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Environmental impact statement

This work describes the development of an innovative analytical strategy to monitor the uptake of gadolinium based contrast agents used for magnetic resonance imaging by plants and higher organisms. The results show an uptake of gadolinium by *Zygnema*, *Lemna minor*, *Lepidium sativum* and *Daphnia magna*. These findings, we believe, will be of great interest to the reader. They prove that external absorption of gadolinium, which in ionic form is highly toxic, by plants and animals is possible. That means that it might also reach the human food chain. We believe that this will be of interest not only because of the newly developed bioimaging method, but also because of the highly important background.

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ARTICLE

Gadolinium-Uptake by Aquatic and Terrestrial Organisms – Distribution Determined by Laser Ablation Inductively Coupled Plasma Mass Spectrometry

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Gadolinium (Gd) based contrast agents (CA) are used to enhance magnetic resonance imaging. As a consequence of excretion by patients and insufficient elimination in wastewater treatment plants they are detected in high concentrations in surface water. At present, little is known about the uptake of these species by living organisms in aquatic systems. Therefore the uptake of gadolinium containing chelates by plants and animals grown in exposed water or on soil irrigated with exposed water was investigated. For this purpose two types of plants were treated with two different contrast agents. The uptake of the Gd contrast agents was studied by monitoring the elemental distribution with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). This technique allows the multi-elemental analysis of solid samples with high resolution and little sample preparation. The analysis of *L. minor* showed that the uptake of Gd correlated with the concentration of gadodiamide in the water. The higher the concentration in the exposed water, the larger the Gd signal in the LA-ICP-MS acquired image. Exposure time experiments showed saturation within one day. The *L. minor* had contact with the CAs through roots and fronds, whereas the *L. sativum* only showed uptake through the roots. These results show that an external absorption of the CA through the leaves of *L. sativum* was impossible. All the analyzed parts of the plant showed Gd signal from the CA; the highest being at the main vein of the leaf. It is shown that the CAs can be taken up from plants. Furthermore, the uptake and distribution of Gd in *Daphnia magna* were shown. The exposure via cultivation medium is followed by Gd signals on the skin and in the area of the intestine, while the uptake via exposed nutrition algae causes the significantly highest Gd intensities in the area of the intestine. Because there are hints of negative effects for human organism these findings are important as they show that Gd based CAs may reach the human food chain via plants and animals growing in contaminated water or plants growing in fields which are irrigated with surface water.

Introduction

In 2005, about 20 million applications in magnetic resonance imaging (MRI) were performed worldwide using gadolinium (Gd) based contrast agents (CA)[1]. GdCAs usually consist of Gd^{3+} ions chelated with aminocarboxylates[2]. Every year approximately 1100 kg Gd are released into the sea and waterways[3]. It originates from hospitals and radiology practices. Due to its high water solubility, the chelates pass wastewater treatment plants mainly unhindered[2]. Previous studies showed that about 90% of the gadolinium is still present in the effluent after wastewater treatment, mostly in their original chelated form[4,5].

In one of our last studies we found a concentration as high as 990 ng L^{-1} of total Gd near the outlet of a wastewater treatment plant in the Teltow channel in Berlin[6]. Because free lanthanide ions are very toxic *in vivo*[7], the subsequent questions to answer are whether CAs are taken up by aquatic organisms and, if so, how factors such as concentration, chemical species of the Gd complexes or exposure time influence their transport and distribution in the organism. Can GdCAs even reach the human food chain?

Although concentrations of anthropogenic gadolinium are to date are rather low in surface water (in the range of 100-1100 ng/L), further input by radiology practices and hospitals can be expected[5,6].

Little is known about the interaction of Gd-based CAs with biological systems. Conventional techniques for elemental analysis (atomic absorption spectrometry AAS, inductively coupled plasma optical emission spectrometry ICP-OES, inductively coupled plasma mass spectrometry ICP-MS) have been used for determination of total Gd concentrations or Gd species in different biological samples[2], often hyphenated to separation systems such as high-

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performance liquid chromatography (HPLC). However, the mentioned methodologies do not provide spatial resolved information and therefore the precise distribution of the element remains unknown.

