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In situ remediation efficacy of hybrid aerogel adsorbent in model aquatic culture of *Paramecium caudatum* exposed to Hg(II)

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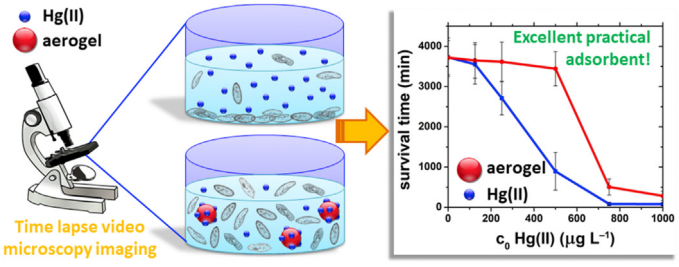
HIGHLIGHTS

- *Paramecium caudatum* used as bio-indicator of Hg(II) toxicity.
- *Paramecium* cultures monitored by time lapse video microscopy imaging.
- Quantitative exposure-effect relationship established for Hg(II) toxicity.
- Silica-gelatin aerogel effectively protects *Paramecium* from Hg(II).

GRAPHICAL ABSTRACT



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ABSTRACT

Silica-gelatin hybrid aerogel of 24 wt% gelatin content is an advanced functional material suitable for the high performance selective adsorption of aqueous Hg(II). The remediation efficacy of this adsorbent was tested under realistic aquatic conditions by exposing cultures of *Paramecium caudatum* to Hg(II) and monitoring the model cultures by time-lapse video microscopy. The viability of *Paramecium* was quantified by analyzing the pixel differences of the sequential images caused by the persistent movement (motility) of the cells. The viability of *Paramecium* displays a clear exposure-response relationship with Hg(II) concentration. Viability decreases with increasing Hg(II) concentration when the latter is higher than 125 μg L^{-1}. In the presence of 0.1 mg mL^{-1} aerogel adsorbent, the viability of the cells decreases only at Hg(II) concentrations higher than 500 μg L^{-1}, and 220 min survival time was measured even at 1000 μg L^{-1} Hg(II). The effective toxicity of Hg(II) is lower in the presence of the aerogel, because the equilibrium concentration of aqueous Hg(II) is low due to adsorption, thus *Paramecium* cells do not uptake as much Hg(II) as in the un-remediated cultures. Video imaging of *Paramecium* cultures offers a simple, robust and flexible method for providing quantitative information on the effectiveness of advanced materials used in adsorption processes for water treatment.

1. Introduction

Heavy metal pollution is an ever growing problem in natural waters due to increasing anthropogenic activities, e.g. mining, metallurgical processes, waste management (Carolin et al., 2017;

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Joseph et al., 2019; Vareda et al., 2019). The effective removal of aqueous heavy metal compounds has an outstanding importance, because all living organisms are eventually exposed to these harmful compounds through the aquatic food chain (Herman et al., 2020a). Adsorption technologies are promising for the effective immobilization and removal of heavy metal compounds from natural waters (Maleki and Hüsing, 2018; Xu et al., 2018; Zhou et al., 2018; Aschermann et al., 2019; Garcia-Gonzalez et al., 2019; Ponomarev et al., 2019; Vareda and Duraes, 2019; Wang et al., 2020).

Mercury is one of the most toxic heavy metals associated with serious health damage even at very low concentrations of exposure. Owing to its high toxicity and high bioaccumulation factor up to 10^6 in the aquatic food chain, a large variety of high performance and selective adsorbents have recently been developed for the removal of aqueous Hg(II) (Yap et al., 2018; Fu et al., 2019; Gai et al., 2019; Lone et al., 2019; Rodríguez-Mata et al., 2019; Alguacil and López, 2020; Moronshing et al., 2020). The evaluation and the benchmarking of the performance of these adsorbents are most often realized by batch experiments performed under sterile laboratory conditions. Only a handful of the new advanced functional materials are tested in natural waters, such as filtered lake water, groundwater, seawater or tap water (Starr and Cherry, 1994; Cantrell et al., 1995; Blowes et al., 1997; Mercier and Pinnavaia, 1997; Mahmoud et al., 2000; Arica et al., 2003; Weisener et al., 2005; Ahmed, 2008; Lo et al., 2012). However, these experiments do not provide information on the ability of the adsorbent to decrease the contaminant uptake and increase of the survival of aquatic organisms. In spite of the large number of publications on novel, high performance aqueous heavy metal adsorbents, no systematic study has been reported to prove the practical suitability of these adsorbents for environmental remediation by utilizing model systems that include living aquatic organisms.

