting in a gap in evaluating the environmental impact of intricate compound combinations and emerging technologies. This limitation underscores the need for improved tools and methodologies to comprehensively assess the ecological footprint of evolving industrial processes and technologies. Therefore, it is highly encouraged to address the challenge of integrating the DTA-based ecotoxicological data for complex mixtures into LCIA calculations [11,20].

Numerous researchers have explored how ecotoxicology could be more valuable and better integrated into environmental decision-making partly based on peer-reviewed literature [15,16,18]. A set of reporting requirements was proposed by Hanson et al. [21] aiming to offer clarity regarding matters such as the test chemical, experimental design, conditions, chemical identification, test organisms, etc. While the application of LCIA tools is increasingly prevalent in diverse developments of environmentally friendly technologies [22,23], in current scientific literature it is even rare to find assessments considering the ecotoxicological aspects of material flows from specific, emerging technologies, utilizing DTA data for LCIA [24]. In recent years some efforts have been made in order to characterize the environmental impacts of scandium production from rare earths tailings to secondary sources based on LCIA approaches [25–30], however none of these studies investigated direct toxic effect on the ecosystem.

In our previous publication, efforts were undertaken to establish an ecotoxicity toolkit specifically designed for assessing the ecotoxicological impacts of a laboratory-scale Sc recovery technology utilizing acid-resistant nanofiltration (arNF) [31]. Conducting and verifying the feasibility of the novel Sc recovery technology based on arNF at the laboratory scale in terms of environmental efficiency enhancement was essential prior to the implementation of the scaled-up pilot version of this technology to optimize process parameters, minimize environmental and financial risks, and ensure long-term cost-effectiveness. In our present study, we have enhanced this established ecotoxicity toolkit by incorporating the terrestrial plant root and shoot elongation test method, the *Daphnia magna* bioaccumulation test, and the genotoxicity evaluation. The main goals of this research were (i) to comprehensively assess the direct toxicity and genotoxic potential of the pilot-scale arNF technology, which itself represents a unique novelty in the environmental efficiency characterization of a Sc recovery technology, as to our knowledge, a survey comparable to this has not been conducted so far, especially in the case of a technology projecting industrial-scale application, (ii) to verify the applicability and to ascertain whether the environmental efficiency could be reliably predicted based on the laboratory-scale study-derived ecotoxicity toolkit for the DTA of the scaled-up version of the arNF Sc recovery technology. Additionally, (iii) we deemed it essential to facilitate a comparison of the environmental efficacy between the laboratory-scale and pilot-scale technology processes. The DTA results of an established pilot-scale arNF technology for Sc recovery may serve as a benchmark for better comparison with upcoming LCIA evaluation outcomes driven by bibliometric ecotoxicity results for individual contaminants of complex waste samples similar to the ones investigated in our study or even with the LCIA outcome of the very same technology.

2. Materials and methods

2.1. Technology input material

The Sc-containing (~81 mg Sc/L) acidic liquid waste used in the pilot-scale filtration process was obtained from an ore processing manufacturer located in the Netherlands. The acid waste (AW) was dark blue to green colored with a pH of 0.26. The elemental composition of AW is detailed in Table S1.

2.2. Experimental setup of the pilot-scale filtration technology and the origin of the samples

A pilot-scale technological experiment was carried out to assess the feasibility of the applied filtration process for the treatment of the Sc-containing acid waste of the manufacturer. The pH of the original AW was adjusted to pH = 1.5 under stirring by adding caustic soda (30 % w/w). The reaction mixture was stirred for 24 h before settling for 48 h. Afterwards, three consecutive filtration steps were carried out: microfiltration (MF), ultrafiltration (UF) and nanofiltration (NF). MF of the pH-adjusted AW (pHa AW) was conducted, using a bag filtration unit (2-EF6-F, Eurowater, Germany) with two filtration bags (size 2, polypropylene, 1 μm nominal removal rate, 17 L volume, 2 bar). After approx. 27 h total filtration time, the filtrate (MFP) was separated from the hydroxide sludge (MFR). Both UF and NF were carried out in cross-flow operation mode using a modified filtration system (Osmo Inspector, Convergence, The Netherlands). For UF, 1812 spiral wound elements (UP150, Microdyn-Nadir, Germany, membrane area: 0.23 m^2, MWCO: 150 kDa) were used with the following filtration parameters: transmembrane pressure (TMP) = 5–20 bar, cross-flow rate = 8 L/min, $T = 25$ °C, 80 % permeate recovery, approx. 108 h total operation time. The average flow rate of UF was approx. 3.7 L/h. For NF, a 2540 spiral wound element (NanoPro A-3014, AMS Technologies, Israel; membrane area: 1.6 m^2, MWCO: 400 Da) was used, where the filtration parameters were as follows: TMP = 35 bar, cross-flow rate = 8 L/min, $T = 25$ °C, 60 % permeate recovery, approx. 31 h total operation time. The retentate production rate was approx. 3.2 L/h. For more detailed description and discussion of the applied technology, please refer to Hedwig et al. [32].

The block flow diagram of the filtration process is shown in Fig. 1. Table 1 presents the details of the differentwaste streams tested with different ecotoxicity assays.

2.3. pH measurements and chemical analysis of the tested samples

The pH of both the liquid phase samples and the aqueous extract was measured in triplicate using a WTW pH 330 pH meter (Wissenschaftlich Technische Werkstätten GmbH, Germany) equipped with a Sentix 81 pH electrode, that was calibrated prior to each measurement according to the operating manual of the pH meter, at pH 4 and pH 7 immersing the electrode in the standard solutions provided by the manufacturer (WTW GmbH, Weilheim, Germany).

Samples were diluted with nitric acid (3 % w/w) using an autodilution system (Simprep, Teledyne Cetac Technologies, USA). Subsequently, the samples were analysed using QqQ-ICP-MS (Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry). Analyses were conducted on an 8800 QqQ-ICP-MS system (Agilent, Switzerland), operated with general-purpose operational settings. For quantification, a calibration curve was measured (0–50 μg/L, seven data points) using multielement standards. To compensate for matrix effects, ^{103}Rh (50 μg/L) was employed as an internal standard. The quantification of various ions, including $^{23}Na^+$, $^{52}Cr^+$, $^{55}Mn^+$, $^{56}Fe^+$, $^{60}Ni^+$, $^{66}Zn^+$, $^{89}Y^+$, $^{137}Ba^+$, $^{139}La^+$, $^{140}Ce^+$, $^{141}Pr^+$, $^{146}Nd^+$, $^{147}Sm^+$, $^{153}Eu^+$, $^{157}Gd^+$, $^{159}Tb^+$, $^{163}Dy^+$, $^{165}Ho^+$, $^{166}Er^+$, $^{169}Tm^+$, $^{172}Yb^+$, $^{208}Pb^+$, ^{232}Th, and $^{238}U^+$, was performed using single-quad mode on the ICP-MS, utilizing helium as the collision gas. Additionally, ions such as $^{24}Mg^+$, $^{27}Al^+$, $^{39}K^+$, $^{45}Sc^+$, $^{47}Ti^+$, $^{51}V^+$, and $^{90}Zr^+$ were measured in triple-quad mass-shift mode, employing O_2 as a reaction gas. The concentration of $^7Li^+$ was determined using single-quad mode without collision or reaction gas. Multi-element standards were measured repeatedly for each series of measurements to ensure accuracy and precision.

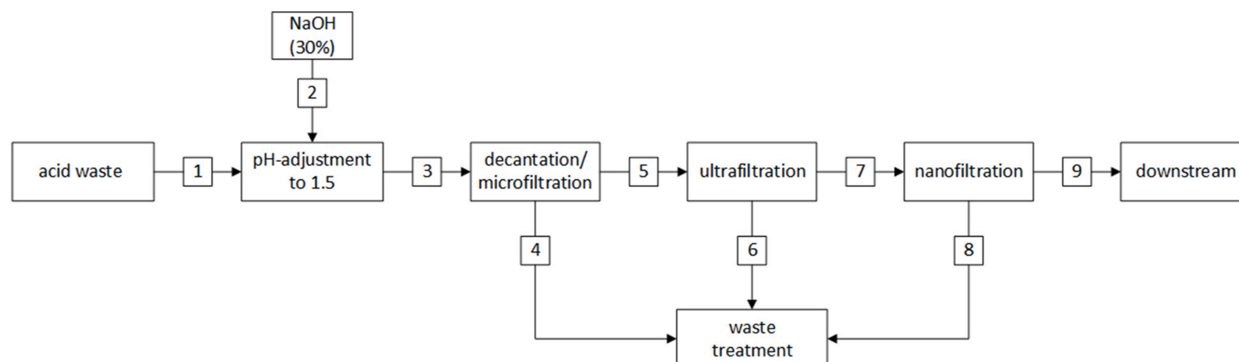

Fig. 1. Acid-resistant filtration process unit block flow diagram.

