

## SPECIES SELECTION FOR PHYTOREMEDIATION OF $^{36}\text{Cl}/^{35}\text{Cl}$ USING ANGIOSPERM PHYLOGENY AND INTER-TAXA DIFFERENCES IN UPTAKE

Neil Willey and Kathy Fawcett

*Centre for Research in Plant Science, Faculty of Applied Sciences, University of the West of England, Bristol, United Kingdom*

*High concentrations of  $^{35}\text{Cl}$  and the radioisotope  $^{36}\text{Cl}$  (produced naturally by cosmic radiation and anthropogenically by U fission and the use of neutron sources) can be problematic in soil, but are potentially amenable to phytoremediation if appropriate plants can be found. Here, results are reported that might aid the selection of plants with unusually high or low uptake of  $^{36}\text{Cl}$ . A residual maximum likelihood analysis was used to estimate, from 13 experiments, relative  $^{36}\text{Cl}$  uptake by 106 species across the angiosperm phylogeny. Nested analysis of variance, coded using a recent angiosperm phylogeny, showed that there were significant inter-species differences in  $^{36}\text{Cl}$  uptake and that species behavior was not independent, but linked through their phylogeny. Eudicots had significantly higher  $^{36}\text{Cl}$  uptake than Monocots and related clades and, in particular the Orders Caryophyllales, Apiales, and Cucurbitales had high uptake while the Poales, Liliales, Brassicales, and Fabales had low uptake. Overall, 35% of the inter-taxa variation in  $^{36}\text{Cl}$  was attributed to the taxonomic ranks of Order and above, a significant phylogenetic effect compared with other elements for which similar analyses have been published. The implications of these findings for selecting plants for phytoremediation of soil contaminated with  $^{35/36}\text{Cl}$  are discussed.*

**KEY WORDS:**  $^{35}\text{Cl}/^{36}\text{Cl}$ , phylogenetic effects, inter-taxa differences

Chlorine (Cl) is highly soluble and has a high diffusion coefficient and a most stable oxidation state of  $-1$  (Bohn *et al.*, 1979). Its movement in soils is determined primarily by mass fluxes of water (for which it can be used as a tracer) and it is readily taken up by plants (White and Broadley, 2001). The residence time of Cl in the rooting zone is determined by net water fluxes. Given the contribution that plant transpiration can make to net fluxes of water in soil, the behavior of Cl in the soil–plant system can be strongly affected by plants. In many ecosystems, especially when evapotranspiration exceeds precipitation, net Cl movement can be upward into the rooting zone (Burns, 1974). The behavior of Cl in the soil–plant system is, therefore, potentially amenable to plant-based control. This is an opportunity for phytoremediation because soil contamination with  $^{36}\text{Cl}$ , a  $\beta$ -emitting radioisotope, and the accumulation of the stable isotope  $^{35}\text{Cl}$  in salinized soils can be significant problems.

$^{36}\text{Cl}$  is a weak  $\beta$ -emitter but has a long half-life ( $3.01 \times 10^5\text{y}$ ). It can have adverse effects inside living organisms and investigations of nuclear waste repositories have noted

Address correspondence to Neil Willey, Centre for Research in Plant Science, Faculty of Applied Sciences, University of the West of England, Coldharbour Lane, Frenchay, Bristol BS16 1QY, UK. E-mail: Neil.Willey@uwe.ac.uk

its importance to long-term assessments of potential doses (Sheppard *et al.*, 1996).  $^{36}\text{Cl}$  is produced naturally by the effects of cosmic radiation on  $^{35}\text{Cl}$ , Ca, and K in the regolith and  $^{40}\text{Ar}$  in the atmosphere (Bentley *et al.*, 1986), but also anthropogenically by neutron bombardment following U fission or the use of neutron sources (White and Broadley, 2001).  $^{36}\text{Cl}$  is released into the environment in trace amounts from nuclear power plants, is produced in great quantities in nuclear explosions, and is a major component of nuclear waste (Sheppard *et al.*, 1996). Methods to decontaminate soils of  $^{36}\text{Cl}$  are, therefore, desirable.

There are soil factors that have been shown to affect  $^{36}\text{Cl}$  transfer from soil-to-plant such as pH, redox potential, and mineral constituents (Coughtrey *et al.*, 1983) but the effects of these variables on  $^{36}\text{Cl}$  transfer are small compared to their effects on other radionuclides, such as U (Huang *et al.*, 1998), Tc (Bennett and Willey, 2003), and Cs (Cremers *et al.*, 1988). There are, however, significant differences in  $^{36}\text{Cl}$  uptake between species of plants (*e.g.*, Yang and Blanchar, 1993) and plant ecophysiologicalists have long classified responses to  $^{35}\text{Cl}$  of a variety of halophytes (plants of salty soils) and glycophytes (plants of nonsalty soils) (Greenway and Munns, 1980). Given that isotopes of Cl can cause radiological and chemical toxicity in soils and that there are known to be significant interspecies differences in the uptake and tolerance of  $^{36/35}\text{Cl}$  by plants, quantifying and predicting interspecies differences might be useful for assessing the potential for plant-based management of soils with problematic  $^{36}\text{Cl}$  or  $^{35}\text{Cl}$  concentrations.