In recent years, various analytical techniques have been applied to create images of the elemental distribution in biological tissues. One of these techniques, the laser ablation ICP-MS (LA-ICP-MS), is a very promising tool for bio-imaging[8], because of the simple and fast sample preparation, the multi-elemental detection capability, the wide dynamic range, and the high spatial resolution of a few micrometers[9-11]. Thus, an elemental mapping at the cellular range is possible[12]. The solid state analysis offers the advantages of solvent-free sample preparation resulting in a lower risk of contaminations and lower values of oxides, hydroxides[13], and polyatomic interferences[14] compared to techniques analyzing liquid solutions such as HPLC ICP-MS. Additionally, the limits of detection (LOD) are lower (in the low µg kg^{-1}-range[15]) than in the presence of solvents due to the fact that no dilution is necessary. For quantification by LA-ICP-MS, a calibration method with a suitable matrix matched standard is necessary[16] to compensate for elemental fractionation and matrix effects[17]. Different quantification approaches like using spiked raw material as a standard, for example homogenated rat brain[18] or plant material in spiked cellulose powder[19], have been discussed in the review of Hare *et al.*[16].

LA-ICP-MS is rapidly becoming the method of choice for a detailed and sensitive monitoring of elemental distributions in biological tissue[20-22]. Becker *et al*. used LA-ICP-MS for imaging tissue sections of human, mouse and rat brain samples[24], but also for mapping essential elements in leaves[25] and the effect on the distribution of nutrient elements after copper exposure[26].

In this work, the uptake of Gd-based CAs in plants and animals was investigated using LA-ICP-MS imaging. The local accumulation and distribution of the two selected MRI-CAs, gadopentetic acid (Magnevist®) and gadodiamide (Omniscan®), was studied on four model systems: *Lemna minor* (duckweed), *Lepidium sativum* (cress), *Zygnema* (filamentous algae) and *Daphnia magna* (water flea). The *L. minor* experiment was carried out to determine the dependence of the Gd uptake on the Gd concentration in nutrition water and exposure time. With *L. sativum*, as a terrestrial plant, the uptake of CAs by the roots and the distribution of Gd in different compartments (leaf, stem, root) was studied. This organism has been used in our previous studies to investigate the uptake of Gd species by use of HILIC-ICP-MS[6]. Experiments with *D. magna*[27,28] were conducted to investigate different uptake routes via cultivation medium and nutrition algae.

The growing of *D. magna* in Gd containing growth medium should show if Gd is taken up directly by the organism. Additionally, one-celled algae *Scenedesmus subspicatus*, which was grown in Gd contaminated growing solution, was used to feed the *D. magna* (in an otherwise Gd free solution). This experiment shall show if Gd is taken up through food. For analyzing the uptake and release of Gd by algae, the filamentous algae *Zygnema* was used instead of the one-celled nutrition algae, due to their easier handling.

Experimental

Chemicals

Swedish Standard (SIS, *Lemna minor* growth medium) was used for *L. minor* cultivation following the OECD test guideline 221. The chemicals sodium nitrate, potassium dihydrogen phosphate, magnesium sulfate heptahydrate, sodium carbonate, iron(III) chloride hexahydrate, sodium molybdate, zinc sulfate heptahydrate, copper(II) sulfate pentahydrate, cobalt(II) nitrate pentahydrate (p. a.), calcium chloride dihydrate (puriss. cryst.), and boric acid (suprapur®) were purchased from Merck (Darmstadt, Germany). Manganese(II) chloride tetrahydrate (p. a.) (Ferak Berlin, Germany) and Na_2EDTA (p. a.) (AppliChem, Darmstadt, Germany) were also used. The contrast agents used in this study were gadopentetic acid (Magnevist®, gadolinium(III)-2-[bis[2-[bis(carboxy-methyl)amino]ethyl]amino]acetic acid, Bayer Schering Pharma, Berlin, Germany) and gadodiamide (Omniscan®, Gadolinium(III)-5,8-bis(carboxylatomethyl)-2-[2-(methylamino)-2-oxoethyl]-10-oxo-2,5,8,11-tetraazadodecan-1-carboxylathydrate, GE Healthcare, Braunschweig, Germany). Sea sand (p. a.) was purchased from Merck (Darmstadt, Deutschland). Deionized water (18.2 MΩ cm) was used for diluting GdCA solutions.

Plant cultivation and sample preparation

***L. minor* – Exposure concentration experiments.** Ten *L. minor* plants per vessel were cultivated in 30 mL SIS medium and exposed to different solutions of gadodiamide containing 0, 0.1, 1, and 10 mg L^{-1} of Gd. After seven days, the plants were washed with deionized water, and then pressed and dried between blotting papers. For LA-ICP-MS measurement the samples were taped on top to a glass slide with adhesive tape (with the tapeless side showing up). The samples were mounted onto glass slides (Thermo Scientific, Germany) and inserted into the ablation cell of the laser system.

***L. minor* – Exposure time experiments.** Fifty *L. minor* plants per vessel were cultivated in 30 mL SIS medium and exposed to gadodiamide (10 mg L^{-1} of Gd) over periods of 0, 1, 2, 5, and 7 days. After exposure, the plant samples were prepared as described above.

