Chemical Elements in Plants and Soil: Parameters Controlling Essentiality

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Chemical Elements in Plants and Soil: Parameters Controlling Essentiality

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To the memory of my father Prof.Otto Fränzle (December 9th, 1932 - August 20th, 2009), distinguished natural scientist, who did not live to see this book in print but whose spirit is felt on every single page

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Chapter 1
The Biological System of Elements

By the Biological System of Elements, originally (Markert 1996) an array of chemical elements was denoted describing their distribution in green plants and abundance correlation among plant species, irrespective of biochemical "roles" (e.g., essentiality as a component of enzymes) and functions. Later on, the present author went to give a causal account which draws on different physicochemical features and necessities of biochemistry – including effects of elements other than C, N, S, O, H and P – and extending the scope of interest to other living beings and their interactions in ecosystems, that is, to stoichiometric ecology.

1.1 Principles of Element Distribution in Plants

In the beginning of the nineteenth century, analytics of plant matter samples started with that of plant ashes. In addition, no methods were available then which could have enabled intact biological materials to be digested for complete, no-loss analyses without burning them before. Hence, volatile elements then could not be detected, let alone quantified in biomass. Elements then found in plant ashes (Fe, Na, K, Ca, etc.) were both abundant and had been discovered in other sources before. As, e.g., no spectroscopic methods whatsoever were at hand earlier than about 1860, technical prospects for trace analysis then were dim at best (there are very few instances of elements detected in environmental samples/spectra prior to their isolation on Earth: helium (in 1868) and technetium (in 1952) were found in stellar spectra before being isolated from or detected in terrestrial minerals rather than synthetized by nuclear methods (Kenna and Kuroda 1962; Kuroda 1998), a third couple of emission lines (first attributed to some postulated new element "nebulium") turned out to be due to a forbidden low-pressure emission line of oxygen atoms). Amounts usually present in plants (or animals) could not be detected or measured for most elements, a problem which could be overcome only during the last decades. Now, however, advanced mass spectrometers (ICP-MS) and similarly sensitive analytic gear provide detection and determination ($<<$ 1 μg/kg DM) limits low enough to find and quantify most elements.

1.1.1 Distribution Patterns of Chemical Elements in Plants

As a rule, differences in (elemental) chemical compositions which exist among different species of (e.g.) plants should be caused by some unlike behaviour/differing processes in uptake or transport. For instance, there may be either active or passive transport of metal ions or other speciation forms of elements (complexes, oxoanions, organoelement species such as kakodylic [dimethylarsinic] acid or methylmetal [M, e.g., Hg, Pb, Tl] compounds/ions), producing different rates and/or equilibria of uptake. In turn abundance correlations among these very plant species appear which are at odds with chemical intuition, that is, a very low, virtually nonexistent abundance correlation in pairs of closely elements one of which is resorbed and shuttled onward to leaves/needles and fruits/seeds in a constant manner whereas the other is transported by ways/carriers

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which depend on the corresponding species while conversely chemically apparently unrelated elements may follow similar paths. Geochemistry, including pH and wetness of soil substrate, thus provides very different patterns of elemental abundances for metals as well as certain non-metals. There are both synergistic and antagonistic relationships between uptake or use of different elements by plants. Of course these latter interactions, which partially represent the response of the plant to local geochemical conditions, in turn change the distribution patterns by mainly antagonistic interactions among essential elements (Kaim and Schwederski 1993); also consider Fig. 1.1:

Negative abundance correlations (e.g., Ca/Mg) may indicate a direct competition for the same binding centers owing to some chemical similarity among the pair of metals (Fig. 1.3). Thus, chemical similarity can bring about both highly positive and highly negative abundance correlations depending on dynamic features: if retention to biomass dominates in the end, similar coordination properties – both concerning binding strength and ligand selectivities – will result in positive abundance correlation whereas control by transport mechanisms, including competition for low-concentration carriers, rather gives a negative correlation. However, it is unlikely that both effects will cancel, producing no discernible abundance relationship across various plant species whatsoever. Notably, Fig. 1.3 does not display dynamic features like a rate of plant growth but "simply" the abundance relationship among the elements and plant species. Thus, Figs. 1.1 and 1.2 cannot be directly compared even for identical pairs of elements.

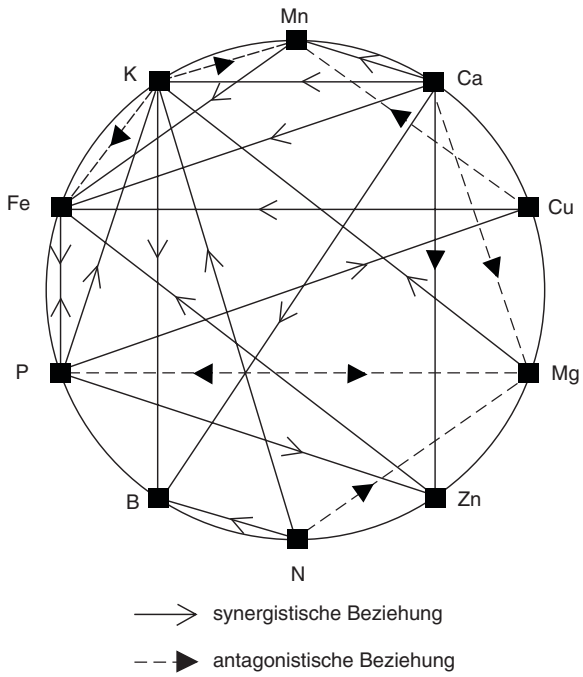

Fig. 1.1 Network of interactions/influences among some essential chemical elements in plants (Kaim and Schwederski 1993). The parameter used for attribution of either synergy or antagonisms to an interelement relationship is rate of plant growth

Local enrichment of certain elements within some plant may be due to both complexation with polymeric components of biomass and to precipitation of solid, insoluble, sometimes even crystallinic phases. Before an element may be enriched or separated in any of these kinds, three other factors contribute to the series of events, besides the conditions of uptake, namely:

