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Chitin as a Sorbent Superior to Other Biopolymers: Features and Applications in Environmental Research, Energy Conversion, and Understanding Evolution of Animals

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Abstract: Chitin is an effective sorbent which can be used in environmental monitoring, beyond obvious applications in withholding metal-containing pollutants from wastewater- or nuclear fuel reprocessing flows, since background levels in (purified) chitin are very low except for a few metals (Fe, Cu, Al, Ti, and Zn). Since retention of M^{x+} and their complexes on chitin depend on an oxidation state, and to a lesser extent the presence of possible ligands or co-ligands, partition between chitin samples exposed to sediment and those exposed to water can be changed by environmental factors such as local biota producing or absorbing/metabolizing effective ligands such as citrate or oxalate and by changes of redox potential. Thermodynamics are studied via log P, using calibration functions log P vs. 1/r or log P vs. $\Sigma\sigma$ (sum of Hammett parameters of ligand donor groups) for di- and trivalent elements not involved in biochemical activity (not even indirectly) and thus measuring "deviations" from expected values. These "deviations" can be due to input as a pollutant, biochemical use of certain elements, precipitation or (bio-induced reduction of $SO_4{}^{2-}$ or CO_2) dissolution of solids in sediment. Biochemical processes which occur deep in sediment can be detected due to this effect. Data from grafted chitin (saturation within $\leq$ 10 min) and from outer surfaces of arthropods caught at the same site do agree well. Log P is more telling than total amounts retrieved. Future applications of these features of chitin are outlined.

Keywords: adsorption to chitin; complex formation; biomonitoring; charge (redox) fractionation; comparison of isolated chitin and arthropod covers; photoredox chemistry on chitin

1. Introduction

1.1. Chitin and Chitosan—A Comparison of Chemical and Sorptive Properties

Although chitosan, that is chitin which has undergone hydrolysis of acetamido side chains, is commonly used in medical applications and as a glue known to be inert (or get slowly dissolved and metabolized) in physiological terms [1,2], it

(a) does not occur in natural conditions and
(b) is much less robust against thermal or oxidative stress than the native chitin.

Chitin does adsorb all electrophilic agents such as metal ions [3], hydrocarbons [4] and anions such as arsenate, sulfate [5] or hexacyanoferrate which produce strong H bridges with OH groups of sugars. Even though the acetylation extent of chitin may vary (some 80–95% [6]), it proved to display reproducible features as a sorbent (for quantitative terms see below). Chitin is difficult to oxidize both by an anode and by chemical agents such as the common agents for saccharide analytic cleavage; chitin itself is completely silent in electrochemical experiments (when dissolved by adding Li salts in dimethyl formamide) even though it does contain trace amounts of redox-active ions (Fe, Ti, Cu). It can be oxidized by certain staining agents such as Ag^+ or OsO_4 only after deacetylation [6] while not being attacked by common reagents used in saccharide cleavage and analysis, such as

IO_4^- or Pb(IV) acetate [7], both of which do react with the monomer, N-acetyl glucosamine. However, alkaline oligoperiodate, identified as $H_2I_2O_{10}^{4-}$, does react, leaving behind chitin nanocrystals [8]. None of the said oxidants will occur in natural surroundings, however. Chitosan and monomers glucosamine and N-acetyl glucosamine are degraded by both IO_4^- and $Pb(CH_3COO)_4$. Pb(IV) acetate does not react with either chitin or (potato) starch, but with chitosan in DMSO/glacial acetic acid [7].

Thus, chitin is the material of choice if one wants to study sorbent properties concerning, e.g., trace metals in environmental analytics. Moreover, many different organisms, including such of meaningful operative surface sizes such as spiders [9], lichens [3,10] or many insects, crabs, and crayfish are fully or partially covered by chitin which can in turn be sampled without necessarily killing the animals or lichens. The surface of grafted chitin surface used for adsorbing metal ions has a diameter of 1 cm and thus an active surface of some 0.78 cm^2. Much smaller areas would suffice in a trade-off for sensitivity. Legs, wings, or mandibles of bigger arthropods will readily exceed this area. Chitin is much more robust towards pyrolysis than other biopolymers [11]. Degradation of natural chitin, containing proteins and metal carbonates, in moist environments, is distinguished by proteins removed first [12] while chitin may persist over >> 10^8 years in some cases [12,13].

1.2. Chemical Peculiarities Which Turn up at Ecotones and Similar Interfaces

Generally speaking, chitin behaves as a very effective sorbent which even retains most metal ions at aq. levels < 1 nM/L ([14] and literature quoted there). Chitin can be dissolved in various solvents with (slow esterification by neat formic or dichloroacetic acids) or without permanent chemical modifications (hexafluoroisopropanol [15], carboxamides or lactams containing high levels of Li salts [14]), permitting production of fibers or films upon addition of water (all these solvents are completely miscible with H_2O). Different oxidation states may differ in their retention properties, such as with di- and trivalent Eu (Eu^{2+} being eluted from chitin by slowly running water or DMF). Thus, photo- or electroredox reactions taking place next to a chitin layer in flowing water will produce a voltage due to a concentration gradient which exceeds the electrokinetic flow potential (zeta potential) by a factor of 10 at least (the pzzp of chitin is pH $\approx$ 4.6 [14], considerably lower than the pH values obtained in water with carboxylate ions or even ethanol amines [$C_{\text{substr.}}$ = 0.1 mol/L]). Aq. arthropods other than water mites, caddisfly larvae [15] or *Asellus aquaticus* would not withstand pH < 3.5 for long either [16]).

Chitin does report on non-equilibrium phenomena next to water-sediment interfaces in a unique manner. These are caused by biological uptake or precipitation of certain elements in sediment [14], distinguishing part of benthic animals exposed to water only or penetrating the sediment-water interface regularly (i.e., antennae and leg tips of crayfish [17]). A rigorous formal analysis of partition data calibrated against "inert" di- and trivalent cations, respectively, yields much more information (see below). In addition, adsorption of M to chitin was studied depending on presence of such ligands which can be expected in soil liquids, ambient (fresh) waters or mining leachates. None of these features is present in chitosan or any other native or modified biopolymer, including cellulose (moss-based biomonitoring) or proteins. Hence, it is worthwhile to describe methods and results concerning chitin within this framework, too.

Although chitin does undergo slow surface hydrolysis, significant change of metal ion adsorption properties is limited to a few elements (Y, Pb, Bi). "Normal" (crustacean) chitin (non-digging crayfish species *Orconectes limosus*) compares well to grafted chitin samples once exposed to boiling water; this was conducted either to euthanize crayfish caught alive for studying adsorption from either water or sediment depending on body parts/organs and their position/function in situ, or for comparison of data using grafted chitin. This does imply hydrolysis is negligible insofar as changes of complexation are concerned. Metals forming very stable ammin-, amine complexes such as Ni and Cu actually display a slight effect on adsorption after heat treatment of chitin samples. To put it the other way round, we still deal with chitin rather than chitosan after the chitin surface or animal had been (briefly) exposed to boiling water (see below). Apart from this, chitin is exceptionally robust in terms of thermal treatment, unlike other biopolymers [3,12].

Using this property of chitin takes one beyond simply deriving some relationship between adsorbed ion quantity and environmental concentrations. In fact, non-equilibrium states can be detected by comparing adsorption at adjacent sites (partition) and using transport. This feature is unique in biomonitoring. This is shown in the following diagrams. This can be observed at a water/inundated sediment interface. When biological activity or precipitation of solids cause "downward" (water→sediment) diffusion, the partition coefficient will increase, meaning more is adsorbed on chitin exposed to sediment (green arrow in Figure 1):

