Proceedings of the 2nd SINO-SUTCH Workshop on the Environmental Behaviour and Ecotoxicology of Rare Earth Elements and Heavy Metals

October 1997, Delft

Editors:

R. Guicherit Ph.D.
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ISBN: 90-6743-46-5

R90-273

This Workshop was organised and sponsored by:

- **The Netherlands Organisation for Applied Scientific Research (TNO)**
- **The National Natural Science Foundation of China (NFSC)**
- **The Ministry of Transport, Public Works and Water Management, National Institute for Coastal and Marine Management (RIKZ)**
- **The Ministry of Transport, Public Works and Water Management, National Institute of Water Management and Waste Water Treatment(RIZA)**
- **Agricultural Research Department(DLO), Research Institute for Agrobiology and Soil Fertility**
- **The Netherlands Metrological Institute (NMI)**
- **Delft University of Technology (TUD)**

These Proceedings were published by:
The Netherlands Organisation for Applied Scientific Research, Institute of Environmental Sciences, Energy Research and Process Innovation (TNO-MEP), The Netherlands.

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May, 1998

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Preface 1

Cunhao Zhang
President National Natural Science Foundation of China

The publication of this Proceedings reflects the success of the Second Sino-Dutch Workshop on the Environmental Behaviour and Ecotoxicoloy of Rare Earth Elements and Heavy Metals. In addition, it witnesses the huge progress of the co-operation between National Natural Science Foundation of China and the Netherlands Organisation for Applied Sciences. Both countries are paying increasing concern on the sustainable development of our economy and society. To promote the production in order to properly feed and clothe the increasing population is China's topmost task. On the other hand, one of our crucial missions is to ensure that the measures we are taking cause no risk to human being and no stress to eco-environment. The development of agriculture and industry depends on the utilisation of fertilisers, agrochemicals, catalysts, synthetic materials, etc. However, their production mobilises numerous nonessential elements, converting them into man-made substances, releasing them into the environment and the human body. Thus, the evaluation of the risks of all relevant substances is a top pre-requisite. This Workshop has made contributions to the understanding of the stress of the rare earth elements and heavy metals to eco-environment. We look forward to future workshops and other collaborating projects.

Preface 2

J.A. Dekker
President, TNO Board of Management

It was for the Netherlands Organisation for Applied Scientific Research TNO a great honour to host and co-sponsor the Second Sino-Dutch Workshop on the Environmental Behaviour and Ecotoxicology of Rare Earth Elements and Heavy Metals. The first workshop which was held in Beijing in January 1996 had enhanced co-operation in the field of research and development between China and The Netherlands and during this workshop Chinese and Dutch scientists had shared new views and research achievements obtained since then. I am confident that the proceedings of the workshop will contribute to a better understanding of a number of relevent issues concerning the use, environmental fate and effects of Rare Earth Elements and Heavy Metals. Research in the field of environmental pollution, in the coming decades, will continue to be relevant for all countries in the world and I am confident that both our organisations will continue and strengthen their co-operation.

Cellular uptake and response to lanthanides

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Abstract

Our recent works showed that the Lns species may enter the cells by the following mechanisms other than phagocytosis:

(1)For Lns' cations, through the pores formed by Ln^{3+} binding.

(2)For the anionic species, the Lns enter the cells through the anion channel.

Whether the Lns cation can penetrate through the calcium channel is still an open question, but Lns ions probably can activate the calcium channel, since the calcium influx is increased by the Lns ions and the calcium channel blocker can inhibit this effect.

Lns binding induces several changes in membrane structure and functions, including: the induced pore formation and subsequent higher permeability, the effects on the lipid peroxidation and the promotion of phospholipid hydrolysis, resulting in the triggering of the cell signalling system.

Lns species were found to influence the oxygen affinity of hemoglobin by promoting the hydrolysis of DPG and increase the affinity and causing hemoglobin molecules to acquire a more open conformation, which results in lower affinity. Furthermore, the animal experiments showed that Lns feeding led to the oxidation of.the heme Fe(II) to heme Fe(III) and thus decreases the oxygen binding capacity.

Introduction

Recently, the duality on the biological effects of nonessential elements have earned increasing interests of biological and chemical scientists. Since the sixties, the Chinese workers found the growth-promotion effect of lanthanides (Lns) to plants and

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animals and on this basis, long-term field experiments have been undertaken to use Lns as additives to fertilizers. The results of around 10% increased harvest are encouraging. If the Lns could be used rationally , it is really a great expectation since it will contribute much to relieve the food shortage and prevent desertification, but increasing scientists are worrying about their negative effects to human health, soil fertility and the eco-environment. Their deep concerns are not groundless. First of all, the analogy of Lns with calcium ion makes Lns cations to compete with calcium ion and inhibit calcium related functions, such as the cell signaling system, the cytoskeleton assembly system, the gene expression ,etc. Previously, the studies on Lns' biological effects have long been focused on their toxicity, but recently, people became more careful to conclude on whether they are beneficial or harmful. In nearly three decades, there has been a lot of studies published, but the conclusions are diverse, even controversial. Several questionable points should still be clarified. Before we make a decision on whether the agricultural applications of Lns are safe, we should answer these questions to reveal and interpret the nature of the plus and minus effects. We expect that the conclusions might lead us to utilize the positive effects and avoid the negative ones. This is probably realizable, because to be positive or negative depends on the concentration, the acting time and the species.

The critical questions we should answer include the followings:

(1) Are the RE species transportable across the biomembrane? If they are, how do they enter the cells? We can speculate that they enter the cell as the disguised essential ions, such as calcium, through the channel and/or by the carrier of the latter. If so, its entrance might influence the transport and the partition of essential ions across the cell membrane. If they cannot penetrate into the intracellular medium, then the problem turns to be how do they work outside the cells to promote the growth or cause damages to the intracellular targets? Although we can suppose that they trigger the intracellular signaling system and affect the gene expression and the protein synthesis, we cannot help to hesitate before several fatal problems, especially those of the cell process, i.e. differentiation, proliferation, division and death (both necrosis and apoptosis).

(2) Why are their biological effects bifacial? Basically, the core problem is how to interpret the nature of their *hormesis* behaviours. Are these superficially converse effects the two extremes of a single process or the results of two or even more different processes? A lot of suspicious points remain ambiguous. Why Lns promote the growth nonspecifically to biological species, from plants to animals, from bacteria to highly evoluted animals or plants? The growth promotion is so universal that we have enough reasons to suspect that is not normal. Perhaps it is just the stimulation effect of toxic substances. Moreover, since the effect is so universal in animals, we cannot help to ask for their effects on human being.

(3) Several groups emphasize that the level of Lns used in the fertilizers and those exposed to the human bodies are so low that their toxicity is negligible. This is of

course a common working principle in drug and pesticide developments. However, the growth-promotion is quite different with the pharmacological activity and the short term administration of drug is also different with the long term exposure. We should reveal the effects of low-dosage-long-exposure and their effects to the future generations. Are the cells and the organisms adapting the RE-rich environment by synthesizing several special biomolecules and thus affecting the future generations? Do the Lns affect the structure and function of nucleic acids, the gene expression and the protein biosynthesis?

In the present report, our recent results on the cellular uptake and response will be outlined.

Uptake and cross-membrane transport and their mechanism

For the cellular uptake of Lns, a lot of experimental results have been accumulated, but the conclusions drawn from these results are diverse. El-Fakahany et al.[1] and Evans[2] reported that Lns are not transportable,, but there is plenty of evidence indicating the cellular uptake of Lns. Among these conclusions, the uptake via internalization is evidently tenable, but this is only for the cases, in which the Lns are in form of insoluble particles[3,4,5] or macromolecule-bound species[6] and the cells have the phagocytosis or endocytosis function. Since Lns are highly reactive in hydrolytic polymerization and binding to proteins, the formation of insoluble particles is highly possible, but the cells with endocytostic function are only a fraction of the cell family. Moreover, the internalization cannot be considered as the ion transportation strictly. Thus, we are interested in the other mechanisms attributed to their across-membrane penetration. It will be more important , since most cells are not able to take up the Lns bound particles or macromolecules. Furthermore, it is important when the concentration of Lns is too low to precipitate, such as in the body fluid. The body fluid contacting the Lns containing solid phase will also contain low concentrations of Lns species. Therefore, we have been focusing our studies on the uptake by erythrocytes which lack the internalizing function. The early work of Szasz et al., has already indicated the uptake of La by erythrocytes[7], but their results are not conclusive because they determined the cell-bound La content which includes the membrane bound La , thus, it cannot reflect the amount of La in the cytosol.

In general, controversial results and conclusions can be traced up to the inconsistency in the methodology and the philosophy. Up to now, we cannot make a universally accepted , sensitive , accurate and clear-cut experimental method to determine the amount of the Lns which has really entered the cells, or, in other words, in the intracellular space, including those in the cytosol and those in the vesicles and nucleus. By the conception, we cannot define and discriminate clearly *uptake, across-membrane transport and penetration*. For this sake, we tried to determine the amount of Lns in the cytosol, and those bound with the membrane, as well as with the membrane lipids and membrane proteins separately.

Mechanism of Lanthanides Uptake by Erythrocytes

According to Yang et al.[8], Lns were found in the cytosol and determined by ICP-MS method after incubation of the human erythrocytes with Ln chlorides. By studying the effect of the presence of the anion channel blocker DIDS on the Lns citrate complex, Sun et al. [9] postulated that the anionic species complex entered the cells via the anion channel. Based on the kinetic data, the rate constants of transport were calculated (Fig 1). However, after comparing the Lns amounts in the cells exposed to Lns species with various charges (Fig.2), Yang et al. concluded that the cationic charged species enter the cells much more easily than the anionic species[8]. Their results are not in

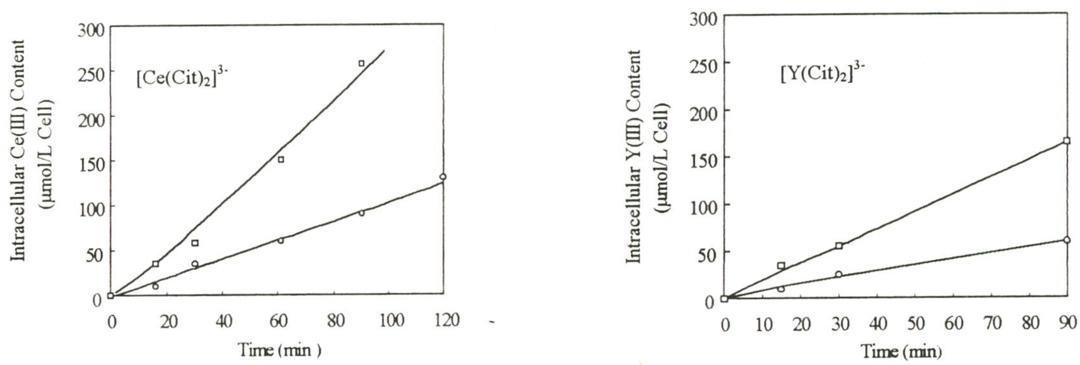


Fig.1 The intracellular content of Ln as a function of the incubation time of human erythrocytes with $[Ln(Cit)_2]^{3-}$ solutions (Ln: Ce, Y)at 25^0C (pH 7.4).
□: in absence of DIDS; o: in presence of 8.6 μmol/L DIDS.

controversy with that of Sun et al., since Yang did not exclude the across membrane transport of anionic species. Recently, the proton induced X-ray emission spectroscopy (PIXE) was used to determine the Lns in the cytosol, membrane, membrane lipids, membrane proteins in erythrocytes and hepatocytes, and those in the nuclei (hepatocytes). The sensitivity of PIXE is lower than ICP-MS but it is sensitive enough and requires very small amount of samples. More importantly, it can be used to determine the relevant elements in a single sample. This is very important, because, as it is mentioned above, we suspect that the entry of Lns ions, either penetrating through the phospholipid bilayer, through the channel, or by the carrier of any essential ion, might influence the intracellular concentration of the relevant essential elements. The most susceptible cation is calcium , while the anion would be chloride.

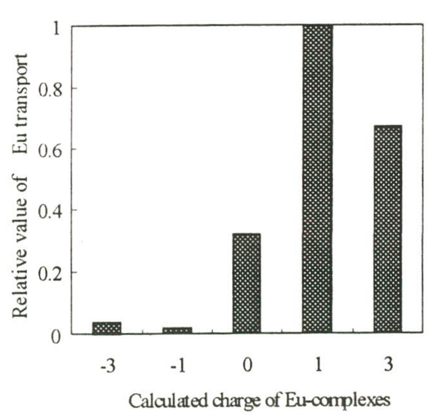


Fig.2 The dependence of Eu^{3+} transport on the electric charge of the complexes

The Gd content of the cytosol after incubating human erythrocytes with 10^{-6}~10^{-5}mol/L increased and depended on the incubation time and the concentration of Gd. The profiles of the cytosolic Gd vs Gd concentration became biphasic in higher concentration indicating two possible mechanisms working in the transportation(Fig.3). Furthermore, we determined the Ce uptake by feeding the rats with Ce^{3+} chloride(20, 80 and 400mg/kg body weight per day). The uptake was also evaluated by determining the Ce content in the erythrocytes and components. The Ce cations were able to enter the cells, and the concentration dependency was also biphasic.(Fig.4)

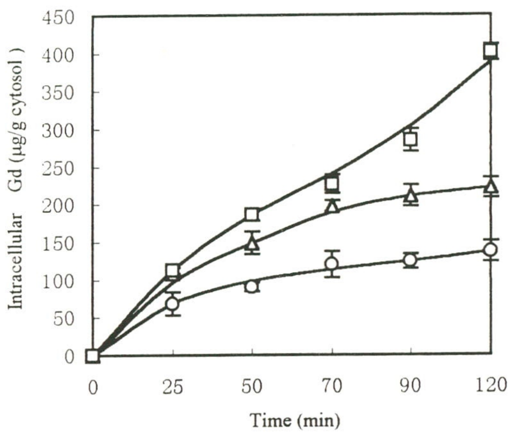
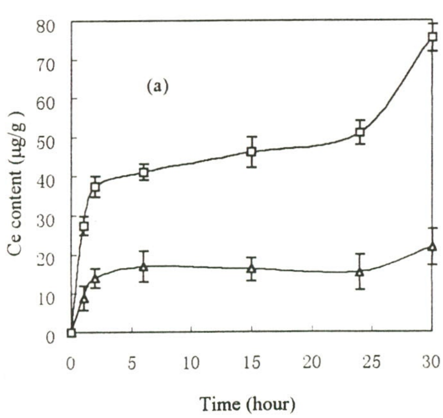


Fig.3 The intracellular content of Gd as the function of the incubation time of human erythrocytes with Gd^{3+} solutions at 25°C (pH 7.4). Gd^{3+} concentrations: (o: 1×10^{-6}mol/L; Δ: 5×10^{-6}mol/L; □:1×10^{-5}mol/L)

In consistence with previous studies [8,9], the *in vitro* incubation experiments showed that only a fraction of the anionic Gd species $[Gd(cit)_2]^{3-}$ do penetrate via anion channels. The anion channel blocker, DIDS was shown to depress the cytosol Gd content significantly, but not totally (Fig.5). There are a significant amount of Lns bound to the membrane components, even more than those entered the cells. A significant amount Lns was bound to the membrane, but was are released from the membrane later. This dynamic state was shown by the PIXE results.Thus we may conclude that the transport through anion channel is one of the ways other than phagocytosis. However, are there any other ways for the transportation of cationic Lns species?

Yang et al. [8] noted a parallel relation between the charge dependencies of the erythrocyte uptake of the Lns and that Lns species induced hemolysis (Fig.6).They



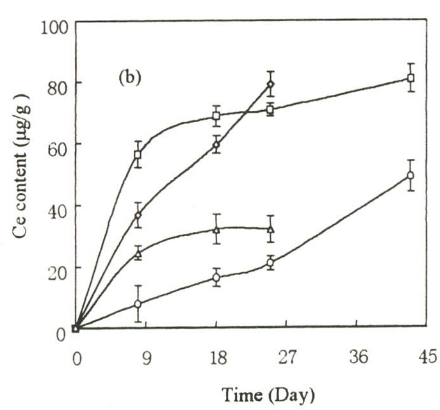


Fig.4 The binding content of Ce in erythrocyte membrane and cytosol of rat as the function of the feeding time.

(a): 400 mg/Kg-day rat, P.O. □: erythrocyte membrane ; Δ : cytosol

(b) 80 mg/Kg-day rat, P.O. ◊: erythrocyte membrane ; Δ : cytosol

20 mg/Kg-day rat, P.O. □: erythrocyte membrane ; O : cytosol

found that the more positive were the more transportable into the cells. They noted a parallel relation between the Lns uptake and the Lns induced hemolysis. The hemolysis induced by the cationic species was also stronger than the anions as the uptake(Fig.6).

That suggests a common mechanism underlying these two effects. As an important clue to interpret this relation, it is noteworthy that the temporal behviour of Lns cations induced hemolysis proceeds through a three stage process. In the first stage the Ln binding does not affect the cell morphology, and no hemolysis occurs, while the final stage is actually the lysis of the erythrocytes and a large amount of broken cells is observed under microscope. The hemolysis in the final stage is irreversible. The features of the second stage are rather characteristic. Generally , the second stage has a

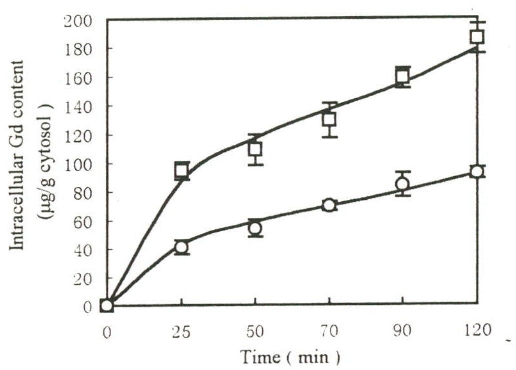


Fig.5 Intracellular Gd content as the function of the time .Incubation of erythrocyte with $[Gd(Cit)_2]^{3-}$ (5×10^{-6} mol/L) at 25°C; [DIDS]=7.0 μmol/L, □: $[Gd(Cit)_2]^{3-}$; o: $[Gd(Cit)_2]^{3-}$ + DIDS

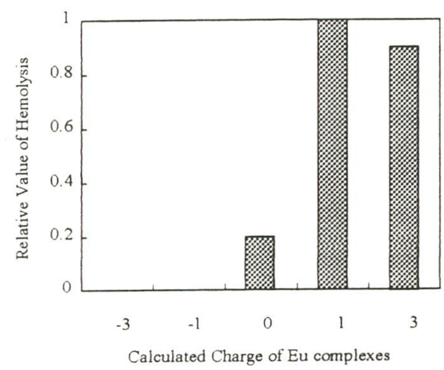


Fig.6 The dependence of Eu^{3+} -induced hemolysis on the electric charge of the complexes.

feature of the repairable hemolysis. The events occuring in this stage were observed as follows:

(1) The cells still keep their integrity and morphology, but become swollen to an extent, indicating the perturbed water and ion balances

(2) The trypan blue molecules in the extracellular media can migrate into the cells

(3) The hemoglobin molecules leak out. That is why we conclude at the hemolysis

(4) However, the hemolysis has two features:

※ The hemolysis is sustainable, i.e. if the hemolyzing cells are washed with the Lns-free culture to remove the Lns adhering to the cell surface and removed to a Lns-free medium , the hemolysis still proceeds

※ If the hemolysing cells are washed thoroughly with EDTA solution, the hemolysis and the entry of trypan blue will be both stopped .

These behaviours displayed only with cationic species and in parallel with the leaking of the REE ions. Based on these results, Yang et al.[8] speculated that the hemolysis in the second stage is due to hemoglobin leaking through the "pores" formed by Lns binding to the membrane. It is reasonable on the basis of the analogy with Ca^{2+} ion. The "calcium shock" was generally used in gene recombination to induce pore formation in the bacterial plasma membranes, and thus admit the plasmid enter the cells[10] Actually, Seelig et al. have observed the domain formation in the phospholipid bilayer induced by Ca^{2+} and La^{3+} [11]. Our recent AFM studies support this postulation. The pores with a size of ca 20nm were observed after incubation the erythrocytes with Gd^{3+}($5x10^{-6}$ mol/L). The pores can be resealed by EDTA treatment. With the anionic species $[Gd(Cit)_2]^{3-}$, no pore formation could be observed, but the surface contour of the cells were changed to a small extent.

The pore formation might be described as a process initiated by Lns cations binding to phospholipids and/or membrane proteins. Although it was reported that without proteins incorporated in the lipid molecules , the Lns were able to bore the membrane, we cannot exclude the contribution of protein binding. The ATM studies strongly supported this point. Anyhow, either lipids only or lipids and proteins both are thus crosslinked or aggregated and accompanied by the conformation changes. Then, their self-assembly mode is altered and phase transitions can occur.

We have conducted a series of experiments to monitor the steps in the process.A series of spin labels, N-doxylstearic acid, nDS(n=5,7,12,16) incoporated in the phospholipid bilayer with their ^{14}N nitroxide moiety located at different depths were used to follow the entrance of RE ions into the membrane. The binding of RE to nDS diminished the ESR signals of these spin labels.The concentration- and time-dependence of the diminished ESR signals of various nDS at various depths displayed the entry of Gd^{3+} into the phospholipid bilayer[12].

Based on the changes of Gd content in the ghosts and lipids and the membrane proteins with reaction time, we can imagine that Lns cations bind at first stage very rapidly to both lipids and proteins to a large extent, rather nonspecifically. Then they are released from the membrane molecules and, perhaps penetrate inward to the cytosol slowly.

A stopped flow study of the whole process was used to follow the events triggered by Gd^{3+} binding to erythrocyte membrane. The intrinsic fluorescence of the membrane proteins was monitored to reveal the conformation changes. The Gd fluorescence was scanned in order to ascertain the binding of Gd with proteins and also the lipids. The results were compared to those obtained with the phospholipids (deproteinized membrane). The results showed that there are two stages. Firstly the Gd^{3+} binds to negative charged sites on the phospholipids (within 20ms, k_{obs}=400~800s^{-1}). It is followed by a slow quenching of fluorescence(20~50ms, k_{obs}=70~200s^{-1}) indicating the binding to membrane proteins and the subsequent slower conformation changes. The protein molecules become more open to the surrounding and several hiden sites become accessible to the Lns ions. Then Gd binds to new sites, which merged as the result of conformation changes. Then the second round conformation changes proceeds. This is similar to the Tb-spectrin system we reported previously[13].. By comparing with that of phospholipds , we can say that the reaction is initiated by the Gd binding to lipids and proteins.The fluorescence increased in the initial stage due to binding to phospholipids mainly and the subsequent quenching is related to the conformation changes of proteins. The slow reaction observed in the final stage is considered to be the phase transition of lipids.. The ^{31}P NMR indicates the phase transition , since the signal of liquid crystal phase changes to hexagonal (Fig 7). Based on the FTIR spectra, the α helix content of the proteins was found to be decreasing from 32% to 23% and the β sheet content increasing from 36% to 45% after La^{3+} binding. This result supports the stacking of proteins(increased β sheet content), which indicates the domain and pore formation.

So far, the Lns ions were known to be able to inhibit the calcium influx by blocking the calcium channel. However, from the PIXE spectra of cytosol as the results of both the *in vitro* and *in vivo* experiments, we found that along with the increased Gd content in the cytosol, the intracellular content of both calcium and chloride increased. It can be explained as the result of the increased permeability of the cell membrane. However, we cannot say definitely that the increased influx is due to the penetration through the pores or the activated calcium and anion channel . An informative result is obtained from the incubation of the erythrocytes with the anion $[Gd(Cit)_2]^{3-}$ and $[Gd(Lac)_2]$. As we discussed above, the anions enter the cells through the anion channel, and thus promote the chloride influx ,but do not affect the calcium cations. Thus we may consider that more chloride ions entered the cells after the anion channel was activated by the anionic Lns species.

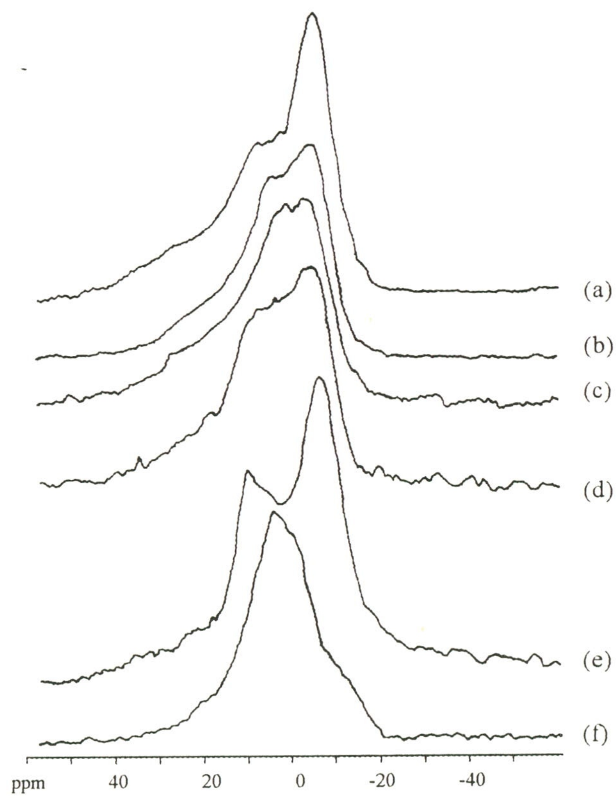


Fig.7 ^{31}P NMR spectra of human erythrocyte membrane lipids .

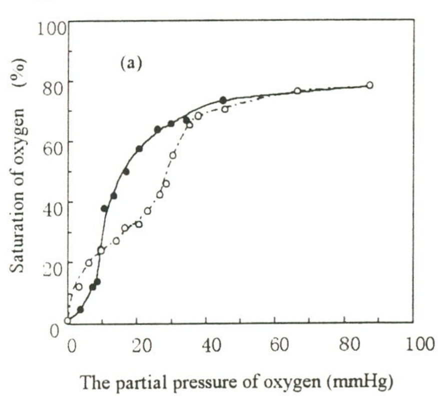
(a): the control; (b): 5.0×10^{-6}mol/L La^{3+};
(c): 1.0×10^{-5}mol/L La^{3+}; (d): 4..5×10^{-5}mol/L La^{3+};
(e): 7.5×10^{-4}mol/L La^{3+}; (f): 1.1×10^{-4}mol/L La^{3+} .

To postulate that the Lns ion can enter the cells through the calcium channel is reasonable, since trivalent Lns cations are very similar to calcium ions indeed. Nevertheless, we cannot make conclusion on this aspect, since no strong supporting evidence exists. Zhang et al.[14] showed that, in the course of incubation of rat skin fibroblasts with Ce^{3+} in concentration of 10^{-5} mol/L ,the first step is characterized by an increasing intracellular Ca content , which can be inhibited by the addition of verapamil, a calcium channel blocker, in the culture media. After 30min. as in the second step, the Ca content decreases .They suggested that Lns cations are able to activate both the calcium channel and the calcium pump.The effects are depending on the lanthanides and the cells. For the rat liver cells, both Gd and Ce promote the Ca influx, but for the fibroblasts , only Gd can promote the Ca influx[14].

The influence on the oxygen affinity of hemoglobin

Although ischemia has been observed in the Lns exposed animals, their effect on the oxygen carrying capacity of hemoglobin has not been studied and reported. By determining the degree of oxygen saturation, Zuo et al.[15] have shown that a low concentration of Lns cations increases the oxygen affinity of hemoglobin after incubation human erythrocytes with Lns, but it turns to be inhibition in higher concentration. The effects of different REE are somewhat different in extent. Among them, Pr^{3+} is the strongest. The oxygen saturation cure is normally in sigmoid form. After incubation with Pr^{3+}, the oxygen affinity in low partial pressures of oxygen becomes higher, but the oxygen binding capacity is significantly lower in high oxygen partial pressures.

Similar results were obtained after feeding the rats with the feedstuff containing $CeCl_3$ at 20mg/kg of body weight. After 40 and 80 days, the hemoglobin was then isolated and the oxygen saturation curves were determined and compared with the control. The curve of the control was in sigmoid form with the O_2 partial pressure at half saturation,p50 = 23mmHg. After feeding with $CeCl_3$ for 40days , the curve changes to a double sigmoid shape and the oxygen affinity in low oxygen pressure increased with the p50 to 32mmHg at 40days. After 80 days , the curve changes to the original sigmoid form again, but the binding capacity was higher than that in the control (Fig. 8).The dependency on the concentration and the incubation time indicated that several mechanisms are possible and might be working simultaneously. First of all, we found that the REE ions promote the hydrolysis of 2,3-DPG, which is the binding bridge between the subunits in the tetrameric hemoglobin. The ^{31}P NMR



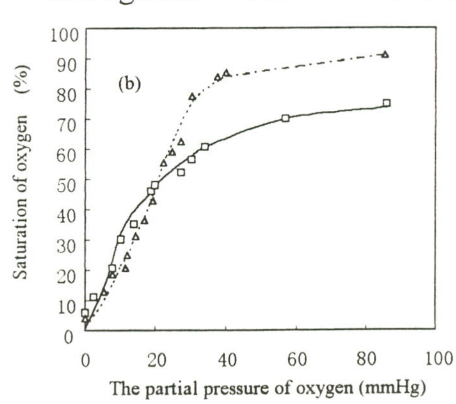


Fig.8 The effect of Ce on the oxygen dissociation curve of hemoglobin.with $CeCl_3$ feeding rats.
$CeCl_3$: 20 mg/Kg-day rat, P.O.
(a): •: feeding $CeCl_3$ 40day; o: Control ; (b):Δ: Control ; □: feeding $CeCl_3$ 80day ;

showed clearly that the 2,3-DPG hydrolyzes and gives inorganic phosphate and 3-phosphoglyceric acid(3PG). It depends on the doses and the exposure time. Since the binding of 2,3-DPG favours the deoxy form of Hb, the loss of one negatively charged phosphate group will increase the oxygen affinity. We have compared the catalytic effectiveness of various Lns cations. Among them, the strongest effect was found in Ce^{3+}. However, further studies showed that the predominant acting species is Ce^{4+}, which is formed by the autooxidation of Ce(III). A correlation between the catalytic effect and the Ce(IV) content was established and the addition of reductant was found to depress the Ce(IV) formation and the hydrolysis both significantly.

The PIXE determinations of the intracellular phosphorus content in *vivo* (Fig.9) revealed that the Lns feeding decreases the intracellular phosphorus content. Among the cytosolic proteins in erythrocyte, hemoglobin is mostly abundant and the leaking out of the inorganic phosphate ions formed as the hydrolytic products will cause a determinable decrease. However, only a small fraction of phosphate dissociates from the hemoglobin tetramers, since the decrease of the intracellular phosphorus is not very significant.

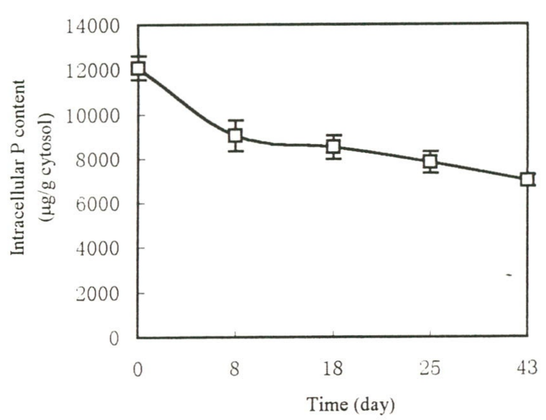


Fig.9 The influence of $CeCl_3$ on the P content in erythrocyte with $CeCl_3$ feeding rats. $CeCl_3$: 20 mg/Kg-day rat, P.O.

Evidently, the promotion of DPG hydrolysis might be the main mechanism underlying the increased oxygen affinity, which occurs in low Lns-hemoglobin ratio. The reduction in oxygen affinity in higher ratio might be interpreted by the changes in second and higher order structure of the Hb tetramer. This is the result caused by Lns binding. By means of ultrafiltration method, the binding thermodynamics was studied. The data can be explained by assuming three categories of binding sites: sites with positive cooperative effects(n=8~12), independent strong binding sites (n=14±2)and the independent weak binding sites(n=44±3). We have studied the second order structure of the hemoglobin isolated from the erythrocytes from the *in vitro* and *in vivo* experiments by means of the FTIR and CD technique. In both cases, the Lns binding caused an α helix content decrease of 10~30%, and a T turn content increase of about 10%.

By means of the stopped flow fluorescence study of the process triggered by Pr^{3+} binding to human hemoglobin, the binding and the subsequent conformation changes were followed. The results showed that at first step, the Lns ions bind to the high affinitive ,perhaps the positive cooperative sites. The cooperativeness indicated that conformation changes to more open to the environment, as shown by intrinsic fluorescence. In the second step, more Lns ions bind to strong, but mostly independent sites and the conformation changes further to make several buried sites exposed to the environment and then further binding and further changes in conformation. All the spectroscopic data support that conformation becomes more loose and more open to the aqueous media at higher Lns-hemoglobin ratio. Evidently, such an influence exists even in from the low ratio.but the influence of DPG hydrolysis predominates in low ratio.

Although the decreased oxygen affinity could be explained as such in the cases of higher Lns-hemoglobin ratio, there must be an additional mechanism, which works in animal body. We found that the oxidation state of a fraction of heme-iron changes from +2 to +3 in the cerium chloride feeding rats. The Fe(III) signals were clearly displayed in both the Mossbauer and ESR spectra of the Hb isolated. This oxidative effect cannot be detected in the erythrocytes incubated with Lns. Evidently, the oxidation of ferrous ion in heme to ferric will depress the oxygenation , as shown by the oxygen saturation curve.

Summarizing remarks

Based on the results of the present works, the interaction between Lns ions and erythrocytes can be summarized in Fig.10. Evidently, this schematic presentation

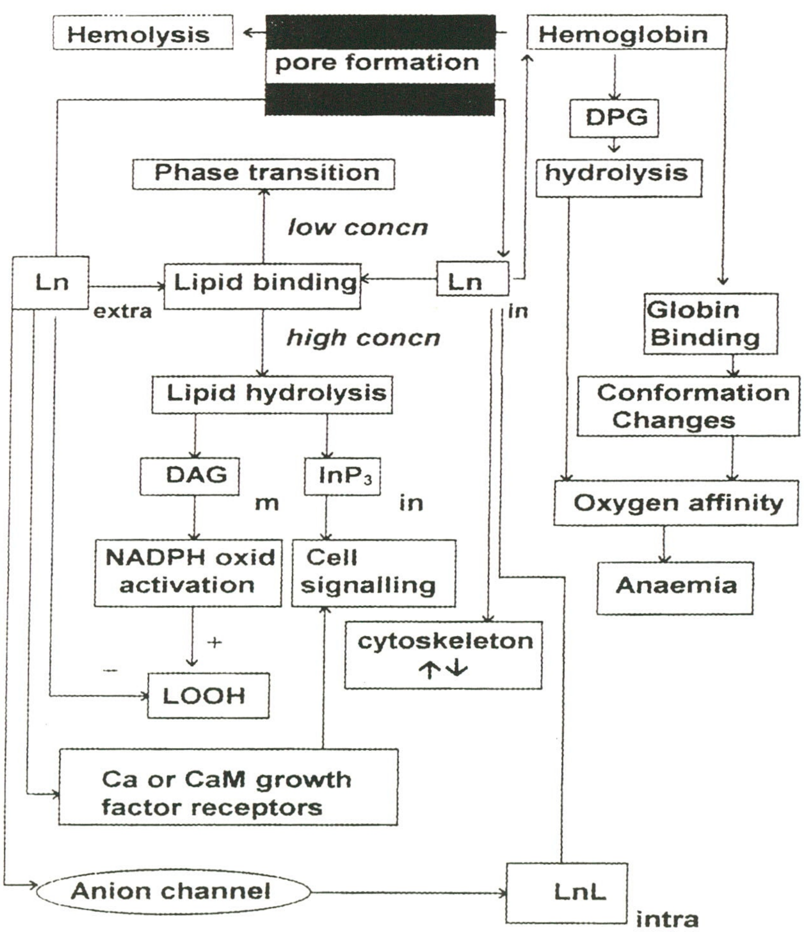


Fig.10

is not a complete one. Several points in the figure are not discussed in the present paper. The influence on lipid peroxidation , both plus and minus is important on the cell life and death (apoptosis). The Lns inducing phospholipid hydrolysis may play an important role in triggering the intracellular signaling system and thus affect the cell proliferation.

