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Mini-Review

Aluminium cycling in the soil-plant-animal-human continuum

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Abstract

A critical review of the literature on Al toxicity in plants, animals and humans reveals a similar mode of Al action in all living organisms, namely interference with the secondary messenger system (phosphoinositide and cytosolic Ca^{2+} signalling pathways) and enhanced production of reactive oxygen species resulting in oxidative stress. Aluminium uptake by plants is relatively quick (across the intact plasma membrane in < 30 min and across the tonoplast in < 1 h), despite huge proportion of Al being bound in the cell wall. Aluminium absorption in the animal/human digestive system is low (only about 0.1% of daily Al intake stays in the human body), except when Al is complexed with organic ligands (eg. citrate, tartarate, glutamate). Aluminium accumulates in bones and brain, with Al-citrate and Al-transferrin complexes crossing the blood-brain barrier and accumulating in brain cells. Tea plant and other Al-accumulator plant species contain large amounts of Al in the form of non-toxic organic complexes.

Introduction

Aluminium is the most abundant metal and the third most abundant chemical element in the Earth crust. Most of Al is incorporated into aluminosilicate soil minerals, with only small quantities appearing in soluble forms that can influence living organisms (May & Nordstrom 1991). However, solubilisation of Al-containing minerals is enhanced in acidic environments.

Most soils are being acidified continuously due to (i) imbalances in nitrogen, sulphur and carbon cycles (Bolan & Hedley 2003; Tang & Rengel 2003), (ii) excess uptake of cations over anions (Tang & Rengel 2003), (iii) use of ammonia- and amide-containing fertilizers (Mahler *et al.* 1985), (iv) nitrogen-fixation by legumes (Bolan *et al.* 1991; Coventry & Slattery 1991; Tang & Rengel 2003), and (v) acidic depositions from the atmosphere (Alewell 2003).

Acidic atmospheric depositions (mainly sulphuric and nitric acids) are caused by emissions into atmosphere of sulphur dioxide, nitrogen oxides and ammonia. These acids lower pH, increase Al concentration in surface waters (Bi 2001), decrease biodiversity in aquatic ecosystems (Driscoll *et al.* 2003), and enhance acidification of sensitive soils (Blaser *et al.* 1999; Herman *et al.* 2001; Jönsson *et al.* 2003), especially in forests because of a filtering effect of tall vegetation (Fowler *et al.* 1999). However, decreased atmospheric emissions in recent decades in Europe (Alewell 2001; Herman *et al.* 2001) and the USA (Gbondo & Driscoll 2002) have resulted in partial, albeit slow, recovery of aquatic and terrestrial ecosystems (Herman *et al.* 2001). Part of the reason for slow recovery is a 'memory effect' of high acidification load from the earlier part of 20th century (1965-1985) (Blaser *et al.* 1999). In addition, soil acidification is still continuing, at least at some locations in Europe (Jönsson *et al.* 2003).

Increasing Al concentration in surface waters due to acidification (Bi 2001) resulted in Al toxicity to fish (Roux *et al.* 1996) and other aquatic organisms (Witters 1998), like benthic invertebrates (Herrmann 2001) when toxic thresholds were exceeded. In soil, Al is toxic to earthworms at acidic pH (Phillips & Bolger 1998) as well as to a range of other soil animals (Markich *et al.* 2002). In terrestrial ecosystems, Al toxicity to plants in acid soils is a major factor limiting

crop production and yield in acid soils worldwide (Foy 1988).

Despite decades of extensive effort to decipher the mechanism(s) of Al toxicity to plants, the primary cause remains largely speculative (Rengel 1992a, 1996; Horst 1995; Matsumoto 2000; Rengel & Zhang 2003). Similarly, there is continuous controversy regarding modes of Al toxicity to animals as well as possible involvement of Al in the Alzheimer's disease and other neurodegenerative diseases in humans (eg. Candy *et al.* 1992; Kruck 1993; Armstrong *et al.* 1996; Savory *et al.* 1996; Makjanic *et al.* 1998; Yokel 2000; Flaten 2001; Campbell 2002). Regardless of the type of living organism, there are similar main reasons for these controversies (i) technical difficulties associated with determining ionic speciation of Al in a given medium, (ii) problems with detecting transport of Al across the plasma membrane of intact cells, and (iii) ascertaining the intracellular localisation of Al.

In this review, I will highlight recent progress in understanding the transport of Al from soil to plants to humans and will critically examine implications of Al location in cells and organelles in Al toxicity. For other aspects of Al toxicity and resistance, readers interested in plants are referred to Kochian (1995), Rengel (1996), Matsumoto (2000), Barcelo & Poschenrieder (2002) and Rengel & Zhang (2003). Aluminium toxicity in animals has been reviewed by Appelberg *et al.* (1992), Battarbee (1994), Nayak & Chatterjee (1999) and Crichton *et al.* (2002), whereas the role of Al as a human neurotoxin has been discussed by Armstrong *et al.* (1996), Srinivasan *et al.* (1999), Yokel (2000), Flaten (2001), Yokel & McNamara (2001), Campbell (2002) and Rondeau (2002).

Symptoms of aluminium toxicity to living organisms

Despite substantial differences in some aspects of Al toxicity in various living organisms, there are certain common points (Strong *et al.* 1996; Yokel 2000). In plants, animals and humans, Al interferes with basic cellular functions, like the phosphoinositide and intracellular Ca^{2+} signalling pathways, which are, as secondary messengers, involved in a myriad of cellular metabolic functions.

In murine strain mice (Haug *et al.* 1994), rat hippocampal slices (Breakwell & Publicover 1994) as well as human neuroblastoma cells (Wood *et al.* 1994), Al exposure disturbs the phosphoinositide signalling pathway by acting on guanine nucleotide-binding proteins (G-proteins) and a phosphatidylinositol-4,5-diphosphate specific phospholipase C. In addition, Al decreases the accumulation of inositol phosphates [especially inositol-1,4,5-triphosphate (IP_3)] and, by changing the membrane phospholipid composition, can also affect the phospholipase C in murine neuroblastoma cells (Shi & Haug 1992; Shi *et al.* 1993) and wheat root cells (Jones & Kochian 1995).

In murine (Shi & Haug 1992; Haug *et al.* 1994) as well as human neuroblastoma cells (Wood *et al.* 1994) Al disturbs intracellular Ca^{2+} homeostasis and diminishes Ca^{2+} rises, thus affecting the action of cytosolic Ca^{2+} activity ($[Ca^{2+}]_{cyt}$) as a secondary messenger. In plant cells, all but one report published so far demonstrated an increase in $[Ca^{2+}]_{cyt}$ as a consequence of Al toxicity (Rengel & Zhang 2003), eg. in intact wheat (Zhang *et al.* 1998; Zhang & Rengel 1999) and rye roots (Ma *et al.* 2002), excised barley roots (Nichol & Oliveira 1995), Arabidopsis root hairs (Jones *et al.* 1998a), tobacco suspension cells (Vitorello & Haug 1996) and protoplasts isolated from wheat roots (Lindberg & Strid 1997). On the other hand, Jones *et al.* (1998b) reported a rapid decrease in $[Ca^{2+}]_{cyt}$ in BY-2 tobacco cell culture upon exposure to Al. Hence, all plant experimental system tested so far responded to the Al toxicity stress by *altering* the cytosolic Ca^{2+} homeostasis (as predicted by Rengel 1992b).

The other common feature of Al toxicity in plant and animal/human cells is increased production of reactive oxygen species (ROS) resulting in the oxidative stress. In mammalian cells Al can potentiate Fe-induced oxidative stress through increased production of ROS (Crichton *et al.* 2002), which have been implicated in neurological disorders (Strong *et al.* 1996; Campbell 2002). In plants, Al also causes increased production of ROS (Yamamoto *et al.* 2002) as well as lipid peroxidation (Yamamoto *et al.* 2003), with the former being a potential cause of root growth inhibition upon exposure to Al (Yamamoto *et al.* 2003).

