BIOAVAILABILITY OF LANTHANIDES TO FRESHWATER ORGANISMS

SPECIATION, ACCUMULATION AND TOXICITY
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BIOAVAILABILITY OF LANTHANIDES TO FRESHWATER ORGANISMS

SPECIATION, ACCUMULATION AND TOXICITY

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Calvin: Why do you suppose we’re here?
Hobbes: Because we walked here.
Calvin: No, no ... I mean here on earth.
Hobbes: Because earth can support life.
Calvin: No, I mean why are we anywhere? Why do we exist?
Hobbes: Because we were born.
Calvin: Forget it.
Hobbes: I will, thank you.

BILL WATTESON (1990) WEIRDOS FROM ANOTHER PLANET!

One fine day the chaos subsides
Bleeds into awareness and a lifetime of surprise
The beauty of your mother’s eyes, the pain when you fall
You drink it in and marvel at it all, but you never really figure it out

STEVE HOGARTH (1994) BRAVE

voor Janjje & Henk
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SUMMARY

BIOAVAILABILITY OF LANTHANIDES TO FRESHWATER ORGANISMS
SPECIATION, ACCUMULATION AND TOXICITY

Lennart Weltje

The lanthanides consist of a group of fifteen homologous metals and together with scandium (Sc) and yttrium (Y) they are known as the rare earth elements (REE). Contrasting to what this name suggests they are not rare at all and lanthanides can be found in most soils and sediments in quantities comparable to those of Pb and Cd. However, their soluble concentrations are strongly limited by the low solubility products of lanthanides with carbonate and phosphate. The increasing industrial uses of lanthanides are accompanied by increasing emissions, which mainly end up in the aquatic environment. Therefore, it should be investigated if these emissions cause adverse effects on aquatic biota. This is the background of the research described here.

Since the low solubility and high reactivity of trivalent lanthanides constrain the solution composition and set-up of experiments, it was decided to pay extra attention to solution chemistry and the possible losses of lanthanides due to adsorption. Solution chemistry was monitored mainly by means of speciation calculations (chapters 4, 5, 6 and 7), and to this end stability constants of lanthanide complexes were collected from the literature or estimated from available data (appendix I). Since pH is a dominant factor in metal speciation, its role was evaluated (chapters 4, 6 and 7), and its value buffered (chapters 4 and 5) and/or measured (chapters 3, 4, 5, 6 and 7). By using the lanthanum radiotrace, $^{149}$La, adsorption of lanthanum was demonstrated to membrane filters (chapter 4) and to glass vessels (chapter 5). In both cases the LaEDTA complex contributed to the sorbed amount, possibly next to the free La$^{3+}$ ion. Adsorption to glass caused a significant reduction in lanthanum solution concentrations; yet by incorporating this process into a mathematical model for lanthanum uptake and elimination by exponentially growing duckweed (Lemna minor), experimental data were explained satisfactorily.

The measurements on L. minor (chapter 5), but also data from the literature (chapter 2) and measurements on field-collected plants (chapter 3) show high bioconcentration factors (BCF) of lanthanides in plants, i.e. between 1,000 and 1,000,000 L kg$^{-1}$ dry wt. BCFs of heavier lanthanides tend to be lower than those of lighter lanthanides. Although water and sediment concentrations from various locations were not significantly different, a variation of over two orders of magnitude was observed in the sediment-rooting plant Potamogeton pectinatus (chapter 3). This variation was not present in L. minor, which has no contact with sediment and is genetically more homogeneous. Contact with the sediment and genetic variation appear to be responsible for the large variation in P. pectinatus. Biomagnification of lanthanides from plants to snails in the field is low, with maximum values of 5.5 kg kg$^{-1}$ dry wt (chapter 3).
Toxicity studies with lutetium and the luminescent bacterium *Vibrio fischeri* were aimed at testing if lanthanides comply with the free-ion model (chapter 6). This was indeed the case, since total dissolved lutetium concentrations, including organically complexed lutetium, could not explain the observed toxicity, while the calculated free Lu$^{3+}$ ion concentrations could. The relationship between chemical characteristics and toxicity to *V. fischeri* for eleven lanthanides was studied in chapter 7. It was shown that heavier lanthanides are more toxic than lighter ones, up to two orders of magnitude. The increase in toxicity is a smooth function of atomic number. Lanthanide toxicity is comparable to those of well-known heavy metals Cd, Cu, Pb and Zn. Because concentrations of free lanthanide ions in natural waters are low, adverse effects on biota are expected under extreme conditions only.
SAMENVATTING

BIOBESCHIKBAARHEID VAN LANTHANIDEN VOOR ZOETWATERORGANISME

SPECIATIE, ACCUMULATIE EN TOXICITEIT

LENNART WELTZJE

De lanthaniden (atomenummers 57 tot en met 71) bestaan uit een groep van vijftien sterk ver-
wante metalen. Samen met scandium (Sc) en yttrium (Y) staan ze bekend onder de naam
zeldzame aardmetalen. In tegenstelling tot wat deze naam doet vermoeden zijn lanthaniden ech-
ter niet zeldzaam en ze zijn dan ook aan te treffen in de meeste bodems en sedimenten in
hoeveelheden vergelijkbaar met die van lood en cadmium. De concentraties van lanthaniden in
water zijn echter zeer laag, doordat ze erg slecht oplossen in de aanwezigheid van fosfaat en car-
bonaat, en die anionen zijn in de meeste wateren in relatief hoge concentraties aanwezig. De in-
dustriële toepassingen van lanthaniden zijn de laatste jaren sterk toegenomen en daardoor ook de
uitstoot van lanthaniden naar het milieu. Het merendeel van deze emissies komt direct of indirect
in zoetwater terecht. Het is niet bekend of deze emissies negatieve effecten hebben op zoet-
waterorganismen. Het in dit proefschrift beschreven onderzoek richt zich op het beantwoorden
van deze vraag.

De slechte oplosbaarheid van de lanthaniden en het feit dat de opgeloste driewaardige lan-
thanide-ioni gemakkelijk reageren, maakt het experimenteren met deze metalen in waterige
oplossingen niet eenvoudig. Extra aandacht is daarom geschonken aan de chemische samenstel-
ling van de oplossingen en aan de mogelijke afname van de lanthanideconcentratie in de oplossin-
g door adsorptieverschijnselen. De chemische samenstelling van de oplossingen werd
voorafgaand aan de experimenten doorgerekend met behulp van een speciatieprogramma om te
voorkomen dat de oplosbaarheid van de lanthaniden in de experimenten werd overschreden
(hoofdstukken 4, 5, 6 en 7). Voor een succesvolle uitvoering van deze berekeningen zijn de
evenwichtsconstanten nodig van de chemische reacties die de lanthaniden kunnen aangaan in
water. De benodigde constanten werden verzameld uit de literatuur of geschat aan de hand van
bekende waarden voor andere reacties (appendix I). Aan de hand van de speciatieberekeningen
kon de samenstelling van de oplossing aangepast worden voordat het experiment werd uit-
gevoerd. De rol die de pH speelt in al deze reacties werd Æéevaluerd in de hoofdstukken 4, 6 en
7. pH metingen werden gedaan in alle experimentele hoofdstukken (3, 4, 5, 6 en 7) en een pH
buffer werd gebruikt om grote zwakkingen in de zuurgraad te voorkomen (hoofdstukken 4 en 5).
Door gebruik te maken van radioactief lanthaan (het γ-straling uitzendende radioisootop 149La)
kon aangetoond worden dat lanthaan adsorbeert aan 0,22 μm membraan filters, die gebruikt
worden om oplossingen te steriliseren (hoofdstuk 4) en ook aan de glazen bakjes die gebruikt
werden in experimenten met eendekroos (hoofdstuk 5). In beide gevallen levert het LaEDTA
complex een bijdrage aan de hoeveelheid geadsorbeerde lanthaan, waarschijnlijk naast het vrije
SAMENVATTING

La$^{3+}$-ion. De adsorptie van lanthaan aan de glaswand zorgde voor een significante afname van lanthaan in de oplossing. Door deze afname mee te modeleren kon de opname en uitscheiding van lanthaan door exponentieel groeiend klein kroos (*Lemna minor*) bevredigend verklaard worden.

De metingen aan kroos (hoofdstuk 5), maar ook gegevens uit de literatuur (hoofdstuk 2) en metingen aan planten uit het vrije veld (hoofdstuk 3) laten zien dat lanthaniden sterk door waterplanten worden geaccumuleerd. De verhouding tussen de lanthanidenconcentraties in de planten en die in het water, de zogenoemde bioconcentratiefactor (BCF), loopt uiteen van 1.000 tot 1.000.000 L·kg$^{-1}$ drooggewicht. Daarbij hebben de zwaardere lanthaniden meestal iets lagere BCF waarden dan de lichtere lanthaniden. De metingen aan lanthaniden in oppervlaktewater, sediment en poriewater op vijf locaties in Nederland toonden geen significante verschillen aan tussen de locaties. De lanthanidenconcentraties in schedefonteinkruid (*Podarcus pectinatus*), een plant die in sediment wortelt, varieerden echter meer dan twee ondergroottes tussen de locaties (hoofdstuk 3). Deze verschillen werden niet gevonden voor klein kroos (*L. minor*), dat door zijn drijvende bestaan geen contact heeft met het sediment en bovendien bestaat uit genetisch zeer homogene populaties. Deze twee aspecten lijken verantwoordelijk te zijn voor de gevonden verschillen tussen deze plantensoorten. Biomagnificatie, het doorgeven van stoffen in de voedselketen, van lanthaniden in waterplanten aan zoetwaterslakken in het vrije veld, blijkt met een maximumfactor van 5,5 kg·kg$^{-1}$ drooggewicht niet groot (hoofdstuk 3).

Toxiciteitsexperimenten met luminescerende bacteriën (*Vibrio fischeri*) en lutetium waren er op gericht te testen of de lanthaniden in dit systeem aan het vrije-ionmodel voldoen (hoofdstuk 6). Volgens het vrije-ionmodel wordt de opname en giftheid van metalen bepaald door hun vrije ionen in de oplossing; in het geval van lanthaniden dus het La$^{3+}$-ion. Inderdaad bleek dat het vrije Lu$^{3+}$-ion de toxiciteit goed kon verklaren, in tegenstelling tot het totaal aan opgelost lutetium, waaronder ook complexen van lutetium met organische liganden. De relatie tussen de chemische eigenschappen en toxiciteit van elf lanthaniden voor *V. fischeri* werd onderzocht in hoofdstuk 7. Aangetoond werd dat lanthaniden met een hoog atoomnummer giftiger zijn dan lanthaniden met een laag atoomnummer en dat de toename in toxiciteit een functie is van het atoomnummer. Het verschil tussen het minst en het meest giftige lanthanide, respectievelijk lanthaan en lutetium, bedroeg maximaal twee ondergroottes. De giftheid van lanthaniden voor *V. fischeri* is vergelijkbaar met die van de bekende ‘zware metalen’ cadmium, koper, lood en zink. Omdat de vrije-ionconcentraties van lanthaniden in natuurlijke wateren over het algemeen laag zijn, worden negatieve effecten op organismen slechts verwacht onder extreme omstandigheden, zoals in water met lage concentraties aan opgelost organisch materiaal, een lage pH en weinig fosfaat en carbonaat.
ZUSAMMENFASSUNG

BIOVERFÜGBARKEIT VON LANTHANOIDEN FÜR SÜßWASSERORGANISMEN
SPEZIATION, AKKUMULATION UND TOXIZITÄT

LENNART WELTJE


Messungen an Wasserlässen (Kapitel 5), aber auch Daten aus der Literatur (Kapitel 2) und Messungen an Pflanzen aus dem Freiland (Kapitel 3) zeigten, dass Lanthanoide in großem Umfang von Wasserpflanzen akkumuliert werden. Das Verhältnis von Lanthanoidekonzentrationen in Pflanzen und im Wasser, der sogenannte Bioakkumulationsfaktor (BCF), liegt zwischen 1.000 und 1.000.000 Lkg$^{-1}$ Trockengewicht, wobei die schwereren Lanthanoide zumeist etwas niedrigere BCF-Werte aufweisen als die leichteren Lanthanoide. Messungen von Lanthanoide in Oberflächengewässer, Sediment und Porenwasser an fünf Probenahmestellen in den Niederlanden ergaben keine signifikanten Unterschiede zwischen den untersuchten Stellen. Die Lanthanoidekonzentrationen in Kamm-Laichkraut (*Potamogeton perennis*), einer sedimentwurzelnden Pflanze, varierten jedoch über mehr als zwei Größenordnungen zwischen den Probenahmestellen (Kapitel 3). Solche Unterschiede fanden sich nicht für die kleine Wasserlinse (*L. minor*), welche durch ihre freischwimmende Lebensweise nicht in Kontakt mit dem Sediment steht und zudem aus genetisch äußerst homogenen Populationen besteht. Diese beiden Aspekte scheinen verantwortlich für die festgestellten Unterschiede zwischen den beiden Pflanzenarten zu sein. Die Biomagnifikation, d.h. die Weitergabe von Stoffen in der Nahrungskette, von Lanthanoide aus Wasserpflanzen in Süßwasserschnecken im Freiland war mit einem maximalen Faktor von 5,5 kgkg$^{-1}$ Trockengewicht nicht besonders hoch (Kapitel 3).

CHAPTER 1

GENERAL INTRODUCTION
CHAPTER I

This chapter introduces the substances, which are the focus of this thesis: the lantha-
nide elements. Aspects of their chemistry and occurrence in freshwater systems are
described, as well as their interactions with plants and invertebrates. These interac-
tions, i.e. uptake and toxicity, are controlled by availability and form the heart of the
research described in this work.

The current research project was started, because large amounts of lanthanides
are brought into the environment by man’s activity, while their fate and impact on
ecosystems is largely unknown. This implies that here the lanthanides are studied
from an ecotoxicological point of view. After introducing the lanthanides and de-
scribing their environmental fate in freshwater ecosystems, analytical aspects are
briefly discussed and the scope of this thesis is given.
1.1 The lanthanides

1.1.1 General

Two groups of elements stand aside from – or rather below – the Periodic System as Mendeleev originally designed it (see Fig. 1-1). These are the lanthanides (Ln) and the actinides (An). From these two groups of metals, the actinides are relatively well-known, because they contain the nuclear fuel elements uranium (U, atomic number Z = 92) and plutonium (Pu, Z = 94). The lanthanide elements are not so well known, as suggested by the origin of their name, the Greek word lanthanin, which means ‘hidden’ (Choppin, 1989). However, anyone who watches television or uses a cigarette lighter has most likely had an encounter with a member of the lanthanide series, since they are used in the manufacturing of television screens and flints, respectively. More recently, it was shown that the brand new five Euro banknote has a built-in luminescent lanthanide complex for protective reasons (Suyver and Meijerink, 2002). So it might seem that the lanthanides are hidden, but in fact, they are widespread and commonly used in our present-day society.

<table>
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<td>178</td>
<td>A e</td>
<td>179</td>
<td>Rf</td>
<td>177</td>
<td>Db</td>
<td>178</td>
<td>Sg</td>
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</tbody>
</table>

Fig. 1-1. The Periodic Table with in group 3, in bold, the lanthanides and in italics elements without a stable isotope

1.1.2 Electronic configurations and elemental relationships

Figure 1-1 presents the Periodic Table with in group 3, in bold, the lanthanides. The series starts with lanthanum (La, Z = 57) and ends with lutetium (Lu, Z = 71). As the Periodic Table is designed according to the physical structure of atoms, i.e. their electron configuration, there must be something odd with the lanthanides (and the actinides) to place them outside the Table. Indeed, in Mendeleev’s Periodic Table there was no place for them, because they did not follow the
general rule of adding electrons to outer shells with increasing nuclear charge. Instead, electrons are added to the inner 4f shell (see Table 1-1 for electronic configurations). Because these inner electrons are well shielded, they are virtually not involved in chemical bonding. This, and the fact that all lanthanides commonly favour the (III) oxidation state, results in great similarity among these elements (Schijf, 1992; Seaborg, 1993; Henderson, 1996). In fact, they behave so similarly that their separation was a long-standing challenge in analytical chemistry, until the last half century, when improved ion-exchange and solvent extraction techniques became available (Topp, 1965; Cotton, 1991; Choppin, 1993). The success of separation, i.e. the purity of the isolated lanthanides, was in the beginning primarily determined by instrumental neutron activation analysis (INAA).

Table 1-1. Chemical characteristics of the lanthanides. Electronic configuration is given for the neutral element. $R = \text{ionic radius, } CN = \text{coordination number}$

<table>
<thead>
<tr>
<th>symbol</th>
<th>element</th>
<th>$Z$</th>
<th>electronic configuration</th>
<th>$R_{\text{Ln}^{3+}}$ (Å)</th>
<th>earth crust concentration (mol/kg $^{3+}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>La</td>
<td>lanthanum</td>
<td>57</td>
<td>(Xe)6s5d$^1$</td>
<td>1.045</td>
<td>2.81·10$^4$</td>
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<tr>
<td>Ce</td>
<td>cerium</td>
<td>58</td>
<td>(Xe)6s$^2$5f$^1$</td>
<td>1.010</td>
<td>4.74·10$^4$</td>
</tr>
<tr>
<td>Pr</td>
<td>praseodymium</td>
<td>59</td>
<td>(Xe)6s$^2$5f$^2$</td>
<td>0.997</td>
<td>6.53·10$^3$</td>
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<td>Nd</td>
<td>neodymium</td>
<td>60</td>
<td>(Xe)6s$^2$5f$^3$</td>
<td>0.983</td>
<td>2.88·10$^4$</td>
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<td>Pm</td>
<td>promethium</td>
<td>61</td>
<td>(Xe)6s$^2$5f$^4$</td>
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<td></td>
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<td>Sm</td>
<td>samarium</td>
<td>62</td>
<td>(Xe)6s$^2$5f$^5$</td>
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<td>1.32·10$^3$</td>
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<tr>
<td>Gd</td>
<td>gadolinium</td>
<td>64</td>
<td>(Xe)6s$^2$5f$^7$</td>
<td>0.938</td>
<td>3.94·10$^3$</td>
</tr>
<tr>
<td>Tb</td>
<td>terbium</td>
<td>65</td>
<td>(Xe)6s$^2$5f$^8$</td>
<td>0.923</td>
<td>7.55·10$^3$</td>
</tr>
<tr>
<td>Dy</td>
<td>dysprosium</td>
<td>66</td>
<td>(Xe)6s$^2$5f$^9$</td>
<td>0.912</td>
<td>3.20·10$^3$</td>
</tr>
<tr>
<td>Ho</td>
<td>holmium</td>
<td>67</td>
<td>(Xe)6s$^2$5f$^{10}$</td>
<td>0.901</td>
<td>7.89·10$^3$</td>
</tr>
<tr>
<td>Er</td>
<td>erbium</td>
<td>68</td>
<td>(Xe)6s$^2$5f$^{11}$</td>
<td>0.890</td>
<td>2.09·10$^3$</td>
</tr>
<tr>
<td>Tm</td>
<td>thulium</td>
<td>69</td>
<td>(Xe)6s$^2$5f$^{12}$</td>
<td>0.880</td>
<td>3.08·10$^3$</td>
</tr>
<tr>
<td>Yb</td>
<td>ytterbium</td>
<td>70</td>
<td>(Xe)6s$^2$5f$^{13}$</td>
<td>0.868</td>
<td>1.85·10$^3$</td>
</tr>
<tr>
<td>Lu</td>
<td>lutetium</td>
<td>71</td>
<td>(Xe)6s$^2$5f$^{14}$</td>
<td>0.861</td>
<td>4.57·10$^4$</td>
</tr>
</tbody>
</table>

a data taken from Lide (1994), b Pm has no stable or long-lived isotopes, hence no natural background

As a result of imperfect shielding of the nuclear charge with each additional 4f electron, the ionic radius ($R$) of the Ln$^{3+}$ ions gradually decreases along the series (see Table 1-1). This effect is known as the 'lanthanide contraction' (Kuiper, 1969; Choppin, 1989; Brown et al., 1990) and is illustrated by Fig. 1-2, where $R$ is plotted against $Z$. The decrease of $R$, but constancy of valence, i.e. 3+, leads to an increasing charge density with $Z$. This has some interesting consequences for their chemical behaviour, for heavier lanthanides with higher charge densities have thus an in-
increased affinity for negatively charged groups, as is shown by Fig. 1-3, where \( \log \beta_n \), the logarithm of the first hydroxide stability constant, is plotted \( \text{versus} \ R \). Most chemical characteristics of the lanthanides are a smooth function of charge density, and therefore of \( R \) and also of \( Z \). Although the common oxidation state among the lanthanides is \( 3^+ \), exceptions are noted. The only other environmentally relevant oxidation states occur for cerium (Ce, \( Z = 58 \)), which can adopt the \( 4^+ \) oxidation state in relatively oxidising conditions, and europium (Eu, \( Z = 63 \)), which can adopt the \( 2^+ \) oxidation state under relatively reducing conditions (Henderson, 1996).

![Fig. 1-2. Ionic radius (R) for coordination number 6 of trivalent lanthanides (Ln³⁺) \( \text{versus} \) atomic number (Z), showing the ‘lanthanide contraction’. For comparison, the ionic radii of Eu⁴⁺ and Ce⁴⁺ are also shown. Ionic radii from Lide (1994).](image)

Together with the elements scandium (Sc, \( Z = 21 \)) and yttrium (Y, \( Z = 39 \)), also from group 3, the lanthanides are often referred to as the rare earth elements (REE), a confusing name, as these elements are not rare at all (see next section on Natural occurrence). Lanthanides share a number of chemical characteristics with Sc and Y and they are often found together in minerals (Brown \textit{et al.}, 1990). Completing group 5 are the actinides, with whose trivalent members, \( \text{e.g.} \) americium (Am, \( Z = 95 \)), the lanthanides share a number of characteristics as well. All actinides are radioactive and strongly associated with man’s nuclear activities. Lanthanides are often used as analogues to study the biochemical and long-term environmental behaviour of actinides (Boniforti, 1987; Bierkens and Simkiss, 1990; McCarthy \textit{et al.}, 1998), which are difficult to work with, due to their emission of \( x \), \( \beta \)- and \( \gamma \)-radiation, long half-lives, decay chains and radiation dose when taken up in the human body. Nonetheless, results from lanthanide studies should only be extrapolated to actinides when oxidation states match, \textit{i.e.} are trivalent. In general this would be the case for actinium (Ac, \( Z = 89 \)) and the actinides beyond Pu. As mentioned above, Ce can occupy the \( 4^+ \) oxidation state and could thus be possibly matched with tetravalent thorium (Th, \( Z = 90 \)), U and Pu. However, the latter analogy is hampered by differences in redox sensitivities of Ce and the aforementioned actinides (Choppin, 1991 and 1995).
1.1.3 Natural occurrence

Fourteen of the fifteen lanthanides occur in nature. Promethium (Pm, Z = 61) is the only lanthanide without a stable isotope; the Pm radioisotope with the longest half-life is $^{145}$Pm with $t_{1/2} = 17.7$ y. Promethium has been available at the origin of our solar system, but due to the short half-lives of its isotopes it quickly decayed away. It originates from fission of uranium. To illustrate the commonness of the naturally occurring lanthanides, their concentrations in the earth’s crust (Lide, 1994) are compared on a mo/kg basis with those of two well-known metals, lead (Pb, Z = 82) and cadmium (Cd, Z = 48). This shows that concentrations of La, Ce and neodymium (Nd, Z = 60) are higher than those of Pb, and that even the least abundant lanthanides, thulium (Tm, Z = 69) and Lu, have concentrations exceeding those of Cd. Through weathering and erosion of rocks, lanthanides become distributed in soil, sediment and water. Significant amounts can be found in minerals such as monazite (a phosphate ore), bastnaesite and xenotime (Brown et al., 1990; Henderson, 1996).

Because of their chemical similarity, lanthanides stay together as a group as they are distributed in the environment. Their concentrations in environmental samples display a typical pattern, which is strongly conserved. It can be described as a log-linear decrease of concentration with atomic number (Z) and, in addition, the odd-numbered elements have lower concentrations than the nearby even-numbered ones, known as Oddo-Harkins’ rule (Oddo, 1914; Harkins, 1917). This universal phenomenon originates from the nucleosynthesis of elements, whereby the formation of nuclei with an even number of protons is favoured over those with an odd number, for the latter are slightly less stable (Henderson, 1996). The typical saw-tooth pattern in environmental samples (Goldberg et al., 1963; Markert, 1987; Potts, 1995) when lanthanide concentrations are logarithmically plotted against Z, is shown in Fig. 1-4 for the earth’s crust (data given in Table 1-1). In general, the highest concentrations are found for Ce, the first even-numbered lanthanide, while the lowest concentrations are found for the last two odd-numbered lantha-
nides, Tm and Lu, whose concentrations are about two orders of magnitude lower than those of Ce.

Interpreting lanthanide concentration patterns is often preceded by normalising the data to the composition of a reference material, such as shale or chondrite, which yields a straight and horizontal line if no fractionation has occurred (Portts, 1995; Tjiv et al., 1983). Hence it removes the overwhelming Oddo-Harkins' effect (Schiff, 1992). If fractionation did occur, it may be easier detected after normalisation. Especially the redox-sensitive Ce and Eu may show deviating behaviour from the other lanthanides, e.g. a relative enrichment or depletion, which are called positive or negative anomalies, respectively. Anomalies arise when Ce$^{3+}$ is oxidised to Ce$^{4+}$ or alternately when Eu$^{3+}$ is reduced to the more mobile Eu$^{2+}$ form. Such non-trivalent lanthanides tend to segregate from the other strictly trivalent group members. Consequently, these anomalies may provide geologists with information on the prevailing environmental (redox) conditions during which a mineral or sediment was formed (Wright et al., 1987; Henderson, 1996). For interpretation of biological data on lanthanides in terms of availability, uptake or species comparisons, normalisation should rather be carried out with local environmental concentrations, such as those in surrounding water or sediment.

Tables 1-2 and 1-3 present concentrations of lanthanides in abiotic phases (surface water, pore water and sediment) of freshwater ecosystems as collected from the literature. Data are given only for the least and most abundant lanthanide, e.g. Lu and Ce, since other lanthanides will have concentrations in between those two. Data on waters with a salinity above 0.5‰ (seas, oceans, most estuarine waters) were not considered in composing Tables 1-2 and 1-3, since they fall beyond the scope of this thesis (i.e. are not representing fresh water). To facilitate comparisons, surface water data were selected to represent 'dissolved' concentrations, i.e. those passing at least a 0.45-μm filter. While such a filter limits the amount of suspended matter, which usually contains high lanthanide concentrations (somewhat below those of sediment), it still al-
Table 1-2. Dissolved concentrations (i.e. passing at least a 0.45-μm filter) of Ce and Lu in freshwater and pore water (nmol L⁻¹).

<table>
<thead>
<tr>
<th>surface water</th>
<th>filter (μm)</th>
<th>Lu (nmol L⁻¹)</th>
<th>Ce (nmol L⁻¹)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Mead (US)</td>
<td>0.45</td>
<td>0.00025</td>
<td>0.021</td>
<td>Hodge et al. (1996)</td>
</tr>
<tr>
<td>Twente canal (NL)</td>
<td>0.45</td>
<td>-</td>
<td>3.1</td>
<td>de Boer et al. (1996)</td>
</tr>
<tr>
<td>Lake IJsselmeer (NL)</td>
<td>0.45</td>
<td>0.017</td>
<td>0.093</td>
<td>de Boer et al. (1996)</td>
</tr>
<tr>
<td>Lake Bwa (JP)</td>
<td>0.45</td>
<td>0.0035</td>
<td>0.053</td>
<td>Haraguchi et al. (1998)</td>
</tr>
<tr>
<td>Amazon mouth (BR)*</td>
<td>0.22</td>
<td>0.0064-0.0073</td>
<td>0.75-0.93</td>
<td>Sholkovitz (1995)</td>
</tr>
<tr>
<td>Hudson (US)</td>
<td>0.22</td>
<td>0.0039</td>
<td>0.44</td>
<td>Sholkovitz (1995)</td>
</tr>
<tr>
<td>Mississippi (US)</td>
<td>0.22</td>
<td>0.0035</td>
<td>0.53</td>
<td>Sholkovitz (1995)</td>
</tr>
<tr>
<td>Connecticut (US)</td>
<td>0.45</td>
<td>0.0047-0.0054</td>
<td>0.22-0.29</td>
<td>Sholkovitz (1995)</td>
</tr>
<tr>
<td>Connecticut (US)</td>
<td>0.22</td>
<td>0.0041-0.0088</td>
<td>0.086-0.68</td>
<td>Sholkovitz (1995)</td>
</tr>
<tr>
<td>Fly (PNG)</td>
<td>0.22</td>
<td>0.0017</td>
<td>0.25</td>
<td>Sholkovitz et al. (1999)</td>
</tr>
<tr>
<td>Sepik (PNG)</td>
<td>0.45</td>
<td>0.0029</td>
<td>0.35-0.39</td>
<td>Sholkovitz et al. (1999)</td>
</tr>
<tr>
<td>Mullica, Delaware (US)</td>
<td>0.45</td>
<td>0.0047-0.029</td>
<td>0.17-5.0</td>
<td>Elderfield et al. (1998)</td>
</tr>
<tr>
<td>11 rivers (UK)</td>
<td>0.45</td>
<td>0.0032-0.027</td>
<td>0.24-4.8</td>
<td>Elderfield et al. (1998)</td>
</tr>
<tr>
<td>Tuscany streams (IT)</td>
<td>0.45</td>
<td>0.004-12*</td>
<td>0.14-2-781*</td>
<td>Protano and Riccobono (2002)</td>
</tr>
<tr>
<td>Yangtze (CHI)</td>
<td>0.45</td>
<td>0.017-0.040</td>
<td>0.79-1.4</td>
<td>Peng and Wang (1997)</td>
</tr>
<tr>
<td>Lake Tyrfjorden (N)</td>
<td>0.45</td>
<td>-</td>
<td>5.1</td>
<td>Solh et al. (1985)</td>
</tr>
<tr>
<td>Nieuwe Maas (NL)*</td>
<td>0.45</td>
<td>0.0034</td>
<td>0.34</td>
<td>Tijank and Yland (1998)</td>
</tr>
<tr>
<td>Arouit (CAM)</td>
<td>0.20</td>
<td>-</td>
<td>10-11*</td>
<td>Dupré et al. (1999)</td>
</tr>
<tr>
<td>Songhua, Dongting, Yangtze (CHI)</td>
<td>0.45</td>
<td>0.0037-0.023</td>
<td>0.36-1.7</td>
<td>Wang et al. (1997c)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pore water</th>
<th>surface water</th>
<th>filter (μm)</th>
<th>Lu (nmol L⁻¹)</th>
<th>Ce (nmol L⁻¹)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nieuwe Maas (NL)*</td>
<td>0.45</td>
<td>0.0034</td>
<td>0.34</td>
<td>Tijank and Yland (1998)</td>
<td></td>
</tr>
</tbody>
</table>

* salinity 0.3‰, † high values, related to acidic streams in a metal-mining area, ‡ salinity 0.22-0.24‰, § high value due to high DOC concentration, ~35 mg L⁻¹, * salinity 0.28‰. Abbreviations: BR = Brazil, CAM = Cameroon, CHI = China, IT = Italy, JP = Japan, N = Norway, NL = The Netherlands, PNG = Papua New Guinea, UK = United Kingdom, US = United States.

The passage of some non-dissolved lanthanides, e.g. those present in colloids (see Sholkovitz, 1992). Furthermore, it appeared that data on pore water concentrations from freshwater sediments are extremely scarce (only one reference found) and secondly, that most data are from recent years, reflecting both the analytical progress in lanthanide determination and the growing interest in these elements. The Lu concentration in the earth’s crust (Table 1-1) is higher than those found in sediment, but the value for Ce lies in the range found for sediments. This may imply that Lu is geochemically more mobile than Ce (and other lanthanides will probably show intermediate behaviour). Compared to their concentrations in sediment, lanthanides have very low dissolved concentrations (compare Tables 1-2 and 1-3), hence high Kₚ values (sediment-water partition coefficients, in L·kg⁻¹). The low dissolved concentrations reflect the low solubility...
INTRODUCTION

Table 1-3. Concentrations of Ce and La in freshwater sediment (mmol/kg$^1$ dry weight)

<table>
<thead>
<tr>
<th>sediment</th>
<th>La (mmol/kg$^1$)</th>
<th>Ce (mmol/kg$^1$)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linsley Pond (US)</td>
<td>-</td>
<td>0.56-0.80</td>
<td>Cowgill (1973a)</td>
</tr>
<tr>
<td>Niagara shed (CAN)</td>
<td>0.0011-0.0025</td>
<td>&lt;0.036-0.24</td>
<td>Dickman and Rygiel (1996)</td>
</tr>
<tr>
<td>Fly (PNG)</td>
<td>0.0021</td>
<td>0.51</td>
<td>Sholkovitz et al. (1999)</td>
</tr>
<tr>
<td>Sepik (PNG)</td>
<td>0.0022</td>
<td>0.34</td>
<td>Sholkovitz et al. (1999)</td>
</tr>
<tr>
<td>Tagus (P)</td>
<td>-</td>
<td>0.62-1.4</td>
<td>Freitas et al. (1993)</td>
</tr>
<tr>
<td>IJ (NL)</td>
<td>-</td>
<td>0.53-11$^1$</td>
<td>Mars (1989)</td>
</tr>
<tr>
<td>Songhua, Dongting, Yangze (CHI)</td>
<td>0.00057-0.011</td>
<td>0.036-4.5</td>
<td>Wang et al. (1997c)</td>
</tr>
<tr>
<td>12 locations (NL)</td>
<td>0.00051-0.0014</td>
<td>0.20-0.71</td>
<td>van Son (1994)</td>
</tr>
<tr>
<td>7 major rivers (CHI)</td>
<td>0.00011-0.0024</td>
<td>0.12-0.88</td>
<td>Zhu et al. (1997)</td>
</tr>
<tr>
<td>Nieuwe Maas (NL)</td>
<td>0.0014</td>
<td>0.37</td>
<td>Tijink and Yland (1998)</td>
</tr>
</tbody>
</table>

$^1$ high concentrations due to emissions of catalyst industry. Abbreviations: CAN = Canada, CHI = China, NL = The Netherlands, P = Portugal, PNG = Papua New Guinea, US = United States

of lanthanide-phosphates with $K_c \sim 10^{-25}$ mol$^{-1}$L$^2$ (Liu and Byrne, 1997), lanthanide-carbonates with $K_c \sim 10^{-8}$ mol$^{-1}$L$^3$ and lanthanide-hydroxides with $K_c \sim 10^{-24}$ mol$^{-1}$L$^4$ (Martell and Smith, 1997), as well as the high affinity of lanthanides for sediment components. Because PO$_4^{3-}$, CO$_3^{2-}$ and OH$^-$ are ubiquitous ligands occurring in reasonable concentrations, they strongly limit aqueous lanthanide concentrations. This has important consequences for lanthanide bioavailability, as will be discussed in the section Bioavailability and uptake.

1.1.4 Emissions

Because of the widespread uses of lanthanides in our society, they are emitted to the environment by many different sources. Some striking examples of emission sources are: automotive catalysts emitting La, Ce and Nd (Helmers, 1996; Ballach, 1997; Rauch et al., 2000), hospital effluents containing Gd, which is used in Magnetic Resonance Imaging (Bau and Dolski, 1996; Kümmerer and Helmers, 2000) and artificial fertiliser manufacturing and application in agriculture will distribute all fourteen naturally occurring lanthanides (Drabback et al., 1987; Volold et al., 1999; Gorbanov et al., 1992; Todorovskiy et al., 1997). Uranium fission in nuclear power plants or nuclear bomb testing yields the radioisotopes $^{148}$La ($t_{1/2} = 40.3$ h), $^{146}$Ce ($t_{1/2} = 285$ d) and $^{147}$Pm ($t_{1/2} = 2.62$ y), amongst others (Kuiper, 1969; Agekina et al., 1998). From the mentioned sources, the catalyst and artificial fertiliser industry are the most important ones. In addition, indications have been found that the mobility of naturally present lanthanides increases as a result of acid rain (Nesbitt, 1979).

The main emission route is to surface water (Slooff et al., 1993), where the majority of lanthanides ends up in sediment, due to the low lanthanide solubility. When waterways are dredged and the material dumped on land, lanthanide emissions are moved to terrestrial envi-
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Environments. The two main producers of artificial (i.e. phosphate) fertilisers in the Netherlands introduced about 355 tons of the lanthanides La, Ce, prasodymium (Pr, Z = 59) Nd and samarium (Sm, Z = 62) in surface water in 1994 (Sneller et al., 2000). For other emission sources, no quantitative data are available.

It is expected that emissions of lanthanides will increase, because their number of applications is still growing (Hedrick, 1996). The global annual demand of REE, the majority of which are lanthanides, in the late eighties was estimated to be 30,000 tons (Hirano and Suzuki, 1996) and for 2000 it was estimated to be 50,000 tons (Zhu, 1999). The increasing demand for lanthanides, their growing number of applications and accompanying emissions, call for data concerning their concentrations in and effects on ecosystems (Janus et al., 1994). As with all metals, it is thereby important to differentiate between the natural background and anthropogenic enrichments (Struijs et al., 1997). This may be achieved relatively easy for lanthanides, because anthropogenic enrichments with single elements will appear as positive anomalies after normalising the concentrations to a local background sample. Obviously, simultaneous enrichment with all lanthanides, such as with artificial fertiliser, cannot be detected this easily, but has to be judged against uncontaminated samples. Finally, it must be noted that the availability of emitted lanthanides may differ from (is probably higher than) that of naturally present lanthanides, since the latter are at least partly incorporated in the crystalline matrix of sediments.

1.1.5 Concentrations in plants and invertebrates

An overview of lanthanide concentrations in freshwater plants and invertebrates, as they were collected from the literature, is given Tables 1-4 and 1-5. These concentrations were determined with various techniques, which will be dealt with in section 1.2. During collection of the data it was noted that most of the environmental-biological research on lanthanides has been conducted with terrestrial plants, marine organisms and vertebrates (i.e. fish and mammals). This selection of research subjects originates mainly from aspects related to human health in combination with the use of lanthanides as actinide analogues. Firstly, many studies were aimed at estimating Ln (or rather An) uptake in man by consumption of vegetables, meat, marine fish and molluscs, and secondly, uptake and effect studies of Ln on mammals were conducted for extrapolation to man. Freshwater invertebrates and plants are hardly consumed, if at all, and have therefore not been studied from a human health point of view. Also, they have not been studied for extrapolation to man.

From the values in Tables 1-4 and 1-5 it seems that Ln concentrations in freshwater invertebrates are approximately of the same order as those in freshwater plants. However, this preliminary conclusion is based on relatively few data, especially for invertebrates. The study of Cowgill (1973b) stands out as reporting consistently high concentrations for plants. Considering their date of publication, 1973, the analytical quality of the data may be questioned. Landolt and Kandeler (1987) derived their values from Kovács et al. (1984) so the former entry may be ignored. Bionta concentrations lie in between those of water and sediment (compare with Tables
Table 1.4. Lanthane concentrations (μmol kg⁻¹ dry weight) in freshwater plants

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>La</th>
<th>Ce</th>
<th>Pr</th>
<th>Nd</th>
<th>Sm</th>
<th>Eu</th>
<th>Gd</th>
<th>Tb</th>
<th>Dy</th>
<th>Ho</th>
<th>Er</th>
<th>Tm</th>
<th>Yb</th>
<th>La ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aquilis filicinoides</em></td>
<td>2.8</td>
<td>6.6</td>
<td>&lt;0.8</td>
<td>0.4</td>
<td>0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.14</td>
</tr>
<tr>
<td><em>Ceratophyllum demersum</em></td>
<td>38</td>
<td>76</td>
<td>60</td>
<td>5.9</td>
<td>1.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.32</td>
</tr>
<tr>
<td><em>Ceratophyllum submersum</em></td>
<td>2.7-297</td>
<td>12-147</td>
<td>1.2-29</td>
<td>8.5-100</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Daphnia pulex</em></td>
<td>261-462</td>
<td>575-895</td>
<td>26-47</td>
<td>93-137</td>
<td>42-147</td>
<td>14-41</td>
<td>18-27</td>
<td>20-36</td>
<td>7.5-10</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hydrocharis morsus</em></td>
<td>0-3.1</td>
<td>1.6-9.1</td>
<td>0-1.5</td>
<td>10-14</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lemna minor</em></td>
<td>0.93</td>
<td>1.7</td>
<td>0.23</td>
<td>0.94</td>
<td>0.19</td>
<td>0.44</td>
<td>0.2</td>
<td>0.028</td>
<td>0.2</td>
<td>0.033</td>
<td>0.088</td>
<td>0.014</td>
<td>0.08</td>
<td>0.013</td>
</tr>
<tr>
<td><em>Lemna trisulca</em></td>
<td>6.3</td>
<td>16</td>
<td>3.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td><em>Lemna minor</em></td>
<td>6.5</td>
<td>14</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Myriophyllum spicatum</em></td>
<td>14.63</td>
<td>56-125</td>
<td>5.6-31</td>
<td>94-212</td>
<td>3</td>
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<tr>
<td><em>Myriophyllum verticillatum</em></td>
<td>2.7-6.8</td>
<td>8.6-16</td>
<td>2.3-11</td>
<td>0.040-1.7</td>
<td>6</td>
<td></td>
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<tr>
<td><em>Nuphar advena</em></td>
<td>166-377</td>
<td>502-895</td>
<td>26-50</td>
<td>62-137</td>
<td>57-165</td>
<td>18-41</td>
<td>9.2-25</td>
<td>17-41</td>
<td>7.5-12</td>
<td>2</td>
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<tr>
<td><em>Nuphar advena</em></td>
<td>197-455</td>
<td>496-795</td>
<td>25-50</td>
<td>67-130</td>
<td>78-146</td>
<td>17-36</td>
<td>7.4-27</td>
<td>14-41</td>
<td>7.5-9.8</td>
<td>2</td>
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<tr>
<td><em>Potamogeton argus</em></td>
<td>135-316</td>
<td>483-729</td>
<td>19-43</td>
<td>69-130</td>
<td>62-127</td>
<td>19-42</td>
<td>5.7-27</td>
<td>8.4-30</td>
<td>6.4-8.7</td>
<td>2</td>
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<tr>
<td><em>Potamogeton crispus</em></td>
<td>237-308</td>
<td>446-730</td>
<td>17-25</td>
<td>62-65</td>
<td>78-100</td>
<td>27-30</td>
<td>7.4-12</td>
<td>17-32</td>
<td>6.4-8.1</td>
<td>2</td>
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<tr>
<td><em>Potamogeton crispus</em></td>
<td>1.3-1.9</td>
<td>0.24-1.1</td>
<td>0.97-1.8</td>
<td>0.25-0.37</td>
<td>6</td>
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<tr>
<td><em>Potamogeton pectinatus</em></td>
<td>4.6-31</td>
<td>4.3-11</td>
<td>1.1-2.3</td>
<td>4.2-17</td>
<td>3</td>
<td></td>
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<tr>
<td><em>Potamogeton perlatus</em></td>
<td>2.1-31</td>
<td>4.1-68</td>
<td>1.1-6.0</td>
<td>5.6-34</td>
<td>3</td>
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<tr>
<td><em>Potamogeton pusillus</em></td>
<td>1.43</td>
<td>0.61</td>
<td>19</td>
<td>64</td>
<td>68</td>
<td>24</td>
<td>11</td>
<td>47</td>
<td>5</td>
<td>2</td>
<td></td>
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<tr>
<td><em>Stratiotes aloides</em></td>
<td>1.2-16</td>
<td>1.9-35</td>
<td>0.6-3.4</td>
<td>0.0-12</td>
<td>3</td>
<td></td>
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Table 1-5. Lanthanide concentrations (μmol kg\(^{-1}\) dry weight) in freshwater invertebrates

<table>
<thead>
<tr>
<th>invertebrate species</th>
<th>La</th>
<th>Ce</th>
<th>Pr</th>
<th>Nd</th>
<th>Sm</th>
<th>Eu</th>
<th>Gd</th>
<th>Tb</th>
<th>Dy</th>
<th>Ho</th>
<th>Er</th>
<th>Tm</th>
<th>Yb</th>
<th>La</th>
<th>ref.</th>
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<tbody>
<tr>
<td><em>Lymnaea stagnalis</em></td>
<td>0.30-0.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Planorbarius corneus</em></td>
<td>0.014-0.23</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Trissoclada larue</em></td>
<td>0.40-1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Corbicula fluminea</em> tissue</td>
<td>7.4</td>
<td>11</td>
<td>1.6</td>
<td>5.3</td>
<td>1</td>
<td>0.13</td>
<td>0.83</td>
<td>0.13</td>
<td>0.62</td>
<td>0.12</td>
<td>0.24</td>
<td>0.059</td>
<td>0.17</td>
<td>0.057</td>
<td>2</td>
</tr>
<tr>
<td><em>Corbicula fluminea</em> shell</td>
<td>0.58</td>
<td>0.86</td>
<td>0.14</td>
<td>0.42</td>
<td>0.066</td>
<td>0.066</td>
<td>0.064</td>
<td>0.12</td>
<td>0.06</td>
<td></td>
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<td></td>
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<tr>
<td><em>Corophium multitesticum</em></td>
<td>9</td>
<td>14</td>
<td>1.8</td>
<td>6.1</td>
<td>1</td>
<td>0.33</td>
<td>0.89</td>
<td>0.13</td>
<td>0.74</td>
<td>0.12</td>
<td>0.3</td>
<td>0.059</td>
<td>0.20</td>
<td>0.057</td>
<td>2</td>
</tr>
</tbody>
</table>

1.2 and 1.3. The concentration ratios of biota to water are > 10,000 L·kg⁻¹ dry weight and those of biota to sediment are < 0.1 L·kg⁻¹ dry weight, respectively. Furthermore, when going through Table 1-4 from La to Lu, the amount of missing values increases as a consequence of analytical problems, *i.e.* concentrations lie beyond detection limits. In addition, more values are missing for the odd numbered elements, which is associated with lower concentrations as well.