L. sativum. Seeds of *L. sativum* were cultivated for nine days on commercial sea sand. The sand was prior mixed with gadopentetic acid solution containing 100 mg L^{-1} of Gd. This way, after drying, sea sand containing 10 mg kg^{-1} of Gd was obtained. Sand was chosen as growth medium to allow accurately controlled Gd exposure and for an easy removal without breaking the roots.

During their growth, *L. sativum* were irrigated with tap water once a day for nine days. This schedule allows the plants to reach a reasonable size and a continuous uptake of the CAs through the roots for the whole time of the experiment.

After collection, the plant were dried and pressed between blotting papers. For LA-ICP-MS analysis, the procedure described above was followed.

***Zygnema*.** *Zygnema* were cultivated in a gas wash bottle for permanent air supply in 200 mL WC-medium[29] („Woods Hole modified CHU 10“, see Electronical Supplementary Material, table

2) and exposed to gadodiamide with a Gd concentration of 50 mg L^{-1}. The air supply was regulated by a membrane pump. After seven days of exposure, a part of *Zygnema* was filtered, washed with deionized water and dried and pressed like described for *L. minor*.
Another batch of *Zygnema* was cultivated with 200 mL Gd-free nutrition solution. After three days of exposure, the algae samples were prepared as described for *L. minor*.

D. magna. *D. magna* were cultivated in Elendt-M4-medium according to OECD guideline 211[30] (see Electronical Supplementary Material, table 1). The different compounds were solved in the parent solutions I-VIII.2. The nutrition solution was produced by mixing the parent solutions.
The water fleas were fed with the single-celled algae *Scenedesmus subspicatus*, which was cultivated in the WC-medium.

***D. magna* – Uptake via cultivation medium surrounding.** The daphnia was exposed to gadopentetic acid with a Gd concentration of 100 mg L^{-1} for 19 hours without feeding. After exposure, the daphnia was washed in deionized water to remove the growth solution from its surface and then dried on air. For the LA-ICP-MS measurement, the daphnia was fixed on glass slides with adhesive tape. The whole organism was ablated. Because the sample had different thickness on each point, the ablated material and the laser focus is not the same over the measurement. Thus, the result only gives a hint for the Gd distribution.

***D. magna* – Uptake via nutrition algae.** The one-celled algae (*Scenedesmus subspicatus*) were exposed to gadopentetic acid with a Gd concentration of 50 mg L^{-1} in WC-medium and air supplied by a pump. Due to growth of the algae the solution was diluted to a Gd concentration of 28 mg L^{-1}. This concentration was calculated in consideration of added CA and the volume of the nutrition medium. After filtration and washing the algae, daphnia was exposed to it for 19 hours and then prepared for measurement as described for *D. magna* – Uptake via cultivation medium surrounding.

Instrumentation

An optical image of the *L. minor* fronds was made with a Stemi Mikroskop DV4 (Zeiss, Oberkochen, Germany) before ablation.

LA-ICP-MS. A commercial laser ablation system (NWR 213, ESI, Portland, Oregon), equipped with a beam expander and an adjustable laser with a spot size between 4 and 250 µm, was coupled to an ICP sector field MS (Element XR, Thermo Fisher Scientific, Germany). Please note that the presented studies could also be carried out with a quadrupole ICP-MS. The ICP-MS was synchronized with the LA unit in external triggering mode. The aerosol was transported by helium or argon at a flow rate of 1 L min^{-1}. Argon was added as sample gas at a flow rate around 0.75 L min^{-1}. The ICP was tuned daily for maximum signal intensity, keeping the oxide ratio (ThO/Th) below 1%. Samples were completely ablated by adjacent single line scans under optimal LA-ICP-MS conditions. The isotope ^{158}Gd was selected for analysis of the distribution of CAs in biological tissue and was measured in low resolution. For each line, a gas blank was
measured for 30 s. The average of every blank was subtracted from all signals of the respective line. The optimized parameters are shown in Table 1.

Table 1: Optimized parameters of the LA-ICP-MS system.