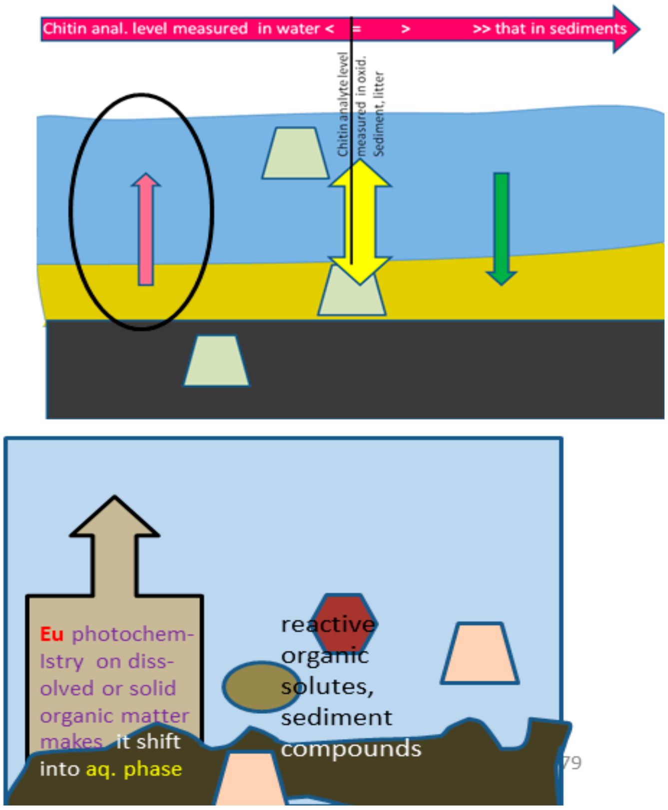
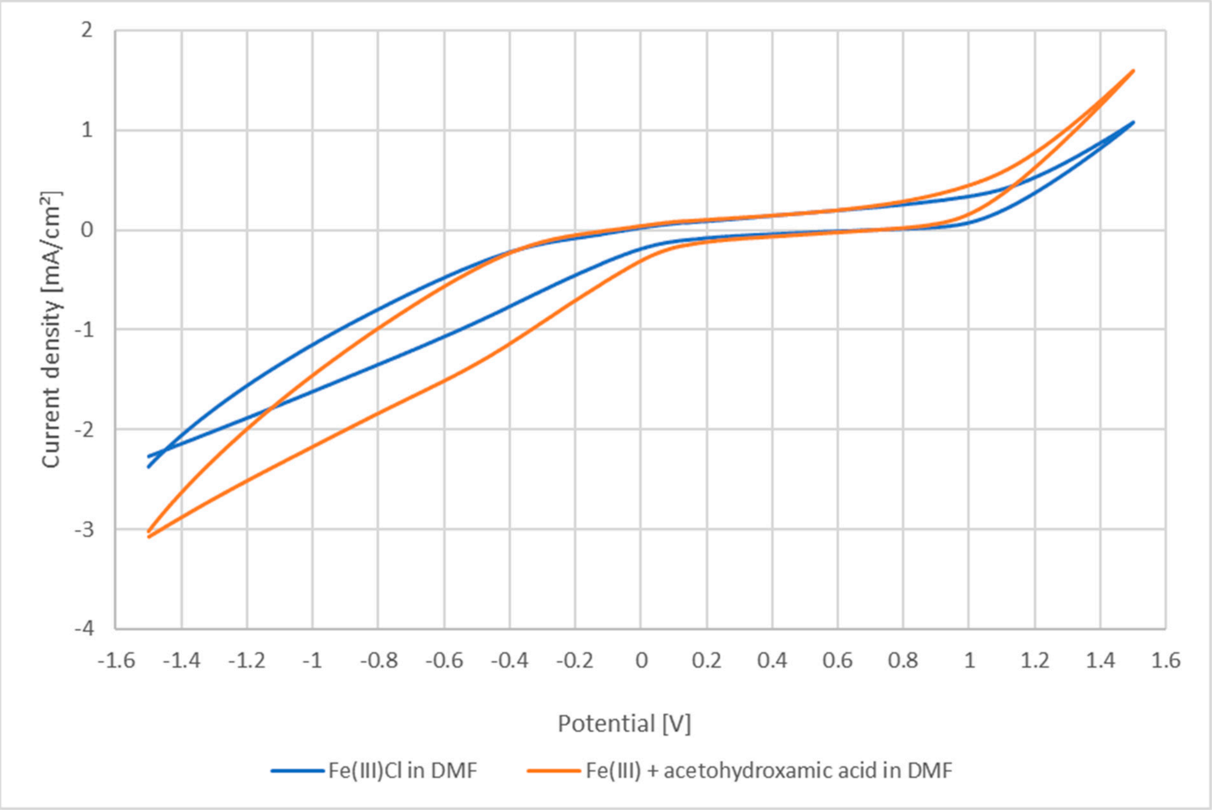


Figure 1. Partition of metals on chitin flakes embedded in water (**top**) and sediment (**below**) and corresponding effects concerning europium (Eu) co-illuminated with organic matter and photo-oxidizing it. Violet upward-pointing arrow refers to removal of Ba and Pb from sediment and depletion on the chitin flakes located there. The green downward arrow may be due to either input from above (limnetic or aerosol) or uptake deeper in the sediment. By this method one can detect processes which occur at least 40 cm from the interface, including methanogenesis linked to Ni which otherwise is too scarce to effect it without aerial input in ombrotrophic bogs. It does not matter whether this is the bottom of some water pool or its beach.

2. Materials and Methods

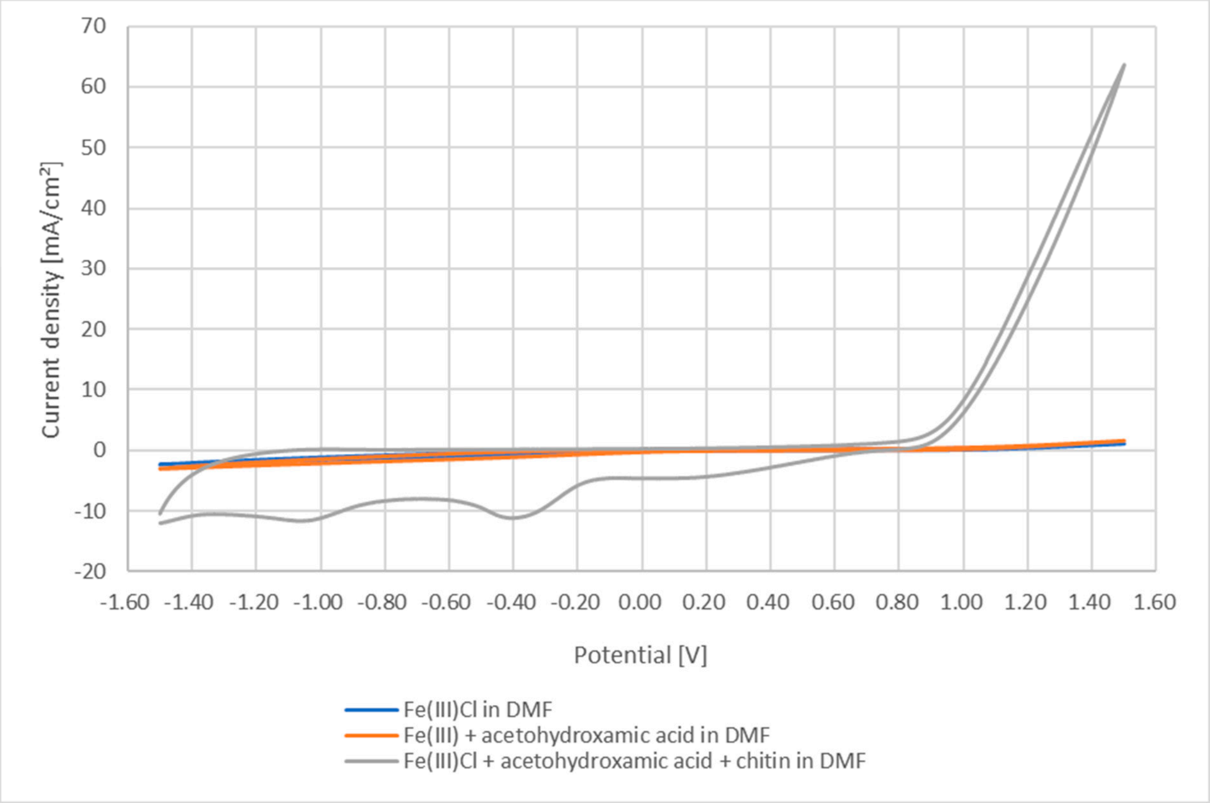
The procedure of sample processing and extraction of data was described before ([14,17,18]). Surface dissolution is conducted using $LiClO_4$ solutions in DMF, and absorption of this solute by cation exchanger resins kept in home-built Nylon baglets [17]. Backward ion exchange is achieved by flushing the bag several times by 1% aq. nitric acid subsequent to ion exchange which permits direct ICP-MS measurements (and re-use of the ion exchanger bag if wanted [17,18]). Amounts of reagent and periods of time in the workup steps were conducted according to the optimization procedures given by [14,17,18]. ICP-MS was undertaken by a Perkin-Elmer DR-Ce, not using the reaction cell. Detection limits were exceeded by far also for most REEs and other elements. A total of 26 elements was studied, omitting those which are abundant in biomass, yet do not bind to chitin (alkali metals, Mg, Ca) [14]. Solutions of chitin prepared in this manner do also bind metal ions up to a comparable amount. When doing electrochemistry with such a solution, metal ions (if exceeding chitin saturation), chitin adducts and complex chitin adducts all do contribute to an electrochemical signal which can be determined by means of cyclic voltammetry. An example is given in Figure 2:

Whereas both metal (e.g., Fe like in this example) ions and complexes can be adsorbed to chitin, the latter may or may not adsorb without exchange of ligands other than halides. This enhancement effect was demonstrated recently in a simple experiment by these authors: after dissolving (anhydrous) $FeCl_3$ in 40 mL DMF (2.7 mM/L), same amount (i.e., 100 µmol = 7.5 mg) of solid acetohydroxamic acid $CH_3CON(H)$-OH (causing immediate color change, meant to represent exudates from soil fungi or bacteria) and finally 20 mL of a saturated solution of shrimp chitin in DMF (1.6 M $LiClO_4$) were added.



(**a**)

Figure 2. *Cont.*



(**b**)

Figure 2. (**a**): Electrochemical behavior of $FeCl_3$ dissolved in DMF (cp. [18,19]) as such and after adding acetohydroxamic acid. (**b**): Superposition of all three redox systems between −1.5 and + 1.5 V vs. SCE. Chitin adsorption does markedly increase measured current by organizing the redox system near electrode. Smell of solution indicates that a terminal ($\varepsilon \geq 0.9$ V vs. SCE) increase in irreversible oxidation current is due to decay product dimethyl amine (for its oxidation properties cp. [20]).