Up until now, no essentiality of biological function of any of the lanthanides has been established and consequently, it remains unclear what the lanthanide content of organisms means. The only conclusion that may be drawn from organism concentrations is that lanthanides are apparently available for uptake from the environment. The next section goes into bioavailability and uptake and the ratio of biota concentrations to those in the surrounding environment. Effects that lanthanides may have on biota are dealt with in the section *Toxicity and beneficial effects.*

### 1.1.6 Bioavailability and uptake

Before going into discussing the bioavailability of lanthanides, a clear definition of bioavailability must be given, since this term is not always used consistently. In this thesis, bioavailability or biological availability of a substance is defined as ‘the form or forms of a substance present in the environment, which may be taken up or associate with an organism’. It may seem trivial, but sometimes it is rather difficult to assess if lanthanides are truly associated with an organism’s outside or merely present in attached (sediment) particles (Sansone *et al.*, 1998; Freitas *et al.*, 1993), in gut particles or associated with other organisms, like fungi and algae (*Aspergillus*), growing on its outside. Furthermore, the definition does not account for potential availability, *i.e.* the Ln fraction that becomes available when the readily available lanthanides are taken up and equilibria start shifting. As such, kinetic effects are ignored. The definition also implies that bioavailability depends on the organism under consideration and not only on the chemistry of a substance. Due to different exposures, caused by differences in feeding, breathing, burrowing, outer surface permeability, mobility, surface-volume ratio and so on, bioavailability will have its own value for each organism and substance. Therefore, one rather studies the chemical availability of substances, as this is organism independent and easier to quantify.

Chemical availability of metals in aquatic systems is operationally defined. For sediments, it usually implies a chemical extraction with water, calcium chloride, ammonium acetate, a sequential extraction with increasingly stronger extractants (see Leleyter *et al.*, 1999, Sun *et al.*, 1998) or the collection of pore water (Tijink and Yland, 1998), assuming the Ln’s present in the extract or pore water to be available. For surface water and pore water, the available Ln’s are usually considered those which are present in the dissolved state, that is passing a 0.45-μm filter. A further step is to define available species (usually the free Ln⁺-ion). If the overall composition of the system is sufficiently known, the available fraction or species may be estimated by equilibrium speciation calculations. Biological and chemical availability of Ln’s is linked through metal mobility. If a metal is highly mobile it may reach an organism easily and adsorb to it or be taken up by drinking and passive diffusion through skin and gills. If food is the dominant source for Ln
CHAPTER I

intake, the relation between bioavailability and chemical availability is weaker and chemical methods to assess bioavailability do not necessarily apply.

In freshwater systems, most of the lanthanides (~99%) are present in or bound to sediment and suspended matter (see Table 1-3) and considered largely as unavailable (see above). A minor fraction is dissolved in sediment pore water and surface water. The dissolved concentrations of lanthanides depend on redox state, pH, presence of organic and inorganic ligands, total Ln concentration, competing metals, etc. in the system. In freshwater solution, lanthanides are for about 95% associated with dissolved organic matter (DOM), often expressed in dissolved organic carbon (DOC), which mainly consist of humic and fulvic acids, that bind Ln’s to their carboxylic and phenolic groups (Torres and Choppin, 1984; Choppin, 1986; Haraguchi et al., 1998; Düpš et al., 1999). The major role of DOC has two important consequences: (i) it increases the solubility of Ln’s by counteracting precipitation, and (ii) it increases the mobility of Ln’s in the environment by serving as a transport vector (Torres and Choppin, 1984; Choppin, 1986; McCarthy et al., 1998). The rest of the dissolved Ln’s is present in a free ionic form ( LnIII ) or in an inorganically complexed form (e.g. Ln[OH]2+, Ln(CO3)42−). Again, the concentration of each species depends on pH, ligand concentration, ionic strength and so on. Availability of dissolved species decreases in the following order: free ion, inorganic complex, and finally organic complex. This was demonstrated by Sun et al. (1997a) for the uptake of La and Gd in the presence and absence of organic ligands by the freshwater alga Chlorella vulgaris. Uptake of Gd by the marine alga Ulva lactuca was strongly reduced by increasing carbonate concentrations (Stanley Jr. and Byrne, 1990). Uptake of La by the freshwater alga Somecosmus pannonicus was reduced at a lower pH and also at a higher Ca concentration (Demon et al., 1988 and 1989), probably reflecting cationic competition. The sediment-bound fraction is usually considered not to be available, but may become so when circumstances change and equilibria are disturbed. This happens for instance when sediment particles are ingested by organisms, or when sediment-rooting plants exude protons (H+) or chelating substances (e.g. malate, citrate) to increase the availability of another trivalent metal, the essential, but poorly soluble iron (Mori, 1999). As such, the sediment may be considered a source for lanthanides in water. Lanthanides present in biota may become available to other organisms feeding on them, or alternately, they are released again when organisms die and decompose. Further attention in this section is focused on the dissolved lanthanides.

Studying the speciation of lanthanides in freshwater, i.e. their distribution over different chemical forms, leads to a more detailed insight into bioavailability. The labile Ln fraction, including the free Ln ion, may be measured with a sensitive technique such as voltammetry (Lee et al., 1997). Lanthanide speciation may also be assessed by combining chromatographic separation with inductively coupled plasma - mass spectrometry (ICP-MS) (Haraguchi et al., 1998). Another option is to calculate speciation, which requires full characterisation of the system and a database of equilibrium constants. Calculating speciation is usually very well possible for artificial (laboratory) systems where all components and their concentrations are defined. For natural systems, there is always the problem of heterogeneous organic material, which complexes many metals,
INTRODUCTION

including the lanthanides. Furthermore, speciation calculations on natural waters demands a large analytical effort to characterise the system in terms of its components and also simplifications considering the DOM present (Dupré et al., 1999). In laboratory studies, DOM is usually replaced by organic ligands such as NTA, EDTA or citrate, enabling the full support of speciation calculations. For various divalent metals, it has been shown that the free-ion activity (or concentration) is most indicative of their uptake by and toxicity to aquatic organisms. This principle is known as the free-ion activity model (FIAM) (Morel, 1983), and started with the pioneering work of Sunda and Guillard (1976) on copper and algae. For lanthanides, this model has not yet been tested.

Uptake of Ln is often controlled by surface adsorption (Demon et al., 1988; Stanley Jr. and Byrne, 1990), caused by the high reactivity of the trivalent Ln ions (Bingler et al., 1989), combined with the fact that for many biota the outer surface is negatively charged. The degree of Ln accumulation can often be related to an organism’s surface area (Palumbo, 1963). Adsorption of Ln from food in the digestive tract is low, which explains their use as internal markers (Brown et al., 1990). After the initial sorption of Ln to the cell wall they may be transported into the cell, possibly through Ca channels, which have a higher affinity for the trivalent Ln than for divalent Ca. This has led to the ‘super-calcium’ status of La and the frequent use of this element for blocking Ca uptake (Evans, 1990). This relationship works both ways, and it has been shown that high Ca concentrations lower the uptake of La in algae (Demon et al., 1989). Another possibility for Ln to enter the cell is by formation of hydrophobic complexes, which are able to pass the cell membrane by simple diffusion. Elimination of Ln is likely to follow the routes previously established for other metals, through active excretion (ion channels, faeces, urine, slime, moulting), by reproduction (eggs and juveniles) and growth dilution. Furthermore, since Ln’s are also adsorbed on the outer surface, they may be released when the solution pH decreases, or when the concentration of competing metals or ligands increase. Such a route is not under control of organisms, but instead a passive and imposed means of elimination. From the literature, no information is available on Ln elimination by freshwater organisms.

Uptake and elimination finally result in a steady-state situation, which is characterised by the bioconcentration factor (BCF), describing the ratio of Ln concentrations in biota versus those in water. As discussed in section 1.1.4, these values for Ln lie approximately between 1,000 and 100,000 L·kg⁻¹ dry weight. Assuming the organism to be in equilibrium with its environment, measurements of biota and water samples allow for a calculation of these values. Wolterbeek and van der Meer (1996) report BCFs of 1,800 to 6,000 and 5,800 to 46,000 L·kg⁻¹ dry weight for La, Ce, Nd, Sm, Eu and Lu in the freshwater plants Azolla filiculoides and Ceratophyllum demersum, respectively. The higher values for the latter species were attributed to its fully submerged existence in contrary to the floating life of the former. However, these values must be considered as conservative estimates, because their water concentrations are based on 2-μm filtrates and are therefore likely to contain some non-dissolved lanthanides (Sholkovitz, 1992). Kryshev and Sazykina (1994) report BCFs of 2,400-4,600 L·kg⁻¹ fresh weight in molluscs and 3,000-24,000
L·kg\(^{-1}\) dry weight in various aquatic plants for the radionuclides \(^{140}\)La and \(^{141,144}\)Ce. BCFs for the radionuclides \(^{141,144}\)Ce in unspecified water plants ranged from 50 to 810 L·kg\(^{-1}\) plant (fresh or dry weight not specified) (Hermann et al., 1975). Calculations on the biota and pore water data of Tijink and Yland (1998) on all naturally occurring Ln (except Eu) resulted in BCFs of 14,000 to 37,000 L·kg\(^{-1}\) dry weight for the bivalve Corbicula fluminea (tissue) and 17,000 to 42,000 L·kg\(^{-1}\) dry weight for the crustacean Corophium multiretusum. Values for Eu were much lower, 2,400 and 6,000 L·kg\(^{-1}\) dry weight, respectively, which is probably related to the divalent state of Eu in reducing sediment (see also chapter 3). The calculated BCFs decrease slightly with atomic number, which was also reported for marine algae by Stanley Jr. and Byrne (1990). Biota-to-sediment accumulation factors (BSAFs) range from 0.017 to 0.040 and from 0.028 to 0.047 kg·kg\(^{-1}\) dry weight for Corbicula fluminea and Corophium multiretusum, respectively. In this case, a higher BSAF of 0.063 kg·kg\(^{-1}\) dry weight was found for Eu in Corophium multiretusum. The Eu value for Corbicula fluminea did not deviate from other La’s.

1.1.7 Toxicity and beneficial effects

Both negative and positive effects of lanthanides on biota have been reported. Negative effects are related to toxicity, which occurs for any given element, provided that a sufficiently high concentration is applied (Paracelsus’ law). Even then, effects may be due to indirect action, e.g. by precipitation of lanthanides with required nutrients like phosphate or carbonate, or by increasing the available concentrations of more toxic metals by displacing them from ligands or adsorptive sites. Other mechanisms of toxic action are described below. Beneficial effects of lanthanides have been described only for plants, and are almost exclusively reported by Chinese researchers. This is related to the application of lanthanide-containing fertilisers in Chinese agriculture. Mechanisms, which may lead to beneficial effects, are discussed below.

The most likely mechanisms of toxicity of Ln are through interactions with the micronutrients Ca\(^{2+}\), Mg\(^{2+}\), Fe\(^{3+}\), Fe\(^{2+}\) and Mn\(^{2+}\). In biota, lanthanides substitute for them (Evans, 1990), causing ineffective proteins or micronutrient deficiencies. For Lemma trisula it was demonstrated that the Ca influx was inhibited by 12 h of exposure to La, which caused disturbances in chloroplast movement (Talka and Gabrys, 1993). Substitution for other ions has been demonstrated for terrestrial organisms, but data are lacking for freshwater biota. According to Nieboer and Richardson (1980), lanthanides belong to class A metals, which seek oxygen-containing ligands and functional groups, such as OH\(^{-}\) and PO\(_4\)\(^{3-}\). In biota, these groups are ubiquitous and offer many binding sites to Ln. This binding may cause biochemically ineffective macromolecules, but on the other hand, may act as a detoxifying mechanism.

Table 1-6 gives data on the toxicity of lanthanides to freshwater invertebrates, as they were collected from the literature. No data on aquatic plants were found. Most data in Table 1-6 (~75%) relate to water fleas (daphnids) and the lighter lanthanides. Furthermore, the majority of the data relates to acute toxicity, which seems somewhat outdated in present-day environmental risk assessment. The highest values, hence lowest toxicity, are found for strongly complexed Gd.
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Table 1-6. Effect concentrations of lanthanides (in mmol·L⁻¹) for freshwater invertebrates

<table>
<thead>
<tr>
<th>invertebrate species</th>
<th>lanthanide</th>
<th>endpoint</th>
<th>mmol·L⁻¹</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Carphobranchus elegans</em></td>
<td>La</td>
<td>LC₅₀ (24 h)</td>
<td>9.7</td>
<td>1</td>
</tr>
<tr>
<td><em>Daphnia carinata</em></td>
<td>La</td>
<td>LC₅₀ (24 h)</td>
<td>0.0035-0.0089</td>
<td>2</td>
</tr>
<tr>
<td><em>Daphnia carinata</em></td>
<td>La</td>
<td>LC₅₀ (48 h)</td>
<td>0.00031-0.0089</td>
<td>2</td>
</tr>
<tr>
<td><em>Daphnia hyalina</em></td>
<td>LaClₓ·xH₂O</td>
<td>LC₅₀ (48 h)</td>
<td>0.00076</td>
<td>3</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>La</td>
<td>LC₅₀ (48 h)</td>
<td>0.0068</td>
<td>3</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>La</td>
<td>LC₅₀ (21 d)</td>
<td>0.0040</td>
<td>4</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>La</td>
<td>LOEC (21 d)</td>
<td>≥0.0020</td>
<td>4</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>La</td>
<td>EC₅₀ (21 d)</td>
<td>&gt;0.0048</td>
<td>4</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>La</td>
<td>EC₅₀ (48 h)</td>
<td>0.17</td>
<td>5</td>
</tr>
<tr>
<td><em>Taijifex tubifex</em></td>
<td>La</td>
<td>LC₅₀ (24-48 h)</td>
<td>0.24-0.24</td>
<td>6</td>
</tr>
<tr>
<td><em>Taijifex tubifex</em></td>
<td>La</td>
<td>LC₅₀ (96 h)</td>
<td>0.21</td>
<td>6</td>
</tr>
<tr>
<td><em>Daphnia hyalina</em></td>
<td>Ce</td>
<td>LC₅₀ (48 h)</td>
<td>0.00071</td>
<td>3</td>
</tr>
<tr>
<td><em>Daphnia hyalina</em></td>
<td>CeClₓ·xH₂O</td>
<td>LC₅₀ (48 h)</td>
<td>0.00081</td>
<td>3</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Ce</td>
<td>EC₅₀ (48 h)</td>
<td>0.16</td>
<td>5</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>CeClₓ·xH₂O</td>
<td>LC₅₀ (48 h)</td>
<td>0.0048</td>
<td>3</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Pr</td>
<td>EC₅₀ (48 h)</td>
<td>0.064</td>
<td>5</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Nd</td>
<td>EC₅₀ (48 h)</td>
<td>0.0097</td>
<td>5</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Nd</td>
<td>NOEC (21 d)</td>
<td>0.011</td>
<td>5</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Sm</td>
<td>EC₅₀ (48 h)</td>
<td>0.051</td>
<td>5</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Gd</td>
<td>EC₅₀ (48 h)</td>
<td>0.043</td>
<td>5</td>
</tr>
<tr>
<td><em>Carphobranchus elegans</em></td>
<td>Gd(CPA-DO3A)⁺</td>
<td>LC₅₀ (72 h)</td>
<td>52</td>
<td>7</td>
</tr>
<tr>
<td><em>Carphobranchus elegans</em></td>
<td>Gd(DTPA)²⁻</td>
<td>LC₅₀ (72 h)</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td><em>Carphobranchus elegans</em></td>
<td>Gd(HP-DO3A)²⁺</td>
<td>LC₅₀ (72 h)</td>
<td>288</td>
<td>7</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Dy</td>
<td>EC₅₀ (48 h)</td>
<td>0.056</td>
<td>5</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Dy</td>
<td>NOEC (21 d)</td>
<td>&lt;0.0012</td>
<td>5</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Dy</td>
<td>NOEC (21 d)</td>
<td>&gt;0.013</td>
<td>5</td>
</tr>
</tbody>
</table>

* complexes with very high stability constants, used in magnetic resonance imaging, † range in LC₅₀ values due to the use of different media, ‡ immobility, almost equal to mortality, § mortality and fitness, ¶ reproduction. References: 1 = Tataru et al. (1998), 2 = Barry and Meehan (2000), 3 = van Urk (1977), 4 = Bögers et al. (1998), 5 = den Ouden (1995), 6 = Khangarot (1991), 7 = Williams et al. (2000). Abbreviations: LC₅₀ = concentration causing 50% mortality, EC₅₀ = concentration causing 50% effect, NOEC = no observed effect concentration, i.e. the highest concentration not significantly different from the control, LOEC = lowest observed effect concentration, i.e. the lowest concentration significantly different from the control

Comparison of the data is hampered by their different nature (LC₅₀, EC₅₀, LOEC and NOEC), the use of different endpoints (mortality, mobility and reproduction) and different exposure durations (varying from 24 h to 21 d). While some studies base their endpoint calculations on analytically determined values (and without exception report severe La precipitation), others relate effects to nominal concentrations. The most sensitive species seem to be the closely related water
CHAPTER I

fleas *Daphnia hyalina* and *D. carinata*. Nevertheless, the lowest value in Table 1-6 (310 nM for *D. carinata*), is still very high compared to the values found for Ce in natural freshwater (Table 1-2). However, it must be kept in mind that this value of 310 nM relates to 50% of the animals dying in 48 hours.

In some papers, the possibility is discussed that lanthanides may fulfil an essential role in plants (Cowgill, 1973b; Ozaki *et al.*, 1997). While these trends are merely based on statistical correlation, essentiality of lanthanides has never been proven. Dieloff *et al.* (1995a) proposed that under some circumstances phosphate might reach toxic levels for plants, which can be suppressed by precipitation with lanthanides, hence leading to enhanced performance. Another cause of stimulating effects of lanthanides on plant growth may be the inhibition of Cd uptake. This toxic element frequently enters an organism through calcium channels, which are blocked very effectively by *La*³⁺ and other lanthanides (Evans, 1990). Craig *et al.* (1999) demonstrated that Cd uptake in midge larvae (*Chironomus stagnalis*) was inhibited for 73% and 92% by 10 and 100 µM La, respectively. In *Lemma minor*, 1 mM La inhibited the uptake of 1 µM Cd by 51% and that of 1 µM Tl by 25% (Kwan and Smith, 1991). Finally, enhanced performance may be due to the general phenomenon called ‘hormesis’ (Stebbing, 1982) and has been shown for an array of chemicals. Hormesis is most likely caused by an (over) stimulation of an organism’s metabolism as a result of exposure to low concentrations of toxic chemicals. As such, it may be classified as a response to a toxic substance.

Finally, Gabrashanska *et al.* (1989) concluded that Ce, among other elements, plays a decisive role in the pathogenesis of trematode infections of freshwater snails. In different snail species, the infection caused either a decrease or an increase of Ce tissue concentrations. Yet, it is unknown how the Ce concentration is influenced by the infection.

1.2 Methodological aspects of lanthanide analysis

1.2.1 General

From the literature review in the previous section, it appears that Ln data from laboratory studies frequently suffer from poor quality. With respect to the evaluated field data it was noticed that many studies report only the most abundant Ln’s, i.e. the lighter ones and the heavier ones with even numbers, which is obviously related to analytical difficulties (see for instance how Table 1-4 is filled). Therefore, special emphasis was put on the quality of the data obtained in this work. This is reflected in the applied analytical techniques, the choice of organisms and the methodology and design of experiments. This section discusses these aspects, before the scope of the thesis is given. First the analytical techniques for Ln determinations are described and then the experimental design is discussed.
1.2.2 Analytical techniques

Lanthanide concentrations in environmental matrices, such as water, sediment and organisms (see Tables 1-2 to 1-5) can be determined with several analytical techniques, including ICP-MS, INAA, isotope dilution - thermal ionisation mass spectrometry (ID-TIMS), ICP - atomic emission spectrometry (ICP-AES) and by means of γ- and/or β-radiation emitting radioisotopes. The latter does not refer to analysing Ln’s in natural samples, but to the application of tracers in laboratory experiments. The other techniques can be applied to both laboratory and field samples.

The number of Ln determinations in natural samples greatly increased with the development of ICP-MS. Its high sensitivity and simultaneous multi-element measurement enabled accurate determinations of all fourteen naturally occurring Ln’s. ICP-MS measurement requires the complete (pressure) digestion of a sample, by means of a combination of H2O2 and strong acids (i.e. HF, HCl, HNO3 and HClO4) all of high purity. Due to isobaric overlap in the mass spectrum, Ln determinations may be hampered by oxide and hydroxide formation of the many barium (Ba, Z = 56) isotopes, and in case of the heavier Ln’s, by oxide and hydroxide formation of the lighter Ln’s. In cases of high Ba concentrations, a chemical separation of Ln’s from Ba may be applied to improve Ln determinations. It is also possible to exactly quantify the Ba concentration and the degree of oxide and hydroxide formation, so that interferences may be calculated and corrected for. For water samples, high resolution ICP-MS is currently the best technique available, because it unravels overlaps, while its sensitivity is high enough to measure directly the low natural aqueous lanthanide concentrations (see Zhu, 1999). For Ln determinations in solid samples, e.g. sediment and biota, ICP-MS is at present the most widely used technique, followed by INAA.

INAA is another multi-element technique, that works well for the determination of about eight Ln’s in solid samples (Tjoee et al., 1983; Laul and Gosselin, 1989). INAA has the advantage of not requiring the dissolution of a solid sample, and thus has no difficulties associated with incomplete digestions or the possibility of introducing contaminations by the chemicals applied. However, samples must be pulverised, since INAA requires sample homogeneity, in the interest of measurement geometry. Water cannot be measured routinely by INAA, since samples may easily heat up to 70°C during irradiation, and the resulting pressure build-up within the sample vessel poses the risk of spreading its radioactive contents. Hence, determining Ln’s in water samples with INAA requires to concentrate the sample volume into a solid form (Woltersbeek and van der Meer, 1996). Not all Ln’s are easily measured with INAA, which is related to sample composition, element specific abundance, isotopic composition, cross section, half-lives and characteristics of the emitted γ-rays, amongst others (for an overview, see Laul and Gosselin, 1989). More abundant elements, notably phosphor (P, Z = 15), sodium (Na, Z = 11) and bromine (Br, Z = 35), cause strong interferences, such as a high Compton background and overlapping peaks, thus complicating the recording and interpretation of Ln γ-spectra (Tjoee et al., 1983; Kawamoto et al., 1998). In addition, fission of U in the sample is accelerated by irradiation and
the resulting fission products may interfere with the measurements. To improve the measurement and increase the number of Ln’s to be determined, sample digestion and a chemical group separation are needed (a combination of techniques called radiochemical neutron activation analysis, RNAA).

ID-TIMS is a very sensitive technique for measuring the ten poly-isotopic Ln’s (the application of isotope dilution requires multiple isotopes per element). However, at present ID-TIMS cannot be used in a routinely fashion, since sample preparation is laborious and the technique as a whole is expensive and time-consuming. Determining Ln’s with techniques other than ICP-MS or INAA (e.g. ICP-AES) always requires a chemical group separation and preconcentration steps to remove interferences from other elements and facilitate detection (Markert and Zhang, 1991a). The growing interest in Ln measurements in environmental matrices called for certified reference materials, and recently they have become available for sediment, aquatic organisms (Kramer et al., 1999) and water (Verplanck et al., 2001).

In laboratory studies, the use of γ-ray emitting radionuclides is an unmatched technique to follow Ln behaviour. Its exceptional sensitivity, combined with the fact that γ-rays can be measured without sample digestion, is unique. It even allows in vivo measurements of metal uptake in invertebrates (Weltje et al., in preparation) and plants (Krüger et al., 1999). Radionuclides emitting β-radiation may provide highly sensitive measurements as well. However, samples usually need to be digested before detecting low-energy β-radiation with liquid-scintillation counting (LSC).

1.2.3 Experimental design and methodology

The experimental work in this thesis can be divided into the following parts (chapters in parentheses): field study (3), laboratory accumulation studies with plants (4 and 5) and short-term laboratory toxicity studies with microorganisms (6 and 7). Each of these parts is characterised by a set of techniques and methods that were specifically chosen to reduce variation in the data. Chapter 2 is a literature study and is therefore not considered here.

For the field study of chapter 3, the chosen method for Ln analysis is (HR)-ICP-MS, since it enables determination of all naturally occurring Ln’s in all matrices (see above). Note-worthy is the sampling of sediment pore water with an in situ technique. Apart from the fact that Ln pore water data from freshwater systems are non-existent in the international literature, the applied sampling technique is supposed to yield values that best represent the true pore water composition (Winger and Lasier, 1991). Among the sampled organisms is the free-floating plant Lemna minor, whose populations are usually composed of genetically identical individuals. These plants thus hardly possess variation among individuals and take up Ln’s from a single source, i.e. surface water.

A γ-emitting radionuclide was used in chapters 4 and 5 to study La behaviour in nutrient solution. Chapter 4 focuses on the adsorption of La to different filters during the sterilisation of the solution, whereby cellulose filters are used as a model for passive uptake of La by plant cell walls. In addition, it should be noted that the filter showing the lowest adsorption of La in
chapter 4 was used in chapter 3 for the filtration of surface water. Chapter 5 deals with the uptake and elimination of La by *L. minor* and La adsorption to the glass vessel. Experiments are complemented with speciation calculations to avoid using oversaturated solutions and to study the relation between La speciation and adsorption and uptake. All La fractions are quantified, so that the completed mass balances can be used as an integral part of the compartment modelling of La kinetics. Maintaining solution pH and solution volume was achieved by the use of a buffer and reducing evaporation by partly closing the vessels, respectively. Further changes in solution chemistry are reduced by increasing the solution-to-organism ratio. Biological variation is minimised by choosing a clonal organism (*L. minor*), maintaining a constant growth rate and working at a pH and La concentration which is not adversely affecting the organism.

In chapters 6 and 7, short-term toxicity experiments are described using the bacterium *Vibrio fischeri*. Since these organisms originate from a single strain, their biological variation is limited, hence their widespread use as a standard toxicity test. Positive control treatments with Cu were included and before the experiments were started, the condition of the bacteria was assessed. A simple inorganic medium was chosen that has hardly any complexing capacity for Ln’s and its pH was precisely set (without introducing a pH buffer). Since the applied volumes are too small to be sampled, experiments rely heavily on the speciation calculations. In this case, they are the sole method of analysis. Stability constants for all equilibria were carefully selected from recent literature to complete the database (appendix I).

1.3 Scope

The aim of this work, as stated at the beginning of this chapter, was to gain insight into the environmental fate of lanthanides and their impact on freshwater ecosystems. Since this is a very comprehensive and general goal, three specific aspects were chosen to be dealt with in this thesis: availability, bioconcentration and toxicity. These aspects are critical in assessing the ecotoxicological impact of lanthanides, and also coincide with knowledge gaps, which arose from the literature review given in the previous sections of this chapter. The three chosen aspects and the way they are investigated in this thesis are discussed in logical order, i.e. first availability, then bioconcentration and finally toxicity. The poor solubility of the lanthanides plays a dominant role in the selected aspects, because chemical-biological interactions are causally linked to external metal (species) concentrations. In many of the previous laboratory studies (see sections 1.1.5 and 1.1.6), lanthanide solubility did not receive the attention it deserved and, consequently, led to flaws in experimental set-ups and sometimes to overinterpretation of data. In field studies, on the other hand, analytical difficulties are regularly encountered in quantifying the natural concentrations of heavier Ln’s in organisms and particularly in water. In the latter studies, the focus of attention was therefore mostly on the lighter Ln’s.

*Availability.* The question of which chemical species is or are available for uptake and/or are responsible for toxicity is addressed in five chapters. In chapter 2, the importance of the
soluble Ln fraction is assessed for higher plants, by comparing BCFs of terrestrial and aquatic plants on the basis of soluble concentrations, under the assumption that these should be equal. As this is done by literature review, the condition is that enough data are available to be able to make such a comparison. With measured field data on plants, snails, surface water and sediment pore water (chapter 3), it was attempted to draw conclusions on the importance of both water sources for the uptake of all naturally occurring Ln’s by sediment-rooting plants and also on the importance of surface water and food (plants) for the uptake of Ln’s by snails. For this purpose, the smoothness of lanthanide BCF patterns and the possibly deviating behaviour of Ce in oxic surface water and that of Eu in reducing pore water is used. In addition, the relationship between oxidation state and availability is studied for Ce$^{4+}$, Eu$^{2+}$ and trivalent Ln’s in free-floating and sediment-rooting freshwater plants. Addressing these questions requires accurate measurements of plant, snail and water concentrations of all Ln’s by ICP-MS. The relation between La speciation in nutrient solution and adsorption to cellulose filters, amongst others (chapter 4) can give information on the availability of La species for passive uptake (adsorption) by plant cell walls. In the same solution, La availability and speciation was studied by uptake experiments with the higher freshwater plant Lemma minor (chapter 5). The measurements in chapters 4 and 5 require the use of a technique more sensitive than ICP-MS, i.e. the application of the radiotracer $^{152}$La, which enables us to quantify sub-picomole quantities of La. Furthermore, a full chemical characterisation of the solution is needed, as well as a collection of stability constants for the chemical equilibria involved. In addition, the solution must uphold its sterility, pH and volume, i.e. pH changes and evaporation must be minimised. In chapter 6, the availability of the heaviest lanthanide Lu is tested in a luminescent microbial bioassay, so that conclusions can be drawn on the Lu species responsible for observed effects. Requirements to be met are the use of an exposure medium whose complexing capacity is easily manipulated and its speciation accurately calculated, hence the required equilibrium constants must be available and reliable.

Bioconcentration. BCFs provide information on the extent to which freshwater organisms concentrate Ln’s from surrounding water and therefore if emitted Ln’s are likely to end up in biota. Chapter 2 gives a review of collected and calculated BCFs of Ln’s in higher plants, which requires a certain amount of data to draw a general conclusion. In chapter 3, BCF’s were calculated from field measurements on biota and water concentrations for all naturally occurring Ln’s. From these data, (dis)similarities among lanthanide BCFs may be picked up, as well as a sense of the natural variability. The analyses require sensitive measurements of plant, snail and water concentrations by ICP-MS, especially of the heavier Ln’s. Quantifying the La uptake rate, elimination rate and growth rate of Lemma minor plants (chapter 5) allows for the calculation of a dynamic BCF, which can be compared with steady-state BCFs from life-long exposed duckweed plants from the field (chapter 3). This type of information is needed to validate short-term experiments for extrapolation to the field. Conditions of the La uptake experiments have been described above. A good comparison requires that BCFs from the field truly represent an equilibrium state between plant and water.
**Introduction**

Toxicity. In three chapters, the toxicity of Ln’s is addressed. Growth of La-exposed freshwater plants (*Lemna minor*) was tested against that of an unexposed group (chapter 5). Requirements for these experiments have already been described. Biological variation was limited, since duckweed grows vegetatively and thus consists of genetically identical individuals, i.e. a clone. Validity of the free-ion activity model (FIAM) is studied for Lu in a short-term bacterial toxicity test (chapter 6). The FIAM is mainly based on divalent metals, and was never tested for lanthanides. The speciation calculations require stability constants for all Lu equilibria involved. In this same bioassay, the effects of eleven lanthanides and Sc are compared (chapter 7), to study if the toxicity of these elements is equal or possibly a function of chemical characteristics. To prevent the use of oversaturated solutions, the experiments are supported by speciation calculations, which require stability constants for all lanthanides and Sc equilibria involved. In addition, a comparison with Cd, Cu, Pb and Zn toxicity is made (chapters 6 and 7). Because the toxicity of these ‘heavy’ metals is well established, one can easily rank the Ln’s, which are often considered ‘exotic’ metals, and give them a place in ecotoxicology. This comparison requires that the toxicity of all elements involved is determined under the exact same conditions.
CHAPTER 2

UPTAKE AND BIOCONCENTRATION OF LANTHANIDES IN HIGHER PLANTS:
LINKING TERRESTRIAL AND AQUATIC STUDIES

CHAPTER 2

Abstract

Literature data on lanthanide concentrations in terrestrial and aquatic higher plants are discussed in view of lanthanide availability, bioconcentration and biological effects. In general, the lanthanide distribution pattern of terrestrial plants reflects that of their host soils. This involves a characteristic decrease of lanthanide concentrations with increasing atomic number, where lanthanides with even atomic numbers are more abundant than those with odd numbers. Anomalies in this pattern are the elements cerium and europium (both with concentrations lower than expected) and promethium, which has no stable or long-lived isotopes.

Bioconcentration factors (BCFs) of lanthanides in terrestrial plants relative to soil are generally low (0.00005 - 0.1 kg dry soil·kg⁻¹ dry plant), whereas BCFs in freshwater plants relative to water are high (100 - 46,000 L·kg⁻¹ dry plant). The observed difference in BCFs between terrestrial and aquatic plants is caused by the physical-chemical properties of lanthanides, i.e. their low solubility. For soil ecosystems, this results in low concentrations of dissolved lanthanides in the soil solution, which are available for plant uptake. When BCFs for terrestrial plants are based on the soil solution concentration, they become fully comparable with those observed in aquatic plants.
2.1 Introduction

The lanthanides comprise a group of fifteen elements with atomic numbers 57 to 71, which occur in all natural environments, with the exception of promethium (Pm, Z = 61) which has no stable or long-lived isotopes and, consequently, no natural occurrence anymore. Through weathering and erosion of mother rock, lanthanides become distributed in soil, sediment and water. Background concentrations of individual lanthanides are typically 0.16 - 50 mg kg$^{-1}$ dry soil (Bowen, 1979; Coughtry and Thorne, 1983; Kabata-Pendias and Pendias, 1985) while in the soil solution, concentrations range from 0.04 - 71 µg L$^{-1}$ (Diaslof et al., 1996; Wang et al., 1997b). In sediments, the concentrations of individual lanthanides are 10 - 100 mg kg$^{-1}$ dry sediment (Cowgill, 1973a; Janus et al., 1994) and in freshwater concentrations range from 0.001 - 3 µg L$^{-1}$ (Bowen, 1979; Coughtry and Thorne, 1983; Wolterbeck and van der Meer, 1996; Peng and Wang, 1997). From these concentrations the majority can be attributed to three lanthanides with low atomic numbers, i.e. lanthanum (La, Z = 57), cerium (Ce, Z = 58) and neodymium (Nd, Z = 60).

The lanthanides show a similar chemical behaviour, usually a smooth function of atomic number (Evans, 1990). Combined with their natural occurrence, this results in a typical concentration pattern in the environment, showing higher abundance of lanthanides with lower atomic numbers relative to the heavier lanthanides, and also greater abundance of the even-numbered lanthanides compared to the odd-numbered ones. The latter is known as Oddo-Harkins’ rule (Cowgill, 1973a; Evans, 1990). Environmental concentrations of even-numbered lanthanides decrease approximately log-linearly with increasing atomic number as do the odd-numbered ones (Marken, 1987).

In nature, lanthanides are present in a 3$^+$ valence state. Cerium and europium (Eu, Z = 63) can also occur in oxidation states of 4$^+$ and 2$^+$, respectively. For this reason, both elements regularly show anomalies in the distribution pattern. The environmental chemistry of lanthanides is dominated by their low solubility. Fluorides, carbonates, phosphates and hydroxides may form neutral lanthanide complexes with low solubility products, resulting in low dissolved concentrations in the aqueous phase of ecosystems. In solution, lanthanides may be complexed with inorganic ligands (e.g. hydroxide, carbonate, sulphate) and organic ligands (e.g. humic and fulvic acids). These aspects are of particular interest when the availability of lanthanides to organisms is considered.

Lanthanides have no known biological function and are therefore considered non-essential metals. However, they can be found in virtually all organisms. Terrestrial plants tend to reflect the lanthanide distribution of the soils they grow on (Tjoep et al., 1983; Ichihashi et al., 1992). Concentrations of individual lanthanides in terrestrial plants range from 0.0001 to 140 mg kg$^{-1}$ dry weight (Lal et al., 1979; Ichihashi et al., 1992; Rikken, 1995). Aquatic plants are also known to take up lanthanides from their environment. The relation between aquatic plants and the associated water is more difficult to establish, because very often only a handful of the fourteen lanthanides can be detected in water samples (Cowgill, 1973a; Kovács et al., 1984).
CHAPTER 2

In this paper, plant-lanthanide relationships are considered for both terrestrial and freshwater environments. Aspects of availability, uptake, bioconcentration and biological effects are discussed and some general conclusions are drawn.

2.2 Materials and methods

A literature search was performed to obtain data on lanthanides related to higher plants for both terrestrial and freshwater ecosystems. Studies confined to relating terrestrial plant concentrations to total soil concentrations are not discussed here, because good reviews of these data already exist, e.g. Coughtry and Thorne (1983), Kabata-Pendias and Pendias (1985) and Rikken (1995). Instead, the search was focused on aquatic studies and studies which related available soil or nutrient solution concentrations to those in plants. Terrestrial plant experiments conducted in nutrient solutions provide a means to study lanthanide bioconcentration, without the difficulties associated with the soil matrix. In addition, it is felt that this provides a sounder basis to compare lanthanide uptake by both terrestrial and aquatic plants.

Bioconcentration factors (BCFs) were either taken from the studies or calculated from the data presented therein. The BCF is calculated as the concentration in mg/kg dry weight plant material, divided by the concentration in the environment (mg/kg dry weight soil or mg/L water).

2.3 Results and discussion

2.3.1 General remarks

From the literature studied, some general observations are characteristic of lanthanide behaviour in plants. Firstly, lanthanides are mainly concentrated in the roots of plants (Sun et al., 1997b; Diatloff et al., 1995c; Diatloff et al., 1995d; Pham Thi Huynh et al., 1997). This observation can be explained by fast surface adsorption, which appears to be the controlling mechanism for lanthanide uptake (Marcialoniene, 1980; Kryshev and Sazykina, 1994). In plants, lanthanides are transported from the roots to the shoots and may also enter the plant cell. The latter was a topic of discussion, but evidence for lanthanide presence in plant cells was found by Robards and Robb (1974), Van Steveninck et al. (1976) and Wolterbeek and van Die (1980) among others. The restriction of both uptake through the cell membrane and lanthanide transport to the shoots are considered plant-protection mechanisms from adverse effects of lanthanides (Diatloff et al., 1995a).

Bioconcentration factors for terrestrial plants relative to soil are low (~ 0.001 kg dry soil/kg dry plant), whereas BCFs for freshwater plants relative to water are high (~ 1,000 L/kg dry plant). This topic will be discussed further in the section Bioconcentration. At higher concentrations lanthanides inhibit plant growth (Pickard, 1970; Van Steveninck et al., 1976; Diatloff et al., 1995b), see also the section Effects in plants. Finally, lanthanides, but especially lanthanum, interfere with calcium
and magnesium metabolism in plants (Pickard, 1970; Nair et al., 1989; Rengel, 1994; Ishikawa et al., 1996).

2.3.2 Distribution patterns

In almost all terrestrial plant studies the characteristic lanthanide distribution, as indicated in the introduction, is shown (e.g. Tjioe et al., 1983; Markert, 1987; Markert and Zhang, 1991b; Rikken, 1995; Ozaki et al., 1997). For aquatic plants, data on this distribution are scarce. However, for one freshwater plant, data are available to construct such a curve. Figure 2-1 shows the distribution pattern of nine lanthanides in the waterlily (Nymphaea odorata) and the sediment it roots in (data taken from Cowgill, 1973a). The odd-numbered elements 63, 65, 67, 69 and 71 (promethium is 61) were not detected in the waterlily nor in sediments and none of the lanthanides were detected in twenty-fold concentrated water. The observed pattern does not indicate disagreement with Oddo-Harkins’ rule. Compared to the sediment, the heavier lanthanides appear to be slightly enriched in the waterlily. This might be related to a higher availability of the heavier lanthanides, which have higher solubilities, for instance in the presence of phosphates (Liu and Byrne, 1997).

![Graph](image)

**Fig. 2-1.** Distribution pattern of nine lanthanides in the waterlily (Nymphaea odorata) and the sediment it roots in, against atomic number, Z (data from Cowgill, 1973a). For further explanation see text.

For terrestrial plants various degrees of fractionation were observed, leading to both enrichment and depletion of certain (groups of) lanthanides (Milton et al., 1944; Wytenbach et al., 1996; Wang et al., 1997a; Ozaki et al., 1997). From terrestrial studies it is apparent that Ce and Eu often cause negative anomalies in the distribution pattern (Laat et al., 1979; Guo et al., 1996). The Ce-anomaly is caused by the Ce$^{4+}$ valence state in soils, which is apparently less available for plant uptake (Wytenbach et al., 1996; Ozaki et al., 1997). The Eu anomaly in plants is probably related to the Eu$^{2+}$ valence state and is often already present in the host soil.

Furthermore, Ozaki et al. (1997) showed a seasonal variation of lanthanide concentrations in ferns, with the highest concentrations occurring in springtime. Volokh et al. (1990) showed that plants can reflect environmental pollution with lanthanides, which is caused by air emissions from
Chapter 2

phosphate fertiliser producers. Peresedov et al. (1997) established a clear relationship of decreasing lanthanide concentrations in pine needles and soil with increasing distance from phosphoric fertiliser industries.

2.3.3 Bioavailability

Uptake of metals by plants takes place via the soil solution (Hunter et al., 1987), sediment pore water and/or the surrounding freshwater. It is thereby assumed that the free-metal ion is the available chemical species for biota. Some evidence to support this hypothesis for lanthanides can be found in the studies of Sun et al. (1997b) and Knaus and El-Fawaris (1981), who showed that for lanthanide-EDTA complexes in solution, BCFs in roots were approximately one order of magnitude lower than BCFs from experiments with non-chelated lanthanides. Interestingly though, were the higher La and gadolinium (Gd, Z = 64) concentrations in wheat shoots when chelated forms of these elements were offered in the nutrient solution (Sun et al., 1997b). The same was observed for Pm BCFs in shoots of beans (Iessington et al., 1963). These data indicate that plant-lanthanide relations are complex and also depend on various plant factors. Another factor that influences the availability of lanthanides is soil or solution pH. At lower pH, more lanthanides are present in both nutrient and soil solution (Coughtrey and Thorne, 1983; Dietloff et al., 1995c; Dietloff et al., 1996; de Boer et al., 1997). Scott-Russell (1966) showed that uptake of Ce by peas from solution was higher at lower pH.

