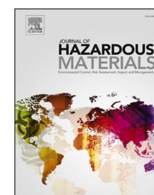
Journal of Hazardous Materials 458 (2023) 131930

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat



Research Paper

Phytoremediation, recovery and toxic effects of ionic gadolinium using the free-floating plant *Lemna gibba*

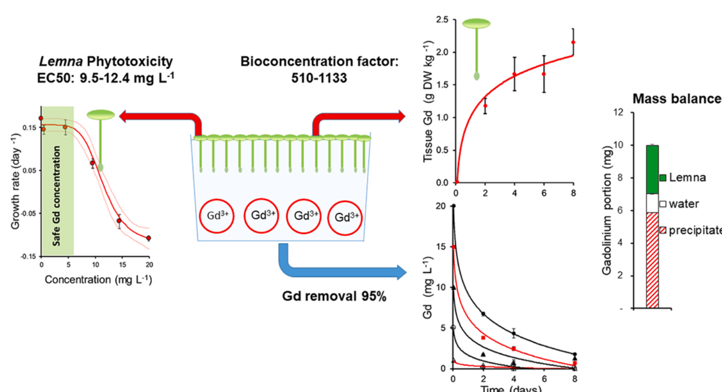
Sándor Szabó [a,*,1], Györgyi Zavanyi [a,b,1], Gergő Koleszár [a,b], Dahlia del Castillo [a], Viktor Oláh [c], Mihály Braun [d]

[a] Department of Biology, Institute of Environmental Sciences, University of Nyíregyháza, P.O. Box 166, H-4401 Nyíregyháza, Hungary
[b] Doctoral School of Biological Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary
[c] Department of Botany, University of Debrecen, Egyetem Square 1, H-4032 Debrecen, Hungary
[d] Isotope Climatology and Environmental Research Centre (ICER), Institute for Nuclear Research, Debrecen, Hungary

HIGHLIGHTS

- Ionic gadolinium (Gd) resulted in 50% growth inhibition at 12.4 mg L^{-1} for *Lemna*.
- Up to 2.5 g Gd kg^{-1} accumulated in *Lemna* biomass.
- Gd removal efficiency in water was 95% in 8 days.
- Plant-related Gd removal was 17–37% for *Lemna*.
- From *Lemna* ash, 23.2 g Gd per kg can be reached.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Wagner L. Araújo

Keywords:
Accumulation
Bioconcentration, *Lemna*
Macrophyte
Rare earth element
Removal
Toxicity

ABSTRACT

The biosorption and recovery of ionic gadolinium (Gd) from contaminated water by the free-floating duckweed *Lemna gibba* was studied. The highest non-toxic concentration range was determined as (6.7 mg L^{-1}). The concentration of Gd in the medium and in the plant biomass was monitored and a mass balance was established. Tissue Gd concentration of *Lemna* increased with increasing Gd concentration of the medium. The bioconcentration factor was up to 1134 and in nontoxic concentrations up to 2.5 g kg^{-1} Gd tissue concentration was reached. *Lemna* ash contained 23.2 g Gd kg^{-1}. Gd removal efficiency from the medium was 95%, however, only 17–37% of the initial Gd content of the medium accumulated in *Lemna* biomass, an average of 5% remained in the water, and 60–79% was calculated as a precipitate. Gadolinium-exposed *Lemna* plants released ionic Gd into the nutrient solution when they were transferred to a Gd-free medium. The experimental results revealed that in constructed wetlands, *L. gibba* is able to remove ionic Gd from the water and can be suitable for bioremediation and recovery purposes.

* Corresponding author.
E-mail address: szabo.sandor@nye.hu (S. Szabó).
[1] SS and GZ contributed to the manuscript equally

https://doi.org/10.1016/j.jhazmat.2023.131930
Received 4 April 2023; Received in revised form 1 June 2023; Accepted 23 June 2023
Available online 25 June 2023
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Journal of Hazardous Materials 458 (2023) 131930

1. Introduction

In the 21th century, the rising trend in the use of lanthanides has led to the release of these elements to the environment in continuously increasing amounts [10]. Among lanthanides, gadolinium (Gd) was the first that had reached one order of magnitude higher ambient concentration in the hydrosphere compared to its historic level [4]. High anthropogenic Gd inputs were detected in those densely populated areas where highly advanced healthcare systems extensively used magnetic resonance imaging (MRI) [17]. For MRI purpose at a global level, 22–66 tons of Gd are used annually as Gd-based contrast agents (GBCAs) [17]. Trought urination by the patients, cumulatively an estimated 4500 tons of Gd has entered wastewater treatment plants (WWTP) in form of GBCAs up to 2021 [8]. At WWTP effluent outfalls, Gd concentrations in the range of 200–1100 μg Gd L^{-1} have been reported [4]. In aquatic ecosystems over the long term, toxic Gd^{+3} has a potential to be released from the chelates [5,18] due to microbial and UV degradation. Beyond GBCAs from health care facilities, industrial release of ionic Gd to the environment could also be significant [10,26,40]. In mining areas, elevated blood lanthanide concentrations of local residents, including Gd, correlated well with the increased soil concentrations [21]. Therefore, it is very urgent to study the toxicological and bioaccumulation characteristics of ionic Gd in more detail. Studies have already investigated the adverse effects of ionic Gd on a wide scale of aquatic organisms like bacteria, algal species, Cnidaria, Rotifera, Crustacea and mussels [13,14,37]. Under marine conditions, the bioaccumulation potential of macroalgae (*Phucus, Gracillaria, Osmundea, Ulva*) for ionic Gd has also been reported recently [11,28].

It is well known that phytoremediation with freshwater aquatic plants is an effective and inexpensive method for removing pollutants from the environment. Far exceeding marine macroalgae, free-floating plants show the most rapid growth and best harvestable characteristics. In addition, they are also capable of accumulating heavy metals [2, 24,39] therefore, they are also used to clean industrial wastewaters with a high heavy metal content [1,32]. Despite their ecological importance and phytoremediation potential in freshwater ecosystems, only one single publication has investigated the biofiltration potential for ionic Gd using tropical species so far [15]. In addition, until now, not a single study has addressed the phytotoxicity of Gd on freshwater macrophytes. The Gd phytoremediation potential of aquatic plants grown in temperate regions has also been unexplored yet.