Table 1
Samples from the different filtration steps of the pilot-scale filtration technology process.

Sample name	Sample abbreviation	Sample description	Sample number
Acid waste	AW	Ore related Sc containing acid waste (pH = 0.26)	1
pH-adjusted acid waste	pHa AW	Ore related Sc containing acid waste after pH adjustment by 30 % w/w NaOH (pH = 1.5)	3
Microfiltration permeate	MFP	Filtrate of microfiltration step of the pH-adjusted (pH = 1.5) Sc containing acid waste	5
Microfiltration retentate	MFR	Thickened sludge of microfiltration step of the pH-adjusted (pH = 1.5) Sc containing acid waste	4
Ultrafiltration permeate	UFP	Permeate after ultrafiltration of MFP (permeate recovery: 80 %)	7
Ultrafiltration retentate	UFR	Retentate after ultrafiltration of MFP	6
Nanofiltration permeate	NFP	Permeate after nanofiltration of UFP	8
Nanofiltration retentate	NFR	Retentate after nanofiltration of UFP	9

2.4. *Environmental impact assessment*

The liquid waste samples were diluted for testing with the growth medium necessary for the cultivation or maintenance of a particular test organism. In each toxicity test, a negative control was included, functioning as both a reference point and quality control within the experiment. All test organisms were selected based on their previously proven sensitivity to heavy metals [33–36] and the examples in the field of testing complex wastes, wastewaters and environmental samples, such as construction product leachates [37], wastewaters [38,39], and foundry sludge leachates [40]. The selection of the applied ecotoxicity test methods with test organisms of 3 different trophic levels was also based on their performance in our previous study [31]. The aquatic plant test organism has been replaced with the terrestrial plant, *S. alba*, with easier cultivation and a shorter necessary exposure period. Additionally, ecotoxicity characterzation was complemented with genotoxicity assessment and *D. magna* bioaccumulation studies based on the recommendations of Hennebert et al. [41].

2.4.1. *Aliivibrio fischeri bioluminescence inhibition assay*

The bacterial strain (NRRL B-111 77) used in the study was cultivated and maintained in the laboratory under axenic conditions. The routine maintenance of the *A. fischeri* bacterial strain and the protocol of the bioluminescence inhibition test was described by Fekete-Kertész et al. [31]. The luminescence intensity was measured with a Fluostar Optima BMG Labtech microplate reader after 30 min incubation time in three parallels. As a negative control, distilled water was applied. In order to validate the data and assess the sensitivity of the *A. fischeri* cell culture, copper sulfate was included as a reference toxicant and measured in each series of measurements.

2.4.2. *Daphnia magna lethality and immobilization assay*

An inhouse *D. magna* colony was used in a series of experiments. The maintenance of the *D. magna* colony was described by Fekete-Kertész et al. [31]. The *D. magna* acute lethality and immobilization tests were performed as described in the OECD (Organization for Economic Co-operation and Development) 202 [42] test protocol in three parallels. Distilled water was applied as a negative control. To check the sensitivity of the *D. magna* culture, acute toxicity tests were performed with potassium dichromate ($K_2Cr_2O_7$) as a reference toxicant at intervals of approximately six months. The sensitivity range of *D. magna* culture to $K_2Cr_2O_7$ was within the limits (EC_{50}, 24 h = 0.6–2.1 mg/L) set by the OECD 202 guideline [42].

2.4.3. *Daphnia magna bioaccumulation assay*

To assess the bioaccumulation potential of different metals in the case of all samples from the filtration technology 10 *D. magna* individuals were exposed to a particular dilution of each sample in triplicates for 96 h. Experimental conditions were the same as described in Section 2.4.2. In order to be able to comprehensively assess the bioaccumulation potential in each sample with different levels of toxicity, the dilution factor corresponding to the EC_{20} value was applied based on the conventional *D. magna* lethality test results. At the end of the exposure period, alive daphnids were preserved by applying a series of exsiccation steps by increasing ethanol concentration (30, 50, 70, 90 and 100 w/w %) in 20-min cycles. After the final exsiccation step, daphnids were air-dried on filter paper and transferred into 0.5 mL microcentrifuge tubes. The total weight of the exsiccated daphnids per sample parallel was determined with a semi-micro balance (Series 360 EP 225 SM-DR, d = 0.01/0.1 mg) manufactured by Precisa Gravimetrics AG (Switzerland). The metal contents of the preserved samples were measured using QqQ-ICP-MS (Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry) as described in Section 2.3.

To calculate the bioconcentration factor (BCF), we utilized QqQ-ICP-MS to determine the total concentration of each metal present in daphnids exposed to water samples collected from distinct test systems containing a specific diluted technology sample. The metal content within daphnids was quantified in g/metal units, and these values were standardized to the total dry body weight of the daphnids from each respective sample, yielding values expressed as g metal/kg of dry body weight. Additionally, we determined the concentration of various metals within the assembled test systems using QqQ-ICP-MS, given in g/L units. By dividing the metal concentrations within the daphnids by those within the aqueous test systems, we computed the BCF, represented in L/kg dry body weight (eq (1)). In short the bioconcentration factor (BCF, L/kg (dry weight) was calculated from the ratio of the total concentration in

daphnids exposed through water (eq (1)) (i.e., g metals kg dry weight^{-1}) and the dissolved water concentration (C_{water}, g metals/L) [43].

$$\boldsymbol{BCF}\,(\mathbf{L}/kg) = \frac{C_{Daphnia(aqueous\ exposure)}}{C_{water}} \quad \text{(eq 1)}$$

2.4.4. *Sinapis alba* root and shoot elongation assay

The *S. alba* root and shoot elongation tests were carried out based on the OECD 208 [44] test protocol and its modification by Leitgib et al. [45]. Twenty *S. alba* seeds, exhibiting over 90 % germination ability, were positioned on a filter paper disc moistened with 2.5 mL of the sample in darkness within a glass Petri dish (10 cm in diameter, 2 cm height) at 23 ± 1 °C for a duration of 3 days in darkness. Distilled water served as the negative control and was used for diluting the samples. The lengths of both roots and shoots were measured using a ruler, and the averages were computed for each Petri dish. Inhibition (%) was calculated based on the measured data in comparison to the control. To assess the sensitivity of the *S. alba* seeds, acute toxicity tests were conducted using copper sulfate as a reference toxicant approximately every 3 months.

2.5. Genotoxicity assessment

2.5.1. Ames (*Salmonella typhimurium*) reverse mutation test

This method is used to assess chemicals for their genotoxic potential, especially for inducing point mutations [46]. The applied *S. typhimurium* bacterium strain, TA100 was purchased from TRINOVA BIOCHEM GmbH (Germany). The TA100 *S. typhimurium* strain has been employed for over four decades to identify mutagenic compounds in a diverse range of samples, including chemicals, pharmaceuticals, cosmetics, biocides, water and environmental specimens [47].

The strain stemming from agar slant cultures was inoculated in 10 mL of LB growth medium, and then the cultures were grown overnight (18 h at 37 °C, 160 rpm). Sodium azide (NaN_3) dissolved in distilled water was used as positive control. The test protocol was designed for a total of three concentrations in triplicate plus positive and negative controls.

The experiments were carried out based on the plate incorporation method of Maron and Ames [46] without metabolic activation. 1 mL of sterile liquid sample was mixed into 9 mL of glucose minimal agar medium (914 mL distilled water, 17 g agar, 20 mL 50x Vogel-Bonner salt solution, 50 mL 40 % glucose solution) or 0.1 g of sterile solid samples was mixed into 10 mL of glucose minimal agar medium. 100 μL of histidine/biotin solution (10 mL biotin solution: 3.1 mg biotin +25 mL distilled water, 100 μL histidine solution: 0.5 mg biotin +25 mL distilled water), then 100 μL of bacterium cell culture was spread over the surface evenly. After 48 h at 37 °C the number of colonies per plate and per dose was counted by eye and compared with the number of spontaneous revertant colonies obtained in the negative control plates. The frequency of mutation is determined by the following formula: frequency of mutations = number of revertants in the sample per plate/spontaneous revertants per plate.