In aqueous systems, lanthanide-phosphate, -hydroxide and -carbonate solubility products limit the amount of lanthanides in solution. For this reason concentrations of dissolved lanthanides are often approaching the analytical limits of detection. Calculated sediment-water partition coefficients for La, Ce and Nd for freshwaters in The Netherlands, indicate that the concentration factor for these lanthanides in sediments is circa 4×10^3 L·kg⁻¹ (van Wezel et al., 1997). Coughtrey and Thorne (1983) calculated values of 5×10^3 to 2×10^3 L·kg⁻¹ for sediment-water partition coefficients. Calculations on the data of Zhang et al. (1997) showed that for different Chinese soils only 0.07% of the present lanthanides was water extractable. It is obvious that these high partition coefficients are the underlying reason for the large differences between terrestrial plants (BCF related to total soil concentrations) and water plants (BCF related to water concentrations).

2.3.4 Bioconcentration

Bioconcentration of lanthanides from soil by terrestrial plants has been reviewed by Coughtrey and Thorne (1983) and Rikken (1995). With some exceptions, BCFs for lanthanides in terrestrial plants relative to soil are in the order of 5×10⁻¹ to 1×10¹ kg dry soil·kg⁻¹ dry plant. Higher BCFs, between 1 and 10, were found for hickory trees (Carya spp) and ferns (Robinson et al., 1958; Ozaki et al., 1997). BCFs for the waterfowl relative to sediment range from 1.0 to 5.5 kg dry sediment·kg⁻¹ dry plant (see also Fig. 2-1).

Bioconcentration factors for lanthanides in freshwater plants relative to water are presented in Table 2-1. As the table shows, more data are available for lighter lanthanides (La - Nd), than for the heavier ones (Pm - La). This is due to a combination of their natural occurrence and analytical difficulties. The high BCFs for *C. demersum* compared to *A. filicaulis* from the same ditch, could be
the result of different exposure; while *C. demersum* is fully submerged, *A. filicinoides* is free floating (Wolterbeek and van der Meer, 1996).

**Table 2-1.** BCFs (in L·kg⁻¹ dry weight) for different lanthanides in aquatic plants relative to freshwater

<table>
<thead>
<tr>
<th>plant species</th>
<th>La</th>
<th>Ce</th>
<th>Pr</th>
<th>Nd</th>
<th>Prn</th>
<th>Sm</th>
<th>Eu</th>
<th>Lu</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chara trichiata</em></td>
<td></td>
<td></td>
<td>2,600</td>
<td>3,000</td>
<td>&lt;6,000</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Polymotrema perfoliatum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>average of 32 species</td>
<td>7,100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>average of 4 species</td>
<td>3,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>average of n species</td>
<td>5,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>average of n species</td>
<td>4,600</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
</tbody>
</table>

* Prn data were obtained by using radionuclides, † based on wet weight, which implies that for dry weight the BCF is approximately 20-fold higher. References: 1 = Wolterbeek and van der Meer (1996), 2 = Kowack et al. (1984), 3 = Marcqulie (1980), 4 = Timofeeva-Rysovskaya et al. (1961), 5 = Kryshen and Sazykina (1994), 6 = Coughtrye and Thorne (1983), 7 = Polikarpov (1966)

In Table 2-2 the BCFs for terrestrial plants relative to aqueous media *i.e.* freshwater, nutrient solution and soil solution are presented. It is clear from these tables that BCFs for lanthanides in aquatic plants are high (1×10⁵ - 5×10⁶ L·kg⁻¹ dry plant) and match those in terrestrial plants, when the latter are related to solution concentrations. This suggests that terrestrial and aquatic plants behave similarly towards lanthanides. Moreover, and also in view of bioavailability, it is more relevant to compare terrestrial plant concentrations with soil solution concentrations, than with total soil concentrations. Also, fractionation of lanthanides by terrestrial plants should be judged against soil solution concentrations.

In the literature it is implicitly assumed that BCFs are independent of the lanthanide concentration in solution. Unfortunately, there are not enough data (plant lanthanide concentrations as a function of lanthanide concentration in solution) available to test this hypothesis.


**Chapter 2**

**2.3.5 Effects in plants**

Solution concentrations of <10 μM La, Ce and ytterbium (Yb, Z = 70) severely inhibit plant growth (Kinraide et al., 1992; Diatloff et al., 1995b, 1995c, 1995d; Ishikawa et al., 1996). When lanthanides are taken up by plants they can disturb calcium and -to a lesser extent- magnesium metabolism (Nair et al., 1989). Some authors suggest that calcium interference of the lanthanides, and especially lanthanum, is the cause for their toxicity. Velasco et al. (1979) suggests that lanthanides replace the essential element boron from its active sites and thus induce boron deficiency. Diatloff et al. (1995d) demonstrated manganese deficiency in mungbean plants exposed to solutions containing Ce concentrations above 0.63 μM.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>La</th>
<th>Ce</th>
<th>Nd</th>
<th>Gd</th>
<th>Dy</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alnus nitida</td>
<td>1,200</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zea mays b</td>
<td>23,000</td>
<td>18,000</td>
<td>15,000</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Triticum spp. c</td>
<td>930 d</td>
<td>1,400 d</td>
<td></td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Zea mays d</td>
<td>&gt;1,300</td>
<td>20,000</td>
<td></td>
<td></td>
<td>4,5</td>
<td></td>
</tr>
<tr>
<td>Vigna radiata e</td>
<td>&gt;21,000</td>
<td>36,000</td>
<td></td>
<td></td>
<td>4,5</td>
<td></td>
</tr>
</tbody>
</table>

+ based on freshwater, b based on soil solution, c based on nutrient solution, d based on wet weight, which implies that for dry weight the BCF is approximately 20-fold higher. References: 1 = Kraus and El-Fawaris (1981), 2 = Wang et al. (1997b), 3 = Sun et al. (1997b), 4 = Diatloff et al. (1995c), 5 = Diatloff et al. (1995d)

Besides toxic effects, greater biomass and increased root growth were observed after exposure to low lanthanide concentrations (Velasco et al., 1979; Diatloff et al., 1995d; Pham Thi Huynh et al., 1997). The mechanism for this stimulatory effect is yet unknown (for discussion see also Diatloff et al., 1995a). An explanation could be the occurrence of hormesis: a regulatory overcorrection by biosynthetic control mechanisms, following exposure to a low toxicant concentration (Stebbing, 1982). It also has been suggested that lanthanides increase uptake and transport of phosphates (Evans, 1990). In China, farmers apply lanthanide-containing fertilisers to improve their crop (Brown et al., 1990; Hong et al., 1997).

Only recently, two lanthanide binding proteins, probably glyco-proteins, were identified in the terrestrial fern Dicranopteris dichotoma (Guo et al., 1996) which is known to accumulate lanthanides up to 3,358 mg·kg⁻¹ in its leaves (Wang et al., 1997a). Whether this protein is induced by exposure to lanthanides and acts as a detoxification mechanism, remains to be solved.

**Acknowledgements**

The author is grateful to Prof.dr.ir. Jeroen de Goeij for helpful comments on a previous version of this paper.
CHAPTER 3

LANTHANIDE CONCENTRATIONS IN FRESHWATER PLANTS AND MOLLUSCS, RELATED TO THOSE IN SURFACE WATER, PORE WATER AND SEDIMENT

CHAPTER 3

Abstract

Industrial emissions of lanthanides to aquatic ecosystems increase, but the knowledge of the environmental fate of these metals is limited. This paper focuses on the distribution of lanthanides in freshwater ecosystems, describing lanthanide partitioning between sediment, water and biota. Since lanthanides are often used as oxidation state analogues for actinides, their distribution may provide insights in long-term behaviour of the radioactive transuranics as well. Concentrations of all 14 naturally occurring lanthanides were measured by ICP-MS in Sago pondweed (*Potamogeton perfoliatus*), common duckweed (*Lemma minor*), seven different mollusc species (tissue and shell), two sediment fractions (< 2 mm and < 63 μm), surface water and sediment pore water from five locations in The Netherlands.

In all samples the typical ‘saw-tooth’ lanthanide pattern was observed, which implies that lanthanides are transported as a coherent group through aquatic ecosystems. Typical deviations from this pattern were found for Ce and Eu and could be explained by their redox chemistry. The variation in concentrations in abiotic fractions was limited, i.e. within one order of magnitude. However, variations up to three orders of magnitude were observed in biotic samples, suggesting different affinities among organisms for lanthanides as a group, with significant differences only among molluscs and pondweed samples, associated with sampling location. For *P. perfoliatus* it was shown that pore water was the most important lanthanide source and for snails, food (plants) seems to be the dominant lanthanide source. Lanthanides were not equally distributed between mollusc shell and tissue and the ratio of lanthanide concentrations in shell and tissue proved to be dependent on the sampling location. Shells contained much lower concentrations, and were relatively enriched in Eu and to a lesser extend also in Ce.

Bioconcentration factors for lanthanides in plants and snails relative to surface water were typically between 10,000 and 100,000 L·kg⁻¹ dry matter, while sediment-water partition coefficients were between 100,000 and 3,000,000 L·kg⁻¹ dry matter. There was a low extent of biomagnification in the plant-to-snail system, with a maximum biomagnification factor of 5.5. Many distribution coefficients displayed a trend of slight decrease with atomic number, which may be attributed to the general increase of ligand stability constants with atomic number, keeping the heavier lanthanides preferentially in solution.
FIELD CONCENTRATIONS OF LANTHANIDES IN FRESHWATER ECOSYSTEMS

3.1 Introduction
The 14 naturally occurring lanthanide elements with atomic numbers $Z = 57-60$ and $Z = 62-71$, form a group of trivalent metals, which show a typical concentration pattern in environmental samples (Vickery, 1953; Goldberg et al., 1963; Piper, 1974; Markert, 1987). The pattern can be described as a log-linear decrease of concentration with atomic number and, in addition, the odd-numbered elements have lower concentrations than the nearly even-numbered ones, known as Oddo-Harkins’ rule (Oddo, 1914; Harkins, 1917). The logarithm of the abundance against atomic number displays a saw-tooth pattern, which is strongly conserved as the lanthanides disperse from indigenous rocks to soils and sediments, water and eventually biota. Conservation of this pattern results from the great similarity in chemical behaviour among the lanthanides. To correct for the substantial differences in abundance of individual lanthanides, their concentration patterns are often normalized to the composition of chondritic meteorites or shale, resulting in a straight and horizontal line if no fractionation, relative to the normalizing material, occurs (e.g. Tijoe et al., 1983; Schiff, 1992). Deviations from a straight and horizontal line may provide geologists with information on geochemical changes during formation, weathering and transport of the material. Using normalization and the strong correlation between the lanthanides, Markert et al. (1989) formulated a mathematical model to calculate and predict lanthanide concentrations in soil and plant samples, from the known concentration of La ($Z = 57$), the first lanthanide. In addition, normalization can reveal additional enrichments of a single or more lanthanides, which may be caused by anthropogenic input or originate from an indigenous rock composition, differing from that of chondrites or shale. In past years, anthropogenically caused enrichments have increased due to the growing uses and applications of (mostly single) lanthanides in various types of industries, resulting in contamination of mainly the aquatic environment (Sneller et al., 2000).

Common exceptions to the typical lanthanide pattern are Ce ($Z = 58$) and Eu ($Z = 63$), which exhibit a $4^+$ and $2^+$ oxidation state, respectively, next to the $3^+$ oxidation state common for all lanthanides. These transitions in oxidation state influence the behaviour of Ce and Eu (for instance, Ce$^{4+}$ in oxic water forms the very insoluble CeO$_2$) and cause so-called ‘anomalies’ for these two elements in the typical lanthanide pattern (de Baar et al., 1991; Erel and Stolper, 1993). Redox cycling of Ce and (co)precipitation of lanthanides in general, has shown to be closely linked with iron and manganese (Elderfield and Sholkovitz, 1987; de Baar et al., 1988; Bau, 1999).

Shale-normalized lanthanide patterns of biota provide no insight into availability and bioconcentration of lanthanides by organisms, but instead give information on the degree of similarity between lanthanide concentrations in organism and shale. To obtain information on availability and bioconcentration of lanthanides, it is crucial to relate or normalize concentrations in organisms to those in the environmental phase they take up lanthanides from, e.g. water phases for plants and foodstuff and water for animals.

The importance of a metal’s chemical mobility is the link to its bioavailability. Changes in metal mobility may therefore be reflected in its concentration in an organism. Since lanthanides
can be determined in practically all biota (Bowen, 1979), they must be available for uptake. Bioconcentration factors (BCFs) of lanthanides in algae, plants and molluscs from the field, relative to water, have been measured, reaching values of up to 3,000,000 L·kg\(^{-1}\) dry weight in marine algae (Hou and Yan, 1998; Weltje, 1998c; Tijink and Yland, 1998). These values are among the highest recorded for metals (Hou and Yan, 1998) and are due to the low solution concentrations combined with the appreciable amount of lanthanides taken up. In physiological studies, lanthanides and especially La are frequently used to inhibit the uptake of Ca\(^{2+}\), which has a similar ionic radius, but a lower charge density than the trivalent lanthanide ion (Evans, 1990). Although lanthanides are taken up by organisms, they have no established biological function and are therefore considered non-essential metals. However, some studies reported beneficial effects of lanthanides on plant growth and in China lanthanide-containing fertilisers are applied (for a discussion see Dieloff et al. (1995a)).

The present work aims at gaining insight into the concentrations and partitioning of lanthanides in freshwater ecosystems, with an emphasis on the following compartments: sediment (the fractions < 2 mm and < 63 μm), sediment pore water, surface water, plants (rooting in sediment and free-floating on the water surface), snails (which feed on plant parts) and bivalves (which are filter-feeders). The organisms chosen have different exposure routes and it is therefore expected to find differences among them, judged against lanthanide concentrations in the environmental compartment from where they take up lanthanides. As a starting point hypotheses are formulated to be tested with field measurements. The hypotheses are divided into two groups: those considering abiotic behaviour and those considering interaction with biota. Ranked in increasing order of detail, they are as follows:

**Abiotic behaviour**

1. Lanthanides behave as a group, because of their chemical similarity. This results in largely the same concentration pattern in abiotic samples (i.e. water and sediment) and therefore comparable sediment-water partition coefficients.
2. If differences occur in sediment-water partition coefficients within the lanthanide group, they are small and a smooth function of atomic number, as are their chemical characteristics.
3. Lanthanide pore water concentrations are higher than surface water concentrations, due to the lower pH in the former.
4. Exceptions to the hypotheses 1-3 may be Ce, whose behaviour can deviate in (oxic) surface water, and Eu, whose behaviour can deviate in (anoxic) pore water.
5. Lanthanide concentrations in the sediment fractions < 2 mm and < 63 μm are expected to be approximately equal, because lanthanides are considered primarily part of the particle matrix.

**Interaction with biota**

6. Lanthanide concentrations in floating plants are the result of uptake from surface water (uptake from air is considered negligible).
7. Lanthanide concentrations of sediment-rooting (whole) plants are the result of uptake from both surface water and pore water.
8. Lanthanide concentrations in snails are the result of uptake from surface water (bioconcentration) and food, i.e. plants (biomagnification).
9. Lanthanide concentrations in filter-feeding bivalves are the result of uptake from surface water, with possibly a contribution from pore water for sediment-burrowing species.
10. Lanthanides are equally distributed between mollusc shell and tissue, implying the shell gland has no selectivity in passing on lanthanides from tissue to shell.
11. Possible small differences in BCFs within the lanthanide group are a smooth function of atomic number, as are their chemical characteristics.

Testing these hypotheses aims at obtaining more insight into the consequences of lanthanide partitioning between water and sediment for uptake and bioconcentration in freshwater organisms. Another aim is to identify the dominant uptake route for lanthanides in sediment-rooting plants (i.e. surface water or pore water) by studying the BCFs and using the possibly anomalous behaviour of Ce and Eu. The BCF, based on the dominant uptake route, is expected to have similar values for all lanthanides, and thus to display a pattern with less or less pronounced anomalies. The implicit assumption here is that availability is independent of oxidation state, e.g. Eu$^{3+}$ and Ce$^{4+}$ are equally available as trivalent lanthanides. Also, biomagnification of lanthanides is hardly studied. Knowledge on these topics is required to assess the environmental fate and impact of lanthanides in aquatic ecosystems. In addition, it may provide insight in the long-term behaviour of the radioactive transuranic elements, i.e. actinides, for lanthanides are generally considered to be analogues for actinides, provided they have the same oxidation state (Boniforti, 1987; Bierkens and Simkiss, 1990; Choppin, 1991).

### 3.2 Materials and methods

#### 3.2.1 Sampling locations

Five locations in The Netherlands (indicated in Fig. 3-1), four of them in the highly industrialized region around the city of Rotterdam, were selected on the basis of the occurrence of Potamogeton pectinatus and secondly for their different characteristics; the latter were anticipated to result in different lanthanide concentrations and/or concentration patterns. All sampling locations are situated in the catchment of the Rhine and Meuse rivers and hence the sediments originate from the same source, a well-mixed riverine flood plain. Samples were taken in August and September 2000, the time when *P. pectinatus* has its highest biomass (van Wijk, 1988). At all locations, temperature of surface water and sediment, and pH of surface water and pore water were measured (see Table 3-1). The locations are shortly characterised as follows: 1) Veluwemeer, Elburg. A large and open freshwater body, well-mixed and mainly used for recreational purposes. This *P. pectinatus* population has been well characterised by van Wijk (1988 and 1989) and serves as a reference to the Rotterdam populations. The sediment is sandy. 2) Botlekpark, Rozenburg. A small, shallow and stagnant drinking pool used by cows, 50 m from the river Nieuwe Waterweg, the entrance to the Rotterdam harbour. In this river, the main part of industrial lanthanide emis-
Fig. 3-1. Map of The Netherlands (NL = The Netherlands, D = Germany, B = Belgium). Arrows with numbers indicate sampling locations (1 = Veluwemeer, 2 = Botelopark, 3 = Kralinge Plas, 4 = Nieuwe Maas, 5 = Charlois).

...s to the Dutch environment occurs (Tijink and Yland, 1998), and here it is expected to find higher concentrations of all lanthanides. Sediment contains some sand. 3) Kralinge Plas, Rotterdam. A ditch in a recreational area, 25 m from the main lake, located within the municipality of Rotterdam; the sediment is rich in organic matter. Possibly higher lanthanide concentrations may be found here, depending on the previous land use. 4) Nieuwe Maas, Rotterdam. One of the branches of the river Rhine with many ships travelling from inland to the Rotterdam harbour and vice versa. This river undergoes tidal influence from the North Sea and this water is therefore the most dynamic of the five sampling sites and is slightly brackish. The local difference between high and low tide is approximately 1.7 m. The sediment is sandy. 5) Charlois, Rotterdam. A small and stagnant ditch beside a provincial road, which in addition serves as a drinking spot for horses. This site may be influenced by traffic related input of La and Ce (Ballach, 1997; Rauch et al., 2000). Sediment is very rich in organic matter and strongly anoxic.

Table 3-1. Temperature and pH of surface water and pore water from all sampling locations

<table>
<thead>
<tr>
<th>location</th>
<th>surface water</th>
<th></th>
<th>pore water</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp. (°C)</td>
<td>pH (SD, n = 2)</td>
<td>Temp. (°C)</td>
<td>pH (SD, n = 2)</td>
</tr>
<tr>
<td>Veluwemeer</td>
<td>21</td>
<td>8.72 (0.01)</td>
<td>21</td>
<td>7.55 (0.21)</td>
</tr>
<tr>
<td>Botelopark</td>
<td>19</td>
<td>8.61 (0.04)</td>
<td>19</td>
<td>7.37 (0.33)</td>
</tr>
<tr>
<td>Kralinge Plas</td>
<td>20</td>
<td>7.89 (0.05)</td>
<td>19</td>
<td>7.12 (0.12)</td>
</tr>
<tr>
<td>Nieuwe Maas</td>
<td>18</td>
<td>8.17 (0.08)</td>
<td>18</td>
<td>7.52 (0.08)</td>
</tr>
<tr>
<td>Charlois</td>
<td>19</td>
<td>7.30 (0.04)</td>
<td>18</td>
<td>6.92 (0.01)</td>
</tr>
</tbody>
</table>
3.2.2 Sampling procedures

Surface water samples \((n = 3)\) were filtered through 0.45-µm membranes (Sartolon polyamide, i.e. nylon 6, Ø 50 mm, Sartronius) using the polycarbonate Anlia Pressure Filtration System (Schleicher & Schuell, Dassel, Germany), which had been thoroughly rinsed with diluted 65% HNO\(_3\) (Merk, Suprapur) and Milli-Q water (Millipore-Waters, Milford, MA, USA). Nylon membranes were chosen, because sorption of La and other metals to this material is negligible (Weltje et al., 2003). Filters were replaced with plastic tweezers before a new sample was taken or when clogging of the filter made it necessary. With at least five repetitions, a volume of ca. 200 mL was collected in 250-ml polyethylene (PE) bottles, which had been thoroughly rinsed with diluted 65% HNO\(_3\) (Merk, Suprapur), then Milli-Q water and equilibrated with sample. Samples were acidified with 500 µL 70% HNO\(_3\) (J.T. Baker, Ultrex Ultrapure Reagent) and stored in a dark cool place. The pH of surface water was directly measured in the bulk \((n = 2)\), using a wireless Mess-Stab 656 pH meter (Knick, Berlin, Germany), equipped with an Inlab 417 electrode (Mettler-Toledo, Greifensee, Switzerland).

Pore water samples \((n = 3)\) were extracted \textit{in situ} by using Rhizcon Soil Moisture Samplers (MOM type, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). This type of technique is thought to yield samples that best reflect the true pore water composition (Winger and Lasier, 1991). A Rhizcon sampler consists of a 10-cm microporous polymer rod (Ø 2.5 mm, average pore size 0.1 µm), which is reinforced with glass-fibre epoxy wire and attached to a tube (polyvinyl chloride (PVC) outside, PE inside) fitted with a Luer-Lock connector. The Luer-Lock enabled the attachment of a syringe, which had been thoroughly rinsed with diluted 65% HNO\(_3\) (Merk, Suprapur) and Milli-Q water. The Rhizcon sampler was inserted into the sediment, ensuring that the porous rod was completely below the sediment surface. Vacuum was applied by retracting and bracing the syringe plunger. After 5 to 15 minutes the syringe contained 9 mL of pore water. This volume was collected in 10-ml PE bottles, which had been thoroughly rinsed with diluted 65% HNO\(_3\) (Merk, Suprapur), then Milli-Q water and equilibrated with sample. Samples were acidified with 20 µL 70% HNO\(_3\) (J.T. Baker, Ultrex Ultrapure Reagent) and stored in a dark cool place. Additional samples were taken for immediate pH measurements \((n = 2)\), using a wireless Mess-Stab 656 pH meter (Knick, Berlin, Germany), equipped with an Inlab 417 electrode (Mettler-Toledo, Greifensee, Switzerland).

Sediment samples \((n = 3)\) were collected with a bottom sampler according to Ekman-Birge (Hydro-bios, Kiel, Germany). Approximately 200 mL of sediment was collected from the sampler in 250-ml PE bottles, which had been thoroughly rinsed with diluted 65% HNO\(_3\) (Merk, Suprapur) and Milli-Q water. Samples were stored in a dark cool place. All sediments were anaerobic, judged by their dark grey colour and the smell of H\(_2\)S, which also came from the pore water samples.

Sago pondweed (Potamogeton pretensis) samples \((n = 3)\) were collected with a rake and washed in local water to remove attached sediment. Both roots and shoots were included. Aufwuchs (algae, fungi etc. growing on the plants) was removed as much as possible. Plants were put
in plastic bags, which were placed in a freezer.

Common duckweed (*Lemma minor*) samples (*n* = 2) were collected with a small plastic sieve, after which non-desired material was removed with plastic tweezers. The resulting material was put into plastic vials, which were placed in a freezer. *L. minor* was present at three locations (2, 3 and 5). At location Charlois (5) samples consisted of a mixture of *L. minor* and giant duckweed (*Spirodela polyrhiza*).

Several species of molluscs were sampled (*n* = 2 to 20), depending on which species was found at the sampling site, snails (with sampling site in brackets): *Potamopyrgus antipodarum* (1), *Lymnaea stagnalis* (5), *Radix exita* (1, 2), *Physella acuta* (5), *Planorbis cornus* (5), *Planorbus planorbid* (5) and at location Nieuwe Maas (4) bivalves *Dreissena polymorpha* and *Corbicula fluminea*. Molluscs were hand-collected and put into plastic vials with native water. They were allowed to empty their guts for 3 to 5 h, after which shells were cleaned on the outside with a soft plastic brush and molluscs were put into plastic vials, which were placed in a freezer.

### 3.2.3 Analysis

Surface water and pore water samples were directly measured by HR-ICP-MS (Finnigan MAT Element, Bremen, Germany) equipped with a Teflon concentric nebulizer. $^{115}$In was used as an internal standard and for optimizing the operating parameters and ion lens voltages. In order to reduce the risk of contamination, all work on the water samples was carried out on a clean bench; this included the ICP-MS sample introduction system and nebulizer. Before measurement, all containers and chemicals were checked for contamination by analysis of blank values. Nebulizer, spray chamber, torch, Teflon tubes etc. were cleaned by rinsing with 7 M HNO$_3$ (J.T. Baker, Uitrex Ultrapure Reagent) for 24 h and then with ultrapure water. Recoveries were checked with standard additions for all lanthanides.

Sediment was dried at 80°C for 40 h, crushed with pestle and mortar and sieved (Analytical Sieve Shaker AS 200 control ‘g’, Retsch, Haan, Germany) through a 2-mm mesh stainless steel sieve. About one third of the material passing the sieve was kept apart for analysis and the remainder was sieved through a 63-μm mesh stainless steel sieve. The material passing this sieve (i.e. silt) was also sampled for analysis. In between samples, sieves were cleaned with pressure air. Prior to digestion, the sediment fraction < 2 mm was milled (Vibratory Disc Mill RS 1 of Specialty steel, Retsch, Haan, Germany, at 700 rpm for 60 s), which was not necessary for the sediment fraction < 63 μm. Due to the nature of the sediment matrix (partly silicate) and the way in which lanthanides are incorporated in minerals, such as monazite, apatite etc. and Fe-Mn constituents (Zhang et al., 1998), it was the sample type most difficult to digest. Digestion of silicate requires HF and this introduces the problem of lanthanide-fluoride precipitation, which in turn is solved by adding boric acid. A fine-tuned combination of acids, digestion time and microwave heating programs (adapted from Ivanova et al., 2001a) eventually gave satisfying results for the reference material. About 0.1 g dry sediment was placed in a polytetrafluoroethylene (PTFE) pressure vessel and 4 mL 65% HNO$_3$, 2 mL 30% H$_2$O$_2$ and 1 mL 40% HF (all Merck, Suprapur)
were added. Vessels were closed and left standing overnight after which a microwave (MLS 1200 mega, Milestone, Bergamo, Italy) program was applied (Table 3-2, Program 1). After cooling down for 30 min, 0.5 mL of 40% HF was added and microwave Program 1 was applied again. After cooling down for another 30 min, 5 mL of boric acid solution (5.00 g H$_3$BO$_3$, Merck pro analysis in 100 mL. Milli-Q water) was added to dissolve precipitated fluorides and microwave Program 2 was applied. The sample was brought to a volume of 50 mL with Milli-Q water in volumetric polymethylpentene (PMP) flasks (VIT-LAB, Secheim-Jugenheim, Germany). For analysis, this was diluted tenfold with 3% HNO$_3$ (Merck, Suprapur).

### Table 3-2. Microwave programs for digestion procedures

<table>
<thead>
<tr>
<th>Program 1</th>
<th>Program 2</th>
<th>power step</th>
</tr>
</thead>
<tbody>
<tr>
<td>time (min)</td>
<td>time (min)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>250 W</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>400 W</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>500 W</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>650 W</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>300 W</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>ventilation</td>
</tr>
</tbody>
</table>

Lanthanide concentrations in total sediment digests were measured with a quadrupole Perkin-Elmer Elan 6000 ICP-MS equipped with a cross-flow nebulizer. Prior to the measurements, the ICP-MS was optimized for oxide formation, by means of the ratio CeO$^+$/Ce$^+$, and for formation of double-charged ions, by means of the Ba$^{2+}$/Ba$^+$ couple (for more details on instrumental parameters see Ivanova et al., 2001b). External calibration was performed with multi-element standard solutions (Merck IV and CLMS-1, SPEX). Each sample was measured with three repetitions to generate a measurement average with standard deviation. Mathematical corrections for isotopic overlap were applied, using the built-in Perkin-Elmer software routines. The reference material BCR 667 Estuarine Sediment (European Commission, Institute for Reference Materials and Measurements (IRMM); Geel, Belgium) was used, which is part of a series of reference materials especially prepared for lanthanide determinations in environmental matrices (Kramer et al., 1999).

Plant material was dried at 60°C overnight and *P. pectinatus* was milled (45 s at 700 rpm, Vibratory Disc Mill RS 1 of Specialty steel, Retech, Haan, Germany). It was not necessary to mill *L. minor* material as this was much easier digestible than *P. pectinatus* material. About 0.2 g dry plant material was placed in a PTFE pressure vessel and 4 mL 70% HNO$_3$ (J.T. Baker, Ultrex Ultrapure Reagent), 2 mL 30% H$_2$O$_2$ and 1 mL 40% HF (both Merck, Suprapur) were added and microwave Program 1 was applied. After cooling down, the samples were brought to a volume of 50 mL with Milli-Q water in volumetric PMP flasks (VIT-LAB, Secheim-Jugenheim, Germany). This was diluted two- or tenfold with 3% HNO$_3$ (Merck, Suprapur) for analysis. For *L. minor* there was usually less material available and hence amounts of added acid and correspond-
CHAPTER 3

ing volumes were halved. Lanthanide concentrations in plant digests were measured as described for the sediment, using the reference materials SRM 1515 Apple Leaves (National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA) and BCR 670 Aquatic Plant (European Commission, IRMM, Geel, Belgium). The latter material consists of L. minor plants (Kramer et al., 1999).

Molluscs were separated with tweezers under a dissecting microscope into shell and soft tissue. Soft tissue was freeze dried (ALPHA 1-4, Christ, Osterode, Germany). About 0.2 g dry tissue was placed in a PTFE pressure vessel and 4 mL 70% HNO₃ (J.T. Baker, Ultrex Ultrapure Reagent) and 2 mL 30% H₂O₂ (Merck, Suprapur) were added and microwave Program 2 was applied. After cooling the samples down they were brought to a volume of 50 mL with Milli-Q water in volumetric PMP flasks (VIT-LAB, Secheim-Jugenheim, Germany). This was diluted two- or tenfold with 3% HNO₃ (Merck, Suprapur) for analysis. If there was little material available then the amounts of added acid and corresponding volumes were halved. Shells were dried at 60°C and crushed; shells of bivalves were milled (at 700 rpm for 30 s, Vibratory Disc Mill RS 1 of Specialty steel, Retsch, Haan, Germany). Digestion was the same as for soft tissue. Lanthanide concentrations in mollusc digests were measured as described for sediment, using the reference material BCR 668 Mussel Tissue (European Commission, IRMM, Geel, Belgium) (Kramer et al., 1999).

3.2.4 Data handling and statistics

In order to achieve some insight into the differences between locations, concentrations of lanthanides in all analysed samples from the five sampling locations were subjected to one-way analysis of variance (ANOVA), using the software package Prism, Version 2.01 (GraphPad Software, San Diego, CA, USA). A significant ANOVA (p < 0.05) was followed by a post-test, Tukey’s test, comparing pairs of locations. To remove the overwhelming Oddo-Harkins’ effect in the lanthanide concentration pattern of our samples, concentrations in each sample were divided by the average shale concentrations (taken from de Baar et al., 1985 and 1988). This procedure provides a better resolution for studying the differences between samples. It was not our objective to study differences between our samples and shales.

3.3 Results

3.3.1 General

Some lanthanide concentration measurements were based on the analysis of a single isotope only, i.e. 139La, 140Ce, 141Pr, 149Tb, 150Ho, 150Tm and 175Lu, due to either their occurrence as single isotope elements (Pr, Tb, Ho and Tm) or a clear dominance of one of the isotopes (in case of 139La, 140Ce and 175Lu, which have abundances of 99.9%, 88.5% and 97.4%, respectively). The concentration of the remaining seven lanthanides was measured by analysis of multiple isotopes:
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143, 144, 145\textsuperscript{Nd}, 147, 149, 152\textsuperscript{Sm}, 151, 153\textsuperscript{Eu}, 155, 156, 157, 158\textsuperscript{Gd}, 163, 164\textsuperscript{Dy}, 166, 167\textsuperscript{Er} and 172, 173, 174\textsuperscript{Yb}. This serves as an additional quality check of the measurements, for under ideal conditions, i.e. without interferences, different isotopes of the same element should result in identical concentrations. The measurements showed that for plant and snail samples 152\textsuperscript{Sm} and 158\textsuperscript{Gd} always gave higher concentrations than the other isotopes of these elements. This was caused by interferences, most likely due to oxide formation of 136\textsuperscript{Ba}, 138\textsuperscript{Ce} and 139\textsuperscript{La} isotopes, respectively. Consequently, results based on 152\textsuperscript{Sm} and 158\textsuperscript{Gd} were excluded from further analyses. For the remaining isotopes and other lanthanides, concentrations were practically identical and therefore analytical results were selected on basis of the isotope with the lowest measurement error.

![Graph](image)

Fig. 3.2. Lanthanide surface water concentrations, left in mmol L\textsuperscript{-1} versus atomic number (symbols are averages ± SEM, n = 3), and right divided by shale concentrations

3.3.2 Water analyses

In Fig. 3-2, lanthanide concentrations of the surface water samples are presented. Lu was excluded from the surface water analyses, for its concentration measurements could not be reproduced with sufficient accuracy, e.g. sample concentrations were below the detection limit. For the other lanthanides, recoveries of standard additions ranged from 92\% to 107\%. Variation among locations was well within one order of magnitude. A one-way ANOVA revealed no significant differences between locations (\(p = 0.943\)). Deviations from the typical pattern were observed for Eu (\(Z = 63\)) (higher than expected at Charlois and Nieuwe Maas) and Gd (\(Z = 64\)) (higher than expected at Nieuwe Maas). Concentrations of Ho (\(Z = 67\)) seemed to be somewhat high.

Results for the pore water analyses are presented in Fig. 3.3. Variation among locations was also within one order of magnitude. Again, one-way ANOVA found no significant differences between locations (\(p = 0.199\)). Deviations from the typical pattern are observed for Ce (\(Z = 58\)) (lower than expected at Nieuwe Maas) and Eu (higher than expected at Kralingse Plas, Charlois and Nieuwe Maas). Also, a systematic enrichment with heavy lanthanides is observed.

Apart from Ce, pore water concentrations are always higher than the corresponding surface water concentrations, which is associated with the lower pH in pore water (see Table 3.1).
To be able to compare locations and study general trends, the ratio of lanthanide concentrations in pore water and those in surface water was plotted against pH of pore water and surface water, respectively (see Fig. 3-4 for concentration ratio against pH of pore water). This shows that Eu has the highest ratio (ca. 8) and Ce the lowest (< 1, and quite variable), while the other lanthanides have intermediate ratios (between 3 and 6). Except for Ce, the ratio decreases with increasing pH of both pore water and surface water. The deviating behaviour of Ce and Eu is probably related to their different oxidation states, as mentioned in the Introduction and will be dealt with in the Discussion.
**FIELD CONCENTRATIONS OF LANTHANIDES IN FRESHWATER ECOSYSTEMS**

![Graph](image)

**Fig. 3-5.** Lanthanide concentrations in sediment < 2 mm, left in mmol·kg⁻¹ dry sediment versus atomic number (symbols are averages ± SEM, n = 3), and right divided by shale concentrations.

**3.3.3 Sediment analyses**

Table 3-3 presents the values for the sediment reference material BCR 667. The results of the sediment analyses are presented in Figs. 3-5 and 3-6 for the two analysed fractions, < 2 mm and < 63 μm, respectively. The figures show the typical lanthanide concentration pattern and very little variation among locations (no significant ANOVA, p = 0.836 and 0.678, respectively). The differences between locations were a factor of three at most. Lanthanide concentrations in the sediment fraction < 63 μm were somewhat higher than those in the sediment fraction < 2 mm. For the locations Kralinge Plass, Botlekpark and Charlois this difference was very small, but for the locations Nieuwe Maas and Veluwemeer the concentrations in the sediment fraction < 63 μm were 2 to 4 times higher than in the sediment fraction < 2 mm. Furthermore, the locations Nieuwe Maas and Veluwemeer are the ones with the lowest concentrations in the sediment fraction < 2 mm and among the highest in the sediment fraction < 63 μm.

![Graph](image)

**Fig. 3-6.** Lanthanide concentrations in sediment < 63 μm, left in mmol·kg⁻¹ dry sediment versus atomic number (symbols are averages ± SEM, n = 3), and right divided by shale concentrations.
Fig. 3-6 (right) shows a depletion of Eu (relative to shale) in Nieuwe Maas and Veluwemeer sediment < 63 μm. These samples were the only ones in which a Eu depletion was observed. Nieuwe Maas sediment < 63 μm, displays a systematic enrichment with lighter lanthanides.

### Table 3.3. Lanthanide concentrations in reference materials. Certified values with uncertainty in parentheses and measured values with standard deviations (\( n = 3 \)) in parentheses

<table>
<thead>
<tr>
<th>Ln, Z</th>
<th>BCR 667</th>
<th>BCR 668</th>
<th>BCR 670</th>
<th>SRM 1515</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eutrophic Sediment</td>
<td>Estuarine Sediment</td>
<td>Mussel Tissue</td>
<td>Aquatic Plant</td>
</tr>
<tr>
<td></td>
<td>certified</td>
<td>measured</td>
<td>certified</td>
<td>measured</td>
</tr>
<tr>
<td>La, 57</td>
<td>200 (9)</td>
<td>183.5 (0.6)</td>
<td>578 (48)</td>
<td>519 (7)</td>
</tr>
<tr>
<td>Ce, 58</td>
<td>405 (19)</td>
<td>396.8 (1.2)</td>
<td>633 (83)</td>
<td>562 (4)</td>
</tr>
<tr>
<td>Pr, 59</td>
<td>44 (4)</td>
<td>45.4 (0.2)</td>
<td>87 (9)</td>
<td>83 (2)</td>
</tr>
<tr>
<td>Nd, 60</td>
<td>173 (10)</td>
<td>168.2 (0.6)</td>
<td>378 (41)</td>
<td>356 (8)</td>
</tr>
<tr>
<td>Sm, 62</td>
<td>31.0 (1.5)</td>
<td>32.73 (0.15)</td>
<td>74 (7)</td>
<td>68 (3)</td>
</tr>
<tr>
<td>Eu, 63</td>
<td>6.6 (0.5)</td>
<td>7.10 (0.10)</td>
<td>18.4 (1.6)</td>
<td>17.3 (0.9)</td>
</tr>
<tr>
<td>Gd, 64</td>
<td>280 (1.0)</td>
<td>34.9 (0.4)</td>
<td>82 (6)</td>
<td>90 (3)</td>
</tr>
<tr>
<td>Tb, 65</td>
<td>4.3 (0.2)</td>
<td>4.74 (0.06)</td>
<td>10.2 (1.1)</td>
<td>11.3 (1.2)</td>
</tr>
<tr>
<td>Dy, 66</td>
<td>24.7 (1.4)</td>
<td>25.40 (0.14)</td>
<td>55 (4)</td>
<td>50 (2)</td>
</tr>
<tr>
<td>Ho, 67</td>
<td>4.8 (0.3)</td>
<td>5.02 (0.04)</td>
<td>11 (4)*</td>
<td>11.0 (1.1)</td>
</tr>
<tr>
<td>Er, 68</td>
<td>14.0 (1.1)</td>
<td>13.92 (0.10)</td>
<td>27 (3)</td>
<td>27 (2)</td>
</tr>
<tr>
<td>Tm, 69</td>
<td>1.9 (0.2)</td>
<td>2.03 (0.02)</td>
<td>2.8 (0.4)</td>
<td>3.5 (0.2)</td>
</tr>
<tr>
<td>Yb, 70</td>
<td>12.7 (0.6)</td>
<td>12.80 (0.15)</td>
<td>16 (3)*</td>
<td>15.2 (1.2)</td>
</tr>
<tr>
<td>Lu, 71</td>
<td>1.86 (0.11)</td>
<td>1.92 (0.03)</td>
<td>2.22 (0.22)</td>
<td>2.6 (0.3)</td>
</tr>
</tbody>
</table>

* indicative values

### 3.3.4 Plant analyses

For plant samples measured values for the reference materials BCR 670 and SRM 1515 agreed well with preliminary certified and/or indicative values (Table 3-3). The results of the Sago pondweed (P. pectinatus) analyses are illustrated in Fig. 3-7. Variation among locations was about two orders of magnitude. A one-way ANOVA revealed significant differences between locations (\( p = 0.010 \)), namely Charlois versus Nieuwe Maas and Kralingse Plas versus Nieuwe Maas. Deviations from the typical pattern are observed for Eu (higher than expected at Kralingse Plas and Charlois). Lighter lanthanides and especially La (Z = 57) are enriched in Nieuwe Maas plants.

The results of the analyses of L. minor are presented in Fig. 3-8. Note that the sample of location Charlois was a mixture of L. minor and S. polyrhiza, a closely related duckweed species. Variation among locations was about a factor of three. A one-way ANOVA revealed no significant differences between locations (\( p = 0.461 \)). Deviations from the typical pattern are observed only for Eu (higher than expected at Charlois). With the exception of location Kralingse Plas, lanthanide concentrations in L. minor are lower than those in P. pectinatus.
3.3.5 Mollusc analysis

Digestion of mollusc tissue and shell (a calcium-carbonate matrix) presented no problems, judged by the values for the reference material BCR 668 (Table 3-3). Results of the analyses of mollusc tissues are presented in Fig. 3-9. For location Charlois, the measurements of the closely related species *Planorhynchus planorhynchus* and *Planorhynchus cornus* were combined, and referred to as *Planorhynchus* (a), since there were only few specimens for each species available. Variation among locations was about two orders of magnitude. A one-way ANOVA revealed significant differences between locations (*p = 0.012*), namely Kralingse Plas (*L. stagnalis*), Charlois (*Planorhynchus* (a)) and Nieuwe Maas (both species) all versus Veluwe Meerdijk. Deviation from the typical pattern was observed only for Ce (lower than expected at Nieuwe Maas for both species present there).
Four samples are slightly depleted of Eu: Botlekpark (R. ovata), Charlois (R. ovata and P. australis) and Veluwemeer (R. ovata). Most samples show a systematic enrichment with lighter lanthanides. The results of the analyses of mollusc shell are presented in Fig. 3-10. Variation among locations was over two orders of magnitude. A one-way ANOVA revealed significant differences between locations \( (p = 0.007) \), namely Kralingse Plas (L. stagnalis), Charlois (Philobranchia) and R. ovata and Veluwemeer (R. ovata) all versus Botlekpark (R. ovata).

Deviations from the typical pattern were observed for La (higher than expected at Nieuwe Maas for both species and at Charlois for P. australis) and Eu (higher than expected at Kralingse Plas,
Charlois (all three species), Nieuwe Maas (D. polymorpha) and Veluwemeer (P. antipodarum)). It must be noted that for all mollusc species lanthanide concentrations in tissues were always higher than those in shells.

![Graph showing lanthanide partition coefficients](image)

**Fig. 3.11.** Lanthanide partition coefficients (*K*_i) in L·kg⁻¹ dry sediment, based on the sediment fraction < 2 mm and surface water (left) or pore water (right) versus atomic number (symbols are averages ± SD)

3.3.6 Sediment-water partition coefficients

In Fig. 3-11, the calculated partition coefficients (*K*_i) for lanthanides between the sediment fraction < 2 mm and surface water (left) and pore water (right) are plotted against atomic number. Fig. 3-12 presents these *K*_i values for the sediment fraction < 63 μm. Overall, values for *K*_i lie typically between 100,000 and 3,000,000 L·kg⁻¹ dry sediment. Again, Ce and Eu are exceptions, having lower *K*_i values (Ce only in surface water).

