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Compartmentalisation of uranium and other elements in plant biomass: A review of literature and results from the RP1 Constructed wetland filter

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Abstract

Key Words: uranium; *Eleocharis sphacelata*; *Cyperaceae*; constructed wetland filter; plant composition; plant biomass; plant uptake; literature review

A review of the literature on U uptake by plants has shown there to be a paucity of data, especially for hydrophytes. In many instances, data are compromised by a lack of quality assurance protocols to determine the level of contamination of shoots and roots by soil and/or dust. In addition, there is poor understanding of U absorption by plants, response, translocation and storage. Where U is labile and present in relatively high concentrations in a substrate, plants are capable of translocating U to the shoot but with higher concentrations generally found in the root. The U content of plants growing in contact with a background level of U in soil/sediment is commonly <0.1 mg U/kg DM.

Samples of *Eleocharis sphacelata* encompassing young and mature shoots, and roots were collected from a number of sites in the RP1 CWF during May–September 1997 and their elemental composition determined. The effectiveness of washing plant tissue to remove surface contamination was investigated.

Our work with *E. sphacelata* showed roots to contain significantly higher concentrations of Ti, Fe, Al, Co, Mo, Pb and U compared with shoots. However, our results imply that adhering soil, and the presence of a Fe plaque on roots, contributed to these elevated concentrations. Similarly, there was evidence to suggest that washing was partly successful in removing contamination from shoots although contamination was less pronounced than for roots. Unwashed shoots were typified by having enhanced concentrations of Ti, Fe, Al, Pb and U.

Young shoots were characterised by having significantly higher concentrations of P, Cu and Mo and lower concentrations of Mg, Ca, S, Ni and Pb compared with mature shoots. Despite a strong relationship between the distribution of sediment U in the RP1 CWF and path length, there was no evidence of a U concentration gradient in plant tissue.

The mass of U associated with *E. sphacelata* biomass is small in relation to the quantity of U retained by the RP1 CWF from the treatment of RP2 mine water. At any one time during the sampling interval, the U in *E. sphacelata* biomass accounted for less than 0.1–4.2% of retained U, but over the three year operational time of the RP1 CWF a much larger portion of retained U will have been turned over in organic matter. The determination of organic matter turnover rate and the fate of U associated with plant material remains a fundamental requirement to understanding the function and future performance of wetland filters at Ranger. Our work highlights the difficulties inherent in studying plant uptake of U *in situ* and stresses the appropriateness of using controlled environmental conditions for future work to elucidate U uptake and turnover.

1 Introduction

Previous studies have discussed the design, function, performance and sediment properties of the Retention Pond #1 Constructed Wetland Filter (RP1 CWF) at Ranger Uranium Mine (leGras & Klessa 1997; Klessa et al 1998). In summary, the majority of the U load from Retention Pond #2 (RP2) water passing through the RP1 CWF is polished of which between two-thirds and three-quarters is thought to be accounted for in sediment. Of this sediment U, about half the total is potentially labile based upon its extraction by 0.5 M $NaHCO_3$ (Klessa et al 1998).

In contrast, little is known about the capacity of biota, particularly plants, to absorb and compartmentalise U in wetland systems. For artificial wetlands at Ranger, this is important not only for understanding the factors influencing the annual efficiency of operation of a CWF to clean mine waste waters but over the longer term the performance of sentinel wetland systems will likely depend upon the turnover of plant biomass C and the provision of new adsorption sites in sediment from the humification of organic C.

Quality assurance procedures are essential to ensure that the possibility of contamination of plant material, especially roots, by soil is checked and accounted for. This is particularly important in radiological studies since a small degree of contamination of plant samples by soil or sediment can easily give rise to erroneous conclusions on biological uptake. Surprisingly, few radiological studies concerned with quantifying plant uptake appear to have addressed the need for soil contamination indicators exceptions being Ibrahim & Whicker (1988) and Sheppard & Evenden (1990) who used Ti and Dreesen et al (1978) who relied upon Al. An example of such a problem is shown in Figure 1.1. The data derives from samples of shoots and roots of *Eleocharis* spp. taken from the RP1 CWF in 1996 and shows a strong relationship ($p<0.001$) between U and Fe contents. Since the concentration of Fe in plants is generally of the order of <100 mg/kg dry matter (DM) (Mengel & Kirkby 1978), and is measured in mineral soil at the percentage level (ie commonly 1–5% Fe), the presence of Fe contents in plant tissue orders of magnitude greater provides strong evidence of contamination.

Potentially, there are several problems associated with determining the concentration of an element in macrophytes *arising from biological uptake* in a contaminated environment, particularly one characterised naturally by a low concentration ratio between plant biomass and sediment. First, there is the risk of cross contamination of plant material by the substrate in which the plant grows. This often becomes a serious problem when studies involve the sampling of below-ground biomass. Second, McBride & Noller (1995) drew attention to problems associated with sampling wetland plants with attached periphyton. The presence of periphyton on the surface of shoots of submerged macrophytes may make a significant contribution to the apparent concentration of a metal in macrophyte tissue if the periphyton is not adequately removed. Periphyton are efficient at removing heavy metals from solution (Vymazal 1984) particularly in low alkalinity waters which favour the precipitation of metals inside algal biofilms (Liehr et al 1994). However, periphyton may only be removed with difficulty. For example, McBride & Noller (1995) noted that only around 30–40% of periphyton was removed using physical dislodgement by sonification but advised against chemical procedures to achieve more efficient removal because of the risk of cell component loss. Third, the submerged portions of wetland plants may provide a surface on to which products of precipitation and suspended solids from the water column may form and collect. Iron plaque may also form on root and rhizomes surfaces of wetland plants (Taylor

&Crowder 1983; Otte et al 1989) thus providing a surface for metal adsorption (Twining 1993) and constituting as much as 6.4% of root dry weight as Fe (Crowder & Macfie 1986).

Currently ***eriss*** does not have a standard procedure for vetting plant samples for *soil* contamination. In this regard, titanium (Ti) is a useful signature and has long been used in field-based trace element studies. Its usefulness as an indicator of soil contamination derives from its relatively high concentration in soil, especially tropical soils, compared to that in plants. The average Ti content of Australian soils is 0.6% with >3% found in tropical environments (Stace et al 1968). In comparison, Ti concentration in crops range from 0.1–20 mg/kg DM (Scott et al 1971) and 0.15–80 mg/kg DM (Kabata-Pendias & Pendias 1984). As a guideline for mixed herbage, Scott et al (1971) provide a value of >2 mg Ti/kg DM as indicative of soil contamination. Sheppard & Evenden (1990) quote a mean (±SD) blueberry leaf Ti content of 4.00 (± 2.11) and a median concentration ratio (ie plant Ti content/soil Ti content) of 0.0016.

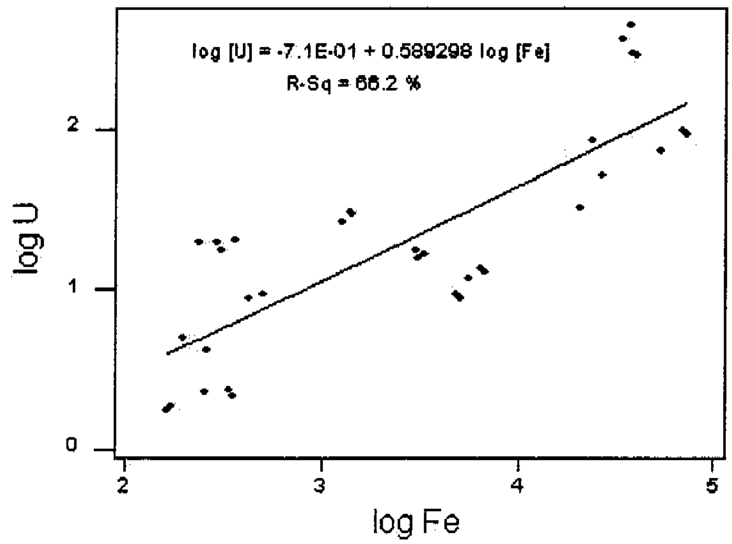

Fig 1.1 The relationship between U and Fe content in contaminated *Eleocharis* spp. shoot and root samples

The main objective of the work described here was to provide an overview of the importance of wetland plants, particularly *Eleocharis* which dominates the RP1 CWF, as a sink for U and other contaminants derived from RP2 water processed by the wetland. Information thus gleaned from the study would provide a basis for developing strategic research with respect to the role of macrophytes in constructed wetland filters. The results contained in this report relate to the 1997 Dry season when the RP1 CWF was last used to treat restricted release zone (RRZ) water from Ranger mine. More specifically the study had the following aims with special reference to U content:

1. To determine the effect of RP2 water on the composition of *Eleocharis*.
2. To compare the composition of shoots and roots of *Eleocharis*.

3. To examine the influence of path length (ie location within the RP1 CWF) on the composition of *Eleocharis*.
4. To assess the importance of plant maturity as a factor determining composition.
5. To recognise a signature of contamination of plant tissue by sediment and to ascertain its degree thereby maximising quality assurance in the reporting of results.

2 Methods

2.1 Sampling

Plant samples were taken from a number of sites in the RP1 CWF and over a number of dates during the 1997 Dry Season (Table 2.1). The first sample, taken on 7 May preceded the start of the operational period in the wetland which began on 27 May and ended on 8 October (ERA 1998). With the exception of samples of algae (identified as *Chara spp*) taken from Cells 1 and 4 on 7 May, all other plant samples were identified exclusively as *Eleocharis sphacelata* which dominates the RP1 CWF. *E. sphacelata* shoots were sampled using stainless steel secateurs by cutting approximately 5 cm above the sediment-water interface. On some occasions, separate samples of young and mature shoots were taken, arbitrarily defined by <30 cm and >30 cm heights respectively. Roots were sampled from whole plants (whose shoots were separated and retained for analyses) by cutting and lifting sods with a stainless steel spade. However, the difficulty of removing intact plants *in situ* is such that it was unlikely that the whole root mass of individual plants was harvested. On three occasions, namely 20 June, 22 July and 4 September, fresh weight yields of *E. sphacelata* shoots were measured within a 1 m^2 quadrat in Cell 5 to provide an estimate of plant shoot biomass in the wetland filter. All plant samples were placed in sealed polythene bags for transport to the laboratory.

Table 2.1 Dates of sampling of *E. sphacelata* and sample types

Sample type	Cell 1	Cell 4		Cell 5	Cell 7		Cell 8
		inlet	outlet		inlet	outlet	
Washed young shoots	7 May	7 May	7 May		7 May	7 May	
	20 June			20 June			
	4 Sep						
Washed mature shoots	7 May	7 May	7 May	22 July	7 May	7 May	22 July
	20 June						
	22 July	22 July					
	4 Sep	4 Sep		4 Sep			4 Sep
Washed composite shoots	13 May	13 May		13 May	13 May		13 May
							20 June
Unwashed mature shoots	22 July	22 July		22 July			22 July
Washed roots	13 May	13 May		13 May	13 May		13 May

2.2 Sample preparation, digestion and analyses

Within two hours of sampling, plant samples were washed initially under tap water and finger rubbed gently under flowing water without rupturing the tissue to remove adhering periphyton and plaque. This was followed by rinsing under deionised water. Samples specifically retained as 'unwashed' did not undergo any washing. Stems and roots were then cut into approximately 1 cm lengths using stainless steel scissors and the plant material placed in aluminium foil trays for drying in a forced-draught oven set at 40°C. After drying, each sample was passed through (<0.5 mm) a *Culatti* grinding mill and stored in polycarbonate screw-top containers.

On one occasion (20/5/97), the unwashed sheath was removed from mature stems of *E. sphacelata* sampled from Cell 5 and retained separately for analysis. Unfortunately, its associated samples (ie 'unwashed stem minus sheath' and 'washed stem minus sheath') were inadvertently lost.

Approximately 0.5 g milled plant material was placed in teflon digestion vessels containing inner linings to which 5 mL concentrated *Aristar* grade HNO_3 was added. Samples were then digested in a *Questron Q Wave-1000* microwave using programmed pressure control and 1000 W power. The program was as follows with dwell time at each step of 2 min:

1. 5 min ramp to 20 psi and dwell.
2. Zero ramp to 40 psi and dwell.
3. 2 min ramp to 60 psi and dwell.
4. Five successive ramps of 2 min with increments of +20 psi and corresponding dwell times to reach a maximum of 160 psi.

After cooling, the contents of the digestion vessels were transferred to 50 mL volumetric flasks and made up to the mark with *Milli-Q* deionised water. A 5 mL aliquot of each solution was then placed in a series of 50 mL HDPE bottles and further diluted with 45 mL *Milli-Q* deionised water. An internal standard containing In and Tl was then added to the solutions.

Each microwave digestion batch, consisting of 12 samples, contained a blank, three separate reference plant sample (repeated on each run) with the remainder composed of duplicated plant samples for analyses. The standard reference materials consisted of *Platihypnidium ripariodes* (CRM 061) and tomato leaves (SRM 1573). The internal standard was derived from a large bulked sample of washed and milled (<0.5 mm) *Eleocharis sphacelata* stems taken from Cell 5 on 20 June 1997 and prepared in the same way as samples . For each run, oven-dry (OD) moisture contents of subsamples of reference and unknown plant samples were determined by drying overnight at 105°C.

Digests were analysed using ICP-MS and ICP-AES for Mg, Ca, P, Ti, Mn, Fe, Al, S, Co, Ni, Cu, Zn, Mo, Cd, Pb and U. All results are expressed on an OD weight basis.

2.3 Data handling

Before applying statistical methods, such as analysis of variance which rely upon normally distributed data, data was first checked for normality using the Ryan-Joiner test. Unless stated to the contrary, data was transformed to achieve a normal distribution using logarithms.

3 Results

3.1 Reference materials

The results from analysing the standard and internal reference materials are given in Tables 3.1 & 3.2. Reference materials CRM 061 and SRM 1573 have certified values for Mn, Cu, Zn, Cd and Pb; and P, Ca, Mn, Fe, Cu, Zn, Pb and U respectively. Results for P, Ca, Mn, Cu, Cd and Pb are excellent and within certified values. In addition, all the reference materials showed good reproducibility for the macronutrients (ie Ca, Mg, S and P).

Of the remaining certified elements, the poor result for the U assay of SRM 1573 is not surprising because of the dilution rate used in preparing the extracts for ICP-MS determination. The concentration of U in the diluted extract of SRM 1573 is approximately 0.07 µg/L which is only around three times the detection limit (ie 0.02 µg/L). While the mean Zn result for SRM 1573 was good, consistently high values, around 100 µg/g greater than the certified value, were obtained with CRM 061. However, this was not caused by contamination during digest preparation and handling. Rather the similar variability around the Zn means for the standard (Table 3.1) and internal (Table 3.2) reference materials infers errors in the determination of Zn by ICP-MS. Recoveries of Fe (70%) and Al (16 and 30%) reflect incomplete digestion by HNO_3 and the use of HF would likely improve their extraction (White 1996). The recovery of Ti based upon an uncertified concentration in CRM 061 was not quantitative with only around 5% accounted for.

3.2 Effect of maturation on shoot composition

Analytical results for young and mature shoots sampled on 7 May, 20 June and 4 September 1997 are listed in Appendix A (Tables A1–A3) and shown graphically in Figures A1–A12. A two-way analysis of variance (ANOVA) of the effects of maturation and sampling site on the composition of plant tissue was limited to the data derived from the 7 May sampling (Table A1 and Figures A1–A4).

The mean treatment effect of maturation on plant composition is summarised in Table 3.3. Young shoots were characterised by having significantly higher concentrations of P, Cu and Mo and lower concentrations of Mg, Ca, S, Ni and Pb compared to mature shoots. Data from the later samplings (Tables A2 & A3) showed the same pattern in differences between young and mature shoots for the macronutrients (ie Mg, Ca, S and P) but not necessarily for the micronutrients (ie Ni, Cu and Mo) and Pb.

3.3 Composition of shoots vs roots

The complete results are contained in Appendix B (Table B1 and Figures B1-B4). A summary of the mean composition (±standard error) of shoots and roots, derived from a two-way ANOVA (with site and plant part as factors), is given in Table 3.4.

Roots contained significantly higher concentrations of the metals Ti, Fe, Al, Co, Mo, Pb and U. Compared to shoot composition and with the exception of Fe, these enhanced metal concentrations were around an order of magnitude greater. In the case of Fe, concentrations were two orders of magnitude higher with a mean of about 4%. In contrast, shoots contained greater concentrations of Mg and Ca than roots.

Table 3.1 Elemental composition (mg/kg DM) of reference plant samples (n = 12) and 95% confidence intervals for certified values

Sample	Value	Mg	P	Ca	Ti	Mn	Fe	Al	S
CRM 061	Mean	3620	8133	15497	41	3708	6198	2794	3987
	SD	61	456	302	32	55	595	204	118
	CV (%)	1.7	5.6	2.0	77.9	1.5	9.6	7.3	3.0
	Certified	–	–	–	–	3771	–	–	–
	95% CI	–	–	–	–	78	–	–	–
	Uncertified	3920	9208	16938	779	–	9302	17148	2300
SRM 1573	Mean	6743	3462	27844	12.8	232	496	367	8951
	SD	105	56	487	1.6	4	26	44	256
	CV (%)	1.6	1.6	1.7	12.5	1.9	5.3	11.8	2.9
	Certified	–	3400	30000	–	238	690	–	–
	95% CI	–	200	300	–	7	25	–	–
	Uncertified	7000	–	–	–	–	–	1200	–

Sample	Value	Co	Ni	Cu	Zn	Mo	Cd	Pb	U
CRM 061	Mean	43.0	396.2	773	695	3.94	1.33	60.6	1.11
	SD	10.6	83.4	236	118	1.38	0.28	4.8	0.10
	CV (%)	24.7	21.0	30.5	16.9	34.9	20.7	7.9	9.2
	Certified	–	–	720	566	–	1.07	64.4	–
	95% CI	–	–	31	13	–	0.08	3.5	–
	Uncertified	–	–	–	–	–	–	–	–
SRM 1573	Mean	0.5	1.8	11	70	0.60	2.9	5.8	0.036
	SD	0.1	0.5	2	9	0.15	0.2	0.2	0.020
	CV (%)	20.5	25.7	18.0	12.6	25.1	7.6	3.7	55.7
	Certified	–	–	11	62	–	–	6.3	0.061
	95% CI	–	–	1	6	–	–	0.3	0.003
	Uncertified	0.6	–	–	–	–	3	–	–

Table 3.2 Elemental composition (mg/kg DM) of internal standard (n = 17)

	Mg	P	Ca	Ti	Mn	Fe	Al	S
Mean	5230	822	2580	0.390	120	142	37.3	11041
SD	211	54	115	0.527	5	31	24.1	501
CV (%)	4.0	6.6	4.4	135.1	4.1	21.6	64.6	4.5

	Co	Ni	Cu	Zn	Mo	Cd	Pb	U
Mean	0.049	0.53	1.48	17.7	0.32	0.020	0.16	4.76
SD	0.010	0.35	0.31	2.7	0.12	0.030	0.06	0.34
CV (%)	20.4	66	20.9	15.3	37.5	147.3	37.5	7.1

Table 3.3 Mean composition (mg/kg DM) of young and mature *Eleocharis sphacelata* shoots sampled on 7/5/97

Element	Young shoots	Mature shoots	SE	Significance (p)
Mg	2914	4078	297	0.05
P	1317	570	151	0.02
Ca	920	2628	328	0.02
log Ti	-0.5845	-0.7342	0.0615	NS
S	4273	8638	637	0.008
log Mn	2.3754	2.5762	0.0560	NS
log Fe	2.2517	2.0474	0.0595	NS
log Al	1.3963	1.2586	0.1005	NS
log Co	-0.9816	-1.0618	0.0652	NS
log Ni	-0.2083	0.1288	0.0322	0.02
Cu	5.567	2.900	0.366	0.007
Zn	27.09	22.14	2.25	NS
Mo	0.309	0.156	0.019	0.004
log Cd	-1.6363	-1.8133	0.1184	NS
Pb	0.146	0.430	0.051	0.02
log U	-0.1901	-0.3782	0.0637	NS

Table 3.4 Mean composition (mg/kg DM) of shoots and roots of *Eleocharis sphacelata* sampled on 13/5/97

Element	Shoot	Root	SE	Significance (p)
Mg	3962	2471	188	0.005
P	690	479	62	NS
Ca	2190	546	202	0.004
log Ti	0.2353	1.0352	0.0667	0.001
log S	3.8291	3.7859	0.0386	NS
log Mn	2.6414	2.4662	0.0521	NS
log Fe	2.6435	4.5755	0.1105	0.001
log Al	2.3872	3.2268	0.0864	0.002
log Co	-0.7844	0.3154	0.1166	0.003
log Ni	-0.1304	0.3243	0.1254	NS
Cu	3.91	8.30	1.62	NS
log Zn	1.4009	1.9194	0.1414	NS
Mo	0.24	1.30	0.18	0.02
log Cd†	0.0146	0.2691	0.0889	NS
log Pb	-0.3649	0.7597	0.1139	0.002
log U	0.8410	2.0072	0.0708	0.001

†A value of 1 was added to Cd concentrations to account for a zero (ie not detected) result in the data prior to taking logarithms. The means and SE as given above require to be corrected if back-transformed.

3.4 Effect of washing on shoot composition

Data is given in Appendix C in tabular form (Table C1) and means are graphed for each element in Figures C1–5. A two-way ANOVA (ie site and sample treatment) was conducted on the data, and means for the washed and unwashed shoots are summarised in Table 3.5.

The relative effect of washing on plant composition of each sample was determined by calculating the ratio of the treatment effect for each element (Table 3.6). Hence, elements whose loads had been assimilated by the plant rather than being derived from surface contamination will show ratios of around unity. The data is not normally distributed (ie skewed towards low values) and could not be transformed to give a normal distribution using common transformations. However, a Kruskal-Wallis χ^2 test (ie one-way) in which ratios were ranked showed there to be a significant difference ($p<0.001$) between elements. In general, unwashed samples were typified by having enhanced concentrations of Ti, Fe, Al, Pb, and U (Table 3.6) with the latter three metals being significantly higher than in washed shoots (Table 3.5).