Ciliates in general are fundamental components of aquatic ecosystems due to their key role in decomposing organic matter and transferring energy to higher tropic levels. Several ciliate species are sensitive to aqueous heavy metal compounds, which makes them excellent bioindicators of this type of chemical pollution. Due to their small size, short generation time and rapid response to perturbation, they can conveniently be utilized as whole-cell bioassays and bioindicators for chemical contamination. A recent meta-analysis of several toxicological studies point out that ciliates are especially sensitive to aqueous Hg(II) (Vilas-Boas et al., 2020). Among many ciliate species, *Paramecium caudatum* is one of the most frequently used in laboratory toxicity studies involving heavy metal compounds (Kvitek et al., 2009). The biology, morphology and behavior of this species is well-documented (Rao et al., 2006). *Paramecium caudatum* feeds on bacteria and tolerates organic matter; therefore, they adapt well to laboratory conditions and are easy to culture (Alves et al., 2016). Their ciliary motion is an excellent indicator of their viability that can be quantified by video microscopy imaging. The sensitivities of *Paramecium caudatum* against many heavy metal compounds are well-documented in several publications (Gong et al., 2014; Vilas-Boas et al., 2020). *Paramecium caudatum* shows medium tolerance to aqueous Hg(II), which can advantageously be utilized to establish exposure-response relationship in a wide concentration range regarding this contaminant.

In the present study, the objective was to test the *in situ* remediation efficacy of a novel hybrid aerogel for the adsorption of Hg(II) under quasi-realistic aquatic conditions using *Paramecium caudatum* as a model bioindicator unicellular organism. Aerogels are excellent adsorbents due to their open porous structures, high porosity, versatile surface chemistry and possibility to incorporate surface functional groups (Vareda et al., 2020). Several reviews have recently reported the application of aerogels in environmental technology (Maleki and Hüsing, 2018). Silica-gelatin aerogel of 24 wt% gelatin has excellent performance for the removal of aqueous Hg(II) under conditions of practical applications in environmental engineering, as discussed in detail in our previous publication (Herman et al., 2020b). Therefore, the objective was to test its effectiveness by exposing *Paramecium* cultures to increasing concentrations of Hg(II) and using the adsorbent for *in situ* remediation. The cultures were continuously monitored by time lapse video microscopy imaging and the motility of the cells was analyzed quantitatively. The toxicity of aqueous Hg(II), the biocompatibility of the aerogel and the efficacy of the aerogel for improving the survival of *Paramecium* cells were studied under identical experimental conditions.

2. Materials and methods

2.1. Chemicals

$HgCl_2$ monoelement standard solution (ISO analytical grade, trace metal impurity max. 0.002%) of 1000 mg L^{-1} was purchased from Scharlau. All aqueous solutions and suspensions were prepared with ultrafiltered water ($\rho = 18.2$ MΩ cm provided by Milli-Q from Millipore). The primary precursor materials of the aerogel adsorbent are tetramethyl orthosilicate (TMOS) from Fluka, and household gelatin. Gelatin was purchased from Dr. Oetker KG (Germany). According to the specifications of the manufacturer, this is food grade Type A gelatin with average molecular mass of 150 kDa, reference No. L B86754/01.

2.2. Silica-gelatin hybrid aerogel

Silica-gelatin hybrid aerogel of 24 wt% gelatin content was synthesized by the sol-gel technique utilizing co-gelation. Briefly, gelatin and $(NH_4)_2CO_3$ were dissolved in hot water (ca. 45 °C). TMOS was dissolved in methanol and added to the gelatin solution under stirring. The mixture was poured into a cylindrical mold for gelation (24 h). The gel was placed into methanol for 24 h to remove water. Next, methanol was replaced by acetone in four 24 h soaking steps. Finally, acetone was extracted with liquid CO_2, then the gel was dried with supercritical CO_2. The detailed description of the synthesis was given in our previous publication (Herman et al., 2020b).

A representative scanning electron microscopic (SEM) image of the microstructure of the silica-gelatin hybrid aerogel is shown in Fig. 1. The hybrid aerogel is built from primary globules with characteristic diameters of $d_{globule} = 40–100$ nm. According to N_2 adsorption-desorption porosimetry measurements, the dry silica-gelatin aerogel is a mesoporous material with some macropores (specific surface area: $S_{BET} = 290 \pm 30$ m^2 g^{-1}; mean pore size: $d_{pore} = 20$ nm; total pore volume: $V_{pore} = 1.7$ cm^3 g^{-1}).

For the remediation experiments presented in this paper, the aqueous suspension of the aerogel was prepared by wet grinding by using the same protocol as in the case of the previously reported batch adsorption studies (Herman et al., 2020b). The applied protocol is given in the description of the toxicity experiments in Section 2.4. The size distribution of the suspended aerogel particles used in this study is given in Fig. 1.

2.3. Paramecium caudatum culture

Stock cultures of *Paramecium caudatum* (Ehrenberg, 1833) were originally collected from a natural pond and subsequently reared under laboratory conditions (Rao et al., 2006; Wichterman, 2012).