![Graph showing lanthanide partition coefficients](image)

**Fig. 3.12.** Lanthanide partition coefficients (*K*_i) in L·kg⁻¹ dry sediment, based on the sediment fraction < 63 μm and surface water (left) or pore water (right) versus atomic number (symbols are averages ± SD)
Both sediment fractions show the same pattern, with $K_f$ values for the fraction < 63 μm being higher than those of the fraction < 2 mm, due to the higher concentrations in the former fraction. Within the same sediment fraction, the values for $K_f$ based on surface water are higher than those based on pore water, due to the lower concentrations in surface water. $K_f$ values based on pore water show a decrease with atomic number for both sediment fractions (right side of Figs. 3-11 and 3-12).

Exceptions to the above-mentioned pattern are $K_f$ values for Ce and Eu, which are all lower for surface water (apart from location Botlekpark) and in case of Eu also for pore water. The trend shown for Ce and Eu is related to their redox chemistry as already mentioned in the Introduction. The behaviour of these two elements will be discussed later.

![Graph](image)

**Fig. 3-13.** Bioconcentration factors (BCF) for lanthanides in *L. minor* based on surface water concentrations in L·kg⁻¹ dry plant material versus atomic number (symbols are averages ± SD)

### 3.3.7 Bioconcentration factors for plants

Bioconcentration factors (BCFs) for *P. pectinatus* were calculated on basis of both surface water and pore water, because this species is exposed via both water phases, whereas *L. minor* is only exposed via surface water. In Fig. 3-13 the BCFs of lanthanides in *L. minor* are given. There seems to be hardly any variation between locations and between lanthanides. On average the value is about 10,000 L·kg⁻¹ dry plant material, with an exception for Ce, whose value is about 3,000 L·kg⁻¹ dry plant material.

*P. pectinatus* BCFs based on surface water are given in Fig. 3-14 (left side) and range from 5,000 to 300,000 L·kg⁻¹ dry plant material. With the exception of location Kralingse Plas, *P. pectinatus* BCFs are higher than those for *L. minor* and show much more variation between locations, i.e. two orders of magnitude. Anomalous behaviour is observed for Ce (apart for location Kralingse Plas less pronounced than for *L. minor*) and Eu (for the locations Nieuwe Maas, Charlois and Veluwemeer). *P. pectinatus* BCFs based on pore water (Fig. 3-14, right side) show an almost straight line, slightly decreasing with atomic number and only interrupted by Eu, except at location Kralingse Plas. BCF values range from 1,000 to 300,000 L·kg⁻¹ dry plant material and vary
over two orders of magnitude between locations. Values for Eu range from 800 to 22,000 L·kg\(^{-1}\) dry plant material.

![Graph](image1)

**Fig. 3-14.** Bioconcentration factors (BCF) in L·kg\(^{-1}\) dry plant material for lanthanides in *P. pectinatus*, based on surface water concentrations (left), and pore water concentrations (right) versus atomic number (symbols are averages ± SD).

3.3.8 Bioconcentration factors for molluscs

BCFs for snail and bivalve tissue were calculated on the basis of surface water concentrations (Figs. 3-15 and 3-16). In addition, biomagnification of lanthanides in snails was evaluated by calculating the ratio of snail tissue concentrations and *P. pectinatus* concentrations (Fig. 3-17).

Snail BCFs based on surface water range from 5,000 to 200,000 L·kg\(^{-1}\) dry snail tissue. All species from all locations show a deviation for Ce, but only at Charlois a deviation is observed for Eu, for all three snail species present there. Bivalve BCFs based on surface water range from 3,000 to 30,000 L·kg\(^{-1}\) dry bivalve tissue and show a slight decrease with atomic number (Fig. 3-16). Both bivalve species, which are from the same location, show a deviation for Ce.

![Graph](image2)

**Fig. 3-15.** Bioconcentration factors (BCF) for lanthanides in snail tissue based on surface water concentrations in L·kg\(^{-1}\) dry snail tissue versus atomic number (symbols are averages ± SD).
However, Eu is lower in *C. fluminensis* than in *D. polymorpha*. The lanthanides Ho (Z = 67) and Tm (Z = 69) also show deviations. This is not understood, but since the pattern is so consistent with their abundance, it might be an artifact.

Biomagnification of lanthanides from plant (*P. pectinatus*) to snail tissue displays a smooth decrease with atomic number (Fig. 3-17). Values range from 0.43 to 5.5 kg dry plant material·kg⁻¹ dry snail tissue. Especially for location Kralingse Plas, there is an Eu deviation. At location Kralingse Plas the values for both Eu and Gd (Z = 64) are lower than expected. The BCF for Eu in *P. antipodarum* from Veluwemeer is contrastingly higher than those of other lanthanides.

Next to the BCFs, the ratio of lanthanide concentrations in shell and tissue was plotted against atomic number (Fig. 3-18). Values range from 0.009 to 0.4 kg dry shell material·kg⁻¹ dry mollusc tissue. All species show a distinct, positive Eu deviation. Five species have a slightly higher ratio
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for Ce. Some species show an increase of the shell-tissue ratio with atomic number. Values for ratios in *P. aucta* from location Charlois should be interpreted with care, for they are very much scattered and have large errors (see Fig. 3.18).

![Graph showing shell-tissue ratios of lanthanides for all mollusc species](image)

**Fig. 3.18.** Shell-tissue ratios of lanthanides for all mollusc species in kg dry shell/kg dry mollusc tissue versus atomic number (symbols are averages ± SD)

### 3.4 Discussion

#### 3.4.1 Concentrations of lanthanides in surface water, pore water and pH

Surface water concentrations of individual lanthanides were between 1 pM and 2 nM, displaying the classic lanthanide pattern. The same pattern is observed for pore water, which contains concentrations of individual lanthanides between 3 pM and 1 nM. No significant differences between locations were observed for either surface or pore water. Results from de Boer et al. (1996) for 0.45-μm filtered surface water from The Netherlands and those from Tijink and Yland (1998) for filtered surface and pore water from the river Nieuwe Maas are in good agreement with the values found in the present study. Concentrations reported by Zhu et al. (1998) for 0.45-μm filtered surface water from the Rotterdam harbour, are one order of magnitude higher, which may be caused by lanthanide discharges of the phosphate ore processing industries upstream.

Tijink and Yland (1998) reported that lanthanide pore water concentrations are generally higher than surface water concentrations, which is in agreement with the findings and expectations (hypothesis 3) of the present work; Ce being the only exception. Ce behaviour was expected to deviate (hypothesis 4), but in the opposite direction, since in oxic surface waters Ce(III) is easily oxidized to Ce(IV), which in turn forms the highly insoluble CeO₂ and is thereby removed from the water column (de Baar et al., 1988, 1991). A possible explanation for the present findings might be that surface water samples included suspended particles < 0.45 μm, i.e. colloids, on which preferential precipitation of CeO₂ has occurred, which may be released after acidifying the sample. Indeed it was shown for river water that colloidal material is preferentially enriched in Ce (Sholkovitz, 1992, 1993) and from data on lake water (Hodge et al., 1996) it was calculated that
Ce is the second most concentrated lanthanide by suspended particulate matter > 0.45 μm. If water samples do include suspended particles, then it would be advisable to use filters with smaller pore sizes in order to exclude such particles and really study dissolved Ce concentrations (see Sholkovitz, 1992). In this respect, filtration rate versus accumulation of residue on the filter should be mentioned, because it may influence the size differentiation of the filtration procedure. With the field equipment used here, the filtration rate of natural water could not be controlled, and in some cases clogging of the filter occurred, after which the filter was replaced. Consequently, an influence on the size differentiation of particles cannot be ruled out. However, as it would merely exclude some particles < 0.45 μm, it would work in a favourable direction for analyzing dissolved Ce concentrations.

For Eu, a different behaviour was expected in pore water (hypothesis 4), where reduction of Eu³⁺ to the more mobile Eu²⁺ form may take place. Considering the stability field of Eu²⁺ in water: pH ≥ 6 and Eh ≤ -0.36 V at 25°C (Sverjensky, 1984; Brookins, 1989), and the prevailing conditions in our pore waters, Eu reduction is likely to have occurred (Sverjensky, personal communication). The pH and temperature conditions have been summarised in Table 3-1, and for Eh a value of at least -0.48 V (Lide, 1994) was derived from the H₂S smell of the pore water samples. Such strongly reducing conditions are caused by organic matter decay in the sediment. The observation that Eu has the highest pore water - surface water concentration ratio (Fig. 3-4) and the negative Eu anomalies in the bioconcentration factors (Figs. 3-14 and 3-16) are further evidence that reduction occurs, because it means that the bioavailability of Eu has obviously changed. Previously, positive Eu anomalies in water have only been reported for submarine hydrothermal vents (e.g. Michard, 1989; Olivarez and Owen, 1991), whereas the existence of Eu(II) species at low temperature reducing environments was ruled out (de Baar et al., 1988). Unfortunately, Michard (1989) and Olivarez and Owen (1991) describe no mechanism, such as Eu³⁺ reduction, to explain their observations on the increased Eu concentrations. Erel and Stolper (1993) explain the positive Eu anomalies of solid phases by increased Eu adsorption, due to the slightly higher first hydroxide stability constant of Eu, compared to those of its neighbours Sm and Gd.

Increased Eu concentrations were indeed observed in pore waters of three locations (Kralingse Plas, Charlois and Nieuwe Maas) and also for surface water at location Charlois, which could mean that the water there is not well-mixed and partly anoxic. The anoxic state is strongly related to the presence of decaying organic matter of which particularly the Charlois sediment was very rich. Directly after sampling of Charlois pore water a fine brown precipitate was formed, probably iron oxide, which acts as a scavenger for lanthanides (Bau, 1999). The formation of this precipitate reflects the anoxic condition of the sediment, and the fact that during sampling it was not retained, e.g. by working under N₂-gas.

The peak for Gd (Z = 64) in the river Nieuwe Maas (right side of Fig. 3-2) is probably caused by the application of gadopentetic acid, Gd(DTPA)³⁻, in magnetic resonance imaging (MRI), as described for rivers which drain densely populated and industrialised areas in Central
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Europe and North America (Bau and Dulski, 1996). For the deviations of Ho no explanation was found. The systematic enrichment of pore water with heavier lanthanides can be explained by the increase of solution complexation with Z.

The lanthanide concentration ratio of pore water versus surface water as shown in Fig. 3-4 clearly points out Ce and Eu as the two extremes of the lanthanide series. Eu has the highest ratio as expected from the increased mobility of its 2+ oxidation state in anoxic pore water, and Ce the lowest as a result of the high surface water concentrations as discussed above. The other lanthanides show intermediate results, and resemble the trend of Eu (see Fig. 3-4). As the pH of pore water increases, the concentration ratio goes toward unity. The pH values for surface waters and near-neutral values for pore waters (Table 3-1) were in good agreement with those found by Tijnik and Yland (1998) and by van den Berg et al. (2000). Furthermore, a higher pH value in pore water was associated with higher pH values in surface water ($r^2 = 0.77$, linear regression), but the latter pH values span a wider range.

3.4.2 Lanthanide concentrations in sediment

Sediment may be considered the source for trace metals, such as lanthanides, in pore water and surface water (van den Berg et al. 2000, see also the Introduction). Concentrations of lanthanides in the sediment fraction < 2 mm showed very little variation between locations. Veluwemeer had the lowest concentrations and can therefore successfully be considered as the proposed reference location (see Materials and methods). Botlekpark and Charlois had the highest lanthanide concentrations. From Botlekpark this was anticipated, but the origin of the higher concentrations at Charlois is unknown. At this location, only higher concentrations of La and Ce were expected (see Materials and methods). The values for Nieuwe Maas were slightly lower than those reported by Tijnik and Yland (1998).

The sediment fraction < 63 µm contained approximately the same lanthanide concentrations as the sediment fraction < 2 mm for three locations (confirming hypothesis 5). In this respect lanthanides differ from many other trace metals (e.g. Hg), which are not only present in the matrix itself, but also absorbed from the aqueous phase, sometimes with elevated levels due to industrial releases. In these cases trace metal concentrations of the sediment particles are a function of the specific surface. Moreover, when pH and salinity change, absorbed trace metals may be desorbed (de Groot et al., 1971). For the locations Nieuwe Maas and Veluwemeer however, the sediment fraction < 63 µm did contain higher lanthanide concentrations than the sediment fraction < 2 mm, which may be attributed to a change in sample composition during the sieving process. The 63-µm sieve excludes part of the sand (mainly silicates) which contains low lanthanide concentrations. During sampling it was already observed that sediments from Nieuwe Maas and Veluwemeer contained more sand than those of other locations (see Materials and methods). In fact, the < 2 mm sediment fractions from Nieuwe Maas and Veluwemeer have the lowest lanthanide concentrations (Fig. 3-5), whereas their < 63 µm fractions are among those with the highest lanthanide concentrations (Fig. 3-6). The dependence of lanthanide concentrations on
Chapter 3

Grain size was also shown for sediments by Zhang et al. (1998) and for soils by Minarik et al. (1998). In the sediment fraction < 63 μm there is slightly more variation, but still there are no significant differences between locations.

For several samples of both analysed sediment fractions, a systematic depletion of heavier lanthanides is shown, most notably for Nieuwe Maas sediment < 63 μm (Fig. 3-6). This is opposite to the observation on pore waters, which are enriched in heavier lanthanides (Fig. 3-3). Furthermore, the only samples depleted of Eu (relative to shale) were found in sediment < 63 μm, while pore waters are generally enriched in Eu. These observations make sense if sediment is considered the source for lanthanides in pore waters.

The small variation in the sediment data is probably due to the fact that all sampling locations are situated in the catchments of the rivers Rhine and Meuse (see Fig. 3-1) and hence all samples originate from the same source. This may imply that the lanthanide concentrations studied here are reflecting the natural background.

3.4.3 Lanthanide concentrations in plants

Concentrations in P. pectinatus showed significant variations between locations, which were not observed for the water or sediment samples. Possible explanations for this variation include: (1) biological variation, causing individuals and populations to differ in uptake of lanthanides (2) differences in lanthanide availability, i.e. speciation, in the surface and/or pore waters (3) remains of attached fine sediment (silt) on plants causing higher lanthanide concentrations (Sansone et al., 1998) (4) the result of uptake from two different lanthanide sources, i.e. surface water and pore water (Ernst, 1994; Greger and Kautsky, 1993). The deviations for Ce and Eu in the pattern agree with those found in the pore water samples, suggesting this may be the main source for lanthanides in P. pectinatus. Lanthanide concentrations reported by Cowgill (1973b) for Potamogeton crispus and Potamogeton praehensus are about five times higher than those in plants from Nieuwe Maas, which is the location with the highest concentrations (Fig. 3-7). With respect to the date of this work (1973), it should be noted that the analytical quality of the data may not be able to meet present day requirements, and interpretation should be done with care. Lanthanide concentrations in P. pectinatus as measured by Kovács et al. (1984) fit nicely into the range found in the present study.

For L. minor, the results did not show significant differences, but this concerns only three locations instead of five for P. pectinatus. Besides, L. minor has only one of the four sources of variation named for P. pectinatus, namely differences in lanthanide availability, i.e. speciation, in the surface waters. Regarding the other three sources of variation it must be noted that L. minor populations are usually made up of one genotype, they are not in contact with sediment and take up lanthanides only from surface water. The mixture of two duckweed species at Charlois, i.e. L. minor and S. polyrrhiza, is not expected to produce abnormal results, since these species are closely related (Landolt and Kandeler, 1987) and experience exactly the same exposure. The anomaly found for Eu at location Charlois corresponds, as expected, with the one found in surface water.
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(Confirming hypothesis 6). Concentrations in *Lemna trisulca*, a closely related, but submerged living duckweed species, were approximately 6 times higher than the values found for *L. minor* in the present study (Kovács *et al.*, 1984). Values for the reference material BCR 670 Aquatic Plant (see Table 3-3), which is in fact *L. minor*, are 2 to 3 times higher than the values found in the present work.

On average, *L. minor* contains lower lanthanide concentrations than *P. pectinatus*. Besides the physiological differences between the two species, this may also be attributable to their different exposure routes. *L. minor* floats on the water surface, whereas *P. pectinatus* is fully surrounded by surface water and roots in sediment where it is in contact with pore water (Ernst, 1994) which contains higher lanthanide concentrations, associated with a lower pH. Both species are in contact with suspended particles in surface water, which may adhere to their surface (Sansone *et al.*, 1998). If such particles are not completely removed during sample preparation, they will add to a plant’s lanthanide concentration. Because the surface capacity of *L. minor* and *P. pectinatus* for uptake of such particles may differ, it can contribute to the differences in lanthanide concentrations found between them.

3.4.4 Lanthanide concentrations in mollusc tissue and shell

In a few mollusc tissue samples some grains of sand were found in solution after the digestion procedure. These grains were either present in the mucus layer of the snail body or in the digestive tract of the molluscs, but were not digested during the analytical procedure. They may have been leached to a certain extent, but no appreciable contribution is expected to the measured concentration in the tissue (see discussion on sediments). Significant variation between locations was found, which may be attributed to differences between the seven mollusc species, biological variation within species, differences in lanthanide availability between locations and the different lanthanide sources (uptake routes) involved. Molluscs can take up lanthanides from surface water by drinking (passive and active), filter-feeding (for bivalves only), diffusion through their permeable skin, from sediment pore water (in case of sediment burrowers, like *C. fluminarum*) and from feeding on plants (for snails only). The Ce deviations observed for tissues of both bivalve species at location Nieuwe Maas coincide with the Ce deviation in pore water, which was anticipated for the sediment-burrowing *C. fluminarum*. However, it was not expected for *D. polymorpha*, which lives attached to rocks and not in direct contact with sediment. Concentrations reported in the literature for Ce in *L. stagnalis* and *P. corneus* were twice as low and two orders of magnitude lower than the values from the present work, respectively (Gabrashanska *et al.*, 1989). Values for lanthanides in *C. fluminarum* tissue as measured by Tijink and Yland (1998) at location Nieuwe Maas were about 5 times the values found in the present study. If this is attributable to temporal changes (our samples were taken 3 years later) or if it merely reflects a heterogenous distribution of lanthanides at this location, is unknown.

Concentrations in tissues are higher than those in shells. This was also found by Tijink and Yland (1998) for *C. fluminarum*. Thereby the differences in density of these samples must be noted,
as well as the implicit assumption of a homogeneous lanthanide distribution within them. Further, the species with the highest shell concentration does not necessarily have the highest tissue concentration, although the lowest lanthanide concentrations in shell and tissue were both measured in *L. stagnalis* from location Kralingse Plas. There is more variation between locations for shell than for tissue concentrations and, in addition, more deviations from the typical lanthanide pattern are observed in shells. This emphasizes the importance of analyzing the shell and tissue of molluscs separately. Apart from the sources of variation for molluscan tissue, it may be possible that the exterior shell, as a non-living tissue, is prone to unregulated elemental exchange with the surrounding water. For instance, lanthanides may substitute for Ca$^{2+}$ in the CaCO$_3$ matrix of which shells predominantly consist. This substitution has been shown for marine foraminifera, and its dependence on the Ln$^{3+}$ ionic radius was emphasized (Palmer, 1985). For marine shells it has been shown that the outer shell layer (the periostracum; organic material) contains higher lanthanide concentrations than the inner shell layer (Ohde, 1998), implying that the lanthanide distribution within shells is not homogeneous.

The deviation for Ce in *C. fluminarum* shells at location Nieuwe Maas is related to the deviation in pore water, but the Ce anomaly in *P. acuta* shells from location Charlois cannot be explained. For Eu there were deviations for six species at four locations, namely Kralingse Plas, Charlois, Nieuwe Maas and Veluwemeer. Apart from the location Veluwemeer, these deviations coincide with those found in pore water, surface water and in *P. pestinarius*. Ohde (1998) reported concentrations for seven lanthanides in marine shells, which fall within the range of the values found in the present work. Values for *C. fluminarum* shells reported by Tijink and Yland (1998) for location Nieuwe Maas were comparable to those from the present work, but they did not report a Ce anomaly.

Partitioning of lanthanides between tissue and shell as shown in Fig. 3-18, revealed a positive Eu anomaly for all samples and for five samples also a positive Ce anomaly. This means that, in contrast to hypothesis 10, the lanthanides are not equally distributed between molluscan shell and tissue. Either the shell gland is not passing on all lanthanides equally, or Eu is enriched in the shell after it is formed. For some species an increase of the shell-tissue ratio is observed with atomic number. This might be explained by the general increase of stability constants with atomic number and thus the presence of ligands (e.g. carbonate) in the shell.

### 3.4.5 Sediment-water partition coefficients

In general, partition coefficients are defined for equilibrium situations. This can safely be assumed for sediment and pore water, which is a stable environment. For surface water this is not always the case. Surface water is more dynamic, because it experiences water movements (e.g. water flow, tides, wind), temperature differences and all kinds of input (e.g. rain, run-off water, biota, substances). Therefore, $K_p$ values based on pore water are considered more reliable than the ones based on surface water. For surface water it would make more sense to calculate $K_p$ values on the basis of suspended matter instead of sediment. Also when comparing bulk sediment...
concentrations as in this paper, one is aware that this represents both the inert crystalline matrix of in essence grounded igneous rocks (notably sand, but also clay minerals), as well as the more environmentally reactive phases such as carbonate minerals, to some extent also clay minerals, and above all surface coatings and adsorbed phases.

Overall, $K_j$ values for all lanthanides lie between 100,000 and 1,000,000 L·kg$^{-1}$ dry sediment (confirming hypothesis 1). The partition coefficients for pore water clearly show the different behaviour of Eu, as expected (hypothesis 4, see also the right side of Figs. 3-11 and 3-12). Its reduction from oxidation state $3^+$ to $2^+$ results in an increased mobility, thus higher pore water concentrations and therefore lower $K_j$ values. The other lanthanides, all having oxidation state $3^+$ in pore water, display a smooth pattern of $K_j$ decreasing with atomic number (confirming hypothesis 2). This may be attributed to the general increase of lanthanide-ligand stability constants with atomic number (Bingler et al., 1989; Martell and Smith, 1998), which preferentially keeps the heavier lanthanides in solution. Furthermore, pore water $K_j$ values based on the sediment fraction $< 63 \mu$m are more variable than those based on the sediment fraction $< 2$ mm. This may be related to the larger surface area and the more dynamic character of small particles, for they are easily transported by water movements and thus may not reside long enough to achieve equilibrium with the local pore water.

Partition coefficients for surface water (left side of Figs. 3-11 and 3-12) do not display such a clear pattern and are more scattered than those for pore water. This may be related to the more dynamic character of surface water - especially at locations Nieuwe Maas and Veluwemeer - relative to pore water. In addition, the pattern is disturbed by possible artifacts in the measurements of Ho in surface water as already mentioned in Results.

For Ce, higher $K_j$ values were expected in surface water (hypothesis 4), where its oxidation state can change from $3^+$ to $4^+$, usually resulting in Ce removal from the water column by precipitation of CeO$_2$. However, Ce concentrations in surface water were unexpectedly high (see previous discussion), leading to lower $K_j$ values. The low $K_j$ values for Eu in surface water (with the exception of location Botlekpark) were not expected and could mean that water at the sampling locations was not well-mixed, i.e., partly anoxic. Furthermore, surface water $K_j$ values based on the sediment fraction $< 63 \mu$m are less variable than those based on the sediment fraction $< 2$ mm. This is opposite to the situation for pore water and may be related to the resemblance of the sediment fraction $< 63 \mu$m to suspended matter.

Tijink and Yland (1998) reported $K_j$ values for location Nieuwe Maas for sediment and pore water between 300,000 and 1,000,000 L·kg$^{-1}$ dry sediment and for suspended matter and surface water between 80,000 and 3,000,000 L·kg$^{-1}$ dry matter. These values are in the same range as the values found in the present work. $K_j$ values calculated by Coughtrey and Thorne (1983) are between 5,000 and 20,000 L·kg$^{-1}$ dry sediment, hence lower than the values found in the present work. However, in their calculations they used values for soil instead of values for sediment and therefore they are considered not representative nor fully comparable with the values obtained in this work.
3.4.6 Bioconcentration factors for plants

The BCFs for \( L.\ minor \) (Fig. 3-13) show very little variation between locations, for reasons discussed above. The only deviations are noted for Ce (confirming hypothesis 6). Again the Ce behaviour in \( L.\ minor \) is likely to be related to the redox chemistry of this element. Apparently, Ce\(^{4+}\), as solid CeO\(_2\) or precipitated on (iron oxide coated) dissolved suspended matter is less available for uptake from surface water.

BCFs for \( P.\ pectinatus \) were calculated on the basis of both surface water and pore water (Fig. 3-14), because \( P.\ pectinatus \) can take up metals from both these phases (Ernst, 1994; Gregor and Kaunsky, 1993). Both BCF measures show considerable variation, about two orders of magnitude; those based on pore water slightly more than those based on surface water. In calculating the BCF, \( P.\ pectinatus \) concentrations are combined with concentrations in surface and pore water, which have very little variation. Consequently, the variation in BCFs does not originate from the variation in water concentrations, but from the variation in lanthanide concentrations in \( P.\ pectinatus \), which have been discussed previously.

The dominant uptake route for lanthanides in \( P.\ pectinatus \) may be found by using the anomalous behaviour of Ce and Eu. The BCF based on the dominant uptake route, should result in similar values for all lanthanides, thus in a pattern with less or less pronounced anomalies. This implies an assumption that availability is independent of oxidation state, because the lanthanides were measured as total concentrations irrespective of oxidation state. From Fig. 3-14 it is concluded that pore water is the dominant uptake route for lanthanides in \( P.\ pectinatus \), for it produces a smooth pattern with only one anomaly. A contribution from surface water cannot be ruled out, and so hypothesis 7 was neither confirmed nor rejected. The lower BCF for Eu can be explained by the lower affinity that plants have for divalent Eu compared to the other trivalent-lanthanides, which are considered to be among the most reactive metals known (Bingler et al., 1989). Therefore, the assumption that availability is independent of oxidation state is not valid. This was also concluded above for the BCF of Ce in \( L.\ minor \). The lower BCFs for heavier lanthanides (confirming hypothesis 11) may be attributed to the general increase of lanthanide- ligand stability constants with atomic number (Bingler et al., 1989; Martell and Smith, 1998), which preferentially keeps the heavier lanthanides in solution. The decrease in BCF with atomic number was also found for a marine diatom and the lanthanides Ce, Gd and Yb (Bingler et al., 1989).

The BCFs for aquatic plants found in this study are in the same range as those found for surface water by Wolterbeek and van der Meer (1996) and Weltje (1998c), but one order of magnitude higher than those of Kovács et al. (1984).

3.4.7 Bioconcentration factors and biomagnification in molluscs

Figs. 3-15 and 3-16 give the BCF for snail and bivalve tissue versus surface water. All molluscs have an obvious relationship with surface water, either due to the fact that they are filter-feeders (the bivalves \( C.\ flaminus \) and \( D.\ polymorpha \)) or that they take in water through drinking or while
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eating, as shown for the snail *L. stagnalis* (de With, 1996). Besides, the mollusc skin is permeable for water and provides another possible uptake route for lanthanides. The BCF pattern of snails is very similar to that of *P. pectinatus*, having a negative Ce anomaly and for location Charlois also a negative Eu anomaly. *C. fluminam* from Nieuwe Maas also shows the negative Eu anomaly like *P. pectinatus* from that location. BCF values for plants and molluscs are of the same order. The patterns for *C. fluminam* and *D. polymorpha* (Fig. 3-16) are the same, with the exception of Eu. This can be related to the different exposure of these organisms. While *D. polymorpha* is in contact with surface water only, *C. fluminam* lives in sediment and thus the Eu concentration reflects the influence of pore water (confirming hypothesis 9). BCFs for *C. fluminam* based on pore water as determined by Tijink and Yland (1998) for location Nieuwe Maas are comparable to the values found in the present work.

The snails (*L. stagnalis, P. antiparum, R. ovata, P. acuta, P. cernus* and *P. planorbis*) feed on (rotting) plant parts and on *Aufwuchs growing on it* (Landolt and Kandel, 1987; Ludwig, 1993). Therefore the transfer from plant food to snail tissue, i.e. biomagnification, was studied (Fig. 3-17). Lanthanide concentrations in *P. pectinatus* were used for calculating biomagnification factors. The patterns are very smooth (confirming hypothesis 11), the only exception being Eu, especially at location Charlois. This might be related to the fact that rotting plant parts are linked to reducing circumstances were Eu is divalent. The results emphasize the importance of food as a lanthanide source for snails. Most values are above unity, but below 5, suggesting that the extent of biomagnification in this food chain is low. A contribution from surface water to lanthanide concentrations in snails cannot be ruled out, and so hypothesis 8 was neither confirmed nor rejected. Cowgill (1973a) reported the biomagnification of lanthanides in the plant-louse *Rhaphalothrom nymphora* relative to leaves of the waterlily *Nymphaea odorata*. The lanthanides La, Ce, Pr, Sm, Gd, Er and Yb had higher concentrations in the plant-louse (maximum a factor of two), but those of Nd and Dy were lower than those in plant leaves. However, considering their date of publication (1973), the analytical quality of these data should be reassessed.

3.5 Conclusions

The samples taken for this study may be considered representative for background concentrations in The Netherlands, judged by the lack of significant variation in sediment concentrations, sampled at five locations with different characteristics. There was also no significant variation in concentrations of lanthanides in surface water or pore water. However, the biota (plants and molluscs) showed significant variation in lanthanide concentrations for the various locations, comprising about two orders of magnitude. This may be attributed to inter- and intra-specific biological variation, differences in lanthanide speciation and the involvement of different lanthanide sources for organisms, e.g. surface water, pore water and food for snails. In all samples, the typical saw-tooth pattern for lanthanide concentrations was observed, in agreement with hypothesis 1. Except for Ce, lanthanide concentrations in pore water were higher than in surface
water, which is associated with a lower pH in pore water (confirming hypothesis 3). The sediment fractions < 63 μm and < 2 mm showed only minor differences, which is in agreement with hypothesis 5.

As a result of their redox chemistry, Ce and Eu show a deviating behaviour from the other lanthanides (confirming hypothesis 4), in all sample types, except for sediment. The resulting anomalies can be used to identify important sources of lanthanides for snails and sediment-rooting plants. By relating organism concentrations to the possible sources they take up lanthanides from, i.e. by calculating bioconcentration or biomagnification factors, anomalies should become smaller or disappear. For common duckweed a relation was established between surface water and plant concentrations (confirming hypothesis 6). For Sago pondweed it was shown that pore water was the most important lanthanide source and for snails, food (plants) seem to be the dominant lanthanide source (neither confirming nor rejecting hypotheses 7 and 8). For bivalves the lanthanide source is probably surface water, but pore water seemed to be involved too for a sediment-burrowing species (confirming hypothesis 9).

Lanthanides are not equally distributed between mollusc shell and tissue (in contrast to hypothesis 10). Shells contain much lower concentrations, and are relatively enriched in Eu. BCFs for lanthanides in plants and snails relative to surface water lie typically between 10,000 and 100,000 L·kg⁻¹ dry matter, while sediment-water partition coefficients are one order of magnitude higher. There is a low extent of biomagnification in the plant-to-snail system, with a maximum biomagnification factor of 5.5. Many distribution coefficients showed a trend with atomic number, in agreement with hypotheses 2 and 11. This is related to the chemical characteristics of the lanthanides, i.e. the general increase of ligand stability constants with atomic number, keeping the heavier lanthanides preferentially in solution as complexes.

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CHAPTER 4

ADSORPTION OF La, Zn, Cu, Mn AND K SPECIES TO MEMBRANE FILTERS
DURING THE STERILISATION OF A DUCKWEED (LEMNA MINOR L.)
NUTRIENT SOLUTION

This chapter is based on:
Abstract

Filtration of a solution may decrease its metal concentrations through adsorption of metal species to the filter. Processes such as filter-sterilising nutrient solution and filtration of field water are sensitive to these sorption artifacts, yet basic data on the affinity of different filters for metals is lacking. This paper describes the adsorption of five metals to eight types of 0.2-µm membrane filters, used for sterilising a plant (Lemna minor L.) culture medium. Filters of cellulose acetate, cellulose nitrate, mixed cellulose ester, nylon, polyamide, polycarbonate, polyester and polyvinylidene fluoride were tested for their affinity towards mono- (K), di- (Mn, Cu, Zn) and trivalent (La) metals. Metal concentrations were quantified using radioisotopes and speciation was calculated.

Results showed that metals had the lowest affinity for polycarbonate and nylon filters, and the highest for cellulose and polyester type filters. Furthermore, it was shown that the metal load on cellulose filters correlated best with free ion concentrations (indicating electrostatic attraction), while loads on other filters correlated better with total metal concentrations. Filtering 5 mL solution of pH 5 did not affect its metal concentrations, ranging from 10 nM (La) to 49 µM (K). To minimize filtration artifacts, we propose to use polycarbonate or nylon filters, especially when dealing with low volumes of high pH, and low metal species concentrations.
4.1 Introduction

Sterilisation of aqueous solutions, such as nutrient media used for culturing and testing algae and higher plants, is done by filtration through pore size 0.2 µm (Dickenson, 1992; Waterhouse and Hall, 1995), autoclaving at 121°C or by combining the two (OECD, 2000). These techniques are applied in order to avoid undesired influences of bacteria, fungi or (other) algae in otherwise controlled experiments. The idea is to kill the microorganisms by autoclaving or separate them from solution by filtration, but not to affect the chemical composition of the medium itself. Both methods have drawbacks and a choice for either of them should be based on the specific further use and characteristics of the sterilised solution. Microorganisms are killed in autoclaved solutions, while filter sterilisation removes them from solution. Autoclaving increases the solution’s temperature to 121°C, so it needs to be cooled before use, while filtered solutions are ready for immediate use. Sterile filtration of small volumes through a syringe filter requires a large differential pressure and yields less filtrate than autoclaving, since some solution is lost in the void volume of the filter and filter holder (Menzies et al., 1991a; Chérif et al., 1998). Another disadvantage of filtration is that sterile filters are relatively expensive and non-sterile filters require sterilisation before they can be used. A disadvantage of autoclaving is that the high temperature can cause irreversible chemical reactions to occur. Examples are the escape of CO₂ (g) from the solution’s carbonate system and the damaging effect of autoclaving on ethylenediaminetetraacetic acid (EDTA) (Huebert and Shay, 1992).

Possible damage to organic chemicals could be the decisive factor for choosing filtering instead of autoclaving a solution (Waterhouse and Hall, 1995). While there are only few organic substances present in nutrient solutions, e.g. a metal chelator, like EDTA and optionally a pH buffer, such as 2-morpholinoethane sulfonic acid (MES), the main part of the medium is made up of inorganic components. It is not likely that inorganic medium components, except the above-mentioned carbonate, suffer from autoclaving, but it has been shown that membrane filters have some affinity for cations and metal complexes (Chapman, 1999; Horowitz et al., 1996; Menzies et al., 1991b; Marvin et al., 1970). The speciation and charge of a metal are consequently of great importance. Some data concerning the influence of filtration on metal concentrations in solution are available, but they do not reveal if metals are adsorbed to the filter or are retained because they are present in or sorbed to particles exceeding the pore size (Horowitz et al., 1996; Ankley and Schubauer-Berigan, 1994; Winger et al., 1998). Consequently, there is a need for basic data evaluating the compatibility of different filter types with various metals in solution (Chapman, 1999; Horowitz et al., 1996; Wenzel and Wieshammer, 1995). Such data may also be of use in studies on natural waters, where filtration is used to separate dissolved metals from particulates.

The purpose of this work was to evaluate the suitability of eight filter types for the sterilisation of nutrient solutions, with respect to the adsorption of mono-, di- and trivalent metal cations or complexes. Chemical speciation of the metals was calculated to assess its importance for adsorption, while the use of radioisotopes permitted sensitive measurements of metal concentra-
tions in both filters and filtrates. The results and their validity for other metal containing solutions, e.g. natural waters, are discussed in view of the speciation of metals in solution.

4.2 Materials and methods

4.2.1 Nutrient solution

The nutrient solution evaluated in this assessment was adapted from SIS (1995) and was designed for the sterile culturing and testing of common duckweed (*Lemna minor* L.). This medium has minimal concentrations of nutrients compared to other culture media (e.g. Gorham's medium (Gorham, 1950) and for an overview of media see Landolt and Kandeler, 1987), but resembles those observed in natural environments of *L. minor*. Its composition is given in Table 4-1. Adaptations made to the original medium are 1) replacement of pH buffer 3-morpholinopropane sulfonic acid (MOPS) by MES, for lower pH, 2) omission of Na2CO3, because the main carbon source for *L. minor* is CO2 from air (Landolt and Kandeler, 1987), 3) addition of NaVO3, to supply plants with the essential element vanadium, and 4) addition of a small amount of lanthanum (La), making up a final La concentration of 10 nM.

Table 4-1. Composition of *Lemna minor* nutrient medium, adapted from SIS (1995), concentrations in mol·L⁻¹ (M)

<table>
<thead>
<tr>
<th>component</th>
<th>concentration (M)</th>
<th>component</th>
<th>concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO3</td>
<td>5.00×10⁻⁴</td>
<td>CuSO4·5H2O</td>
<td>2.00×10⁻⁴</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>4.92×10⁻⁵</td>
<td>Co(NO3)2·6H2O</td>
<td>3.44×10⁻⁸</td>
</tr>
<tr>
<td>MgSO4·7H2O</td>
<td>3.04×10⁻⁴</td>
<td>NaVO3</td>
<td>8.20×10⁻⁸</td>
</tr>
<tr>
<td>CaCl2·2H2O</td>
<td>2.45×10⁻⁴</td>
<td>FeCl3·6H2O</td>
<td>3.11×10⁻⁶</td>
</tr>
<tr>
<td>H3BO3</td>
<td>1.62×10⁻⁵</td>
<td>Na2EDTA·2H2O</td>
<td>5.37×10⁻⁵</td>
</tr>
<tr>
<td>MnCl2·4H2O</td>
<td>1.01×10⁻⁶</td>
<td>MES·H2O</td>
<td>2.34×10⁻⁵</td>
</tr>
<tr>
<td>ZnSO4·7H2O</td>
<td>1.74×10⁻⁷</td>
<td>LaCl3·7H2O</td>
<td>1.00×10⁻⁸</td>
</tr>
<tr>
<td>Na2MoO4·2H2O</td>
<td>4.13×10⁻⁸</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* the metal of this component was spiked with radioisotopes

Lanthanum is normally not present in a nutrient solution (other than as a chemical impurity), but was added to incorporate a trivalent metal in the experiments and be able to study its sorption behaviour next to that of mono- and divalent metals. Iron (Fe), the only trivalent metal present in the original medium, is less suitable with respect to the required radiochemical sensitivity (see Discussion). In addition to La, we chose copper (Cu), zinc (Zn), potassium (K) and manganese (Mn) for this work, since at irradiation they produce suitable γ-emitting isotopes, regarding both half-lives and characteristic photo peaks (Table 4-2).
LA ADSORPTION TO MEMBRANE FILTERS

Table 4-2. Radioisotopes of K, Mn, Cu, Zn and La with half-lives (h or d), characteristic photopeaks (keV) used for detection (Blaauw, 1996), specific activities (GBq·mol⁻¹) and activity concentrations (kBq·L⁻¹) at the start of the experiment

<table>
<thead>
<tr>
<th>Radioisotope</th>
<th>Half-life</th>
<th>Characteristic Photo Peak (keV)</th>
<th>Specific Activity (GBq·mol⁻¹)</th>
<th>Activity Concentration (kBq·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>⁴²K</td>
<td>12.4 h</td>
<td>1,524.58</td>
<td>35.1</td>
<td>1,730</td>
</tr>
<tr>
<td>⁵⁶Mn</td>
<td>2.58 h</td>
<td>846.81; 1,810.77</td>
<td>492</td>
<td>497</td>
</tr>
<tr>
<td>⁶⁴Cu</td>
<td>12.7 h</td>
<td>1,345.78</td>
<td>11,500</td>
<td>230</td>
</tr>
<tr>
<td>⁶⁵Zn</td>
<td>244 d</td>
<td>1,115.52</td>
<td>7.82</td>
<td>1.36</td>
</tr>
<tr>
<td>⁶⁹mZn</td>
<td>13.8 h</td>
<td>438.63</td>
<td>57.7</td>
<td>10.0</td>
</tr>
<tr>
<td>¹⁴⁰La</td>
<td>1.68 d</td>
<td>1,596.54</td>
<td>115,000</td>
<td>1,150</td>
</tr>
</tbody>
</table>

4.2.2 Production of radioisotopes

Concentrated stock solutions of the metal salts, all reagent grade chemicals, were prepared in Milli-Q water (Millipore-Waters, Milford, MA, USA). From these solutions, three samples were prepared in quartz vials and irradiated for 24 h at a neutron flux of 1.60×10¹⁷ m⁻²·s⁻¹ in the Hoger Onderwijs Reactor at the Interfaculty Reactor Institute, Delft, The Netherlands. The samples contained: 2.0×10⁻⁸ mole CuSO₄·5H₂O plus 1.74×10⁻⁷ mole ZnSO₄·7H₂O in 500 µL to yield the radioisotopes ⁶⁴Cu, ⁶⁵Zn and ⁶⁹mZn (a metastable ⁶⁵Zn isotope), 4.92×10⁻⁶ mole KH₂PO₄ in 500 µL to yield ⁴²K and 1.0×10⁻⁸ mole LaCl₃·7H₂O in 100 µL to yield ¹⁴⁰La, respectively. The radioisotope ⁵⁶Mn was prepared by irradiating a polythene capsule containing 1.01×10⁻⁶ mole MnCl₂·4H₂O in 500 µL for 1 h at a neutron flux of 4.40×10¹⁶ m⁻²·s⁻¹. Samples were cooled down for 3 h to eliminate short-lived radioisotopes such as ³⁸Cl, which has a half-life of 37.2 min. Specific activities of the isotopes at the starting time of the experiment are given in Results.

4.2.3 Handling of nutrient solution and filters

The irradiated solutions were added to a volumetric flask, already containing the non-radioactive solution components in approximately 500 mL Milli-Q water, and the volume was adjusted to one litre. The pH was adjusted to 5.00 ± 0.01 with 0.2 N HNO₃ and 0.1 N NaOH. This results in a small net addition of nitrate or sodium, but due to the relatively high concentrations of these ions in the original nutrient solution (5.0×10⁻⁴ M and 5.1×10⁻⁴ M, respectively) and their weak interactions with other components, influence on medium speciation was insignificant (as shown by speciation calculations, see below). Finally, the nutrient solution was mixed thoroughly by gently shaking the flask.

The experiment included eight types of hydrophilic membrane filters: cellulose acetate, CA (Sartorius, Nieuwegein, The Netherlands), cellulose nitrate, CN, nylon, NY, and polyester, PE.
(Fisher Scientific, ’s-Hertogenbosch, The Netherlands), mixed cellulose ester, MCE, polycarbonate, PC, and polyvinylidene fluoride, PVF (Millipore, Etten-Leur, The Netherlands) and polyamide, PA (Schleicher & Schuell, ’s-Hertogenbosch, The Netherlands) with pore size 0.2 or 0.22 µm and a diameter of 25 mm. Polyamide filters were punched out of 50-mm diameter filters. All filter types were readily available from laboratory suppliers and needed no pre-treatment before filtration. Filters were handled with tweezers and placed in a filter holder of either polysulphone or polycarbonate. The filter holder with the membrane was screwed tightly on a syringe and exactly 5 mL medium was pipetted into the back of the syringe. The medium was pushed through the membrane and the final 2 mL were collected in pre-weighed tubes. Then, the membrane was removed from the filter holder and put into a tube which was preweighed with a dry membrane. Also, 2 mL of unfiltered medium was pipetted in duplicate in pre-weighed tubes. All tubes and samples were weighed on an analytical balance (Mettler-Toledo, Greifensee, Switzerland) with an accuracy (standard deviation) of 0.00002 g, to be able to correct for attached medium on the filters and differences in sample weight.

4.2.4 Activity measurements

Samples were measured with a well-type detector of high-purity germanium with a full width - half maximum (FWHM) of 2.4 keV at 1332 keV (60Co). Filling height corrections were made to account for differences in geometry of the samples. For interpreting γ-ray spectra, 24Na was taken into account, next to the six intentionally-produced radionuclides, because 24Na is nearly always produced during irradiations as a result of the omnipresent Na. Gamma-ray spectra were interpreted according to Blaauw (1994).