Table 3.5 Mean composition (mg/kg DM) of washed and unwashed shoots of *Eleocharis sphacelata* sampled on 22/7/97

Element	Washed	Unwashed	SE	Significance (p)
Mg	5614	5438	404	NS
P	799	789	21	NS
Ca	2404	2578	102	NS
log Ti	-0.9519	0.1782	0.3791	NS
S	9007	8616	477	NS
log Mn	2.5832	2.7332	0.0454	NS
log Fe	2.2694	2.7834	0.1280	NS
log Al	1.7411	2.5245	0.1249	0.02
log Co	-0.7778	-0.4472	0.1130	NS
Ni	21.9	20.0	1.1	NS
Cu	6.2	6.7	1.1	NS
log Zn	1.3561	1.3805	0.0443	NS
Mo	0.42	0.56	0.05	NS
Cd	1.38	1.16	0.06	NS
log Pb	-0.7504	-0.1437	0.1319	0.05
log U	0.8120	1.5815	0.1598	0.05

Table 3.6 Mean ratios of concentrations of elements in unwashed:washed plant samples.

Mg	P	Ca	S	Ti	Mn	Fe	Al
1.0	1.0	1.1	1.0	16.5	1.5	4.4	7.9

Co	Ni	Cu	Zn	Mo	Cd	Pb	U
2.6	0.9	1.1	1.1	1.3	0.8	5.6	8.4

3.5 Differences between sampling sites

An analysis of the *treatment* effect of sampling site on plant composition was limited to the balanced data sets of 7/5/97 (Table A1; Figs A1–4), 13/5/97 (Table B1; Figs B1–4) and 22/7/97 (Table C1); Figs C1–4) which had been subjected to two-way ANOVA as previously described. Significant ($p<0.05$) effects were restricted to the following sampling dates and elements (with significance shown in parenthesis);

7/5/95	log Ti (0.004); log Mn (0.03); log Co (0.05); log Ni (0.02); log Cd (0.03)
13/5/95	log Mn (0.02); log Pb (0.02); log U (0.02)
22/5/97	P (0.007), log Mn (0.01); Cd (0.034)

Means are given in Table 3.7. Relatively high concentrations of Mn were found in *Eleocharis* samples from Cell 1 on all three sampling dates. The high Mn concentration in *Eleocharis* tissue sampled on 7/5/97 from Cell 1 was also matched by significantly higher Co and Ni contents.

Table 3.7 Mean composition (mg/kg DM) of plant material at sampling sites.

(i) 7/5/97 sampling

Site	log Ti	log Mn	log Co	log Ni	log Cd
Cell 1	-1.0112^{a}	2.8980^{b}	-0.6433^{c}	0.2276^{b}	-1.5920^{a}
Cell 4 inlet	-0.7826^{ab}	2.5462^{a}	-1.2803^{a}	0.0027^{a}	-2.0965^{a}
Cell 4 outlet	-1.1618^{a}	2.2563^{a}	-1.2218^{ab}	-0.1311^{a}	-0.8372^{b}
Cell 7 inlet	0.0938^{c}	2.3033^{a}	-0.8678^{bc}	-0.1603^{a}	-1.8678^{a}
Cell 7 outlet	-0.4348^{b}	2.3752^{a}	-1.0952^{ab}	-0.1377^{a}	-2.2304^{a}
SE	0.0972	0.0886	0.1030	0.0509	0.1872

(ii) 13/5/97 sampling

Site	log Mn	log Pb	log U
Cell 1	2.9485^{c}	-0.2750^{a}	1.3480^{b}
Cell 4	2.7634^{bc}	-0.3077^{a}	0.8606^{a}
Cell 5	2.2767^{a}	0.9792^{c}	1.9121^{a}
Cell 7	2.4703^{ab}	0.5963^{bc}	1.5628^{bc}
Cell 8	2.3100^{a}	-0.0060^{ab}	1.4369^{b}
SE	0.0823	0.1801	0.1120

(iii) 22/7/97 sampling

Site	P	log Mn	Cd
Cell 1	1037.0^{c}	3.0866^{c}	0.96^{a}
Cell 4	611.3^{a}	2.7827^{b}	1.56^{c}
Cell 5	746.3^{b}	2.2999^{a}	1.44^{bc}
Cell 8	780.6^{b}	2.4635^{a}	1.08^{ab}
SE	29.5	0.0652	0.08

Note that for a given element and sampling date, means followed the same subscript are not significantly ($p>0.05$) different.

3.6 Relationships in composition between U and other elements

Relationships between elements in the composition of *E. sphacelata* were investigated initially by correlation using 'washed shoot' data (n = 30) as a precursor to regression analysis. Correlations were conducted on normal data and the matrix is shown in Table 3.8.

The log U content of washed shoots was positively and significantly related to Mg, log Cd, log Fe, log Al and log Co concentrations. Possible indicators of contamination by particulates and/or sediment, namely log Ti, log Al and log Fe, were strongly and positively correlated to one another. Notably, however, Mn (logged) was not related to either U (logged) or the aforementioned indicators of surface contamination.

Further analysis was conducted by incorporating root, and unwashed shoot and sheath results into the data set (n = 40) to test the hypothesis that degree of contamination, as determined by relationships between indicators, and between indicators and U, holds irrespective of the plant part analysed. The corollary to this is that the U content of plant samples is determined by the degree of dilution of the internal, uncontaminated fraction by an external, contaminated fraction which has a large influence on U concentration.

Inclusion of the root, and unwashed shoot and sheath results led to a marked increase in explained variance for linear regression relationships between U and contamination indicators (Fig 3.1). In addition, explained variance was improved greatly between Al and Ti (Fig 3.1). However, incorporation of the extra data for Fe led to a non-normal distribution for both untransformed and logged transformed values. This arose because the Fe content of washed roots and the unwashed sheath were around two orders of magnitude greater than in other plant parts. The very high Fe content of the former did not show a concomitant increase in U concentration and for these reasons the data were omitted from regression (Fig 3.1).

Table 3.8 Correlation matrix of normalised data for washed shoots (n = 29). Asterisks show significance at the 5, 1 and 0.1% confidence levels.

	Mg	log P	Ca	log Ti	log Mn	log Fe	log Al	S	log Co	log Ni	log Cu	log Zn	Mo	log Cd	log Pb	log U
log P	-0.41**															
Ca	0.75***	-0.70***														
log Ti	0.08	-0.22	0.12													
log Mn	0.33	-0.17	0.44*	0.27												
log Fe	0.14	0.13	-0.00	0.60***	0.27											
log Al	0.22	-0.06	0.13	0.68***	0.39	0.76***										
S	0.63***	-0.43*	0.68***	-0.20	-0.04	-0.15	-0.08									
log Co	0.08	0.29	-0.06	0.36	0.45*	0.70***	0.65***	-0.18								
log Ni	0.57***	-0.10	0.24	-0.24	0.16	-0.09	0.08	0.29	0.20							
log Cu	-0.07	0.66***	-0.48**	-0.08	-0.00	0.28	0.10	-0.36	0.60***	0.24						
log Zn	-0.48**	0.50**	-0.55**	0.21	-0.36	0.31	0.28	-0.33	0.50**	-0.11	0.54**					
Mo	0.24	0.52**	-0.25	-0.01	0.01	0.37*	0.25	-0.05	0.49**	0.29	0.74***	0.28				
log Cd	0.40*	0.00	0.11	-0.16	-0.09	0.05	0.16	0.26	0.17	0.80***	0.21	0.12	0.19			
log Pb	-0.14	-0.29	0.17	0.23	-0.02	0.30	0.41*	0.11	0.29	-0.06	-0.11	0.46*	-0.35	0.16		
log U	0.46**	0.14	0.16	0.26	0.28	0.65***	0.73***	0.24	0.68***	0.31	0.34	0.26	0.54	0.40*	0.13	

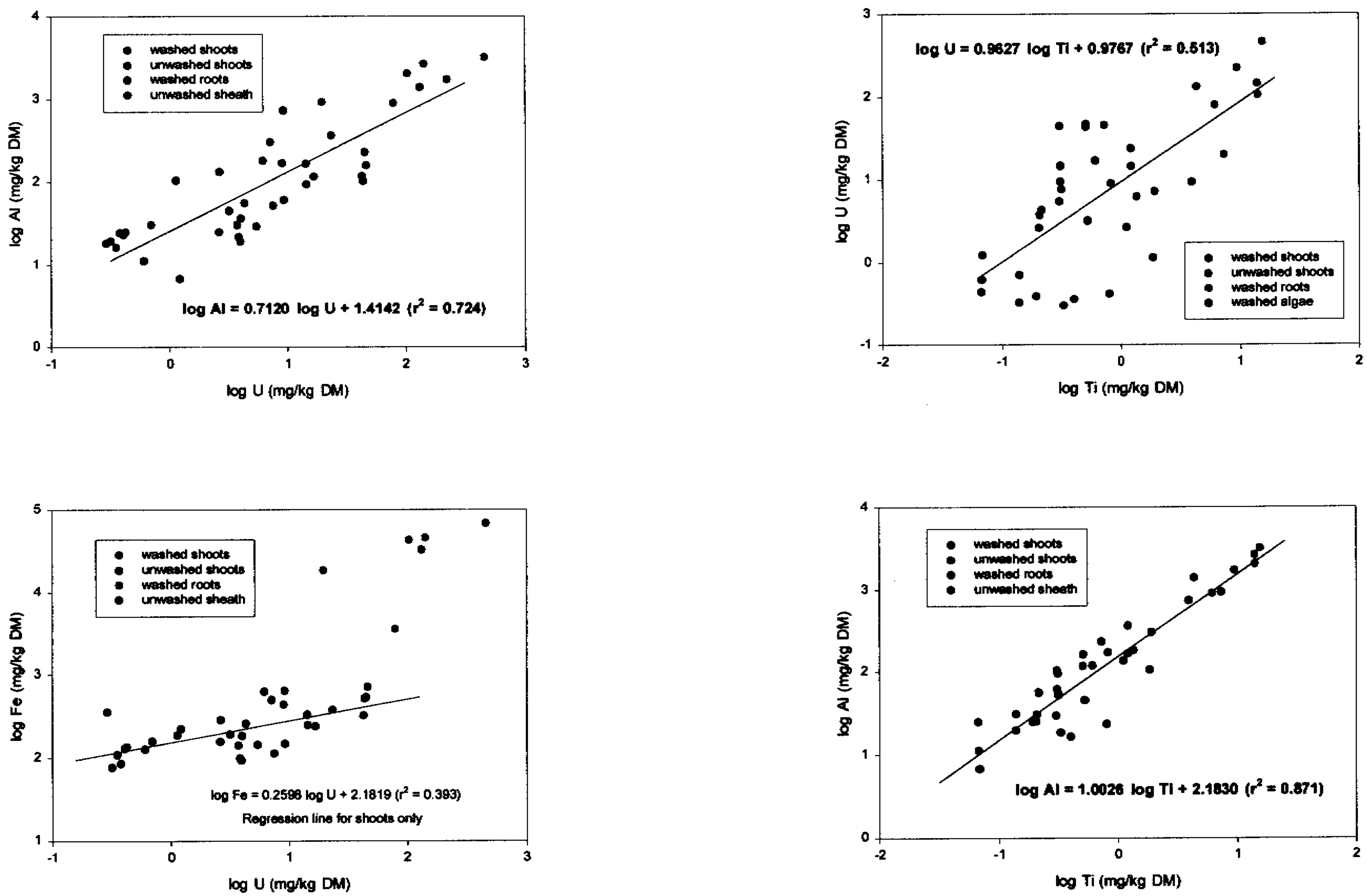

Fig 3.1 Relationships between U, Fe, Al and Ti in plant tissue

4. Discussion

4.1 Review of the literature

The soil/sediment of the RP1 CWF is characterised by high concentrations of total U which decrease exponentially as a function of path length and depth (Klessa et al 1998). Other metals including Mn and Co also show a similar pattern to their distributions in RP1 CWF sediments (Klessa et al 1998). Mean total U contents in the surface 0–1 cm and 0-10 cm depths range from 90–570 and 20–134 mg/kg respectively. In comparison, the typical and average concentration ranges of total U in soil are 1-4 and 1–2 mg/kg (Harmsen & de Haan 1980) and it is concentrations of this order which have provided the baseline for many comparative studies of U uptake by plants between uncontaminated (ie control) and contaminated sites. Indeed, claims have been made in the literature that total U concentrations in soil of around 10 mg/kg, which approaches background concentration, may be toxic to plants although this is likely spurious and probably nearer 300 mg/kg (Sheppard et al 1992).

Critical assessment of our data was undertaken by reviewing the literature on the composition of hydrophytes and U uptake by plants. With the exception of one paper (Greenway 1997) dealing with the N and P content (0.94%) of *E. sphacelata* in contact with wastewater, no relevant published work on this species was found. In the absence of data for *E. sphacelata*, information was obtained on other species of *Cyperaceae* from reviews by Oatridge & Noller (1991) and Vymazal (1995) which we have abstracted and present abridged in Tables 4.1a, b. It is important to note, however, that these data derive almost exclusively from cool, northern hemisphere conditions. The original references cited by these reviewers providing these data are listed in Appendix D. In addition, data on the mean composition of three grasses sampled from the Magela flood plain (ARRI 1987) are given in Table 4.2.

E. sphacelata shoots had a similar composition to other species of *Cyperaceae* (Table 4.1) and to Magela flood plain grasses (Table 4.2) but with some exceptions. The mean concentrations of Mg and S were higher in *E. sphacelata* reflecting their contact with RRZ water in the RP1 CWF which is approximately 4 mM $MgSO_4$. However, the Mg and S contents of *E. sphacelata* were not abnormal compared to plants in general which contain around 0.5% Mg and 0.2–0.5% S (Mengel & Kirkby 1978). Except for Mn, and possibly Ni, the roots of *E. sphacelata* had a higher heavy metal and U content than shoots, the relative difference (based on means) being Fe>Pb>U>Cd>Co>Zn>Mo>Cu>Ni. Notably, the degree of enrichment of *E. sphacelata* roots by metals, especially Fe, Pb, U and Cd, both quantitatively and relatively (to shoots) was generally greater than cited for other species of *Cyperaceae* (Table 4.1) and for Magela flood plain grasses (Table 4.2). In the case of Fe, however, Vymazal (1995) cites a number of papers where concentrations >4% Fe in above ground biomass have been found in a range of wetland plants although this strongly suggests some form of surface contamination. The latter, in the form of Fe plaque, is commonly found on the roots of hydrophytes and may act as a chemical barrier to the uptake of heavy metals (Taylor & Crowder 1983; Otte et al 1989). For example, McLaughlan et al (1985) found 4.8% Fe in unwashed roots of *Agrostis gigantea* growing in mine tailings which decreased to 2.1% after rinsing three times with deionised water. Respective concentrations in shoots were 0.13% and 0.04%.

There is a paucity of data on the U content of wetland plants including *Eleocharis* spp. and, overall, a poor understanding of soil-plant relationships for U and daughter radionuclides

Table 4.1a A comparison of published data on the composition of *Cyperaceae*, and *Eleocharis sphacelata* from this study. 1. Cadmium, cobalt, copper, manganese, molybdenum, nickel, lead, uranium and and zinc contents. Data abstracted from Outridge & Noller (1991) and Vymazal (1995) and references cited in Appendix D.

Cd (mg/kg)	Co (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Mo (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	U (mg/kg)	Zn (mg/kg)	Species
0.00–11	0.04–17	0.14–55	34–6880	0.14–87	0.85–23	0.30–35	0.05–1.1	11–250	Range: freshwater vascular plants[1]
1.0	0.32	7.9	370	12	4.2	6.1	0.50	52	Median
1.9	3.4	13	730	18	6.2	8.1	0.45	66	Mean
1.3–2.5	1.4–5.4	9.7–16	480–990	11-26	4.9–7.6	6.3–9.9	0.06–0.84	54–78	CI (95%)
-	-	-	55–460	-	-	-	-	-	*Carex acutiformis*[2]
-	0.3	1.5	479	-	19.3	-	-	24	*Carex gracilis*[3]
0.02	-	3.0	211	-	7.5	-	-	33	*Carex hudsonii*[3]
<1.0	1.5–1.9	2.0–2.7	-	-	1.0–1.5	3.2–3.9	-	22–29	*Carex lacustris*[4]
-	-	-	-	-	-	-	-	15	*Carex pendula*[5]
-	-	3.1–7.3	440–743	-	-	-	-	14–27	*Carex rostrata*[6]
		(12–17)	(280–365)					(20–24)	
-	*6.7*	*5.6*	*970*	*0.29*	*2.5*	-	-	*63*	*Carex stricta*[7]
0.12	0.01	5.4	189	-	14.3	-	-	48	*Carex vesicaria*[2]
0.4–11.0	-	7–1900	79–5400	-	5–1200	6–150	-	43–200	*Eleocharis acicularis*[8]
-	-	*170*	-	-	-	*76*	-	-	*Eleocharis acicularis*[9]
0.7–1.5	-	4.3	-	-	2.3–3.4	5.0–9.0	-	41–52	*Eleocharis dulcis*[10]
-	-	11	>400	5.0	-	-	-	20	*Eleocharis quandrangulata*[11]
-	-	-	-	19	-	-	-	-	*Eleocharis smallii*[12]
-	-	55	-	-	-	1.9	-	250	*Eleocharis sp.*[13]

Cd (mg/kg)	Co (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Mo (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	U (mg/kg)	Zn (mg/kg)	Species
-	-	-	674	-	-	-	-	-	*Schoenplectus lacustris*[2]
<0.5	0.04	-	-	-	<6.0	-	<0.1 *(<0.1)*	11 *(19-29)*	*Scirpus acutus*[14]
<0.5–7.4	-	-	-	-	*<6.0*	-	*<0.1*	*10–37*	*Scirpus americanus*[14]
-	-	-	-	-	-	-	-	*520–7000*	*Scirpus fluvitans*[15]
-	0.13	-	-	-	5.4	-	-	-	*Scirpus lacustris*[2]
-	-	11	200	-	-	-	-	-	*Scirpus lacustris*[16]
-	*5.6*	*4.8*	-	*0.55*	*1.7*	-	-	*50*	*Scirpus lacustris*[8]
0.2–0.7 (0.4–6.0)	-	3–12 (4–40)	-	-	-	-	-	15–80 (30–440)	*Scirpus maritimus*[17]
<0.5	-	-	-	-	*<6.0–140*	-	*<0.1*	*28–37*	*Scirpus validus*[14]
0.19	-	-	-	-	-	-	0.63	35	*Scirpus sp.*[18]
0.00–1.7	0.05–0.66	1.5–22	69–1377	0.09–0.87	0.20–27	0.04–1.6	0.03–47	11–44	Range; washed shoots; this study†
0.06	0.13	4.5	296	0.34	1.2	0.18	3.9	24	Median
0.07	0.14	4.6	346	0.37	2.1	0.19	3.2	24	Mean
0.03–0.16	0.11–0.19	3.5–6.0	261–459	0.30–0.44	1.2–3.6	0.14–0.26	1.8–5.7	21–27	CI (95%)
0.005–4.0	0.92–4.4	1.8–14	129–579	0.69–1.8	0.66–6.4	1.0–57.1	19.9-460	22-303	Range; washed roots; this study
0.51	1.7	9.7	291	1.4	2.1	4.2	104	82	Median
1.3	2.4	8.3	343	1.3	2.7	15.7	161	136	Mean

Italicised values refer to plants sampled from either wetlands used for wastewater treatment or contaminated environments. Values in parenthisis refer to roots. All other values refer to shoots. †With the exception of Mo, means and confidence levels (CI) are derived from log transformations.

Refs 2–8, 11 & 17 are cited by Vymazal (1995) and the remainder by [1]Outridge & Noller (1991); [2]Allenby (1967); [3]Bican et al (1982); [4]Murdoch & Capobianco (1979); [5]Horovitz et al (1974); [6]Bernard & Bernard (1989); [7]Seidel (1966); [8]Miller et al (1983); [9]Heisey & Damman (1982); [10]Pancontinental Mining Co (1981); [11]Boyd & Vickers (1971); [12]Linn et al (1975); [13]Friant (1979); [14]Wells et al (1980); [15]Nicholas & Thomas (1978); [16]Guilizzoni (1975); [17]Otte et al (1991); [18]Sprenger & McIntosh (1989)

Table 4.1b A comparison of published data on the composition of *Cyperaceae*, and *Eleocharis sphacelata* from this study. 2. Calcium, magnesium, phosphorus, sulphur and iron contents. Data abstracted from Vymazal (1995) and references cited in Appendix D.