- Speciation of elements next to its rhizosphere, respectively
- Mechanisms and kinetics of uptake by roots (or fungal mycelia) or leaves (especially in aquatic plants)
- Mobility inside the plant, controlled, e.g., by phosphate in the xylem

For example, the relatively large amounts of Rb present in plants may be involved in chemical signalling much like Na or K and will obviously contribute to osmoregulation, but the latter effect does not render Rb essential because it can be replaced by other ions (or even organic compounds such as glycerine) for this purpose, and other, (more) specific uses are not obvious from analysis alone. Although some chemical details of paleobiochemistry may be inferred from appropriate fossile samples such as chitin in amber inclusions, analytical data will never reveal what element actually was required by some extinct organism. Though differences in essentiality patterns among protist, animals, plants or fungi are well-known for now (Table 2.1), and "genetic clocking" allows for temporal reconstruction of the separations of their common ancestors (Feng et al. 1997), the corresponding changes among essentiality patterns upon evolutionary radiation are not accessible. This holds the more for results of thorough geochemical changes during evolution or for such extinct organisms which apparently do not fit into patterns and categories of recent-time

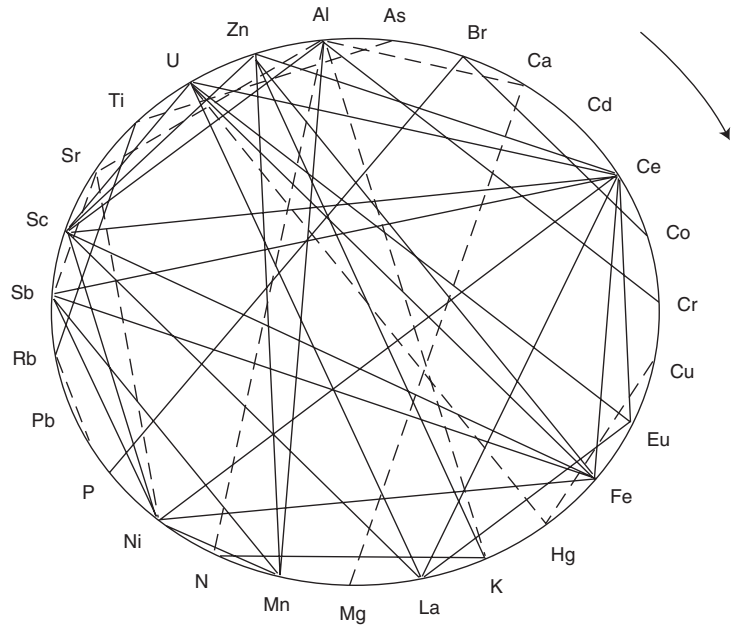

Fig. 1.2 Highly positive (*straight connection lines*) and highly negative (*broken connection lines*) abundance correlations among pairs of chemical elements in 13 species of plants (from Markert 1996)

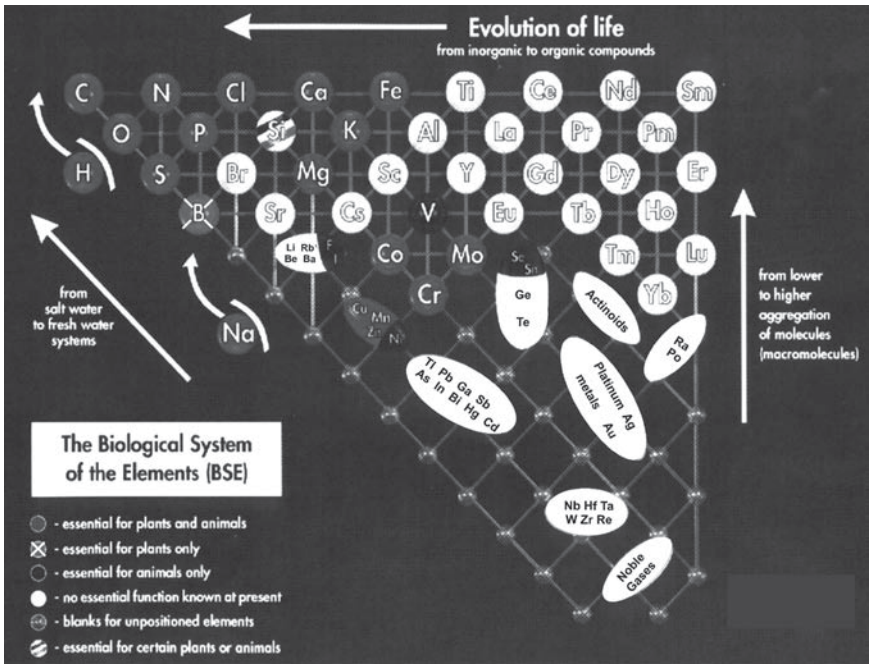

Fig. 1.3 The Biological System of Elements (Markert 1994a). The diagram shows relationships among the elements together with their corresponding essential functions (*colours*), extent of biochemical functions and the corresponding capacity to form macromolecules by condensation reactions (*vertical arrow at right side of diagram*). Whereas in "pure" geochemistry oxophilic metals produce the most complicated condensation products, i.e., clay minerals, there was a shift towards non-metal-based structures during chemical and biological evolution which afforded polymeric structures based on the latter (C, N, O) (*horizontal arrow to the left*). The diagonal arrow refers to changes of concentration from ocean- to freshwater. There is substantial decrease of concentrations in some elements (Mg, Sr, Cl, Br) from ocean to freshwater requiring them to be enriched by biomasses if their biochemical use is to be continued. Such kinds of enrichment can only be accomplished by means of certain biochemical features which involve properties and/or components of the corresponding biogenic materials – many of which are specific for one species at least in their particular combination

taxonomy at all (e.g., Venda organisms from the uppermost Precambrian).

Once, however, biocatalytic essentiality or chemical signalling with no chance to replace the components by other elements have been identified, matters change radically. Now, life is about reproducing some very complicated chemical gear – including accumulation and appropriate speciation of all the elements which are essential to that species – in an autocatalytic manner. From this point of view, a green plant is a device which produces and employs carbon compounds to obtain more C and increase its throughput/fixation rates of C (and all other essential elements) by (usually) identical reproduction of first the corresponding reactants (rubisco, chlorophyll and all the other proteins involved) and then that of metastructures (cells, entire organisms). Growth and reproduction thus correspond to *autocatalysis*.