Plants

Inhibition of root growth is one of the earliest and most dramatic symptoms exhibited by plants suffering from Al stress; this symptom has been observed within minutes of exposure to micromolar concentrations of Al in solution cultures (Blancaflor *et al.* 1998; Sivaguru & Horst 1998; Ryan *et al.* 1993; Vazquez *et al.* 1999; Zhang *et al.* 1998, 1999; Ahn *et al.* 2001;

Ma *et al.* 2002). The root apex is a critical site of perception and expression of Al toxicity. Inhibition of root growth occurs only after the distal part of the elongation zone within the apex (Sivaguru & Horst 1998; Kollmeier *et al.* 2000) was exposed to Al.

Root growth is a complex and dynamic process, making it likely that a number of biochemical and physiological processes may have already been altered prior to Al-induced inhibition of root growth. Among these processes, the important ones are disturbance of cytoplasmic Ca^{2+} and pH homeostasis (for a recent review see Rengel & Zhang 2003), inhibition of the H^+-ATPase activity in the plasma membrane of the root tip cells (Ahn *et al.* 2002, 2004; Rengel & Zhang 2003), exacerbated oxidative stress (Yamamoto *et al.* 2002, 2003), and disruption of cytoskeleton dynamics (Sivaguru *et al.* 1999; Schwarzerova *et al.* 2002). These changes may directly or indirectly underlie the observed Al toxicity symptoms on plants. Indeed, neutralisation of the membrane surface charge in the root tips of Al-sensitive wheat genotype (due to a decrease in H^+-ATPase activity and H^+ transport, Ahn *et al.* 2004) was accompanied by inhibited root growth (Zhang *et al.* 1999), suggesting a link between growth inhibition and alteration of the plasma membrane properties by Al exposure.

Animals and humans

An excellent compilation of metal toxicity for many aquatic and soil animals has recently been published (Markich *et al.* 2002), identifying large differences among animal species in sensitivity to Al toxicity. For example, Al was toxic to benthic invertebrates when inorganic Al concentration reached 0.1-0.3 mg L^{-1}, but large differences in the severity of toxic effects were noted among species (Roux *et al.* 1996).

Different symptoms of Al toxicity were found in different animal species. In freshwater mussels, the filtering action was severely impaired by a short-term (1 h) as well as long-term (15 days) exposure to Al at neutral pH (Kadar *et al.* 2002). In pond snail *Lymnaea stagnalis*, Al altered voltage-activated sodium currents in neurons (Csoti *et al.* 2001), suggesting interference with electrophysiology of ion channels in the plasma membrane. In earthworms, cocoon production was inhibited by Al toxicity (Phillips & Bolger 1998).

Aluminium can be toxic to humans at both environmental and therapeutic levels. The human exposure to Al is primarily dietary, even though occupational (industrial) exposure (eg. Polizzi *et al.* 2002) and medically administered Al (including medications like antacids, phosphate-complexation treatments like Al trihydroxide, Al-contaminated dialysis fluids, etc.) can represent substantial proportion of Al intake (Yokel & McNamara 2001).

The association between Al and dialysis encephalopathy is well established (eg. see Van Landeghem *et al.* 1997). Administration of Al-containing phosphate-complexation treatment to dialysis patients can cause acute toxicity (Desroches *et al.* 2000) and may be the major factor in renal osteodystrophy (Vanholder *et al.* 2002) and Al accumulation in brain (Candy *et al.* 1992). Also, acute Al toxicity of iatrogenic origin is well documented (Desroches *et al.* 1999).

Aluminium is associated with the inflammatory responses (Platt *et al.* 2001), mediated by interleukins and other inflammatory cytokines (Campbell 2002). In addition, Al increases Fe-induced oxidative injury in brain tissues (Yokel 2000; Campbell 2002; Crichton *et al.* 2002), resulting in lipid peroxidation and cytotoxicity of free-oxygen radicals (Strong *et al.* 1996). Aluminium affects brain neurotransmitters by decreasing the release and subsequent breakdown of 5-hydroxytrytamine (Kumar 2002), by binding to acetylcholine (Matlaba *et al.* 2000) and by increasing acetylcholinesterase activity in brain (Zatta *et al.* 2002).

The neuronal response to Al exposure can be morphologically distinguished into two types: (i) formation of intraneuronal neurofilamentous aggregates, or (ii) induction of significant neurochemical and neurophysiological perturbations without formation of neurofilamentous aggregates (Strong *et al.* 1996).

The neurofibrillary tangles (first described by Alzheimer in 1907) and the senile plaques represent the two principle neuropathologic lesions identified in the brains of patients with Alzheimer's disease. Aluminium contributes to accumulation of insoluble amyloid beta protein (causing senile plaques, Armstrong *et al.* 1996) and hyperphosphorylated tau in brain tissue (leading to formation of neurofibrillary tangles and neuropil threads, Armstrong *et al.* 1996) and to some extent mimics the deficit of cortical cholinergic neurotransmitters (Matlaba *et al.* 2000; Platt *et al.* 2001), all of which are characteristics of Alzheimer's disease (Yokel 2000). However, the Al involvement in Alzheimer's disease and other neurological disorders has remained controversial over the years (eg. Candy *et al.* 1992; Kruck 1993; Armstrong *et al.* 1996; Savory *et al.* 1996; Makjanic

et al. 1998; Yokel 2000; Flaten 2001; Campbell 2002). Epidemiological studies could not provide a conclusive evidence of the causality of the frequently obvious connection between Al and Alzheimer's disease, whereas appropriate physiological studies (eg. employing weakly radioactive ^{26}Al isotope and accelerated mass spectrometry detection, see below) of Al effects under physiological conditions have only recently become possible (eg. Moore *et al.* 2000; Ward *et al.* 2001).

Aluminium speciation

Aluminium makes complexes with numerous ligands, especially oxygen-donor ones. Many of these complexes also tend to hydrolyse and form polymeric species in aqueous solution, resulting in complex coordination chemistry of Al in the environmental samples and biofluids. In addition, complexation constants for Al and common ligands (like citrate, phosphate and hydroxide) are uncertain at best (Harris *et al.* 1996), making reliable Al speciation quite a challenge. Speciation of Al is further hampered by technical difficulties in separating and reliably quantifying various Al species. The time factor also needs to be considered because polynuclear Al species can be depolymerised by organic ligands into uncondensed monomeric Al species over time (Masion *et al.* 2000).

Large differences in toxicity of various Al species (from none to extreme) to living organisms make speciation of Al a necessity. Special attention needs to be paid to bioavailable Al species because they can cause toxicity. All ecotoxicology research should therefore move from expressing total concentrations of Al and other metals to assessing the concentrations of relevant bioavailable metal species (eg. see Witters 1998).

Analytical techniques for determining aluminium speciation in environmental samples and biofluids

Determining Al speciation is a two-step process, comprising separation and quantification. Numerous pitfalls exist along this analytical path, eg. (i) extraneous metal contamination, (ii) unrecognized aspecific binding of metals to proteins, (iii) unwanted interactions with separation equipment such as chromatography columns and ultrafiltration membranes (Van Landeghem *et al.* 1998a), and (iv) interference problems during quantification.

Hybrid techniques (for a review see Sanz 1998b) combine the capacity to separate ionic species with specific analytical capacity to quantify them. Attempts to avoid the separation step and rely only on the capacity of the ion spray and triple quadrupole-ICP-MS to reliably quantify different Al species present together in a single sample were not successful (Sharp *et al.* 1997).

Separation techniques

Ion chromatography using size exclusion (either by gel filtration or by HPLC, Rollin & Nogueira 1997) and cation exchange column can be useful in separating Al complexes commonly occurring in the environment (eg. fluo, oxalate, citrate) (Kerven *et al.* 1995b; Borrmann & Seubert 1996, Hils *et al.* 1999; Mitrovic & Milacic 2000; Tsunoda *et al.* 2001) and the blood serum (Rollin & Nogueira 1997; Van Landeghem *et al.* 1998b). The size exclusion is especially suitable for separating organic Al complexes (Rollin & Nogueira 1997; Hils *et al.* 1999). Keeping the temperature of a size exclusion/cation exchange column below 10 °C can minimize decomposition of neutral or negatively charged Al-fluoride and Al-citrate complexes (Busch & Seubert 1999).