We can conclude at the following points:

1.The Lns species may enter the cells by the following mechanisms:

(1) Internalization: This is important for the insoluble particles or the biopolymer bound species. It occurs when the Lns species encounter the cells with endocytosis or phagocytosis function only, such as lung macrophagocytes, the Kupfer cells, etc.

(2)Through the pores formed by Lns binding: This mechanism is important for the soluble cationic species, which can strongly bind to the membrane lipids and the proteins.It is not a specific effect of Lns only. Even calcium ions can "bore" the pores, but a much higher concentration is necessary.

Evidently, these two mechanisms are not the normal across-membrane transportation of metal ions.

(3)For the anionic species , the Lns enter the cells through the anion channel.

(4)Whether the Lns cations can penetrate through the calcium channel is still an open question, but Lns ions can probably activate the calcium channel, since the calcium influx is increased by the Lns ions and the calcium channel blocker can inhibit this effect.

2. Lns binding induces changes in membrane structure and functions. These changes are known to be affected by the following reactions:

(1)The Lns ion binding induced pore formation leading to higher permeability

(2)The Lns ions inhibit or promote the lipid peroxidation depending on the concentration and the species. This is a fatal oxidative damage to the cells. The reactive oxygen species thus generated are fatal to the cells.

(3)The Lns ions promotes the hydrolysis of phospholipids and trigger the cell signaling system and perturb the cell process.

3.Lns influence the oxygen affinity of hemoglobin by:

(1)promoting the hydrolysis of 2,3-DPG, and increase the O_2 affinity

(2)inducing the hemoglobin molecules to acquire a more open conformation, which results in lower affinity.

(3)oxidizing the heme Fe(II) to heme Fe(III) and thus decreasing the oxygen binding capacity. This was found in animal experiments only.

Up to now, how to determine rare earths in very low concentrations and very small amount of samples is still a problem, which affects the discussion of the biological effects and their dose-dependence. The ICP-MS is generally used since it is highly sensitive, but it requires a larger amount of sample. For PIXE determinations, a very small sample is enough, but its sensitivity is lower than ICP-MS method. Perhaps the neuron-activation analysis can be used to solve the two requirements, but we still need a method which enable us to scan the rare earth in a cell, for instance in the cell flowmetric studies.

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The Distribution of Different Speciation of Rare Earth Elements in Plant and Plant Cells*

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Abstract

Bioaccumulation of light, medium and heavy rare earth elements and their NTA or EDTA complexes by wheat and rice seedling was investigated simultaneously. The thermodynamic and kinetic results all showed that the bioaccumulation values in the root and above ground part (stem & leaves) of the wheat and rice seedling were positively correlated with the concentration of the rare earth elements in the culture solution. The order of the different speciation bioaccumulation values in root was: ion speciation > NTA-complexes > EDTA-complexes indicating that ion speciation of rare earth elements were the most available speciation for the root of plant . For the above ground part the order was : ion speciation < NTA-complexes < EDTA-complexes. Thus, the EDTA -complexes were the most available speciation for the above ground part of plant. In the different parts of wheat and rice , the order of bioccumulation values was: root > stem & leaves .

The distribution of La, Gd and Y in cells of wheat seedling's root and above ground parts was also investigated by sequential centrifuging. The results showed that 70-80% of the Rare Earth Elements of different speciation in root was deposited in the cell wall. The distribution order of the different REE speciation in the cells of root and above ground parts mostly was cell wall > mitochondria > nucleus ≈ nucleoprotein > soluble fraction.

Keywords: Rare Earth Elements (REEs), Speciation, Distribution, Plant and Plant cell

INTRODUCTION

In China, increasing amount of Rare Earth Elements (REE) are applied to cropland as micro-element fertilizers because of their ability to enhance yields and improve qualities of crops [1]. A problem arises since the usage of REE can result into their accumulation in crops and their transfer into the food chain. Unfortunately, the details of accumulation and the factors affecting these processes are still unclear.

It is well known that speciation has a large effect on the bioavailability of REE in soil, and there also exist several factors that can change the speciation of REE in soils especially organic ligands, but whether RE-chelated complexes will be taken up by plant or remain in soil is still unknown. One hypothesis is that chelation formed in the soil reduces REE uptake dramatically.

* This Project is supported by National Natural Science Foundation

The alternative possibility is that the complexed REE in the soil can increase its solubility and promote REE convection and diffusion and hence potential uptake.

In this study, we used a chemically-defined nutrient solution and the organic ligands EDTA (ethylenediamine tetraacetic acid) and NTA (nitrilotriacetic acid) to control the speciation of REE in a test solution in order to study the effects of speciation on the bioavailability of REE. The test crops were wheat *tritiicum* and rice , the important crops in china as well as in other countries of the world. The distribution of REE in wheat cell and the effects of speciation on the distribution was also investigated.

MATERIALS AND METHODS

Chemicals

Lanthanum, Gadolinium and Yttrium were selected as representatives of light, medium and heavy REEs respectively. REE stock solutions with a concentration of 1.00 mg • mL^{-1} were prepared separately from $La(NO_3).nH_2O$, Gd_2O_3 and $Y(NO_3)_3 \cdot 4H_2O$. The REE , EDTA and reagents such as HCl, HNO_3 and $HClO_4$ used were all of analytical grade.

Calculation of REE Speciation

The speciation of REE in the nutrient solution were calculated by the computer program MINTEQA[2] . To ensure that the calculation results of each dissolved REE speciation in the solution were as accurate as possible, we carefully selected the thermodynamic data base from the appropriate documents[3,4].

Test Plants

Wheat and rice were chosen as the test plants and the study was conducted in a green house with a photo period of 14h/day. Temperature during the photo period was between 22-25 ℃ and 17-18 ℃ during the night . The seeds were sterilized with 2% NaClO for 30 minutes and washed thoroughly with sterilized distilled water. They were placed in controlled - environmental chamber at 25±1℃ (wheat) or 27±1℃ (rice) for germination. After 3 days, germinated seeds were transplanted to the culture disk which contained 2L of the nutrient solution and stored there for 14 d before treatments were initiated.

Bioaccumulation Experiments

Kinetic Experiment

Ten seedlings (8-18 cm high) were placed into each of the 100mL-beakers used for the experiment. Beakers were filled to 80 mL with the 1 mg • L^{-1} REE nutrient solution with (except for the control) addition of definite concentration of organic ligands , which can ensure the concentration of RE-Organic complex to occupy more than 98% of total REE speciation concentration in the nutrient solution as calculated by the MINTEQA program. The pH of the test solution was kept at constant of 6.0 ± 0.1 and the test solutions were replaced every two days .

Samples were collected at different time intervals and were divided into the root and the aboveground parts (stem and leaves) , then transferred to 50 ml beakers and digested by HNO_3 and $HClO_4$ separately. The final solution were measured by the ICP-AES.

Thermodynamic Experiment

Different concentrations of REE (0.5- 4.0 mg • L^{-1}) were added in the test solution with and without organic ligands; seedlings were selected and grew under the same conditions as the kinetic experiment. The experiment was carried out to obtain the equilibrium with a growing period of 32 days. Samples were collected and determined with the method described in the kinetic experiment.

The separation methods of REE in plant cell

40 wheat seedlings were placed in culture solutions which contained 4 mg • L^{-1} REEs or 4 mg • L^{-1} RE-EDTA complexes respectively for a 32-day bioaccumulation experiment. Samples were collected and the roots of wheat were washed by deioned water for several times, root and above ground parts were separated and homogenized in 0.1 mol • L^{-1} Tris-HCl solution at 4 ℃respectively. After homogenizing, solutions were separated by centrifugation according to the methods shown in Fig .1 .

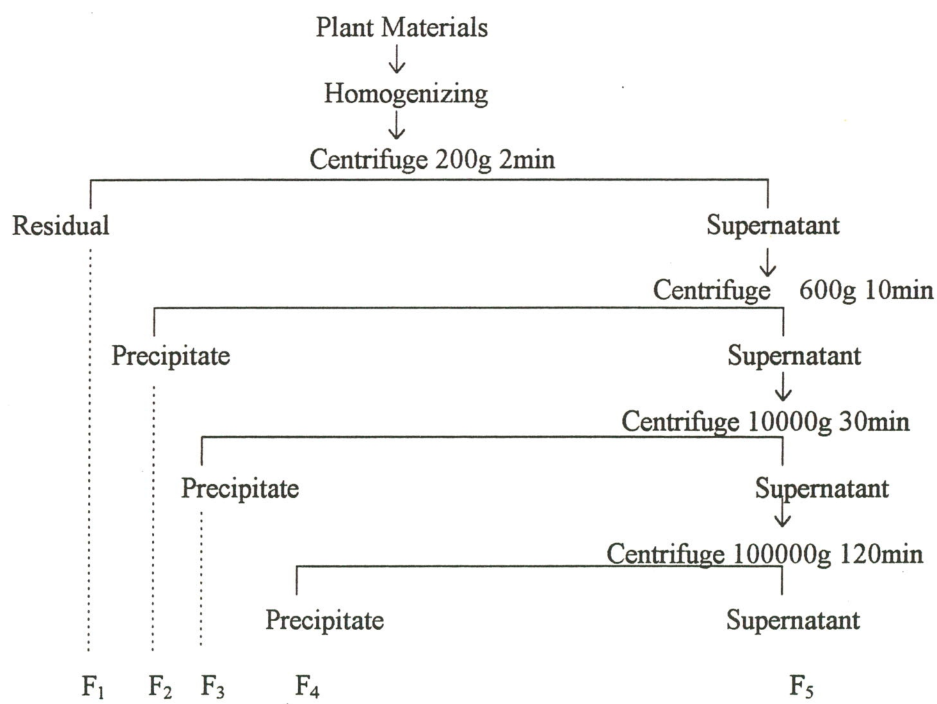


Figure 1 . Schematic diagram of the separation methods by centrifuging

RESULTS

Kinetic Experiment

The wheat seedlings were cultured in the nutrient solution with different REE speciation. The bioaccumulation of REE in wheat seedlings is shown in Fig. 2.

Bioaccumulation Values mg/kg

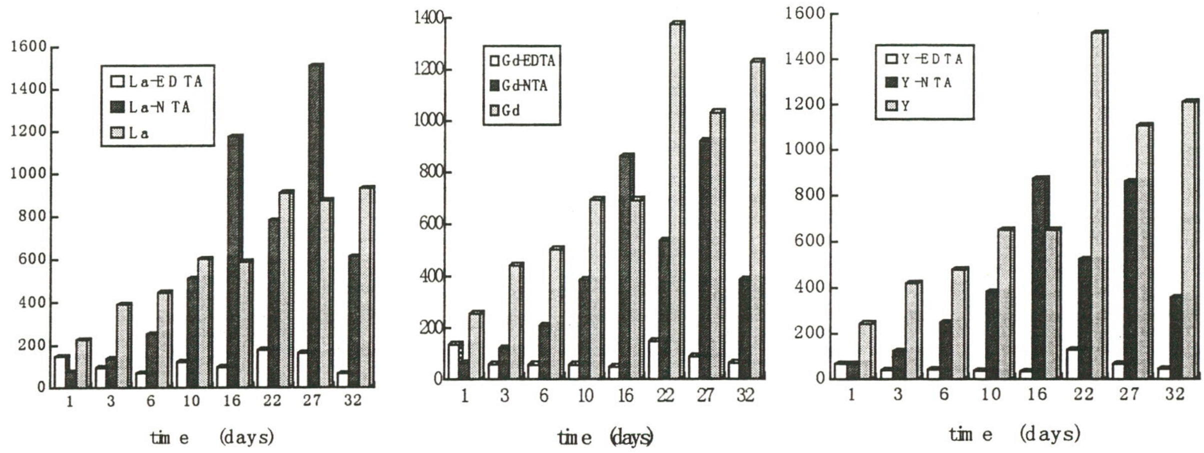


Figure 2. The bioaccumulation of different REE speciation in wheat root

Bioaccumulation Values mg/kg

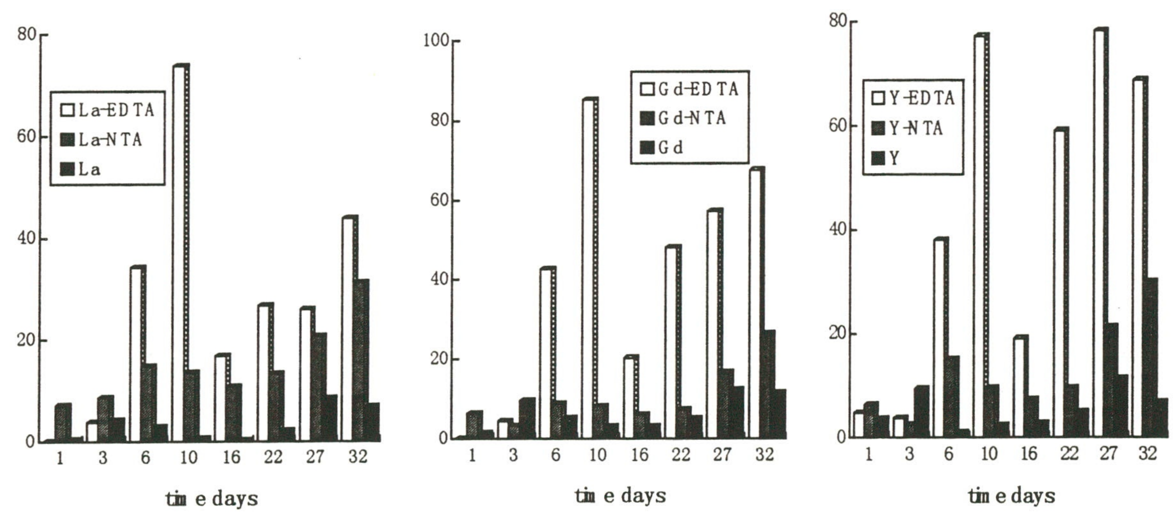


Figure 3. The bioaccumulation of different REE speciation in wheat above ground parts (stem and leaves)

As shown in Fig .2, wheat samples treated with different REE speciation all had high levels of REEs in the root. The highest bioaccumulation values of REE in root were 9.30×10^2, 1.38×10^3 and 152×10^3 mg • kg^{-1} for La、Gd and Y respectively. However large differences occurred when organic ligands were added into test solutions. Compared with ionic speciation, addition of organic ligands in test solutions reduced the concentrations of REE in root greatly. The bioaccumulation values of RE-EDTA speciation were at least two times lower than those of ionic speciation in each sample and the equilibrium was almost reached in three days. All these data indicate that the bioavailability of REE changed greatly when organic ligands were added to test solutions. The explanation may be that RE-Organic complexes were more stable and difficult to absorb by wheat roots, The order of bioaccumulation values of wheat root for different speciation REE was: ionic speciation> RE-NTA complex > RE-EDTA complex.

Fig. 3 shows a different pattern of bioaccumulation. The addition of organic ligands had a minor effect on the bioaccumulation values at the beginning of the experiment (1-3 day); REE bioaccumulated in the aboveground part of wheat increased with time, and the bioaccumulation values were similar among different speciation REE. After 3 days, the bioaccumulation values of ionic speciation were significantly lower than those of complexed speciation. This results suggests that addition of organic ligands could prompt the transportation of the REEs from the root to the aboveground part (stem and leaves) of wheat compared with speciation of ionic REE, this was just the contrary of the accumulation pattern of the root.

Bioaccumulation Values mg/kg

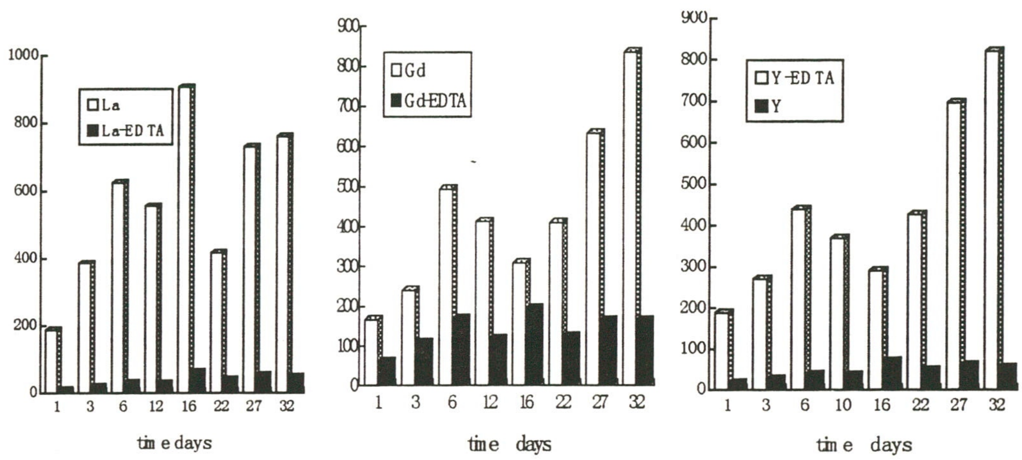


Figure 4. The bioaccumulation of REE in rice root with and without EDTA

Bioaccumulation Values mg/kg

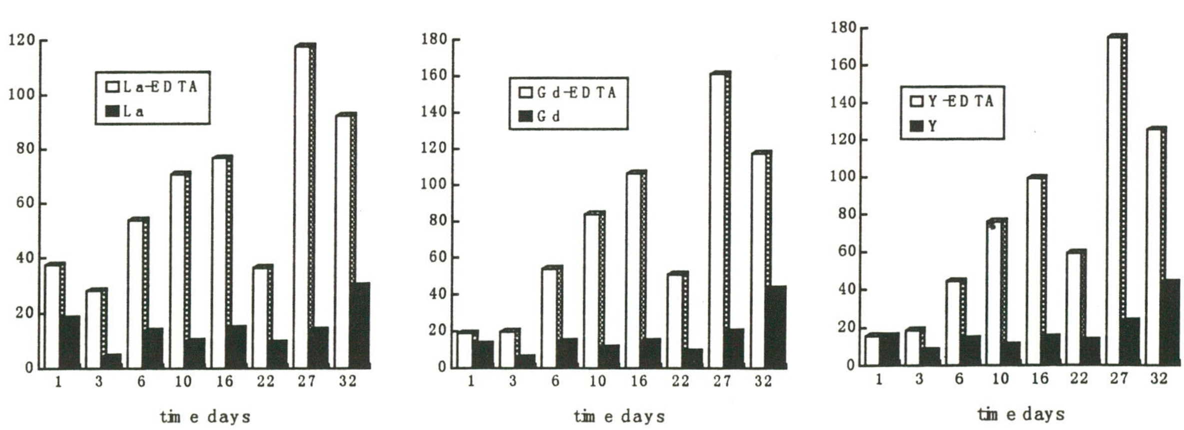


Figure 5. The bioaccumulation of REE in rice aboveground part (stem and leaves) with and without EDTA

Fig. 4 and 5 show the bioaccumulation results of rice for different speciation REE. From the data shown in these two figures, we can see that results obtained were similar to those with wheat

Thermodynamic Experiment

The thermodynamic experiment data showed similar results to the kinetic experiment. Fig. 6 shows that the concentrations of REE in wheat's root correlated well with the concentrations of REE in the nutrient solution. By using liner regression at the range of experiment concentrations (0 - 4.0 mg • L^{-1}) , the thermodynamic bioaccumulation process can be described with the following equation:

$$C_B=KC_w+b \qquad (1)$$

In which:

C_B is the REE concentrations in wheat root (mg • g^{-1});

C_w is the REE concentration in nutrient solution (mg • L^{-1});

K is bioaccumulation factor;

b is a constant.

In this equation, we can use the bioaccumulation factor (K) to assess the accumulation abilities of different speciation of REE in wheat's root. Regression equations in Table 1 show that the order of bioaccumulation factors was ionic speciation > RE-NTA complex > RE-EDTA complex. This indicated the same results as the kinetic study that organic ligands could reduce the bioaccumulation values of REE in wheat roots.

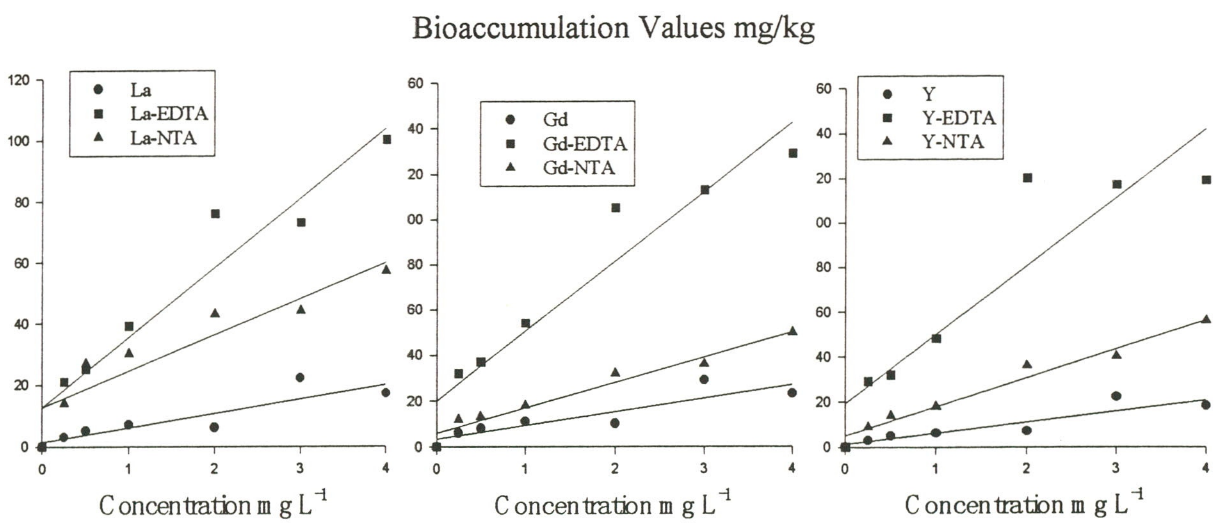
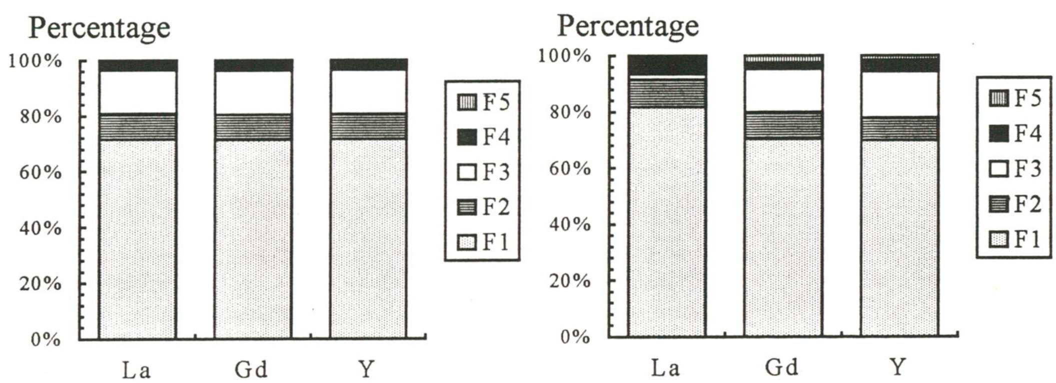


Figure 6. Relationship between the REE concentrations in test solutiosn and their bioaccumulation values in wheat root

Table 1. Thermodynamic Regression Equations of REE bioaccumulated in wheat root and REE in test solution

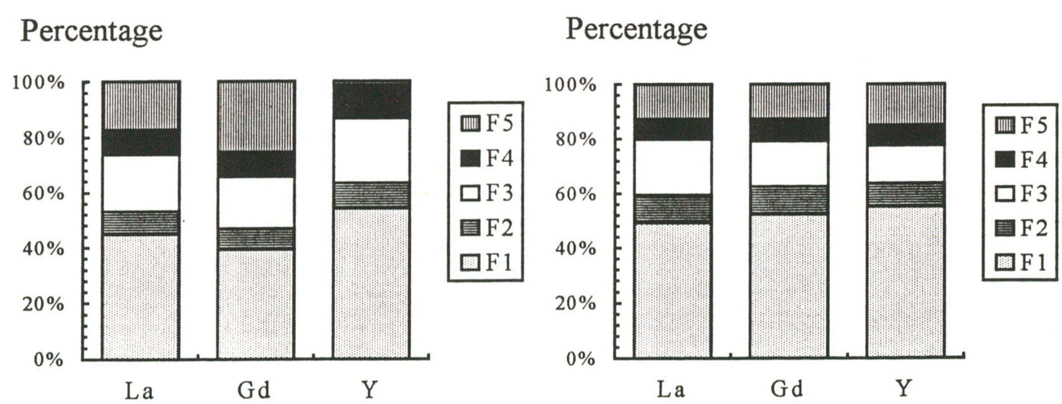
Speciation	Elements	Regression Equations	r^2
Ionic	La	C_B=0.788C_w+0.525	0.92
	Gd	C_B =0.877 C_w +0.547	0.92
	Y	C_B =0.751 C_w +0.450	0.94
RE-EDTA	La	C_B =0.160 C_w +0.056	0.88
	Gd	C_B =0.027 C_w +0.007	0.90
	Y	C_B =0.011 C_w +0.006	0.77
RE-NTA	La	C_B =0.531 C_w -0.062	0.93
	Gd	C_B =0.319 C_w -0.053	0.98
	Y	C_B =0.226 C_w -0.043	0.98

The distribution of REEs in plant cell



Ionic speciation RE-EDTA complex speciation

Fig. 7 The distribution percentages of REEs in cells of wheat root



Ionic speciation RE-EDTA complex speciation

Fig.8 The distribution percentages of REEs in cells of wheat above ground parts stem and leaves

The distribution percentages of ionic REE in wheat root cell are shown in Fig.7; the legends 1 to 5 represent five parts of the cell: F1 cell wall , F2 cell nucleus, F3 mitochondrion , F4 nucleus protein and F5 soluble fraction. As shown in Fig.7 and Fig .8 , the percentage of F1 was the highest among the five parts of the cell, it contributed for about 70% of the total REE concentration in wheat root cell and about 40% in wheat above ground part cell. The second part was F3 except for RE-La in wheat root cell; the soluble fraction F5 contributed the least to the total REE concentration . These results indicate that most of the REE were associated with the cell wall and deposited at this site, only a small (soluble fraction F5) can enter the cell and be transferred to the stem and leaves of the wheat. The order of distribution of different speciation of REE in the cells of root and above ground part mostly was cell wall > mitochondria > nucleus ≈ nucleoprotein > soluble fraction.

DISCUSSION

Addition of a chelation agent (EDTA) quit successfully alleviated REE uptake. The relative importance of chelation compounds in reducing metal uptake was studied by Srinivas and Singh[5] who reported that the uptake of chelated metal is less than that of its ionic form. Evidence for the role of chelation in the detoxification of metals also comes from in vitro studies. Neet et al.[6] found that metal-induced inhibition of yeast hexokinase activity was reversed by EDTA. These authors argued that the formation of metal-ATP complex was responsible for inhibition of hexokinase activity and that amelioration was due to the formation of stable complexes. In the present study also the uptake of REEs in the presence of chelation agents was also less in root probably because of the formation of a stable REE-chelate complex which is poorly absorbed by wheat root compared with ionic forms of REE.

In this study, the root and the aboveground parts (stem and leaves) demonstrated a different pattern of bioaccumulation. The reason may be related to the assimilation, transportation and detoxicification mechanisms of the different speciation. The thermodynamic experimental data (Table 1) suggest that addition of EDTA had no effect on the correlation relationships between REE concentrations in the root and their concentrations in the nutrient solution; they were both correlated very well ($r^2 > 0.7$ Table 1). This fact indicates that ionic speciation and RE-EDTA complex speciation may both transport directly across the external cell membrane of root; RE-EDTA complex speciation was more easily transported to the stem and leaves compared with ionic speciation.

The distribution of different REE speciation in wheat root cell and above ground parts cell also indicates for both speciation that most of REE entering the root cell were associated with the cell wall and deposited at the site. Considering the small concentration of the soluble fraction F5, there was only small REE fraction that can be transferred to the stem and leaves of the wheat. This could be the reason why the bioaccumulation values of REE in wheat root were much higher than those in wheat above ground parts.

CONCLUSIONS

In this study, addition of EDTA can dramatically reduced the bioaccumulation values in the root. While, the bioaccumulation values in the stem increased. Considering the different parts of wheat, the root had higher bioaccumulation values than that in stem. During the range of experiment concentrations (0-4.0 mg • L^{-1}), both ionic and complexed speciation REE in nutrient solution had positive linear correlation with their concentrations in the root. All these indicate that EDTA had high effects on the bioavailability of REE through controlling their speciation in the nutrient solution and their transportation processes from the root to the stem . The distribution of different speciation of REE in wheat cell indicates that most of the REE entering the wheat cell were associated with cell wall, only a small fraction of REE could be transferred to above ground parts.

Acknowledgment: This research work was supported by National Natural Science Foundation of Jiangsu Province Grant No. **BK**: 95019209

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Research on the ecology of Chinese soil-plant systems polluted by heavy metals

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ABSTRACT

The research on the ecology of Chinese soil -plant systems polluted by heavy metals may be roughly divided into 4 stages: investigation and research of farmlands contaminated by heavy metals, investigation of background values of heavy metal elements in soil and research on environmental capacities of heavy metals in soil-plant system, combined pollution of heavy metal elements and its ecological impacts, and control and restoration of soil contaminated by heavy metals.

The most typical large-scale soil pollution by heavy metals in China is was discovered in 1975 in the Zhangshi wastewater irrigation area in Shenyang. In 1980s, research on environmental capacities was launched and 5 heavy metal elements were chosen as targets. The types of studied soil included red soil, meadow dark soil, and meadow brown soil. In the investigation on background values of soil elements, heavy metals and rare earth elements were screened as the most important chemicals.

Research on the ecological impacts of heavy metals has been developed from individual element pollution to the combined pollution of elements, and the combined pollution of heavy metals with organic compounds. Today, the impact of pollution on plants at the level of cells is also investigated.

In China, ecological restoration is an important objective for the control and recovery of contaminated soil, it should be further strengthened as an effective technology .

KEY WORDS: Soil-plant system; Heavy metals; Pollution; Ecology.

A soil-plant system has the function of transforming solar energy into bio-chemical energy. When the system is polluted by organic and inorganic chemicals, its biomass productivity will be influenced, or even deprived in the most serious cases. Pollutants accumulated in the soil environment can migrate into air, water, and organisms, affect the biological qualities of agricultural products, and bring damage to human health in direct or indirect ways(Gao, et al., 1986). The pollution of soil-plant system is closely related to the sustainable development of agriculture.

In China, the occurrence of pollution, the migration, transformation and degradation of pollutants in soil-plant system, their eco-toxicological effects, and the recovery of contaminated soil are important topics in environmental research.

Heavy metals (HM) pollution of soil is defined as the excessive input of HM into a soil eco-system and the deterioration of a soil environment by the increasing of pollutant contents. The main resources of HM are wastewater irrigation, disposal and agricultural usage of solid wastes, pesticides and chemical fertilizers and atmospheric precipitation, with discharge and irrigation of wastewater into farmland being the most important factors[2]. With the rapid development of wastewater treatment, it can be predicted that the sludge from sewage treatment facilities will be a very important source of pollution in soil .

This research is of significant importance to establish environmental standards for the control of food-chain pollution and the exploitation of purification functions for soil-plant systems.

1. Research on HM pollution of farmland

The earliest study on HM pollution of farmland in China is on Cd in the Zhangshi wastewater irrigation area located in Western Shenyang. Since 1962, wastewater from urban districts of Shenyang was used to irrigate paddy rice fields in this area. In 1974, Institute of Applied Ecology, Academia Sinica monitored a high concentration of Cd in rice grains grown in this area. In 1975, a comprehensive investigation on Cd pollution was carried out within an area of 2800 ha.

1.1 Cd pollution of soil and rice in Zhangshi irrigation area

Investigation on the source of Cd showed that the main source of Cd was the Shenyang Smeltery whose annual Cd discharge into the sewage system was 10 T before effective control. Irrigation with the wastewater polluted by Cd caused serious accumulation of Cd in soil and plants.

Inside the irrigation area, Cd concentration in soil and paddy rice decreased from the upper to lower reaches, nevertheless, the overall level of pollution was more serious than that of Jintsukawa, Japan (Table 1).

Table 1 Comparison of Cd Content in Rice Between Zhangshi Irrigation Area and Jintsukawa, Japan[3]

Cd Content in Rice (mg/kg, in dry weight)	Jintsukawa 1971 — 1976 (%)	Zhangshi Irrigation Area, 1982 ,(%)		
		Upper Reaches	Lower Reaches	Average
<0.4	61.8	18.18	86.36	40.90
0.4～0.99	29.2	15.90	9.09	13.63
1.0～1.99	7.7	22.70	4.52	16.66
>2.0	1.2	43.18	0	28.78

The plant-soil Cd ratio is considered as an important parameter to describe the migration ability of Cd from soil to plant, it depends on the characteristics of soil and the type of plant. Table 2 presents the ratios for different wastewater irrigation areas.

Table 2 Rice Cd/Soil Cd Ratio of Main Wastewater Irrigation Areas in China

Irrigation area	Rice Cd / soil Cd
Zhangshi, Shenyang	0.13～0.42
Tianjin	0.09～0.13
Guangxi	0.002～0.003

In the Zhangshi irrigation area, pH of soil is usually less than 6; at this pH, more than 80% of Cd in soil exist in the forms of $CdCl_2$ or $CdCl^-_3$ which are more easily to be assimilated by plant [4].

From the analysis of soil samples, it was observed that the 0-10 cm layer kept the 30.7% of the total amount of Cd stored in the area, while the 0-30 cm layer kept 77-86.6% of Cd. In this area, the accumulation of Cd in top soil and sediment of irrigation channels was about 18 t by 1986.

When compared with other heavy metals such as Cu and Pb, Cd has a very high migration ability from soil to crop grain. For paddy rice, the migration rate of Cd in the Zhangshi area was 8.6 times that of Pb. For different crops, the abilities to assimilate Cd followed the order of sunflower > rice > corn > soybean.

In the Zhangshi area, the average content of Cd in 9 vegetables was 0.76 mg/kg in dry weight, 5.6 times that of the clean soil nearby.

1.2 The accumulation of Cd in human body and animals

The Cd content of animal products from HM polluted area were also higher than the clean area because of high content of HM in forage crops. In the Zhangshi area, the Cd content in pork and pig kidney were 8 and 460 times the neighbouring clean soil respectively.

By the analysis of Cd in drinking water and food produced in Cd polluted area, daily assimilation of human body can be calculated. In the Zhangshi area, the daily input of Cd would be 557.77ug in average, 32 times that of the neighbouring clean area.

As a result of Cd accumulation, significant differences of Cd contents in blood, hair and urine of people living in polluted area were observed when comparing with the neighbouring clean area (Table 3).

Detection of Cd in fetus viscera showed that Cd content of placenta was 2.4 times that of the clean area, similarly, Cd content in liver and kidney of fetus were 2.2 times that of the clean area. Therefore, a potential risk of transportation of Cd from mother's body to fetus exists in Cd polluted area.

Table 3 Cd contents in blood, hair, and urine of people living in the Zhangshi Area[3]
(Data from 1980)

	Blood (ug/L)		Hair (ug/kg)		Urine (ug/L)	
	Average	SD	Average	SD	Average	SD
Zhangshi	1.06	0.21	0.14	—	13.26	0.37
Control	0.42	0.25	0.07	—	2.13	0.17

1.3 Environmental factors influencing the impacts of HM in soil.

In China, the farmland area contaminated by Cd is about 13000 ha, spreading from the north to the south part. According to the literature, factors influencing the impacts of Cd and other HM in soil include:

a. physical and chemical properties of soil, for instance, if the pH of soil >7.2, the impacts of Cd, Pb, and Hg to plant will be significantly reduced;
b. forms of HM in soil;
c. type of the crops; and
d. presence of other elements in soil, for example, the existence of Zn can evidently reduce the uptake of Cd and other HM by crops.