In the case of the Ames reverse mutation test, the reported means for historical solvent controls were 75–200 CFU for TA100 [47], which requirements were met in our experiments, where solvent control spontaneous revertant colony values ranged from 89 ± 4 to 195 ± 8. The number of revertant colonies in the NaN_3 containing positive control agar plates also met the requirements of the standard Ames Plate Incorporation method [48]. When evaluating the results of the standard Ames plate incorporation method, the „CR Criterion" (a concentration-related increase of the revertants) and the "Fold rule Criterion" (the increase compared to the concurrent control using the strain-specific fold increase criterion; e.g. > 2-fold increase compared to solvent control) were applied [47]. A sample was labelled „possible genotoxicity can not be excluded" when both criteria were satisfied, considered „inconclusive" when only one of the two criteria was met, and categorized as „potentially not genotoxic" when neither criterion was fulfilled.

2.5.2. Ames MPF assay

The Ames microplate format (MPF™) test kit was purchased from Xenometrix (Allschwil, Switzerland). TA98 and TA100 tester strains (purchased from TRINOVA BIOCHEM GmbH, Germany) were applied in compliance with OECD Guideline 471 [49]. The TA98 an TA100 auxotroph mutant cultures were grown overnight at 37 °C, shaken at 250 rpm in the Ames MPF growth medium until reaching $OD_{600} > 2$ optical density of the cell suspension. The cultures were exposed to six doses of the tested technology samples (10 μL) in sterile conical tubes in the Ames MPF exposure medium (240 μL) for 90 min at 37 °C, shaken at 250 rpm. After the exposure period, the cultures were diluted using histidine-free media with a pH indicator (Ames MPF reversion indicator medium, 2.6 mL), and the contents of the conical tubes were transferred into the wells of a 96-well microplate (50 μL/well). This was followed by a 48-h incubation period at 37 °C. During this period, the cells that have mutated back to the wild-type genotype, either spontaneously or as a result of exposure to the test chemical, divide. As a result, cellular metabolism reduces the pH of the medium, changing the indicator colour from purple to yellow. Upon completion of the incubation period, 96-well plates were scored by differentially counting colored wells spectrophotometrically by a DIALAB EL800 reader at 490 nm in order to avoid operator bias and obtain highly accurate data. These yellow revertant wells are counted for each dose and compared to the zero dose [50]. The assessment criteria vary for cultures with low and high spontaneous revertant rates. If the negative control value was ≤30, a 2 to 3 times increase from the negative control at a given dose was considered weak positive, while responses exceeding three-fold were classified as positive. At least two adjacent doses with significant increases ($p < 0.05$) or a significant increase at the highest non-toxic dose level were required. For negative control values > 30, a 1.5 to 2.5 times increase from the negative control at a given dose was considered weak positive, and responses surpassing 2.5-fold were classified as positive. Similar to the previous criteria, at least two adjacent doses with significant increases ($p < 0.05$) or a significant increase at the highest non-toxic dose level were necessary [50].

2.5.3. SOS chromotest

The SOS ChromoTest is a colourimetric assay to detect DNA-damaging (genotoxic) agents [51]. The SOS-ChromoTest™ Kit was purchased from Eco Test S.L. (EPBI™ distributor) and was performed as described by EPBI [52]. Shortly, SOS bacteria (engineered *Escherichia coli*, where the β-galactosidase gene is tethered to the SOS DNA repair promoter) were incubated for 16 h at 37 °C before the test, and a suspension with OD 0.05–0.06 (at 630 nm) was applied for testing. A two-fold dilution series was prepared from each sample in 10 % DMSO (dimethyl sulfoxide) in 0.85 % saline. A two-fold 4-nitroquinoline-1-oxide (4-NQO, 10 μg/mL) dilution series was applied as a positive control (concentration-dependent blue colour should be observed). Only the diluent (negative control) and the tested samples (blank) were also applied as controls. We applied SOS-ChromoTest as direct contact test without pH adjustment. Each well of the 96-well microplate contained a final volume of 10 μL sample. 100 μL bacterial suspension was added to each well, and the OD was measured at 630 nm and 405 nm (background colour) immediately after addition, and the plate was incubated at 30 °C for 2 h. After incubation, 100 μL of a mixture of blue chromogen and alkaline phosphatase substrate was added to each well and incubated for 2 h at 37 °C. Final absorbance was measured at 630 nm to determine β-galactosidase production (β-gal, SOS induction, genotoxicity) and at 405 nm to determine bacteria viability (G, alkaline phosphatase activity) by a DIALAB EL800 reader. Measured ODs were corrected against the absorbance at the start (to remove colour interference), and all calculations were done with the corrected values: β-gal = Sample $OD_{630\ nm\ corr}$/Negative control $OD_{630\ nm\ corr}$, G = Sample $OD_{405\ nm\ corr}$/Negative control $OD_{420\ nm\ corr}$, induction factor (IF) = β-gal/G, and survival rate) = G*100 (should be higher than 80 %, may be accepted if it is higher than 70 %, otherwise the sample iy cytotoxic and the results are invalid). We considered IF > 2 as genotoxic, and if a concentration-response relationship can be found for at least two consecutive concentration levels, 1.5 < IF < 2 as marginally genotoxic, and IF < 1.5 as not genotoxic [53,54].

2.6. Data evaluation and statistical analysis

In the case of the ecotoxicity test results, the inhibition percentage (H%) for each ecotoxicity endpoint was calculated by comparing it to the respective control. Effective Concentration (EC_{20}, EC_{50}) values were computed using OriginLab 2018 software, representing concentrations causing a 20 % or 50 % decrease in the test endpoint compared to the control. The calculations utilized the Logistic function fitting ($y = A_2 + (A_1\text{-}A_2)/(1 + (x/x_0)^p)$), where A_1 is the initial value, A_2 is the final value, x_0 is the center, and p is the power. EC values are presented in dilution factor units, indicating the minimum dilution required for the original sample to induce a maximum inhibition of 20 % or 50 % in the test endpoint.

To identify statistically significant effects ($p < 0.05$), a one-way analysis of variance (ANOVA) was performed using STATISTICA 13® software. Cochran's C test was employed to assess the homogeneity of variances. The Newman-Keuls test ($p < 0.05$) was then used to determine the statistical significance between different treatments or dilutions. The significant effects are marked with lowercase letters in the figures and tables in alphabetical order, where "a" is the smallest value. Values signed with the same letter indicate that there was no significant difference between them.

The results of the ecotoxicity assays were used to assess the environmental efficiency of the pilot-scale arNF technology in terms of toxicity attenuation. This was achieved by calculating the relative toxicity, which was done by dividing the dilution factor corresponding to the EC values of a sample by the dilution factor corresponding to the EC values of the initial AW sample (e.g. y = 50 %, when the dilution factor required for 20 % inhibition is halved).

3. Results and discussion

3.1. Chemical analysis of the pilot-scale arNF technology samples

The received AW contained more than 30 different elements, with concentrations ranging from mg/L to multiple g/L [32]. In total the concentrations of measured elements in the AW summed up to ~57 g/L. Most prevalent elements were Fe (31.6 ± 0.6 g/L), Mn (6.1 ± 0.1 g/L), Al (4.6 ± 0.2 g/L) and Ti (4.4 ± 0.1 g/L). Furthermore, considerable amounts of Zr (2.15 ± 0.05 g/L), V (2.03 ± 0.02 g/L), Cr (1.27 ± 0.01 g/L) and Nb (0.89 ± 0.02 g/L) were present. Aside of these higher concentrated elements, traces of Ba (179 ± 7 mg/L), Ni (67 ± 4 mg/L), Pb (45 ± 1 mg/L) and naturally occurring radioactive material (NORM) were found (Th: 114 ± 3 mg/L, U: 26.0 ± 0.5 mg/L).

Adjusting the pH to 1.5 lead to precipitation of the majority of Nb (−100 %), Th (−95 %), Ti (−100 %), U (−86 %) and Zr (−100 %; AW vs. UFP, Table S1). The concentration of other elements decreased to lesser extend (on average −25 %), being slightly more than expected based on the dilution factor through NaOH addition of 1.2 (AW vs UFP, Table S1). In exchange for the precipitated metals, the sodium content increased drastically, from <LOD to approx. 30 ± 3 g/L (AW vs. UFP, Table S1). Thus, the total element concentration reached ~64 g/L (UFP, Table S1). The intermediate fractions (pHa AW and MFP) contained still higher amounts of the precipitated elements, although most likely in suspended/colloidal form and not dissolved. This assumption is based on the drastical reduction of these elements' concentrations after ultrafiltration (MWCO: 150 kDa). Interestingly, elemental concentrations in the UFR ressembled the original AW, except for considerable amounts of additional sodium in the UFR (AW vs. UFR, Table S1).