Therefore, the aim of this study was to find a safe Gd concentration range for biofiltration in order to maintain vitality of the free-floating plant (*Lemna gibba*) for extended periods. To this end, we first determined the phytotoxicity of ionic Gd on *Lemna* under a wide range of analyte concentrations. Secondly, we examined the Gd removal capacity of the species under the highest non-toxic concentration range in order to mimic optimized industrial biofiltration conditions. Our further aim was to investigate the bioconcentration potential and mobilization kinetics of Gd in the plants. Thus, the uptake and release kinetics of Gd between the nutrient medium and the plants was followed in time too.

Table 1
Summary of Gd toxicity and biofiltration experiments.

No. of exp.	Aims of experiment	Gd^{3+} in the water (mg L^{-1})	Pot volume (L)
1	Phytotoxicity Bioaccumulation Gd removal	0–20	0.3
2	Kinetics of bioaccumulation Kinetics of Gd removal	5	2.0
3	Kinetics of Gd release	5 and 10, then Gd-free solution	2.0 0.2

2. Material and Methods

2.1. Laboratory experiments

2.1.1. Plant collection

Specimens of the duckweed *Lemna gibba* L. (subsequently termed as *Lemna*) were collected from Igice canal near Nyíregyháza (NE Hungary, N 47.996376°, E 21.734152°).

2.1.2. Pre-incubation and experimental conditions

The plants were pre-incubated for 30 days under experimental conditions on a general purpose "BS" medium [3] that was supplemented by adding 10 mg N L^{-1} as NH_4NO_3. For optimal plant growth, phosphorus was added as K_2HPO_4 to a final concentration of 2.0 mg P L^{-1} and a supply of micronutrients was ensured by adding 0.1 mL L^{-1} TROPICA supplier micronutrient solution. The cultures were placed into a temperature-controlled (24–26 °C) water bath and were incubated under 210–230 μmol m^{-2} s^{-1} photon flux density, 16-h light/8-h dark. The toxicity and exchange kinetics (i.e., accumulation and release) of ionic Gd between free-floating aquatic plants and the medium were investigated in three subsequent experiments. Ionic gadolinium was added to the culture medium in form of $GdCl_3$ (Sigma Aldrich). The pH of the medium was adjusted to 6.5 where 95% of Gd was in form of Gd^{3+} in the medium [9]. In each experiment, four culture pots as experimental units were used per treatment. The purpose and details of the three experiments are summarized in Table 1.

2.1.3. Toxicological traits, bioaccumulation, Gd removal

Experiment-1 was designed to determine the toxicological characteristics, removal and accumulation of Gd^{3+} in *Lemna* under a wide range of initial analyte concentrations.

The plants (500 ± 5 mg fresh weight (FW) were exposed under experimental conditions for 8 days in pots (9 cm in diameter) containing 0.3 L nutrient solution. In parallel, pots with the same treatments but without plants were used as blanks to track the possible Gd precipitation. Initial Gd concentrations in the medium were 0, 1, 5, 10, 15 and 20 mg L^{-1} by adding 1000 mg Gd^{3+} L^{-1} stock solution. Water samples (10 mL) were taken for Gd analyses from each pot on the initial day and on the 2nd, 4th and 8th days, respectively. After 8 days, the plants were harvested, and the fresh weight (FW) of duckweed fronds from each treatment was measured. Subsamples of 50–100 mg FW from the initial and 8th days were taken for determination of tissue chlorophyll and carotenoid concentration. For determining the dry weights (DW), the plant samples were dried at 105ºC until constant weight was achieved (within 24 h). Relative growth rates (RGR) of the cultures were calculated as follows: $RGR = (\ln DW_8 - \ln DW_0)/t$ in which DW_8 and DW_0 are the dry weights after 8 days and at time zero, respectively, and t is the time in days (8). RGR based on chlorophyll content of the plants was calculated from the final and initial total chlorophyll contents. After acidic digestion of the dried plants, Gd content of the samples was determined (see 2.2.).

2.1.4. Kinetics of bioaccumulation, Gd removal

Experiment-2 was designed to monitor the uptake kinetics of ionic Gd into *Lemna* under 5 mg L^{-1} initial Gd concentration that proved to be non-toxic within 8 days exposure according to the first experiment. *Lemna* plants (10 g FW, plus controls without plants) were exposed for 8 days under experimental conditions in 2 L pots (11 ×11.5 ×18 cm DxWxL) containing 2 L nutrient solution. The initial nominal Gd concentration in the medium was 5 mg L^{-1}. Samples (15 mL) were taken from the solutions at the initial day and after 1, 2, 4, and 8 days, and then analysed for Gd concentration. Plant samples (2000 ± 5 mg FW) from each cultivation pot were taken at the beginning of the experiment and after 2, 4, 6 and 8 days. After 8 days, all plants from each cultivation pot were harvested, and FW and DW were measured. After acidic digestion of the plants, Gd content was determined (see 2.2.).