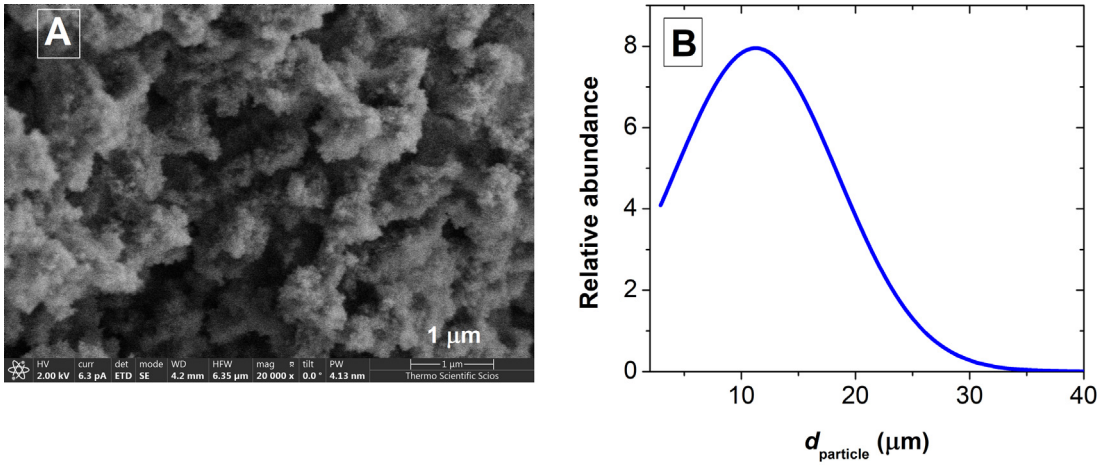

Fig. 1. Panel A: Scanning electron microscopy (SEM) image of silica-gelatin hybrid aerogel of 24 wt% gelatin content (×20 K magnification). Panel B: Size distribution of the suspended aerogel particles prepared by wet grinding. The size of the particles was measured by laser diffraction light scattering (LDLS).

Paramecium cultures were maintained in dried vegetable medium containing bacteria and yeast as food. (The food was a mixture of bacteria species in order to mimic the microflora of the natural pond.) Fresh cultures were initiated by seeding 500 mL of an aquatic medium containing dried vegetables with 10 mL of a stationary phase of a stock *Paramecium* culture (ca. 10,000 cells/mL). The cultures were maintained at room temperature (25 ± 2 °C) with a photoperiod of 14 h light and 10 h dark (Rao et al., 2006; Minter et al., 2011). The pH of the original culture was naturally between 5 and 7. After 2 days, the success of the propagation was checked by optical microscopy. In order to obtain a filtered culture, free of suspended organic matter, *Paramecium* cells were transferred to the darkened bottom part of a half-darkened flask. The bottom part of the flask was separated by using a cotton wool in order to limit the light and the O_2 access of *Paramecium* cells, while the upper portion of the flask was filled with clean, stagnant water. After storing this flask under natural sunlight at room temperature for 1 day, the *Paramecium* cells moved through the wet cotton wool into the filtered, clean water. Cells were collected from this medium for the toxicology studies.

2.4. Toxicity and remediation experiments

Two sets of experiments are designed. In the first set of experiments, *Paramecium* cultures are exposed to aqueous Hg(II) in the concentration range of 125–1500 μg L^{-1}. In the second set of experiments, exactly the same conditions are used, but silica-gelatin aerogel is added to the exposed cultures. Therefore, the comparison of the viability of *Paramecium* cultures in the two sets of experiments quantitatively reveals the remediation efficacy of the silica-gelatin aerogel adsorbent.

The toxicity of aqueous Hg(II) against *Paramecium* was tested by exposing *Paramecium* cultures to different concentrations of Hg(II) in the range of 125–1500 μg L^{-1}. The investigated concentration range was chosen based on the documented sensitivity of *Paramecium caudatum* (Vilas-Boas et al., 2020). Hg(II) stock solutions (0.625–10.00 mg L^{-1} for Hg(II)) were diluted from the monoelement standard solution of 1000 mg L^{-1} $HgCl_2$ (Scharlau) with Milli-Q water. Hg(II) containing *Paramecium* cultures were prepared by mixing the calculated aliquots of the appropriate Hg(II) stock solution and the filtered cell culture.

In order to test the biocompatibility of the silica-gelatin hybrid aerogel, the suspended aerogel was mixed with filtered *Paramecium* cultures to obtain a final aerogel concentration of 0.1–0.5 mg mL^{-1}. The aerogel suspension was prepared by using a well-established protocol, reported previously (Herman et al., 2020b). The dry aerogel was wetted and ground in Milli-Q water for 10 min by using a Potter-Elvehjem tissue grinder. This suspension was sonicated in a sonicator bath (ARGO LAB DU-32) for 15 min and then stirred for 20 min at 300 rpm on a magnetic stirrer using a 1.0 cm Teflon-coated rod. This protocol ensures that the properties, e.g. the particle size distribution of the different batches of the aerogel suspensions are uniform (cf. Fig. 1).

The efficacy of the hybrid aerogel in increasing the survival of *Paramecium* was tested by adding the aerogel adsorbent at a final concentration of 0.1 mg mL^{-1} to *Paramecium* cultures containing Hg(II) in concentrations between 125 μg L^{-1} and 1000 μg L^{-1}.

All experiments were carried out in 24-well cell culture plates (TPP, Sigma-Aldrich) which enabled the simultaneous study of 24 individual samples at the same time. Thus the control, the Hg(II) toxicity and the remediation tests were carried out simultaneously, under identical experimental conditions. Extra care was taken to ensure the identical conditions of the cell cultures in the different types of experiments. 800 μL of the filtered *Paramecium* culture was added to each of the 24 test wells that already contained altogether 200 μL of the appropriate Hg(II) stock solution and/or the aerogel suspension. In the resulting 1.0 mL sample the average number of *Paramecium* cells was ca. 2000. In control samples the Hg(II) solution or the aerogel suspension was replaced with 9.0 g L^{-1} saline (NaCl solution). The different types of experiments were arranged in a random order in the plate. All experiments were performed in several independent replicate sets in different plates.