The further line of argument is focussed on land plants for reasons of comprehensive data sets available here, yet there also are data for limnetic plants, as well as bacteria and animals. In terrestrial plants, there is a well-defined pathway for uptake and transport of metal ions rather than the chance to establish an equilibrium of element concentrations between water and biomass making use of almost the entire interface like in aquatic plants: for terrestrial plants, few, mainly epiphytes (Strasburger and Sitte 1991), are capable of taking up salts by leaves, otherwise it takes place by way of roots only. Although some (essential) non-metals such as sulfur (as SO_2) and nitrogen (both [either] as NH_3 or various nitrogen oxides) can be absorbed, used and metabolically transformed within the leaves, this pathway can be neglected for metals and so-called semimetals in higher plants and geochemically realistic conditions. In mosses, there are "colloquial" amounts of essential metals, say 35 μg/g DM of Zn and 3 μg/g DM of Cu even if there is no atmospheric deposition (extrapolation to zero). This points to a similar way of uptake to meet essential demands between mosses and vascular plants even though there are no roots in the former. Mosses give away amino acids and peptides when exposed to drought stress, causing weathering of underlying material with the apparent result of complexes to be resorbed by the mosses quite efficiently.

Speciation, uptake and transport alike depend on chemical properties and possible chemical and biochemical transformations of the corresponding element. Most of the essential non-metals (N, B, P, S, in addition Mo) are absorbed as oxoanions in their highest oxidation states whereas Cl is used as an elemental anion, that is, as Cl^-. Yet, it must be noted that some 40 – 50 % of absorbed nitrate N are converted into amino acids in the microroots already, as are – sometimes even larger – shares of other metal or non-metal oxoanions such as CrO_4^{2-}. In higher plants, substantial uptake of metals or semimetals *by leaves* would occur only if there are fairly persistent volatile forms which could be admitted to the stomata, except or particles within hydrometeors small enough to pass the stomata (or soluble in water, e.g., sea salt spray). However, permethyl compounds of Hg, As or Se have tropospheric lifetimes of minutes rather than hours given their reactivities towards OH radicals, much like peralkyls of Sn, Pb or Mo, W hexacarbonyls which are released from anaerobic layers of domestic dump pits (Feldmann 1999).

1.1.2 Biochemical Essentiality of Elements in the Light of Enzymatic Reactions

It goes without saying that occurrence of such relationships among elements does not depend on their essential functions, either of one of the involved elements or of both (for all the investigated kinds of organisms?) or even of neither. Rather, non-essential elements are likely to reveal the effects of chemical binding to plant biomass even more clearly because the influences by element-specific regulation or transport mechanisms should be less pronounced than with either essential or highly toxic elements (cp. the role of chaperons in sequestration, transport and elimination of Cu, Ni, Zn [essent.] and As, Cd, Pb [toxic] in both several plants [Tottey et al. 2005; Vernay et al. 2006] and kinds of bacteria). Farago (1986) and Clemens et al. (2002) give a very detailed picture of the processes which occur during binding of chemical elements in plants. Farago's work deals with the responses of different plants – including metal ion hyperaccumulators – towards variations of soil metal contents, focussing on morphology and mode of function of roots influencing resorption kinetics. In Farago's list, there are six dominant non-metals and besides these three macronutrients (K, Mg and Ca) and a larger number of "essential micronutrients" (Fe, Cu, Mn, Zn, Mo, Co, V, Na, Rb, B, Si, Cl, I, Se) while other elements (Ni, Al, Sr, Sn, Cr, Br and F) are considered "beneficial or of restricted essentiality" (there is some disagreement with this list, for example Ni is known to be a component of plant

enzymes including urease controlling the nitrogen cycle while Al [although accumulated by some plants like certain ferns and black tea; Kaim and Schwederski 1993;Markert 1996] and Cr are counted among plant toxins otherwise. The actual role of Rb is uncertain up to this day).

Concerning a high tolerance towards Cu, high concentrations of proline (a proteinogenic amino acid) in roots of Cu-adapted populations of the bog-plant *Armeria maritima* are presumed to be involved. Different authors (Still and Williams 1980; Farago 1986) agree that the effective fractionation between Co and Ni in *Hybanthus floribundus* may not be effected by the hydroxycarboxylic acids produced by their roots only; in addition, most of Ni in the leaves is water-soluble (-extractable) hence probably in a low-molecular state of binding. These examples – which could be amply extended – already give proof that coordination chemistry is most important for understanding the processes occurring with metallic elements in the biota. But we shall soon notice that this is not the complete story.

In the 1840s, Liebig laid the fundaments of *Agrikulturchemie* (agricultural chemistry), dealing with the question which amounts of which chemical elements are necessary to grow and maintain higher (terrestrial) plants. In 1860, iron became the first trace element established to be essential for higher plants, to be followed by essentiality determinations for Mn, B, Zn and Cu between 1922 and 1931; lateron Mo and Cl were added to this list (Marschner 1986). In 1939, Arnon and Stout coined the term mineral nutrient. According to other investigations some 13 chemical elements, among them seven metals (K, Mg, Mo [or W], Mn, Fe, Cu and Zn) are essential for sustaining life of almost all living beings, higher plants in addition need B, Cl and Ca to yield a total of (at least) 16. Some of them or their endosymbionts, e.g., N_2-assimilating rhizobacteria in root nodules of leguminoses (Co) or the fungus components of lichens (V), also require Na, Si, Co or V.

In those early days (nineteenth and early twentieth century) there were just empirical studies on growth impediments brought about by lack of some purportedly essential element, without control and counterchecks. For this purpose, Sachs (around 1882) introduced hydroponic culture method, because the composition of aqueous or other solutions can be better and more easily controlled than that of the multiphase solid "soil". Around 1900, biochemistry was extended beyond analyses of main components of biological materials into traces (e.g., determination of Ce in [animal] biomasses). Then essentiality of Fe, Mg, etc. became linked to compositions of some chemical components of (e.g.) plants, for example by identification of chlorophyll as a (porphyrin) complex of Mg.

