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# Gadolinium based contrast agents in the aquatic environment and the possibilities of their removal

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## Abstract

In this dissertation, gadolinium-based contrast agents were studied in the view of their possible distribution in the aquatic environment. The ICP-MS and ICP-OES methods were developed and validated for the determination of rare earth elements, in particular gadolinium, in river and waste water and *Chlorella kessleri* algae biomass and selected sorbents. River and hospital waste water from the surroundings of Pardubice and Hradec Králové were analyzed to evaluate the contribution of gadolinium of anthropogenic origin to the so-called gadolinium anomaly. An increased content of gadolinium of anthropogenic origin in hospital waste water has been demonstrated. In laboratory experiments, the ability to capture gadolinium contrast agenst (Dotarem®, MultiHance®) and control $Gd(NO_3)_3$ by viable algae of *Chlorella kessleri* and selected sorbents that may be present in the aquatic environment or are usable for sewage treatment technologies was assessed. Significant differences were found in the bioaccumulation of gadolinium compounds from real hospital waste water and from defined laboratory experiments. It was found that the adsorption ability differs for the different forms of gadolinium as well as for the individual sorbents that were included in the study (dead biomass of *Chlorella kessleri*, active carbon, humic acids, lake sediment).

## Abstrakt

V rámci této disertační práce byly studovány kontrastní látky na bázi gadolinia s ohledem na jejich možné šíření ve vodním environmentu. Byly vyvinuty a validovány metody ICP-MS a ICP-OES pro stanovení prvků vzácných zemin, zejména gadolinia ve vodách říčních a odpadních a v biomase řasy *Chlorella kessleri* a vybraných sorbentech. Byly analyzovány říční a nemocniční odpadní vody z okolí Pardubic a Hradce Králové s cílem vyhodnotit příspěvek gadolinia antropogenního původu k tzv. gadoliniové anomálii. Zvýšený obsah gadolinia antropogenního původu v nemocničních odpadních vodách byl prokázán. V laboratorních pokusech byla hodnocena schopnost vázat kontrastní látky (Dotarem®, MultiHance®) a kontrolní $Gd(NO_3)_3$ živou řasou *Chlorella kessleri* a vybranými sorbenty, které se mohou vyskytovat ve vodním prostředí nebo jsou využitelné v technologiích čistíren odpadních vod. Byly nalezeny významné rozdíly v bioakumulaci sloučenin gadolinia z reálných nemocničních odpadních vod a z definovaných laboratorních pokusů. Bylo zjištěno, že schopnost adsorbovat se liší pro různé formy gadolinia i pro jednotlivé sorbenty, které byly zahrnuty do studie (mrtvá biomasy řasy *Chlorella kessleri*, aktivní uhlí, huminové kyseliny, jezerní sediment).

## Keywords

Rare earth elements, Gadolinium, Bioaccumulation, Biosorption, Contrast agents, Anthropogenic gadolinium, ICP-MS, ICP-OES

## Klíčová slova

Prvky vzácných zemin, Gadolinium, Bioakumulace, Biosorpce, Kontrastní látky, Antropogenní gadolinium, ICP-MS, ICP-OES

# Table of Contents

**INTRODUCTION** ..... **6**

**1. THEORETICAL PART** ..... **7**

1.1 ELEMENTS OF RARE EARTHS AND GADOLINIUM IN THE ENVIRONMENT ..... 7

1.1.1 Gadolinium of anthropogenic origin ..... 8

1.2 REMOVAL OF METALS FROM WASTE WATERS ..... 8

1.2.1 Biosorption ..... 8

1.2.2 Bioaccumulation ..... 9

**2. EXPERIMENTAL PART** ..... **10**

2.1 CHEMICALS AND SAMPLES ..... 10

2.2 PREPARATION OF SAMPLES ..... 11

2.3 INSTRUMENTATION ..... 12

**3. RESULTS AND DISCUSSION** ..... **13**

3.1 VALIDATION METHOD ..... 13

3.2 RARE EARTH ELEMENTS IN HOSPITAL AND RIVER WATERS ..... 14

3.2.1 Rare earth elements in Matiční Lake ..... 16

3.3 BIOACCUMULATION OF GADOLINIUM FORMS IN .....

CHLORELLA KESSLERI BIOMASS ..... 18

3.3.1 Pilot phase of bioaccumulation experiment ..... 18

3.3.2 Extended bioaccumulation experiment ..... 19

3.4 REMOVAL OF GADOLINIUM SUBSTANCES USING OTHER SORBENTS ..... 22

**4. CONCLUSION** ..... **24**

**LIST OF REFERENCES** ..... **25**

**LIST OF STUDENTS' PUBLISHED WORKS** ..... **28**

# Introduction

The environment is the space in which the organism lives and is tied to by a system of bonds representing all external natural and cultural influences which give him favorable living conditions. The environment is formed of inanimate, living and socio-economic influences and components which simultaneously and continuously cooperate and through negative feedback protect itself from adverse effects. Man produces many chemicals to provide him a comfortable life but disrupt the natural balance in the environment. Great attention should be payed to heavy metals that have negative health effects.

Rare earth elements (REE) seem less sigifnificant from this point of view, but their importance for different technologies continues to grow. Gadolonium based contrast agents (GdCAs) are widely used in medical diagnostic methods, especially in magnetic resonance imaging (MRI). The GdCAs have to be very stable in the body. Due to their high stability, they pass the patient body in an unmetabolized form through a sewerage system into a waste water treatment plant. Then still untransformed, they are spread in the water environment. In surface water, gadolinium content is gradually increasing over the natural background. Gadolinium forms disseminate to other environmental compartments and enter into food chains. Higher concentrations of gadolinium of anthropogenic origin are recorded in drinking water and it is not yet clear what the risks are to the human population (Ersoy 2007), (Künnemeyer et al. 2009).

New analytical methods are developed for the determination of gadolinium forms in environmental and biological samples that allow following their fate. The GdCAs can be monitored in the waste water treatment process that can help to optimize the cleaning process and remove them. An interesting option in the waste water treatment technology is the use of various biosorbents including algae that accumulate easily a relatively large amount of toxic metals through biosorption and bioaccumulation. Due to good sorption ability, wide accessibility and financial affordability, biosorbens are frequently used for biological and environmentally friendly waste water treatment (Chojnacka 2010), (Velásquez and Dussan 2009).

The objectives of this work are:

(i) To develop and validate ICP-MS and ICP-OES methods for the determination of rare earth elements, in particular gadolinium, in river and waste water and *Chlorella kessleri* algae biomass and selected sorbents with as simple a sample preparation as possible.

(ii) To evaluate the contribution of gadolinium of anthropogenic origin (the gadolinium anomaly) for surface and hospital waste water from the East Bohemia region.

(iii) To evaluate, in laboratory experiments, the ability to capture gadolinium contrast agenst (Dotarem®, MultiHance®) and control $Gd(NO_3)_3$ by fresh algae of *Chlorella kessleri* and compare with capability to remove gadolinium forms from hospital waste water.

(iv) To test capability of selected sorbents that may be present in the aquatic environment or are usable for sewage treatment technologies was assessed (e.g. dead biomass of *Chlorella kessleri*, active carbon, humic acids, river sediment) to adsorb gadolinium compounds.

# 1. Theoretical Part

## 1.1 Elements of rare earths and gadolinium in the environment

Gadolinium is a member of lanthanides – a group of 15 elements ranging from lanthanum to lutetium with atomic numbers 57 – 71 (La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb a Lu). Together with scandium and yttrium, a group of rare earth elements (REE) is formed. All REEs exhibit similar chemical and physical properties (Zawisza et al. 2011), (Lindner et al. 2015), (Kanazawa and Kamitani 2006), (Jordens et al. 2013). REE can be used as a component of catalyst mixtures, polishing compounds for polishing glass, mirrors or silicone chips, and from the production of luminophores for compact discs (Toda 2006), (Hatch 2012), (Mancheri et al. 2013), (Liang et al. 2005).

Gadolinium has the atomic number 64 and is the only element of the REE family that is ferromagnetic at low temperatures. Gadolinium has interesting metallurgical properties, improves the workability and resistance of Cr, Fe and related alloys at high temperatures. Gadolinium-yttrium garnet is used for the production of magneto-optical films. Gadolinium doped Ce is the only crystal used as a scintillator in medical imaging technology. Gadolinium in the form of stable complexes plays a significant role in medicine as contrast agents in imaging techniques (Ewa Bobrowska-Grzesik 2013), (Ersoy 2007), (Li et al. 2014). Gadolinium and other lanthanides in ionic forms can bind to S-transferase, dehydrogenase, kinase, ATPase, and glutathione coenzyme enzymes and inhibit calcium ions in biological processes. They can influence calcium channels, leading to adverse effects associated with blood clotting, contraction of muscles, nerve impulses, etc. (Ramalho et al. 2016), (Rogosnitzky and Branch 2016), (Sherry et al. 2009).

The most commonly used contrast agents (CAs) are Gd chelates, which must be very stable to avoid the release of toxic $Gd^{3+}$ ions. After intravenous administration, the gadolinium chelates, are eliminated from the body in the renal pathway in the range of 70 – 90 minutes in the unchanged form. (Ersoy 2007), (Li et al. 2014). The contrast agents are divided according to the structure of the chelate to linear and cyclic and to ionic and nonionic. Linear chelates are very flexible, open chains that do not provide a strong bond with $Gd^{3+}$. Cyclic chelates, on the other hand, bind $Gd^{3+}$ very strongly; the ion is closed in a circle resulting in higher stability of the complex and less dissociation susceptibility. The non-ionic linear GdCAs include OptiMARK® and Omniscan®, the ionic linear GdCAs are Magnevist® and MultiHance®. Another group is the non-ionic cyclic GdCA (Gadovist® and ProHance®). The only ionic cyclic GdCA is Dotarem® (Telgmann et al. 2013), (Rogosnitzky and Branch 2016).

The stability of chelates can be influenced by environmental conditions, pH values, the presence of other metal ions or ligands and their concentrations, the presence of ions such as $Zn^{2+}$, $Cu^{2+}$ and $Ca^{2+}$ should be mentioned, because transmetallation can occur, i. e. the binding of these ions to the complex instead of the free $Gd^{3+}$ (Telgmann et al. 2013), (Cacheris et al. 1990), (Ersoy 2007). The toxic potential of GdCA and their risks have not yet been fully investigated (Lindner et al. 2015).

