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RADIONUCLIDE AND METAL BIOREMEDIATION FROM AQUATIC ENVIRONMENT BY ALGAE

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Abstract

Three element were chosen for this investigation, radioactive carbon ^{14}C in form of ^{14}C-urea and two element; chromium III and cobalt to study their biosorption and desorption mechanisms by two algal species; *Sargassum linifolium* and *Dunaliella salina*, in relation to their surface area. Active transport was the mechanism for ^{14}C, Co and Cr uptake by the two algal species. *Sargassum* showed higher accumulation ratios than living algal cells; the reverse was true for *Dunaliella*. The antagonistic action between metal ions (radionuclide) binding to different algal cell surface was obtained by mixing ^{14}C with Cr and ^{14}C with Co in living algal cells of the two tested algae, while synergistic action was obtained by mixig Cr and Co in the living algal species. Non-interactive action was shown by mixng ^{14}C with Cr or Co in dried algal material. ^{14}C-uptake by living cells of *Sargassum* and *Dunaliella* ameliorated cell vitality; this effect was higher in *Dunaliella* than in *Sargassum*. Chromium showed adverse effect than cobalt, both Co and Cr altered the metabolic pathway of chlorophyll formation. Siderophore formation increased the adsorption power of algal cell-wall especially *Sargassum* alga. The biosorption effect of these radionuclides was due to surface characteristics. Desorption mechanism was fast from *Sargassum* surfaces, while it was slower in *Dunaliella*. Dried Sargassum can be used successfully for bioremediation of ^{14}C, Co and Cr from the contaminated sea water (even, at low concentration) up to five elution times, while living *Dunaliella* could be used for the removal of these elements from lakes. To ameliorate the adsorptive power of dried *Sargassum*, its surface area must be increased and the media must be iron-free.

Introduction

Along the Mediterranean coasts, a number of nuclear energy reactors have been established in France, Spain …etc. Nuclear reactors release gaseous radioisotopes, mainly ^{3}H, ^{14}C, ^{133}I and ^{135}I and liquid effluents containing mainly ^{3}H (Halim, 1987). Aqueous effluents produced during nuclear power and defense activities frequently contain radionuclides. These effluents obtained from various sources, including reactor coolant water, evaporator condensate and fuel reprocessing wastes. Metal contained within these wastes include mainly the fission product strontium ^{90}Sr and caesium ^{137}Cs which possess particular health hazards. In addition, about nine radioactive metals enter the Suez Canal and Mediterranean Sea either directly or indirectly and cause radioactive pollution to the marine environment (Gazso, 2001).

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According to radiation hazards, several international and national organizations have established to set of guidelines for the safe handling of radioactive materials. The recommendations include: the maximum permissible dose, principles of radiation protection, personal monitoring, survey meters and waste disposal (Saleh, 1987). In this investigation, radionuclide wastes bioremediation from the marine environment was studied.

Among the aquatic food chains, algae may absorb radioisotopes from the aquatic environment, both ionic and particulate forms of radionuclide, and both passive and active mechanisms of uptake may be involved.

In natural waters, the uptake of a given isotope in relation to other fission products may be quite different from that on land. For example, planktonic algae were found to accumulate ^{144}Ce to a much greater extent than ^{137}Co, whereas for land plants the reverse is true (Rice and Willis, 1959).

Observations of surface adsorption were firstly discussed in 1949 by Spooner; this adsorption is recently known as biosorption (surface adsorption). Once occurred, such radioisotopes may be more or less firmly bound or readily eluted. For example nitrosyl ruthenium apparently can form nonexchangeable complexes involving colloidal iron on the surface of diatom cells, although in the absence of iron, this does not occur (Jones, 1960). This phenomenon is recently known as siderophore.

Application of biotechnology for the treatment of various forms of radionuclides liquid wastes has many advantages such as; environment friendliness, self-reproducibility and adaptability, recycling of bio-products, specificity and good cost benefit ratios (Gazso, 2001).

