ORIGINAL PAPER

CHITIN ADSORPTION IN ENVIRONMENTAL MONITORING: NOT AN ALTERNATIVE TO MOSS MONITORING BUT A METHOD PROVIDING (LOTS OF) BONUS INFORMATION

STEFAN FRAENZLE[1,2], MICHAEL ERLER[1,2], FELIX BLIND[1,2], LKHAGVA ARIUNTSETSEG[3], DASHDONDOG NARANGARVUU[3]

Manuscript received: 11.06.2019; Accepted paper: 31.07.2019; Published online: 30.09.2019.

Abstract. *Although moss-based biomonitoring of environmental pollutants (heavy metals, POPs, reactive nitrogen, and sometimes other items) is well established after 50 years of a history starting in Scandinavia, the method now faces a crisis also in terms of funding. This latter is due to the* ***assumption*** *that precision and reproducibility of this biomonitoring method are too poor to provide information on small recent changes of situation well after the large changes had occurred, that is, around 1990. In addition, moss-monitoring requires that conditions where mosses can live and grow be constantly kept over a longer period of time; measurements in the dark or at sites where there are high levels of herbicides, radioactivity, acids, etc. cannot be accomplished at all; proper identification of used moss species is crucial, too. Hence moss-monitoring should be* ***supplemented*** *by other methods which – unlike passive biological or synthetic filters of atmospheric deposition – can provide information on local transport (both by airborne deposition and underground biological uptake or precipitation, plus reductive dissolution of sulfates) at ecotones such as the water/sediment interface at bottom of water-bodies or at some shore. Studies of adsorption to* ***chitin****, which originally had been applied for fractionation of radioactive waste and for withholding trace concentrations of heavy metals from wastewaters, can fill this gap as more robust "dead" (grafted) chitin is used. Adsorption is fast, and even minute traces of analytes can be recovered. Analytics are cheap and straightforward, requiring no digestion of samples. In understanding the data, some calibration line(s) for partition of both di- and trivalent metal ions (best: such which are not involved in biological processes) between water- and sediment-exposed chitin samples are constructed then to identify "deviations" from this expectation. These can be due to biological uptake of trace elements (Ni by methanogenes, plants, Mo, certain more "exotic" bioelements), precipitation (Fe, Mn), reductive dissolution (sulfates of Ba, Pb, Eu exposed to ascending* CH_4*) or, of course, atmospheric or aquatic pollution from above. As a rule, chitin-related partition of toxic non-metals (As, Se) does respond to different environmental conditions, vegetation covers less than metal ions will do. Grafted chitin can be fitted to robots like drones transporting it to hazardous sites where organisms (arthropods, lichens) would not survive. Of course, data of this kind can be combined with pieces of information from biomonitoring (are there organisms controlling transport of certain elements?, is there eutrophication of open waters?) and biogeochemistry.*

[1] International Institute (IHI) Zittau, Department of Biology and Environmental Sciences, 02763 Zittau, Germania. E-mail: stefan.fraenzle@tu-dresden.de.
[2] Technische Universität Dresden, Theoretical and Environmental Chemistry Research Group, 01062 Dresden Germany.
[3] National University of Mongolia, Faculty of Biology, 210604 Ulaanbaatar, Mongolia.

Keywords: *biomonitoring – adsorption to chitin – workup by dissolving this biopolymer – trace determination of heavy metals – understanding thermodynamics of phase transfer, biological uptake in the open environment – correlations with complex formation constants, ionic radii.*

1. INTRODUCTION

Beginning with experiments aiming at retention of most fissiogenic radionuclides other than ^{137}Cs from dissolved nuclear fuel rods [1], the sorbent properties of chitin were applied in wastewater purification and similar activities tackling a broad range of environmental pollutants, sometimes including organics [2, 3]. Unlike other biopolymers, chitin is known to dissolve in several polar organic solvents like formic, dichloroacetic acids – without any additive but causing slow esterification of the polysaccharide OH groups – and in carboxamides, lactames when Li salts are added, then without any chemical changes; nor would Li ions block access of some analyte or complex ligand from this (e.g., DMF/Li^+) solution.

The first author (SF) and his team showed during the last years that retention of metal ions and –complexes on crab chitin does occur down to the nM/L range, and that solutions of chitin will bind other metal ions or –complexes from DMF solutions up to a saturation level of some 40 µM/g chitin [4]. For most "common" transition metal ions, this is roughly the dissolved level in ocean water [5] hence washed chitin from marine organisms like Arctic swimming crab *Pandalus borealis* (the most common "donor") will provide very low background levels, except for Al, Ti, Fe [3, 4, 6] and the metals which are most involved in arthropod enzymes, oxygen handling, Cu, and Zn.

A single chitin strand consists of some 2,000 – 3,000 monomer units, allowing for co-binding of many different metals to the same strand. The only metal to approach saturation on a chitin interface in environmental settings is Zn [7]; some elements undergo rather fast diffusion from surface into the bulk chitin, once again including Zn [3]. Thus, if samples are processed only months after they had been taken, one must make sure that uppermost some 15 µm of chitin are dissolved and passed to analytical gear. Amounts of process chemicals are chosen appropriately [8]. Partition on chitin plates which were immersed into the different phases for some time (≈ 10 min) is shown in the following figures (Figs. 1a-1c):