Ca (%)	Mg (%)	P (%)	S (%)	Fe (mg/kg)	Notes
0.11	0.15	0.35	-	-	*Bolboschoenus maritimus*[19]
(0.02–0.04)	(0.06–0.11)	(0.40–0.52)			
0.08–0.63	0.10–0.17	0.28–0.35	-	-	*Bolboschoenus maritimus*[20]
0.36	0.65	0.07	-	-	*Carex acutiformis*[21]
0.15–0.33	-	-	-	-	*Carex acutiformis*[2]
-	-	0.12	-	-	*Carex aquatilis*[22]
0.08	0.20	0.15	-	-	*Carex aquatilis*[23]
-	0.14	-	-	-	*Carex aquatilis*[24]
-	-	0.2–0.3	-	-	*Carex aquatilis*[25]
0.16–0.33	-	-	-	-	*Carex gracilis*[26]
0.19–0.25	0.08–0.10	-	-	-	*Carex gracilis*[27]
0.27	0.33	0.12		-	*Carex gracilis*[21]
-	0.13–0.21	0.09–0.21	-	--	*Carex gracilis*[26]
-	-	0.12–0.38	-	-	*Carex gracilis*[27]
-	-	-	-	443	*Carex gracilis*[3]
-	-	-	-	193	*Carex husdoni*[3]
0.18	0.33	0.007	-	-	*Carex lacustris*[21]
-	0.13	0.13	-	-	*Carex lacustris*[24]
0.22	0.09	0.14	-	-	*Carex lacustris*[28]
-	-	0.16	-	-	*Carex lacustris*[29]
0.25	0.12	0.17	-	-	*Carex lacustris*[30]
0.32	0.14	0.17	-	-	*Carex lacustris*[31]

Ca (%)	Mg (%)	P (%)	S (%)	Fe (mg/kg)	Notes
-	-	0.25	-	-	*Carex lacustris*[32]
0.44	-	-	-	-	*Carex lacustris*[24]
0.42	0.10	0.09	-	-	*Carex lanuginosa*[24]
-	-	0.45	-	-	*Carex lanuginosa*[32]
-	-	0.14–0.40	-	-	*Carex lyngbyei*[33]
-	-	-	-	61	*Carex pendula*[5]
-	-	0.10	-	-	*Carex rostrata*[34]
-	-	0.18	-	-	*Carex rostrata*[35]
-	-	0.14–0.29	-	-	*Carex rostrata*[36]
0.30	0.10	0.20–0.30 (0.20)			*Carex rostrata*[37]
-	-	-	-	42–84 (2346–6900)	*Carex rostrata*[6]
0.48	0.14	0.17	-	-	*Carex stricta*[31]
-	*0.21*	*0.22*	-	*3800*	*Carex stricta*[7]
-	-	0.15	-	-	*Carex tenuiflora*[38]
-	-	0.15		-	*Carex trisperma*[38]
0.18	0.08	0.20	-	-	*Carex vesicaria*[27]
-	-	-	-	520	*Carex vesicaria*[3]
-	-	0.20	-	-	*Carex spp.*[39]
-	-	0.24	0.28	-	*Eleocharis acicularis*[40]
-	-	-	-	3600–59000	*Eleocharis acicularis*[8]
-	-	-	0.15	-	*Eleocharis quadrangulata*[40]

Ca (%)	Mg (%)	P (%)	S (%)	Fe (mg/kg)	Notes
0.20	0.07	0.10	-	918	*Eleocharis quadrangulata*[11]
0.07–0.25	0.06–0.15	0.23–0.34	-	-	*Schoenoplectus lacustris*[20]
0.09–0.38	-	0.16–0.49	-	-	*Schoenoplectus lacustris*[41]
0.11	-	0.23	-	-	*Schoenoplectus lacustris*[42]
0.16	-	0.03	-	-	*Schoenoplectus lacustris*[43]
0.30	-	0.23	-	-	*Schoenoplectus lacustris*[44]
0.45–0.64		0.13–0.30	0.55–0.68	-	*Scirpus americanus*[45]
-	-	0.18	0.59	-	*Scirpus americanus*[40]
-	0.21–0.33	-	-	-	*Scirpus americanus*[46]
-	-	0.19	-	-	*Scirpus cyperinus*[47]
0.40	*0.10*	*0.20*	-	*780*	*Scirpus lacustris*[4]
-	-	-	-	129	*Scirpus lacustris*[3]
0.07–0.36	0.23–0.67	0.04–0.23	0.37–1.32	76–691	Range; washed shoots; this study
0.18	0.43	0.08	0.73	182	Median
0.20	0.43	0.09	0.76	204	Mean
0.17–0.23	0.39–0.47	0.07–0.10	0.67–0.86	160–259	CI (95%)
0.04–0.08	0.23–0.27	0.02–0.07	0.48–0.76	17986–67356	Range; washed roots; this study
0.05	0.24	0.05	0.63	42195	Median
0.05	0.25	0.05	0.62	41098	Mean

[19]Dykyjová (1989); [20]Dykyjová (1973); [21]Kovács (1976); [22]Auclair (1982); [23]Chapin et al (1975); [24]Auclair (1977); [25]Ulrich & Burton (1988); [26]Květ & Ostrý (1988); [27]Dykyjová & Květ (1982); [28]van Dyke (1972); [29]Klopatek (1978); [30]Bernard & Solsky (1977); [31]Linn et al (1973); [32]Bernard & Bernard (1977); [33]Kistritz et al (1983); [34]Solander (1983); [35]Verhoeven (1983); [36]Ho (1979); [37]Bernard & Hankinson (1979); [38]Small (1972); [39]Richardson et al (1976); [40]Boyd (1970); [41]Dykyjová (1973); [42]Misra (1938); [43]Bernatowicz (1969); [44]Vavruška (1966); [45]Boyd (1970a); [46]Boyd (1969); [47]Garten (1978)

Table 4.2 A comparison of the composition of three grasses (ARRI 1988) with *Eleocharis sphacelata*

	Pseudoraphis spinescens		*Hymenachne acutigluma*		*Oryza meridionalis*		*Eleocharis sphacelata* (ie this study)	
Element	shoots	roots	shoots	roots	shoots	roots	shoots	roots
P (%)	0.08	0.05	0.22	0.08	0.11	0.07	0.09	0.05
S (%)	0.27	0.28	0.36	0.28	0.16	0.21	0.76	0.62
Ca (%)	0.14	0.12	0.16	0.24	0.16	0.17	0.20	0.05
Mg (%)	0.15	0.12	0.26	0.19	0.19	0.20	0.43	0.25
Mn (µg/g)	180	70	200	70	470	370	346	343
Fe (%)	0.38	0.47	0.22	0.87	0.27	1.2	0.02	4.1
Cu (µg/g)	26	76	9.8	88	9.6	27	4.6	8.3
Zn (µg/g)	45	53	37	39	41	61	24	136
Cd (µg/g)	0.07	0.13	0.05	0.07	0.05	0.06	0.07	1.3
Pb (µg/g)	0.41	0.75	0.20	2.3	0.85	2.4	0.19	15.7
U (µg/g)	0.37	0.46	0.08	0.54	0.06	0.61	3.2	161

(Mortvedt 1994). In the work reported here, the concentration range of U in shoots of *E. sphacelata* span the values cited by Outridge & Noller (1991) for a variety of wetland plants (Table 4.1) and in Magela flood plain grasses (Table 4.2) but the median (3.9 mg/kg) and mean (3.2 mg/kg) concentrations of U provided here for *E. sphacelata* are at least three times higher than cited by Oatridge & Noller (1991) for freshwater plants sampled from contaminated environments (ie <0.1–1 µg/g DM). Similarly, the concentration range of *Nymphæa violacea* foliage sampled from mildly contaminated sediment in Djalkmara billabong (containing 31–103 mg/kg total U) ranged between 0.13–0.38 mg/kg DM (Hancock 1994), more than an order of magnitude less than in *E. sphacelata* from the RP1 CWF. In addition, Garten (1981) found a U concentration of 0.132 mg/kg in an unidentified species of *Eleocharis* sampled from a shoreline of a contaminated pond containing 30 mg/kg total U in sediment.

A number of studies have measured U concentrations in plants sampled from the natural environment and these generally fall into one of the following categories; biogeochemistry including exploration for U anomolies (Cannon 1952, 1960; Sheard 1986a,b); soil contamination (Garten 1979;) and radiological surveys of agricultural crops. Other work has examined the uptake of U under controlled conditions including field trials, pot experiments and lysimeter studies, and the plant physiological effects of U. A summary of publications under each category is given in Table 4.3. Early work on biogeochemical relationships between U in plants, and soil and parent material has been reviewed by Dunn et al (1985) and their main conclusions are referred to here.

A large part of the literature dealing with U uptake by plants is difficult to relate directly to our results because of the expression of U concentration in terms of a U mass per unit mass of plant ash and the omission in most papers of ash yield per unit mass of plant DM. Dunn et al (1985) in summarising the findings of their review indicate that normal background levels of U in plant ash range from 0.5–2 mg/kg and which was later confirmed by Zafrir et al (1992). Assuming an ash content of around 0.05 g/g DM, this implies a background concentration range of about 0.02–0.1 mg U/kg DM which is within the lower range of <0.1–1.1 mg/kg DM cited by Outridge & Noller (1991) for uncontaminated sites. According to Dunn et al (1985), >2 mg U/kg ash in plants sampled from the natural environment may imply a U province. The highest concentration of U in plant tissue cited by Dunn et al (1985) comes from a survey by Cannon (1952) who measured 7400 mg U/ kg ash in *Sarcobatus* (greasewood) roots. Concentrations in excess of 2000 mg U/kg ash also appear to be commonly recorded in the twigs and needles of conifers including lodgepole pine, western red cedar and black spruce (Dunn et al 1985). Very high concentrations of U have also been recorded by Titaeva et al (1979) on sites impacted by naturally elevated radiation where concentrations ranged from 30–13820 mg U/kg ash in a variety of plant species and different plant parts[1]. Sheard (1986a,b) compared the U content of plants from uraniferous and non-uraniferous regions. Most of the plant species sampled showed significant differences in their U content between these regions with the highest concentrations being found in nonvascular plants such as mosses and lichens. Concentration ranges for the same plant species from the U mineralised and unmineralised regions were 0.13–2.8 and 0.006–0.05 mg U/kg DM[2]. In addition, several studies have noted relatively high concentrations of U in plants growing in Histosols in the vicinity of U mineralisation. For example, Sheppard & Thibault (1984) found 12.3 and 29.0 mg U/kg ash[3] respectively in *Sphagnum* spp. and *Umbilicaria muhlenbergii* and Lopatkina et al (1970) noted high concentrations in plants (100–>200 mg U/kg ash) from peat bogs[4]. Higher concentrations of U in older tissue of *Sphagnum* spp. were ascribed to its greater ash content compared with young tissue (Sheppard & Thibault 1984).

Table 4.3 Bibliography of plant studies measuring U uptake and content (excluding lichen & algae)

Subject	References
Biogeochemistry	Cannon 1952, 1960; Dean 1960; Lopatkina et al 1970; Sheard 1986a,b; Sheppard & Evenden 1990; Sheppard & Thibault 1984; Titaeva et al 1979; Zafrir et al 1992
Soil contamination	Dreesen et al 1978; Estabrook et al 1985; Garten 1979; Garten 1981; Garten et al 1981; Ibrahim & Whicker 1988, 1992; Moffat & Tellier 1977
Agricultural crops	Lal et al 1983; Smith et al 1982
Field trials, pot expts & lysimeter studies	Adams et al 1975; Dreesen & Cokal 1984; Kaur et al 1989; Lakshmanan & Venkateswarlu 1988; Saric et al 1995; Schreckhise & Cline 1980; Sheppard et al 1983, 1984, 1989 1992; Sheppard & Evenden 1992; Van Nettan & Morley 1982ab,1983
Plant physiology	Koul et al 1983; Sela et al 1988

Studies of U uptake by plants on contaminated sites have included growth on tailings (Dreesen et al 1978; Moffat & Miller 1977), and in the vicinity of current or rehabilitated radioactive waste ponds (Garten 1981; Garten et al 1981), or U mining or milling operations (Ibrahim & Whicker 1988). In general, U concentrations in plants growing on contaminated areas have been found to be elevated. For example, differences in shoot U content of as much as two orders of magnitude were shown between control (0.36 mg U/kg DM) and contaminated sites (3.3–33 mg U/kg DM)[5] by Ibrahim & Whicker (1988) although the latter noted that samples probably retained a significant source of soil contamination despite washing. Garten (1981), Garten et al (1981) and Dreesen et al (1978) reached similar

[1] U contents of ashed soil samples (which by inference were organic) ranged between 3900–184800 mg/kg.

[2] Mean soil U contents were 0.01 and 0.06 mg/kg respectively.

[3] Ashed soil U content was 98 and 36 mg/kg respectively.

[4] U content of peat ranged from 130–325 mg/kg DM.

[5] The total U content of the control soil was 4 mg/kg and 14–41 mg/kg in the contaminated soil.

conclusions, attributing a significant portion of measured U in plants growing on contaminated sites to contamination from adhering sediment or windblown tailings. However, in the case of Garten's work the maximum concentration of U in *Eleocharis* spp. was not unusually large, amounting to 0.26 mg/kg[6]. On U tailings containing around 15–20 mg U/kg, the U content of various grasses were <0.1 mg/kg DM which were an order of magnitude higher than on the control sites (Moffat & Tellier 1977).

Many studies examining plant uptake of U have used pot experiments to quantify effects by manipulating the rooting substrate. These have either involved using soil naturally high in U (Van Nettan & Morley 1982a,b; 1983), contaminated soil (Dreesen & Cokal 1984;) adding a uranyl salt to a previously uncontaminated soil (Kaur et al 1988; Lakshmanan & Venkateswarlu 1988) or adding a U isotope to the substrate as a tracer (Schreckhise & Cline 1980). Other work has relied upon cropping field plots on soil naturally high in U (Saric et al 1995) and using cropped lysimeters (Sheppard et al 1983; 1984; 1989; 1992; Sheppard & Evenden 1992). However the results from some of these studies must be treated with caution for a variety of reasons. First, the effect of a restricted root volume on absorption rates and on the chemical environment of the rhizosphere can be marked and bear no relation to conditions and plant response in the field. Second, the addition of relatively high rates of U, depending on form, can cause confounding effects on other limiting factors (eg pH[7], availability of nutrients), and may induce a change in the factors dictating the solubility of U and its availability for uptake. Third, abnormally high[8] or varying[9] rates of nutrition may also confound plant response to U.

In a pot experiment. the response of barley shoots to soil containing 3–313 mg U/kg ranged between 1.3–16 mg U/kg DM (Van Nettan & Morley 1982a). A similar study, using oats, found the partitioning of U (in concentration) to be in the order roots>seeds>stalks and in the ranges 0.6–131, 0.3–1.5 and 0.1–3 mg U/kg DM[9] (Van Nettan & Morley 1982b). Radish also showed higher concentrations in the root (1–14 mgU/kg DM) compared with stalks (0.5–2 mg U/kg DM)[10] (Van Nettan & Morley 1983). However, in each of these experiments by Van Nettan & Morley, plant response was not simply dependent on soil U content.

The effect of soil buffering properties on U availability was investigated by Sheppard et al (1983). In a poorly buffered sand, a linear response in U uptake was shown by Swiss chard to uranyl nitrate (0–25 mg U/kg in soil) leading to concentrations as high as 120 mg U/kg DM in whole plant tissue. Overall, U offtake by Swiss chard was about 80 times higher from sand compared to peat (Sheppard et al 1983). Sheppard et al (1989) drew attention to the problems of soil adherence which were estimated to be of the order of 0.01 g dry soil/g DM on the aerial parts of field crops and discussed the implications to setting and applying concentration ratios for radionuclides.

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[6] Maximum sediment total U content was 86 mg/kg.

[7] Sheppard et al (1992) recorded pH 2.1 in a soil which showed depressed germination and which had received 10,000 mg U/kg as uranyl nitrate (prepared from U_3O_8 and HNO_3). The possible effect of U toxicity was confounded by the effect of U treatment on pH.

[8] For example, Saric et al (1995) used an application rate every 10 days in their field experiment of 4 g N/m² (= 40 kg N/ha) over a period of at least 4 months for sunflower!

[9] Soil total U content was 1–574 mg/kg.

[10] Soil total U content was 2–560 mg/kg

Kaur et al (1988)[11] and Lakshmanan & Venkateswarlu (1988) used uranyl nitrate to supplement U. In the absence of data either on soil U concentration or application rate, the data provided by the former is impossible to interpret. However, their work demonstrates that for a variety of plants the concentration of U in roots was between 2–10 times higher than in aerial parts although no quality assurance method to account for soil contamination is described. Interpretation of data provided by Lakshmanan & Venkateswarlu (1988) is also restricted by most U concentrations expressed on a fresh weight (FW) basis. However, the U content of a variety of crops grown in U amended soil (21.2 mg total U /kg) did not differ much from the control (2.1 mg total U /kg) and were <0.04 mg U/kg FW. Rice showed the highest U concentration in straw (1.7 mg/kg DM) in the order straw>husk>grain but differences in U content of rice growing in the U treated and control soils were small (Lakshmanan & Venkateswarlu 1988). Saric et al (1995) found a U content in a variety of crops which ranged from 0.03–1.2 mg/kg[12]. In addition, mature leaves were found to contain around double the U content than young leaves.

Little attention has been paid to the physiology of U uptake by higher plants. Evidence suggests that U can be sequestered efficiently in the root (Koul et al 1983) where it may outcompete Mg for cell wall binding sites and be prevented from moving to the shoot (Sela et al 1988). Other studies which has examined algae (Crist et al 1988) and microbial cells (Premuzic et al 1991) have also demonstrated that the various components and structures of cell walls which characterise these organisms have a propensity to adsorb and retain metals including UO_2^{2+}. In lichen, the uranyl cation appears to be absorbed more efficiently than neutral (phthalate) or anionic (oxalate) forms with the relative affinity of UO_2^{2+} correlated with the stability of monocarboxylic and dicarboxylic ligands ie $Cu^{2+} \approx UO_2^{2+} > Ni^{2+}$ (Boileau et al 1985).

In conclusion, a review of the literature of U uptake by plants has emphasised a lack of consistency in findings borne out to a large extent from problems with soil contamination and from poor methodologies particularly in pot and field based experiments. Little is known about the biological mechanisms affecting the uptake of U in higher plants and its translocation from root to shoot. For wetland plants, the role of foliar absorption as opposed to root uptake of U has not been elucidated and the toxicity of U in contaminated sediment to higher plants has received scant attention. However in relation to our study of U uptake by *Eleocharis* spp., the review has verified the following points

- under conditions where U is labile and present in relatively high concentrations in a substrate, plants are capable of translocating U to the shoot but with higher concentrations generally being found in the root.
- the U content of plants growing in contact with a background level of U in soil/sediment is commonly <0.1 mg U/kg DM.
- adherance of metals to aerial plant parts but especially roots is a major source of contamination especially in radionuclide studies and can prove very difficult to remove. In most studies, U contamination has been ascribed to soil and/or dust. Hence, the apparent U content of plant tissue may be raised or diluted depending on the U content of soil or sediment.

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[11] Kaur et al (1988) used uranyl nitrate as the sole nutrient supplement which gave rise to varying rates of N nutrition.

[12] Saric et al (1995) do not define whether the U is expressed on a DM or ash basis. Mean total soil U content was 17 mg/kg.

4.2 Contamination of plant tissue

Given the significant relationships (Fig 3.1) between U and indicators of contamination (ie Ti, Fe and Al) our results suggest at least two causes of contamination of *E. sphacelata*. First, sediment adherence contributed to the apparent U concentration of plant tissue as shown by the strong correlation between Al and Ti. Ti is not absorbed markedly by plants because the low solubility of Ti-containing secondary minerals (Brookins 1987) dictate low concentrations in natural waters (Hem 1989) and soil pore water. In highly weathered soil, Ti exists principally as polymorphic TiO_2 (rutile, anatase and brookite) and as mixed oxides in the forms of ilmenite ($FeTiO_3$), sphene ($CaTiSiO_5$) and perovskite ($CaTiO_3$) (Taylor et al 1983). According to Hem (1989) in a review of thermodynamic data, solubility of Ti (IV) is at a maximum at pH <2 (≥1000 µg/L) with the formation of the aqueous TiO^{2+} or $Ti(OH)_2^{2+}$ species. At pH >3, $Ti(OH)_4^0$ becomes the dominant form with a decrease in solubility of Ti between pH 4–8 to around 150 µg/L. In soil solution, the concentration of Ti will be buffered. Hem (1989) notes, however, that there is a general lack of thermodynamic data on aqueous Ti which prevents a more rigorous appraisal of its behaviour in natural systems. In contrast to Ti, Al is absorbed by plants with availability increasing at <pH 5.5 and accumulation occurring in the root (Mengel & Kirkby 1978). The Al content of plants is generally around 200 mg/kg DM (Mengel & Kirkby 1978) although in our work shoots had a mean (±SD) concentration of 88 (±137) mg Al/kg DM compared with a Ti content of 0.57 (±0.80) mg/kg DM. Consequently our results imply a continuum in the degree of contamination by sediment (Fig 3.1) as shown by the correspondance of roots and unwashed shoots to the relationship shown between Ti and Al for washed shoots, and that washing was only partly successful in removing soil. In turn, this infers that adhering sediment contributed towards the U content of plant tissue.

A second cause of contamination is implied by the behaviour of Fe which was different to other indicators in terms of its contribution to the composition of roots and unwashed sheath tissue (Fig 4.1) and in its relationship with U (Fig 3.1). Iron was present probably as a plaque, especially on roots, and possibly as a ferrihydrite deposit on periphyton attached to the shoot. The high Fe content on the exterior of the shoot (ie as shown by sheath tissue) was effectively diluted in unwashed shoots so that the other principle factors determining the apparent plant tissue composition, namely plant absorption and sediment contamination, assumed greater influence. Iron oxyhydroxide may coprecipitate U and also provide a surface for U adsorption (Hsi & Langmuir 1985; Bruno et al 1995) and thus has the potential to influence the apparent composition of plant tissue, when present as coatings, by sequestering metals from the water column or pore water.

The relative degree of contamination of plant tissue by either adhering soil or iron oxyhydroxide has been impossible to quantify in our study. The method used to digest plant material was most likely not to have been quantitative in dissolving soil Ti (see p 6) and neither Al nor Fe can be substituted confidently as soil indicators because of the problem of separating plant absorption from soil adherence. However, if 200 mg Al/kg DM is taken as a cut-off point for contamination, the majority of washed plant samples with the notable exception of roots, were below this concentration (Fig 3.1). The corresponding trigger Ti concentration is approximately 1.3 mg/kg (Fig 3.1). The Fe content of plant material was probably supplemented by soil adherence and oxyhydroxide precipitate with washed roots and the unwashed stem sheath showing obvious contamination (Fig 3.1).

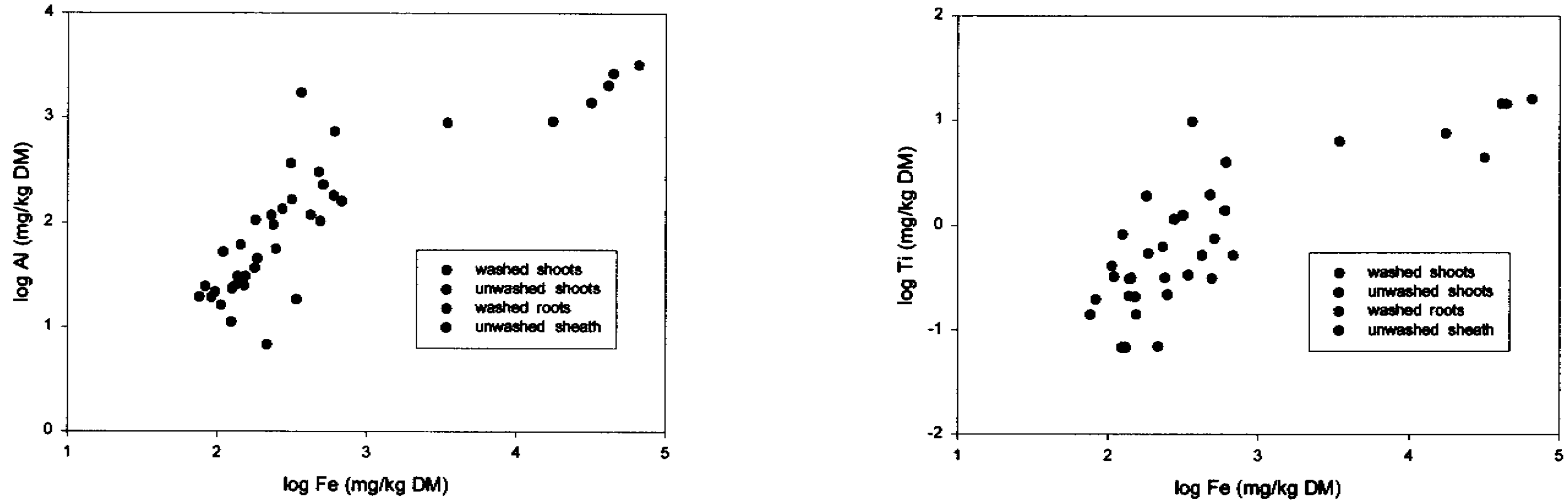

Fig 4.1 Relationships between Al and Ti, and Fe in plant tissue

4.3 Compartmentalisation of U and other elements by *E. sphacelata* in the RP1 CWF

Estimates of above ground *E. sphacelata* biomass production were made during the sampling period in Cell 5 of the RP1 CWF. Surface coverage over the Dry season ranged from 10–25% and FW yields from 1.30–1.75 kg/m². Taking the maximum yield, and assuming homogeneity over the whole CWF, this equates to a DM production[13] of 15.34 t. Uptakes are summarised in Table 4.4 based on mean, minimum and maximum concentrations in washed and unwashed shoots, and washed roots. In the absence of below ground FW yield data, a 1:1 ratio of above:below ground biomass has been assumed.

