Ascophyllum nodosum (L.) Le Jolis as a bioindicator of radioactivity in the Irish Sea

Bioindicators
Radioactivity
Ascophyllum nodosum (L.) Le Jolis
Irish Sea

Bio-indicateurs
Radioactivité
Ascophyllum nodosum (L.) Le Jolis
Mer d'Irlande

Geoffrey Stanley BOURNE and David John ASSINDER

School of Ocean Sciences, University of Wales, Menai Bridge, Anglesey, Gwynedd LL59 5EY, Wales, UK.

ABSTRACT

The response of *A. nodosum* to a sudden increase in radioactivity analogous to a nuclear incident is examined in the field. ^{137}Cs, ^{241}Am and $^{239+240}Pu$ showed net accumulation with exposure time, unlike natural ^{228}Th, which was used as a control. Caesium had the highest accumulation rate followed by americium and finally plutonium. Younger plant sections were found to accumulate all the radionuclides significantly faster than older plant sections. Americium and plutonium were accumulated significantly faster in frond tissue than in vesicle tissue. No significant difference could be identified for caesium. These results and their implications for future monitoring surveys and modes of uptake are discussed.

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RÉSUMÉ

Ascophyllum nodosum (L.) Le Jolis en tant qu'indicateur de la radioactivité en Mer d'Irlande

La réaction de *A. nodosum* a une augmentation soudaine de la radioactivité analogue à une explosion nucléaire est étudiée sur le terrain. ^{137}Cs, ^{241}Am et $^{239+240}Pu$ montrent une nette accumulation à mesure que le temps d'exposition augmente, à l'opposé du ^{228}Th naturel, qui a été utilisé comme référence. Le caesium a le taux d'accumulation le plus élevé, suivi dans l'ordre par l'américium et par le plutonium. On a trouvé que des sections de plantes plus jeunes accumulent plus rapidement dans les tissus de frondaison que dans le tissu vésiculaire. On n'a pu identifier aucune différence significative pour le caesium. Ces résultats et leurs conséquences pour des enquêtes et contrôles à venir, et des modes d'exploitation ont été discutés.

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INTRODUCTION

The safe environmental discharge of contaminants requires a complete understanding of their physico-chemical and biological fates. Protection of biological resources is essential because of potential feedback to man through the food chain. Since the commissioning of the Sellafield nuclear fuel reprocessing facility (Cumbria, England) in the 1950s, biological samples from the Irish Sea have been analysed for their radionuclide content (*e.g.* Dunster, 1956; 1958; Mauchline, 1963). Annual surveys of the radioactivity in surface and coastal waters of the British Isles, which rely heavily on biological samples, are conducted by MAFF: The Ministry of Agriculture, Fisheries and Food (Mitchell, 1969-1977; Hunt, 1979-1989).

Certain biological materials are powerful monitoring aids and are termed bioindicators. Monitoring programmes based on bioindicators have three main advantages over those based on large seawater samples (Portmann, 1976; Phillips, 1978): 1) they give an indication of the biologically available contaminant levels; 2) they often possess high concentration factors, permitting quicker, simpler and cheaper analytical techniques; 3) they provide a time integrated average of short-term fluctuations in contaminant levels, thereby compensating for irregular discharge patterns or variations in the physical and chemical properties of seawater. Monitoring programmes may therefore be established which comprehensively monitor pollution and the biosphere, whilst still assessing public radiation exposure.

Macroalgae are sessile and respond to contaminant levels in the water column (Phillips, 1978). They are thus well suited to map the transport of aqueous discharges (*e.g.* Mitchell, 1967-1977; Hunt, 1979-1989). Heterotrophic organisms tend to be mobile and accumulate elements predominantly from their food (Pentreath, 1976). Concentrations in heterotrophic organisms in bioindicator studies should therefore only be examined with dietary and mobility considerations.

Brown macroalgae (Phaeophyceae) are powerful bioindicators because they often show concentration factors of several thousands relative to the surrounding seawater and because they cannot regulate their accumulation processes for many trace elements (Fuge and James, 1974). Radionuclide levels in brown algae should therefore be dependent on the levels in seawater. Brown algae have proved very useful bioindicators of marine environmental radioactivity (Nilsson *et al.*, 1981). In particular *Fucus* spp. and *Ascophyllum nodosum* (L) Le Jolis have been used as such on many occasions (*e.g.* Cross and Day, 1981; Thompson *et al.*, 1982; Cosson *et al.*, 1984; Germain and Miramand, 1984; Nilsson *et al.*, 1984; Mitchell, 1967-1977; Hunt, 1979-1989).