2. Background values and environmental capacities of heavy metals in soil

2.1 Background values of elements in soil

The background values of elements in soil depend on the intrinsic chemical components, the structures, and the characteristics of soil elements without the effects of human activities especially modern industrial pollution. Background values of elements are usually a compilation of statistics results obtained from a range of specific values and they are different for different regions[5].

Investigation on background values of soil elements results from a complex systematic engineering approach in which distribution of sampling sites, procedure of sampling, methodology for chemical analyses, quality control of sample analyses and data treatment are decided according to general principles and the regional circumstances[5]. The investigation can be divided into two phases:

Phase I, forming out of overall scheme, including main research objectives, design of overall technological scheme, quality management, expected output, and analysis of benefits.

Phase II, implementation of the research, including collection of samples, preparation and management of samples, sample analyses, and data treatment[6].

Institute of Applied Ecology carried out the investigation on background values of soil elements in Liaoning Province. The schematic diagram of the research is described in Fig.1.

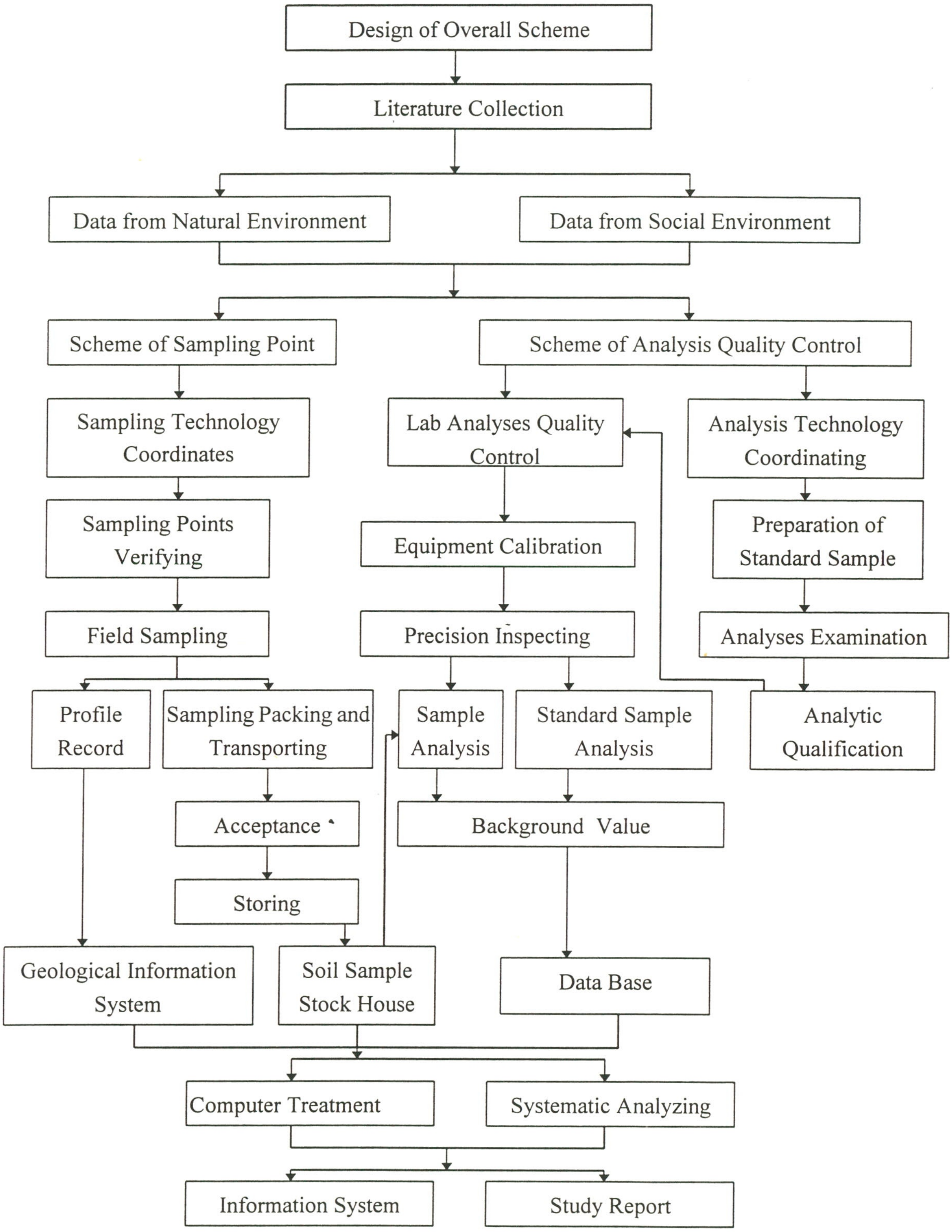

Fig. 1 Schematic Diagram of Study on Background Value of Soil Elements in Liaoning Province [8]

Investigation on background values of soil elements in China began in 1973 and finished in 1990, in this research about 4000 sampling sites were investigated. The main results on heavy metals and rare earth elements are presented in Table 4[5].

Table 4 Background Values of Some Soil Elements in China (A Layer)[5]

mg/kg

Atomic Number	Element	Minimum value	Maximum value	Arithmetic mean	Standard Deviation
23	V	0.46	1264	82.4	32.68
24	Cr	2.20	1209	61.0	31.07
25	Mn	1.00	5888	583	362.80
27	Co	0.01	93.9	12.70	6.40
28	Ni	0.06	627	26.90	14.36
29	Cu	0.33	272	22.60	11.41
30	Zn	2.60	593	74.20	32.78
42	Mo	0.10	75.10	2.00	2.54
48	Cd	0.001	13.4	0.097	0.079
50	Sn	0.10	27.60	2.60	1.54
51	Sb	0.002	87.60	1.21	0.676
80	Hg	0.001	45.90	0.065	0.080
81	Tl	0.036	2.38	0.62	0.216
82	Pb	0.68	1143	26.0	12.37
57	La	0.26	242	39.7	14.40
58	Ce	0.02	265	68.40	23.48
59	Pr	0.10	40.50	7.17	2.83
60	Nd	0.05	100	26.40	8.65
62	Sm	0.004	20.10	5.22	1.764
63	Eu	0.01	5.15	1.03	0.328
64	Gd	0.19	16.80	4.60	1.47
65	Tb	0.005	3.10	0.63	0.260
66	Dy	0.07	14.4	4.13	1.309
67	Ho	0.04	3.04	0.87	0.279
68	Er	0.13	9.37	2.54	0.813
69	Tm	0.04	1.40	0.37	0.115
70	Yb	0.02	7.68	2.44	0.786
71	Lu	0.002	1.90	0.36	0.108

2.2 Environmental capacities of HM in soil

The environmental capacity of a soil represents the highest loading of pollutants acceptable by this soil without environmental damage and deterioration of yield and biological quality of agricultural products. The environmental capacity is usually related to the specific interval of time and environmental standards [7].

In China, research on environmental capacity of soil has been going on for 10 years. Besides HM, some organic pollutants, such as mineral oil, were also included. The main soil types studied were red rice soil in semitropical zone, meadow dark soil and meadow brown soil in warm temperate zone.

2.2.1 The framework of research on environmental capacity

a. Background values of HM in soil were studied.
These values can be provided by other research, however, supplement and rectification are usually necessary.

b. Studies on ecological effects of HM in soil, included:
- — influences of HM on agricultural yields of the crops;
- — accumulation and distribution of HM in plants, and the effects on biological quality of farm products;
- — effects on diversity and bio-chemical activity of soil microorganisms; and
- — migration to surface and ground water and their effects on water qualities.

c. Determination of critical concentrations for environmental capacities
As an ecological system, soil consists of numerous sub-systems and keeps a close connection with the exterior environment. The critical concentrations of HM in soil should be determined considering eco-environmental effects and critical concentrations of HM for individual sub-systems (Table 5 & 6).

Table 5 The basis for determination of soil critical concentration [9]

System	Soil-plant system		Soil-microbe system		Soil-water system	
Contents	Human health effects	Crop effects	Biological effects		Environmental effects	
			Biological index	Amount of microbe	Ground water	Surface water
Objective	Prevention of food pollution	Maintaining productivity and economic benefits	Keeping regular circulation of soil ecosystem		No water environmental pollution	
Standards	National food hygienic standards	Reducing of yield and change of physiological parameters	Change for at least one of bio-chemical parameters	Change of microbe count	Ground water standard	Surface water standard
Classifying of standard		Reducing yield 10% 20%	>10% >25%	>50% >30% >10%		

Table 6 Critical concentrations of HM for 3 soils (mg/kg)

Soil type	Cd	As	Hg	Pb	Cr^{6+}
Meadow dark soil	2.8	21	0.4	300	Background+3.5
Meadow brown soil	2.0	30	0.2	300	Background+5.0
Red paddy rice soil	1.1	45	—	230	—

d. Determination of environmental capacities

On the basis of critical concentrations and the balance of HM in soil, the annual capacities or annual permitted inputs of HM, with a dimension of g/ha, can be calculated. Table 7 presents water quality for irrigation according to annual capacities of HM.

Table 7. Water quality for irrigation determined on the basis of environmental capacities[9] (mg/L)

Soil	Annual irrigation amount	Element	Suggested water quality: For 50-year irrigation	Suggested water quality: For 100-year irrigation	National standard
Meadow dark soil	$15000m^3$ /a • ha	Hg	0.00026	0.00015	<0.001
		Cd	0.0096	0.0056	<0.005
		As	0.130	0.117	0.05
		Cr	2.247	1.691	0.1
		Pb	0.874	0.44	0.1
Meadow brown soil	$12000m^3$ /a • ha	Hg	0.00037	0.0002	
		Cd	0.0076	0.0049	
		As	0.0725	0.0533	
		Cr	0.422	0.363	
		Pb	1.039	0.630	
Red rice soil	$15000m^3$ /a • ha	Cd	0.0036	0.0025	
		As	0.126	0.066	
		Pb	0.337	0.508	

As an ecological research, the environmental capacity varies significantly in accordance with the regional differences of the natural system. With the changes of environmental properties of the regions, the ecological effects, the critical concentrations, the mass balances and the accepted annual inputs of HM in soil fluctuate accordingly.

2.2.2 The limitation of environmental capacity

The environmental capacity is undoubtedly an important factor in HM research. However, there are still problems to solve in further studies, such as:

a. Effects of combined pollution
Ecological effects are the basis for determination of critical concentrations; when pollutants are co-existing of pollutants, their effects will be more complicated than those of individual pollutants.
b. The survey in longer term is essential to obtain more reliable results of environmental capacities.
c. Further research indicated that the type of heavy metal compounds has obvious effect on the results. So, what compound should be used in the research, and the determination for standard types of compounds are very important.
d. Along with the changes of biological indicator, the pollution processes and the environmental factors, the soil environmental capacity must be regarded as a value fluctuating in a range.

3. Combined pollution by HM and their ecological effects

Under natural conditions, pollution of HM is usually complex because of the co-existence of more than one element. The ecological effects of HM become more complicated owing to the antagonistic, additive and synergistic actions among the elements. The environmental standards formulated on the basis of research for individual pollutant must be revised. In China, research on combined pollution is an urgent need for the solution of practical environmental problems.

3.1 Ecological effects of combined pollution by HM
The ecological effects of combined pollution include the behaviour and concentration of pollutants, the physical-chemical properties of soil and the type of crops.

3.1.1 The behaviour of HM
A long term research showed that Cd has the strongest migrating ability among numerous heavy metals; about 70% of Cd sorbed by soil can enter soil solution by desorption and then be assimilated by plant. Similarly, about 50% of As can be desorbed from soil[10]. Cd and As are usually considered as important factors of combined pollution. Moreover, the toxicity of Cd and As can be strengthened by the co-existence of other metal elements. When As exists at a considerable concentration in soil, the sorption-desorption process of HM will become more complicated[11].

3.1.2 The effects of pollutant concentration
Yu et al., (1995)[12] studied the sorption competition between Zn and Cd. When Zn/Cd >10, Cd has an increasing effect on the sorption of Zn onto soil, however, if this ratio is less than 10, Cd can limit the sorption of Zn. In the research on HM absorption by paddy rice, Cd and Cu showed relation of antagonism at low concentrations but of synergism at high concentrations[13] .

3.1.3 Effects of soil properties
Chen (1996) [2]reported the sorption of Cd onto four types of soil in presence of Cu, Pb and Ni. Under the same experimental condition, the sorbed amount of Cd by four soils presented decreasing tendencies because of the competition of Cu, Pb and Ni. However,

the decreasing amounts of Cd sorbed by four soils were only from 0 to 14% when comparing with sorption experiment in which only Cd was used. It is obvious that the differences mainly arise from the various properties of the 4 soils, such as pH, mineral components, and the cation exchangeable capacity (CEC).

3.1.4 Effects of crops

Zhou et al., (1994)[14-15] found that the joint effects of Cd and Zn varied for different crops. The accumulation of Cd and Zn displayed the relation of antagonism in corn seeds, but of synergism in soybean. For paddy rice, the joint effects of Cd and Zn depended not only on their concentrations in soil, but also on the different tissues of the rice plant.

3.2 Combined pollution of HM and organic pollutants

3.2.1. Combined pollution and eco-effects of HM and petroleum hydrocarbons

In a soil-paddy rice system, hydrocarbons can restrain the absorption of Cu and Cd by paddy rice. However, there was no remarkable effects of Cd and Cu on the accumulation of hydrocarbon in paddy rice. An antagonistic joint effect was observed when hydrocarbons, Cd and Cu existed together, and the joint actions between hydrocarbons-Cd and hydrocarbons-Cu were also antagonistic respectively[13] .

3.2.2 Combined eco-effects of HM, phenanthrene and paclobutrazol

Gong (1995)[16] reported the combined eco-effects of Cd, Zn, phenanthrene, and paclobutrazol on soil microorganism under laboratory conditions. Complex joint actions among the four pollutants were observed. Phenanthrene was the main factor influencing the activity of microorganism, followed by Cd, Zn, the combined effects of Zn-phenanthrene and phenanthrene-paclobutrazol.

3.2.3 Combined eco-effects of HM and surfactants

Luo (1997)[17] reported the combined eco-effects of Cd-LAS, Cd-CTAB, and Cd-Tween 80 on the physiological characteristics of wheat under the condition of solution culture. Surfactants were found to be able to accelerate the accumulation of Cd in wheat leave with the order of CTAB>LAS>Tween 80. The level of lipid peroxidation in leaf cells under combined pollution was obviously higher than that of single Cd presence.

3.3 The injury mechanism of HM on soil-plant system

Under Cd stress, the accumulation of superoxide radical and malondialdehyde (MDA) in wheat leaves was significantly enhanced, but the activity of superoxide dismutase was apparently decreased; the lipid peroxidation of cellular membrane was stimulated by endogenous active oxygen radicals.

Experimental results suggested that the mechanism of Cd effects on wheat was the degradation of free radical scavenging system and the lipid peroxidation of cellular membrane[17].

4. Control and recovery of soil contaminated by HM

In China, research on control and recovery of HM contaminated soil has obvious characteristics of ecological engineering since its beginning in 1980'.

4.1 Control and recovery technology

In the comprehensive control and treatment of soil pollution in the Zhangshi irrigation area[7], Wu, et al., (1985) created a special model of cultivation and the application of lime and Calcium-Magnesium phosphate fertilizer for the prevention of Cd harmfulness. By this measure, the Cd content in rice grain can be effectively dropped to meet the national standard. Wang[18], (1995) studied the controlling effects of the technique mentioned above on combined HM pollution . Comparing with control, the absorption of Cd by rice and wheat was reduced by 31.5~55%, meanwhile, the absorption of Pb in four crops decreased by 23.4~75.8%.

Huang et al., [19](1986) reported the ecological restoration of a Cd contaminated land using poplar trees. Within a growth period, the decrease of soil Cd was 0.6~1.2 ppm through the absorption by plant. Moreover, poplar had satisfactory economic benefits higher than those of rice cultivation.

4.2 Harmless utilization of soil polluted by HM in agriculture

Harmless utilization is one of important channels for the reform of polluted soil. By this technology, the excessive entrance of pollutants into food chain and human body can be avoided and satisfactory social and economic benefits can be obtained.

In the Zhangshi irrigation area, a successful utilization model of polluted land includes the construction of seed multiplication and ornamental plant bases [20]. In southern China, the income of mulberry plantation and silkworm breeding on polluted land is 2 ~ 3 times more effective than rice cultivation.

Owing to the shortage of land resources and the necessity for protecting people health, the effective control and recovery of polluted land will be a continuous important task in China.

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Rare Earth Elements and Their Bioavailability in the Aquatic Environment*

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Abstract

In this paper , the environmental behavior and bioavailability of Rare Earth Elements (REE) were investigated and a method to investigate the speciation of REE and their bioavailability was also established by using the geochemical computer model MINTEQ. The results indicate that the existing speciation of REE in the aquatic environment had a large effect on their biovailabilities , the ionic speciation of REE were the main speciation which can be easily bioaccumulated by aquatic organisms and the presence of organic acid ligands in the aquatic environment can reduce the bioavailability of REE to algae. For fish, the internal organ was the main part bioaccumulating REE and played an important role in the detoxification process. There was no biomagnification effects during the bioaccumulation process of REE through the food chain. Environmental factors such as pH and organic ligands dominated the release of REE in sediment. The results of studying the REE distribution and chemical partitioning in a model aquatic ecosystem suggested that most of REE (mainly anthropogenic inputs) migrated into sediment. When spiked REE entered the sediment, they mainly existed in acid extractable speciation and this speciation which had a high correlation relationship with REE bioavailability.

Key words: Rare Earth Elements (REE) , Speciation , Bioavailability

Introduction

In China, Rare Earth Elements (REE) are used widely in agriculture, forestry, animal husbandry and aqua-culture in which they are contained in bactericides, micro-element fertilizers or animal food . As a result, more and more REE are getting into the aquatic environment in dissolved forms which usually have higher bioavailability for organisms. Unfortunately, the effects of these elements on the aquatic environment are not clearly known.

Until now, considerable work has been devoted to establish the background values of REE in the environment, their behavior in soil and their biological effects on crops [1-5] , but studies on the biological effects of REE on aquatic organisms and their behavior in the aquatic environment have just begun, so very few has been reported. The limited information available mainly focuses on the acute or chronic toxicity of this group of elements to several fish species.

Under normal circumstances, REE transported into the aquatic environments can't be degraded, so the information of the REE speciation, distribution, bioavailability and transportation in rivers and lakes are essential to understanding the environmental behavior in the aquatic environment. The research on these aspects is becoming more and more important in China.

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* This research work is supported by the National Natural Science foundation of China

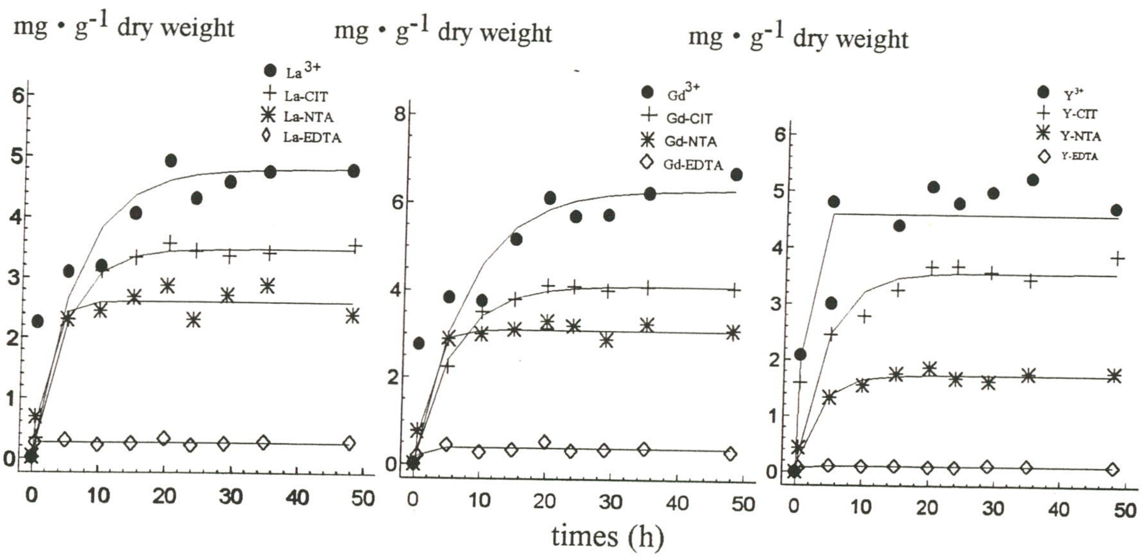
In this paper, we review our recent research work about the speciation of REE in the aquatic environment and their bioavailability in order to get a comprehensive understanding of REE environmental behavior and effects on aquatic organisms.

The Speciation of REE in the Aquatic Environment

Because of the difficulty of determining the real concentrations of REE speciation in the aquatic environment, a chemically-defined test medium has been developed in this study. In this medium, the concentrations of inorganic ions were fixed and the speciation of REE can be controlled by adding organic ligands such as EDTA (ethylenediamine tetraacetic acid), NTA (nitrilotriacetic acid) etc.; the existing speciation were calculated by the geochemical computer program MINTEQA[6]. To ensure that the calculation results of each dissolved REE speciation in the medium were as accurate as possible, we carefully selected the thermodynamic data from the appropriate literature [7-8].

The Bioaccumulation and Elimination of REE in aquatic organisms

As shown in Fig.1, the bioaccumulation of different speciation of REE by the alga (*Chlorella vulgaris Beijerinck*) was studied.



(Total REE concentration was 1.0 mg • L^{-1})

Figure 1. The bioaccumulation kinetics processes of different speciation REE by algae

According to Fig.1, the bioaccumulation process included two steps, the first step which may be dominated by adsorption process by algae surface was faster , the second step which indicated the transportation of REE into algae cell was slower. This two-step process can be described by the following equations according to the surface coordination model[9] :

$$\frac{dC_b}{dt} = k_1 C_w - k_2 C_b \qquad (1)$$

In which:

C_w: concentration of REE in water (mg • L^{-1});

C_b: concentration of REE in algae (mg • g^{-1}dry weight);

k_1: uptake rate constant($L \cdot g^{-1} \cdot h$);

k_2: elimination rate constant (h^{-1});

t: time (h).

Integration gives: $$C_b = \frac{k_1}{k_2} C_w (1 - e^{-k_2 t}) \qquad (2)$$

Division by C_w gives: $$\frac{C_b}{C_w} = \frac{k_1}{k_2}(1 - e^{-k_2 t}) \qquad (3)$$

At steady-state, the bioconcentration factor (BCF $g \cdot L^{-1}$) can be defined as

$$BCF = \frac{k_1}{k_2} = \frac{C_{b(t \to \infty)}}{C_{w(t \to \infty)}} \qquad (4)$$

According to the BCF (Table 1) calculated by non-linear regression equations (3) and (4), when total concentrations of REE were kept constant, the BCF of different speciation still differed from each other largely, indicating that speciation had a large effect on the bioavailability of REE to algae. In this experiment, the BCF of RE-EDTA complexes were the lowest compared with other speciation of REE. The order of BCF from high to low was RE^{3+} > RE-Cit > RE-NTA > RE-EDTA complex, this order was just opposite to the order of their 1:1 RE-Organic complex stability constants. This suggests that free ionic speciation was important for controlling REE bioavailability in the aquatic environment.

Table 1. Bioconcentration factors (BCF) and correlation coefficients of the equation 2

Elements		organic ligands			
		no ligand	Cit	NTA	EDTA
La	BCF	4.8	3.48	2.6	0.26
	r^*	0.88	0.99	0.95	0.91
	$logK^{**}$	--	7.17	10.47	15.50
Gd	BCF	6.36	3.16	3.12	0.38
	r^*	0.89	0.98	0.98	0.87
	$logK^{**}$		7.38	11.35	17.73
Y	BCF	4.6	3.55	1.74	0.11
	r^*	0.9	0.98	0.98	0.87
	$logK^{**}$		7.75	11.42	18.1

* correlation coefficients

** stability constant

The bioaccumulation and elimination of five light REE (La, Ce, Pr, Nd, Sm) were investigated in carp (*Cyprinus carpio.L*). The bioaccumulation experiment was carried in a period of 43 days, when equilibrium was reached , the fishes were placed in clean water for the elimination experiment.

Table 2 Variations of bioaccumulation factor s of REE in carp exposed to mixed REE at pH 6.0

Tissues	Elements	Bioaccumulation Factors L • kg^{-1} wet weight					
		3d	8d	15d	29d	36d	43d
Muscle	Ce	0.60	1.01	0.82	1.46	0.49	0.22
	La	0.60	0.56	0.50	1.20	0.27	0.83
	Nd	0.48	0.48	0.38	0.86	0.14	0.62
	Pr	0.66	1.16	0.66	1.66	0.33	0.33
	Sm	0.67	0.90	0.59	1.45	0.55	1.10
Skeleton	Ce	1.49	2.02	4.00	4.52	1.12	5.94
	La	0.40	0.56	0.70	1.23	0.96	3.66
	Nd	0.35	0.52	0.79	0.86	0.24	2.28
	Pr	2.32	3.31	4.47	6.13	3.64	8.11
	Sm	0.98	1.10	4.28	2.95	1.45	5.50
Gills	Ce	5.83	13.0	7.81	13.4	14.8	12.8
	La	3.86	12.8	4.99	12.6	13.8	13.5
	Nd	2.94	10.9	3.69	7.15	11.2	9.73
	Pr	4.64	11.1	7.62	19.0	12.6	8.94
	Sm	4.16	15.9	6.64	12.2	18.8	16.0
Internal organ	Ce	128	163	387	804	44.0	608
	La	130	167	399	828	45.2	602
	Nd	104	131	315	634	32.0	451
	Pr	121	153	368	750	37.5	530
	Sm	152	194	460	978	49.3	705

The results of bioaccumulation are listed in Table 2 in terms of bioaccumulation factor (BF) which is defined as the concentration of REE in fish tissue (mg • kg^{-1}) divided by the concentration of REE in the test water (mg • kg^{-1}). It was observed that at a certain exposure time, the BF of different REE in a given tissue were slightly different. According to the BF, the bioaccumulation ability of different tissues varied greatly and could be divided into two groups: one group which BF values between 100-1000 L • Kg^{-1} was for internal organ only, another group which BF values varied between 0.35-20 L • Kg^{-1} included gills, muscle and skeleton. For most REE, the BF values of internal organ were approximately two orders of magnitude higher than that of gills, muscle and skeleton. The order of bioaccumulation factor was internal organ >>gills >skeleton >muscle;, this indicates that the internal organ was the main metabolic pathway of the REE bioaccumulated in carp.

The elimination processes of light REE in gills, muscle and skeleton were similar and could be divided into two periods — a fast elimination period following a slower loss period. This elimination pattern suggests that the light REE which bioaccumulated in fish tissues may exist in two forms, one was unbound REE with fish tissues which accounted for more than 50%-70% of total tissue REE concentrations, another was the REE which bound tightly in fish tissues. Accordingly, we could divide the fish tissues into two compartments, one was associated with the rapid release of unbound REE and the other is associated with the subsequent slower release of bound REE in tissues. So, the elimination curves of REE can be fitted mathematically as the sum of two exponential functions (equation 5) according to a two-compartment model[10]:

$$C(t) = Ae^{-mt} + Be^{-nt} \qquad (5)$$

In which

$C(t)$ = the concentration of REE retained by the tissue at time t;

A, B are intercepts of each exponential phase;

m, n are slopes of each exponential phase and related to the biological half-lives (T_b) of different forms of REE.

Table 3. lists the corresponding biological half-lives which were calculated from the relations $T_{b1}=\ln2/m$, $T_{b2}=\ln2/n$ and the percentages of each form of REE in different compartments. Element La had the highest biological half-lives in skeleton with 693 d and 166 d in muscle . For bound form , considering the differences among the fish tissues ,the biological half-lives of most REE in higher skeleton and muscle than those in gills is reasonable .

Table 3. The biological half-lives and percentages of different forms of light REE in different tissues of Carp.

Tissue	Element	Unbound form		Bound form		R*
		Half-lives (d)	Percentage	Half-lives (d)	Percentage	
Skeleton	Ce	0.24	47.5	23.9	52.4	0.89
	La	0.21	73.8	693	26.2	0.94
	Nd	0.43	77.8	99.0	22.2	0.98
	Pr	2.24	62.7	71.5	37.3	0.99
	Sm	0.34	77.4	86.6	22.6	0.99
Gills	Ce	0.13	83.7	25.7	16.3	0.98
	La	0.11	84.7	14.7	15.3	0.99
	Nd	0.23	87.0	12.4	13.0	0.99
	Pr	0.16	77.0	34.7	23.0	0.98
	Sm	0.28	85.2	23.1	14.8	0.99
Muscle	Ce	0.14	85.2	25.7	14.8	0.98
	La	0.08	96.5	166	3.5	0.98
	Nd	0.10	96.4	86.6	3.6	0.98
	Pr	0.06	87.9	85.6	3.6	0.98
	Sm	0.23	89.1	49.5	10.9	0.98

* The correlation coefficients of releasing equations according to a two-compartment model

The elimination process of REE from internal organ can also be divided into two periods, in the first period the concentrations of REE increased and reached maximum values at the end of the second day. In the second period, REE released from internal organ and the kinetics of elimination can be described by the one-compartment model as follows:

$$C(t)=C(i)e^{-kt} \qquad (6)$$

In which

C(i) = the maximum concentration of REE in the internal organ.
k = the biological elimination rate constant.

The biological half-lives (T_b) of REE in internal organ can be calculated by equation 7:

$$T_b = \ln 2/k \qquad (7)$$

The corresponding T_b of each REE in internal organ are listed in Table 4.

Table 4. Half-lives and correlation coefficients R of REE in internal organ

Elements	Ce	La	Pr	Nd	Sm
Half-lives (d)	8.66	7.07	8.66	6.30	7.07
R *	0.98	0.99	0.99	0.99	0.99

*The correlation coefficients of releasing equations according to a one-compartment model

The correlation coefficients R of the regression analysis were highly significant, hence demonstrating a first-order process during second period of elimination in internal organ . The increasing period before elimination occurred can be explained by the transportation of light REE from gills, muscle and skeleton to internal organ. When equilibrium was not reached, the rate of elimination from gills, muscle and skeleton was faster than that from internal organ and the maximum REE concentrations in internal organ were observed. A similar pattern of metal mobilization has been demonstrated by Zaroogian[11] in the oyster *crassostrea virginica.* Considering the highest BCF of the internal organ in bioconcentration process, we consider it as the important site of REE detoxicification and storage. This agrees with the research of Tu and Wang[12].

The Bioaccumulation and Distribution of REE Through Aquatic Food Chain and Microecosystem

A system of five aquaria (5 L) was used as illustrated in Fig.2. Each aquarium contained 3 L of Freeman standard reference water and was connected with glass siphon. Aquarium I was cultivated with 100 mL of the alga (*Chlorella vulgaris Beijerinck*), aquarium II and IV contained about 200 *Daphnia magna Straus* and aquarium III and V contained five fishes (*Cyprinus Carpio L.*)

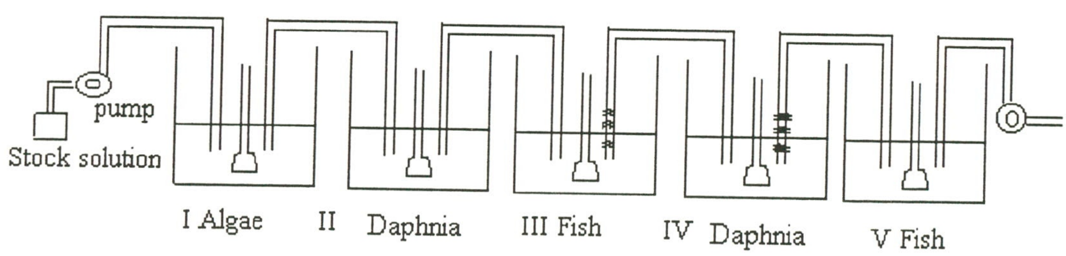


Fig .2. Schematic diagram of the experimental apparatus

This dynamic system simulated a food chain which has three trophic levels: algae, *Daphnia* and fish. In this system, *Daphnia* fed on algae from aquarium I , fish fed on algae and *Daphnia* from aquarium II, so the *Daphnia* and fish can bioaccumulate REE through both test solutions and food chain. Thus, the experimental results of aquarium I,II and III can be used to evaluate the biomagnification effect. *Daphnia* in aquarium IV fed on algae which was cultivated in a separated culture solution, and fish in aquarium V fed on *Daphnia* which was also cultivated in separated culture with no REE. So, the organisms in aquarium IV and V bioaccumulated REE only thorough the aquatic environment. The results of this dynamic experiment are listed in Table 5.

Table 5. The changes of BCF (mg.kg^{-1}) vs time during the dynamic experiment

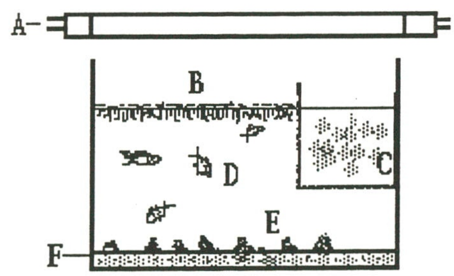
time (week)	element	Algae(I) $\times 10^4$	*Daphnia* (II) $\times 10^3$	Fish (III)	*Daphnia* (III) $\times 10^3$	Fish (V)
1	La	3.08	3.50	150	0.908	72.0
	Gd	4.18	4.03	136	0.737	43.7
	Y	8.90	9.00	274	1.92	115
2	La	2.20	3.30	154	1.37	109
	Gd	3.94	4.40	163	1.27	66.0
	Y	8.23	5.45	412	1.77	186
3	La	1.67	3.18	202	1.94	114
	Gd	1.87	3.32	215	1.79	71.2
	Y	2.58	5.20	235	2.30	121
4	La	1.15	3.74	208	1.60	116
	Gd	1.09	3.30	232	1.12	79.5
	Y	1.84	4.70	204	2.06	128

From the data listed in Table 5, we could find that the order of bioconcentration factors (BCF) of REE in three trophic level food chain (algae, *Daphnia* and fish) was algae > *Daphnia* > fish, and there was no biomagnification effect in the bioaccumulation process. The results also showed that aquatic organisms could take up REE from the test solution and from the food.

The distribution of REE in a duckweed-*Daphnia*-shellfish-fish- sediment microecosystem was also investigated. An aquarium (20×50×50 cm) containing 50 L of filtered lake water was used as shown in Fig. 3. A 2 cm thick layer of sediment was placed at the bottom of the aquarium. A cage (15×15×20 cm) made of nylon web which aperture was 1×1mm was placed in water. The experiment was initiated by filling aquarium with contamination-free filtered lake water.

Shellfish、duckweed and goldfish were added into the aquarium, *Daphnia* were added in the cage placed in water. After the system equilibrated for 1 week, aquarium was spiked with about 1.00 mg • L^{-1} of mixed REE.

As shown in Fig.4, when spiked REE entered this aquatic ecosystem, most of them were accumulated in sediment, duckweed and *Daphnia*, only a small portion of them in the water column , shellfish and goldfish. In this microecosystem, the accumulation trend of five REE (La, Gd, Y, Ce and Sm) in different phases was duckweed ≥ *Daphnia* >sediment > shellfish > goldfish > water. From this figure, we can also find different patterns of accumulating REE by different phases in this ecosystem. Duckweed and *Daphnia* had higher bioaccumulation rates and values, compared with



A:Fluorescent Lamp B:Duckweed C:*Daphnia*
D: Goldfish E: Shellfish F: Sediment

Figure 3 Schematic diagramof microcosm

shellfish and goldfish indicating different mechanisms of bioaccumulation. Shellfish and goldfish mainly depended on sediment and water for their bioaccumulation , while, because of advanced root of duckweed and large surface area of *Daphnia* , adsorption from water controlled most of the bioaccumulation processes.

With this Microecosystem, containing a large amount of sediment, the total amount of REE in sediment was also very large. Thus, the adsorption and desorption of REE by sediment had a large effect on the concentrations of REE in water.