Plants actively regulate the flow of ions into roots and hence to shoots by processes that can differ between species. Until recently, such interspecies differences in ion transfer were primarily regarded as adaptive responses to particular environments, *e.g.*, halophytic adaptations to high-salt soils. However, the differences between plant species are also constrained by their evolutionary descent (phylogeny). Understanding interspecies differences in the soil-to-plant transfer of ions necessitates quantifying these evolutionary constraints. Recently, improved angiosperm (flowering plant) phylogenies have been used to identify significant phylogenetic effects on plant uptake of Cs (Broadley *et al.*, 1999), Cd, Cr, Pb, Zn, Ni, and Cu (Broadley *et al.*, 2001), Ca (Broadley *et al.*, 2003) and a suite of macronutrients (Broadley *et al.*, 2004). This demonstrated that, for concentrations of these elements, plants do not behave independently, but are linked through phylogeny. Further, they show that this phylogenetic linkage can be useful for quantifying and predicting soil-to-plant transfer of ions. Here we use the methods successfully applied to other elements to quantify interspecies differences in  $^{36}\text{Cl}$  uptake. We describe a database of interspecies differences in  $^{36}\text{Cl}$  concentrations, test the hypothesis that there is a phylogenetic component to differences in soil-to-plant transfer of  $^{36}\text{Cl}$  using a recent angiosperm phylogeny, and nominate plant taxa that might merit particular attention in the development of plant-based management of  $^{36/35}\text{Cl}$  contamination of soil.

## METHODS

One hundred and six species were grown and radiolabeled with  $^{36}\text{Cl}$ . Five replicate 12-cm-diameter pots of each species, each with approximately 250 g of Levington's F2S (a loam-based compost with added nutrients and sand; Levington's, Ipswich, UK), were grown in a greenhouse for approximately 7 wk in 16-h days and 8-h nights at *c.* 24°C and 16°C, respectively. Species chosen were primarily fast growing and herbaceous, but also included as wide a range of food crops as practicable. Plants were labeled with  $^{36}\text{Cl}$  in the exponential phase of their growth and before they flowered; hence, some species were

slightly younger and others slightly older than seven weeks. Plants were watered on demand up to the day before labeling.

Fifty ml of 10  $\mu\text{M}$  KCl radiolabeled with 1850 kBq  $^{36}\text{Cl}^{-1}$  was added to the surface of each pot after trial experiments to establish appropriate labeling volumes, carriers, activities, and exposures. Saucers beneath the pots collected any excess solution, allowing it to be re-absorbed, but pots were not watered for 24 h prior to radiolabeling and in general no excess solution appeared in saucers. Plants were harvested after 24 h 1 cm above soil level, dried for at least 48 h at 80°C, and ground.  $^{36}\text{Cl}$   $\beta$ -activity was measured in solutions extracted from ground plant material using the method of Ghosh and Drew (1991) with appropriate standards and blanks. The 106 species were radiolabeled across 13 labeling events. In each event, five replicate pots of the species being labeled were organized in a randomized block design in a radiolabeling arena with light supplemented to *c.* 350  $\mu\text{Em}^{-1}\text{s}^{-1}$ . To provide link species, five replicates of *Pulsatilla vulgaris*, *Beta vulgaris*, *Geranium pyrenium*, *Trifolium pratense*, *Trifolium repens*, *Fragaria vesca*, *Brassica oleraceae*, *Mentha picata*, and *Daucus carota* were labeled in each of two labeling events and for *Ipomea purpurea* and *Nicotinia glauca*, five replicates in each of three labeling events.

Residual maximum likelihood (REML) analysis was run on Genstat 5th ed. for Windows release 4.2 (VSN International, Oxford, UK; Thompson and Welham, 2000) using the program of Broadley *et al.* (1999, 2001, 2003, 2004). It included  $\log_e$ -transformation of original values, then the REML procedure followed by a nested ANOVA coded using the phylogeny of Soltis *et al.* (1999), which was designed for comparative experiments and for the species here is very similar to the more recent APG II grouping (APG II, 2003). Each of the 13 radiolabeling events was used as a separate “block” in the REML analysis and species were used as the “treatments.” To enable comparison with previously published analyses for other elements, the categories “class,” “subclass,” “group,” and “superorder” were used nominally for ranks above the Order although the relationship between the Linnaean hierarchy they derive from and higher taxonomic groups on recent phylogenies is contentious. Normality was tested using the Kolmogorov–Smirnov test on SigmaStat 3.0 for Windows.

## RESULTS

REML analysis provided relative concentration values for the species (treatments) across the different labeling events (blocks). A labeling event (block) was a significant variance component in the REML analysis demonstrating that species (treatment) values could not be compared strictly without taking it into account. Table 1 provides the most taxonomically wide-ranging dataset yet published of relative  $^{36}\text{Cl}$  uptake by plants and gives species values from REML analysis. Given that data have been  $\log_e$ -transformed prior to REML, Table 1 shows that there are large interspecific differences in the uptake of  $^{36}\text{Cl}$  after an acute exposure. *Cucurbita pepo* had the highest absolute  $^{36}\text{Cl}$  concentration at 1,834 Bq/g dry weight while *Maclura pomifera* had the lowest detectable concentration at 0.9 Bq/g dry weight (*Eleagnus multiflora* had an activity not significantly different from background). REML  $^{36}\text{Cl}$  concentrations in all species failed the Kolmogorov–Smirnov test for normality although probability and residual plots indicated that relatively few species were outside the normal distribution. These included *E. multiflora* with a concentration not significantly different from background plus seven species with unusually high concentrations: *Papaver somniferum*, *Silene chalconica*, *Rumex sanguineus*, *Antirrhinum* spp., *Coriandrum sativum*, *Cucurbita maxima*, and *Cucurbita pepo*. Without the values for these species, the values for the remaining 98 species passed the test for normality (Figure 1).