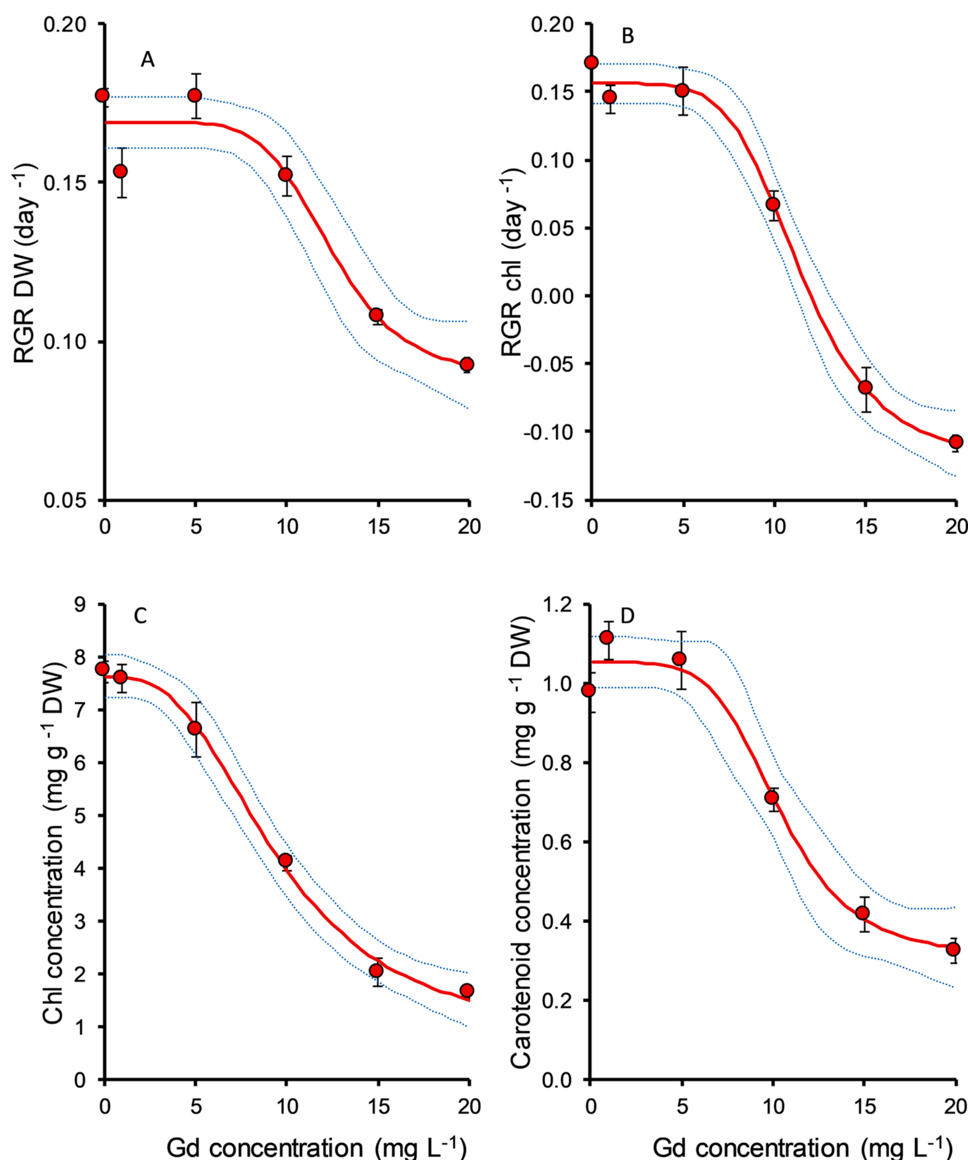

Fig. 1. The impact of free Gd on dry weight-based relative growth rate (RGR) (A), RGR based on total chlorophyll (B); concentration of tissue chlorophyll (C) and carotenoid concentration (D) of *Lemna*. All data are based on dry weight. Means ± SE, dotted lines are 95% confidence intervals, n = 4.

2.1.5. *Kinetics of Gd release*

Experiment-3 was designed to determine the dynamics of ionic Gd leaching from Gd-treated *Lemna*. The plants were cultivated in duckweed enclosures [33,36] for 8 days under experimental conditions in separate pots (11 ×11.5 ×18 cm DxWxL), each containing 2 L nutrient solution. Portions of pre-incubated duckweed fronds (1 g FW) were placed inside the enclosures. As soon as *Lemna* cover reached 100%, overcrowding was avoided by replacing the enclosure with a bigger one. This method allowed us to culture *Lemna* on a static medium under optimal growing conditions avoiding overcrowding as well as algal inhibition [16,33]. The initial Gd concentration in the medium was adjusted to 5 and 10 mgL^{-1} by adding 1000 mg Gd^{3+} L^{-1} stock solution. For both treatments, 5 parallel aquaria were used. On the last day, all duckweed fronds were harvested from each aquarium (10–11 g FW) and rinsed with Gd-free tap water. The excess water was removed from the surface of the plants by using a salad centrifuge. Subsequently, using 20–20 pots (0.2 L), 20–20 portions of Gd^{3+}-pretreated duckweed fronds (1000 ±2 mg FW) were cultivated on Gd-free nutrient medium for 8 days. The initial concentration of Gd in *Lemna* and in the water was determined in 4 parallel samples of both Gd treatments (5 and 10 mgL^{-1}). Then 4 parallel samples of each treatment were collected after 1, 4, 6 and 8 days. The total number of plant and water samples were 40 and 40 respectively. The dry weight of the duckweed fronds from both treatments was measured, and Gd concentration of the fronds was analysed (see 2.2). Samples were also taken from the medium and analysed for their Gd concentration (see 2.2).

2.2. *Analytical methods*

In experiment 1, after extraction and measurement of photosynthetic pigments (chlorophyll a and b, carotenoids), their concentrations were measured [35] and calculated according to Lichtentaler [22] on a dry weight basis. Before determination of Gd, acid digestion was used to dissolve the plant materials (50–200 mg DW) with 5 mL of 67% (m/m) nitric acid (Promochem, suprapure) and 0.5 mL 30% (m/m) hydrogen peroxide (Molar Chemicals). The digestion was carried out using Xpress teflon bombs and Chem Mars 5 microwave system at 165 °C for 20 min. Digested samples were diluted to 10 mL by ultra-pure water (Synergy UV Milli-Q). Ashing of dried plant samples in experiment 2, was carried out using laboratory furnace (Nabertherm). Dried plant samples

Table 2
The calculated effective Gd concentrations (EC) with 95% confidence intervals in brackets (95% CI) that resulted in 10%, 20% and 50% inhibition of the respective test endpoints (dry weight- and chlorophyll based relative growth rates, and chlorophyll and carotenoid concentration) by the end of the 8 days-long treatments. Data are in mg L^{-1}.