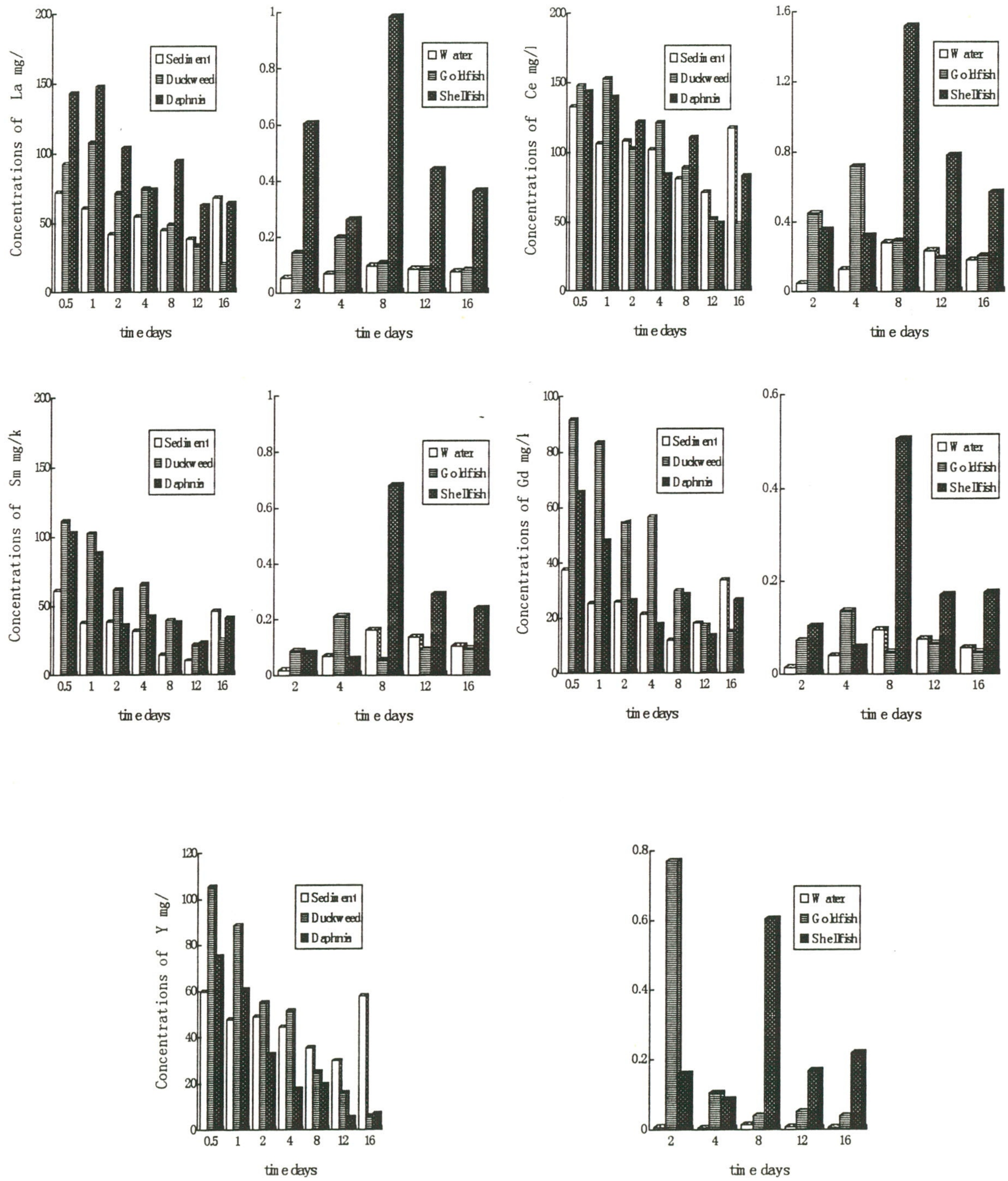


Fig 4. The distribution of REE in the different compartments of microecosystem

The Speciation of REE and Their Bioavailability in Sediment

The speciation and chemical behavior of REE in sediment and their bioavailability were largely depend on the environmental factors such pH and organic ligands. Normally for heavy metals , because of the hydrolysis effect (with the increasing of pH). The amount of metal released from the sediment will decrease. When adding organic ligands in solution, the amount of metal released from the sediment will increase because of the formation of soluble metal organic complexes. The release curves of REE in Fig.5 also demonstrated this rule.

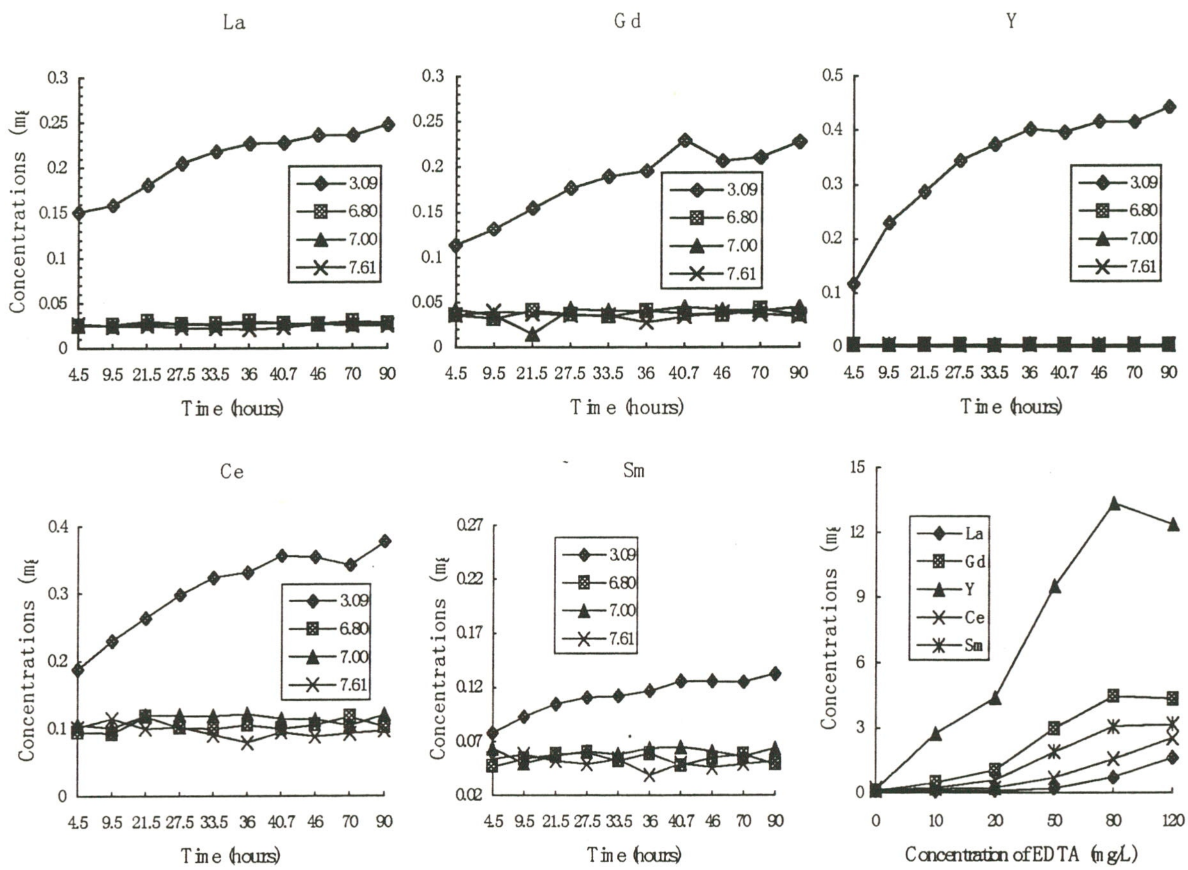


Fig. 5. The release of REE in sediment at different pH and concentrations of EDTA

Table 6. Chemical sequential extraction procedures for different speciation of REE in sediment

Procedure	Extraction Operation	Speciation
1	0.5 g sediment extracted with 25mL culture medium for 24 hours	Water Soluble
2	Residue extracted with 0.1 M HCl (pH=1.0) at 25 ℃	Acid Extractable
3	Residue extracted with 30% H_2O_2/HNO_3 (v/v 5:3 pH=2) in water bath at 85℃ with agitation	Organic/sulfides bound

In order to study the relationship between speciation and bioavailability, sediment samples spiked with REE were sequentially extracted into various operationally defined speciation. REE bound with the water soluble (WS), acid extractable (AE) and organic/sulfide bound speciation (OS) were extracted based on the procedures presented in Table 6. REE in the residual phase were not assessed as they are normally essentially unavailable to organisms[12].

Results from the sequential extraction procedure (Fig 6.) revealed principally the partitioning of spiked REE among three phases (AE,OS and WS) of the sediments under this investigation. On average, the following order could be arranged for the REE studied: AE>OS>WS. For each levels of spiked REE, the percentage of acid extractable speciation were at least more than 70%, in most cases, while, the contents of organic/ sulfide bound speciation were 1-2 times higher than that of water soluble speciation.

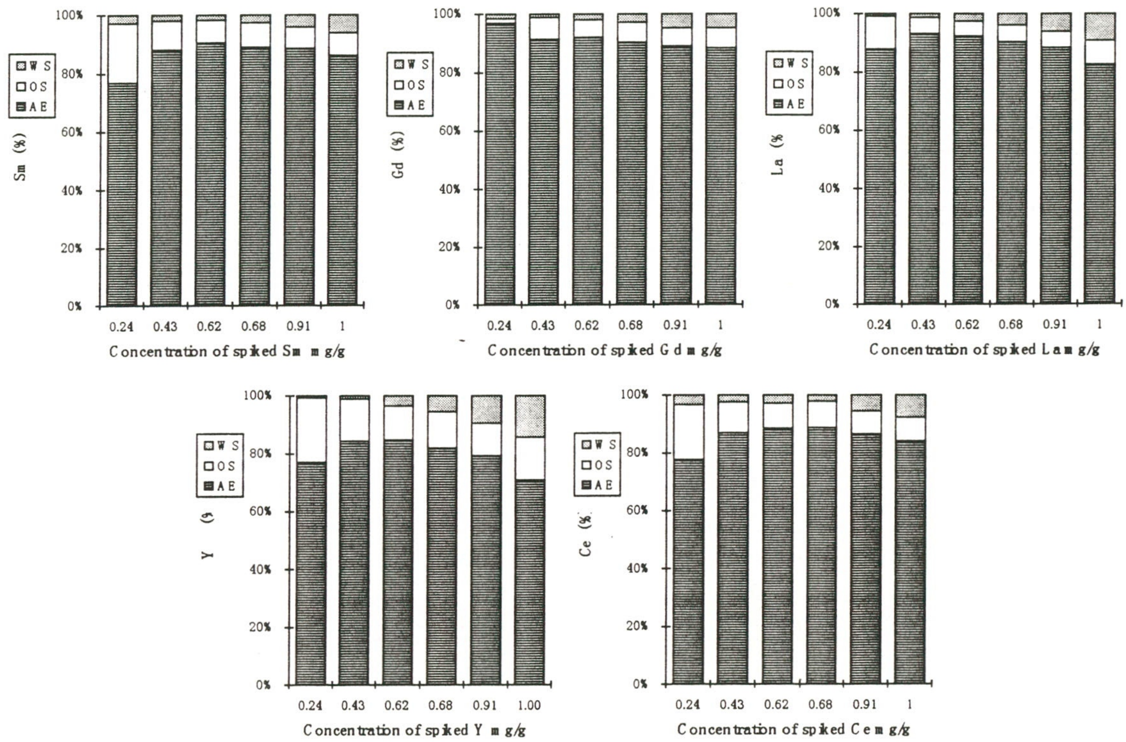


Figure 6. The relative distribution of REE among three phases in sediment

An artificial system was employed to simulate bioaccumulation process of REE speciation from sediment by algae, As shown in Fig.7, the large tube was a 600mL glass beaker, sediments were placed at the bottom of the tube; the small tube was placed in big tube and sealed on the lower end with a 0.45 μm filter membrane. Culture solution (pH 6.0) was added with the ratio of water: sediment 50:1.

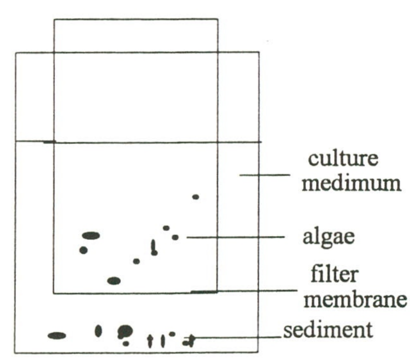


Figure 7. Experimental apparatus used to study chemical speciation of REE and their bioavailabilties to algae

In this experiment a simple relationship between REE concentrations in sediment and concentrations in organisms was obtained. Correlation equations of bioaccumulation a of various REE speciation have been calculated for green alga (*Chlorella Vulgarize*

Beijerinck). The data are listed in Table 7.

Table 7. Relationship between REE bioaccumulation by algae and various REE speciation

Element	Water Soluble	r^2	Organic Sulfide bound	r^2	Acid Extractable	r^2
La	C_B=95.20C_R-0.71	0.99	C_B=201.2C_R-6.39	0.92	C_B=11.77C_R-3.78	0.77
Gd	C_B=157.6C_R-1.0	0.88	C_B=181.4C_R-6.79	0.91	C_B=10.35C_R-3.89	0.74
Y	C_B=69.20C_R-0.10	0.99	C_B=148.4C_R-9.78	0.94	C_B=8.31C_R-6.66	081
Ce	C_B=107.9C_R-1.31	0.99	C_B=173.5C_R-8.95	0.89	C_B=10.39C_R-4.33	0.71
Sm	C_B=145.1C_R-1.12	0.99	C_B=168.7C_R-6.31	0.89	C_B=9.98C_R-3.16	0.70

C_B is the bioaccumulation values of REE in algae,
C_R is the concentrations of REE speciation in sediment.

The best correlations were generally obtained between the speciation of water soluble and the REE concentrations in algae. The results of this study suggest that water soluble speciation is good indicator of REE uptake by algae. Because it is easy to analysis , this method will provide a better estimation of food-chain contamination by water soluble REE than total REE loading alone.

The results of this study indicated that the water soluble speciation was the most important speciation for evaluating the bioavailiability to alga. Because most green algae exist in the aquatic environment as suspended particulates, the REE released into the water body should be accumulated by algae more easily than other speciation in sediments. The experimental data also shows that most of spiked REE existed in acid extractable speciation, so the factors such as pH、chelation agents in water、oxidation、reduction conditions etc. which had high effects on the transformation of REE speciation in sediment are also important to have a comprehensive understanding of the relationships between REE speciation and their bioavailiability to algae.

Conclusions

In summary, the bioavailability of different speciation of REE varied greatly, in the aquatic environment. Ionic speciation of REE can be easily bioaccumulated by algae and in sediment, acid extractable speciation was very important to estimate the bioavailability of REE. Internal organ of fish was the main part for detoxicification of REE bioaccumulated in fish and there was not any biomaginification effect observed during bioaccumulation process of REE through food chain. The aquatic organisms could take up REE from test solution and food. When spiked REE entered a microecosystem , most of them distributed in sediment and the changes of environmental factors had a large effect on the release of REE from sediment to water .

Acknowledgment: This research work was supported by National Natural Science Foundation .

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Distribution of rare earth species in rabbit blood and some biological effects

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Abstract

The distribution of rare earth species in rabbit blood and their biological effects on erythrocyte have been investigated by ultrafiltration and chromatography technique and determined by ICP-MS, ^{31}P、^{1}H NMR methods. The rare earth mostly bound to micromolecular ligands ($M_w<10^3$) and the concentration decreased quickly with metabolism. Chromatography studies indicated that the rare earth can bind to immunoglobulin G(IgG), transferrin(Tf), albumin(Alb) species, but mostly bound to Tf. The ^{31}P、^{1}H NMRspectra and ICP-MS results of erythrocyte showed that no transmembrane behaviours occurred under the safe doses(in vivo: 0.05~5.0mg/kg, in vitro:0.05~10μg/mL).

Key words: La, Eu, distribution, biological effects, rabbit blood.

Introduction

Along with the widespread applications of rare earth(REE) in agriculture and medicine, REE can probably enter our body through food. Since the toxicity of REE is related to their species in the organism[1], it is very important to know the distribution of REE species in blood and their effects on the erythrocyte[2]. Jackson et al., [3] studied REE species in human plasma by computer modelling and suggested that REE only bind to Tf; but because of the complexity of plasma, this hypothesis needs some experimental basis. Do REE enter the cell?

[#] Surported by the National Natural Science Fundation of China.

Many investigations have been done[4] indicating that REE cannot enter normal cell. But some biological effects showed that REE may participate in the metabolism of organisms[5,6,7]. There are now much controversy about transmembrane behaviours of REE[8]. Here the distribution of rare earth species in rabbit plasma and their effects on erythrocyte have been investigated.

Experimental

The following apparatus were used:

T-124 high centrifugal machine and UV-922(Contron), Phastsystem Automated Electrophoresis System and Grading Fraction System (Pharmacia), POEMS type ICP-MS(TJA), Varian Unity 400 NMR spectrometer.

Materials:

Male Kunming rabbits of 3.2kg were purchased from the animal lab of Bethune Medical University. Eu_2O_3 、 La_2O_3 and In (99.99%). DEAE-Sephadex A_{50} and Sepharyl S-200 were purchased from Pharmacia. Chemicals for solutions such as tris(hydroxymethyl) aminomethane(Tris) and sodium citrate were purchased from Huamei (China).

Methods:

1. Plasma samples

(1)Rare earth nitrate(La,Eu)were injected to rabbits through the abdominal cavity or intravenous injection. The rabbit blood was collected with stainless steel needles in plastic syringes and placed in plastic tubes containing ACD anticoagulant. After centrifugation (4000rpm,10min), the blood plasma was ready for ultrafiltration 、 ICP-MS and content of protein. In vitro, we collected the normal plasma first, then added some rare earth nitrate and warmed 1h or 4h at 37°C. The cells were treated as in method 2. (2)After ultrafiltration with the series membranes(one thousand, fifty thousand and one million), the four fractions $F_1(M_w<1\times10^3)$, $F_2(10^3<M_w<10^4)$, $F_3(10^4<M_w<5\times10^4)$ and $F_4(M_w>5\times10^4)$ were obtained. (3)Using the above ultrafiltration fraction F4 three main proteins (IgG 、 Tf 、 Alb) were obtained by separation on DEAE-Sephadex A-50(1.4cm×30cm, flow 0.3mL/min, 0.05M Tris-HCl, 0-0.5M NaCl gradient, pH 7.5), the albumin fraction was further separated on Sepharyl S-200(1.6cm×70cm, 0.0025M Tris-HCl, flow 0.3mL/min, pH 7.5). The REE content of the three species were analyzed by ICP-MS(in 1% HNO_3, 5μg/L $In(NO_3)_3$ solution).

2. Erythrocyte samples

(1) In vivo: the erythrocyte obtained from method 1 were first washed with EDTA (1.6mM,5~6times,1min/per time), then washed with an isotonic saline solution (5~6times, 1min/per time). After each washing, we centrifugated and removed the above clear solution to clear away the REE, which was outside the cell. We added 20%D_2O to the enriched cells for ^{31}P、 ^{1}H NMR; or added 3.8mL water to 0.2mL enriched erythrocyte. After swelled for 30min, the solution was centrifugated at 10000rpm for 10min, and the red solution was used for ICP-MS and protein content.

(2) In vitro: we collected the normal erythrocyte first, and added some rare earth nitrate, warmed 1h or 2.5h at 37°C, then treated them as above.

Results and Discussion

1. Metabolic curves

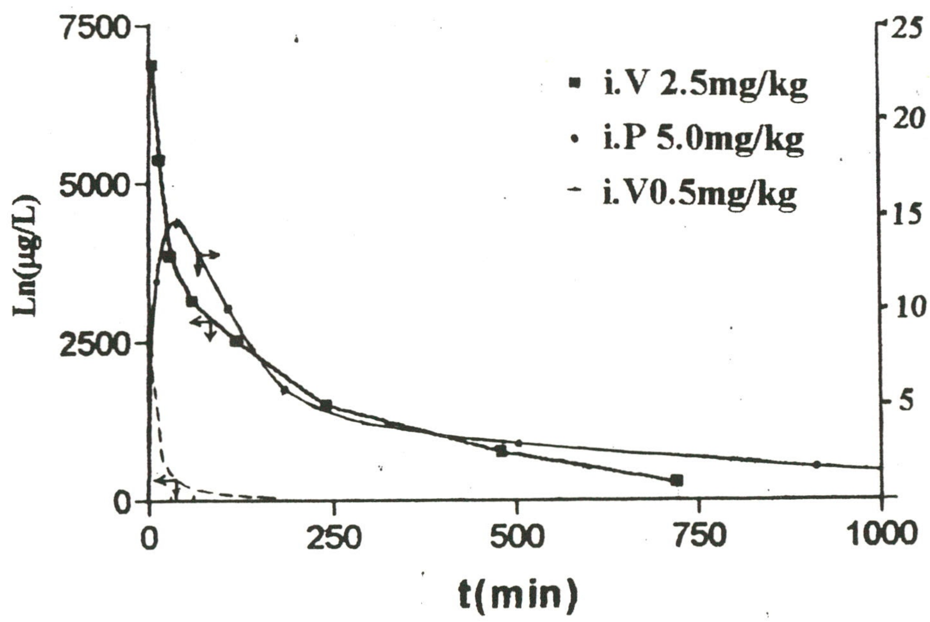

Figure 1 Metabolic curves of the Ln content in plasma

[a] i.V-intravenous injection, i.P-abdominal cavity injection

Three metabolic curves with different modes and different doses are shown in Figure 1. We can see that: (1) when injected by i.V, the REE concentration falls quickly. But if the dose is high enough, the concentration of REE will maintain at a high level for several hours; (2) when injected by i.P, the concentration of REE will reach the highest level during 1~1.5 hours, and then fall off slowly, the concentration of REE will maintain at a low level for a long time. This was because the phosphate or bicarbonate of REE would be absorbed gradually. So, we can determine the mode、 dose and time for taking blood for the needs of our experiment according to the metabolic curves.

2. Distribution of REE in rabbit plasma

(1) Ultrafiltration

Table 1 ICP-MS data of rabbit plasma

mode and dose*		T(°C)	t(h)	Lanthanum(μg/L)			
				F_1 ($<10^3$)	F_2 ($10^3<M_w<10^4$)	F_3 ($10^4<M_w<5\times10^4$)	F_4 ($>5\times10^4$)
In vitro	1.8×10^{-5}M	37°C	4h	1352.38	45.27	6.58	428.25
	1.8×10^{-5}M	37°C	1h	2667.50	67.20	5.86	110.34
i.P	0.5mg/kg		1h	86.42	3.07	1.37	22.04
i.V	0.025mg/kg		0.5h	17.80	0.82	0.89	2.67
i.V	2.5mg/kg		12h	11.69	0.037	0.043	0.605

* $La(NO_3)_3$ or $Eu(NO_3)_3$

Table 1 shows results of ultrafiltration. There is little REE in fractions F2 and F3; REE mostly exist in F1、 F4, with the amount of REE in F1 being more than that in F4. This is probably because the content of micromolecular ligands in plasma was much higher than that of proteins. The total content of micromolecular ligands in plasma was 412.2mmol/L; and the total content of protein species was 1.38mmol/L. So, there could be a competition of REE between them. According to the in vivo data, we can also draw a conclusion that REE get away from blood by metabolism with micromolecular ligands.

(2) Separation of proteins containing REE

Using the two columns(DEAE-Sephadex A-50 and Sepharyl S-200) the rabbit plasma(from in vivo) were separated, and the three proteins IgG、 Tf、 Alb containing REE were obtained. They were determined using SDS-Page(sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and then analyzed by ICP-MS. Results are listed in Table 2.

Table 2 REE content of each protein species in 1L plasma(ng/L)

Ree	IgG	Tf	Alb
Eu	100.4*	269.8	59.3

* Mean value(n=3).

The data in Table 2 showed that the rare earth can bind to IgG、 Tf and Alb species, but mostly bound to Tf.

3. The interaction of REE with erythrocyte

(1) ICP-MS results

Table 3 In vivo data of rabbit erythrocyte

mode and dose	(mg/kg)	Lanthanum(μg/L) control	5min	30min	1h	4h	24h
i.P	0.5	0.22			0.18	0.11	0.13
$La(NO_3)_3$	5.0	0.28				0.20	0.20
	0.025	0.016	0.017	0.017			
i.V	0.25	0.019	0.018	0.016	0.017		0.017
$Eu(NO_3)_3$	2.5	0.018	0.017	0.020	0.013	0.025	0.014

In vivo, when rare earth nitrate were injected to rabbits by i.V or i.P, the concentration of REE in erythrocyte did not change, just at the same level as the control, indicating that no transmembrane behaviours occurred either in different doses or at different times.

In vitro, the effect of REE on erythrocyte was investigated under the different doses(0.05μg/mL、 0.5μg/mL 、 5μg/mL 、 0.05mg/mL、 0.01mg/mL、 0.1mg/mL、 0.25mg/mL、 0.5mg/mL) and different warming times(1h, 2.5h). When the concentration of rare earth nitrate $Ln(NO_3)_3$(Ln=La,Eu) was under 0.01mg/mL, the results of ICP-MS were the same as the control(La: 0.22±0.05μg/L, Eu: 0.020±0.005μg/L), indicating that no transmembrane behaviours occurred. When the concentration of $Ln(NO_3)_3$ was higher than 0.01mg/mL, there was some haemogobin leaking out. It was probably cell lysis happening. The higher the dose, the heavier was the cell lysis.

(2) ^{31}P、 ^{1}H NMR results

The ^{31}P NMR spectra of rabbit erythrocyte are shown in Figure 2. Compared with the reference[9], the ^{31}P NMR spectra were composed of three group of peaks of DPG、 ATP and Pi. Because Eu^{3+} is a shift reagent, if it got in the cell, the chemical shift would change to upfield and the peaks would broaden. From

Figure 2 we can see that there were no any change of each peak either in different doses or at different time, indicating that Eu^{3+} did not get into the cell.

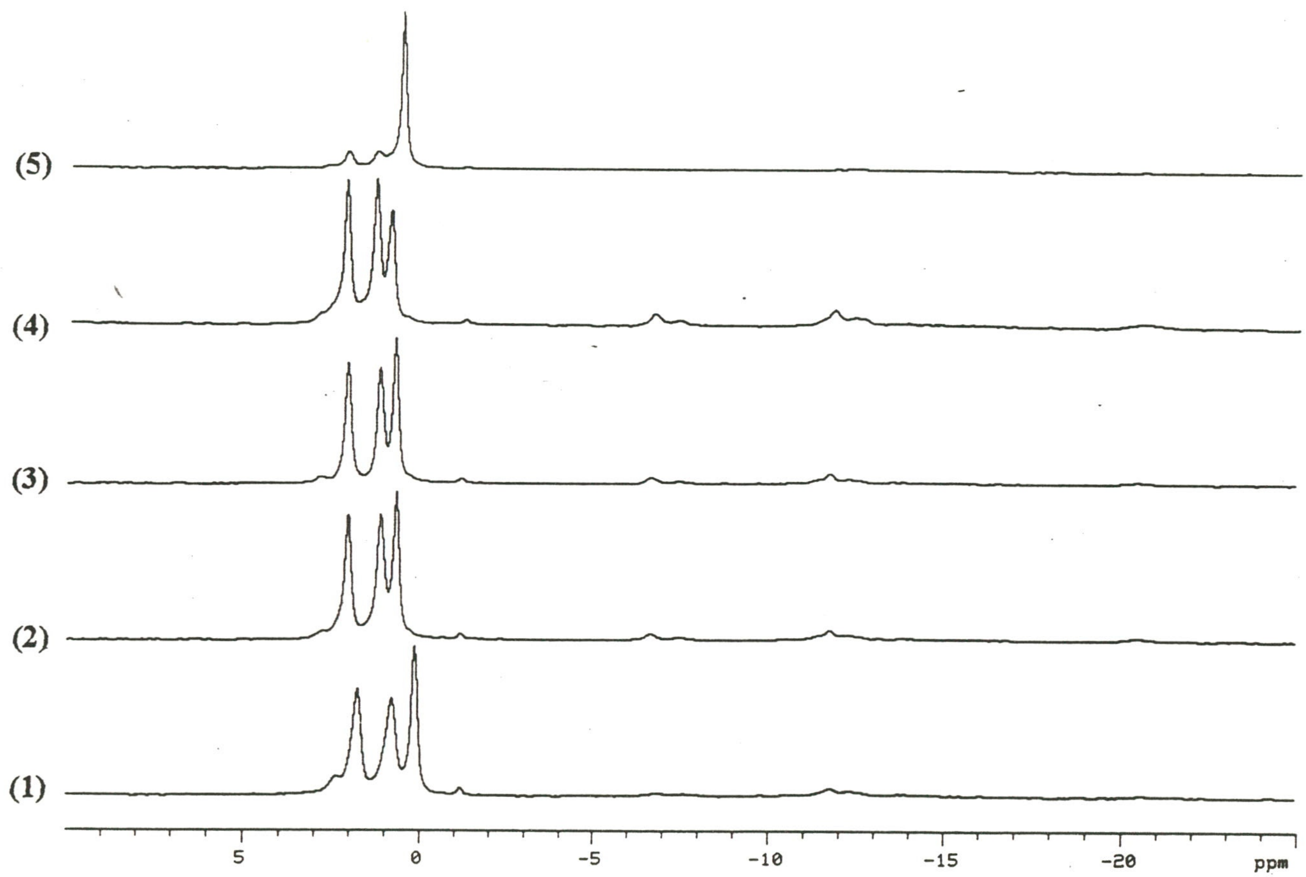

Figure 2 ^{31}P *NMR spectra*

(1) Normal rabbit erythrocyte

(2) i.V 2.5mg/kg $Eu(NO_3)_3$*, 1h*

(3) i.V 2.5mg/kg $Eu(NO_3)_3$*, 8.5h*

(4) In vitro 0.5µg/mLEu$(NO_3)_3$*, 2.5h*

(5) Normal rabbit erythrocyte, two days

The ^1H spin-echo NMR spectra of rabbit erythrocyte are shown in Figure 3 and Figure 4. From these spectra, the small molecules(motion fast) can be observed, the macromolecules(such as proteins、 membrane, which move slowly) cannot be observed. Similarly as in ^{31}P spectra, if Eu^{3+} got in the cell, the chemical shifts of small molecule would change. No such change was observed, indicating that Eu^{3+} didnot interact with small molecular ligands. The above NMR results show that no transmembrane happened in our experiments.

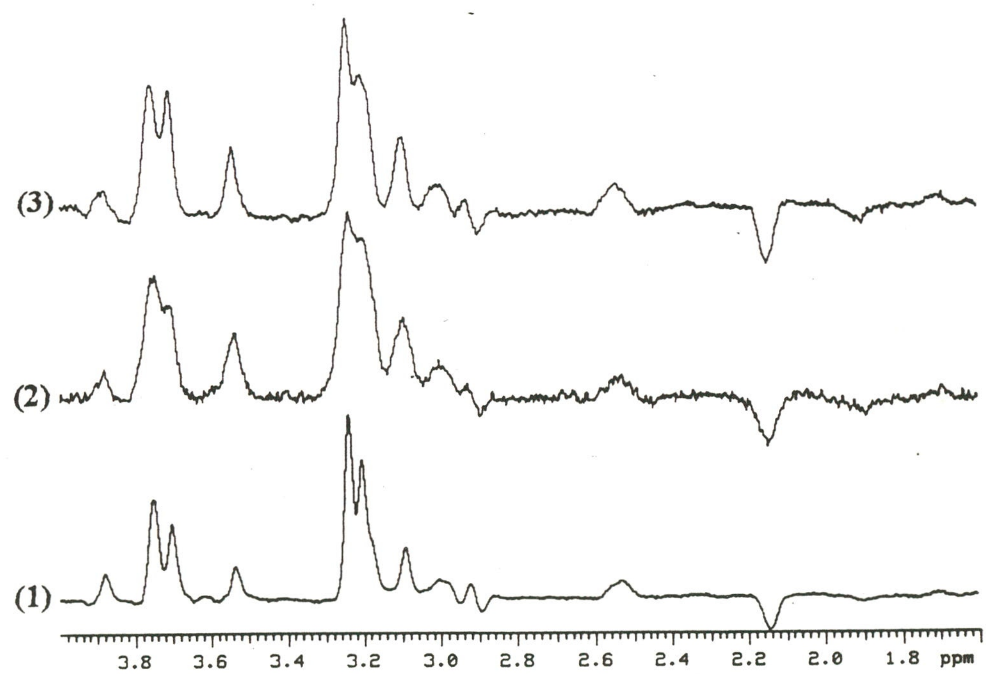

Figure 3 ^1H NMR spectra(in vivo)

(1) Normal rabbit erythrocyte

(2) i.V 2.5mg/kg $Eu(NO_3)_3$, 1h

(3) i.V 2.5mg/kg $Eu(NO_3)_3$, 8.5h

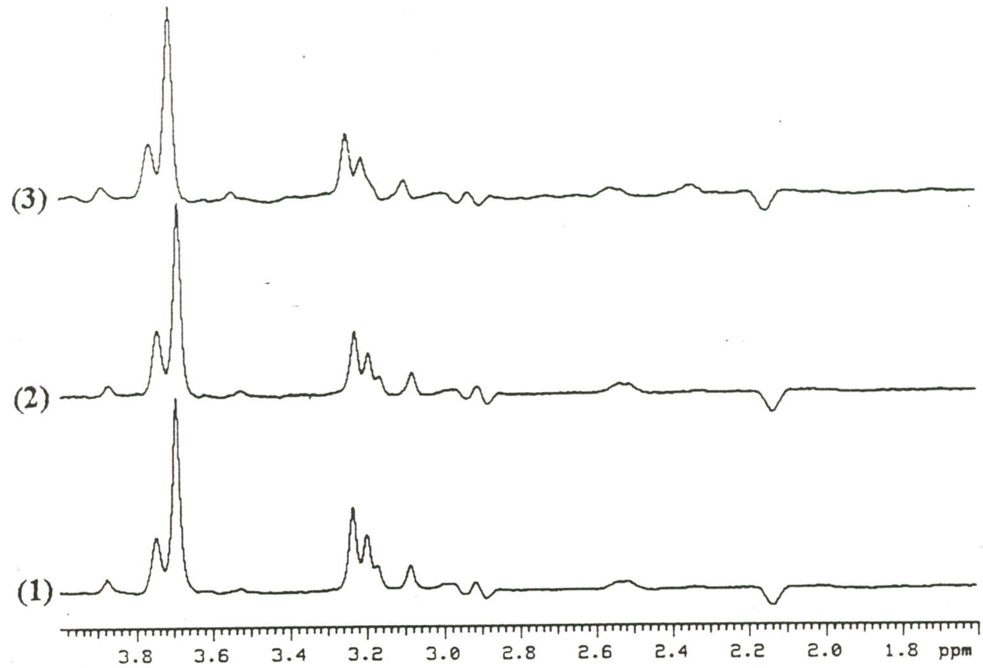

Figure 4 [1]*H NMR spectra(in vitro,2.5h)*

(1) Normal rabbit erythrocyte

(2) 5μg/m Eu(NO$_3$)$_3$

(3) 0.5μg/mLEu(NO$_3$)$_3$

Conclusions

1. When Ln(NO$_3$)$_3$ was given to rabbit in vivo, the REE mostly bound to micromolecular ligands(M_w<10^3) and the concentration fell quickly with metabolism.

2. By using ultrafiltration and chromatography techniques, we found that REE can interact with the proteins IgG、 Tf、 Alb, and among them, Tf species had a higher REE content.

3. In vivo, no transmembrane behaviours happened either in different doses(0.025~5.0mg/kg) or at different times(5min~24h). In vitro, under 0.01mg/mL Ln(NO$_3$)$_3$(Ln=La,Eu) concentration, no transmembrane behaviours happened; if the concentration of Ln(NO$_3$)$_3$ was higher than 0.01mg/mL, somehaemoglobin leaked out.