During nanofiltration, most (multivalent) elements were retained to some degree and thus concentrated (e.g. Mn: +20 %, Al: +170 %), while monovalent ions, such as Na^+ were depleted (−33 %; UFP vs. NFR, Table S1). The permeation of Na against its concentration gradient, led to higher Na levels in the nanofiltration permeate (NFP) than in the corresponding NFR (50 ± 1 g/L vs. 20 ± 1 g/L, NFP vs. NFR, Table S1). When comparing the original AW to the NFR, in some cases precipitation/removal compensated for concentration during NF, leading to similar concentrations in the two streams (Fe: −2%, Mn: −13 %, V: −5%, Pb: −18 %; AW vs. NFR, Table S1). Those elements that were mostly removed through pH adjustment or even depleted during NF, were consequently found reduced/

absent in the NFR (Ba: −71 %, Nb, Zr, Ti: −100 %, Th: −83 %, U: −90 %; AW vs. NFR, Table S1). Elements that were little precipitated, but well retained, were thus more abundant in the NFR than in the AW (Cr: +61 %, Al: +93 %, Ni: +49 %; AW vs. NFR, Table S1). The total element concentration in NFR reached 74 g/L (Table S1).

The NFP was relatively rich in Na (50 ± 1 g/L), Fe (25.4 ± 0.5 g/L), Mn (5.7 ± 0.2 g/L), V (1.34 ± 0.03 g/L) and Al (1.20 ± 0.05 g/L; Table S1). Additionally, traces of Ba (89 ± 7 mg/L), Cr (290 ± 20 mg/L), Ni (49 ± 9 mg/L) and Pb (35 ± 4 mg/L) were found. Thus, total element concentration in the NFP was higher than in other fractions and exceeded 80 g/L (Table S1). Nonetheless, in comparison to the AW, most elements were removed via precipitation or NF, whereas the average element concentration was drastically lower (−70 % on average excl. Na; Table S1).

3.2. Ecotoxicity characterization of the pilot-scale arNF technology samples

To conduct a thorough ERA, aquatic and terrestrial test organisms from different trophic levels (*A. fischeri* bioluminescent bacterium, *D. magna* freshwater crustacean, and *S. alba* terrestrial plant) were chosen. *D. magna* bioaccumulation studies and genotoxicity assessment were also carried out based on the *Salmonella typhimurium* (Ames) plate incorporation method, the AMES MPF Assay and the SOS Chromotest to ascertain potential environmental risks in case of accidental leakage of the technology samples into the environment.

3.2.1. Aliivibrio fischeri bioluminescence inhibition assay

In the context of the *A. fischeri* bioluminescence inhibition test, similar trends were observed for EC_{20} and EC_{50} values (Fig. 2). However, based on the EC_{20} values, the initial pH adjustment did not lead to a statistically significant reduction in toxicity (AW EC_{20} = 503x, pHa AW EC_{20} = 454x), unlike the case with the EC_{50} values (AW EC_{50} = 236x, pHa AW EC_{20} = 120x). Turning attention to the mitigating effects on toxicity resulting from consecutive application of different filtration steps, it was found that the permeate sample obtained after microfiltration (MFP) did not exhibit reduced toxicity according to EC_{20} values (MFP EC_{20} = 449x). In contrast, toxicity was significantly alleviated after ultrafiltration (UFP EC_{20} = 171x), with no further improvement observed upon subsequent nanofiltration (NFP EC_{20} = 164x). Nevertheless, the toxicity of the ultrafiltration retentate (UFR EC_{50} = 69x) was comparable to that of the ultrafiltration permeate (UFP EC_{50} = 85x) based on EC_{50} values and nanofiltration permeate (NFP EC_{50} = 60x) samples reaching the lowest dilution needed to reach 20 % inhibition (least toxic samples). Notably, the concentration of toxic elements was more pronounced after micro- and nanofiltration processes. The EC_{50} results exhibited a closely analogous pattern of sample toxicity compared to the EC_{20} findings (Fig. 2).

A. fischeri is has been employed to evaluate the toxicity of airborne heavy metal pollution, along with assessing heavy metal contamination in wastewater, surface waters, and sediments. Its previously proven sensitivity towards metals [34] explains its feasibility for the sensitive detection of the adverse effects of the multi-metal component technology samples from arNF. Based on the results of the *A. fischeri* bioluminescence inhibition assay on the samples from pilot-scale arNF, this method is recommended as a general screening method for samples of similar composition and with aggregated toxicity effects. Other advantages of using the standardized *A. fischeri* for the assessment of industrial technology samples such as robustness of the test systems, shortexposure times,

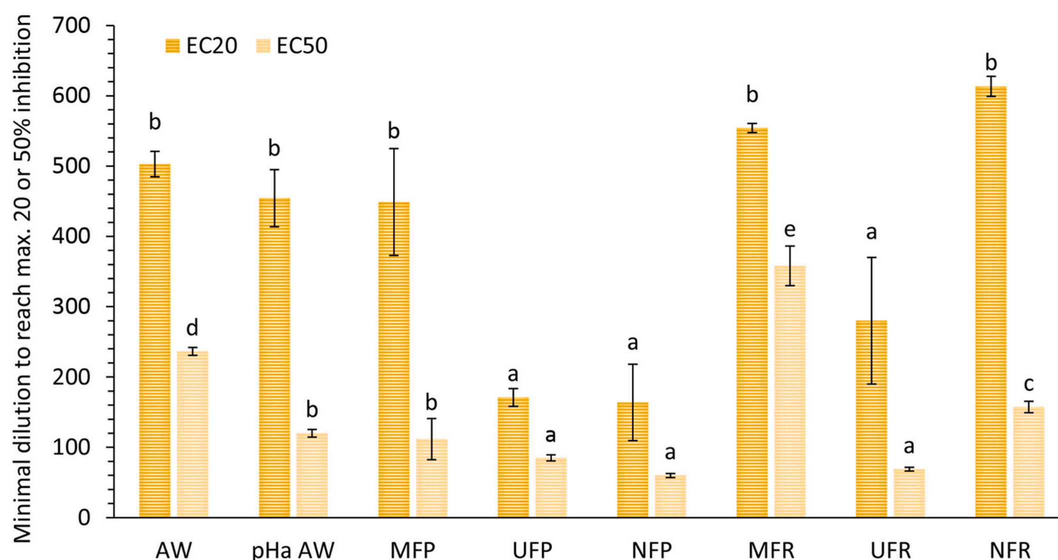

Fig. 2. EC_{20} and EC_{50} values given in dilution factor units determined for the samples of the pilot-scale arNF technology process in the case of *A. fischeri* bioluminescence test. In the diagram statistical significance distinctively for EC_{20} and EC_{50} values is marked by lower case letters. The significant effects are marked with letters in the figures and tables in alphabetical order, where “a" is the smallest value. Values signed with the same letter indicate that there was no significant difference between them.

hence time- and cost-effectiveness for general industrial application have been already discussed by Fekete-Kertész et al. [31].

3.2.2. *Daphnia magna lethality and immobilization assay*

In the context of the *D. magna* lethality test, the EC_{20} and EC_{50} values determined for each technological sample exhibited consistent trends across all applied exposure times (Fig. 3). Generally, adjusting the pH of the original acidic waste (AW) sample led to a significant reduction in toxicity. However, the introduction of an additional microfiltration step did not yield a further statistically significant decrease in toxicity of the permeate when compared to the results of the pH-adjusted AW sample (AW pHa and MFP, except at 48 h). Conversely, ultrafiltration resulted in a modest reduction in toxicity compared to microfiltration, though this effect did not reach statistical significance in all instances.