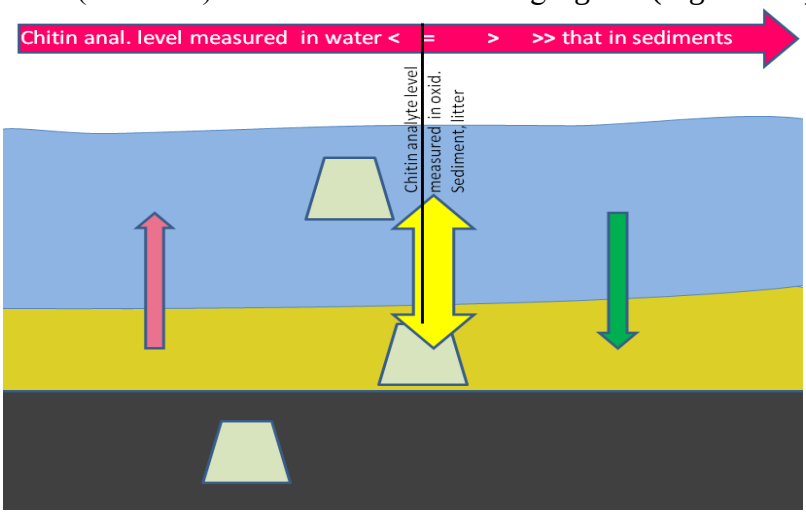

Figure 1a. Adsorption of analytes to chitin does depend on both "intrinsic" chemical partition among water, top- and (sulfate-) reducing layers of sediment.

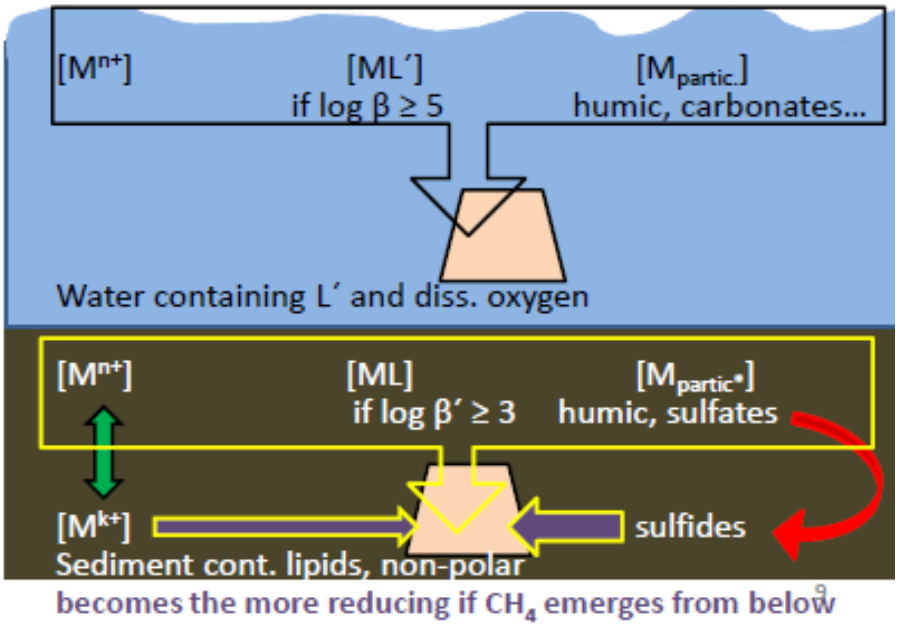

Figure 1b. Details of partition including speciation with ligands dissolved in water and sediment and redox processes in the latter phase.

Figure 1c. Actual setup (Mongolia, June 2017). Small depression (diameter about 1.25 m, 90 cm deep, little light getting in) in a drying bog, N Mongolia. Marks point to chitin-plates on glass support, exposed to water (left) and sediment (bottom right). Photograph by Beate Noack.

It is possible to do chitin-based measurement in very small water bodies including phytotelmas (Fig. 2) which is important in understanding microbiota particularly as such water pools surrounded by either wood, leaves (e.g., *Bromeliaceae*) or even chitin (fungi) are distinguished by specific, sometimes specialized fauna and flora.

Figure 2. Chitin-based sampling of a water pool (≈ 7 cm Ø, ≈ 1.5 cm deep) located on a fungus in Mongolia; August 2018. Photograph by SF. Outlets of small springs and methane vents (in bogs) were studied in the same manner.

2. MATERIALS AND METHODS

The method of chitin-based sampling and monitoring is described elsewhere in more detail [7]. Equilibration between chitin interface and sampled phase is fast (≤ 10 min), and the small size of sampling devices permits investigations on a cm-scale spatial resolution (see above), including studies of vertical redox gradients. Spatial resolution can be further increased by focusing on more abundant elements; then about 1 mm^2 interface will do but information based on trace element partition features is lost, of course. For methods of analysis of data, see discussion section.

3. RESULTS

Near Zittau (Germany), there was an opportunity for direct comparison of (previously obtained) moss monitoring results [6, 9]. With time elapsing, it became likely that Ni and other metals then still detected at the (bog or other) surface meanwhile had migrated several tens of cm downward, thereby enabling methanogenesis in a patchy manner. The results on that site are given in Table 1.

Table 1. Levels of metals in water and on chitin placed in water and sediment, respectively, for sites without and with methanogenesis (from [6]); pH = 4.8 (site A) and 4.6 (site B), conductivity about 15 µS/cm, oxidizing conditions in overhead water but not right inside the "exhaust" formed by CH_4 bubbles.