### 1.1.1 Gadolinium of anthropogenic origin

The spreading of rare earth elements in the Earth system is largely affected by anthropogenic influences. In contrast to the natural ratios of rare earth elements, the gadolinium content is gradually increasing. So-called positive gadolinium anomaly is encountered not only in waste water treatment plants, surface and coastal areas of developed countries but also in drinking water (Elizalde-González et al. 2017). Positive gadolinium anomaly is described practically all over the world. It is associated with the use of contrast agents for magnetic resonance. After examination of the patient, GdCA is excreted in non-metabolised form within a few hours into hospital sewerage from where it enters the waste water treatment system and from here into surface and groundwater (Lindner et al. 2013), (Lindner et. al 2015), (Brünjes et al. 2016), (Birka et al. 2016). For assessing anthropogenic impacts on the propagation of rare earth elements, it is common to relate their content to a standard that represents the average composition of the earth's top crust. In practice, the Australian shale PAAS (Post-Archean Australian Shale) geological standard is often used in this normalization process (Piper and Bau 2013).

## 1.2 Removal of metals from waste waters

Conventional methods to remove heavy metals from the environment, such as chemical precipitation, membrane separation, evaporation or ion exchange, are not very effective, and can also be expensive and environmentally hazardous (Chojnacka 2010), (Edris et al. 2012).

An appropriate alternative to conventional techniques for removing or recovering metals from contaminated waste water can be using of biomass. Both live and dead biomass cells capture toxic metals very efficiently through biosorption and bioaccumulation (Chojnacka 2010), (Velásquez and Dussan 2009), (Kaduková and Virčíková 2005), (Gadd and Rome 1988), (Cho et al. 1994).

### 1.2.1 Biosorption

In biosorption, a sorbate, e.g. pollutants (metals) is bound on specific surface sites of the material of biological origin, not into living biomass. The sorbate captured on the surface of the biological material is then regenerated, sorbate is obtainable with the eluent and reused. It is very important to carefully select a desorption agent whose low volume removes all sorbate from the biomass, while preserving the sorption properties of the biosorbent. Biosorption is not metabolic dependent, it occurs in dead biomass and there are no problems with metabolic products. Non-living biomass is not affected by the toxic effects of metals, so biosorption may take place over a wider range of working conditions than would be appropriate for living cells. In comparison with bioaccumulation, the biosorption is established at a faster rate, ranging from a few minutes to hours at optimum pressure and temperature. Biomass can be used repeatedly several times, is capable of concentrating the metal about 1000 times, and has been described for use in biohydrometalurgy and biogeochemistry (Mattuschka and Straube 1993), (Volesky et al. 1993), (Ahluwalia and Goyal 2007), (Vijayaraghavan and Yun 2008).

For the sorption of heavy metals, the use of non-living biomass of algae, ferns and aquatic plants is used as a sorbent. Particularly effective sorbents are, for example,

various kinds of fungi, yeasts etc. (*Rhizopus*, *Aspergillus*, *Streptoverticilium* and *Saccharomyces)*. Bacteria have also a high sorption potential (*Bacillus sp*., *Pseudomonas*, *Zoogloea*, *Streptomyces*). Among the very good candidates for biosorption process, marine photoautotrophic microorganisms. whose sorptive activity is comparable to the green algae of the genus *Chlorella.* These algae are excellent metal sorbents, their easy availability and the possibility of cultivation in laboratory conditions is also an advantage (Volesky et al. 2003), (Ahluwalia and Goyal 2007), (Ahalya et al. 2003), (Chojnacka 2010), (Kaduková and Virčíková 2005).

### 1.2.2 Bioaccumulation

The mechanism of bioaccumulation is more complex than biosorption. It is an active process dependent on the cellular metabolism of living cells. The activation energy of bioaccumulation is about 63 $kJ.mol^{-1}$, which is about 3times higher than the activation energy required for biosorption. Bioaccumulation takes place in two phases. The first phase is fast and is identical to biosorption, and in a subsequent slower phase the sorbate is transported to the interior of the cells. In the intracellular space, metals are bound to cytoplasmic ligands, phytochelatins and metallothioneins. In this process, it is possible to achieve a lower concentration of pollutants because the cells provide binding sites both on the surface and inside the cell. By bringing bio-accumulation of some of the pollutants into the interior of the cell, the binding sites present on the surface are released so that other substances can be bound to the surface. Gradual growth of biomass makes possible to bind even more pollutants. Bioaccumulation allows a lower residual concentration of pollutants in the environment than biosorption. (Chojnacka 2010), (Posten and Chen 2016).

# 2. Experimental Part

## 2.1 Chemicals and samples

All the reagents used were of analytical-reagent grade. Demineralized water was further purified using the SG Ultra Clear system (SG Water, USA). 14.4 mol.l$^{-1}$ $HNO_3$ (LachNer, Czech Republic) was distilled in sub-boiling distillation equipment (BSB 939 IR, Germany). A commercially available multi-element stock standard solution containing 100 mg.l$^{-1}$ of elements "A" (La, Ce, Nd and Pr) and 20 mg.l$^{-1}$ of elements "B" (Dy, Er, Eu, Gd, Ho, Lu, Sc, Sm, Tb, Tm, Y and Yb) (Analytika, the Czech Republic) and single-element standard solution (1.000 ± 0.002) g.l$^{-1}$ of Gd (the Analytika, Czech Republic) and In (SCP Science, Canada) were used for instrument calibration and sample spiking. Standard solutions containing Gd (in μg.l$^{-1}$) 500, 100, 50, 20 and 10 for the ICP-OES determination and 1.0, 5.0, 10, 15 and 20 for the ICP-MS analysis were prepared. The ICP-OES standards and blanks were acidified with $HNO_3$ to final concentration 3.5 mol.l$^{-1}$. The ICP-MS multi-element standards containing (all in μg.l$^{-1}$) 0.1, 1.0, 2.5, 5.0, 10 and 25 of elements "A" and 0.02, 0.2, 0.5, 1.0, 2.0 and 5.0 of elements "B" were used. Each of the ICP-MS blanks, standards, spikes and samples contained an internal standard In in the final concentration of 0.5 μg.l$^{-1}$and 0.14 mol.l$^{-1}$ $HNO_3$. Injection solution of contrast agent Dotarem® (Acidum gadotericum, 0.5 mol.l$^{-1}$, lot number: 13GD111B; Geubert, USA) and MultiHance® (Dimeglumine gadobenas, 0.5 mol.l$^{-1}$, lot number: S3P273A; Bracco Imaging, Germany) were used together with $Gd(NO_3)_3.6\ H_2O$ (analytical-reagent grade, Sigma Aldrich, Co., USA) in batch experiments. Analytical-reagent grade $NaNO_3$, $CaCl_2.2\ H_2O$, $MgSO_4.7\ H_2O$, $K_2HPO_4$, $NaCl_2$, $KH_2PO_4$, EDTA, KOH, $FeSO_4.7\ H_2O$, $H_3BO_3$, $MnCl_2.4\ H_2O$, $MoO_3$, $CuSO_4.5\ H_2O$, $Co(NO_3)_2.6\ H_2O$, $H_2SO_4$ (96 %) (all LachNer, the Czech Republic) were used for preparation of the Bold-Basal/Bristol Medium (BBM) (Andersen 2005). Fresh water algae *Chlorella kessleri* were obtained as a suspension in the growth medium from the Fycological Laboratory (University of South Bohemia, České Budějovice, the Czech Republic). Hospital waste waters were sampled (2 l each) at the University Hospital of Hradec Králové (the Czech Republic) in the waste pipe of the magnetic resonance workplace (MR) and in the waste water treatment plant (WWTP) in January 2015 and March 2015. River water samples were collected from rivers in Eastern Bohemia (in May 2015; 10 places). Quality control material METRANAL® 8 Green Algae (Analytika the Czech Republic) and certified reference material (CRM) BCR®-670 Aquatic plant (IRMM, Belgium) were used for validation of ICP-MS and ICP-OES methods. As solid adsorbents, humic acids prepared by acid precipitation of their Na salt Humitan (Humatex, the Czech Republic), the active carbon CHEZACARB 5H (Unipetrol, the Czech Republic), the inactivated biomass *Chlorella kessleri* obtained in dried form (Institute of Botany of the Czech Academy of Sciences, Třeboň, the Czech Republic) and the lake sediment (collected from the Matiční Lake, Pardubice, the Czech Republic) were used.

## 2.2 Preparation of samples

The algae *Chlorella kessleri* were cultured in a sufficient amount for the following experiments under artificial white light 590 nm (Osram 18 W/840, Osram, Germany) for 12 h a day (from 7 to 19 h) in the laboratory at the room temperature in a BBM containing essential nutrients. About 50 ml of the algae suspension (about $4 \times 10^6$ cells $ml^{-1}$) was inserted into the 500ml sterile Erlenmeyer flask and made up to the final volume of 200 ml with: (i) growth of BBM medium; (ii) growth medium BBM with addition of Gd as $Gd(NO_3)_3$, Dotarem®, MultiHance® about concentrations 2, 20, 100 $\mu g.l^{-1}$; (iii) waste water from the building of a MR workplace from the Faculty Hospital in Hradec Králové; (iv) waste water from the WWTP from the Faculty Hospital in Hradec Králové. The algae were cultivated under artificial light (12-h period) for defined periods (0.5, 1, 2, 3, 7, 10, 14, 21 and 33 days). After cultivation, the algae samples were harvested using the Eppendorf Centrifuge 5804 R (Eppendorf AG, Germany; 5 min, 10 °C, speed 3000 rpm, the rotor GH 3.2). The algae was rewashed three times with DW and dried in a laboratory oven UM 400 (Memmert, Germany; 48 h, 70 °C). Dried algae (precisely about 50 mg) were inserted with distillatively purified $HNO_3$ (6 ml) to the microwave oven SpeedwaveTM MWS-2 (vessels DAC-70S, 100 barr, 1450 W; Berghof, Germany), left in an open vessel (30 min) and then decomposed (160 °C, 5 min, 80 % of power; 200 °C, 10 min 80 % of power and 10 min without heating). The mineralised samples were filled up with DW in 25ml volumetric flasks and stored in acid-washed polyethylene bottles at -20 °C until analysis. The digested algae and sample blanks were diluted tenfold with DW and the internal standard In was added (0.5 $\mu g.l^{-1}$) before for the ICP-MS analysis. In the ICP-OES analysis, the samples were used without further dilution.