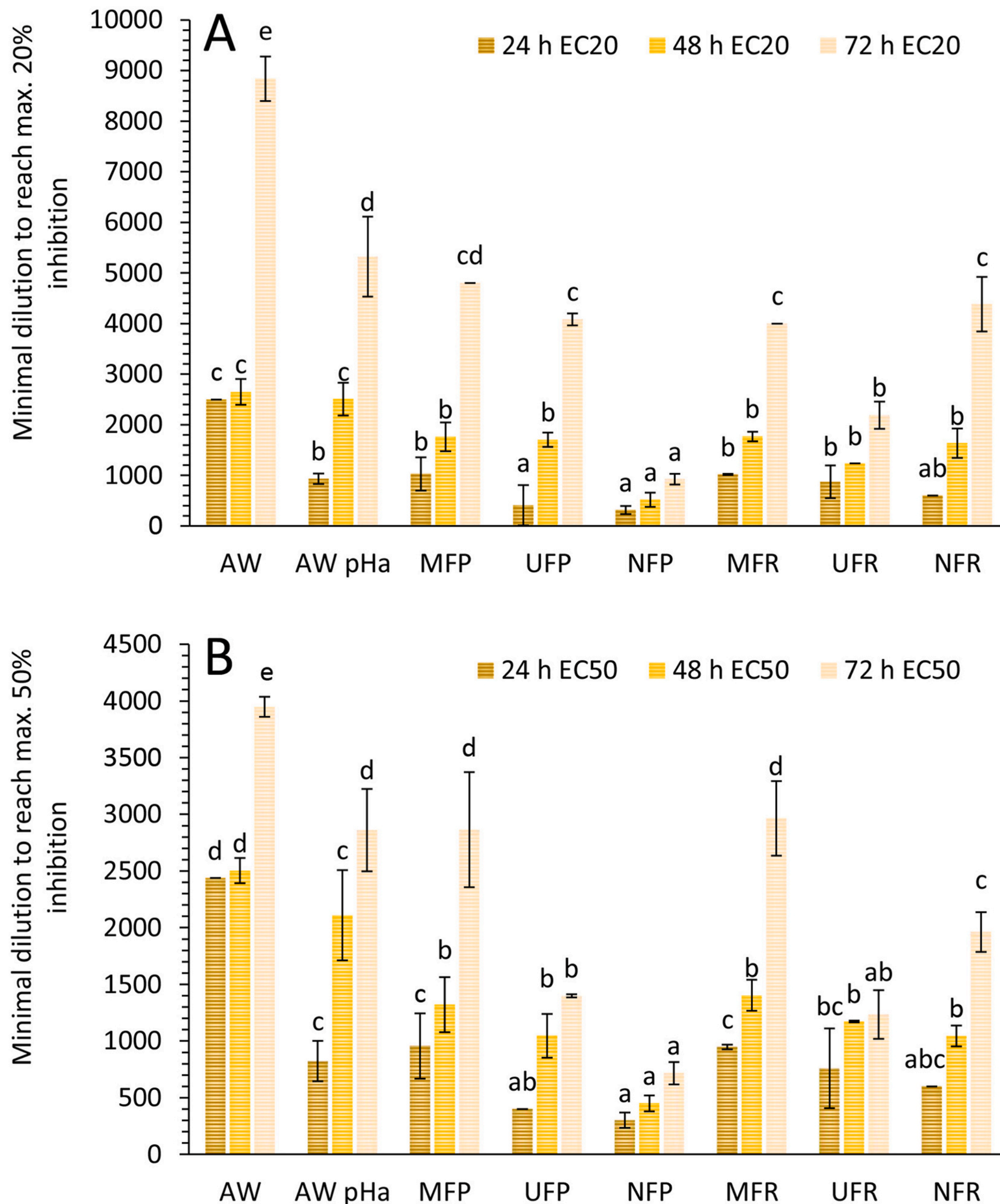

Fig. 3. EC_{20} and EC_{50} values given in dilution factor units determined for the samples of the pilot-scale arNF technology process in the case of the *D. magna* lethality test. In the diagram statistical significance distinctively for 24, 48 and 72 h exposure time is marked by lower case letters. The significant effects are marked with letters in the figures and tables in alphabetical order, where "a" is the smallest value. Values signed with the same letter indicate that there was no significant difference between them.

The most pronounced attenuation of toxicity was observed after nanofiltration in the case of the permeate samples (NFP). Relative to the minimal dilution required to achieve a 20 % inhibition (EC_{20}) values after 24, 48, and 72 h of exposure, the initial EC_{20} values of 2500x, 2647x, and 8838x times dilution of the AW sample were reduced to 314x, 519x, and 925x times dilution, respectively. These results indicate that based on the 72-h exposure, the NFP sample needs to be diluted 10 times less to avoid exceeding the 20 % inhibition rate compared to the original input material, the acidic waste (AW). Comparing the EC_{50} values between AW and NFP samples reveals a ratio of only 5.5 times, but NFP is still the least toxic sample among all shown by the EC_{50} values (Fig. 3).

Examining the EC_{20} and EC_{50} values of the retentate samples, the outcomes of both the *A. fischeri* bioluminescence test and the *D. magna* lethality test demonstrate consistency (Figs. 2 and 3). In both ecotoxicity test systems, it was evident that the retentate following microfiltration (MFR) exhibited the highest toxicity among the three retentate samples. A substantial reduction in toxicity was observed in the case of the ultrafiltration retentate (UFR) when compared to MFR. Given that economically valuable elements are predominantly concentrated in the nanofiltration retentate (NFR) sample, one might anticipate a pronounced toxicity level due to the elevated content of toxic elements in NFR.

3.2.3. *Daphnia magna* bioaccumulation assay

Bioconcentration factors (BCFs) were calculated for Ti, V, Cr, Mn, Fe, Ni, Zr, Nb, Ba and Pb due to exposure to the samples from the pilot-scale arNF technology (Table 2). Examining the log BCF (dry weight) values statistically significant alteration in bioavailability in *D. magna*, hence bioconcentration potential was revealed due to a simple pH adjustment of the original AW technology sample in the case of Ti, Fe and Zr. Log BCF for Ti increased from 2.88 to 3.68, for Fe from 2.96 to 3.47 and for Zr from 2.57 to 3.36. Based on these results, pH adjustment – a generally recommended sample pretreatment procedure in advance to ecotoxicity testing in tha case of extreme sample pH – should be avoided as it was revealed, that this intentional modification of pH essentially modified metal bioavailability and sample toxicity. Considering the permeate samples of the three consecutive filtration steps, a significant decrease in BCF values was observed in the case of Cr, Mn. Fe, Ba and Pb comparing the values of MFP and NFP. Metal contents of the daphnids are collected in Table S2.

There is extensive and well-documented literature on single metals' toxicity on terrestrial [55] and aquatic organisms [56], in particular *D. magna* [57–59], however there are limited information on the combined effects of metal mixtures in complex environmental compartments [56]. This knowledge gap in metal risk assessment has been recognized by the U.S. Environmental Protection Agency (USEPA) [60] and also by the task force of the United Nations Environment Programme: Global Guidance on Environmental Life Cycle Impact Assessment Indicators (GLAM project) [11]. Analyzing bioaccumulation or toxicity outcomes in the context of metal combinations is intricate due to potential chemical interactions with media components and physiological processes causing metal biotransformations, and competition at the toxicity site(s) [56]. While the biotic ligand model (BLM) is a widely accepted tool for quantitatively modelling the binding manner of metals in biological systems and how these processes are affected by environmental factors resulting in altered transformation and bioavailability, it has been shown that the behavior of several metal mixtures deviates from the predictions made by the process of competitive inhibition of metal binding to organisms as described by the BLM [61]. These limitations associated with predicting the toxicity of metal mixtures solely from individual metal exposure studies further emphasize the significance of the DTA approach.

Table 2
Log BCF values of the samples from the different filtration steps of the pilot-scale filtration technology process based on the D. magna bioaccumulation test. The significant effects are marked with letters distinctively for each metal in alphabetical order, where “a" is the smallest value. Values signed with the same letter indicate that there was no significant difference between them.