ICP-MS	Element XR
RF power (W)	1200 – 1300
Plasma gas (L min^{-1})	15
Sample gas flow (L min^{-1})	0.69 – 0.85
Auxiliary gas flow (L min^{-1})	0.8 – 1.0
Mass resolution m/Δm	300
Number of passes	1
Number of runs	250 – 320
Measured isotopes	^{158}Gd
Laser ablation system	New Wave NWR-213
Wavelength (nm)	213
Transport gas flow rate (L min^{-1})	1
Laser fluence (J cm^{-2})	0.7 – 23.5
Repetition rate (Hz)	20
Spot size (µm)	4 – 50
Scan speed (µm s^{-1})	10 – 50
Distance between lines	Spot diameter – 10 %

Results and discussion

In one of our last studies we showed an anthropogenic entry of Gd-based CAs into surface water[6]. Because their effect on aquatic and human life is unknown, we investigated the uptake of these CAs by plants and higher organisms. This will be of major importance when evaluating possible consequences for human health if these compounds reach the human food chain.
The following experiments were designed to clarify whether Gd-based CAs are taken up by plants and animals and to characterize the uptake efficiency and distribution depending on contaminant concentration and exposure time. The applied Gd concentrations were very high compared to those in nature, but were chosen in order to obtain enough accumulation after short exposure times.
Gd distribution is shown in the LA-ICP-MS intensity profiles.
The question whether the CA is preserved, will be discussed later.

Concentration dependent uptake of Gd by *L. minor*

L. minor (duckweed) was exposed to different concentrations of gadodiamide over seven days. The high complex formation constant of Gd with DTPA-BMA (log K = 16.85[4]) allows us to assume that the complex remains stable during the potential uptake by the plant[6], which was conducted in mild experimental conditions. A replacement of Gd by any of the essential nutrients from the nutrient solution (e. g. Ca^{2+}, Cu^{2+} and Zn^{2+}, Fe) is unlikely due to much lower complexation constant of the respective DTPA-BMA complex[31].
Figure 1 shows the optical images and the Gd distribution of *L. minor* after exposure to different CA concentrations.
The images of all exposed plants show Gd signal. The Gd signal intensity increases with the concentration of Gd in the cultivation solution of the respective sample. The control sample shows intensities between 0 and 500 cps and was used as blank. It should be noted that the carrier material (adhesive tape) around the

fronds gave no signal. The Gd signal in the middle of the fronds of the exposed plants is low, whereas higher intensities in the proximity to the edges correlate to increasing Gd concentrations in the cultivation solutions. The plant exposed to 10 mg L^{-1} of Gd has the highest concentration in the area where the two fronds overlap; this is due to a higher amount of ablated material.

The root threads are visible at the bottom of the LA-ICP-MS images for Gd concentrations of 1 and 10 mg L^{-1} (intense filamentous area). The root of the plant exposed to 0.1 mg L^{-1} of total Gd does not show significant ^{158}Gd signals in the LA-ICP-MS image. This indicates a low uptake through the roots.

The comparison of the intensities of the different elemental distributions shows a clear trend. The mean intensity in the distribution images of exposed *L. minor* is proportional to the concentration of Gd in the exposure solution. Therefore, higher gadodiamide concentrations in the cultivation solution lead to more Gd uptake. However, the spatial distribution of Gd does not change with the concentration.

At this point it is important to mention that these and the following results give an insight into the Gd distribution in plants assuming that Gd is on the same plane throughout the examined sample. The compensation of thickness differences with internal standards is subject of ongoing research.

L. minor is a swimming aquatic plant. The plants have small air-filled cavities under the fronds to float on water so that only the edges touch the water. The root threads are mainly used for stabilization and the nutrients are mainly absorbed through the fronds.

Therefore, the CAs can be taken up through the roots or by the fronds when they are in contact with the cultivation solution. In all exposed plants, the ^{158}Gd intensities on the edges are four times higher compared to those detected in the middle of the fronds.

The higher intensities of Gd in *L. minor* at higher exposure concentrations suggests that the uptake is diffusion-driven until equilibrium is reached. That means, the higher the concentration the higher is the uptake of Gd by the organism. Since the beginning of using CAs for MRI examinations the amount of Gd, released to environment, is increasing continuously. Therefore, the plants living in the contaminated surface are permanently exposed to Gd.