Metal	Site A (no methanogenesis				Site B (methano-genesis)			
	Water [µg/l]	Adsorption from water to chitin [µg/l]	Adsorption from sediment to chitin [µg/l]	Quotient; log Q	Water [µg/l]	Adsorption from water to chitin [µg/l]	Adsorption from sediment to chitin [µg/l]	Quotient; log Q
Divalent ions								
Ba	2.5	0.325	0.29		4.2	0.32	0.66	2.06; +0.31
Pb	1.80	1.01	0.52	0.52; -0.29	2.10	0.48	0.825	1.72; 0.23
Cd	0.08	0.08	0.08	1.0; 0	0.09	0.07	0.08	1.14; 0.06
Ca	500	1,450	1,200	0.83; -0.08	950	950	1,215	1.28; +0.11
Co	0.14	0.095	0.09	0.94;-0.03	0.15	0.095	0.11	1.16; + 0.07
Cu	3.3	8.95	5.2	0.58; -0.24	2.40	2.60	5.95	2.29; +0.36
Mg	160	75	70	0.93; -0.03	130	70	70	1.0; 0
Mn	4.20	0.74	0.57	0.74; -0.13	4.80	0.53	0.945	1.78; 0.25
Ni	1.10	1.25	1.03	0.82; -0.09	0.61	1.10	2.10	1.91; 0.27
Sr	2.50	0.75	0.70	0.93; -0.03	3.20	0.68	1.135	1.67; 0.23
Zn	13.4	120.5	97.25	0.81; -0.10	8.60	78.9	116.3	1.48; 0.17
Trivalent ions								
Al	190	10	10	1.0; 0	220	10	15	1.5; 0.18
Ce	0.13	0.115	0.115	1.0; 0	0.17	0.095	0.085	0.9; -0.04
Cr	0.68	51.9	50.9	0.98; -0.01	1.00	50.75	52.2	1.03; +0.01
Fe	330	45	45	1.0; 0	220	45	100	2.22; 0.36
La	0.06	0.055	0.045	0.8; -0.1	0.07	0.04	0.035	0.88; -0.05
Bi	0.15	0.16	0.115	0.72; -0.15	0.02	0.08	0.07	0.88; -0.05
Other elements								
Ag	0.02	0.04	0.035	0.88; -0.05	0.03	0.045	0.045	1.0; 0
As	1.80	4.45	4.45	1.0; 0	1.60	4.40	4.95	1.13; 0.05
Sb	0.11	0.055	0.06	1.1; 0.04	0.16	0.055	0.055	1.0; 0

In methanogenesis, both Ni and Co are involved. As and Sb do not respond to differing conditions as they do not form cations then forming complexes. In addition, these data were used to derive adsorption equilibria between aq. solution and chitin depending on ion radius and oxidation state.

EVALUATION OF METAL-ION BINDING AFFINITIES TO CHITIN

Measurements in Rownia pod Snieżką (bog, located directly at Polish/Czech border in Sudety mountains in between Mt. Sniezka (E of bog area) and Loučna hora (W), some 1,420 m ab. SL) concerning both aq. and chitin-based concentrations of metal-ions – both members of Irving-Williams series and trivalent ions such as La, Ce, Cr, Bi – showed there are clusters of such ions each in comparable amounts, namely some 30 nM/L (aq.) for M^{2+} and about 1 nm/L for the said M^{3+}.

Table 2. Trivalent ions at Rownia pod Snieżką bog, both in water and chitin-samples. Concentration range ≈ 10^{-9} M/L [6].

Metal	Aq. concentration [µg/L]	Aq. conc. [nM]	Level on chitin [nM/L nitric acid eluate]	Reciprocal radius [nm^{-1}]
La	0.065	0.47	0.36	9.56
Ce	0.15	1.1	0.75	9.90
Bi	0.15	0.72	0.8	8.47
	0.02	0.1	0.38	
Co	0.145	2.46	1.43	13.33
Cd	0.085	0.75	0.7	10.2

Table 3. Trivalent ions at Rownia pod Snieżką bog, both in water and chitin-samples. Concentration range ≈ $10^{-7.5}$ M/L [6].

Metal	Average aq. concentration [µg/L]	Aq. conc. [nM]	Level on chitin [nM/L nitric acid eluate]	Reciprocal radius [nm^{-1}]
Ba	3.35	24	2.3	7.45
Sr	2.85	34	9	8.94
Mn	4.5	82	11.5	11.0
Ni	1.1	19	21.5	13.5
Cu	2.85	45	41	14.29
	2.4	38	41	
	3.3	52	140	

In the second set of metal ions – all of which are divalent – retention to/by chitin from solutions containing very similar levels of the said ions does once again follow the Irving-Williams series as one would expect: Ba << Sr ≈ Mn(II) < Ni < Cu(II). The corresponding regression $\log_{retention}$ against reciprocal ion radius is presented in Fig. 3.

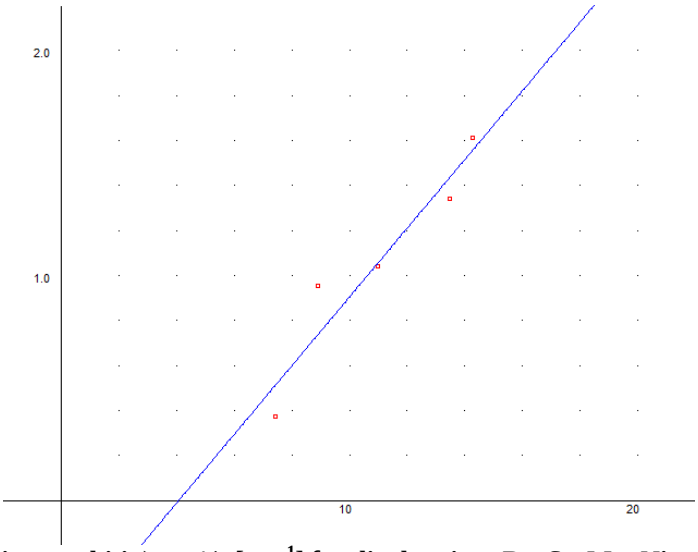

Figure 3. log (retention on chitin) vs. 1/r [nm^{-1}] for divalent ions Ba, Sr, Mn, Ni, and Cu at similar aq. concentrations.

That is,

$$\log [M_{chitin}] = 152.7/r - 0.623 \text{ [all data in nM/L]}, \quad (1)$$

using the averaged retention value for Cu.

Thus it is feasible to calculate regressions for retention of these ions to chitin vs. either x, c [10, 11] or simply the ion radius.

