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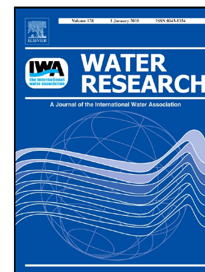


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**Can aquatic macrophytes be biofilters for gadolinium based contrasting agents?**

Mihály Braun[a#], Györgyi Zavanyi[b], Attila Laczovics[c], Ervin Berényi[c], Sándor Szabó[b]

[a] Isotope Climatology and Environmental Research Centre (ICER), Institute for Nuclear Research, Hungarian Academy of Sciences, Debrecen, Hungary, 4026 Debrecen, Bem tér 18/C

[b]Department of Biology, University of Nyíregyháza, H-4401 Nyíregyháza, POBox 166, Hungary

[c]Department of Medical Imaging, University of Debrecen, H-4032 Debrecen, Nagyerdei krt. 98., Hungary

# Corresponding author:

Mihály Braun

braun.mihaly@atomki.mta.hu

# Abstract

The use of gadolinium-based contrasting agents (GBCA) is increasing because of the intensive usage of these agents in magnetic resonance imaging (MRI). Waste-water treatment does not reduce anthropogenic Gd-concentration significantly. Anomalous Gd-concentration in surface waters have been reported worldwide. However, removal of GBCA-s by aquatic macrophytes has still hardly been investigated.

Four aquatic plant species (*Lemna gibba*, *Ceratophyllum demersum*, *Elodea nuttallii*, *E. canadensis*) were investigated as potential biological filters for removal of commonly used but structurally different GBCA-s (Omniscan, Dotarem) from water. These plant species are known to accumulate heavy metals and are used for removing pollutants in constructed wetlands. The Gd uptake and release of the plants was examined under laboratory conditions. Concentration-dependent infiltration of Gd into the body of the macrophytes was measured, however significant bioaccumulation was not observed. The tissue concentration of Gd reached its maximum value between day one and four in *L. gibba* and *C. demersum*, respectively, and its volume was significantly higher in *C. demersum* than in *L. gibba*. In *C. demersum*, the open-chain ligand Omniscan causes two-times higher tissue Gd concentration than the macrocyclic ligand Dotarem. Gadolinium was released from Gd-treated duckweeds into the water as they were grown further in Gd-free nutrient solution. Tissue Gd concentration dropped by 50% in duckweed treated by Omniscan and by Dotarem within 1.9 and 2.9 days respectively. None of the macrophytes had a significant impact on the Gd concentration of water in low and medium concentration levels (1-256 µg $L^{-1}$). Biofiltration of GBCA-s by common macrophytes could not be detected in our experiments. Therefore it seems that in constructed wetlands, aquatic plants are not able to reduce the concentration of GBCA-s in the water. Furthermore there is a low risk that these plants cause the accumulation of anthropogenic Gd in the food chain.

**Keywords:** gadolinium, contrasting agent, biofiltration, macrophytes, uptake, leaching

42 # 1. Introduction

43 The development of new technologies in the last quarter century has led to the steadily
44 increasing use of rare earth elements (REE) and their consequent release into the environment
45 (Du and Graedel, 2011; Tepe et al., 2014). The occurrence of abnormally high concentrations
46 of REE in the hydrosphere was first reported by Bau and Dulski in 1996 (Bau and Dulski,
47 1996). They detected positive Gd anomalies in surface waters, ground water and in tap water
48 in the area of Berlin. Further research supported the view that the positive Gd anomaly is by no
49 means a local phenomenon. It is characteristic of those metropolitan areas where a high number
50 of magnetic resonance imaging (MRI) studies are performed, due to a developed health care
51 system (Kulaksiz and Bau, 2011, 2007; Tepe et al., 2014).

52 MRI studies using gadolinium-based contrasting agents (GBCA) were introduced to
53 medical diagnostics in 1988 (de Haën, 2001). These contrasting agents have high paramagnetic
54 properties, they are water-soluble and highly stable to prevent any interaction of toxic $Gd^{3+}$
55 with the body. For the purpose of an MRI study, a contrast agent containing 1000-3000 mg Gd
56 is injected into the human bloodstream and extracted via the kidneys as urine within 24-48
57 hours (Swan et al., 1999). During the twenty years since the introduction of MRI contrasting
58 agents, an estimated 200 million applications of gadolinium-based contrasting agent (GBCA)
59 have been performed worldwide (Hao et al., 2012). For contrast agent applications,
60 approximately 22–66 tons of Gd is used each year globally (Kulaksiz and Bau, 2011).