Log BCF [L/kg dw]	Ti	V	Cr	Mn	Fe
AW	2.88 ± 0.07 a	3.69 ± 0.01 b	2.99 ± 0.04 b	3.14 ± 0.04 bc	2.96 ± 0.07 b
pH adj	3.68 ± 0.05 b	3.95 ± 0.04 c	3.32 ± 0.05 c	3.19 ± 0.06 c	3.47 ± 0.04 de
MFP	3.98 ± 0.10 c	4.03 ± 0.01 d	3.34 ± 0.07 c	3.19 ± 0.09 c	3.48 ± 0.06 de
UFP	nd	3.89 ± 0.00 c	3.38 ± 0.05 c	3.09 ± 0.06 bc	3.39 ± 0.03 d
NFP	nd	3.37 ± 0.05 a	2.45 ± 0.06 a	2.09 ± 0.01 a	2.46 ± 0.14 a
MFR	3.48 ± 0.01 ab	3.92 ± 0.03 c	3.54 ± 0.04 d	3.51 ± 0.02 d	3.53 ± 0.01 e
UFR	2.73 ± 0.01 a	3.68 ± 0.04 b	2.95 ± 0.12 b	2.97 ± 0.00 b	2.86 ± 0.03 ab
NFR	nd	3.89 ± 0.03 c	3.14 ± 0.03 b	2.98 ± 0.05 b	3.2 ± 0.04 c
	Ni	**Zr**	**Nb**	**Ba**	**Pb**
AW	2.87 ± 0.06 a	2.57 ± 0.07 ab	nd	3.18 ± 0.03 b	4.26 ± 0.03 e
pH adj	2.80 ± 0.00 a	3.36 ± 0.14 c	3.53 ± 0.06 b	3.35 ± 0.04 c	4.12 ± 0.06 d
MFP	2.93 ± 0.09 a	3.64 ± 0.11 e	3.94 ± 0.07 c	3.42 ± 0.08 c	4.12 ± 0.02 d
UFP	2.77 ± 0.04 a	nd	nd	3.19 ± 0.03 b	3.72 ± 0.02 b
NFP	nd	nd	nd	2.63 ± 0.09 a	3.23 ± 0.02 a
MFR	nd	3.26 ± 0.04 bc	3.45 ± 0.01 b	3.57 ± 0.00 d	3.99 ± 0.00 c
UFR	2.84 ± 0.21 a	2.34 ± 0.11 a	2.97 ± 0.23 a	2.90 ± 0.01 a	3.64 ± 0.01 b
NFR	nd	nd	nd	3.44 ± 0.02 c	3.81 ± 0.03 b

3.2.4. Sinapis alba root and shoot elongation assay

Based on the EC_{20} values for shoot elongation inhibition in *S. alba*, significant toxicity mitigation was achieved through pH adjustment of the acidic waste (AW (toxicity (AW $EC_{20\ shoot} = 1284x$, pHa AW $EC_{20\ shoot} = 415x$), a mitigation further enhanced by microfiltration (MFP $EC_{20\ shoot} = 244x$) for shoot elongation (Fig. 4). However, the application of ultra- and nanofiltration did not yield a statistically significant additional reduction in toxicity of permeates. The toxic impact of the retentate samples based ont he EC_{20} values exhibited an ascending pattern: MFR=UFR < NFR.

Similar trends were observed in the case of root elongation inhibition in *S. alba* as seen in shoot inhibition (AW $EC_{20\ root} = 2302x$, pHa AW $EC_{20\ root} = 419x$, (MFP $EC_{20\ root} = 370x$). However, the degree of toxicity attenuation was more pronounced in root elongation inhibition when comparing the effect of AW to the treated technological samples. In general, according to the EC_{20} and EC_{50} values for shoot and root elongation inhibition, NFP proved to be the less toxic treated technological sample (NFP $EC_{20\ root} = 189x$, NFP $EC_{20\ shoot} = 175x$), while NFR demonstrated higher toxicity compared to other treated samples (Fig. 4).

While the effect of some toxic metals, e.g. As, Cd, Cu, Fe, Ni, Pb, Zn on S. alba is well documented [62–64], the effect of a large set of metals and metalloids, in particular, rare earth elements (RERs) are scarcely described [65]. Predicting metal mixture toxicity on

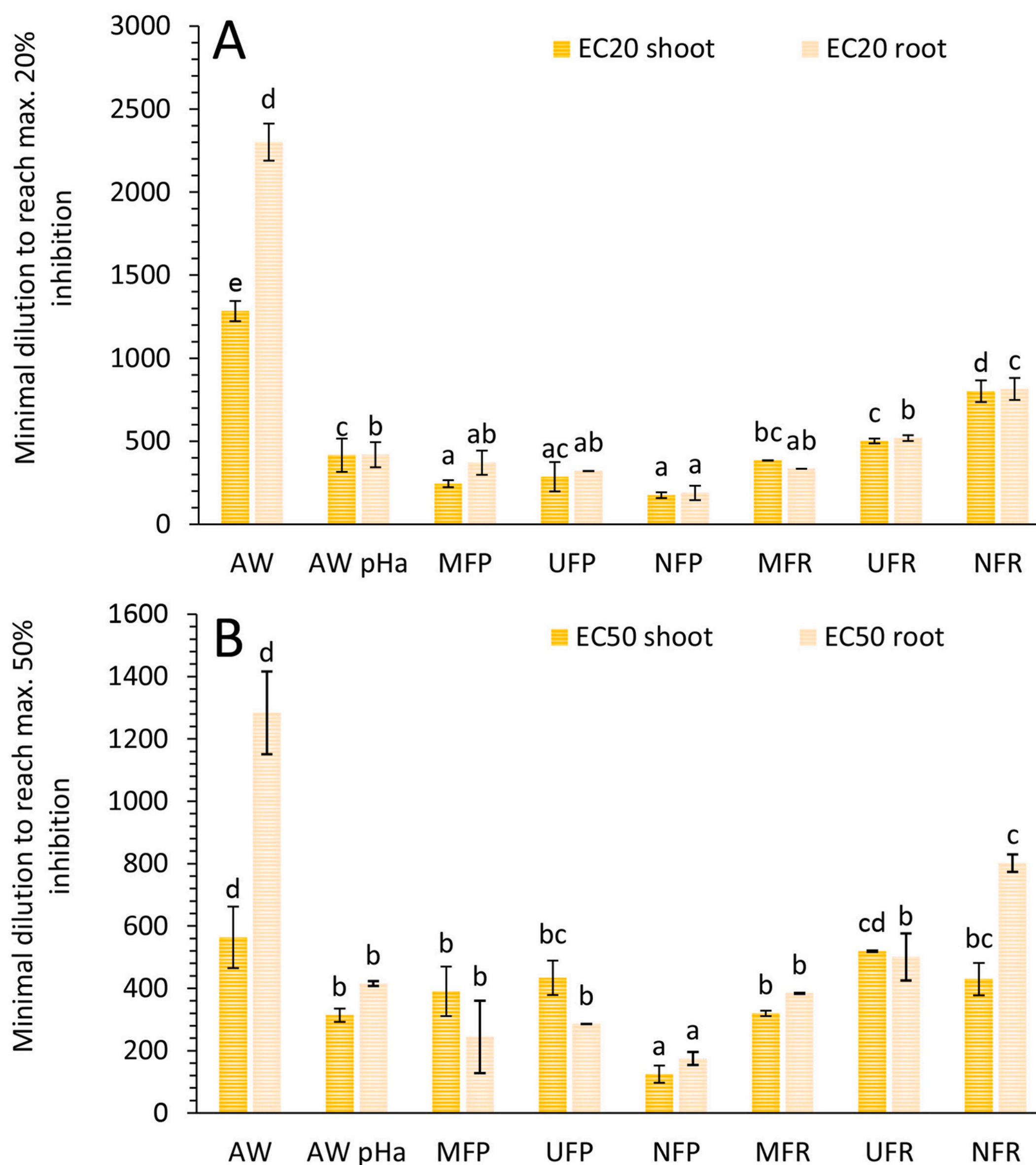

Fig. 4. EC_{20} and EC_{50} values given in dilution factor units determined for the samples of the pilot-scale arNF technology process in the case of the *S. alba* root and shoot elongation assay. In the diagram statistical significance distinctively for 24 and 48 h exposure time is marked by lower case letters. The significant effects are marked with letters in the figures and tables in alphabetical order, where "a" is the smallest value. Values signed with the same letter indicate that there was no significant difference between them.

plants is even more biased by the fact that a variety of metals (Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Se, Zn) are essential nutrients that are required for various biochemical and physiological functions [66], while others (Al, Sb, As, Ba, Be, Bi, Cd, Ga, Ge, Au, In, Pb, Li, Hg, Ni, Pt, Ag, Sr,Te, Tl, Sn, Ti, V, U) have no biological functions and considered as non-essentials [67,68]. In the case of *S. alba*, where essential metals in physiologically beneficial concentration may mitigate the toxicity of metals that have been proven to exert adverse effect on seed germination, root and shoot elongation or plant physiology, predicting mixture toxicity in LCIA approaches is considered even more critical [11].