Hospital waste waters were sampled in January 2015 and March 2015. The samples were filtered through filter paper K4 (Papermill Perštejn Keseg & Rathouský, Pernštejn, the Czech Republic) and divided. One part was used directly in the growth experiment, the second part was acidified (0.14 $mol.l^{-1}$ $HNO_3$) and stored in sterile, acid-cleaned polyethylene bottles at -20 °C up to analysis. Samples of waste water were decomposed using 20 ml of the sample together with 6 ml of distilled nitric acid and the same temperature program as for algae. The cooled decomposed samples were transferred to volumetric flasks and made up to 50 ml with DW. The samples were stored in acid washed polyethylene vials up to analysis at -20 °C, prior to analysis diluted tenfold with DW and the internal standard In was added in the final concentration of 0.5 $\mu g.l^{-1}$.

River water samples were collected from rivers in May 2015. The samples were transported to the laboratory in a cooling box (about 4 °C), filtered, acidified and stored as detailed above. The river samples were analyzed without digestion after the addition of internal standard In (final concentration of 0.5 $\mu g.l^{-1}$).

As solid adsorbents, humic acids, the active carbon, the inactivated biomass *Chlorella kessleri* obtained in dried form and the lake sediment were used. The lake sediment was more of a sandy character with a higher proportion of inorganic components and it was not characterized in terms of its chemical composition. The lake sediment was dried in a laboratory oven (Memmert, Germany; 48 h, 105 °C) and sieved (sieve 2 mm mesh). The 0.2 g of a solid adsorbent was weighed into 250ml volumetric flasks and made up with 200 ml of Gd solutions (final concentration of Gd

2, 20, 100 μg.l$^{-1}$) and inserted into a laboratory shaker (Vibramax 100 Heidolph, Germany) for time according to an experiment (0.5, 1, 2, 3, 6 and 12 h). The samples were filtered through filter paper K4. Liquid parts were acidified (0.14 mol.l$^{-1}$ $HNO_3$) and stored in acid-cleaned polyethylene bottles at -20 °C up to analysis. The adsorbent parts were dried at 70 °C in the laboratory oven for 12 h. About 0.2 g of the solid adsorbents with 6 ml distilled nitric acid were left for 30 min in an open vessel and then decomposed and treated in the same way as the algae samples.

About 0.3 g of the certified reference material BCR®-670 Aquatic plant or the quality control material METRANAL® 8 Green Algae with 6 ml distilled nitric acid was left for 30 min in an open vessel and then decomposed and treated in the same way as the algae samples. The METRANAL® 8 material was spiked before the decomposition with the Gd solution to a final concentration of 1 μg.l$^{-1}$ and in one case 0.2 μg.l$^{-1}$. In the ICP-OES analysis, the samples were used without further dilution.

## 2.3 Instrumentation

The orthogonal time-of-flight ICP-MS spectrometer Optimass 8000 (GBC Scientific Equipment, Australia) equipped with the concentric nebulizer MicroMist (0.4 μl.min$^{-1}$) coupled with the 70 ml thermostaic (10 °C) cyclonic spray chamber (both Glass expansion, West Melbourne, Australia) was used for analysis of Gd in algae samples and all of REE in river and waste water samples. The operating conditions of the ICP-MS analysis were adjusted to compromise the sensitivity and resolution of the instrument for $^{238}U$ as well as to obtain the minimal $LaO^+/La^+$ and $UO^+/U^+$ ratios (usually less than 4 %): sample flow-rate 0.5 ml.min$^{-1}$; plasma power 1250 W; plasma, auxiliary and nebulizer gas flow-rates were 12, 0.8 and 0.98 ml.min$^{-1}$, respectively, and multiplier gain 2400 V. The sensitivity of 50000 counts.s$^{-1}$ and 55000 counts.s$^{-1}$ for 1 μg.l$^{-1}$ (mass integrated peak) and resolution of 1500 and 1650 were attained for $^{139}La$ and for $^{238}U$. The external calibration with the internal standard In was used for quantification. A peak area mode, five-second data acquisition time and ten replicates were used for measurement. Selected unwanted ranges of m/z were excluded (10 – 44.5; 55 – 57 and 78 – 81) from detection using the device "smart gate". Working isotopes ($^{139}La^+$, $^{140}Ce^+$, $^{141}Pr^+$, $^{146}Nd^+$, $^{147}Sm^+$, $^{153}Eu^+$, $^{158}Gd^+$, $^{159}Tb^+$, $^{164}Dy^+$, $^{165}Ho^+$, $^{166}Er^+$, $^{169}Tm^+$, $^{172}Yb^+$, $^{175}Lu^+$) were selected with regard to possible isobaric overlaps of interfering ions with the same mass (Krejčová et al. 2012). Their selection was carried out using both a spectral library integrated in equipment software, and a mass spectrum of samples.

The analysis of Gd in mineralized samples of algae was carried out with the sequential, radially-viewed ICP-OES spectrometer Integra XL (GBC Scientific Equipment, Australia), equipped with a concentric nebulizer and a glass cyclonic spray chamber (both Glass Expansion, West Melbourne, Australia). The measurement conditions were optimized based on Gd signal-to-background ratios. The analytic emission was based on the difference between the emission intensity measured on the top of the peak and the background near the peak. The emission lines used were Gd I 336.223 nm and Gd I 342.247 nm. The operating conditions of the ICP-OES analysis were as follows: plasma power 1000 W, sample flow-rate 1.5 ml.min$^{-1}$, plasma, auxiliary and nebulizer gas flow-rates were 10, 0.6 and 0.65 ml.min$^{-1}$, respectively, photomultiplier voltage 600 V, view height of 6.5 mm, ten replicated readings on-peak 1s and fixed point background correction.

# 3. Results and discussion

Firstly, appropriate materials were prepared, i.e. freshwater algae were cultivated in the laboratory enriched in Gd in various ways. The next step was the development and validation of ICP-OES and ICP-MS methods for the determination of REEs, in particular Gd, and waste water and *Chlorella kessleri* algae biomass and selected sorbents with a simple sample preparation as possible.

## 3.1 Validation method

Applicability and validity of the ICP-MS and ICP-OES instrumentations were evaluated based on a limit of detection and quantification (LOD, LOQ), sample blanks, analysis of reference materials and the recovery study. The LODs and LOQs for ICP-MS determination were evaluated as the concentration of three times and ten times, resp. the standard deviation of intensity measured near the monitored ion peak for the standard solution of ten replicates. The LODs and LOQs of ICP-OES determination were estimated as the concentration of three times and ten times, the standard deviation of the intensity of the background correction measured for the standard in ten replicates. These instrumental values were multiplied by a dilution factor related to the sample preparation steps prior to analysis. Depending on the single REE, the procedural LOQs for the ICP-MS analysis of river waters (all in $\mu g.l^{-1}$) ranged from 0.010 for Tm to 0.016 for Ce. The LOQs for the analysis of waste waters (all in $\mu g.l^{-1}$) ranged from 0.026 for Tm to 0.040 for Ce. In the case of Gd in the algae samples and sorbent (all in $mg.kg^{-1}$), the LOQ of 0.090 and 0.023 was achieved for the ICP-MS resp. 8.7 for algae samples the ICP-OES of 342.247 nm. Both the ICP-MS and ICP-OES LOQs were suitable for the analysis of Gd in the algae samples spiked with Gd just prior to the mineralized step. In other cases, the less demanding and readily operated ICP-OES was not suitable. The Gd contents in sample blanks, which included a calibration sample blank, a growth sample blank (only the BBM medium under the batch experiment condition) and a mineralization sample blank (only pure $HNO_3$ treated with the decomposition step) were below the LOD and confirmed the absence of contamination risk in the process. The REE contents in the sample blanks were determined below the corresponding LODs and confirmed the absence of contamination risk in the process.

The ICP-MS and ICP-OES validation were performed through a recovery study and repeatability (as a relative standard deviation, RSD) of calibration standards, spiked algae samples and reference materials.

For the calibration standards of ICP-MS (0.1 $\mu g.l^{-1}$ and 1 $\mu g.l^{-1}$ of elements "A"/0.02 $\mu g.l^{-1}$ and 0.2 $\mu g.l^{-1}$ of elements "B") analyzed randomly throughout the study, recoveries 91.9 – 102 % and RSD 2.3 – 4.5 % were obtained. The waste water treatment plant, sample was spiked with REE (final concentrations were 0.1 $\mu g.l^{-1}$ and 1 $\mu g.l^{-1}$ of elements "A" + 0.02 $\mu g.l^{-1}$ and 0.2 $\mu g.l^{-1}$ of elements "B"), decomposed in the microwave oven (n = 10) and recoveries were found to be 91.7 – 103 %, RSD were 2.9 – 7.9 %. The recoveries for the Gd standards for algae and biosorbents (1 and 10 $\mu g.l^{-1}$, n = 10) were 94.4 – 101 % and RSD 2.1 – 2.9 %, which were analyzed randomly throughout the study. The recovery for algae (cultivated only in the BBM and enriched with $Gd(NO_3)_3$, the final concentration 1, 2 and 10 $\mu g.l^{-1}$, n = 10) were 96.9 % (RSD 3.1%), 93.6 % (RSD 4.2 %) and 94.8 % (RSD 4.0 %). The quality

control material METRANAL® 8 was spiked with $Gd(NO_3)_3$ (2 and 10 $\mu g.l^{-1}$ [1]of Gd in the final digests; n = 10), Dotarem® and MultiHance® (10 $\mu g.l^{-1}$ [1]of Gd in the final digests; n = 4) before the mineralization step. The recoveries were obtained 93.8 – 101%, RSD 1.2 – 7.6 %. The certified value for Gd in the certified reference material BCR ®-670 Aquatic Plant was 0.098 ± 0.008 $mg.kg^{-1}$ which was in good agreement with the found value of 0.092 ± 0.008 $mg.kg^{-1}$ for the ICP-MS analysis for $^{158}Gd$. The achieved recoveries, repeatabilities and LODs endorsed further use of the method.

For the calibration standards of ICP-OES (10 $\mu g.l^{-1}$ and 20 $\mu g.l^{-1}$ of Gd) analyzed randomly throughout the study, recoveries 99.8 and 104 % and RSD 3.9 and 7.1 % were obtained. The recovery for algae (cultivated only in the BBM and enriched with $Gd(NO_3)_3$, the final concentration 10 and 20 $\mu g.l^{-1}$, n = 10) were 94.5 % (RSD 3.1%), 102 % (RSD 2.7 %). The quality control material METRANAL® 8 was spiked with $Gd(NO_3)_3$ (10 and 20 $\mu g.l^{-1}$ [1]of Gd in the final digests; n = 10), before the mineralization step. The recoveries were obtained 94.9 % and 92.6 %, RSD 3.9 % and 6.2 %. The ICP-OES analysis of BCR®-670 did not have a sufficient detection capability. The achieved recoveries, repeatabilities together with the limits of detection.