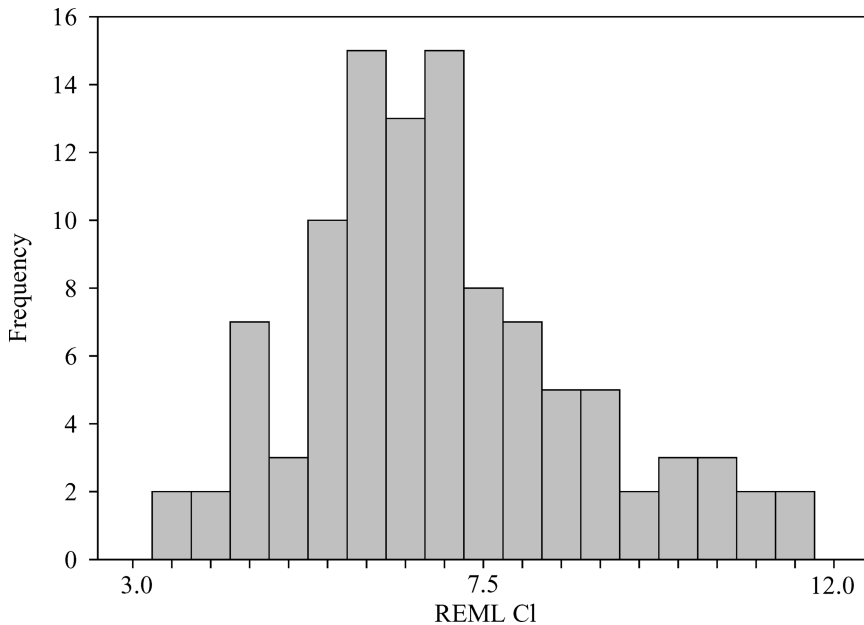
**Table 1** REML concentrations of  $^{36}\text{Cl}$  in 106 species of angiosperm after acute exposure and organized according to the phylogeny of Solitis *et al.* (1999)

Class	Sub-class	Group	Superorder	order	Scientific name	Common name	REML [CI]	Group	Superorder	Order	Scientific name	Common name	REML [CI]
MAGNOLIID COMPLEX				Laurales	<i>Cubycanthus occidentalis</i>	Western spice bush	8.60				<i>Salvia officinalis</i>	Sage	7.63
					<i>Chimonanthus praecox</i>	Wintersweet	6.24				<i>Anthrinum</i>	Snapdragon	12.54
PALAEOHERBS				Piperales	<i>Peperomia hederiaefolia</i>	Ivy peperomia	9.37				<i>Digitalis ambigua</i>	Large Yellow foxglove	7.59
					<i>Peperomia rotundifolia</i>	Round-lyd peperomia	10.26				<i>Digitalis purpurea</i>	Wild foxglove	7.97
					<i>Houtynia cordata</i>	Houtynia	9.09		Asterid 2	Asterales	<i>Aster</i>	Michaelmas daisy	7.67
		Monocots	Commelinoid		<i>Areca lutescens</i>	Areca palm	8.45				<i>Cenaurea</i>	Cornflower	10.94
MAGNOLIIDS				Arecales	<i>Phoenix</i>	Date	6.08				<i>Helianthus annuus</i>	Sunflower	11.88
				Commelinales	<i>dactylifera</i>	Blue spiderwort	8.20				<i>Helianthus debilis</i>	Sunflower 'Vanilla Ice'	11.80
					<i>Commelina coelestis</i>	Umbrella plant	9.25				<i>Lactuca sariva</i>	Lettuce	10.40
					<i>Cyperus zumita</i>	Bronze sedge	5.98				<i>Tithonia rotundifolia</i>	Mexican sunflower	9.06
					<i>Carex comans</i>	Pendulous sedge	4.79			Apiales	<i>Angelica hispanica</i>	Angelica	6.80
					<i>Carex pendula</i>	Sedge	3.98				<i>Apium graveolens</i>	Celery	8.97
					<i>Carex stricta</i>	Rye grass	9.23				<i>Coriandrum sativum</i>	Coriander	14.03
					<i>Lolium perenne</i>	Northern sugar cane	8.69				<i>Daucus carota</i>	Carrot	11.70
					<i>Sorghum vulgare</i>	Wheat	7.59				<i>Hedera helix</i>	Ivy	4.99
					<i>Triticum aestivum</i>	Durum wheat	8.40				<i>Pittosporum</i>	Pittosporum	4.95
				Zingiberales	<i>Triticum durum</i>	Canna lily	6.49	Rosids	Basal	Saxifragales	<i>Bergenia</i>	Bergenia	6.59
					<i>Canna indica</i>	Maranta	10.25				<i>Bergenia cordifolia</i>	Bergenia	6.64
				<i>Maranta species</i>	Ginger	8.31				<i>Bergenia purpurescens</i>	Bergenia	6.44	
				<i>Zingiber officinale</i>	Devil's Ivy	7.55				<i>Heuchera micrantha</i>	Alum-root	6.44	
		Non-Commelinoid		Alismatales	<i>Scindapsis aureus</i>					<i>Heuchera sanguinea</i>	Heuchera	7.37	