Later on, about 1935, the first metalloproteins were isolated and identified as such (Höhne 1980). Like it had been done in earlier (though often somewhat speculative) work on pathways of sugar synthesis or nitrogen assimilation in plants, it now became obvious to compare functions of these very metal (ions or complexes thereof) in (methods and principles of) technical chemical catalysis to those observed or presumed in biological systems, e.g., Cu or V in oxidation catalysts or several metals in hydrolases. Later advances in both spectroscopy and trace analysis were to reveal the presence (and insubstitutable function) of metal ions also in many of such enzymes which had been isolated and even crystallized (rendering them accessible to both crystallographic investigation and XRF analysis) long before: one peculiar example is the identification (Dixon et al. 1975) of nickel (12 atoms per enzyme molecule!) in jackbean urease which had been crystallized by Sumner already in 1926. As for the required amounts of essential elements, there are tremendous differences both in concentrations within one species (Mg or Ca vs. Mo or Co) and among different species. This poses some problem of interpretation of the BSE: it is conceivable that corresponding differences are due to unlike "weights" of single catalytic or other functions of some metal ion among the species and taxa (giving rise to poor correlations between abundances of two elements in the set of 13 species in each case), yet some part of the observed differences may be rather due to blunt biological coordination chemistry (bioinorganic chemistry) or the necessity of organisms to reproduce in order to maintain life or the corresponding species. Both rules of bioinorganic chemistry and the criterion of reproduction (as an act of autocatalysis, chemically speaking) provide specific limiting conditions, contribute to fractionation and (possibility of) catalytic functions, or either exclude the latter.

1.1.2.1 How Do Chemical Elements Shape Biology, Biochemistry?

About one third of all the biochemical transformations in any organism, including those of nucleic acids, aromatic

compounds and nitrogen speciation forms, are brought about by metalloenzymes and thus are metal-complex-catalyzed in a certain way. Hence, the ability of anabolic metabolism is controlled by metal availability, as is transfer of elements, proper nutrition, etc. within trophic chains. Thus element flows – both bound to food and obtained from the "free" environment – can also shape ecosystems, often in a subtle way: the balance between ruminants, sheep and other hoofed animals such as deer, antelopes in open grassland or savanna (wildebeests!) may as well be controlled by the Mo/Cu ratios in soil (antagonistic toxicity to which hoofed animals are sensitive to very different extent) as by direct depletion of certain elements in grasses, leaves and other food. Among consumer organisms with different trace element demands – different in terms of both identity (stony corals need Sr while other planktivores do not, some fungi or animals do depend on administration of V or Co, respectively, while others, even closely related creatures, do not) and of amount – the abilities to settle in a certain area or ecosystems by exploiting some producer- or lower-level-consumer species living there already obviously are unlike, with chances to compensate for lacking materials from ambient water or by soil ingestion limited by either dilution of the elements or inability to mobilize them, that is, in any case, by limited or mediocre complex formation in (attempted) sequestration of the said elements (considering metal ions mainly for the moment). The elements may be constant in amount/concentration but will be differently retained or extracted, owing to, e.g., the competitive exclusion principle even though sequestration agents may be identical in rather different organisms, e.g., hydroxamates in fungi and soil bacteria.

1.1.2.2 Metal Ions and Their Relationship Towards Biocatalysis

Reactivities of other ligands such as N_2, NO or CN^- which are or get bound to metal complexes or metalloproteins are likewise influenced by the $E_L(L)$ of the coligands (Chatt et al. 1980a; Rehder 1991). Accordingly, there is also a relationship between binding properties of a central ion as defined by Eq. 2.4 and its catalytic properties which extends to metalloproteins. Thus *essentiality patterns* – including biocatalytic activities – *can be directly linked with chemical properties of biorelevant metal ions*. Another issue that arises here is whether or inhowfar features of biological uses of metal ions (biocatalysis) match the "optima" for promoting the same reaction which are known from technical or bench-scale catalytic chemistry or else differ somehow. Of course, a meaningful comparison does imply the non-biological reactions to occur in similar to physiological conditions, also. We already mentioned one conspicuous example before: transport (including reversible attachment to metal ions) of molecular oxygen in biology is effected by either Fe (haemoglobin, haemerythrin) or Cu (haemocyanin) rather than Co; many more such "discrepancies" are listed in tab. 1.1. Activation of CO_2 by coordination towards electron-rich metal centers (Ni(I), Co(I)) and/or reduction to carbonyl- besides carbonatoligands is known for long (Floriani 1983), likewise reductive terminal addition to alkene or alkyne ligands causing chain extension and eventually direct carboxylation of phenolates or carbanionoids (Li or Mg organyls). On the other hand, there is not yet a model (whether using Mg or any other metal ion) complex which mimics the function of rubisco, that is, can add CO_2 to partially oxidized organic molecules splitting their C–C backbones. The coordination chemistry of formaldehyde at V(II) [vanadocene] or Zr sites (Floriani 1983) interaction can be considered to mimic the interactions between (aldo-)sugars and metal centers even though neither V nor Zr are used for related purposes in biochemistry.

In Table 1.1, reactions or transport modes of some 30, usually small, biorelevant molecules are listed together with the metal ions which effect these reactions in (a) biochemistry and (b) catalytic inorganic chemistry, giving an impression of how abundant differences are between "procedures" in biochemistry and chemical technology even after several biollion. years of evolution. Among the substrates in this list small molecules and "simple" functional groups do prevail over larger ones or even macromolecules for the simple reason that, because the behaviour of the former is better understood also in terms of quantum chemistry, "optimum" catalysts (last column in Table 1.1) can be pinpointed there more easily. This table forms some semi-theorical background for a theoretical analysis of limiting conditions set by both evolution and geochemistry.