In this investigation three of the hazards nuclide found in the aquatic environment was chosen, viz: cobalt (Co), chromium (Cr III) and radioactive carbon (^{14}C). Biosorption and desorption of these radionuclide, by the marine macroalga *Sargassum linifolium* as representative for marine environment and *Dunaliella salina*, the unicellular alga as representative for lakes environment in relation to their surface area, were investigated. Also, the effect of these radionuclide on cell activities, in addition to siderophores production were also investigated, aiming to find safe, economic and practical mechanism for radionuclide removal with the recycling of biosorbant either from marine environment or from lakes.

Materials and Methods

Algae materials

Sargassum linifolium (Phaeophyta) was collected from Abu Qir locality in Alexandria. Ten grams fresh weight samples were washed thoroughly with sterilized distilled water several times and bacterial detection was carried out as described by Bekheet and Syrett (1977). Fresh samples of *S. linifolium* used as biosorbant were transferred to flasks containing 50 mL medium prepared as

described by Shaban (1981). ^{14}C-urea, chromium sulphate ($Cr_2(SO_4)_3$) and cobalt chloride ($CoCl_2$) were added (individually) to this living material and deionized water was used for media preparation.

Dried samples of *S. linifolium* were prepared (in parallel with the fresh samples) by drying in an oven at 60ºC, then ground to a size in range of 500-700μm and stored in a desiccator.

The unicellular alga, *Dunaliella salina* (Chlorophyta), was the second biosorbant alga used in this investigation. It was obtained from Lake Mariut of Alexandria, purified and identified according to Masjuk (1972). The alga was cultivated on MH medium described by Loeblich (1982). The cultures were maintained at 25ºC under light intensity of 4000 lux and aerated with sterilized air enriched with 5 per cent CO_2 for 12 days. The algal cells were harvested at the mid of the exponential phase of growth, subjected to starvation for 24 hours, then diluted to give a suspension containing $7x10^6$ cell mL^{-1} (Bekheet *et al*., 1984). At each sampling time, the algal suspension was filtered through the GF/C glass microfilter. The supernatents were discarded and the remaining filter discs with the pellet (cells) were subjected for measuring the tested metal or ^{14}C. Dried *Dunaliella* cells were prepared as for *Sargassum*.

Radionuclide preparation

Radionuclide (^{14}C-urea), and chromium or cobalt were added to flasks containing fresh *Dunaliella* or *Sargassum* as well as to dried algal cells of both algae. Heavy metal concentrations (5-15 mg/L) were added by filtration to the sterilized stock medium through 0.2 m nitrocellulose membrane of $K_2Cr_2O_7$. The flasks were agitated at 200 rpm and 25ºC±2 in rotatory shaker, irradiance (200 mol/$m^{-2}s^{-1}$). The pH of the flasks was adjusted at 5 and the flasks kept for 10 hours or 36 hours incubation periods then filtered through 0.45 m membrane filters and finally acidified and kept for analysis using the method described by Valdmar and Leite (2000). Control experiments were carried out simultaneously.

Cobalt, chromium and ^{14}C-urea were introduced to stock medium singly as well as in mixture: ^{14}C plus Co,: ^{14}C plus Cr and Cr plus Co; the second treatment was injected after 1 hour of injecting the first treatment. Experiments were stopped after 36 hour (Terry and Stone, 2002).

The radioactivity in pellet (living), residue (non-living) and filtrate of the two investigated algae was measured by Beckman LS 200B Liquid Scintillation Counter as described by Price (1983).

Determination of sorption surface areas

To calculate the surface area of *Dunaliella salina* algal cells (living and dried), the method described by Tien (2002) was used. The mean dimensions of the cell were measured by haemocytometer (assuming that algal shape approximates to a cylinder), and its surface areas were calculated by the equation:

$2\pi r (r + h)$, where: π is a constant = 3.14; r is the radius of the cell and h is the height. The total surface area per unit volume was calculated by multiplying the average surface area per unit cell by the total number of cells. The surface area of dried *Sargassum* material was also calculated from the mean dimension of the dried particles as mentioned above.