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Assessment of the potential toxicity of lanthanum (la) for aquatic organisms

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Abstract

The toxic potential of Lanthanum was assessed in short-term experiments with bacteria (Pseudomonas putida) and fresh water algae (Scenedesmus subspicatus), and in prolonged toxicity tests with Daphnia magna (21-day reproduction test) and fish (Cyprinus carpio). The experimental designs were based on international guidelines. Lanthanum was applied as lanthanumchloride and analytical support of the test concentrations was based on analyses of samples taken during the various tests. The lanthanum concentrations were far from stable during any of the tests performed. The instability of the lanthanum concentrations was contributed to the fact La^{+++} precipitated out of the water phases by forming insoluble salts with CO_3^{--} present in all test media, but most dramatically with PO_4^{--} present in the media used in the algal and bacterial tests. The exponential decline of dissolved lanthanum concentrations resulted in relative low average exposure concentrations. However, the repetitive exposure to the higher initial dissolved lanthanum concentrations in the semi-static tests with fish and Daphnia magna caused significant effects on survival of these organisms. Hence, toxicity parameters based on the geometric means of the highest and the lowest measured concentrations tended to overestimate the toxic potential of lanthanum for aquatic organisms. The reproductive capacity of Daphnia magna appeared to be the most sensitive parameter for possible toxicity of lanthanum with a NOEC value of 150 µg/l.

Keywords: Aquatic toxicity Lanthanum Lanthanides Rare earth metals Daphnia magna Carp Bacterial toxicity Fresh water algae Invertebrate reproduction Prolonged fish toxicity

Introduction

Lanthanum is one of the rare earth metals (REMs). The REMs consist of a group of metals defined as lanthanides and the elements Yttrium and Scandium. Environmental contamination by REMs is a concern by the production of phosphoric acid from natural sources. The main sources consist of sedimentary and igneous rock. The igneous rocks normally have a much higher content of REMs than the sedimentary rocks. Further the content of REMs depends on the origin of the rocks and total concentrations can vary from 100 to 6000 mg/kg.

The waste stream of a phosphoric acid plant consists of a gypsum slurry. This slurry is formed by suspending gypsum from the "repulp" filter in the phosphoric acid plant in river water. The slurry can be divided into a liquid and a solid phase. The liquid phase can be regarded as a saturated gypsum solution in which a number of impurities are solubilized. REMs are mainly present as insoluble salts (phosphates or fluorides) and therefore concentrations of free REMs are believed to be relatively low. There exist hardly any data on the potential toxicity to aquatic organisms of REMs in case of chronic exposure. Hence, the primary objective of the studies reported here was to assess the toxic potential of Lanthanum as a representative of the REMs in different trophic levels of the aquatic environment, including bacteria, fresh water algae, crustaceans and fish. In addition, the behaviour of Lanthanum introduced as a soluble salt (chloride) was examined in the various test media applied.

Materials and methods

Test substance : Lanthanum (LaCl3.7H2O), a colourless crystalline powder supplied by Fluka (Product No. 61490) with a purity of > 99.0%.

The test media used in the different tests were based on those prescribed by the ISO Standards for acute toxicity testing (1) and the algal growth inhibition test (see Table 1).

Table 1 *Compositions of the test media used in the various experiments.*

Test medium 1 Carp and D. magna[1]	**ISO**	**Test medium 2 Fresh water Algae**	**ISO**	**Test medium 3 P. putida**	**Bacteria 1**
Ca^{2+}	80 mg/l	NH_4Cl	15 mg/l	$NaNO_3$	500 mg/l
Mg^{2+}	12 mg/l	$MgCL_2.6H_2O$	12 mg/l	K_2HPO_4	120 mg/l
Na^+	15 mg/l	$CaCl_2.2H_2O$	18 mg/l	KH_2PO_4	60 mg/l
K^+	3 mg/l	$MgSO_4.7H_2O$	15 mg/l	$MgSO_47H_2O$	200 mg/l
Cl^-	145 mg/l	KH_2PO_4	1.6 mg/l	$C_6H_{12}O_2.H_2O$	2 mg/l
SO_4^{2-}	49 mg/l	$FeCl_3.6H_2O$	80 µg/l	Fe (III) citrate	0.5 mg/l
HCO_3^-	47 mg/l	$Na_2EDTA.2H_2O$	100		
		H_3BO_3	185		
		$MnCl_2.4H_2O$	415		
		$ZnCL_2$	3		
		$CoCl_2.6H_2O$	1.5		
		$CuCl_2.2H_2O$	0.01		
		$Na_2MoO_4.2H_2O$	7		
		$NaHCO_3$	50		
Hardness	218 mg $CaCO_3$	Hardness	24 mg $CaCO_3$/l		

1 The test medium used in the reproduction test with D. magna contained additional trace elements as prescribed for M7-medium by Elendt (2).

A pretest was performed to monitor the course of dissolved lanthanum concentrations in de-ionized water (Milli-Q), ISO-medium and the bacterial medium. The nominal concentration was 100 mg of $LaCl_3.7H_2O$ per litre in the first two media and 125 mg/l in the bacterial medium.

The various test solutions were prepared starting from a stock solution prepared by dissolving a weighed amount of $LaCl_3.7H_2O$, corresponding with 100 mg La, per litre medium. Preparation of the test solutions used in the bacterial test started with a stock solution of 100 mg $LaCl_3.7H_2O$ per litre corresponding to 37.5 mg La/l. The pH of this solution was adjusted to 7.8. The stocks were then stirred overnight (at least 20 hours) to reach a stable Lanthanum concentration. After stirring, the pH was adjusted to 7.8 and the stock solution was filtered through a 0.45 μm filter. The filtrate was then tested as a 100 % saturated solution and used for the preparation of the lower test concentrations. At the start of the test, all test solutions were clear. There was no indication of precipitation of the test material.

Test organisms:

The bacterial multiplication test was performed with Pseudomonas putida, an aerobic gram-negative bacterial species from the genus Pseudomonas, being a natural inhabitant of surface waters. The bacteria originated from a stock culture stored in liquid nitrogen. The stock cultures had been supplied by the BGA, Berlin, Germany and belonged to the strain: MIGULA, Berlin 33/2 (DSM 50026).

The fresh water algal test was performed with Scenedesmus subspicatus (strain: 86.81 SAG) obtained from our own laboratory cultures.

Neonates of Daphnia magna (Crustacea, Cladocera) (Straus, 1820) not older than 24 hours were collected from our own laboratory culture. The culture was kept at a temperature between 18 and 22°C. The culture was fed daily with a suspension of fresh water algae (Chlorella pyrenoidosa).

Carp (Cyprinus carpio) were supplied by Zodiac, proefacc, "De Haar Vissen", L.U. Wageningen, the Netherlands. Mean fish length was 3.1 cm (s.d. = 0.11 and n= 10) and mean fish weight was 0.90 g (s.d. = 0.14 and n= 10). The carp were fed daily with Trouvit. In the batch of fish used for the test, mortality during the seven days prior to the start of the test was less than 5%. Water quality parameters were checked weekly.

Experiments:

The studies with lanthanum were conducted during the period of January to March, 1995. The test procedures were in compliance with those described in the DIN 38412 Guideline Part L 8, 1991 (3), and the OECD guidelines for Testing of Chemicals, guidelines Nos. 201 (4), 202 Updated draft (5) and 204 (6). Further, all studies described in this paper were conducted in compliance with the most recent edition of the OECD Principles of Good Laboratory Practice.

Bacterial cell multiplication inhibition test:

Bacteria originating from a 7-day old stock culture of Pseudomonas putida were incubated in pre-culture medium for ca. 7 hours. Subsequently the extinction of the monochromatic radiation was measured at 436 nm for a 10 mm layer of the prepared calibration solutions and of the bacterial suspension, respectively. Formazin standard suspension was used for calibration. On the basis of the values measured, the final turbidity value of the bacterial suspension was adjusted up to TU/F/436 nm = 50. The actual test was performed in 100 ml Erlenmeyer flasks at an incubation temperature of 21 + 1°C for 16 ± 1 hours. Three parallel dilution series in 100 ml Erlenmeyer flasks covered with aluminium caps were prepared from the formulated test substance stock solution. An extra flask containing 32.8 mg/l lanthanum was made up identically, but without including the bacterial suspension to check for bacterial influence. During incubation the bacteria were kept in suspension by continuous shaking.

Fresh water algal growth inhibition test:

The algae test was started with an initial cell density of 2 x 104 cells/ml. Test duration was 72 hours and the algal suspensions were continuously exposed to light of TLD-lamps of 18 Watt (Philips, Spain) yielding 7000 - 8000 lux in an incubator.

The temperature of incubator was controlled between 21 and 22 °C. During incubation the algal cells were kept in suspension by continuous shaking. Exponentially growing algal suspensions were exposed to 10, 18, 32, 56 and 100 % (v/v) of a saturated solution of lanthanum in test medium. The experiment consisted of three replicas of each test concentration, six replicas of the blank-control and one replica at the highest test concentration without algae. At 24, 48 and 72 hours cell densities were determined by spectrophotometric measurement of samples at 720 nm using a Lambda Spectrophotometer (Perkin-Elmer, Illinois, USA), with a cuvette of 5 cm path-length. Algal medium was used as blank. The pH decreased with increasing La concentration and ranged from 6.7 at the highest concentration to 8.4 in the untreated control.

21-Day Reproduction test with Daphnia magna:

The daphnids were exposed individually from the start of the 21-day exposure period. Ten neonate daphnids were used per test concentration, while the untreated control group consisted of 20 daphnids. Each test vessel (8 x Ø4 cm, all-glass covered by a perspex plate) contained 50 ml of test solution. The study was

performed under semi-static conditions, i.e. the whole volumes of test solutions were renewed three times a week.
Daily, defined volumes of different Chlorella pyrenoidosa suspensions were added as feed for the daphnids. The ration was 1.106 cells/ml/day during the whole test, except for day 3 and 4 (0.85.106 cells/ml/day) and day 5 to day 13 (0.5.106 cells/ml/day). There was no record of feeding on day 19. Every workday, the number of living, immobile or dead parental daphnids were recorded. Dead daphnids were removed when observed.
Further recordings included the presence of eggs in the brood pouch, the appearance of unhatched eggs and every workday the number of newborn young was counted and the condition of the young recorded. Thereafter the young were removed. The pH ranged within 7.0 and 8.4 and differences in pH between old and fresh solutions only incidentally exceeded 0.5 units. The oxygen concentration in all test solutions remained > 7 mg/l during the whole exposure period. Temperature of the medium varied between 20 and 21 °C. Room temperature remained within the range of 19 to 23 °C. The mortality of the parental daphnids in the controls did not exceed 20%. The average cumulative number of young per female in the controls after 21 days was 109 ± 34.7.

21-Day Prolonged toxicity test with carp:

Ten fish were exposed per concentration and control with a loading of ca. 0.9 g of fish/litre. Illumination consisted of a 16 hours photoperiod daily. The test period was 21 days. Test vessels (20 litres) were all glass covered by a removable glass plate. The study was performed under semi-static conditions, i.e. the whole volumes of test solutions were renewed three times a week. Feeding happened twice a day with Trouvit; ration during the first week was 0.36 ± 0.04 g/vessel/day, the second week it was 0.44 ± 0.04 g/vessel/day and the third week it was 0.36 ± 0.04 g/vessel/day. The pH ranged from 7.6 to 8.1 in the freshly prepared solutions. In general, pH ranged between 7 to 8. Oxygen concentration remained > 6 mg/l except for some incidental partial or total drops of aeration. During the main part of the study, temperature of the test medium measured in the blank control varied from 20 to 21 °C.
Daily the fish were observed. Dead fish were removed when found. Fish were considered to be dead when no reaction was observed after touching the caudal peduncle combined with the absence of visible breathing movements.

At the end of the test the surviving fish were rapidly killed by exposing them to ca. 1.2% ethylene glycol monophenylether in water. Then fish were weighed and measured individually (not individually identified). The fish were measured from the front to the the caudal peduncle. The purpose of the test was to determine the lowest concentration at which effects of the test substance were recorded for the first time (threshold level) and the highest concentration at which the substance still has no effect (NOEC).

Analysis of lanthanum (La) concentrations:

During the short-term experiments with bacteria and algae, samples were taken directly after filtration of the starting solution (day -1) and at the start and the end of the exposure.

During the 21-day studies, samples for analysis of actual test concentrations were taken directly after filtration of the starting solution (day -1), on days 0, 7 and 14 from the freshly prepared test solutions, and on day 21 from the remaining test solutions.

All samples were filtered through a 0.45 μm filter (except the freshly prepared test solutions which were already filtered) and acidified as follows: 4 ml of HNO3 (65 %) and 12 ml of HCl (30 %) made up to 100 ml with sample, thus 84 ml sample or comparable volume ratio for a final sample volume of 50 ml. All samples were stored in a refrigerator at ca. 4 °C until they were sent to KEMIRA, Denmark for analysis. These analyses were not performed under GLP conditions.

Determination of the lanthanum concentration was performed by optical ICP at 408.672 nm. Standards were adjusted to the different requirements so there was always one standard lower and one standard higher than the actual analysis. Standards were prepared from a stock solution containing 1000 ppm La. The stock solution was prepared from La2O3 (BDH with a purity of 99.9%) using Suprapura acids and millipore water. The acid content in the standards was adjusted so the standards and analysis contain the same concentration of acids. The calibration of the instrument was controlled by control solutions prepared from Perkin-Elmer's ICP calibration standard-1 which contained 10 ppm La. Samples from untreated test media were spiked with La and subsequently analysed to check for any interferences in the La-signal from elements present in the test media.

Data handling:

Exposure concentrations:

The average exposure concentrations during the 21-day studies were based on the geometric means of three 72-hour intervals and six 48-hour intervals:

$(6 \times (Cmf \times Cm48)^{1/2} + 3 \times (Cmf \times Cm72)^{1/2})/9$, where Cmf is the average concentration measured in freshly prepared solutions and Cm48 and Cm72 are average concentrations measured in the 48-hour or 72-hour old media, resp.

Bacterial cell multiplication inhibition test:

The percentage of cell multiplication inhibition for each tested concentration was calculated as follows:

$$I = \frac{Bc - Bn}{Bc - Bo} * 100$$

I	Cell multiplication inhibition in %
Bn	Measured biomass (extinction) at the end of the test period for the nth concentration of test material
Bc	Measured biomass (extinction) at the end of the test period in the control batch
Bo	Measured biomass (extinction) at the start of the test (t=0) in the control batch

Fresh water algal growth inhibition test:

A calibration curve expressing cell density against extinction was used to calculate the cell densities from the extinctions measured in the various test solutions at different points in time during the test period.

The area below the growth curve was calculated using the formula:

$$A = \frac{N1-N0}{2} \times t1 + \frac{N1+N2-2N0}{2} \times (t2-t1) + \frac{Nn-1+Nn-2N0}{2} \times (tn-tn-1)$$

The average specific growth rate (μ) for exponentially growing cultures was calculated as:

$$\mu = \frac{\ln Nn - \ln N1}{tn - t1}$$

A = area
N0= nominal number of cells/ml at the start of the test
N1= measured number of cells/ml at t1
Nn= measured number of cells/ml at tn
t1= time of first measurements after beginning of the test
tn= time of nth measurement after beginning of the test

21-Day Reproduction test with Daphnia magna:

The values for reproduction observed at various concentrations of the test substance in water were expressed as mean number of living young per parent. For each concentration the results of reproduction were tested for normality and for homogeneity of variance. Furthermore, these data were statistically tested using an ANOVA test followed by mean comparison tests (Tukey Test and Williams' Test).

The overall threshold level of effect and the overall NOEC were determined on basis of these statistics. The EC50 (immobilisation) and the EC50 (reproduction) were calculated.

21-Day Prolonged toxicity test with carp:

Statistical analysis was performed on body weights and lengths recorded at the end of the study. The data were tested for normality and for homogeneity of variance. Furthermore, these data were statistically tested using an ANOVA test followed by the Williams' test and the Tukey method of multiple comparison of the means.

Results

In the pretest the recoveries of free lanthanum in ISO-medium showed an exponential decline during the first 6 hours of incubation reaching a final level of ca. 0.7 mg/l (see figure 1). This level was reached almost instantly after mixing of LaCl with the medium used in the bacterial growth test. Further, lanthanum appeared to be hydrolytically stable for at least 96 hours in de-ionized water. Therefore, we decided to use a semi-static system in the prolonged toxicity tests and a pre-incubation of the stock-solutions for a period of 24 hours to reach a stable La-concentrations.

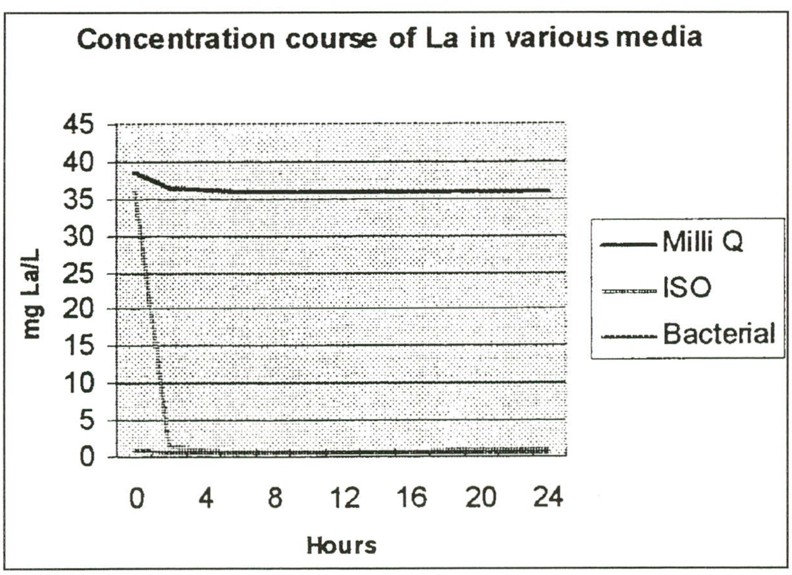

Figure 1 Lanthanum concentrations during incubation in different aqueous media

Measured concentrations:

Table 2 summarizes the results of chemical analysis of the samples taken during the various tests.

Table 2 *Concentration ranges of lanthanum measured in samples taken during the various tests.*

Test group	Exposure range (mg La/l)	Mean [La'] mg/l	Exposure range (mg La/l)	Mean [La'] mg/l[1]	Exposure range (mg La/l)	Mean [La'] mg/l[1]
Control	-	-	-	-	-	-
1	nd - 3.90	-	0.01 - 0.51	0.15	0.04 - 0.48	0.19
2	nd - 6.61	-	0.05 - 1.05	0.28	0.03 - 0.98	0.26
3	nd - 11.1	-	0.11 - 2.50	0.48	0.03 - 2.27	0.38
4	7.8 - 19.1	12.2	0.07 - 5.46	-.67	0.18 - 12.1	1.48
5	31.1 - 34.3	33	2.93 - 7.95	4.83	6.51	6.51

nd = below detection level

[1] Average exposure concentrations based on the geometric means of three 72-hour intervals and six 48-hour intervals.

Table 2 does not include concentrations measured in the bacterial multiplication inhibition test as no detectable lanthanum concentrations were found in any of the samples. This was probably due to instantaneous precipitation of lanthanum with the phosphate present in the test medium.

In contrast to what was expected based on the pretest, the concentration of lanthanum hardly decreased the 24-hour period of stirring prior to the start of the exposure or the renewal of the test solutions. Although the method of preparation was kept standard in all tests, there was a relative high variation between the recoveries of lanthanum in the freshly prepared solutions especially during the fish test. Analysis of the samples taken from the various dilutions showed that measured concentrations were in agreement with the dilution steps. The actual lanthanum concentrations decreased substantially during the periods between renewals, but an exponential decline as seen in the pre-test was recorded only in the higher dilutions of the filtrates.

Bacterial cell multiplication inhibition test:

No inhibition of cell multiplication was observed in the test with P. putida. The toxicity threshold value (EC10) of lanthanum could not be determined. The inoculum used in the control batch was multiplied by a factor 92 within the test period. The EC10 and EC50 values of the reference substance 3,5-dichlorophenol determined for Pseudomonas putida were 14.4 and 25.0 mg/l respectively.

Fresh water algal growth inhibition test:

Based on results of a range-finding test the EC50 for growth inhibition was estimated at ca. 5 mg/l and the EC50 for growth rate reduction at ca. 3 mg/l. All growth curves in the lanthanum treated solutions tested in the final EC50-test showed a retarded cell growth during the 72-hour period when compared to the exponentially growing controls. The pattern of growth observed in the range-finding test was repeated in the final study, i.e. algal growth increased to normal levels during the first 24 hours but further increase did not follow the normal exponential growth.
Inhibition of cell growth increased with increasing La concentration and reached almost 100 % at 34 mg/l (see Table 3). Growth rate reduction increased with increasing concentration and additional reduction of growth rate was recorded at all La concentrations during the last 48 hours of exposure reaching 100 % reduction at 19 mg/l.

Table 3 *Percentage inhibition of cell growth and percentage reduction of growth rate.*

Initial concentration La (mg/l)	Cell growth Inhibition 0-72 h (%)	Growth rate Reduction 24-72 h (%)	Growth rate Reduction 0-72 h (%)
3.9	46.9	33.6	21.3
6.6	32.2	49.2	19.5
11	40.9	67.8	27.7
19	66.0	100	54.4
34	99.5	100	100.0

21-Day Reproduction test with Daphnia magna:

The extent of parental mortality increased with increasing La-concentration. All parental Daphnia died within the first 4 days of exposure at the highest concentration, whereas all survived the 21-day test period at the lowest test concentration. The average reproductive capacities of Daphnia exposed to the solutions containing 0.48 to 4.8 mg La/l were almost equally suppressed during the first 18 days of exposure (see Table 4). After the total exposure period of 21 days, the average numbers of offspring at these concentrations were still ca. 20 % below the controls and almost 40 % below the average number recorded at the lowest test concentration. However, the standard deviations were rather high due to relatively large variations between individual reproduction values. Consequently, statistical analysis failed to show significant effects on reproduction comparing the values of the different groups (Tukey test, P=0.05) or those of the treated groups versus the control (Williams test, P=0.05). During the total exposure period of 21 days, no significant (>10%) immobilisation (including mortality) of newborn young was observed at any of the test concentrations. Also, no biologically significant numbers of unhatched eggs were observed during the reproduction phase.

Table 4 Cumulative mean number of living young per concentration[1].

Concentration (mg/l)	Day 14		Day 18		Day 21	
	Mean	%	Mean	%	Mean	%
Blank	57.6	100	89.2	100	109	100
0.15	68.6	119	99.5	112	131	120
0.28	27.6	48	50.1	56	91.4	84
0.48	43.3	75	65	73	87.3	80
0.67	40.2	70	50.2	56	84.8	78

21-Day Prolonged toxicity test with carp:

At the highest concentration tested (100 %) all fish exposed died during the first 4 days of exposure. During the remaining test period, no additional mortality or other effects were observed until day 12. On day 12 all fish were found dead at 45 % of the saturated La-solution. This mortality was related with a rather high recovery of lanthanum in a sample taken on day 14 from the 45%-solution prepared on day 11, i.e. 12.1 mg/l. No effects were seen in the remaining test solutions during the remaining part of the exposure period.

No significant differences in body weight or length were observed between the various test groups (Tukey test, $p=0.05$) or between any of the treated groups and the control (Williams' test, $p=0.05$).

Discussion and conclusions

The lanthanum concentrations were far from stable during any of the tests performed. The instability of the lanthanum concentrations was contributed to the fact La^{+++} precipitated out of the water phases by forming insoluble salts with CO_3^{--} present in all test media, but most dramatically with PO_4^{--} present in the media used in the algal and bacterial tests. This counted for the instantaneous precipitation and disappearance of dissolved lanthanum in the water phases of the bacterial solutions. Instability of the test concentrations was also more prominent in the algae test, where the lower measured concentrations had decreased to levels below detection at the end of the 72-hour test period. The higher instability in the algae test was probably related to binding of La^{+++} to the surface of the algal cells, as the decline of dissolved lanthanum concentrations was most prominent in the solutions with continuous algal growth, a factor that also may have to some extent played a role in the reproduction test with Daphnia magna. However, also in the fish toxicity test where no algal cells were introduced an exponential decline of actual La-concentrations was recorded during the first 48 hours of each period of renewal.

The most marked difference was observed in the behaviour of lanthanum during the 24-hour pre-incubation between the pretest and the toxicity tests. Where the actual concentration of lanthanum measured directly after dissolving in the media was in agreement with nominal during the pretest, precipitation and significantly lower recoveries were found in comparable samples taken during the toxicity tests. After dissolving, the lanthanum concentrations in 100%-filtrates used during the toxicity tests decreased rather slow during the 24-hour incubation period and also much slower during the exposure periods than in the solutions prepared in the pretest. Further, the dissolved lanthanum concentrations measured in the dilutions of the filtrate showed an exponentially decline in contrast to the dissolved lanthanum concentrations in the filtrate itself (100%). This may be explained by the fact that the dissolved lanthanum concentrations had more or less stabilized in the filtrate during the 24-hour pre-incubation period, whereas dilution of the filtrate with fresh test media induced a destabilization of the lanthanum concentrations by introducing additional precipitation of lanthanum with the anions present in the fresh media (CO_3^{--} and PO_4^{--}).

The exponential decline of dissolved lanthanum concentrations resulted in relative low average exposure concentrations. However, the repetitive exposure to the higher initial dissolved lanthanum concentrations in the semi-static tests with fish and Daphnia magna are probably the major cause of effects on survival of these organisms. At the higher average exposure concentrations these repetitive peak exposures probably masked any long-term sublethal effects by more severe acute effects. At the lower average exposure concentrations the longer term effects may have probably been caused mainly by these repetitive peak exposures. Hence, toxicity parameters based on the geometric means of the highest and the lowest measured concentrations tend to overestimate the toxic potential of lanthanum for aquatic organisms.

The reproductive capacity of Daphnia magna appeared to be the most sensitive parameter for possible toxicity of lanthanum with a NOEC value of 150 µg/l. Comparing this value to those known for other metals like zinc, lead, copper, cobalt, mercury and cadmium, it proves to be significant higher. According to literature the EC16 for reproductive impairment for these metals are all below 100 µg/l: 70 µg/l for zinc, 30 µg/l for lead, 22 µg/l for copper, 10 µg/l for cobalt, 3.4 µg/l for mercury and 0.17 µg/l for cadmium (7). Further, cadmium and zinc are also more toxic to other aquatic organisms than lanthanum including both vertebrates and invertebrates (8,9). It can therefore be concluded that the toxicity of lanthanum is low in comparison with other metals.
Table 5 summarizes the values for the various toxicity parameters.

Table 5 *Toxicity of lanthanum in fresh water algae, crustaceans and fish.*

Toxicity parameters	Concentrations expressed in mg La/l		
	Algae[1] S.capricornutum	Crustaceans D.magna	Fish C.carpio
72-hour EC50	13-16		
72-hour EC10	1.3-1.4		
21-day LC50		0.556	> 5
95% fiducial limits		0.419 - 0.916	
21-day EC50		> 0.67	-
LOEC		0.28	-
NOEC		0.15	0.38

1 Based on initially measured concentrations.

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INITIAL ASSESSMENT OF THE EFFECTS OF APPLYING RARE-EARTH ELEMENTS ON SOIL INTEGRITY

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Abstract

The potential effects of applying rare-earth elements as micro-fertilizer on the integrity of soil ecosystem are screened in this study. Through plots experiments, the influence of REEs on soil pH, Eh, cation exchange capacity (CEC), soil content of organic matter (OM), concentration of soil available nitrogen (AN) and phosphorus (AP), as well as the enzymatic activities of soil urease was examined after application of different doses of REEs. No obvious influence of REEs application on soil pH, Eh, CEC, OM could be observed. The results show that REEs application may interfere the supplement and metabolism of soil available nitrogen and phosphorus in higher doses in plot experiments. The findings were confirmed by pot experiments, where soils from experimental plots were used and REEs were applied in concentrations of 0-30 $mg.kg^{-1}$ of soil.

Key Words: rare-earth elements; soil chemistry; nutrients; soil integrity

Introduction

In China, extensive application of REEs-containing fertilizer for agriculture begins in the 70's. Research works agree on that REEs in trace amount can increase crop and cotton production[1], improve the bioavailability of calcium and manganese in soil, stimulate synthesis of chlorophyll, improve the seedling and seedling development[2], etc. While there are intensive discussions on the beneficial effects of applying REEs for different plants, on the mechanism of the stimulating effects, as well as on the environmental aspects of REEs, less is known about the harmful effects of REEs on the integrity of agricultural soil. Some research works have illustrated that application of REEs to agricultural soil can improve plant uptake of nitrogen[3] and phosphorus[4]. In contrary, other researchers stated that REEs are irrelevant to the metabolism of nitrogen and phosphorus[5] in soil-plant system and application of REEs may interfere the metabolism of nutrients in soil and, therefore, reduce their bioavailability[6]. It is obvious that previous conclusions made on the effects of REEs on soil integrity were contradictory.

In this paper the influence of REEs on physico-chemical properties of soil and on bioavailability of soil nitrogen and phosphorus have been screened, in order to further elucidate the long-term effects of applying REEs-containing fertilizer on agricultural soil ecosystems.

Methods

Four plots of 12 m^2 each located near Beijing were used for the experiment. These plots were planted with *Spring corn* and sprayed with 0, 16, 32 and 64 $mg.m^{-2}$ of a REEs-containing fertilizer (commercial name: Chang-Le) during seedling. Soil samples were taken before REEs application and after the harvest for chemical and biochemical analysis.

In the pot experiment, soil was taken from the experimental plot. The soil was dried and passed through a 1 mm sieve. Each 0.5 kg of soil was put into a ϕ12x12 cm plastic pot and 10 seedlings of *Spring corn* were planted. REEs were applied in concentrations of 1, 5, 10, 20 and 30 $mg.kg^{-1}$ of soil after the seeds had sprouted and grown for a few days. Soil was sampled both before and three weeks after the REEs application.

The composition of REEs in the fertilizer, concentration of the spraying solution, as well as the elementary ratio between La and other elements are shown in Table 1.

Table 1 Composition and concentrations of REEs in applied fertilizer

Element	mg/g fertilizer	Concentration ($\mu g.L^{-1}$)	La/RE
La	132.49	39746	
Ce	63.25	18975	2.1
Pr	26.39	7918	5.0
Nd	40.01	12004	3.3
Sm	4.02	1206	33.0
Gd	2.46	738	53.9
Dy	0.05	16	2484.1
Total	268.62	80587	

Soil samples were dried at room temperature and they were ground and sieved before analysis. The pH, soil redox potential (Eh), soil cation exchange capacity (CEC), content of soil organic mater (OM), soil available nitrogen (AN) and phosphorous (AP) were measured, according to recommended methods[7,8]. Enzymatic activities of soil urease were measured following the methods proposed by Wu[9].

Considering the spatial and temporal variations of the soil ecosystem itself, the effects were assumed to be positive if (a) the differences of measured values after REEs application exceeded two folds the standard deviation of the background value (here it refers to the standard deviation of 4 experimental plots before REEs application, N=4); (b) there should be a dose-dependence relationship; and (c) the measured differences should be more significant in the top layer of soil than in deeper layer because REEs were known to retain mostly in top soil.

Results and discussions

It was found that the soil pH was fairly constant before and after REEs application. For example, the averaged soil pH before application of REEs was 7.68 ± 0.31 (N=4) while the maximum change of pH after application was only 0.46 pH units for samples of top 20 cm, well within two folds of standard deviation. The results are shown in Table 2, and no influence of REEs application on soil pH should be expected.

Table 2 Influence of REEs on soil pH

Depth (cm)	[1]Soil pH (N=4)	Change after REEs application ($pH_{after}-pH_{before}$)[2] *0*	*16*	*32*	*64*
20	7.68 ± 0.31	-0.31	-0.23	0.1	0.46
40	7.80 ± 0.21	-0.89	-0.26	0.22	-0.12
60	7.75 ± 0.22	-0.39	-0.37	0.1	0.16
80	7.53 ± 0.38	-0.73	-0.21	0.58	0.61
100	7.44 ± 0.24	-0.71	0.10	0.26	0.48

[1] Mean and standard deviation for 4 plots before REEs application.
[2] Differences of the measured values before and after application in each treatment.

For some soil samples, soil Eh changed after REEs application, as shown in Table 3. Since there was no obvious dose-dependent or depth-dependent changes of soil Eh, therefore the changes in soil Eh should be due to natural variation or inaccuracy in measurements. No influence of REEs application on soil Eh can be expected according to the measured values.

Table 3 Influence of REEs on soil Eh (mv)

Depth (cm)	Soil Eh(mv) (N=4)	Changes after REEs application ($Eh_{after}-Eh_{before}$)* *0*	*16*	*32*	*64*
20	137 ± 4	1	7	7	-2
40	127 ± 8	14	9	3	26
60	130 ± 7	13	3	3	2
80	130 ± 8	0	2	10	-14
100	134 ± 7	11	-10	0	9

* See Table 2

For most of soil samples treated with REEs, CEC decreased after application while this parameter showed less variation in the control. In the experiments, REEs were applied in forms of nitrates which are soluble in water. One can expect a decrease in soil CEC because they can replace abundant cations, such as potassium and calcium, on the surface of soil particulate and cause a decrease in soil CEC. The changes after REEs application were significant only for some samples, but for most of the samples the changes were within the standard deviation. Also, there were no dose-dependent and/or depth-dependent changes in the experiments. Therefore the influence of REEs application on soil CEC could not be confirmed in this study. The results are shown in Table 4.

Table 4, Influence of REEs on soil cation exchange capacity ($mg.kg^{-1}$)

Depth (cm)	Soil CEC (N=4)	Changes after application ($CEC_{after}-CEC_{before}$)*			
		0	***16***	***32***	***64***
20	199 ± 7	1	-17	-5	-3
40	168 ± 4	2	-8	-3	-3
60	154 ± 5	6	-7	-10	-5
80	154 ± 7	-1	-7	-7	-12
100	154 ± 6	11	-15	-1	-6

* See Table 2

Irregular changes in the content of soil organic matter were observed and there was a significant increase in soil organic matter in top 0-40 cm for treatments with 16 and 64 $mg.m^{-2}$ of REEs. For soil samples treated with 32 $mg.m^{-2}$ of REEs and for soil samples in deeper layers, there were no significant changes. Increase in soil OM after REEs application could be the consequences of the stimulation effects of REEs on soil fauna and flora. Since there were no dose-dependent changes, one can hardly reach a conclusion that REEs application could affect soil OM. Further research is needed in order to observe the possible effects of REEs on soil OM metabolism. The results are shown in Table 5.

Table 5, Influence of REEs on content of soil organic matter (OM, %)

Depth (cm)	Soil OM (%) (N=4)	Changes after application ($OM_{after}-OM_{before}$)*			
		0	***16***	***32***	***64***
20	0.87 ± 0.18	0.04	0.30	-0.18	0.65
40	0.76 ± 0.06	0.24	0.63	0.07	0.53
60	0.77 ± 0.20	0.08	-0.05	0.18	0.22
80	0.66 ± 0.18	0.16	0.35	0.02	0.06
100	0.80 ± 0.12	0.62	0.11	-0.65	-0.24

* See Table 2

The concentration of soil available nitrogen decreased after application of REEs, in contrary to control where it shows increasing or relatively stable values (Table 6). Changes in measured values after REEs application were significant, especially for higher dosages. Dose-dependent relationship could be observed for the top 20 cm soil samples, where increased dosage of REEs caused lower concentration of soil available nitrogen. The results are shown in Table 6.

It was surprisingly observed that enzymatic activity of soil urease followed the similar changes as soil available nitrogen. Fig.1 shows the changes of soil available nitrogen and soil urease for samples of top 20 cm. Concentrations of soil available nitrogen increased of about 11 percents in control group, while it decreased of 19.7, 32.5 and 46.4 percents in treatments of 16, 32, and 64 $mg.m^{-2}$, respectively. Similarly, enzymatic activity of soil urease increased of about 20 percents in the control, while it decreased of 15.4, 31.3 and 54.5 percents in treatments of 16, 32 and 64 $mg.m^{-2}$, respectively. For both soil available nitrogen and soil urease, influence of REEs was dose-dependent at top 20 cm.

