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# Assessment of acute and chronic ecotoxicological effects of aqueous eluates of stone wool insulation materials

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## Abstract

**Background** Stone wool is an inorganic mineral insulation material increasingly used to reduce the climate impact of buildings. The acute and chronic ecotoxicological potential of stone wool eluates have been studied in a battery of standardized laboratory ecotoxicological tests. The experiments were conducted with stone wool test materials in fibrous and milled form, with and without the presence of organic binder. For the preparation of eluates, the OECD protocol on the transformation/dissolution of metals and metal compounds was applied. The resulting eluates were used in acute tests, i.e., bioluminescence test with *Aliivibrio fischeri* (DIN EN ISO 11348-1:2009), algae growth test with *Desmodesmus subspicatus* (OECD No. 201) and immobilization test with *Daphnia magna* (OECD No. 202), as well as chronic tests, i.e., the *Daphnia magna* reproduction test (OECD No. 211) and the nematode growth and reproduction test with *Caenorhabditis elegans* (ISO 10872:2010).

**Results** While no acute or chronic ecotoxicological effects of the eluates were observed for fibrous stone wool material, the milled test materials showed some chronic effects on aquatic invertebrates. Depending on the test materials and concentrations of milled stone wool used in the eluate preparation, these chronic effects included significant stimulation or inhibition of daphnid reproduction and nematode growth. The chemical analysis conducted in parallel to the ecotoxicological assessment indicated no leaching of organic substances from the applied binder or mineral oils and no formation of nanoparticles by the milling of stone wool. Furthermore, ICP-MS and ICP-OES analysis of eighteen elements revealed that only aluminum and nickel could be quantified in the eluates, at concentrations of approximately 750 µg/L and 7 µg/L, respectively.

**Conclusions** Based on the present ecotoxicological assessment, eluates from stone wool fibers cannot be considered as chemically hazardous to the aquatic environment. However, additional investigations of the ecotoxicological potential of the milled material and the environmental exposure of stone wool products are necessary for a complete evaluation of potentially negative effects of stone wool insulation materials.

**Keywords** Aquatic ecotoxicology, Construction product, Elution, *Aliivibrio fischeri*, *Desmodesmus subspicatus*, *Daphnia magna*, *Caenorhabditis elegans*

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## Background

One of the biggest challenges in the coming years will be to slow down anthropogenic climate change and reach the goal of the 2015 United Nations Paris agreement. This agreement aims to keep the increase of average global temperature well below 2 °C compared to pre-industrial levels, with a target maximum increase of 1.5 °C [5]. According to the fifth report by the Intergovernmental Panel on Climate Change (IPCC), in 2010 buildings represented approximately 20% of global energy-related greenhouse gas emissions and 30% of total global final energy consumption [26]. By representing approximately one-third of this consumption [26], space heating is a major contributor to climate change. Nevertheless, according to the European Commission about 75% of EU buildings are currently energy inefficient [9]. Therefore, an effective measure towards keeping the Paris agreement is to decrease the climate impact of buildings using proper thermal insulation during construction or renovation campaigns [20, 27, 39].

Currently, the main commercially applied insulation products are organic petroleum-based foams and synthetic mineral wools [1, 20]. The high production volumes and the widespread applications of these materials demand the evaluation of their potential impacts on the environment. Especially when considering inorganic mineral insulation materials like stone wool or glass wool, ecotoxicological knowledge is very limited. In fact, no published studies on the ecotoxicity of mineral wool could be obtained in a search of scientific literature, while the evidence currently summarized in the REACH dossier indicates no ecotoxicological effects. Vitreous mineral wool fibers, as the basic component of the various insulation products, are registered under REACH (EC 1907 [10]) as "Man-made vitreous (silicate) fibers [MMVF] with random orientation with alkaline and alkali earth oxides ($Na_2O + K_2O + CaO + MgO + BaO$) content greater than 18% by weight and fulfilling one of the CLP Regulation Annex VI Note Q conditions". As mentioned in their registered name, MMVF Note Q fibers are composed mainly of silicate and oxides, leading to compositions that are similar to soda-lime silicate glass and basaltic glass for glass wool and stone wool, respectively. The chemical composition of the fibers is intensely monitored as it is essential for their biosolubility, which means the products dissolve in body fluids and are quickly cleared from the lungs. This accounts for the fact that they are not classified as carcinogenic [16]. Nevertheless, considering the expected increasing production and use of insulation materials, ecotoxicological evaluations of commercially available products are required in order to avoid unintended effects on the environment.

For construction products, ecotoxicological testing is often not straightforward due to the limited water solubility as well as heterogeneous properties and complex compositions of these materials. However, the usefulness of testing aqueous eluates of construction products to assess ecotoxicological implications of these materials has been reported repeatedly [2, 12, 18, 23, 25, 37]. Recently, Heisterkamp et al. [17] have shown in an inter-laboratory test with 29 European laboratories that ecotoxicity testing of construction product eluates with four aquatic test systems (luminescent bacteria, green algae, daphnia and zebrafish eggs) is adequately reproducible and a suitable method to study the environmental impact of chemicals released by test materials. An important step in the process is the selection of an elution protocol, which is appropriate for the particular test material. A wide range of standardized elution protocols are available, for example the one stage batch test (DIN EN 12457-1), the dynamic surface leaching test (CEN/TS 16637-2:2014) or the column leaching test (CEN/TS 16637-3:2016). Lillicrap et al. [25] used the OECD guideline No. 29 on the transformation/dissolution of metals and metal compounds [28] for producing eluates from silica fumes which are used in high-strength concrete. As the stone wool test materials are composed of silica and metal oxides, it was decided to likewise apply the OECD No. 29 protocol for the elution procedure in this study. Additionally, the transformation/dissolution protocol provides the advantages of a relatively long contact time between aqueous medium and test material, favorable for sparingly soluble compounds like basaltic glasses, and the usage of a standardized freshwater as the elution medium. Thus, the application of the eluates in ecotoxicity tests was possible with minimal modifications.

The aim of the present study was to analyze if acute and chronic ecotoxicological effects can be induced by eluates of stone wool. A fibrous and two milled stone wool samples were prepared from a commercial stone wool insulation product. The preparatory steps depended on the respective sample and included sieving, milling and heating of the stone wool to remove binder and mineral oils, which are used to adhere the mineral fibers together for commercial use. The test battery applied covered species from several trophic levels, namely the luminescent bacterium *Aliivibrio fischeri*, the green algae *Desmodesmus subspicatus*, the planktonic crustacean *Daphnia magna* and the nematode *Caenorhabditis elegans*. Concomitantly, chemical analyses were performed to detect a potential release of organic and inorganic substances from the test material.

## Materials and methods

### Test materials and their preparation

All test materials were prepared from a common stone wool insulation product sample. The chemical composition of the bulk sample was analyzed by X-ray fluorescence spectroscopy, according to the methods described by Barly et al. [3]. Briefly, the organic constituents were removed by heating to 590 °C for 20 min. Next, the sample was milled and 0.75 g of sample was mixed with approximately 9 g of $Li_2B_4O_7$. The mixture was melted into a pellet with an electric furnace (X-300, Katanax, Canada) and subsequently analyzed with a ARL Advant'X 2095 XRF IntelliPower (Thermo Fisher Scientific, USA). In this way the stone wool was found to contain 42.0% $SiO_2$, 18.8% $Al_2O_3$, 0.7% $TiO_2$, 6.2% $Fe_2O_3$, 19.3% CaO, 8.6% MgO, 1.8% $Na_2O$, 0.8% $K_2O$, 0.8% $P_5O_5$ and 0.2% MnO. Subsamples of the product sample were then used to prepare the test materials of this study. An overview of the test materials used for elution is given in Table 1. Briefly, a stone wool fiber sample without binder as well as milled stone wool samples with and without cured phenol–urea–formaldehyde (PUF) binder were included in the experiments (PUF binder composition as described in Okhrimenko et al. [32]).

Approximately 300 g of each material were prepared according to Table 1. To remove the organic binder and mineral oils from the materials, stone wool samples were heated at 590 °C for 20 min. This temperature was chosen to prevent crystallization of the amorphous fibers. Next, test material # 1 was sieved to exclude any material greater than 63 µm to remove non-fiber material left over from the production process ("shots") in order to generate more homogenous test materials. Additionally, to simulate a worst-case scenario with maximum leaching from the test materials, stone wool fibers with and without binder (test materials # 2 and # 3) were milled for 45 s in a tungsten carbide vessel in order to increase their surface area.

To characterize the test materials, their specific surface area was determined via the Brunauer–Emmett–Teller (BET) method. Prior to BET measurements samples were degassed under vacuum at 50 °C for 2 h. Afterwards, nitrogen adsorption isotherms were recorded in the relative pressure range $0.1 < P/P_0 < 0.3$ at 77 K using Quantachrome Autosorb-1 analyzer.

### Preparation of eluates

To characterize the ecotoxicological potential of the test materials, eluates of the test materials were prepared based on the OECD No. 29 Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media [28]. Briefly, the test materials were weighted (1 mg, 10 mg or 100 mg) and suspended in brown glass bottles in 1 L medium (standardized freshwater; Additional file 1: Table S1). Subsequently, the glass bottles were placed on a laboratory shaker set to 100 rpm and left shaking in the dark at room temperature for the elution period of 7 d. Afterwards, the eluates were filtered (0.45 µm nitrocellulose filter, OE 67, Whatman, UK) and then used for chemical analyses (within max. 72 h) and ecotoxicological testing (within max. 24 h).

To determine the effects of pH on the dissolution of metals from the test materials, a pH screening test at pH 6, pH 7 and pH 8 was carried out prior to ecotoxicological examinations (for more details see Additional file 1: Text S1).