Variable	EC10	EC20	EC50	RSE	pseudo-R^2
RGR DW	8.7 (6.7–10.8)	9.9 (8.1–11.8)	12.4 (10.0–14.8)	0.0134	0.93
RGR Chl	7.6 (6.3–8.9)	8.8 (7.9–9.8)	11.5 (10.2–12.8)	0.0238	0.98
Cc Chl	4.4 (3.1–5.8)	5.8 (4.6–7.1)	9.5 (7.6–11.4)	0.5531	0.98
Cc Car	6.7 (4.4–9.1)	7.9 (6.29.6)	10.3 (8.7–12.0)	0.1014	0.96

(500–600 mg DW) at day 8 were gradually heated for 8 h up to 550 °C and were kept at 550 °C for 4 h. Ash masses were measured by analytical balance. Total ash content was dissolved in 50 mL 1% nitric acid. Water samples were filtered through membrane syringe filters (Ministart NY 25 mm, Sartorius) with pore size of 0.45 µm. Nitric acid (0.1 mL 67% m/m) was added to 10 mL filtered water sample. The samples were analysed for Gd using an Agilent 8800 inductively coupled plasma mass spectrometer (ICP-MS). Integration time was 1 s for the ^{157}Gd isotope. Tune mode was MS/MS, and collision cell was used with 7 mL/min He flow. NIST traceable monoelement Gd standard (CPAChem, 1000 mg L^{-1}) was used for preparing calibration (0.1, 1, 10 and 100 µg L^{-1}) and quality check (50 µg L^{-1}) solutions. The limit of quantification was 0.05 µg L^{-1} for water samples, and 0.05 mg kg^{-1} for plant samples. The instrumental parameters were detailed in Table A1.

2.3. Data analysis

Gadolinium removal efficiency (R%) was calculated by using its initial concentration of the water (C_0, mg L^{-1}) and its concentration at day 8 (C_8, mg L^{-1}). R%= 100 * (C_0-C_8)/C_0.

Plant-related Gd removal efficiency (R_{plant} %) was calculated through initial Gd concentration (C_0, mg L^{-1}) and volume (V) of the medium; and the total Gd content of the plants at day 0 and 8 (Gd_{plant0}, Gd_{plant8} mg DW), as follows: R_{plant}%= 100 * (Gd_{plant8} -Gd_{plant0})/(C_0 *V).

Bioconcentration factor (BCF), defined as the ratio between Gd concentration in the plants ($Gdcc_{plant}$ as mg Gd kg^{-1} DW), and the initial Gd concentration in water (C_0, mg Gd L^{-1}) was calculated as follows: BCF= $Gdcc_{plant}$ / C_0.

Mass balance for Gd was estimated using the calculated amounts of Gd in water and in plants at the respective sampling day. The amount of precipitated (residual) Gd_{res} was estimated as the difference between the summarized initial Gd amount in the water and plants ($Gd_{j,0}$), and the summarized amount of Gd in the water and plants on the given sampling day.

$$Gd_{tot} = \sum_{j=1}^{2} Gd_{j,N} + \sum_{j=1}^{2} \sum_{i=1}^{N-1} Gd^{*}_{j,i} + Gd_{res}$$

Abbreviations are the followings:

N = Number of days

$$i = \{1; \ldots; N\} \quad N = 8$$

$$j = \left\{ \begin{array}{l} 1 = Plant \\ 2 = Water \end{array} \right\}$$

i = Number of days when the subsamples were taken.

j = Type of the sample.

Gd_{tot} = Total amount of Gd in the experiment.

$Gd^{*}_{j,i}$ = Amount of Gd removed by the subsamples.

Gd_{res} = Residual amount of gadolinium (not explained by the measurements) was considered as precipitate.

Table 3
Gd removal in the water (R%), plant-related Gd removal (R_{plant}%), bio-concentration factor (BCF) and their standard errors (SE) in *Lemna* cultures cultivated for 8 days on 1–20 mg L^{-1} initial Gd in experiment 1.

Gd (mg L^{-1})	R%	SE	$R\%_{plant}$	SE	BCF	SE
0						
1	99.0	0.00	37	1.86	1093	116
5	99.8	0.00	21	0.87	510	51
10	86.9	4.98	17	0.29	515	28
15	95.7	1.40	22	0.38	926	13
20	91.2	0.71	24	0.59	1133	17

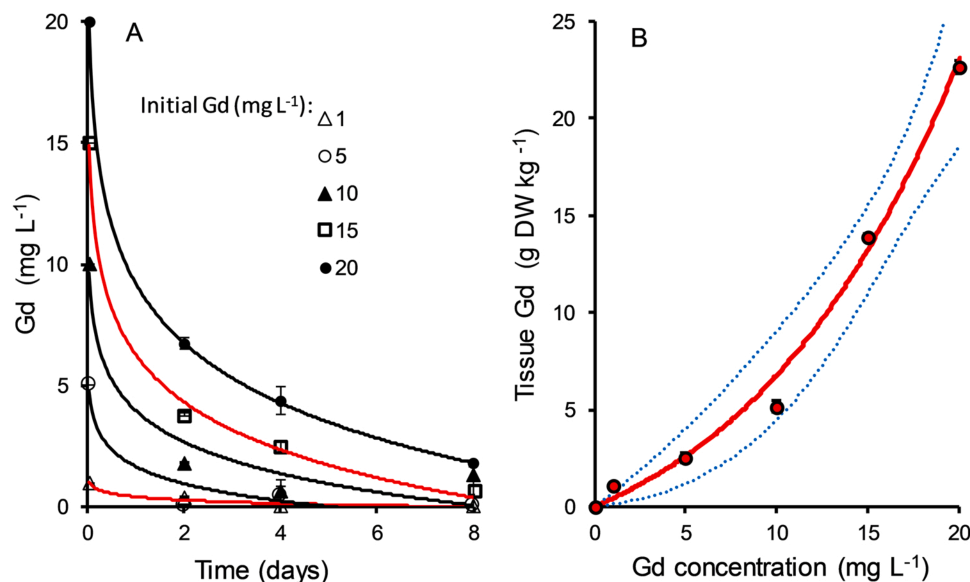

Fig. 2. The change Gd concentration of the water in the presence of *Lemna* (A). The impact of free Gd concentration of the medium on tissue Gd concentration of *Lemna* plants (B). Means ± SE, dotted lines are 95% confidence intervals in Fig2B, n = 4.