The latter functions are

$$[M_{chitin;\ aq.}] = 152.7/r - 0.623 \quad (2)$$

(M^{2+}, 30 nM/L; the second value referring to concentrations in nitric acid eluate after twice ion exchange) and

$$[M_{chitin;\ aq.}] = 644.5/r - 6.365 \text{ [}M^{3+}\text{, 1 nM/L, same conditions]} \quad (3)$$

The empirical relationship between local chemistry and adsorption to chitin then is done by linking the two equally shaped equations to each other (see below). For data from different sites in Mongolia concerning the aftermath of permafrost degradation [7].

4. DISCUSSION

4.1. ANALYSIS OF PARTITION EQUILIBRIA

Like with pairs of different solvents [12, 13], there is a phase transfer energy for any ion between water (which happens to be the usual comparison solvent in the above case) and sediment; for immiscible pairs of strongly ionizing solvents (e.g., water/nitromethane) the partition coefficient can be directly calculated from this:

$$\text{Log P} = \Delta G^{\#}/5.7 \text{ at } 25°C \text{ [kJ/mol]} \quad (4)$$

Unfortunately, the former phase transfer energy is known mainly for mono- (alkali metals, quaternary ammonium- or phosphonium salts, Cu(I), Ag, Tl [13]) and some divalent ions [12] (of which Ba, Zn, Cd, Cu, and Pb can be studied by chitin adsorption also) only even for very common solvents such as methanol, acetone, acetonitrile, DMF, or DMSO let alone such ones immiscible with water, with sizable error bars. However, both solvation of metal ions by water, binding to sediment organics and to chitin can be regarded as processes of complexation; thus one would expect that e.g. many divalent ions (alkaline earths ≠ Be, 3d M^{2+} ions Mn…Zn) would behave according to the Irving-Williams series [14] (Fig. 4) which, like similar rules for trivalent ions (Al, Fe, Cr, other transition metal ions, REEs) states a general series of stabilities[1] relationship between log β or log P, respectively, and the inverse ion radius (for the respective oxidation state and likely spin[2] state).

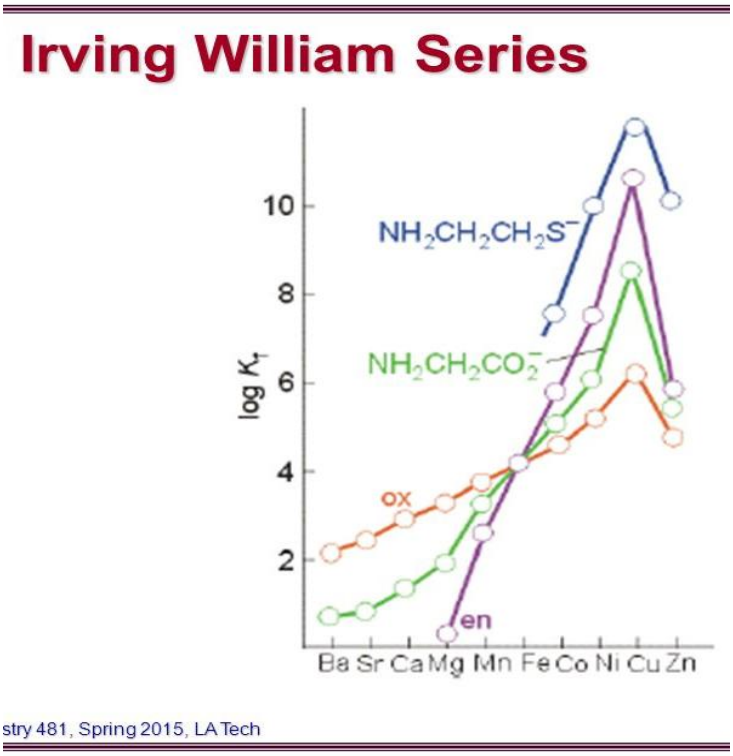

Figure 4. The Irving-Williams series of complex stabilities for bidentate L = ethylene diamine (en), glycinate, oxalate (ox), and aminoethanethiolate. Note that log β for glyc usually is arithmetic mean between values for en and ox: formally you can obtain glyc$^-$ by cutting the C-C bonds of both ox and en and rejoin the radicals cross-wise. The same holds for the electrochemical ligand parameter E_L(L) [6, 15]. For Fe(II), x = 0 as log β = const for unlike E_L(L).

[1] It should be noted that among these many are involved in biochemical activities. Because biologically most important alkaline earths Mg and Ca do just reversibly and weakly bind to chitin, chitin from marine arthropods can be used for this analytical purpose after just washing it. The log β/ion radius relationship for divalent ions does not hold beyond the original IW series M^{2+} members (Mg…Ba, Mn…Zn), V, Cr and Eu which is important when understanding behavior of highly toxic M(II) ions like Cd, Hg, Pb, Pd. For Pb many partition data on chitin are available while Cd (fortunately) is often close to detection limit on chitin at least for water (i.e., log $P_{chitin} > 0$) and Hg, Pd were not addressed so far.

[2] Holds for 3d- and 4d-ions mainly; depending on ligands ion radii may differ considerably by some 10%, and so will 1/r. Differences of ion sizes between oxidation states n and (n±1) may even be considerably larger, e.g. with di- and trivalent Cr, Eu, and Fe

Knowing the latter a series of "bio-inert" ions can be investigated for log P_{chitin} at a given site. Then one looks for the "outliers" and their possible reasons, including pollutions. For some metals like Pb, REEs, where biological functions other than toxicity by enzyme inhibition are non-existent or rare (for LREEs in general [16], and [17] for a second methanol-oxidizing system which works on nM/L levels of La already) pollution by atmospheric deposition is the most likely reason. However, onset of biochemical transformations which call for particular conditions (e.g., strongly reducing) and photochemistry (Eu, possibly Fe and Cu) must likewise be considered.