2.5. Time lapse video imaging

In order to follow the motility of *Paramecium* under the different chemical conditions, the cell culture plates were placed into a zenCell Owl incubator microscope apparatus (innoME GmbH), that is compatible with the standard culture vessels. The device is equipped with 24 independent miniaturized cameras, thus it is capable of the automated, simultaneous monitoring of the 24 cell cultures with real time data capturing and visualization on PC. (The technical specifications of the microscope apparatus are the following: 10 × magnification, 1.2 mm × 0.9 mm field of view, 5 MP image resolution by CMOS camera, 2592 × 1944 display resolution per microscope.) *Paramecium* cultures were monitored for 24 h with 5 min of recording intervals.

In order to follow the motility of *Paramecium*, the sequential

microscopic images were subtracted from each other by using the ImageJ software (National Institutes of Health, USA). The difference between two successive scans is given in pixel number. The pixel number was converted into viable cell number.

Statistical analysis of survival times was conducted using unpaired *t*-test in IBM® SPSS® Statistics v22.0 software. Statistical significance was determined at a p value of 0.05.

2.6. Elemental analysis using ICP-OES

The Hg(II) concentration of aqueous samples was measured after centrifugation (20 min, 3500 rpm, VWR MegaStar 1.6 R). Mercury concentration was measured by inductively coupled plasma optical emission spectrometry (Agilent Technologies ICP-OES SVDV 5100). The ICP-OES experimental parameters are given in Table S1 in the Supporting Information. Samples were acidified with HNO_3 and measured immediately after preparation. Five-point calibration series was applied, diluted from monoelement standard solution. Intensity values were collected at three different wavelengths. Concentration was calculated based on the optical lines that gave the best signal-to-background ratio.

The concentrations of at least 3 independent parallel samples were averaged. Relative standard deviation (RSD) was calculated.

3. Results and discussion

3.1. Adsorption of Hg(II) by silica-gelatin aerogel

The characteristics of the adsorption of aqueous Hg(II) by silica-gelatin aerogel of 24 wt% gelatin content have rigorously been investigated in batch adsorption experiments. Detailed results and appropriate discussions are given in our previous publication (Herman et al., 2020b). The summary is as follows. The selectivity of the adsorbent for Hg(II) has been demonstrated, and adsorption isotherms has been measured. The adsorption isotherm of Hg(II) follows the Langmuir model, and the adsorption capacity of the 24 wt% hybrid aerogel is 209 mg g^{-1}. The adsorption equilibrium is established in 15 min contact time with the adsorbent. The adsorbent can effectively be regenerated and reused. The optimum pH for removing Hg(II) is 6.0. It has been verified that the adsorbent is effective in natural pond water, as well. The most important experimental results reflecting the performance of the aerogel adsorbent are summarized in the Supporting Information of the present article.

Overall, rigorous laboratory tests with appropriate controls have proved that silica-gelatin aerogel of 24 wt% gelatin content is an effective adsorbent of aqueous Hg(II) under conditions of practical applications in environmental engineering. Therefore, the investigation of the effectiveness of this adsorbent in the remediation of living aquatic cultures is beneficial to facilitate technology development.

3.2. Remediation of paramecium cultures

The motility of the *Paramecium* cells is directly proportional to their viability in the culture (Soldo et al., 1970; Evans and Nelson, 1989). In order to quantify the motility of the cells, the pixel differences of the sequential video microscopic images of the culture were calculated by using image analysis and plotted as function of observation time. The difference between the subsequent video microscopy snapshots is caused by the displacement of *Paramecium* cells during the observation period. The cessation of the quantifiable difference between successive images means the zero motility, thus, the total destruction of *Paramecium* cells. The effect of the different chemical treatments on the survival of *Paramecium* can be estimated by carrying out the image analysis on the complete image sequence collected during the 24 h observation period of the culture (Nagy et al., 2017).

Representative motility curves recorded in the Hg(II) toxicity tests are shown in Fig. 2. The quick, spiral-like, ciliary motion of the *Paramecium* cells results in the large amplitude fluctuation of the calculated motility curve. In order to facilitate the visualization of the data, the curves were smoothed by applying the adjacent-averaging method (Fig. 2).

The decrease of the motility of *Paramecium* due to the toxicity of Hg(II) is visible in the reconstructed videos at higher Hg(II) concentrations. The time when the number of viable *Paramecium* cells decreases to 10% of the initial number of cells in the culture was approximated based on the reconstructed videos (Table 1).

In order to increase the robustness of data evaluation the motility curves were analyzed. The cease of the motility, i.e. the total destruction of the culture, was quantified by fitting a linear trendline to the original (unsmoothed) data points of the motility curve by using the Levenberg-Marquardt least-squares algorithm. In each experiment, the fit of the trendline was started at the start of the visible monotonous decrease of motility. The x-axis intercept of the trendline was calculated from the estimated slope and y-axis intercept of the trendline. The x-axis intercept gives the time when the motility of the culture decreases to zero, i.e. the number of the viable individual cells decreases to zero. This time is termed the "survival time" of *Paramecium* and it is used to quantify the viability of the cultures.