The samples were measured twice: immediately after the experiment (counting time 15 min per sample) and again after 6 days (counting time 4 h per sample) to optimise results for the long-lived isotopes 140La and 65Zn (see half-lives in Table 4-2). The two measurements were combined to produce final activities of radionuclides in the samples, calculated back to the starting time of the experiment. If the activity of a radioisotope was equal to or below the detection limit, its activity was estimated as 20% of the detection limit, corresponding to a detection probability of 50%, and with a standard deviation of twice the detection limit (Blaauw, 1993). Finally, activities (Bq) were converted to moles. To be able to calculate propagated errors in the metal amounts, an arbitrary error of 1% in stock solution concentrations was assumed.

4.2.5 Statistics and data handling

Data were reported as weighted means with standard errors of the mean (SEM, \( n = 2 \)). Standard errors were calculated using common error propagation rules. A one-way analysis of variance (ANOVA) was applied to test differences between filters and between filtrates and unfiltered medium. This implies that values were assumed to come from a Gaussian population. This assumption is reasonable, as there are many independent error sources involved. A significant ANOVA (\( p < 0.05 \)) was followed by a Tukey test to compare all pairs of means. Pearson’s cor-
relation coefficients were calculated for metal amounts and charges on filters against total metal and free ion concentrations in solution. Finally, linear regression of logarithmically-transformed metal amounts on filters against logarithmically-transformed metal concentrations in solution was performed. The software program Prism, version 2.01 (GraphPad Software, San Diego, CA, USA) was used for all calculations.

4.2.6 Speciation calculations
To be able to assign chemical forms to each of the metals in the medium, equilibrium speciation calculations were performed with the software program CHEAQS (Verweij, 2000). Information from these calculations, i.e. the speciation of a metal in solution, may help to explain its sorption behaviour. Some changes and additions to the program’s stability constants database were made. Six constants for MES were added (Good et al., 1966; Balikungeri, 1989) and twelve constants for La were added or replaced (Millero, 1992; Lee and Byrne, 1992; Lee and Byrne, 1993; Liu and Byrne, 1997; Bonal et al., 1998).

Precipitation equilibria were included in the calculations and CO₂ (g) saturation of the solution was assumed, using the ambient partial pressure of this gas, $p_{CO₂}$ of 3.5×10⁻⁴ atm (Stumm and Morgan, 1996). This assumption seems reasonable, since stock solutions and the final nutrient solution were open to exchange with the air above at all times. The from CO₂ diffusion derived carbonate in solution (total concentration 12.6 µM) is predominantly associated with H⁺ (95.25% H₂CO₃ and 4.73% HCO₃⁻) and only for about 0.01% and 0.02% with Mg²⁺ and Ca²⁺, respectively. Furthermore, calculations for a closed system showed that no significant change occurred in ionic strength, nor in the speciation of any of the metals studied. Redox equilibria, which are important for Mn speciation, amongst others, were not included in the calculations, since Mn oxidation takes place at a rate too slow to be relevant within the time-frame of our experiments (Stumm and Morgan, 1996).

4.3 Results
4.3.1 Measurements
The activity measurements of filters and filtrates, obtained with a filter holder of either polysulphone or polycarbonate, showed excellent agreement, and no losses of activity occurred. Hence, the filter holder material had no detectable influence on metal sorption to the filters. Therefore, observations obtained with different filter holders were treated as replicates. Specific activities were calculated from measurements of unfiltered medium at the starting time of the experiment and are given in Table 4-2. Combining specific activities with metal concentrations (Table 4-1) yields the activity concentrations, which are also given in Table 4-2. Zinc-65 had the lowest activity concentration (1.36 Bq·mL⁻¹) and ⁴²K the highest (1,730 Bq·mL⁻¹). Because of the low ⁶⁵Zn
activity concentration and associated analytical difficulties, Zn data are calculated and presented primarily on basis of the $^{69}$mZn measurements.

Metal concentrations in filtrates and unfiltered medium are given in Fig. 4-1. The length of the error bars indicates that measurements of Cu and Zn were less reproducible than those of La, K and Mn, which is related to the activity concentrations (Table 4-2). Significant differences were detected only for Mn between three filtrate types and the unfiltered medium. This is probably due to the somewhat high value for unfiltered medium and could not be explained by significant adsorption of Mn to the three filter types concerned. Moreover, Fig. 4-1 shows that no significant losses occurred during filtration for any of the metals.

Results from the filter measurements are presented in Fig. 4-2. Activities on filters were corrected for attached medium by calculating the attached volume from the weight measurements (varying roughly from 8 µl for polycarbonate to 80 µl for mixed cellulose ester) and subtracting the corresponding unfiltered medium activity of that volume. In three cases (once each for Mn, Zn and Cu), correction procedures resulted in negative values for metal amounts, reflecting uncertainties in weighing, but more importantly in activity measurements. This results from some activity measurements being equal to or below the detection limit (see section 4.2.4), while weights were established easily. However, negative values were not significantly different from zero (see Fig. 4-2). Although measurements of the filtrates showed no decrease in metal concentrations,
adsorption of La, K and Mn to filters was significant and clear differences between filters were observed (indicated with different letters in Fig. 4-2). All eight filter loads for La were significantly different from zero, but for K only four filters had significant loadings (CA, CN, MCE, PVF), as was the case for Mn (CA, CN, PE, PVF). For Cu and both Zn isotopes, no significant adsorption to filters or differences between filters were found. As nylon belongs to the polyamides group, one would expect the same results for the nylon and polyamide filter. Apart from La, which has a significantly higher loading on polyamide, this was indeed the case.

4.3.2 Speciation and adsorption

Table 4-3 gives the relevant chemical species of K, Mn, Cu, Zn and La in nutrient solution at pH 5.0 as calculated by CHEAQS. The corresponding calculated concentrations of the free metal ion are 49.1 µM for K⁺, 135 nM for Mn²⁺, 0.039 pM for Cu²⁺, 98.6 pM for Zn²⁺ and 11.6 pM for La³⁺. The free ion is for these metals quantitatively the most important cationic species. In case of La there is one other cationic species in a significant amount, namely La(SO₄)⁺, with a concentration of 7.6 pM. While K is almost exclusively (~ 99.8%) present in a free cationic form, Cu, Zn, La and Mn are mainly (> 86%) present as negatively charged EDTA complexes (Table 4-3). This could explain why Mn and La show affinity for the same filters (notably polyester and polyvinylidene fluoride), which differ from those to which K adsorbs (Fig. 4-2).
on the speciation of Cu and Zn, similar results were expected as for La. However, none of the 
Cu and Zn filter measurements differed significantly from zero.

Table 4-3. Speciation of K, Mn, Cu, Zn and La in nutrient solution at pH 5.0, calculated with 
CHEAQS (Verweij, 2000). Note that only values ≥ 0.1 mole percent are given

<table>
<thead>
<tr>
<th>metal</th>
<th>species</th>
<th>% of total for each metal</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>K⁺</td>
<td>99.78</td>
</tr>
<tr>
<td></td>
<td>KSO₄⁻</td>
<td>0.16</td>
</tr>
<tr>
<td>Mn</td>
<td>Mn²⁺</td>
<td>13.32</td>
</tr>
<tr>
<td></td>
<td>MnSO₄</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>MnEDTA₂⁻</td>
<td>83.82</td>
</tr>
<tr>
<td></td>
<td>MnHEDTA⁻</td>
<td>2.37</td>
</tr>
<tr>
<td>Cu</td>
<td>CuEDTA₂⁻</td>
<td>97.25</td>
</tr>
<tr>
<td></td>
<td>CuHEDTA⁻</td>
<td>2.75</td>
</tr>
<tr>
<td>Zn</td>
<td>ZnEDTA₂⁻</td>
<td>97.75</td>
</tr>
<tr>
<td></td>
<td>ZnHEDTA⁻</td>
<td>2.19</td>
</tr>
<tr>
<td>La</td>
<td>La³⁺</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>LaEDTA⁻</td>
<td>99.54</td>
</tr>
<tr>
<td></td>
<td>LaHEDTA⁻</td>
<td>0.27</td>
</tr>
</tbody>
</table>

To establish the role of the free metal ion in adsorption, different approaches were tried to relate 
speciation with adsorption. Firstly, we compared sorbed amounts with the amount of free metal 
ion passing the filter, denoted by the dotted lines in Fig. 4-2. For K and Mn, the sorbed amounts 
were much lower than the amount of free metal ion passing the filter, but the opposite was true 
for La on most filters (Cu and Zn showed no significant adsorption). This means that (negatively 
charged) EDTA complexes of La contribute directly to the observed filter load by adsorption, or 
indirectly by supplying the free ion by dissociation.

Secondly, the correlation of metal amounts on filters with total metal and free ion concen-
trations in solution was studied. In evaluating the outcome of such calculations, it must be kept 
in mind that free ion concentrations have an extra uncertainty introduced by the speciation cal-
culations. The same correlation analyses were made for filter load expressed as charges, to study 
if relationships improved by accounting for mono-, di- and trivalency of the metals. Both total 
and free metal concentrations in solution were highly correlated ($r^2 > 0.85$) with metal load on all 
types of filters. For polyamide (PA) no correlation coefficient could be calculated, for there were 
too few data left after the exclusion of two negative values. The highest correlation coefficients 
($r^2 > 0.999$) were observed for cellulose type filters (cellulose acetate, cellulose nitrate, mixed 
cellulose ester) and free metal concentrations. Metal loads on the other -artificial polymeric- filter 
types (nylon, polycarbonate, polyester, polyvinylidene fluoride) correlated best ($0.853 < r^2 < 
0.999$) with total metal concentrations in solution. Fig. 4-3 shows the data for these two groups
of filters on a logarithmic scale. The metal load on artificial polymeric filters against total metal concentrations in solution are satisfactorily described by linear regression, but the trend for cellulose filters and free metal concentrations was significantly non-linear (runs test, \( p = 0.003 \)), see also Fig. 4-3. Converting metal amounts on filters to charges on filters weakened most correlations, and even made those of the nylon filters non-significant (\( \chi^2 = 0.49 \)).

![Log-log plot of metal load on filter in pmol versus metal concentrations in solution in pM. M = metal. Closed symbols: cellulose type filters against free metal concentrations (average ± SEM, \( \bar{n} = 3 \)), open symbols: artificial polymeric filter types against total metal concentrations (average ± SEM, \( \bar{n} = 4 \) or 5)](image)

In Table 4-4, the metal load on each filter is given as a percentage of the metal amount passing the filter, in total moles retained on the filter and in positive charge equivalents. If the metal load on the filter would mainly consist of di- and trivalent metals, then values in the column ‘positive charges’ would be 2 to 3 times greater than those in the column ‘moles’. However, the values in these columns are almost equal (values for nylon and polyester produced the largest differences, i.e. 1.4 and 1.2, respectively). This shows that monovalent K is almost exclusively responsible for the metal load on the filters, for moles correspond almost one to one with positive charge equivalents.

### 4.4 Discussion

#### 4.4.1 Solution composition

Lanthanum is not a regular component of culture solutions and its addition has consequences for other solution components. The concentration of La itself is very low (10 nM) and direct influences on other medium components are not expected. However, the EDTA concentration of the medium had to be increased from the original 3.76 µM to 5.37 µM to prevent La from precipitating with phosphate (\( K_c = 10^{-21.7} \text{ mol}^2\text{ L}^{-2} \) at 25°C and I = 0.0 M; Liu and Byrne, 1997).
Increasing the EDTA concentration hardly influences the speciation of Cu, Zn, Co and Fe, because they are bound to EDTA for more than 95% already. However, the higher EDTA concentration did change the speciation of Mn. The concentration of free Mn$^{2+}$ decreased from 0.65 to 0.14 µM and the concentration of MnEDTA$^{2-}$ increased from 0.32 to 0.85 µM. As both species are still present in a substantial and measurable fraction of the total Mn present, it was considered acceptable. La was chosen to represent trivalent metals instead of Fe, which is the only trivalent metal present in the original medium. The reason is that $^{59}$Fe, the radioisotope produced during Fe irradiation, is less suitable for low concentration measurements than $^{140}$La; the activity concentration of $^{59}$Fe after 24 h of irradiation would be only ~1 kBq·L$^{-1}$, against 1,150 kBq·L$^{-1}$ for $^{140}$La (see Table 4-2).

Table 4-4. Metal amount on the filter in percentage of metal in the filtered volume (5 mL). For the five metals summed, total moles and corresponding positive charges on the filter are given

<table>
<thead>
<tr>
<th>filter type</th>
<th>% metal on filter</th>
<th>five metals summed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
<td>Mn</td>
</tr>
<tr>
<td>cellulose acetate</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>cellulose nitrate</td>
<td>1.6</td>
<td>0.2</td>
</tr>
<tr>
<td>mixed cellulose ester</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>nylon</td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>polyamide</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>polycarbonate</td>
<td>0.04</td>
<td>0.1</td>
</tr>
<tr>
<td>polyester</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>polyvinylidenefluoride</td>
<td>0.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*a data for $^{69m}$Zn are used, $^{b}$ calculated as K + Mn + Cu + Zn + La, negative values excluded, $^{c}$ calculated as K + 2(Mn + Cu + Zn) + 3·La, negative values excluded, $^d$ negative value (see 4.3 Measurements)

4.4.2 Adsorption to filters

It was anticipated that the filters have some cation exchange capacity (CEC) and thus would electrostatically bind free metals and positively charged complexes (Menzies et al., 1991b; Wenzel and Wieshammer, 1995; Räsänen and Stenius, 1997). Fig. 4-3 supports this suggestion for cellulose type filters. From Table 4-4 it can be derived from the similarity in values of the columns ‘moles’ and ‘positive charges’ that sorption of the monovalent cation K$^{+}$ is almost exclusively responsible for the metal load on the filters. Furthermore, considering the similarity in La and Mn sorption to the filters it is hypothesized that di- and trivalent metals bind to specific sites on polyester filters or, alternatively, that polyester has some affinity for negatively charged metal-
LA ADSORPTION TO MEMBRANE FILTERS

EDTA complexes. The highest metal load for Cu (although not significant), of which > 99% is associated with EDTA (Table 4-3), was on the polyester filters as well. Our results agree with those of Salbu et al. (1985), who found that the anions are more prone to sorption on non-ionic polymers than cations.

Polycarbonate and nylon showed the lowest affinity for metals and are as such recommended for the filtering of culture media and other metal containing solutions. Thereby it is recommended to pre-equilibrate filters in the solution to be filtered and so saturate possible binding sites (Salbu et al., 1985; Danielsson, 1982). Our results are in agreement with those of Wenzel and Wieshammer (1995) and Wenzel et al. (1997) who found that nylon membranes had no effect on metal concentrations at pH values between 3 and 5, presumably due to the low CEC, and also with Taylor and Shiller (1995) who advocate the use of polycarbonate filters because of the low surface area. Cellulose type filters retained the highest metal load (Table 4-4), which was expected, for they can be considered depth filters with a relatively high active surface (Danielsson, 1982; Bailey, 1990). Winger et al. (1998) found that metal concentrations in so-called peepers fitted with cellulose membranes were always lower than in pore waters collected with other techniques. For La, a loss of 5% was reported by Sholkovitz (1992) using mixed cellulose ester filters.

Overall, there were significant differences between filters, but no significant decreases of metal concentrations in the filtrates (maximum loss for the sum of five metals was 3.5%, polyester filter, Table 4-4). These results have to be considered in view of the filtered volume (5 mL), the total solution composition (only five of the ten metals present were measured) and the solution pH (5.0). Filtering a smaller volume is more likely to produce significant differences in metal concentrations in filtrates, because, at equal concentrations, the metal amount passing the filter is smaller. Since membrane filters have a certain, saturable capacity to bind cations (CEC), the amount of metals that may adsorb is best conceived an absolute value, independent of filtered volume. Consequently, the loss of metal (species) from solution is more important for small samples, especially when there is too little sample to pre-equilibrate the filter and saturate its capacity. Other -non-spiked- metals in our solution (Na, Ca, Mg, Co and Fe) will probably adsorb to filter sites as well, thus contributing to the total metal load, but this was not quantified here.

The relatively low pH of our solution causes considerable competition between protons and metal cations for filter binding sites, thus reducing metal adsorption. Increasing the pH is likely to result in a higher metal load on the filters (Wenzel and Wieshammer, 1995; Stumm and Morgan, 1996) for acidic groups on the filter will deprotonate and become negatively charged. Such an increase of CEC with pH was demonstrated for mixed cellulose filters (Menzies et al., 1991b) and even for nylon filters, significant adsorption of Cu and Pb occurred at pH 6.5 and higher (Winger et al., 1998). To assess the role of pH on free metal concentrations, a titration calculation of our solution was performed with CHEAQS, increasing the pH step-wise from 3 to 9. Since the objective was to study changes in solution complexation, solids were not allowed to form (in this pH range it would only affect La by formation of LaPO4). Fig. 4-4 shows that the speciation of K does not depend on pH, but concentrations of Mn²⁺, Zn²⁺, La³⁺ and Cu²⁺
monotonically decrease as pH increases. In short this means that increasing solution pH will lower free ion concentrations, and ultimately removes metals from solution by precipitation, hence filtration artifacts are expected to be more pronounced at higher pH values. This statement is especially relevant for cellulose filters (see Fig. 4-3), but may also apply to other filter types (see above).

4.4.3 Implications for natural waters

The results presented in this paper are relevant for natural water sampling, because this usually includes a filtration step. Although natural water samples are generally filtered over 0.45 µm, giving the -operationally defined- dissolved metal concentration (Chapman, 1999; Horowitz et al., 1996), adsorption phenomena such as described here are primarily related to the filter material and not its pore size. This stems from the fact that our solution contains virtually no particulates, but instead only truly dissolved metals. However, pore size will gain importance when, in solution, particles are present that may clog the filter, thereby influencing the effective pore size and flow rate (Horowitz et al., 1996). In addition, it must be noted that the pore size 0.45 µm, used to separate dissolved metals, has often been questioned (Chapman, 1999; Horowitz et al., 1996; Stumm and Morgan, 1996), since it still allows for passage of some bacteria (Shirey and Bissonnette, 1991) and colloids (Chapman, 1999; Horowitz et al., 1996; Sholkovitz, 1992), which are often enriched in metals. Therefore, the use of 0.2-µm membranes is advocated, since it reduces the amount of colloids and, more importantly, it sterilises the sample, thus preventing the occurrence of microbially-mediated changes in the water (Stumm and Morgan, 1996). Clogging, however, is more likely to occur in 0.2-µm filters than in 0.45-µm filters.

Especially at high pH, small sample volumes and low metal (species) concentrations, the choice of filter type is a crucial issue. The pH value of water samples depends strongly on sample
type (fresh or salt surface water, ground water, soil or sediment pore water, fog water) but ranges at least from 2.3 to 8.3 (Menzies et al., 1991b; Wenzel and Wieshammer, 1995), including those pH values for which adsorption is expected to play an important role (Stumm and Morgan, 1996). Small sample volumes occur for instance during collection of sediment pore water by syringe extraction (Ankley and Schubauer-Berigan, 1994), soil solution extraction (Menzies et al., 1991a) and particularly during fog water collection, where samples may be as small as 0.2 mL (Chérif et al., 1998). Furthermore, filter choice is of major importance when samples are taken for speciation studies, where care must be taken to minimize the influence of filtration on metal solution equilibria. Concentrations of metal species, e.g. the free metal ion, are often in the nM to pM range, while the highest metal amount on a filter measured in this study was already 3.9 nanomoles. This value must, however, be considered a lower estimate, since only five of the ten metals present were measured.

4.5 Conclusions

Differences were shown for the adsorption of K, Mn and La to different filter types, although no significant decrease in metal solution concentrations was observed. Nylon and polycarbonate filters displayed the lowest adsorption capacity for metals and are thus recommended for filtration of metal-containing solutions. Alternately, the highest amount of metals (3.9 nanomoles) was retained by the cellulose nitrate filter. Polyester filters appear to have a higher affinity for divalent and trivalent metals and/or their negatively charged metal-EDTA complexes. The free metal ion appears to dominate adsorption to cellulose type filters, indicating an electrostatic bonding mechanism. Finally, studying metal sorption greatly benefits from the use of complementary tools like radioisotopes and metal speciation calculations.

Acknowledgements

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CHAPTER 5

ACCUMULATION AND ELIMINATION OF LANTHANUM (La) BY DUCKWEED

(LEMNA MINOR L.) AS INFLUENCED BY ORGANISM GROWTH

AND La SORPTION TO GLASS

This chapter is based on: Weltje, L., Brouwer, A.H., Verburg, T.G., Wolterbeek, H.Th., and
J.J.M. de Goeij (2002) Accumulation and elimination of lanthanum by duckweed (Lemna minor
L.) as influenced by organism growth and lanthanum sorption to glass. Environmental Toxino-
Chapter 5

Abstract

Lanthanide emissions to the environment increase as a result of the growing industrial applications of these elements. However, robust data to evaluate the environmental fate of lanthanides are scarce. This paper describes the accumulation and elimination of lanthanum (La) by common duckweed (Lemna minor L.). Speciation modelling was performed to assure that solubility products were not exceeded. It also showed that La was predominantly associated with ethylenediaminetetraacetic acid (EDTA). Lanthanum concentrations in plants, medium and the amounts sorbed to glass vessels were quantified by using the radioisotope $^{149}$La. The amount of La adsorbed on the glass reached values of 25% of the total La present. A model was formulated to describe La uptake in exponentially growing duckweed in the presence of an adsorptive surface.

Growth-induced dilution appeared more efficient in lowering plant La concentrations than actual elimination. An elimination study revealed two compartments, of which the smallest eliminated 50 times faster than the bigger compartment, which eliminated mainly by growth dilution. The average bioconcentration factor was 2,000 L/kg$^f$ fresh weight and 30,000 L/kg$^d$ dry weight, comparable to those of other higher plants. At the applied concentration of 10 nM, no effects were observed on duckweed growth. However, the high bioconcentration factor warrants monitoring of lanthanide emissions.
5.1 Introduction

Lanthanides (57 ≤ atomic number ≤ 71) are elements without a biological function, although beneficial effects on plants have been reported (Brown et al., 1990; Velasco, 1997). They comprise a chemically homogeneous group of 15 metals, one of which, promethium (Pm), has only radioactive isotopes (maximum $t_{1/2} = 17.7$ y) hence no natural occurrence. The other fourteen lanthanides are ubiquitous in most soils in reasonable quantities. For instance, the concentration of lanthanum (La), the first lanthanide element, in the earth's crust is approximately equal to that of lead (Pb) and higher than that of cadmium (Cd) (Brown et al., 1990; Bowen, 1979). In nature, lanthanides exist mostly in a 3+ oxidation state and form slightly soluble complexes with e.g. phosphate (Liu and Byrne, 1997) and carbonate (Martell and Smith, 1997). Lanthanides can also be found in practically all biota. Natural background concentrations of La in terrestrial plants range from 1.1 to 1.8 mmol·kg⁻¹ dry weight (Markert and Zhang, 1991a) and for aquatic plants of the genus Lemma (duckweed) values are reported of 0.93 and 6.3 mmol·kg⁻¹ dry weight (Kramer et al., 1999; Kovács et al., 1984). In physiological studies, lanthanides are frequently used to inhibit the uptake of free ionic calcium (Ca²⁺), which has a similar ionic radius, but lower charge density than the trivalent lanthanide ion (Evans, 1990; Tlalka and Gabryś, 1993). For duckweed, La³⁺ has also shown to inhibit the uptake of Cd²⁺ and free ionic thallium (Tl⁺) (Kwan and Smith, 1991).

Due to increasing industrial uses of lanthanides and lanthanide-containing ores, large amounts of these elements are emitted to the environment, particularly to the aquatic environment. The main sources of lanthanide emissions are the fertiliser and catalyst industries. The two main producers of artificial fertiliser in The Netherlands introduced about 100 tons of La in surface water in 1994 (Smelt et al., 2000). Nevertheless, lanthanides received little attention in environmental studies so far. Since the final impact of the emissions is largely unknown, knowledge is required on the environmental fate of lanthanides in aquatic ecosystems. Currently, a lack of reliable data exists on availability, uptake and effects of lanthanides in aquatic organisms. Some studies were performed, but they suffered from flaws, such as substantial precipitation as a result of exceeding lanthanide phosphate or carbonate solubility products in the applied media (Maas and Botterweg, 1993; Bogers et al., 1998; Barry and Meehan, 2000). Although steady state bioconcentration factors (BCFₛ) for lanthanides in aquatic plants from the field have been frequently measured, reaching values of up to 46,000 L·kg⁻¹ dry weight (Welte, 1998e), little is known about the actual uptake and elimination processes. Knowledge of the behaviour of one of the lanthanides can easily be extrapolated to other members of this series, for their chemistry is quite similar. In addition, it can help to predict the environmental fate of the radioactive actinides, since trivalent members of this series share a number of chemical characteristics with the lanthanides (Miekeley et al., 1994).

This work describes the uptake and elimination of La by common duckweed (Lemna minor L.). L. minor is a widespread, free-floating higher freshwater plant, which is easy to handle in the laboratory, has a high vegetative growth rate (Landolt and Kandel, 1987), and is known for its accumulative behaviour towards metals (Jenner and Janssen-Mommen, 1989). In our experi-
ments, the mass balance for La was completed by quantifying the La concentrations in medium, plants and the amounts of La adsorbed onto glass vessels. All data were used to formulate a model with which the rates of La transport between the compartments could be described. Experiments were supported by speciation calculations to assure that no oversaturated solutions were used.

5.2 Materials and methods

5.2.1 General

To study La kinetics in duckweed, different exposure regimes were applied (described below). The following conditions apply to all experiments. Plants were cultured axenically and exposed in a climate chamber at a light intensity of 10,000 lux and a day-night regime of 16:8 h. Relative humidity was circa 70% and the temperature 25 ± 2°C. The exposure substrate was a sterilised low-level nutrient medium, adapted from SIS (1995), with an initial pH of 5.05 ± 0.05. Its composition is given in Table 5-1.

Table 5-1. Composition of *Lemma minor* nutrient medium, adapted from SIS (1995), concentrations in mol L⁻¹ (M)

<table>
<thead>
<tr>
<th>component</th>
<th>concentration (M)</th>
<th>component</th>
<th>concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>5.00×10⁻⁴</td>
<td>CaSO₄·5H₂O</td>
<td>2.00×10⁻⁴</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>4.92×10⁻⁵</td>
<td>Co(NO₃)₂·6H₂O</td>
<td>3.44×10⁻⁴</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>3.04×10⁻⁴</td>
<td>NaVO₃</td>
<td>8.20×10⁻⁴</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>2.45×10⁻⁴</td>
<td>FeCl₃·6H₂O</td>
<td>3.11×10⁻⁴</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>1.62×10⁻⁵</td>
<td>Na₂EDTA·2H₂O</td>
<td>5.37×10⁻⁴</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>1.01×10⁻⁶</td>
<td>MES·H₂O⁵⁺</td>
<td>2.34×10⁻⁵</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>1.74×10⁻⁷</td>
<td>LaCl₃·7H₂O⁶⁺</td>
<td>1.00×10⁻⁴</td>
</tr>
</tbody>
</table>

* a 2-morpholinoethane sulfonic acid, a pH buffer, b Lanthanum component; not present in control treatment of experiment 1 and elimination phase of experiment 3.

The applied La concentration was 10 nM, which is about 10 times above the natural background concentration for fresh water (Bowen, 1979), but close to levels found in 0.45-µm filtered surface water of the Rotterdam harbour (Zhu et al., 1998), which is polluted with lanthanides, and the river Rhine (de Boer et al., 1996). All chemicals used were of pro analysis quality. Before preparing test solutions, speciation calculations were performed with the software program CHEAQS (Verweij, 2000), to assure that La remained in solution even if the pH would rise half a unit during the experiments. This demonstrated the necessity of increasing the ethylenedi-

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aminetetraacetic acid (EDTA) concentration from the original 3.76 μM to 5.37 μM. Plants were exposed in 200 ± 1 mL medium in glass vessels, which were closed with a glass lid to minimise evaporation, but allow limited gas exchange. Plant fresh weight was determined after spin drying by 3,000 rpm for 10 min at room temperature to remove attached and free-space water. This method yields highly reproducible measurements, contrary to dry blotting (Wolterbeek et al., 2000). Fresh weight measurements were used to construct growth curves.

5.2.2 Experiment 1. La uptake from refreshed medium

Twenty glass vessels with medium and 10 nM La and 20 glass vessels with La free medium were inoculated with 12 duckweed fronds (3 to 5 colonies). Plants in vessels without La served as a control group to compare their growth rate with La exposed plants and study possible toxicity or growth stimulation of La. At times 48, 96, 144 and 192 h, plants were transferred to vessels with fresh medium (with or without La). At times 0, 4, 8, 24, 45, 55, 72, 94, 124, 168 and 216 h, pH of the medium was measured, fresh weight was determined and La concentrations in media and plants and La amounts on glass vessels were measured. All measurements were performed in duplicate.

5.2.3 Experiment 2. La uptake from non-refreshed medium

Twenty-seven glass vessels with medium and 10 nM La were inoculated with 12 duckweed fronds (3 to 5 colonies). In addition, three vessels with medium but no plants were followed in time, to check for possible precipitation and constancy of pH. The first 48 h were thus the same as for experiment 1. At times 0, 1, 3, 5, 24, 48, 92, 120 and 167 h, pH of the media was measured, plant fresh weight was determined and La concentrations in media and plants and La amounts on glass vessels were measured. All measurements were performed in triplicate.

5.2.4 Experiment 3. La uptake from non-refreshed medium and elimination in refreshed medium

Twenty-eight glass vessels with medium and 10 nM La were inoculated with 12 duckweed fronds (3 to 5 colonies). In addition, three vessels with medium but no plants were followed in time, to check for possible precipitation and constancy of pH. The first 48 h were the same as for experiments 1 and 2. To study elimination, plants from 15 vessels were gently lifted off the surface after 48 h of accumulation and transferred, without spin-drying, to vessels with La free medium. This medium was refreshed at times 55, 72, 120 and 144 h to keep the La concentration in medium as low as possible and thus prevent backflow of La to plants. At times 0, 5.5, 12, 24, 48, 55, 72, 96, 144 and 169 h, pH of the media was measured, fresh weight was determined and La concentrations in media and plants and La amounts on glass vessels were measured. Measurements were performed in duplicate (accumulation) or triplicate (elimination).
5.2.5 $^{140}$La production and activity measurements

Lanthanum was spiked with the radionuclide $^{140}$La ($t_{1/2} = 40.3$ h), which emits $\beta$ and $\gamma$ radiation (main $E_\gamma$ are 329, 487, 816 and 1596 keV). $^{140}$La was prepared by irradiating a quartz vial containing a solution of 0.1 mM LaCl$_3$·7H$_2$O (Aldrich, purity 99.999%) in Milli-Q water (Millipore-Waters, Milford, MA, USA) at a neutron flux of $1.60 \times 10^{17}$ m$^{-2}$s$^{-1}$ for 24 h in the Hoger Onderwijs Reactor of our institute. The solution was allowed to decay for 3 h to eliminate the short-lived chloride isotope $^{36}$Cl ($t_{1/2} = 37.2$ min) and was subsequently added to the rest of the medium components to make up a final La concentration of 10 nM.

Activity of $^{140}$La was measured in media and plants by $\gamma$-detection on a 1480 Wizard 3" gamma counter (Wallac Oy, Turku, Finland) using an energy window of 15 to 2000 keV. Automatic background correction was applied. For measuring $^{140}$La activity on glass vessels a plane NaI(Tl) detector (type 12S12, Harshaw–Bicron, Newbury, OH, USA) of 7.62 cm × 7.62 cm was used, placed in a lead-shielded container. The detector was calibrated with a sample of known activity and the same geometry as the vessels. Lanthanum activities were directly converted to La amounts, which was justified, as the La background concentration of the L. minor culture was about 9 pmol·g$^{-1}$ fresh weight (measured with inductively coupled plasma mass spectrometry, results not shown) and therefore had no effect on the initial specific activity.

5.2.6 Data modelling

Experimental data on duckweed growth and La concentrations in media, plants and glass vessels were described according to the model depicted in Fig. 5-1, assuming first order kinetics for all processes.

![Diagram](image)

**Fig. 5-1.** Model for describing La concentrations in L. minor, medium and on glass vessels. Definition of symbols: $\mu$ = exponential growth rate constant (h$^{-1}$), $k_1$ = uptake rate constant (L·g$^{-1}$·h$^{-1}$), $k_2$ = elimination rate constant (h$^{-1}$), $k_3$ = adsorption rate constant (L·m$^{-2}$·h$^{-1}$), $k_4$ = desorption rate constant (h$^{-1}$).

Duckweed growth, as fresh weight increase, was described by an exponential growth curve (Equation 1):

$$W_t = W_0 \cdot e^{\mu t}$$  \hspace{1cm} (1)

in which $t$ is time (h), $W_t$ is fresh weight at time $t$ (g), $W_0$ is fresh weight at $t = 0$ (g) and $\mu$ is the exponential growth rate constant (h$^{-1}$). Lanthanum concentrations in plants (on a fresh weight
LA UPTAKE AND ELIMINATION BY LEMNA MINOR

\[
\frac{d[La]_p}{dt} = k_1 \cdot [La]_{m}, - (k_2 + \mu) \cdot [La]_p, \tag{2}
\]

\[
\frac{d[La]_{m}}{dt} = \frac{W}{V} \cdot \left( k_2 \cdot [La]_p - k_1 \cdot [La]_{m} \right)^{- \frac{1}{s}} \left( k_1 \cdot [La]_p - k_3 \cdot [La]_{m} \right), \tag{3}
\]

\[
\frac{d[La]_s}{dt} = k_4 \cdot [La]_{as} - k_5 \cdot [La]_s, \tag{4}
\]

\[L_{atm} = [La]_p \cdot W, + [La]_{m} \cdot V + [La]_s \cdot S \tag{5}\]

in which \([La]_p,\) is the La concentration in plants as a function of time (nmol g\(^{-1}\)), \([La]_{m},\) is the La concentration in medium as a function of time (nmol L\(^{-1}\)), \([La]_s\) is the La concentration adsorbed to the glass surface as a function of time (nmol m\(^{-2}\)), \(k_1\) is the uptake rate constant (L g\(^{-1}\) h\(^{-1}\)), \(k_2\) is the elimination rate constant (h\(^{-1}\)), \(k_3\) is the adsorption rate constant (L m\(^{-3}\) h\(^{-1}\)), \(k_4\) is the desorption rate constant (h\(^{-1}\)), \(V\) is medium volume, a constant (0.2 L), \(S\) is glass surface, a constant (0.0157 m\(^{-2}\)) and \(L_{atm}\) is the sum of the La amounts in the three compartments as a function of time, which should be constant (2 nmol). The value for \(\mu\) was estimated first by fitting Equation 1 to duckweed fresh weight data, and then values for \(k_1\) to \(k_3\) were estimated from the data using Equations 2 to 5. From these estimates the dynamic bioconcentration factor, \(BCF_{dyn}\) (L kg\(^{-1}\) fresh weight) was calculated with Equation 6 (Jager and Hamers, 1997).

\[
BCF_{dyn} = \frac{1000 \cdot k_1}{k_2 + \mu} \tag{6}
\]

In experiment 1, the La medium concentration was kept approximately constant by regular transfer of plants to new vessels with fresh medium. Hence, only Equations 1 and 2 apply. The analytical solution for this system is given by Equation 7 (Wolterbeek et al., 1995).

\[
[La]_p = \frac{[La]_{atm} \cdot k_1}{\mu + k_2} \left( 1 - e^{-\left(\mu + k_2\right)t} \right) \tag{7}
\]

in which \([La]_{atm}\) is the average exposure concentration, calculated by linear regression from measurements of La concentrations in medium. The other variables have already been defined above.

Obviously, the model of Fig. 5-1 simplifies our experimental system. For instance, duckweed is expected to consist of multiple compartments, e.g. cell walls, protoplasts and vacuoles (Wolterbeek et al., 2000; Walker and Pitman, 1976). To obtain more information on compartmental behaviour of La in duckweed, elimination was studied (experiment 3), since distinguishing compartments is much easier during an elimination phase (Jager and Hamers, 1997; Looser et al., 2000). In addition, the system was simplified by frequently refreshing the elimination medium, keeping its La concentration close to 0 nM and thus prevent backflow from eliminated La to
duckweed as well as La adsorption by glass. Two plant compartments could be distinguished, and were described with the model depicted in Fig. 5-2 and by Equations 8 to 10.

\[
\frac{d[La]_{p1}}{dt} = k_3 \cdot [La]_{p2} - (k_3 + k_5 + \mu) \cdot [La]_{p1},
\]

\[
\frac{d[La]_{p2}}{dt} = k_4 \cdot [La]_{p1} - (k_4 + \mu) \cdot [La]_{p2},
\]

\[
[La]_{p1} = [La]_{p1,0} + [La]_{p2,0}
\]

in which \([La]_{p1,0}\) and \([La]_{p2,0}\) are La concentrations in duckweed compartment \(p_1\) and \(p_2\) as a function of time, respectively (nmol-g\(^{-1}\)), and \(k_3\) and \(k_5\) are the rate constants describing La transport from the first to the second compartment and back, respectively (h\(^{-1}\)). The other variables have already been defined above.

**Fig. 5-2.** Model for describing elimination of La from *L. minor*, consisting of two compartments, \(p_1\) and \(p_2\). Definition of symbols: \(\mu\) = exponential growth rate constant (h\(^{-1}\)), \(k_3\) = elimination rate constant (h\(^{-1}\)), \(k_4\) = transport rate constant from \(p_1\) to \(p_2\) (h\(^{-1}\)), \(k_5\) = transport rate constant from \(p_2\) to \(p_1\) (h\(^{-1}\)).

Linear and non-linear least squares regressions were performed with Prism, version 2.01 (GraphPad Software, San Diego, CA, USA) and numerical analyses with Scientist, version 2.01 (MicroMath Inc., Salt Lake City, UT, USA).

**5.3 Results and discussion**

**5.3.1 Growth and toxicity**

During the course of the experiments duckweed growth was purely exponential, indicating that growth-limitation under the present conditions is not occurring. Exponential growth was one of the conditions for application of the proposed models. From Table 5-2 it can be seen that growth rate constants (\(\mu\)) are not significantly different between experiments, indicating the reproducibility of the experimental and culturing conditions. Differences in \(W_0\) reflect variations in duckweed fresh weight at the start of the experiments and do not influence \(\mu\) (Table 5-2).

Simultaneously tested control cultures in experiment 1, in which the most intensive exposure to La occurred, had growth rates equal to exposed cultures (Table 5-2). This leads to the conclusion that an initial medium concentration of 10 nM La, which is mainly associated with
EDTA, has no adverse or beneficial effects on duckweed growth in 9 days. The associated maximum concentration of La in *L. minor* without effects was 12 nmol g⁻¹ fresh weight.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$W_0$ ± s.e. (g)</th>
<th>$\mu$ ± s.e. (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (La exposed)</td>
<td>0.009 ± 0.003</td>
<td>0.0191 ± 0.0016</td>
</tr>
<tr>
<td>1 (control)</td>
<td>0.011 ± 0.002</td>
<td>0.0185 ± 0.0009</td>
</tr>
<tr>
<td>2 (La exposed)</td>
<td>0.014 ± 0.002</td>
<td>0.0190 ± 0.0007</td>
</tr>
<tr>
<td>3 (La exposed)</td>
<td>0.021 ± 0.002</td>
<td>0.0186 ± 0.0007</td>
</tr>
</tbody>
</table>

### 5.3.2 Speciation and availability

Speciation calculations showed all La to be in solution up to pH 5.6 (initial pH 5.05) and for more than 99.9% associated with EDTA. They also showed that a substantially higher pH or an EDTA concentration below 5.37 μM would result in precipitation of LaPO₄ ($K_{sp} = 10^{-29.7}$ mol²L⁻² at 25°C and $I = 0.0$ M; Liu and Byrne, 1997). The free La³⁺ ion concentration at pH 5.0 at the start of the experiment was calculated to be 12 pM, i.e. 0.12% of the total La concentration. While some researchers assume that for *Lemna* the free metal ion is the species available for uptake (Huebert and Shay, 1992), there is also evidence for uptake of complexed metals by *Lemna* and other higher plants. The results of Buckley (1994) for copper (Cu) and *L. minor* indicated that next to the free Cu²⁺ ion, some Cu was taken up as organic complexes. For La and hydroponically grown *Triticum sp.*, it was shown that uptake of La by roots decreased in the presence of EDTA, while uptake in the shoots increased (Sun *et al.*, 1997b). Vasil *et al.* (1998) showed that uptake of Pb by *Brassica juncea* was directly correlated with EDTA uptake and also that Pb is transported in co-ordination with EDTA in xylem exudate and Wolterbeek *et al.* (1988) suggested simultaneous uptake of Cd and EDTA by *Solanum hypericium*, possibly as CdEDTA²⁻ complex. It must be noted that these observations on metal availability are of course subject to metal-complex lability. As our experiments cannot discriminate between uptake of ionic La³⁺, which is supplied by dissociation of the LaEDTA complex, and the complex itself, both possibilities are considered here. Work is in progress to resolve this question.

### 5.3.3 La in medium and on glass vessels

Values for medium pH in treatments with or without plants were all within the desired range, between 5.0 and 5.5, thus assuring the abovementioned speciation calculations to be valid. Precipitation is therefore not expected. However, [La]ₘₚ in the vessels without plants decreased slightly (Fig. 5-3b), which is probably due to adsorption of La onto the glass vessels (see for instance Fig. 5-4b). The decrease of [La]ₘₚ in the vessels with plants (Fig. 5-3) is due to adsorption
Fig. 5-3. Lanthanum concentrations (in nM) in media with plants (closed symbols) and without plants (open symbols) versus time (in h). Symbols are averages with standard errors (n = 2 to 9). If error bars are not visible, they fall within the symbols. Data from experiments 1, 2 and 3 are presented in a, b and c, respectively. The vertical dashed lines in a and c indicate medium renewal and the solid lines are fits.

Fig. 5-4. Mass balances (in nmol) versus time (in h). Totals (closed dots) are the sum of La amounts in duckweed (squares), medium (asterisks) and glass vessels (open dots). Symbols are averages with standard errors (n = 2 or 3). If error bars are not visible, they fall within the symbols. Data from experiments 1, 2 and 3 are presented in a, b and c, respectively.

on the glass, and, more importantly, due to La uptake by duckweed (Fig. 5-4). This underlines the importance of combining substance transport between (closed) compartments with a mass balance. While at the start all La is in the medium, it can decrease to 20% at the end of the experiment (Fig. 5-4b). In the first experiment, where medium was renewed, it is clear that with the increase of the transferred biomass, the decrease of the La medium concentration goes ever
faster (Fig. 5-3a). Since the net La concentration in medium is decreasing at the applied refreshing rate, it would make sense to increase either the refreshing rate or the refreshed volume, corresponding to the amount of transferred biomass, and achieve a better semi-constant exposure. While the net La concentration in medium of experiment 1 is decreasing, the average exposure concentration (depicted by the solid horizontal line in Fig. 5-3a) was assumed to be constant. This means that by applying Equation 7, the real exposure is underestimated at the beginning and end of this experiment.

The mass balances (Fig. 5-4) show that amounts of La on glass can measure up to 25% of total La present. From all measurements, La on glass was the most variable, which may be attributed to a lower γ-detection efficiency associated with sample geometry, and differences in adsorptive capacity among glass vessels. Estimated values for the adsorption rate constant $k_s$ in experiments 2 and 3 are $0.117 \pm 0.039$ and $0.055 \pm 0.007 \text{ L.m}^{-2}\text{h}^{-1}$, respectively. For the desorption rate constant $k_d$ a value of $0.019 \pm 0.009 \text{ h}^{-1}$ was estimated in experiment 2, while in experiment 3 it took negative values and was consequently set to 0. This partly explains the lower value for $k_d$ in experiment 3, which, since desorption is not occurring, represents a net adsorption. Loos et al. (2010) also incorporated a net adsorption rate in their bioaccumulation model, but did not support it with data on metal amounts on the glass.