## 3.2 Rare earth elements in hospital and river waters

Natural concentrations of REE in the river environment are related to the geological composition of the bedrock and exhibit a typical concentration pattern (Weltje et al. 2002). Gd of anthropogenic origin deviates from this pattern. The amount of natural Gd can be estimated from the neighbouring elements, usually Sm and Tb, by interpolation (Bau et al. 2006), (Morteani et al. 2006), (Kulaksiz and Bau 2007). In the present study, various water samples were analyzed in order to estimate the ratio of natural and anthropogenic Gd. The REE concentrations found were normalized to the post-Archean Australian Shale (PAAS) geological standard (Piper and Bau 2013). A very simple equation was used for Gd anomalies ($Gd_{anom}$): $Gd_{anom} = Gd_{PAAS-total}/Gd_{PAAS-natural} = Gd_{PAAS-total}/(0.33Sm_{PAAS} + 0.67Tb_{PAAS})$, where $Gd_{PAAS-natural}$ is the normalized natural background Gd concentration estimated by interpolation between Sm and Tb, $Gd_{PAAS-total}$ is the normalized total concentration found in the samples, $Sm_{PAAS}$ and $Tb_{PAAS}$ are the normalized concentrations of the neighbouring elements used for interpolation. The ratio of $Gd_{PAAS-total}/Gd_{PAAS-natural}$ reveals the presence of the anthropogenic Gd but also depends on the natural background Gd concentration, the threshold for this ratio indicating the Gd anomaly is 1.5 (Bau et al. 2006). Table 1 presents the results for Gd. In addition to the determined concentrations, the content of natural and anthropogenic Gd, the percentage of natural Gd and Gd anomalies are calculated. This table shows the difference in anthropogenic Gd between waste water and river waters. The input of anthropogenic Gd is 0.856 – 1.510 $\mu g.l^{-1}$ for waste water and 0.0139 – 0.0270 $\mu g.l^{-1}$ for river waters. The content of REE in the samples normalized to the PAAS standard, are depicted in Figure 1 and shows differences in dependence for hospital wastes and river water.

For all samples analyzed, a Gd anomaly was found and the difference between hospital waste water and river water. The highest values were found for water collected directly in MR sewage pipes (126 and 195), slightly lower in waste water treatment plants (140 and 146), where MR waste water is mixed with other waste

water streams and the composition is also time dependent on operation MR. When compared to a threshold of 1.5, all river water samples showed a Gd anomaly. There are two hospitals in the East Bohemian region that have a workplace with MR (Hradec Králové and Pardubice). The highest ratio (3.37) was obtained for samples from the Elbe River in the Valy, which lies downstream of the city of Pardubice. The second highest value was found on the Elbe in Kunětice (3.14), located between Hradec Králové and Pardubice. Ratios from other sampling sites (on the river in front of hospitals) ranged from 2.28 to 3.10. The Man-Whitney U-test revealed a statistically significant difference between the ratios for river water sampled before and behind the hospitals on the flow (p-value 0.0058).

Positive Gd anomaly found in waters in the Berlin area was reported: Gd concentrations of 0.013 – 1.069 $ng.l^{-1}$ were found in river waters, 0.116 – 1.160 $ng.l^{-1}$ in surface waters, 1.410 $ng.l^{-1}$ in waste water (Knappe et al. 2005). Higher concentrations of Gd and Gd of anthropogenic origin will probably continue to increase in waters after treatment in WWTPs, as well as in surface waters, as currently operated WWTP technologies are unable to purify waste water from stable complexes of GdCA.

**Table 1:** Results of Gd analysis in waste water and river waters

| **Code** | **Sampling place** | Gd $\mu g.l^{-1}$ | $Gd_{antrop.}$ $\mu g.l^{-1}$ | $Gd_{natural}$ $\mu g.l^{-1}$ | $Gd_{natural}$ % | $Gd_{anomaly}$ |
|---|---|---|---|---|---|---|
| H | MR, January 2015 | 3.99 | 0.856 | 0.007 | 0.79 | 126 |
| H | MR, March 2015 | 7.04 | 1.51 | 0.008 | 0.51 | 195 |
| H | WWTP, January 2015 | 4.31 | 0.925 | 0.007 | 0.71 | 140 |
| H | WWTP, March 2015 | 4.06 | 0.871 | 0.006 | 0.68 | 146 |
| 1 | Seč dam | 0.065 | 0.014 | 0.006 | 43.6 | 2.28 |
| 2 | Chrudimka, Nemošice | 0.071 | 0.015 | 0.006 | 41.7 | 2.40 |
| 3 | Chrudimka, Pardubice | 0.120 | 0.026 | 0.008 | 30.4 | 3.27 |
| 4 | Confluence Elbe and Chrudimka, Pardubice | 0.094 | 0.020 | 0.007 | 32.4 | 3.10 |
| 5 | Loučná, Počáply | 0.091 | 0.020 | 0.008 | 39.9 | 2.51 |
| 6 | Orlice, Hradec Králové | 0.720 | 0.015 | 0.007 | 43.7 | 2.28 |
| 7 | Elbe, Předměřice | 0.080 | 0.017 | 0.008 | 45.6 | 2.19 |
| 8 | Elbe, Hradec Králové | 0.110 | 0.024 | 0.008 | 34.8 | 2.87 |
| 9 | Elbe, Kunětice | 0.105 | 0.023 | 0.007 | 31.8 | 3.14 |
| 10 | Elbe, Valy | 0.126 | 0.027 | 0.008 | 29.7 | 3.37 |

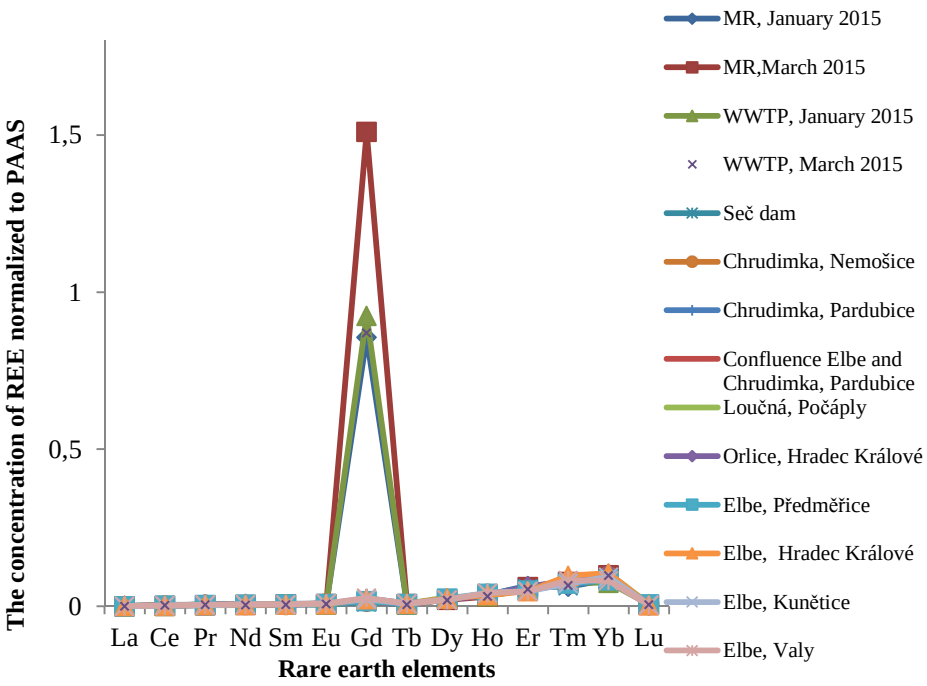


**Figure 1:** Content of REE in analyzed samples normalized to PAAS.

### 3.2.1 Rare earth elements in Matiční Lake

The lake sediment from Matiční Lake was analyzed together with water samples. Samples were taken by employees of University of Pardubice, Institute of Environmental and Chemical Engineering. There was one sampling from each specific location. The content of REE normalized to the PAAS standard (chapter 3.2) for samples from Matiční Lake is shown in Figure 2. In addition to the determined concentrations, the content of natural and anthropogenic Gd, the percentage of natural Gd and Gd anomalies are calculated. The Gd content of the lake sediment is 7.75 – 11.7 $\mu g.kg^{-1}$ and in the lake water 0.113 – 0.130 $\mu g.l^{-1}$, which correspond with analysis of water taken from the Chrudimka River near Matiční Lake as diseribed in the previous section of 0.120 $\mu g.l^{-1}$. The table shows the difference in Gd anomaly between lake sediment (2.1 – 2.6) and lake water (3.2 – 3.5). The Man-Whitney U-test revealed a statistically significant difference between the ratios for river water sampled before and behind on the flow the hospitals (p-value of 0.0222). Based on these different values, it can be assumed that the anthropogenic Gd is present in a soluble form in water rather than adsorbed on the sediment. According to the observations, the lake sediment is of a sandy nature and does not contain more sludge and organic matter, which could be adsorbed substances such as GdCA.