61 After the elimination from human bodies, these chelates pass smoothly through sewage
62 treatment plants and are discharged by their effluents into the hydrosphere (Möller and Dulski,
63 2010a). The elevated concentration of Gd does not decrease significantly during waste water
64 treatment (Lawrence et al., 2009; Möller and Dulski, 2010b). The reason for this phenomenon
65 is either their high solubility (they are not absorbed by organic particulate matter), or resistance

66 to microbial degradation (Lawrence et al., 2009; Verplanck et al., 2005). Therefore, Gd
67 complexes are eventually passed into rivers and lakes, bypassing waste water treatment plants
68 (WWTP). In addition, in those riverside cities, where the water is supplied by bank-filtered
69 wells (e.g. Berlin, London), anthropogenic Gd can even be detected in tap water (Kulaksiz and
70 Bau, 2011), since bank filtration does not prevent the migration of Gd-complexes into the
71 hydrosphere (Möller et al., 2000). The extent of the anthropogenic Gd anomaly depends on the
72 population density in the river catchment, the level of the health care system and the ratio of
73 Gd-contaminated discharge from WWTPs to uncontaminated natural river discharge (Bau et
74 al., 2006; Kulaksiz and Bau, 2007; Merschel et al., 2015). From the rivers, these micropollutants
75 eventually reach coastal seawater (Kulaksiz and Bau, 2011, 2007; Nozaki et al., 2000). A
76 positive Gd anomaly can be detected in bays worldwide e.g. North Sea (Kulaksiz and Bau,
77 2007), Mediterranean Sea (Rabiet et al., 2009), Pacific Ocean at San Francisco, USA (Hatje et
78 al., 2016), Nagoya, Japan (Zhu et al., 2004), Brisbane, Australia (Lawrence, 2010).

79 It is well known that phytoremediation with aquatic plants is an effective and inexpensive
80 method for removing pollutants from the medium. In temperate regions, free floating emergent
81 (*L. gibba*) (Körner and Vermaat, 1998, Ran et al 2004) and submerged plants (*Ceratophyllum*
82 *demersum, Elodea canadensis, E. nuttallii*) are able to grow rapidly in water containing high
83 concentration of nitrogen and phosphorus (Pietro et al., 2006). Moreover they highly
84 accumulate heavy metals (eg. Cr, Cd, Ni, U, Pb). Therefore they are used for removing
85 pollutants from industrial wastewater effluents in constructed wetlands (Khan et al. 2009).

86 In spite of several studies that have shed light on the hydrological and geological
87 behaviour of the anthropogenic Gd complexes, only a few studies deal with the impact of Gd
88 complexes on aquatic organisms (Kulaksiz and Bau, 2013; Merschel and Bau, 2015). No single
89 publication addresses the issue of whether GBCA-s can be accumulated by aquatic macrophytes.

The question remains completely open whether aquatic macrophytes are able to remove anthropogenic Gd complexes.

Therefore, the aim of this study is to examine the Gd removal potential of aquatic plants (*L. gibba*, *C. demersum*, *E. nuttallii* and *E. canadensis*). A further aim is to investigate the accumulation possibilities and mobilisation kinetics of GBCA-s in freshwater macrophytes.

# 2. Material and methods

*2.1. Plant collection*

Shoots of submerged rootless species (*C. demersum*) and free-floating species (*L. gibba*) were collected from ditches near Nyíregyháza, (NE Hungary). Submerged rooted species *E. canadensis* and *E. nuttallii* were collected from the river Bodrog and from the Eastern Principal Channel in the surroundings of Hajdunánás (NE Hungary), respectively (Fig. 1).