By using radionuclides it is relatively easy to quantify the La amount on the glass vessel and thus complete the mass balance, which was typically 98 ± 8%. With other analytical methods of metal detection it would be much more elaborate to quantify the amount of metals lost, but as the present study shows, adsorption can have a substantial influence on the applied concentration. To minimize this influence, containers with lower adsorption capacity for La should be applied.

5.3.4 Modelling La in duckweed

The accumulation of La in duckweed (Figs. 5-5b and 5-5c) was well described by the proposed model of Equations 1 through 5. This model assumes an exponentially growing organism in a closed system, accumulating a substance in the presence of an adsorptive surface. The resulting pattern is similar to the one described for uptake of nonpersistent chemicals (Widranarko and van Straalen, 1996). As a result of uptake and adsorption, the medium concentration of La is going down. Due to the decreasing medium concentration, La in duckweed is not reaching equilibrium. However, this does not preclude calculation of a dynamic bioconcentration factor, $BCF_{aw}$ (Eqn. 6, Table 5-3). Equation 7, which assumes constant exposure, did not describe La concentrations in duckweed satisfactorily, because the net exposure concentration was not (semi-)constant but still decreasing and hence equilibrium was not reached. This explains the obvious misfit of Equation 7 with the experimental data (Fig. 5-5a). Refreshing the medium did not result in higher La concentrations in plants (compare Fig. 5-5a with Figs. 5-5b and 5-5c), but in fact made modelling of these discontinuous data less straightforward. Therefore, application of an unrefreshed medium is advocated, which enables the integral use of a mass balance in model-
Chapter 5

Fig. 5.5. Lanthanum concentrations in L. minna (in nmol g⁻¹ fresh weight) versus time (in h). Symbols are averages with standard errors (n = 2 or 3). If error bars are not visible, they fall within the symbols. Data from experiments 1, 2 and 3 are presented in a, b and c, respectively. The solid lines are fits. The vertical dashed lines in a and c indicate medium renewal. The observation represented with an open symbol in a was considered an outlier and consequently excluded from fit.

ling the data, in turn leading to a better description of exposure. The model can be applied under the following conditions: 1) growth must remain exponential during exposure, 2) no substance is lost and 3) substance transport is satisfactory described with first order kinetics.

The first five hours gave rise to a deviation from the model predictions (Figs. 5-5a to 5-5c) which slightly overestimated La concentrations in duckweed. The cause for this small delay in uptake, which was replicated in all experiments, is yet unknown. It may be related to transferring the plants at the beginning of the experiment from the culture to test solution.

The maximum concentration of La in duckweed in our experimental system, 12 nmol g⁻¹ fresh weight, is reached at t ≈ 50 h (Fig. 5-5b). At t = 216 h in experiment 1, the La amount in duckweed was 4 nmol (Fig. 5-4a), which equals the total amount of La present in 400 mL medium (the contents of two vessels). At the end of experiment 2, about 60% of La in medium were taken up by duckweed (Fig. 5-4b).

The data of the elimination phase in experiment 3 were in excellent agreement with the proposed two-compartment model of Equations 8 to 10 (Fig. 5-5c). The second compartment, p₂, contained more La, 6.86 ± 1.50 nmol g⁻¹ fresh weight, and appeared to eliminate slower than p₁, the first compartment, which contained 4.50 ± 1.53 nmol g⁻¹ fresh weight. Values for the transport rate constants k₅ and k₆ were 0.157 ± 0.020 and 0.019 ± 0.005 h⁻¹, respectively.
5.3.5 Uptake and elimination rate constants and bioconcentration factors

In Table 5-3, the calculated values for uptake and elimination rate constants ($k_1$ and $k_2$) and the resulting dynamic bioconcentration factor ($BCF_{dynamics}$) with their respective standard errors are given. For reasons stated above, the rate constants from the accumulation phases of experiments 2 and 3 are considered more reliable than those from experiment 1. This can also be concluded from the calculated standard errors. The deviating value for $k_2$ from the elimination phase of experiment 3 will be discussed separately. Values for $k_1$ range from 0.049 to 0.056 L.g$^{-1}$.h$^{-1}$ and are not significantly different. Furthermore, because $\mu > k_2$, it is concluded that growth-related dilution is more important than elimination sensu stricto in lowering the plant La concentration. This stresses the great importance of considering plant growth rates in uptake and elimination studies, something not always recognized (Nigam et al., 1998). However, in experiment 1, $k_2 > \mu$, but this is due to the fact that by applying Equation 7 to these data, the elimination rate constant must partly compensate for the decreasing medium concentration. The true elimination rate is smaller than the growth rate.

Table 5-3. Estimated values and standard errors (s.e.) for $k_1$, the uptake rate constant, $k_2$, the elimination rate constant (Eqns. 2-5, 7) and $BCF_{dynamics}$ the dynamic bioconcentration factor (Eqn. 6). Units in parentheses

<table>
<thead>
<tr>
<th>experiment</th>
<th>$k_1 \pm$ s.e. (L.g$^{-1}$.h$^{-1}$)</th>
<th>$k_2 \pm$ s.e. (h$^{-1}$)</th>
<th>$BCF_{dynamics} \pm$ s.e. (L.kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. accumulation</td>
<td>0.050 ± 0.012</td>
<td>0.024 ± 0.012</td>
<td>1.145 ± 0.416</td>
</tr>
<tr>
<td>2. accumulation</td>
<td>0.049 ± 0.004</td>
<td>0.006 ± 0.003</td>
<td>1.917 ± 0.267</td>
</tr>
<tr>
<td>3. accumulation</td>
<td>0.056 ± 0.003</td>
<td>0.007 ± 0.003</td>
<td>2.198 ± 0.286</td>
</tr>
<tr>
<td>3. elimination</td>
<td>$^a$</td>
<td>0.339 ± 0.049</td>
<td>$^a$</td>
</tr>
</tbody>
</table>

$^a$ uptake not occurring, so $BCF_{dynamics}$ is not calculable, see text, $^b$ value estimated with Eqns. 8 to 10.

The elimination rate constant from the elimination phase of experiment 3, is circa 50 times higher than the average $k_2$ value. This rate constant represents a small and fast eliminating compartment, which could not be distinguished in the accumulation phase. This fast elimination might be caused by transferring the plants -without spin-drying- to medium without La and the presence of uncomplexed EDTA therein, which has a high affinity for La ($logK_2 = 17.92$ at 25°C and $logK_3 = 0.0$ M; Martell and Smith, 1997). Judged by its value it is likely that $k_2$ from the elimination phase represents a different elimination process than $k_2$ calculated from the accumulation phase.

Approximately 60% of the La taken up are only slowly eliminated by duckweed, mainly by growth-dilution. This can be seen in Fig. 5-4c, where the amount of La in plants remains almost constant in the second half of the elimination phase. The mass balances of the other experiments show that the amount of La in duckweed steadily increases, while concentrations decrease (compare Figs. 5-4a and 5-4c with 5-5a and 5-5c). This is illustrative for growth-induced dilution. The calculated $BCF_{dynamics}$ for La in duckweed ranges from 1,145 to 2,198 L.kg$^{-1}$ fresh weight (Table 5-3).
CHAPTER 5

Given that the fresh to dry weight ratio of duckweed is approximately 15 (results not shown), this means a $BCF_{La}$ between 17,175 and 32,970 L·kg$^{-1}$ on a dry weight basis, indicating the high accumulating potential of duckweed for La. This value for <i>L. minor</i> has the same order of magnitude as previously reported $BCF_{La}$ for La in other higher aquatic and terrestrial plants (for an overview, see Weltje, 1998c).

5.4 Conclusions

In summary, it can be stated that <i>L. minor</i> takes up La from medium as LaEDTA complex and/or as ionic La$^{3+}$, reaching a $BCF_{La}$ of 32,970 L·kg$^{-1}$ on a dry weight basis. Elimination study revealed a large (60%) slow and small (40%) fast compartment. The large compartment eliminates mainly by means of maintaining a high growth rate. The proposed model incorporating a mass balance rule and exponential growth of the exposed organism, agrees well with the experimental data. Neither stimulating nor toxic effects of 10 nM complexed La on duckweed growth in 9 days were observed. However, considering the high $BCF_{La}$ values, effects may occur under constant exposure as uptake continues at the rate reported here. The use of radionuclides in uptake studies such as described here, can reveal ‘missing’ compartments of the mass balance, e.g. the glass vessels to which metals may adsorb. Support by speciation calculations in applying difficult substances in culture media, reveals the possible exceeding of solubility products. Refreshing the medium did not result in higher La concentrations in plants, but instead made the modeling of these data less straightforward.

Acknowledgements

The authors are grateful to their colleagues Wim den Hollander, Koos Kroon and Joris Haftka for assistance with γ-detection, irradiations and experiments, respectively, and to Heike Heidenreich (IHI Zittau, Germany) for ICP-MS measurement of La in <i>L. minor</i>. The comments of Prof.dr. Bernd Markert (IHI Zittau, Germany) and two anonymous reviewers are gratefully acknowledged. Part of this work was presented at the SETAC-Europe conference in Leipzig, Germany, 25-29 May 1999.
CHAPTER 6

LUTETIUM (LU) SPECIATION AND TOXICITY
IN A MICROBIAL BIOASSAY WITH VIBRIO FISCHERI.
A TEST OF THE FREE-ION MODEL FOR LANTHANIDES

This chapter is based on:
CHAPTER 6

Abstract
The validity of the free-ion model was tested in a bioassay with the bacterium Vibrio fischeri for the element lutetium (Lu), the heaviest member of the lanthanide series. The free-ion model is mainly based on experimental evidence concerning divalent metals and synthetic ligands and has not been tested with a member of the trivalent lanthanides.

The bioluminescence response of V. fischeri, a measure for its metabolic activity, was studied at different Lu concentrations in a 0.355 M NaNO₃ solution of pH 5.50, in the presence and absence of natural and synthetic organic ligands (citrate, malate, oxalate, acetate, EDTA and NTA). All ligands were tested separately to assure that their concentrations would not cause adverse effects themselves. This led to the exclusion of acetate, for which toxic concentrations were needed to sufficiently complex Lu. Free Lu³⁺ concentrations and activities were calculated with the speciation program CHEAQS, after extension of the database with the relevant equilibria involving Lu.

Our results confirmed the free-ion model for Lu, i.e. calculated free Lu³⁺ concentrations or activities had a more apparent relationship with the bioluminescence response of V. fischeri than total dissolved Lu concentrations. However, a contribution of inorganic Lu-complexes (approximately 4% of total dissolved Lu) could not be ruled out. Lutetium in its free form is more toxic than La³⁺, Cd²⁺ or Zn²⁺, and approximately equally toxic as Cu²⁺. The EC₅₀ of free Lu³⁺ after 15 min of exposure is 1.57 ± 0.05 μM and the EC₅₀ at infinite exposure is estimated as 1.35 ± 0.01 μM. Although the pH increased during the experiments from the initial 5.50 to 6.30, it was shown by calculations that the influence on Lu speciation was limited.
6.1 Introduction

The speciation of a metal in solution has great consequences for its availability and effects on aquatic organisms (Allen et al., 1980). Here, speciation is defined as the different physicochemical forms of a metal that together make up its total concentration in solution (adopted from Florence, 1989). Ground-breaking was the work of Sunda and Guillard (1976), who showed that copper toxicity to phytoplankton was best explained by Cu$^{2+}$ activities. These and other well-defined studies with mainly synthetic ligands (e.g. NTA, EDTA) and divalent metals, such as cadmium, copper and zinc, led to formulation of the free-ion model or free-ion activity model, which states that uptake and toxicity of cationic trace metals are regulated by the free-ion activity (Morel, 1983). According to Campbell (1995), the biological response elicited by a dissolved metal is usually a function of the (hydrated) free-metal ion concentration, $M^{n+} (\text{H}_2\text{O})_n$. Note that the former definition refers to ‘activity’ and the latter to ‘concentration’. Concentration $c$ and activity $a_i$ of a species $i$ are related through $a_i = c \cdot \gamma_i$ in which $\gamma_i$ is the dimensionless activity coefficient, which is a function of ionic strength $I$ (in molL$^{-1}$). Further, at infinite dilution ($I = 0.0$ M) values for concentration and activity are equal ($\gamma_i = 1$) and at constant ionic strength, activity of the free ion is a linear function of concentration (Stumm and Morgan, 1996). Testing the free-ion model and possibly extending its validity for trivalent metals, such as the lanthanides, in the presence of natural organic ligands, is a meaningful contribution to aquatic toxicology.

Studying and testing the free-ion model was greatly enhanced by the development of commercially available speciation modelling software and the collection and evaluation of equilibrium constants for metals with all kinds of (in)organic ligands. In this respect, the ‘Critically selected stability constants database’ by Martell and Smith (1997-1998) should be mentioned.

In general, there are three types of approaches to assess the speciation of a metal: 1) analytical chemical methods, 2) biological methods and 3) computational methods. Chemical methods often suffer from problems with detection limits, most notably for ambient concentration levels. In addition, the quality control of analytical measurements is often a problem, since chemical equilibria are easily disturbed. Biological methods (such as bioassays) give in fact the most relevant information for environmental research (e.g. Timmermans et al. (2001), who used diatoms as iron speciation monitors), but unfortunately these methods do not give a quantitative insight in the concentration and precise nature of the toxic substance. Computation of the speciation may be a suitable approach, provided that the relevant equilibria are sufficiently known, including the equilibrium constants (Turner, 1995). For synthetic solutions this is usually the case. Computation is also a cost-effective and fast method and one can easily explore different scenarios (e.g. by means of titrations) and identify the most important parameters that determine the final speciation of a metal. In our specific case, chemical analysis was not a serious option, because of the low volumes (< 1 mL) available for analysis (see Materials and Methods). We have chosen to calculate the speciation in our samples in combination with bioassays. This will allow
use to quantify toxic levels of the relevant species (i.e. the free-metal ion) at low cost and in little time.

Whereas a body of evidence exists to support the free-ion model for divalent metals such as cadmium, copper, lead, nickel and zinc (for an overview see Campbell, 1995), information on trivalent metals, e.g. iron (see Sunda, 2001) and aluminium (Parent et al., 1996) is scarce, and application of the free-ion model has never been described for elements of the lanthanide series. The lanthanides comprise fifteen homologous trivalent metals, ranging from lanthanum (La, $Z = 57$) to lutetium (Lu, $Z = 71$). The industrial uses and applications of lanthanides are still increasing (Hedrick, 1997), and so are the accompanying emissions to the environment. The larger part of the emissions ends up in aquatic environments (Sneller et al., 2000), but only little is known about the effects of lanthanides on aquatic biota. Therefore, lanthanides have received more attention in the past few years. Some aquatic toxicity studies were performed, but because of the low solubility products of lanthanides with phosphate and carbonate, which are present in most artificial media, substantial precipitation occurred in these experiments (e.g. Bogers et al., 1998; Barry and Meehan, 2000). This made it difficult to relate toxic effects to total dissolved concentrations, let alone to free-ion concentrations. Another problem is that not many speciation programs have lanthanide stability constants in their database. For vertebrates it was suggested that toxicity of gadolinium (Gd, $Z = 64$) is related to the free-ion concentration (Cacheris et al., 1990), but Jackson et al. (1990) found no correlation between Gd toxicity and free-ion concentrations. Stanley Jr. and Byrne (1990) found a clear relation between concentrations of free Gd$^{3+}$ and Gd uptake by marine algae in carbonate manipulated media, but could not completely explain their observations based on Gd$^{3+}$ alone.

This work aims at testing the free-ion model for Lu, the heaviest lanthanide, in a bioassay with the luminescent bacterium Vibrio fischeri Beijerinck 1889 (formerly known as Photobacterium phosphorum). This organism is widely used in a non-substance-specific test system known as the Microtox$^\text{®}$ system (Azur Environmental, Carlsbad, CA, USA). Briefly, during metabolism of its substrate the bacterium is not utilising all available ATP energy and converts a fixed portion of this energy into light (luminescence). Conversely the amount of luminescence is proportional to the overall metabolism, and at lower luminescence there is lower metabolism. Hence at increasing exposure to a toxic substance, the luminescence decreases accordingly as indicator of toxic stress.

Data on complexation experiments with Gd, Cu, Ni, Pb and Zn demonstrated that toxic effects on V. fischeri correlate best with free metal ions (Codina et al., 1993; Thomulka et al., 1997, Villaescusa et al., 1998; Davies et al., 1998). These data and the possibility to expose V. fischeri in a simple nitrate medium, whose metal complexing capacity and thus the metal speciation, is easy to manipulate by addition of ligands, made us choose for this organism. In addition, an extensive literature search was performed to collect reliable equilibrium constants for Lu with several organic and inorganic ligands.
6.2 Materials and methods

6.2.1 Experimental

*Vibrio fischeri* is a marine bacterium, and therefore usually exposed in a NaCl solution. However, it can also be exposed in a 0.355 M (or 3.02%) NaNO₃ solution, which reflects freshwater speciation conditions (McCloskey et al., 1996). The ionic strength, however, is intermediate between freshwater and seawater. Since we were interested in Lu effects in freshwater, we chose this NaNO₃ solution. An additional advantage is that nitrate has hardly a complexing capacity for lanthanides (Bonal et al., 1998) and has thus a limited influence on the free-ion concentration. The presence of Na⁺ and the high ionic strength are necessary for undisturbed performance of the bacteria (Watanabe and Hastings, 1986).

For preparing NaNO₃ solutions we used a 99.8% stock of J.T. Baker, and the source for Lu was an ICP stock solution (10,000 ppm of 99.999% Lu₂O₃, in 4% HNO₃, CPI International, Santa Rosa, CA, USA). The amount of NO₃⁻ that was introduced through the use of this stock was accounted for in preparing the final NO₃⁻ concentrations. The pH of the medium was adjusted to 5.50 ± 0.10 with HNO₃ (Merek, Suprapur) and NaOH (J.T. Baker, 98.6%), using a Metrohm 744 pH meter, equipped with a Pt-electrode. Amounts of added acid and base were noted to make volumetric corrections and also to account for added Na⁺ and NO₃⁻ in the speciation calculations. All solutions were prepared with Milli-Q water (Millipore-Waters, Milford, MA, USA).

Using the above medium, we prepared two Lu series: 1) a logarithmic concentration range of Lu without ligands (10 concentrations between 0.15 μM and 2.56 mM), and 2) two Lu concentrations (5 μM and 50 μM) each with six concentrations of each of the following organic ligands: ethylenediaminetetraacetate (EDTA, Sigma Chemicals & Co, ca. 99%), nitrilotriacetate (NTA, Sigma Chemicals & Co, ca. 99.5%), citrate (J.T. Baker, 99.8%), malate (Aerof, 99%), oxalate (Merck, 99.5%) and acetate (J.T. Baker, 99.5%). All six ligands were also tested as pure substances (in the same way as the first Lu series), to make sure that no toxic ligand concentrations were used, and hence that the observed effects could be attributed to Lu and were not due to the applied ligand concentration. Ligands were chosen on basis of their differences in complexing capacity for Lu (see Table 6-1) and their different charges in fully dissociated state: 4- for EDTA, 3- for NTA and citrate, 2- for malate and oxalate and 1- for acetate. The speciation of the ligand and its actual charge will of course be determined by the degree of protonation at the applied pH (in this study 5.50), and is accounted for in the calculations. In addition, it should be noted that citrate, malate, oxalate and acetate are naturally occurring ligands, while EDTA and NTA are artificial ones.

A volume of 150 μL of the prepared test concentration was pipetted into white 96 wells plates (Luminoscan LB 96P WMP 25). All treatments were replicated three times. Each series had its own control treatment, consisting of a 0.355 M NaNO₃ solution of pH 5.50. In each plate, a Cu (Johnson Matthey, AAS standard solution, Specpure®, 1,000 ppm in 5% HNO₃)
concentration series was added as positive control. The plate filled with test solutions was put into the luminometer with automatic injector (Labsystems Luminoscan RS).

Lyophilized bacteria (*V. fischeri*, NRRL B-11177, Microtox® Acute Reagent, Azur Environmental, Carlsbad, CA, USA) were prepared for the experiments as described by Hamers et al. (2001). First, the condition of the bacteria was tested by studying performance of the control treatment during 5 min. If the bacteria condition is satisfactory (a small increase of light emission followed by a slow decrease), 75 μL of the bacteria suspension (in 0.355 M NaNO₃, of pH 5.50) was injected into each well of the plate. Exactly 7.5, 15, 22.5 and 30 min after injection the luminescence of each well was measured, expressed in relative light units (RLU). Measuring the bioluminescence response is an indirect way of assessing the metabolic rate of the bacteria, and as such a valuable endpoint (Campbell and Striebig, 1999). The exposure concentrations used in the calculations were the final concentrations in the wells, *i.e.* after addition of the bacteria suspension.

Because of reported problems with pH control in this biotest system (Carlson-Ekevall and Morrison, 1995; Villaescusa et al., 1998), we checked the pH at the end of the experiment by collecting the contents of three replicate wells (ca. 650 μL) with a pipet and measuring the pH in an Eppendorf vial with a micro pH meter (Knick 646, equipped with Pt-electrode). Also, the pH of the bacteria suspension was measured.

### 6.2.2 Chemical equilibrium program CHEAQS

For the speciation calculations we chose the program CHEAQS (a program for calculating CHemical Equilibria in AQuatic Systems), because it provides a fairly extensive and well-documented database (including Lu, Cu, acetate, EDTA and NTA). A background document is available with, for each equilibrium constant, the literature reference and any conversions made. The database is easily changed or extended with user-friendly routines. The program and the background document can be downloaded for free from the Internet (Verweij, 2000). Generally, CHEAQS, and similar programs, work as follows:

1. For the total concentrations entered, the program considers which equilibria should be taken into account.
2. An initial assumption is made of the free concentration of each cation and ligand.
3. Using this assumption, a first guess is made of the ionic strength I of the solution. This value is then used to calculate conditional equilibrium constants (*i.e.* for I > 0.0 M) by using the Davies equation.
4. The speciation is calculated; the entered total concentrations are compared with the calculated total concentrations. This leads to a better estimate of the free concentrations of each cation and ligand. If the calculated total concentration is close enough to the entered total concentration, the program finishes the calculations; otherwise, it returns to step 3.
The program settings for the equilibrium calculations were as follows:

- solids were allowed to precipitate in case of oversaturation (to check if solubility products were exceeded);
- CO₂ saturation of the solution was assumed (using a pCO₂ of 3.5×10⁻⁴ atm, corresponding to the ambient concentration of this gas), which in turn determined the concentration of free CO₃²⁻ in solution. In a laboratory, the actual pCO₂ may be higher due to the presence of (exhaling) workers, but since our experiment was performed in a closed luminometer, this effect was deemed negligible;
- ionic strength was calculated rather than fixed;
- redox equilibria were not included (because they were not relevant under the prevailing conditions);
- pH was entered (as measured for each test concentration);
- for all other components, the added total concentration was entered;
- the convergence criterion was set to 1×10⁻⁶ (0.0001%).

6.2.3 Equilibrium constants

Most of the equilibrium constants in the CHEAQS database are taken from Martell and Smith (1998) and some from Turner et al. (1981). However, where we felt certain that recent literature of high quality was not yet included in the database, we added or replaced equilibria. The following sources were used: Iroh et al. (1985), Millero (1992), Lee and Byrne (1992), Haas et al. (1995), Liu and Byrne (1998), Bonai et al. (1998), Diakonov et al. (1998), Klangness and Byrne (2000) and Schif and Byrne (2001). When necessary, logK values were converted from step-wise to total stability constants (i.e. logQ values) and corrected to infinite dilution (I = 0.0 M) using the Davies equation in CHEAQS. Two complexation constants and one solubility product of Lu were not found in the above-mentioned sources. To be able to include these equilibria in the calculations, the complexation constants were estimated from available data for other lanthanides (taken from Martell and Smith, 1998) by extrapolating a linear regression curve (an example is given in Fig. 6-1). We feel that this is a justified approach, since most chemical characteristics of the lanthanides are a smooth and systematic function of atomic number (Henderson, 1996). Table 6-1 lists all Lu complexation constants and solubility products we used, including notes on the conversions made. A value for the solubility product of Lu₂(ox)₃ could not be found in the literature, nor be estimated from available data (n = 4). However, precipitation of Lu₂(ox)₃ was not expected in our experiments, since the product [Lu]⁺[ox]⁻ was always below the values for other lanthanide-oxalate solubility products (Martell and Smith, 1997). For citrate, oxalate and malate, which are not present in the standard database of CHEAQS, we also added stability constants for the association of H⁺ and Na⁺ with these ligands (data from Martell and Smith, 1997).
Table 6-1. Total stability constants (log$_2$$\beta$) and solubility products (log$_2$$K$) of La, appropriate at I = 0.0 M and T = 25°C.

<table>
<thead>
<tr>
<th>complex</th>
<th>log$_2$$\beta$</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>La(OH)$_2$$^+$</td>
<td>6.72$^+$</td>
<td>Klangness and Byrne, 2000</td>
</tr>
<tr>
<td>La(OH)$_3$$^+$</td>
<td>12.32$^+$</td>
<td>Lee and Byrne, 1992</td>
</tr>
<tr>
<td>La(OH)$_3$$^+$</td>
<td>18.14$^+$</td>
<td>Lee and Byrne, 1992</td>
</tr>
<tr>
<td>La(OH)$_4$$^-$</td>
<td>24.05</td>
<td>Haas et al, 1995</td>
</tr>
<tr>
<td>La$_2$(OH)$_4$$^+$</td>
<td>14.60</td>
<td>estimated value$^b$</td>
</tr>
<tr>
<td>La(CO$_3$)$_2$$^+$</td>
<td>8.00</td>
<td>Liu and Byrne, 1998</td>
</tr>
<tr>
<td>La(H$_2$CO$_3$)$_2$$^+$</td>
<td>12.22</td>
<td>Millero, 1992</td>
</tr>
<tr>
<td>La(CO$_3$)$_2$$^+$</td>
<td>13.93</td>
<td>Liu and Byrne, 1998</td>
</tr>
<tr>
<td>La(NO$_3$)$_2$$^+$</td>
<td>-0.076</td>
<td>Bonal et al, 1998</td>
</tr>
<tr>
<td>La(Ac)$_2$$^+$</td>
<td>2.49$^+$</td>
<td>Martell and Smith, 1998</td>
</tr>
<tr>
<td>La(Ac)$_3$$^+$</td>
<td>4.22$^+$</td>
<td>Martell and Smith, 1998</td>
</tr>
<tr>
<td>La(Ac)$_4$$^+$</td>
<td>5.30$^+$</td>
<td>Martell and Smith, 1998</td>
</tr>
<tr>
<td>La(NTA)$_2$$^+$</td>
<td>14.24$^2$</td>
<td>Martell and Smith, 1998</td>
</tr>
<tr>
<td>La(NTA)$_3$$^+$</td>
<td>23.37$^d$</td>
<td>Martell and Smith, 1998</td>
</tr>
<tr>
<td>La(NTA)$_4$(OH)$_2$$^+$</td>
<td>20.54$^d$</td>
<td>Martell and Smith, 1998</td>
</tr>
<tr>
<td>La(EDTA)$_2$$^+$</td>
<td>22.30$^d$</td>
<td>Martell and Smith, 1998</td>
</tr>
<tr>
<td>La(EDTA)$_3$$^+$</td>
<td>22.53</td>
<td>estimated value$^e$</td>
</tr>
<tr>
<td>La(Cit)$_2$$^+$</td>
<td>9.670</td>
<td>Martell and Smith, 1998</td>
</tr>
<tr>
<td>La(Cit)$_3$$^+$</td>
<td>16.22$^f$</td>
<td>Iroh et al, 1985</td>
</tr>
<tr>
<td>La(Mal)$_2$$^+$</td>
<td>6.36$^f$</td>
<td>Martell and Smith, 1998</td>
</tr>
<tr>
<td>La(Mal)$_3$$^+$</td>
<td>10.37$^f$</td>
<td>Martell and Smith, 1998</td>
</tr>
<tr>
<td>La(Ox)$_2$$^+$</td>
<td>6.96</td>
<td>Schijf and Byrne, 2001</td>
</tr>
<tr>
<td>La(H$_2$Ox)$_2$$^+$</td>
<td>7.05$^g$</td>
<td>Schijf and Byrne, 2001</td>
</tr>
<tr>
<td>La(Ox)$_3$$^+$</td>
<td>11.77</td>
<td>Schijf and Byrne, 2001</td>
</tr>
<tr>
<td>La(Ox)$_4$$^+$</td>
<td>13.95$^h$</td>
<td>Martell and Smith, 1998</td>
</tr>
<tr>
<td>La(Ox)$_4$$^+$</td>
<td>14.68$^h$</td>
<td>Martell and Smith, 1998</td>
</tr>
<tr>
<td>solid</td>
<td>log$_2$$K$</td>
<td>La(OH)$_3$$^+$</td>
</tr>
<tr>
<td></td>
<td>25.00</td>
<td>Dhakonov et al, 1998</td>
</tr>
<tr>
<td></td>
<td>La$_2$(CO$_3$)$_3$$^+$</td>
<td>30.80</td>
</tr>
</tbody>
</table>

La = lanthanide, Ac = acetate, Cit = citrate, Mal = malate, Ox = oxalate, $^*$ calculated from hydrolysis constants using a log$_K$ of 13.997 at I = 0.0 M (Martell and Smith, 1998), $^b$ using Ln data (n = 7) from Martell and Smith (1998), $^c$ converted to total stability constants using a log$_K$ of 10.329 for HCO$_3$ at I = 0.0 M (Martell and Smith, 1998), $^d$ converted from I = 0.1 M with the Davies eqn., $^e$ using Ln data (n = 10) from Martell and Smith (1998) converted to total stability constants and extrapolated I = 0.0 M with the Davies eqn., $^f$ converted from I = 0.05 M with the Davies eqn. after conversion to total stability constants, using a log$_K$ of 3.92 for H(Ox)$_2$ at I = 0.05 M (Schijf and Byrne, 2001), $^g$ converted from I = 1.0 M with the Davies eqn., determined at 20°C, $^h$ using Ln data (n = 7) from Martell and Smith (1998).
6.2.4 Data handling and statistics

The response data, i.e. relative light units (RLU), were converted to percentages of the control response, of which the average (n = 3) was set to 100%. This was done for each exposure period. Data of the Lu-ligand experiments were combined. Concentration-response relations were described with the Weibull model, given by Equation (1).

\[
R_C = \frac{R_0}{\left(\frac{C}{E_{C50}}\right)^b} \quad (1)
\]

wherein \(R_C\) is the performance as a function of the concentration (in % of control performance), \(R_0\) is the control performance (100%), \(C\) is the final Lu concentration in the well (mol·L\(^{-1}\)), \(E_{C50}\) is the Lu concentration for which the response is 50% (mol·L\(^{-1}\)) and \(b\) is the shape or slope parameter of the concentration-response curve. To be able to compare our results with those from other toxicity studies, \(E_{C50}\) calculations are primarily based on free Lu\(^{3+}\) concentrations instead of activities. However, any conclusion based on concentrations is equally valid for activities, because all our solutions are of the same ionic strength (I = 0.34 M) in which case the activity coefficients are constants. Free Lu\(^{3+}\) concentrations may be converted to activities by multiplying concentrations with the activity coefficient \(\gamma_{\text{Lu}^{3+}} = 0.0607\) (as calculated with the Davies equation in CHEAQS).

\[
E_{C_{50}} = \frac{E_{C_{50,\text{a}}}}{1 - e^{-kt}} \quad (2)
\]

Using the \(E_{C_{50}}\) values of the four exposure times (7.5, 15, 22.5 and 30 min), the \(E_{C_{50}}\) at infinite exposure was calculated with Equation (2). From this equation the uptake rate \(k\) may also be de-
rived. In Equation (2), $EC_{50}$ is the $EC_{50}$ as a function of exposure time (mol·L$^{-1}$), $EC_{50,I}$ is the $EC_{50}$ at infinite exposure (mol·L$^{-1}$), $k$ is the uptake rate (min$^{-1}$) and $t$ is the exposure time (min).

All linear and non-linear least squares regressions were performed with the software package Prism, release 2.01 (GraphPad Software, San Diego, CA, USA).

6.3 Results and discussion

6.3.1 Toxicity of Lu in the absence of organic ligands

The toxicity of Lu from the experiments in the absence of organic ligands was expressed in total dissolved concentrations and calculated free Lu$^{3+}$ concentrations. The difference between these two measures is basically the amount of Lu complexed by NO$_3^-$, which was about 4% (the sum of all hydroxide- and carbonate-species is 0.34% and 0.02%, respectively). Consequently, the concentration-response curves and calculated $EC_{50}$ values for total dissolved Lu and free Lu$^{3+}$ at each of the four exposure times are very close (see Table 6-2). Table 6-2 also shows that the standard error of the $EC_{50}$ is decreasing with increasing exposure times. This is probably related to Lu accumulation not having reached equilibrium in all individuals when the first measurements were made, causing variation in responses. This coincides with the increase in the value for $k$, the slope of the concentration-response curve, as given in Table 6-3 (for a discussion see also Appendix II). Slope values for total dissolved Lu and free Lu$^{3+}$ are practically identical, which is expected in this virtually non-complexing medium. Figure 6-2 gives the concentration-response curve for free Lu$^{3+}$ after 15 min of exposure. It shows that the slope of the curve is very steep, indicating some sort of threshold at approximately 1 μM, above which the performance is almost completely inhibited. Comparison with other metals, studied under the exact same conditions by McCloskey et al. (1996), showed that free Lu$^{3+}$ is more toxic than for instance free

Table 6-2. Values for $EC_{50}$ ± standard error (μM) for free Lu$^{3+}$ concentrations and total dissolved Lu concentrations in the presence and absence of organic ligands, calculated with Equations (1) and (2).

<table>
<thead>
<tr>
<th>time (min)</th>
<th>Lu$^{3+}$</th>
<th>Lu$^{3+}$</th>
<th>total Lu</th>
<th>total Lu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no ligands</td>
<td>with ligands</td>
<td>no ligands</td>
<td>with ligands</td>
</tr>
<tr>
<td>7.5</td>
<td>2.16 ± 0.10</td>
<td>3.09 ± 0.36</td>
<td>2.27 ± 0.11</td>
<td>23.05 ± 5.53</td>
</tr>
<tr>
<td>15</td>
<td>1.57 ± 0.05</td>
<td>2.03 ± 0.24</td>
<td>1.64 ± 0.05</td>
<td>16.05 ± 4.34</td>
</tr>
<tr>
<td>22.5</td>
<td>1.44 ± 0.04</td>
<td>1.62 ± 0.19</td>
<td>1.51 ± 0.04</td>
<td>13.37 ± 3.95</td>
</tr>
<tr>
<td>30</td>
<td>1.37 ± 0.04</td>
<td>1.36 ± 0.16</td>
<td>1.43 ± 0.04</td>
<td>11.38 ± 3.58</td>
</tr>
<tr>
<td>$\infty$</td>
<td>1.35 ± 0.01</td>
<td>1.25 ± 0.09</td>
<td>1.41 ± 0.01</td>
<td>11.18 ± 0.77</td>
</tr>
</tbody>
</table>
Table 6-3. Values for $k \pm$ standard error as a function of time for free Lu$^{3+}$ concentrations and total dissolved Lu concentrations in the presence and absence of ligands, calculated with Equation (1)

<table>
<thead>
<tr>
<th>time (min)</th>
<th>Lu$^{3+}$</th>
<th>La$^{3+}$</th>
<th>total Lu</th>
<th>total Lu</th>
</tr>
</thead>
<tbody>
<tr>
<td>no ligands</td>
<td>with ligands</td>
<td>no ligands</td>
<td>with ligands</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>2.06 ± 0.26</td>
<td>0.93 ± 0.12</td>
<td>2.06 ± 0.26</td>
<td>0.56 ± 0.12</td>
</tr>
<tr>
<td>15</td>
<td>3.77 ± 0.94</td>
<td>0.89 ± 0.14</td>
<td>3.77 ± 0.93</td>
<td>0.47 ± 0.11</td>
</tr>
<tr>
<td>22.5</td>
<td>4.04 ± 0.69</td>
<td>0.90 ± 0.16</td>
<td>4.03 ± 0.69</td>
<td>0.43 ± 0.11</td>
</tr>
<tr>
<td>30</td>
<td>4.31 ± 0.56</td>
<td>0.92 ± 0.17</td>
<td>4.30 ± 0.56</td>
<td>0.41 ± 0.11</td>
</tr>
</tbody>
</table>

Ca$^{2+}$ and Zn$^{2+}$, but less toxic than free Pb$^{2+}$ (EC$_{50,35}$ ± standard errors are 19 ± 4, 28 ± 6 and 0.46 ± 0.05 μM, respectively). In the same study, an EC$_{50}$ ± standard error for free La$^{3+}$ was reported of 322 ± 31 μM, which implies that free Lu$^{3+}$ is approximately 200 times more toxic as this first member of the lanthanide series.

![Graph](image)

Fig. 6-2. Luminescence in percentage of control versus calculated free Lu$^{3+}$ concentrations after 15 min exposure. Symbols are averages ($n = 3$) with standard error bars. If error bars are not visible, they fall within the symbols. Dotted line is a fit ($r^2 = 0.97$) of Equation (1). The control treatment without Lu is plotted on the left segment of the X-axis.

Figure 6-3 shows the EC$_{50}$ for free Lu$^{3+}$ as a function of exposure time, from which the EC$_{50}$ was calculated with Equation (2). This value is also given in Table 6-2, as well as the EC$_{50}$ for total dissolved Lu concentrations. Again, the EC$_{50}$ values for free Lu$^{3+}$ and total dissolved Lu are very similar. For both types of Lu concentrations, the value ± standard error for uptake rate $k$ were calculated as 0.131 ± 0.005 min$^{-1}$. The models from Equations (1) and (2) agreed very well with the data.

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6.3.2 Toxicity of ligands and Cu

Experiments with the pure ligands showed no toxicity in the concentration ranges tested, except for acetate. In Fig. 6-4 the data for acetate and EDTA are given, the latter as an example of the five ligands used in the complexation experiments. At concentrations higher than 1 mM, acetate has toxic effects on V. fischeri. $EC_{50}$ and $EDTA$ values with standard error bars, calculated with Equation (1). The solid line is a fit ($r^2 = 0.999$) of Equation (2) and the dotted line is the calculated $EC_{50}$ (µM), given in Table 6-2.

![Graph showing EC50 for calculated free Lu3+ concentrations versus exposure time. Symbols are EC50 values with standard error bars, calculated with Equation (1). The solid line is a fit (r² = 0.999) of Equation (2) and the dotted line is the calculated EC50 (µM), given in Table 6-2.](image)

![Graph showing luminescence in percentage of control versus EDTA (open symbols) and acetate (closed symbols) concentrations after 15 min exposure. Symbols are averages (n = 5) with standard error bars. If error bars are not visible, they fall within the symbols. The dotted line through the acetate observations is a fit (r² = 0.96) of Equation (1). The control treatment without ligand is plotted on the left segment of the X-axis.](image)
complexing capacity (see also Table 6-1) is too low at non-toxic concentrations and hence the free-ion model cannot be studied.

The \( EC_{50,2} \pm \) standard error for free \( \text{Cu}^{2+} \), which is the positive control in our experiments, was \( 1.04 \pm 0.17 \, \mu M \) \( (r^2 = 0.96) \). This value is in good agreement with the value of \( 1.23 \pm 0.10 \, \mu M \), found by McCloskey et al. (1996) under the same conditions, namely in a 0.355 M NaNO\(_3\) medium of pH 5.35 after 15 min of exposure. The outcome of this positive control experiment means that the luminescence response, hence the quality of the bacteria, is ascertained.

### 6.3.3 Toxicity of \( \text{Lu} \) in the presence of organic ligands

Data from the experiments with \( \text{Lu} \) in the presence of organic ligands were combined to fit concentration-response curves (Eqn. 1) on the basis of both total dissolved and free \( \text{Lu}^{3+} \) concentrations. Data for 15 min exposures and the fits to them are given in Figs. 6-5 and 6-6 for total dissolved \( \text{Lu} \) and free \( \text{Lu}^{3+} \), respectively. Tables 6-2 and 6-3 give the values for \( EC_{50,2} \) \( EC_{50,0} \) and \( b \) for all exposure times.

![Graph showing luminescence in percentage of control versus total dissolved Lu concentrations after 15 min exposure.](image)

**Fig. 6-5.** Luminescence in percentage of control versus total dissolved Lu concentrations after 15 min exposure. Symbols are averages \( (n = 3) \) with standard error bars. Data from experiments of \( \text{Lu} \) in the presence of organic ligands. The dotted line is a fit \( (r^2 = 0.27) \) of Equation (1). The control treatment without \( \text{Lu} \) is plotted on the left segment of the X-axis.

Figure 6-5 clearly shows that for total dissolved \( \text{Lu} \) concentrations the fit to the data is poor. For \( \text{Lu} \) (final concentrations 3.3 \( \mu M \) and 33 \( \mu M \)) in the presence of organic ligands, the full range of responses between 0 and 100% is possible. The observations shift towards a more apparent relation between performance and concentration when the latter is expressed as free \( \text{Lu}^{3+} \) (Fig. 6-6). The fit improves significantly, indicated by the \( r^2 \) value, which increases from 0.27 to 0.75. Some observations seem to deviate from the concentration-response relation, notably for citrate and for EDTA. Concerning citrate it must be noted that there is no consensus yet on the kind of
lanthanide complexes which are formed with this ligand (see Wood, 1993). The EC\textsubscript{50} values of the Lu experiments in the presence of organic ligands, presented in Table 6-2, show the same trend as for Lu in the absence of organic ligands: in time they decrease and the standard error becomes smaller. The EC\textsubscript{50} values for free Lu\textsuperscript{3+} are in good agreement with those from the Lu experiment in the absence of organic ligands, whereas the EC\textsubscript{50} values for total dissolved Lu concentrations from both experiments are far apart. These observations lead to the conclusion that for Lu (and most likely also for other members of the lanthanide series) the free-ion activity model is valid. While this conclusion is based on free Lu\textsuperscript{3+} concentrations, it is also valid for Lu\textsuperscript{3+} activities, because all solutions were of the same and constant ionic strength (I = 0.34 M), in which case the Lu\textsuperscript{3+} activity is directly proportional to the Lu\textsuperscript{3+} concentration.

![Fig. 6-6. Luminescence in percentage of control versus calculated free Lu\textsuperscript{3+} concentrations after 15 min exposure. Symbols are averages (n = 3) with standard error bars. Data from experiments of Lu in the presence of organic ligands. The dotted line is a fit (r\textsuperscript{2} = 0.75) of Equation (1). The control treatment without Lu is plotted on the left segment of the X-axis.](image)

As a second remark, it must be noted that because of the equilibrium c\textsubscript{eq}P\textsubscript{f} = [Lu(OH)\textsuperscript{3+}][Lu\textsuperscript{3+}]\textsuperscript{3}[OH\textsuperscript{−}]\textsuperscript{3} and the constancy of pH, thus constant OH\textsuperscript{−} concentration, the ratio of free Lu\textsuperscript{3+} and Lu(OH)\textsuperscript{3+} concentrations is fixed. This means that the concentration-effect curve based on Lu(OH)\textsuperscript{3+} has the same shape and statistically an equally good fit as that based on free Lu\textsuperscript{3+} (shown in Fig. 6-6). The same holds for other OH (i.e. pH) dependent Lu species and in fact also for Lu-nitrate and Lu-carbonate species, because the NO\textsubscript{3} concentration and CO\textsubscript{2} partial pressure are constants as well. Consequently, our experiments cannot discriminate between effects of free Lu\textsuperscript{3+} and other inorganic Lu species. This statement holds for many experiments originally designed to test the free-ion model. For this reason, in Fe\textsuperscript{3+}-studies, the concept of Fe\textsuperscript{3+} is used, comprising all dissolved inorganic Fe(III) species considered available for uptake by algae (Gerringa et al., 2000; Sunda, 2001). For Lu we may conclude that the species
responsible for the effect is or are at least proportional to the free Lu$^{3+}$ concentration. Furthermore, Fig. 6-7 shows that the concentrations of other inorganic species at the applied pH are low compared to that of free Lu$^{3+}$, suggesting a bigger role for the latter in establishing the observed effects. Still, a concept such as Lu$^3$, to denote all potentially available inorganic Lu species, could prove very useful in lanthanide studies.