The main redox transition of $[Fe(CH_3CO\text{-}N(H)O)]^{2+}$ at −0.4 V remains at its position but becomes both stronger and irreversible, indicating desorption of Fe^{2+} from chitin. In the original solution there are just two species, namely partially hydrated ${FeCl_2}^+$ and $[FeCl_4]^-$ solvated by DMF [21]. The effect of enhancing electrochemical activity may be important for both fuel cell applications and electroanalytical sensor methods (see below).

2.1. Technique of Sampling

Sampling was conducted by either grafted chitin [14] and/or collecting arthropods [17,22], lichens [10]. The former method is preferred for reasons of both

(a) protection of possibly endangered species and
(b) full control of exposition times while

The chitin sample can be located in either sediment or open water (it is recommended to mark chitin flakes placed in gravel- or other sediment by colored plastic strips or the like to improve the chances of retrieving them). Grafted chitin thus duplicates exposition features of “live” chitin whether or not the animal is a benthic one. Additionally, grafted chitin can be used in conditions by far exceeding those which would permit animals to survive, like excessive heat, cold, insecticides, radioactivity, etc., for most aquatic animals pH < 3.5 [16]. The latter fact allows detection of traces of contaminants, as well as determination of non-equilibrium states due to “uncommon” metabolic activities (i.e., such catalyzed by elements less common in biology). Typical examples are Ni (methanogenesis) or V (haloperoxidases, N_2 fixation when Mo is depleted) or precipitation/dissolution of insoluble salts. The latter may include biochemical activity, too, particularly when Ba or Pb sulfates are dissolved by reduction consuming H_2, CH_4, or other organics in inundated sediment (see below).

The sampling is least invasive; in fact, the technique was designed to sample the same individuals of living arthropods (cricket *Gryllus assimilis*) several times, just etching the surface a little bit without intoxicating or killing the animal (both for protection concerns and to obtain a baseline for a single animal before beginning active biomonitoring) [14,22]. The DMF/Li^+ solution (employing the most simple and convenient tertiary carboxamide available) can reach/suspend chitin concentrations of some 30 g/L solvent at $\geq$ 2 M/L Li^+ (Li salt = nitrate, chloride, bromide, perchlorate, trifluoromethane sulfonate but not acetate or other carboxylates which do not suspend chitin in DMF even at similar Li levels). This method was selected and optimized (times, concentrations) after experiments conducted in my workgroup to obtain films of materials dissolving surface chitin and its sorbates without working in a liquid medium gave disappointing results: f.e., when melting ε-caprolactam or δ-valerolactam (m.p. = 40 °C) and adding Li salts decomposition and carbonization invariably occurred when adding solid Li salts to the lactam melts before even making contact with chitin. The end was to finally remove the solidified lactam/nylon film containing chitin and the analytes from the surface. Hence the experiment could not be completed into testing the retention to some organic film ripped from the chitin support [23].

Such solutions of chitin in DMF ($LiClO_4$ as a conducting salt) are completely silent in electrochemical terms (ε in between −2.6 up to +1.73 V vs. SCE (onset potential of oxidative solvent breakdown in DMF and similar carboxamides) although there are traces of metals in native chitin which might undergo redox transitions within this range (10–20 ppm Fe, some 15 ppm Ti, 1.5 ppm Cu). Redox transitions do only occur after addition of metal salts or complexes and can be used to study adsorption to (dissolved) polymer strands by adding little by little metal salt until the original transition in DMF turns up (saturation; effects of added or present ligands can be studied in the same manner).

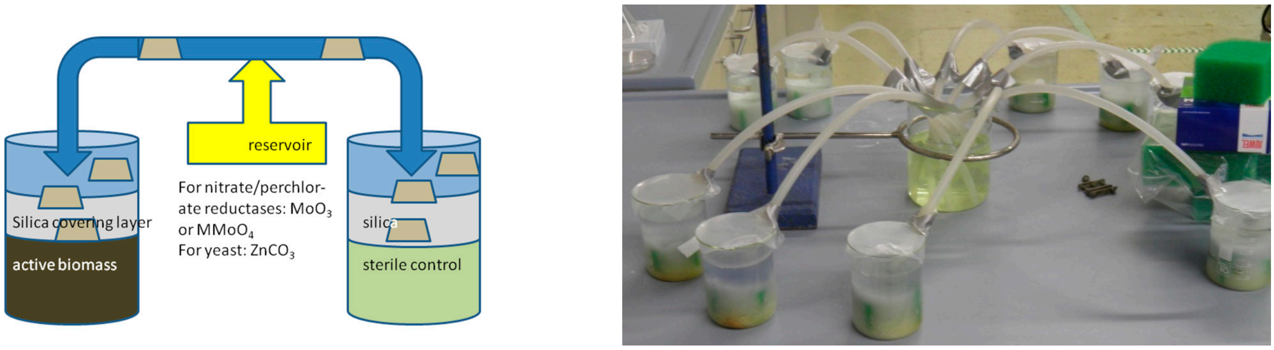
2.2. Processing of Data

Thus, meaningful partition coefficients are defined using sets of elements (di- or tri- or tetravalent metals, As, Sb, Se, Ge, etc.) which are not involved in biology. Partition coefficients P of such elements are plotted against functions which relate to complex formation constants β of "similar" complexes vs. Hammett's σ constant or reciprocal ion radii 1/r. The equations depend on the assumption that

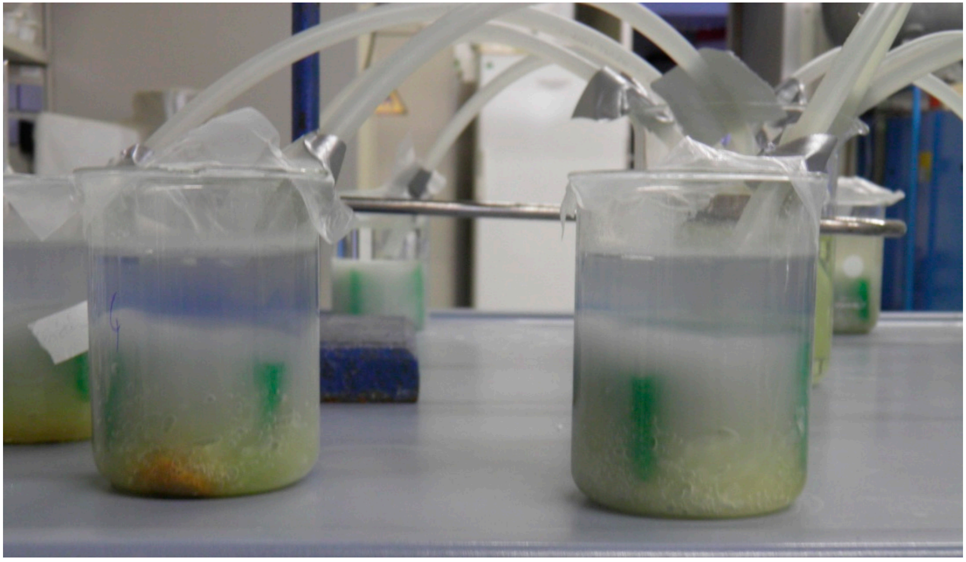
$$\log P \sim \log \beta(ML) = a \times \log \beta + b \tag{1}$$

for some "test" or "equivalent" ligand L. a and b are given in a table below; the sign a

Then, corresponding data for "active" elements are introduced (e.g., Cu, Zn) to calculate differences vs. the expected value. The same kind of shift happens when equilibrium is perturbed by photochemical processes (mediated Eu (bottom of Figure 1), or U, then changing partition of either element) or by external redox processes, e.g., responding to strongly reducing conditions (Figure 6). These effects can be correlated with observations of enzyme-related product formation/substrate consumption along the interface. Examples from our work include CH_4 formation vs. Ni distribution (open field: a bog located right on the Polish–Czech border W of Mt. Śniezka (Sudety Mountains)) and nitrate reduction vs. uptake of Mo (model study done in our lab; Figure 3):



(central beaker with Mo stock solution connected to both active and inert [control] vessels)



(active [left] and inert [right] vessels)

Figure 3. Transfer of Mo from central solution (pale yellow solution in central beaker in top right picture) via filled tubes to beakers where there is glucose, nitrate and nitrate reductase (bacterial pellets) or the latter is missing (sterile control). When there is biological activity, nitrate will be depleted while the levels of Mo inside silica layer are 2.5–2.9 times those on chitin placed in aq. supernatant. In controls (right), $P_{\text{chitin;sedim./chitin;H2O}} < 1.5$. Once nitrate is depleted, glucose keeps on reducing Mo turning the supernatant blue (bottom picture, left). Chitin flakes within tubes were omitted, transport occurring simply by diffusion, the "pump effect" of evaporation (and oxidation by O_2 rather than NO_3^-) being limited by Parafilm® covers.

Possible reasons for non-equilibrium according to Figure 1 include photochemistry, biological/biochemical activity involving less-common and less-abundant metals and redox reactions which may cut linkage between chitin strands in either solid polymers or dissolved chitin.