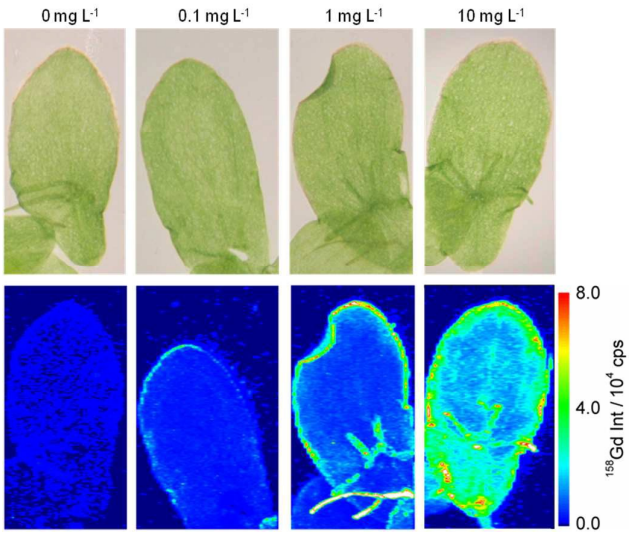

Figure 1: Microscope images and LA-ICP-MS intensity profiles of ^{158}Gd in *L. minor* fronds after seven days of exposure to cultivation solutions of different concentrations of gadodiamide contrast agent. Gd concentration in the cultivation solutions: 0, 0.1, 1, and 10 mg L^{-1}. Ablation parameters: laser fluence 0.7 J cm^{-2}, spot size 50 µm, scan speed 25 µm s^{-1}, ablation gas He, ablated area 2.5 × 3.9 mm^2.

Time dependent uptake of Gd by *L. minor*

To investigate the hypothesis that the uptake of Gd is diffusion-driven, the dependence on uptake time was studied in *L. minor*. We wanted to determine whether the duration of exposure had an effect on the distribution and concentration of Gd in the plants. Therefore, *L. minor* was exposed to gadodiamide with a Gd concentration of 10 mg L^{-1} for 0, 1, 2, 5 and 7 days.

In Figure 2, the microscope and LA-ICP-MS images of *L. minor* fronds are shown. The lateral veins and plant cells are visible. The image of the five day sample also shows root filaments.

In the control sample (0 days, no GdCA addition) no Gd was detected. After one day of exposure, Gd is detectable in the plant. Like before, the highest intensities of Gd were detected at the edges of the fronds due to the direct contact to the cultivation solution. The distribution and the Gd intensity in the fronds do not change with increasing exposure time, which suggests that saturation or equilibrium of the GdCA between the cultivation solution and the plant is reached. A time dependent accumulation of Gd in the *L. minor* plants after one day is not observable. This reinforces the assumption that the uptake of Gd based CAs by *L. minor* is a diffusion-driven process. This finding was the motivation for further plant experiments with terrestrial plants.

Regardless of the uptake mechanism, these experiments clearly show that the introduction of Gd into the human food chain is possible via plants that were exposed to contaminated water.

Distribution of Gd in *L. sativum*

With the experiments with *L. minor* plants, we studied the uptake of Gd through the roots and the surface of the fronds. To investigate the entry route of Gd only through the roots, further investigations were performed with a terrestrial plant, the garden cress *L. sativum*. Leaves, stems and roots were analyzed separately.

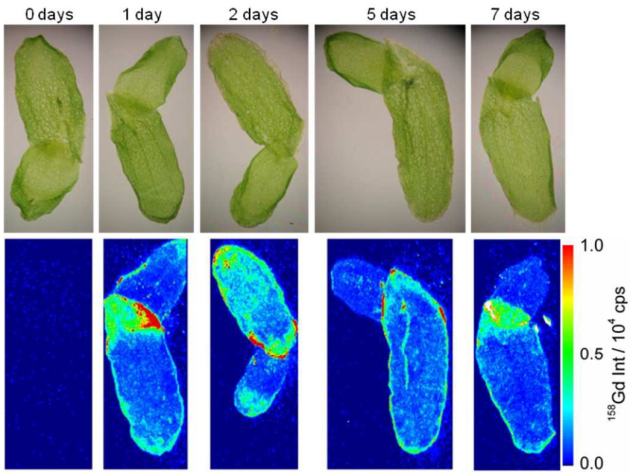

Figure 2: Microscope images and LA-ICP-MS intensity profiles of ^{158}Gd in *L. minor* fronds after 0, 1, 2, 5, and 7 days (from left to right) of exposure to gadodiamide

cultivation solutions (10 mg L^{-1} of Gd). Ablation parameters: laser fluence 1.5 J cm^{-2} (0 – 2 days), 1.0 J cm^{-2} (5 & 7 days), spot size 25 µm, scan speed 50 µm s^{-1}, ablation gas Ar, ablated area (1.5 – 2.6) × 5.0 mm^2

L. sativum was chosen as model organism, because the leaves are small but still have discernable structures. In addition, they can be easily grown on nutrient-poor soils. In order to prove the diffusion-driven hypothesis, all accessible parts of the *L. sativum* plant were analyzed by LA-ICP-MS after exposure to GdCA spiked water.

Figure 3 shows the optical images of a complete *L. sativum* plant, as well as LA-ICP-MS images monitoring ^{158}Gd in three different parts of the plant. Gd was detected in all analyzed parts. The highest average intensities were found in the leaf veins and on the left edge of the leaf. Also, the root presented high and homogeneously distributed Gd signal. As has been described for the *L. minor* leaves, the overlapping of roots led to higher Gd responses. The high accumulation of Gd on the left edge of the leaf was also caused by overlapping of several layers of tissue. The lowest average intensities were measured in the stem. Interestingly, the central part of this sample showed higher accumulation of Gd than the borders.