*2.2. Pre-incubation and experimental conditions*

The pre-incubation under experimental conditions lasted for 20 days. Duckweed fronds and apical shoots of submerged plants (*E. canadensis, E. nuttallii, C. demersum*) were placed in 20 L aquaria separately, containing growth medium, modified from Smart and Barko (Smart and Barko, 1985). For the submers plants (*E. canadensis, E. nuttallii, and C. demersum*), nitrogen and phosphorus concentrations were adjusted to 2 mg N $L^{-1}$ and 0.4 mg P $L^{-1}$, and for duckweed (*L. gibba*) to 10 mg N $L^{-1}$ and 2 mg P $L^{-1}$ respectively. Nitrogen was added as $NH_4NO_3$, phosphorus as $K_2HPO_4$. Supply of micronutrients was ensured by adding TROPICA Supplier micronutrient solution with a 10,000 times dilution. The concentrations after dilution were: Fe 0.07, Mn 0.04, Zn 0.002, Cu 0.006 and Mo 0.002 mg L-1 respectively (Szabó et al., 2003). During pre-incubation, the solution was renewed twice a week. After the pre-incubation period, the length of the shoots was reduced

115 to 150 mm. The incubation experiments are described in the section 2.2. During incubation,
116 culture pots were set under controlled temperature water bath at 22-25°C. Illumination was
117 provided by Philips 400 W metal halide lamps with 16 h light and 8h dark regime.
118 Photosynthetically active radiation (PAR) was 220 $\mu molm^{-2}s^{-1}$ measured on the water surface.
119

120 *2.3. Analytical methods*

121 Simple open-vessel acid digestion was used to dissolve the plant materials. For the
122 measurements, 100-200 mg of dried plant samples were weighed into glass beakers. The
123 digestion was carried out with 5 mL of 67% (m/m) nitric acid (Promochem, suprapure) and
124 5 mL 30% (m/m) hydrogen peroxide (Molar Chemicals). The samples were spiked with 25 µL
125 100 $mgL^{-1}$ terbium solution (C.P.A. Ltd.) as an internal standard (ISTD) and heated slowly to
126 90°C. This temperature was maintained up to 60 min until the solid remains of the samples
127 completely disappeared. When the dissolution was completed, the samples were evaporated to
128 dryness and washed to 50 mL volumetric flasks with 1% (m/v) nitric acid. The concentration
129 of ISTD was 50 $\mu gL^{-1}$ in the sample solutions.

130 Water samples were filtered through membrane syringe filters with pore size of 0.45 µm
131 (Hydrophilic, Ministart NY 25 mm, Sartorius). From the filtered sample, 50 mL was measured
132 and spiked with 25 µL 100 $mgL^{-1}$ Tb solution for setting the concentration of ISTD to 50 $\mu gL^{-1}$.
133 Analysis of gadolinium was performed using an Agilent 8800 inductively coupled plasma mass
134 spectrometer (ICP-MS). Integration time was 1 s for the $^{157}Gd$ isotope and 0.5 s for the $^{159}Tb$.
135 Tune mode was MS/MS and collision cell was used with 5 $mLmin^{-1}$ helium flow. The limit of
136 quantification (LOQ) of Gd was ≤0.05 $\mu gL^{-1}$ (RSD <5%). Recovery of the ISTD was between
137 95 and 105%. The parameters used in this analysis are shown in Table 2. The concentration of
138 Gd was expressed as $\mu gkg^{-1}$ fresh weight in the case of plant materials and as $\mu gL^{-1}$ in the case
139 of solutions.

140

141 **Table 1**
142 Instrumental parameters for the analysis of Gd by ICP-MS/MS

| **Instrumental parameter** | |
|---|---|
| RF power (W): | 1550 |
| Plasma gas flow ($Lmin^{-1}$): | 15 |
| Auxiliary gas flow ($Lmin^{-1}$): | 0.7 |
| Nebuliser: | Micromist |
| Nebuliser gas flow ($Lmin^{-1}$): | 0.65 |
| Dilution gas flow ($Lmin^{-1}$): | 0.40 |
| Sample flow rate ($mLmin^{-1}$): | 0.1 |
| Replicates: | 10 |
| Tune mode: | MS/MS |
| He flow rate ($mLmin^{-1}$): | 5 |
| Octopole bias (V): | -18 |

143

144 *2.4. Gadolinium mobilisation experiments*

145 The mobilisation of Gd contrasting agents into aquatic macrophytes was investigated in
146 four subsequent experiments. Two world widely used commercial available GBCAs were
147 used in the experiments **(Fig.2)**. Omniscan (gadodiamide) is a linear, non-ionic type of GBCA,
148 while Dotarem (gadoterate meglumine) is a macrocyclic, ionic one (Rogosnitzky and Branch,
149 2016). Three culture pots were used per treatment in each experiment. The survey of
150 experiments is detailed in Table 2.