Partition of LREEs La, Ce, and Sm (and of Y, Gd which behave differently because displaying eight- rather than ninefold coordination patterns) in a microcosm (a fish-tank containing Lemnaceae [duckweed], daphnia, small shellfish and goldfishes [*C.carassius auratus*)] among sediment, water and biomass was studied before [18] using, however, considerably higher levels of REEs (1 mg/L each, that is, between some 7 and 11 μM) were introduced, with most (between 82 and 97%) of the metals ending up in sediment. Partition of LREEs between sediment and (water + biomass) can be described by the following equation (applies for La, Ce and Sm only):

$$\text{Log } P_{\text{sedim./(water + biomass)}} = 842.5/r - 5.724\ [\text{pm}^{-1}], \quad (5)$$

that is log P ≈ 1.5

Duckweed does accumulate REEs [18] (eq.5 below derived from own calculations):

$$\text{Log BCF} = 285/r - 0.559, \quad (6)$$

while both in vitro (own experiments) and in vivo Mg in chlorophyll is readily replaced by REEs, producing highly reactive but short-lived yellow-orange Eu porphyrin photosensitizer complexes in case of europium. Our work refers to quite similar conditions, with the water containing living beings once again.

Selectivity of extractions of REE ions from mixed oxides by chitin was studied before in our group [8]. Introducing the latter equation and the reported ion radii for trivalent La, Ce, Sm, Eu, Gd, and Yb into these equations provides rules pertinent to fractionation among REEs; it is evident from the above equation that retention to chitin at a given aq. level does increase with decreasing ion radius, that is (lanthanoid contraction) with increasing Z. For the pair Gd/Ce(III), enrichment should be about 5-fold, for Yb/La about 20-fold, while corresponding quotients Eu/Ce and Eu/La, relevant for understanding partition in the dark would be 2.65 and 4.4, respectively. The M^{3+} equation gives an effective electrochemical ligand parameter (see [10] for definition) of – 0.124 V, meaning log β ≈ 4.2 for Eu(III) complex of monomeric N-acetyl glucosamine.

All these data correspond to small amounts of loading of binding positions which suggests almost proportional increase of adsorption with aq. concentration. Hence for the redox couple $Eu^{II/III}$ on chitin (≈ 97% photochemical reduction in given conditions) $[Eu^{2+}]_{chitin;aq.} = 152.7/117 - 0.623 = 10^{0.68} = 4.8$ nM/L in nitric acid eluate or on native chitin and $[Eu^{3+}]_{chitin;aq.} = 644.5/94.7 - 6.365 = 10^{0.41} = 2.6$ nM/L, that is, although only 3% of Eu are still trivalent owing to illumination when there are substrates susceptible to H atom abstraction or conditions of thermo(bio-)chemical reduction by H_2 or CH_4, 280/7.4 ≈ 38% of Eu bound to chitin are still trivalent. The surface redox potential measured in a DMF/Li^+ solution will yet not differ significantly. Given the range of linear response concerning adsorption (cp. this range of concentrations and the above log β value which implies that most binding positions are vacant), estimates can be done for lesser reduction or photoreduction (or higher total Eu) also.

The total value of 7.4 nM/L eluate corresponds to 45.6 pMol in total or some 70 pMol (10.6 ng) Eu/0.8 cm^2 regarding a recovery rate of some 65%. The removed (by surface dissolution) chitin mass is some 1.5 mg then, with a maximum binding capacity on surfaces of some 40 µMol/g chitin almost regardless of kind of metal ion [2, 4] and whether the metal ion is in its di- or trivalent oxidation state. Additional data are now gathered in this lab for both aq. (buffered to pH 4.7, like Rownia pod Snieżką bog) and 50% aq. DMF solutions containing various ligands which are observed in soil solutions (buffer: aniline/anilinium[1] trifluoroacetate)

For the samples taken in N Mongolia in 2017, except for the rather narrow and dark bog pothole (Fig. 1b), regressions do fit much better assuming (photogenerated) Eu(II) than native Eu(III) (Fig. 5). Cerium on chitin was at or below determination limit in several cases, but the above correction for [Eu/La] on chitin still means levels of Eu in these waters (being much less acidic than the Sudety Mountains bog [pH ≈ 7.5 vs. pH 4.7]) are much higher than those of La. Probably photochemical processes do constantly (or periodically each daytime) leach Eu from the sediment ceiling into water where photochemistry keeps on due to intermittent oxygen- or UV reoxidation of Eu(II)[2] which both are slow compared to photochemical (Eu-reducing) attack on organic matter. Accordingly most of Eu is divalent (Fig. 5) [5]:

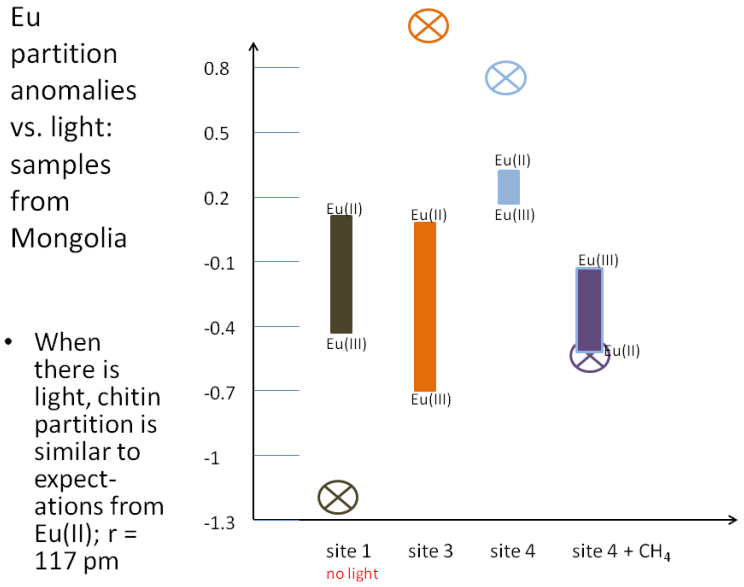

Figure 5. log P_{chitin} for Eu calculated for Eu(II) and Eu(III) from regressions for the sites log P = a/r + b and actual values.