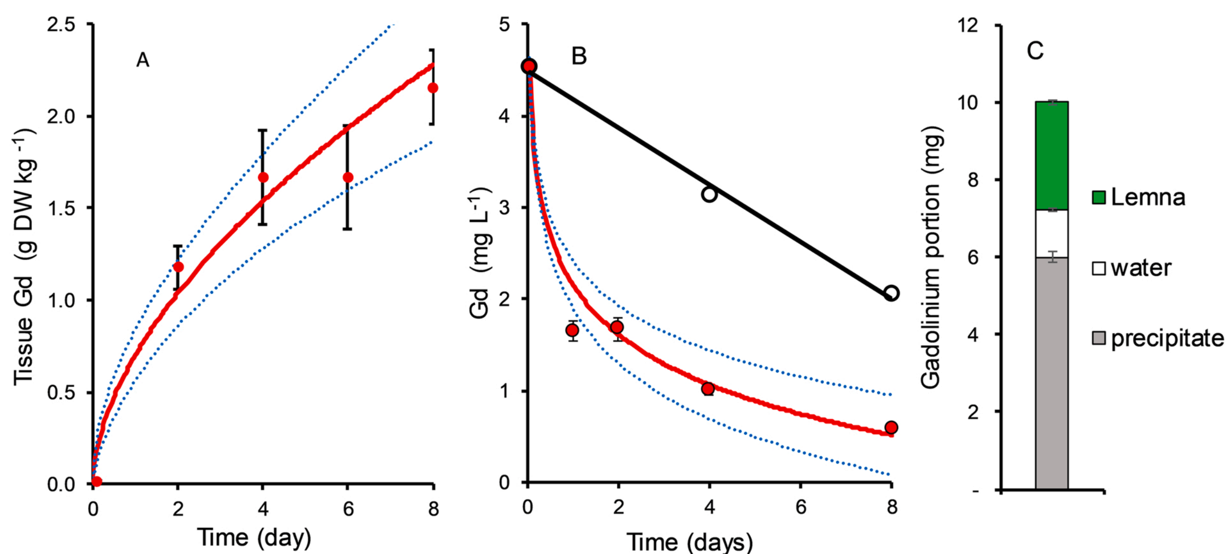

Fig. 3. The change in Gd concentration of *Lemna* (A) and of water in the presence (full circles) and absence (open circles, n = 1) of *Lemna* (B). Gd distribution between *Lemna* water and precipitate at day 8 (C). Plants were cultivated on 5 mg L^{-1} initial gadolinium. Means ± SE, dotted lines are 95% confidence intervals, n = 4.

2.4. Statistics

Gd concentration-dependent plant responses in terms of DW- and chl content-based RGR, and chlorophyll and carotenoid concentrations in the biomass were described by 4-parameter log-logistic functions using results from the 1st experiment. The functions were fitted using the "drc"-package (version 3.0–1, [30]) in RStudio (version 2022.12.0.353, [31]). After visual inspection of the model fittings, pseudo-R^2 and effective concentrations resulting in 10%, 20% and 50% inhibition (EC_{10}, EC_{20} and EC_{50}, respectively) in the respective toxicity endpoint were calculated based on the fitted regression models by means of the "drc"-package's "cor" and "ED" functions.

Nonlinear models were fitted in time-dependent Gd uptake studies. Regression models were calculated by LAB Fit curve-fitting software (Silva and Silva 2018). Leaching of ionic Gd from *Lemna* was described by an exponential decay function using the "drc"-package (version 3.0–1, [30]) in RStudio (verion 2022.12.0.353, [31]).

3. Results

3.1. Toxicological characteristics

There was no significant difference in the growth rates (RGR_{DW}, RGR_{chl}) of cultures growing in the range of 0–5 mg Gd L^{-1}. With increasing Gd concentration, plant growth rate and tissue chlorophyll concentration showed a significant decrease above 5 mg Gd L^{-1} ($P < 0.001$; PC). The calculated 50% growth inhibition for RGR_{DW} and RGR_{chl} was at 12.4 and 11.5 mg Gd L^{-1} (Fig. 1 AB); and the EC_{50} for tissue chlorophyll and carotenoid concentrations were at 9.5 and 10.3 mg Gd L^{-1} respectively (Fig. 1CD, Table 2). At 15 mg Gd L^{-1}, RGR_{chl} became negative and plants treated with 20 mg Gd L^{-1} showed significant mortality.

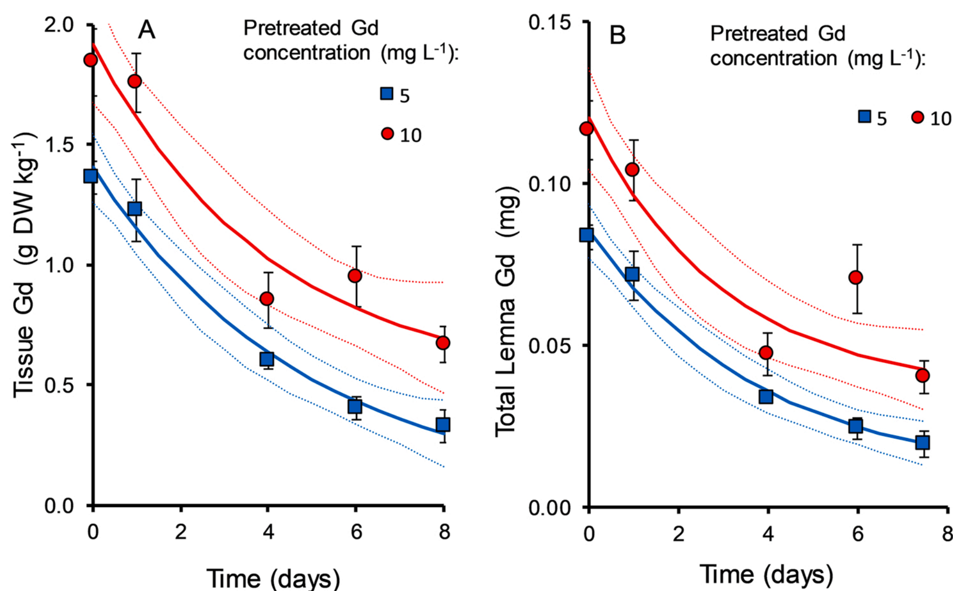

Fig. 4. The change in tissue Gd concentration (A) and total Gd content (B) in Gd-treated (5 and 10 mg L^{-1}) *Lemna* cultures grown on Gd-free media. The concentration data are based on dry weight. Means ± SE, dotted lines are 95% confidence intervals, n = 4.