A. nodosum produces one set of vesicles each spring at the apical tips (Baardseth, 1970). The age of a plant section is shown by the number of vesicles between it and the apical tip (Fig. 1). Haug *et al.* (1974) found higher copper and zinc levels in older plant sections, due to a constant accumulation rate. Bourne and Assinder (in prep.) found higher plutonium and americium activities in older plant sections, but higher caesium levels in younger tissues, possibly due to caesium's chemical similarity to potassium which is closely linked to metabolism. They also investigated activity variations in *A. nodosum* with tissue type, season and distance from Sellafield.

This information on radionuclide distributions in algae under present conditions is important, however knowledge of their response to sudden increases in environmental radioactivity is an essential requirement for monitoring programmes. Single radionuclide effects can be successfully studied in aquaria (*e.g.* Bonotto *et al.*, 1989), however nuclear incidents could release many radionuclides (*e.g.* Chernobyl), possibly leading to competition between nuclides for macroalgal uptake sites (Phillips, 1978). In this study, the response of *A. nodosum* to a sudden increase in environmental radioactivity, analogous to a nuclear incident, was examined in the field.

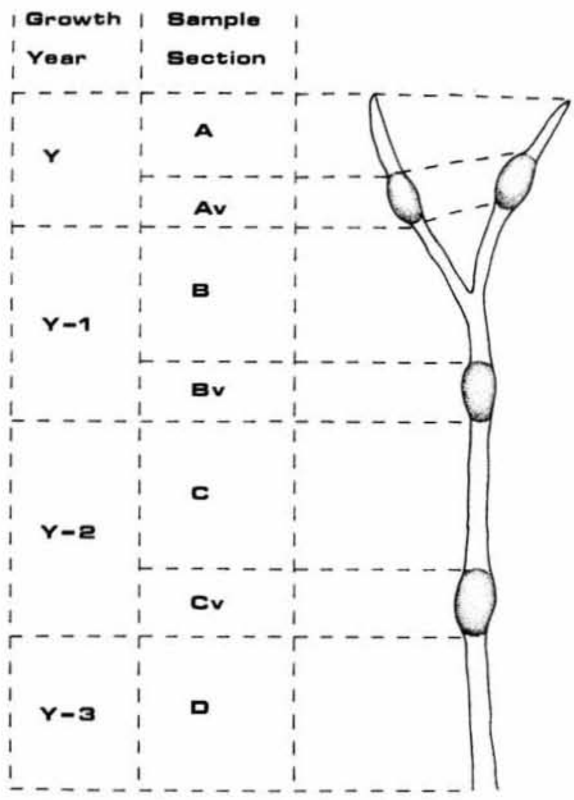


Figure 1

Sample sections and annual growth of Ascophyllum nodosum *(L) Le Jolis sampled in year Y.*

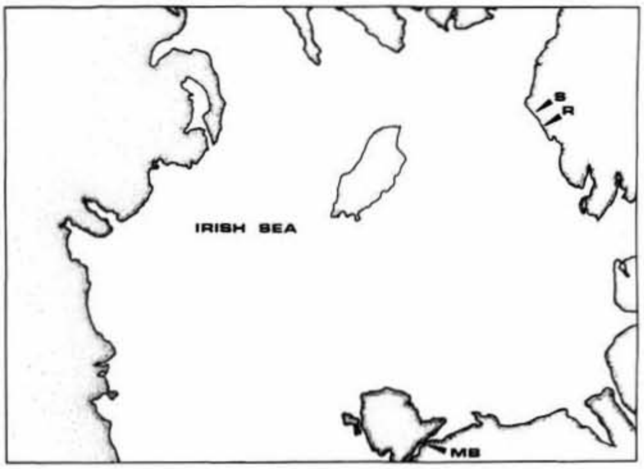
Échantillons et croissance annuelle de l'*Ascophyllum nodosum* (L.) Le Jolis pris en l'année Y.