Table 4.4 Uptake by *E. sphacelata* shoots and roots

	Washed shoots			Unwashed shoots			Roots		
Element	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
U (kg)	0.005	0.14	0.72	0.14	1.15	3.44	0.30	2.47	7.06
Mg (kg)	35.3	65.7	103.2	76.9	82.3	92.2	34.4	37.4	40.6
P (kg)	6.24	14.8	34.7	8.77	11.9	15.8	3.33	7.25	10.5
Ca (kg)	10.1	30.3	55.4	33.2	39.0	43.4	6.10	8.26	11.6
S (kg)	56.8	117.4	202.7	107.2	130.4	140.2	72.1	94.0	114.7
Mn (kg)	1.06	7.01	21.1	3.71	11.2	26.2	1.95	5.19	8.76
Fe (kg)	1.17	3.87	10.6	4.80	14.3	40.4	272.3	622.2	1019.8
Al (kg)	0.10	1.46	11.2	1.77	8.95	25.9	13.4	29.1	47.9
Ni (kg)	0.004	0.09	0.41	0.26	0.30	0.35	0.01	0.03	0.10
Cu (kg)	0.02	0.09	0.34	0.05	0.10	0.16	0.03	0.13	0.22
Zn (kg)	0.16	0.39	0.67	0.24	0.39	0.62	0.33	2.06	4.59
Co (g)	0.80	3.02	10.2	2.60	10.7	34.1	14.0	37.0	66.1
Mo (g)	1.35	5.62	13.4	4.84	8.40	12.0	10.5	19.6	27.5
Cd (g)	ND	5.52	26.6	0.18	17.4	21.0	0.07	19.4	60.8
Pb (g)	0.58	4.28	24.4	3.56	17.7	47.1	15.4	87.1	863.2

It has been estimated that between 1995–96 a total of 250 kg U was retained by the RP1 CWF from the treatment of RP2 water of which 64–77% was accounted for in sediment (Klessa et al 1998). By comparison, the proportion of retained U associated with *E. sphacelata* biomass is small and constituted less than 0.1–4.2% of retained U at any one time during the sampling interval. However, the mass of U compartmentalised by *E. sphacelata* spanning the three years the RP1 CWF has operated (ie 1995–97) will be significantly and proportionately greater since the larger portion of this U is turned over in organic matter. Similarly, the proportion of Mg and S accounted for in *E. sphacelata* biomass at any one time in the RP1 CWF is small amounting to less than 0.2 & 0.3% respectively of input. Determining the importance of organic matter turnover to the fate of U within wetland filters remains a primary objective in current environmental chemistry research.

[13] Area of RP1 CWF excluding sump = 5.88 ha

5 Conclusions

A review of the literature has shown there to be a paucity of data on U uptake by plants, especially hydrophytes. In many instances, data are compromised by a lack of quality assurance protocols to determine the level of contamination of shoots and roots by soil and/or dust. In addition, there is poor understanding of U absorption by plants, response, translocation and storage. Where U is labile and present in relatively high concentrations in a substrate, plants are capable of translocating U to the shoot but with higher concentrations generally found in the root. The U content of plants growing in contact with a background level of U in soil/sediment is commonly <0.1 mg U/kg DM.

Our work with *E. sphacelata* showed roots to contain significantly higher concentrations of Ti, Fe, Al, Co, Mo, Pb and U compared with shoots. However, our results imply that adhering soil, and the presence of a Fe plaque on roots, contributed to these elevated concentrations. Similarly, there was evidence to suggest that washing was only partly successful in removing contamination from shoots although, in the case of shoots, contamination was less pronounced. Unwashed shoots were typified by having enhanced concentrations of Ti, Fe, Al, Pb and U.

Young shoots were characterised by having significantly higher concentrations of P, Cu and Mo and lower concentrations of Mg, Ca, S, Ni and Pb compared with mature shoots. Despite a strong relationship between the distribution of sediment U in the RP1 CWF and path length, there was no evidence of a U concentration gradient in plant tissue.

The mass of U associated with *E. sphacelata* biomass is small in relation to the quantity of U retained by the RP1 CWF from the treatment of RP2 mine water. At any one time during the sampling interval, the U in *E. sphacelata* biomass accounted for less than 0.1–4.2% of retained U, but over the three year operational time of the RP1 CWF a much larger portion of retained U will have been turned over in organic matter. The determination of organic matter turnover rate and the fate of U associated with plant material remains a fundamental requirement to understanding the function and future performance of wetland filters at Ranger. Our work highlights the difficulties inherent in studying plant uptake of U *in situ* and stresses the appropriateness of using controlled environmental conditions for future work to elucidate U uptake and turnover.

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Appendix A

Table A1 Composition (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 7/5/97

Sample	Mg	P	Ca	Ti	Mn	Fe	Al	S	Co	Ni	Cu	Zn	Mo	Cd	Pb	U
Cell 1; new shoots; rep 1	2311.8	2009.9	764.0	0.00	448.1	148.5	16.8	4174.5	0.310	1.41	6.39	34.3	0.63	0.024	0.18	0.70
Cell 1; new shoots; rep 2	2311.8	1910.5	773.9	0.21	479.1	162.9	35.8	4097.2	0.340	1.28	5.84	34.2	0.34	0.044	0.19	0.72
Cell 1; new shoots; rep 3	2285.2	1964.0	777.7	0.21	435.1	157.4	38.8	3933.2	0.185	0.87	4.82	27.5	0.20	0.042	0.18	0.68
Mean	*2302.9*	*1961.5*	*771.8*	*0.14*	*454.1*	*156.3*	*30.5*	*4068.3*	*0.278*	*1.19*	*5.68*	*32.0*	*0.39*	*0.037*	*0.19*	*0.70*
Cell 1; old shoots; rep 1	4429.3	503.5	3148.8	0.00	1390.6	137.5	26.9	6480.9	0.213	2.77	4.56	12.1	0.41	0.015	0.63	0.49
Cell 1; old shoots; rep 2	4374.9	515.4	3094.5	0.00	1368.8	128.8	20.7	6372.1	0.200	2.63	4.63	11.8	0.12	0.026	0.72	0.41
Cell 1; old shoots; rep 3	4272.7	557.4	3019.9	0.20	1370.3	131.9	26.1	5966.7	0.144	1.80	3.66	22.8	0.12	0.012	0.66	0.39
Mean	*4359.0*	*525.4*	*3087.7*	*0.07*	*1376.6*	*132.8*	*24.6*	*6273.2*	*0.186*	*2.40*	*4.28*	*15.6*	*0.22*	*0.018*	*0.67*	*0.43*
Cell 4 inlet; new shoots; rep 1	3160.2	1001.1	1192.9	0.32	323.5	80.5	14.9	4605.4	0.057	0.60	4.96	22.3	0.31	0.000	0.08	0.34
Cell 4 inlet; new shoots; rep 2	3141.6	1017.3	1168.1	0.06	327.1	82.5	17.0	4550.6	0.059	0.63	4.85	23.3	0.34	0.000	0.08	0.36
Cell 4 inlet; new shoots; rep 3	3208.9	945.9	1217.0	0.21	335.0	88.9	41.5	4209.6	0.041	0.46	4.15	21.1	0.33	0.025	0.13	0.46
Mean	*3170.2*	*988.1*	*1192.7*	*0.19*	*328.5*	*84.0*	*24.5*	*4455.2*	*0.053*	*0.56*	*4.65*	*22.2*	*0.33*	*0.008*	*0.09*	*0.39*
Cell 4 inlet; old shoots; rep 1	3201.7	819.1	1668.9	0.00	363.9	66.5	10.8	7256.5	0.057	2.02	2.77	16.8	0.15	0.000	0.49	0.32
Cell 4 inlet; old shoots; rep 2	3150.9	786.1	1638.5	0.00	372.5	65.9	9.8	7129.0	0.061	2.10	2.84	25.1	0.13	0.000	0.50	0.32
Cell 4 inlet; old shoots; rep 3	3234.3	731.6	1647.9	0.42	393.4	97.0	37.2	7320.0	0.039	1.28	2.03	25.6	0.09	0.023	0.53	0.34
Mean	*3195.6*	*778.9*	*1651.8*	*0.14*	*376.6*	*76.4*	*19.3*	*7235.2*	*0.052*	*1.80*	*2.55*	*22.5*	*0.12*	*0.008*	*0.51*	*0.32*

Sample	Mg	P	Ca	Ti	Mn	Fe	Al	S	Co	Ni	Cu	Zn	Mo	Cd	Pb	U
Cell 4 outlet; new shoots; rep 1	2967.4	1168.5	685.5	0.00	138.9	234.2	4.5	4927.3	0.076	0.59	6.25	27.3	0.23	0.045	0.12	1.28
Cell 4 outlet; new shoots; rep 2	2979.3	1206.3	686.0	0.00	135.0	220.7	4.5	5002.4	0.073	0.57	6.30	26.8	0.22	0.118	0.12	1.26
Cell 4 outlet; new shoots; rep 3	2928.4	1135.1	640.6	0.21	128.2	200.3	11.3	4869.0	0.050	0.37	4.80	26.2	0.16	0.127	0.18	1.13
Mean	*2958.4*	*1170.0*	*670.7*	*0.07*	*134.0*	*218.4*	*6.8*	*4932.9*	*0.067*	*0.51*	*5.78*	*26.8*	*0.21*	*0.097*	*0.14*	*1.23*
Cell 4 outlet; old shoots; rep 1	5055.1	561.0	3566.4	0.00	247.7	130.4	10.7	11786.7	0.041	0.46	1.67	11.8	0.20	0.035	0.25	0.70
Cell 4 outlet; old shoots; rep 2	4940.1	560.0	3492.2	0.00	242.0	126.4	9.6	11612.3	0.087	2.13	3.39	16.9	0.14	0.042	0.45	0.56
Cell 4 outlet; old shoots; rep 3	4960.9	674.4	3769.6	0.20	238.8	118.6	13.1	11589.1	0.034	0.61	1.50	35.4	0.13	0.581	0.40	0.60
Mean	*4985.4*	*598.5*	*3609.4*	*0.07*	*242.9*	*125.1*	*11.1*	*11662.7*	*0.054*	*1.07*	*2.19*	*21.4*	*0.16*	*0.219*	*0.37*	*0.62*
Cell 7 inlet; new shoots; rep 1	3157.1	1523.5	939.8	3.19	168.3	191.1	111.2	3744.5	0.099	0.53	6.06	34.5	0.22	0.000	0.24	1.20
Cell 7 inlet; new shoots; rep 2	3126.9	1444.4	937.2	1.49	170.0	197.0	112.0	3712.6	0.166	0.63	7.61	40.8	0.35	0.003	0.26	1.39
Cell 7 inlet; new shoots; rep 3	3058.9	1521.1	900.2	1.01	170.6	159.1	89.8	3645.1	0.054	0.26	4.00	26.1	0.21	0.050	0.18	0.83
Mean	*3114.3*	*1496.3*	*925.7*	*1.90*	*169.7*	*182.4*	*104.3*	*3700.7*	*0.106*	*0.47*	*5.89*	*33.8*	*0.26*	*0.017*	*0.23*	*1.14*
Cell 7 inlet; old shoots; rep 1	3489.9	415.6	1761.8	1.50	237.4	131.8	17.8	7568.0	0.254	1.37	4.90	40.0	0.11	0.000	0.41	0.52
Cell 7 inlet; old shoots; rep 2	3550.1	398.1	1770.3	0.53	237.5	126.0	19.9	7810.5	0.166	1.05	3.12	24.3	0.04	0.000	0.28	0.37
Cell 7 inlet; old shoots; rep 3	3359.3	407.0	1724.7	0.40	239.8	122.8	31.4	7517.2	0.099	0.59	3.45	22.7	0.11	0.032	0.50	0.35
Mean	*3466.4*	*406.9*	*1752.3*	*0.81*	*238.2*	*126.9*	*23.0*	*7631.9*	*0.173*	*1.01*	*3.82*	*29.0*	*0.09*	*0.011*	*0.40*	*0.41*

Sample	Mg	P	Ca	Ti	Mn	Fe	Al	S	Co	Ni	Cu	Zn	Mo	Cd	Pb	U
Cell 7 outlet; new shoots; rep 1	3029.9	1174.9	1081.7	0.52	226.4	367.4	29.1	4180.9	0.136	1.05	6.26	21.5	0.35	0.000	0.16	0.30
Cell 7 outlet; new shoots; rep 2	3024.8	1240.6	1041.2	0.28	222.6	347.9	11.7	4199.1	0.133	0.40	6.48	22.0	0.37	0.000	0.05	0.29
Cell 7 outlet; new shoots; rep 3	3025.9	1190.3	996.9	0.20	217.3	325.2	13.9	4247.1	0.088	0.23	4.72	18.4	0.37	0.038	0.05	0.30
Mean	*3026.9*	*1202.0*	*1039.9*	*0.33*	*222.1*	*346.8*	*18.2*	*4209.0*	*0.119*	*0.56*	*5.82*	*20.6*	*0.37*	*0.013*	*0.09*	*0.30*
Cell 7 outlet; old shoots; rep 1	4309.0	519.7	2940.0	0.04	252.3	111.5	11.7	10192.9	0.066	0.71	1.82	44.0	0.20	0.000	0.21	0.38
Cell 7 outlet; old shoots; rep 2	4432.7	524.4	3078.7	0.98	255.7	113.2	25.8	10678.4	0.059	1.74	1.90	13.6	0.16	0.000	0.23	0.35
Cell 7 outlet; old shoots; rep 3	4409.7	571.1	3092.8	0.20	252.1	96.8	11.0	10295.6	0.037	0.39	1.26	9.1	0.21	0.008	0.17	0.35
Mean	*4383.8*	*538.4*	*3037.2*	*0.41*	*253.4*	*107.2*	*16.2*	*10389.0*	*0.054*	*0.95*	*1.66*	*22.2*	*0.19*	*0.003*	*0.20*	*0.36*

Table A2 Composition (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 20/6/97

Sample	Mg	P	Ca	Ti	Mn	Fe	Al	S	Co	Ni	Cu	Zn	Mo	Cd	Pb	U
Cell 1; new shoots; rep 1	4459.7	1371.7	1826.2	0.41	568.9	504.8	110.2	5475.9	0.64	2.07	13.65	33.3	0.63	0.001	0.12	44.14
Cell 1; new shoots; rep 2	4540.5	1409.1	1876.1	0.21	574.1	494.6	94.5	5581.9	0.61	2.07	13.77	33.8	0.60	0.017	0.11	44.10
Mean	*4500.1*	*1390.4*	*1851.2*	*0.31*	*571.5*	*499.7*	*102.3*	*5528.9*	*0.63*	*2.07*	*13.71*	*33.5*	*0.62*	*0.009*	*0.12*	*44.12*
Cell 1; old shoots; rep 1	6188.4	1447.4	2304.4	0.62	422.9	727.8	174.3	8912.8	0.58	1.36	15.38	28.4	0.93	0.020	0.17	47.43
Cell 1; old shoots; rep 2	6026.4	1382.4	2261.3	0.41	415.3	653.9	142.8	8707.7	0.54	1.27	15.19	29.3	0.81	0.008	0.16	46.14
Mean	*6107.4*	*1414.9*	*2282.8*	*0.51*	*419.1*	*690.8*	*158.6*	*8810.3*	*0.56*	*1.32*	*15.29*	*28.8*	*0.87*	*0.014*	*0.17*	*46.78*
Cell 4; new shoots; rep 1	4221.9	969.5	1618.1	0.62	882.4	235.4	117.6	8443.3	0.14	1.05	5.15	24.8	0.53	0.041	0.10	17.18
Cell 4; new shoots; rep 2	4214.2	987.9	1632.6	0.62	886.1	232.2	116.0	8254.4	0.13	0.99	4.92	21.6	0.56	0.020	0.10	16.75
Mean	*4218.0*	*978.7*	*1625.4*	*0.62*	*884.3*	*233.8*	*116.8*	*8348.9*	*0.13*	*1.02*	*5.04*	*23.2*	*0.55*	*0.031*	*0.10*	*16.97*
Cell 4; old shoots; rep 1	5508.0	458.4	3207.1	0.20	632.4	129.2	28.7	12132.0	0.11	0.59	1.89	10.7	0.31	-0.007	0.04	5.66
Cell 4; old shoots; rep 2	5535.0	434.2	3242.6	0.41	630.9	150.8	29.7	12144.2	0.11	0.72	1.91	10.8	0.34	0.011	0.03	5.25
Mean	*5521.5*	*446.3*	*3224.8*	*0.30*	*631.6*	*140.0*	*29.2*	*12138.1*	*0.11*	*0.65*	*1.90*	*10.8*	*0.33*	*0.002*	*0.04*	*5.45*
Cell 5; new shoots; rep 1	3205.9	1358.3	888.0	0.21	69.8	155.8	24.1	7237.1	0.06	0.32	2.16	31.4	0.53	0.036	0.07	2.83
Cell 5; new shoots; rep 2	3194.3	1361.0	883.7	0.21	68.5	151.9	25.4	7288.0	0.07	0.48	2.00	28.9	0.44	0.018	0.07	2.43
Mean	*3200.1*	*1359.7*	*885.8*	*0.21*	*69.2*	*153.8*	*24.8*	*7262.5*	*0.06*	*0.40*	*2.08*	*30.1*	*0.48*	*0.027*	*0.07*	*2.63*
Cell 8; old+new shoots; rep 1	4108.3	1665.4	998.3	0.21	185.4	139.2	29.6	6717.1	0.27	1.68	22.37	40.6	0.70	0.112	0.10	3.64
Cell 8; old+new shoots; rep 2	4086.1	1720.9	981.6	0.21	183.2	138.1	30.6	6628.3	0.28	1.71	22.38	41.5	0.75	0.120	0.09	3.85
Mean	*4097.2*	*1693.2*	*990.0*	*0.21*	*184.3*	*138.6*	*30.1*	*6672.7*	*0.27*	*1.70*	*22.37*	*41.0*	*0.72*	*0.116*	*0.09*	*3.75*

Table A2 Composition (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 4/9/97

Sample	Mg	P	Ca	Ti	Mn	Fe	Al	S	Co	Ni	Cu	Zn	Mo	Cd	Pb	U
Cell 1; new shoots; rep 1	2556.2	2264.4	659.9	0.003	189.5	93.9	20.6	6199.1	0.15	3.92	10.14	40.0	0.39	0.20	0.10	3.97
Cell 1; new shoots; rep 2	2518.7	2260.7	659.9	0.003	188.1	90.4	17.5	6166.8	0.15	4.06	9.92	36.9	0.45	0.17	0.08	3.97
Mean	*2537.4*	*2262.6*	*659.9*	*0.003*	*188.8*	*92.2*	*19.1*	*6182.9*	*0.15*	*3.99*	*10.03*	*38.5*	*0.42*	*0.19*	*0.09*	*3.97*
Cell 1; old shoots; rep 1	5448.4	1167.1	2356.2	0.845	1188.1	519.5	232.2	9251.8	0.66	9.35	5.72	23.7	0.38	0.60	0.25	45.47
Cell 1; old shoots; rep 2	5429.0	1179.2	2347.5	0.624	1178.2	521.3	223.8	9155.2	0.67	9.17	5.81	23.9	0.42	0.59	0.26	45.66
Mean	*5438.7*	*1173.2*	*2351.9*	*0.734*	*1183.1*	*520.4*	*228.0*	*9203.5*	*0.67*	*9.26*	*5.77*	*23.8*	*0.40*	*0.60*	*0.25*	*45.57*
Cell 4; old shoots; rep 1	5405.7	556.5	2850.2	0.425	842.5	115.5	59.4	8066.2	0.15	27.28	3.52	15.2	0.37	1.48	0.08	7.78
Cell 4; old shoots; rep 2	5401.5	525.8	2820.0	0.214	824.9	106.1	44.0	7979.1	0.14	25.88	3.34	15.2	0.35	1.41	0.08	7.40
Mean	*5403.6*	*541.2*	*2835.1*	*0.319*	*833.7*	*110.8*	*51.7*	*8022.6*	*0.15*	*26.58*	*3.43*	*15.2*	*0.36*	*1.44*	*0.08*	*7.59*
Cell 5; old shoots; rep 1	5175.8	568.2	2214.3	0.005	125.8	95.7	19.1	13122.0	0.06	11.86	2.45	11.4	0.31	0.68	0.25	3.77
Cell 5; old shoots; rep 2	5217.5	622.0	2252.3	0.000	126.6	99.8	24.0	13307.0	0.06	11.76	2.51	17.0	0.31	0.69	0.28	3.92
Mean	*5196.6*	*595.1*	*2233.3*	*0.002*	*126.2*	*97.7*	*21.6*	*13214.5*	*0.06*	*11.81*	*2.48*	*14.2*	*0.31*	*0.69*	*0.26*	*3.85*
Cell 8; old shoots; rep 1	5085.1	743.6	1968.9	0.218	183.8	255.6	52.2	8156.0	0.11	20.00	8.11	20.8	0.63	1.44	0.14	4.32
Cell 8; old shoots; rep 2	5131.9	738.8	1996.9	0.216	187.8	247.1	57.9	8276.5	0.11	20.46	8.25	21.7	0.74	1.47	0.15	4.31
Mean	*5108.5*	*741.2*	*1982.9*	*0.217*	*185.8*	*251.3*	*55.1*	*8216.3*	*0.11*	*20.23*	*8.18*	*21.2*	*0.69*	*1.45*	*0.14*	*4.31*