Desorption of adsorbed radionuclide

The algal material after treatment with radionuclide was introduced into 125 ml Erlenmeyer flasks containing 100ml eluent. The flasks were shaked at 200 rpm, then the samples were centrifuged and the total metal concentration was assayed for Cr and Co in eluent by atomic absorption spectroscopy and by Scintillation counter for ^{14}C. Eluent used for this experiment were $CaCl_2$ (0.05M) in HCl for cobalt removal (Volesky and Kuyucak, 1988); H_2SO_4 (0.6M) for chromium (Amorim *et al*., 2003); and NaOH (0.1) for^{14}C (Vilar *et al*., 2007). All the desorption experiments were carried out in algal-packed columns with diameter / length ratio of 1/15 (Yang and Volesky, 1999).

The amount of adsorption (Q_{ads}) and desorption (Q_{des}) were calculated as described by Amorim *et al*. (2003) using the equations:

$$Q_{ads} = (Ci - Cf) . V/M$$

$$Q_{des} = C_{des}. V/M$$

Where: Q_{ads}= Experimental amount of adsorption of element (mg/g),

Q_{des}= Experimental amount of desorption of element (mg/g),

C_{des}= Concentration of desorbed metal (mg/l),

Ci= Initial concentration (mg/l),

Cf= Final equilibrium concentration,

V= Volume of solution (l),

M= weight of biomass (g).

Siderophores formation and chlorophyll estimation

Iron-deficient media for culturing *Sargassum* and *Dunaliella* was prepared as described by Neilands (1983). The effect of this deficiency on Co, Cr and ^{14}C-radionuclide adsorption was tested (relative to the control) and accumulation ratios were calculated. Chlorphylls were estimated according to Jeffrey and Humphrey (1975).

Results and Discussion

Active transport was the mechanism of ^{14}C, Cr and Co uptake by both *Sargassum linifolium* and *Dunaliella salina* of this investigation, (Table 1), since the accumulation ratios of these nuclides under the low and high concentrations used exceeded 1.

Both *Sargassum* and *Dunaliella* used exhibited a number of metabolic-dependent and metabolic-independent processes of uptake and accumulation of radionuclide (Gazso, 2001) and heavy metals (Gadd, 1990; Chaisukant, 2003 and

Akhtar *et al*., 2004). The tendency of *Sargassum* (living and dried) to accumulate ^{14}C was greater than its tendency to cobalt and chromium (Table 1). Non-living algal material exceeded the living material by about 7-fold in ^{14}C uptake; with both cobalt and chromium only 2-fold was increased over the living material. High adsorptive power for dried cells was for ^{14}C and less efficiency was observed for chromium and cobalt treatments.

Table (1): Bioaccumulation of ^{14}C, Co and Cr in *Sargassum linifolium* and *Dunaliella salina* in living materials and in dried cells. Values are percent of total concentrations used.

Algal species			Treatment					
			^{14}C		Co		Cr	
			10h	36h	10h	36h	10h	36h
S. linifolium	Living	Cells	82.00	89.80	76.25	82.14	73.41	80.40
		Medium	18.00	10.20	23.75	17.86	26.59	19.60
		C/M	4.50	8.80	3.21	4.60	2.76	4.10
	Dried	Cells	91.70	97.35	91.10	90.99	79.70	90.19
		Medium	8.30	2.65	8.90	9.01	10.30	9.81
		C/M	11.02	36.70	10.2	10.09	8.70	9.19
D. salina	Living	Cells	85.34	91.00	73.27	87.70	72.61	87.50
		Medium	14.66	9.00	26.73	12.30	27.39	12.5
		C/M	5.82	10.11	2.74	7.13	2.65	7.0
	Dried	Cells	85.98	85.78	82.56	83.61	83.05	80.08
		Medium	14.02	14.22	17.44	16.39	16.95	19.92
		C/M	6.13	6.03	4.73	5.10	4.90	4.02