Anion chromatography using a gradient elution based on chloride start eluent and perchlorate as final eluent can effectively separate neutral or cationic from negatively charged Al complexes (Borrmann & Seubert 1999). A combination of anion and cation chromatography for more complete separation of Al species is recommended (Borrmann & Seubert 1999). An anion-exchange column and fast protein liquid chromatography (FPLC) can be used to separate low-molecular-weight complexes with Al in the serum (Polak *et al.* 2001a) or to separate negatively charged Al-citrate complexes from neutral Al-citrate species and Al^{3+} in solution (Bantan *et al.* 1998). Hydrolysable polyphenol-bound Al complexes can be separated using Amberlite XAD-7 resin, whereas Chelex 100 can be used for separation of cationic Al species in tea infusions (Erdemoglu *et al.* 2000a).

Quantification techniques

After separation of Al species, quantification can be achieved using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Bantan *et al.* 1998; Berube & Brule 1999), ICP-Mass Spectrometry (ICP-MS) (Sanz 1998a; Berube & Brule 1999; Hils *et al.* 1999; Tsunoda *et al.* 2001), Graphite Furnace Atomic Absorption Spectrometry (GFAAS) (Sanz 1998a; Berube & Brule 1999), Zeeman AAS

(Rollin & Nogueira 1997) or Electrothermal AAS (ET-AAS) (Van Landeghem *et al.* 1997; Mitrovic *et al.* 1998; Van Landeghem *et al.* 1998b; Mitrovic & Milacic 2000; Polak *et al.* 2001a, b). Electrospray tandem Mass Spectrometry (ES-MS-MS) can also be used to detect low-molecular-weight Al complexes (eg. Al-phosphate, Al-citrate, and ternary Al-citrate-phosphate complexes, Polak *et al.* 2001a), or to reliably quantify Al-malate and Al-citrate in the same sample (Polak *et al.* 2001b). Flame atomic absorption spectrometry was also used occasionally (Erdemoglu *et al.* 2000a, b), even though this technique is less sensitive to Al than other atomic spectroscopy techniques (Rengel 1996).

The techniques like ICP-MS, quadrupole-ICP-MS and double focusing ICP-MS (DF-ICP-MS) offer a significant increase in sensitivity to Al over ICP-AES. In addition, DF-ICP-MS is four times more sensitive than quadrupole-ICP-MS in detecting Al in biofluids (Marchante *et al.* 1999). Using DF-ICP-MS, detectable levels of Al were found in the blood serum from uraemic patients, but not in healthy subjects (Bayon *et al.* 1999).

Other techniques used for quantification of Al species in a range of environmental samples and biofluids are nuclear magnetic resonance (^{27}Al NMR) spectroscopy (Kerven *et al.* 1995a; Masion *et al.* 2000; Wu & Wang 2000; Perry & Shafran 2001; Song *et al.* 2003) and various electrochemical methods. More prominent electrochemical methods are oscillopolarography for labile monomeric Al species (Bi *et al.* 1999), amperometry for 'free Al' [$Al^{3+} + Al(OH)^{2+}$] (Downard *et al.* 1997), automated potentiometry for hydrolysis of Al ions in solutions (Perry & Shafran 2001), derivative adsorption chronopotentiometry on a hanging mercury drop electrode for various Al fractions, especially monomeric Al (Wang *et al.* 2001a), and adsorption stripping voltammetry for monomeric Al in water (Wang *et al.* 2001b) and for complexes between Al and acetylcholine *in situ* (Matlaba *et al.* 2000). For further information on electrochemical as well as atomic spectroscopy techniques used to determine Al species after the separation step, readers are referred to recent reviews (Das & Chakraborty 1997; Sanz 1998b; Van Landeghem *et al.* 1998a; Bi *et al.* 2001b).

Post separation-column reaction with Tiron (Borrmann & Seubert 1996; Busch & Seubert 1999) or pyrocatechol violet (Flaten & Lund 1997) followed by UV photometry is frequently employed. Pyrocatechol violet can also determine Al_{13} complex (Hils *et al.* 1999) that is extremely toxic to plants (Parker *et al.* 1989; Kinraide 1997).

Reactive Al in water is often determined using 8-hydroxyquinoline method followed by 15-s extraction of chelated forms into an organic solvent (Dixon & Gardner 1998). Keeping reaction times with 8-hydroxyquinoline short (3 s) allows separation of reactive Al from the non-toxic AlF^{2+} complexes (Fairman & Sanz 1996), even though the success may not be guaranteed (Simpson *et al.* 1997). Flow-injection analysis can be critical in ensuring precise control of the reaction time, which is important for determining Al species in equilibrium systems (Simpson *et al.* 1997; Pyrzynska *et al.* 2000). Using 8-hydroxyquinoline-5-sulphonic acid in the post column mix with the separation buffer after separation of cations via capillary electrophoresis can result in increased sensitivity, especially for studying Al-F complexes (Zhu & Kok 1998). However, the 8-hydroxyquinoline method (and its variants), as well as some other chromogenic and fluorometric analytical techniques, eg. ferron (Jallah & Smyth 1998), pyrocatechol violet (Flaten & Lund 1997; Hils *et al.* 1999), chrome-azurol S (Simpson *et al.* 1997) or morin (Lian *et al.* 2003), can only be useful in kinetic reaction studies to operationally define Al fractions, but actual speciation within these fractions is frequently uncertain. Even when such separation of Al species was achieved (Tsunoda *et al.* 2001), the sensitivity of ICP-MS as a quantification technique was better than that of the 8-hydroxyquinoline-5-sulphonic acid method.

Computation of aluminium speciation

In addition to a range of analytical techniques for separation and quantification of Al species, researchers resorted to using computer programs, like MINEQL (e.g. Bi 2001; Bi *et al.* 2001a) and GEOCHEM (e.g. Kerven *et al.* 1995a; Stevens *et al.* 1997; Jallah & Smyth 1998; Naumann & Horst 2003), to calculate the activities of individual Al species in aqueous systems and biofluids based on an assumed equilibrium condition and on the set of thermodynamic constants for the reactions under study (Parker *et al.* 1995). However, uncertainties associated with the thermodynamic constants (Harris *et al.* 1996) make computational speciation of complex, Al-containing aqueous solutions imprecise, yielding only rough estimates of activities of Al species.

Aluminium species in environmental samples and biofluids

Toxicity of aluminium species

Bioavailability and thus toxicity of various Al species to living organisms is different. Aluminate ion $Al(OH)_4^-$ is non-toxic to fish (Poleo & Hytterod 2003) and plants (Kinraide 1990), even though relatively high aluminate concentration at pH > 9.0 can be toxic to wheat (Ma *et al.* 2003). Aluminium trihydroxide is non-toxic to plants (e.g. Yoshimura *et al.* 2003) and humans (it is used frequently as a phosphate-complexing agent, e.g. Van Landeghem *et al.* 1998b). The monomeric Al species $Al(OH)^{2+}$ and $Al(OH)_2^+$ are non-toxic to plants (Kinraide 1997), but no definite information could be found for animal/human cells (even though these Al species were included in the sum of Al species presumed toxic to aquatic organisms, Bi 2001). Aluminium-phosphate is non-toxic to plant (Takita *et al.* 1999) and animal/human cells (because it is present in serum of healthy individuals, Polak *et al.* 2001a). Aluminium complexes with sulphate are harmless to plants (Kinraide 1997) and presumably also to animals and humans.

Aluminium complexes with organic acids are non-toxic to plants (see Matsumoto 2000). However, in animals and humans, Al-citrate complex is present in substantial amounts in serum of healthy individuals (Bantan *et al.* 1999b; Polak *et al.* 2001a), but is also (i) the main Al complex in the cerebrospinal fluid of acutely Al-intoxicated dialysis patients (Van Landeghem *et al.* 1997), and (ii) one of the two major forms of Al taken up across the plasma membrane of brain cells (Yokel *et al.* 2002). Hence, Al-citrate in animal/human cells may not be toxic itself, but may represent a vehicle for delivering Al to sensitive sites, whereby upon changes in Al speciation, toxicity may occur. Among other Al-organic complexes, lipophilic ones (like Al-acetylacetonate and Al-maltolate) are cytotoxic to murine neuroblastoma cells (Zatta & Zambenedetti 1996), Al-maltolate is toxic to neuronal and astroglial cells (Levesque *et al.* 2000), and Al-lactate causes toxicity in mice brain by affecting acetylcholine metabolism (Zatta *et al.* 2002). In contrast, binding of Al to organic ligands eliminates Al toxicity to aquatic organisms (Bi *et al.* 2001a).