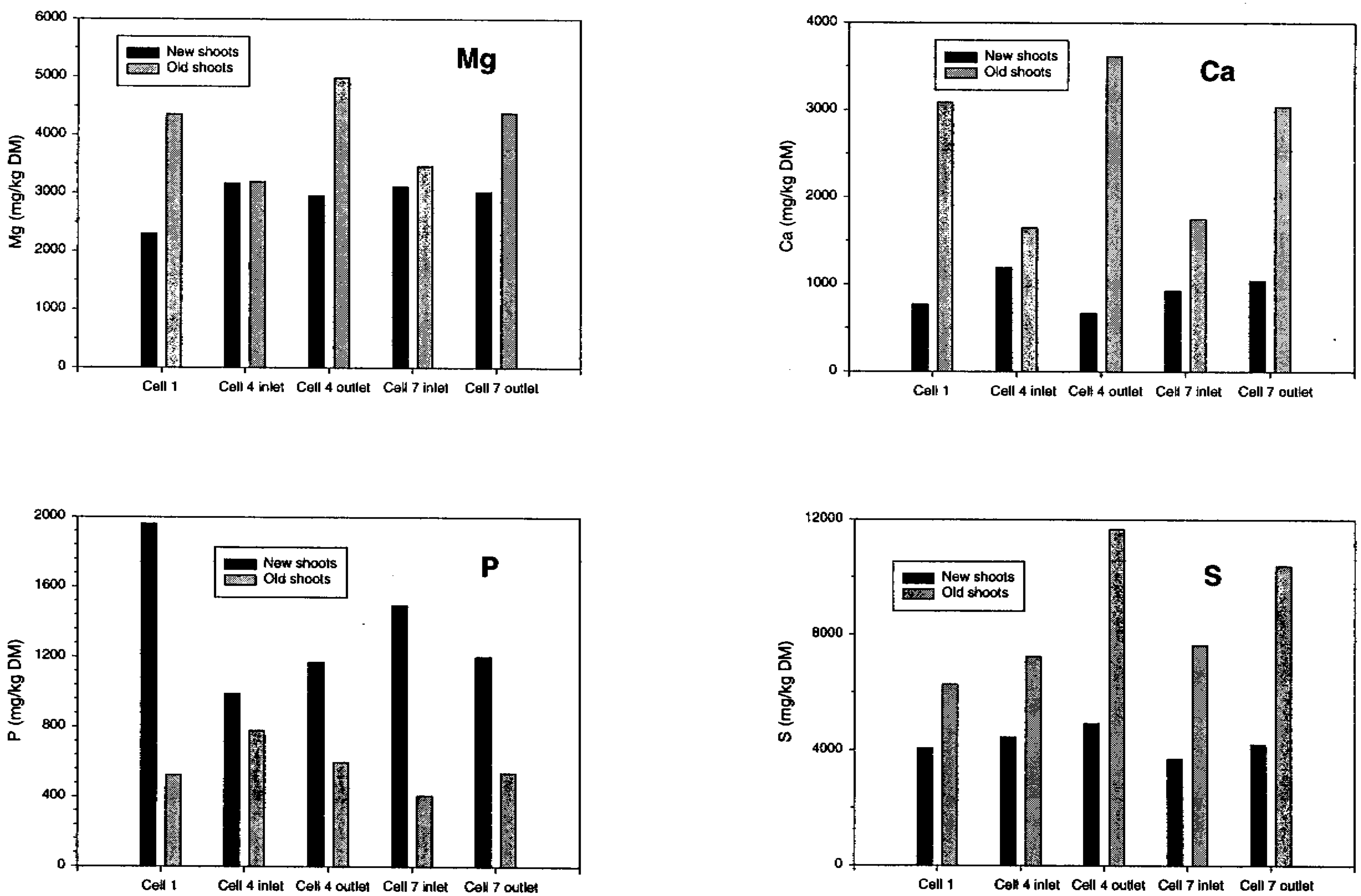


Fig A1 Mg, Ca, P and S contents (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 7/5/97

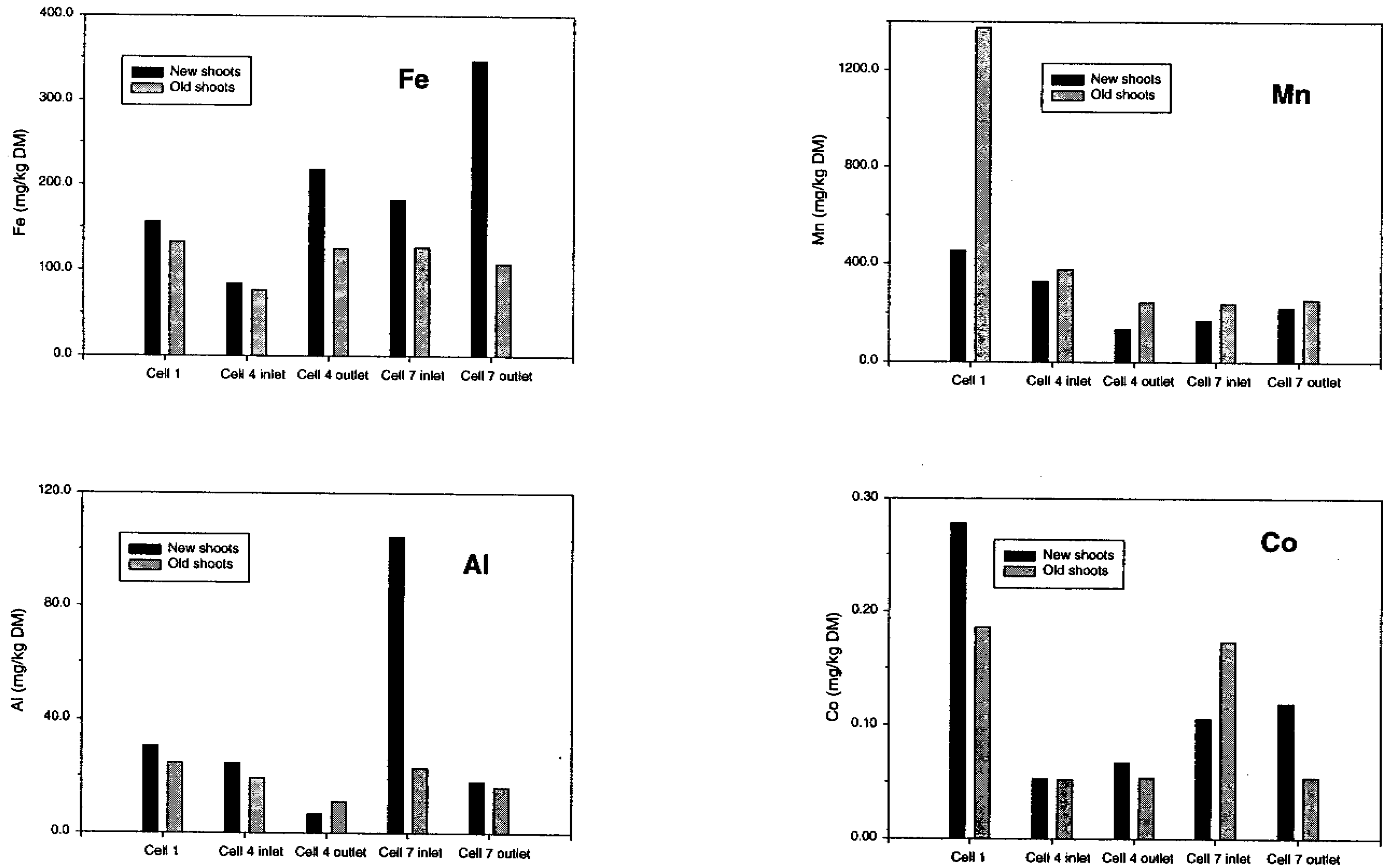


Fig A2 Fe, Mn, Al and Co contents (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 7/5/97

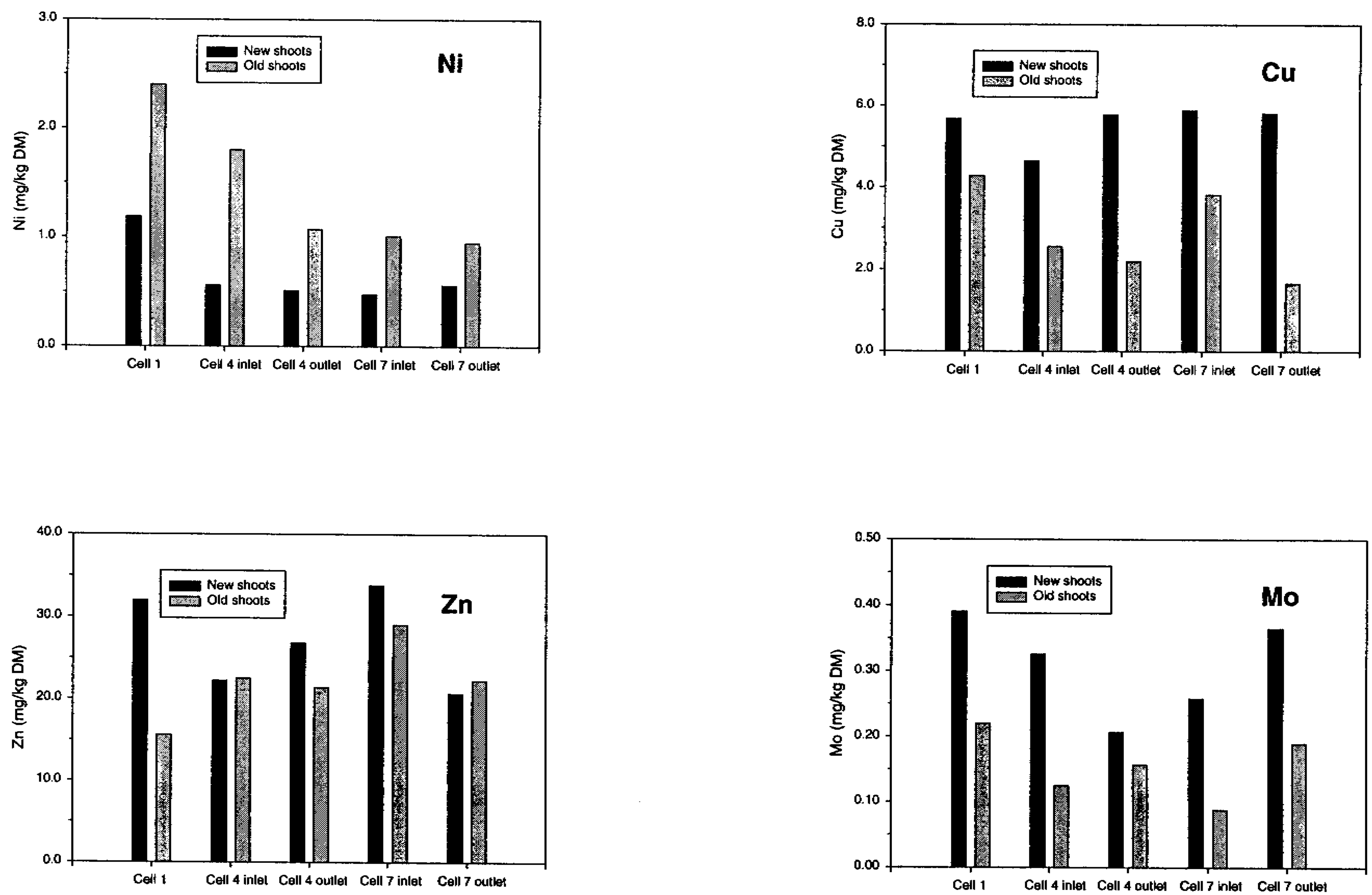


Fig A3 Ni, Cu, Zn and Mo contents (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 7/5/97

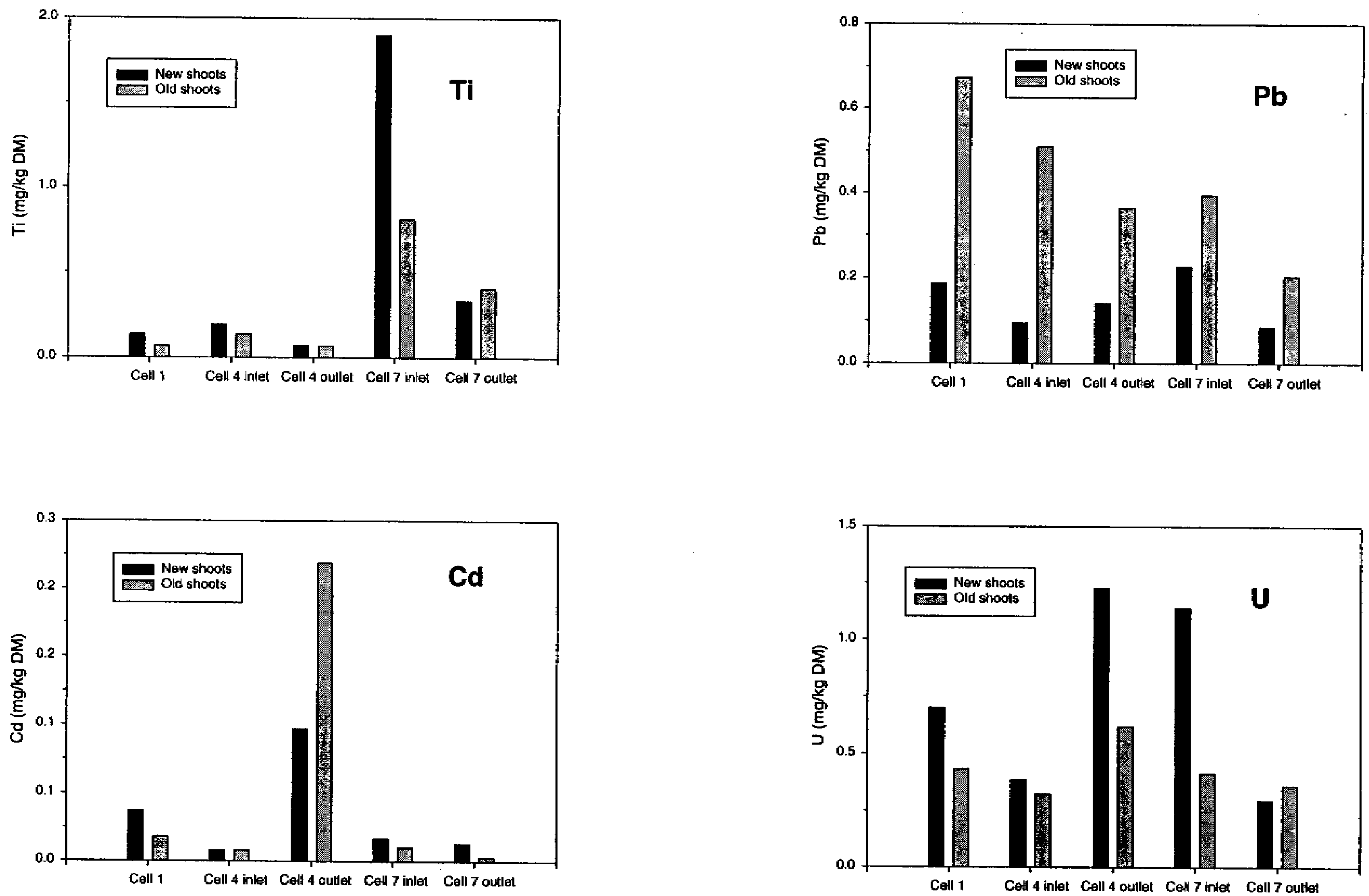


Fig A4 Ti, Pb, Cd and U contents (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 7/5/97

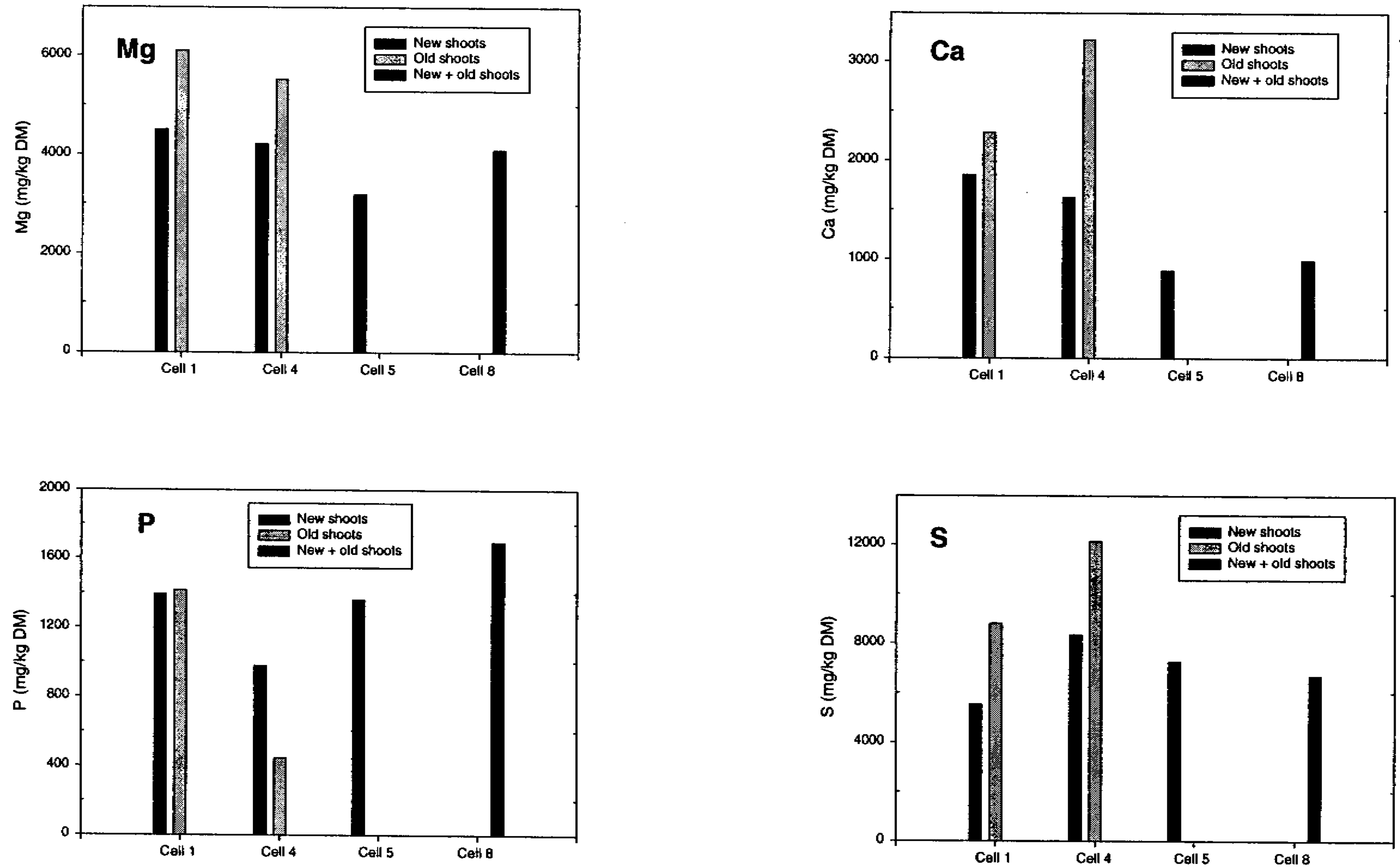


Fig A5 Mg, Ca, P and S contents (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 20/6/97

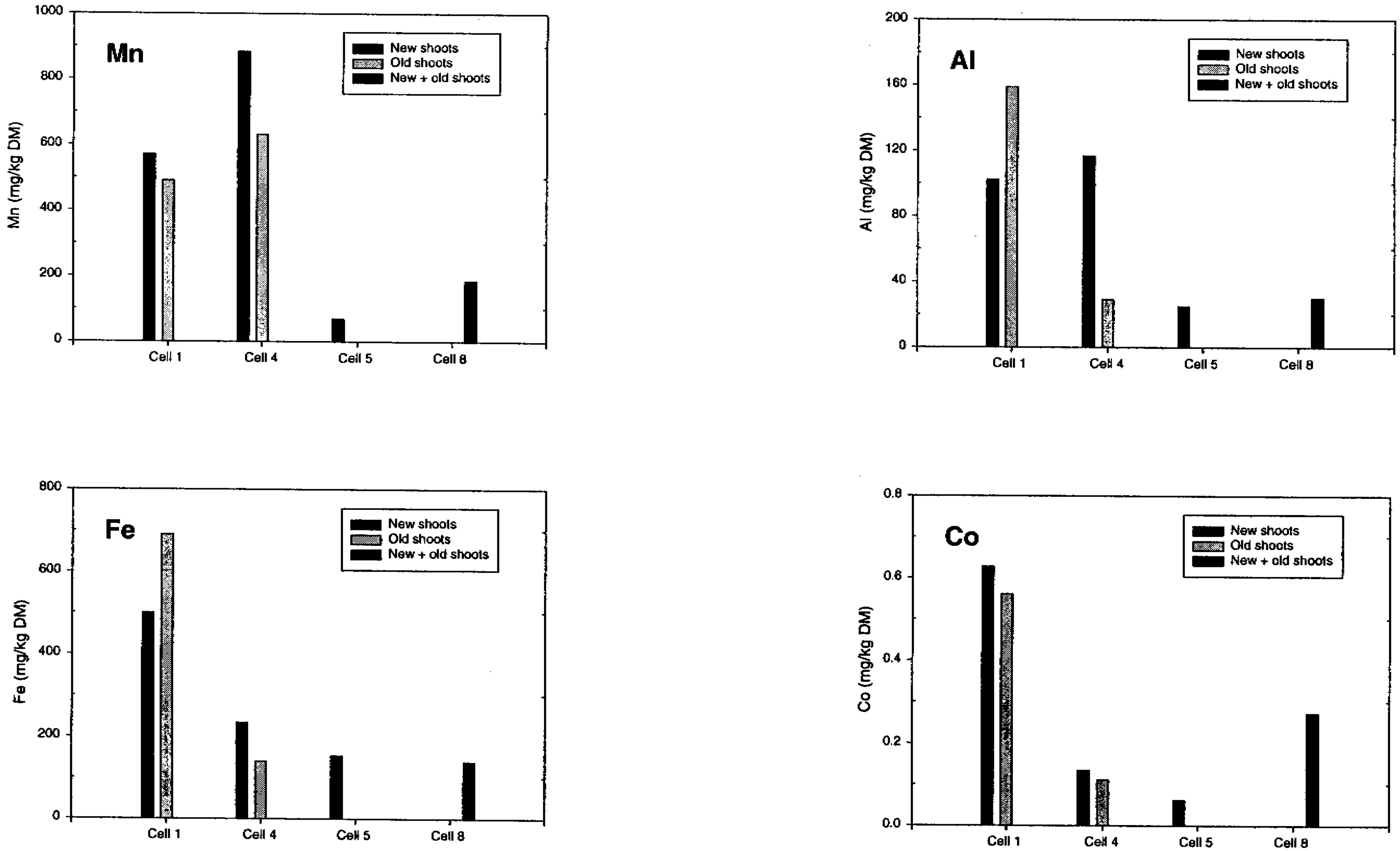


Fig A6 Mn, Fe, Al and Co contents (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 20/6/97