	<i>Philodendron hastatum</i>	Elephant's ear	7.64	Rosid 1	Geraniales	<i>Geranium pyrenarium</i>	Pyrenian cranesbill	8.74
Asparagales	<i>Asparagus officinalis</i>	Asparagus	9.18		Brassicales	<i>Alyssum montanum</i>	Alyssum	7.89
	<i>Crocsmia masonorum</i>	Montbretia	6.15			<i>Alyssum petraeum</i>	Alyssum	7.73
Dioscoreales	<i>Tigridia pavonia</i>	Peacock flower	6.91			<i>Alyssum saxatile</i>	Alyssum	3.38
	<i>Dioscorea japonicus</i>	Yam	10.04			<i>Brassica oleracea (rape)</i>	Rape	10.45
Liliales	<i>Allium ameloprasum</i>	Leek	7.65			<i>Brassica oleracea (cabbage)</i>	Cabbage	6.85
	<i>Allium cepa</i>	Onion	6.56			<i>Tropaeolum perigrinum</i>	Canary creeper	7.84
	<i>Allium schoenoprasum</i>	Chives	5.22		Malvales	<i>Cistus palhinhae</i>	St. Vincent Cistus	5.49
	<i>Allium tuberosum</i>	Garlic chives	4.47			<i>Althea rosea</i>	Hollyhock	8.35
	<i>Lilium formosanum</i>	Lily	6.49			<i>Malva sylvestica</i>	Common mallow	11.24
Proteales	<i>Banksia robur</i>	Dwarf banksia	7.77		Sapindales	<i>Pistachia chinensis</i>	Chinese pistachio	9.12
	<i>Grevillea robusta</i>	Silk oak	6.91			<i>Pistachia lentiscus</i>	Mastic	4.21
Ranunculales	<i>Papaver pilosum</i>	Hairy poppy	6.18			<i>Ruta graveolens</i>	Rue	6.85
	<i>Papaver somniferum</i>	Opium poppy	13.26	Rosid 2	Myrtales	<i>Callistemon subulatus</i>	Tonghi bottle-brush	5.09
	<i>Pulsatilla vulgaris</i>	Pasque flower	7.02			<i>Clarkia bottea</i>	Clarkia	6.83
Caryophyllales	<i>Beta vulgaris</i>	Beet	10.75			<i>Oenothera hookeri</i>	Giant Yellow Evening Primrose	7.57
Core	<i>Dianthus gratinopoulis</i>	Cheddar pink	7.68			<i>Oenothera tetragona</i>	Evening Primrose	5.16
	<i>Dianthus seguiri</i>	Pink	6.89		Malpighiales	<i>Hypericum olympicum</i>	'Sundrops'	4.81
	<i>Gypsophila elegans</i>	Baby's tears	7.44			<i>Hypericum perforatum</i>	Dwarf St. John's Wort	7.82

**Table 1** REML concentrations of  $^{36}\text{Cl}$  in 106 species of angiosperm after acute exposure and organized according to the phylogeny of Soltis *et al.* (1999) (Continued)

Class	Subclass	Group	Superorder	Order	Scientific name	Common name	REML [CI]	Superorder	Order	Scientific name	Common name	REML [CI]
					<i>Gypsophila paniculata</i>	Baby's tears	7.07		Fabales	<i>Lupinus angustifolius</i>	Lupin	8.42
					<i>Silene chalcidonia</i>	Campion	12.43			<i>Medicago lupulina</i>	Black medik	5.71
					<i>Rheum tataricum</i>	Rhubarb	8.83			<i>Trifolium arvense</i>	Hare's foot clover	6.53
					<i>Rumex acetosa</i>	Sorrel	7.99			<i>Trifolium pratense</i>	Red clover	6.44
					<i>Rumex sanguineus</i>	Bloodwort	13.14			<i>Trifolium repens</i>	White clover	7.77
					<i>Camellia sinensis</i>	Tea	5.75		Rosales	<i>Humulus japonicus</i>	Japanese hop	7.78
					<i>Ipomoea purpurea</i>	Purple morning glory	10.29			<i>Elaeagnus multiflora</i>	Elaeagnus	-8.51
					<i>Nemophila menziesii</i>	Baby blue eyes	6.05			<i>Maclura pomifera</i>	Osage orange	-2.88
					<i>Lycopersicon esculentum</i>	Tomato	10.52			<i>Morus alba</i>	White mulberry	6.91
					<i>Nicotiana glauca</i>	Yellow tree tobacco	7.99			<i>Fragaria vesca</i>	Strawberry	7.82
					<i>Nicotiana sylvestris</i>	Tobacco 'Only the Lonely'	9.86			<i>Celtis occidentalis</i>	Celtis	9.73
					<i>Solanum sisymbriifolium</i>	Solanum	7.70			<i>Pilea caderii</i>	Pilea	7.79
					<i>Mentha piperata</i>	Peppermint	7.04		Curcubiales	<i>Cucurbita maxima</i>	Pumpkin 'Blue Hubbard'	12.71
					<i>Mentha spicata</i>	Spearmint	7.87			<i>Cucurbita pepo</i>	Pumpkin	13.66



