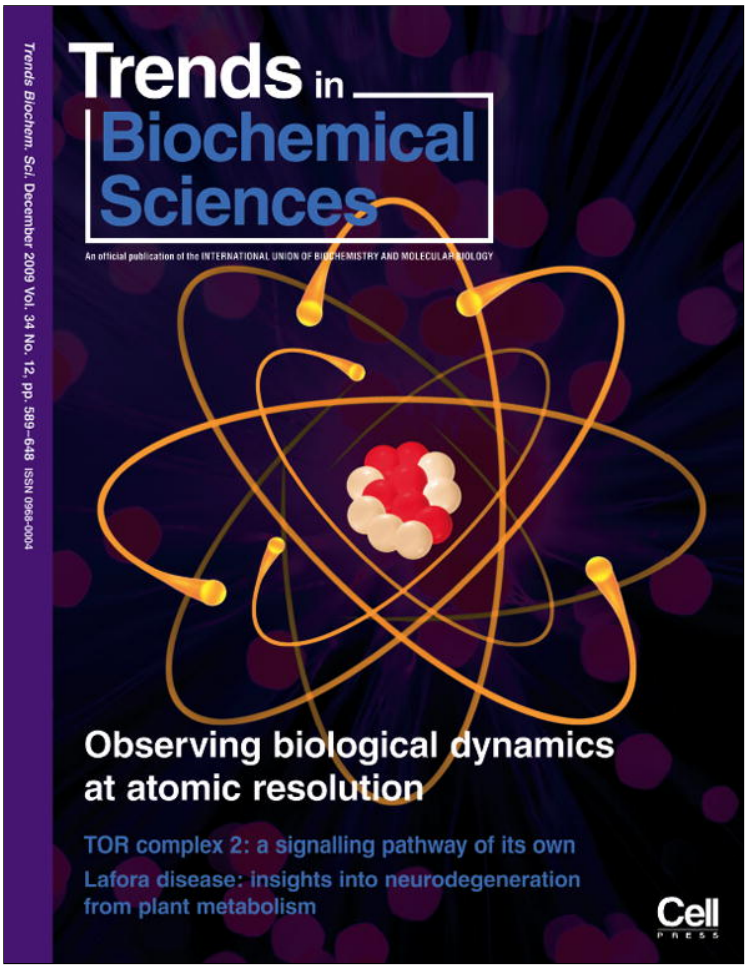

Opinion

Darwin, natural selection and the biological essentiality of aluminium and silicon

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If one was asked to produce a set of 'Trump Cards™' based upon 'Forces of Nature Defining Life on Earth' then which card would be 'Top Trump'? I was recently chastised on the Darwin Today website for suggesting Darwin and 'natural selection' rather than, for example, Newton and 'gravity'. Although there is no denying the significance of gravity, my argument in favour of natural selection is simply that gravity is just one factor that contributes towards an outcome which ultimately is defined by natural selection. Both the beauty and the brilliance of natural selection are reflected in its omnipotence to explain the myriad observations of life and, as I will affirm herein, its explanation of the biological essentiality of aluminium and silicon is no exception.