Table 6, Influence of REEs on soil available nitrogen ($mg.kg^{-1}$)

Depth (cm)	Soil AN (N=4)	Changes application (AN_{after}-AN_{before})*			
		0	***16***	***32***	***64***
20	21.3 ± 3.5	2.2	-1.4	-7.5	-11.7
40	19.0 ± 2.7	0.8	-7.3	-7.8	-4.2
60	20.7 ± 2.1	-0.4	-4.2	-13.6	-10.2
80	15.3 ± 5.7	6.7	-6	-10	-11.8
100	19.1 ± 4.2	5.5	-13.1	-2.8	-11.3

* See Table 2

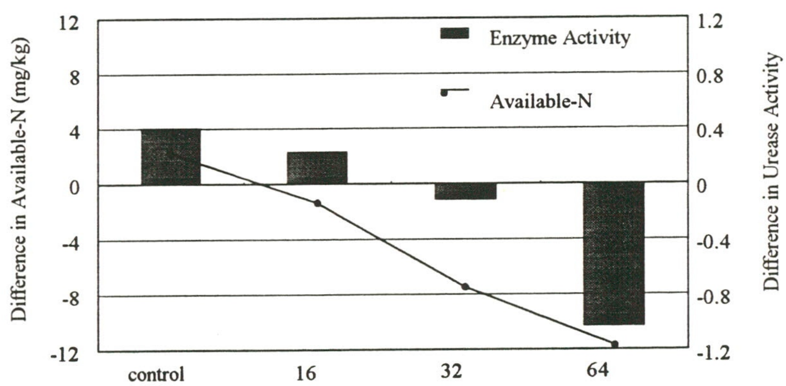

Fig. 1 Influences of REEs on soil available nitrogen and enzymatic activity of Urease

To verify the influence of REEs on soil available nitrogen and soil urease, pot experiments were carried out. The results are shown in Fig. 2. Based on the results, REEs could promote the activity of soil urease and increase the concentration of available nitrogen when applied in lower dose (<1 mg/kg soil). The activity of soil urease was inhibited in higher doses of REEs (>5 mg/kg soil), where the available nitrogen concentration decreased with increasing REEs concentration. The results from pot experiments supported the findings in plot experiments.

From both plot and pot experiments, the results indicated an influence of REEs on supplement and metabolism of soil available nitrogen. This finding is obviously inconsistent with the observation that REEs can promote plant growth. Therefore it is necessary to further examine the contradictions and to give explicit mechanisms.

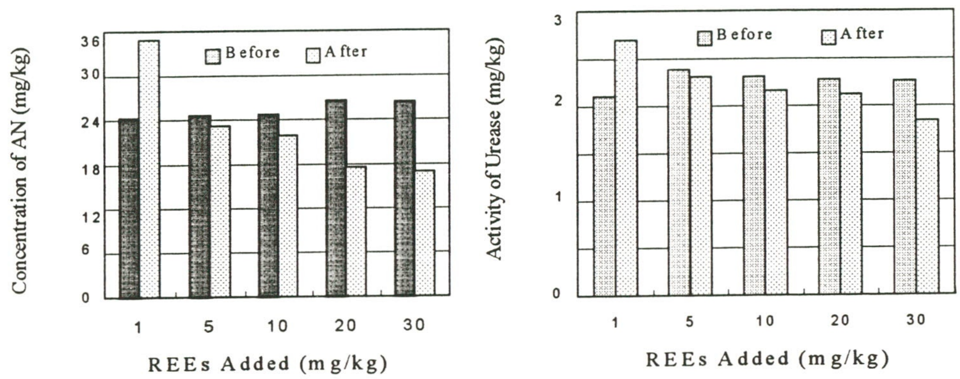

Fig. 2, Influence of REEs on soil available nitrogen and enzymatic activity of soil urease in pot experiemnts

The influence of REEs on soil available phosphorus was also obvious in top 20 cm. As demonstrated in Table 7, soil available phosphorus increased in the control plot and showed a slight increase even in treatment with 16 $mg.m^{-2}$. But it decreased significantly after application 32 and 64 $mg.m^{-2}$ of REEs. There was an obvious dose-dependent relationship between the dosages of REEs and changes in AP after application. For samples of deeper layers, the changes were neither significant nor dose-dependent. As stated before, the effects of REEs on concentration of soil available phosphorus could be estimated.

Table 7, Influence of REEs on soil available phosphorus (mg/kg)

Depth	Soil AP	Changes after application (AP_{after}-AP_{before})*			
(cm)	(N=4)	*0*	*16*	*32*	*64*
20	12.6 ± 1.3	1.9	0.3	-2.4	-6.0
40	11.5 ± 2.0	0.0	-2.4	-1.2	--
60	6.9 ± 0.7	4.5	0.2	1.4	0.2
80	9.6 ± 1.2	-2.4	-0.7	1.7	-0.5
100	11.0 ± 1.3	-0.5	-2.4	-2.1	-2.1

* See Table 2

The possible mechanism behind the influences of REEs on soil available phosphorus could be related to the formation of insoluble salt of REEs phosphates, which causes the decrease in concentration of total soluble phosphorus. This is highly possible considering REEs in concentration as high as 80 $mg.L^{-1}$ was brought to react with several $mgs.L^{-1}$ of soluble phosphate in soil solution. Besides, surface precipitation of REEs can occur where phosphates in the minerals are exposed. Nevertheless, this finding needs further confirmation.

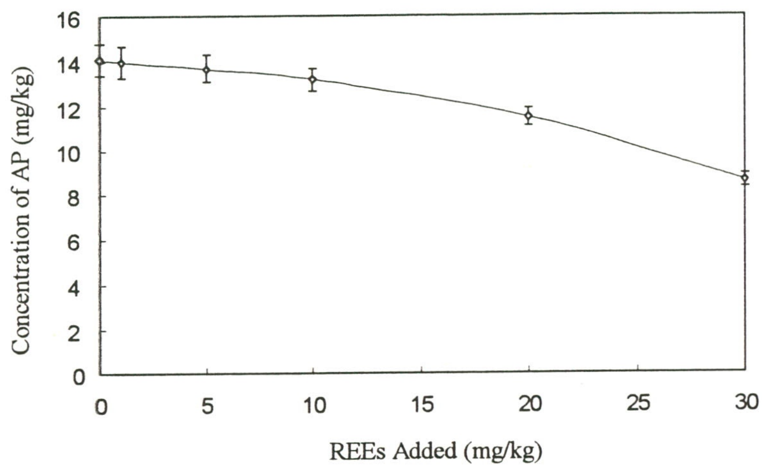

Fig. 3 Influence of REEs on soil available phosphorus in pot experiments

In pot experiments, the results showed that concentration of soil available phosphorus decreased significantly after REEs application in concentration as high as 30 $mg.kg^{-1}$. There was a negative correlation between concentration of AP and concentration of REEs added to the soils ($r = -0.961$, $p<0.05$).

Based on both plot and pot experiments, one can conclude of an influence of REEs on soil available phosphorus. Again, the results obtained with respect to the supplement of soil available phosphorus in the study were inconsistent with the promoting effects of REEs observed in previous researches.

Conclusions

In this study, the influence of REEs on soil integrity, with respect to soil pH, Eh, CEC, OM, AN, AP, as well as enzymatic activity of soil urease, were screened. Based on the results and base-lines, we concluded that REEs may interfere the supplement and/or metabolism of soil available nitrogen and phosphorus. Since this statement is obviously contradictory to the previous studies, it is necessary to carry out research on the interaction between REEs in fertilizer and the cycle of major soil nutrients.

Acknowledgment:

This work was supported by China Natural Science Foundation. The authors are grateful for the help of Ms. Mei Ma and Ms. Ping Hao.

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Research on the Scavenging Effects of Rare Earth Elements on Superoxide Anion Radical by Pulse Radiolysis Technique*

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Abstract

Using pulse radiolysis technique, the interaction between rare earth elements and superoxide anion radical was studied. It was found that rare earth nitrates, such as $La(NO_3)_3$, $Pr(NO_3)_3$, $Nd(NO_3)_3$, $Sm(NO_3)_3$, $Y(NO_3)_3$ and $Yb(NO_3)_3$ can significantly scavenge the superoxide anion radical ($O_2^{\bullet-}$) produced by pulse radiolized formate aqueous solution saturated with oxygen. The clear rate was between 28% to 92% for different rare earth elements at $5\times10^{-4} mol\cdot l^{-1}$ concentration. The distinguished dose-effect relationship existed between the rare earth nitrates concentration in the system and the $O_2^{\bullet-}$ clear rate.

Key Words: Rare earth nitrates, Superoxide Anion Radical, Scavenging Effect

* This project was supported by National Science Foundation of China and State Educational Committee of China.

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Introduction

The rare earths have been widely used in agriculture, livestock, aquatic and forestry in the People's Republic of China, since its stimulating effects on the animals and plants. And recently, they are also widely applied to medical science for preventing and treating cancer, anti-inflammation, anti-bacteria and treating burn [1-2]. A great attention has been paid to the biological effects and toxicological mechanisms of rare earth compounds. Ji et al.[3] and Cui et al. [4] separately verified the antimutagenicity and anticarcinogenicity of rare earth compounds in vivo and in vitro by bio-test.
Oxygen toxicity is defined as injurious effects because of activated oxygen species, also referred to as oxygen free radicals or oxyradicals [5-6]. The one, two, and three electron reduction products of molecular oxygen (O_2) are of particular interest. They are the superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), the hydroxyl radical ($^{\bullet}OH$), singlet oxygen and alkoxyl radical. The effects of rare earth compounds on enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and lipid peroxidation process which are all related to active oxygen were reported [7-8]. But the influence of rare earth compounds on oxygen free radicals is rarely reported[9]. This study was focused on the interaction between rare earth nitrates, such as $La(NO_3)_3$, $Pr(NO_3)_3$, $Nd(NO_3)_3$, $Sm(NO_3)_3$, $Y(NO_3)_3$ and $Yb(NO_3)_3$ and superoxide anion radical produced by pulse radiolysis method.

Materials and Methods

Rare earth oxides were supplied by Changchun Institute of Applied Chemistry, Chinese Academy of Sciences. The purity of them were more than 99.9%. Sodium formate, potassium thiocyante and other chemical agents used were all GR grade. N_2O and O_2 gas were high purity, water used was deionized-distilled water. The rare earth nitrates solution were prepared by dissolving the rare earth oxides in nitric acid and diluting with water.

The pulse radiolysis experiments were undertaken on a BF-5 model linear accelerator (Institute of Medical Apparatus and Instrument of Beijing, China),(200 ns electron pulse), 5 MeV which were delivered on the sample solutions contained in a quartz cell (1×1×1.5cm), the strength of electron beam was 200mW, pulse width was 2μs, doses of 8.5Gy was determined by potassium thiocyante solution saturated with N_2O by using syringe bubbling. All solutions were prepared just before use and were exposed to the minimum of light and bubbled with O_2 10min for test. $O_2^{\bullet-}$ was detected under 250nm wavelength.

Results and Discussion

The primary products formed by irradiation of aqueous sodium formate saturated with O_2 with high-energy pulse electron beam are H_2O_2, H_3O^+, OH^- and H_2[10], represented by follow equation:

$$H_2O \sim\sim\longrightarrow e^{-\bullet}_{aq},\ {}^\bullet OH,\ H^\bullet,\ H_2,\ H_2O_2,\ H_3O^+,\ OH^-$$

When excess $HCOO^-$exits, $HCOO^-$ transforms various radicals to $O_2^{\bullet-}$ [10], which is represented as follow:

$${}^\bullet OH + HCOO^- \longrightarrow H_2O + CO_2^{\bullet-}$$

$$H^\bullet + HCOO^- \longrightarrow H_2 + CO_2^{\bullet-}$$

$$CO_2^{\bullet-} + O_2 \longrightarrow O_2^{\bullet-} + CO_2$$

$$e^{-\bullet}_{aq} + O_2 \longrightarrow O_2^{\bullet-}$$

The concentration of $O_2^{\bullet-}$ produced in the system was detected under 250nm wavelength ($\varepsilon = 2 \times 10^3 mol^{-1} \cdot l \cdot cm$). The amount of $O_2^{\bullet-}$produced was determined according to the optical density value of the system detected under 250nm wavelength. Figure 1 reveals the growth of $O_2^{\bullet-}$ from irradiation of 0.10M HCOONa solution (pH 6.5) saturated with O_2, and 500 data points were collected. Generally $O_2^{\bullet-}$could auto-disproportionate quickly, it is stable in the test period of 800μs. The inhibition effect of $La(NO_3)_3$ on the production of $O_2^{\bullet-}$ was clearly seen in figure 2 with 28% clear rate which was defined as $(H_{control} - H_{test})/H_{control}$

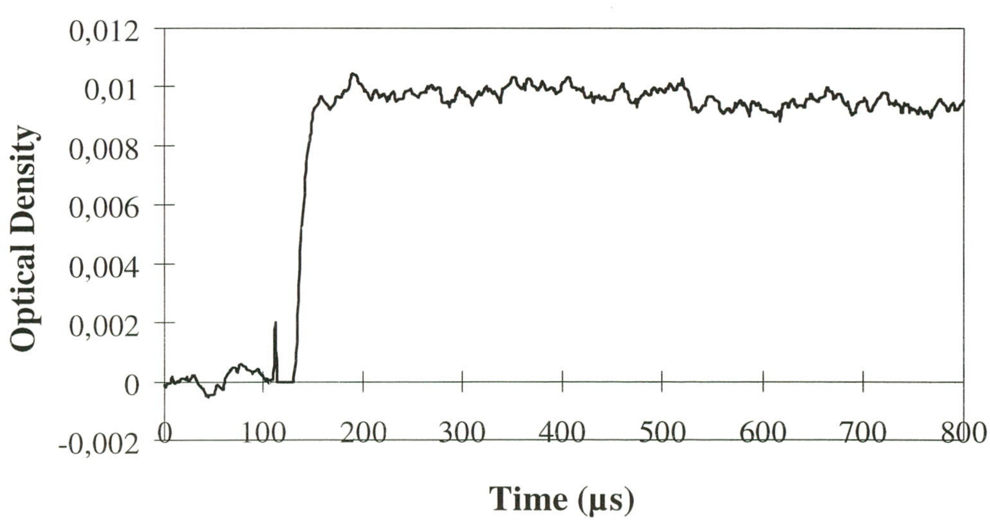

Figure 1 *The Production of Superoxide Anion Radical by the radiolysis of Sodium Formate*

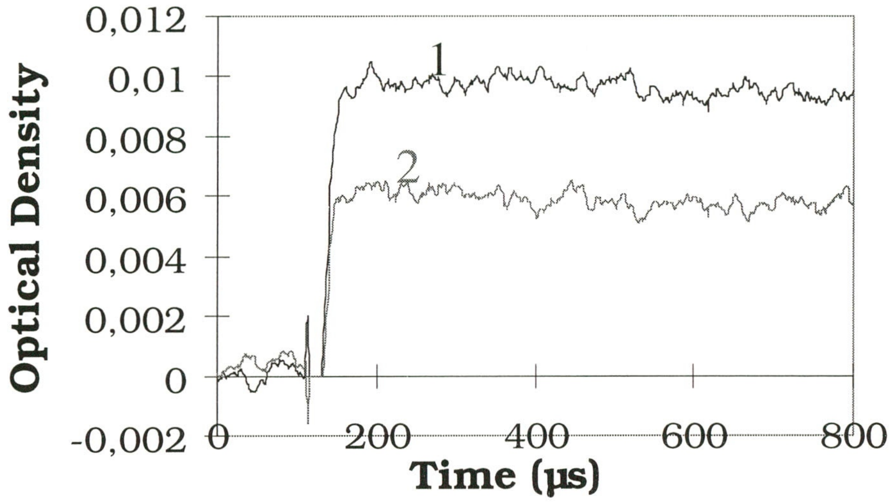

Figure 2 *The Scavenging Effect of $La(NO)_3)_3$ to Superoxide Anion Radiacal. 1: 0.01 mol/L HCOONa solution; 2: 0.01 mol/L HCOONa solution containing $5*10^{-4}$ mol/L $La(NO_3)_3$*

The influence of pH of the system on the inhibition effect of $La(NO_3)_3$ on the production of $O_2^{\bullet-}$: When testing solutions containing same concentration of $La(NO_3)_3$ ($5 \times 10^{-4} mol \cdot L^{-1}$) were carried out at different pH, the results were obtained as Figure 3. It showed that the pH of the system obviously affected the production of $O_2^{\bullet-}$. But during a small range, such as 4.50-6.50, and 7.25-7.95 we tested , there was no great difference. Therefore we chose all the systems at the same pH, 6.5, to carry out the experiment.

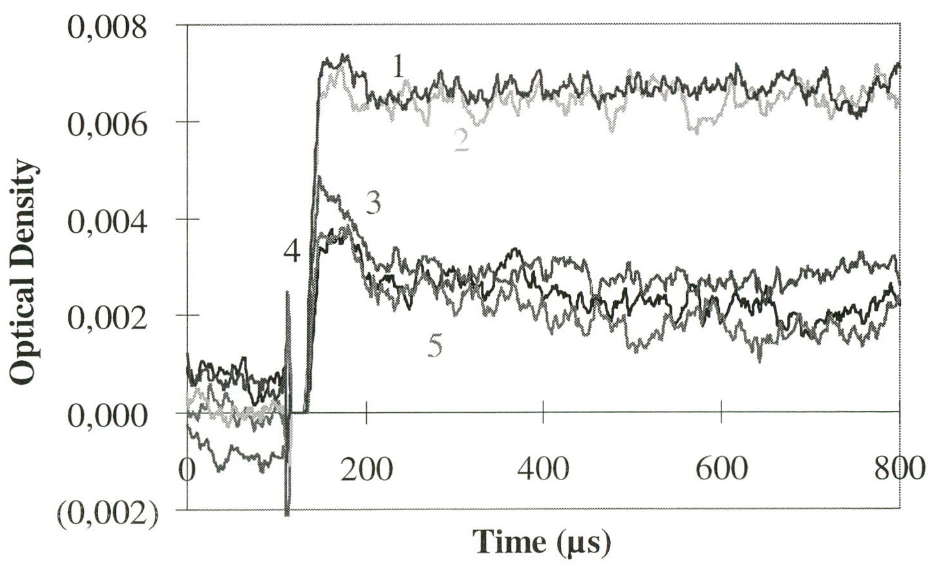

Figure 3 *The Influence of pH on the Scavenging Effect of $La(NO_3)_3$ to Superoxide Anion Radical. 1: pH = 7.25; 2: pH = 7.95; 3: pH = 6.50; 4: pH = 5.45 and 5: pH = 4.50*

The production of $O_2^{\bullet-}$ in the presence of $La(NO_3)_3$ with different concentrations: On pulse radiolysis of aqueous O_2–saturated solutions of sodium formate in the presence of $La(NO_3)_3$ with different concentrations, a distinguish inhibition effects on the production of $O_2^{\bullet-}$ related to the concentration of $La(NO_3)_3$ were observed (Fig. 4). The inhibition effects of $La(NO_3)_3$ increased with the increase of its concentration. This dose-dependent relationship indicated that the inhibition effect was mainly due to $La(NO_3)_3$.

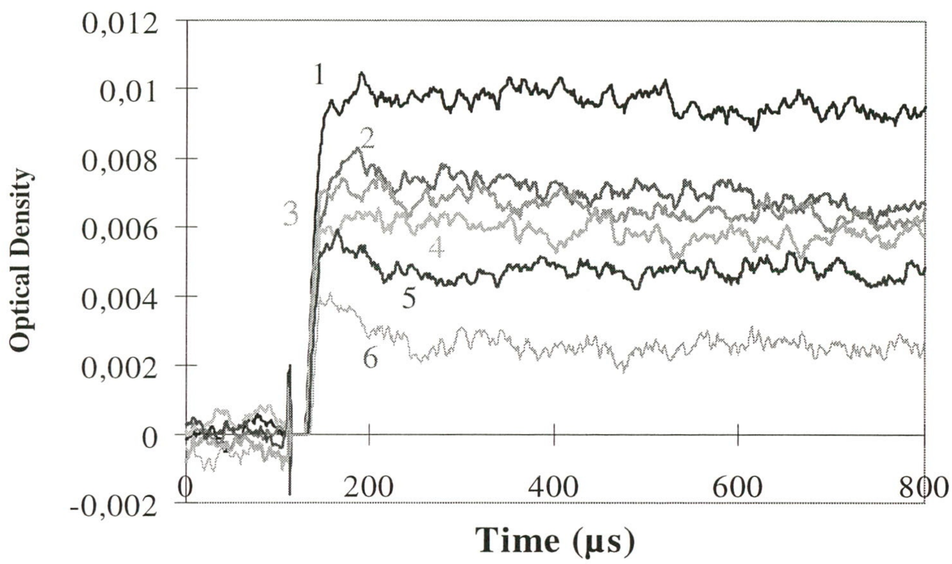

Figure 4 *The Inhibition Effects of $La(NO_3)_3$ with Different Concentrations on the Production of Superoxide Anion Radical.*
The concentrations of $La(NO_3)_3$ are: 1): zero as control; 2): $5*10^{-5}$ mol/L 3): $1*10^{-4}$ mol/L; 4): $5*10^{-4}$ mol/L; 5): $1*10^{-3}$ mol/L and 6): $5*10^{-3}$ mol/L, respectively

The production of $O_2^{\bullet-}$ in the presence of different rare earth nitrates at same concentration: Investigating the influence of different rare earth elements on the production of $O_2^{\bullet-}$, we prepared the test solution containing the same concentration ($5 \times 10^{-4} mol \cdot L^{-1}$) of different rare earth nitrates. It has been found that all these rare earths inhibit the production of $O_2^{\bullet-}$ with the similar mode as $La(NO_3)_3$, the clear extent was different. We calculated the clear rate of different rare earth elements on $O_2^{\bullet-}$ as shown in Table 1.

Table 1 *The Scavenging Effects of Different Rare Earth Elements on $O_2^{\bullet-}$ Produced by Pulse Radiolysis of Sodium Formate.*

Rare earth Nitrates	Clear rate on $O_2^{\bullet-}$ (%)
$Y(NO_3)_3$	86.1
$La(NO_3)_3$	28.6
$Pr(NO_3)_3$	90.5
$Nd(NO_3)_3$	75.3
$Sm(NO_3)_3$	92.8
$Yb(NO_3)_3$	42.2

The superoxide dismutase (SOD) are a group of metalloenzymes that catalyze the disproportionation of $O_2^{\bullet-}$ to yield H_2O_2 as follow[11]:

$$2\,O_2^{\bullet-} + 2H^+ \longrightarrow H_2O_2 + O_2$$

SOD as considered to play a pivotal antioxidant role, its importance is indicated by its presence in all aerobic organisms. It has been reported[9] that La^{3+} can increase the activity of SOD and illustrated as that La^{3+} can stabilize the active conformation of bovine blood SOD. Our results indicated that not only rare earths can increase the activity of SOD and therefore increase the clearance of $O_2^{\bullet-}$, but also they themselves can scavenge $O_2^{\bullet-}$ and take the role of anti-oxidant. This could be the chemical basis of rare earths applied for treating free radical related diseases. This work provide a direct basis for the interaction between rare earths and superoxide anion radical in vitro.

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Exchangeable Rare Earth Elements in Some Soils of China

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Abstract

Exchangeable rare earth elements (EXREE) of sixteen typical soils in China were determined by high-resolution inductively coupled plasma mass spectrometry. Results showed that the EXREE contents of the samples ranged from 3.71 to 82.33 mg/kg with an average of 18.4 mg/kg, which represents roughly 10 percent of the average value of total REE in tested soils. This results is about nine-fold high compared with the content of EXREE extracted with $Mg(NO_3)_2$. When the data of EXREE were normalized by the data of REE in chondrites, positively abnormal contents of Ce in acidic soils, negatively abnormal content of the same element in alkaline soils, and negatively abnormal contents of Eu for all soils were observed.

Key Words: rare earth elements, soil, exchangeable

Introduction

Rare earth elements (REE) have been used for agriculture for more than two decades in China. Fertilizers containing nitrate of REE or a mixture of complexes of REE (mainly La and Ce) have been manufactured. Reported yield increases of various crops treated with these fertilizers range from 5-15%[1]. The areas for applying REE in agriculture have been extending on a large scale in recent years. Although the mechanism of the growth-promoting effects of REE have not been clearly revealed, there are experimental works showing that REE have some effects on organisms in some ways. The investigation on the forms of REE in soil and their relationship with the bioavailability are important to understand the effect of REE.

On the basis of sequential fractionation, several studies on the distribution of REE in soils have been reported by Chinese researchers[2-4]. Zhu and Liu have reported the soluble REE contents of numerous soils in eastern China. They used NaOAc/HOAc as extractant at pH 4.8[5]. After correlation with field responses in crops to REE, they determined the critical values of soluble REE contents in soils. Other researchers have used H_2SO_4 plus H_2O_2[6], aqua regia[7] to extract REE. Several materials have been used to extract exchangeable REE (EXREE) in soil such as $Mg(NO_3)_2$[2], $Ca(NO_3)_2$[8], $MgCl_2$[4], and NH_4OAc[3].

Analytical techniques used to measure the extracted REE from soils include colorimetric methods[3,6], inductively coupled plasma atomic emission spectroscopy (ICP-AES)[2,7], instrumental neutron activation analysis (INAA)[4], and inductively coupled plasma mass spectrometry (ICP-MS)[8]. Although ICP-MS has been recognized for its high sensitivity together with its simplicity for REE analysis, attempts to determine trace levels of extracted REE in solutions encountered considerable difficulties. A large partion of the concentrations of EXREE in soils extracted with 0.1 $Ca(NO_3)_2$ was below the detection limit (0.05 mg/kg)[8]. So, there is a need for a more powerful instrument. In this paper we report the use of high resolution ICP-MS to measure EXREE in some Chinese soils.

Materials and Methods

Sampling

Sixteen typical soil profiles of China were collected, air-dried and ground to pass 60-mesh (0.28-mm) sieve. Total REE (TREE) were determined by ICP-MS (quadrupole type, PQ Plus II, VG Co.), and other soil properties were measured using standard methods[9]. Informations on studied soils are shown in Table I.

Table I. Informations on studied soils

Soil name		Code	Locality	Parent material	pH	Total REE (mg/kg)
Chinese name	FAO name					
Red soil (upland)	Haplic acrisols	RE-U	Jiangxi	Q_4[1)] red clay	5.2	126.9
Red soil (wild)	Haplic acrisols	RE-W	Jiangxi	Q_4 red clay	5.5	132.5
Lateritic red earth (forest)	Haplic acrisols	LR-F	Guangdong	Granite	4.2	482.2
Lateritic red earth(upland)	Haplic alisols	LR-U	Guangdong	Granite	5.0	733.1
Paddy soil	Cambisoil Anthraquic	P(GD)	Guangdong	Granite	5.7	815.1
Calcarous purplish soil (upland)	Calcalric regosols	CP-U	Sichuan	Calcareous purplish sandstone	8.7	185.4
Calcareous purplish soil (wild)	Calcalric regosols	CP-W	Sichuan	Calcareous purplish sandstone	8.3	191.2
Paddy soil	Calcalric regosols	P(SC)	Sichuan	Calcareous purplish sandstone	8.4	169.6
Fluvoaquic soil	Calcaric fluvisols	FA	Henan	Yellow river alluvial deposit	8.8	181.9
Manured loessial soil	Cumulic anthrosols	ML	Shaanxi	Loess	8.5	187.5
Yellow cultivated soil	Calcaric cambisols	YC	Shaanxi	Loess	8.7	173.6
Dark loessial	Calcaric Kastanozems	DL	Gansu	Loess	8.7	182.6
Paddy soil	Cambisols	P(SX)	Shanxi	Diluvium	7.4	194.7
Residual slain soil	Haplic solonchaks	RS	Xingjiang	Alluvial deposit	9.0	130.7
Brown desert soil	Haplic gypsisols	BD	Xingjiang	Alluvial deposit	8.2	137.1
Grey desert soil	Calcaric cambisols	GD	Xingjiang	Alluvial deposit	9.5	172.0

1) The Quaternary Period

Sample preparation

Triplicates samples (1.0g) of soil sample were placed in plastic test tubes and 40 mL of 1M NH_4OAc containing 5mM of EDTA (ethylene diamine tetraacitic acid) was added. The tubes were shaken for two minutes and then the solid phase was separated from the liquid phase by centrifugation with RCF 12000g. The solutions were usually diluted ten-fold. ^{114}In was added as an internal standard. The samples were collected in pre-cleaned polypropylene bottles with a tightly fitted screw cap, and kept in a refrigerator until analysis.

Instrument

The measurements were carried out using a double focusing type of ICP-MS with high resolution supplied by VG Elemental, Winford, Cheshire, England. The torch box and the interface region are basically similar to those used in quadrupole type instruments. The double focusing mass spectrometer consists of a 70° electrostatic sector for energy focusing and 35° laminated magnetic sector for mass separation. Operating conditions and analytical parameters were essentially those recommended by the manufacturer.
The ultrasonic nebulizer (USN) used in this study was provided by Applied Research Laboratories, En Vallaire, CH-1024, Ecublens, Switzerland. A peristaltic pump was used to deliver the sample solution to the oscillating surface of quartz plate. The temperature of the heated tube and the condenser was kept at 120 °C and 1 °C respectively. The sample introduction rate was adjusted to 2-4 mL/min. The HR-ICP-MS operating conditions are given in reference [10].

Standard

Since the liner range of working curves for heavy metals is wide (from ng/L to mg/L), a single internal standard (In) with concentration 0.1 mg/L was used. Two mixed external standard solutions containing 5-500 μg/L of each REE were used. The working standards were prepared from SPEX Multi-Element Plasma Standards (XSTC-1 and XSTC-13), supplied by SPEX Industries, Inc., 3880 Park Ave., Edison, NJ., USA.

RESULTS AND DISCUSSION

Selection of operation condition

Quadrupole type ICP-MS can not eliminate the interference of ^{137}BaO with ^{153}Eu in mass spectra besides its lower sensitivity. The main purpose of the development of high resolution ICP-MS is to resolve molecular overlaps due to matrix species[11]. Two methods may be used to overcome the overlap problem. The first one was the use of high resolution mode (set the resolution at 7500). It was possible to separate both peaks completely, but this approach suffered a serious loss of sensitivity. The second one was the use of a mathematical correction. First, the concentration of Sm was determined using both ^{147}Sm and ^{152}Sm, the latter overlapped with BaO. Then the magnitude of the interference of BaO on Eu was estimated from the difference of the Sm values derived from ^{152}Sm (Sm+BaO) and ^{147}Sm. It should point out that this correction was possible only when the ratio of Ba/Eu in solution was less than around 10000. The former method was used in this work. As a compensation of the loosing of

sensitivity, an ultrasonic nebulizer was used which improved the sensitivity about ten times.
GSD-2 and GSD-6, which were geological reference materials provided by the Institute of Geophysical and Geochemistry Prospecting (IGGP), China, were used to check the reproducibility of the method. The relative standard deviation of six results obtained during three months was better than 12% for the light REE, 20% for the heavy REE.

Content of EXREE in soils

Among the sixteen soils, thirteen soils were collected from agricultural areas and three soils were collected from arid-desert areas. The thirteen agricultural soils can be divided into two groups: acidic and alkaline soils. The contents of EXREE in acidic soils were relatively higher than those in alkaline soils (see Fig. 1). The highest one (82.33 mg/kg) was from the sample of lateritic red earth (upland, Guangdong) with granite as parent material. The lowest one (3.71 mg/kg) was from the sample of brown desert soil (Xingjiang) with Alluvial deposit as parent material. The contents of EXREE in arid-desert soils (alkaline soils) were low except the for sample of grey desert soil (see Fig. 1, C).
The contents of EXREE in the soils investigated varied widely. The total EXREE ranged from 3.71 to 82.33 mg/kg with an average of 18.4 mg/kg, which is roughly 10 percent of average value of total REE in tested soils. This results is about nine-fold high compared with the content of EXREE extracted with $Mg(NO_3)_2$[2]. The contents of different REE in solution also varied widely. The highest concentration was found for Ce, ranging from 0.77 to 39.79 mg/kg, and the lowest one was Lu, ranging from 0.002 to 0.17 mg/kg.
In a water-culture experiment, Wu[12] found that low concentration of REE (0.1 to 1 mg/L) of REE in water solution enhanced the root initiation of cucumber and mungbean, but higher concentration of REE (higher than 5 mg/l) had inhibitions. Diatloff et al.[13,14] showed that mungbean growth was reduced to 70% by 0.42 μM La and to 56% by 0.19 μM Ce when growth in diluted, continuously flowing nutrient solutions comparable in composition to soil solutions. If the amounts of EXREE measured in the present study are taken as "available" REE, the EXREE in most of the soils tested would be sufficient to affect crop growth, and as for the acidic soils, might be harmful to some plants. So, EXREE in soils should not be ignored when applying REE to the crops, i.e. some soils may already have enough REE available to plants. Since REE are basically toxic heavy metals and have not been proved as essential elements to plants, the delicate balance between amounts causing beneficial and amounts causing harmful effects need to be considered in applying REE in agriculture.

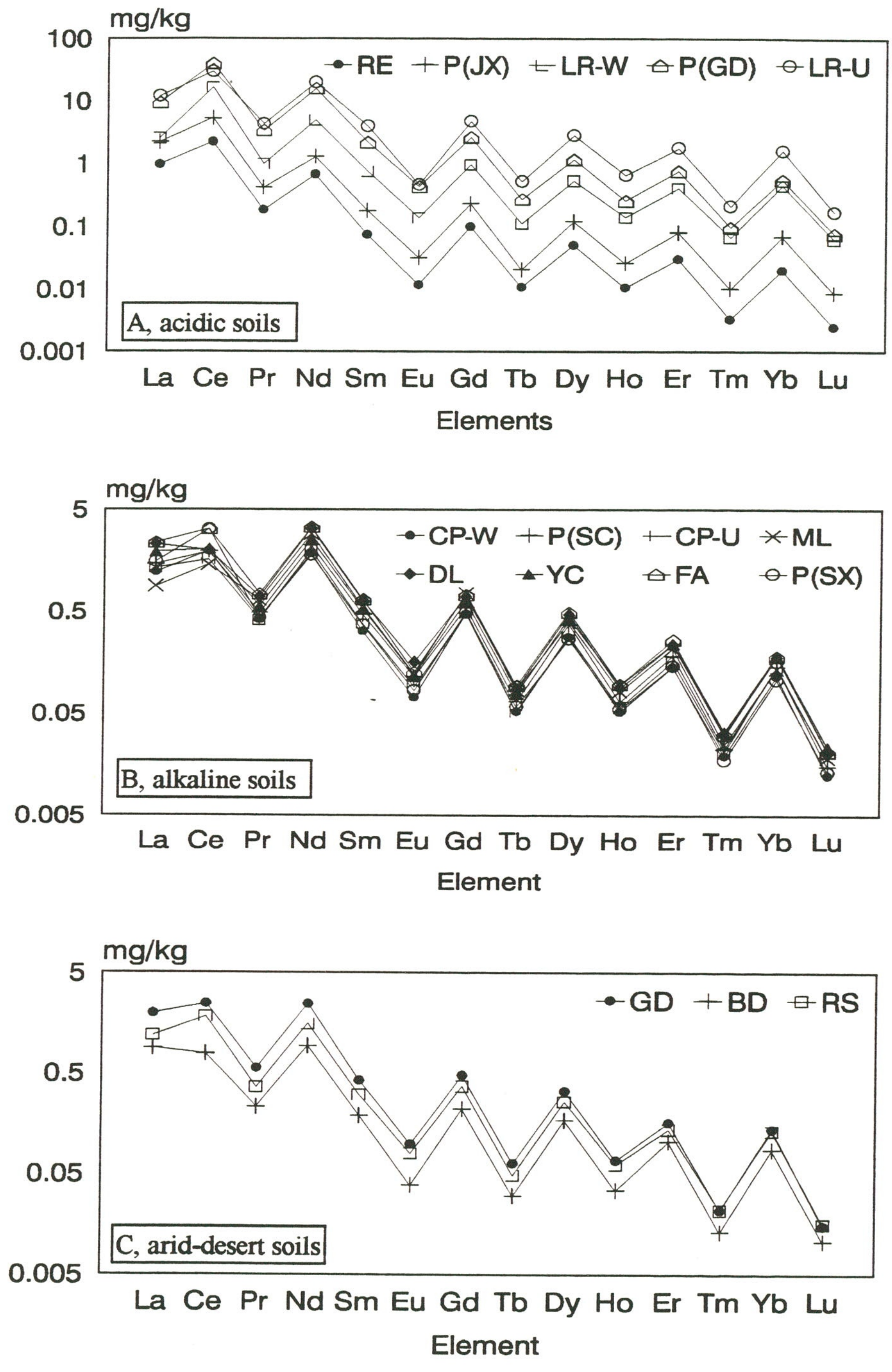

Fig. 1. Exchangeable REE in soils. For soil code, see Table 1.