MATERIALS AND METHODS

In May 1989, whole, live samples of *A. nodosum*, attached to substrate, were transported from Menai Bridge (North Wales) to the more radioactively contaminated Ravenglass estuaries (Cumbria, England), 10 km south of British Nuclear Fuels' reprocessing plant at Sellafield (Fig. 2). Samples were fixed in wire-mesh cages at the natural tidal height as shown by the indigenous population (Fig. 3), single whole plants were sampled after 0, 6, 18, 30, 42, 53, 92, 132 and 182 days.

Samples were sectioned according to age (damaged material leading to discontinuous growth is obvious and discarded), sub-sectioned into frond and vesicle tissue (Fig. 1) and oven-dried at 70°C. Dry weights were recorded after homogenising the samples. ^{137}Cs and ^{241}Am activities were determined by gamma spectrometry using a HPGe detector on samples ranging from 0.29 to 12.98 g with an average of 3.00 g.

Samples were ashed overnight at 450°C. Yield monitors, ^{228}Th and ^{236}Pu, were added before mixed nitric-



←Figure 2

The Irish Sea showing the relative positions of Sellafield (S), Menai Bridge (MB) and Ravenglass Estuaries (R) [1 cm = 25 miles].

La Mer d'Irlande montrant les positions relatives de Sellafield (S), Menai Bridge (MB) et les estuaires Ravenglass (R) [1 cm = 25 milles].

Figure 3

A. nodosum *still attached to substrate fastened in wire-mesh cages with fronds floating free of cage at natural tidal height as shown by the indigenous population.*

A. nodosum encore, collant à des substrats attachés dans ces cageots en mailles métalliques avec frondaisons flottant à l'extérieur des cageots au niveau naturel de la marée comme indiqué par la population indigène.

hydrochloric acid leaching and iron(III) hydroxide coprecipitation. Thorium and plutonium were separated by anion exchange chromatography (based on Wong, 1971) and electroplated onto stainless steel discs from a mixed oxalate-chloride medium. Alpha spectrometry was performed using silicon surface barrier detectors.

RESULTS AND DISCUSSION

Tables 1-4 show ^{137}Cs, ^{228}Th, ^{241}Am and $^{239+240}Pu$ activity variations in plant sections with exposure time. For ease of comparison, whole plant specific activities were calculated for each radionuclide. For the Menai Bridge ($t = 0$) and Ravenglass estuaries samples ($t = 0$), these values were in accordance with previous data (Hunt, 1979-1989; Bourne and Assinder, in prep.). Figures 4-7 show graphs of whole plant specific activities against exposure time. Regression analyses of activity against time, both before and after logarithmic transformation, were performed. Samples containing radionuclide activities not significantly different from zero were treated as zero activity. The increase in biologically available radioactivity was defined as the activity difference between the Menai Bridge sample and the Ravenglass sample (both sampled at $t = 0$). Times taken for plants to accumulate half of this activity increase (Ta) were calculated for each radionuclide from the regression equation.

Caesium, americium and plutonium showed net accumulation with increasing exposure time. Caesium was accumulated the fastest and logarithmically ($p < 0.001$), Ta = 15 days. The highest recorded caesium activity was 153.12 Bq/kg in the apical tip (section A) of the sample exposed for 182 days, compared with the whole plant specific activity of 103.31 Bq/kg (an apical tip: whole plant activity ratio of 1.48).

Americium was accumulated linearly with time ($p < 0.001$) and slower than caesium, Ta = 65 days. The highest recorded activity was 89.54 Bq/kg, again found in the apical tips of the plant exposed for 182 days, compared with the whole plant specific activity of 57.38 Bq/kg (a ratio of 1.56).

Plutonium was accumulated linearly with time ($p < 0.001$) and slower than caesium and americium, Ta = 90 days. The highest recorded activity was 80.51 Bq/kg, compared with a whole plant specific activity of 52.49 Bq/kg (a ratio of 1.53).

Thorium showed no significant accumulation with time. Caesium, americium and plutonium are present in the Ravenglass estuaries at elevated levels relative to Menai Bridge because of their presence in Sellafield discharges. ^{228}Th is a natural radionuclide not significantly enriched in Sellafield discharges and therefore should not be present at elevated levels in the Ravenglass estuaries. The major problem with using algae as bioindicators is that competition for binding sites occurs at high concentrations leading to underestimation of contamination (Phillips, 1978). If competition for binding sites had occurred, a decrease in ^{228}Th specific activity with increasing exposure time would have been expected, this did not occur.