The difference in accumulation ratio between dried and *in vitro* cells of *Sargassum* was due to the large surface area of dried algal materials; the adsorptive power was high and accordingly the accumulation ratio was greater than in living algal materials. In the contrary, the accumulation ratio in living cells of *Dunaliella* exceeded that of the dried cells (Table 1). After 36 hour of incubation with either ^{14}C, Co or Cr, the accumulation ratio was about 3-fold that after 10 hour incubation. In dried cells, the accumulation ratio was almost constant for each element within the 36 hour incubation period. In the unicellular algae, *Chlorella vulgaris* and *Scenedesmus obliquus*, dried algal cells had high adsorption power for metals at very low concentration, and to accumulate them with large quantities, via active transport (Centinkaya Donmez *et al*., 1999). Shafik and Alaa (1995) found that: living *Scenedesmus obliquus* removes large amounts of phosphorous from water by an active transport system.

Terry and Stone (2002) pointed out that living microalgae have an advantage to be used as biosorbant, due to metabolic uptake and continuous growth. They postulated that the microalga *Scenedesmus abundans* significantly

outperformed dried algae in metal adsorption. Moreover, Pena-Castro *et al*. (2004) found that *Scenedesmus incrassatulus* adsorbed chromium VI efficiently by living cells because the uptake of chromate could be favored by actively growing alga. The results of this investigation agree with that of Pena-Castro (2004) for *Dunaliella* microalga.

In *Sargassum*, the accumulation ratio was higher when chromium was mixed with cobalt and with cobalt when mixed with chromium (about 2-fold) than when cobalt or chromium was present singly (Table 2). No significant difference was obtained when Cr was added prior to Co. When Co was added prior to ^{14}C-urea, the accumulation ratio of ^{14}C was increased by 0.50% while, addition of ^{14}C prior to Cr decreased them. The effect of chromium (trivalent metal) on ^{14}C and Co was strongly marked; chromium ratio itself was ameliorated.

Table (2): Combined effect of ^{14}C, Cr and Co on *Sargassum linifolium* and *Dunaliella salina* bioaccumulation (by either living or dried) algal materials. The first element was added 1 hour before the addition of the second element, and then incubated together for 36 h.

Algal species			Treatments											
			^{14}C prior to Cr		Cr prior to ^{14}C		^{14}C prior to Co		Co prior to ^{14}C		Co prior to Cr		Cr prior to Co	
			^{14}C	Cr	Cr	^{14}C	^{14}C	Co	Co	^{14}C	Co	Cr	Cr	Co
S. liumifolium	Living	C	88.5	77.3	79.o	86.1	86.7	73.0	81.0	88.3	85.7	89.1	89.0	86.2
		M	11.4	22.6	20.9	10.8	13.2	26.9	19.9	11.6	14.2	10.8	10.9	13.7
		A	7.7	3.42	3.77	8.20	6.53	2.71	4.06	7.61	6.02	8.21	8.15	6.29
	Dried	C	95.8	89.1	87.5	96.8	95.0	88.6	88.4	96.0	90.0	88.1	89.0	88.0
		M	4.14	10.8	12.5	3.18	4.98	11.3	11.5	3.92	10.0	11.8	10.9	11.9
		A	23.1	8.21	7.00	30.4	19.0	7.83	7.63	24.5	9.00	7.46	8.13	7.39
D. salina	Living	C	90.5	86.5	88.0	87.8	90.6	87.2	87.7	88.9	89.4	86.0	87.7	87.9
		M	9.47	13.4	11.9	12.2	9.31	12.7	12.2	11.0	10.5	13.9	12.2	12.0
		A	9.56	6.42	7.38	7.20	9.74	6.85	7.16	8.06	8.51	6.19	7.15	7.29
	Dried	C	82.5	84.3	81.1	83.3	83.0	81.1	79.9	81.4	80.9	82.0	82.5	80.0
		M	17.4	15.6	18.8	16.6	16.9	18.8	20.0	18.5	19.0	17.9	17.4	19.9
		A	4.74	5.39	4.31	5.02	4.90	4.31	3.98	4.38	4.26	4.56	4.73	4.02