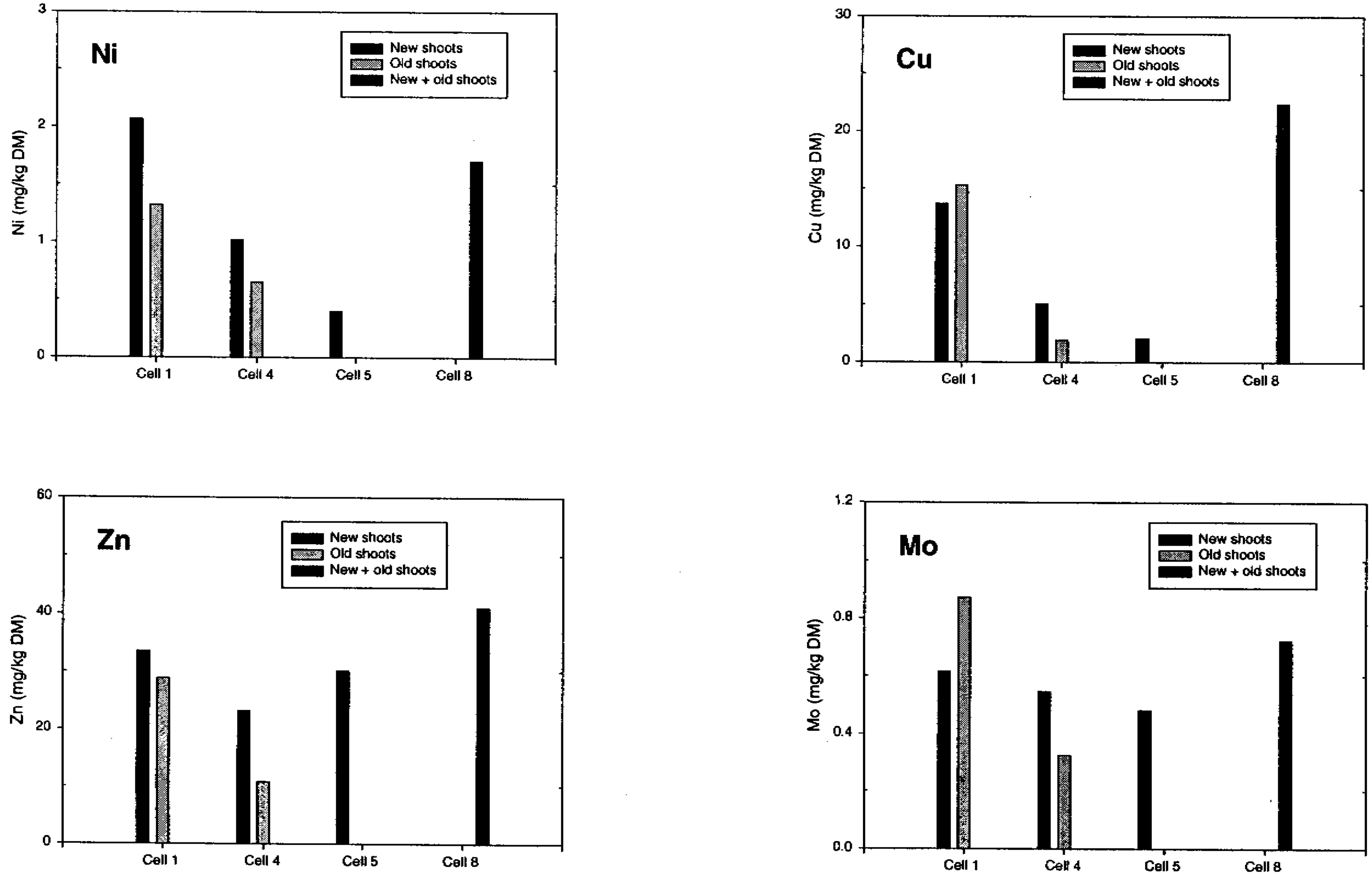


Fig A7 Ni, Cu, Zn and Mo contents (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 20/6/97

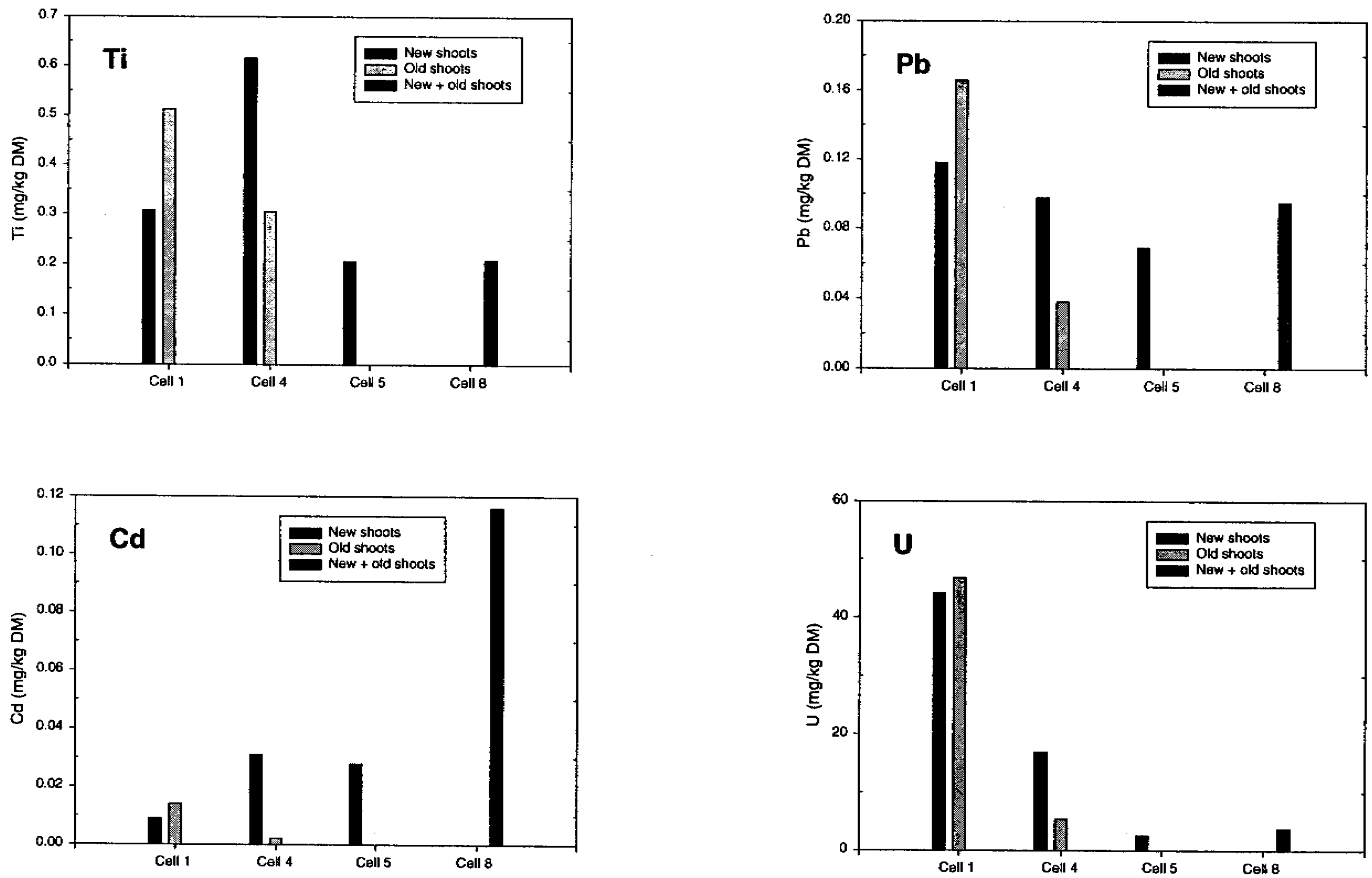


Fig A8 Ti, Pb, Cd and U contents (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 20/6/97

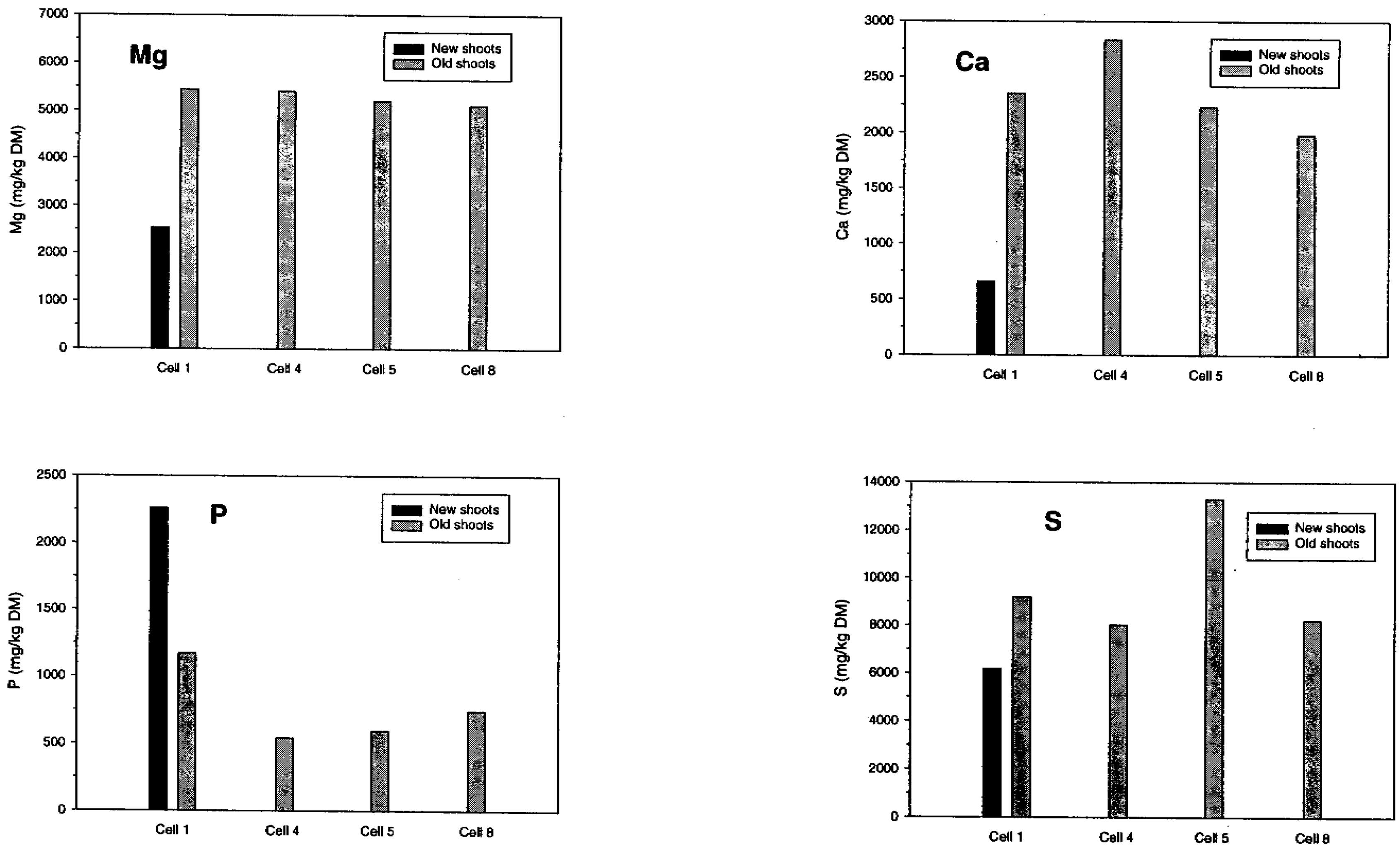


Fig A9 Mg, Ca, P and S contents (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 4/9/97

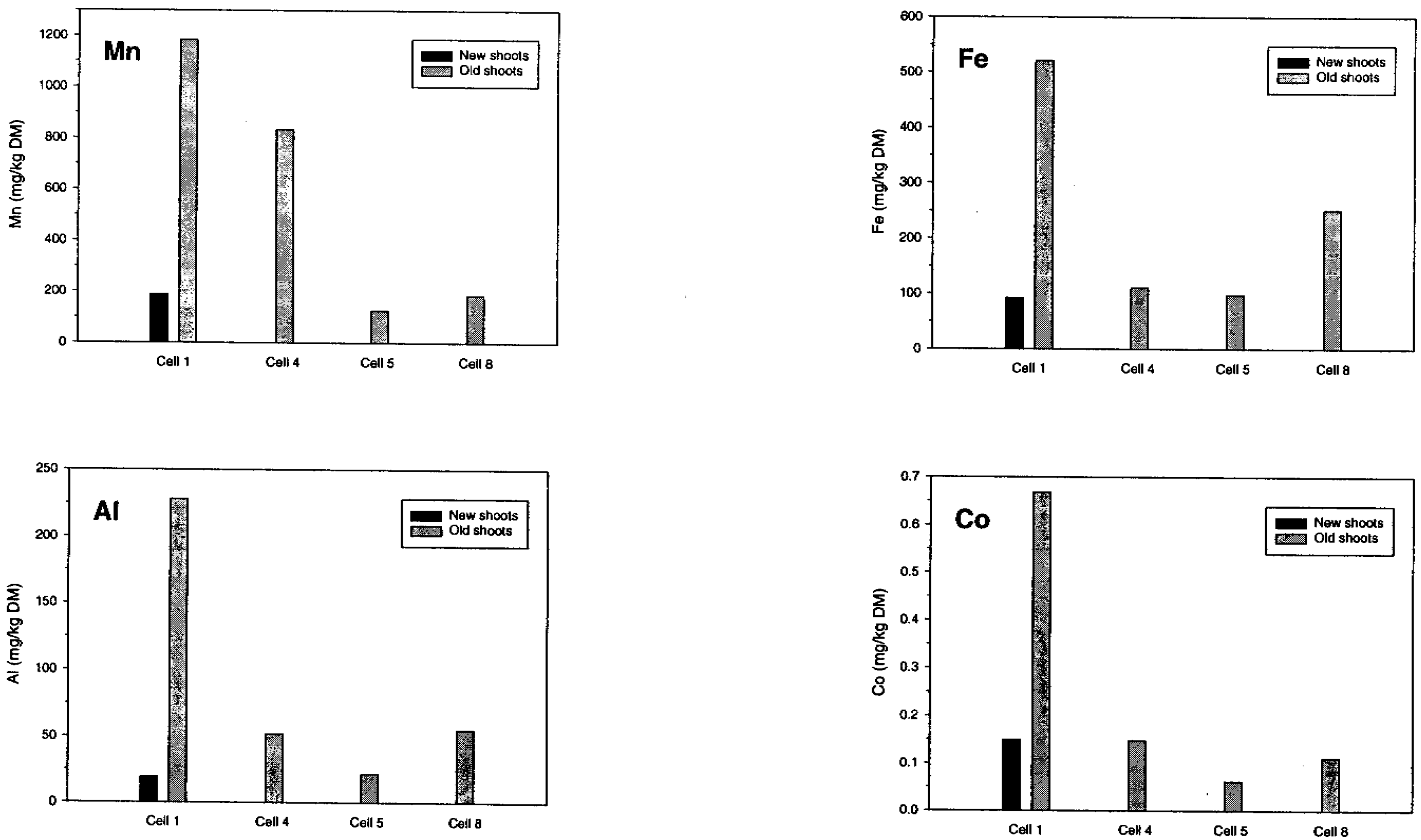


Fig A10 Mn, Fe, Al and Co contents (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 4/9/97

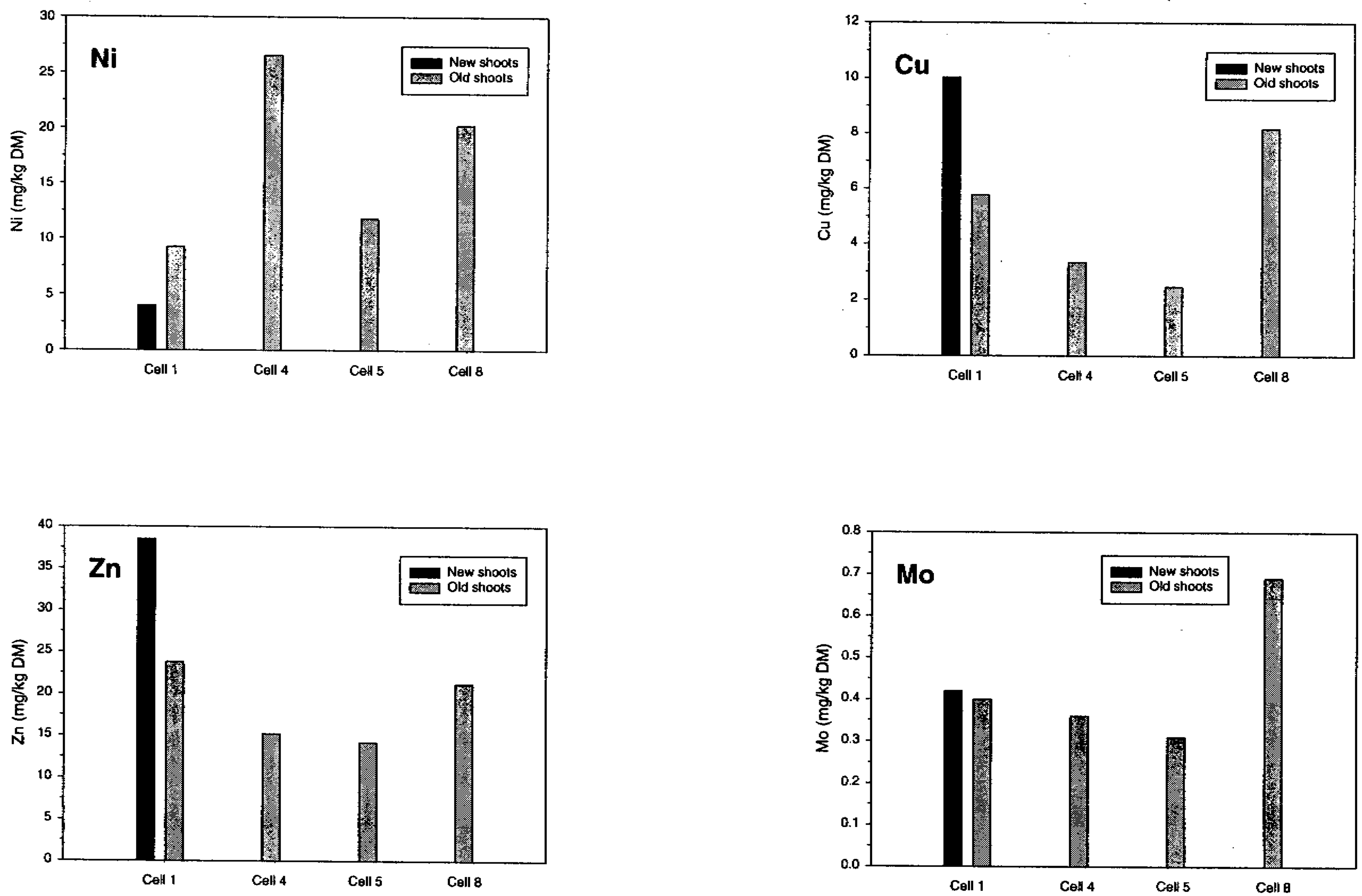
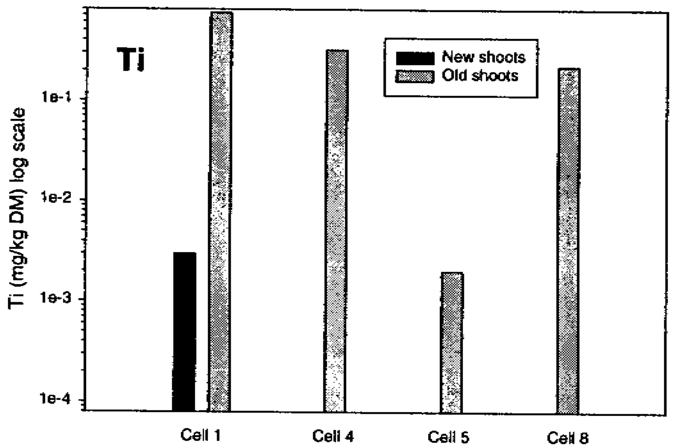
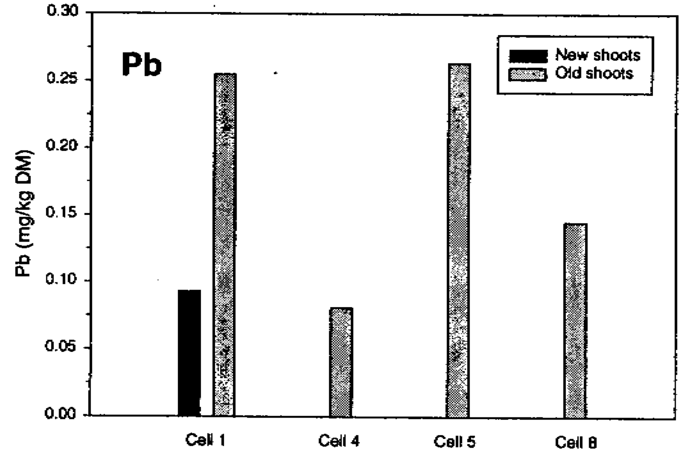
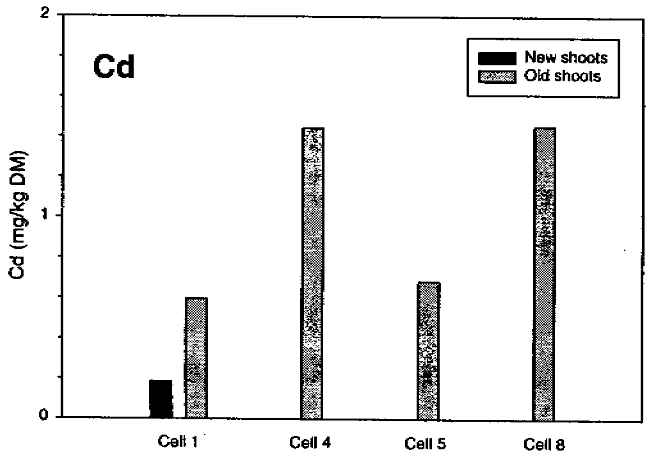