For the ecotoxicological tests, the medium with pH 8 (Additional file 1: Table S1) was chosen for the elution, as no clear differences in metal release at different pH values were observed (Additional file 1: Figs. S1 and S2) and using pH 8 medium brought methodological advantages (see also Additional file 1: Text S2). Medium without the addition of test material, treated in the same way as the samples, was used as a procedural blank and negative control in the chemical analysis and ecotoxicity testing. Generally, the aim was to use the eluates as unmodified as possible in the ecotoxicity tests. However, some modifications were necessary to provide optimal conditions for the test organisms. Thus, in the bioluminescence bacteria test NaCl (20 g/L; Bernd Kraft, ≥ 99.5%) was added to the filtered eluates; for the algae growth inhibition test the concentrations of two medium constituents ($CaCl_2 \times 2\ H_2O$ (Carl Roth, ≥ 99%) and $MgSO_4 \times 7\ H_2O$ (Carl Roth, ≥ 99%)) were reduced by 80% (Additional

**Table 1** Overview of test materials and preparation steps

| Test material number | Test material | Preparation steps | Binder |
|---|---|---|---|
| # 1 | Stone wool fibers without binder | Heat treatment at 590 °C for 20 min; sieving to < 63 µm | Removed by heat treatment |
| # 2 | Milled stone wool fibers without binder | Heat treatment at 590 °C for 20 min; milling | Removed by heat treatment |
| # 3 | Milled stone wool fibers sample with binder | Milling | PUF (3.5% (w/w)) |

file 1: Table S2) and micronutrients were added to the test solutions (Additional file 1: Table S3); in the acute and chronic *Daphnia* tests the eluates and blanks were aerated for 18 h before starting the tests; and in the chronic *Daphnia* test selenium dioxide (7 µg/L; AppliChem, ≥ 99%) was added to the test solutions.

For chemical characterization of the eluates, additional eluates with a concentration of 100 mg/L were prepared in duplicates for each test substance with pH 8 medium (Additional file 1: Table S1) and according to the methods described above. The samples for DLS (dynamic light scattering) analysis did not require further preparation and were measured within 48 h after filtration. The samples for ICP-MS (inductively coupled plasma mass spectrometry) and ICP-OES (inductively coupled plasma optical emission spectrometry) analysis were immediately acidified after filtration by adding 0.1% nitric acid (subboiled) and were subsequently measured within 72 h. The samples for analysis of NPOC (non-purgeable organic carbon) were immediately acidified by adding 0.01% hydrochloric acid (suprapure) and measured within 48 h.

## Chemical analysis

### *Elemental concentrations in the eluates*

*ICP-MS* The elements antimony (Sb), arsenic (As), boron (B), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), lead (Pb), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), tin (Sn), uranium (U), vanadium (V), and zinc (Zn) were analyzed in the undiluted eluates by ICP-MS. The stable mass lines used for the quantification of the elements are given in Additional file 1: Table S4. A quadrupole ICP-MS system (Elan DCR-e, PerkinElmer, Germany) operating at 1100 W plasma power and 1.05 L/min nebulizer gas flow was used for the analysis. Between each measurement, the instrument was flushed with 1% nitric acid (subboiled) for 15 s to avoid any memory effects. For each element, a linear calibration ranging from 0.1 to 100 µg/L was prepared (except 1 to 1000 µg/L for Cu and Zn). Prior to the measurements, yttrium (Y) and thulium (Tm) were added as internal standards to each calibration solution and eluate to achieve a concentration of 10 µg/L. Limits of detection (LOD) and limits of quantification (LOQ) are given in Additional file 1: Table S5.

*ICP-OES* Due to its occurrence in higher concentrations, aluminum (Al) was analyzed by ICP-OES in the undiluted eluates. The emission wavelength of 237.312 nm (Additional file 1: Table S4) was used for quantification and a linear calibration between 250 and 1000 µg/L was prepared. The ICP-OES system (Agilent 720 ICP-OES, Agilent, USA) was set to a plasma gas flow of 15 L/min, auxiliary gas flow of 1.5 L/min and radio frequency power of 1200 W. Between each measurement the instrument was flushed with 1% nitric acid (subboiled) for 15 s.

### *Non-purgeable organic carbon content of the eluates*

To check for leaching of organic substances from the test materials, the organic carbon concentration in the eluates was determined as NPOC with a TOC analyzer (TOC-L, Shimadzu, Japan), assuming that leaching organic compounds are mostly non-volatile. Information on calibration and instrument settings are provided in Additional file 1: Table S6, while LOD and LOQ are given in Additional file 1: Table S7.

### *Analysis of nanoparticles*

To check the eluates for the presence of nanoparticles which might have been formed during milling, dynamic light scattering (DLS) was performed with a Zetasizer Nano ZS ZEN 3600 instrument (Malvern, UK; laser $\lambda = 633$ nm) in a glass cuvette (PCS1115, Malvern, UK). For the particle size calculations (intensity distribution and number distribution), the refraction index (0.06) and the absorption (0.001) of $SiO_2$ was used and subsequently particle concentrations (particles per mL) were determined. Both experimental replicates for the eluates were analyzed separately and, if any particles were found, another independent sample was drawn from the eluate and the measurement was repeated to confirm results (technical replicate).

## Ecotoxicological testing

All ecotoxicological tests were carried out according to OECD or ISO guidelines with laboratory organisms cultured at the University of Duisburg-Essen, Germany, in the department of Aquatic Ecology. The culturing conditions are described in Additional file 1: Text S3).

### *Acute effects*

*Luminescence inhibition test* The inhibition of bacterial luminescence testing was based on DIN EN ISO 11348-1 [7]. Briefly, the exposure solutions were inoculated with cells of *Aliivibrio fischeri* and the bacterial luminescence was monitored over 30 min in the negative control (elution medium), in the eluates (100 mg/L) and two positive controls ($ZnSO_4$ and $K_2Cr_2O_7$). As the eluates were diluted 1:2 with *A. fischeri* inoculate, the actual exposure concentrations of the eluates and the positive controls are halved in this test. The validation criteria for this test are summarized in Additional file 1: Table S13.

The positive controls applied in the test were prepared by dissolving 219.8 mg/L $ZnSO_4 \times 7\ H_2O$

(Merck, ≥ 99%) or 22.6 mg/L $K_2Cr_2O_7$ (Sigma-Aldrich, ≥ 99%) in the elution medium. NaCl (Bernd Kraft, ≥ 99.5%) was added to positive controls, the negative control and filtered eluates to achieve a concentration of 20 g/L. All exposure solutions were cooled to 15 °C. An aliquot of 100 µL frozen *A. fischeri* suspension (FAU 2500 ± 500) was thawed and mixed with 0.5 mL of 20 g/L NaCl solution at 15 °C. After 15 min, another 11.5 mL of 20 g/L NaCl solution was added to this mixture and the bacterial suspension was homogenized by vortexing. The test was conducted in dark 24-well plates (Microplate, Greiner Bio-One) and 0.5 mL of the bacterial suspension was distributed to each well. The luminescence in each well was determined with a plate reader (Hidex Sense Microplate Reader, Hidex Oy, Finland) equipped with a photomultiplier tube (spectral range 230–850 nm). Four replicate wells for each test solution were set up by adding 0.5 mL of the test solution to the bacterial suspension in the wells. Luminescence measurements were repeated after 5 min, 15 min and 30 min incubation time.

*Algae growth inhibition test* Algae growth toxicity testing was based on OECD guideline 201 [30], as applied earlier (e.g., [22]). Briefly, eluates were inoculated with cells of the green algae *Desmodesmus subspicatus* and algae growth was monitored over 72 h in the negative control (elution medium), three eluate concentrations (1 mg/L, 10 mg/L and 100 mg/L) and a positive control ($K_2Cr_2O_7$). The validation criteria for this test are summarized in Additional file 1: Table S13.

To evaluate the sensitivity of the test system, a reference test with $K_2Cr_2O_7$ was conducted prior to testing the eluates. Five different concentrations of $K_2Cr_2O_7$ (between 0.05 and 4 mg/L) were prepared in modified elution medium (Additional file 1: Table S2). Likewise, eluates were prepared from the three sample materials, using the modified elution medium (Additional file 1: Table S2). Prior to starting an algae test, 80 µL of micronutrient supplement solution (see Additional file 1: Table S3) were added to each liter of medium (for positive reference solutions, negative controls and test eluates) to provide sufficient micronutrients for algae growth. All tests were carried out in 24-well plates (Tissue Culture Plate; VWR Chemicals) and the six replicates of each treatment were distributed on the plate in a semi-randomized design. The set-up of each plate included six replicates of negative controls. To start a test, in each well 2 mL of exposure solution were well mixed with algae inoculum (Additional file 1: Table S14) to achieve initial biomasses of *D. subspicatus* between $9.84 \times 10^3$ and $1.47 \times 10^4$ cells/mL (Additional file 1: Table S14). Algae growth was monitored every 24 h by fluorescence measurements with a plate reader (multimode reader Infinite M200, Tecan, Switzerland) set to the parameters stated in Additional file 1: Table S15. Fluorescence was subsequently converted to number of algae cells using a calibration curve (Additional file 1: Fig. S3) and to biomass using the factor of $3.5 \times 10^{-8}$ mg dry weight per cell, as suggested for *D. subspicatus* by OECD 201. The exposure period was 72 h and the pH value in each treatment was monitored at exposure start and end. Between fluorescence measurements, the plates were covered with Parafilm (Bemis, USA) and kept at room temperature on a laboratory shaker (Celltron shaker, Infors HT, Switzerland) set to 100 rpm, which was placed under a light source providing approximately 2220 lx (measured with a LI-250A light meter, LI-Core Biosciences, USA). Thus, the light intensity was significantly lower than the 4440 to 8880 lx recommended in the OECD guideline 201.

*Daphnia magna immobilization test* The *Daphnia magna* immobilization test was based on OECD guideline 202 [29], as applied earlier (e.g., [24]). Briefly, daphnids were exposed for 48 h to a negative control (pure elution medium), three eluate concentrations (1 mg/L, 10 mg/L and 100 mg/L) per test material and a positive control ($K_2Cr_2O_7$). The mobility of the animals was recorded after 48 h of exposure. The validation criteria for this test are summarized in Additional file 1: Table S13.

To determine the sensitivity of the test system, a reference test with $K_2Cr_2O_7$ as a reference substance was applied prior to testing the eluates. Five different concentrations of $K_2Cr_2O_7$ (between 0.32 and 3.2 mg/L) were prepared in the elution medium. Prior to starting a test, the exposure solutions were aerated for 16 to 18 h, to provide sufficient oxygen conditions for the test animals. Afterwards, four replicates of 20 mL exposure medium for each treatment were measured into 50-mL glass beakers. Five juvenile daphnids (< 24 h) were carefully placed in each beaker, transferring as little medium as possible to the exposure vessels. The test animals were visually checked for activity after the transfer, before placing the beakers in the dark at room temperature. After 48 h, the mobility of *D. magna* was visually inspected. According to OECD guideline 202, daphnids were considered immobile if they did not move within 15 s of gentle agitation of the exposure vessel, thus this endpoint includes both dead and alive immobilized animals. Oxygen concentration and pH of the different treatments were measured before the exposure start and after the exposure end.