The retention by chitin does also depend on oxidation state of an element. Thus, it will respond to redox or photoredox processes by changes of partition. Accordingly, some reduced (Eu^{2+}) or oxidized ($HCrO_4^-$) forms of an element can be removed/extracted from the chitin layer by some slow flow of solvent, sometimes facilitated by light (reduction of Eu, U) or presence of a natural catalyst such as MnO_2 that promotes air oxidations of Ce, Cr [24], Cl^-, phenols [25], alkyl benzenes, etc., with both solid Mn phases [26] and Mn^{2+}, although weakly adsorbing to chitin [22]). Then, two electrodes placed on either side of the percolated chitin pack will record different potentials due to the concentration drop (scheme Figure 4, practical setup Figure 5 below):

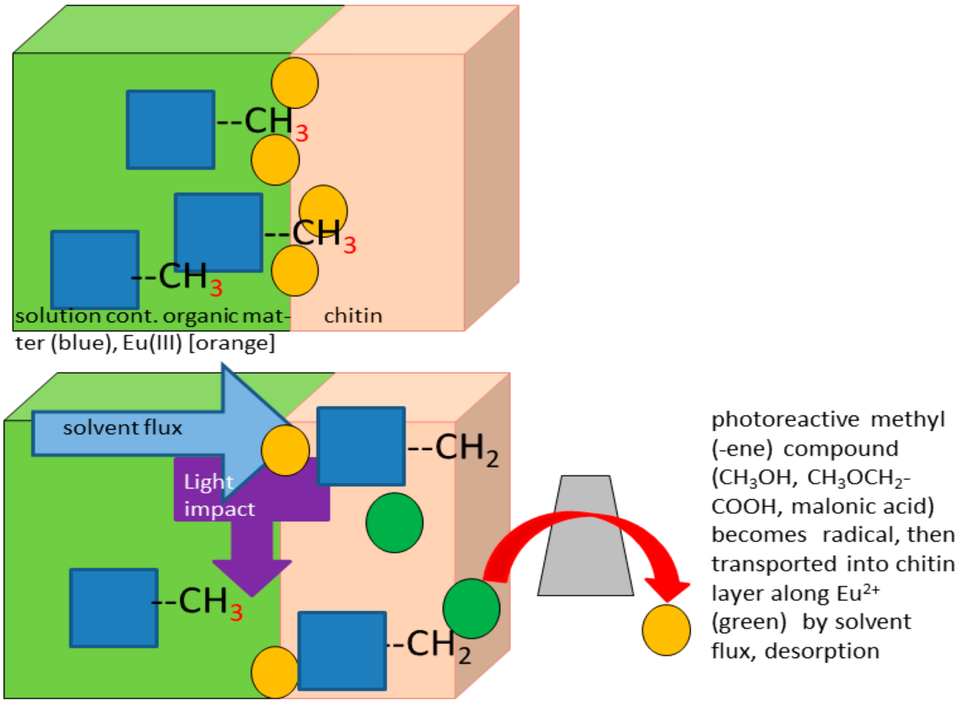


Figure 4. Eu can only pass the solvent/chitin-layer interface if reduced, otherwise it is retained by chitin surface.

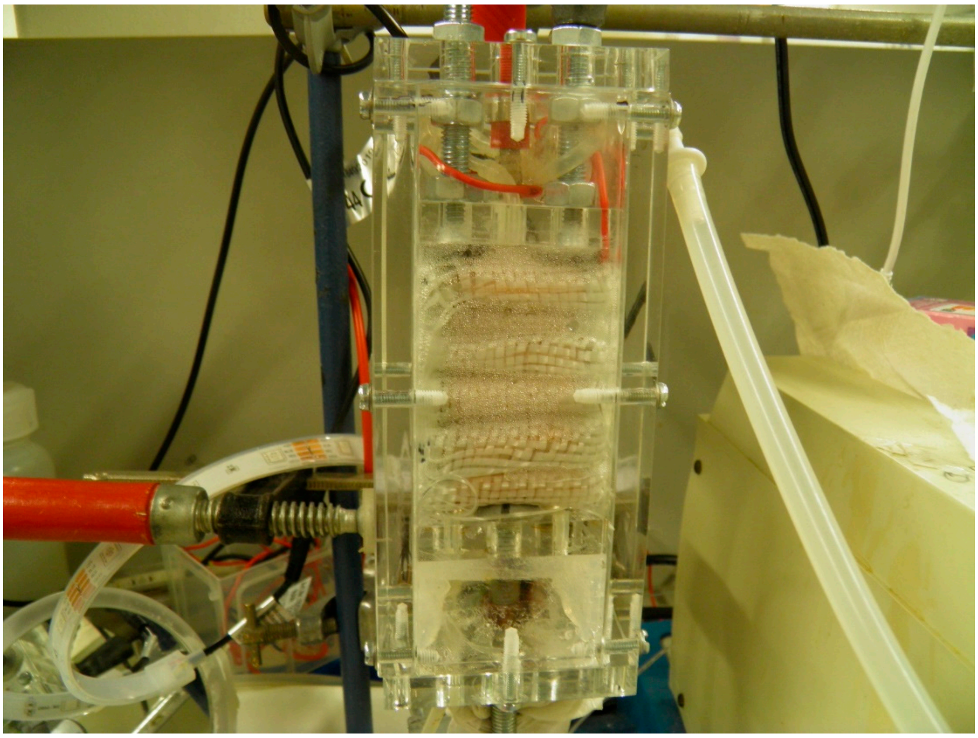
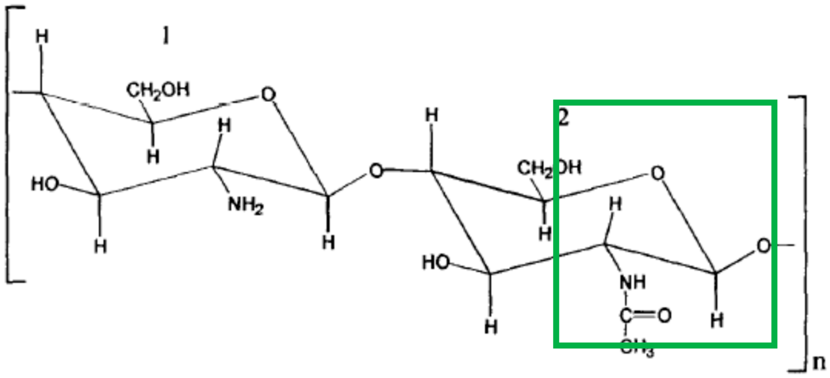


Figure 5. Design of a photo-assisted fuel-cell where excited Eu(III) does abstract hydrogen atoms from organic matter while the metalliferous product Eu^{2+} loses contact with/adsorption to chitin and is eluted "downward" to create an electrochemical potential like shown in Figure 3. When a reductant like ethanolamine spreads by diffusion in an open cell to reach Eu(III) at different times, initial potentials can be >> 100 mV implying that > 99% of Eu are reduced by LED-light before the diffusion front reaches the counter-electrode also. The folded material is glass fiber fabric which was selected to be both inert and transparent for visible light (UV radiation is not required).

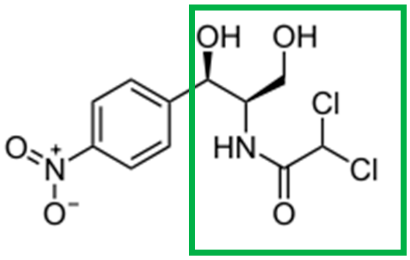
Here, sensor technology and energy conversion—meant to induce fuel cell conversion of organic compounds containing CH and NH bonds while not requiring Pt group metals for CH bond activation and storing some light energy by making Eu^{2+} and H_2 (in a secondary step)—do merge.

Beyond the statement that there is retention down to aq. M^{x+} levels < 1 nM/L, it is also feasible to compare chitin to a small ligand molecule the dative structure of which is very similar to that of chitin. One such ligand for which many complex formation constants are known is antibiotic agent chloramphenicol. Except for very simplified models of bidentate C-hydroxo/carboxamide (probably O-) binding such as N-formyl ethanolamine or N-acetylethanolamine, the best-investigated ligand for which a reasonable number of complex formation constants are available is the antibiotic agent chloramphenicol ([27], introduced into clinical applications back in 1949), cp. the key structural motifs which are relevant for M^{n+} complexation. The similarity of binding modes is shown in Figure 6a,b.



(**a**)

vs. chloramphenicol (bottom)



(**b**)

Figure 6. (**a**): Chitin, displaying a free amino group at left saccharide ring (<15% of total N in natural chitin samples, almost 100% in chitosan). (**b**) Chloramphenicol: bidentate binding of metal ions via OH and carboxamide. For complex stabilities see Table 1, note similarity of binding motif. Dichloroacetamide will display a slightly increased NH acidity as compared with acetamide groups, thus possibly enhancing binding of metal ions like Cu, Pt (cp. complexation properties of substituted hydantoins).

Table 1. List of chemicals and materials used.

Substance	Supplier	Purpose	Remarks
chitin	Sigma-Aldrich, Taufkirchen, Germany	sorbent	purified, background levels see text. No electrochemical signal unless metal salts were added
Lithium perchlorate	Sigma-Aldrich, Taufkirchen, Germany	Dissolution of chitin, conducting salt in CV	"battery-grade" purity
Cation exchanger resin	Amberlite H-120, Supelco, Bellefonte, PA, USA	Fixation and transfer of analytes	Checked for trace metal background, nothing detected. Can be re-used several times after leaching ions bound from chitin solution. Retrieval rate about 70% [18]
Nitric acid	Merck Suprapur, Darmstadt, Germany		
Crayfish *Orconectes limosus*			Caught in local waters, also exuvia were studied
Eu(III) trifluoromethanesulfonate	Sigma-Aldrich, Taufkirchen, Germany	Photooxidation reagent	
Solacor glue	Boldt & Co., Wermelskirchen, Germany	Fixation of chitin flakes	Photo-hardening, metal-free glue, rather resistant towards organic solvents including DMF

For the latter monomeric ligand the following complex formation constants (aq., I = 0.1 M, T = 25 °C) do hold (Table 2) ([27], except for lead and iron):

Table 2. Complex formation constants of M^{2+} ions, Fe(III) with chloramphenicol ([24] and other sources).