151

152 **Table 2**
153 Characteristics of Gd-CA mobilisation experiments

| **Number of Experiment** | **Species** | **Contrast agent** | **Gd in the water (μg $L^{-1}$)** | **Analysed sample type** | **Pot volume (L)** |
|---|---|---|---|---|---|
| 1 | *L. gibba*<br>*C. demersum*<br>*E. canadensis,*<br>*E. nuttallii* | Dotarem | 1 | water | 2.0 |
| 2 | *L. gibba* | Dotarem | 1-256 | water, plant | 0.3 |
| 3 | *L. gibba*<br>*C. demersum* | Dotarem<br>Omniscan | 256 | plant | 2.0 |
| 4 | *L. gibba* | Dotarem<br>Omniscan | 256 till 8 days<br>Gd-free solution | water, plant | 2.0<br>0.2 |

154

155 *2.4.1. Impact of macrophytes on the Gd concentration of the water*

156 Experiment 1 was designed to measure the change in Gd concentration of the nutrient

157 solutions in the presence of four different macrophytes. 10 g biomass of macrophytes (*L. gibba*,

158 *C. demersum, E. nuttallii, E. canadensis*) were cultivated in 2 L aquaria containing 2 L culture

159 medium described above. Gadolinium concentration in the medium was adjusted to 1 µg $L^{-1}$ by

160 adding Dotarem. Three aquaria were used per treatment. Gd-treated macrophytes were

161 cultivated under experimental conditions for 6 days. Samples were taken from the solutions at

162 the start of the experiment (control), and at the $1^{st}$, $2^{nd}$, $4^{th}$ and $6^{th}$ days. Samples were filtered

163 immediately using a 0.45 µm membrane and analysed for Gd concentration by ICP MS.

164

165 *2.4.2. Change in tissue Gd concentration*

166 Experiment-2 was designed to determine the change in tissue Gd concentration in duckweed

167 (*L. gibba*) under a wide range of Gd concentrations.

168 Duckweed fronds (1000±2mg) were cultivated for 8 days in pots containing 0.3 L nutrient

169 solution. Initial Gd concentrations in the medium were adjusted to 1, 2, 4, 8, 16, 64, 128 and

170 256 µg $L^{-1}$ by adding Dotarem. Each treatment was replicated three times, meaning that 24 pots

171 were used. Samples were taken from the solutions at the start of the experiment and on the $8^{th}$

172 day. Samples were filtered on 0.45 µm membrane and analysed for Gd concentration by ICP

173 MS. After the plants were harvested, the fresh and dry weight of the duckweed fronds of each

174 treatment was measured using an analytical balance. Samples were dried at 105ºC until constant

175 weight was achieved (within 24 hours). The chemical composition of the duckweed fronds for

176 Gd was analysed by ICP MS, after acid digestion.

177

178 *2.4.3. Gd mobilisation into macrophytes*

179 Experiment-3 was designed to determine the infiltration dynamics of two different forms of Gd
180 complexes into two freshwater macrophytes (*L. gibba; C. demersum*). Fronds of *L. gibba* (10 g
181 fresh weight) and *C. demersum* shoots (40 g fresh weight) were cultivated for 8 days under
182 experimental conditions in aquaria, each containing 2 L nutrient solution. The initial Gd
183 concentration in the medium was adjusted to 256 $\mu g\ L^{-1}$ by adding either macrocyclic Dotarem
184 or open-chained Omniscan Gd complexes. Each treatment was replicated three times, meaning
185 that 12 aquaria were used. Plant samples (2000±2mg fresh weight) from each aquarium were
186 taken at the beginning of the experiment, after 12 hours, and after 1, 2, 4 and 8 days. The plant
187 material was dissolved by acid digestion and the concentration of Gd in the duckweed fronds
188 was determined by ICP MS.
189
190 *2.4.4. Leaching of Gd complexes*
191 Experiment-4 was designed to determine the dynamics of two Gd complexes during leaching
192 from Gd-treated duckweed (*L. gibba*). Duckweed fronds were cultivated for 8 days under
193 experimental conditions in separate aquaria, each containing 2 L nutrient solution. A PVC-tube
194 (9 cm length) with a diameter of 5 cm was placed vertically in each aquarium. It served as a
195 duckweed enclosure (Szabó et al., 2003). Portions of pre-incubated duckweed fronds (3.00 g
196 fresh weight) were placed inside the enclosures. This method allowed us to culture duckweed
197 on a static medium under optimal conditions avoiding overcrowding as well as algal inhibition
198 (Szabó et al., 2003, 2010). The initial Gd concentration in the medium was adjusted to 256 $\mu gL^{-}$
199 $^{1}$ by adding either Dotarem or Omniscan Gd complex forms. For both treatments, six aquaria
200 were used. For the optimal growth of duckweed, 10 mg N $L^{-1}$ and 2 mg P $L^{-1}$ ($NH_4NO_3$,
201 $K_2HPO_4$) were supplemented in the medium on days three and six. On the last day, all duckweed
202 fronds were gathered from each aquaria (10-11 g fresh weight) then rinsed with tap water.
203 Water was removed from the surface of the plants by using a salad centrifuge.