#### *Chronic effects*

*Daphnia magna reproduction test* The *D. magna* reproduction test was based on OECD guideline 211 [31], as

described earlier [42]. Briefly, the reproductive output of daphnids was monitored over an exposure period of 21 d for a negative control (pure elution medium), three eluate concentrations (1 mg/L, 10 mg/L and 100 mg/L) per test material and a positive control ($K_2Cr_2O_7$). The validation criteria for this test are summarized in Additional file 1: Table S13.

To verify the sensitivity of the test system, a reference test with $K_2Cr_2O_7$ was conducted. Five different concentrations of $K_2Cr_2O_7$ (between 0.05 and 0.5 mg/L) were prepared in modified Aachener Daphnia Medium (ADaM, see Additional file 1: Table S11). For the eluate toxicity testing, eluates were freshly prepared. After completing the elution and filtering, 100 µL of selenium dioxide ($SeO_2$) stock solution (70 mg/L) was added to each liter to achieve a concentration of 7 µg/L in the exposure solutions [21]. Prior to starting a test, the exposure solutions were aerated for 16 to 18 h, to provide sufficient oxygen conditions for the test animals. Subsequently, ten replicates of 50 mL exposure medium for each treatment were added to 50 mL glass beakers. One juvenile daphnid (< 24 h) was carefully placed in each beaker, transferring as little medium as possible to the exposure vessels. The test animals were visually checked for activity after the transfer. During the 21-day exposure period, the test animals were fed regularly with fresh *D. subspicatus* cells (for details see Additional file 1: Text S3). The exposure medium was renewed once a week using eluates which were freshly prepared, filtered, supplemented with $SeO_2$ and aerated. The oxygen concentration and pH in old and new exposure media were measured at every exposure medium change. Eluates of material #1 were tested first, followed by parallel testing of eluates of the materials #2 and #3. Due to this testing regime, the positive and negative controls were the same for eluates #2 and #3.

*Caenorhabditis elegans (Nematoda) growth, fertility and reproduction test* The *Caenorhabditis elegans* test was based on ISO 10872 [19], as described earlier [38]. Briefly, first-stage larvae (L1) were exposed for 96 h to a negative control (pure elution medium), three eluate concentrations (1 mg/L, 10 mg/L and 100 mg/L) of each of the three test materials and a positive control (benzylcetyldimethylammonium chloride; BAC-C16). As the eluates were diluted 1:2 with *Escherichia coli* feed stock, the actual exposure concentrations were halved. After the exposure period the growth, fertility and reproductive output of the nematodes were determined. The validation criteria for this test are summarized in Additional file 1: Table S13.

To evaluate the sensitivity of the test system, a reference test with BAC-C16 as a reference substance was conducted. Seven different concentrations of BAC-C16 (between 2.5 and 40 mg/L) were prepared in pH 8 elution medium. For the eluate toxicity testing, eluates were freshly prepared as described above. To start a test, a 5- to 6-day-old Nematode growth medium (NGM) plate with abundant hermaphrodites was selected, the nematodes were washed from the plate using M9 medium (Additional file 1: Table S16) and synchronized using alkaline hypochlorite solution to obtain first-stage larvae (L1). The synchronization procedure is described in more detail in Additional file 1: Text S4. Prior to starting each test, between 9 and 41 L1 were randomly selected and transferred to an Eppendorf tube using a binocular (Olympus SZX9). The volume inside the tube was adjusted to 1 mL with M9 medium and 0.5 mL Rose Bengal solution (0.3 mg/L) were added to stain the nematodes and simplify the recovery of the animals. The tube was placed inside a water bath at 80 °C for 15 min to kill and straighten the nematodes. The body length of these "initial" larvae was determined at the microscope (Olympus BH2) by taking pictures (Moticam 2300; Motic Microscopy) and measuring with the Motic Images Plus 2.0 software. These measurement results were averaged to determine the mean initial body length of the larvae used in the tests (Additional file 1: Table S17). Simultaneously, an *E. coli* feed stock was prepared as described in Additional file 1: Text S5.

The experiment was set up in 12-well plates (Tissue Culture Plate; VWR Chemicals) by adding 0.5 mL feed stock and 0.5 mL test solution (eluates, negative and positive control) to four replicate wells each. Under the binocular, ten moving L1 were randomly selected and added to each well. The well plates were sealed with Parafilm (Bemis, USA) and incubated for 96 h at 20 ± 0.5 °C in the dark (Incubator IPP 55; Memmert GmbH, Germany). The tests were terminated by adding 0.5 mL Rose Bengal solution (0.3 mg/L) to all wells. The plates were resealed and placed in an oven for 15 min at approximately 80 °C. Plates were subsequently stored at 8 °C for up to 14 days before evaluation of the test results. Under a binocular, the adult nematodes were transferred to a microscope slide and placed under the microscope, whereas pictures of all gravid nematodes and males were taken with a camera (Moticam 2300; Motic Microscopy). The body length was determined as described for the "initial" L1 above. Number of male nematodes was recorded and the fertility of the animals was assessed by counting all gravid nematodes. A nematode was considered as gravid when at least one egg was detected inside its body. To determine the reproduction (i.e., the number of offspring per gravid hermaphrodite) all offspring in the wells was counted.

### Data evaluation

The concentration–response graphs and $EC_{50}$ calculations of the reference tests were prepared using the GraphPad Prism software (Version 9.1.2; GraphPad Software, USA). The other figures were created in RStudio (Version 1.3.1073; "ggplot2" package Version 3.3.5), which was also used for the evaluation of statistical differences between the treatments ("car" package Version 3.0-11, "FSA" Version 0.9.1). Normality and homogeneity of variance of the data were always examined and this information was used to decide whether to apply a parametric (ANOVA) or non-parametric (Kruskal–Wallis) test. If these overall tests yielded significant results ($p < 0.05$), they were followed by post hoc tests (Dunnett´s test for parametric data and Dunn´s test with Holm $p$-adjustment for non-parametric data). Limits of detection (LOD) and limits of quantification (LOQ) for the analytical data were determined in RStudio with the help of the "chemCal" package (Version 0.2.2), according to the calibration method and DIN 32 645 [6]. According to this guidance, the limit of detection is the concentration above which it is possible to decide whether the amount of analyte in the analysis sample is higher than in the blank sample. It therefore marks the decision limit for the presence of an analyte. In the DIN guidance, the limit of quantification is the concentration at which the relative uncertainty of the analytical results reaches a predefined value. Thus, the limit of quantification denotes the limit for determining the amount of an analyte in the analysis sample.

## Results

### Characterization of the test materials and their eluates

#### *Specific surface area*

The results of the BET testing showed that milling increased the specific surface area of the stone wool (Additional file 1: Table S18). Thus, the milled stone wool samples had a surface area that was approximately 50–90% larger than the surface area of the non-milled fibers.

#### *Chemical analysis and nanoparticle determination of the eluates*

The results for the pH screening test are described in Additional file 1: Text S2 and shown in Additional file 1: Figs. S1 and S2. As for the elemental concentrations in the eluates, only Al and Ni were detected in concentrations above the LOQ (Additional file 1: Table S5; Fig. 1). Additionally, B and Mn could be detected but not quantified in the eluates of the milled stone wool (LOD < concentrations B and Mn < LOQ), whereas the other elements analyzed were not detected in the eluates (Additional file 1: Table S5). Ni was detected in the blank elution medium in a concentration of approximately 4 µg/L and reaching approximately 7 µg/L in the stone wool eluates. Al concentrations in the blanks were below the LOD, while the elution from stone wool led to Al concentrations up to approximately 750 µg/L. Generally, Al and Ni concentrations were elevated in the stone wool eluates, with the trend being more pronounced for the milled materials (#2 and #3) as compared to the intact fibers (#1). Comparing eluates from milled stone wool, Al and Ni concentrations were higher if no binder was present (#2 > #3).

The detected concentrations of NPOC were above the LOQ (Additional file 1: Table S7; Fig. 2), however the NPOC concentration in the stone wool eluates did not exceed the organic carbon concentration of the blank. Thus, the cured PUF binder and mineral oils did not leach from the stone wool during the elution.

DLS measurements yielded the detection of nanoparticles for only one technical replicate (milled stone wool without binder) (Additional file 1: Table S19). However, this finding was not confirmed either by the analysis of a technical replicate or by the experimental replicate.

### Ecotoxicological testing

#### *Acute effects*

*Luminescence inhibition* None of the tested eluates significantly affected the bioluminescence of the bacteria, at any exposure period (Fig. 3). A maximum bioluminescence inhibition of about 17% was registered for the eluates. In contrast, the bioluminescence inhibition of the positive control using potassium dichromate was within the range of the validation criteria (i.e., 20 to 80%). The effect of the positive control using zinc sulfate was above this range only after 30 min of exposure. Additionally, the adjustment factor of the bioluminescence test did not meet the validation criteria of DIN EN ISO 11348-1 (Additional file 1: Table S20; Fig. 3). With a factor of 1.5 (for 15-min contact time) and 1.8 (for 30-min contact time), the mean adjustment factor in the negative control was above 1.3 and the deviations of the individual adjustment factors in the control replicates from the mean adjustment factor were commonly above 3%.