The similarity in the apical-tip whole-plant ratios for each radionuclide in the sample exposed for 182 days may be fortuitous because this degree of similarity does not extend to most samples. In general, however, these ratios are greater than unity, probably due to the formation of new binding sites at the apical tips and therefore greater accumulation potential relative to older tissues.

Variations in accumulation rates between plant sections were analysed by regression analyses. Logarithmic regressions of

Table 1

Variations in ^{137}Cs activity (Bq/kg) in A. nodosum *sections with increasing exposure time including activity of an indigenous Ravenglass sample (R) and weighted specific activities (M).*

Variations de l'activité de ^{137}Cs (Bq/kg) des sections de *A. nodosum* avec des temps d'exposition progressivement plus longs, incluant l'activité d'un échantillon indigène Ravenglass (R) et des activités spécifiques pondérées (M).

Days exposed	Plant section							Average
	A	Av	B	Bv	C	Cv	D	M
0	N/D	32.67	36.00	35.88	28.74	28.88	33.88	32.22
6	29.54	43.89	42.60	37.37	N/D	N/D	54.32	35.31
18	67.76	55.46	42.90	54.64	32.37	61.26	33.89	46.40
30	58.30	63.59	51.29	55.03	58.73	53.99	71.19	56.99
42	83.89	126.47	78.92	71.56	66.12	70.14	67.68	82.66
53	75.22	82.13	54.13	66.29	63.70	74.53	120.79	70.97
92	100.78	110.33	94.41	77.61	94.41	86.28	57.50	90.85
132	84.69	78.77	81.69	104.67	66.27	103.46	90.13	87.63
182	153.12	135.92	110.66	96.75	104.28	71.23	89.71	103.31
R	N/S	94.20	80.70	120.42	84.95	111.50	64.01	84.36

N/D: not detected; N/S: no sample; all counting errors < 10 %.

N/D : non détecté ; N/S : aucun échantillon ; toutes erreurs de comptage < 10 %.

Table 2

Variations in ^{241}Am activity (Bq/kg) in A. nodosum *sections with increasing exposure time including activity of an indigenous Ravenglass sample (R) and weighted specific activities (M).*

Variations de l' activité de ^{241}Am (Bq/kg) des sections de *A. nodosum* avec des temps d'exposition progressivement plus longs, incluant l'activité d'un échantillon indigène Ravenglass (R) et des activités spécifiques pondérées (M).

Days exposed	Plant section							Average
	A	Av	B	Bv	C	Cv	D	M
0	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
6	9.74	14.94	8.30	8.20	7.79	N/D	N/D	8.63
18	27.03	18.92	14.46	6.00	12.50	16.63	29.90	15.33
30	30.42	8.04	23.59	11.44	28.24	17.21	26.13	17.02
42	N/D	20.45	18.08	N/D	22.00	N/D	26.58	14.07
53	39.65	24.53	24.69	29.62	31.96	17.09	40.08	27.79
92	35.16	30.40	40.83	28.30	47.64	24.01	41.38	36.40
132	61.01	35.58	58.72	30.24	21.36	35.23	80.01	43.61
182	89.54	70.99	72.78	37.27	67.24	32.27	59.38	51.38
R	N/S	32.92	43.95	60.61	60.49	55.56	49.45	51.11

N/D: not detected; N/S: no sample; all counting errors < 10 %.

N/D : non détecté ; N/S : aucun échantillon ; toutes erreurs de comptage < 10 %.

caesium activities in two plant sections (C and Cv) were not significantly different from zero. This was due to the labelling of undetectable radionuclide activities as zero, an activity level which is very improbable. In the following discussion, therefore, regression analyses on caesium accumulation in sections C and Cv are based on only eight observations, the zeros in each having been ignored. The regression gradients (*i. e.* accumulation rates) were compared using a simple t-test (Lee and Lee, 1982). T-test significance results are given in Tables 5-7.