The *Paramecium* motility curves measured at different Hg(II) concentrations in the absence and in the presence of the aerogel adsorbent are shown in Fig. 3 together with the fitted linear trendlines. The relatively large standard deviation (SD) of the estimated survival times is the result of the large fluctuation in the motility curves caused by the quick displacement of *Paramecium* cells. However, it is the quick displacement of the *Paramecium* cells that makes the quantification of their motility possible by using a simple and robust image analysis protocol for the evaluation of the time lapse video images.

The survival times corresponding to the fitting of the motility curves in Fig. 3 are shown as function of Hg(II) concentration in Fig. 4. The survival times calculated by data fitting (Fig. 4) are in excellent agreement with the survival times approximated based on the reconstructed videos (Table 1).

The evaluation of the Hg(II) toxicity experiments carried out in the absence of the aerogel adsorbent reveals the following features. The survival of *Paramecium* shows a clear dose-effect relationship. As seen in Fig. 4, 125 μg L^{-1} of aqueous Hg(II) does not have significant toxicity, but a higher concentration of Hg(II) causes the expressed reduction of the survival time. The survival time steeply decreases in an approximately linear manner with the increase of the Hg(II) concentration from 125 μg L^{-1} to 750 μg L^{-1}. In the case of 750 and 1000 μg L^{-1} Hg(II) concentrations, the total destruction of the *Paramecium* population was detected after 70–80 min (Fig. 4).

The biocompatibility of the aerogel was tested in cultures containing the adsorbent in different concentrations (0.1, 0.25 and 0.5 mg mL^{-1}) in the absence of Hg(II). None of the applied aerogel concentrations caused the decrease of the survival time of the *Paramecium* cells during the observation period. (The survival time of the *Paramecium* cells in the presence of 0.1 mg mL^{-1} aerogel is the first point in Fig. 4, where c_0Hg(II) = 0.) The aerogel concentration of 0.1 mg mL^{-1} was found to be optimal for testing the efficiency of the adsorbent in cultures exposed to Hg(II). A higher adsorbent concentration is not feasible for developing an environmental engineering technology, and also, the quantitative evaluation of video microscopy data is less reliable at high aerogel concentrations by using the simple image analysis protocol.

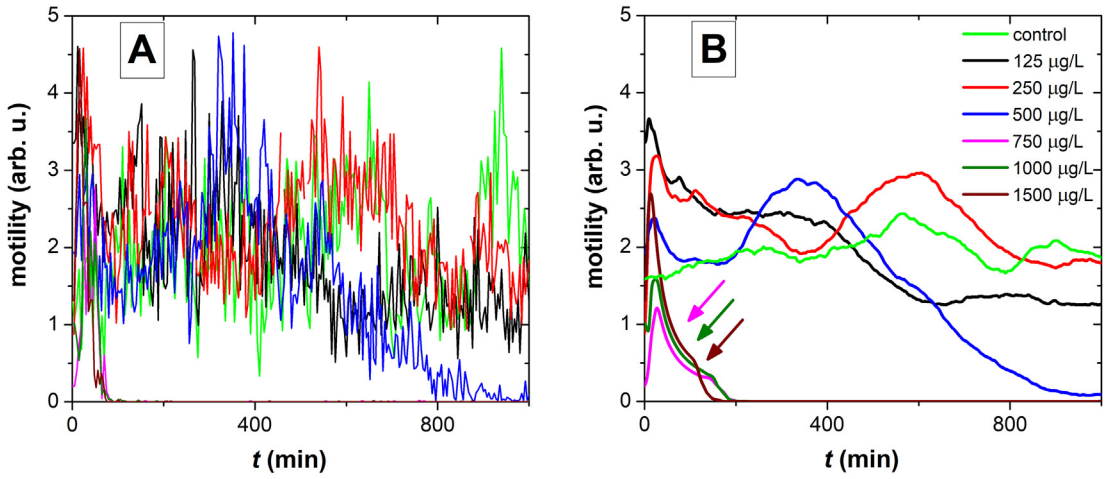

Fig. 2. The motility of *Paramecium* cells in cultures exposed to different concentrations of aqueous Hg(II) visualized by plotting the pixel differences of the sequential video microscopy images of the cultures as function of observation time. The total destruction of a culture occurs when the motility drops to zero. The Hg(II) concentrations are shown in the legend. Panel A shows the original data, and panel B shows data smoothed by applying the adjacent-averaging method.