*Algae growth inhibition* In the algae growth tests, no significant inhibition of algae growth for the entire concentration range of the eluates was observed, except for milled stone wool without binder at a concentration of 10 mg/L with a mean growth reduction by 6% (Fig. 4). In contrast, exposure to eluates from 100 mg/L milled stone wool with binder caused a mean increase in algae growth of 4%. In comparison, the potassium dichromate showed a highly significant growth inhibition thus demonstrating the sensitivity of the test system. However, the algae growth tests did not meet all validation crite-

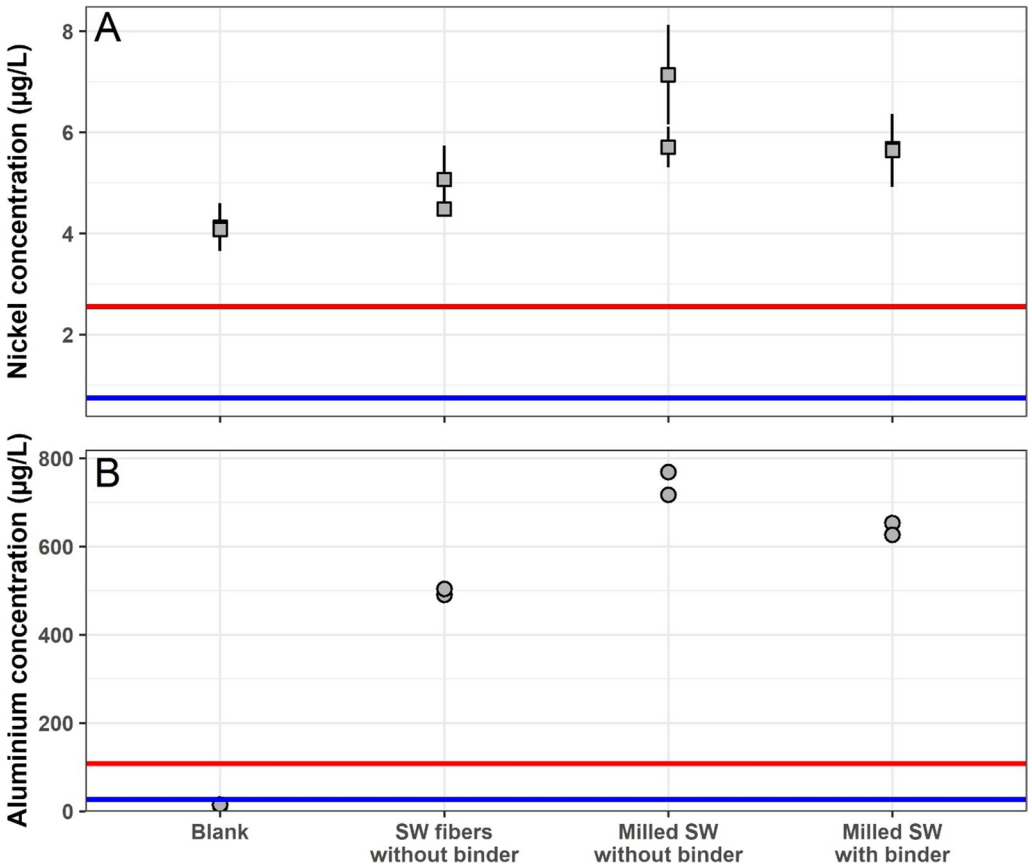


**Fig. 1** Nickel (**A**; squares) and aluminum (**B**; circles) concentrations as mean and standard error of three measurements each for two experimental eluate replicates, as detected by ICP-MS (for Ni) and ICP-OES (for Al). Limits of detection (LOD; blue line) and limits of quantification (LOQ; red line) for the two elements are indicated. Eluates were prepared according to OECD No. 29 with 100 mg/L sample material and a 7-day elution period

ria of OECD 201 (Additional file 1: Table S21). The algae growth reference test with potassium dichromate yielded an $EC_{50}$(72 h) value of 2.7 mg/L (Additional file 1: Fig. S4), which is above the range of 0.85 to 1.12 mg/L reported by Paixão et al. [34]. Additionally, the increasing factors in the control cultures were mostly below 16 and the mean coefficient of variation for section-by-section specific growth rates was above 35% in all tests. The lower growth in the control cultures (i.e., increasing factor < 16) can be explained by the light source, which provided a lower light intensity than specified in OECD 201 (2220 lx by the light source of this study vs. 4440 to 8880 lx recommended in the OECD guideline No. 201). The high variation between section-by-section specific growth rates was most likely caused by high algae cell concentrations at the test start.

*Immobilization of D. magna* For all concentrations tested, the stone wool eluates did not cause any significant immobilization of *D. magna* after 48 h of exposure (Fig. 5). In contrast, the *Daphnia* immobilization reference test with potassium dichromate yielded an $EC_{50}$(24 h) value of 1.6 mg/L and an $EC_{50}$(48 h) value of 1.0 mg/L (Additional file 1: Fig. S5), which is in accordance with results from inter-laboratory tests [8]. Thus, all validation criteria according to OECD 202 were fulfilled.

### *Chronic effects*

*Reproduction of D. magna* Following the 21-day exposure, the eluates of the three test materials showed different effects on the reproduction of *D. magna* (Fig. 6). The eluates of the non-milled stone wool fibers without binder had no significant effect on the daphnids reproduction in all tested concentrations. In contrast, for milled stone wool without binder the reproduction tended to decrease with increasing concentration, resulting in a significant effect at the highest concentration (100 mg/L). The reproduction of the exposed test animal of this treatment was on average 60% lower than in the control culture. An opposite effect was found for the eluates from milled stone wool with binder (1 mg/L and 10 mg/L), which led to significant increase of *D. magna* reproduction compared to the negative control. For these treatments, the

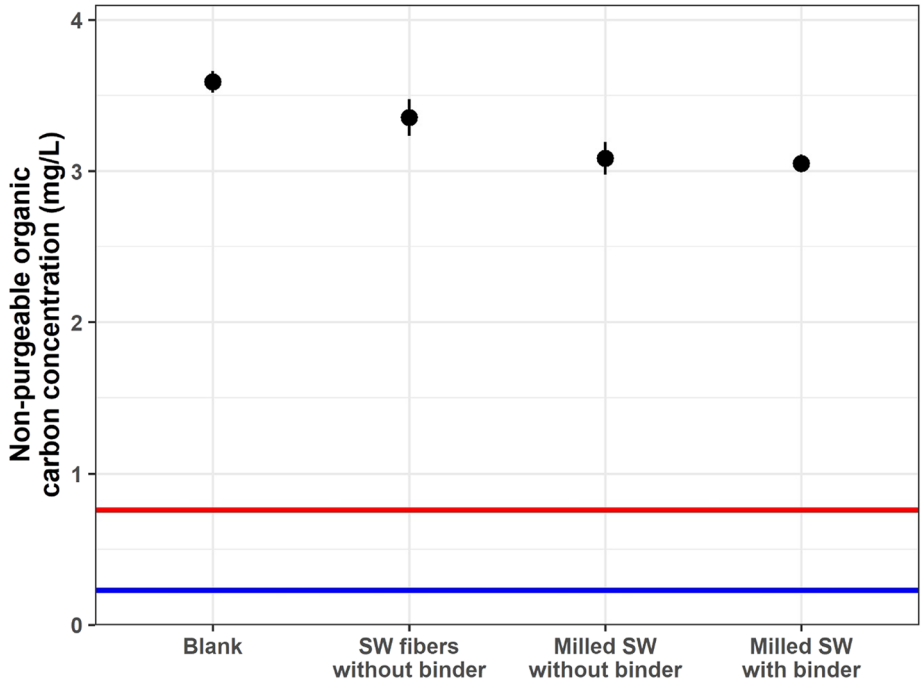


**Fig. 2** Non-purgeable organic carbon contents in the eluates as means and standard errors of three measurements of two experimental eluate replicates (total $n = 6$). Limit of detection (LOD; blue line) and limit of quantification (LOQ; red line) are indicated. Eluates were prepared according to OECD No. 29 with 100 mg/L sample material and a 7-day elution period

average increase in reproduction was approximately 50% and 40%, respectively.

The validation criteria of OECD 211 were met in the tests. However, in three of the five tests the applied concentrations of the positive control substance (potassium dichromate) were too low to cause any significant effects on the daphnids (Fig. 6). Therefore, a full reference test was conducted. The reference testing with potassium dichromate resulted in an $EC_{50}$(21 d) value of 125 µg/L (Additional file 1: Fig. S6), which was slightly lower than the 95% confidence limits for the $EC_{50}$(21 d) of 160 to 220 µg/L, as reported by Gopi et al. [15].

*Nematode growth and reproduction* As in the chronic *D. magna* reproduction test, the effects on growth and reproduction of *C. elegans* differed among the eluates of the three test materials (Fig. 7). Although the median effect on growth as compared to the negative control was always low (< 10%), statistical tests revealed that the eluates of milled stone wools caused significant growth inhibition in the following treatments: eluates from stone wool without binder (0.5, 5 and 50 mg/L) and with binder (5 mg/L). Meanwhile, eluates from stone wool fibers (50 mg/L) and milled stone wool with binder (50 mg/L) led to a significant stimulation of nematode growth.

Considering reproduction, only the exposure to the 5 mg/L eluate from milled stone wool with binder led to significant effects on the nematodes reproduction as compared with the negative control (Fig. 8). Generally, the variability of the results for reproduction was greater than the variability of the results for growth. Combined with the relatively small number of replicates ($n = 4$), none of the other reproduction inhibitions caused by the stone wool eluates were significant. Nevertheless, a general trend of increased inhibition of reproduction for the eluates from milled stone wool is noticeable.

The strong effects of the positive control on growth and reproduction of the nematodes demonstrated the sensitivity of the test system. In the reference test with BAC-C16 the $EC_{50}$(96 h) for fertility, growth and reproduction of *C. elegans* were determined at 34.7 mg/L, 15.4 mg/L, 9.5 mg/L (Additional file 1: Fig. S7) and therefore, the $EC_{50}$(96 h) value for growth inhibition was within the limits set by the test guideline [19]. The nematode growth test met most of the validation criteria (Additional file 1: Table S22), except for the tests with milled stone wool fibers with binder, where the mean recovery in the control was above the validation limits of 120%.