Regarding Al-F complexes, early reports indicated their non-toxicity to plants (Cameron *et al.* 1986; MacLean *et al.* 1992), but recent work suggested increasing toxicity of Al-F complexes with their increasing positive charge (Kinraide 1997; Stevens *et al.* 1997, 1998). The Al-F complexes are non-toxic to fish and other aquatic organisms (Bi *et al.* 2001a). In human cells, the Al-F complex resulted in the lowest Al accumulation and had the lowest toxic effect on viability of neuronal and astroglial cells compared to the other three Al complexes studied (maltolate, lactate and chloride) (Levesque *et al.* 2000).

It remains to be established whether non-toxicity of certain Al species and complexes is related to (i) a lack of uptake across the plasma membrane, or (ii) decreased (or an absence of) the positive charge (eg. plant toxicity of ions generally increases with positive charge, Kinraide 1997). Indeed, polynuclear hydroxy-Al complexes with a high positive charge (eg. Al_{13}, $AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$, Parker & Bertsch 1992) are more toxic to plants than monomeric, less charged species (Comin *et al.* 1999). The Al_{13} species is also toxic to bacteria (Amonette *et al.* 2003), but no information could be found on its potential toxicity to animal/human cells.

Aluminium speciation in water

Aluminium in water is mainly bound to the colloidal matter (55%) compared to the soluble fraction (23%), with the rest being in a particulate form (Gundersen & Steinnes 2003). However, depending on the source of raw water (water destined for drinking, but before treatment), up to 80% of Al can be in the particulate form (Schintu *et al.* 2000). After water treatment (coagulation, flocculation and filtration, which results in 'finished water' that is used for drinking) most Al (between 40 and 62%) was in the monomeric form (Schintu *et al.* 2000).

Polyaluminium chloride or aluminium sulphate is regularly used in treatment of water for drinking. New coagulant has been produced recently by combining polyaluminium chloride and polysilicic acid (polyaluminium silicate chloride). It has an increased coagulation capacity and results in a lower proportion of Al_{13} species compared with polyaluminium chloride (Song *et al.* 2003), and may therefore be better to use.

The regular drinking water may contain substantial amounts of Al (average about 70 μg L^{-1}). However, bioavailability of Al from drinking water is estimated to be low, with only 0.3% of the total Al amount ingested in water being absorbed into the body (Yokel & McNamara 2001). This appears to be a small percentage given potentially high proportion of bioavailable monomeric Al forms in drinking water (eg. up to

62%, Schintu *et al.* 2000). However, even with a high bioavailability of Al in drinking water, Al absorption from water may still be a small proportion of the total amount of Al ingested. For example, regular antacid users ingest gram amounts of Al daily, which may be three to four orders of magnitude greater than ingestion of Al from drinking water (see Flaten 2001; Yokel & McNamara 2001).

Out of a range of Al species in drinking water in the Quebec study [total Al, total dissolved Al, monomeric organic Al, monomeric inorganic Al, polymeric Al, Al^{3+}, $Al(OH)^{2+}$, AlF^{2+}, $AlH_3SiO_4^{2+}$, $Al(SO_4)^{+}$], a significant relationship was found between the long-term exposure of subjects to monomeric organic Al and Alzheimer's disease (Gauthier *et al.* 2000). Many other studies (nine out of thirteen studies considered) also found a positive relationship between Al in drinking water and Alzheimer's disease (for a review see Flaten 2001).

Aluminium speciation in plants

In the plant sap of *Sempervivum tectorum* and *Sansevieria trifasciata*, Al-citrate and Al-aconitate were the only low-molecular-weight Al complexes detected (Bantan *et al.* 1999a). In contrast, type of Al species supplied in the root medium influenced the Al form transported in the xylem sap of Al-tolerant Chinese cabbage (*Brassica rapa pekinensis*). When Al^{3+} was the predominant Al form in the nutrient solution, Al complexation to malate occurred in the root tissue, and Al-malate was the dominant form in the xylem sap (70%), with Al^{3+} being the other fraction (30%), whereas supplying Al to the solution as Al-citrate or Al-malate resulted in these same forms being exclusively present in the xylem sap (Polak *et al.* 2001b).

Aluminium speciation in food

Aluminium speciation in food has been reported relatively rarely (for a review see Marchante *et al.* 1999), even though a need for more research of that type has been emphasised. Clearly, the variety of foods and a range of Al-complexing ligands represent a challenge in trying to speciate Al in realistic food mixtures. Speciation of Al in simulated food solutions (pH 4–8) suggested that most Al is present in the organic complexes, most of which have relatively high bioavailability and thus potential toxicity to humans (Bi 1996). Most work on Al species in food solutions was done on dietary organic acids (Venturini & Berthon 1998, 2001; Desroches *et al.* 1999; Desroches *et al.* 2000; Dayde *et al.* 2003); see the section on Al uptake by animal and humans.

Cooking and preparing food in Al-containing containers result in migration of Al into the food, especially in acidic and salty environments (Gramiccioni *et al.* 1996). Al-complexing agents (e.g. organic acids, fluoride, OH^{-}, etc.) can also increase release of Al from Al-containing cooking utensils (Bi 1996).

Aluminium speciation in human and animal biofluids

The main low-molecular-weight Al complexes in blood serum of healthy human subjects were Al-citrate, Al-phosphate and ternary Al-citrate-phosphate complexes (Bantan *et al.* 1999b; Polak *et al.* 2001a). The relative ratio of these complexes varied between subjects tested, but no specific difference was found between patiens on continuous ambulatory peritoneal dialysis and healthy subjects (Polak *et al.* 2001a).

In serum, Al is mainly transported in the complex with high-molecular-weight Fe-transporting protein transferrin (Harris *et al.* 1996), whereas albumin (Harris *et al.* 1996; Rollin & Nogueira 1997) and ferritin (Ward *et al.* 2001) do not appear to have a role in Al binding in serum. Increased Fe load in humans may decrease Al binding to transferrin; this effect is not due to interference by Fe in Al absorption in the gastrointestinal tract, but rather due to direct Fe competition with Al for binding to transferrin in blood (Van Landeghem *et al.* 1998b). Hence, a negative correlation (albeit relatively weak) was found between transferrin saturation with Fe and Al levels in serum (Van Landeghem *et al.* 1998b).

Occupational exposure to Al can increase the proportion of Al bound to high-molecular-weight complexes (largely transferrin) compared to Al bound in low-molecular weight complexes in serum (Rollin & Nogueira 1997). Microultrafiltration through a membrane filter (e.g. with a cut-off point of 30 kDa) can be used to effectively separate the transferrin-type high-molecular-weight Al complexes from low-molecular-weight ones in serum (Polak *et al.* 2001a). However, even a small proportion of Al bound to low-molecular-weight complexes at high total Al concentration caused by occupational exposure means that relatively high amounts of low-molecular-weight Al complexes are present in serum of such patients compared with healthy individuals. High content of low-molecular-weight Al complexes in serum sug-

gests high toxicity because of ease by which these complexes can be transferred into cells (Rollin & Nogueira 1997).

The main form of Al in the cerebrospinal fluid of acutely Al-intoxicated dialysis patients is Al-citrate/silicate (Van Landeghem *et al.* 1997), without any Al-transferrin complexes detected. This result is in contrast to Al-transferrin complex being the important form of Al uptake into brain cells across the blood-brain barrier (Van Landeghem *et al.* 1998a; Yokel 2000, Yokel & McNamara 2001).

Analytical techniques for measuring aluminium in cells and tissues

Analytical techniques for quantifying Al in cells and tissues can be divided into two groups (i) for measuring Al *in situ* in tissue sections and individual cells, and (ii) for determining total Al content in cells, tissues and organs.