**Table 2:** Results of Gd analysis in lake sediment and lake water

| **Code** | Gd | $Gd_{antrop.}$ | $Gd_{natural}$ | $Gd_{natural}$ | Gd anomaly |
|---|---|---|---|---|---|
| sediment | | $\mu g.kg^{-1}$ | | % | |
| s1 | 11.7 | 2.52 | 0.973 | 38.6 | 2.59 |
| s2 | 10.6 | 2.27 | 0.935 | 41.2 | 2.43 |
| s3 | 9.26 | 1.99 | 0.835 | 42.0 | 2.38 |
| s4 | 7.75 | 1.66 | 0.813 | 48.9 | 2.05 |
| s5 | 8.52 | 1.83 | 0.767 | 41.9 | 2.38 |
| s6 | 9.75 | 2.09 | 0.964 | 46.1 | 2.17 |
| s7 | 8.81 | 1.89 | 0.885 | 46.8 | 2.13 |
| water | | $\mu g.l^{-1}$ | | % | |
| w1 | 0.121 | 0.026 | 0.008 | 31.6 | 3.16 |
| w3 | 0.116 | 0.025 | 0.007 | 28.3 | 3.53 |
| w6 | 0.130 | 0.028 | 0.008 | 29.6 | 3.38 |
| w7 | 0.113 | 0.024 | 0.008 | 31.5 | 3.18 |

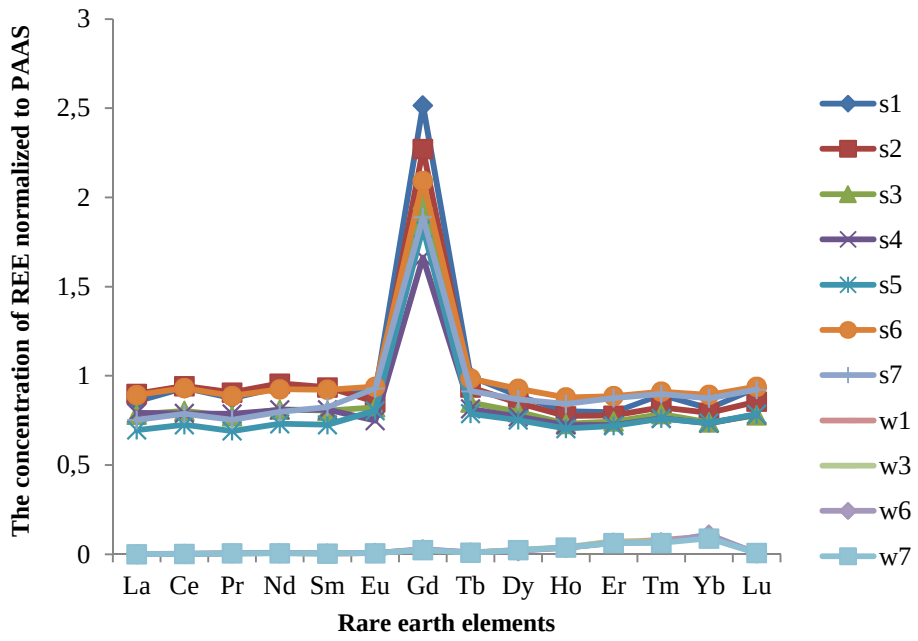


**Figure 2:** Content of REE in samples from Matiční Lake normalized to PAAS standard (s1 – s7 sediments, w1, 3, 6, 7 waters).

## 3.3 Bioaccumulation of gadolinium forms in *Chlorella kessleri* biomass

In the study, the cyclic ionic Dotarem® and linear MultiHance®, the most frequently applied and most stable Gd chelates, differing in thermodynamic stability constants (MultiHance® 22.6, Dotarem® 28.8, Morcos 2008), were used. $Gd(NO_3)_3$ represents an ionic form of Gd. The $Gd^{3+}$ and Gd agents differ significantly in their structures and a dissimilar affinity to adsorbents can be expected.

In biosorbent characterization or technological studies, metal concentrations ranged from milligrammes to grams per litre (Chojnacka 2010). In the case of bioconcentration studies, the initial concentration was 100 $\mu g.l^{-1}$ of Gd (Hao et al. 1996), (Hao et al. 1997), (Hao et al. 1998), (Yang et al. 1999). Our study aims to reflect the Gd concentration in hospital waste water (up to tens of $mg.l^{-1}$) and surface waters (below $\mu g.l^{-1}$) (Morteani et al. 2006), (Künnemeyer et al. 2009). Fresh algae were spiked with 2, 20 or 100 $\mu g.l^{-1}$of Gd.

The work was divided into two parts, which differed in the substance used, its concentration and the time of cultivation of algae. The aim of the first pilot phase was to determine whether the *Chlorella kessleri* algae were able to capture $Gd^{3+}$ ions from the solution. In a positive finding, thus detectable amount of Gd in biomass, it was possible to continue in the extended experiment. The experience gained in the pilot phase further resulted in a workflow adjustment. Evaluation of experiments was based on a bioconcentration factor (concentration of Gd in algae to concentration of Gd in medium: $BCF = c_{algae} / c_{medium}$) and on evaluation of sorption efficiency.

### 3.3.1 Pilot phase of bioaccumulation experiment

In the pilot phase of work algae were cultured in five replicates in BBM medium with the addition of 100 $\mu g.l^{-1}$ Gd in the form of $Gd(NO_3)_3$, next on in the waste water from the MR sewage piping and in the waste water from the WWTP discharge in the University Hospital in Hradec Králové.

The evaluations of the results were done with the known concentration of Gd in the medium at the beginning of the experiment and with the known content of the Gd in the biomass in the end of the experiment. The growth medium in the end of the experiment was not analysed. The results are summarized in Table 3.

Different BCF values (all in $l.kg^{-1}$) were found in algae cultivated in BBM medium with the addition with $Gd^{3+}$ of 100 $\mu g.l^{-1}$(1100), in the MR (2300) and WWTP waste water (4400). These differences can be influenced by significantly different Gd concentrations in the enriched BBM medium (100 $\mu g.l^{-1}$) and approximately 2 $\mu g.l^{-1}$ in real waste water. The cause may also be the presence of different chemical forms of Gd in the growth medium. In the case of enriched BBM medium, it is only $Gd(NO_3)_3$. The original unchanged forms of contrast agents are probably present in the waste water from MR, but the Gd species spectrum may be more varied on the effluent from the WWTP. At cleaning in WWTPs, waste water is subjected to physico-chemical and biotransformation processes that can lead to chemical changes in Gd species in waste water (Telgmann et al. 2013). The presence of unspecified Gd specimens including possible original contrast agents is assumed. Hao et al. studied the effect of REE chemical species on the bioconcentration capacity of *Chlorella vulgarize beijerinck* and noted a high dependence on chemical specimens where the presence of organic

ligands resulted in reduced algal deposition. The authors do not report a specific BCF (Hao et al. 1997).

From the decrease concentration of Gd during the experiment ($c_{medium\text{-}decrease}$ = ($c_{medium\text{-}before}$- $c_{medium\text{-}after}$) / $c_{medium\text{-}before}$ × 100), it is clear that the algae was able to remove 15 – 38 % of the present Gd regardless of its form (Table 3). From this pilot phase bioaccumulation experiment, the following conclusions can be made: (i) in the algae, such an amount of Gd was obtained that it can be reliably analyzed by ICP-OES, in a laboratory-simpler way than ICP-MS, (ii) measurable results were also obtained in algae the cultivated in waste water, (iii) the procedure for washing and centrifuging of algae after cultivation has been revised and shortened

**Table 3:** Analysis of Gd in algae in the pilot phase of the experiment

| Growth medium[a] | $c^{b}_{medium\text{-}before}$ $\mu g.l^{-1}$ | $c^{b}_{medium\text{-}after}$ $\mu g.l^{-1}$ | $c^{b}_{algae}$ $mg.kg^{-1}$ | BCF $l.kg^{-1}$ | $c^{c}_{medium\text{-}decreace}$ % |
|---|---|---|---|---|---|
| 1 BBM + Gd | 100 | 62.7 ±5,1 | 81.5 ±6,8 | 1210 | 37.3 |
| 2 BBM + Gd | 100 | 83.2 ±7,2 | 84.7 ±8,1 | 1020 | 16.8 |
| 3 BBM + Gd | 100 | 70.1 ±8,1 | 79.8 ±5,9 | 1140 | 29.9 |
| 4 BBM + Gd | 100 | 68.3 ±5,9 | 75.2 ±5,9 | 1100 | 31.7 |
| 5 BBM + Gd | 100 | 78.6 ±4,9 | 80.3 ±9,1 | 1020 | 21.4 |
| MR, January 2015 | 2.10 ±0,21 | 2.02 ±0,19 | 5.22 ±0,59 | 2580 | 38.1 |
| MR, March 2015 | 273 ±0,26 | 2.30 ±0,25 | 4.73 ±0,38 | 2060 | 15.8 |
| WWTP, January 2015 | 164 ±0,16 | 1.32 ±0,11 | 5.23 ±0,53 | 5130 | 19.5 |
| WWTP, March 2015 | 143 ±0,12 | 0.93 ±0,10 | 3.49 ±0,33 | 3750 | 35.0 |

a) algae were cultured in: (i) BBM medium with Gd of 100 $\mu g.l^{-1}$; (ii) waste water from MR workplaces; (iii) waste water from WWTP; b) Gd was determined in growth medium before and after the experiment in algae; c) $c_{medium\text{-}decreace}$ = ($c_{medium\text{-}before}$- $c_{medium\text{-}after}$) / $c_{medium\text{-}before}$ × 100.

### 3.3.2 Extended bioaccumulation experiment

After the successful pilot phase of the experiment, the experiment was expanded to include real gadolinium contrast media, additional concentration levels and shorter times. The algae suspension used for batch experiments represented about 0.12 g of dry biomass or about 4 x $10^6$ cells $ml^{-1}$. The algae was let to grow in the BBM medium with an addition of $Gd(NO_3)_3$, Dotarem®, MultiHance® (the concentration of Gd 100, 20, 2 $\mu g.l^{-1}$) for defined periods (0.5, 1, 2, 3, 7, 10, 14, 21 and 33 days). The final weight of the harvested algae was determined. The contact time varied in a relatively wide range from 30 min to 33 days to describe if and how much the algae was influenced by the presence of Gd compounds compared to the blank algae grown without any Gd chemicals. Over time, an increase of biomass about twice the weight used at the beginning of the experiment was noticed, as shown in Figure 3. The graphs show that there are not too large differences in the biomass amounts obtained for each substance and for their different concentrations. Compared to the blank biomass experiment, the algae growth is about 20 % lower. The effect of the different concentrations of Gd substances is not very significant and is probably hidden in the reproducibility of the biomass growth.

Furthermore, the intake of Gd substances by living *Chlorella kessleri* biomass was investigated. The ability and effectiveness of the living cells of *Chlorella kessleri* to remove the Gd nitrate and two Gd contrast agents from artificial water solution have been explored. The biomagnification process is influenced with more parameters especially the starting metal / substance concentration, algae dosage and contact time. The algae are a living, dynamic system which grows and dies during the contact time. The harvested biomass includes both living and dead *Chlorella* cells. As the biomass gradually grows, the metal captured on the cell surface or inside the cells bound is spread into a large amount of biomass and consequently diluted during the experiment.

In the Figure 4 (a), there are concentrations of Gd in the biomass grown in the $Gd(NO_3)_3$ and Gd-based contrast agents Dotarem® and MultiHance® media containing 100 $\mu g.l^{-1}$ of Gd in the beginning of the experiment. The concentration of 100 $\mu g.l^{-1}$ was chosen to find more reliably Gd in the algae in comparison with lower concentration levels. The concentration of Gd in the biomass may be considered as an uptake capacity.