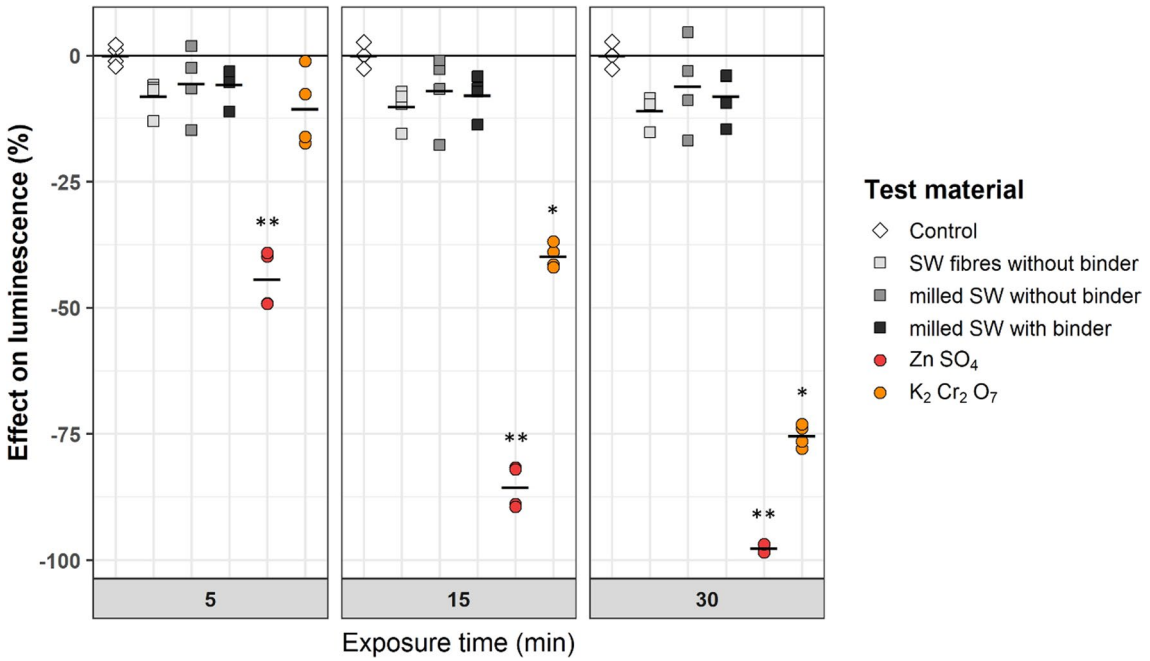


**Fig. 3** Results of the bacterial bioluminescence inhibition test for the negative control (white diamond), the eluates (100 mg/L) of the three test materials (grey squares) and the positive controls (zinc sulfate and potassium dichromate; red and orange circles) after 5, 15 and 30 min of exposure, as individual values and means of $n = 4$ replicates (black dash). Effects were calculated in comparison to the mean value of luminescence in the negative control and positive effect values show luminescence stimulation, while negative values show luminescence inhibition. Asterisks denote significant differences between the treatments and the negative control (Dunn's test: *: $p < 0.05$, **: $p < 0.01$)

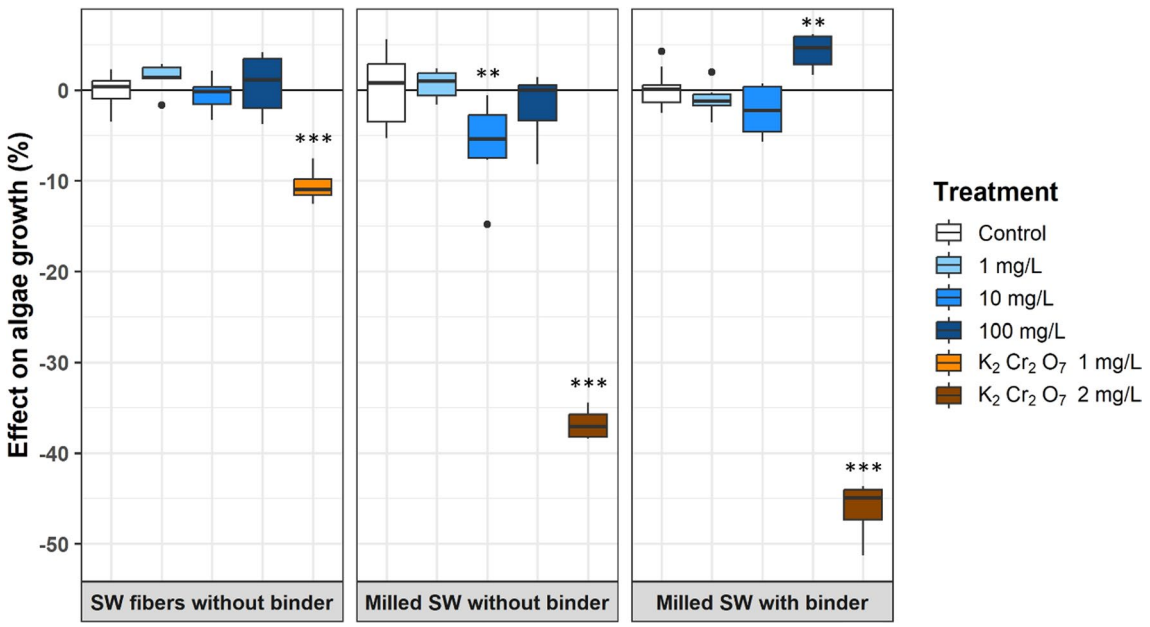


**Fig. 4** Results of the algae growth inhibition test for the negative control (white), the eluates of three concentrations (1, 10 and 100 mg/L) of each of the three test materials (blue colors) and the positive control (potassium dichromate; orange colors) as boxplots. Boxes depict the lower and upper quartile as well as the median; whiskers show the standard deviation of mean of $n = 6$ replicates and dots represent outliers. Effects were calculated in comparison to the mean value of algae growth in the negative control and positive effect values show algae growth stimulation, while negative values show growth inhibition. Asterisks denote significant differences between the treatments and the negative control (Dunnett's test: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$)

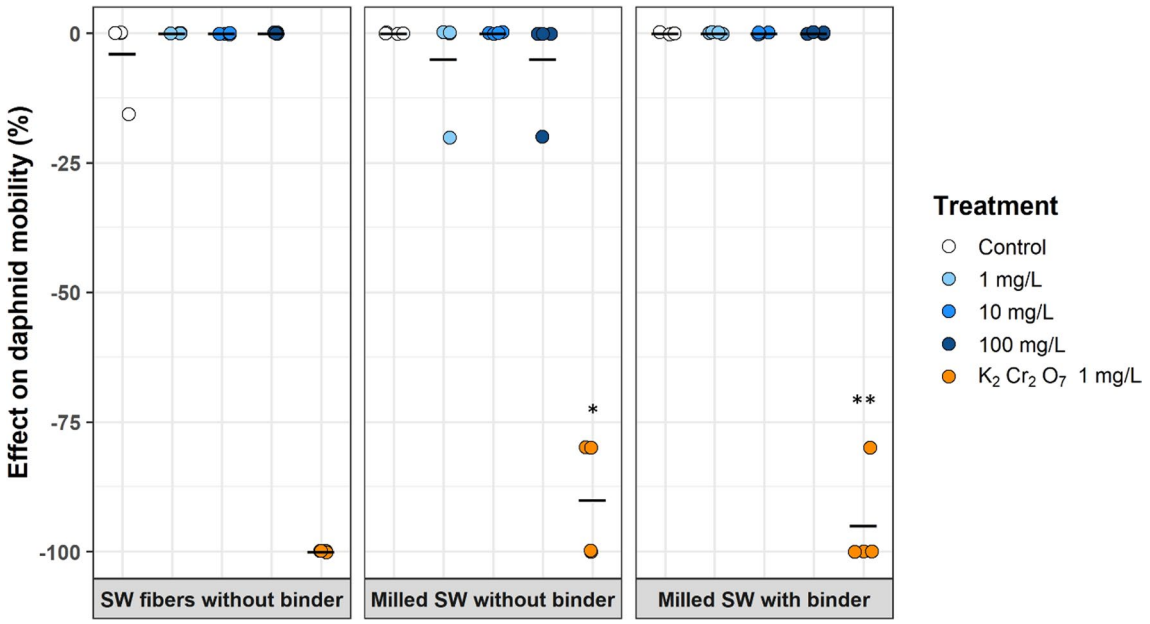


**Fig. 5** Acute effects (48 h) on *Daphnia* mobility for the negative control (white), the eluates of three concentrations (1, 10 and 100 mg/L) of each of the three test materials (blue colors) and the positive control (potassium dichromate; orange color) as individual values and means of $n = 4$ (black dash). Effects were calculated in comparison to the mean value of *D. magna* mobility in the negative control and negative effect values show daphnid immobilization

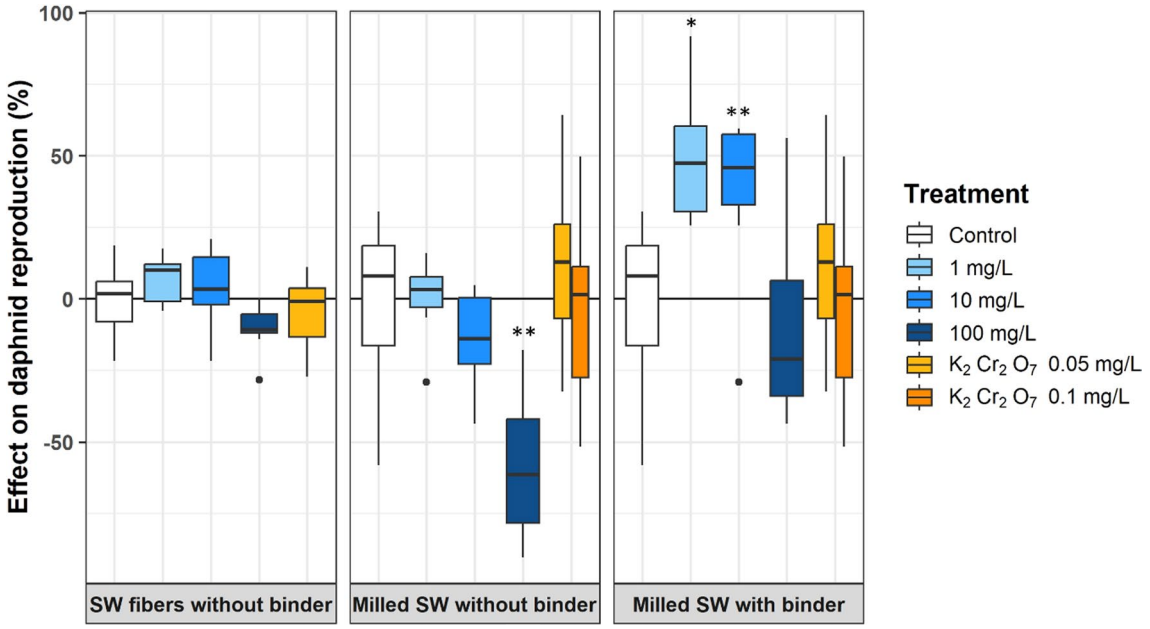


**Fig. 6** Chronic effects (21 d) on *D. magna* reproduction for the negative control (white), the eluates of three concentrations (1, 10 and 100 mg/L) of each of the three test materials (blue colors) and the positive control (potassium dichromate; orange colors) as boxplots. Boxes depict the lower and upper quartile as well as the median; whiskers show the standard deviation of mean of $n = 10$ replicates and dots represent outliers. Effects were calculated in comparison to the mean number of offspring in the negative control and positive effect values show reproduction stimulation, while negative values show reproduction inhibition. Asterisks denote significant differences between the treatments and the negative control (Dunn´s test: *: $p < 0.05$, **: $p < 0.01$)