M^{2+}	Log k	Remarks
Pb	3.26	Pb does accumulate on chitin more than other metals [9,13]
Cd	3.93	background on chitin essentially zero
Mn	3.54	
Ni	3.33	Detection of methanogenesis
Zn	3.16	
Fe(III)	4.13	different oxidation state

The corresponding calculated electrochemical ligand parameter is—0.154 V (bidentate binding). This value would correspond to log k ≈ 5.7 for binding of Cu(II) to chitin using the formula introduced by this author [28]:

$$\text{Log } \beta = x \times E_L(L) + c \quad (2)$$

with x and c tabulated in [28,29]. Equation (2) is formally equivalent to (1) since there is some linear relationship between Hammett´s constant σ and the electrochemical ligand parameter [30,31], as defined by Lever [31] (there are also other scales/benchmark redox systems but these comprehend far less ligands), whereas x and c are linearly correlated and thus can be traced to Equation (1) by corresponding coefficients (suggested in [30] without actually revealing correlation equations). Understanding LREE binding to chitin is most important because it offers the only sizable dataset for trivalent ions (besides of Al, Cr, Bi) unless there is biocatalytic activity associated with some of them [32–35], and because of its application in fuel cells and sensor devices (see below).

However, both x and the slope of the latter equation (not explicitly given by [30])

$$E_L(L) = e_{nd} \times \Sigma\sigma + f_{nd}\ [V] \quad (3)$$

(parameters calculated by SF [36]) depend on denticity d and charge of ligand. Thus, a couple of parameters is required rather than a single set, given in the following Table 3:

Table 3. Relationship between σ and electrochemical ligand parameter for different kinds of ligands.

Kind of Ligands	No. of Ligands in Regression	e [Σσ]	f [V]	Remarks
Monodentate, neutral	7	0.278	0.124	N-, P-, As-, S- and C- (isocyanides) donors, which all are π-bonding (NH_3, aliphatic amines do not correlate properly)
	7	0.265	0.133	omitting pyridine, plus $AsPh_2(CH_3)$
Monodentate anions	15	0.593	−0.402	Halides, pseudohalides including CN^-, OH^-, carboxylates, etc., not H^-
Bidentate, different charges	6	−0.185	−0.176	Negative slope, also includes neutral L en (ethylene diamine)

The advantage of (1) is that many more Hammett constants σ are available (e.g., [28]) than electrochemical ligand parameters [10,29,31].

Most Hammett parameters were taken from literature [31] while complex stability constants for benzene-1,2-dicarboxylic (ortho-phthalic) acid anion, salicylate, and maleate (M = La, Nd, and Eu [37]) gave the following additional values for σ′ (Table 4):

Table 4. Hammett constants of different ligands (calculated using [13]).

Ligand (Bidentate)	Σσ	σ′	Σ of "Normal" Ligands	
o-phthalate	−0.36	−0.18	Carboxylate 0, OH −0.37	
salicylate	−0.40	OH (aromatic) = −0.22		
maleate	−0.30	Acrylic carboxylate −0.15		Cannot be broken up as definition applies to bidentate ligands

An actual chitin polymer strand has M* ≈ 400.000, and the structural motif binding M^{x+} is present about 2000 times. Hence, actual complex formation constants (log) $\beta_{M;chitin}$ are much higher than for complexation by chloramphenicol, agreeing to the fact that even pMol/l levels cause measurable adsorption of element ions.

Chitin-based biomonitoring does require reliable and fast adsorption. In our experiments, equilibrium was usually reached within 10 min. Earlier work had shown that even traces of 'heavy metals', including radionuclides [38] could be removed from water flows and slurries, causing us to explore chitin application chances in environmental analytics. Background levels of metals in commercial chitin obtained by shrimp peeling (*Pandalus borealis*; Sigma-Aldrich, Taufkirchen, Germany) are very low, permitting detections of traces of contaminants in ambient matter, too: levels of Al, Ti, and Fe are some 10–20 µg/g chitin; Cu and Zn come with about 1.5 µg/g regardless of the significant role of either metal in arthropods, while important toxic metals [22] such as Be, Cd, and U cannot easily be detected in the native material, implying almost any trace will be pinpointed. Binding capacity is about 40 µmol/g for most metals, while values for Al [22], Ce or Eu [39], V or Pb [14,22,40] are considerably higher. Most metals for which some biological role was established recently (e.g., [41]) bind rather well, so do Bi and certain others for which no (catalytic) biological role was detected so far.

3. Results

3.1. Photoredox Processes and Retention

Of course, complexation can alter the extent/equilibrium of metal ion retention. Many ligands, however, contain “free” terminal oxygen or nitrogen (carboxylate-, sulfate groups, unbound nitriles in AAN or cyanoanilines, etc.) which form H bonds with OH groups of the polysaccharide. Then, complexation will rather enhance retention of M^{x+} to chitin. Since retention of metal ions by chitin is not just “all (most metal ions, -complexes, down to < 1 mM/L) or nothing (alkali metals, Mg, Ca)”, binding rather depends on oxidation states, too; there can be both energy conversion (subsequent to redox- or photochemical changes of oxidation state) and some effect—not generally a perturbation—on adsorption for analytical purposes. Because levels of these ligands used to be low, this does not compromise results on M in normal lakes or ponds. However, it should be noted that the above change of partition might also extend to LREEs as the latter, especially La and Nd, are involved in oxidation of methanol [33,34] and other primary alcohols run by aerobic proteobacteria [35]; that is, this will happen right in the uppermost sediment layer. Except for this case, REEs can be taken—such as and adding to Al, Cr, Bi—to represent physiologically inert M^{3+} for production of such a calibration function. With M^{2+}, it can be Sr, Ba, Co, Ni (at sufficiently high potentials which preclude methanogenesis [40]), Pb, or Cd. Mg and Ca do not bind to chitin, whereas Mn, Cu, and Zn are involved in all life-forms (and thus shall generally “deviate” from the regression obtained in the above Equation (1) in favor of a stronger adsorption within sediment). However, Ba and Pb can be mobilized once there is sulfate reduction. There are no difficulties with coarse, gravel-like sediments or beaches, but fine-grained sediments are more difficult to understand if inundated. Elements commonly occurring in oxidation states +IV and higher normally do not take part in biochemistry (except for Mo, V, and sometimes W [40]).

3.2. Relationship between Donor Groups and Log σ_{para} or Log P Constants for Metal Coordination to Chitin

It is an interesting case to compare chitin in its “physiological place”—covering aquatic or moisture-exposed arthropods (like crayfish, shrimps, ants or bumblebees building nests in moist soil)—to isolated and grafted chitin samples: will adsorption patterns match insofar as measurements on living beings can be attributed to sample either the aq. phase or sediment (which means crayfish to be studied must not dig tunnels in which they live, hide or even reproduce). As can be seen from Table 4, differences against the simple aq. system are small enough to analyze adsorption chemistry/selectivity along the common way of reasoning but large enough to observe effects due to local chemistry. There are no indications for isotopic fractionation of multi-isotopic elements during chitin adsorption; for example, determinations of Sm content on chitin surfaces using isotopes ^{147}Sm, ^{149}Sm, and ^{152}Sm, respectively, provide the same result assuming the normal isotopic composition of samarium [18]; (this does likely permit to reconstruct the whereabouts of chitin-covered organisms by means of Pb [41] isotopic signature). Solvent impact may alter chitin structure [42]; acidity values of potential ligands (protonated forms thereof) in DMF are given in [43] and were used besides own measurements to derive Equations (9) and (10).

Given the binding groups of chitin (or that in any other polymeric ligand) are either obvious (say, in polyacrylonitrile, all being -CN) or can (must) be inferred from complex formation studies, the following equation (4) can be used to accomplish, with (5), referring to partition and assumed to be equivalent to (4), polymer-metal ion interactions which are taken to happen by complexation, with or without additional ligands involved:

$$\text{Log } \beta = a \times \sigma_{\text{para}} + b \text{ and} \tag{4}$$

$$\text{Log P} = \text{a} \times \Delta\sigma_{\text{para}} \tag{5}$$

where σ_{para} is Hammett´s constant (for para substitution and log P denotes the partition between chitin samples immersed into sediment and adjacent water (e.g., at bottom of some pond or creek or at a beach or bog pool lining), tacitly assuming metal binding to chitin occurs by complexation as with ligands dissolved in some solvent (this is to account for the large differences in metal retention by chitin and by either chitosan or cellulose). Data for meta substitution (e.g., [31]) are linearly correlated with σ_{para}; however, considerably less values are available for σ_{meta}. Some σ_{para} values must be modified as metal ions may bind to ligands in manners not available to withhold the aromatic groups such as phenyl or naphthyl. This also holds for carboxamide or 3-ketoenolate ligands which interact via O with metal ions, rather than forming CC bond, as well as carboxylates, nitrate, sulfate, etc. which would form esters or acid containing a C-O-E or HO-E single bond chain whereas REE^{3+} will bind via two O atoms, which is why there are different descriptions for REE ions.