3.2.5. Toxicity attenuation evaluation

Based on the results of the ecotoxicity assays the efficiency of the applied pilot-scale arNF technology was assessed in terms of toxicity attenuation. According to the relative toxicity of the UFP and NFP samples compared to the technology input material (AW) (Fig. 5), in terms of EC_{20} values, the consecutive steps of ultrafiltration and nanofiltration lowered toxicity by 66 % and 73 %, respectively, as determined by the *A. fischeri* bioluminescence inhibition assay (30 min). Toxicity attenuation rates were also assessed using *D. magna* (24 h) and *S. alba* root and shoot elongation inhibition tests (72 h): results showed reductions of 84 % and 87 %, 86 % and 77 %, and 78 % and 86 % in toxicity, respectively. These reductions were statistically significant compared to the original input material (AW) across all tests.

Nevertheless, it is important to highlight that while the toxicity attenuation achieved with the pilot-scale arNF technology was slightly less pronounced compared to the laboratory-scale arNF technology (the toxicity of AW was decreased by 96 %) [31], as indicated by the results of the *A. fischeri* bioluminescence inhibition and *D. magna* lethality tests, the toxicity attenuation achieved at pilot-scale remains notably high based on the NFP EC_{20} values (73–87 %). It is crucial to note that the comparison between the results of these two different technology experiments is not directly comparable due to evident timely variations in the composition of the acid liquid waste (AW) provided by the waste owner and the slight modifications in technology parameters in the case of the scaled-up experiment.

These findings demonstrate that the consecutive filtration steps effectively mitigate toxicity, making the waste and intermediate byproducts safer for environmental handling. The ecotoxicity test methods employed were also proven to be suitable for characterizing the environmental efficiency of the pilot-scale arNF technology process for ore processing related acidic liquid waste. We recommend using the term “industrial ecotoxicology” for this type of ecotoxicological toolkit application. Although “green toxicology” exists, it covers a broader methodology, including green and sustainable product development [7], the application of fast, easy-to-apply toolkits for the assessment of technological performance at the bench, pilot or even industrial scale deserves a separate and less broad term that can be applied for similar methodologies recommend also by Römbke [69].

3.3. Genotoxicity evaluation

The Ames plate incorporation method [70–72] and SOS Chromotest [73,74] have been widely employed in evaluating the genotoxicity of various industrial waste streams and waste leachates. Conversely, the high-throughput Ames MPF method is less commonly utilized in industrial toxicity assessments [75]. Hence, we decided to investigate its suitability for our purposes.

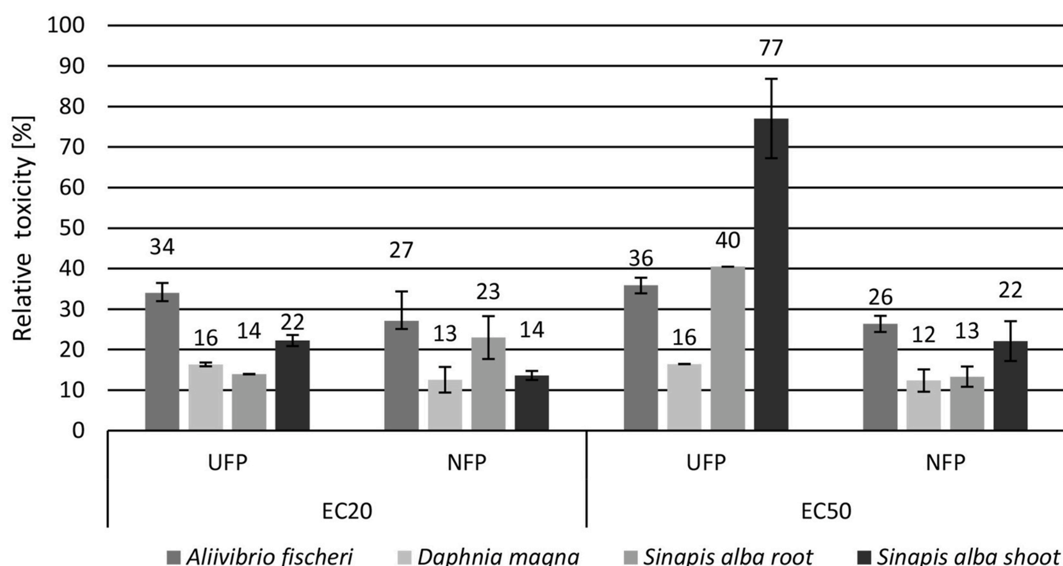

Fig. 5. Toxicity attenuation based on EC_X values in the applied ecotoxicity test systems. Relative toxicity was calculated by dividing the dilution factor corresponding to UFP and NFP samples with the dilution factor of the initial AW sample (e.g. y = 50 %, when the dilution factor required for 20 % inhibition is halved). In the case of the *D. magna* lethality test the results of the 24-h-long exposure were used.

3.3.1. Ames (Salmonella typhimurium) reverse mutation test

The result of the 50x dilution of the AW, MFR, UFR, NFR and the 100x dilution of the AW samples were invalid due to severe cytotoxic effects. According to the results presented in Table 3, possible genotoxic effects cannot be excluded based on the CR and Fold rule Criteria in the case of the applied TA100 strain. The highest revertant rates compared to solvent control were found in the case of UFP, UFR, NFP and NFR samples, however, it has to be noted that concentration dependence was weak and the increase in revertant colony number compared to the concurrent solvent control was slightly above 2 (typically 2.02–2.34) (Table 3).

Earlier research has shown that, in addition to radionucleotides, certain heavy metals exhibit genotoxic properties. Nickel (Ni) and lead (Pb), even at low concentrations, have been demonstrated to induce chromosomal aberrations in *Allium sativum* [76]. Furthermore, contamination of natural surface water samples with a mixture of heavy metals led to an increase in chromosome aberration frequency in the root meristem of *Allium cepa* and elevated levels of DNA breaks in peripheral blood erythrocytes of *Oreochromis niloticus* [77]. Hussein Kehinde et al. [78] also reported the genotoxic effects of heavy metal contaminated environmental samples on different fish species. Considering that metals are concentrated in the retentate samples and in the nanofiltration permeate, it is reasonable to expect potential genotoxic effects in the UFR, NFR and NFP samples.

3.3.2. Ames MPF assay

The cytotoxic effect on the *S. typhimurium* TA100 strain was robust across all samples in the small-volume test system using a liquid medium, rendering the results of the concentrated samples unreliable.

3.3.3. SOS chromotest

All samples exhibited a strong cytotoxic effect on the SOS Bacteria, therefore, the results of the concentrated samples are invalid. However, we can put our samples in order based on the dilution at which they are non-cytotoxic and non-genotoxic: NFP (64 times dilution) < AW pHa = MFP = MFR = UFR (128 times dilution) < AW = UFP = NFR (256 times dilution). A reduction of cytotoxicity was observed in the NFP sample, and AW pHa, MFP, MFR and UFR were also less cytotoxic than the original AW. It is also notable that in most samples at least 128–256 times inhibition is needed to ensure non-cytotoxic and non-genotoxic effects.

3.4. Recommendations on the integration of DTA results into the LCIA and the green toxicology concept

While numerous authors have verified that the methods intended for categorizing products under the Classification, Labelling, and Packaging Regulation (CLP) [79–81] are inappropriate for testing wastes [82], a unified testing strategy remains elusive. Furthermore, combining an appropriate array of biological test methods and chemical analyses is imperative for the comprehensive ecotoxicological characterization of wastes [69,83,84]. To address these issues, our DTA results may offer reliable information for decision-makers involved in waste management, product design, and regulatory compliance. Authors are convinced that by using DTA results in LCIAs, decision-makers can make more informed choices to mitigate environmental impact and promote sustainable practices. Considering the pivotal role of Life Cycle Impact Assessment (LCIA) in determining the most environmentally friendly pathway for a given product, our ecotoxicity assessment results can contribute to evaluating the impact of input and output parameters associated

Table 3
Effect of the arNF samples on the ratio of revertants per plate in the standard Ames plate incorporation assay. The significant effects are marked with letters distinctively for each sample alphabetically, where "a" is the smallest value. Values signed with the same letter indicate that there was no significant difference between them. Red color highlights values that exceed the threshold value of the Fold Rule Criterion (FRC), while the numbers written in bold indicate those cases where the value closely approaches the FRC threshold.