Caesium was accumulated in the order A > Av > B > Bv > C > Cv > D. Younger sections accumulated caesium significantly faster than older sections (Tab. 5). No significant difference in accumulation rates was identified between the frond and vesicle tissue. Americium was also accumulated fastest by younger tissues, in the order A > B > C > D > Av > Bv > Cv. Accumulation of americium was significantly faster in frond compared to vesicle tissue (Tab. 6). Plutonium was also accumulated significantly faster in younger tissues, in the order B > A > Av > D > C > Bv > Cv. Plutonium, like americium was accumulated significantly faster in frond compared to vesicle tissue (Tab. 7). No comparable data have been identified in the literature.

Table 3

Variations in $^{239+240}Pu$ activity (Bq/kg) in A. nodosum *sections with increasing exposure time including activity of an indigenous Ravenglass (R) sample and weighted specific activities* (M).

Variations de l' activité de $^{239+240}Pu$ (Bq/kg) des sections de *A. nodosum* avec des temps d'exposition progressivement plus longs incluant l'activité d'un échantillon indigène Ravenglass (R) et des activités spécifiques pondérées (M).

Days exposed	Plant section							Average
	A	Av	B	Bv	C	Cv	D	M
0	4.86	2.27	4.08	4.88	6.29	4.41	6.40	4.82
6	11.43	7.00	10.44	5.87	6.71	4.04	9.39	8.39
18	13.42	14.84	12.16	7.21	10.00	10.00	11.78	10.77
30	17.29	11.75	14.62	7.58	13.31	12.28	20.49	11.93
42	18.56	12.13	15.14	9.45	14.44	8.41	15.23	13.35
53	19.00	14.39	14.76	8.72	17.80	10.65	18.11	14.32
92	31.63	31.88	38.03	16.42	28.18	11.35	19.85	28.50
132	34.80	33.13	55.21	29.46	45.76	43.00	43.33	40.68
182	80.51	64.77	78.12	35.66	51.27	29.30	47.13	52.49
R	N/S	38.16	42.87	58.84	75.04	81.14	54.04	60.38

N/D: not detected; N/S: no sample; all counting errors < 10 %.

N/D : non détecté ; N/S : aucun échantillon ; toutes erreurs de comptage < 10 %.

Table 4

Variations in ^{228}Th activity (Bq/kg) in A. nodosum sections with increasing exposure time including activity of an indigenous Ravenglass sample (R) and weighted specific activities (M).

Variations de l'activité de ^{228}Th (Bq/kg) des sections de *A. nodosum* avec des temps d'exposition progressivement plus longs incluant l'activité d'un échantillon indigène Ravenglass (R) et des activités spécifiques pondérées (M).

Days exposed	Plant section							Average
	A	Av	B	Bv	C	Cv	D	M
0	3.15	0.58	1.29	1.12	0.91	0.89	1.21	1.08
18	1.87	1.04	0.98	0.91	1.10	1.76	1.63	1.19
42	0.38	0.51	0.50	0.80	0.66	1.01	1.55	0.66
92	0.67	0.74	0.70	0.85	1.21	1.23	1.09	0.86
132	3.31	1.56	1.78	1.29	2.02	1.34	2.46	1.85
182	3.01	2.80	2.72	1.57	2.77	1.89	2.47	2.36
R	N/S	1.73	1.12	2.44	2.00	4.52	1.80	1.87

N/D: not detected; N/S: no sample; all counting errors < 10 %.

N/D : non détecté ; N/S : aucun échantillon ; toutes erreurs de comptage < 10 %.

No significant differences were identified between the caesium accumulation rates of frond and vesicle tissues. Americium and plutonium, however, are accumulated significantly faster by frond tissue. Caesium is chemically similar to potassium which is linked to metabolism. Vesicles provide buoyancy enabling the plant to photosynthesise optimally when submerged (Aleem, 1969). They were originally suggested to have a special metabolic role (Zeller and Neikirk, 1915) although this has been disputed (Sifton, 1945; Dromgoole, 1981). If vesicles play a role in metabolism then their relative enrichment of caesium compared to americium and plutonium may be explained. It is possible, however, that the different accumulation patterns observed between frond and vesicle tissue are due to chemical or physical differences between the radionuclides studied and not to physiological variations.