Important *in situ* techniques used for measuring Al accumulation in plant and animal/human tissues, cells and cell compartments are: energy-dispersive X-ray analysis (EDXMA, see e.g. Peri & Good 1990; Memon *et al.* 1981; Marienfeld & Stelzer 1993; Ownby 1993; Marienfeld *et al.* 1995; Echlin 1996), particle induced X-ray emission (PIXE, Watt 1996; Schofield *et al.* 1998; Irigaray *et al.* 2000; Clark *et al.* 2001), secondary ion mass spectrometry (SIMS, Candy *et al.* 1992; Chaussidon *et al.* 1993; Lazof *et al.* 1994; Jones *et al.* 1997; Mangabeira *et al.* 1999; Florent *et al.* 2001; Kishikawa *et al.* 2003) and laser microprobe mass analysis (LAMMA, Peri & Good 1990; Kuhn *et al.* 1995; Iancu *et al.* 1996; Reusche *et al.* 1999; Marienfeld *et al.* 2000). Sometimes a combination of techniques is used for a maximum effect, eg. to determine Al in neuritic plaques in Alzheimer's patients, PIXE (for trace element concentrations) was combined with Scanning Transmission Ion Microscopy (for imaging features in the tissue), and Rutherford Backscattering Spectrometry (to characterize the tissue matrix) (Watt 1996).

Techniques for measuring total Al in plant and animal specimens are: Graphite Furnace Atomic Absorption Spectroscopy (GFAAS, Zhang & Taylor 1989, 1990; McDonald-Stephens & Taylor 1995; Archambault *et al.* 1996; Vitorello & Haug 1996; Ranau *et al.* 1999; Taylor *et al.* 2000; Baffi *et al.* 2002), Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Asano *et al.* 2002; Baffi *et al.* 2002; Rahil *et al.* 2002; Chan & Lo 2003; Juranovic *et al.* 2003; Lesniewicz & Zyrnicki 2003; Piczak *et al.* 2003), ICP-Mass Spectrometry (ICP-MS) (Baffi *et al.* 2002; Nystrom *et al.* 2002; Adamo *et al.* 2003; Chan & Lo 2003; Noel *et al.* 2003); colourimetric and fluorometric techniques (Reid *et al.* 1996; Vitorello & Haug 1996, 1997; Yoshimura *et al.* 2003), ion chromatography (Rincón & Gonzales 1992), and ^{27}Al NMR (Nagata *et al.* 1992; Ma *et al.* 1997; Watanabe *et al.* 1998; Xia & Rayson 1998; Shen *et al.* 2002; Yoshimura *et al.* 2003).

Techniques for measurement of aluminium in situ

The theoretical principles of the *in situ* analytical techniques are beyond the scope of the present review; readers can find more information in Rengel (1996). Briefly, all these techniques rely on bombardment of the specimen with a beam of either ions (SIMS and PIXE), electrons (EDXMA) or photons (LAMMA), causing either ejection of surface atoms/ions that are analysed by mass spectrometry (SIMS and LAMMA) or knocking out of some shell electrons that give rise to element-specific primary X-rays (EDXMA and PIXE). The EDXMA and SIMS are superior to the other two and will be considered further.

Potential advantages of SIMS are high elemental sensitivity, 3-D analysis and capacity to provide isotopic information, whereas a disadvantage is non-uniform ion ejection (sputtering) that may cause different cell constituents to sputter at different rates (Linton *et al.* 1980), resulting in lateral resolution of SIMS being insufficient for identification of subcellular structures. However, Lazof *et al.* (1994) estimated that lateral resolution for SIMS was in the order of 2 μm (as for comparison, the cell wall of epidermal root cells might be several micrometers thick, Marschner 1995). Other methods used to study concentration of Al *in situ* can potentially have better spatial resolution (down to 0.1 μm for EDXMA, Linton *et al.* 1980) but, due to limitation of sample preparations and the tear-drop shape of the primary electron beam penetrating biological samples, the minimal limit of lateral resolution for EDXMA may be considerably greater, possibly exceeding even the diameter of the individual cell (see discussion in Lazof *et al.* 1994, 1997). In contrast, Marienfeld & Stelzer (1993) suggested that the spatial resolution of EDXMA is sufficient for distinguishing between cytoplasm and the nucleus, but that small vesicles in meristematic and

differentiating cells may interfere with measurements of the cytoplasmic mineral content.

Assuming no spectral interferences, a detection limit for Al of around 1 μg g^{-1} (37 nmol g^{-1}) can be achieved by SIMS (Lazof *et al.* 1994, 1997), which is at least one (Linton *et al.* 1980) or even two orders of magnitude lower than for EDXMA (Marienfeld & Stelzer 1993).

There are several recent advancements in the traditional SIMS technique. Resonance Ionisation Mass Spectrometry (RIMS) is superior to SIMS in Al quantification because RIMS alleviates the isobaric interference problems with compounds like CNH (Jones *et al.* 1997). Time-of-Flight SIMS is also more sensitive than traditional SIMS and is expected to be important in quantifying Al concentrations in biological specimens (Kishikawa *et al.* 2003).

The new nanoSIMS technology, currently used in the author's laboratory to determine Al transport across the wheat root-cell plasma membrane, is considerably better (lateral resolution of 50 nm and sensitivity about 0.1 μg Al g^{-1} tissue) than SIMS. This lateral resolution allows distinguishing Al accumulated in the cell wall from Al that has crossed the plasma membrane and is situated in the cytosol or in subcellular compartments.

Localisation of aluminium in animal and human cells and tissues in situ

The dynamic SIMS technique was used to detect Al accumulation in cortical pyramidal neurons of dialysis patients (without Alzheimer's diagnosis) who were given large doses of Al-containing phosphate-binding agents (Candy *et al.* 1992). Electrothermal atomic absorption spectroscopy revealed increased Al content in brains of Alzheimer's patients compared to controls (Kruck 1993). Aluminium (as indicated by the fluorescent morin staining) was concentrated in white matter of the medial striatum, corpus callosum, and cingulate bundle (Platt *et al.* 2001) (see also Senitz & Bluethner 1990). The nuclear microscopy technique, however, failed to detect Al in pyramidal neurons of Alzheimer's disease patients (Makjanic *et al.* 1998), leading authors to suggest that the previous findings of Al in neurofibrillary tangles were caused by contamination or elemental redistribution during preparation of tissue for analysis.

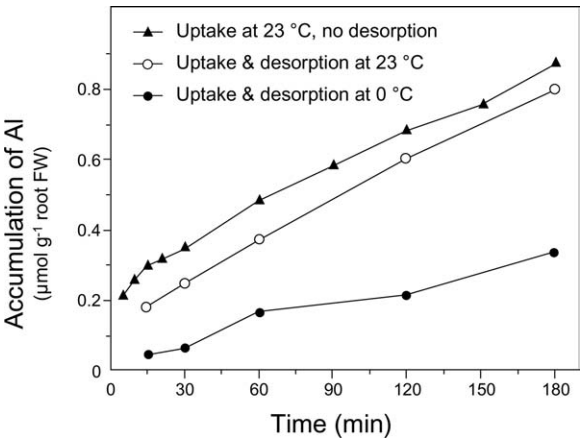

Figure 1. Aluminium accumulation in the wheat (*Triticum aestivum*) root tips. After Al exposure at different temperatures, roots were desorbed in 0.5 mM citrate (pH 4.5) for 30 min. Compiled from Zhang & Taylor (1989, 1990).

Uptake of aluminium

Characterisation of Al transport across biological membranes has been hindered by the complex aqueous coordination chemistry of Al and the lack of sensitive analytical techniques for detecting the low levels of Al associated with subcellular compartments. Hence, the long exposure times (>24 h) and concentrations of Al high enough to cause precipitation or polymerisation of Al had to be used. For plant cells, there are additional challenges because the cell wall (which accumulates large amounts of Al) cannot generally be separated from the cell symplasm (which has only a relatively low concentration of Al). Desorption of Al from the cell wall cannot be achieved nowhere near to completion in roots (Figure 1) and algal cells (Table 1) regardless of the desorption method used. Hence, the rates of Al uptake were grossly overestimated (Figure 1, Table 2).