[1] Formal acidity constants of ammonium-, pyridinium-, quinolinium-, and anilinium salts in water and DMF and mixtures thereof are almost identical [19], and aniline, unlike amines, does not undergo photooxidation by Eu(III) in either water or DMF [20]. Other acids (neutral or anionic, e.g. HSO_4^- or Hox^- or $Hsucc^-$) are much weaker in DMF than in water [19]

[2] O_2 oxidation in/of neutral aq. Eu^{2+} solutions is fast while photochemical reoxidation (CTTS) does take place at some 320 nm, with maximum Φ (0.072) at 334 nm [21]. The latter process is enhanced by the fact that these studies were done at high-mountain altitudes (some 1,650 up to > 2,000 m ab. SL) in summer; besides the water samples were saturated to over-saturated with dioxygen (90 – 130 % rel. sat.).

4.2. CONDITIONS OF CALIBRATION

Generally speaking, this kind of statistical treatment of chemical properties of elements calls for some fairly large number of elements to be measured. In this work, it was 26 but studying several more would be advantageous, notwithstanding the possibility that some will be sometimes near to or below determination limit in this setup while binding to chitin can be taken for granted except for Mg, Ca. Here, some "blanks" were seen with Ce, Cd, U, and V (detection limits exceeded for some of the samples only). As all biological uptake, formation (aquoxides) or dissolution (sulfates, carbonates) of insoluble phases and photochemical reduction will alter patterns and extents of interaction with chitin, the elements studied should be classified according to these features, producing the following graph (Fig. 6).

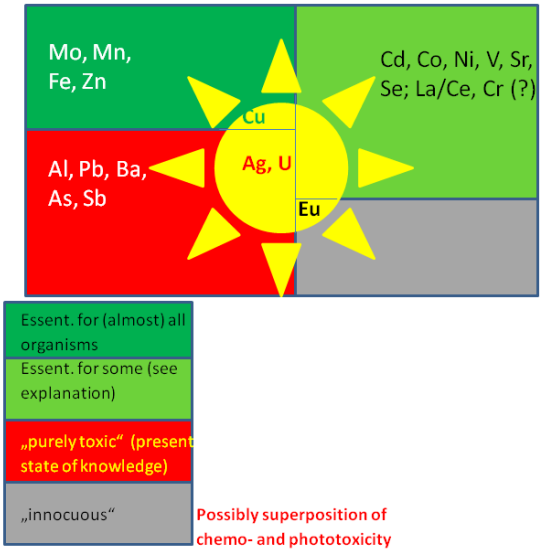

Figure 6. Classification of chemical elements for biological function and photochemical properties [21]. The question mark refers to the fact that an essential biological rule of Cr could not yet be substantiated for any species. Classification of LREEs relies on recent findings concerning methylotrophic bacteria [16] rather than earlier speculations presuming a rather general (i.e., encompassing most of the more abundant) REE essentiality in plants [22]. Hickory and other Carya (e.g., pecan *Carya illinoinensis*) species do enrich REEs in their leaves [23] but as yet no biological function is established concerning REEs in higher plants.

Eu-based photochemistry is being intensely studied by these authors [7, 20] with respect to both fuel-cell applications which replace (the rarer, i.e. Ru, Ir) PGMs by photochemical CH bond cleavage and to chemical evolution, spatial distribution of Eu among environmental compartments depending on photochemical reduction. As this effect is unique to Eu[1] among the lanthanoids (uranyl(VI) dication UO_2^{2+} behaves in a roughly comparable manner), a fractionation of Eu vs. other REEs (most abundant: La, Ce, Nd, Yb of which only Ce undergoes redox processes in environmental conditions) both within and among environmental compartments and concerning chitin plates exposed to either can be predicted. In fact, this is used by our group to study environmental photochemistry related to sensitization by europium [20]. Many biological and biogenic products, however, are inert

[1] Comparable photochemistry might be anticipated with Yb(III) which is more abundant than Eu(III) in most samples, but only in far ultraviolet not penetrating to surface of Earth. Ce(IV) photoreduction occurs by another mechanism, involving LMCT states [21] rather than f orbitals (which are completely vacant in Ce(IV)) or f→d transitions.

towards photochemical CH abstraction by Eu(III), e.g. amino acid sarcosine, mannose, certain carboxylic acids whereas very similar or phosphorylated compounds do react [20]. Pb does respond to conditions of sulfate reduction much as Ba, Eu although reduction products should be expected to be insoluble, too. While the main product of sulfate reduction on inundated grassland might be colloidal S_8 ("milk of sulfur") rather than H_2S, it should be emphasized that all Fe, Zn, Cd, and Pb, Sb were transferred to chitin from a solid two-phase mixed Zn/FePb-sulfide mineral ("Schalenblende") slurry [4], that is, from solids in previous experiments, too.

Partition of REEs in a fish-tank including its sediment was studied before [18]. Yang et al. (1999) reported on partition of light REEs La, Ce, Sm (and Y, Gd which, however, differ with respect to average coordination numbers [24]) which occurs in some microcosm (fish-tank) containing biomass (duckweed, daphnia waterfleas (kept separately in a net cage C to avoid their being eaten by fishes), small shellfish, goldfish *C.carassius auratus*).

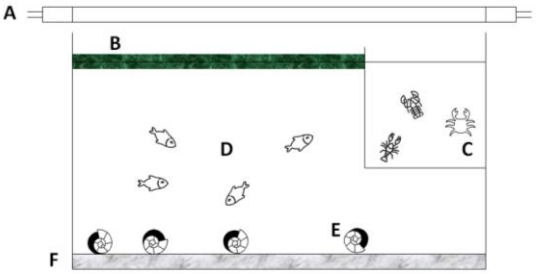

Figure 7. Testbed for LREE partition in a microcosm: A – fluorescent lamp, B – duckweed, C – crustacean, D – goldfish, E – Shellfish, F - sediment [18].