Table 1
The time when the number of viable *Paramecium* cells exposed to different concentrations of Hg(II) decreases to 10% of the initial number of cells in the culture in the absence and in the presence of the aerogel adsorbent. The data in this table are approximated based on the videos reconstructed from the microscopy images. The ±SD values are approximated from replicate experiments.

c_0 Hg(II) (μg L^{-1})	Hg(II) toxicity test	Hg(II) + aerogel
0 (control)	viable culture	viable culture
125	viable culture	viable culture
250	viable culture	viable culture
500	750 ± 110 min	viable culture
750	75 ± 10 min	420 ± 45 min
1000	65 ± 10 min	220 ± 25 min

The efficacy of the aerogel in increasing the survival of *Paramecium* was studied under identical experimental conditions that were used to test the toxicity of aqueous Hg(II). Fig. 4 shows the survival time of *Paramecium* as function of Hg(II) concentration in the presence of 0.1 mg L^{-1} aerogel adsorbent. The results explicitly prove that the application of the aerogel adsorbent significantly improves the survival time of the *Paramecium* in a large range of aqueous Hg(II) concentrations. As a result of the addition of the aerogel, the majority of the *Paramecium* cells are viable during the total observation period at Hg(II) concentrations up to 500 μg L^{-1} (Table 1 and Fig. 4). Comparing the survival time vs. Hg(II) concentration data measured in the absence and in the presence of the aerogel adsorbent reveals, that the starting point of the steep decrease of the survival of *Paramecium* is shifted from 125 μg L^{-1} to 500 μg L^{-1} Hg(II) concentration in the presence of the aerogel. The mortality of the *Paramecium* cells becomes significant starting at 500 μg L^{-1} Hg(II) concentration in the remediated cultures, in contrast to the steep decrease of survival starting at 125 μg L^{-1} Hg(II) in the Hg(II) toxicity tests. Another important difference between the survival of *Paramecium* in the absence and in the presence of the aerogel is, that a much steeper decrease of survival is observed with the increase of the Hg(II) concentration in the presence of the aerogel. The explanation of this phenomenon is the following.

In the absence of living cells, the equilibrium concentration of aqueous Hg(II) in the medium used for the cultivation of *Paramecium* is much lower in the presence of 0.1 mg L^{-1} aerogel than the total Hg(II) concentration in the system. This is due to the distribution equilibrium between Hg(II) adsorbed on the aerogel and dissolved in the aqueous medium. The adsorption isotherm of Hg(II) measured under these conditions is shown in Fig. 5. In accordance with the laws of adsorption, the equilibrium concentration of aqueous Hg(II) [c_{Eq}Hg(II)] increases steeply in a non-linear manner as function of the initial (total) Hg(II) concentration [c_0Hg(II)] in the presence of the adsorbent. The remaining concentration of Hg(II) [i.e. c_{Eq}Hg(II)] is shown in Fig. 4 with green markers.

Naturally, the survival time of the *Paramecium* cells is inversely proportional to the amount of Hg(II) taken up by the cells. Evidently, the adsorption equilibrium lowers the amount of aqueous Hg(II) that can be taken up by the cells and contribute to toxicity. In the simultaneous presence of living cells and the aerogel adsorbent, complex distribution equilibria are established, and these govern the proportionation of Hg(II), as remaining aqueous Hg(II), adsorbed Hg(II) and Hg(II) taken up by the cells. Due to these complex equilibria, the survival time of *Paramecium* cells shows a very steep decrease when it is contrasted to the total concentration of Hg(II) in the case of the aerogel treated samples (Fig. 4). Up to 500 μg L^{-1} total Hg(II) concentration, the aerogel adsorbent keeps the effective Hg(II) concentration below the toxic level, but at higher total Hg(II) concentrations, the amount of aqueous Hg(II) taken up by the cells reaches the dose that causes the destruction of *Paramecium* cells.

3.3. Toxicity of Hg(II)

Based on the present results, the approximate LC50 value for the toxicity of aqueous Hg(II) to *Paramecium* for 24 h survival is higher than 125 μg L^{-1} in the un-remediated cultures, and it is ca. 500 μg L^{-1} in the presence of the aerogel adsorbent. Aqueous Hg(II) toxicity has previously been studied under several conditions. Due to the large variation of the experimental conditions, the reported LC50 values are significantly different from each other (Nyberg and Bishop, 1983; Madoni et al., 1992; Miyoshi et al., 2003; Liu et al., 2017). A recent meta-analysis of the various toxicological studies conducted on ciliates as model organisms arrived to the following conclusions (Vilas-Boas et al., 2020). *Paramecium caudatum* is one of the most frequently used species for assessing the toxicity of aqueous heavy metal compounds. Interestingly, the sensitivity analysis across ciliates taxa showed that *Paramecium caudatum* is not the most sensitive ciliate to Hg(II). By analyzing several