204 Subsequently, portions of duckweed fronds (2.000 ± 0.002 g fresh weight) were
205 cultivated on Gd-free nutrient media for 21days. The initial concentration of Gd was determined
206 by three parallel sample. Then 3 parallel samples of each GBCA treatments were collected after
207 12 hour, and after 1, 1.5, 4, 8, 11, 16 and 21 days. The dry weight of the duckweed fronds from
208 both treatments was measured and after acid digestion the Gd concentration of the fronds was
209 analysed by ICP MS. Samples were also taken directly from the solutions and then were filtered
210 and analysed for their Gd concentration.

211

212 *2.5. Statistical analysis*

213 One-way analysis of variance (ANOVA) was used to test the effects of the investigated factors
214 (e.g. concentration of Gd in the water, incubation time). Levene's test was applied to check the
215 homogeneity of variances. In the case of heteroscedasticity, a Kruskal-Wallis test was used
216 instead of ANOVA. These analyses were performed using SPSS 16.0 software. Linear
217 regression analysis was used to investigate the uptake of Gd at different concentration levels.
218 Nonlinear models were fitted in the case of time dependent uptake studies. Regression models
219 were calculated by LAB Fit curve-fitting software.

220

221 # 3. Results

222 *3.1. The impact of macrophytes on the Gd concentration of the nutrient solution*

223 The Dotarem-spiked nutrient solution was stable under experimental conditions, the Gd-
224 concentration was not changed by any physical or chemical processes (e.g. precipitation,
225 sorption, evaporation). Recovery of Gd in the nutrient solution was 1.07±0.03 $\mu gL^{-1}$ at the
226 beginning of the experiment measured by ICP-MS (Fig.3). The concentration of control
227 samples had not changed significantly (ANOVA, $F_{(2,6)}$=1.478, p=0.301) within 6 days, the

228 average concentration of Gd remained 1.1±0.4 $\mu gL^{-1}$. Variances were homogeneous as checked
229 by the Levene-test ($W_{(2,6)}$=0.350, p=0,718).

230 Variances were significantly different in the case of *L. gibba* ($W_{(2,3)}$=8.353, p=0.018)
231 and *C. demersum* ($W_{(2,6)}$=6.082, p=0.036). Therefore, comparisons were done using a
232 nonparametric Kruskal-Wallis test. The concentration of Gd was not changed significantly by
233 *L. gibba* ($Chi^2_{(2)}$=1.689, p=0.430), but *C. demersum* might have an effect on the conentration
234 of Gd ($Chi^2_{(2)}$=5.956, p=0.051).

235 Since the variances were homogeneous (*E. nuttallii*: $W_{(2,6)}$=0.801, p=0.492; *E.*
236 *canadensis*: W(2,6)=0.954, p=0.437), ANOVA was applied to the comparisons in the case of
237 *Elodea* species. Significant differences could not observed for *E. nuttallii* ($F_{(2,6)}$=0.066,
238 p=0.963). A significant difference was found for *E. canadensis* ($F_{(2,6)}$=33.878, p=0.001), the
239 correlation coefficient between time and Gd-concentration was -0.987, suggesting that this
240 macrophyte may lower the concentration of Gd in water.