Water volume (pH = 6.65 ± 0.15), obtained from a "typical eutrophic lake from China" and filtered, was 50 L, that of sediment 2 l (50*20*2 cm), meaning volume densities in extraction/partition have to be corrected by a factor of 25 and $\Delta \log P = 1.4$. That is, except for a higher (biomass- and number-) share of fishes this does represent the very kind of system we did investigate. Table 4 gives the partition of REEs in the microcosm [18]:

Table 4. Partition of REEs, Y in the model ecosystem.

Metal	REE in sediment [%]	REE in water [%]	REE in biomass [%]	Log P $_{sedim./(water + biomass)}$ [-]	Log P$_{water/biomass}$ [-]	Reciprocal ion radius [nm^{-1}]
La	90.95	8.24	0.81	1.00	1.01	9.56
Ce	92.48	7.06	0.46	1.09	1.19	9.90
Sm	97.64	1.93	0.43	1.62	0.65	10.30
Gd	82.01	16.97	1.02	0.66	1.22	10.78
Y	87.80	11.69	0.51	0.85	1.34	10.87

There were indications that LREEs first were absorbed and then re-discharged by the sediment. Accordingly, we can evaluate 1) the free transfer energy of LREE ions from water (the added biomass does contain just 5-10% of the combined inventory, and except for the mussels there is no direct contact between sediment and biomass[1]) into sediment while 2) the remaining difference RT*c gives an idea of partition behavior of involved ligands between water and sediment, with the sediment behaving much like an nonpolar solvent such as toluene.

[1] Previous work in the first author´s lab [4] dealt with partition of other elements among model sediment (chromatographic silica amended with minerals or insoluble salts) and exoskeletons of living crickets *Gryllus assimilis*

The involved ligands which were produced and delivered by plant roots or fungal mycelia or derived from degradation of lignin and similar organics (humic acids) and do bind REEs effectively use to be intermediately polar (log $k_{OW} \approx \pm 1$, e.g. citric, oxalic, lactic, hydroxamic and malic acids), but not extremely polar (amino acids [log $k_{OW} \approx -3$] do not appreciably bind to REEs [25], hence can be expected to be absorbed by sediment only feebly ($\Delta G_{transf} > -10$ kJ/mol).

This empirical equation for partition of LREEs La, Ce and Sm (derived by SF using the data from [18], omitting Y and Gd for different coordination numbers) is

$$\text{Log } P_{\text{sedim./(water + biomass)}} = 842.5/r - 7.124 \text{ [pm}^{-1}\text{]}$$

at starting levels of $[\text{LREE}]_{tot}$ = 1 mg/L per element ≈ 7 µM except for Y, with the above correction for volume

$$= \text{Log } P_{\text{sedim./(water + biomass)}} = 842.5/r - 5.724 \text{ [pm}^{-1}\text{]} \quad (7)$$

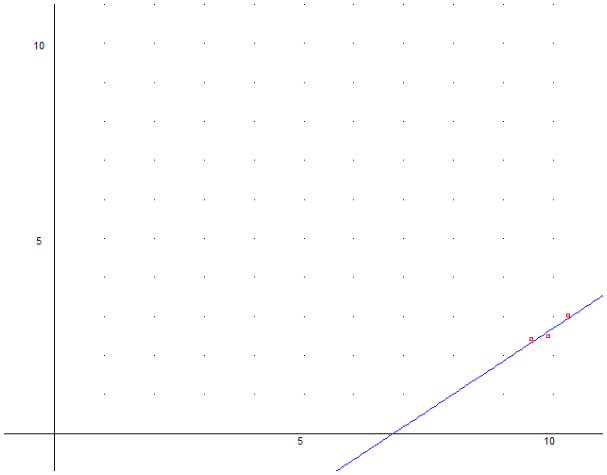

Figure 8. Regression of sediment/water+biomass partition vs. reciprocal ion radius.

Partition on chitin among plates exposed to different yet connected environmental compartments was studied before giving parameters for an equivalent formula [5]

$$\text{Log } P_{\text{chitin(sedim./water + biomass)}} = a/r + b \quad (8)$$

which differs between di- and trivalent ions; for the latter a ≈ 20 – 70 and b ≈ ± 0.4 [5]. Here, aq. levels of REEs are about 1 nM, rather.

The actual partition in such systems, allowing for complexation and other chemical processes in either compartment, is shown in Fig. 1b above. The ratio of environmental REE concentrations between experimental work by Yang et al. [18] and "normal" situations is of order 10^4. Obviously both above equations are related to each other by

$$\text{Log } P_{\text{sedim./(water + biomass)}} = 842.5/r - 5.724 \text{ [pm}^{-1}\text{]} = (a/r + b) + c.$$

Empirically, for trivalent ions and different environmental situations including peat-bog pools of similar pH (about 7), a is 20…70, thus

A + (b + c + 5.724)*r = 842.5, with r ≈ 100 pm for LREEs (104.5 pm for La decreasing to 94.7 for Eu) and c being an approximation for ligand partition: (b + c + 5.724)*r ≈ 800 or b + c ≈ 2.3, that is c about 2 – 3 .

As partition is considered only, total concentrations in either phase do not matter as long as they are a) "dilute" and b) sufficient to enable retention by chitin to a measurable extent. Both limiting conditions do apply here. It should be emphasized that unlike with local binding in moss monitoring, diffusion across the chitin plates does give a deeper look into processes which occur below in the sediment or indicate surface pollution. The vertical range is 50 cm at least, judged e.g. from onset of methanogenesis requiring a Ni level which is much higher than that in fresh *Sphagnum* mosses unless for additional pollution in earlier times as evidenced by [8].