Equation (3) can be rearranged to calculate an effective value of σ or $\Sigma\sigma$ even without knowing the binding functions, or in cases where M ligation differs from binding modes to aromatics, such as carboxamide or 3-ketoenolate ligands:

$$\text{Log } \beta - \text{b} = \text{a} \times \sigma_{\text{para}} \text{ and thus} \tag{6}$$

$$\sigma_{\text{eff}} = (\log \beta - \text{b})/\text{a} \tag{7}$$

Hence, log P represents the difference of local chemical conditions between the two phases connected by an ecotone while log β refers to complexation of the ion in the same solvent. This does mean the equilibrium state shown in Figure 1 (yellow double arrow) can exist for log $P \neq 0$ for respective parameters and ion sizes.

3.3. Effects of Boiling Water on M Retention

Generally speaking, chitin is very robust against both thermolysis (wet or dry) [12,13] and oxidation [7,14]. This holds for both chemical, using the colloquial reagents for poly- or monosaccharide analysis (which, however, do readily react with chitosan, see above) such as periodate or Pb(IV) acetate, and electrochemical processes (own experiments in DMF solvent containing Li^+). Yet, acetamide groups may be labile towards (hot) water hydrolysis (chitosan is prepared by rough (e.g., warm NaOH) hydrolysis from chitin [2,6]). Such hydrolysis might occur when chitin is exposed to hot (boiling) water [17]. Effects from this were studied in both grafted chitin and crayfish (this is the regular method of euthanizing them, rather than applying chemicals). As a rule, the layer from which metal ions are mobilized by DMF/Li^+ becomes thicker, and thus more metal ions are leached. Certain metals, however, show the opposite behavior, namely when amino groups produced by acetamide hydrolysis do better to bind metal ions, e.g., with Y, Ni or Cu. Complexation of trace metals essential to microorganisms (too) by chitin or chitosan apparently do block growth/multiplication of pathogenic bacteria [44] or yeasts [45].

3.4. Effects of Ligands on M Retention

While effects of non-specified ligands were considered before in this paper, adsorption depending on ligand additions was studied for a couple of elements also [18] in the test medium (water/DMF 50/50 v/v, buffered to $H_0 = 5.8$ by adding anilinium salts. We did study this for M = Mn^{2+}, Ni^{2+} [46], and six REEs (i.e., La, Ce, Sm, Eu, Dy, and Yb [18], also covering Mn). The only ion which generally does lose contact with chitin interfaces upon addition of ligands known or expected to be present in the soil solution (delivered by plant roots, fungal mycelia, or soil bacteria) is Sm(III) introduced as trifluoromethane sulfonate salt, like the other REEs [18]. The buffer was chosen to circumvent the problem that most buffers either do precipitate REEs or else undergo photooxidation by Eu(III)* while retention by chitin depends on pH or H_0 [14,22]. La and Eu do better bind to chitin when glycine was added, while retention of both Ce and HREEs Dy, Yb is increased by adding anyone of many ligands, including humic acids. Some of them are photoactive with Eu(III) while others are not. This will change results of partition measurements (Figure 1 bottom), but it is included in calibration as these measurements were conducted in very dim light.

3.5. Comparison to Chitin on Living Benthic Animals and Their Exuvia

Data on antennae (comparing to open water) and other parts of benthic, non-digging crayfish *Orconectes limosus* showed highest accumulations (BCF values) for elements such as Bi, V, and light REEs from water [17] while sediment/water partition on both chitin flakes and crayfish cover parts which penetrate into sediment (leg tips, telson) is most pronounced for LREEs La, Ce. Knowing that adsorption of "integer" complexes prevails over that of M^{x+} for $M \neq Sm$ in many/most cases [18], $\Sigma\sigma_{para} = -0.44$ for chitin in pure water and calculations from log P of "bioinert" metals in different local waters are feasible. They give an idea of the size of changes caused by presence of (possibly ligating) organics in water and/or sediment of local waters. Samples were obtained in upper Lusatia, Saxony, FR Germany, representing mainly small ponds, lakes, and (lignite, gravel) quarry ponds, and beside small rivers (data from [17], here Table 5); water depth in sampling was 1 m or less:

Table 5. Partition factor P for chitin flakes and comparison telson/antennae, leg tips/antennae for calculation of effective $\Sigma\sigma$ (calculation from a, b for Al, La and Ce. $\Sigma\sigma$ does refer to aq. phase while $\Sigma\sigma'$ denotes conditions in top sediment layers (leg tips, chitin flakes). La^{3+} does accumulate on flakes (FA) much more rapidly than in (leg tips of) living animals (LA) likewise immersed in sediment except when there is lignite. Animals were caught and exuvia collected next to the shore at water depths ≤ 1 m [17]. Exuvia were not "boiled", thus site VII gives a direct comparison on the effect of thermal treatment of chitin.

Site	Description of Site	$\Sigma\sigma$	$\Sigma\sigma'$	Remarks	La Partitions (P) [–]
I	Pond, former lignite pit (12 m max. depth), exuvia	−0.574	−0.532		LA 1.27 FA 0.39
II	Small river	−0.522	−0.475		LA 1.45 FA 6.00
III	Small lake inhabited by tench (strongly digging fishes, introducing O_2 and NO_3^- into sediment); exuvia	−0.566	−0.412	highest value of $\Sigma\sigma'$	LA 0.09 FA 1.32
IV	Shallow pond, very rich in SO_4^{2-}; many exuvia could be sampled	−0.621	−0.508	REEs form sulfatocomplexes; lowest value of $\Sigma\sigma$	LA 0.63 FA 10.93
V	Small river	−0.401	−0.441	pH > 8; highest value of $\Sigma\sigma$, hard sediment	LA 2.19 FA 9.83
VI	Lake, former lignite pit (41 m deep)	−0.470	−0.573	pH > 8	LA 2.60 FA 0.42
VIIa	Flooded former basalt quarry, exuvia	−0.611	−0.446		LA 0.36 FA 0.295
VIIb	Flooded former basalt quarry, living crayfish	−0.582	−0.459	Either value of $\Sigma\sigma$ is very similar to those of VIIa	LA 0.29 FA 4.47

Antennae (*Orconectes limosus*) are distinguished by $\Sigma\sigma$ somewhat lower than for both pure water-chitin and leg tips penetrating top sediment layers; the shift generally is negative and of order −0.1 to −0.2. It is difficult to explain this value into much detail, yet this does mean local organic contributions will hardly change or limit application of the method. The two sets of effective $\Sigma\sigma$ are related according to Equation (8)

$$\Sigma\sigma = -\,0.321\,\Sigma\sigma' - 0.666 \tag{8}$$

obtained from linear regression of the above values. Accordingly, partition between (a) leg tips and (b) telson (getting contacted with sediment while normally not penetrating it) on one side and antennae (exclusively exposed to water given the living habits of this crayfish species) is correlated but in a negative way, indicating that telsons of *O. limosus* are kept in water for most of the time, unlike leg tips.

4. Discussion

Table 6 gives the parameters required for calculation/prediction of complex formation constants (empirical data: [47,48]) of several trivalent metals, including Al, Cr and many REEs. For Eu [48], but in theory also other REEs such as Ce, Yb, Sm, or Nd using appropriate solvents, electrochemical data from [47,49] can be used to calculate complex formation constants in DMF. However, there are some difficulties: the reduction signal of $Yb^{3+/2+}$ [48] in DMF does match that of the polysaccharide protons of chitin in same solvent at −1.5 V whereas Ce gives poorly reproducible data on adsorption unless the redox state is tightly controlled. Reduction of Sm is feasible when there are ligands, though a and b in Equations (3) and (7) are empirical parameters, which in turn were calculated by this author using tables of complex formation constants (data from [42,43]). The sum of Hammett parameters (taken from [31]) for the ligand donor sites (e.g., 0 [carboxylate]–0.66 [-NH_2 group] for glycinate or anthranilate, −0.75 for sarcosinate or −0.37 for glycolate or lactate, 2 × [−0.66] = −1.32 for ethylene diamine, etc.) can be compared to partition for environmental sites. For HOX acids (except of water, H_2O_2, HSO_4^-) (e.g., nitric, formic, benzoic, methanesulfonic, hydroxamic acids, phenol, glyconitrile, or HCO_3^-) the following equation applies (own calculation):

$$\mathrm{pKa} = 9.97 - 12.48\ \sigma_X \qquad (9)$$

Table 6. a and b parameters for mono- to tridentate ligands and certain trivalent ions Al, Cr, several REEs. The values in square brackets for a_{1d} and b_{1d} hold for OX^- donors excluding nitrate (carboxylates, sulfate, carbonate, phenolate), for bidentate ligands complex formation constants for glycinate, glycolate, oxalate, or hydroxamates were used. For chitin, $\Sigma\sigma \approx -0.44$ and bidentate binding do hold.

M(III)	a_{1d}	b_{1d}	a_{2d}	b_{2d}	a_{3d}	b_{3d}
Al			12.48	6.92		
Cr			−8.50	4.52		
Y			2.57	5.67		
La	[−10.08]	[6.90]	8.32	6.21	−2.52	3.09
Ce	−8.00 [−11.50]	2.89 [7.58]	3.70	2.56	−2.58	3.31
Pr			3.95	2.57	−2.74	3.35
Nd			4.02	2.60	−2.77	3.43
Sm	[−11.07]	[7.54]	8.08	6.02		
Eu	−8.98 [−11.92]	3.44 [7.97]	9.45	7.02		
Gd	[−11.55]	[7.71]	9.91	7.05		
Dy	[−14.55]	[8.90]	9.65	7.04		
Tm	[−12.58]	[8.02]				
Yb	[−13.22]	[8.29]				
Lu	[−12.23]	[7.86]	10.59	7.30		

CN = 6; CN = 9; variable CN; CN = 8, orange: tentative values.