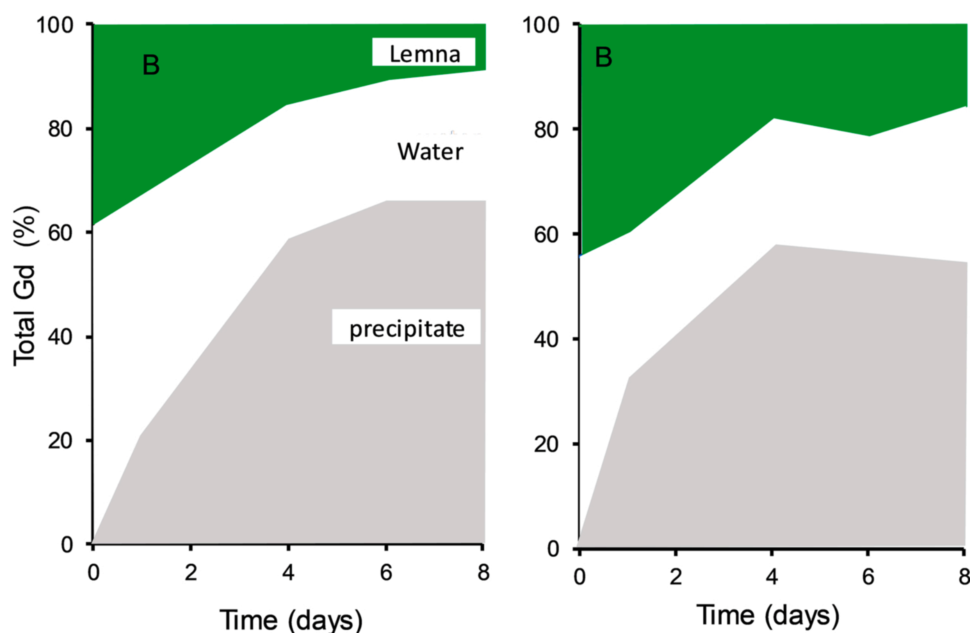

Fig. 5. Distribution of Gd between *Lemna*, water and precipitate in the leaching experiment. *Lemna* had been grown for 8 days on 5 (A) and 10 mg Gd L^{-1} (B) culture media before getting transferred to Gd-free medium.

Table 4
Effective inhibitory concentrations (EC50%) of ionic Gd on various aquatic organisms.

Species	Taxon	EC50% (mg L^{-1})	Publication
Sceletonema costatum	*Diatoma*	4.7	[37]
Pseudokirchneriella subcapitata	*Chlorophyta*	3.11	[13]
Hydra attenuata	*Cnidaria*	2.55	[13]
Hydra attenuata	*Cnidaria*	0.1	[6]
Brachionus calyciflorus	*Rotifera*	1.12	[13]

3.2. Gd removal

The Gd concentration of *Lemna* tissues increased with increasing Gd concentration of the water (Fig. 2). After 8 days of incubation, in the range of 17–37% of the total Gd was detected in the biomass (Table 3). In the cultures growing on the highest Gd concentration, the tissue Gd concentration was 1879 times higher than in control cultures grown on Gd- "free" nutrient solution (Table 3). The bioconcentration factor of plants for gadolinium was ranged between 510 and 1133 based on dry weight. The removal efficiency of the plants decreased with increasing the Gd concentration (Table 3).

Gd concentration of the nutrient solution showed a drastic change in the presence of *Lemna*. The best-fit equation for Gd removal kinetics from the water was a modified negative logarithmic function (Fig. 2). In the examined concentration range (1–20 mg L^{-}), the Gd concentration decreased by an average 89% in just 4 days and by 95% at the end of the experiment (Fig. 2, Table A2.). Despite the sharp drop in the Gd concentration of the culture media, by the end of the experiment only 17–37% of the initial Gd content of the media was present in the plant biomass, an average 7% remained in the water and the largest part (62–79%) was calculated as precipitation (Table A3.).

3.3. Kinetics of Gd accumulation and removal

Under nontoxic conditions up to 2.5 g kg^{-1} for Gd tissue concentration was reached and the best-fit equation for Gd uptake kinetics was a power function. The concentration increased rapidly within two days, however, an equilibrium had not been reached within the 8 days (Fig. 3. A). The best-fit equation for Gd removal kinetics from the water was a modified negative logarithmic (Fig. 3. B). The concentration decreased by 63% in just 2 days and by 87% at the end of the experiment. At the last day, only 27.9 ± 1.4% of the initial Gd content of the medium was present in the plant biomass and the largest part was calculated as precipitation (60.0 ± 1.4%) Fig. 3. C). At the last day, ash content of the dried plants was 10.57 ± 0.16% and ash contained 23.18 ± 2.07 g Gd kg^{-1}.

Table A1
Instrumental parameters for the analysis of Gd by Agilent 8800 ICP-MS/MS.

Plasma		Lenses		MS/MS		Cell	
RF power (W)	1550	Extract 1 (V)	0	Q1 Mass Gain	126	He flow rate (mL·min^{-1})	7
RF matching (V)	1.8	Extract 2 (V)	-175	Q1 Mass Offset	126	Octopole Bias (V)	-18
Sampling depth (mm)	10	Omega Bias (V)	-90	Q1 Axis Gain	0.9987	Octopole RF (V)	190
Plasma gas flow rate (L·min^{-1})	15	Omega Lens (V)	10.6	Q1 Axis Offset	0.18	Energy Discrimination	4.0
Auxiliary gas flow rate (L·min^{-1})	0.7	Q1 Entrance (V)	3.0	Q1 Bias (V)	0		
Type of nebuliser	Micromist	Q1 Exit (V)	3.0	Q1 Prefilter Bias (V)	-16		
Carrier gas flow rate (L·min^{-1})	1	Cell Focus (V)	1.0	Q1 Postfilter Bias (V)	-22		
Make up gas (L·min^{-1})	0.2	Cell Entrance (V)	-40	Q2 Mass Gain	125		
Peristaltic pump (rps)	0.1	Cell Exit (V)	-60	Q2 Mass Offset	126		
S/C temperature (°C)	2	Deflector (V)	-4.8	Q2 Axis Gain	1.0000		
		Plate Bias (V	-60	Q2 Axis Offset	-0.03		
				Q2 Bias	-14		

Table A2
The change of Gd concentration (means, standard errors (SE)) of the water with the presence of *Lemna* in experiment 1. Data are in (mg L^{-1}).