Natural selection defines the biologically essential elements

This year we celebrate the 200th anniversary of the birth of Charles Darwin and 150 years since publication of the seminal work 'On the Origin of Species' [1]. In a recent lucid overview of the last 150 years of natural selection Mark Pagel illustrated how Darwin's theory is, in equal measures, omnipresent and omnipotent [2]. I intend to affirm this view in my field of the natural sciences, inorganic biochemistry. My resolve to do so emanated from my experience of a book which I read and reviewed for *TiBS* in the early 1990s. This seminal work [3], along with a sister publication by the same authors [4], not only informed my view of biochemical evolution but provided me with an all too logical framework upon which I have been able to build an understanding of the biological essentiality of the elements. I have formed the view that natural selection, as a force of nature, is as important in biochemical evolution as it is in speciation and, in turn, in explanations of the living world. At its most fundamental level natural selection is a competition in which winners and losers are defined by selection pressures which act upon competitors that are constrained within specific boundaries or arenas. In many ways the latter, the selection arenas, help to explain the universality of natural selection in that no one single set of selection pressures will dominate across all or even many selection environments. The selection criteria which result in elements being chosen for biochemical functions and ultimately, thereby, define their biological essentiality are myriad and their successes are niche driven. Whereas the latter ensures that no single criterion dominates selection across all biological milieus, the evidence of biochemical evolution to date highlights that the abundance of an element is of critical importance to its ultimate biological essentiality. However, as will be discussed for aluminium and silicon, the abundance of an element in the lithosphere only becomes a major driving force for its selection for biochemical purpose when abundance is commensurate with the availability of biologically reactive forms of the element. Selection acts upon the biological reactivity of an element through pressures such as reaction thermodynamics and reaction kinetics. The former are exemplified by equilibrium constants which define properties of reaction products such as solubility and complex stability whereas kinetic constraints influence how (bio)chemical equilibrium is approached and ultimately which biochemical pathways predominate. The paramount importance of kinetics over thermodynamics in determining biochemical fate reflects the influence of other varied selection pressures such as concentrations of reactants, products, competitors and interferences as well as physical constraints acting as compartments, for example, membranes, or fluid flow acting as transport systems, such as the bloodstream. These are examples of the kinetic barriers, which through continually disrupting and disturbing myriad attempts to approach chemical equilibrium, define living systems and their continuous evolution. At present, reaction constants which have been determined experimentally at thermodynamic equilibrium remain the only quantitative tools to predict the fate of, for example, a metal ion in a biological system [5]. However, by their own definitions, such constants, whether prefixed with such terms as 'effective' or 'association' or 'dissociation', etc. are only truly predictive in (hypothetical) biological systems approaching or at chemical equilibrium. Simple computational models of non-equilibrium binding of metals have begun to contribute to understanding of the fate of metal ions in multiligand systems that are far from equilibrium [6] and it will be models of this ilk which will eventually be used to demonstrate *in silico* the natural selection of biologically essential elements.

Frausto da Silva and Williams in their two major works in this field have provided a thorough background and an in depth understanding of the selection pressures which

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act upon biochemical evolution [3,4]. Herein I will try to expand upon their ideas to show how natural selection is the architect of biological essentiality. How it acts within a framework of 'the economical use of resources' to build self-sustaining biological systems from abundant and biologically available elements. While, during the evolution of life on Earth, the lithospheric abundances of the elements have remained approximately constant, I will argue that the same is not true of their availabilities for selection for nascent biochemistry. The interplay between abundance, biological reactivity and biological availability is the story of aluminium and silicon in biochemical evolution.

Introducing aluminium and silicon

Aluminium is the most abundant metal and the third most abundant element in the Earth's crust. In spite of its abundance, aluminium has no essential role in any biochemical system in any extant organism [7]. By contrast, silicon, the second most abundant element of the lithosphere after oxygen and widely regarded as an essential element, has no known biochemistry to describe its requirement by biota [8]. Non-essential aluminium is biologically reactive with a surfeit of known, if inimical, biochemistry whereas silicon's essentiality appears to be based upon a complete lack of bioorganic chemistry (no Si–C, Si–O–C bonds) and an extremely limited bioinorganic chemistry. Although the paradoxically 'lacking' and 'limited' biological essentialities of the ubiquitously abundant aluminium and silicon respectively are probably open to many possible explanations, they are equally well described by the force of natural selection acting upon biochemistry through evolutionary time.

Natural selection of aluminium

Today, aluminium's lack of an essential biological role might be attributed to either its non-participation in the natural selection of the essential elements or its selection out of biological systems due to a time-served, but ultimately unsuitable, biochemistry. Although the latter is the oft-cited explanation, for example, aluminium's slow ligand exchange rates are suggested to preclude any efficacy as a metal co-factor for enzymes [9], it is the former, its non-selection, which best survives detailed scientific scrutiny. However, if aluminium has been a direct participant in natural selection and has ultimately been selected out of successful biochemical pathways, then what is it about its biological reactivity which renders it an unsuitable element for a biochemical role in at least one form of life?