Distribution of EXREE

The contribution of each REE to the total EXREE was quite different. The contents of light REE were 5 to 14 times higher than that of heavy REE with an average of 7. La, Ce, Nd, accounted for over 77% of the total EXREE measured. Cerium was the most abundant REE, accounting for 39% of the total EXREE in average, and ranged in concentration from 0.77 to 39.79 mg/kg. Lutetium (Lu) was the smallest in contribution, accounting for 0.16% of the total EXREE, and raging from 1 μg/kg to 0.17 mg/kg.

As compared with chondrite, there is a fractionation of REE in the rock and ore forming processes that results in an enrichment or deficit for some of REE. This characteristic of parent rocks is transferred to soils and the fractionation of REE is continued in the soil-forming process. If the contents of REE in soils were normalized by the contents of REE in chondrites, the enrichment or deficit for some of the REE would be observed for the most of soils. There have been some reports on abnormal phenomena (enrichment or deficit) of Ce and Eu in soils[15,16]. The abnormal Ce and Eu distributions varied between the soils due to different soil properties, mainly soil pH. When the data of EXREE were normalized by the data of REE in chondrite, those abnormal phenomena were also observed (see Fig. 2). As for the acidic soils , a positive abnormal content of Ce was observed (see Fig. 2, A), whereas for the alkaline soils, a negative one was observed (see Fig. 2, B and C). The reason is probably that Ce^{4+} is more easily reduced to Ce^{3+} under acidic conditions, and Ce^{3+} is much more easily extracted than Ce^{4+}. The negative abnormal contents of Eu for all of soils tested is probably caused by the deficit of Eu in the soils.

CONCLU SION

The combination of ICP-MS with an ultrasonic nebulizer resulted in an analytical system of excellent detection power. Although the concentration of some REE in soils were as low as μg/kg levels , it was determined with high precision by direct sample introduction. No sample pre-treatment, such as chemical separation and/or pre-concentration, was required. The amounts of EXREE in soils have certain effects both on the plant and environment, and more attention should be paid to the possible consequences caused by applying REE in agriculture.

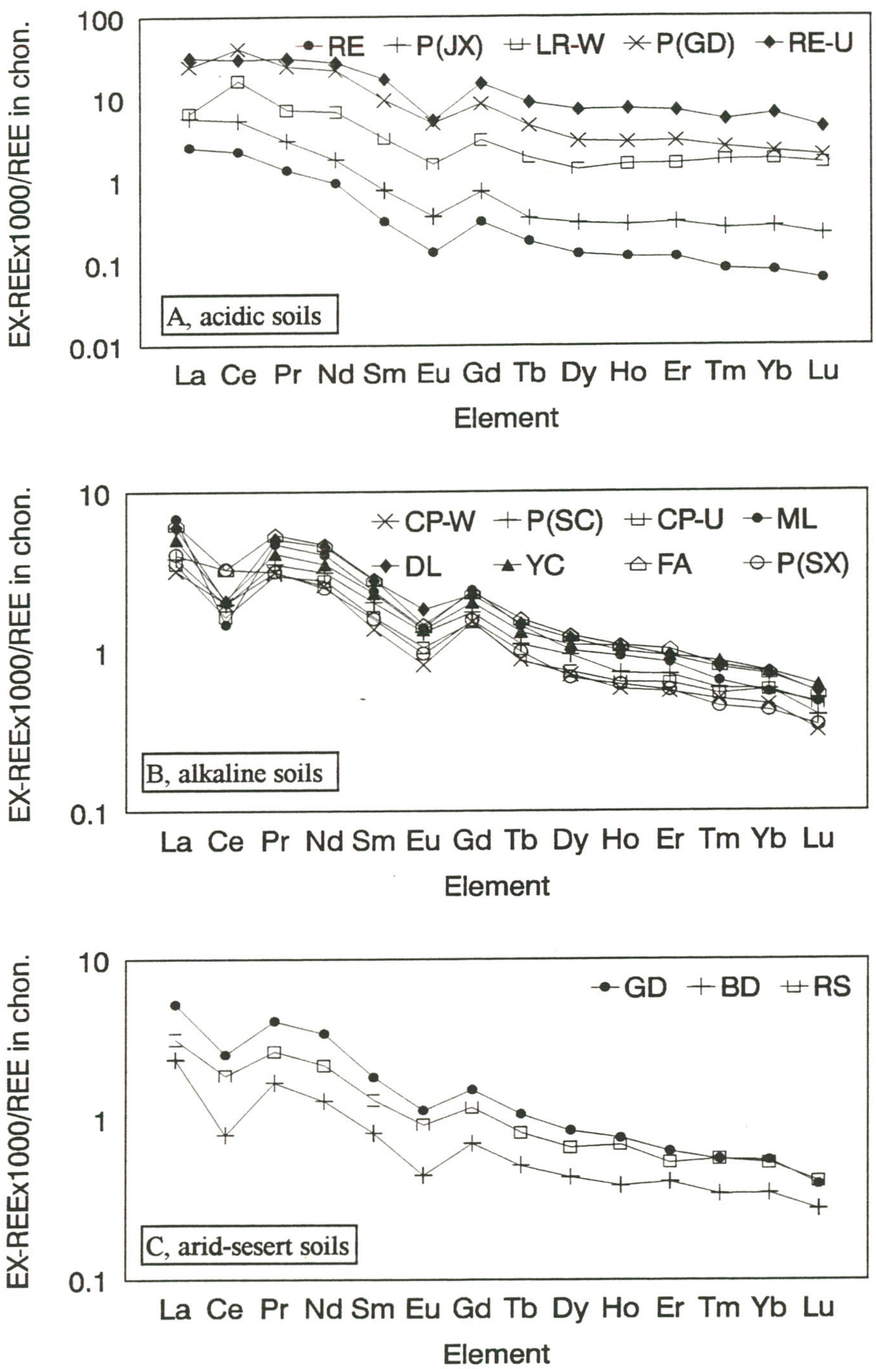

Fig. 2. Chondrite normalized abundance of exchangeable REE in some soils.
For soil code, see Table 1.

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Primary analysis of metals using isotope dilution ICP-MS

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Abstract

Primary analytical methods are methods for which the relationship between measurement quantity and signal is completely described and understood and for their results a complete uncertainty statement can be given. They also require the existence of an equation which describes what is measured without (significant) empirical correction factors.
Similar to the application of reference materials, primary methods may be applied for quality control of the results obtained by other methods.
Isotope dilution analysis applying an inductively coupled plasma mass spectrometer (ID-ICP-MS), is potentially a primary method for elemental analysis. It is based on the direct comparison of ratios of known and unknown number of atoms. It is fully understood and transparent with a clear relationship between what is measured and what is intended to be measured with the results expressed in the SI unit for the amount of substance. Consequently, if the measurement including the sample preparation is carried out correctly, the potential for accuracy is high.
As an example of the accuracy that may be obtained, the results are presented of a laboratory intercomparison organized under the supervision of the CCQM (Consultative Committee on Amount of Substance) regarding the determination of the Pb content in water.

Introduction

The contents of trace metals in a large number of environmental materials is subject to national and international (European) legislation. For an effective implementation of the legislation, a high quality of the analyses performed is of paramount importance. However, results of laboratory intercomparisons [*e.g. 1,2*] show in a number of cases large differences in the individual results, occasionally more than 100%. The fact that the relationship between the acclaimed experience of the laboratory and the accuracy of the result is weak [1], emphasises the need for an improvement of quality control. The quality of the analysis may be assessed by the laboratory using (certified) reference materials. However, intercomparisons show that use of reference materials not always leads to an improvement in the results [3]. Causes may be that the reference values are known and laboratories tend to work toward these values, and that the composition (trace metal contents and matrix constituents) of the reference material not fully matches the composition of the samples under investigation. As an alternative for reference materials, the concept of reference measurements is proposed [3]. The method by which reference measurements may be performed are called primary methods. These methods have the following characteristics:

1. the physical and chemical measurement processes of the method are completely described and understood;
2. the equation which describes the relationship between the contents and measurement signal should be without (significant) correction factors.

Correction for phenomena such as suppression, enhancement or (spectral) interference of the signal should therefore not be necessary.
Consequently, for the results obtained by a primary method, a complete uncertainty budget may be assessed.
Analytical methods that are considered to be potentially primary methods for amount of substance measurement are gravimetry, titrimetry, coulometry, isotope dilution mass spectrometry and other methods are under consideration.
Isotope dilution analysisapplying inductively coupled plasma mass spectrometry (ICP-MS) is especially suitable for the determination of trace metals at environmental relevant concentration levels ($\mu g.l^{-1}$ and below).

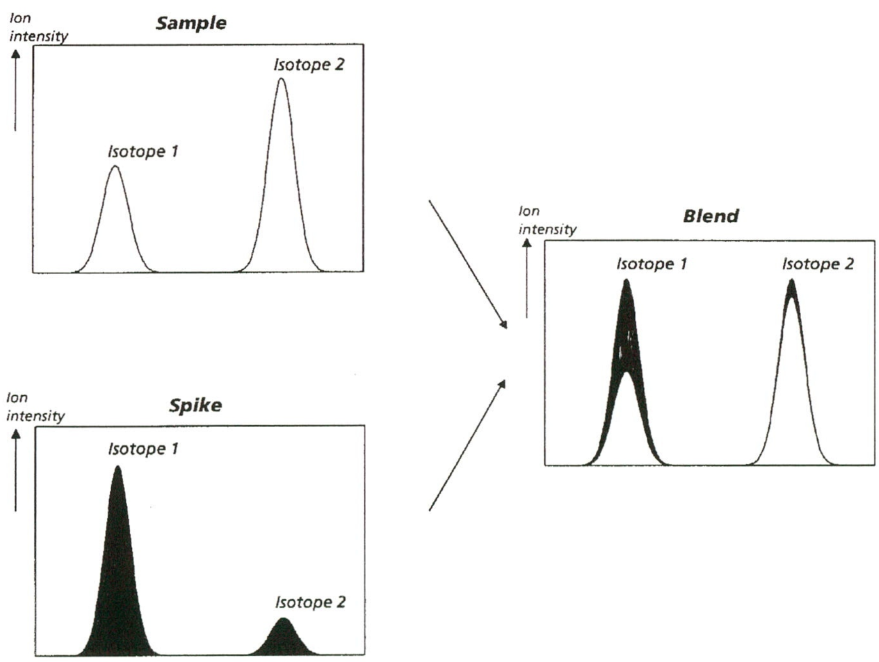

Figure 1 Principle of isotope dilution analysis

Elemental isotope dilution mass spectrometry

Isotope dilution analysis is based on the ratio measurement of two isotope signals of the element under investigation. This allows a very accurate determination of the element contents since matrix interferences, *i.e.*, signal suppression or enhancement, and signal instability will be of the same magnitude for both isotopes.

In its simplest form, isotope dilution mass spectrometric analysis consists of the following procedure (schematically shown in Figure 1). To an aliquot of the sample an aliquot of a solution (the spike) is added which contains the element of interest with one enriched isotope (the reference isotope). The resulting blend is analyzed for its ratio of the contents of a selected isotope to the contents of the reference isotope.

Blending N_x moles of the element from the sample with N_y moles of the element from the spike, for an element consisting of two isotopes (A and B) with a known isotopic distribution the following equation holds[1]:

$$\frac{N_x}{N_y} = \frac{N_x^A + N_x^B}{N_y^A + N_y^B} = \frac{R_x + 1}{R_y + 1}\frac{N_x^B}{N_y^B} \qquad (1)$$

where:

N_x^A, N_x^B = are the number of moles of isotope A resp. B from the sample added

N_y^A, N_y^B = are the known number of moles of isotope A resp. B from the spike added

R_y = the known isotope ratio in the spike

R_x = the known isotope ratio in the sample

For the isotope ratio in the blend, R_B, the following equation holds:

$$R_B = \frac{N_x^A + N_y^A}{N_x^B + N_y^B} = \frac{R_x N_x^B + R_y N_y^B}{N_x^B + N_y^B} \qquad (2)$$

and, therefore:

$$\frac{N_x^B}{N_y^B} = \frac{R_y - R_B}{R_B - R_x} \qquad (3)$$

Combining equations 1 and 3, it follows that:

1 For elements consisting of more than two isotopes the calculations become slightly more complicated.

$$\frac{N_x}{N_y} = \frac{R_y - R_B}{R_B - R_x} \frac{R_x + 1}{R_y + 1} \qquad (4)$$

When m_x grams of sample is blended with m_y grams of spike, the element contents in the sample, c_x, may consequently be calculated from:

$$C_x = \frac{R_y - R_B}{R_B - R_x} \frac{R_x + 1}{R_y + 1} \frac{m_y}{m_x} C_y \qquad (5)$$

where:

c_x	=	the element contents in the sample
c_y	=	the known element contents in the spike
m_x	=	the mass of sample used for blend preparation
m_y	=	the mass of spike used for blend preparation

Since mass spectrometers suffer from mass discrimination, the observed ratio in the blend, $R_{B(obs)}$, has to be corrected for this effect to obtain the "true" ratio, R_B. To that end, the following equation is applied:

$$R_B = K.R_{B(obs)} \qquad (6)$$

where K is the mass discrimination factor. The magnitude of this factor has to be determined empirically. Although K deviates no more than a few percent from 1 for isotope dilution analysis with ICP-MS, the correction is significant and, consequently, the primary property of the method is reduced.
The influence of mass discrimination may be decreased by applying additionally so-called "reverse" isotope dilution analysis. Reverse isotope dilution analysis is performed by preparing a blend of the spike and a solution containing an accurately known element contents (assay standard solution). Similar to normal isotope dilution analysis, the following relationship may be derived:

$$C_y = \frac{K.R'_{B(obs)} - R_z}{R_y - K.R'_{B(obs)}} \frac{R_y + 1}{R_z + 1} \frac{m_z}{m'_y} C_z \qquad (7)$$

where:

c_z	=	the known element contents in the assay standard solution
m'_y	=	the mass of spike used for blend preparation
m_z	=	the mass of assay standard solution used for blend preparation
R_z	=	the known isotope ratio in the assay standard solution
$R'_{B(obs)}$	=	the observed isotope ratio in the blend

Combining equations 5 to 7, it follows that:

$$C_x = \frac{R_y - K.R_{B(obs)}}{R_y - K.R'_{B(obs)}} \frac{K.R'_{B(obs)} - R_z}{K.R_{B(obs)} - R_x} \frac{R_x + 1}{R_z + 1} \frac{m_x}{m_z} \frac{m_y}{m'_y} C_z \qquad (8)$$

The natural isotopic distribution for most of the elements does not vary much and, therefore, $R_x \approx R_z$. When choosing the masses for preparation of the blends such that the ratio's in the blends, $R_{B(obs)}$ and $R'_{B(obs)}$, are close, from equation 8 it can directly be seen that the accuracy of the determination of the element contents in the sample is hardly influenced by the accuracy of the value of the mass discrimination factor. Furthermore it can be seen from this equation that, in case the ratio's in the blends are close, any systematic error in the observed ratio's as well as any systematic error in the isotopic composition of the element in the spike, will have little effect on the final result.

Therefore, reverse isotope dilution analysis will increase the primary property of the method and the accuracy of the analysis.

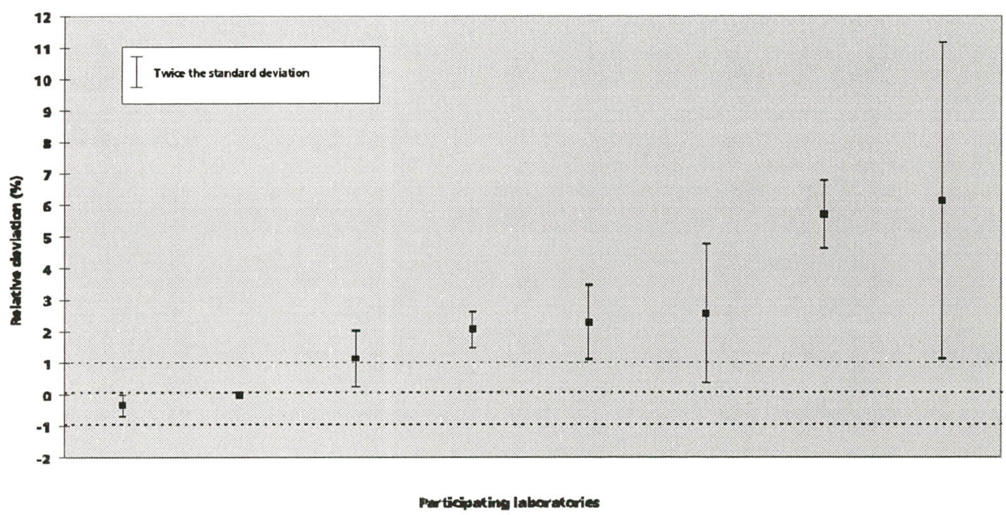

Figure 2 1994 CCQM intercomparison for Pb (contents is 49,38 µg.g^{-1})

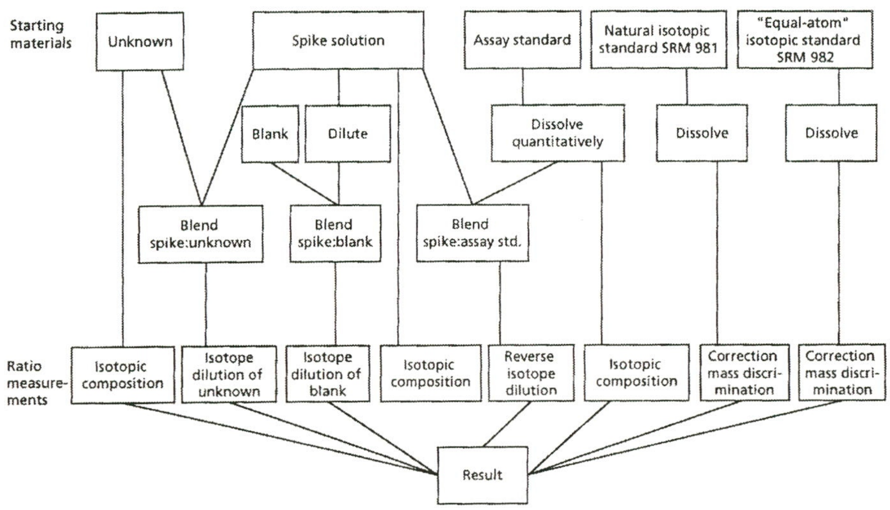

Figure 3 *Isotope dilution protocol for the determination of the Pb contents in water*

Laboratory intercomparison for the determination of the Pb contents in water by means of ID-ICP-MS

Based on the above described method of isotope dilution analysis combined with reverse isotope dilution, in 1996-1997 under the supervision of the CCQM (Consultative Committee on Amount of Substance) a laboratory intercomparison was organized by NIST (US National Institute of Standards and Technology) for the determination of the Pb contents in a water. Ten metrology laboratories participated in the intercomparison. The sample was gravimetrically prepared and consisted of acidified water with a high Pb content ($g.g^{-1}$-level). A deviation of the result of less than 1% from the gravimetrical value was the target set by the CCQM. In order to realize this target, a detailed analysis protocol was drafted [3] that the laboratories had to adhere to strictly. A protocol was found necessary, because the results of a laboratory intercomparison organized in 1994 showed large deviations from the gravimetrical value (see Figure 2). Although this earlier intercomparison included AAS techniques, the results obtained by isotope dilution analysis did not prove to be better.

A summary of the protocol is shown in Figure 3 and a detailed description of the protocol is given by Watters *et al* [3].
Apart from the sample, each laboratory received a spike solution, a blank solution, an assay standard (a piece of high-purity lead), and reference materials with accurately known isotopic compositions (NIST SRM 981, natural isotopic standard, and NIST SRM 982, "equal atom" isotopic standard).

To obtain traceable and accurate Pb contents, the preparation of the assay standard solutions and of the blends was performed gravimetrically. Two assay standard solutions and in each case four blends were prepared.
Unlike for most elements, the natural isotopic composition of Pb varies strongly. For that reason, the isotopic composition of the unknown and of the assay standard had to be determined. The results of these analyses were corrected for mass discrimination by analysing the natural isotopic standard NIST SRM 981. For the analysis of the blends, a standard (NIST SRM 982) was analyzed with an isotopic composition more close to that of the blends.

The results (averages and 95% confidence intervals) of the laboratory intercomparison are shown in Figure 4. As can be seen, when comparing with the results obtained in the previous intercomparison (Figure 2), the accuracy has improved significantly. Each laboratory, apart from one, has reported an average result within 1% of the gravimetrical value and, moreover, the average result is in a number of cases not significantly different from the gravimetrical value.

Conclusions and discussion

The result of the laboratory intercomparison shows that for the determination of Pb by means of ID-ICP-MS, very accurate results may be obtained when the analyses are carried out according to the protocol described.
A similar protocol may also be applied to other elements with two or more isotopes, such as most of the lanthanides. The signal of the isotopes should be free of spectral interference, or a simple mathematical correction for the interference should be possible. For most of the elements the natural variation in the isotopic composition is negligible. Therefore no natural isotopic standard is required and no measurements for the determination of the isotopic composition of the element in the sample and assay standard have to be performed and the protocol may be simplified.

Although the accuracy of the method has still to be proven for the analysis of "real-life" samples, with lower element contents and a less "clean" matrix, literature results [4, 5] are very promising.

The method is an elaborate and therefore not suitable for routine analysis. However, in case of demonstrated accuracy of the method, measurement results obtained by this method may serve as a reference for results obtained by other methods.

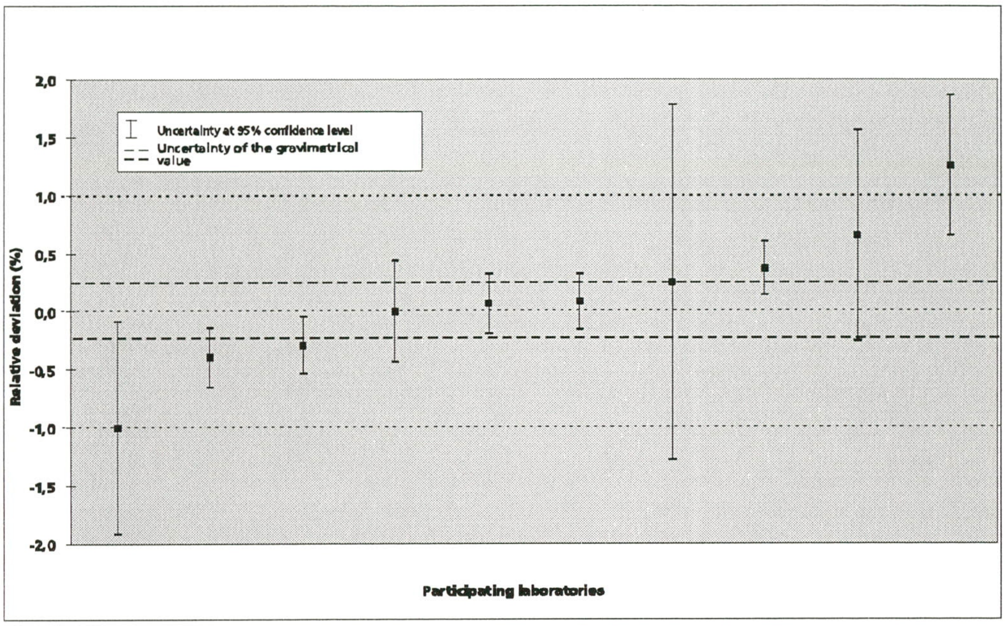

Figure 4 *1997 CCQM intercomparison for Pb (contents is 10,40 µg.g^{-1})*

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Uptake and bioconcentration of lanthanides in higher plants: linking terrestrial and aquatic studies

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Abstract

Literature data on lanthanide concentrations in terrestrial and aquatic higher plants are discussed in view of lanthanide availability, bioconcentration and biological effects. 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 were lanthanides with even atomic numbers are more abundant than those with odd numbers. Anomalies in this pattern are cerium and europium (both with concentrations lower than expected) and promethium which has no stable 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 they are high in freshwater plants (BCFs of plants relative to water are 100 - 46,000 $l.kg^{-1}$ dry plant). The observed difference in BCFs between terrestrial and aquatic plants is caused by the physico-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.

Keywords: lanthanides, rare earth elements (REEs), uptake, accumulation, bioconcentration, BCF, availability, higher plants, macrophytes, soil, sediment, water, solubility, toxicity.

Introduction

The lanthanides comprise a group of fifteen elements with atomic numbers 57 - 71, which occur in all natural environments, with the exception of promethium (Pm, atomic number 61) which has no stable isotopes and, consequently, no natural occurrence. 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 [1, 3, 19] while in the soil solution, concentrations range from 0.04 - 71 $\Phi g.l^{-1}$ [7, 51]. In sediments, the

concentrations of individual lanthanides are 10 - 100 $mg.kg^{-1}$ dry sediment [4, 18] and in freshwater concentrations range from 0.001 - 3 $\Phi g.l^{-1}$ [1, 3, 52, 32]. From these concentrations the majority can be attributed to three lanthanides with low atomic numbers, *i.e.* lanthanum (La, 57), cerium (Ce, 58) and neodymium (Nd, 60). The lanthanides show a similar chemical behaviour, usually a smooth function of atomic number [12]. 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 [4, 12]. Environmental concentrations of even numbered lanthanides decrease approximately log-linearly with increasing atomic number as do the odd numbered ones [27].
In nature, lanthanides are present in a 3+ valence state. Cerium and europium (Eu) 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.* carbonate, sulphate), organic ligands (*e.g.* humic and fulvic acids) and, at high pH, with hydroxyl-ions. 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 up till now. However, they can be found in virtually all organisms. Terrestrial plants tend to reflect the lanthanide distribution of the soils they grow on [45, 16]. Concentrations of individual lanthanides in terrestrial plants range from 0.0001 to 140 $mg.kg^{-1}$ dry weight [24, 16, 38]. 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 [4, 22].
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.

Materials & 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.* Coughtrey & Thorne [3], Kabata-Pendias & Pendias [19] and Rikken [38]. 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 more sound 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 $mg.kg^{-1}$ dry weight plant material, divided by the concentration in the environment ($mg.kg^{-1}$ dry weight soil or $mg.l^{-1}$ water).

Results and discussion

General remarks
From the literature studied, some general observations seem characteristic of lanthanide behaviour in plants. Lanthanides are mainly concentrated in the roots of plants [43, 9, 10, 34]. This can be explained by fast surface adsorption, which seems to be the controlling mechanism for lanthanide uptake [26, 23]. 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 & Robb [39], Van Steveninck *et al.* [46] and Wolterbeek & Van Die [53] 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 [6].
Bioconcentration factors for terrestrial plants relative to soil are low (~ 0.001), whereas BCFs for freshwater plants relative to water are high (~ 1000). This topic will be discussed further in the section *Bioconcentration*. At higher concentrations lanthanides inhibit plant growth [35, 46, 8], see also the section *Effects in plants*. Finally, lanthanides, but especially lanthanum, interfere with calcium and magnesium metabolism in plants [35, 30, 37, 17].

Distribution patterns
In almost all terrestrial plant studies the characteristic lanthanide distribution, as indicated in the introduction, is shown (*e.g.* Tjioe *et al.* [45]; Markert [27]; Markert & De Li [28]; Rikken [38]; Ozaki *et al.* [31]). For aquatic plants, data on this distribution are scarce. However, for one freshwater plant, data are available to construct such a curve. Figure 1 shows the distribution pattern of nine lanthanides in the water-lily (*Nymphaea odorata*) and the sediment it roots in (data taken from Cowgill [4]). The odd numbered elements (63 - 71, promethium is 61) were not detected in the water-lily 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 seem to be slightly enriched in the water-lily. This might be explained by the higher solubility product of heavier lanthanide-phosphates [25].

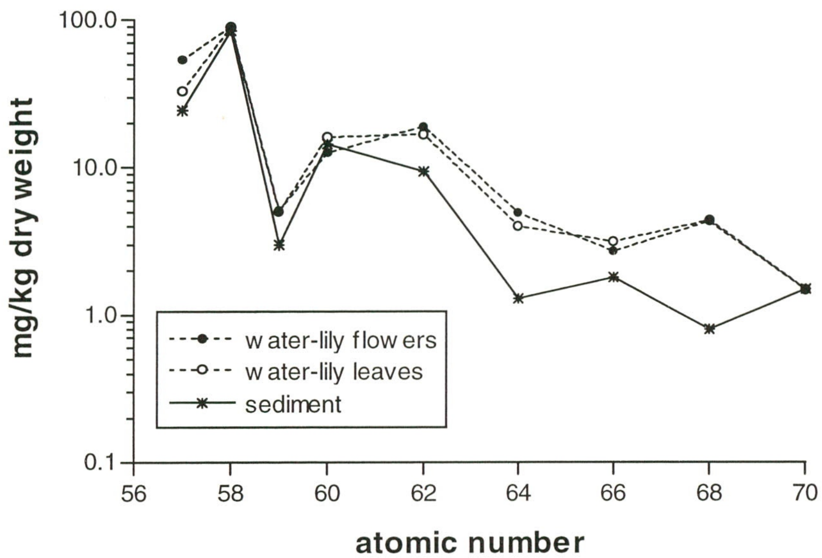

Figure 1 *Distribution pattern of nine lanthanides in the water-lily and the sediment it roots in (data from Cowgill [4]). Further explanation is given in the text*

For terrestrial plants various degrees of fractionation were observed, leading to both enrichment and depletion of certain (groups of) lanthanides [29, 54, 50, 31].
From terrestrial studies it is apparent that Ce and Eu often cause negative anomalies in the distribution pattern [24, 13]. The Ce-anomaly is caused by the Ce 4+ valence state in soils, which is less available to plant uptake [54, 31]. 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.* [31] showed a seasonal variation of lanthanide concentrations in ferns, with the highest concentrations occurring in springtime. Volokh *et al.* [49] showed that plants can reflect environmental pollution with lanthanides, which is caused by air emissions from phosphate fertiliser producers. Peresedov *et al.* [33] established a clear relationship of decreasing lanthanide concentrations in pine needles and soil with increasing distance from phosphoric fertiliser industries.

Bioavailability
Uptake of metals by plants takes place via the soil solution [15], sediment pore water and/or the surrounding freshwater. It is thereby assumed that the Afree≅ 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.* [43] and Knaus & El-Fawaris [21], who showed that for lanthanide-EDTA complexes in solution, bioconcentration factors 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) concentrations in wheat shoots when chelated forms of these elements were offered in the nutrient solution [43]. The same was observed for Pm BCFs in shoots of beans [11]. 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 [3, 9, 7, 5]. Scott-Russell [41] showed that uptake of Ce by peas from solution was higher at lower pH.

In aqueous systems, lanthanide-phosphato, -hydroxo and -carbonato solubility products limit the amount of lanthanides in solution. For this reason concentrations of dissolved lanthanides are often below the detection limits. Van Wezel *et al.* [47] calculated sediment/water partition coefficients for La, Ce and Nd in Dutch freshwater, indicating that the concentration factor for these lanthanides in sediments is circa 4.10^7 $l.kg^{-1}$. Coughtrey & Thorne [3] calculated values of 5.10^3 to 2.10^5 $l.kg^{-1}$ for sediment/water partition coefficients. Calculations on the data of Zhang *et al.* [55] 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).

Bioconcentration

Bioconcentration of lanthanides from soil by terrestrial plants has been reviewed by Coughtrey & Thorne [3] and Rikken [38]. With some exceptions, BCFs for lanthanides in terrestrial plants relative to soil are in the order of 5.10^{-5} to 1.10^{-1} kg dry soil.kg^{-1} dry plant. Higher BCFs, between 1 and 10, were found for hickory trees (*Carya spec.*) and ferns [40, 31]. BCFs for the water-lily relative to sediment range from 1.0 to 5.5 kg dry sediment.kg^{-1} dry plant (see also Fig. 1).

Table 1 *BCFs (in $l.kg^{-1}$ dry weight) for different lanthanides in aquatic plants relative to freshwater.*

Plant species [ref.]	La	Ce	Pr	Nd	Pm 1)	Sm	Eu	Lu
Ceratophyllum demersum [52]	>24000	46000		>5800		39000	32000	14000
Azolla filiculoides [52]	>1800	4000				2600	3000	<6000
Spirodela polyrhiza								
Ceratophyllum submersum [22]	10^3-10^4	10^3-10^4	10^2-10^4	>10^4				
Hydrocharis morsus [22]	1000	10^2-10^3	100	>10^4				
Lemna trisulca [22]	1000	1000	1000					
Myriophyllum spicatum [22]	10^3-10^4	10000	10^3-10^4	>10^4				
Potamogeton pectinatus [22]	1000	1000	10^2-10^3	>10^4				
Potamogeton perfoliatus [22]	10^2-10^3	10^3-10^4	10^2-10^3	>10^4				
Stratiotes aloides [22]	10^2-10^3	10^2-10^4	10^2-10^3	>10^4				
Elodea canadensis [26]		1000*						
Potamogeton perfoliatus [26]		210*						
thirty-two species pooled [44]		7100						
four species pooled [23]	3000	22000						
average freshwater plants [3]		5000*						
average freshwater plants [36]					4600			

1) Pm data were obtained by using radiotracers

* Based on wet weight, which implies that for dry weight the BCF is approximately 20-fold higher

Bioconcentration factors for lanthanides in freshwater plants relative to water are presented in Table 1. As the table shows, more data are available for lighter lanthanides (La - Nd), than for the heavier ones (Pm - Lu). This is due to a combination of their natural occurrence and analytical difficulties. The high BCFs for *C. demersum* compared to *A. filiculoides* from the same ditch, could be the result of different exposure; while *C. demersum* is fully submerged, *A. filiculoides* is free floating [52].
In Table 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 (1.10^2 - 5.10^5 $l.kg^{-1}$ 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.

Table 2 *BCFs (in $l.kg^{-1}$ dry weight) for different lanthanides in terrestrial plant roots relative to freshwater, nutrient solution or soil solution.*

Plant species [ref.]	La	Ce	Nd	Gd	Dy
Alnus rubra [21] 1)					1200
Zea mays [51] 2)	23000	18000	15000		
Triticum spec. [43] 3)	930*			1400*	
Zea mays [9, 10] 3)	>1300	20000			
Vigna radiata [9, 10] 3)	>21000	36000			

1) Based on freshwater 2) based on soil solution 3) based on nutrient solution

* Based on wet weight, which implies that for dry weight the BCF is approximately 20-fold higher

Effects in plants
Solution concentrations of <10 µM La, Ce and ytterbium (Yb) severely inhibit plant growth [20, 8, 9, 10, 17]. When lanthanides are taken up by plants they can disturb calcium and -to a lesser extent- magnesium metabolism [30]. Some authors suggest that calcium interference of the lanthanides, and especially lanthanum, is the cause for their toxicity. Velasco *et al.* [48] suggests that lanthanides replace the essential element boron from its active sites and thus induce boron deficiency. Diatloff *et al.* [10] demonstrated manganese deficiency in mungbean plants exposed to solutions containing >0.63 µM Ce.
Besides toxic effects, greater biomass and increased root growth were observed after exposure to low lanthanide concentrations [48, 10, 34]. The mechanism for this stimulatory effect is yet unknown (for discussion see also Diatloff *et al.* [6]). An

explanation could be the occurrence of hormesis: a regulatory overcorrection by biosynthetic control mechanisms, following exposure to a low toxicant concentration [42]. It also has been suggested that lanthanides increase uptake and transport of phosphates [12]. In China, farmers apply lanthanide-containing fertilisers to improve their crop [2, 14].
Only recently, two lanthanide binding proteins, probably glyco-proteins, were identified in the terrestrial fern *Dicranopteris dichotoma* [13] which is known to accumulate lanthanides up to 3358 ppm in its leaves [50]. 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. J.J.M. de Goeij for helpful comments on a previous version of this paper.