1.1.3 Soil and Geochemistry: Support and Storage/Buffer System for Biology

1.1.3.1 General Geochemical Considerations

Soil is also mentioned in the title of this book because it is not just a mechanical support for terrestrial plants but both a source – tapped via the roots, with or without

Table 1.1 Selected biochemical key transformations promoted (catalyzed) by metal ions and corresponding technical–chemical processes (Ochiai 1968; Riedel 2004) in comparison

Substrate	Reaction	Enzyme or carrier	Product	Group(s) of organisms which accomplish this biotransformation	Metal ions employed in biochemistry	Metal ions "best" employed in technical catalysis
H_2	Disproportionation, oxidation	Hydrogenase	H_2O	Archaea, clostridia	**Ni**, Fe (either **Ni** + Fe or Fe only)	**Ni** (PGMs)
CO	Oxidation	CO dehydrogenase (rather to be called: CO oxidase)	CO_2	Archaea	**Ni, Fe**	**Ni, Fe**, Cu
CO_2	Reduction	Formate dehydrogenase	Various		**Zn** (in clostridia: Fe + W, Se)	**Zn**
	Hydratation	Carboanhydrase	HCO_3^-	Almost all organisms	**Zn,** Cd, Co	**Zn**
CO_2	Fixation to ribulose-1,5-bisphosphate (C-site carboxylation)	Ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco)	3-Phosphoglycerate (2 times)	Green plants, chemolithoautotrophs	Mg	**Ni (see above)**
	Fixation to phosphoenolpyruvate	PEP carboxylase	Oxaloacetate	Green plants (C_4 plants)	Mn	**?**
O_2/O_2^-	Transport	Haemoglobin, myoglobin, haemerythrin, haemocyanin	Transport only	Aerobic heterotrophs	Fe (-porphyrins; in animals, fungi), Cu (molluscs, arthropods)	Co
O_2^-	Disproportionation	Superoxide dismutase	O_2 and H_2O_2	Aerobics	Mn + Cu	V
H_2O_2	Disproportionation	Catalase	O_2 and H_2O	Aerobics	V, Mn, **Fe, Se**	**Fe** (solid-state reaction at oxide interfaces)
N_2	Reduction	Nitrogenase	NH_3	Bacteria (symbiotic), cyanobacteria	**Mo + Fe,** V + Fe, Fe only[a]	**W, Mo**
NO	Intercellular communication		(no product)	Metazoans	Fe	Cu
NO	Fixation to CH-acids or corresponding enolates		Hydroxamates	Fungi, (soil) bacteria	Cu	Fe, Mo
NO_3^-	Reduction	Nitrate reductase	NO_2^-	Plants, animals, soil bacteria, yeasts (if undergoing "nitrate respiration"	**Mo**	**Mo**
NO_2^-	Reduction	Nitrite reductase	N_2, N_2O or NO	Plants, bacteria	Fe, **Cu**	Mo or **Cu**

(continued)

Table 1.1 (continued)

Substrate	Reaction	Enzyme or carrier	Product	Group(s) of organisms which accomplish this biotransformation	Metal ions employed in biochemistry	Metal ions "best" employed in technical catalysis
N_2O	Reduction		N_2	Some soil bacteria	Fe, **Cu**	**Cu** (generally speaking, N_2O bound to metal ions loses O very readily)
SO_4^{2-}	Reduction	Sulfate reductase	H_2S (after several intermediates, including phosphorylation)	Sulfate reducing bacteria, plants	**Fe** (FeS cluster)	("optimum" cannot be determined as there is no function-reproducing model complex yet reported)
SO_3^{2-}	Oxidation	Sulfite oxidase	SO_4^{2-}	Aerobic organisms	**Fe or Mo** (sulfite oxidases which rely upon molybdopterin	V, **Fe**
AsO_4^{3-}	Reduction	Arsenate reductase (most often also acting as a phosphatase)	As(III), organo-As compounds	Both eucaryotes and prokaryotes, e.g., *Staphylococcus aureus*	Mo + Fe + **Zn** (combined, employing glutathione)	**Zn (?)**
Hal^- (Hal = Cl, Br or I)	Oxidation	Haloperoxidase	$OHal^-$	Several fungi, marine algae	**V,** Fe, (Se)	**V, Mo**
HPO_4^{2-}	Phosphorylation	Phosphate ligase, – kinase	(Oligo)phosphoric acid esters, e.g., phosphorylated alcohols, sugars	All organisms	Mg or **Zn** (alkalinic), sometimes Fe(II) or Mn(II) (acidic)	**Zn**
Polyphosphates, nucleoside triphosphates (NTPs)	Hydrolysis, transfer of chemical energy, activation towards nucleophiles	ATPase and similar NTPases; nucleases	Nucleoside monophosphates	All organisms	Mg	Zn, Co
Guanidine (arginine sidechain)	Hydrolysis	Arginase	Urea and derivatives, e.g., ornithine	Green plants, animals (urea cycle)	Mn (animals), Ni (green plants)	Co
Methyl groups (nonacidic)	Oxidation		$HCOO^-$ and formylated compounds		**Co**; Zn, Se	**Co**, Cu
Methyl groups CH_3X (CH-acidic, e.g., acetyl-CoA) + CO_2	Carboxylation	Malonyl-CoA-synthase and related enzymes	$CH_2X\text{-}COO^-$	All organisms (catabolic direction of Krebs cycle)	Mn	Cu
Esters, enol ethers	Nucleophilic cleavage by NH_3OH^+	Anthranilate synthetase, using chorismate, glutamine and NH_3OH^+	Hydroxamates	Fungi, soil bacteria	Mg (optional)	Fe
Methyl groups + CO	Reduction	Insertion	CH_3COO^- and esters thereof	*Moorella thermoacetica*	Ni or Cu	Rh, Co (Monsanto or Reppe processes)