Plutonium and americium activities have been shown to increase with increasing age of the plant material due to the constant accumulation of the elements with time (Bourne and Assinder, in prep.). At the conclusion of this experiment, however, the trend was reversed. New binding sites are continually produced as plants grow. These should accumulate radionuclides at the same rate as new material in the indigenous Ravenglass population. Activities found in growing plant sections should therefore tend to the activity found in the same section of plants comprising the indigenous population. Tables 1-4, however, show that younger, growing tissues after six months exposure contain more caesium and about twice as

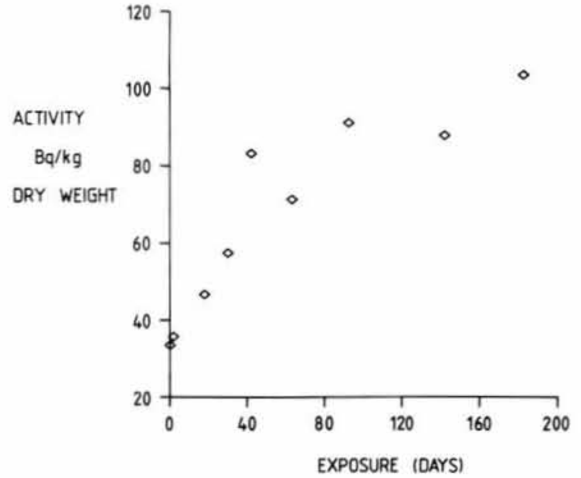


Figure 4

Whole plant ^{137}Cs specific activity (Bq/kg) with increasing exposure time.

Activité spécifique (Bq/kg) des plants ^{137}Cs entiers avec temps d'exposition progressivement plus long.

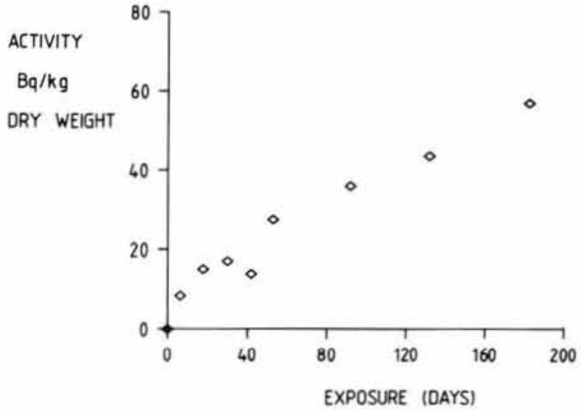


Figure 5

Whole plant ^{241}Am specific activity (Bq/kg) with increasing exposure time.

Activité spécifique (Bq/kg) des plants ^{241}Am entiers avec temps d'exposition progressivement plus long.

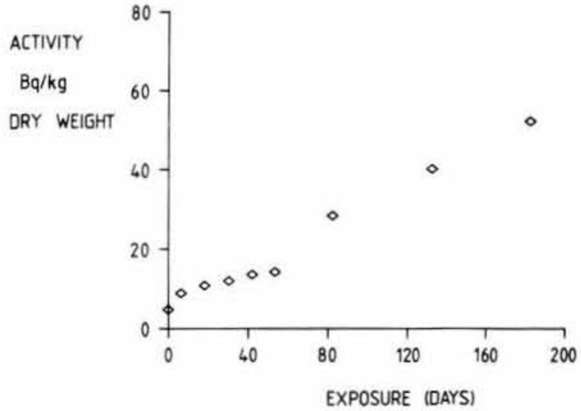


Figure 6

Whole plant $^{239+240}Pu$ specific activity (Bq/kg) with increasing exposure time.

Activité spécifique (Bq/kg) des plants $^{239+240}Pu$ entiers avec temps d'exposition progressivement plus long.

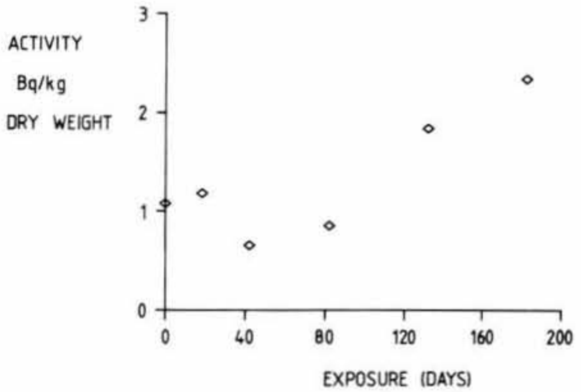


Figure 7

Whole plant ^{228}Th specific activity (Bq/kg) with increasing exposure time.