Recent studies on Al uptake across the plasma membrane of plant cells (Reid *et al.* 1996; Taylor *et al.* 1996, 2000) have focused on the giant-cell green alga *Chara corallina*, in which surgical separation of the cell wall from the cytoplasm can be achieved, thus eliminating problems inherent in measuring exceedingly small amounts of intracellular Al in the very high background of apoplasmic Al. In brief, a single giant cylindrical algal cell (approx. 0.8 mm diameter and up to 80 mm length) is mounted into a 3-compartment chamber and the middle compartment exposed to the Al-containing solution. Upon termination of the uptake period, cell ends which were not in contact with

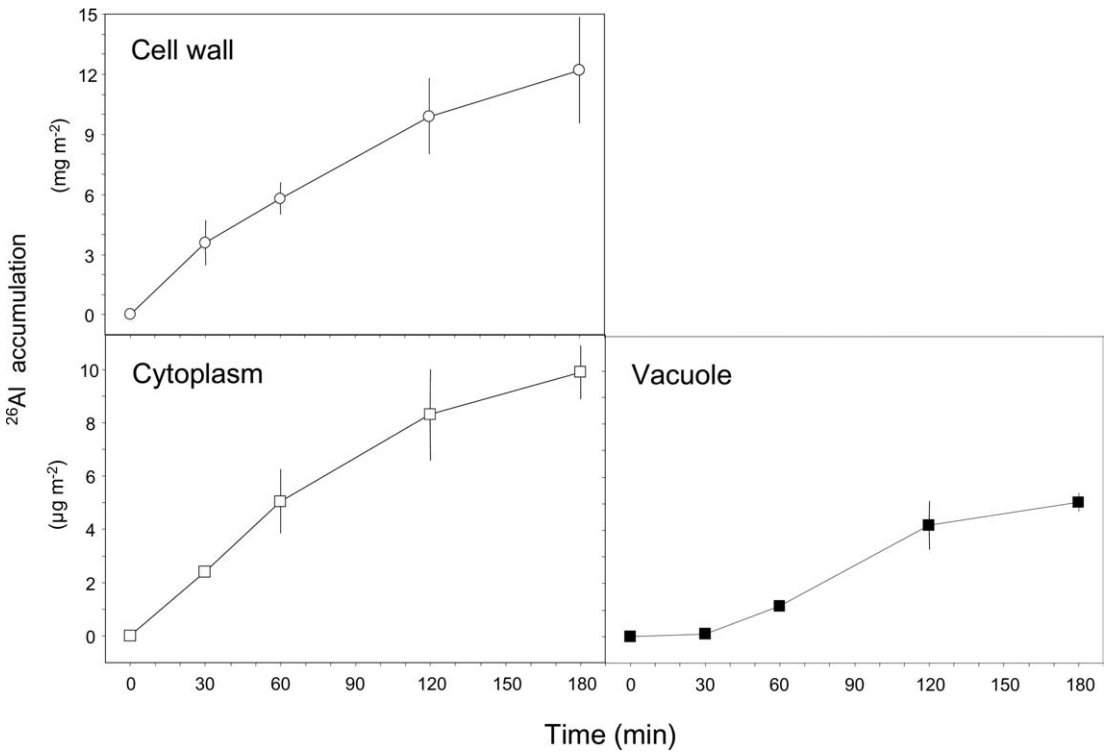

Figure 2. Short-term accumulation of ^{26}Al within subcellular fractions (cell wall, cytotoplasm and vacuole) of single cells of *Chara corallina* as measured by Accelerated Mass Spectrometry. Cells were exposed to 50 μM $AlCl_3$ in 0.4 mM $CaCl_2$ spiked with 75 pCi of ^{26}Al (pH 4.3), followed by desorption for 30 min in 1.0 mM citric acid (4 °C, pH 4.3) (to remove exchangeable Al from the cell wall) and surgical separation of cell wall, cytoplasm and vacuole. Note different units for ^{26}Al accumulation in the cell wall (top graph) and the cytoplasm or vacuole (bottom two graphs). Values represent the mean ± SE of three replicates. Modified from Taylor *et al.* (2000).

Table 1. The ratio of Al content in the cell wall and the symplasm of giant-cell alga *Chara corallina*. Cells were exposed to artificial pond water containing 10 to 100 μM Al for up to 7 days in a number of experiments. After termination of Al treatment, desorption of cell wall Al was performed by a method indicated, followed by surgical separation of the cell wall and the intracellular content. Modified from Reid *et al.* (1996).

Al content in cell wall / / Al content in the symplasm	Desorption method
60	1.5 h 5 mM citrate/$Ca(OH)_2$, pH 5.0
22	4.5 h 10 mM citrate/$Ca(OH)_2$, pH 4.4
28	2 h 10 mM $CaCl_2$ + 1 mM citrate/NaOH, pH 4.5
>20	2 h 1 mM $LaCl_3$ + 2 mM citrate/$Ca(OH)_2$, pH 4.4
30	2 h 1 mM $La(NO_3)_3$, pH 4.4

Table 2. Rates of Al uptake across the plasma membrane of plant cells

Species	Method	Al uptake rate $\mu mol\ g^{-1}\ FW\ h^{-1}$	References
Triticum aestivum (wheat)	GFAAS	0.3–0.4	Zhang & Taylor (1989, 1990); Archambault *et al.* (1996)
Glycine max (soybean)	SIMS	0.3	Lazof *et al.* (1994)
Chara chorallina	fluorimetry	$0.01–0.3 \times 10^{-3}$	Reid *et al.* (1996)
Chara chorallina	^{26}Al and AMS	up to 0.005×10^{-3}	Taylor *et al.* (1996, 2000)

GFAAS = Graphite Furnace Atomic Absorption Spectroscopy.
AMS = Accelerator Mass Spectrometry.

Al solution at all are cut, syringe needle inserted, and cell content flushed out by rapidly injecting deionized water through the cell lumen (Reid *et al.* 1996). Such advantage of physical separation of cell wall and the cytosol (Reid *et al.* 1996) (Table 1), especially when coupled with increased sensitivity due to usage of radioactive isotope ^{26}Al (Taylor *et al.* 2000), may represent the best model system for quantifying the magnitude and timing of Al flux across the plasma membrane of plant cells (Table 2).

Labelling with the radioactive isotope of aluminium

An accurate quantification of kinetics of transmembrane uptake of Al in intact organisms, cells, organelles and/or membrane vesicles can be achieved using a suitable tracer. The ^{26}Al is the only Al isotope suitable for tracer work (e.g. Taylor *et al.* 2000). It is a weak β and γ emitter, with the long half-life (approximately 7.3×10^5 y). The conventional mass spectrometry is not sensitive enough for measuring ^{26}Al. In contrast, Accelerator Mass Spectrometry (AMS) allows acceleration of atomic and molecular ions to MeV energies as opposed to KeV, providing sufficient sensitivity to resolve ^{26}Al from ^{27}Al at atom ratios as low as 10^{-14} and a detection limit in the 10^{-17} g range (see Taylor *et al.* 2000). Hence, combining ^{26}Al with AMS detection provides sufficient sensitivity to detect Al in subcellular fractions isolated from cells exposed to realistic environmental Al concentrations for a short period of time.

The ^{26}Al isotope has been used to monitor Al uptake by plant species *Gleditsia triacanthos* and *Pinus taeda* (Schaedle *et al.* 1989) and by internodal cells of the giant algae *Chara corallina* (Taylor *et al.* 1996, 2000). The ^{26}Al-AMS is also beginning to play a role in animal and human physiology, including studies of Al absorption in the human gastrointestinal tract (Priest *et al.* 1998; Jugdaohsingh *et al.* 2000; Moore *et al.* 2000) and uptake of Al by brain and other animal and human tissues (Yokel & McNamara 2001).

Aluminium species transported across the plasma membrane and existing in the symplasm

It is unclear at present which Al species cross the plasma membrane due to uncertainty in Al speciation. Negative charges in the cell wall of plant cells as well as on the plasma membrane of all cells would alter the Al speciation in vicinity of these cellular structures compared with Al speciation in the bulk solution. Therefore, determining the identity of Al complexes in contact with the plasma membrane and the time-course of their transfer into the cytosol are beyond the limits of the current experimental techniques.