The LA-ICP-MS Gd distribution patterns in the *L. sativum* samples are consistent with the results recently reported after ICP-MS analysis of the digested parts of the plant[5].

The root shows homogeneous distribution of Gd as expected, most likely due to the uptake of the CA through the bark around the root and the subsequent transport through the secretory tissue to the leaf. For the same reason, especially the veins stand out by showing high Gd intensities. From the main leaf vein the CA can be distributed into the whole system of veins, resulting in the observed increased Gd intensity in the leaf.

Due to the predominant distribution of Gd in the water transport paths, it is assumed that the CA is transported and distributed by water. This assumption corresponds to the idea of a diffusion-driven uptake.

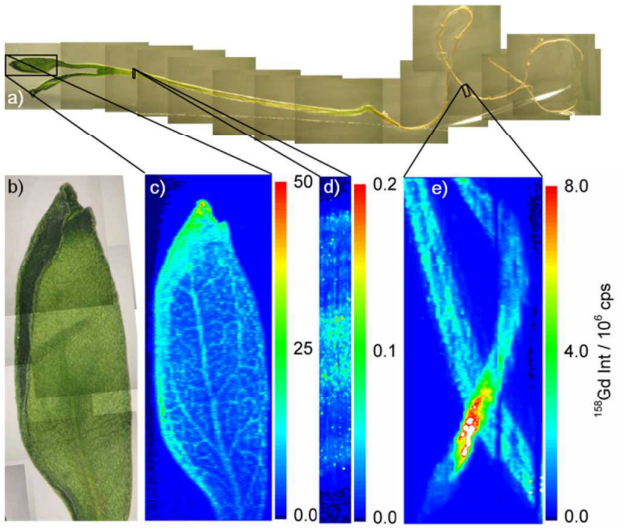

Figure 3: a) and **b)** microscope images of a *L. sativum* plant exposed to gadopentetic acid (10 mg L^{-1} of Gd) for nine days; **c)** LA-ICP-MS intensity profile of ^{158}Gd in the leaf; ablation parameters: laser fluence 5.7 J cm^{-2}, spot size 50 µm, scan speed 15 µm s^{-1}, ablation gas He, ablated area 1.9 × 5.3 mm^2; **d)** LA-ICP-MS intensity profile of ^{158}Gd in the stem; ablation parameters: laser fluence 9.7 J cm^{-2}, spot size 4 µm, scan speed 10 µm s^{-1}, ablation gas He, ablated area 1.0 × 7.6 mm^2; **e)** LA-ICP-MS intensity profile of ^{158}Gd in the root; ablation parameters: laser fluency 23.5 J cm^{-2}, spot size 8 µm, scan speed 10 µm s^{-1}, ablation gas He, ablated area 3.6 × 9.0 mm^2

The question whether the CA is stable during uptake by plant or metabolizes has already been answered[5]. After five days of exposure to *L. sativum,* the CA was found with no or minor modifications.

The uptake of the CA through the root is still very surprising, because it was not expected that such large molecules can pass through the protective system of the root. Normally, terrestrial plants prevent the uptake of large molecules and uncontrolled uptake of substances – even water – with the casparian band. The casparian band is a membrane consisting of lignin and endodermin in the endodermis. It blocks the water and substance transport through the apoplastic space to the vascular cylinder of the root. The casparian band forces all absorbed substances from the apoplastic into the symplastic space, where they are selected. If the chelate surrounding the metal ion is similar to the siderophore – the uptake chelate for the needed metals – the plant can take up not essential metal ions[32].

In this work, we investigated *L. sativum* seedling. Presumably, in these young plants, the casparian band is not fully established[33], and thus the substances can be easily taken up through the apoplastic space[34]. So the Gd can reach the human food chain via terrestrial plants exposed to CAs.

Uptake of Gd from cultivation medium surrounding by *D. magna*

The uptake and the distribution of Gd from CAs were already shown at various representatives of the flora (aquatic and terrestrial). Additionally, the Gd uptake and distribution should be studied in a more complex aquatic animal model organism, the daphnia, due to other uptake and transport mechanisms.

Daphnia is a very important bio-monitor, because it can take up dissolved and solid contaminants, by gills and the digestive system. Thus, Daphnia is able to take up Gd-containing contrast agents directly from the water, or alternatively by its nutrition in which contrast agents are incorporated.