Day 0	Day 2	SE	Day 4	SE	Day 8	SE
1	0.39	0.27	0.01	0.00	0.01	0.00
5	0.06	0.03	0.41	0.22	0.01	0.00
10	1.79	0.04	0.66	0.46	1.31	0.50
15	3.80	0.04	2.46	0.23	0.65	0.21
20	6.72	0.23	4.35	0.58	1.76	0.14

Table A3
Distribution of Gd (in mg) between water, *Lemna* and precipitate after 8 days of exposure in experiment 1.

Gd (mg L^{-1})	Water	SE	*Lemna*	SE	Precipitation
1	0.003	0.000	0.111	0.006	0.186
5	0.003	0.000	0.316	0.013	1.181
10	0.393	0.149	0.524	0.009	1.934
15	0.194	0.063	0.996	0.017	3.247
20	0.527	0.043	1.439	0.035	3.991

3.4. Kinetics of Gd release

In the leaching experiment, when the *Lemna* cultures had been grown on 5 and 10 mg Gd L^{-1} for 8 days, and then were further cultivated on Gd-free nutrient solution, the total Gd content of the plants showed an attenuated decrease. By the 8th day, 65–77% of the total accumulated Gd was released from *Lemna* into the nutrient solution (Fig. 4). Leaching of ionic Gd from L. gibba could be described by an exponential decay equation. The highest released Gd concentrations in the water were 0.69 ± 0.17 and 0.73 ± 0.13 mg L^{-1} in pots where the plants previously cultivated on 5 and 10 mg L^{-1} Gd had been grown respectively. However, the total Gd content of the nutrient solution showed a steady decrease between the initial and 8th days. Mass balances of ionic Gd in the duckweed fronds and in the water were determined. The total Gd (100%) was initially present in 5 and 10 mg L^{-1} Gd treated plants and in the water. After eight days there was a net flux of Gd from the duckweed into the water (77%, 64%), and from the water to the precipitate fraction. On the last day, 53–65% of the total Gd introduced by the plants ended up in the precipitate fraction (Fig. 5, Fig. A4).

4. Discussion

4.1. Toxicity of Gd

Our results showed that ionic Gd had no significant effect on *Lemna* growth up to 5 mg Gd L^{-1}), but above 10 mg L^{-1} Gd proved to be toxic. In contrast to Gd-based contrasting agents in our previous study [7], the measured phytotoxicity endpoints showed a sigmoid inhibition pattern with increasing Gd concentration, and at 20 Gd L^{-1} they showed strong mortality. The toxicity of free Gd was most sensitively indicated by the RGR based on total chlorophyll content of the plants, showing that chlorophyll content is a highly responsive indicator of phytotoxicity [25]. A recent study investigating the biosorption potential of water hyacinth (*Eichornia crassipes*) brought similar results, where 5 mg L^{-1} ionic Gd concentration did not affect significantly the overall health of the plants [15]. In the present study, the toxicity of Gd was much higher comparing to the very similar rare earth element cerium that had an EC_{50} higher than 70 mg L^{-1} in case of the duckweed *Lemna minor* [41]. However, in our study, we used more than two orders of magnitude lower phosphate concentration (0.06 mM). Since phosphate can precipitate with lanthanides [12], using extremely high phosphate concentrations (e,g., 7.35 mM, [41]) may easily mask the actual toxicity of REE-s resulting in unrealistically low toxicity. The calculated toxicity of Gd was slightly lower for *Lemna* than for diatoms or green algal species that have approximately 50% lower EC50% values [13,37]. On the other hand, Cnidaria and Rotifera taxa showed much higher sensitivity to ionic Gd with up to two orders of magnitude lower EC50 value comparing to *Lemna* ([6,13], Table 4).

4.2. Gd accumulation of the plants

The significance of this study was that we found a nontoxic Gd concentration range between 1 and 5 mg Gd L^{-1}, where biofiltration of

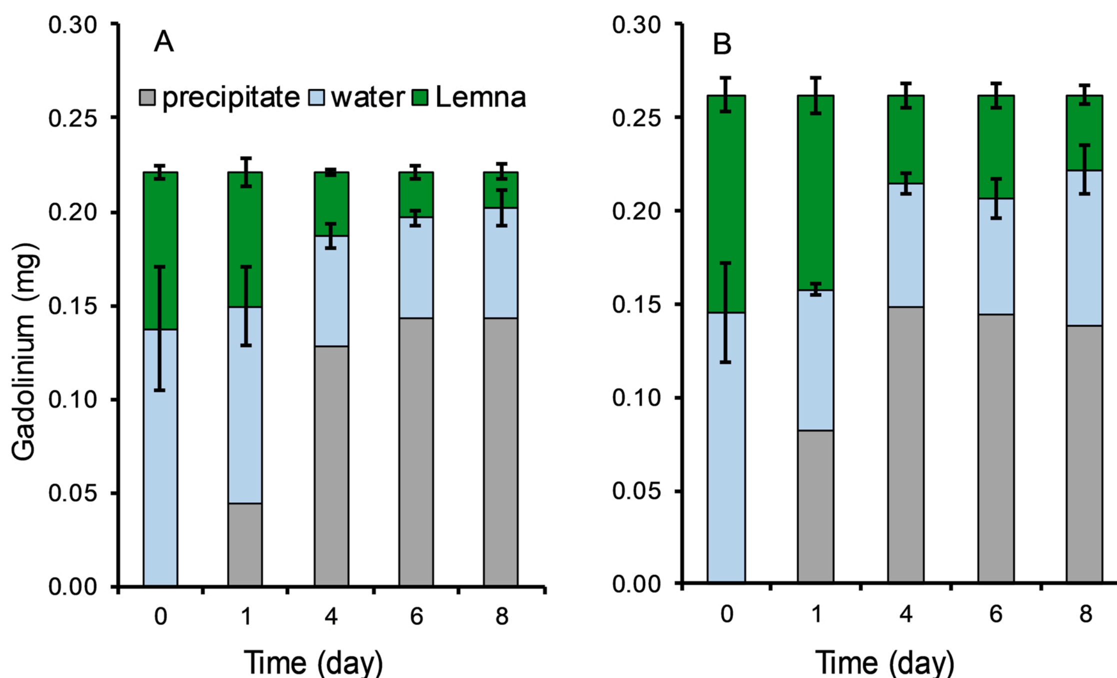

Fig. A4. Distribution of Gd between *Lemna*, water and precipitate in the leaching experiment. *Lemna* had been grown for 8 days on 5 (A) and 10 mg Gd L^{-1} (B) culture media before getting transferred to Gd-free medium.