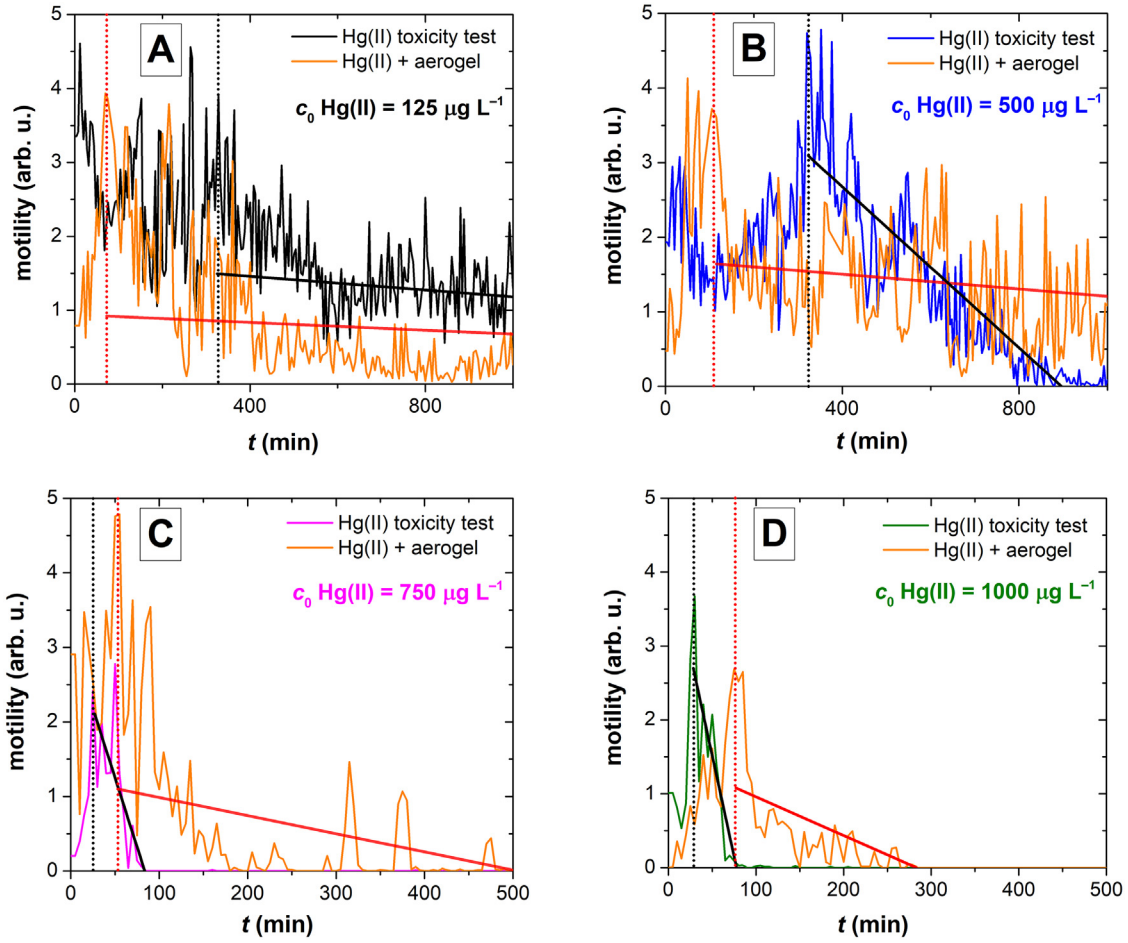

Fig. 3. The motility of *Paramecium* cells exposed to different concentrations of Hg(II) in the absence and in the presence of the aerogel adsorbent. The start of the monotonous decrease of the motility of *Paramecium* cells is marked by a vertical dashed line in each curve. The continuous black lines show the results of the fitting of the motility curves recorded in the Hg(II) toxicity tests. The continuous red lines show the results of the fitting of the motility curves recorded in the remediation tests. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

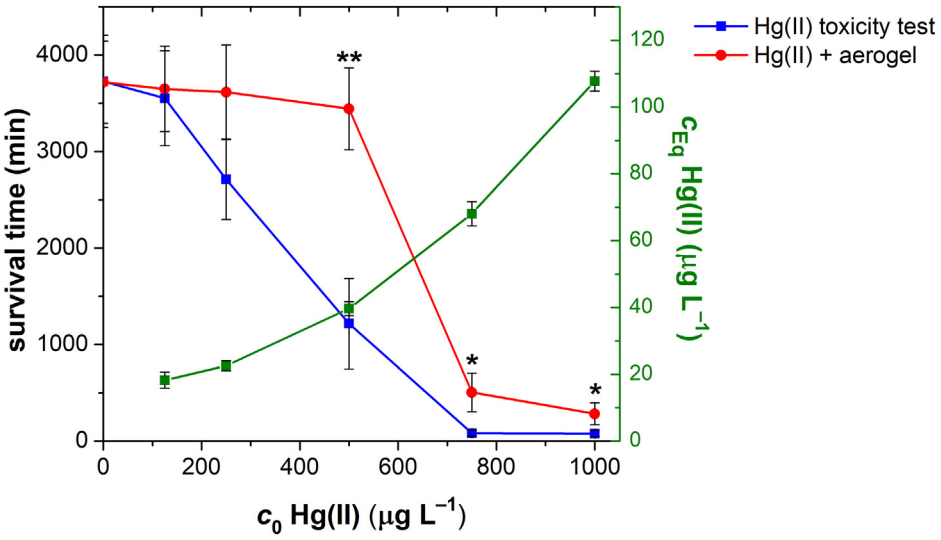

Fig. 4. The survival time of *Paramecium* cells as function of aqueous Hg(II) concentration. The BLUE markers correspond to the Hg(II) toxicity experiments carried out in the absence of the aerogel adsorbent, and the RED markers correspond to the remediation experiments carried out in the presence of 0.1 mg mL^{-1} aerogel adsorbent under otherwise identical conditions. The markers and error bars represent the compiled results of independent replicate experiments. Significance levels at *: $p < 0.05$ and **: $p < 0.01$. The GREEN markers show the equilibrium aqueous Hg(II) concentration as a function of the initial Hg(II) concentration established in the presence of 0.1 mg mL^{-1} aerogel adsorbent in the cell free culture medium (cf. Fig. 5). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