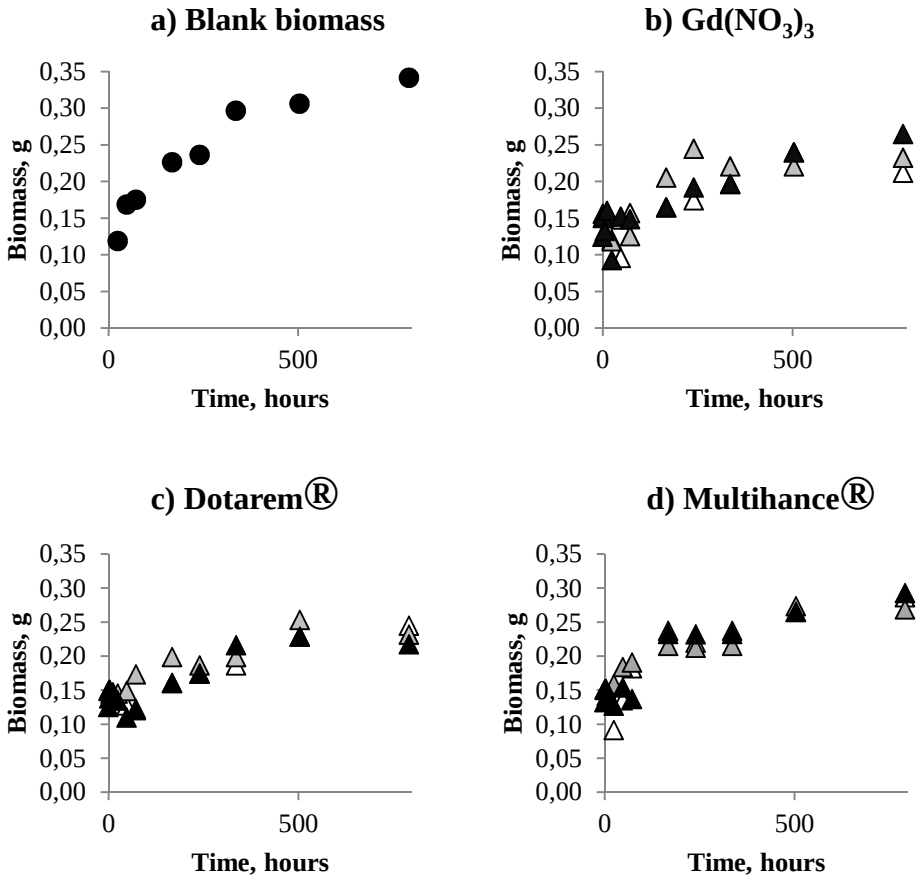


**Figure 3:** The *Chlorella kessleri* biomass growth in the presence of Gd compounds. The blank biomass growth (-●-), i.e. *Chlorella kessleri* cultivated in the pure BBM medium (a) is presented together with *Chlorella kessleri* cultured in the BBM medium with (b) $Gd(NO_3)_3$ and Gd-based contrast agents (c) Dotarem® and (d) MultiHance® in the concentrations of Gd 100 -▲-, 20 -◭-, 2 - △- $\mu g.l^{-1}$).

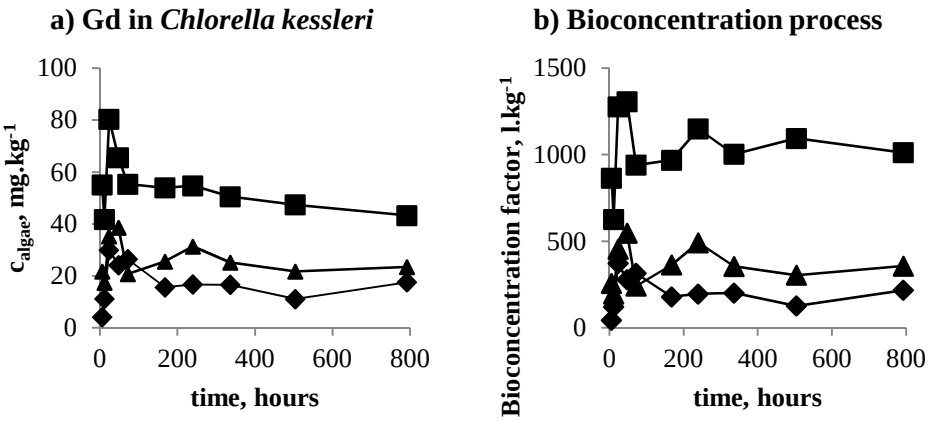


**Figure 4:** The uptake of Gd compound by living *Chlorella kessleri* biomass. The concentrations (a) of Gd in harvested *Chlorella kessleri* and the corresponding bioaccumulation factors (b) are presented for $Gd(NO_3)_3$ (-■-), Dotarem® (-♦-) and MultiHance® (-▲-) for the initial Gd concentration 100 µg.l$^{-1}$). The results are presented as the average values from three repeated algae biomass analysis from two growth experiments. The relative standard deviations varied in the range from 10 to 15 %.

The final shape of the curves is given by mechanisms that may be contradictory in results. Logically, the metal concentration in the solution decreases as the metal or their compound are removed from the solution and increases in the *Chlorella kessleri* biomass. As the biomass grows, the captured metal is "diluted" and the concentration in the biomass decreases. In other words, at the beginning, the uptake peak is observable and then the decrease as the algae biomass passes through the individual growth phases. Basically, it corresponds to the batch system, with varying amounts of stacked biomass.

As shown in Figure 4 a), we found the maximal concentration of Gd approximately after 24 – 48 h and reached (in mg.kg$^{-1}$) from 30 for Dotarem®, 35 for MultiHance® up to 80 for $Gd(NO_3)_3$. The concentration decreased in the end of the experiment to about half to one-third maximum value, i.e., (in mg.kg$^{-1}$) 11 for Dotarem®, 21 for MultiHance® and 43 for $Gd(NO_3)_3$. Hao et al. (1997) have studied the uptake of Gd, La and Y by *Chlorella vulgarize* within 2 days. They have found an increase in biomass concentration which has been around 5 mg.g$^{-1}$ and stable after 10 – 20 h. The same trend in the curves as for the concentrations was observed for the bioaccumulation factors as a function of time (see Figure 4 b). The maximal bioconcentration factors (the concentration in algae to the concentration in medium: $BCF = c_{algae} / c_{medium}$) were found about after 24 – 48 h and reached (in l.kg$^{-1}$) from 374 for Dotarem®, 454 for MultiHance® up to 1277 for $Gd(NO_3)_3$. Then, the values decreased at the end of the experiment to about half to one-thirdy maximum value, i. e. , (in l.kg$^{-1}$) 217 for Dotarem®, 354 for MultiHance ® and 1012 for $Gd(NO_3)_3$. The BCF values obtained for $Gd(NO_3)_3$ corresponded with those found in the work, 1000 – 1200 l.kg$^{-1}$ (Bendakovska et al. 2016).

For technological wastewater treatment purposes, a quick capture of metal on a sorbent and a rapid equilibrium achievement is very important. In our case, the maximal fixed Gd was reached approximately after 24 h. However, in terms of monitoring of the fate of substances in the environment and their possible transition entering to another trophic level in the food chain, long-term information is important.

In the case of *Chlorella kessleri*, Gd remained built in the algae even after a month and was not released back into the solution.

## 3.4 Removal of gadolinium substances using other sorbents

In addition to the capture of Gd substances on living *Chlorella kessleri* biomass, the effectiveness of their sorption on four other adsorbents inactivated *Chlorella kessleri* biomass, humic acids, activated carbon and lake sediment was used. The choice of sorbents and their content in the liquid phase reflects their presence in the aquatic environment or in technological applications. Compared to the experiment with living *Chlorella kessleri* biomass, the experimental time was shortened to 12 h due to the expected shorter course of sorption without the need to monitor the growth of algae biomass. The concentration of the Gd 100 $\mu g.l^{-1}$ was used to find more reliably Gd in the adsorbents in comparison with lower concentration levels.

In Figure 5 for each tested sorbent (a) dried *Chlorella kessleri* biomass, (b) humic acids, (c) activated carbon and (d) lake sediment, the adsorption effectiveness expressed in percentage of the adsorbed amount of the substance are displayed. Different sorption abilities of the tested sorbents were observed. The dry biomass *Chlorella kessleri* showed the highest adsorption effect depending on the adsorbed Gd substances from 30 (Dotarem®) to 91 % ($Gd(NO_3)_3$). The adsorption efficiency for activated carbon was found in the range from 65 % for Dotarem® to 93 % for $Gd(NO_3)_3$. Humic acid also showed the best capture of 89 % $Gd^{3+}$ ions from $Gd(NO_3)_3$ and the lowest for Dotarem® (39 %). The lake sediment adsorbed about 51 % of $Gd^{3+}$ ions and 21 % for Dotarem® and 18 % for MultiHance®. In the living *Chlorella kessleri* biomass, 42 % of $Gd^{3+}$ ions from $Gd(NO_3)_3$, 17 % of MultiHance® and 11 % of Dotarem® were captured after 12 h.

Significant differences in adsorbability of Gd compounds were revealed. The highest adsorption efficiency was recorded for $Gd(NO_3)_3$ from which $Gd^{3+}$ ion was captured on the sorbent surface by its functional groups. In the end of the 12 hours experiment, from 51 to 91 % of nitrate was adsorbed depending on the adsorbent used. The lowest adsorbed amount from 21 to 65 % was observed for Dotarem® that has a cyclic structure, a non-ionic character, and is the most stable of all three of the monitored substances. The adsorption efficiency from 18 to 69 % for MultiHance® with the linear structure was somewhere between $Gd(NO_3)_3$ and Dotarem® efficiencies. The results obtained for each of the above mentioned adsorbents together with those for living *Chlorella kessleri* algae in the end of the experiment after 12 hours are summarized in Table 4. Our results seem to be comparable with those of González et al. (Gonzalez et al. 2017). According to them, the adsorption of Gd-contrast agents on activated carbon depends on a method of carbon preparation but also on a composition of solution from which adsorbates are removed. They have found for Dotarem®, Magnevist® and Primovist® the adsorption effieciencies from 41 to 91 % in water solution and from 13 to 80 % for adsorption from an artificial urine.