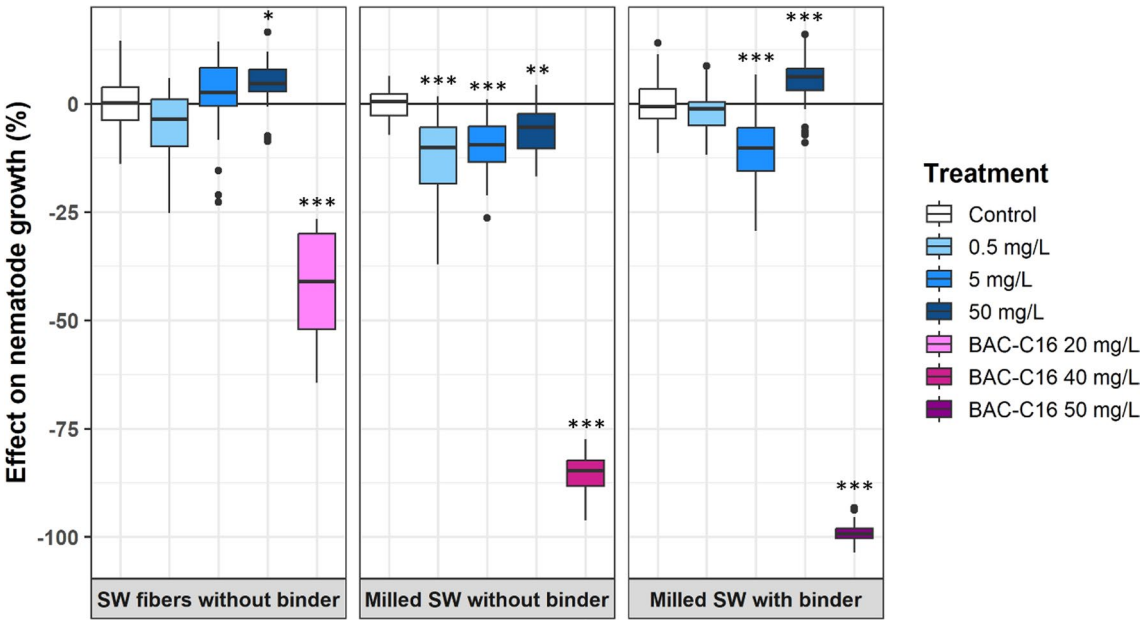


**Fig. 7** Chronic effects (96 h) on growth of *C. elegans* for the negative control (white), the eluates of three concentrations (0.5, 5 and 50 mg/L) of each of the three test materials (blue colors) and the positive control (BAC-C16; violet colors) as boxplots. Boxes depict the lower and upper quartile as well as the median; whiskers show the standard deviation of mean of $n > 10$ replicates and dots represent outliers. Effects were calculated in comparison to the mean growth in the negative control and positive effect values show nematode growth stimulation, while negative values show growth inhibition. Asterisks denote significant differences between the treatments and the negative control (Dunn's test: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$)

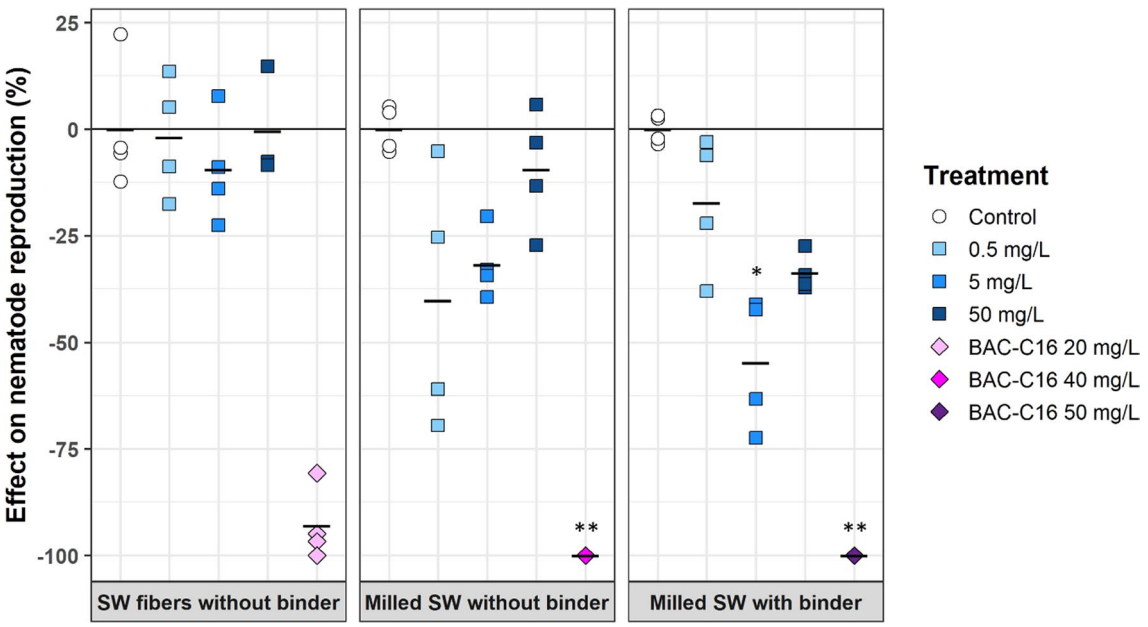


**Fig. 8** Chronic effects (96 h) on the reproduction of *C. elegans* for the negative control (white), the eluates of three concentrations (0.5, 5 and 50 mg/L) of each of the three test materials (blue colors) and the positive control (BAC-C16; violet colors) as individual values and means of $n = 4$ (black dash). Effects were calculated in comparison to the mean reproduction in the negative control and positive effect values show nematode reproduction stimulation, while negative values show reproduction inhibition. Asterisks denote significant differences between the treatments and the negative control (Dunn´s test: *: $p < 0.05$, **: $p < 0.01$)

## Discussion

The aim of this study was to investigate the ecotoxicological potential of biosoluble stone wool. Three stone wool samples (fibrous and milled; with and without binder) were subjected to a 7-day elution according to the OECD transformation/dissolution protocol [28] and the resulting eluates were applied in acute and chronic aquatic ecotoxicity tests. This study followed the general approach of performing an aqueous elution followed by ecotoxicity testing that was recently deemed reproducible and suitable to study the environmental impact of chemicals leached from construction products by an inter-laboratory test [17].

When compared to the fibrous material, the results indicate an increased leaching of Al and Ni from milled materials (up to approx. 750 and 7 µg/L, respectively), most likely due to their increased surface area (Additional file 1: Table S18). The higher surface area has most likely also led to the detection of B and Mn in the eluates of the milled stone wool in non-quantifiable amounts. Additionally, the heat treatment, which was used to remove the organic constituents (binder and mineral oil) from the fibrous (#1) and one of the milled samples (#2), may also lead to alterations in the elution behavior by oxidizing Fe(II) to Fe(III) within the stone wool. Stranghoener et al. [40] have reported differences in the release of metals from vitreous basaltic silicate glass, depending on the thermal history of the glasses and Fe redox state within the material. The authors explain their findings with the different roles of Fe(II) (as network modifier) and Fe(III) (as network former) within silicate glass, where $SiO_4$ tetrahedra are linked by T–O–Si bonds (T = Al, Fe(III)). During the dissolution of oxidized silicate glass, Fe(III)–O–Si bonds seem to be more vulnerable to protonation, breakage and the subsequent formation of non-bridging Si–OH bonds, when compared to Al–O–Si bonds [40]. The high contents of $Al_2O_3$ and $Fe_2O_3$ and structural similarity of vitreous stone wool with basaltic silicate glass, may suggest similar mechanisms for the test materials of this study. This could explain why the release of Al and Ni is the highest for "milled SW without binder".

Generally, the leaching of Al is plausible when considering the compositions of the stone wool, which possesses an $Al_2O_3$ content of 18.8%. The occurrence and concentration of trace elements like Ni in mineral wools is not regularly reported in literature, hampering the comparison of measured concentration in the eluates with literature values. However, it can be assumed that this element occurs in the stone wool only in trace amounts. The analysis of organic carbon in the eluates indicated that the cured PUF binder and mineral oils are not water-soluble, as the NPOC concentration in the "milled SW with binder" eluates was not elevated, when compared to the blanks or the stone wool samples without binder.

The battery of acute ecotoxicological tests in the present study covered several trophic levels by including luminescent bacteria, green algae and invertebrates. No acute ecotoxicological effects were observed for any of the test organisms in the entire test concentration range. Therefore, the experimental results from testing with *A. fischeri*, *D. subspicatus* and *D. magna* indicate that stone wool eluates are not likely to cause acute effects in the aquatic environment. The chronic tests included aquatic invertebrates and showed a number of effects on the different test species. As presented in the results, non-milled stone wool (i.e., "SW fibers without binder") showed no chronic effects on *D. magna* and *C. elegans*. In contrast, eluates of the milled stone wool samples did show chronic effects: eluates of 100 mg/L of "milled SW without binder" inhibited *D. magna* reproduction, while the low concentration eluates of "milled SW with binder" at low concentrations (1 mg/L and 10 mg/L) stimulated daphnid reproduction. Likewise, slight inhibitions of nematode growth (up to 15% inhibition on average) and inhibitions of nematode reproduction (up to 60% inhibition on average) were observed for both milled stone wool eluates. The DSL measurements performed in this study refute a formation of nanoparticles, which could have affected the test organisms. However, the results showed that both the milling (i.e., increase of the surface area) and heat treatment for the removal of organic constituents influence the leaching of Al, Ni and potentially B and Mn from the samples. As the detection of the highest Al and Ni concentrations correlate with the observed chronic effects, these elements might affect reproduction and growth of *D. magna* and nematode.