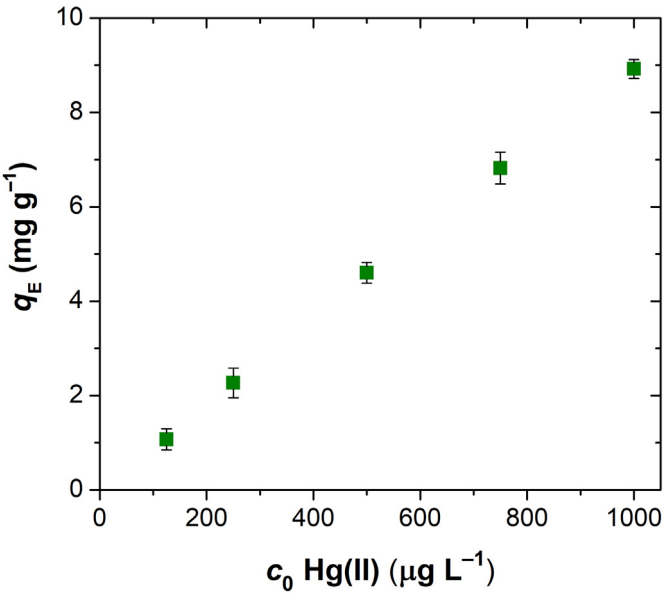

Fig. 5. Experimental isotherm of the adsorption of aqueous Hg(II) by the silica-gelatin aerogel of 24 wt% gelatin. Batch experiments; pH = 6.0; 0.10 mg mL^{-1} aerogel adsorbent.

literature sources, Vilas-Boas et al. concluded that the LC50 value of *Paramecium caudatum* for Hg(II) toxicant is ca. 260 μg L^{-1} determined by measuring mortality. This value is in good agreement with the results of the present study (cf. Figs. 3 and 4); keeping in mind that in the present study the motility of cells was the primary information indicating viability. This is an independent validation of the time-lapse video microscopy based approach for toxicity assessment presented in this article.

Liu et al. has investigated the accumulation and the toxicological mechanisms of aqueous Hg(II) and MeHg in five different *Tetrahymena* species (Liu et al., 2017). *Tetrahymena* are unicellular eukaryotic protozoa that are also frequently used model species in studying the toxicity of heavy metal compounds. Liu et al. used flow cytometry (FACS) and propidium iodide (PI) staining to evaluate membrane damage and elevation of intracellular reactive oxygen species (ROS) induced by Hg(II) and MeHg. After 24 h exposure of 100 μg L^{-1} Hg(II), the cellular functions were intact compared to control groups. A higher concentration of Hg(II) induced the elevation of intracellular ROS concentration, but did not significantly damage the cell membrane. (MeHg is more effective in causing cell death by damaging cell membranes.) Based on these results, we propose that the mechanism of Hg(II) toxicity towards *Paramecium caudatum* is the uptake of the toxic metal ion by the cells and the consequent elevation of intracellular ROS concentration. The present study shows, that silica-gelatin aerogel in the *Paramecium* culture effectively inhibits the uptake of Hg(II) by the cells by adsorbing the ions, which protects the cells form Hg(II) toxicity. Hypothetically, the protective effect of the adsorbent was significantly lower whether Hg(II) were damaging the cell membrane. In this case, the adsorbent and the cell membrane would be in dynamic competition for binding Hg(II), that could effectively be shifted only by applying a very high concentration of the adsorbent. This mechanism can be ruled out based on the present results.

4. Conclusions

The results of the present study unambiguously show that the cultures of the aquatic unicellular organism *Paramecium caudatum* can conveniently be used as a model bioindicator system for testing the *in situ* remediation efficiency of advanced functional materials designed for the removal of aqueous Hg(II). *Paramecium* cultures show a clear exposure-response relationship regarding Hg(II) toxicity. The survival time of the *Paramecium* cells is inversely proportional to the amount of Hg(II) taken up by the cells, that correlates with the equilibrium concentration of aqueous Hg(II) in the culture medium. The need of practical testing during the course of adsorbent development highlights the significance of the presented quasi-realistic aquatic toxicity model system that fulfills the requirements of environmental and chemical engineering technology development. Testing in the presence of a bioindicator organism gives a straightforward answer for the suitability of an adsorbent for environmental applications, for which the chemical laboratory tests carried out under sterile conditions are insufficient.

Credit author statement

Petra Herman: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Alexandra Kiss:Methodology, Validation, Formal analysis, Investigation, István Fábián: Conceptualization, Resources, Supervision, Funding acquisition, József Kalmár: Conceptualization, Validation, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision, Gábor Nagy: Conceptualization, Methodology, Validation, Resources, Data curation, Supervision

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

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