aldehydes	Hydrogen transfer (either direction)	Aldehyde oxidoreductase	Primary alcohols, $RCOO^-$, esters	Yeast, animals	Yeast: Zn, animals: Mo (aldehyde dehydrogenase)	Al, Zr, B (Meerwein-Ponndorf or Tishshenko reactions)
Amino acids	Oxidative NH_2 transfer	Transaminase + pyridoxal phosphate (cofactor)	2-ketoacids, ketones; acceptors: glyoxylate in plants, ketoglutarate in animals	All organisms	No metal	(various degrees of **inhibition** by metal ions [slight with Al, Cu, Zn, pronounced with Fe, REEs])
	Polycondensation	Peptide ligase	Peptides, proteins	All organisms	Zn	Cu
N (nonbranched) carboxylates (fatty acids)	β-Oxidation (abstraction of acetate units)	Shorter-chain carboxylates, eventually: acetate or formate	Acetyl-coenzyme A	Most organisms	**Fe** (bound in ironthio-clusters (ferredoxines)	Thioclusters $\mathbf{MFe_xS_{3,4}}$; identity of M does not matter much
Sugars	Glycolysis: involves phosphorylation and C–C cleavage of sugar molecules	Sugar kinases, etc.	Terminal product: pyruvate	All organisms	Mg	Cp. Phosphorylation (Zn)
Pyruvate	Decarboxylation	Pyruvate decarboxylase	Ethanal	Yeast, some vertebrates (e.g., *Carassius auratus*)	Zn	Cu
	Oxidative decarboxylation		Acetyl-coenzyme A	All organisms		
electrons	Transportation towards some reducible sink	Quinone oxidases and others	Reduced electron acceptor (e.g., water)	All organisms	**Fe** (quinone oxidases); **Fe + Cu** (cytochrome c oxidase)	**Fe + Cu**
Citrate, etc.	Redox cycle which links oligocarboxylates and involves absorption or formation of CO_2	Enzymes of tricarboxylate cycle		All organisms	Fe (aconitase), Mg (succinyl-coenzyme A synthetase), **Mn** (malate oxidoreductase)	For aconitase behaving as anisomerase: Co; **malate oxidoreductase: Mn**[b]
Benzenoid or polycyclic aromatics	Hydroxylation	Various	Phenols, hydroxi-PAHs	Most organisms	**Cu** (laccase), Fe (haem peroxidases)	**Cu**

[a]When reacting with nitrogenase, N_2 and its other substrates (nitriles, isocyanides, ethyne, etc.) get bound to Fe while "model compounds" reducing N_2 at ambient conditions may also contain Fe but rather accomplish N_2 trapping and reduction at Mo, W, V or Re sites (Schrauzer 1975; Chatt et al. 1980b; Rehder 1991).

[b]Although Cu would be a more efficient oxidation catalyst, it does also support decarboxylation much more than the lighter 3d-ions. As a result, malate would be converted into pyruvate rather than oxaloacetate, causing the entire tricarboxylate cycle to collapse. As Mn^{2+} does hardly cause decarboxylation (Pedersen 1948; Hedrick and Sallach 1961), but favors oxidation, it affords a kind of local optimum for this purpose.

Bold letters: Metalloenzymes and technical catalysts agree with respect to given reaction.

assistance by other soil-dwellers like mykorrhiza – and a sink to chemical elements which make their way through some plant before recycling by foliage littering or crackdown of the stem of some tree. This corresponds to a set of element cycles which are usually but partly closed, efficiencies and chemical details being subject to all plant cover, mineral composition of soil and succession/soil stratification during development. In fact, soil is the principal source of elements other than C (atmosphere), sometimes N and S (air also, plus rainwater in polluted areas) and some metals from dust. As we do here compare concentrations of elements in soil and plant (parts) to determine bioconcentration behaviour (BCF values), some general remarks on soil, its origins and vertical structure are due here. By mass, most of soil is composed of mineral phases, often with substantial shares of silica (sand, etc.) and clays, besides metal oxides like Fe_2O_3, Fe_3O_4, MnO_{2-x}, carbonates, phosphates, silicates, etc., with minor shares of water dissolving some salts, organic components and eventually gases (air or reducing gas phases). The organic components, much of them humic acids with phenol, carboxylate, 3-ketoenolate and other functional groups, can bind metal ions, partly leaching them from the mineral phases, partly withholding them from plant roots which produce metal ion sequestrants of their own. Their share, approximated by weight loss during aerobic heating to 500°C, usually is <10 % by mass.

Thus, soil is a chemically – and biologically – highly active multiphase system – which always must be considered as a chemical reactor linked to both plants and hydrology (e.g., springs, creeks, etc. in forests) in order to understand chemical dynamics in individual green plants and larger ecosystems alike and to model it. Moreover, it is dynamic also with time, changing its chemical features often within rather short periods of time, e.g., when a bog converts to solid land, with the assembly of plants undergoing thorough concomitant changes.

In the broad sets of data for soils in different climates and geochemical impacts, e.g., by tillage, the focus mostly rests with elements which are essential to plants which limits the chances to obtain data on fractionation or information to which extent retention of elements is simply due to complex formation in soil. As an exception, element distributions down to –4.0 m from litter level were investigated at Bornhöved (Schleswig-Holstein, northernmost part of FRG) test site (Arenic Umbrisol) also for Al and Ti which both merit particular interest for their combination of high abundance and non-essentiality. In this rather acidic soil a distinct minimum of both Al and Ti (which is considered immobile there) exists in the B horizons under forest between 60 cm and 1.7 m depth (Fränzle and Schimming 2008) while concentrations of Mg, Ca or Mn agree with those above and below except for single, sharply confined enrichments. Accordingly, when sampling plants the roots of which descend to ≥1 m beneath the soil surface external ratios of both elements which readily form carboxylate complexes (Al/Mg, Ca) and others which do not (Ti, Mn) will differ from those elsewhere. Obviously Al and Ti are taken up or otherwise removed from down there. The change in ratios corresponds to a change in plant ratios and in turn to attributions to element clusters of identical BCF used to calculate $E_L(L)_{eff}$. While Ti rarely is contained in any such cluster, although being absorbed by plants, the problem is more pronounced with Al. Regrettably there are no values for REEs from Bornhöved (either leaves or soils), keeping in mind that most of them correlate very strongly with Al abundances in plants.