The differences found in the results are related partly to the different chemical compositions of the individual Gd substances and by the presence of different functional groups on the surface of the adsorbents used in the study. In the case of the fresh algae, metabolic processes and growth phases can play a role.

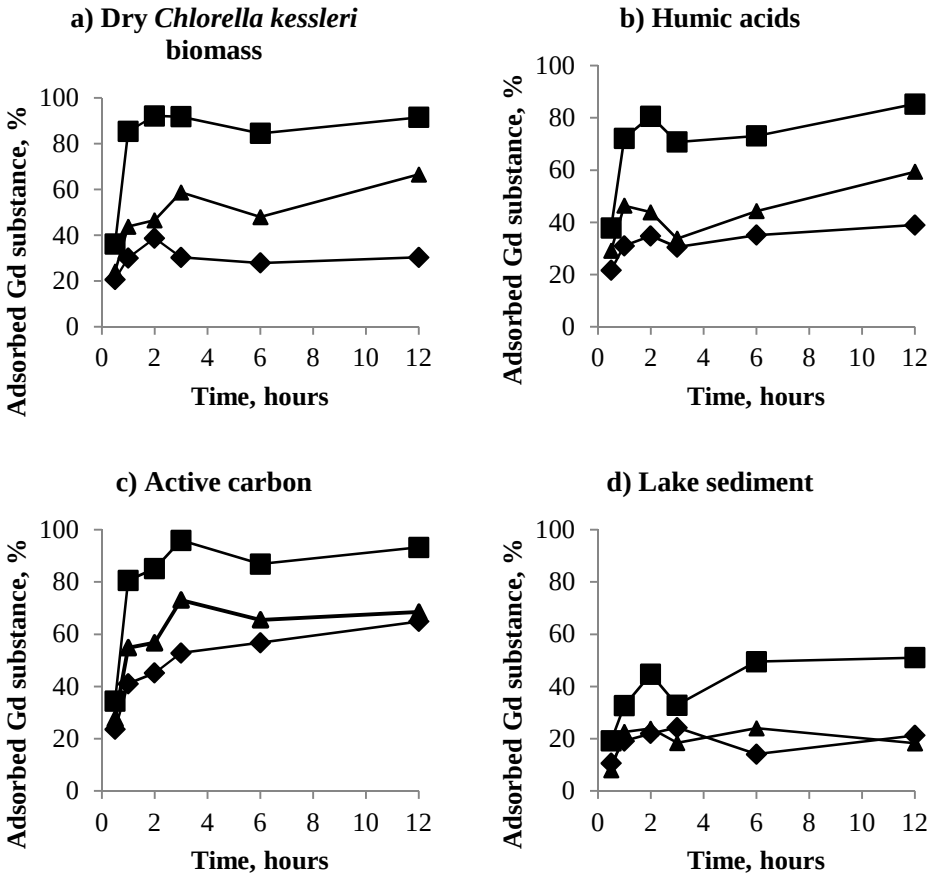


**Figure 5:** The absorption efficiency of Gd compounds on selected adsorbents.
The effectiveness of dry *Chlorella kessleri* biomass (a), humic acids (b), activated carbon (c) and (d) lake sediment in removing of $Gd(NO_3)_3$ (-■-), Dotarem® (-♦-) and MultiHance® (-▲-) for the initial Gd concentration 100 $\mu g.l^{-1}$) is presented. The results are presented as the average values from three repeated experiments. The relative standard deviations varied in the range from 15 to 20%.

**Table 4:** The absorption efficiency of Gd compounds using all adsorbents after 12 hours

| Adsorption efficiency, % | | | | | |
|---|---|---|---|---|---|
| | *Chlorella kessleri* biomass | | Biosorbents | | |
| | Living | Dry | Humic acid | Active carbon | Lake sediment |
| Nitrate | 42 | 91 | 85 | 93 | 51 |
| MultiHance® | 17 | 67 | 59 | 69 | 18 |
| Dotarem® | 11 | 30 | 39 | 65 | 21 |

The results are presented as the average values from three repeated experiments for the dry biomass, humic acids, active carbon and lake sediment. The living algae biomass analysis was repeated three times and analysed algae came from two growth experiments. The relative standard deviations varied in the range from 15 to 20%.

# 4. Conclusion

The GdCA were studied in the view of their distribution in the aquatic environment and their possible removal from the aquatic environment. The ICP-MS methods were developed for REEs in river and waste water. Applicability and validity of the ICP-MS and ICP-OES methods were evaluated based on LODs, LOQs, sample blanks, analysis of reference materials and the recovery study. The ICP-MS LOQs for REE in river waters (0.010 – 0.016) and in WWTP waste waters (0.026 – 0.040) enabled to monitor REEs in both types of samples (all in $\mu g.l^{-1}$). Throughout the study, the recoveries were found 91.7 – 103 % and RSD 2.3 – 7.9 %. Gd in the *Chlorella kessleri* algae and sorbents was analyzed by ICP-MS and ICP-OES method. The ICP-MS LOQs were found for fresh algae 0.090, for other sorbents 0.023 and the ICP-OES LOQ for fresh algae 8.7 (all in $mg.kg^{-1}$). Both methods were validated using of Gd enriched algae and quality control material METRANAL® 8; the recoveries were in the range 93.6 – 105 % for ICP-MS and 92.6 – 102 % for ICP-OES. The CRM BCR®-670 was used for ICP-MS (93.9 %). RSDs were found for both methods less than 10 % through the study. Due to the very good ICP-MS sensitivity, no preconcentration step was necessary. The ICP-OES analysis of Gd in algae due to its suitable detection ability and its lower analytical and time demands was could be preferred.

The REEs were determined in the waters of East Bohemia. The Gd anomaly was evaluated using the ratio of Gd of anthropogenic and natural origin. The anomaly was detected in all collected samples: river waters 2.19 – 3.40, 126 and 195 for MR waste water, 140 and 146 for WWTP effluent. Higher values of the ratio were found for the water taken downstream of the anthropogenic Gd source and lower before the anthropogenic Gd source. The anomaly was evaluated lower for the lake sediment (2.05 – 2.59) than for lake water (3.16 –3.53). Based on these different values, the anthropogenic Gd is probably present in dissolved form rather than adsorbed on the sediment.

The ability to capture GdCA (Dotarem®, MultiHance®) and control $Gd(NO_3)_3$ by fresh algae of *Chlorella kessleri* was studied for algae cultivated in the medium with Gd substances and in hospital waste waters. The BCFs (all in $l.kg^{-1}$) were found approximately 1100 for the medium with $Gd(NO_3)_3$, 220 for Dotarem®, 360 for MultiHance®, 2300 for algae cultured in the MR waste water and 4400 for the WWTP effluent. The increase in algal biomass in the presence $Gd(NO_3)_3$, Dotarem® and MultiHance® was evaluated and no significantly measurable differences were not found between Gd compounds. Compared to a blank experiment, the *Chlorella kessleri* biomass growth was lower with addition the presence of Gd compounds.

The efficiency of adsorption of Gd forms on selected adsorbents (dead biomass of *Chlorella kessleri*, active carbon, humic acids, river sediment) was assessed. The sorption efficiencies decreased in the order $Gd(NO_3)_3$ (42 – 93 %) > MultiHance® (17 – 69 %) > Dotarem® (11 – 65 %) and, in case of sorbents, actived carbon (65 – 93 %) > dry algae biomass (30 – 91 %) > humic acids (39 – 85 %) > lake sediment (18 – 51 %) > fresh algae biomass (11 – 42 %) resp., for the substance used. The differences found in the results are the consequence partly of the different chemical compositions of the individual Gd substances and by the presence of different functional groups on the surface of the adsorbents used in the study. In the case of the fresh algae, metabolic processes and growth phases can play a role.

## List of References


1. Ahalya, N., T. Ramachandra & R. Kanamadi (2003) Biosorption of heavy metals. *Res. J. Chem. Environ,* 7**,** 71-79.
2. Ahluwalia, S. S. & D. Goyal (2007) Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresource Technology,* 98**,** 2243-2257.
3. Andersen, R. A. (2005). *Algal culturing techniques*. Academic press.
4. Bau, M., A. Knappe & P. Dulski (2006) Anthropogenic gadolinium as a micropollutant in river waters in Pennsylvania and in Lake Erie, northeastern United States. *Chemie der erde-Geochemistry,* 66**,** 143-152.
5. Bendakovska, L., A. Krejcova, T. Cernohorsky & J. Zelenkova (2016) Development of ICP-MS and ICP-OES methods for determination of gadolinium in samples related to hospital waste water treatment. *Chemical Papers,* 70**,** 1155-1165.
6. Birka, M., C. A. Wehe, O. Hachmöller, M. Sperling & U. Karst (2016) Tracing gadolinium-based contrast agents from surface water to drinking water by means of speciation analysis. *Journal of Chromatography A,* 1440**,** 105-111.
7. Brünjes, R., A. Bichler, P. Hoehn, F. T. Lange, H.-J. Brauch & T. Hofmann (2016) Anthropogenic gadolinium as a transient tracer for investigating river bank filtration. *Science of The Total Environment,* 571**,** 1432-1440.
8. Cacheris, W. P., S. C. Quay & S. M. Rocklage (1990) The relationship between thermodynamics and the toxicity of gadolinium complexes. *Magnetic resonance imaging,* 8**,** 467-481.
9. Cho, D. Y., S. T. Lee, S. W. Park & A. S. Chung (1994) Studies on the biosorption of heavy metals onto Chlorella vulgaris. *Journal of Environmental Science & Health Part A,* 29**,** 389-409.
10. Chojnacka, K. (2010) Biosorption and bioaccumulation – the prospects for practical applications. *Environment International,* 36**,** 299-307.
11. Edris, G., Y. Alhamed & A. Alzahrani (2012) Cadmium and lead biosorption by Chlorella vulgaris. In *Sixteenth International Water Technology Conference, IWCT*.
12. Elizalde-González, M. P., E. García-Díaz, M. González-Perea & J. Mattusch (2017) Removal of gadolinium-based contrast agents: adsorption on activated carbon. *Environmental Science and Pollution Research***,** 1-12.
13. Ersoy, H. a. R. F. J. (2007) Biochemical safety profiles of gadolinium-based extracellular contrast agents and nephrogenic systemic fibrosis. *Journal of Magnetic Resonance Imaging,* 26**,** 1190--1197.
14. Ewa Bobrowska-Grzesik, J. C., Andrzej Grossman, Joanna Kluczka, Jolanta Trojanowska, Maria Zolotajkin. (2013) Chemical Elements Compendium. ISBN 978-80-86380-66-7.
15. Gadd, G. M. & L. Rome (1988) Biosorption of copper by fungal melanin. *Applied microbiology and biotechnology,* 29**,** 610-617.
16. Gonzalez, L., E. Cundari, L. Leyns & M. Kirsch-Volders (2017) Towards a New Paradigm in Nano-Genotoxicology: Facing Complexity of Nanomaterials' Cellular Interactions and Effects. *Basic & Clinical Pharmacology & Toxicology,* 121**,** 23-29.
17. Hao, S., W. Xiaorong, W. Liansheng, D. Lemei, L. Zhong & C. Yijun (1997) Bioconcentration of rare earth elements lanthanum, gadolinium and yttrium in algae