Gd by free-floating plants could be safely operated. Bioaccumulation factors of Lemnaceae species for heavy metals (e.g. Mn, Cr, Pb, Ni, Cd) are in the range of 100–100,000 [19]. In the present study, the dry matter-based bioconcentration factor of *Lemna* for Gd was very similar to the leaves of water hyacinth [15] or to marine macroalgae [11,28]. However, *Lemna* in our study accumulated more than ten times more Gd in the biomass than marine macroalgae (eg. *Ulva*, *Gracillaria*) [11]. Depending on the initial Gd concentration, in the presence of *Lemna*, the Gd concentration in the nutrient solution showed a 77–99% decrease by the end of the experiments. However, this strong drop cannot be considered simply as Gd removal efficiency of the plants. Only a 17–37% drop in the Gd concentration of the medium was attributed to bioaccumulation by *Lemna*.. Precipitation of Gd from the nutrient solution could have been responsible for the main part of the drop in Gd concentration. Precipitation of Gd can largely be caused by the presence of phosphate [12]. Furthermore, it is also well known that by increasing the pH, the solubility of Gd^{3+} decreases significantly above pH 7. Moreover, above pH 8, most of this fraction turns into an insoluble precipitate of $Gd(OH)_3$ [9,29]. It is well known that algal species utilizing bicarbonate ions as carbon source are able to raise the pH of the medium during their photosynthesis [16,34]. As a result, algae possibly growing in the culture media may cause a drop in Gd concentration of the water due to their pH-increasing effect. Beyond planktonic and epiphytic algae, marine macroalgae [20,23] and submerged macrophytes utilizing bicarbonate ions can also increae the pH strongly [27, 35]. As this results in an apparent Gd removal by precipitation, they may take up Gd from the medium to a much lesser extent than free-floating emergent plants. Therefore, use of submerged plants or macroalgae in biofiltration/ bioaccumulation may not be so advisable even if they induce high apparent drop in the Gd concentration of the medium. Several studies followed the Gd accumulation of macroalgae or free-floating plants over a time-course, using mainly concentration changes in the medium as input data [11,15,28]. Those studies all assumed that Gd removed from solution was absorbed by the plants. However, due to the Gd precipitation as detailed above, removal/uptake models based on water concentration data may lead to unrealistic/idealistic image of the Gd removal efficiency (e.g., 98.5% by *Eichornia* - Kartamihardja et al., [15]; *Ulva lactuca* up to 82% - [11] and 90% - [28]). Here we went further since, beyond water concentration data, the time-course of tissue Gd concentration data was also considered for calculation of the removal efficiency of macrophytes that gave more modest removal efficiency (17–50%). This study is therefore unique in that a complete mass balance was established. It clearly revealed that by the end of the experiment, beyond Gd uptake of the plants, more than 50% of the total Gd got precipitated. A similar result was found in a microcosm study after REE treatment, where the REE-s (including Gd) ended up mostly in the sediment [38].

4.3. *Leaching of Gd from the plants*

Similar to Gd-based contrasting agents in previous studies [7,15], ionic Gd was also released from the plants. The dissolved free Gd, on the other hand, probably left the nutrient solution in the form of an insoluble precipitate, since Gd concentration of the medium continuously decreased. The release of Gd from *Lemna* into the Gd-free solution took place slower in case of the ionic form compared to the complex form (GBCA-s), where the calculated half-life was only 2–3 days [7].

5. Conclusions

Our present study is the first to deal with the toxicity, removal and phytoremediation of ionic Gd using freshwater plants. Based on our experimental results, we can conclude that Gd uptake and accumulation of *Lemna* is relatively fast. Since there was a significant accumulation of free Gd in the model organisms studied, we can conclude that the macrophytes are able to efficiently reduce the concentration of free Gd. Since the accumulated Gd proved to be released from the plants in Gd-free medium, frequent harvesting with 4 days-long intervals is required for optimal Gd removal during phytoremediation. After gradual heating of the dried plants, 23.2 g Gd per kg ash can be reached from *Lemna* biomass cultivated on 5 mg Gd L^{-1} medium respectively. Thus, we obtained promising results for Gd recovery from contaminated waters.

Environmental Implication

From hospital sources, cumulatively 4500 tons of gadolinium (Gd) has entered in surface water in non-toxic chelated form. In aquatic ecosystems over the long term, toxic Gd^{+3} has a potential to be released from the chelates. Industrial and agronomical release of ionic Gd to the aquatic environment could also be significant. In mining areas, blood Gd concentrations of residents increased with increased soil concentrations. Therefore, it is very urgent to study the toxicological and bioaccumulation characteristics of ionic Gd. This is the first study addressing both the toxicological characteristics, and phytoremediation possibilities of ionic gadolinium using the free-floating freshwater plant *Lemna gibba* .

CRediT authorship contribution statement

SS: Conceptualization, Formal analysis, Methodology, Supervision, Visualization, Writing – original draft, Writing. **GZ:** Formal analysis, Investigation, Methodology. **DC:** Formal analysis, Investigation, Methodology. **GK:** Investigation, Methodology, **VO:** Conceptualization, Visualization, Writing – review & editing. **MB:** Conceptualization, Investigation, Methodology, Supervision, Visualization, Weiting – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgment

This study was financed by the Scientific Board of University of Nyíregyháza and by the European Union and the State of Hungary, co-financed by the European Regional Development Fund in the project of GINOP-2.3.2-15-2016-00009 "ICER". GZ and GK received funding from the Doctoral School of Biological Sciences of the Hungarian University of Agriculture and Life Sciences. Timothy Jull is gratefully thanked for linguistic correction.

Appendix

See Tables A1–A3 and Fig. A4.

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