In exploring such a possibility, there should be no doubting the, probably unrivalled, varied and versatile chemistry of this element, testimony to which are myriad applications in modern living in what today can rightly be called an 'Aluminium Age' [7]. Thus, the non-essentiality of aluminium is not easily explained by (bio)chemical inertia. Equally there are abundant examples of the ecotoxicology of aluminium. It is the protagonist in the devastation wreaked by 'acid rain', [10] and its iniquity in the natural environment owes much to it being avidly bound by oxygen-based functional groups, for example out-competing Mg(II) by at least a factor of 10^3 for complexation by ATP [11]. In addition the redox inactive Al(III) is paradoxically a powerful pro-oxidant and at submicromolar concentrations catalyses both iron and non-iron driven redox chemistries. Although the underlying mechanism is unknown, it probably proceeds through aluminium being bound by the superoxide radical anion [12]. Aluminium is also an unlikely, but extremely effective immunogen, with its dual role as both adjuvant and antigen enabling its widespread application in vaccines [13]. Aluminium's potential to participate in biochemical pathways appears boundless and although often disruptive to 'normal' function, can also be benign, for example by substituting for Mg(II) in metal-nucleotide complexes without causing a significant impact upon commensurate reaction efficacies [14].

The myriad ways in which aluminium could have been involved in the natural selection of the elements of biochemistry and, probably fuelled by man-made increases in its biological availability, is involved today, must surely point not to its selection out of biochemical systems but to its non-selection due to its hitherto lack of availability for selection. Further evidence to support such a contention is the lack of footprints left by aluminium in the sands of evolutionary time. For example, when an organism has encountered biologically available cadmium (Cd(II)), its response is an up-regulation of the transcription of proteins, such as metallothioneins, which are capable of binding and detoxifying the metal. There is evidence in DNA that non-essential heavy metals such as cadmium have at a previous time in biochemical evolution been both encountered and selected out of biochemistry. Such evidence is completely lacking for aluminium [7]. At the present time in biochemical evolution, aluminium remains a silent visitor in biota and its presence is disguised by any number of Trojan horse-like complexes. There are no known designed-for-purpose mechanisms by which aluminium is specifically either kept out of or removed from biota, nor is there evidence in biochemical evolution of significant encounters between biota and biologically available aluminium. Therefore, even allowing for its ubiquity within the Earth's crust, the logical explanation of the non-essentiality of aluminium must be that biochemical evolution has proceeded in the absence of biologically-reactive forms of the metal.

But how has the by far most abundant metal in the Earth's crust remained hidden from biochemical evolution? There are powerful arguments, many of which influenced Darwin's own thinking [15], which identify natural selection as acting upon geochemistry as it acts upon biochemistry. I have argued previously that the lithospheric cycling of aluminium, from the rain-fuelled dissolution of mountains through to the subduction of sedimentary aluminium and its re-emergence in mountain building, depends upon the 'natural selection' of increasingly insoluble mineral phases of the metal [7]. The success of this abiotic cycle is reflected in the observation that less than 0.001% of cycled aluminium enters and passes through the biotic cycle. In addition, only an insignificant fraction of the aluminium entering the biotic cycle, living things, is biologically reactive. However, my own understanding of such an explanation of how life on Earth evolved in the absence of biologically available aluminium was arrived at by a somewhat serendipitous route! In studying the acute toxicity of aluminium in Atlantic

salmon I discovered that the aqueous form of silicon, silicic acid, protected against the toxicity of aluminium [16]. Subsequent work showed that protection was afforded through the formation of hydroxyaluminosilicates (HAS) [17] which, intriguingly, are one of the sparingly soluble secondary mineral phases of the abiotic cycling of aluminium! The discovery that silicic acid was a geochemical control of the biological availability of aluminium, though now seemingly obvious in hindsight, was a seminal moment in my understanding of the bioinorganic chemistry of aluminium, and although it helped me to understand the non-selection of aluminium in biochemical evolution, it also provided me with a missing link in the wider understanding of the biological essentiality of silicon.