(Chlorella Vulgarize Beijerinck): Influence of chemical species. *Chemosphere,* 34**,** 1753-1760.
18. Hao, S., W. Xiaorong, W. Qin, W. Liansheng, C. Yijun, L. Zhong & C. Mi (1998) The species of spiked rare earth elements in sediment and potential bioavailability to algae (Chlorella Vulgarize Beijerinck). *Chemosphere,* 36**,** 329-337.
19. Hao, S., W. Xiaorong, H. Zhaozhe, W. Chonghua, W. Liansheng, D. Lemei, L. Zhong & C. Yijun (1996) Bioconcentration and elimination of five light rare earth elements in carp (Cyprinus carpio L.). *Chemosphere,* 33**,** 1475-1483.
20. Hatch, G. P. (2012) Dynamics in the Global Market for Rare Earths. *Elements,* 8**,** 341-346.
21. Jordens, A., Y. P. Cheng & K. E. Waters (2013) A review of the beneficiation of rare earth element bearing minerals. *Minerals Engineering,* 41**,** 97-114.
22. Kaduková, J. & E. Virčíková (2005) Comparison of differences between copper bioaccumulation and biosorption. *Environment International,* 31**,** 227-232.
23. Kanazawa, Y. & M. Kamitani (2006) Rare earth minerals and resources in the world. *Journal of alloys and compounds,* 408**,** 1339-1343.
24. Knappe, A., P. Moller, P. Dulski & A. Pekdeger (2005) Positive gadolinium anomaly in surface water and ground water of the urban area Berlin, Germany. *Chemie Der Erde-Geochemistry,* 65**,** 167-189.
25. Krejčová, A., T. Černohorský & M. Pouzar (2012) O-TOF-ICP-MS analysis of rare earth elements, noble elements, uranium and thorium in river-relating species. *International journal of environmental analytical chemistry,* 92**,** 620-635.
26. Kulaksiz, S. & M. Bau (2007) Contrasting behaviour of anthropogenic gadolinium and natural rare earth elements in estuaries and the gadolinium input into the North Sea. *Earth and Planetary Science Letters,* 260**,** 361-371.
27. Künnemeyer, J., L. Terborg, B. r. Meermann, C. Brauckmann, I. Möller, A. Scheffer & U. Karst (2009) Speciation analysis of gadolinium chelates in hospital effluents and wastewater treatment plant sewage by a novel HILIC/ICP-MS method. *Environmental science & technology,* 43**,** 2884-2890.
28. Lawrence, M. G., J. Keller & Y. Poussade (2010) Removal of magnetic resonance imaging contrast agents through advanced water treatment plants. *Water Science and Technology,* 61**,** 685-692.
29. Li, R., Z. Ji, C. H. Chang, D. R. Dunphy, X. Cai, H. Meng, H. Zhang, B. Sun, X. Wang, J. Dong, S. Lin, M. Wang, Y.-P. Liao, C. J. Brinker, A. Nel & T. Xia (2014) Surface Interactions with Compartmentalized Cellular Phosphates Explain Rare Earth Oxide Nanoparticle Hazard and Provide Opportunities for Safer Design. *ACS Nano,* 8**,** 1771-1783.
30. Liang, T., S. Zhang, L. Wang, H.-T. Kung, Y. Wang, A. Hu & S. Ding (2005) Environmental biogeochemical behaviors of rare earth elements in soil–plant systems. *Environmental Geochemistry and Health,* 27**,** 301-311.
31. Lindner, U., J. Lingott, S. Richter, N. Jakubowski & U. Panne (2013) Speciation of gadolinium in surface water samples and plants by hydrophilic interaction chromatography hyphenated with inductively coupled plasma mass spectrometry. *Analytical and bioanalytical chemistry,* 405**,** 1865-1873.
32. Lindner, U., J. Lingott, S. Richter, W. Jiang, N. Jakubowski & U. Panne (2015) Analysis of Gadolinium-based contrast agents in tap water with a new hydrophilic

interaction chromatography (ZIC-cHILIC) hyphenated with inductively coupled plasma mass spectrometry. *Analytical and bioanalytical chemistry,* 407**,** 2415-2422.
33. Mancheri, N., L. Sundaresan & S. Chandrashekar (2013) Dominating the World China and the Rare Earth Industry. *National Institute of Advanced Studies***,** 1-74.
34. Mattuschka, B. & G. Straube (1993) Biosorption of metals by a waste biomass. *Journal of Chemical Technology and Biotechnology,* 58**,** 57-63.
35. Morcos, S. K. (2008) Extracellular gadolinium contrast agents: Differences in stability. *European Journal of Radiology,* 66**,** 175-179.
36. Morteani, G., P. Möller, A. Fuganti & T. Paces (2006) Input and fate of anthropogenic estrogens and gadolinium in surface water and sewage plants in the hydrological basin of Prague (Czech Republic). *Environmental geochemistry and health,* 28**,** 257-264.
37. Piper, D. Z. & M. Bau (2013) Normalized rare earth elements in water, sediments, and wine: identifying sources and environmental redox conditions. *American Journal of Analytical Chemistry,* 4**,** 69.
38. Posten, C. & S. F. Chen (2016) Microalgae Biotechnology. Springer.
39. Ramalho, J., R. Semelka, M. Ramalho, R. Nunes, M. AlObaidy & M. Castillo (2016) Gadolinium-based contrast agent accumulation and toxicity: an update. *American Journal of Neuroradiology,* 37**,** 1192-1198.
40. Rogosnitzky, M. & S. Branch (2016) Gadolinium-based contrast agent toxicity: a review of known and proposed mechanisms. *Biometals,* 29**,** 365-376.
41. Sherry, A. D., P. Caravan & R. E. Lenkinski (2009) Primer on gadolinium chemistry. *Journal of Magnetic Resonance Imaging,* 30**,** 1240-1248.
42. Telgmann, L., M. Sperling & U. Karst (2013) Determination of gadolinium-based MRI contrast agents in biological and environmental samples: A review. *Analytica Chimica Acta,* 764**,** 1-16.
43. Toda, K. (2006) Recent research and development of VUV phosphors for a mercury-free lamp. *Journal of alloys and compounds,* 408**,** 665-668.
44. Velásquez, L. & J. Dussan (2009) Biosorption and bioaccumulation of heavy metals on dead and living biomass of Bacillus sphaericus. *Journal of Hazardous Materials,* 167**,** 713-716.
45. Vijayaraghavan, K. & Y.-S. Yun (2008) Bacterial biosorbents and biosorption. *Biotechnology advances,* 26**,** 266-291.
46. Volesky, B., H. May & Z. Holan (1993) Cadmium biosorption by Saccharomyces cerevisiae. *Biotechnology and Bioengineering,* 41**,** 826-829.
47. Volesky, B., J. Weber & J. Park (2003) Continuous-flow metal biosorption in a regenerable Sargassum column. *Water Research,* 37**,** 297-306.
48. Weltje, L., H. Heidenreich, W. Zhu, H. T. Wolterbeek, S. Korhammer, J. J. de Goeij & B. Markert (2002) Lanthanide concentrations in freshwater plants and molluscs, related to those in surface water, pore water and sediment. A case study in The Netherlands. *Science of the total environment,* 286**,** 191-214.
49. Yang, X., D. Yin, H. Sun, X. Wang, L. Dai, Y. Chen & M. Cao (1999) Distribution and bioavailability of rare earth elements in aquatic microcosm. *Chemosphere,* 39**,** 2443-2450.
50. Zawisza, B., K. Pytlakowska, B. Feist, M. Polowniak, A. Kita & R. Sitko (2011) Determination of rare earth elements by spectroscopic techniques: a review. *Journal of Analytical Atomic Spectrometry,* 26**,** 2373-2390.

## List of Students' Published Works

### Articles dealing with the topic of Ph.D. thesis published in journals with IF

1. BENDAKOVSKÁ, Lenka, KREJČOVÁ, Anna, ČERNOHORSKÝ, Tomáš, ZELENKOVÁ, Jana, Development of Method for Determination of Gadolinium in Samples Related to Hospital Wastewater Treatment, *Chemical Papers*, 2016, 70, 1155-1165.
2. BENDAKOVSKÁ, Lenka, KREJČOVÁ, Anna, WEIDLICH, Tomáš, Sorption and biosorption of Gd-based contrast agents in the water environment, *Chemical Papers*, 2019, 1-9.

### Other articles of the author published in journals with IF

1. BENDAKOVSKÁ, Lenka, KREJČOVÁ, Anna, ČERNOHORSKÝ, Tomáš, ZVONÍČKOVÁ, Kateřina, ICP-oTOF-MS analysis of platinum in algae and hospital wastewater, *Chem. Listy*, 2014, 108, 154-159.
2. KREJČOVÁ, Anna, ČERNOHORSKÝ, Tomáš, BENDAKOVSKÁ, Lenka, A practical approach to non-spectral interferences elimination in inductively couple plasma optical emission spektrometry, *Chemical Papers*, 2016, 70, 669-684.
3. PATOČKA, Jan, BENDAKOVSKÁ, Lenka, KREJČOVÁ, Anna, ČERNOHORSKÝ, Tomáš, RESANO, Martín, BĚLINA, Petr, Thallium in spruce needles: a comparison of the analytical capabilities of spectrochemical methods, *Analytical Methods*, 2017, 9, 705-715.

### Other articles of the author published in journals without IF

1. MOULISOVÁ, Alena, BENDAKOVSKÁ, Lenka, KOŽÍŠEK, František, VAVROUŠ, Adam, JELIGOVÁ, Hana, KOTAL, Filip, Pesticidy a jejich metabolity v pitné vodě: jaký je současný stav v České republice? *Vodní hospodářství*, 2018, 68(7): 4-10.