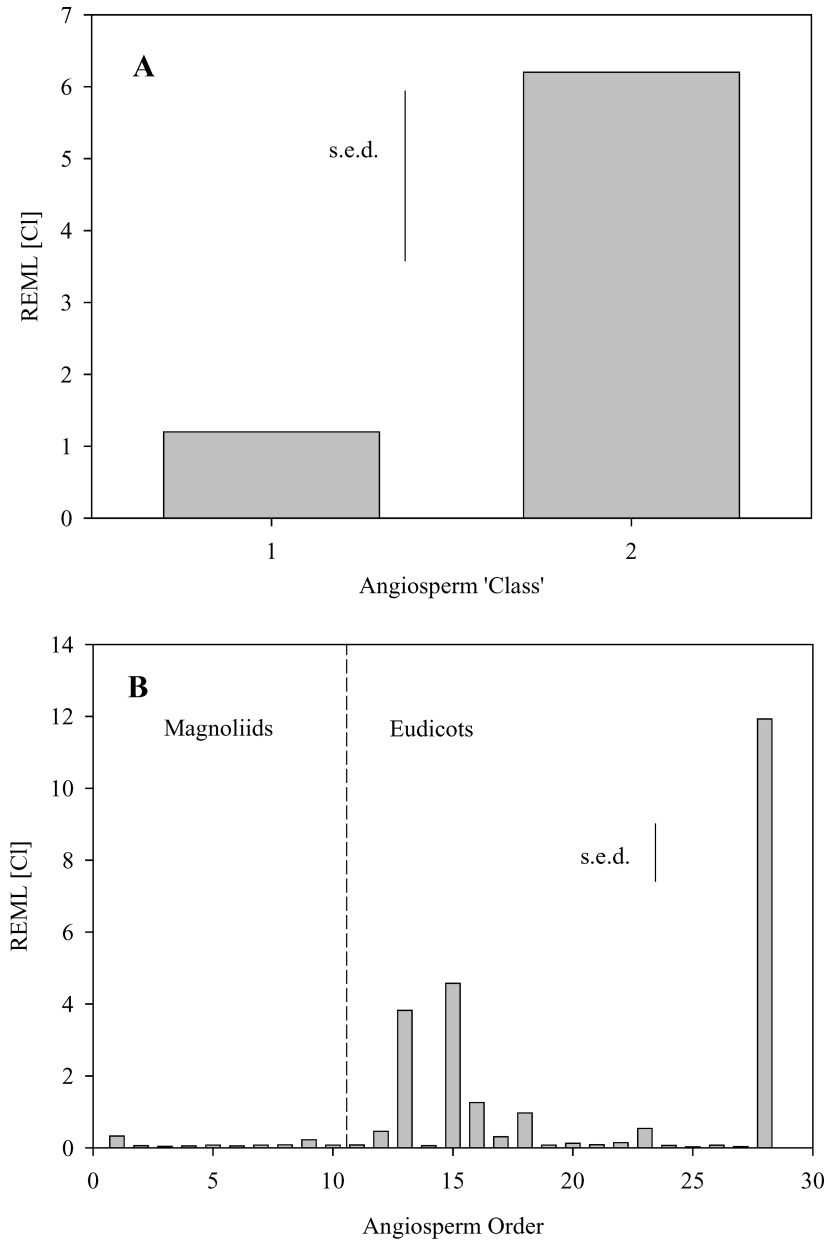
**Figure 1** The frequency distribution of REML <sup>36</sup>Cl concentrations for 98 species of angiosperm acutely exposed to <sup>36</sup>Cl.

This suggests that, in the field, <sup>36</sup>Cl concentrations across most plant species after acute exposures will be log<sub>e</sub>-normally distributed, but also that there are some species that might have unusual <sup>36</sup>Cl uptake.

Hierarchical ANOVA revealed that there were some significant effects of taxonomic rank on relative <sup>36</sup>Cl concentrations in plants (Table 2), in particular at the Ordinal level. Approaching 35% of all differences in uptake were associated with Order or above. This distribution of variance contrasts strongly with that of elements such as N and P for which there is very little effect of taxonomic rank on concentration, *i.e.*, almost all variance is at the rank of species. Overall, the Eudicot “clades” (branches of common descent on the evolutionary tree) had significantly higher relative <sup>36</sup>Cl concentrations than monocot and

**Table 2** Results of hierarchical ANOVA on mean concentration of <sup>36</sup>Cl in 106 species of angiosperm coded with the phylogeny of Soltis *et al.* (1999) and using ranks above Order nominally

Taxonomic level	df	Sum of squares × 100	% SS	Mean square	Variance ratio
Class	1	7.47	2.53	2.49	1.49
Subclass	3	1.39	0.47	0.466	0.28
Group	3	2.72	0.92	1.36	0.82
Superorder	4	12.39	4.20	3.1	1.86
Order	15	77	26.11	5.14	3.08
Family	22	25.6	8.68	1.17	0.7
Genus	30	125	42.39	4.17	2.5
Residual	26	43.3	14.68	1.67	
Total	105	294.87			