Fig A11 Ni, Cu, Zn and Mo contents (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 4/9/97







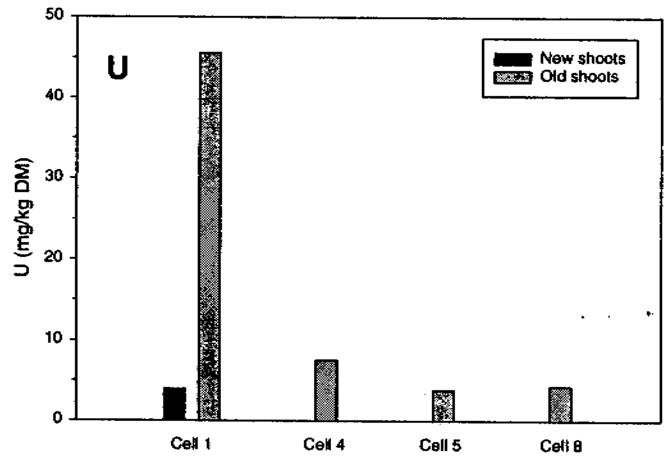


Fig A12 Ti, Pb, Cd and U contents (mg/kg DM) of young and mature shoots of *Eleocharis sphacelata* sampled on 4/9/97

Table B1 Composition (mg/kg DM) of shoots and roots of *Eleocharis sphacelata* sampled on 13/5/97

Sample	Mg	P	Ca	Ti	Mn	Fe	Al	S	Co	Ni	Cu	Zn	Mo	Cd	Pb	U
Cell 1; shoot; rep 1	4058.4	1107.5	1794.3	1.74	1380.1	619.2	192.3	6008.9	0.51	1.20	4.35	21.0	0.35	0.040	0.29	6.49
Cell 1; shoot; rep 2	4042.5	1095.2	1792.2	1.01	1345.6	603.1	166.9	5968.9	0.46	1.09	4.17	20.4	0.36	0.018	0.27	5.93
Mean	*4050.5*	*1101.3*	*1793.2*	*1.38*	*1362.8*	*611.2*	*179.6*	*5988.9*	*0.48*	*1.15*	*4.26*	*20.7*	*0.35*	*0.029*	*0.28*	*6.21*
Cell 1; root; rep 1	2325.4	732.5	414.6	6.89	586.6	18315.0	933.4	5287.1	1.72	1.89	4.47	22.2	0.69	0.033	1.00	80.27
Cell 1; root; rep 2	2223.8	660.2	391.3	5.63	571.1	17657.9	834.7	5113.1	1.72	2.02	4.51	22.0	0.69	0.012	1.03	79.68
Mean	*2274.6*	*696.3*	*402.9*	*6.26*	*578.9*	*17986.4*	*884.1*	*5200.1*	*1.72*	*1.96*	*4.49*	*22.1*	*0.69*	*0.022*	*1.01*	*79.97*
Cell 4; shoot; rep 1	4316.3	429.3	2656.5	1.00	634.4	296.0	125.9	6402.3	0.09	0.49	1.45	12.7	0.31	0.015	0.12	2.51
Cell 4; shoot; rep 2	4307.9	460.0	2682.3	1.27	635.8	260.7	140.7	6340.4	0.09	0.62	1.60	14.4	0.24	-0.001	0.15	2.79
Mean	*4312.1*	*444.7*	*2669.4*	*1.13*	*635.1*	*278.4*	*133.3*	*6371.3*	*0.09*	*0.56*	*1.53*	*13.6*	*0.27*	*0.007*	*0.14*	*2.65*
Cell 4; root; rep 1	2697.6	220.9	555.9	7.38	521.5	69116.9	980.4	4732.1	0.96	0.67	1.87	30.0	0.91	0.003	1.83	19.71
Cell 4; root; rep 2	2660.5	219.6	553.8	7.58	537.9	65595.4	852.0	4790.9	0.89	0.65	1.70	29.5	0.84	0.008	1.71	20.01
Mean	*2679.0*	*220.3*	*554.8*	*7.48*	*529.7*	*67356.2*	*916.2*	*4761.5*	*0.92*	*0.66*	*1.79*	*29.7*	*0.88*	*0.005*	*1.77*	*19.86*
Cell 5; shoot; rep 1	4550.8	618.0	2999.8	1.24	278.5	317.1	157.9	11164.4	0.09	0.18	1.82	22.3	0.08	0.098	1.65	14.80
Cell 5; shoot; rep 2	4517.5	633.6	3013.0	1.25	276.5	324.9	171.4	11106.9	0.09	0.21	1.91	22.4	0.10	0.084	1.54	14.21
Mean	*4534.2*	*625.8*	*3006.4*	*1.24*	*277.5*	*321.0*	*164.7*	*11135.7*	*0.09*	*0.20*	*1.87*	*22.4*	*0.09*	*0.091*	*1.59*	*14.50*
Cell 5; root; rep 1	2311.1	597.2	514.0	21.58	131.3	47677.3	2558.1	7689.0	1.97	3.26	13.52	424.9	2.62	5.657	76.63	628.00
Cell 5; root; rep 2	2354.3	596.5	498.6	9.98	126.4	43487.4	3768.5	7469.9	0.92	1.45	5.89	181.2	1.02	2.375	37.49	291.76
Mean	*2332.7*	*596.9*	*506.3*	*15.78*	*128.9*	*45582.3*	*3163.3*	*7579.4*	*1.45*	*2.36*	*9.70*	*303.0*	*1.82*	*4.016*	*57.06*	*459.88*

Sample	Mg	P	Ca	Ti	Mn	Fe	Al	S	Co	Ni	Cu	Zn	Mo	Cd	Pb	U
Cell 7; shoot; rep 1	3890.3	423.3	1771.4	3.95	303.9	634.3	705.6	4540.4	0.30	1.92	2.71	40.4	0.29	0.195	1.50	12.38
Cell 7; shoot; rep 2	3823.6	403.9	1737.4	4.00	296.0	608.4	750.4	4441.3	0.28	1.37	2.56	33.1	0.24	0.136	0.62	6.30
Mean	*3857.0*	*413.6*	*1754.4*	*3.97*	*299.9*	*621.3*	*728.0*	*4490.8*	*0.29*	*1.65*	*2.64*	*36.7*	*0.27*	*0.166*	*1.06*	*9.34*
Cell 7; root; rep 1	2619.9	398.0	765.2	14.31	286.1	41591.5	2646.8	6218.3	2.03	2.95	6.69	107.4	0.59	0.849	8.67	82.44
Cell 7; root; rep 2	2683.4	436.3	772.8	14.05	295.5	42797.6	2605.3	6341.7	6.71	9.84	21.83	378.4	2.19	2.837	20.78	203.54
Mean	*2651.7*	*417.1*	*769.0*	*14.18*	*290.8*	*42194.5*	*2626.0*	*6280.0*	*4.37*	*6.39*	*14.26*	*242.9*	*1.39*	*1.843*	*14.72*	*142.99*
Cell 8; shoot; rep 1	3056.3	864.4	1724.0	1.95	223.6	486.0	300.7	7322.1	0.10	1.09	9.27	43.8	0.24	ND	0.23	7.19
Mean	3056.3	864.4	1724.0	1.95	223.6	486.0	300.7	7322.1	0.10	1.09	9.27	43.8	0.24	ND	0.23	7.19
Cell 8; root; rep 1	2415.4	428.1	501.5	13.73	190.1	33318.8	2097.0	7385.4	4.03	2.38	9.80	80.6	1.73	0.522	4.39	106.66
Cell 8; root; rep 2	2423.5	500.5	491.4	14.86	182.9	31422.3	1951.6	7033.4	3.51	1.89	12.70	82.7	1.70	0.500	3.97	101.29
Mean	*2419.4*	*464.3*	*496.4*	*14.30*	*186.5*	*32370.5*	*2024.3*	*7209.4*	*3.77*	*2.13*	*11.25*	*81.6*	*1.72*	*0.511*	*4.18*	*103.97*

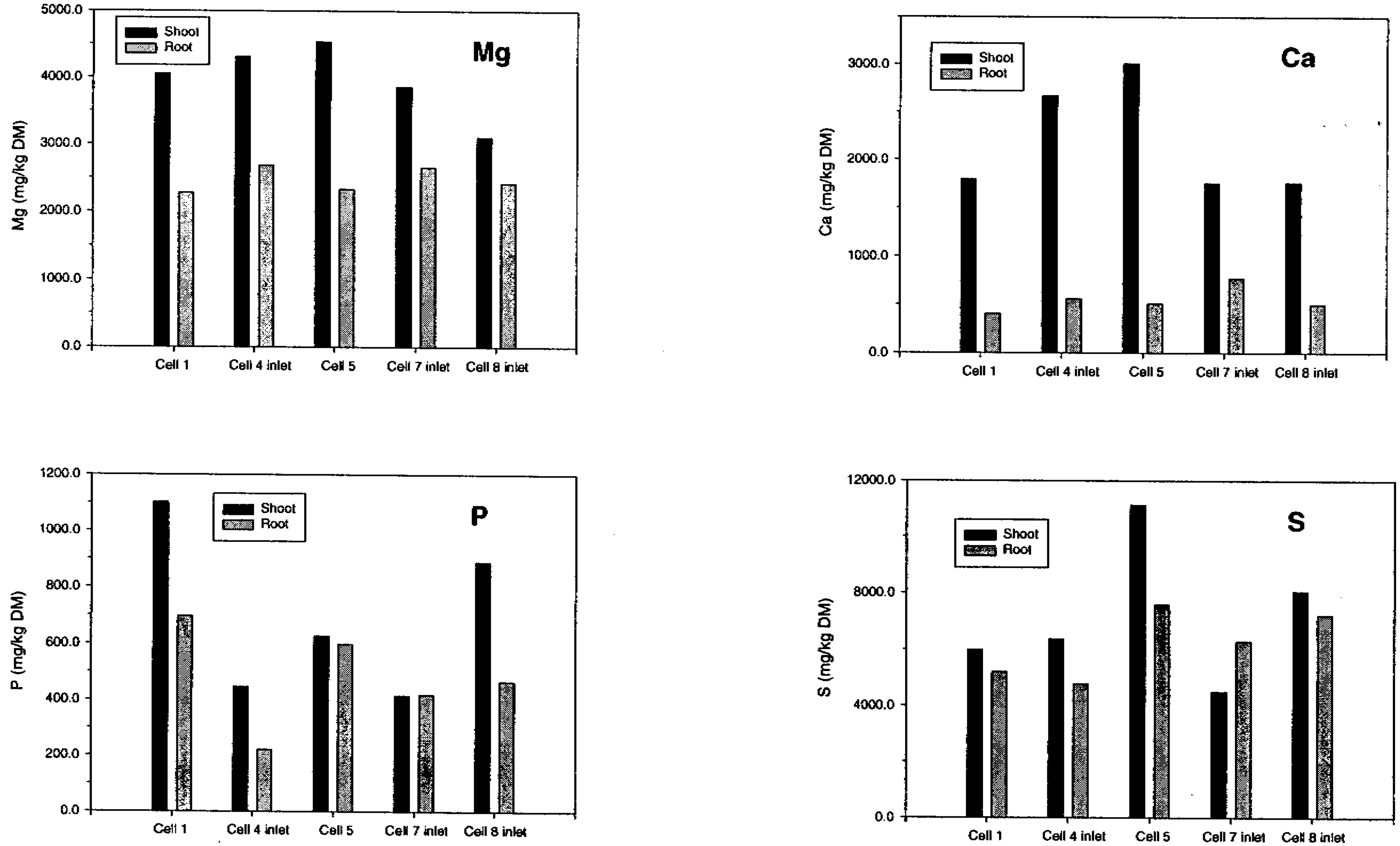


Fig B1 Mg, Ca, P and S contents of shoots and roots of *Eleocharis sphacelata* sampled on 13/5/97

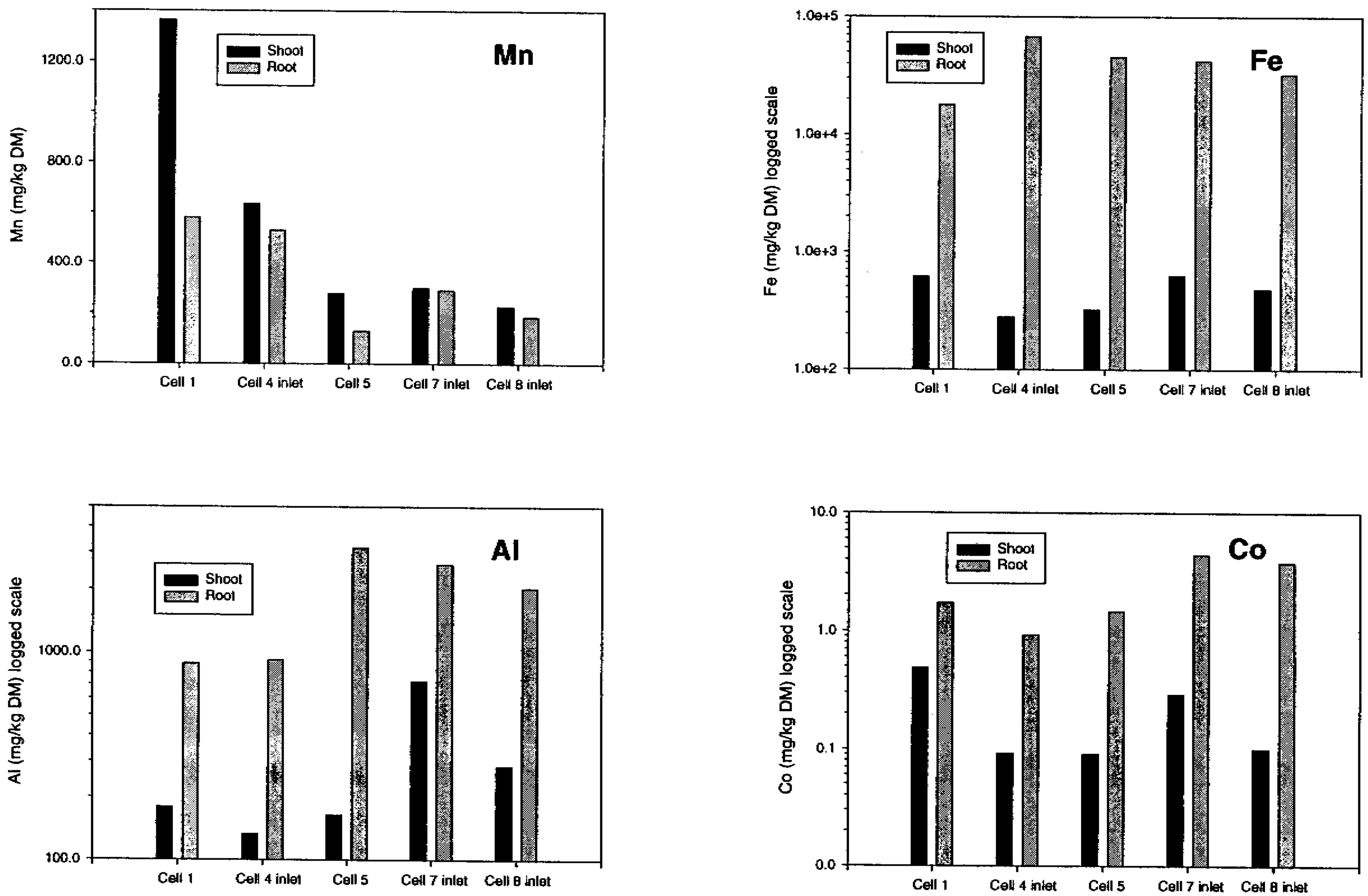


Fig B2 Mn, Fe, Al and Co contents (mg/kg DM) of shoots and roots of *Eleocharis sphacelata* sampled on 13/5/97

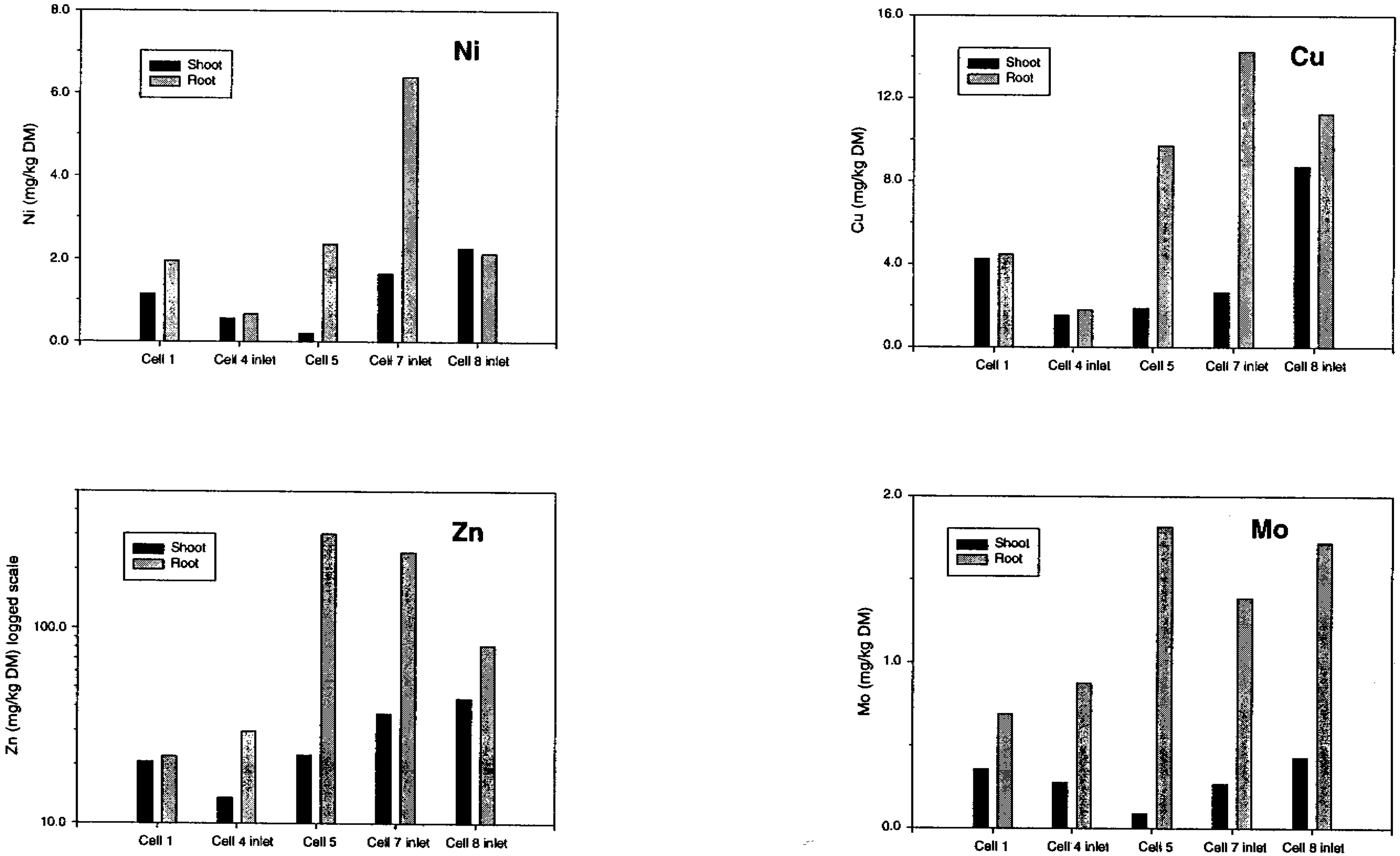


Fig B3 Ni, Cu, Zn and Mo contents (mg/kg DM) of shoots and roots of *Eleocharis sphacelata* sampled on 13/5/97

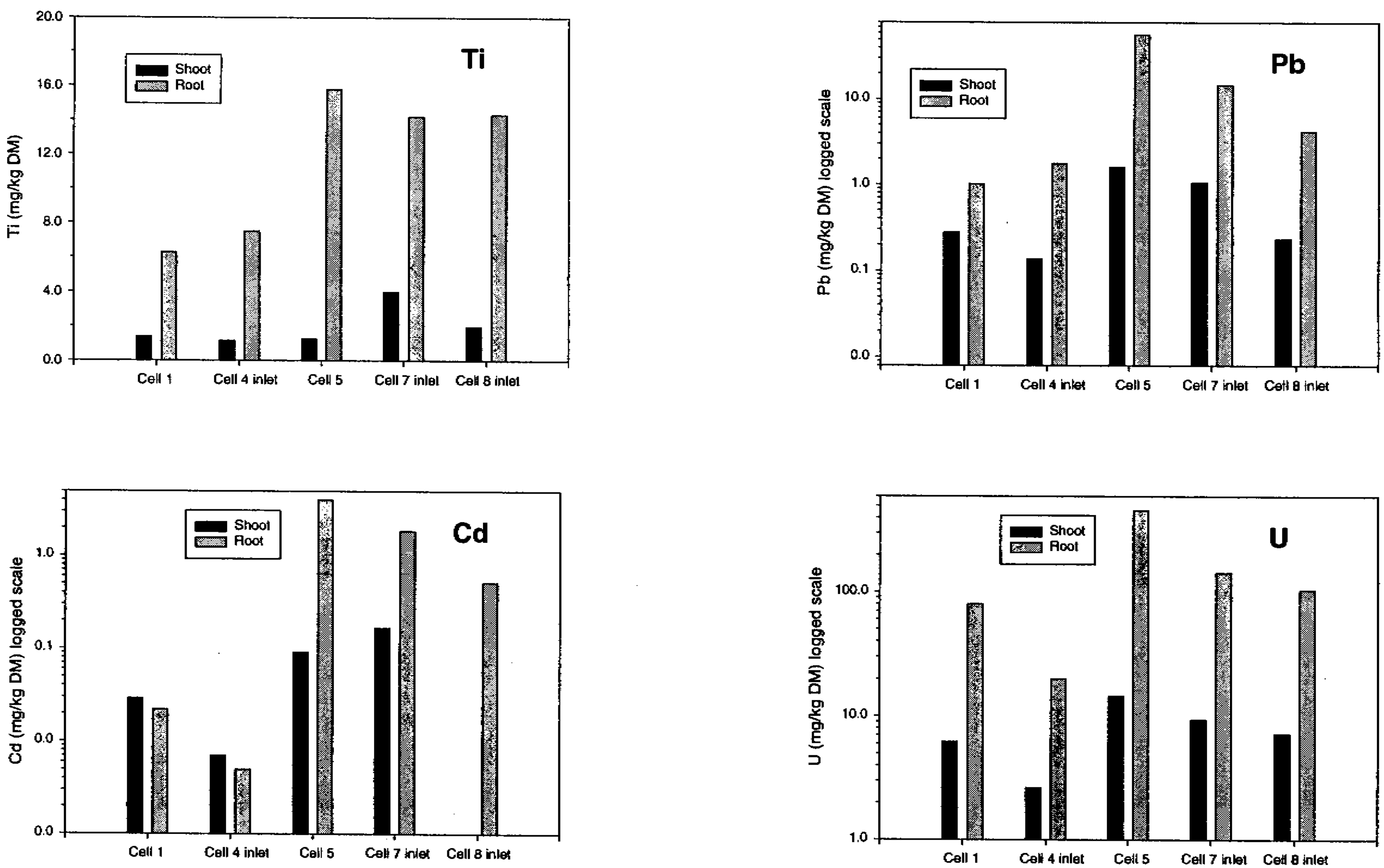


Fig B4 Ti, Pb, Cd and U contents (mg/kg DM) of shoots and roots of *Eleocharis sphacelata* sampled on 13/5/97