Upon transfer of Al species from outside of the plasma membrane into the symplasm, speciation of Al is altered because of higher pH (near-neutral) in the cytosol compared to the medium outside the cell. Hence, cytosolic Al is expected to be complexed with hydroxyl ions, high-molecular-weight substances, phosphate-containing compounds, and/or Ca-dependent regulatory cytosolic protein calmodulin (see Martin 1988; Taylor 1991; Rengel 1996). Nevertheless, a small proportion of cytosolic Al may remain in the free ionic form in dynamic equilibrium with a larger pool of complexed Al. Increasing the pool of complexed Al over time would inevitably result in an increase in the pool of the free ionic Al that may potentially affect cellular structures and processes after a prolonged exposure to Al. Concentrations of free Al in the nanomolar to sub-nanomolar range *in vitro* have affected polymerization of tubulin (Macdonald *et al.* 1987; Martin 1988) and activity of protein kinase C (see Rengel, 1996); these concentrations of free Al may be expected to occur in the cytosol of Al-intoxicated cells.

Aluminium uptake across the plasma membrane of plant cells

Transport of ^{26}Al across the plasma membrane of *Chara corallina* showed a clear maximum at pH 4.3 (176 ng m^{-2} min^{-1}) (Taylor *et al.* 2000), with rates of transport approximately an order of magnitude lower at pH 3.7 and 5.2, suggesting that Al^{3+} may be the main species transported. A decrease in uptake at pH 5.2 was caused by a lowered proportion of Al^{3+}, whereas lower uptake at pH 3.7 was likely due to competition with H^+ (cf. Kinraide 1993).

When total Al was kept constant, but $\{Al^{3+}\}$ was reduced in the presence of sulphate and citrate, rates of Al transport across the plasma membrane of *Chara corallina* increased by 2.1-fold in the presence of SO_4^{2-} and by 2.0-fold in the presence of citric acid (Taylor *et al.* 2000), suggesting that complexes such as $AlSO_4^+$, $AlCit_{H-1}^-$, or $AlCit^0$ may be readily transported across the plasma membrane. Indeed, in animal and human cells, Al-citrate is one of the two major forms of Al taken up across the plasma membrane of brain cells (Yokel *et al.* 2002).

In contrast to animal cells, plant cells have the vacuole as a relatively large organelle where toxic ions are stored to decrease their metabolic impact (Rengel 1997). In *Chara corallina*, transport of Al into the vacuole was limited during the first 30 min of exposure, then increased to a rate that represented a large portion of Al across the plasma membrane (Figure 2) (Taylor *et al.* 2000). This suggests that (i) Al may need to accumulate in the cytoplasm before transport to vacuole can occur, and/or (ii) induction of a transport system capable of moving Al across the tonoplast may be required. Given predictions about the limited solubility of Al in the cytoplasm and its propensity to bind to biological ligands (Martin 1988; Taylor 1991; Rengel 1996; Taylor *et al.* 2000), such a transport system is likely to facilitate transport of organic Al complexes into the vacuole.

Aluminium absorption in the animal/human digestive tract and uptake by cells and tissues

The average daily Al intake by US adults is 8–9 mg for males and around 7 mg for females (Pennington & Schoen 1995). In Italian population, daily Al intake ranges from 2.5 to 6.3 mg (Gramiccioni *et al.* 1996), and in UK is 3.4 mg on average (Ysart *et al.* 2000). The 'normal' daily intake of Al is defined as 5 mg Al by the World Health Organisation (WHO 1996). About 0.1% of daily amounts of Al taken stays in the body (less than 10 μg), whereas the rest is excreted (Priest *et al.* 1998).

Bioavalability of Al (which determines Al uptake, retention in the body and thus Al toxicity) is vastly different in different sources, ranging from 0.012% in antiperspirant applications and 0.3% in drinking water to 1.5% from inhaled airborne soluble Al and virtually 100% from intramuscular injections (e.g. some vaccines contain Al) (Priest *et al.* 1998; Stauber *et al.* 1999; Yokel 2000; Yokel & McNamara 2001). Bioavailability of Al in food, however, is influenced by numerous factors (see section on Al speciation in food) and would have to be determined on a case-by-case basis.

Dietary acids, by their intrinsic acidity and coordinating capacity, can extend solubility of Al^{3+} and thus increase Al bioavailability during longer sections of the gastrointestinal tract, and also help Al^{3+} diffusion across the intestinal epithelium through the formation of neutral complex species (Desroches *et al.* 2000; Dayde *et al.* 2003) (in small intestine alkaline pH would normally eliminate any Al absorption). Hence, these dietary acids, e.g. glutamic acid, an essential amino acid and a common food additive (Dayde *et al.* 2003); malic acid, added to many beverages and processed foods as well as present in vegetables and fruits (Venturini & Berthon 2001) and tartaric acid, found commonly in fruits and industrial drinks and food (Desroches *et al.* 2000) can serve as organic ligands, enhancing Al absorption in the gastrointestinal tract and thus aggravating Al toxicity. It is therefore advisable to avoid simultaneous ingestion of these organic ligands when using any therapeutic aluminium salt (Venturini & Berthon 2001).

Absorption of Al from drinking water in the gastrointestinal tract of rats was dependent not only on the concentration of Al in water, but even more so on the Al-binding capacity of food components in the stomach determining the Al bioavailability (Glynn *et al.* 1999a). Indeed, when the stomach content of rats was fractionated for Al, large portion of Al ingested in acidic water (pH 2.5–3.0) was found to be insoluble, even when citrate, fluoride and silicate as Al-complexing agents were included in the water (Glynn *et al.* 2001). In other studies with rats and mice, however, addition of fluoride caused higher Al absorption from the gastrointestinal tract (Allain *et al.* 1996). Hence, bioavailability of Al cannot be measured in water or food before ingestion because large changes may occur in the gastrointestinal tract.

The study employing radioactive ^{26}Al isotope and quantification by accelerated mass spectrometry showed that only 0.06 to 0.1% of ingested Al was absorbed in the gastrointestinal tract of healthy aged patients after 1 h of drinking Al-laced fruit juice. However, the proportion of Al absorbed was 1.64 times greater in aged-matched Alzheimer's sufferers, suggesting an increased capacity of Alzheimer's disease patients to take up Al from food (Moore *et al.* 2000).

Aluminium concentration in blood serum is increased upon administering Al to the animals (Glynn *et al.* 1999b). Over time, Al accumulates in liver (Lote *et al.* 1995; Glynn *et al.* 1999b; Swegert *et al.* 1999; Esparza *et al.* 2000), brain (Candy *et al.* 1992; Swegert *et al.* 1999), heart (Swegert *et al.* 1999) and especially in bones (Glynn *et al.* 2001; Vanholder *et al.* 2002), affecting their mechanical properties (Hui & Ellers 1999).

An important component of Al uptake into the brain cells across the blood-brain barrier is likely to be transferrin-receptor mediated endocytosis (Van Landeghem *et al.* 1998a; Yokel 2000; Yokel & McNamara 2001), even though Al-transferrin complexes are not always found in the cerebrospinal fluid (Van Landeghem *et al.* 1997). An additional pathway of Al entry is uptake of Al-citrate complex into the mouse brain endothelial cells via a carrier, probably a monocarboxylate transporter or organic anion transporter (Yokel *et al.* 2002). Interestingly, uptake of Al-citrate is 70% greater than uptake of citrate, even though citrate is one of primary substrates for transporters suggested to be involved in Al-citrate transport. Neither the monocarboxylate transporter 1 (MCT 1) nor anion exchanger was involved in Al-citrate uptake by brain cells (Yokel *et al.* 2002).

Aluminium is effluxed from the brain tissues as Al-citrate (Yokel 2000), probably via a carrier (Yokel & McNamara 2001). However, Al accumulates in the brain tissues, potentially causing compounded damage (Yokel 2000).

Excretion of Al from the body can be via faeces in healthy humans (Priest *et al.* 1998), but is also renal [and may be enhanced by citrate (Lote *et al.* 1995) or fluoride (Chiba *et al.* 2002)], with <2% being excreted in bile (Yokel & McNamara 2001). Intravenous infusion of citrate can facilitate renal excretion of Al (Lote *et al.* 1995).