**Figure 2** Mean REML concentrations of  $^{36}\text{Cl}$  after acute exposure in plant taxa coded using the angiosperm phylogeny of Soltis *et al.* (1999). s.e.d = standard error of the difference maximum-minimum,  $n$  = number of species measured with five replicates of each. **A:** Classes, s.e.d. = 2.24; 1 = Magnoliids ( $n = 29$ ), 2 = Eudicots ( $n = 77$ ). **B:** Orders, s.e.d. = 3.62; 1 = Laurales ( $n = 2$ ), 2 = Piperales ( $n = 3$ ), 3 = Alismatales ( $n = 2$ ), 4 = Arecales ( $n = 2$ ), 5 = Commelinales ( $n = 1$ ), 6 = Poales ( $n = 8$ ), 7 = Zingiberales ( $n = 3$ ), 8 = Asparagales ( $n = 3$ ), 9 = Dioscorales ( $n = 1$ ), 10 = Liliales ( $n = 5$ ), 11 = Ranunculales ( $n = 3$ ), 12 = Proteales ( $n = 2$ ), 13 = Caryophyllales ( $n = 9$ ), 14 = Ericales ( $n = 2$ ), 15 = Apiales ( $n = 6$ ), 16 = Asterales ( $n = 6$ ), 17 = Solanales ( $n = 6$ ), 18 = Lamiales ( $n = 6$ ), 19 = Saxifragales ( $n = 4$ ), 20 = Geraniales ( $n = 1$ ), 21 = Myrtales ( $n = 4$ ), 22 = Brassicales ( $n = 6$ ), 23 = Malvales ( $n = 3$ ), 24 = Sapindales ( $n = 3$ ), 25 = Malpighiales ( $n = 2$ ), 26 = Rosales ( $n = 7$ ), 27 = Fabales ( $n = 5$ ), 28 = Cucurbitales ( $n = 2$ ).



allied clades (Figure 2A). Although there was much variation around mean values, all the taxa with high relative  $^{36}\text{Cl}$  concentrations are on the Eudicot clades. There is evidence that these differences arise from particular Orders of plants with high and low uptake of  $^{36}\text{Cl}$  (Figure 2B). On the Eudicot clade, the Caryophyllales ( $n = 9$ ), the Asterid II Order Apiales ( $n = 6$ ) and the Rosid II Order Cucurbitales ( $n = 2$ ) have high uptake, helping to explain the high mean value of the Eudicot clade. It is also notable that the Eudicot Orders Asterales ( $n = 6$ ) and Lamiales ( $n = 6$ ) have above-average  $^{36}\text{Cl}$  uptake. The Poales (Grasses and allies) and Liliales (Lilies and allies), both represented quite well in the dataset with  $n = 8$  and  $n = 5$ , respectively, are primarily responsible for low uptake by the monocot clades and contain no taxa with high  $^{36}\text{Cl}$  uptake. Well-represented Eudicot groups with relatively low  $^{36}\text{Cl}$  uptake include the Rosales ( $n = 7$ ), Brassicales ( $n = 6$ ), and Fabales ( $n = 5$ ) (Figure 2B).

## DISCUSSION AND CONCLUSIONS

Table 1 provides the largest reported comparison of  $^{36}\text{Cl}$  uptake between different plant taxa. However, conclusions must be drawn from the data with care because the plants were subjected to a short pulse of  $^{36}\text{Cl}$  and the species used are only a small subset of the flowering plants. Plants take up a high proportion of their minerals during the exponential growth phase, so pulsing  $^{36}\text{Cl}$  into them in this phase is likely to reflect something of their relative uptake rates. However, the relationship between pulse length and  $^{36}\text{Cl}$  concentration that might be attained at the end of a growth period is not clear. So, the data in Table 1 are, perhaps, most applicable to acute exposure to  $^{36}\text{Cl}$ , but it is likely that they will also be relevant to chronic exposure. It is notable that pulses of availability in  $^{36}\text{Cl}$  to plants occur in periods when evapotranspiration exceeds precipitation and values from acute exposures are thus potentially of direct relevance to some field conditions.

Many of the species included in experiments for Table 1 were herbaceous annual plants and the relative number of species sampled from each clade does not reflect exactly the relative number of species on the clades. Broadley *et al.* (2003), investigating phylogenetic effects for Ca, designed a species-sampling regime that did reflect exact relative numbers of species on clades but concluded that, with in excess of 206 taxa, it was no more powerful in identifying higher level phylogenetic effects than one that was simply spread across the clades. Although the species used here do not reflect exactly the relative numbers on the angiosperm clades and are clearly only a small subset of all angiosperm species, their spread and number is probably sufficient to enable us to at least assess the higher level taxonomic sources of difference in the dataset. Table 1 includes relatively few woody or aquatic species but many crop plants. Therefore, it has, a bias toward plants that might contribute to human radiation doses via food, but includes values for many plants that might be useful in phytoextraction or phytoremediation.