This does describe adsorption performance of chitin somewhat influenced by ambient ligands which may bind to REEs and other metal ions. This can either produce some complex withhold in its dissolved state (e.g., with Sm) and/or (on the contrary) facilitate/increase adsorption by stronger adsorption of large complex molecules. Ligands may even act as anchor groups connecting metal ions and chitin, such as with glycine and other amino acids [18,22]. Values used for calibration and in Equations (4), (6)–(8) were calculated and most of them (the pertinent ones) are given in Table 6 (additional values can be obtained from Equation (3), Table 4 and data in [28]).

These data then are used to identify binding sites from observed sequences of binding preferences, e.g., BCF values following Equation (2).

This gives an account for differences in binding of REEs in different organs of a plant or animal or fungus; here, we limit our focus of attention to chitin. Chitin is one of the ligands where the modified formula must be used for its containing carboxamide ligands. Interestingly, metal retention to chitin does produce an additional reductive half-wave in DMF/Li^+ solutions regardless of the kind of metal which was bound [14,17]. It is attributed to an increased acidity of sugar OH groups caused by complexation. This transition is located at −1.50 V vs. SCE which translates into −1.07 V vs. NHE in DMF (the larger difference vs. SCE owing to the higher proton affinity of this solvent) and thus $pK_{a(M^{x+}\text{-}OH);\,dmf} \approx 18$, comparable to anion acids (HSO_4^-, $HC_2O_4^-$) [43] or glycine (own measurements) in this solvent. Using the equations

$$pK_{a(dmf)} = 23.48 - 22.91\ \sigma_{para} \tag{10}$$

$$pK_{a(dmf)} = 10.82 - 9.12\ \sigma_R \text{ for RCOOH} \tag{11}$$

derived by us for acidities of oxoacid solutions in DMF, one can estimate σ_{para} for the metal-loaded OH groups to be + 0.24 (comparing to metal-free OH = −0.37). By now, there are no σ_{para}-based regressions for all the metals studied concerning their interactions with chitin, but there is a focus on those which are most important in our own work.

4.1. Photoassisted Redox Reactions Occurring When Adsorbed to Chitin

Eu(III) can promote photooxidation (cleavage of CH bonds) of organics and become reduced also when it is adsorbed to chitin [50]. Besides being injected by vegetation (oxalic acid also by lichens [10]), chemical species enhancing or bridging adsorption of Eu can be subject to degradation by bacteria (humic acids also by earthworms). Potential changes between the two chitin-modified electrodes thus must be anticipated and are going to be studied in our continuing experiments (also see outlook). Applications are concerned with sensors rather than fuel cells.

Among established substrates of photooxidation by Eu(III), the only one to enhance adsorption is glycine while humic acid, caffeic acid, citric and malonic acid all do reduce binding of Eu towards chitin. Generally speaking, chitin´s adsorption capacity for Eu (and Ce, Dy, Pb) is considerably higher than for other ions including other REEs [19,37]. Hence, one would predict that glycine photo-processing to afford ethanolamine, HCN and CO_2 [51] would mainly occur at the chitin/solvent interface while the photooxidations of the other substrates take place in solution mainly. However, retention means an electrode placed at the top of chitin or right in it will maintain a rather positive potential, then acting as an anode while the leached Eu^{2+} gives a downstream electrode potential more positive than standard (cp. [48,50]) and thus makes the downstream electrode the cathode. We use Ta (foil or wire) as an electrode material [50] to avoid both

(1) heterogeneous-catalytic electrotransformations of organic compounds (to be anticipated on Pt) and
(2) electrode corrosion (sizable with Cu).

4.2. Change of Chitin Functions during Evolution

The distribution of chitin among taxa [52] does strongly suggest it was present in many animals or their predecessor taxa in late Precambrian much before any hard structures other than mouthpieces (of putative mollusk *Kimberella*) were formed from it, omitting open issues on taxonomy of late Precambrian organisms capable of active movement and on details of the Cambrian explosion. When there was chitin way back in the Precambrian with common antecessors of certain pairs of taxa separating then (i.e., 550–575 mio. y BP) or even earlier (fungi and animals) while totally or mainly absent in others, genetic data of either can be used to determine when it came into being even though chitin biosynthetic pathways in fungi and in animals differ somewhat while those in animals are conserved. However, the sorbent properties of chitin and the origins of mouthpieces combine to yield a meaningful interpretation: in the very beginnings, namely feeding, or retrieval of trace mineral nutrients partially relied on placing chitin fibers/appendices into the sediment. Covering structures, antennae, or legs would form only later (i.e., during Cambrian at the earliest [12,13,52–55]). Yet, given the data on adsorption extent [3,14,22] and kinetics [10,18,22], combined with the chance to leach them from some chitin surface by either acid or complexation, that is, by licking. Besides organisms such as *Hallucigenia sparsa*, it is telling that gill baskets of lancelets also contain chitin, which otherwise is rare in "genuine" vertebrates [55].

In previous and further course of evolution, chitin-bearing organisms developed many modes of interactions with those displaying cell walls that consist of other materials. Later, symbioses rendered chitin and its adsorption features available to organisms uncapable of making it themselves, as in plants (algae, cyanobacteria) teaming with fungi. Plants (*Archaeoplastida*) and cyanobacteria now make use of chitin´s unique properties by way of (mostly obligate) symbiosis with fungi (lichens). In arboreal mykorrhiza there is another version of the same effect, with mykorrhiza known to be involved in transport of metals, too, implying adsorption and desorption of ambient metal ions by chitin in mycelia. Different kinds/materials of cell walls are also shown in the diagram (Figure 7):

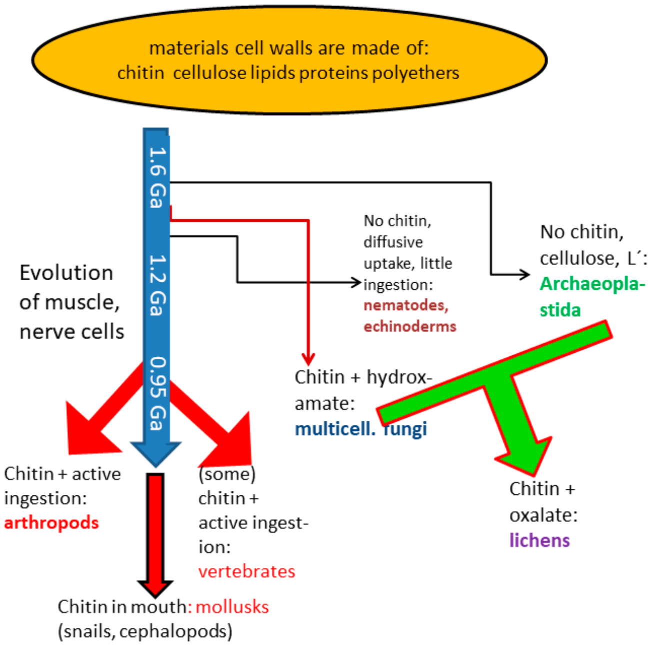


Figure 7. Chitin acts as a nutrient- and metal-retrieving sorbent within soft sediment (Ediacaran creatures purported to represent early animals used to be benthic or dwell within the sediment rather than swimming). (Multicellular) fungi (yeasts do not make chitin) use the same effect in mycelia. Here, uptake, transport, and release are likewise controlled by redox processes. REE contents in lichens may be rather high. Other kinds of cell walls existing in bacteria (*B. subtilis*) can discriminate and enrich terminal HREEs Tm, Yb, and Lu against all lighter REEs and other trivalent ions.