The slope values from the Lu experiments in presence of organic ligands (Table 6-3) do not show such a clear trend as for the Lu experiment in absence of organic ligands, because in combining observations of the different ligands, additional variation is introduced. Another source of variation are the speciation calculations, notably the equilibrium constants. This causes the data to be more scattered around the fitted curve and consequently the slope is more variable.

Values ± standard error for uptake rate $k$ are $0.068 ± 0.008$ and $0.087 ± 0.010$ min$^{-1}$, for free Lu$^{3+}$ and total dissolved Lu, respectively. These values are about half of those for the Lu experiment in the absence of organic ligands as mentioned above. This implies that in the experiments with Lu in the presence of organic ligands the $EC_{50}$ is reached more slowly. This might be related to complexation (kinetics) differences of the used media, but how exactly is yet unknown.

6.3.4 Influence of pH

Measurements of pH revealed that it shifted upward during the experiments from the initial 5.50 ± 0.10 to an average value of 6.30 ± 0.39. This is apparently due to the addition of the bacteria (and possibly substances attached to them), because the bacteria suspension, which was also prepared with a NaNO$_3$ solution of pH 5.5 (see Materials and methods), had an average pH of 6.95 ± 0.12 at the end of the experiment. However, it is anticipated that the pH increased gradually and therefore that the observations after 7.5 and 15 min are representative of the initial speciation conditions. Nevertheless, because pH is one of the major factors affecting metal speciation, we studied how this pH shift would alter the speciation of Lu in our system.

Figure 6-7 presents the speciation of 3.33 μM Lu in a 0.355 M NaNO$_3$ medium as a function of pH (titration calculated with CHEAQS). The figure shows that in the pH range 4.50 to 6.50 there is virtually no change, and free Lu$^{3+}$ is the dominant species. Speciation calculations were also made for media containing organic ligands (10 μM for EDTA, NTA and citrate, and 100 μM for oxalate and malate) and 3.33 μM Lu, in the pH range 5.5 to 6.3. The calculations showed that for malate and oxalate there was no significant change in free Lu$^{3+}$ concentrations, but for EDTA, NTA and to a lesser extent for citrate, the free Lu$^{3+}$ concentration decreased with increasing pH. However, the outcome of these calculations strongly depends on the ratio between the Lu concentration and that of the ligand. Anticipating a gradual increase of pH, we consider the results based on the observations at 7.5 and 15 min robust. Data obtained at 22.5 and 30 min may be less reliable for they approach pH values at which Lu complexation starts to shift and other species, i.e. Lu(CO$_3$)$_2$, start to form.
A possible solution to the poor pH control is the addition of a pH buffer. This means the introduction of a new component in the NaNO₃ medium, which should be included in the speciation calculations. Most pH buffers have some affinity for metals and will therefore complex part of the free metal concentration. However, stability constants of metals with buffers are hardly available (for Lu these values are lacking). The addition of a buffer will also influence the ionic strength of the solution and thereby influence speciation. With respect to the complexing capacity and the pH chosen, the best candidate for our system would have been the Good-buffer MES or 2-morpholinoethanesulfonic acid (Good et al., 1966). Considering the afore-mentioned and also the pH increase in our experiments (on average 0.8 units), which did not influence Lu speciation too much, we feel that not introducing a buffer is justified. However, for other metals, medium compositions and/or different pH values the application of a buffer might be necessary. This should be judged against the speciation of a metal against pH.

6.4 Conclusions

From the experiments described in this paper, the following conclusions may be drawn:

1. The results of the experiments with Lu in the presence of EDTA, NTA, citrate, oxalate and malate are supporting the free-ion activity model for Lu and *V. fischeri*, which implies that the Lu species responsible for toxicity is or are at least proportional to the free Lu⁺ concentration. However, a contribution from Lu-hydroxide, -nitrate and -carbonate complexes cannot be ruled out.

2. Acetate could not be tested as a complexing ligand for Lu, for the concentrations that were needed caused toxic effects (EC₅₀ ± standard error is 6.54 ± 0.26 mM).
3. The toxicity of Lu, expressed as EC_{50} ± standard error for free Lu^{3+}, is 1.57 ± 0.05 μM. The toxicity of Lu is thus higher than those of Cd^{2+} and Zn^{2+}, but comparable to Cu^{2+}, determined under the same conditions.

4. Compared to the EC_{50} of La, the lightest lanthanide, Lu is 200 times more toxic. The cause for this difference is yet unknown, and will be focused on in the next chapter.

Acknowledgements
The authors thank Dr. Bert Wolterbeek, Prof.dr.ir. Jeroen de Goeij and Dipl. Biol. Martina Duft (IHI Zittau, Germany) for their valuable comments on a previous version of this manuscript.
CHAPTER 7

TOXICITY OF ELEVEN LANTHANIDES AND SCANDIUM (Sc)
TO THE BACTERIUM Vibrio fischeri

This chapter is based on: Welte, L., Verhoof, L.R.C.W., Wolterbeek, H.Th., and J.J.M. de Goeij (to be submitted) Toxicity of eleven lanthanides and scandium to the bacterium Vibrio fischeri.
CHAPTER 7

Abstract

The toxicity of scandium (Sc) and eleven lanthanides: La, Ce, Nd, Sm, Eu, Gd, Tb, Dy, Er, Tm and Lu, was studied in a bioassay with the bacterium *Vibrio fischeri*. The bioluminescence response, i.e. metabolic activity, of *V. fischeri* was studied at different concentrations in a fully inorganic aqueous 0.355 M NaNO₃ solution of pH 5.50. Toxicity was expressed as the EC₅₀ in total dissolved concentrations. Experiments were supported by speciation calculations to prevent the use of oversaturated solutions.

Results showed a systematic pattern in lanthanide toxicity, with the heaviest lanthanide, Lu, being the most toxic and the lightest lanthanide, La, the least toxic. The difference in toxicity is a factor ranging from 30 to 100, its magnitude depends on the length of exposure. The toxicity range of the lanthanides coincides with the range for Cd, Ca, Pb and Zn. Chemical properties, such as charge density and the first hydrolysis constant, are highly correlated ($r^2 > 0.85$) with lanthanide toxicity. Furthermore, it appears that lanthanides with a low charge density have slower binding or uptake kinetics than those with high charge densities.

All lanthanides induce hormesis and thus stimulate bacterial metabolism at low concentrations, whereby hormesis increases with exposure time. Finally, the toxicity of lanthanides is correlated with their abundance in the earth’s crust ($r^2 = 0.73$), with rarer elements being more toxic than abundant ones. Consequently, the scaling of toxicity by abundance gives a value in the same order of magnitude for all lanthanides. Sc toxicity is of the same order of magnitude as those of middle-weight lanthanides, but the relation between Sc toxicity and chemical characteristics clearly deviates from that of the lanthanides.
7.1 Introduction

The lanthanides are a homologous group of fifteen trivalent metals, ranging from lanthanum (La, \(Z = 57\)) to lutetium (Lu, \(Z = 71\)). Lately, they received more attention, because their industrial uses and accompanying emissions increase in the, mainly aquatic, environment (Sneller et al., 2000). To be able to assess the impact of lanthanide emissions on aquatic ecosystems and ultimately to set environmental quality standards, toxicity data are required. However, few reliable data are available so far. Some aquatic toxicity studies have been performed, but because of the low solubility products of lanthanides with phosphate (\(K_p \approx 10^{-20} \text{ mol}^2\cdot\text{L}^2\), Lü and Byrne, 1997) and carbonate (\(K_p \approx 10^{-16} \text{ mol}^2\cdot\text{L}^2\), Martell and Smith, 1998), which are present in most artificial media, substantial precipitation occurred in these studies (e.g. Maas and Botterweg, 1993; Bogers et al., 1998; Barry and Mechen, 2000). An additional cause for precipitation may be the pH value, since lanthanide solubility products with hydroxide are low as well (\(K_p \approx 10^{-22} \text{ mol}^2\cdot\text{L}^2\), Martell and Smith, 1998). Occurrence of precipitation makes it difficult to relate toxic effects to total dissolved lanthanide concentrations, whereas adverse effects may have also been caused by deficiencies due to precipitation of required nutrients (i.e. phosphate and carbonate).

In addition, there is no insight in the toxicity of individual lanthanides and the possible differences among them. Therefore, and because of their chemical similarity, the lanthanides are sometimes treated as a group, without differentiation between the individual elements (e.g. Tijink and Yland, 1998). In our previous work on the toxicity of Lu (chapter 6) it was concluded by comparison with literature data on La (McCloskey et al., 1996) that Lu, the heaviest lanthanide, was about 200 times more toxic than La, the lightest lanthanide. Therefore, the present work was initiated, in which the toxicity of eleven lanthanides is studied in a biotest. It concerns experiments with *Vibrio fischeri*, a luminescent microorganism, which is widely used in a non-substance-specific test system known as Microtox® (Azur Environmental, Carlsbad, CA, USA).

It is expected that the toxicity data exhibit a systematic pattern with atomic number, since most chemical properties of the lanthanides do so, and toxicity is derived from those. Consequently, it is attempted to relate toxicity data to chemical properties (following McCloskey et al., 1996; Tatara et al., 1998; Wolterbeek and Verburg, 2001). Such a relationship can provide insight in the toxic action and predict toxicity of the four lanthanides not included in this work and maybe also for scandium (Sc, \(Z = 21\)) and yttrium (Y, \(Z = 39\)). The latter two elements, together with the lanthanides, form the group of the rare earth elements (REE). To study if Sc behaves the same as the lanthanides, it was included in the present work.

In accordance with previous work (chapter 6), the experimental set up was supported by speciation calculations to check if solubility products of lanthanides with carbonate and hydroxide are not exceeded. Phosphate was not present in the applied medium (for further details, see *Materials and methods*). Toxicity of copper (Cu) was determined both as a positive control and as a reference element for comparison with the lanthanides. An extensive literature search was performed to collect reliable equilibrium constants of lanthanides with inorganic ligands present in the test solution.
7.2 Materials and methods

7.2.1 Experimental

*Vibrio fischeri* Beijerinck 1889 (formerly known as *Photobacterium phosphorum*) is a marine bacterium, and therefore usually exposed in a NaCl solution. However, it can also be exposed in a 0.355 M (or 3.02%) NaNO₃ solution, which, according to McCloskey et al. (1996) reflects freshwater speciation conditions. The ionic strength, however, is intermediate between freshwater and seawater. Since the focus of this work is on lanthanide effects in freshwater, we chose the NaNO₃ solution. An additional advantage is that nitrate has hardly a complexing capacity for lanthanides (Bonal et al., 1998; see Appendix B for equilibrium constants) and therefore the lanthanides are mostly present as free ions, i.e. Ln⁺(H₂O)ₓ. The presence of Na⁺ and the high ionic strength are necessary for undisturbed performance of the bacteria (Watanabe and Hastings, 1986).

All solutions were prepared with Milli-Q water (Millipore-Waters, Milford, MA, USA) and NaNO₃ (99.8%, J.T. Baker). For La, Ce, Nd, Sm, Eu, Tb and Dy their respective AAS stock solutions (1,000 ppm in 5% HNO₃, Specpure, Johnson Matthey) were used, as well as for Er (1,000 ppm in 5% HNO₃, 99.9%, BDH) and Sc (1,000 ppm in 5% HNO₃, Specpure, J.T. Baker). For Gd and Sm, oxides (>99.9%, Koch-Light Laboratories, Colnbrook, Bucks, UK) were dissolved in 5% nitric acid (Merck, Suprapur) and for Lu an ICP stock solution was used (10,000 ppm of 99.999% Lu₂O₃ in 4% HNO₃, CPI International, Santa Rosa, CA, USA). The NO₃⁻ introduced with the nitric acid, the AAS and ICP stock solutions was accounted for in preparing the final NO₃⁻ concentrations, such that all test solutions had the same NO₃⁻ concentration. The pH of the medium was adjusted to 5.50 ± 0.10 with HNO₃ (Merck, Suprapur) and a NaOH (J.T. Baker, 98.6%) solution, using a Mettler 744 pH meter, equipped with a Pt-electrode. Amounts of added acid and base were noted to make volumetric corrections and also to account for added Na⁺ and NO₃⁻ in the speciation calculations.

For each of the 11 above-mentioned lanthanides and Sc, five concentrations were prepared in logarithmic steps between ca. 0.15 μM and ca. 3.2 mM (exact concentrations differed somewhat between elements). A volume of 150 μL of the prepared test concentration was pipetted into thoroughly washed, white 96-wells plates (Luminoscan LB 96P WMP 25). All treatments were replicated three or four times. Each series had its own control treatment, also in threefold or fourfold, consisting of a 0.355 M NaNO₃ solution of pH 5.50. In each plate, a Cu (AAS standard solution, 1,000 ppm in 5% HNO₃, Specpure, Johnson Matthey) concentration series was added as positive control. The plate filled with test solutions was put into the luminometer with automatic injector (Labsystems Luminoscan RS).

Freeze-dried bacteria (*V. fischeri*, NRRL B-11177, Microtox® Acute Reagent, Azur Environmental, Carlsbad, CA, USA) were prepared for the experiments as described by Hamers et al. (2001). First, the condition of the bacteria was tested for 5 min by studying performance of a separate control treatment. If this yielded satisfactory results, 75 μL of the bacteria suspension (in 0.355 M NaNO₃ of pH 5.50) was injected into each well of the plate. After 7.5, 15, 22.5 and 30
min of exposure, the luminescence in each well was measured, expressed in relative light units (RLU). Measuring the luminescence is an indirect way of assessing the metabolic activity of the bacteria (Sillanpää and Oikari, 1996) because the emitted light is a by-product of their metabolism. Reduced luminescence is therefore a typical toxicological response in this organism. The lanthanide, Cu and Sc concentrations used in the concentration-response calculations were those in the wells, after dilution by addition of the bacteria suspension.

Because of reported problems with pH control in this biotest system (Carlson-Ekvall and Morrison, 1995; Villaescusa et al., 1998), we checked pH by collecting the contents of three replicate wells (ca. 650 μL) after the test with a pipet and measuring pH in an Eppendorf vial with a micro pH meter (Knick 646, equipped with Pt-electrode). Also, the pH of the bacteria suspension was measured.

7.2.2 Chemical equilibrium program CHEAQS

For the speciation calculations we used the software program CHEAQS (Verweij, 2000), because it is user-friendly and provides a fairly extensive and well-documented database, that includes the lanthanides, Sc and Cu. The speciation calculations were used to set the highest lanthanide concentration without exceeding solubility products of either lanthanide carbonates or lanthanide hydroxides. Consequently, calculations were done before the test solutions were prepared. After preparing the solutions, calculations were repeated using the pH value as set and considering the added amounts of acid (for NO₃⁻) and base (for Na⁺). The program settings for the equilibrium calculations were as follows:

- solids were allowed to precipitate in case of oversaturation (to check if solubility products were exceeded);
- CO₂ saturation of the solution was assumed (using a pCO₂ of 3.5×10⁻⁴ atm, corresponding to the ambient concentration of this gas) which in turn determined the concentration of free CO₃²⁻ in solution. In a laboratory, the pCO₂ may be higher due to the presence of (exhaling) experimenters, but since our experiment was performed in a closed luminometer, this effect was deemed negligible;
- ionic strength was calculated rather than fixed;
- redox equilibria were not included (because they were not relevant under the prevailing conditions);
- pH was entered (as set for each test concentration);
- for all other components, the added total concentration was entered;
- the convergence criterion was set to 1×10⁻⁶ (0.0001%).
7.2.3 Equilibrium constants

Most of the equilibrium constants in the CHEAQS database are taken from Martell and Smith (1998) and some from Turner et al. (1981). In addition, we added equilibria or replaced equilibrium constants using recent literature of high quality not yet included in the database. Sources used are: Millero (1992), Lee and Byrne (1992), Liu and Byrne (1998), Bonal et al. (1998) and Klungness and Byrne (2000). When necessary, logK values were converted from step-wise constants to total stability constants (i.e. logβ values) and corrected to infinite dilution (I = 0.0 M) using the Davies equation in CHEAQS.

Some stability constants or solubility products could not be found in the above-mentioned sources. To be able to include these equilibria in the calculations, the complexation constants were estimated from available data for other lanthanides (taken from Martell and Smith, 1998) by extrapolating a linear regression curve. This is a justified approach, as most chemical characteristics of the lanthanides are a smooth and systematic function of atomic number (Henderson, 1996). The lanthanide stability constants used are listed in Appendix IA, Ib and II; and those for Sc in Table 7-1.

Table 7-1. Total stability constants (logβ) and solubility products (logK) of Sc with inorganic ligands, appropriate at I = 0.0 M and T = 25°C. Stability constants for lanthanides with OH-, CO₃²⁻ and NO₂⁻ are given in Appendix IA, Ib and II.

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<tr>
<td>Sc(OH)³⁺</td>
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<tr>
<td>Sc(OH)⁴⁻</td>
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</tr>
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</tr>
<tr>
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<td>1</td>
</tr>
<tr>
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<td>10.10</td>
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</tr>
<tr>
<td>Sc(NO₂)⁵⁻</td>
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</tr>
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<td>solid</td>
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<tr>
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</tbody>
</table>

References: 1 = Martell and Smith (1998); 2 = Turner et al. (1981). Data on Sc(NO₂)⁴⁻ were not included, converted from I = 4.0 M with the Davies equation

7.2.4 Data handling and statistics

The performance data, i.e. relative light units (RLU), were converted to percentages of the control performance, of which the average (σ = 3 or 4) was set to 100%. This was done for each exposure period. Concentration-response curves were described with Equation (1). This linear logistic model (Hockstra, 1993), takes into account a possible stimulation of performance, i.e. horemsis (Stebbing, 1982) that may occur at low toxicant concentrations.
In Equation (1), $R_p$ is the performance as a function of the toxicant concentration (in % of control performance), $R_c$ is the control performance, $C$ is the toxicant concentration (M), $EC_{50}$ is the toxicant concentration for which the response is 50% (M), $b$ is the dimensionless slope parameter of the concentration-response curve and $H$ is the dimensionless hormesis parameter of the concentration-response curve, which must be greater than 0 for meaningful interpretation. If $H < 0$, its value was set to 0 and the non-linear regression fit was performed again. For $H = 0$, the curve equals the normal logistic concentration-response curve.

All linear and non-linear least squares regressions and correlations were performed with the software package Prism, Version 2.01 (GraphPad Software, San Diego, CA, USA).

7.3 Results and discussion

7.3.1 General

The quality of the experiments was evaluated by testing the condition of the bacteria before starting the experiments and by studying the response of the bacteria to Cu, the positive control. Both tests gave satisfying results, i.e. bacteria were properly awoken from their freeze-dried state and started to emit enough light for a quantitative and reproducible measurement (typically more than 100 RLU in a control well) and secondly, they responded in a concentration-dependent manner to Cu by decreased light-emission, whereby the calculated $EC_{50}$ for total dissolved Cu, 1.57 ± 0.32 μM after 15 min of exposure, was identical to a previously reported value, 1.62 ± 0.13 μM (McClokey et al., 1996).

The pH of the test solutions was measured to check if the speciation calculations were still applicable. Although pH of the test solutions increased after addition of the bacteria suspension (on average 0.8 unit), there was no significant influence on lanthanide speciation up to pH 6.7. This was checked upon by speciation calculations on La and Lu, using the titration option in CHEAQs (results not shown). It was decided not to use a pH buffer, 2-morpholinoethanesulfonic acid (MES) would be appropriate for the work described here, since buffers generally have some affinity for metal ions and will therefore influence metal speciation and thus the exposure conditions. Furthermore, to quantify the exact influence of a pH buffer like MES on lanthanide speciation, equilibrium constants are needed, which are not available for lanthanides and MES. Moreover, organic substances in the test solution may serve as a carbon source for the bacteria and thereby influence performance. For more discussion concerning the influence of pH on lanthanide speciation, see chapter 6.
A potential problem in the applicability of the speciation calculations to the test solutions may be the introduction of substances into the medium, together with the bacteria. It may be possible that there are substances such as NaCl attached to the bacteria in their freeze-dried state, but this was not tested. For now it is assumed that such an influence is negligible and that therefore the speciation calculations may be considered valid and applicable.

### 7.3.2 Toxicity of lanthanides and Sc

The toxicity of the lanthanides was expressed as the EC₅₀ based on total dissolved concentrations. The EC₅₀ was chosen, because it is statistically the most robust point of the concentration-response curve, since it lies on the steepest part of the graph. Total dissolved concentrations were chosen, because in our previous work it was shown that the lanthanide species responsible for the effect may be the free ion or one or more dissolved inorganic species, which, under the chosen conditions, are all proportional to the free ion (chapter 6). However, since stability constants of hydroxide and carbonate increase with Z, while those for nitrate decrease with Z, relative concentrations of inorganic species across the whole lanthanide series are shifting. Therefore, the speciation at the EC₅₀ is studied for all lanthanides included in the present work. In addition, this may yield information on the contribution of certain species to the observed toxicity.

![Graph](image)

**Fig. 7.1.** Speciation of lanthanides (concentration in M) versus Z at 50% effect. For hydroxide and carbonate the concentrations of their lanthanide complexes were summed.

The range of toxicity within the lanthanide series spans two orders of magnitude, with La being the least and Lu the most toxic element, with EC₅₀ values ranging from 0.22 mM to 2.2 μM (after 7.5 min of exposure). This maximum difference, a factor of 100, decreases to a factor of 54 (after 15 min of exposure) and further to a factor of 30 (after 22.5 and 30 min of exposure). The toxicity changed with atomic number as anticipated (see Introduction). Compared to more common heavy metals tested under the same conditions, it appears that the lighter lanthanides are about equally toxic as Cd and Zn and the heavier ones as Cu and Pb (data by McCloskey *et al.*, 1996).
The speciation of lanthanides against Z at 50% effect is shown in Fig. 7-1. For hydroxide and carbonate the concentrations of their lanthanide complexes were summed. Fig. 7-1 shows that at 50% effect the total concentration is decreasing with Z, as well as concentrations of the free Ln$^{3+}$ ion, the nitrate and the carbonate complexes. The hydroxide complex concentrations remain approximately constant. Furthermore, correlations between log-transformed concentrations and Z showed that all, but the hydroxide complexes are negatively correlated with Z (the correlation for hydroxide complexes was not significant). In Fig. 7-2, the same data are presented, but now as percentages of the total dissolved concentration against Z. It shows that free Ln$^{3+}$ concentrations are almost equal to total concentrations (ranging from 79% to 96%, going from La to Lu), and that hydroxide and carbonate complexes are below 0.4% and 0.03%, respectively, of the total concentration. Free-ion concentrations increase with a factor of 1.2, hydroxide-complexes with a factor of 43 and carbonate and nitrate complexes with a factor of 5.5 and 0.20, respectively. Fig. 7-2 also shows the relative increase of hydroxide and carbonate species with Z, due to the increasing affinity for these ligands. On basis of these data we may conclude that effects of the free ion cannot be separated easily from those of its nitrate and carbonate complexes. Since hydroxide-complex concentrations remain approximately constant, their role in establishing toxicity appears to be of minor importance, because lanthanides have chemical properties that vary systematically with Z and toxicity is expected to be derived from these. Considering the above, we may use the total dissolved concentrations to express toxicity.

![Fig. 7-2. Speciation of lanthanides in percentage of total dissolved concentrations at the EC50 versus Z. For hydroxide and carbonate the concentrations of their lanthanide complexes were summed](image)

Speciation calculations on Sc predicted more than 50% of the dissolved concentration associated with hydroxide, contrary to the lanthanides. Furthermore, since the solubility product for scandium hydroxide is $10^{-30.7}$ mol$^3$L$^{-4}$ (Martell and Smith, 1998), which is much lower than those for lanthanide-hydroxides (see Appendix I), precipitation of Sc(OH)$_3$ was predicted for the three highest concentrations. Precipitation was indeed observed for the highest concentration, which
was consequently left out of the experiment. The two other concentrations, 6.64 and 66.4 μM, were clear solutions and therefore included in the test. However, after three months a white precipitate was observed in these solutions. This is in agreement with the used solubility product, which concerns an aged hydroxide precipitate, namely ScO(OH), and therefore the calculations present a worst case scenario. For now it is presumed that the precipitate was formed very slowly and that during exposure all Sc was dissolved. The toxicity of Sc is of the same order of magnitude as those of middle-weight lanthanides.

![Graph](image)

**Fig. 7-3.** Concentration-response curve of Er after 15 min of exposure showing no hormesis. Closed symbols are observations ($n = 3$). The dotted line is a fit ($r^2 = 0.99$) of Equation (1) with $H = 0$

**Fig. 7-4.** Concentration-response curve of Gd after 30 min of exposure showing hormesis. Closed symbols are observations ($n = 3$). The dotted line is a fit ($r^2 = 0.99$) of Equation (1) with $H > 0$

The concentration-response curve regressions showed that all lanthanides invoke hormesis (parameter $H$ from Equation (1) > 0) at one or more exposure times and thus stimulate the bacterial metabolism at low concentrations. The reason for this is unknown. Sc and Cu did not induce hormesis. Typical examples of concentration-response curves without and with hormesis are shown in Figs. 7-3 and 7-4. Six lanthanides, La, Ce, Nd, Th, Er and Lu, showed no hormesis at
some of the exposure times (11 out of 24 cases). Apart from Er, it appeared that for these lanthanides the hormesis response is confined to longer exposures, 22.5 and 30 min. From these observations it is concluded that hormesis 'develops' during the experiments. This is confirmed by observations on the remaining lanthanides, which showed increasing hormesis (value for $H$ increases) as exposure prolonged.

Table 7.2. Chemical characteristics of lanthanides, Sc, Cu, Zn, Cd and Pb. Ionic radius for Ln$^{3+}$ ion with coordination number 6

<table>
<thead>
<tr>
<th>element</th>
<th>atomic number, Z</th>
<th>ionic radius, $R_c$ (Å)$^a$</th>
<th>-log concentration in earth crust (mol/kg$^c$)$^b$</th>
<th>log$_{10}$δ$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sc</td>
<td>21</td>
<td>0.745</td>
<td>3.310</td>
<td>9.700</td>
</tr>
<tr>
<td>Cu</td>
<td>29</td>
<td>0.730</td>
<td>3.025</td>
<td>6.500</td>
</tr>
<tr>
<td>Zn</td>
<td>30</td>
<td>0.740</td>
<td>2.970</td>
<td>5.000</td>
</tr>
<tr>
<td>Cd</td>
<td>48</td>
<td>0.950</td>
<td>5.875</td>
<td>3.900</td>
</tr>
<tr>
<td>La</td>
<td>57</td>
<td>1.045</td>
<td>3.552</td>
<td>5.187</td>
</tr>
<tr>
<td>Ce</td>
<td>58</td>
<td>1.010</td>
<td>3.324</td>
<td>5.657</td>
</tr>
<tr>
<td>Nd</td>
<td>60</td>
<td>0.983</td>
<td>3.541</td>
<td>5.817</td>
</tr>
<tr>
<td>Sm</td>
<td>62</td>
<td>0.958</td>
<td>4.329</td>
<td>6.157</td>
</tr>
<tr>
<td>Eu</td>
<td>63</td>
<td>0.947</td>
<td>4.881</td>
<td>6.237</td>
</tr>
<tr>
<td>Gd</td>
<td>64</td>
<td>0.938</td>
<td>4.404</td>
<td>6.167</td>
</tr>
<tr>
<td>Tb</td>
<td>65</td>
<td>0.923</td>
<td>5.122</td>
<td>6.357</td>
</tr>
<tr>
<td>Dy</td>
<td>66</td>
<td>0.912</td>
<td>4.495</td>
<td>6.407</td>
</tr>
<tr>
<td>Er</td>
<td>68</td>
<td>0.890</td>
<td>4.679</td>
<td>6.477</td>
</tr>
<tr>
<td>Tm</td>
<td>69</td>
<td>0.880</td>
<td>5.512</td>
<td>6.607</td>
</tr>
<tr>
<td>Lu</td>
<td>71</td>
<td>0.861</td>
<td>5.340</td>
<td>6.727</td>
</tr>
<tr>
<td>Pb</td>
<td>82</td>
<td>1.190</td>
<td>4.170</td>
<td>6.400</td>
</tr>
</tbody>
</table>

$^a$ data from Lide (1994), $^b$ lanthanide data from Klangness and Byrne (2000), Sc, Cu, Zn, Cd and Pb data from Martell and Smith (1998)

7.3.3 Lanthanide and Sc toxicity related to chemical characteristics

To study if the toxicity of lanthanides and Sc to V. fischeri has a relation with their chemical characteristics, EC$_{50}$ values for total dissolved concentrations were judged against chemical properties. Table 7.2 gives the ionic radius $R_c$ (in Å for coordination number 6) and the logarithm of the first hydroxide affinity constant, log$_{10}$δ$_{\mathrm{O}}$ next to the atomic number Z. For comparison, these data are also given for Cu, Zn, Cd and Pb. The charge of an ion, $\bar{q}$, divided by $R_c$ gives its charge density. Because $\bar{q}$ is $3^+$ for Sc and all lanthanides and $R_c$ decreases as the lanthanides become heavier (the so-called 'lanthanide contraction', see Table 7.2) the charge density increases accordingly. This increase leads to an enhanced electrostatic affinity in the lanthanide series for negatively charged groups. This can also be seen in Table 7.2, where log$_{10}$δ$_{\mathrm{O}}$ increases as the lanthanides become smaller.
Figure 7-5 gives the relation between toxicity and charge density for all four exposure times. The figure shows a clear relation between these two variables ($r^2 = 0.92$ for 15 min exposure data).

![Figure 7-5](image)

**Fig. 7-5.** EC₅₀ values (in M) of eleven lanthanides and Sc for four exposure times (see insert) against charge density (i.e. ionic charge, $3^+$, divided by ionic radius in Å for CN = 6, given in Table 7-2).

Indeed for such short exposures, maximum 30 min, a good relationship with charge density is expected, because this property relates to instantaneous interaction of a molecule with a binding site. At longer exposures the type of interaction may change and the relationship with charge density could become weaker. Sc, which is a much smaller ion, is clearly not part of this relationship. Figure 7-5 also shows that the EC₅₀ for the lighter lanthanides decreases much more in time than those of the heavy lanthanides, suggesting faster binding or uptake kinetics for the latter.

In Fig. 7-6, the EC₅₀ for 15 min of exposure against log $\log_{10}(\beta)$, are given for the lanthanides and for comparison also for Cd, Cu, Pb and Zn. The value for Sc lies beyond this graph ($\log_{10}(\beta)$ value for Sc is 9.70). The relation between EC₅₀ and $\log_{10}(\beta)$ for lanthanides has about the same correlation as charge density ($r^2 = 0.87$), which was anticipated since $\log_{10}(\beta)$ and charge density are highly correlated. McCloskey et al. (1996) and Tatara et al. (1998) found that $\log_{10}(\beta)$ was a good descriptor of metal toxicity (of mainly divalent metals) to the bacterium *V. fischeri* and the nematode *Caenorhabditis elegans*. They explained this by the fact that $\log_{10}(\beta)$ reflects the binding tendency of metals to intermediate ligands, containing oxygen atoms. Such ligands are ubiquitous in biological systems and metals seeking them are defined as class A metals (Nicolae and Richardson, 1988). Bacterial cell walls and membranes contain many oxygen-containing ligands and thus provide many binding sites for such metals (Beveridge and Doyle, 1989). This line of thought leads to the conclusion that the binding of lanthanides, which are typical class A metals, to such ligands results in a toxic effect on bacterial metabolism. Interestingly, the difference between La and Lu affinity for hydroxide is a factor of 35, which corresponds very well with the factor 30 that lies between their respective EC₅₀ values at 22.5 and 30 min of exposure.
7.3.4 Lanthanide and Sc toxicity related to natural abundance

Håkanson (1980) proposed that metal toxicities may be ordered by metal abundances in the earth’s crust. The idea is called the abundance principle and proposes that less abundant metals are more toxic than more abundant ones. In a review, this idea was confirmed by Wolterbeek and Verburg (2001). The abundance pattern of lanthanides follows Oddo-Harkins’ rule, which states that even-numbered elements are more abundant than the odd-numbered ones and, in addition, there is a decrease of abundance with atomic number. This leads to the typical saw-tooth pattern of lanthanides in environmental samples (e.g. Markert, 1987). Figure 7-7 presents the EC$_{50}$ for 15 min of exposure versus the negative logarithm of elemental abundance of lanthanides,
Sc, Cu, Zn, Cd and Pb in the earth’s crust (in mol kg⁻¹). This indeed shows that lanthanide abundance has a reciprocal relation with toxicity, but less pronounced \((r^2 = 0.73)\) than the relation with their chemical characteristics. Sc fits in better than with \(\log_{10}\alpha\), but for the other metals the relationship is worse (compare Figs. 7-6 and 7-7). The found relationship implies that lanthanide toxicity scaled by their natural abundance gives a value in the same order of magnitude for all lanthanides. Therefore, only few elements have to be measured (e.g. La, Sm, Eu) to be able to assess the impact of all 14 naturally occurring lanthanides. This is a unique feature for these elements, because due to their chemical similarity they ‘move’ as a group through the ecosystem (chapter 3).

7.4 Conclusions

From the experiments with *V. fluvialis* described in this paper, the following conclusions may be drawn:

1. There is a systematic pattern in toxicity of the lanthanide elements.
2. Toxicity differences between the least and most toxic lanthanide, La and Lu, respectively, range from 30 to 100, depending on the length of exposure.
3. Chemical properties, such as charge density and first hydrolysis constant have a high correlation with lanthanide toxicity.
4. Lanthanides with a low charge density have slower binding or uptake kinetics than lanthanides with a high charge density.
5. Lanthanides induce hormesis and thus stimulate bacterial metabolism at low concentrations, whereby hormesis increases with exposure time.
6. The toxicity of lanthanides relates well to their abundance in the earth’s crust, with rarer elements being more toxic than more abundant ones.
7. The toxicity range of the lanthanides coincides with the toxicity range of Cd, Cu, Pb and Zn.
8. Sc toxicity is of the same order of magnitude as those of middle-weight lanthanides. The relation between Sc toxicity and chemical characteristics clearly deviates from that of the lanthanides.

Acknowledgements

The authors thank Ir. Timo Hamers (Toxicology Group, Wageningen University) for technical advice and use of the luminometer.
CHAPTER 8

GENERAL DISCUSSION
Although this thesis is built up of self-containing chapters, each having their own discussion, the scope of this study requires a general discussion going into the overall consistency of the results and applied methods, as well as giving an account of the underlying problems encountered in lanthanide studies. The aims of this work have been outlined in the scope of the Introduction (section 1.3). In the present chapter, the obtained results are summarised and placed in a broader perspective. The discussion follows the same logical order as the scope, preceded by a brief methodological section. First, aspects of pure chemical nature are dealt with: solubility and speciation. Investigation of these aspects had not been explicitly formulated in the scope, but proved to be a necessity in estimating Ln bioavailability. The discussion continues with availability, uptake and bioconcentration of lanthanides to and by organisms. The final aspect being discussed is the toxicity of lanthanides for organisms after they have been taken up. Finally, an outlook is given, with recommendations for risk assessment and future lanthanide studies.
8.1 Methodology

Since the quality of data was given extra priority in this work (see section 1.2), the used methods deserve some attention, before the results are discussed. Three methods were assessed to quantify Ln (species) concentrations: (HR)-ICP-MS (chapter 3), γ-emitting radiotracers (chapters 4 and 5), and speciation calculations (chapters 4, 5, 6 and 7). Speciation calculations and their quality will be comprehensively discussed in the next section, so here we will focus on the analytical techniques. For measuring the whole suite of Ln’s in both solid and liquid natural field samples, (HR)-ICP-MS is the technique of choice (for a discussion and comparison with other techniques, see section 1.2). This proved to be a valid statement, considering that the only measurements causing analytical problems were those of Lu in surface water, which could not be carried out with sufficient accuracy. For the laboratory studies on La behaviour in nutrient solution and its uptake by duckweed we used the γ-emitting radiotracer $^{149}$La, which allows even smaller amounts of La to be quantified than is possible with HR-ICP-MS. The strength and sensitivity of this radiotracer technique are unparalleled. For instance, it does not require the destruction of a sample. In addition, small samples of nutrient solution (~100 μL) may be taken regularly, enabling a dynamic description of La redistribution between solution, plants and adsorptive surfaces. In the experiments described in chapters 4 and 5, the application of $^{149}$La enabled completing the mass balance for La, and revealed the occurrence of La losses (i.e. to filters and glass vessels). Integrating such information in the compartment modelling of La strengthens the explanatory power of the model (see chapter 5).

From a biological perspective, we have chosen to work with organisms consisting of defined populations, having limited genetical variation (chapters 3, 5, 6 and 7). These organisms, Lemma minor and Vitis vinifera, introduce thus little biological variation in the experiments. The advantage is that less organisms and test containers are needed to achieve the same statistical power as with genetically variable populations. For the extrapolation of laboratory data on genetically homogeneous populations to the field or for their use in risk assessment, one should consider that in nature genetical variation may be larger. Although this was clearly not the case for Ln concentrations in L. minor (chapter 3), variation of up to two orders of magnitude was found for Ln concentrations in Potamogeton pectinatus. However, part of the variation may have been caused by an artifact, i.e. attached fine sediment particles on the roots of this plant. A more comprehensive discussion on the use of clones versus genetically variable populations in ecotoxicological research can be found in Appendix II of this thesis.
8.2 Solubility and speciation

8.2.1 General

The aquatic chemistry of the lanthanides controls their availability and has therefore great importance for the interpretation of uptake and toxicity data. To understand the limitations of lanthanide solubility on the quality of experimental data and to prevent Ln precipitation would greatly enhance progress in this field. This may be concluded from the lack of good laboratory data on lanthanide uptake and effects in freshwater plants and invertebrates (see chapter 1), which is basically caused by the use of oversaturated solutions. After discussing the consequences of precipitation, solutions are provided to handle or avoid this problem.

8.2.2 Precipitation in laboratory experiments

Nutrient solutions and culture media, which are routinely used for the exposure of organisms, often contain phosphate, carbonate or hydroxide at concentrations too high to allow even small Ln concentrations in solution (see also Dianloff et al., 1993). Figure 8-1 (from Weltje, 1998b) gives an example of EuPO₄ precipitation from duckweed (Lemma minor L.) culture medium of pH 4.8, containing 0.1 μM Eu, 1.0 mM PO₄ and 2.5 μM EDTA (for a full solution characterisation see Jenner and Jansen-Mommen, 1993). Eu behaviour is followed in time by using the γ-ray emitting radiotracers 152Eu and 154Eu. The figure shows that equilibrium is reached only after 120 h. This time is probably needed for the settlement of microscopic EuPO₄ particles. When most of the test substance, in this case Eu, is precipitated, it makes hardly sense to relate accumulation or observed effects to added or nominal concentrations. Precipitated Ln’s are probably not available for uptake, but may dissolve again when the solution composition changes. If experimental exposure concentrations are not constant, they should at least be monitored. This re-
requires measurements with a technique like ICP-MS, which would make such experiments relatively expensive. Techniques like INAA or ICP-AES are not sensitive enough for this purpose, since they would require at least a 10 to 100 fold increase in sample volume and additional evaporation, chemical separation and/or preconcentration of the sample before measurement can take place. The use of γ-emitting radiotracers, which are available for many Ln’s, is a superior alternative, as it is much more sensitive and requires no further sample treatment (see section 1.2 and chapters 4 and 5). However, it requires trained personnel, a radiochemical laboratory and associated equipment for measurement. Precipitation could also lead to deficiencies, with regard to the anion involved in the precipitate. This will of course depend on the Ln and anion concentration and the amount of anion required by the organism during the whole experiment. Another problem caused by precipitation is the formation of an adsorptive surface (the precipitate) in the experiment, which may lead to further changes in solution composition.

A possibility to deal with supersaturated solutions is to wait until the solution is in equilibrium (in the example of Fig. 8-1 after 120 h) and then start the experiment. In experiments with radiotracers it may also be possible to incorporate the precipitation (kinetics) in the compartment modelling of the whole experiment (analogous to the approach followed in chapter 5). Disadvantages are that treatments containing different Ln concentrations will also differ in the amount of precipitate formed and consequently in concentrations of the anion with which the Ln precipitates. Such side-effects complicate the interpretation of the outcome of biotests with Ln’s. For duckweed experiments, amongst others, the medium should also uphold its sterility and this becomes harder when an experiment lasts longer, since the chance of infection increases. Therefore, the use of speciation calculations is advocated (as in chapters 4, 5, 6 and 7), enabling the researcher to check the possible exceeding of solubility products, before the experiment takes place. The outcome of the calculations may be used to adjust the composition of the solution where needed.

8.2.3 Speciation calculations and stability constants

As a first approximation, the solubility products of Ln phosphate, carbonate and hydroxide should be checked against the concentrations of these anions in the experimental medium, yielding the maximum Ln$^{3+}$ concentration in solution. For instance, at a concentration of 1.0 μM phosphate, the maximum concentration of La$^{3+}$ in solution may thus be approximated by [La$^{3+}$] = $K_{sp}/[PO_4^{3-}] = 10^{-25.7}/10^{-6} = 10^{-19.7}$ M ($K_{sp}$ from Liu and Byrne, 1997). The precise amount of La in solution depends on pH (determines phosphate protonation), ionic strength (influences activity coefficients and thus the value of conditional stability constants) and the presence of other anions (compete with phosphate for La) and cations (compete with La for phosphate). A speciation program takes all these factors into account (for a detailed description see chapter 6) and calculates the maximum amount of Ln in solution and its chemical form(s). At present such programs, e.g. MINEQL+ (Schecher and McAvoy, 1992) and CHEAQS (Verweij, 2002) are available without cost from the Internet.
Because no experimental work is needed, the use of speciation calculations is a fast and cost-effective way to study and evaluate: i) the chemical form(s) of Ln’s in solution, ii) the possible exceeding of solubility products, iii) the influence of omitting or adding components, and iv) the influence of gradually changing a solution component’s concentration (titration) on Ln speciation. However, some more or less predictable obstacles are conceived. For instance, not all speciation programs have all the Ln’s in their database (CHEAQS does, but MINEQL+ contains only La and Ce), and hence the missing ones must be added, including the relevant equilibrium constants. When Ln’s do occur in the database, the presence and quality of their stability constants should be examined against recent literature. Another problem could be that the chosen medium contains components, which are not present in the database. This means that these too must be added, including the relevant equilibrium constants. The other option, to omit a medium component, will be dealt with later. Unusual solution components, which are present in some media, mostly concern organic substances, like vitamins, amino acids or pH buffers. Nearly all inorganic ligands have been characterised with respect to their complexing behaviour towards Ln’s. Finally it must be noted that speciation calculations might work very well for synthetic solutions, but are hampered by a lack of information on natural waters.

Fig. 8-2. Linear regression ($r^2 = 0.985$, $n = 11$) of Ln phosphate stability constants ($\log_{10} \beta_i$ from Lee and Byrne, 1992) against Ln hydroxide stability constants ($\log_{10} \beta_i$ from Klungness and Byrne, 2000)

Collecting stability constants should be done with great care (according to the garbage in - garbage out principle) and additions to a speciation program’s database usually requires some pre-calculations before the equilibrium constant has the right format (i.e. a total stability constant, valid at $T = 25^\circ \text{C}$ and $I = 0.0 \text{ M}$). Appendix I contains Ln stability constants and solubility products for common ligands as they were used in this thesis and includes references to reliable sources. In selecting hydroxide solubility products it is important to distinguish between fresh precipitates and aged or crystalline precipitates, since the latter have smaller values of up to two orders of magnitude (Diakonov et al, 1998). For the evaluation of Ln(OH)$_3$ precipitation and
considering the time frame of most experiments, the solubility products for fresh precipitates are usually more relevant (see also chapter 7). Unknown stability constants may be estimated with linear free-energy relationships (LFERs) (Lee and Byrne, 1992), inter- or extrapolated from linear regression of stability constants against Z (see chapters 6 and 7) or against ionic radius (chapter 1) or from linear regression of Ln stability constants for one ligand against those of another ligand. The latter option is depicted in Fig. 8-2, which shows the marked correlation between hydroxide and phosphate stability constants of Ln’s. Hence, if all Ln hydroxide constants are known and only some of the phosphate constants, one can estimate the missing constants from this relationship. These estimations work considerably well, because of the coherence of Ln solution complexation behaviour (Henderson, 1996).