The mode of toxic action of Al to aquatic invertebrates has been indicated to be mainly due to ionoregulatory effects [14]. As reported by Gensemer et al. [13], the effect concentrations of Al on *D. magna* reproduction are within the range of the concentrations measured in the "milled SW without binder" eluates (reported $EC_{10}$ = 709 µg Al/L and $EC_{20}$ = 791 µg Al/L; measured concentration in the present study: 700–790 µg Al/L). However, literature shows that Al toxicity largely depends on pH, water hardness and dissolved organic carbon (DOC) in the test medium [13]. Therefore, it is important to consider these parameters when interpreting and comparing ecotoxicological effects of Al. In the test medium used in the present study, the organic carbon content determined as NPOC was approximately 3.5 mg/L, the water hardness was 250 mg $CaCO_3$ eq./L and the pH was set to 8. Gensemer et al. [13] have conducted their tests at lower pH, lower water hardness and with a lower DOC content (pH 6.3, hardness: 140 mg $CaCO_3$ eq./L; DOC:

2 mg/L), therefore the effect concentrations can only be used for a rough comparison and any conclusions drawn from the comparison should be treated with caution. For a more nuanced evaluation the recently published Canadian "Federal water quality guideline for total aluminum" was applied, which describes the derivation of a Federal Water Quality Guideline (FWQG) for the protection of aquatic ecosystems from harmful effects of Al [11]. The FWQG is meant to provide a threshold for an 'acceptable environmental quality' and is derived taking into account the three parameters mentioned above (according to Additional file 1: Formula S1). For the test medium and exposure solutions of this study, a FWQG of approximately 880 µg Al/L was calculated. The measured Al concentration in the "milled SW without binder" was close to the FWQG, however still below this threshold, indicating that Al leaching from this material might affect aquatic life, but would not lead to an 'unacceptable environmental quality'.

In contrast to Al, the toxic mode of action for Ni is not yet very well understood. The main mechanisms reported in literature for the toxicity effects of Ni on invertebrates were due to the disruption of ion homeostasis and oxidative stress by the production of reactive oxygen species [4]. For *D. magna* at 20 °C, chronic effect concentrations of approximately 30, 40 and 70 µg Ni/L have been reported for $EC_{10}$, $EC_{20}$ and $EC_{50}$, respectively [36]. Thus, with a Ni concentration up to 7 µg Ni/L, the metal is unlikely to have caused the *D. magna* reproduction inhibition observed for the 100 mg/L "milled SW without binder" eluate.

All in all, the results do not clearly point to one reason for the observed reproduction inhibition of *D. magna*. This might be due to further environmental factors influencing the bioavailability and toxicity of the metals or due to mixture effects of metals present in the eluates, which cannot easily be disentangled [35], or also due to a combination of the two aspects.

Considering the stimulation of *D. magna* reproduction at lower concentrations of "milled SW with binder", the response pattern may be caused by hormesis. This phenomenon describes the effect that low concentrations of substances may positively affect organisms. Hormesis is presumed to be an adaptive trait, leading to the protection of the organism against repeated exposures to a substance [41]. A reproduction stimulation, similar to the one observed in this study, has been reported by Lillicrap et al. [25] after the 21 d exposure of *D. magna* to eluates of silica fumes and the authors have attributed these stimulations to a hormetic response.

With respect to the chronic effects on the nematodes, results from literature suggest that the observed growth inhibition of *C. elegans* for both milled stone wool materials is not due to Al toxicity, as a concentration of about 800 µg Al/L did not affect the nematode growth [33]. However, the authors found effects of Al on *C. elegans* for the endpoint of reproduction at this concentration [33]. Therefore, the reproduction inhibition (although non-significant) of this study could have been caused by Al.

Most of our acute and chronic ecotoxicity tests included three eluate concentrations (apart from the luminescent bacteria test). Thus, the range of concentrations tested was too low to allow for the calculation of effect concentrations for the investigated organisms and endpoints. Instead, the tests can rather be considered as extended limit tests, which might show trends of concentration-dependent effects by the mineral wool samples. A further restriction applies to the chronic *D. magna* reproduction test, as the potassium dichromate positive controls included in the testing were of too low concentration to induce a reproduction inhibition. Thus, the sensitivity of the daphnids during this experiment could not be confirmed beyond doubt and the test results should be interpreted with care.

## Conclusions

The installation of thermal insulation during construction and renovation is a key to reduce the climate change impact of buildings. However, building insulation materials should not be promoted at the expense of ecosystem health. Therefore, it is necessary to study possible ecotoxicological effects of such materials, including stone wool insulation.

The ecotoxicological assessment presented in this study shows, that stone wool is not likely to cause acute adverse effects in the aquatic environment. Furthermore, non-milled stone wool fibers were found to have no chronic ecotoxicological effects. Caution should be taken once the stone wool fibers are extensively broken down (e.g., by milling), as this increases the materials' surface area, promotes the leaching of metals and may thus affect aquatic invertebrates chronically at high concentrations. Nevertheless, based on the results presented in this study, stone wool insulation products should not be considered a chemical hazard to the aquatic environment.

To conduct a complete risk analysis concerning the environmental effects of stone wool products, more research is necessary, especially to assess the effects of the milled materials, to study the consequences of an oxidizing heat treatment on the elution behavior and to perform environmental exposure studies on stone wool. Such investigations should also consider that in the future the recycling of stone wool is expected to increase, while landfilling of the insulation material will likely decrease. Another aspect not covered by this study are the effects of particles on aquatic organisms, as the present study was focused on chemically induced intrinsic effects.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-023-00727-2.

**Additional file 1: Table S1.** Composition of elution media (pH 6, pH 7 and pH 8) according to OECD Guideline No. 29 (OECD, 2001) and based on DIN EN ISO 6341 (DIN EN ISO, 2013). **Text S1.** Elution and sampling procedure of the pH screening test. **Text S2.** Results of the pH screening test and reasons for choosing pH 8 for the following experiments. **Figure S1.** Aluminum mean concentration and standard error in the eluates (n = 2) at pH 6 (circles), pH 7 (squares) and pH 8 (triangles), as detected by ICP-MS. **Figure S2.** Nickel mean concentration and standard error in the eluates (n = 2) at pH 6 (circles), pH 7 (squares) and pH 8 (triangles), as detected by ICP-MS. **Table S2.** Composition of elution medium used to prepare eluates for the algae growth inhibition test. **Table S3.** Composition of micronutrient supplement solution used in algae test and final concentration of micronutrients in the supplemented eluates. **Table S4.** Elements analyzed by ICP-MS and ICP-OES. **Table S5.** Limits of detection (LOD) and limits of quantification (LOQ) for the analyzed elements. **Table S6.** Instrumental settings for the NPOC measurement. **Table S7.** Limits of detection (LOD) and limits of quantification (LOQ) of the non-purgeable organic carbon analysis. **Text S3.** Culturing conditions for the test organisms. **Table S8.** Composition of liquid culture medium for *Aliivibrio fischeri,* according to on DIN EN ISO 11348-1 (DIN EN ISO, 2009). **Table S9.** Composition of protective medium for storage of luminescent bacteria, according to DIN EN ISO 11348-1 (DIN EN ISO, 2009). **Table S10.** Composition of algae culturing medium, based on Ebert (2014). **Table S11.** Composition of modified Aachener Daphnia Medium (ADaM), based on Klüttgen et al., (1994) and modified by Ebert et al. (1996). **Table S12.** Composition of nematode growth medium (NGM), based on ISO 10872 (ISO, 2010). **Table S13.** Relevant validation criteria of the ecotoxicity tests. **Table S14.** Initial algae biomass used to start the growth inhibition tests. **Table S15.** Parameters of fluorescence measurements in algae test. **Figure S3.** Linear correlation between fluorescence and number of algae cells per mL ($R^2$ = 0.9949). **Table S16**. Composition M9 medium. **Text S4.** Synchronization procedure for *C. elegans*. **Table S17.** Mean initial body length of nematodes determined at test start. **Text S5.** Preparation procedure for the *E. coli* OP50 feed stock. **Table S18.** Specific surface area of the test samples as determined by the BET method. **Table S19.** Results of DLS measurements in each sample analyzed. **Table S20.** Results for the validation criteria in the negative control replicates of the bioluminescence inhibition test. **Table S21.** Results for the validation criteria of the algae growth inhibition tests. **Figure S4.** Acute algae growth inhibition in the reference test with potassium dichromate. **Figure S5.** Acute effects (48 h) on *Daphnia* mobility in the reference test with potassium dichromate. **Figure S6.** Chronic effects (21 d) on *Daphnia* reproduction in the reference test with potassium dichromate. **Figure S7.** Chronic effects (96 h) on growth, fertility and reproduction of *Caenorhabditis elegans* in the reference test with Benzylcetyldimethylammonium chloride (BAC-C16). **Table S22.** Results for the validation criteria in the controls of the nematode growth and reproduction tests. **Formula S1.** Formula to calculate the Canadian Federal Water Quality Guideline (FWQG) for the protection of aquatic ecosystems from harmful effects of aluminum (Environment and Climate Change Canada, 2021) at a given pH value, water hardness (in mg $CaCO_3$ eq./L) and DOC concentration (in mg/L).

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**Author contributions**
ES drafted the manuscript; ES, MN and SZ organized the experimental work; ES, ED, TM-G, LS, VIM, KK, DVO and MN conducted the experimental work; CK, MS and BS designed the study; TCS and BS supervised the study. All authors contributed to the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets of the current study are available from the corresponding author on request.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare the following competing interest(s): Deutsche ROCKWOOL GmbH & Co. KG and ROCKWOOL A/S are manufacturers of stone wool. ES, DVO, and MS are employed by Deutsche ROCKWOOL GmbH & Co. KG and ROCKWOOL A/S, respectively. CK was employed by ROCKWOOL A/S while the practical work was carried out.