Table C1 Composition (mg/kg DM) of unwashed and washed shoots of *Eleocharis sphacelata*

Sample	Mg	P	Ca	Ti	Mn	Fe	Al	S	Co	Ni	Cu	Zn	Mo	Cd	Pb	U
Cell 1; unwashed; rep 1	6097.7	1050.0	2815.6	9.68	1744.5	2708.0	1697.1	8865.9	2.27	17.4	10.28	40.6	0.75	0.74	1.58	227.57
Cell 1; unwashed; rep 2	6087.6	1031.1	2920.3	9.51	1720.3	2636.4	1727.8	9026.1	2.23	17.3	10.52	41.2	0.83	0.80	4.64	221.18
Mean	*6092.6*	*1040.6*	*2867.9*	*9.59*	*1732.4*	*2672.2*	*1712.5*	*8946.0*	*2.25*	*17.4*	*10.40*	*40.9*	*0.79*	*0.77*	*3.11*	*224.37*
Cell 1; washed; rep 1	5063.0	1024.0	2568.3	0.42	859.0	242.3	99.3	9365.9	0.40	17.1	5.74	25.6	0.44	1.16	0.22	14.68
Cell 1; washed; rep 2	5130.2	1042.8	2563.7	0.21	861.4	240.8	88.2	9613.6	0.41	16.9	5.82	24.8	0.54	1.14	0.21	14.51
Mean	*5096.6*	*1033.4*	*2566.0*	*0.31*	*860.2*	*241.5*	*93.7*	*9489.7*	*0.41*	*17.0*	*5.78*	*25.2*	*0.49*	*1.15*	*0.22*	*14.60*
Cell 4; unwashed; rep 1	5080.5	577.2	2788.4	1.24	705.2	372.9	350.5	7052.1	0.24	23.3	4.06	16.0	0.34	1.41	0.26	24.10
Cell 4; unwashed; rep 2	5077.0	581.4	2776.7	1.25	695.8	372.0	379.0	7110.2	0.23	23.0	4.00	15.9	0.30	1.37	0.21	23.38
Mean	*5078.8*	*579.3*	*2782.5*	*1.24*	*700.5*	*372.5*	*364.8*	*7081.2*	*0.23*	*23.2*	*4.03*	*15.9*	*0.32*	*1.39*	*0.24*	*23.74*
Cell 4; washed; rep 1	6771.7	655.1	3014.5	0.21	530.0	144.4	58.9	7127.8	0.13	26.7	3.94	16.6	0.27	1.78	0.10	9.68
Cell 4; washed; rep 2	6690.1	631.5	2960.4	0.41	519.6	145.6	61.7	7006.6	0.13	25.4	3.88	16.8	0.29	1.69	0.09	9.17
Mean	*6730.9*	*643.3*	*2987.4*	*0.31*	*524.8*	*145.0*	*60.3*	*7067.2*	*0.13*	*26.0*	*3.91*	*16.7*	*0.28*	*1.73*	*0.09*	*9.43*

Sample	Mg	P	Ca	Ti	Mn	Fe	Al	S	Co	Ni	Cu	Zn	Mo	Cd	Pb	U
Cell 5; unwashed; rep 1	5307.6	727.4	2476.6	0.41	245.0	314.0	114.0	9159.0	0.18	20.8	3.30	24.7	0.37	1.33	0.71	42.36
Cell 5; unwashed; rep 2	5283.9	716.5	2469.1	0.62	244.2	320.0	121.0	9189.1	0.18	20.9	3.19	24.1	0.42	1.38	1.15	44.48
Mean	*5295.7*	*721.9*	*2472.8*	*0.51*	*244.6*	*317.0*	*117.5*	*9174.1*	*0.18*	*20.8*	*3.24*	*24.4*	*0.39*	*1.36*	*0.93*	*43.42*
Cell 5; washed; rep 1	5729.1	765.2	2353.6	0.64	162.8	190.3	48.1	11295.1	0.08	26.0	3.16	24.2	0.37	1.52	0.27	3.22
Cell 5; washed; rep 2	5719.7	776.0	2336.2	0.42	162.7	186.7	41.4	11321.5	0.08	27.9	3.35	26.9	0.43	1.55	0.31	3.16
Mean	*5724.4*	*770.6*	*2344.9*	*0.53*	*162.7*	*188.5*	*44.8*	*11308.3*	*0.08*	*27.0*	*3.26*	*25.6*	*0.40*	*1.53*	*0.29*	*3.19*
Cell 8; unwashed; rep 1	5303.9	837.6	2196.8	0.85	289.9	441.3	180.4	9283.1	0.17	18.3	9.04	20.9	0.72	1.09	0.40	9.28
Cell 8; unwashed; rep 2	5262.8	789.4	2183.5	0.83	287.4	421.2	161.1	9243.8	0.17	18.6	9.18	21.0	0.71	1.05	0.38	9.04
Mean	*5283.3*	*813.5*	*2190.1*	*0.84*	*288.7*	*431.2*	*170.7*	*9263.4*	*0.17*	*18.4*	*9.11*	*20.9*	*0.72*	*1.07*	*0.39*	*9.16*
Cell 8; washed; rep 1	4930.9	771.0	1716.8	0.00	289.1	181.7	37.1	8186.3	0.24	17.4	11.83	24.4	0.53	1.06	0.16	4.01
Cell 8; washed; rep 2	4877.3	724.4	1715.0	0.00	296.6	180.6	35.7	8136.9	0.12	18.1	11.77	25.0	0.53	1.13	0.19	4.06
Mean	*4904.1*	*747.7*	*1715.9*	*0.00*	*292.9*	*181.2*	*36.4*	*8161.6*	*0.18*	*17.8*	*11.80*	*24.7*	*0.53*	*1.09*	*0.17*	*4.03*

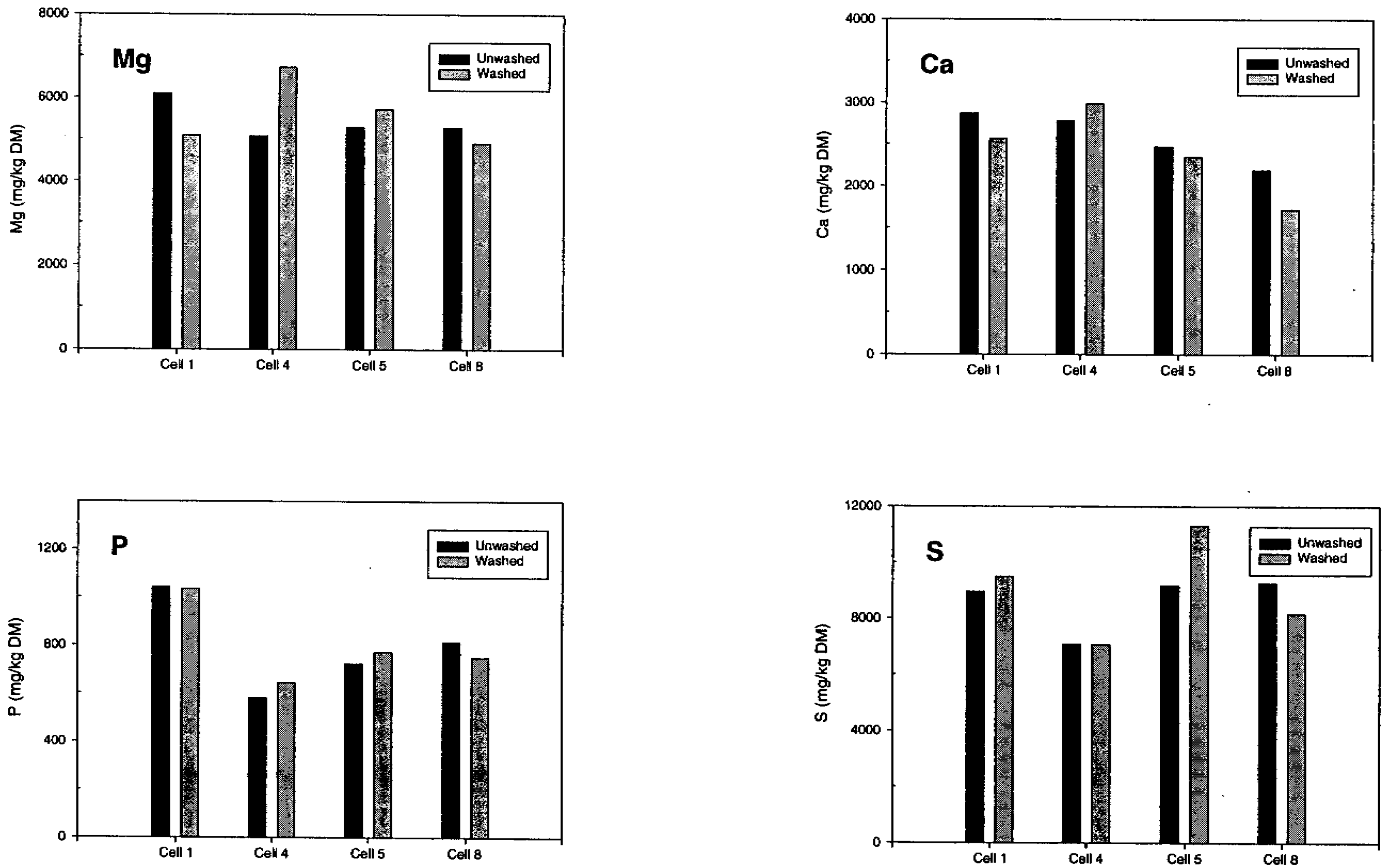


Fig C1 Mg, Ca, P and S contents (mg/kg DM) of washed and unwashed shoots of *Eleocharis sphacelata* sampled on 22/7/97

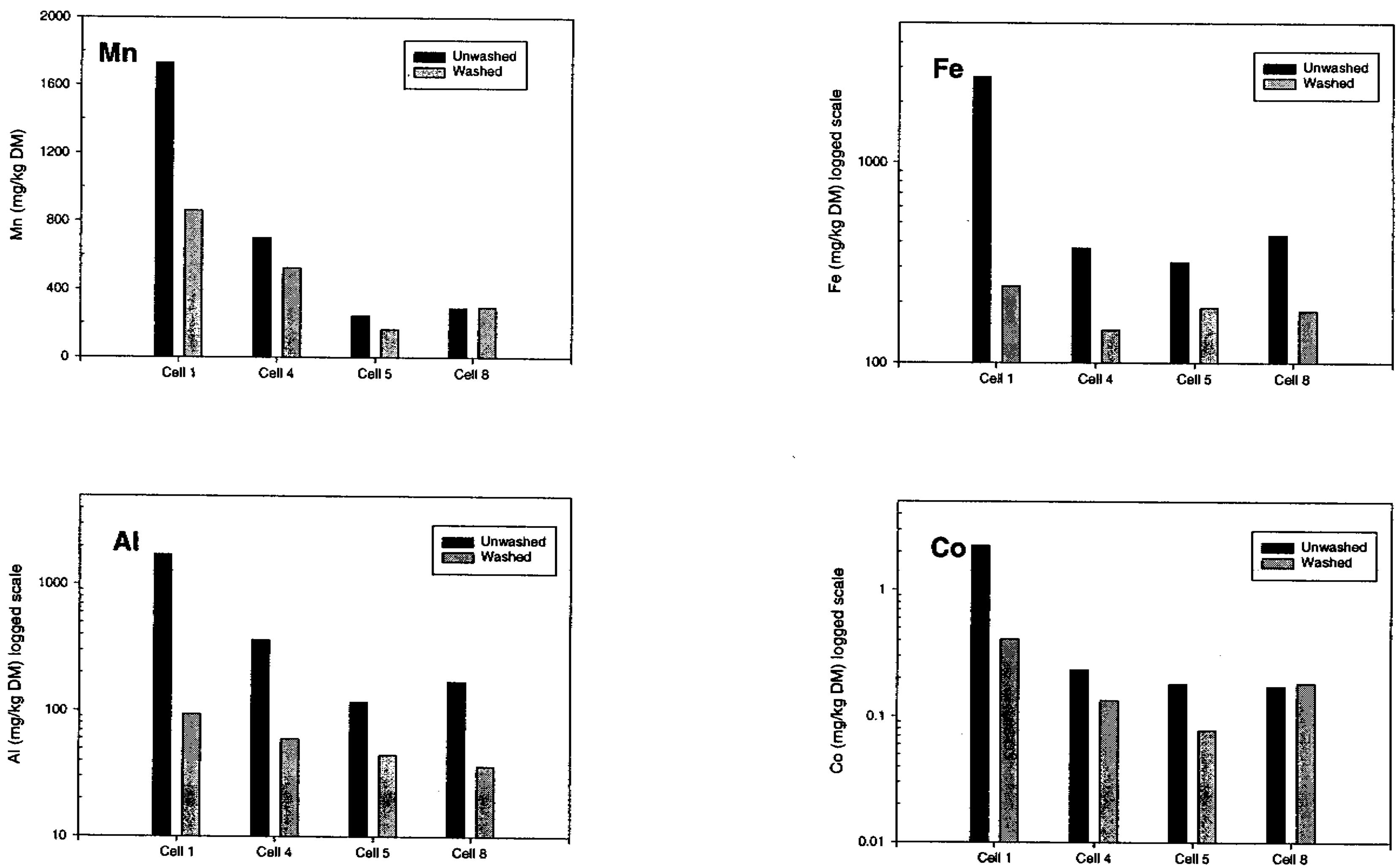


Fig C2 Fe, Mn, Al and Co contents (mg/kg DM) of washed and unwashed shoots of *Eleocharis sphacelata* sampled on 22/7/97

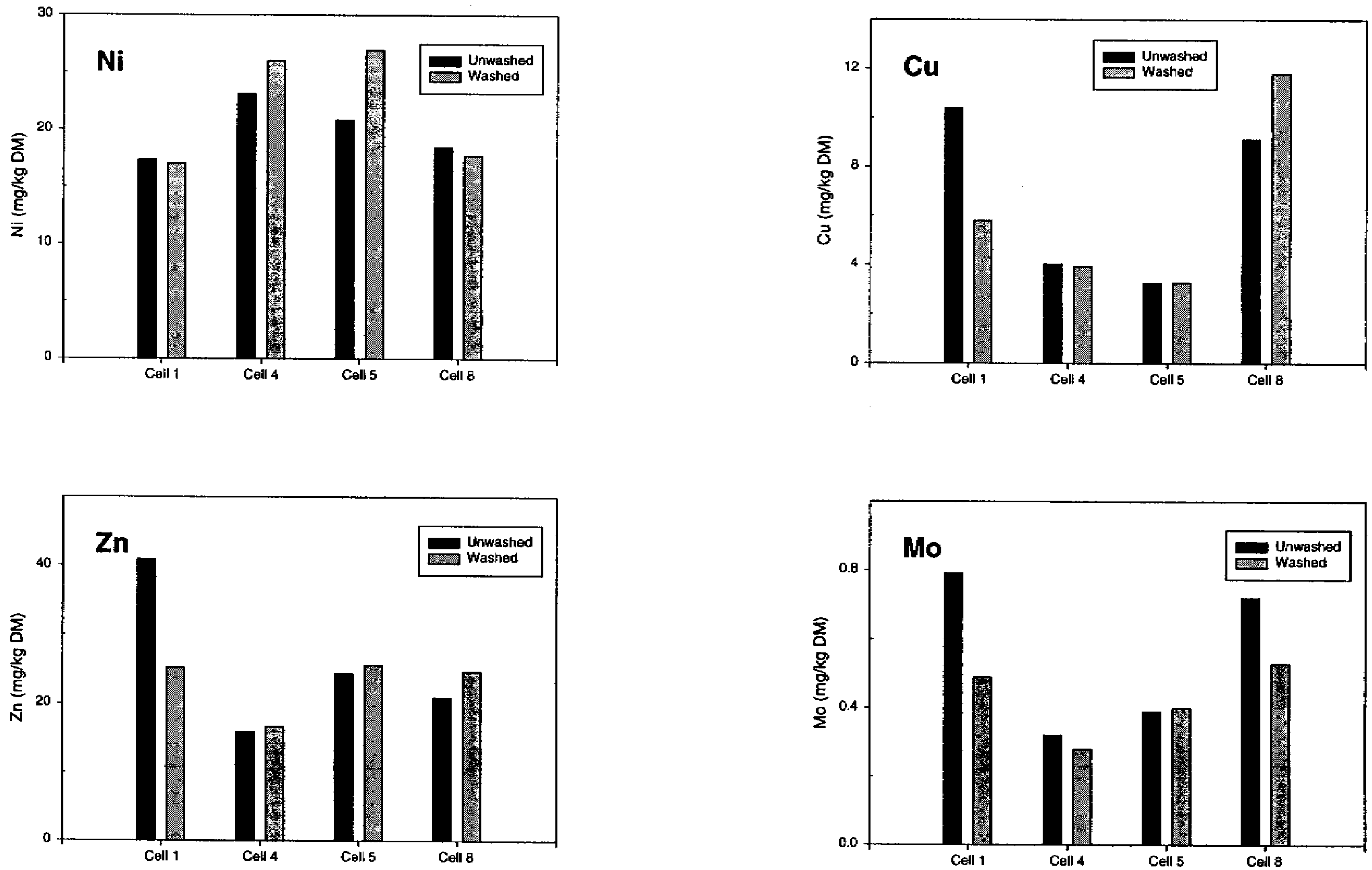


Fig C3 Ni, Cu, Zn and Mo contents (mg/kg DM) of washed and unwashed shoots of *Eleocharis sphacelata* sampled on 22/7/97

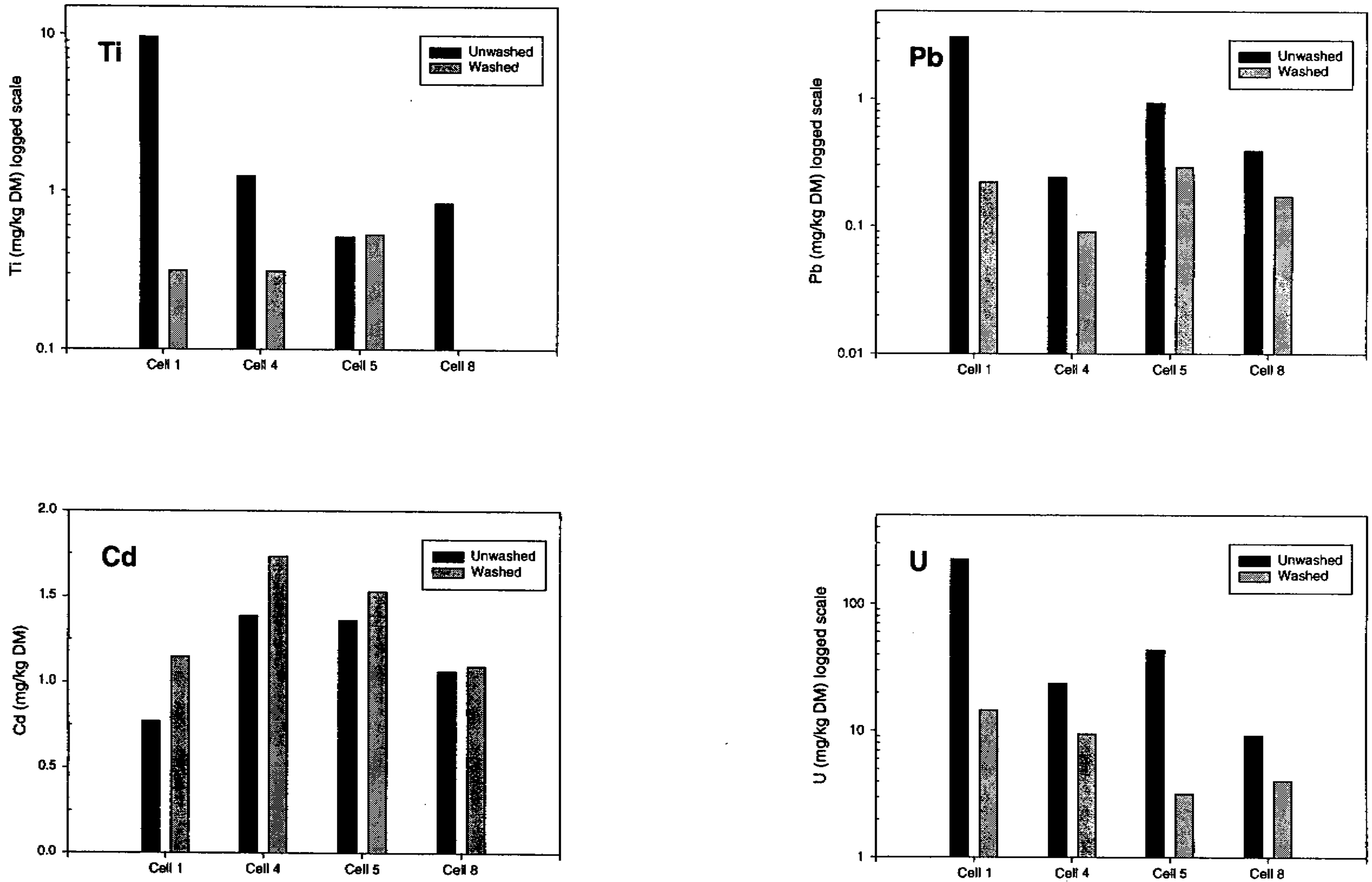


Fig C4 Ti, Pb and U contents (mg/kg DM) of washed and unwashed shoots of *Eleocharis sphacelata* sampled on 22/7/97

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