For most inorganic ligands occurring in nutrient solutions, stability constants with lanthanides are available. Exceptions are borate, vanadate and molybdate, for which no data were found, yet these ligands occur in many plant and algal media (vanadate and molybdate only in very small amounts). Recently, data on Nd complexation by molybdate have become available (Felmy, 1999). From the speciation data, it may be concluded that Ln’s preferentially bind to oxygen-containing ligands such as phosphate, sulphate, carbonate and hydroxide, but not to nitrate. The situation for organic ligands, which comprise a very large and diverse group of ligands, is more complex. Simple ligands such as acetate and oxalate are well studied, and the same holds for artificial ligands such as NTA, EDTA and DTPA, which have high affinities for Ln’s. Beyond these groups, data are more scattered and hence the speciation of a lanthanide in a medium containing such an ‘exotic’ component may not be reliably calculable. If an organism can do without this component for the duration of the experiment, one may consider to omit it or alternately replace it with a known component for the sake of a sound speciation calculation (see also Twiss et al., 2001).

Adding a component or increasing the concentration of an existing component in an artificial medium may be a means of solving precipitation problems in the original medium. This approach was followed in chapters 4 and 5, where the EDTA concentration of the duckweed medium was increased to counterforce the precipitation of LaPO₄. As noted in chapter 4, this had an influence on the speciation of other metals in the medium too, notably on Mn. Consequently, such changes must be made with great care so that the availability of essential metals does not drop beyond the minimal level required. Therefore, performance of organisms in modified nutrient media should always be checked against performance of organisms in the original medium. The experiment thus includes a control (original medium) to the actual control of the experiment (modified medium without Ln). This is important since stressed organisms may show different uptake behaviour and are generally more sensitive to chemicals than unstressed organisms. In our case (chapter 5) L. minor growth remained exponential and no negative effects were found as a result of the increased EDTA concentration.
CHAPTER 8

8.2.4 Natural solutions

Another consequence of the low Ln solubility products with ubiquitous ligands, such as phosphate and carbonate, is that natural water concentrations are hardly increased by industrial emissions. This implies that most natural waters are saturated, which may be expected from the presence of large Ln quantities in sediment (Table 3-2). Only when Lns are emitted as very strong complexes, such as the Gd complexes used in MRI hospital departments, elevated concentrations may be detected in the effluent (chapter 3; Bau and Dulski, 1996). In other cases, the emission can be found primarily in the sediment, which was demonstrated in a few other cases (Mars, 1988; Tijink and Yland, 1998).

Natural water concentrations of the heavier, odd-numbered Ln’s approach the detection limits of even the most sensitive measurement technique, i.e. high-resolution ICP-MS. This problem is related to the low solubility of lanthanides as well and can effectively be solved by preconcentration techniques (Zhu et al., 1998). The major part of the soluble Ln’s in freshwater is associated with DOC. Organic matter thus increases the solubility of Ln’s and thereby their geochemical mobility (Choppin, 1986; McCarthy et al., 1998). It is anticipated that this also increases their biological availability. Recent advances in the speciation modelling of Ln’s, humic acids and DOC (Tipping, 1994; Moermond et al., 2001) will prove to be valuable tools in understanding Ln behaviour in natural systems.

8.3 Availability, uptake and bioconcentration

In chapter 2 it has been shown that by accounting for Ln solubility, the differences in BCFs for terrestrial and aquatic plants, spanning six orders of magnitude, are reduced to about one order of magnitude. This confirms the direct link between Ln solubility and bioavailability, and also the fact that plants from both environments take up Ln’s from the water phase. In chapter 3, it was concluded by using BCF patterns and the redox speciation of Ce and Eu, that sediment-rooting plants take up Ln’s mainly from the sediment pore water. Furthermore, it was demonstrated that oxidation state is a confounding factor in determining Ln bioavailability, with Ce⁴⁺ being hardly available for uptake by duckweed from surface water, probably due to the formation of insoluble CeO₂, and Eu³⁺ in pore water being less available to Plaunaglost pectinatus than other, trivalent Ln’s, probably because it is much less reactive, thereby reflecting the mode of uptake, i.e. passive adsorption. Despite the fact that sediment and water concentrations for Ln’s showed no significant variation, those in P. pectinatus reached two orders of magnitude (chapter 3). This high variation can be explained by a combination of biological factors (variation between populations), chemical factors (differences in Ln availability between locations) and analytical artifacts (remains of attached sediment).

Using total dissolved concentrations is but a first step in approaching availability. More information may be gathered from studying the solution speciation of Ln’s. In chapters 4 and 5, speciation of dissolved La was calculated, while La behaviour was followed with the radioisotope
$^{140}$La. For cellulose filters, which may be considered a simplified model for plant cell walls, it was shown that adsorption (passive uptake) correlated best with free ionic La$^{3+}$, although there was a significant contribution of other La species, notably LaEDTA. If LaEDTA itself was adsorbed or if the complex merely supplied free La$^{3+}$ by dissociation, could not be established. The latter option seems more logical, since cellulose is expected to be negatively charged. Labelling EDTA with the radioisotope $^{14}$C or measuring EDTA species with HPLC could give insight into the uptake of different chemical La species. The same holds for La uptake studies with $L.\ minor$ (chapter 5). In these experiments the amount of La in or on the plant originates mainly from LaEDTA, but the way in which the complex contributes to this load remains unclear. Toxicity studies with $Vibrio fischeri$ did provide convincing evidence for availability of inorganic Lu species, notably free ionic Lu$^{3+}$ (chapter 6). Contrastingly, organic complexes of Lu with NTA, EDTA, citrate, oxalate and malate did not contribute to the observed effect and were thus considered non-available. However, we did not study if Lu in any form entered the cell. It could be envisaged that organic Lu complexes do cross the cell membrane, but do not dissociate and elicit therefore no toxic effect. For terrestrial plants grown in nutrient solution, it was shown that humic and fulvic acids reduce La rhizotoxicity by formation of non-rhizotoxic complexes (Dietloff et al., 1998).

Whereas the previous examples only considered uptake from water, uptake of Ln's from food was taken into account for snails in chapter 3. By comparing the smoothness in BCF patterns on basis of surface water with that based on plant ($P. patinatus$) concentrations, it was concluded that food seemed to be the dominant Ln source for snails. The degree of bioaccumulation in this food chain was limited with the highest calculated value being 5.5. However, the actual contribution of the two uptake routes of Ln's in snails, i.e. food and water, could not be quantified with the present measurements.

The degree of Ln bioconcentration in aquatic plants and invertebrates is high and ranges grossly between 1,000 and 1,000,000 L kg$^{-1}$ dry weight (chapters 1, 2, 3 and 5). Experimental values for dynamic BCFs of La in $L. minor$ (chapter 5) agreed well with steady-state values calculated from field measurements (chapter 3). Consequently, short-term laboratory experiments can provide reliable estimates for BCFs occurring under field conditions. Solution complexation of Ln's increases more with Z than their affinity for biotic ligands, such as plant surfaces. As a consequence, BCFs generally decrease with Z (chapter 3). The same trend has been shown for a marine diatom by Bingler et al. (1989) and for a marine alga by Stanley Jr. and Byrne (1990). The fact that sediment-water partition coefficients displayed the same trend with Z (chapter 3), provides further evidence for the mechanism of passive uptake, i.e. adsorption, of Ln's from water by aquatic organisms.

Because of their high BCFs, plants may be used as monitors for the presence of available Ln's in the aquatic environment (Wolterbeck and van der Meer, 1996). This may prove to be a useful instrument when emitted Ln's can be distinguished from the natural background, for in-
Chapter 8

stance when the emission involves Ln’s, which are enriched in respect to a particular Ln. Also there is a perspective to detect emissions of radioactive Ln’s originating from fission of uranium.

8.4 Toxicity

Two important conclusions on Ln toxicity may be drawn from the work that was described in chapters 6 and 7. These chapters were directed to elucidate i) if Ln’s comply with the free-ion model and ii) if toxicity of the Ln’s is related to their chemical properties or if it is more or less equal among these closely related elements. A chemically simple and easily manipulable test system with the luminescent bacterium *Vibrio fischeri* was chosen to address these questions.

First it was established that Lu, the heaviest Ln, did comply with the free-ion model, with respect to the effects observed in *V. fischeri*. However, other (minor) inorganic Lu species, which are all proportional to the free Lu$^{3+}$-ion, may also have contributed to the observed effects. In the applied experimental set-up, we could not differentiate between inorganic Lu species. However, it must be noted that at least 95% of the total inorganic Lu consisted of Lu$^{3+}$. Analogous to what is commonly done in Fe$^{3+}$-speciation and availability research (Gerringa et al., 2000; Sunda, 2001), it could be useful to introduce the concept of Ln$^{’}$ to denote the sum of all available inorganic Ln species. However, further refinement of the concept is needed, since Ln’s are primarily taken up via adsorption, and the latter aspect shows large differences among the different inorganic Ln species (as shown for Gd uptake by algae in carbonate manipulated media (Stanley Jr. and Byrne, 1990). Because total dissolved Ln in our experiments consist mainly of free ionic Ln$^{3+}$, which is also the most reactive species, effects are primarily attributed to this Ln-form. It followed that the toxicity of free ionic Lu$^{3+}$ is high, i.e. comparable to that of Cu$^{2+}$, an essential, but very toxic element (chapter 6). Secondly, the toxicity of individual Ln is not equal, but progresses with Z and can be related to chemical properties and to their abundance in the earth’s crust (chapter 7). Differences between the most and least toxic lanthanide, Lu and La, respectively, may span two orders of magnitude, depending on the length of exposure (chapter 7). What exactly determines the toxicity of Ln’s to *V. fischeri* was not investigated. Ln toxicity could be accomplished by mechanisms such as the binding of Ln’s to a functional protein in the cell membrane and disturb the protein’s normal functioning or by inhibiting cation (e.g. Ca$^{2+}$) transport across the cell membrane. In case Ln’s do cross the cell membrane, a variety of oxygen-containing ligands can be imagined where Ln’s may bind to and affect normal functioning. In addition it is likely that Ln’s will disturb nutrient cycling by substitution for e.g. Ca$^{2+}$ or Mg$^{2+}$. It seems worthwhile to repeat the toxicity experiments with other (higher) organisms, to see if the conclusions on *V. fischeri* may be generalised.

Because of the low solubility of several inorganic Ln salts (see above), their relatively high affinity for sediment and suspended matter, and their tendency to form complexes with both inorganic and organic ligands, the free ion concentrations in natural freshwaters are low (in the pM to nM range). Assuming that the free ion is the species determining Ln toxicity to aquatic inver-
tebrates and plants, it is obvious that in the field biota will be affected by Ln’s only under extreme conditions. Such conditions can be imagined as a low pH, low dissolved organic matter concentrations and low phosphate, fluoride and carbonate concentrations. Under these circumstances, Ln’s will be largely present as free ions, which easily react with biotic ligands, thereby possibly affecting the organism. The study of Protano and Riccobono (2002) gives details on extremely high Ln concentrations under very low pH values in a metal-mining area, where the effects described above can be imagined. However, the low pH itself and associated increased availability of other metals, e.g. Cu and Zn, will contribute to the eradication of most biota in such streams anyway.

8.5 Outlook

From the results presented in this thesis, ideas for new experiments arise to test some of the conclusions drawn. In addition, questions are left unanswered that may be solved by critical experimental work. For now, the conclusions are limited to one or two organisms, and this number should be increased for a proper evaluation of Ln impacts on freshwater ecosystems. For instance, it should be established if Ln’s show the same progressing toxicity with Z in other organisms, since this could prove very useful in risk assessment. The 14 naturally occurring Ln’s could thus be scaled by their respective toxicities, and these scaled concentrations can be summed into one index, which may then be compared to an environmental quality standard. Summation of scaled concentrations is valid for substances acting in a concentration-additive way, i.e. have the same mode of action (Welte, 1998a). This may be expected for chemically similar elements such as the Ln’s. Since organisms in natural ecosystems will be exposed to all Ln’s simultaneously, this approach has direct relevance to the field.

For future experiments, the gathered stability constants (Appendix I) may be used as input for speciation calculations, aimed at designing test media that do not exceed solubility products. Spiking the Ln’s in solution with γ-ray emitting isotopes has proven to be a very sensitive technique to monitor and model the distribution of Ln’s over various compartments (chapters 4 and 5). These complementary tools, speciation calculations and radioisotopes, can, in concert with cation or anion selective resins, give further insight into the role of chemical speciation in the uptake of lanthanides by aquatic organisms. The relation between different kinds of inorganic Ln species and their availability for uptake by L. minor may be tested in this manner. As the contribution of inorganic Ln complexes to the observed toxicity for V. fischeri in chapters 6 and 7 was not unequivocally proven, it seems useful to test media that differ in inorganic complexing capacity for this species. Such experiments should be aimed at revealing if these complexes themselves are available for uptake or that they are chemically very labile and almost instantly supply the free Ln³⁺ ion.

In addition, uptake of Ln’s from food by snails should be investigated. To establish the contribution of each uptake route (food and water), experiments can be designed with snails ex-
posed to one Ln in the water and another one in the food (plants can be cultured on nutrient solutions containing a Ln). Preferably the applied Ln’s are spiked with γ-ray emitting isotopes, so that uptake and distribution of Ln’s may be measured in vivo and non-destructively. This has the great advantage of being able to measure uptake of Ln’s in a single organism, and hence eliminate biological variation from these experiments. In addition, fewer animals are needed for such experiments.

Whether or not the findings of the work, described in this thesis, are applicable to actinides was not tested and remains for now an open question. To answer the question, simultaneous experiments should be carried out with a Ln on the one hand and an An on the other (the couple Eu-Am is apparent). Prerequisite is the matching of oxidation states, as stated by Choppin (1991 and 1995) and a close monitoring of the redox behaviour, especially when working with reducing sediments. Since the speciation of some actinides, e.g. U, Am, Pu is relatively well studied, calculations on their chemical behaviour are possible and should be done before experimental work is carried out.
APPENDIX I

STABILITY CONSTANTS OF LANTHANIDE COMPLEXES

INORGANIC LIGANDS
IA. HYDROXIDE, CARBONATE, FLUORIDE
IB. NITRATE, PHOSPHATE, SULPHATE, CHLORIDE

ORGANIC LIGANDS
IC. ACETATE, MALATE, OXALATE
ID. CITRATE, NTA, EDTA

SOLUBILITY PRODUCTS
IE. HYDROXIDE, CARBONATE, FLUORIDE, PHOSPHATE, OXALATE
# Appendix Ia

Lanthanide stability constants, \(\log_\beta_d(Ln)\), appropriate at \(I = 0.0\) M and \(T = 25^\circ\)C, 
for hydroxide (OH\(^-\)), carbonate (CO\(_3^{2-}\)) and fluoride (F\(^-\)).

<table>
<thead>
<tr>
<th>Ln(OH(^+))(^{2+})</th>
<th>Ln(OH(^+))(^+)</th>
<th>Ln(OH(^-))(^+)</th>
<th>Ln(OH(^-))(^{2-})</th>
<th>Ln(CO(_3^{2-}))(^+)</th>
<th>Ln(H(CO(_3^{2-}))(^{2+})</th>
<th>Ln(CO(_3^{2-}))(^3-)</th>
<th>LnF(^+))(^2+)</th>
<th>LnF(^2+)</th>
<th>LnF(^3+)</th>
<th>LnF(^-)</th>
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<th>(\text{r})</th>
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<th>(\text{t})</th>
<th>(\text{u})</th>
<th>(\text{v})</th>
<th>(\text{w})</th>
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References: 1 = Klapnness and Byrne (2000), 2 = Lee and Byrne (1992), 3 = Haas et al. (1995), 4 = Martell and Smith (1998), 5 = Lau and Byrne (1998), 6 = Miller (1992), 7 = Schif and Byrne (1999). The species Ce(OH\(^+\))\(^{2+}\), La(OH\(^+\))\(^{2+}\), La\(_2\)(CO\(_3^{2-}\))\(^{4+}\) (ref. 4) are not included. \(\text{p}\) from ref. 2, \(\text{q}\) calculated from hydroxide constants using a \(\log_\beta_d\) of 13.997 at \(I = 0.0\) M (ref. 4), \(\text{r}\) converted from \(I = 3.0\) M with the Davies eqn., \(\text{s}\) converted from \(I = 2.0\) M with the Davies eqn., \(\text{t}\) value seems high compared to other La's, my estimate is 1290, \(\text{u}\) converted to total stability constants using a \(\log_\beta_d\) of 10.329 for HCO\(_3\) at \(I = 0.0\) M (ref. 4), \(\text{v}\) from ref. 4, converted from \(I = 0.1\) M with the Davies eqn., \(\text{w}\) value seems a little high compared to other La's, my estimate is 3.85, \(\text{x}\) converted from \(I = 0.025\) M with the Davies eqn., \(\text{y}\) estimated from data on other La's.
### Appendix IB

Lanthane stability constants, $\log_{10} \beta_i(Ln)$, appropriate at $I = 0.0$ M and $T = 25^\circ$C, for nitrate ($NO_3^-$), phosphate ($PO_4^{3-}$), sulphate ($SO_4^{2-}$) and chloride ($Cl^-$)

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<th>$\text{Ln(NO}_3^3$</th>
<th>$\text{Ln(PO}_4^{3-}$</th>
<th>$\text{LnH}_2(PO}_4^{3-}$</th>
<th>$\text{LnH}_3(PO}_4^{3-}$</th>
<th>$\text{Ln(PO}_4^{2-}$</th>
<th>$\text{LnH}_2(PO}_4^{2-}$</th>
<th>$\text{Ln(SO}_4^{2-}$</th>
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<td>5.30</td>
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<td>5.20</td>
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<td>-0.62</td>
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References: 1 = Bonal et al. (1998), 2 = Lee and Byrne (1992), 3 = Liu and Byrne (1997), 4 = Millero (1992), 5 = Martell and Smith (1998), 6 = Luo and Byrne (2001), 7 = Haas et al. (1995). 4 converted from $I = 1.0$ M with the Davies eqn., value seems high compared to other Ln's, my estimate is 0.69, 4 converted from $I = 0.1$ M with the Davies eqn., after conversion to total stability constants using $\log_{10}$'s 49.1643 (average, $n = 3$) for $HPO_4^{2-}$ and 18.437 (average, $n = 3$) for $H_2PO_4^-$ at $I = 0.1$ M (Martell and Smith, 1997), 7 converted to $\log_{10}$ using 19.573 for $H_2PO_4^-$ at $I = 0.0$ M (ref. 5), d converted from $I = 2.0$ M with the Davies eqn., values seem low compared to other Ln's, my estimates are 3.67 and 5.10, respectively, e value seems high compared to other Ln's, my estimate is 0.01, * estimated from data on other Ln's.
### Appendix IC

Lanthanide stability constants, log$_\beta$(Hn), appropriate at I = 0.0 M and T = 25°C, for acetate (Ac$^-$), malate (Mal$^{2-}$) and oxalate (Ox$^{2-}$)

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<th>Ln(Ac$^-$)$^{3+}$</th>
<th>Ln(Ac$^-$)$^{2+}$</th>
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<th>Ln(Mal$^{2-}$)</th>
<th>Ln(Mal$^{+}$)</th>
<th>Ln(H(Mal)$^{2+}$)</th>
<th>Ln(Ox$^{2-}$)</th>
<th>Ln(Ox$^{+}$)</th>
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<td>2.550</td>
<td>4.120</td>
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<td>5.87</td>
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<td>6.692</td>
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<td>-</td>
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<td>3.813</td>
<td>6.061</td>
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<td>6.732</td>
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<td>4.178&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>-</td>
<td>6.181</td>
<td>9.959</td>
<td>-</td>
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<td>4.128&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>6.862</td>
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<td>-</td>
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<td>4.228</td>
<td>5.301</td>
<td>-</td>
<td>6.361</td>
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<td>-</td>
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<td>11.77</td>
<td>7.052</td>
<td>13.959&lt;sup&gt;1&lt;/sup&gt;</td>
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References: 1 = Martell and Smith (1998), 2 = Martell and Smith (1997), 3 = Schijf and Byrne (2001).<sup>a</sup> converted from I = 0.1 M with the Davies eqn.,<sup>b</sup> converted from I = 2.0 M with the Davies eqn., values seem low compared to other Ln$^3+$, my estimates are 5.05 (Pr), 5.15 (Nd) and 5.40 (Sm), respectively,<sup>c</sup> converted from I = 0.5 M with the Davies eqn.,<sup>d</sup> converted from I = 0.5 M with the Davies eqn. after conversion to a total stability constant, using a log$\beta$ of 4.52 for H(Mal)$^2$: at I = 0.5 M (ref. 2),<sup>e</sup> converted from I = 1.0 M with the Davies eqn. after conversion to a total stability constant, using a log$\beta$ of 4.49 (average, $\sigma$ = 2) for H(Mal)$^2$: at I = 1.0 M (ref. 2),<sup>f</sup> converted from I = 0.05 M with the Davies eqn. after conversion to total stability constants, using a log$\beta$ of 3.92 for H(Ox)$^2$: at I = 0.05 M (ref. 3),<sup>g</sup> converted from I = 1.0 M with the Davies eqn.,<sup>h</sup> converted from I = 0.7 M with the Davies eqn.,<sup>i</sup> converted from I = 1.0 M with the Davies eqn.,<sup>j</sup> estimated from data on other Ln$^3+$.
APPENDIX I

Lanthanide stability constants, log_{10}([Ln]), appropriate at I = 0.0 M and T = 25°C, for citrate (Cit\(^3^+)\), nitritotriacetate (NTA\(^3^+)\) and ethylenediaminetetraacetate (EDTA\(^5^+)\)

<table>
<thead>
<tr>
<th></th>
<th>Ln(Cit)</th>
<th>Ln(Cit)^2</th>
<th>Ln(NTA)</th>
<th>Ln(NTA)OH</th>
<th>Ln(NTA)^2</th>
<th>Ln(EDTA)</th>
<th>LnH(EDTA)</th>
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</thead>
<tbody>
<tr>
<td>Ce</td>
<td>9.332(a)</td>
<td>13.892</td>
<td>12.622</td>
<td>18.402(b)</td>
<td>20.582</td>
<td>18.493</td>
<td>20.396</td>
</tr>
<tr>
<td>Pm</td>
<td>9.632(a)</td>
<td>14.57(b)</td>
<td>13.15(c)</td>
<td>19.35(b)</td>
<td>21.628(b)</td>
<td>19.463</td>
<td>21.06(c)</td>
</tr>
<tr>
<td>Yb</td>
<td>9.65</td>
<td>16.022</td>
<td>14.132</td>
<td>20.422</td>
<td>23.332</td>
<td>22.053</td>
<td>22.40(b)</td>
</tr>
</tbody>
</table>

References: 1 = Martell and Smith (1997), 2 = Itoh et al. (1985), 3 = Martell and Smith (1998). Other Ln-citrate complexes were not included, since their existence is uncertain (see Wood, 1993). \(a\) converted from I = 0.1 M with the Davies eqn., \(b\) T = 20°C, \(c\) converted from I = 0.1 M with the Davies eqn. after conversion to total stability constants, using log\(\beta\)'s for Ln(NTA) at I = 0.1 M; they are also used to calculate the values in the previous column, \(d\) converted from I = 0.1 M with the Davies eqn. after conversion to a total stability constant, using a log\(\beta\) of 15.36 for La(EDTA) at I = 0.1 M (ref. 3), \(e\) converted from I = 1.0 M with the Davies eqn. after conversion to total stability constants, using log\(\beta\)'s for Ln(EDTA) at I = 0.1 M, which were first to 1.0 M; they are also used to calculate the values in the previous column, \(f\) estimated from data on other Ln's.
**APPENDIX IE**

Lanthanide solubility products, \(\log K_s\) appropriate at \(I = 0.0\) M and \(T = 25^\circ\)C

<table>
<thead>
<tr>
<th></th>
<th>Ln(OH)_3</th>
<th>Ln_2(CO_3)_3</th>
<th>LnF_3</th>
<th>LnPO_4</th>
<th>Ln_3(O_3)</th>
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<tr>
<td>La</td>
<td>20.06</td>
<td>33.40</td>
<td>18.700</td>
<td>25.75</td>
<td>28.204^4</td>
</tr>
<tr>
<td>Ce</td>
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<td>27.037^*</td>
<td>19.100</td>
<td>26.27</td>
<td>29.23</td>
</tr>
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<td>Pr</td>
<td>22.33</td>
<td>33.07^*</td>
<td>20.181</td>
<td>26.43</td>
<td>-</td>
</tr>
<tr>
<td>Nd</td>
<td>23.34</td>
<td>33.00</td>
<td>20.300</td>
<td>26.20</td>
<td>31.11</td>
</tr>
<tr>
<td>Pm</td>
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<td>32.69^*</td>
<td>19.75^*</td>
<td>26.20^*</td>
<td>-</td>
</tr>
<tr>
<td>Sm</td>
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<td>32.50</td>
<td>19.181</td>
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<td>32.30</td>
<td>21.900</td>
<td>25.96</td>
<td>-</td>
</tr>
<tr>
<td>Gd</td>
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<td>25.62</td>
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<td>-</td>
</tr>
<tr>
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<td>17.581</td>
<td>25.18</td>
<td>-</td>
</tr>
<tr>
<td>Ho</td>
<td>24.52</td>
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<td>17.081</td>
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<td>-</td>
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<td>31.37^*</td>
<td>18.000</td>
<td>25.13</td>
<td>-</td>
</tr>
<tr>
<td>Tm</td>
<td>24.70</td>
<td>31.18^*</td>
<td>17.081</td>
<td>25.03</td>
<td>-</td>
</tr>
<tr>
<td>Yb</td>
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<tr>
<td>Lu</td>
<td>25.00</td>
<td>30.80^*</td>
<td>16.281</td>
<td>24.75</td>
<td>-</td>
</tr>
</tbody>
</table>

ref. 1  2  2  3  2

References: 1 = Drakonos et al. (1998), data are values for fresh precipitates, 2 = Martell and Smith (1997), 3 = Liu and Byrne (1997). Mixed solids, *i.e.* Ln(OH)(CO_3) and LnCl(CO_3) were not included. ^ converted from \(I = 3.0\) M with the Davies eqn., value seems low compared to other Ln’s, my estimate is 33.26. ^ converted from \(I = 0.1\) M with the Davies eqn., ^ value seems high compared to other Ln’s, my estimate is 18.7, ^ converted from \(I = 0.1\) M with the Davies eqn., \(T = 20^\circ\)C, ^ estimated from data on other Ln’s.
APPENDIX II

ON THE CORRESPONDENCE BETWEEN REACTION NORMS AND CONCENTRATION-RESPONSE CURVES

This chapter is based on WELTJE, L. (accepted) On the correspondence between reaction norms and concentration-response curves. *Entomology.*
Appendix II

Abstract
An attempt is made to further integrate ecotoxicology and evolutionary genetics, disciplines which both study organismic responses to environmental variables. In ecotoxicology, the environmental variable is a chemical and the resulting function a concentration-response curve. Evolutionary geneticists use reaction norms to describe the response of a genotype to environmental variables, such as temperature or salinity. Nevertheless, by making specific choices, concentration-response models and reaction norm models are interchangeable. Recognising this leads to a better understanding of biological responses to toxicants and their underlying genetical basis.

The slope parameter of the concentration-response curve of a single genotype as well as the width of the sensitivity distribution that underlies this curve can be interpreted as a measure of phenotypic plasticity. Interestingly, phenotypic plasticity seems to be inversely defined in ecotoxicology and evolutionary genetics. At present, this hampers the integration of these disciplines, and a consensus should be sought. Since single genotype or clonal populations are much used in ecotoxicology, it seems relevant to explore the implications of the proposed concept with respect to the extrapolation of results from single genotype laboratory toxicity experiments to genetically variable populations in contaminated ecosystems.
REACTION NORMS AND CONCENTRATION-RESPONSE CURVES

Introduction
Since the responses of organisms to environmental variables are studied in both ecotoxicology and evolutionary genetics, the integration of these disciplines seems obvious. Ecotoxicologists and evolutionary geneticists meet each other over genetically based sensitivity differences in responses of organisms to environmental stress. By coupling concentration-response relationships from the former discipline and reaction norms from the latter, a better understanding of the underlying mechanisms for biological responses to toxicants can be achieved. Particular attention is given to the interpretation of the slope parameter of concentration-response curves and reaction norms. This paper discusses the conditions that allow coupling of concentration-response curves and reaction norms, as well as extrapolation of the concept to populations in polluted ecosystems.

Concentration-response curve theory
Traditionally, ecotoxicologists study the performance of organisms at different concentrations of environmental chemicals, usually resulting in the construction of a concentration-response curve. This curve describes the performance of an organism (e.g. growth, reproduction) as a function of the concentration of a chemical (i.e. toxicant) in the environment or in the organism itself. Normally, performance decreases monotonically with increasing toxicant concentrations or, in case of a threshold, the value for undisturbed performance is followed by monotonic decrease. Exceptions to monotonic decreases are caused either by hormesis, a stimulation of performance at low toxicant concentrations (Stebbing, 1982) or by a deficiency of the applied chemical (opposing toxicity). The latter may occur in experiments with essential nutrients (e.g. Zn, Cu) at the lower end of the concentration range. Recently, the inverted U-shape concentration-response curve has been introduced as found in endocrine disruptor studies (see for instance Oehlmann et al., 2000). Here, these cases are not considered, but are merely mentioned to acknowledge the full concentration range and the possible biological responses thereto.

On a logarithmically scaled X-axis (concentration) and linear Y-axis (response or performance), the shape of a concentration-response curve generally follows a sigmoid pattern, comparable to an inverse cumulative log-normal distribution. This curve is characterised by three parameters: i) the maximum or control performance (a scaling parameter, often set to 100%), ii) the EC_{50}, determining the curve’s position on the X-axis and indicating an organism’s sensitivity to a chemical (X usually equals 50), and iii) the slope of the curve, describing its steepness and shape. Although the slope parameter of a concentration-response curve bears some interesting information, most of the attention usually goes to the EC_{50}. The focus of this paper is on the slope parameter and the information it provides. The slope of the sigmoid has various interpretations:

- an indication of absorption, excretion or detoxification rate (WHO, 1978),
- the rate of increase of inhibition by a chemical (Haanstra et al., 1985),
- an indication of mode-of-action of a chemical (Chen and Chiu, 1995),
APPENDIX II

- a measure of response variability among individuals within the exposed population (Forbes and Depledge, 1992).

A steep slope is consequently associated with rapid absorption, low excretion or detoxification rates, a high inhibition increase rate, a certain mode-of-action and a low variability of response within the exposed population, respectively. The first three definitions partly overlap in the sense that they provide a comparative measure of the toxicological nature of a substance. Since they are merely of an indicative nature, they hardly provide insight into a substance's characteristics (see also Smit et al., 2001). The fourth definition stands aside from the others, as it refers to the constitution of the exposed population. This last interpretation of the slope is pursued in the present paper.

In addition, the slope provides information on the range of toxicant concentrations that induce partial inhibition of performance, i.e. the Ecological Dose Range (EDR) as proposed by Haanstra et al. (1985). The value of the slope also depends on the measure of exposure, e.g. environmental or body concentrations (Welčić, 1998a). Frequently, an increase of the slope with time is observed (Haanstra et al., 1985; Crommentuijn et al., 1995a), which might be related to accumulation not having reached equilibrium when the first observations were made or to concentration-dependent changes in availability of the chemical (e.g. sorption to soil or other substrates).

The choice for one of the available sigmoid concentration-response models to describe toxicological data has an interesting consequence. For instance, the logistic model is symmetrical in the Ec50 and thus assumes a symmetrical (i.e. log-normal) distribution of response within a population, whereas e.g. a Weibull model allows for a non-symmetrical or skewed distribution. Consequently and unlike the logistic model, the Weibull model produces less frequently a long right-hand sided tail, which suggests that some performance still occurs at unrealistically high toxicant concentrations. This is the main reason why the Weibull model often fits better to experimental data. Still, mathematical models for concentration-response curves are purely empirical and lack a biological underpinning (van Straalen, 1997).

**Reaction norm theory**

An important issue in evolutionary genetics is to understand how genotypes respond to environmental variables. Dissimilar development of a genotype in different environments leads to phenotypes, which differ in response values. This phenomenon, known as phenotypic plasticity, can increase an organism's environmental tolerance (Lynch and Gabriel, 1987). Phenotypic plasticity is not a characteristic of organisms as a whole, but applies to individual traits (Via, 1987). Schlichting (1989) even states that phenotypic plasticity itself can be considered a trait and is, as such, subject to selection. However, there is no consensus on this issue and it is therefore not considered here (for a discussion see Via et al., 1995). If phenotypic values of different genotypes in a specific environment give rise to differences in fitness, a basis for (natural) selection is provided.
REACTION NORMS AND CONCENTRATION-RESPONSE CURVES

The systematic pattern of response by a genotype to a systematic change in the environment is called the reaction norm of that genotype (Wolterek, 1909; Steams, 1992; de Jong, 1995). The reaction norm can be any function of the environment; the function parameters are genetically determined (Gavrilets and Scheiner, 1993). Selection on the genetically determined reaction norm parameters leads to co-ordinated change in the reaction norm (Gavrilets and Scheiner, 1993). Furthermore, reaction norm shape is entirely variable; sigmoid and linear reaction norms, among others, are possible (Gilbert et al., 1998), and a monotonic decrease with increasing environmental values per se does not apply. If reaction norms of genotypes A and B of one species cross, there is a genotype-by-environment (G×E) interaction. It implies that selection would favour genotype A in one environment and genotype B in another.

The absolute value of the first derivative of a reaction norm in a particular environment can be taken to represent the amount of phenotypic plasticity (de Jong, 1995; Black-Samuelsson and Andersson, 1997). Consequently, if the slope equals zero, there is no plasticity. The steeper the slope, the more sensitive an organism reacts to environmental changes (Falconer, 1990). As with concentration-response curves, most reaction norm curves are purely empirical and lack a scientific mechanistic basis. Exceptions are the growth response of plants to the ratio of red and far red light (Schmitt et al., 1995) and the temperature response in ectotherms, e.g. insects (van der Have and de Jong, 1996).

Joining the concepts

If a toxicant concentration is chosen as the environmental variable, the reaction norm can equal a concentration-response curve (Kammenga, 1995). Correspondingly, in an ecotoxicological experiment the concentration-response curve can equal a reaction norm. To validate this statement, some requirements have to be met. The main condition is the use of one genotype, such as a clone or parthenogen (which coincides with many laboratory toxicity test species). Furthermore, the validity of the statement is restricted to chronic endpoints, such as growth or reproduction, and does not apply to the acute endpoint mortality, because death is not a phenotypic value as such, although a measurement of mortality, such as length of the adult life span, is.

In addition, it must be emphasised that phenotypic plasticity seems to be inversely defined in ecotoxicology and evolutionary genetics. A steep reaction norm slope is associated with a high amount of phenotypic plasticity, whereas for concentration-response curves of single genotypes the opposite is the case: a steep slope means little phenotypic plasticity. This can be demonstrated by considering the two extremes, i.e. no plasticity and infinite plasticity. In the case of the reaction norm, no plasticity gives a horizontal relationship (no change in response value over the applied range of environments) and infinite plasticity a vertical response (immediate value change in a slightly different environment), while for a concentration-response curve, no plasticity is observed as a vertical line at the EC_{50}-concentration (a very narrow sensitivity distribution) and infinite plasticity as a horizontal line (a very tolerant genotype with a wide sensitivity distribution). The present state of opposite definitions hampers the integration of ecotoxicology and evolu-
tionary genetics. For the sake of clarity, a single definition of phenotypic plasticity is followed in this paper: the one from ecotoxicology. Then, the meaning of the slope parameter of a concentration-response curve/reaction norm integrates to the amount of variation in sensitivity of a genotype, resulting from phenotypic plasticity of the response trait for a particular chemical. If different phenotypic values (of different genotypes) relate to different fitnesses, then the environmental chemical is in fact a selection instrument. Selection under the influence of chemical stress has already been proven by studies on metal-tolerant populations of terrestrial plants, invertebrates and micro-organisms (Dueck et al., 1984; Donker and Bogert, 1991; Rutgers et al., 1998) and is obviously associated with the development of pesticide resistance in arthropods. Exposure to environmental toxicants is frequently associated with a loss of genetic variation (the so-called 'cost of tolerance') as demonstrated for natural populations of crayfish (Krane et al., 1999). This finding enables the possibility to use a population’s generic variation as a biomarker for toxicant exposure (Theodorakis and Shugart, 1997).

![Graph](image-url)

**Fig. II-1.** Concentration-response curves for genotypes A and B, showing G×E interaction. Selection favours genotype A below the EC50 and genotype B above the EC50.

If concentration-response curves of single genotypes of one species cross, it implies that one genotype may be more successful than another at environmental concentrations below the point of crossing, while above this threshold, the situation is reversed and hence selection would favour the other genotype (G×E interaction). This is illustrated in Fig. II-1, where genotype A performs better at concentrations below the EC50, while genotype B is the better performer at concentrations exceeding the EC50. If the represented response stands for reproduction, the selection is easily envisaged and consequently the genetic composition of the population will change on either side of the EC50.

Whereas reaction norms deal with responses to external environmental factors, such as light and temperature, toxicants usually exert their effects by entering an organism and interfere with its physiology and biochemistry, so that the environmental toxicant changes from an external to an internal variable. This difference might seem an obstacle for integration of the two concepts. However, it is argued that 'internalisation' of the environmental variable is non-explicitly
incorporated in the concept of the reaction norm. It follows from the idea that an environmental variable can only affect an organism by (in)direct influences on physiological processes. For instance, the environmental variable salinity, which involves a chemical gradient and corresponding physiological characteristics in organisms, such as the uptake and excretion of salt, may be studied as an external variable (Jacobsen and Forbes, 1997). Another example is presented by the water flea *Daphnia longispina*, which shows a plastic response to chemical signals released from its predators by developing defensive morphological and life-history traits. Interestingly, the same response was observed for exposure to the pesticide endosulfan (Barry, 1998).

Another matter, which deserves attention, is the experimental set-up for constructing reaction norms and concentration-response curves, as it represents a potential obstacle for the integration of the two approaches. In the case of reaction norms, organism response is mostly studied in the environment wherein the organisms grew up, while in ecotoxicological experiments organisms are often reared in a clean environment and then placed in a contaminated environment to study their response. This is essentially different, because in the process of growing up in a contaminated environment detoxifying mechanisms may be induced (e.g. metallothionein synthesis after exposure to heavy metals) which influence response in a favourable direction. Reaction norms thus relate to development and usually not to an *ad-hoc* reaction as a result of a sudden change in environment. Thus, for a full comparison, the experimental set-up must be equal and involve some sort of developmental phase, such as a lifecycle test.

![Graph](image)

**Fig. II-2.** Hypothetical concentration-response curve and underlying sensitivity distribution (inset, solid line) with standard deviation (σ) and mean (μ). In a single genotype experiment the standard deviation represents phenotypic plasticity, whereas in an experiment with a three genotype population (with sensitivity distributions indicated by dashed lines) the standard deviation reflects differences in sensitivities among genotypes as well as their phenotypic plasticity. In that case, the solid line distribution is the overall population distribution.
APPENDIX II

Discussion and conclusions

In ecotoxicology, a solid mechanistic base for the concentration-response curve has yet to be formulated. However, progress can be made by integrating the data and knowledge on phenotypic plasticity from evolutionary genetics with existing information from ecotoxicology on the responses of organisms to toxicants and the sensitivity distribution of traits within populations.

For experiments conducted with clones, the slope parameter of the concentration-response curve can be explained in terms of phenotypic plasticity of a chronic endpoint (see Fig. II-2). This additional interpretation is relevant, for clones of organisms are frequently used in both terrestrial and aquatic ecotoxicology. Examples are parthenogens like the nematode Caenorhabditis elegans, the freshwater snail Paludicola antipodarum, the soil collembolan Folsomia candida and the water flea Daphnia magna, but also vegetatively growing organisms like common duckweed ( Lemma minor). Recognising concentration-response curves of single genotypes as reaction norms also emphasises an important limitation for the extrapolation of results from experiments with clones to contaminated ecosystems, because field populations mostly consist of several genotypes and are thus genetically variable. On this subject, research has been undertaken, investigating sensitivity differences of several laboratory clones of a single species to a number of toxicants (Soares et al., 1992; Crommentuyn et al., 1995b; Barata et al., 1998; Mazzeo et al., 1998). Overall, these authors concluded that environmental factors and exposure time were dominant in explaining the variance in clonal response and sensitivity differences between clones were relatively small. The observed small sensitivity differences between clones were partly due to their broad phenotypic plasticity. It can be hypothesised that parthenogens and vegetatively growing organisms show more phenotypic plasticity than sexually reproducing organisms, for they are more dependent on this means to cope with heterogeneous environments (Jacobsen and Forbes, 1997). Furthermore, sensitivity ranking of clones for one particular toxicant did not imply the same rank order for other toxicants. Regarding the latter, Depledge (1994) asserted that mixtures of toxicants may cause erosion of genetic diversity within populations, as different genotypes can be sensitive to different toxicants. In contrast with the previously mentioned studies, Baird et al. (1990) found large sensitivity differences between clones of Daphnia magna in their response to cadmium and 3,4-dichloroaniline. This warrants a further investigation into the sensitivity of laboratory clones (which may have gone through unknown bottlenecks) and their representativeness for field populations.

In ecotoxicological experiments with populations comprising multiple genotypes, the population sensitivity distribution can be thought of as a collection of sensitivity distributions of individual genotypes (see inset of Fig. II-2). Moreover, this illustrates why sensitivity distributions are not necessarily symmetrical on a log scale: it assumes equal frequencies of the most and least sensitive genotypes in a population. Even bimodal population sensitivity distributions can occur for populations made up of two genotypes that differ considerably in their respective tolerances (imagine the middle genotype missing from the inset of Fig. II-2). For a population whose genetical variation with respect to sensitivity for an environmental pollutant is limited, and hence
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the population sensitivity distribution is narrow, conservative environmental quality objectives are required, for small emissions could have a large impact on those populations.

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CURRICULUM VITAE

Lennart Welije was born on January 12, 1968 in Doetinchem, The Netherlands. He attended pre-university education (VWO) at the Gemeentelijke Scholengemeenschap in Doetinchem from 1980 to 1987. In September 1987, he started studying biology at Utrecht University. He received his B.Sc. in 1988 and his M.Sc. in 1993, with majors in evolutionary genetics (supervisor Dr. Gerdien de Jong), soil clean-up policy (supervisor Drs. Angelique van der Schraaf, Environmental Hygiene Inspection South-Holland, Rijswijk) and soil ecotoxicology (supervisor Dr. Leo Posthuma, National Institute of Public Health and the Environment (RIVM), Bilthoven). In addition, he did a minor in environmental science. From October 1993 until October 1995, he worked at the Laboratory for Ecotoxicology, RIVM, on the effects of chemical mixtures on soil invertebrates. In November 1995, he started working at the Faculty of Civil Engineering, Delft University of Technology (TUD), on the toxicity of metals to duckweed. In July 1997, he moved to the Radiochemistry Department of the Interfaculty Reactor Institute, TUD, where he carried out the research described in this thesis. During this period, he was trained as a Radiological Safety Officer, Expert Level 3. In May 2001 he went to Germany, where he works as a Marie-Curie postdoc fellow on the effects of endocrine disruptors on freshwater invertebrates at the International Graduate School (IHI) Zittau (project leader Prof.Dr. Jörg Oehlmann).
PUBLICATIONS RELATED TO THIS THESIS


Welte, L., Verhoof, L.R.C.W., Wolterbeek, H.Th., and J.J.M. de Goeij (to be submitted) Toxicity of eleven lanthanides and scandium to the bacterium *Vibrio fischeri*.

Welte, L., Brouwer, A.H., and H.Th. Wolterbeek (to be submitted) *In vivo* uptake and elimination of europium (Eu) by the pond snail *Lymnaea stagnalis*.

CONFERENCE CONTRIBUTIONS


OTHER PUBLICATIONS


Welte, L. (accepted) Integrating the disciplines of evolutionary genetics and ecotoxicology: On the correspondence between reaction norms and concentration-response curves. Ecotoxicology.


CONFERENCE CONTRIBUTIONS


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