Dilution	AW	MFP	UFP	NFP
50x	0.00 ± 0.00*	**1.90 ± 0.15 c**	**2.28 ± 0.14 c**	**2.68 ± 0.21 c**
100x	0.00 ± 0.00*	1.52 ± 0.11 b	**2.24 ± 0.05 c**	**2.09 ± 0.05 b**
150x	**2.07 ± 0.06 c**	1.31 ± 0.01 a	**1.80 ± 0.08 b**	1.13 ± 0.06 a
200x	1.64 ± 0.11 b	1.27 ± 0.02 a	1.57 ± 0.06 a	0.90 ± 0.06 a
250x	1.68 ± 0.05 b	1.13 ± 0.04 a	1.43 ± 0.22 a	0.99 ± 0.07 a
300x	1.26 ± 0.05 a	nd	nd	nd
Dilution	**AW pHa**	**MFR**	**UFR**	**NFR**
50x	**2.34 ± 0.14 d**	0.30 ± 0.19*	0.00 ± 0.00*	0.00 ± 0.00*
100x	1.56 ± 0.11 c	1.19 ± 0.08 ab	**2.34 ± 0.05 b**	**2.25 ± 0.09 c**
150x	1.34 ± 0.23 b	1.23 ± 0.08 b	**2.02 ± 0.13 a**	**1.83 ± 0.04 b**
200x	1.14 ± 0.06 a	1.03 ± 0.05 a	**1.97 ± 0.08 a**	1.73 ± 0.09 b
250x	1.08 ± 0.10 a	1.01 ± 0.06 a	**1.91 ± 0.05 a**	1.50 ± 0.07 a

*: not applicable due to cytotoxicity

with both the product and its accompanying technology. This holistic approach is crucial for ensuring the long-term sustainability of energy sources, human health, climate, and biodiversity.

Despite a consensus regarding the utilization of USEtox (UNEP/SETAC Scientific Consensus Model), stakeholders continue to engage in discussions regarding the appropriate methodologies for characterizing ecotoxicity in LCIA. The ongoing debate is fueled by both conceptual and practical challenges [84]: the necessity to estimate impacts within an inherently intricate technical and natural system, encompassing numerous chemicals across various environmental compartments, each presenting varying degrees of exposure and species sensitivity. Secondly, the associated impacts must be estimated or extrapolated from restricted data pertaining to ecotoxicological endpoints, frequently only obtainable through laboratory conditions [11]. For the sake of example, the degree of metal toxicity varies based on numerous factors, such as the dosage, exposure route and specific chemical forms of the metals, as well as the age, gender, genetics, and nutritional health of the exposed organisms [67,85] therefore several challenges limit the accurate prediction of these multicomponent metal mixture in complex wastes and environmental compartments.

To facilitate discussions on present ecotoxicity practices in the current waste characterization of industrial processes, this study serves as a component of the broader endeavor aimed at harmonizing ecotoxicity characterization within LCIA and addresses key questions that have been already raised by the Global Guidance on Environmental Life Cycle Impact Assessment Indicators (GLAM) project [80]. These key questions include: (i) How can we incorporate additional ecotoxicity-related impact pathways, exposed organisms, and environmental compartments based on available evidence and data? (ii) How should we manage chemical mixtures in the environment and address mixture toxicity, particularly regarding combined exposure to multiple metals from the same emission source? (iii) Concerning metals and their essentiality, what implications arise when certain emitted metals occur below toxicologically relevant levels for different ecosystems? (iv) While the recognition of metal essentiality exists, it is presently regarded as less pertinent for ecotoxicity characterization, primarily due to constraints in data and the option of separately modelling species-specific benefits from negative effects within the same metal concentration range.

Our results of the *D. magna* bioaccumulation test drew the attention to the fact that especially in the case of multi-metal containing wastes how intentional pH adjustment could alter bioavailability, hence toxicity. Nevertheless, the pH adjustment of waste samples prior to ecotoxicity testing remains a general recommendation of current guidelines on waste risk characterization [86]. The fact that only one out of the three different genotoxicity test methods proved suitable for characterizing samples with extreme pH also highlights the possibility that certain methods may have limitations for specific types of technological and waste samples. Therefore, the development of waste-specific ecotoxicological toolkits becomes even more important. While the high-throughput SOS Chromotest and Ames MPF test are faster and more cost-effective methods compared to the traditional Ames plate incorporation method, in our case, the latter proved to be feasible.

Our findings underscore the necessity for a waste-specific and case-by-case test battery employing harmonized experimental methodologies in the ecotoxicity assessment of diverse waste types. This need is highlighted by the absence of a consensus at the European Union level [87]. This holds significant importance as incorrect classification of the risk and hazard associated with a specific waste can result in improperly assessed environmental and human health risks, along with potential financial repercussions for waste owners. Moreover, our ecotoxicological findings underscore the environmental benefits of NF-based Sc recovery technology over conventional solvent extraction methods, all while avoiding the generation of additional technology waste streams.

We recommend to introduce the term „industrial ecotoxicology" which concept focuses on the assessment of the environmental efficiency of industrial processes and technologies ensuring a holistic and rigorous evaluation process that considers both their intended benefits and potential environmental impacts, thereby promoting the development and adoption of truly sustainable solutions. By integrating industrial ecotoxicology into the evaluation process and into the early phase of technology development, we foster a culture of continuous improvement in green technology development. By identifying areas of concern and addressing them proactively, we can enhance the environmental performance of these technologies over time, leading to more sustainable outcomes, most importantly relying on direct toxicity assessment results over prediction tools.

4. Conclusions

We carried out the chemical and ecotoxicological characterization of byproducts and waste flows from a pilot-scale acid-resistant nanofiltration (arNF) technology experiment to evaluate its environmental efficiency of the treating of acidic liquid waste (AW) from the ore processing industry solely based on filtration techniques. The most important findings of the study were the following:

- The ecotoxicity toolkit established for the environmental risk characterization of the samples from the laboratory-scale arNF technology experiment proved to be feasible in the scaled-up technology experiments.
- All toxicity assays revealed a high and statistically significant reduction in toxicity due to the three filtration steps and proved to sensitively and comprehensively characterize the aggregated effects of the multi-component technology samples.
- Our study underscores the importance of genotoxicity evaluation of samples with naturally occurring radioactive material and revealed no considerable potential risk to human or occupational health in the case of accidental leakage or technology accidents.
- Based on a comprehensive ecotoxicity and genotoxicity assessment of the pilot-scale arNF technology applied to scandium (Sc) recovery, we conclude that this technology can be considered a promising waste valorization approach of Sc production - a critical raw material with significant future potential - with the added benefit of substantial environmental risk mitigation.

We propose a conventional and scientifically rigorous procedure for assessing the ecotoxicity of similar waste streams that can be applied in environmental risk assessment and LCIA of similar waste-reuse and critical raw material (CRM) recoverytechnologies. We

suggest complementing similar ecotoxicity assessments with bioaccumulation studies and genotoxicity assessments to assess the broader potential impact of the applied and generated wastes, mid-term products, and products of similar environmental technologies. Further research may cover other important aspects of exposure (e.g. biomagnification or multigenerational effects), but easy-to-perform and widely available techniques are needed in this field so that they can be included in industrial ecotoxicology studies. The problem of the low (or high) pH in genotoxicity studies should be considered when developing and adapting novel methodologies. In the case of industrial wastes and byproducts with similar characteristics as the samples described in our study, we suggest applying and implementing the laboratory-scale derived ecotoxicity toolkits to scaled-up versions of the technology to follow green ecotoxicology guidelines and the safety-by-design concept. The combination of advanced filtration techniques for the recovery of valuable raw materials could become a valuable addition to various industries, especially when membranes with enhanced water permeability become readily available. For instance arNF could be used for acidic mine tailings or washing water from fly ash treatment in waste incineration plants. By developing safer and environmentally benign technologies for CRM recovery by assessing their environmental risks at all steps of technology development, we can contribute to reducing currently unexplored but valuable waste and enhancing the circular economy in Europe and worldwide.

Data availability statement

Data associated with this study have not been deposited into a publicly available repository. The data that support the findings of this study are available from the corresponding author upon request.

Ethics declarations

Informed consent was not required for this study since it did not involve medical research. During the research the Daphnia magna (water flea) animal test system was used for ecotoxicity testing, which is an invertebrate species; thus, Directive 2010/63/EC does not apply and no authorizations were needed to carry out the work. Nevertheless, we strictly followed the "principle of the Three Rs" of replacement, reduction and refinement (2010/63/EC).

CRediT authorship contribution statement

Ildikó Fekete-Kertész: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Rita Márton:** Investigation, Data curation. **Mónika Molnár:** Supervision, Project administration, Funding acquisition, Conceptualization. **Zsófia Berkl:** Investigation, Data curation. **Sebastian Hedwig:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Viktória Feigl:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33799.

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