The non-selection of silicon

The main biologically reactive form of aluminium is $Al^{3+}_{(aq)}$ [9] and a case has been made that its biological availability, as opposed to its biological reactivity, has, hitherto, precluded its participation in biochemical evolution. Compare this to silicon where the main biologically reactive form, silicic acid, $Si(OH)_{4(aq)}$ [8], has always been available for selection and yet there are few clear examples of the biological essentiality of silicon. I have recently described living things as being permeable to silicic acid [18]. It moves freely across biological membranes and, where conditions are favourable, will equilibrate according to a Donnan equilibrium. As a small, neutral, monomeric molecule, silicic acid closely mimics water in its movement into and out of biota and there can be few if any biological compartments which have not been visited by silicic acid. On the early Earth, silicic acid was uniquely abundant and potentially biologically available; yet today there are very few descriptions of silicon essentiality in biota. Perhaps the most oft-cited examples of the utility of silicon are the 'biosilicifiers' in which silicic acid is concentrated and deposited as amorphous hydrated silica [19]. The forms of deposit range from disordered precipitates in, for example, bone in higher animals, to elaborate exoskeletons in, for example, diatoms and sponges. Intriguingly, all of these silicifications, from simple precipitates to organised structures, are achieved without recognised silicon biochemistry; I have argued that biosilicification is a form of silicic acid homeostasis which evolved as an indirect consequence of silicon's limited and highly specific bioinorganic chemistry [18]. Silicon, unlike aluminium, is an essential element in life [20] in that throughout evolutionary time it has exerted selection pressures which, in spite of there being no silicon biochemistry, are manifested biochemically when silicon is deficient or excluded from a particular environment. With this in mind, what, in the absence of Si–C and Si–O–C bonds, is the mechanism of silicon's biological essentiality? The subtlety of the biological essentiality of silicon is due, at least in part, to the chemical inertia of silicic acid which is the only biologically available form of silicon. It is a weak acid and only loses its first proton at pH approaching 10 [21]. Thus for the majority of environmental and physiological milieus, silicic acid is a small, neutral molecule with no known bioorganic chemistry and an extremely limited bioinorganic chemistry. With respect to the latter, there are only three significant classes of reaction; (i) its autocondensation to give amorphous hydrated silica when its solubility is exceeded at ca 2 mM; (ii) its reaction with aluminium hydroxide to form hydroxyaluminosilicates (HAS); and (iii) its reaction with an excess of molybdate to form the Keggin-like molybdosilicic acid complex. I have argued that of these three, the reaction which has had the greatest impact upon biochemical evolution is that with aluminium hydroxide in forming HAS. I have called this reaction 'a geochemical control of the biological availability of aluminium' and its significance has been to preclude aluminium's participation in the natural selection of the biologically essential elements. Silicon, as silicic acid, has significantly reduced the biological availability of aluminium and in doing so has promoted less abundant metals in the hierarchy of the natural selection of the essential elements. The metals which were selected in the absence of aluminium, and through the course of biochemical evolution are today biologically essential, are also prime targets for substitution or interference by aluminium in the absence of the protection afforded by HAS [22]. Thus, metals such as Mg(II), Fe(II)/(III), Ca(II), Zn(II) and Cu(II) (in tentative descending order of influence by aluminium), which would have been positively selected for in the absence of competition from aluminium (Figure 1), are those which at present are most likely to be affected by a burgeoning burden of biologically available aluminium. For example, recent transcriptomic analyses of aluminium-stressed biotic systems have demonstrated significant changes in gene expression in relation to biochemical pathways which are dependent upon these metals, and, in particular, Fe(II)/(III) and Mg(II) [23,24]. Experimental evidence to support the suggested evolutionary role for silicic acid can also be found in the many experiments which have demonstrated the amelioration of aluminium toxicity in biota by the presence or addition of silicic acid [25–27]. In addition, the symptoms of silicon deficiency, nearly always in the presence of aluminium, are often manifested in biochemical systems which are dependent upon these metals [28]. For example, it is significant that whole-genome expression profiling of the marine diatom *Thalassiosira pseudonana* identified a common set of 84 genes which were induced by both silicon and iron limitation independently [29]. Both of these conditions would be expected to result in increased competition from aluminium and by way of an example, one of these gene families encodes prolyl-4 hydroxylases [30] which are known targets of aluminium toxicity [31]. There are clear examples of how silicic acid, through limiting the biological availability of aluminium, has promoted the natural selection of certain biochemical pathways which might otherwise have been either modified or even prevented by aluminium. For example, burgeoning evidence currently implicates the major intrinsic proteins (MIPs), which include the aquaporins, in the accelerated transport of silicic acid across biological membranes [32]. The primary 'selected' function of the aquaporins is the transmembrane transport of water and their efficacies are dependent upon the actions of metal ions, and in particular Mg(II), Ca(II) and possibly Zn(II) [33]. As might have been predicted, aquaporins are known targets of aluminium