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Policy on metals in the Netherlands

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Abstract

In the Netherlands, the government faces serious environmental problems with at least 6 metals in water, sediment and sewage sludge. For these metals, maximum permissible concentrations (MPCs) in the environment were published in 1997 by the National Institute of Public Health and the Environment (RIVM), using the 'added risk approach'. The risk policy of the Dutch government is focussed on environmental levels below the MPC in the year 2000. At least 6 main diffuse sources of environmental pollution with metals are identified, including corrosion of lead, copper and zinc due to the abundant use of these metals as construction materials. Policy concerning these diffuse sources and other minor sources as well will be announced in the policy document on heavy metals, to be published in 1998.

Keywords: metals, maximum permissible concentration, water, soil, sediment, priority substances, background concentration.

Introduction

The Netherlands is a country surrounded by the North Sea, Belgium en Germany with a total area of 42,000 square km and with about 15 million inhabitants. In a country with such a dense population and with a lot of fresh surface water (below sea level) it is clear that flows of metals into the Dutch environment sooner or later will cause problems in water and sediment, soil and also in sewage systems and sludge.
For instance, for the metal zinc, in 1990 the 380 tonnes of zinc from the corrosion of constructions and houses ended up as 270 tonnes of zinc in sewage sludge and as 110 tonnes in surface water.

Priority substances

In the Netherlands, substances causing problems in the environment are placed on the 'priority substances list'. Now, in 1997, substances on this list are polycyclic aromatic hydrocarbons (PAHs), zinc, copper, lead, chromium, mercury, cadmium, nickel, fluorides and particulate matter (PM). For these substances, the National Institute of Public Health and the Environment (RIVM) in the Netherlands prepares criteria documents, including data on properties, analysis, background concentration, production, application, emission, exposure, effects (no-observed adverse effect levels, NOAELs), the maximum permissible concentration (MPC) and the negligible concentration (NC) in the environment.

Maximum permissible concentration

The MPC for water, sediment and soil means a protection level against adverse effects for 95% of all species in that particular compartment of the environment. It is based on the background concentration and the bioavailability of the substance and is derived according to the 'added risk approach' by the RIVM. The MPC is the addition of the background concentration (Cb) and the maximum permissible addition (MPA): MPC = Cb + MPA (see figure 1).

Cb: background concentration (bioavailability zero)

MPA: maximum permissible addition

MPC: maximum permissible concentration

In formula: MPC = Cb + MPA

NA: negligible addition
NC: negligible concentration
NC = Cb + NA
NA = MPA/100

Figure 1 Added risk approach for metals in the Netherlands

Using this added risk approach, the RIVM [1] published in 1997 MPCs for all metals on the priority list (Table 1). Definitive levels will be discussed in 1998 with the government, the industry and interested non-governmental organisations (NGOs).

Table 1 *MPCs and Cb-values for some metals in water, sediment and soil (RIVM, 1997).*

Metal	Water (µg/l)		Sediment (mg/kg)		Soil (mg/kg)	
	MPC	Cb	MPC	Cb	MPC	Cb
Zn	9.4	2.8	620	140	160	140
Cr	8.7	0.17	1720	100	100	100
Ni	5.1	3.3	44	35	38	35
Hg	0.24	0.01	26	0.3	2.2	0.3
Pb	11	0.15	4800	85	140	85
Cd	0.42	0.08	30	0.8	1.6	0.8
Cu	1.5	0.44	73	36	40	36

As is shown in table 1, for some metals the MPC is very near or the same as the Cb. This means that the MPA by human activities is very low or zero.

Risk policy

The risk policy on priority substances by the Dutch government is split up in a short term and a long term component. Before 2000 the policy is focussed on levels below the MPC; on the long term (before 2010) the NC is the main goal of the policy. In the mean time, we try to keep levels as low as reasonable achievable (alara). This policy is focussed on effects caused by diffuse sources like products. Policy instruments can be legislation, covenants and agreements.
In the present situation in the Netherlands, 6 main diffuse sources of metals in the environment are indentified:

1. construction products: gutters and roofing (zinc)
2. construction products: coating, galvanized steel (zinc)
3. transport and heating of drinking water (copper)
4. animal feed, manure and fertilizers (cadmium, copper and zinc)
5. anti-fouling paints (copper, tin)
6. dry and wet deposition of metals, including transboundary pollution

In 1998 the *'Policy document on heavy metals'* will be published by the government with actions concerning these 6 main sources and 20 other minor diffuse sources as well.

Conclusions

In the Netherlands, a lot of environmental problems are caused by metals . The policy on these metals is based on the data generated by the RIVM using the 'added risk approach'. This approach leads to the MPA, the concentration of the metal that can be added to the background concentration, without exeeding the MPC. For the next decade, 6 main diffuse sources of environmental pollution with metals will be tackled as part of the environmental policy of the Dutch government.

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A perspective of toxic risk assessment for low concentration pollution

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Abstract

China is facing at least two challenges in toxic risk assessment of rare earth elements(REE):

1. The use of biomarkers in toxic risk assessment should be studied.
2. The effects of different doses and conditions should be investigated.

More extensive research including in vivo studies and long term exposure of human are needed.

Key words: rare earth elements (REE); toxic risk assessment.

China is facing at least two challenges in toxic risk assessment of rare earth elements.

1. First, the use of biomarkers in toxic risk assessment should be studied. There is usually a complex pathway from the exposure to a toxicant to the induction of clinical diseases. We should understand the biomarker of exposure and which are the biomarkers of biological effects, especially of subclinical changes.

For cadmium, urinary Cd as a biomarker of exposure, urinary β2-microglobuline and urinary N-acetyl-glucosaminidase are used as the biomarkers of toxic effects.

The Environmental Health Institute of China Academy of Preventive Medicine has suggested the limit values for judgement of health risk:

(1) Cadmium concentration in urine≥15μg/g creatinine.
(2) N-acetyl-glucosaminidase in urine (UNAG):17μg/g creatinine.
(3) β_2-microglobulin in urine (β_2-MG) ≥1000μg/g creatinine.
(4) The rate of combined response in response in population reached 10% or over.

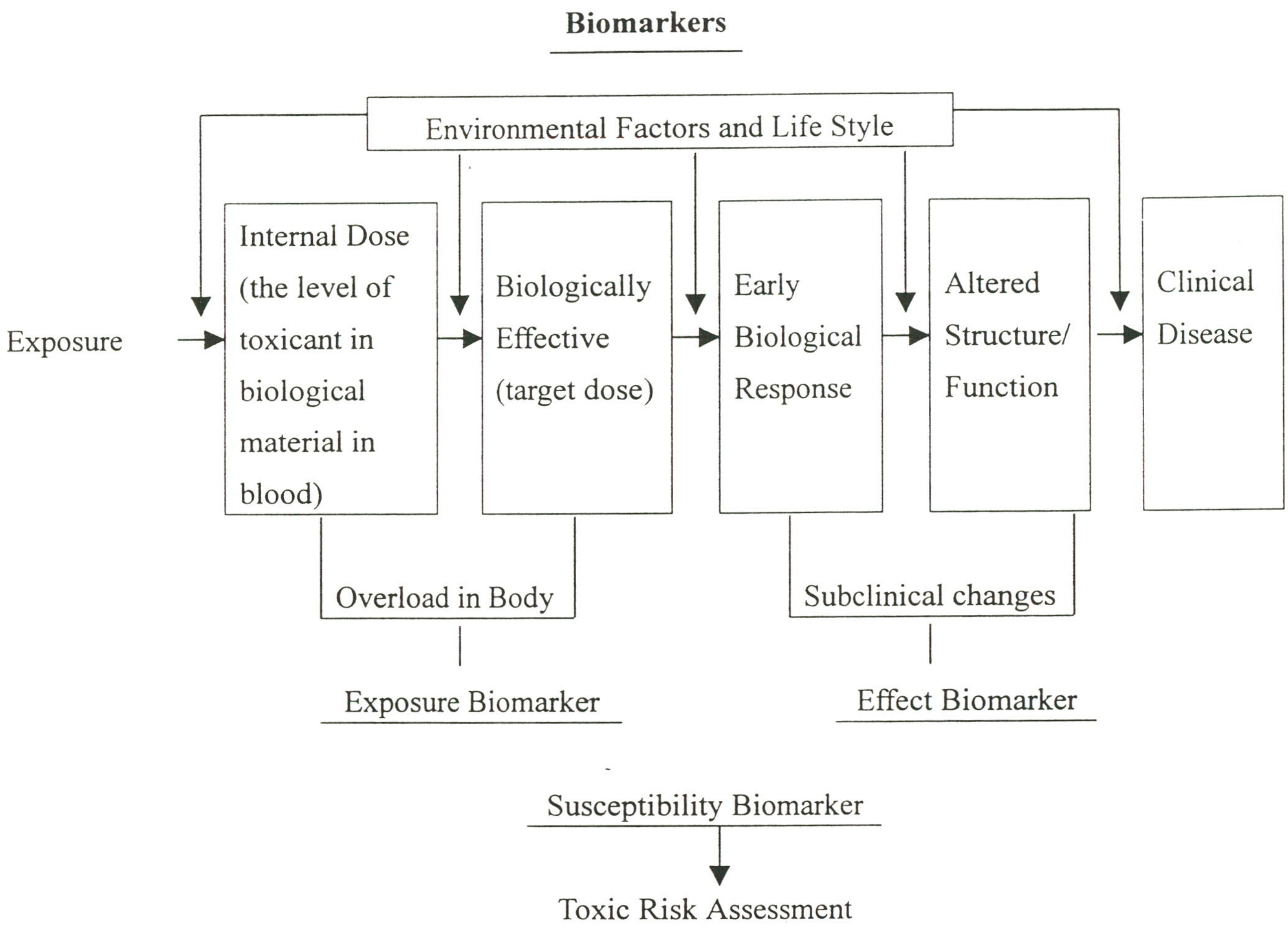

Figure 1 Schematic Model of Biomarkers

Different countries may have different suggestions; a study from Belgium reported that they used five urinary variables as a function of urinary Cd excretion to identify the limit value of urinary cadmium, they also suggested that the limit value to be set at 2μg/g creatinine.

It is therefore necessary to learn from each other and to promote cooperation. Toxic risk assessment for rare earths is more difficult than for cadmium, because no biomarkers have been identified yet. Our primary studies showed that the gastrointestinal tract is the main rout, the biological absorption for REE is very slow. By oral administration, after a single dosing in 72 h, the level of REE in serum were just the same but after a repeated dosing for 30 days, the level significantly increased.

Table 1 The level of La and Ce in mice serum after oral administration of chang Le ($\overline{X}$±SD ng/mL, n=3)

		La				Ce			
		1hr	6hr	24hr	72hr	1hr	6hr	24hr	72hr
A single dosing	5mg/kg	1.12±0.66	0.50±0.10	0.99±0.69	0.66±0.17	1.09±1.01	0.49±0.13	0.60±0.23	0.97±0.33
	50mg/kg	0.55±0.08	0.66±0.11	0.70±0.20	0.71±0.22	0.59±0.19	0.82±0.12	0.81±0.31	0.80±0.39
	Control		0.67±0.08				0.89±0.06		
30 day repeated dosing	5mg/kg		29.5±21.6				3.04±0.75		
	50mg/kg		87.0±31.8				62.4±5.5		

*analyzed by ICP-MS method

"Chang Le" is a common product of REE for agricultural use in China, La $(NO_3)_3$ and $Ce(NO_3)_3$ are two main components, La^{3+} seems to be more easily absorbed then Ce^{3+}. La^{3+} seems to be easily accumulated in the brain. These data indicated that biological dose should be detected and different REE may have different biological doses after exposure to REE.

Table 2 The level of Ce and La in serum and brain of rats after oral administration of the reagent "$CeCl_3$" (2mg/kg)

	serum (ng/mL)	Brain (sea horse) (ng/mL)
Ce^{3+}	0.42	15.1
La^{3+}	36.57	389.9

* The reagent "$CeCl_3$" 2mg/kg in water solution was analyzed by ICPMS method as: Ce^{3+}22μg/mL, La^{3+}0.78μg/mL.

Which biomarker is sensitive to be considered in different testing methods and observation of various effects? For $La(NO_3)_3$(oral administration): neurobehaviral effect is around 0.3mg/kg; pathomorphological effect is over 200mg/kg. I suggest that multiple end points in biological observation should be used for REE threshold level study, the multiple end point consist of the main biological changes are as follows:

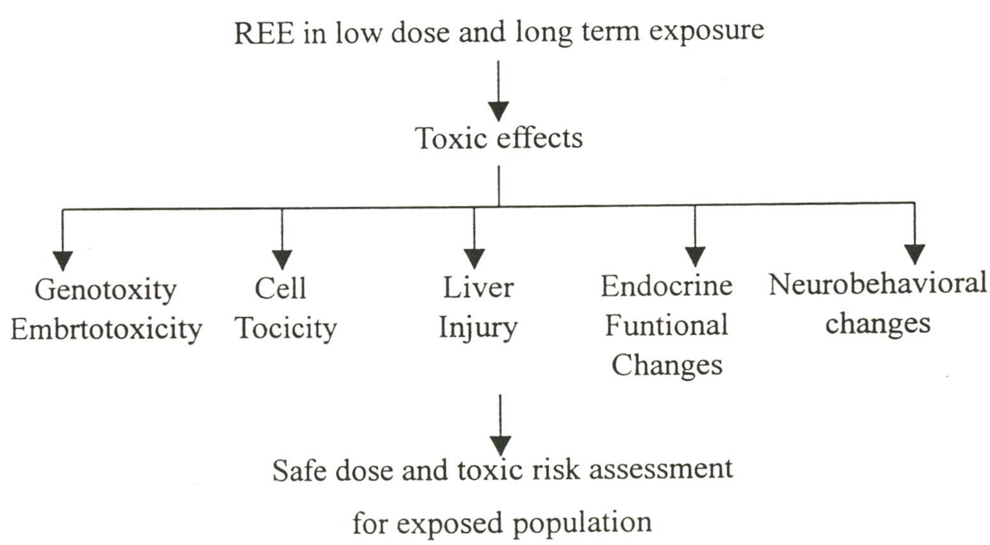

Figure 3 Multiple end points in biological observation

2. Secondly, different doses and conditions should be investigated.

How to decrease the toxic effects (harmful effects) and how to develop the beneficial effects are two important sides in toxicology.

The effective inorganic components combined with effective organic components have much better antimutagenesis. For example, When selenium is combined with a red algae to produce a new drug, selenic acid palysaccharide ester (KS) is better biologically absorbed than the inorganic selenium (as sodium selenite, Na_2SeO_3)and the effect of KS on antiformation of micronuclei is better than the inorganic selenium as well. The low dose of KS can decrease the rate of micronucleated PCE, and for the same effect, sodium selenite (Na_2SeO_3) should be in higher dose.

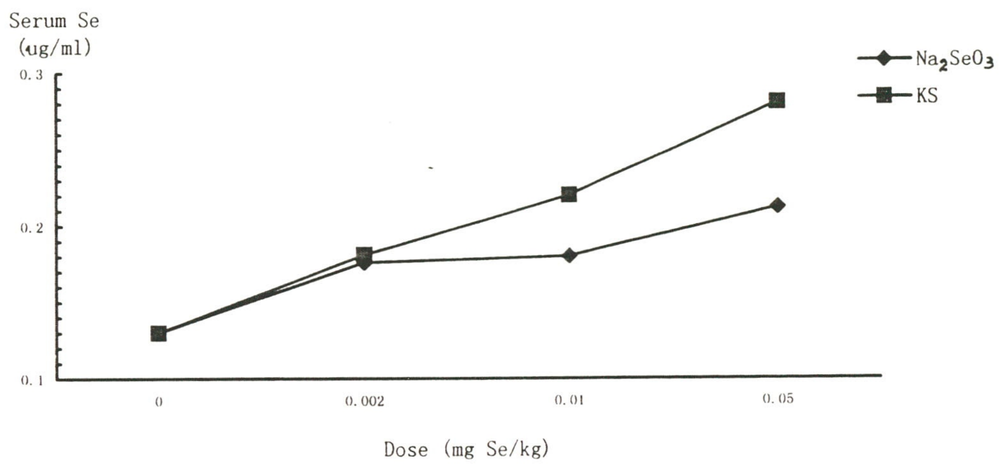

Figure4 Comparison of the selenium levels 6 hours after oral administration of KS and Na_2SeO_3

Table 3 Effects of KS and Na_2SeO_3 on Anti-formation of Micronuclei (Mn) Induced by Cyclophosphamide (CP)

Dose	KS		Na_2SeO_3	
	Serum Selenium (ug/mL)	Micronucleated PCE(‰)	Serum Selenium (ug/mL)	Micronucleated PCE(‰)
0.002mg Se/kg	0.159±0.048	54.0±5.3*	0.157±0.037	62.7±9.7
0.01mg Se/kg	0.221±0.008*	42.4±5.9	0.160±0.021	58.4±5.6
0.05 mg Se/kg	0.282±0.037**	33.0±4.0	0.210±0.072*	55.0±10.1*
CP50 mg /kg	0.130±0.044	61.9±8.5		

Data compared with positive control * $p<0.05$, ** $p<0.005$

Different species of plant have different biological effects. For example, the effect of different species of kiwi grown in different areas on micronuclei formation induced by cyclophosphaminde are different.

These data indicated that different species of plant should be considered in the study of biological effects of REE for agricultural use.

Table 4 Effect of Eight Species of kiwi Juice Supplementation on Micronuclei Formation Induced by Cyclophosphaminde (CP)

Group	Animal number	Number of PCE	NO. of PCE with MN	Micronucleated PCE	P
Positive control (placebo+ CP50mg/kg)	10	10000	488	48.8±2.8	
H.kiwi+CP	8	8000	379	47.4±3.5	>0.05
Chuan No.4 kiwi+CP	9	9000	358	39.8±3.3*	<0.05
FM kiwi+CP	10	10000	365	36.5±3.5**	<0.01
AEB kiwi+CP	10	10000	349	34.9±2.3***	<0.001
Chuan No.3 kiwi+CP	10	10000	346	34.6±2.6***	<0.001
Qin Mei kiwi+CP	9	9000	283	31.4±2.6***	<0.001
Hua Mei(1)kiwi+CP	10	10000	285	28.5±2.0***	<0.001
Hua Mei(2)kiwi+CP	10	10000	272	27.2±1.2***	<0.001
Negative Control (Placebo+Normal saline)	10	10000	19	1.9±0.2	<0.001

PCE/NCE ratio>0.01 Data square root transformation: x= $\sqrt{x+1}$ Data compared with positive control.

Rare earths have been used in humans and in agriculture. However, more extensive research including in vivo and long term exposure human studies is needed.

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An evaluation of methods and environmental quality criteria to calculate critical loads for heavy metals for soils

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Abstract

In order to enable application of the critical load approach in the international heavy metal abatement policies, critical loads of lead, cadmium, copper, zinc, lindane and benzo(a)pyrene have been calculated for Dutch forest soils. The influence of different environmental quality criteria (soil, soil solution and ground water) and calculation methods (steady-state and simple dynamic model) was investigated by calculating critical loads for approximately 18000 forest-soil combinations in 5 x 5 km grid cells derived from detailed soil- and vegetation maps for the Netherlands. Results indicate that the calculated critical loads strongly depend on the type of environmental quality criterium, which serves as a basis for the calculation, and the calculation method. Critical loads based on critical metal contents in the soil and application a steady-state model increased with a decrease in adsorption constant (values were thus highest for acid sandy soils) due to a decrease in metal accumulation. This approach, however, ignores the adverse effects of elevated dissolved metal concentrations on vegetation, soil fauna and (metal leaching to) ground water. Use of critical dissolved metal concentrations, either in soil or groundwater, and applying a steady-state model resulted in critical loads that mainly depended on the precipitation excess. Use of critical dissolved metal concentrations and applying a (simple) dynamic approach implied that the calculated critical loads increased with an increase in adsorption constant (highest for calcareous clay soils). The reason for this is that for a given critical metal concentration in the soil solution, the associated critical metal content in the solid phase increases when the adsorption constant is higher. This in turn lead to an increase in the acceptable accumulation rate during a target period of 100 year, since the difference between the critical and actual metal content increased. Considering the general weak ecotoxicological basis of the quality criteria, more information on these criteria, combined with a time target with respect to protection, is essential to assess the risk of the loads of heavy metals on terrestrial ecosystems.

Introduction

Concern on the dispersion and impacts of heavy metals and persistent organic pollutants (POPs) is large. For example, more than twenty working groups and task forces in

Europe are presently working on policies to prevent air pollution by these substances. In the past years several studies have therefore been carried out to assess critical loads of heavy metals for forest soils and surface waters both on a national scale (ATMODEP study; Bakker, 1995) and a European scale (ESQUAD study; Van den Hout, 1994; Reinds et al, 1995). Based on these studies, DLO Winand Staring Centre and TNO has developed a draft manuals for the calculation of critical loads of heavy metals (De Vries and Bakker, 1996) on soils and surface waters. In this manual, the critical load of heavy metals for soil has been defined as the deposition level, which at steady-state will lead to a metal concentration in a given ecosystem compartment (e.g soil, soil solution, ground water or plants) that equals a given environmental quality objective. The criterium to be used depends on what we want to protect: the soil fauna (soil criterion), the soil vegetation (soils solution criterion) or the human beings that use ground water for drinking water or that consume crops that are grown on the soil.

Here we report the results of a study in which the influence of different environmental quality criteria (soil, soil solution and ground water) and calculation methods (steady-state and simple dynamic models) on calculated critical loads has been investigated.
Critical loads based on a steady-state model were calculated as the net removal (uptake minus litterfall) by forests minus the metal weathering rate plus a critical metal leaching rate. A critical metal leaching rate was calculated by multiplying the precipitation excess with a total metal concentration in the soil solution or in ground water. When criteria for total metal contents in soil solid phase were used, an estimate of the dissolved concentration was made based on a linear partition equation that accounts for both adsorption and complexation processes. An alternative simple dynamic approach was also applied, in which the accumulation until a given or calculated critical metal content in the soil was accepted in a finite time period (100 years) instead of an infinite period (steady-state approach). The study has been limited to critical load calculations for the heavy metals lead (Pb), cadmium (Cd), copper (Cu) and zinc (Zn) for Dutch forest soils. An indication of the environmental risk of the pollutants is presented by the difference between present loads and critical loads.

Methods and data

The influence of different environmental quality criteria and calculation methods was investigated by calculating critical loads for approximately 18000 forest-soil combinations in 5 x 5 km grid cells derived from detailed soil- and vegetation maps for the Netherlands. Calculations were made for the organic layer and the mineral topsoil (top 10 cm) of major forest-soil combinations in each 5 km x 5 km gridcell over the Netherlands. Information on the atmospheric deposition for each grid was derived by using the emission/deposition model TREND. The atmospheric deposition on forests within a grid was then derived by multiplying this deposition with a filtering factor, thus correcting for forest filtering of dry deposition (see De Vries and Bakker, 1996). The critical load of pollutants has been defined as the deposition level that will ultimately lead to pollutant concentrations in various ecosystem compartments (e.g. soil, ground water, vegetation) that equal given environmental quality criteria. Information on the environmental quality criteria, models and input data is summarized below.

Environmental quality criteria
A distinction can be made in effects of heavy metals on soil micro organisms/soil fauna, vascular plants, terrestrial fauna and humans. In this context, criteria can be chosen for the total heavy metal concentrations in (i) the solid phase (organic layer or the mineral top soil), to protect microbiota and soil organisms such as earthworms that consume soil solid material, (ii) the soil solution, to avoid effects on vascular plants, such as elevated uptake and reduced growth, (iii) groundwater, to avoid drinking water contamination and (iv) crops/foliage, to avoid toxic effects on humans consuming the crops (De Vries and Bakker, 1996). In this study, use was made of critical metal concentrations in the soil solid phase, the soil solution and groundwater in order to calculate critical loads (Table 1).

Table 1 *Environmental quality objectives for total metal concentrations in soil, soil solution and ground water (after De Vries and Bakker, 1996).*

Heavy Metal	Soil[1)] ($mg.kg^{-1}$)	Soil solution[2)] ($mg.m^{-3}$)	Ground water[3)] ($mg.m^{-3}$)
Pb	50+L+H	100	15
Cd	0.4+0.007(L+3H)	20	1.5
Cu	15+0.6(L+H)	20	15
Zn	50+1.5(2L+H)	200	150

1) Based on background concentrations in relatively unpolluted areas. Values depend on the clay (lutum) content (L in %) and the organic matter (Humus) content (H in %).

2) Based on ecotoxicological data from laboratory experiments, with culture solutions.

3) Based on background concentrations in relatively unpolluted areas.

Note that the total metal concentrations in soil and ground water are based on background concentrations in relatively unpolluted areas and not on ecotoxicological data.
The reason is that these so-called Dutch target values are used in environmental policy.

Calculation methods
The calculation methods used are all based on several assumptions including equilibrium partitioning, a homogeneously mixed soil system and the occurrence of oxidized circumstances. This implies that the models can only be applied to homogeneous humus layers and upper mineral soil layers of well-drained soils. More information on the assumptions and inherent limitations is given in De Vries and Bakker (1996).

The "default" model used for heavy metals is a steady-state model containing (i) a mass balance equation describing the input-output fluxes of heavy metals, combined with (ii) rate-limited process descriptions for metal cycling due to litterfall and plant uptake, weathering, surface run-off, bypass flow, leaching and (iii) equilibrium descriptions for adsorption and complexation processes, that determine the partitioning of heavy metals between the different soil phases. In this study, the effects of surface runoff and bypass flow were neglected. This implies that the critical load equalled the net metal loss by above- and below-ground forest uptake corrected for litterfall, minus the metal weathering rate (negligible in the humus layer) plus a critical metal leaching rate.

In order to calculate a critical leaching rate, the critical total metal concentration in the soil solution must be known. This may be derived directly (Section 2.1) or indirectly from critical total metal concentrations in soil. The latter approach requires the inclusion of adsorption and complexation processes. The adsorption/ complexation calculations applied in this study were a simple linear equilibrium partition equation that relates the total metal content in the soil to the total metal concentration in the soil solution (De Vries and Bakker, 1996). In this approach, adsorption and complexation are lumped into one partition coefficient. The linear partition (adsorption) constants were estimated from transfer functions with soil properties such as pH, CEC, organic matter and clay content.

The time-period to reach steady-state in the mineral soil can be very long for heavy metals in the mineral soil. Therefore, an alternative (semi-)dynamic method was also applied. In this approach a critical metal accumulation rate (the accumulation of heavy metals in soils until a given critical content in a 100 year time period) was added to the calculated critical load. Using this approach, the present metal status is affecting the critical load, or target load. To avoid confusion with the critical loads calculated with a steady-state model, the term target load is preferred since a definite time target (100 year) is included in the calculation. When environmental quality criteria for the soil solution were used, the associated critical metal concentration in the solid phase was calculated with the adsorption and complexation reactions described above.

Input data
Input data were derived as a function of the substance considered, location (receptor area) and receptor (the combination of land use and soil type). Regarding the type of forest, a distinction was made between pine forests, spruce forests and deciduous forests. Seven major soil groups were distinguished (calcareous and non-calcareous sandy soils, the latter being divided in mineral-poor and mineral-rich soils, loess soils, calcareous and non-calcareous clay soils and peat soils) on the basis of the soil map of the Netherlands 1:50.000. A summarizing overview of the data acquisition approach is given in Table 1. A more detailed overview of the various input data is given in De Vries and Bakker (1996).

Table 2 *Data acquisition approaches for input data.*

Input data	Data acquisition approach
Precipitation	Estimate per grid, interpolating data of weather stations
Interception	Relationship with precipitation amount and forest type
Transpiration	Calculated as a function of precipitation, forest type and soil type
Litterfall/foliar uptake	Relationship with deposition and forest type
Root uptake	Relationship with deposition and forest type
Adsorption	Relationship with soil characteristics such as pH, organic matter content, clay content and CEC based on literature data
Complexation	Relationship with pH based on literature data;

The soil parameters used for the heavy metal model (pH, organic carbon concentration in the soil and soil solution, clay content, CEC and the dissolved Ca concentration of both the organic layer and mineral layer) were based on available data for 200 forested stands for the year 1995. Values for each forest type/soil type in each 5 km x 5 km gridcell were derived by regression relationships with available data on tree species, soil type, modelled atmospheric deposition level etc. A similar approach was used to derive the present heavy metal contents, used in the simple dynamic approach (Leeters et al., 1994).

Results and discussion

Critical loads

Calculated critical loads based on a steady-state model strongly depended on the environmental quality criterium used and the soil type considered as illustrated in Table 3.

Table 3 *Median critical loads of heavy metals for the mineral topsoil as a function of the environmental quality criterium.*

Soil	Critical load $mg.m^{-2}.yr^{-1}$							
	Soil criterium				Soil solution criterium			
	Pb	Cd	Cu	Zn	Pb	Cd	Cu	Zn
Sand	7.1	4.1	208	930	44	9.0	12	106
Loess	8.2	3.6	357	602	54	13	16	131
Clay	2.6	0.70	121	86	46	12	12	112
Peat	15	1.4	548	930	50	12	14	121
Calcareous	0.03	0.07	15	18	48	13	13	119
All	7.0	4.0	205	907	45	9.3	12	108

Use of a critical metal concentration in the soil, while using the steady-state method) resulted in higher critical loads for (acid) sandy soils and lower critical loads for calcareous soils (Table 3). This is because the critical load increases with a decrease in adsorption constant (occurs in the direction from calcareous clay soils to acid sandy soils), due to a decrease in metal accumulation. The adverse effects of elevated dissolved metal concentrations on vegetation, soil fauna and ground water (the latter through metal leaching) are not accounted for in this approach.

Use of critical metal concentrations in the soil solution caused higher critical loads for Pb and Cd and lower critical loads for Cu and Zn as compared to the soil criterion. Furthermore, use of the soil solution criterium resulted in only small differences between the different soil groups (Table 3). This can be expected since adsorption and complexation descriptions are not needed when using critical dissolved metal concentrations, which implies that the critical load mainly depends on the precipitation excess.

Comparison of present loads on forests and critical loads (Table 4) showed that exceedances hardly ever occurred for Cd, Cu and Zn in relation to both the soil and soil solution criterium. Use of a ground water criterium sometimes caused exceedances for Cd but not for Cu and Zn. For those metals, calculated critical loads were quite comparable to those calculated with the soil solution criterium (compare the results in Table 4 and the quality criteria in Table 1). Unlike the other metals, present loads of Pb often exceeded the critical loads when using the soil criterion (Table 4), especially in calcareous soils, clay soils and peat soils. Use of the ground water criterion also caused exceedances at more than 50% of the forested area. Note, however, that the results for the ground water criterium are only indicative, since the assumption of a homogeneous soil system is not valid for a soil profile with a depth until ground water level.

Table 4 *A comparison of median values of modelled present loads (year 1995) and critical loads of heavy metals for the mineral topsoil related to various environmental quality criteria.*

Heavy	Present load ($mg.m^{-2}.yr^{-1}$)		Critical load ($mg.m^{-2}.yr^{-1}$)		
metal	Grid	Forests[1)]	Soil	Solution	Groundwater
Pb	4.5	9.1	7.0	45	6.1
Cd	0.10	0.26	4.0	9.3	0.79
Cu	0.93	2.1	205	12	9.2
Zn	4.0	8.4	907	108	79

1) The modelled deposition on a grid multiplied by a forest filtering factor (see Section 2).

Target loads

The alternative simple dynamic approach showed that the highest target loads were calculated for calcareous clay soils and lowest for acid sandy soils (Table 5). The reason for this is that for a given critical metal concentration in the soil solution, the associated critical metal content in the solid phase increases when the adsorption constant is higher. This in turn lead to an increase in the acceptable accumulation rate during the 100 year period, since the difference between the critical and actual metal content increased. For Cd, Cu and Zn, the calculated target loads were comparable to the critical loads related to the soil solution criterion, especially for the sandy soils. For Pb, however, the target load was much higher (compare Table 4 and 5).

Table 5 *Median target loads for heavy metals for the mineral topsoil.*

Soil group	Target load ($mg.m^{-2}.yr^{-1}$)			
	Pb	Cd	Cu	Zn
Sand	333	10	108	11
Loess	376	17	117	9.3
Clay	1055	26	300	9.2
Peat	75	14	111	12
Calcareous	5424	97	1031	36
All	341	11	11	111

Conclusions

From this study we draw the following conclusions

1. Calculated critical loads based on a steady-state model strongly depend on the type of environmental quality criterium used. More information on the quality criteria to be used is thus essential to gain insight in the risk of the loads of heavy metals on the terrestrial ecosystem.
2. Differences in environmental quality criteria and calculation methods may completely reverse the spatial pattern of calculated critical loads. Critical loads based on critical metal contents in the soil and application a steady-state model increased with a decrease in adsorption constant (values were thus highest for acid sandy soils) due to a decrease in metal accumulation, whereas use of critical dissolved metal concentrations and applying a (simple) dynamic approach implied that the calculated critical loads increased with an increase in adsorption constant (highest for calcareous clay soils).
3. Present atmospheric loads on Dutch forests mainly exceed critical loads of Pb for soil and groundwater and of lindane for soil (organic layer). Results are, however, strongly influenced by the uncertainty in descriptions and input data for adsorption/complexation of heavy metals.

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Conclusions, recommendations and scientific questions related to the 2nd Sino-Dutch Workshop on the environmental behaviour and ecotoxicology of rare earth elements and heavy metals

During the workshop some conclusion were drawn, recommendations were made and questions were posed on the following topics.

Risk Assessment:

For estimating the risk involved in the use of REEs, an assessment has to be made of the magnitude of the consequences as well as the likelihood of adverse effects to happen. Factors of importance are the degree of exposure, (*e.g.* the concentration of REE occurring in the environment) and the adverse affects of REE on man and nature.

Elements and activities to gain insight in the magnitude of environmental pollution and the degree of exposure by REE are:

– Sources of REE.

Activity: Inventorying the amount and frequency of the use of REE in different regions.

– Dispersion and fate of REE in the environment.

Activity: Dispersion modelling to predict the dispersion pathways and receptor areas of REE.

– Behaviour of REE and soil properties.

Activity: Research into the mobility of REE under various conditions, degradation pathways, partitioning (*e.g.* between soil, water, air, sediments, and biota), and speciation in relation to bioavailability).

– Measuring and monitoring.

Activity: Development of a sampling and monitoring strategy to determine the current and future concentration levels of REE in soil, surface/groundwater, air and crop. Also the use of bio-monitoring techniques such as the use of mussels or aquatic plants should be considered.

– Foodchains.

Activity: Identifying the most important routes of intake and analysing the transport routes of REE into the most critical foodchains.

Toxicological aspects

Some outstanding questions in the field of toxicology remain. These may be summarised as follows:

- Are REE essential to organisms and is *e.g.* growth promotion due to hormesis?
- Can species adapt to REE?
- How well are uptake kinetics and elimination mechanisms of REE established? It has, amongst other, to be established if REE elements may be transported through cell membranes and if not, how they affect the organism outside the cell.
- In which chemical species are REE bio-available?
- How should LC50; EC50 and NOEC data be interpreted in view of losses of available REE in the exposure medium due to matrix effects and low solubility of the lanthanides?
- Can we find biomarkers, like metallo-thioneine (or similar protecting proteins) for REE exposure in aquatic organisms, plants and humans?
- Should EQC for REE be established? How should results of experiments conducted over a relatively short period of time and in view of the methodological problems mentioned above be used to establish EQC? What role epidemiological studies may play in establishing EQC?

  In China excellent conditions prevail for such studies; *i.e.* areas with a low environmental concentration of REE vs. areas with a high natural background concentrations and areas where large quantities of REE-fertilisers are being used.

Ecotoxicology of REE

By studying the ecotoxicological effects of REE attention to the following aspects should be paid:

- How does REE interfere with the integrety of the soil ecosystem, with respect to:
  1) physical/chemical and biological aspects
  2) soil metabolism
- How do we "translate" individual NOEC in NOEC for the ecosystem as a whole?
- Are antagonistic or synergystic effects to be expected for REE exposure?
- What interactions between various elements of the ecosystem (microbes, plants, animals) are of importance for the ecotoxicology of REE?