Soil does vary in chemical properties among the different levels while, as a rule, plant roots on solid land penetrate just through oxic layers, except for wet stands (*Alnus*, etc.). Likewise fungi which degrade rotting wood or even lignite can do so only with dioxygen being available. Hence element takeup and partitioning between supporting solids – as a rule, soil – and plant or fungal biomasses will refer to those oxidation states and speciation forms which are stable in presence of O_2, e.g., MoO_4^{2-} rather than thiomolybdates or Mo(III). Nevertheless, reduced forms may be produced and deposited within biomass even if the latter is coupled to air or (by photosynthesis) even produces O_2 itself (e.g., magnetite forming in leaves of green plants [Fränzle et al. 2009]) or become part of bioactive speciation forms (thiomolybdates linked to pterine; Fe(II), Ni(I) in enzymes). This process removes the corresponding metals from the previous state of equilibrium. The bioconcentration factors then are influenced by such secondary reactions, as they are by membrane permeation and the kinds of ligands roots or mycelia give away to soil, to recover the metals along some part of these materials by back-resorption. Metal-processing bacteria are far more effective in metal

turnover in soils, retrieving more than 95% of the compounds delivered to soil, thus cleaving most of Fe(III) then being used as a biological oxidant. In this case, the relative N content of the sequestering agents (siderophores) considerably surpasses that of the remaining biomass (which is hardly relevant given the extent of re-absorption), in stark contrast to the situation with higher plants (≠ grasses) and fungi. In ferric reducers (microorganisms), stoichiometric ratios C/Fe are of order one (Fränzle and Noack 2008), rather than $>10^4$ in "colloquial" plants, and sequestering agents which dissolve and give access to Fe(III) [polyphenols, hydroxamates, generally siderophores] contain about 20 carbon atoms. To maintain the above C/Fe ratio, >95% of the produced siderophore Fe complexes must be really retrieved by the organism.

Among the solid metal oxides (sometimes) accumulating in B horizon as mentioned before, MnO_{2-x} is of paramount importance as a redox catalyst which both will change oxidation states, mobility, bioavailability and toxicity of many metals (Cr, Hg, Ce, V) and non-metals likewise and alter metal binding properties of soil organics by, e.g., cleaving polyphenol sites in soil aromatics (humic acids). Of course, Ru complexes of humic acids (of low molecular weight, like amino acids and other carboxylates, phenols, hydroxamates, etc.) were also investigated with respect to their redox potentials with the end to estimate complex formation constants.

Generally speaking, matter transport in soil is slow (ground water tends to move a few m/year), permitting construction of steep chemical gradients inside soil which influence all mobility, speciation and bioavailability of quite a number of chemical elements. Elements may be kept from takeup from certain soil horizons, or stick to them in vertical transport or be volatalized subsequent to hydride or alkyl formation (Wood 1975; Thayer 1995).

Under strongly reducing conditions, several transition metals may also be mobilized – from decomposing biomass or beginning with certain enzymes such as hydrogenases as precursors – as homoleptic, volatile carbonyl complexes (Mo, Ni, W, not Fe); this reaction (Feldmann 1999) thus extends beyond the small range of transition metals which directly form carbonyl complexes upon contact of the bulk or dispersed (dust, amalgam) metal with CO (Elschenbroich and Salzer 1988).

1.2 Methodology of Inquiries into the Biological System of Elements

1.2.1 *Correlation Analysis of Element Distribution in Multiple Plant Species*

Data reported by, e.g., Bowen (1979), Markert (1996) or Emsley (2001) give an idea on the common ranges of variation of concentrations of most stable (non-radioactive) elements in higher plants, concentrations of some elements are fairly constant while others vary over several orders of magnitude, without any relationship to possible functions like biocatalysis.

In addition, elementary analytics as broad-scoped as this allows for comparisons of metal concentrations beyond taxonomic borders as well as those of spatial, bioclimatic distributions. These comparisons eventually (1989) were merged into a complete set of abundance correlations among 45 chemical elements (including non-metals like B, Si, Cl or Br) for 13 plant species or parts thereof. Markert called this set of abundance correlations the "Biological System of Elements" (BSE, Fig. 1.3).

Owing to the (usually) lower pH of freshwater, avoiding hydroxide or aquoxide precipitation, there is an increase of concentrations of metals forming insoluble (hydr-)oxides with fresh water, which also bears ecochemical implications. This holds for, e.g., most transition metals. From this starting point pathways of chemical and early biological evolution (concerning uptake and usage of metals at least) can be reconstructed.

Returning to the empirical BSE patterns and the list of essential (biocatalytic metal) elements, metal ion properties sensitivity and intrinsic binding stability allow for a relationship to quantitative parameters. How, then, do essential metal ions differ with respect to complex formation stabilities with biomass and/or biological substrates and/or c and x values from those which are not essential (e.g., Al) or others which, although essential, do not directly promote reactions (e.g., Ca)? If parameter sets obtained from Eq. 2.4 can be linked to biological features such as essentiality or abundances in biological materials, the abundance correlations comprising the Biological System of Elements (Markert 1994a, 1996) could be traced back to chemical

properties of plant biomass (general or species-specific ones) *without* needing to know details on chemical methods of transport or binding of these metals. The lack of structural information would not matter so much because the number of different metal-ion-binding groups in biological matter is rather small. This is corroborated by the overview of methods used in this investigation, which are listed and discussed in the forthcoming Chapter 2.

Analogies between the Biological (BSE) and the "classical" chemical (i.e., periodic) system of elements (PSE) are partly due to the fact that similar chemical properties can (not must) bring about common enrichment in plant biomasses. Yet, the biological system of elements does not give another representation of chemical similarities identical to that contained in the PSE because biological functions (or toxicity, respectively) cause selective or even specific reactions towards supplies by certain elements, either binding them more strongly or inactivating or keeping them from the organisms altogether (e.g., by means of phytochelatins or chaperons). Notably, even though the elements get directly linked, with some of these links denoting very strong correlations in the triangular picture above, sometimes also corresponding to chemical similarities, Fig. 1.3 yet does *not* reveal something like "biological groups of elements". On one hand, there are highly correlated abundance distributions among chemically similar elements like REEs (for definition see below), but this also holds for much less similar pairs of elements (e.g., yttrium and vanadium), whereas conversely members of the same group of chemical elements sometimes may not display any statistically meaningful abundance relation to each other; cases of this are the couples P/As (r = – 0.146) or Ca/Ba (r = 0.231).