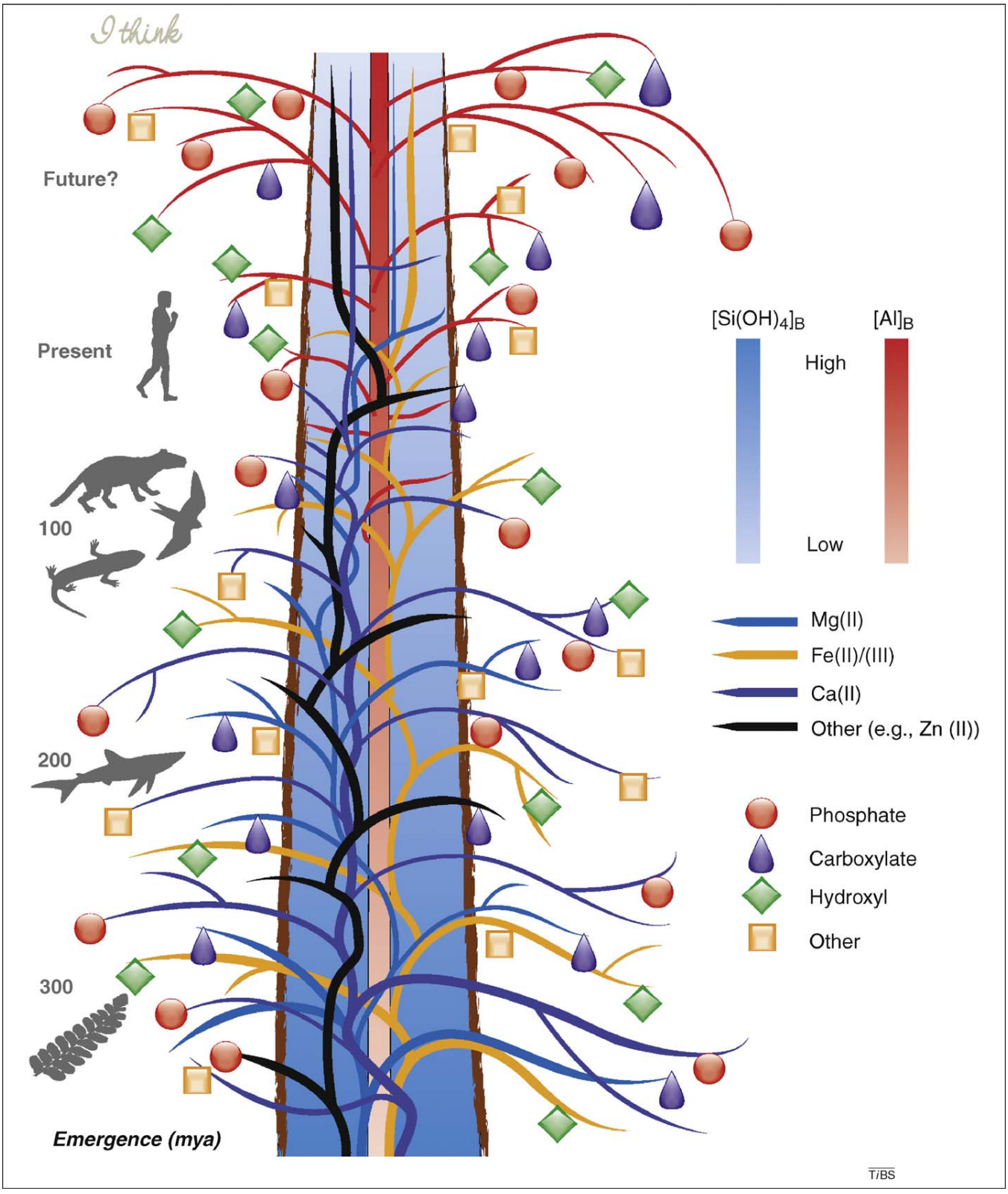

Figure 1. A biochemical tree of life for the natural selection of aluminium. Concomitant with the emergence of higher forms of life are significant concentrations of environmental silicic acid and commensurate insignificant concentrations of biologically reactive aluminium. Silicic acid limits the biological availability of aluminium, and it is unable to compete with Mg(II), Ca(II), Fe(II/III) for binding by phosphate, carboxylate and hydroxyl functional groups. Aluminium is excluded from inorganic biochemistry. Subsequently over tens of millions of years and, recently concomitant with the activities of modern human beings, an increase in the concentration of biologically reactive aluminium occurs. This increase, coincident now and in the foreseeable future with evolutionarily-driven lower levels of environmental silicic acid, displaces essential metals from biomolecules which are integral to vital biochemical processes. Aluminium is now competitive and has become an active participant in biochemical evolution. What then are the implications of such for future biochemistry?

Key: Degree of shading indicates changes through evolutionary time in the environmental concentrations of biologically reactive (i) silicic acid ($[Si(OH)_4]_B$; blue) and (ii) aluminium ($[Al]_B$; red). The extent of coloured branches and their variously-shaped leaves estimates the major metal ion competitors for Al(III) and their preferred functional groups for binding respectively. mya: million years ago.

toxicity and their expression is down-regulated in both plants [34] and animals [35] subjected to aluminium stress. Further, lower rates of urinary silicon excretion in individuals suffering an aluminium overload might be indicative of the disruption of similar biochemical pathways in humans [36,37]. It is intriguing to speculate that by excluding aluminium from biochemical evolution, silicic acid acted to promote aquaporins as important transport proteins for both water and itself.

Beware the natural selection of non-essential elements

The non-selection of aluminium and the partial-selection of silicon in biochemical evolution are themselves subject to the force of natural selection. The power of the latter is not in the delineation of hierarchies of increasing complexities, but rather in how its actions respond to environmental change. The absence of silicon biochemistry does not preclude a biological role for silicon; instead, its roles are best described by its bioinorganic chemistry. The latter played a key role in the non-selection of aluminium through significantly reducing its biological availability. However, the 'aluminium environment' acted upon by natural selection is changing, and through human activities, not least of which has been the technology to extract aluminium metal from its biologically inert ores, biota are experiencing a burgeoning exposure to biologically reactive aluminium. Biochemical evolution now progresses in the presence of aluminium and must account for biologically-reactive aluminium (Figure 1). Some of the early results of such processes have been worryingly obvious, including the death of fish and trees in geographical regions impacted by acid deposition, whereas others, and perhaps those which in particular are linked with the human condition [22], might yet be too subtle to be directly attributable to the participation of biologically-reactive aluminium in the natural selection of the elements of biological essentiality.

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