241

242 *3.2. Change in tissue Gd concentration*

243 Since intensive uptake of Gd from 1 $\mu gL^{-1}$ Dotarem solution was not detected by experiment-1,
244 the maximum concentration of Gd was increased to 256 $\mu gL^{-1}$ in experiment-2. The
245 concentration of Gd in *L. gibba* tissue and in the water phase was measured after 8 days
246 incubation. The average fresh weight of *L. gibba* was 3.2±0.2 g (RSD 6.5%) at the end of the
247 experiment.

248 The Gd concentration of *L. gibba* tissues increased linearly with increasing Gd concentration
249 of the water (Fig.4A). Comparing the initial and final Gd concentration of the solutions, the
250 slope was found to be close to one (0.985±0.006) (Fig.4B). Recovery of Gd was also calculated
251 at the different levels. Their variances (Levene test, $W_{(8,18)}$=1.135, p=0.387), and their averages
252 (ANOVA, F(8,18)=0.761, p=0.640) were similar and *L. gibba* lowered the initial concentration

253 of Gd by 1.45%. The total Gd (100%) was initially present in the water. After 8 days of
254 incubation, 0.80±0.08% of the total Gd was detected in the biomass.
255
256 *3.3. Time dependent uptake of Gd by macrophytes*
257 The uptake kinetics of *L. gibba* was similar in the case of Dotarem and Omniscan (Fig.5), as
258 indicated by the similar equation parameters. The best fit equation was a modified exponential.
259 The concentration increased rapidly (within a day) to the saturation value.
260 **Table 3**
261 Parameters of fitted equations for the time dependent uptake of gadolinium based contrast
262 agents (GBCA) by macrophytes

| Species | GBCA | Model | Parameters | | R |
|---|---|---|---|---|---|
| | | | a | b | |
| *L. gibba* | Dotarem | $y = a*EXP(b/x)$ | 39.57±2.93 | -0.037±0.001 | 0.9678 |
| | Omniscan | (modified exponential) | 39.05±1.96 | -0.037±0.001 | 0.9596 |
| *C. demersum* | Dotarem | $y = a*x^b$ | 25.42±3.37 | 0.460±0.088 | 0.9058 |
| | Omniscan | (power) | 47.21±2.16 | 0.556±0.033 | 0.9811 |

263
264 In the case of *C. demersum,* saturation was not achieved within 8 days. However, the uptake of
265 Omniscan was faster than the uptake of Dotarem (Fig.6). The best fit equation was a power law
266 (Table 3), where the exponents (parameter b) were similar. By the end of the experiment,
267 Omniscan resulted three times higher tissue Gd concentration in *C. demersum* compared to *L.*
268 *gibba*.
269
270 *3.4. Leaching of Gd complexes*
271 When Gd-treated *L. gibba* was placed into clean nutrient solution, the Gd appeared shortly in
272 the water and its concentration increased with time. The release of Gd into the Gd-free nutrient
273 solution was significantly higher from Omniscan-treated duckweed than the duckweed that
274 were cultivated on Dotarem (Fig.7).

275 Parameters of the best fit models are given in Table 4. Leaching of Gd from *L. gibba*
276 could be fitted by an exponential equation for both GBCA. Leaching of Omniscan from the
277 duckweed frond was slightly faster than the leaching of Dotarem. This effect is unambiguous
278 as observed by the change in the Gd concentration of water. The fitted model was a power law,
279 where the exponents (parameter b) were similar. The concentration of Gd on the last day was
280 1.0±0.1 $\mu gL^{-1}$ in the case of Dotarem and 1.6±0.2 $\mu gL^{-1}$ in the case of Omniscan (Fig.7).

281 The decrease in tissue Gd concentration of duckweed was significantly faster (P<0.001,
282 ANOVA) in plants treated by Omniscan than by Dotarem, when placed in the Gd-free nutrient
283 solution. The half-life of Dotarem was calculated to be 2.9 days, and 1.9 days for the Omniscan.

284 Mass balances of GBCA-s in the duckweed fronds and in the water were determined.
285 The total Gd (100%) was initially present in the GBCA treated plants. After eight days there
286 was a net flux of Gd from the duckweed into the water (Dotarem 83%, Omniscan 89%). At the
287 end of the experiment 99% of Dotarem and 100% of Omniscan released from the duckweed
288 plants into the water (Fig.8).