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**References**

1. Abu-Jdayil B, Mourad AH, Hittini W, Hassan M, Hameedi S (2019) Traditional, state-of-the-art and renewable thermal building insulation materials: an overview. Constr Build Mater 214:709–735. https://doi.org/10.1016/j.conbuildmat.2019.04.102
2. Bandow N, Gartiser S, Ilvonen O, Schoknecht U (2018) Evaluation of the impact of construction products on the environment by leaching of possibly hazardous substances. Environ Sci Eur. https://doi.org/10.1186/s12302-018-0144-2
3. Barly SHQ, Okhrimenko DV, Solvang M, Yue Y, Stipp SLS (2019) Dissolution of stone wool fibers with phenol-urea-formaldehyde binder in a synthetic lung fluid. Chem Res Toxicol 32(12):2398–2410. https://doi.org/10.1021/acs.chemrestox.9b00179
4. Brix KV, Schlekat CE, Garman ER (2017) The mechanisms of nickel toxicity in aquatic environments: an adverse outcome pathway analysis. Environ Toxicol Chem 36(5):1128–1137. https://doi.org/10.1002/etc.3706
5. COP 21 (2015) Paris agreement. United Nations Conference of the Parties. https://doi.org/10.1017/s0020782900004253
6. DIN (2008) 32645: Chemische Analytik - Nachweis-, Erfassungs- und Bestimmungsgrenze unter Wiederholbedingungen - Begriffe, Verfahren, Auswertung. Deutsches Institut für Normung e.V.
7. DIN EN ISO (2009) 11348-1: Water quality—determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test)—Part 1: Method using freshly prepared bacteria. Deutsches Institut für Normung e.V.; Normenausschuss Wasserwesen

8. DIN EN ISO (2013) 6341: Wasserbeschaffenheit—Bestimmung der Hemmung der Beweglichkeit von *Daphnia magna* Straus (Cladocera, Crustacea)—Akuter Toxizitäts-Test. Deutsches Institut für Normung e.V.
9. EC (2021) Making our homes and buildings fit for a greener future. In European Commission (pp. 1–3). https://ec.europa.eu/commission/presscorner/detail/en/fs_21_3673
10. EC No. 1907 (2006) Regulation concerning the registration, evaluation, authorisation and restriction of chemicals (REACH), establishing a European Chemicals Agency (ECHA). European Union.
11. Environment and Climate Change Canada (2021) Federal Environmental Quality Guidelines Aluminium. Canadian Environmental Protection Act, 1999. https://www.canada.ca/content/dam/eccc/documents/pdf/pded/feqg-aluminium/Federal-environmental-quality-guidelines-aluminium.pdf
12. Gartiser S, Heisterkamp I, Schoknecht U, Burkhardt M, Ratte M, Ilvonen O, Brauer F, Brückmann J, Dabrunz A, Egeler P, Eisl AM, Feiler U, Fritz I, König S, Lebertz H, Pandard P, Pötschke G, Scheerbaum D, Schreiber F, Soldán P, Weiß R, Weltens R (2017) Results from a round robin test for the ecotoxicological evaluation of construction products using two leaching tests and an aquatic test battery. Chemosphere 175:138–146. https://doi.org/10.1016/j.chemosphere.2017.01.146
13. Gensemer RW, Gondek JC, Rodriquez PH, Arbildua JJ, Stubblefield WA, Cardwell AS, Santore RC, Ryan AC, Adams WJ, Nordheim E (2018) Evaluating the effects of pH, hardness, and dissolved organic carbon on the toxicity of aluminum to freshwater aquatic organisms under circumneutral conditions. Environ Toxicol Chem 37(1):49–60. https://doi.org/10.1002/etc.3920
14. Gensemer RW, Playle RC (1999) The bioavailability and toxicity of aluminum in aquatic environments. Crit Rev Environ Sci Technol 29(4):315–450. https://doi.org/10.1080/10643389991259245
15. Gopi RA, Ayyappan S, Chandrasehar G, Varma KK, Goparaju A (2012) Effect of potassium dichromate on the survival and reproduction of *Daphnia magna*. Bull Environ Pharmacol Life Sci 1(7):89–94
16. Guldberg M, De Meringo A, Kamstrup O, Furtak H, Rossiter C (2000) The development of glass and stone wool compositions with increased biosolubility. Regul Toxicol Pharmacol 32(2):184–189. https://doi.org/10.1006/rtph.2000.1418
17. Heisterkamp I, Ratte M, Schoknecht U, Gartiser S, Kalbe U, Ilvonen O (2021) Ecotoxicological evaluation of construction products: inter-laboratory test with DSLT and percolation test eluates in an aquatic biotest battery. Environ Sci Eur. https://doi.org/10.1186/s12302-021-00514-x
18. Hybská H, Hroncová E, Ladomerský J, Balco K, Mitterpach J (2017) Ecotoxicity of concretes with granulated slag from gray iron pilot production as filler. Materials 10(5):1–12. https://doi.org/10.3390/ma10050505
19. ISO (2010) 10872: Water quality—determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of *Caenorhabditis elegans* (Nematoda). International Organization for Standardization
20. Kalhor K, Emaminejad N (2020) Qualitative and quantitative optimization of thermal insulation materials: Insights from the market and energy codes. J Build Eng 30(February):101275. https://doi.org/10.1016/j.jobe.2020.101275
21. Klüttgen B, Dülmer U, Engels M, Ratte HT (1994) ADaM, an artificial freshwater for the culture of zooplankton. Water Res 28(3):743–746. https://doi.org/10.1016/0043-1354(94)90157-0
22. Knoop O, Woermann M, Lutze HV, Sures B, Schmidt TC (2018) Ecotoxicological effects prior to and after the ozonation of tamoxifen. J Hazard Mater 358(June):286–293. https://doi.org/10.1016/j.jhazmat.2018.07.002
23. Kobetičová K, Černý R (2017) Ecotoxicology of building materials: a critical review of recent studies. J Clean Prod 165:500–508. https://doi.org/10.1016/j.jclepro.2017.07.161
24. Koch C, Sures B (2019) Ecotoxicological characterization of possible degradation products of the polymeric flame retardant "Polymeric FR" using algae and *Daphnia* OECD tests. Sci Total Environ 656:101–107. https://doi.org/10.1016/j.scitotenv.2018.11.207
25. Lillicrap A, Allan I, Friede B, Garmo O, Macken A (2014) Is the transformation/dissolution protocol suitable for ecotoxicity assessments of inorganic substances such as silica fume? Sci Total Environ 468–469:358–367. https://doi.org/10.1016/j.scitotenv.2013.08.035
26. Lucon O, Ürge-Vorsatz D, Zain Ahmed A, Akbari H, Bertoldi P, Cabeza LF, Eyre N, Gadgil A, Harvey LDD, Jiang Y, Liphoto E, Mirasgedis S, Murakami S, Parikh J, Pyke C, VM C (2014) Climate change 2014: mitigation of climate change. In: Edenhofer O, Pichs-Madruga R, Sokona Y, Farahani E, Kadner S, Seyboth K, Adler A, Baum I, Brunner S, Eickemeier P, Kriemann B, Savolainen J, Schlömer S (eds) Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, pp 671–738
27. Moreno RA, Baron R, Bohm P, Chandler W, Cole V, Davidson O, Dutt G, Haites E, Ishitani H, Kruger D, Levine M, Zhong L, Michaelis L, Moomaw W, Moreira JR, Mosier A, Moss R, Nakicenovic N, Price L, Ravindranath NH, Rogner H-H, Sathaye J, Shukla J, Van Wie McGrory L, Williams T (1996) Technologies, policies and measures for mitigating climate change. In IPCC Technical Paper I. https://doi.org/10.1142/9781783265121_0003
28. OECD (2001) Guidance document on transformation/dissolution of metals and metal compounds in aqueous media. In OECD Series on Testing and Assessmenr: Vol. No. 29 (Issue ENV/JM/MONO). Organization for Economic Cooperation and Development
29. OECD (2004) Test No. 202: *Daphnia* sp., Acute Immobilisation Test. In OECD Guidelines for the testing of chemicals. Organization for Economic Cooperation and Development
30. OECD (2006) Test No. 201: Freshwater alga and cyanobacteria, growth inhibition test. In OECD Guidelines for the Testing of Chemicals. Organization for Economic Cooperation and Development
31. OECD (2012) Test No. 211: *Daphnia magna* reproduction test. In OECD Guidelines for the Testing of Chemicals. Organization for Economic Cooperation and Development. https://doi.org/10.1787/9789264070127-en
32. Okhrimenko DV, Thomsen AB, Ceccato M, Johansson DB, Lybye D, Bechgaard K, Tougaard S, Stipp SLS (2018) Impact of curing time on ageing and degradation of phenol-urea-formaldehyde binder. Polym Degrad Stab 152:86–94. https://doi.org/10.1016/j.polymdegradstab.2018.04.001
33. Page KE, White KN, McCrohan CR, Killilea DW, Lithgow GJ (2012) Aluminium exposure disrupts elemental homeostasis in *Caenorhabditis elegans*. Metallomics 4(5):512–522. https://doi.org/10.1039/c2mt00146b
34. Paixão SM, Silva L, Fernandes A, O'Rourke K, Mendonça E, Picado A (2008) Performance of a miniaturized algal bioassay in phytotoxicity screening. Ecotoxicology 17(3):165–171. https://doi.org/10.1007/s10646-007-0179-4
35. Pan J, Pan JF, Diao M (2015) Trace metal mixture toxicity in aquatic organism reviewed from a biotoxicity perspective. Hum Ecol Risk Assess 21(8):2155–2169. https://doi.org/10.1080/10807039.2015.1032211
36. Pereira CMS, Deruytter D, Blust R, De Schamphelaere KAC (2017) Effect of temperature on chronic toxicity of copper, zinc, and nickel to *Daphnia magna*. Environ Toxicol Chem 36(7):1909–1916. https://doi.org/10.1002/etc.3714
37. Rodrigues P, Silvestre JD, Flores-Colen I, Viegas CA, de Brito J, Kurad R, Demertzi M (2017) Methodology for the assessment of the ecotoxicological potential of construction materials. Materials 10(6):649. https://doi.org/10.3390/ma10060649
38. Schertzinger G, Zimmermann S, Grabner D, Sures B (2017) Assessment of sublethal endpoints after chronic exposure of the nematode *Caenorhabditis elegans* to palladium, platinum and rhodium. Environ Pollut 230:31–39. https://doi.org/10.1016/j.envpol.2017.06.040
39. Schiavoni S, D'Alessandro F, Bianchi F, Asdrubali F (2016) Insulation materials for the building sector: a review and comparative analysis. Renew Sustain Energy Rev 62:988–1011. https://doi.org/10.1016/j.rser.2016.05.045
40. Stranghoener M, Dultz S, Behrens H, Schippers A (2020) Far from equilibrium basaltic glass alteration: the influence of Fe redox state and thermal history on element mobilization. Geochim Cosmochim Acta 273:85–98. https://doi.org/10.1016/j.gca.2020.01.005
41. Weltje L, Vom Saal FS, Oehlmann J (2005) Reproductive stimulation by low doses of xenoestrogens contrasts with the view of hormesis as an adaptive response. Hum Exp Toxicol 24(9):431–437. https://doi.org/10.1191/0960327105ht551oa
42. Woermann M, Sures B (2020) Ecotoxicological effects of micropollutant-loaded powdered activated carbon emitted from wastewater treatment plants on *Daphnia magna*. Sci Total Environ 746:141104. https://doi.org/10.1016/j.scitotenv.2020.141104


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