Modelers of  $^{36}\text{Cl}$  behavior in the soil–plant system have previously noted interspecies differences in plant uptake. However, it has not previously been noted that there is a phylogenetic effect in interspecies differences in  $^{36}\text{Cl}$  uptake by plants. The existence of such a signal indicates, primarily, that species are not independent sampling units for  $^{36}\text{Cl}$  concentration, but are linked through phylogeny. Clearly, this has implications for the models and statistical analyses of  $^{36}\text{Cl}$  concentrations that assume that species are independent sampling units but also means that, for maximal efficiency, the search for phytoextraction and phytoremediation candidates should be focused on particular clades of plants. Table 2 shows that interspecies variance can be ascribed to taxonomic units other than the species,

*e.g.*, particular genera. The species is a reproductive unit and there is no *a priori* reason why it should be associated with differences in ion concentration. Table 2 shows that those considering phytoremediation of  $^{36}\text{Cl}$ -contaminated soils might fruitfully think beyond the species unit when trying to categorize plant uptake. The phylogenetic effect on  $^{36}\text{Cl}$  uptake by plants therefore offers a framework for general predictions of  $^{36}\text{Cl}$  uptake by plants. This is useful because measurement of uptake for all species is impractical and general predictions based on phylogenetic position might expedite the search for useful plants. Recognizing groups of plants that have low or high uptake of  $^{36}\text{Cl}$  might enable plants to be used to, respectively, minimize  $^{36}\text{Cl}$  transfer to food/forage or to maximize phytoextraction. However, this will only be the case if the magnitude of variation between species is great enough.

The interspecies differences for  $^{36}\text{Cl}$  are quite large compared to those reported for other plant nutrients. This has been noted previously for  $^{36}\text{Cl}$  as compared to other radionuclides (Coughtrey *et al.*, 1983) and for  $^{35}\text{Cl}$  in plant nutrition (White and Broadley, 2001). At 35% of all interspecies differences, those at the level of Order and above for  $^{36}\text{Cl}$  were greater than those reported for P (6.8%) and N (3.3%) (Broadley *et al.*, 2004), Pb (20%), Cr (23%), Cu (24%), Cd (27%) (Broadley *et al.*, 2001), and Na (23%; Broadley *et al.*, 2004), approaching those for Zn (44%) and Ni (46%) and less than those for K (49%; Broadley *et al.*, 2004) and Ca (63%; Broadley *et al.*, 2003). Overall, therefore, not only is there variation in  $^{36}\text{Cl}$  uptake between species, but its magnitude and the phylogenetic effects it includes provide significant, exploitable variation.

In general, the monocots and associated lineages in the Magnoliid clades have low relative uptake of  $^{36}\text{Cl}$ , including clades with numerous crop plants, *i.e.*, the Poales (cereals) and Liliales (onions and relatives). It also is noteworthy that the Brassicales (cabbage and relatives) and Fabales (beans and relatives), both of which contain numerous crop plants, have low relative  $^{36}\text{Cl}$  values. These Orders might be a source of “safe crops” that could be grown on contaminated soils. All of the Orders with high relative uptake are Eudicots and the Caryophyllales, Apiales, and Cucurbitales contain numerous crop plants (beets, cucurbits, celery, and their relatives, respectively). The data reported here indicate, therefore, that distinguishing between these taxa with low and high relative uptake might be useful to plant-based management of  $^{36}\text{Cl}$ -contaminated land. They will only be useful, however, if their absolute uptake is sufficiently low or high.

$^{35}\text{Cl}$  is a plant micronutrient and is often accumulated to much higher concentrations than is required for normal functioning (Taiz and Zeiger, 2002). Menzel (1965) classified  $^{36}\text{Cl}$  as an isotope that is “strongly accumulated” by plants with soil-to-plant transfer factors of 10 to 1000 and Coughtrey *et al.* (1983) suggested a soil-to-plant transfer factor of 50 for radioecological models. Such transfer factors indicate substantial potential for phytoextraction of  $^{36}\text{Cl}$  from soil, perhaps as great as for any other contaminant, and it has long been known that plant uptake can greatly deplete soil  $^{35}\text{Cl}$  (Wiklander and Andersen, 1974). Like  $^{99}\text{Tc}$ ,  $^{36}\text{Cl}$  is likely to be available in most soils and to be predisposed to phytoextraction. Therefore, the data reported here suggest plants that might have sufficient soil-to-plant transfer of  $^{36}\text{Cl}$  to optimize phytoextraction. In particular, we predict that the best phytoextractors of  $^{36}\text{Cl}$  uptake will be in the Caryophyllales, Apiales, and Cucurbitales. Such transfer factors also indicate that it will be challenging to find “safe crops” for  $^{36}\text{Cl}$ -contaminated soils. We predict, however, that the taxa shown here to have relatively low uptake of  $^{36}\text{Cl}$  are at least the most likely to be a source of “safe crops”. Phytoremediation of  $^{35}\text{Cl}$  necessitates plant tolerance of a high concentration of  $\text{Cl}^-$ , which was not included in the experiments reported here. However, the existence of a phylogenetic signal

in  $^{36}\text{Cl}$  uptake by plants indicates that angiosperm phylogenies might also provide a useful perspective for understanding the uptake and tolerance of  $^{35}\text{Cl}$  in salinized soils.