First, the uptake of dissolved Gd from CAs directly from cultivation medium was investigated. Due to filtration of nutrients from the cultivation medium and breathing through skin and gills, an uptake predominantly via skin but also via digestive system is expected. The Gd intensity distribution and a photo of the examined daphnia are shown in Figure 4. The ^{158}Gd intensity distribution in the body of the daphnia is clearly distinguishable from the background. Increased intensities were found in the whole body of the daphnia. The highest signals are in the area of head and intestines (Figure 4). Thus, daphnia can take up Gd via the digestive system directly from the nutrition solution and also through the skin and the gills via the direct contact with the GdCA in the cultivation medium. Therefore, Gd from CAs can also enter the human food chain by fish.

Uptake and release of Gd from algae

In the following experiment, a daphnia was exposed to Gd via nutrients only. The daphnia was exposed to one-celled algae

(*Scenedesmus subspicatus*), which were cultivated in a Gd containing nutrition medium.

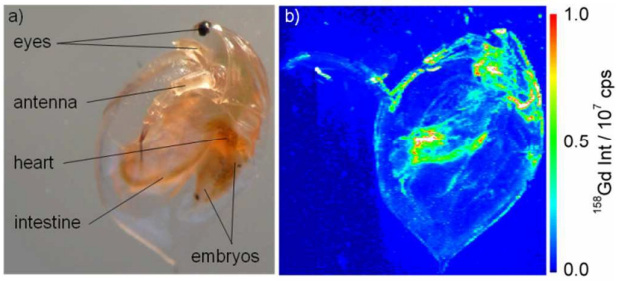

Figure 4: a) Microscope image of a *D. magna* exposed to gadopentetic acid (100 mg L^{-1} of Gd) for 19 hours; **b)** LA-ICP-MS intensity profile of ^{158}Gd in the daphnia; ablation parameters: laser fluence 1.9 J cm^{-2}, spot size 30 µm, scan speed 15 µm s^{-1}, ablation gas He, ablated area 3.5 × 3.2 mm^2; The left antenna – broken during sample preparation – is placed in the left corner of the LA-ICP-MS image.

First, we analyzed filamentous algae due to their easier handling instead of the one-celled algae regarding their Gd uptake. A photo of the algae is shown in

Figure 5 together with the LA-ICP-MS image of ^{158}Gd after seven days of exposure (

Figure 5b) and after three additional days in a Gd-free solution (

Figure 5c).

In

Figure 5b, the Gd intensities are high in the algae and clearly distinguishable from the background. Despite washing after exposure, the CA is preserved on the algae. The Gd distribution shows increased intensities where several filaments overlap, due to increased Gd ablation at these points.

Figure 5c with algae after three additional days in Gd-free solution shows low Gd signals. The comparison of both results from algae shows that the Gd can be released after the uptake, and even after three days the intensities have decreased to a tenth of the initial intensity. Thus, the assumption of a diffusion process driven by a concentration gradient is confirmed.

Uptake of Gd via nutrition algae by *D. magna*

The uptake of Gd from cultivation medium shows a distribution in

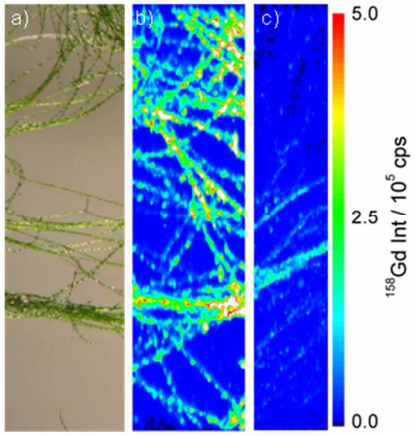

Figure 5: a) Microscope image of *Zygnema* exposed to gadodiamide (50 mg L^{-1} of Gd); **b)** LA-ICP-MS intensity profile of ^{158}Gd in the algae exposed for seven days (50 mg L^{-1} of Gd); ablation parameters: laser fluence 1.2 J cm^{-2}, spot size 15 µm, scan speed 50 µm s^{-1}, ablation gas He, ablated area 0.8 × 3.2 mm^2; **c)** LA-ICP-MS intensity profile of ^{158}Gd in algae exposed for seven days with CA (50 mg L^{-1} of Gd) and three days in Gd-free medium; ablation parameters: laser fluence 7.9 J cm^{-2}, spot size 15 µm, scan speed 50 µm s^{-1}, ablation gas He, ablated area 0.7 × 4.1 mm^2

the intestine and the skin of the daphnia. The algae take up and release the Gd within three days; this time frame was chosen for the following experiment with one-celled algae S. subspicatus. Figure 6 shows the picture and the LA-ICP-MS intensity profile of Gd in a *D. magna* exposed via nutrition algae. Now the highest intensities are in the area of the intestine. The signals are higher than in the remaining tissue by a factor of about 50-300. In the Daphnia exposed via the cultivation medium, the difference between the Gd intensity in the intestine and the remaining tissue was only a factor of about 15-30. Thus, the contrast of Gd signals in different parts of the daphnia exposed with Gd containing nutrition algae is much stronger than in the daphnia exposed with Gd via cultivation medium.