5. Conclusions

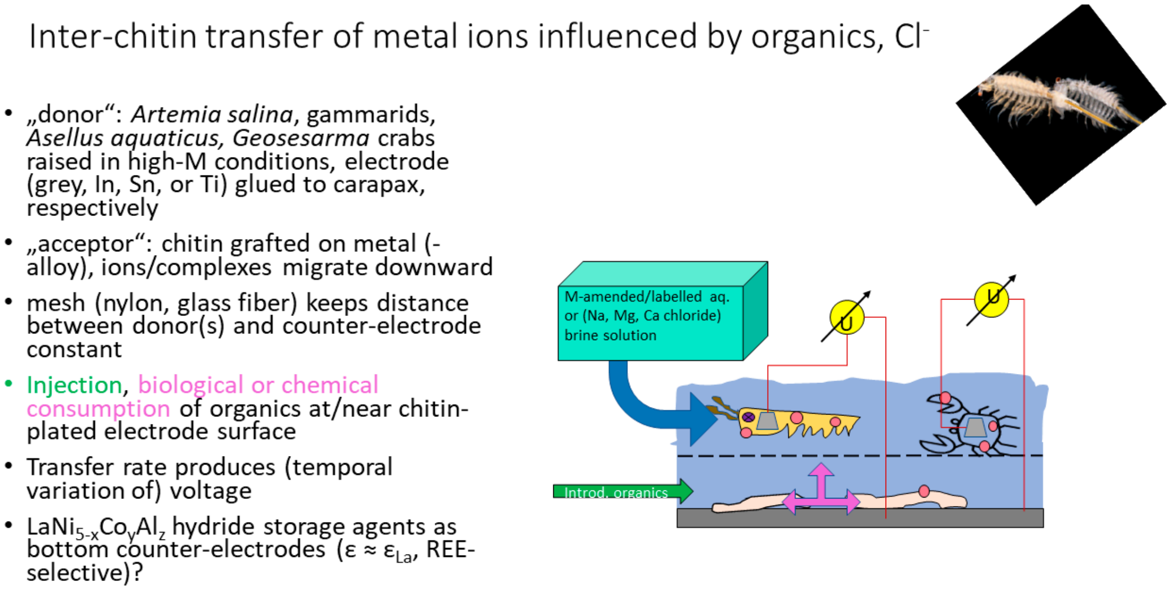
The prevalent role of chitin in terms of forming the outer covers, locomotive organs, and mouthpieces in a number of species which exceeds any other group by far (there are >1.4 mio. recognized species of beetles alone, even disregarding other arthropods) and other biopolymers by orders of magnitude in quantity can be traced back to its sorbent properties [3,5,34,54]. These can be exploited far beyond simple purification of wastewater flows [5,38,54]. Since adsorption depends on the oxidation state of some metal ions, such as Eu, Mn, and U, charge separation producing electrochemical potentials can be achieved by passing a slow solvent flow through a chitin layer where chemical or photochemical reduction [50] or oxidation takes place. Because chitin is plentiful, optically transparent and readily available as scrap, and besides being very robust towards both oxidation and thermolysis, such devices should find their fields of application, e.g., in fuel cells and environmental sensors. It is anticipated that knowledge of such effects will improve understanding of peculiar effects seen in arthropod outer covers such as photovoltaic or thermoelectric activity in hornet *Vespa orientalis* [55].

6. Outlook: Additional Studies in Lab and Open-Field

Studies can be conducted in very different conditions, even without exceeding the limits of “extremophilic” arthropods (*Artemia salina*, crabs and shrimps inhabiting rather hot water pools) or lichens in the Antarctic given the chemical and thermal robustness of chitin. Certain uncommon biotopes and their adapted populations are particularly vulnerable, urging use of the chitin method for studies of local conditions, pollution inputs and metal mobilities, e.g., associated with formation of chloro-, sulfato-, or carbonatocomplexes in cold to mesothermal salinic media. These are inhabited by both arthropods and some fish (*Cyprinodon* spp.).

(a) Studying Salinic Conditions in Water Bodies

Among the vast number of living organisms covered by chitin, brine shrimp *Artemia salina* is easy to grow and keep (endures up to > 20% NaCl), enabling its use as a mobile electrode carrier in the following setup (Figure 8):



Inter-chitin transfer of metal ions influenced by organics, Cl^-

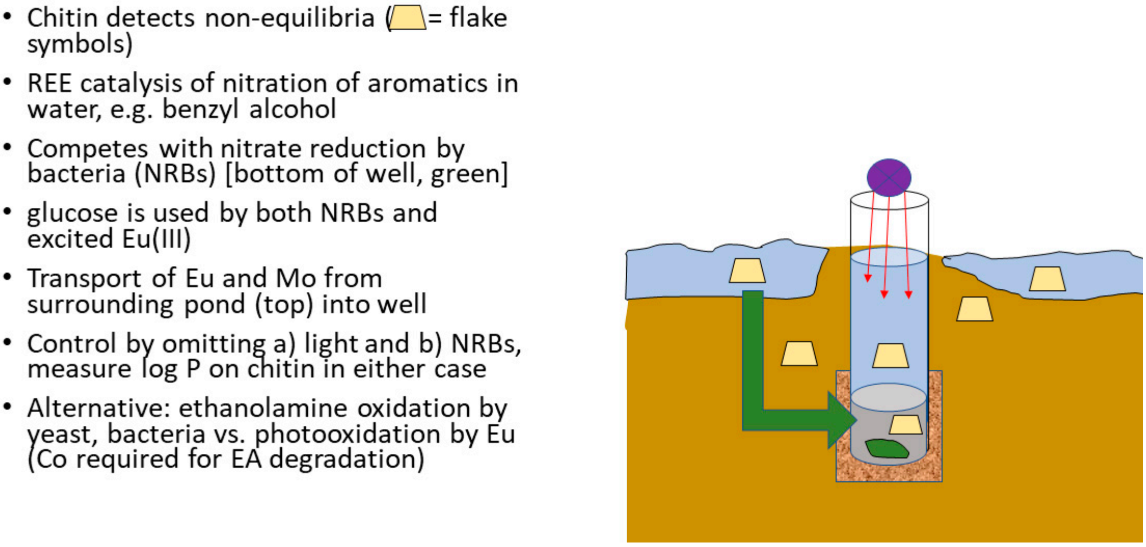
- „donor“: *Artemia salina*, gammarids, *Asellus aquaticus, Geosesarma* crabs raised in high-M conditions, electrode (grey, In, Sn, or Ti) glued to carapax, respectively
- „acceptor“: chitin grafted on metal (-alloy), ions/complexes migrate downward
- mesh (nylon, glass fiber) keeps distance between donor(s) and counter-electrode constant
- Injection, biological or chemical consumption of organics at/near chitin-plated electrode surface
- Transfer rate produces (temporal variation of) voltage
- LaNi$_{5-x}$Co$_y$Al$_z$ hydride storage agents as bottom counter-electrodes ($\varepsilon \approx \varepsilon_{La}$, REE-selective)?

Figure 8. Animals swimming above (**left**) or running on (**right**) the separating mesh are kept at a given distance from the counter-electrode. This does control carry-over of metal ions from metal ions to chitin fixed to bottom electrode.

A “freshwater version” could be realized with gammarids or aq. woodlice (*Asellus aquaticus*) which are notorious for their robustness concerning both acidic pH and chemicals including dissolved chlorine. Other test organisms are crabs inhabiting extreme-condition tiny water pools such as phytotelmata (*Geosesarma* spp.) or—for moderately salinic counterparts —hermit crabs frequently inhabiting tidal rock pools.

(b) Adsorption vs. Transport around and Inside Wells, Springs, and Seepage Sites

Detection of non-equilibrium can be extended to artificial constructions as shown above (Figure 1) for Mo dynamics related to nitrate reductase and likely to wells (Figure 9). Microorganisms and excited Eu(III) compete for the same substrate to be oxidized (glucose, ethanolamine) and nitrate is either used by REE ions to induce (catalyze) nitrations of aromatics [56] such as benzyl alcohol, alkyl phenols or C,C-diphenylglycine. Besides REE catalysis of aromatic nitrations, nitrate can become reduced by nitrate-reducing bacteria (NRBs, chemolithotrophs) whereas chitin flakes in water (top and inside well) and sediment are used to measure partition. Controls are obtained in darkness or omitting living NRBs (Figure 9):



- Chitin detects non-equilibria (⏢= flake symbols)
- REE catalysis of nitration of aromatics in water, e.g. benzyl alcohol
- Competes with nitrate reduction by bacteria (NRBs) [bottom of well, green]
- glucose is used by both NRBs and excited Eu(III)
- Transport of Eu and Mo from surrounding pond (top) into well
- Control by omitting a) light and b) NRBs, measure log P on chitin in either case
- Alternative: ethanolamine oxidation by yeast, bacteria vs. photooxidation by Eu (Co required for EA degradation)

Figure 9. Model of a natural spring where biorelevant trace metals and Eu pass through a sediment layer (e.g., the support of a small island) before re-appearing in a spring or well. Transport and biochemical processes can be surveyed by both chitin adsorption/partition on flakes and photoreduction of Eu.

Solid matrices such as gelatin enable activation of compounds which do not undergo photooxidation in solutions, such as aniline, salicylic acid, and N,N-dimethyl glycine [57]. Both occurrence of this reaction and products may differ depending on the surroundings, including feedback to chitin flakes (Figures 8 and 9). We plan corresponding experiments in the near future.

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Abbreviations

aq.	aqueous, aquatic (refers to living animals)
DMF	N,N-dimethylformamide (solvent)
DMSO	dimethyl sulfoxide (solvent)
E_L(L)	electrochemical ligand parameter; denotes shift of redox potential by replacing member(s) of ligand sphere in $Ru^{II/III}$ system
FUV	far ultraviolet ($\lambda < 300$ nm)
H_0	"effective" pH measured in low-water or nonaqueous solvents. Range about–21 ("magic acid") to > + 30 (strong base solutions in DMSO or liquid ammonia)
HFIP	hexafluoroisopropanol (solvent, does dissolve chitin, extremely transparent in FUV, radiation passes down to some 170 nm)
HREE	heavy rare-earth elements (Z = 66–71 [Dy . . . Lu])
ICP-MS	mass spectrometry using an inductively coupled plasma for sample ionization
LREE	light rare earth elements (Y and Z = 57–63 [La . . . Eu])
M	metal
M*	molecular mass
M^{x+}	metal ion in an unspecified oxidation state x
NRB	nitrate-reducing bacteria
OX^-	oxoanion, e.g., nitrate ($X = NO_2$) or acetate ($X = CH_3CO$)
REE	rare earth elements (Z = 39; 57–71)
$\Sigma\sigma$	sum of Hammett constants for a chemical entity (ligand) interacting with some other via two or more functional groups described by their respective Hammett parameters σ.

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