Owing to biochemical features and processes, there is additional information which distinguishes the BSE from both the PSE and from geochemical descriptions of the elements (Railsback 2003). By the above "deviations" from expectations suggested by the PSE or similar ionic radii, the Irving-Williams series, etc., the BSE in addition contains information on processes of transport and inter-metal fractionation in plants (or other organisms). For example, Irving and Williams (1953) or Sigel and McCormick (1970) point out that complex stability constants and coordination polyhedron geometries of Co^{2+} and Zn^{2+} are most similar to each other, sometimes even allowing for effective replacement of one ion (mainly Zn) by the other in catalytic properties of metalloenzymes (Vallee and Williams 1968). Yet, their abundances in the above set of 13 plant species are entirely unrelated (r = – 0.092). Thus a chemical "fingerprint" is obtained which allows for functional statements based on useful parameters of bioinorganic chemistry, which will be defined in this book lateron.

1.2.2 Fundamentals of the Correlation-Chemical Analysis of Element Abundances

It is not sufficient to investigate amounts and distributions of chemical elements when considering biological materials; an understanding in terms of chemical biology rather needs additional questions to be adressed, including those on functions of trace components (related to essentiality), whether their administration is indeed required to maintain life and fertility, eventually which amounts are required for these purposes.

Corresponding binding and transport processes in turn can be inferred from studies of and similarities with respect to abundance distributions (including BCF values and clusters thereof) both inside some organism and when comparing different species. There is one large group of ubiquitous chemical elements which perfectly match these conditions although, due to common BCF values soil/leaf of order 10^{-3}–10^{-2}, only the more abundant ones (Y, La, Ce, Pr, Nd) can be readily quantified in terrestrial (Markert 1996) or aquatic (Cowgill 1973; Weltje 2003) plants: the REEs (lanthanoides). For this discussion, REEs = La – Nd, (Pm being irrelevant for being a short-lived radioelement [$T_{1/2} \leq 18$ years]), Sm – Yb (Z = 57 – 70), including Sc and Y. As *lutetium* (Z = 71), commonly counted among the REEs, does neither make use of 4f orbital states in redox reactions [there are no Lu(IV) compounds in condensed matter] nor does so in coordination chemistry (it prefers hexacoordinate states to the higher CN values [8 – 11] common in "real" REEs, owing to an irreversibly filled $4f^{14}$ state) and correspondingly gets fractionated from other REEs concerning its correlations to abundances of e.g., Al or V (there is no abundance correlation Lu/V whatsoever, while REE/V or Y/V are highly correlated) and Lu is

enriched together with Ca – unlike La...Yb –, accordingly Lu is not counted among the REEs here.

Otherwise abundances of all essential, non-essential and toxic elements in different plant species were measured and compared (Bowen 1979; Kabata-Pendias and Pendias 1984; Markert 1996) with the latter author calling the inter-species abundance correlations derived from these analyses the Biological System of Elements (Markert 1994a). Besides the REEs and Y, abundances of yet other metals (Al, Ti, V and essential Fe) are linked to each by very highly positive correlation coefficients (Markert 1996).

The present work and book deal with identifying factors which contribute to essentiality in the above manners, trying to put these into quantitative terms if possible. There are three different sources of theoretical reasoning:

- *Stoichiometric network analysis* (SNA)
- *Quantitative* arguments from *coordination chemistry*
- Biochemical applications and implications of *Gibbs's phase rule*

which combine to yield the "*rule of three functions*".

These theoretical frameworks were selected for formal integration and "explanation", making no assumptions other than the ability of living beings to reproduce and that some chemical features of (biocatalytically) essential can be pinpointed which distinguish them from non-essential elements. "Explanation" here means reduction to some theoretical framework in quantitative terms also, that is, constructing a model which can account for the observed effects vs. exclusion of others which are not observed, e.g., non-essentiality of some other elements owing to a semi-empirical description of their chemical properties.

1.2.3 Stoichiometric Network Analysis

Going beyond comparisons of element abundances in various green plants (and possibly, or to some part, also in other organisms, trying to understand food web-based transport of elements possibly and actually controlling essentiality patterns in either participant of a trophic relationship), which may or may not be related to similarities in biochemical pathways, there are also more fundamental (and thus general) principles from chemical physics which likewise apply to living beings and the ways they (can or cannot make) use certain chemical species. The principal feature of living beings controlling their use of matter including chemical elements in metabolism – besides spatial heterogenity – is autocatalytic feedback produced by reproduction or simple cell-budding.

The principal challenge in *"explaining" the BSE* is to account for the relationship between enrichment (bioaccumulation, biomagnification) and function (if there is any). Table 1.1 (below) shows the remarkable abundance of "discrepancies" between those metal ions identified by empirical technical catalytic chemistry to promote/accomplish some transformation most effectively and those used as biocatalysts for these very reactions in biological systems even after almost four billion years of evolution; evolution processes as a rule identify local rather than global optima in the event space even though they can discriminate rapidly among $>>10^{50}$ possible configurations (Rechenberg 1973). Yet this apparently does not imply that the optimum among 30 or 35 possibly suitable and fairly abundant metal ions can be localized in the spatiotemporal frames of terrestrial biological evolution.

1.2.3.1 Biophysical Implications of Gibbs's Phase Rule

Biological systems, in general, are heterogeneous systems, heterogeneous both with respect to coexistence of liquid and solid phases and to chemical compositions of either; however, one cannot increase corresponding complexity arbitrarily without analogous increases in chemical complexity, otherwise the system (that is, the organism) would not be sustained. Because amounts or shares (e.g., for Mg in vertebrates) of metal ions used for catalytic purposes tend to be very small, an enrichment in biological materials can only be identified by correlating it to ligand properties capable of modelling biomass. Empirically, some correlation of abundances and bioconcentration/biofractionation with the electrochemical ligand parameter (of the protein molecule for this case) allows to define *effective* parameter values. These effective values apparently can be treated and used as if a single kind of substance or donor group be responsible for interaction and fractionation in an organ of an organism, and likewise for intracellular enrichment, toxic effects or such obstructing reproduction (Fränzle et al. 2005).