Activité spécifique (Bq/kg) des plants ^{228}Th entiers avec temps d'exposition progressivement plus long.

much plutonium and americium as expected from the reference Ravenglass estuaries sample.

As the Ravenglass sample was taken in May, a seasonal effect is expected. Algae show maximal whole plant concentrations in spring and minimal concentrations in autumn (Phillips, 1978), therefore exaggerating this phenomenon. Possible reasons for this are:

1) There is a difference in accumulation potential between the populations of Menai Bridge and Ravenglass Estuaries.

2) The sample taken at Ravenglass in May might not have been representative of the population. Previous work, however, has indicated good agreement in activities of replicate *A. nodosum* samples when analysed by the method employed here (Bourne and Assinder, in prep.).

3) The activities of all radionuclides present in Sellafield effluent increased during the course of this experiment. This is the most likely explanation as the Sellafield discharge pattern is known to be variable. If this is the case then *A. nodosum* has responded to the increase in activity of the surrounding seawater, proving its value as a bioindicator of marine environmental radioactivity. Ta values quoted earlier for radionuclides would therefore be minimum estimates, however no change in the order of each Ta would be expected.

It is not possible to age other algal species by the method employed here. It is essential, however, to be as specific and consistent as possible when defining samples analysed as part of a monitoring programme. Nilsson *et al.* (1981) identified the age of *Fucus* samples by using the ratio $^{228}Th/^{228}Ra$. It may therefore be possible to age all samples in the future or at least to produce an average age of the sample.

Future monitoring programmes could be based solely on analysis of the newest tissues, these showing the quickest response to radionuclide activity changes in the surrounding seawater.

Table 5

Comparison of ^{137}Cs accumulation rates between plant sections by T-Test. n = 16 in all cases except: 1: n = 15, 2: n = 14.

Comparaison des taux d'accumulation de ^{137}Cs entre sections de plantes par T-test, n = 16 dans tous les cas sauf: 1: n = 15, 2: n = 14.

Plant section	Plant section Av	B	Bv	C	Cv	D
A	2.94**	5.99***	6.92***	5.79[1]***	7.29[1]***	5.94***
Av		2.17*	2.43*	2.12[1]ns	2.98[1]**	2.62*
B			0.10ns	0.11[1]ns	1.03[1]ns	0.85ns
Bv				0.04[1]ns	1.14[1]ns	0.85ns
C					0.85[2]ns	0.70[1]ns
Cv						0.77[1]ns

*ns: no significant difference. *: 0.05 >p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001.*

ns : aucune différence significative. * : 0.05 >p > 0.01 ** : 0.01 > p > 0.001 ; *** : p < 0.001.

Table 6

Comparison of ^{241}Am accumulation rates between plant sections by T-Test. n = 16 in all cases.

Comparaison des taux d'accumulation de ^{241}Am entre sections de plantes par T-test, n = 16 dans tous les cas.

Plant section	Plant section Av	B	Bv	C	Cv	D
A	4.44***	1.74ns	7.83***	1.51ns	8.43***	1.89ns
Av		4.56***	5.09***	2.42*	5.90***	1.73ns
B			10.78***	0.28ns	12.01***	0.84ns
Bv				6.21***	0.71ns	5.27***
C					6.81***	0.45ns
Cv						5.82***

*ns: no significant difference. *: 0.05 >p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001.*

ns : aucune différence significative. * : 0.05 >p > 0.01 ** : 0.01 > p > 0.001 ; *** : p < 0.001.

Table 7

Comparison of $^{239+240}Pu$ accumulation rates between plant sections by T-Test. n = 16 in all cases.

Comparaison des taux d'accumulation de $^{239+240}Pu$ entre sections de plantes par T-test, n = 16 dans tous les cas.

Plant section	Plant section Av	B	Bv	C	Cv	D
A	2.26*	3.05**	10.11***	4.61***	7.88***	3.92**
Av		7.10***	10.34***	2.81*	6.99***	2.01ns
B			22.49***	13.20***	13.25***	10.12***
Bv				13.54***	0.20ns	9.46***
C					6.09***	0.28ns
Cv						5.76***

*ns: no significant difference. *: 0.05 >p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001.*

ns : aucune différence significative. * : 0.05 >p > 0.01 ** : 0.01 > p > 0.001 ; *** : p < 0.001.

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