Plant species that accumulate aluminium in cells and tissues

Many native plant species grow vigorously on acid soils that contain high concentrations of bioavailable Al. For these plant species, Al can be considered a beneficial element (Kinraide 1993; Osaki *et al.* 1997) because growth is stimulated when a high concentration of Al is present in the growing medium (Watanabe *et al.* 1997; Watanabe & Osaki 2002). These plant species can be divided into Al-excluders and Al-accumulators (for a review see Watanabe & Osaki 2002), the latter being of more interest to discussion here. Al-accumulators can have leaf Al concentrations of up to 37 g Al kg^{-1} dry weight (DW) (Masunaga *et al.* 1998), which are concentrations similar to nitrogen and potassium as the two most abundant essential nutrients present in plant tissue.

The Al-accumulator species belong to various families (eg. Euphorbiaceae, Melastomataceae, Rubiaceae, Theaceae) (Owour & Cheruiyot 1989; Masunaga *et al.* 1997, 1998); many are shrubs and trees, but some are herbaceous species. Examples of Al-accumulator plants are *Camellia sinensis* (tea, Owour & Cheruiyot 1989), *Hydrangea macrophylla* (hydrangea, Takeda *et al.* 1985; Osaki *et al.* 1997; Naumann & Horst 2003), *Fagopyron esculentum* (buckwheat, Osaki *et al.* 1997; Shen *et al.* 2002), *Aporusa frutescens*, *Aporusa maingayi*, *Euphorbia acuminata*, *Melastoma laurinum*, *M. corymbosus* (Masunaga *et al.* 1998), *M. malabatrichum* (Osaki *et al.* 1997; Watanabe *et al.* 1997; Osaki *et al.* 1998), *Tibouchina pulchra* (Domingos *et al.* 2003), *Dioclea guianensis* (Izaguirre & Flores 1995) and *Stylosanthes elegans* (Izaguirre *et al.* 2002).

The Al-accumulators may accumulate Al in roots (Osaki *et al.* 1997; Izaguirre & Flores 1995; Izaguirre *et al.* 2002), but exclude it completely from root nodules of N_2-fixing plant species (Izaguirre *et al.* 2002). Most Al-accumulators accumulate Al in leaves, especially old ones (Owour & Cheruiyot 1989; Memon *et al.* 1981; Nagata *et al.* 1992; Masunaga *et al.* 1998; Shen *et al.* 2002). However, accumulation of Al in young, metabolically most productive leaves may occur, for example in *Melastoma malabatrichum* (Osaki *et al.* 1997, 1998; Watanabe *et al.* 1997, 1998) with up to 10 g Al kg^{-1} DW in old and up to 7 g Al kg^{-1} DW in new leaves.

In contrast to other Al-accumulators, in hydrangea Al accumulates in sepals (Takeda *et al.* 1985; Naumann & Horst 2003). Sufficient transport of Al-citrate

into sepals of hydrangea is essential to achieve the commercially desirable colour change from original pink to blue by complexation of the anthocyanin delphinidine (Naumann & Horst 2003).

In Al-accumulators, Al is usually complexed with organic acids or other organic compounds to make it non-toxic. The predominant Al form is Al-catechins in leaves of tea plant (Nagata *et al.* 1992), Al-citrate in hydrangea leaves (Ma *et al.* 1997) and Al-oxalate in buckwheat (Shen *et al.* 2002) and *Melastoma malabatrichum* leaves (Watanabe *et al.* 1998), rendering the high total tissue concentrations non-phytotoxic to the cell cytosol. In addition, the cytosol is protected by Al accumulating predominantly in the cell wall (Memon *et al.* 1981; Shen *et al.* 2002) or vacuoles (Echlin 1996; Shen *et al.* 2002) of tea and buckwheat leaf cells. Binding of Al in the cell wall is mainly to pectic substances, as shown in *Melastoma malabatrichum* (Watanabe *et al.* 1998).

In hydrangea, Al-citrate was the main transporting Al form in the xylem sap (Naumann & Horst 2003) as well as the main Al form accumulating in leaves (Ma *et al.* 1997). In contrast, in *Melastoma malabatrichum*, Al-citrate was the main Al transporting form in the xylem sap, whereas the main form in leaves was Al-oxalate (Watanabe & Osaki 2001, 2002).

Aluminium in the continuum of soil / tea plant / tea drink / humans

Tea drink is the second (after water) most widely drunk beverage in the world. Given that tea plant *Camellia sinensis* is grown on acidic soils and is the Al-accumulator, there is an obvious need to clarify the potential transfer of Al from soil to tea leaves to tea infusions and into a human body.

Increasing soil acidity results in higher bioavailability of Al and higher uptake of Al by tea roots. Indeed, Al accumulation in tea leaves was correlated with $CaCl_2$-extractable (bioavailable) Al in subsoil, and was especially prominent when soil pH dropped below 5.0 (Dong *et al.* 1999). High Al content was present in tea leaves coming from Chinese provinces with highly acidic soils (Wong *et al.* 1998).

In tea leaves, the difference between Al concentration in young and old leaves can be substantial (Table 3) (Nagata *et al.* 1992). Hence, Al content in various tea preparations (e.g. green, oolong, black, brick tea) differs depending on the maturity of leaves collected (only a bud and the two youngest leaves are used for green tea, whereas more mature leaves are collected for other types of tea, Wong *et al.* 1998). The presence of branches (eg. in brick tea) can contribute to Al content in brick tea being more than 4 times higher than in green tea (Wong *et al.* 2003).

Table 3. Aluminium concentration in various parts of *Camellia sinensis* (tea) cultivars grown under diverse environmental conditions.

Plant part	Al content ($g\ kg^{-1}$ *dry weight*)	
	Adapted from Nagata *et al.* (1992)	Adapted from Wong *et al.* (2003)
Buds	0.07	1–2
Young leaves	0.3	2.4–11
Mature leaves	5.5	8–15
Old leaves	8.3	12–18

Brick tea infusions can have total Al concentration of 15 mg Al L^{-1} after 1 h of infusion, whereas other types of tea contain between 0.6 and 1.9 mg Al L^{-1} (Wong *et al.* 2003). Hence, drinking just 0.3 L of brick tea per day would supply the amount of Al estimated as 'normal' (5 mg L^{-1}, WHO 1996). The extraction of Al from tea leaves (1% w/v mixture) is about 35% in the first infusion and about 14% in the second for most common tea types (green, black, oolong) (Wong *et al.* 1998).

Aluminium species in tea infusions

Although high total Al concentrations can be found in tea infusions (like 0.1 mM), most of that Al is complexed so strongly that even Chelex 100 cannot exchange Al from these complexes (Alberti *et al.* 2003), resulting in low chemical and biological reactivity of Al in tea infusions. About 10% of total Al in tea infusions may be bound to fluoride (Erdemoglu *et al.* 2000b). However, the proportion of Al-F complexes increases significantly when simulated gastric juice (pH 2.5) was added to tea infusion (Liang *et al.* 1999). The Al-F complexes decrease absorption of Al by neural cells (Levesque *et al.* 2000).

In black tea infusions, 10–19% of total Al was present as cationic species, whereas 28–33% was present as hydrolysable polyphenol complexes (Erdemoglu *et al.* 2000a). In green tea infusions, the most prevalent complex was Al-oxalate (Liang *et al.* 1999). In contrast, organic compounds of large molecular size were found to complex Al in infusions from some types of tea (Flaten & Lund 1997).

Conclusions

Bioavailability of Al increases in acidic environments. Different chemical species of Al have different toxicity to living organisms, making Al speciation a necessity. Complexes between some organic compounds and Al have high bioavailability and can cross the plasma membrane. The main source of Al in human diet is food, whereas water provides only a minor fraction of Al absorbed. Aluminium accumulates in bones, but also in brain where it causes neurotoxicity. The relationship between common neurological disorders and dietary or environmental exposure to Al is strenuous and requires clarification. Options for minimising medicinal exposure to Al are urgently needed. Further work is required on clarifying various aspects of the transport of Al in the soil-water-plant-human continuum.

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