4.3. COMPARISON BETWEEN CHITIN-AND MOSS-BASED MONITORING

Moss-monitoring relies on topology of moss pads which rather retain solid particles (airborne, from wet deposition, cosmic dust, etc.) than bind by chemical interactions save for cation exchange [26]. Accordingly, proper taxonomic identification of mosses is crucial to obtain any meaningful results. In chitin-based monitoring, analysis of one entire batch of chitin for background elements will do, with 1 kg providing many thousands of individual samples. The method is very fast concerning acquisition of samples [3, 7, 8], and unlike with active biomonitoring the site need not be accessed twice but the time required to obtain common data (weather, pH, water conductivity and so on) will do for providing an appropriate adsorption to chitin.

Given there is efficient adsorption, and chitin was present in *Opisthokonta*, *Bacteria* much before anybody made shells, wings, legs or mandibles from it, which had been its original biological purpose?

Chitin exists in many rather different biological species and taxons, including certain bacteria, mollusks (reinforcing aragonite shells of clams, making beaks and "claws" [attached to arms] of cephalopods) and even chordates (blenniids and other fishes) [27], and hemichordates besides of the familiar fungi, yeasts (with chitin making their outer cell walls) and, of course, all kinds of arthropods like insects, spiders, crabs, millipedes, horseshoe crabs, and extinct ones like trilobites, eurypterids (the oldest known fossil samples still preserving chitin were obtained from a Cambrian sponge [28] and an Ordovician eurypterid [29]). Reinforcing protein fibers which turn up by dissolution in HCOOH are much less stable; their amino acid composition can be evaluated by means of pyrolysis-GC/MS for "really fresh" samples [30]:

Biosynthesis of chitin occurs along somewhat different pathways [31], permitting protection of fungal cultures (champignon, for example) against mites by agents attacking chitin biosynthesis of the latter arachnids. Nevertheless, one look on molecular separation data of the said taxons [32, 33][1] shows that chitin must have originated long before first hard structures were made of it during early Cambrian (*Anomalocaris*) 530 mio. y ago (trilobites and mollusks developed only later), namely about 1 bio. (>> 700 mio.) y BP. Accordingly its original purpose must have been a different one, perhaps associated with obtaining trace elements or other nutrients from sediment which then were removed from the surface by acids. Perhaps (extinct) creatures like *Hallucigenia sparsa* from Middle Cambrian or certain Ediacaran- or Vendian age organisms represent such strategies of obtaining micronutrients.

[1] Divergence times between arthropods and vertebrates are estimated at some 964 mio. y BP [33], that between fungi and animals even at 1.513 bio y [33], near the split of both from plants (with the latter generally lacking chitin) and the origin of plastids [32]. Other estimates for fungus-arthropod- or fungus-animal separations are closer to present, about 850 mio y. This does not change the argument, however.

5. CONCLUSIONS

The methods (chitin-based and moss-monitoring, respectively) do not compete but provide data which are mutually independent and can supplement each other. For some elements, relationships between total and speciation-resolved contents in water and levels detected on chitin exposed to such solutions/suspensions were already investigated (Ni, Mo, several REEs [8, 9]). Living organisms carrying chitin on their outer surfaces (arthropods, lichens, certain "worms", e.g. *Riftia pachyptila*, representing the biggest extant chitin structures in the biosphere) might likewise be used for passive or active biomonitoring but then there is a much smaller range of conditions which can be investigated [33]. Among REEs, photochemistry induced by Eu can be identified.

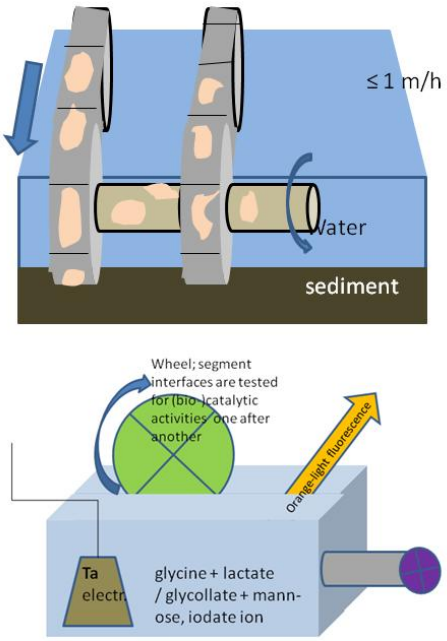

Figure 9. Mobile sampling devices covered by chitin (drones, rovers) and an enzyme activity detector which makes use of the fact that some biogenic compounds, e.g. glucose or dimethylamine or CH_3OH, methoxyacetate are readily photooxidized by Eu(III)* ($\lambda_{exc.}$ = 394 nm) while mannose or N-methylglycine, glycollate are inert in these conditions/reagent mixture [20].

The key purpose of this work, besides of technical applications of Eu-based and chitin-modified photochemistry in activating CH bonds [5, 20] and studying enzyme activities in soil samples is to predict pathways and consequences of climate in regions previously shaped by permafrost.

The advantage that chitin adsorption works beyond the range of biological tolerance considering several dimensions[1] will be fully exploited by replacing living test organisms [4] or the present chitin plates manually located and retrieved by using remotely controlled sampling devices run on rovers such as drones, micro-ROVs (Fig. 9).

Acknowledgements: *The organizing team of 32nd ICP vegetation workshop at Targoviste did an excellent job, and encouraged us to submit this paper in the aftermath. Mrs. Heike Heidenreich (lab staff) is thanked for doing the analyses (ICP-MS) which are not too difficult but must address sometimes tiny traces of metals bound to chitin besides of lots of Li. SF´s partner Dr. Beate Noack did indispensible contributions in fieldwork and documentation.*

[1] Except for pH: adsorption is poor at pH < 3 while there are some aquatic isopods and mites enduring pH 2.5; however, concerning radioactivity (cp. Muzzarelli´s [1] classical experiment!), action of biocides, heat (chitin is more stable than cellulose; for the pyrolysis products see[30]), darkness or cold (notwithstanding lichens) grafted chitin can be applied far beyond limiting conditions of active multicellular life (e.g. [34]) except for acidic pH

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