289 **Table 4**
290 Parameters of fitted equations for the time dependent release of gadolinium based contrast
291 agents (GBCA) from *L. gibba* and increase in water

| **Species** | **GBCA** | **Model** | **Parameters** | | **r** |
|---|---|---|---|---|---|
| | | | **a** | **B** | |
| Plant tissue | Dotarem | $y = a*EXP(bx)$ (exponential) | 58.55±2.61 | -0.241±0.005 | 0.8953 |
| | Omniscan | | 78.71±3.27 | -0.358±0.023 | 0.9725 |
| Water | Dotarem | $y = a*x^b$ (power) | 0.22±0.01 | 0.487±0.030 | 0.9759 |
| | Omniscan | | 0.34±0.02 | 0.485±0.028 | 0.9656 |

292

293 # 4. Discussion

294 *4.1. Gadolinium uptake by macrophytes*

295 Among the macrophytes investigated, several studies have shown that *L. gibba* (Scheffer et al.,
296 2003; Szabó et al., 2003), *E. nuttallii* and *C. demersum* (Lombardo and Cooke, 2003) have high
297 nutrient removal activity. These plants can reduce the macronutrient (N, P) or micronutrient
298 (Fe, Mn) concentration in water by 90% within a few days (Szabó et al., 2010). They can
299 remove lead (Mishra et al., 2006), chromium (Uysal, 2013) nickel (Demirezen et al., 2007),
300 arsenic, boron and uranium (Sasmaz and Obek, 2009) usually with high efficiency. Therefore,
301 our result that neither of the four investigated macrophytes had any significant impact on the
302 Gd-concentration of water was unexpected.

303 However, Gd complexes quickly infiltrated into the body of the macrophytes in
304 proportion to their concentration. It is clear, however, that tissue Gd concentration in the
305 biomass was not higher than the Gd concentration in the medium (bioaccumulation factor <1).
306 Therefore, we can say that neither of the Gd complexes are accumulated into the body of the
307 investigated species and the concentrations in the plant tissue just reflects the concentration of
308 the medium. In contrast, bioaccumulation factors of these macrophythes for heavy metals (e.g.
309 Mn, Cr, Pb, Ni, Cd) are in the range of 100-100000 (Landolt and Kandeler, 1987).

310 The concentration of Gd reached its maximum within one day in *L. gibba* and within four days
311 in *C. demersum,* respectively. The open-chained Omniscan, resulted in two times higher tissue
312 Gd concentration in *C. demersum* than the macrocyclic GBCA Dotarem. Up to now, there is
313 only a single study that deals with the kinetics of GBCA uptake in an emergent aquatic
314 macrophyte (*Lemna minor*) (Lingott et al., 2016). In that experiment, tissue Gd was analysed
315 by laser ablation techniques, even so the results are parallel to our study, since they also found
316 the plants saturated with Gd contrasting agent within 24 hours.

317

*4.2. Leaching of Gadolinium from macrophytes*

Since the GBCA are released from the human body within a few days (Swan et al., 1999), it can be assumed that they show similar mobility in other organisms as well. In the present study, Gd complexes were released from Gd-treated duckweeds into the medium as they were grown further on Gd-free nutrient solution. Tissue Gd concentration dropped by one-half in treated duckweed by about 1.9 days for Omniscan and 2.9 days for Dotarem. Therefore, it can be seen that the mobility of open-chain Gd complexes into and out of the plants is faster than that of macrocyclic ones. In the present study, the release of Gd from *L. gibba* into the Gd free solution took place much faster (half-life time <3 days) than that of macroelements (K, Ca, Mg, S, N; half-life time 40-80 day) measured during decomposition in an earlier study (Szabó et al., 2000). These results suggest that Gd mobilisation into and out of the macrophytes is merely influenced by physical processes (diffusion, differences in concentration) and not any chemical process.

It should be noted that in our study the total Gd concentration was measured both in water and in plant samples. The concentration of GBCAs were not analysed in the samples. If Gd would be released from the complex forms, ligand free Gd could show high ($10^3$-$10^4$) bioaccumulation factor for freshwater plants (*Lemna minor*, *Potamogeton pectinatus*) (Weltje et al. 2002). Since, there was no detectable bioaccumulation in our experiments, and the leaching of Gd from the plant tissue was high, it could be concluded, that Gd still remained in stable complex form during the experiment.