Finally, the data reported here also suggest appropriate plants for biomonitoring of  $^{36}\text{Cl}$ , a potentially useful adjunct to plant-based contaminant management. Those species or taxa with high uptake might be a source of sentinel species for biomonitoring and the frequency distribution in Figure 1 indicates that parametric extrapolations could be made for the majority of species from biomonitoring data. We suggest, therefore, that understanding the phylogenetic signal in plant Cl uptake might be useful for a variety of aspects of  $^{36}/^{35}\text{Cl}$  phytoremediation. Clearly, the database reported here can be improved in many ways, from taxonomic spread to exposure time, but it provides a useful starting point for utilizing the phylogenetic signal in  $^{36}\text{Cl}$  uptake to capitalize on the predisposition of  $^{36}/^{35}\text{Cl}$  to phytoremediation.

## ACKNOWLEDGEMENTS

We would like to thank the UK Food Standards Agency for funding this work and Judy Brown for her help with radioanalysis.

## REFERENCES

- APG (Angiosperm Phylogeny Group) II. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Bot. J. Linn. Soc.* **141**, 399–436.
- Bennett, R. and Willey, N.J. 2003. Soil availability, plant uptake and soil-to-plant transfer of  $^{99}\text{Tc}$ —A review. *J. Environ. Radioac.* **65**, 215–231.
- Bentley, H.W., Phillips, F.M., and Davis, S.N. 1986. Chlorine-36 in the terrestrial environment. In: *Handbook of Environmental Isotope Geochemistry*, pp. 427–480 (Fritz, P. and Fontes, J.C., Eds.). Amsterdam, The Netherlands, Elsevier.
- Bohn, H.L., McNeal, B.L., and O'Connor, G.A. 1979. *Soil Chemistry*. New York, John Wiley & Sons.
- Broadley, M.R., Bowen, H.C., Cotterill, H.L., Hammond, J.P., Meacham, M.C., Mead, A., and White, P.J. 2003. Variation in the shoot calcium content of angiosperms. *J. Exp. Bot.* **54**, 1–16.
- Broadley, M.R., Bowen, H.C., Cotterill, H.L., Hammond, J.P., Meacham, M.C., Mead, A., and White, P.J. 2004. Phylogenetic variation in the shoot mineral concentration of angiosperms. *J. Exp. Bot.* **55**, 321–336.
- Broadley, M.R., Willey, N.J., and Mead, A. 1999. A method to assess taxonomic variation in shoot caesium concentration among flowering plants. *Environ. Pollut.* **106**, 341–349.
- Broadley, M.R., Willey, N.J., Wilkins, J., Baker, A.J.M., Mead, A., and White, P.J. 2001. Phylogenetic variation in heavy metal accumulation in angiosperms. *New Phytol.* **152**, 9–27.
- Burns, I.G. 1974. A model for predicting the redistribution of salts applied to fallow soils after excess rainfall or evaporation. *J. Soil Sci.* **25**, 165–178.
- Coughtrey, P.J., Jackson, D., and Thorne, M.C. 1983. *Radionuclide Distribution and Transport in Terrestrial and Aquatic Ecosystems*, Vol 2. Rotterdam, The Netherlands: AA Balkema.
- Cremers, A., Elsen, A., Peter, de P., and Maes, A. 1988. Quantitative analysis of radiocaesium retention in soils. *Nature* **335**, 247–249.
- Ghosh, G. and Drew, M.C. 1991. Comparison of analytical methods for extraction of chloride from plant tissue using  $^{36}\text{Cl}$  as a tracer. *Plant Soil* **136**, 265–268.
- Greenway, H. and Munns, R. 1980. Mechanisms of salt tolerance in non-halophytes. *Ann. Rev Plant Physiol.* **31**, 149–190.

- Huang, J.W., Blaylock, M.J., Kapulnik, Y., and Ensley, B.D. 1998. Phytoremediation of uranium contaminated soils: Role of organic acids in triggering uranium hyperaccumulation in plants. *Environ. Sci. Technol.* **32**, 2004–2008.
- Menzel, R.G. 1965. Soil-plant relationships of radioactive elements. *Health Phys.* **11**, 1325–1332.
- Sheppard, S.C., Johnson, L.H., Godwin, B.W., Tait, J.C., Wuschke, D.M., and Davison, C.C. 1996. Chlorine-36 in nuclear waste disposal. 1. Assessment results for used fuel with comparison to I-129 and C-14. *Waste Manag.* **16**, 607–614.
- Soltis, P.S., Soltis, D.E., and Chase, M.W. 1999. Angiosperm phylogeny inferred from multiple genes as a research tool for comparative biology. *Nature* **402**, 402–404.
- Taiz, L. and Zeiger, E. 2002. *Plant Physiology*, 3rd ed. MA: Sunderland, Sinauer.
- Thompson, R. and Welham, S.J. 2000. REML analysis of mixed models. In: *The Guide to Genstat, Part 2—Statistics*, pp. 413–503 (Payne, R.W., Ed.). Oxford, UK, VSN International.
- Yang, J. and Blanchar, R.W. 1993. Differentiating chloride susceptibility in soybean cultivars. *Agron. J.* **85**, 880–885.
- White, P.J. and Broadley, M.R. 2001. Chloride in soils and its uptake and movement within the plant. *Annals Bot.* **88**, 967–988.
- Wiklander, L. and Andersen, A. 1974. The composition of the soil solution as influenced by fertilisation and nutrient uptake. *Geoderma* **11**, 157–166.