Gd absorbed by nutrition is not distributed into the whole body of the daphnia in the time frame investigated. Therefore, this experiment shows for the first time that Gd stays in the nutrient algae after being taken up for at least 19 hours and is not fully washed off in the cultivation medium. Secondly, it can be deduced from the significantly higher signals in the intestine that the uptake via nutrition algae is possible. Thus, again it is demonstrated that GdCA can enter the food chain of higher organisms. Algae are an important nutrition for mussels, whereas Daphnia is attractive for fish. In both cases, there is a certain risk that it can enter the food chain of human beings as well. If these results are transferable to humans, everyone takes up little doses of Gd daily via water consumption and nutrition, which are significantly higher than the natural amounts.

Conclusions

In the model organisms *L. minor*, *L. sativum*, *Zygnema* and *D. magna* the uptake of Gd from Gd-based CAs was demonstrated by LA-ICP-MS. The uptake of Gd in the aquatic *L. minor* depends on the concentration of the CA. After one day of GdCA exposure, no further accumulation was observed in the plants, most likely due to

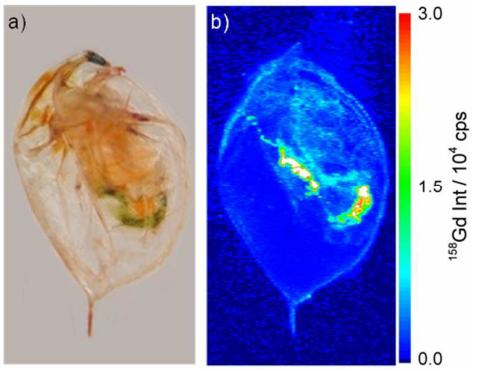

Figure 6: a) Microscope image of a *D. magna* exposed to gadopentetic acid (28 mg L^{-1} of Gd) for 19 hours; **b)** LA-ICP-MS intensity profile of ^{158}Gd in the daphnia; ablation parameters: laser fluence 1.8 J cm^{-2}, spot size 30 µm, scan speed 15 µm s^{-1}, ablation gas He, ablated area 1.9 × 3.3 mm^2

a saturation or equilibrium. In *L. sativum*, the CA enters the plant by the uptake via the roots. This study showed a local accumulation in the leaf veins. The predominant distribution of Gd in the leaf veins and in the top of the leaf was visualized successfully. The stem shows an increase in Gd intensity in the vascular cylinder which transports water to the leaves. The Gd distribution in the roots is nearly homogeneous.

D. magna can take up Gd via different routes. The exposure via the cultivation medium results in Gd signals on the skin and the intestine. In comparison, the uptake via the nutrition algae shows significantly higher Gd intensities in the area of the intestine with a greater contrast to remaining tissue.

Our studies were carried out with Gd concentrations higher than those commonly observed in surface waters. However, we assume that due to the diffusion-driven process, Gd will be taken up until equilibrium is reached, even in lower concentrations. Our results also show that active processes are involved in the distribution, leading to different Gd concentrations in different plant compartments.

These results show that Gd can be absorbed and incorporated by plants and animals. In many surface waters and partly also in the ground water[35] a Gd anomaly was found. It can be concluded that plants and animals living in this water are exposed to higher levels of anthropogenic Gd from CA permanently. But also the fields irrigated and the animals watered with this contaminated water are affected. From there it is only a short way to the human food chain. In view of the fact of increasing Gd concentrations in the water the consequences of the uptake in the different organisms, especially the uptake of the GdCAs by humans through nutrition, should be emphasized. Presently, it is completely unknown if this can cause any risk for living systems, but due to the fact that CAs are highly persistent in waters a monitoring system is required just to document future developments. Prophylactically better water purification in wastewater treatment plants is needed.

In our next study, a quantification approach for laser ablation ICP-MS will be developed in order to get more quantitative information about the amounts of Gd in the different parts of a plant for an even more detailed understanding of uptake and transport mechanisms and kinetics. For the laser ablation, a matrix-matched calibration will be presented in order to minimize the element fractionation effects[17].

Further studies are needed to follow the uptake at low doses over long periods of time by different organisms and the long-term stability of the chelate complexes and their metabolites in the environment. Such data is urgently needed for a more reliable risk assessment and a more objective discussion.

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