# 5. Conclusion

This is the first study dealing with the mobilisation of gadolinium based contrast agent in aquatic macrophytes. Based on our experimental results, the mobilisation of Gd complexes into and out of the freshwater macrophytes is relatively fast. Biofiltration of GBCA-s by common

343 macrophythes could not be detected in our experiments. Since there is no significant
344 accumulation of Gd observed by these aquatic plants, we conclude that in constructed wetlands,
345 aquatic plants are not able to reduce the concentration of GBCA-s in the water. Furthermore
346 there is a low risk that these plants cause any accumulation of anthropogenic Gd in the food
347 chain.

348

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**Highlights**

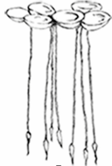
Aquatic plants showed concentration dependent gadolinium infiltration

Fast and complete leaching of gadolinium from macrophythes was detected

Macrophytes could not biofiltrate/accumulate gadolinium based contrasting agents

No risk that macrophytes cause the gadolinium enrichment into the food chain

ACCEPTED MANUSCRIPT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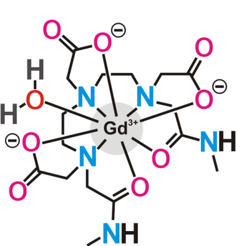
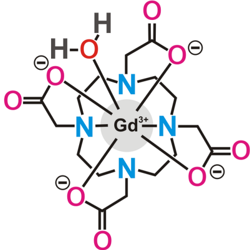

Gd3+
(A)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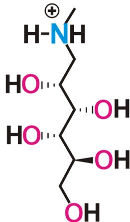

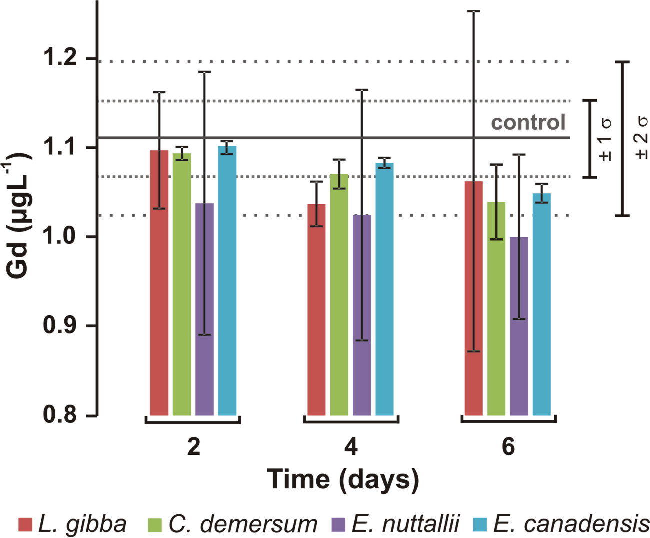

Gd (μgL⁻¹)
1.2
1.1
1.0
0.9
0.8
control
± 1 σ
± 2 σ
2
4
6
Time (days)
L. gibba
C. demersum
E. nuttallii
E. canadensis

A

r = 0.972
slope: 0.774±0.037
intercept: 0.447±3.462

Gd (μgkg$^{-1}$) in *L. gibba* at $T_8$

Gd (μgL$^{-1}$) in water at $T_0$

B

r = 0.999
slope: 0.985±0.006
intercept: 0.003±0.540

Gd (μgL$^{-1}$) in water at $T_8$

Gd (μgL$^{-1}$) in water at $T_0$

A

B

Gd (μgkg$^{-1}$)

80
70
60
50
40
30
20
10
0

0 2 4 6 8

Time (day)

0 2 4 6 8

Time (day)

A

B

Gd (μgkg$^{-1}$)

200 160 120 80 40 0

0 2 4 6 8

Time (day)

0 2 4 6 8

Time (day)

(A)

Gd (μgkg$^{-1}$)

100
80
60
40
20
0

0 5 10 15 20

Time (day)

(B)

Gd (μgL$^{-1}$)

1.6
1.2
0.8
0.4
0.0

0 5 10 15 20

Time (day)

*L. gibba* Water

A

B

Gd (%)

0
20
40
60
80
100

0 5 10 15 20

Time (day)

0 5 10 15 20

Time (day)

*L. gibba* water

MRI

GBCAs in

Accumulation could not be detected

Gd in water

Gd in L. gibba

Distribution

Time, days

GBCAs out