C= Cells; M= Medium; A= Accumulation ratio

Pre-addition of ^{14}C to cobalt negatively affected its accumulation ratio (2.71), in case of adding ^{14}C after Co; cobalt improved ^{14}C-ratio and also its ratio was enhanced (4.06, 7.61 for Co prior to ^{14}C).

Chromium-cobalt interaction was masked perhaps by the effect of ^{14}C-which is taken up by the cells to enter the photosynthetic metabolic pathway, through which it counteracted the effect of other metals. Amado filho *et al*. (1999) postulated that metals were mainly adsorbed on the outer surface of the living algal cell on the basis of the free ion activity.

In dried *Sargassum* material, there was an increase in accumulation ratios with all the treatments used. The least accumulation ratio ranged from 8.21 to 7.00. This again spots the light on the free ion activity hypothesis of algal surface. The above results indicated that: dried cells of *Sargassum* had high adsorption power for ^{14}C with less efficiency for chromium and cobalt.

In *Sargassum stenophyllum*, the cell wall played the main role in cadmium accumulation. The release of metals by this alga has been frequently associated with exudation of metal chelated to polyphenolic compounds known to be present in this genus (Amado filho *et al*., 1999). In this investigation, *Sargassum linifolium* showed also a great sorption capacity for ^{14}C, Co and Cr (especially for ^{14}C) in both one-metal and two-metal systems, suggesting that they are suitable biosorbant for the treatment of water containing these radionuclides. Sorption activity was shown to be dependent on the initial ion concentration but not on the valency of the metal. However, the lower binding capacity for cobalt (by this alga) may be due to functional binding sites with low cobalt biosorbant on its surface.

Phillips (1990) concluded that the initial rapid uptake of an element would be corresponding to extracellular adsorption and/or to passive intracellular uptake (metabolism-independent) involving cell surface adsorption and simple diffusion into cells or intercellular spaces. A slower uptake will be corresponding to metabolism-dependent incorporation in the cell body. Teresa *et al*. (2001) also supported this postulation. On the contrary, Shafik (1993) found that the metabolism of ^{14}C –urea by *Sargassum linifolium* was very fast, since the taken-up ^{14}C –urea found its way to protein and free amino acids within 1 min of incubation, i.e. fast uptake may also lead to metabolic- dependent biosorption.

In *Dunaliella* (Table 2), living cells exceeded the non–living cells in the determined accumulation ratios of the three elements. In living *Dunaliella*, the accumulation ratios for ^{14}C was the highest among the three tested elements, since, it reached the value of 9.74. Again, this is perhaps according to the counteracting effect of ^{14}C for both cobalt and chromium and its enhancing effect on photosynthesis. The interaction between the three nuclides in living cells of *Dunaliella* resembles that of living *Sargassum* dependent on the free ion activity. In dried *Dunaliella* cells, the accumulation ratios were almost constant independent of the valency of the metal. The surface area for dried *Dunaliella* was decreased to about 85% of its living value (Table 6).

The effect of ^{14}C, Co and Cr at different concentrations on chlorophyll contents was shown in Table 3. ^{14}C- uptake by living cells of *Sargassum* and *Dunaliella* ameliorated cell vitality i.e. increased the total chlorophyll contents and kept the chlorophyll ratios constant when different concentrations of ^{14}C- were used. The effect of ^{14}C on *Sargassum* exceeded that of *Dunaliella*. In *Sargassum*, an 8.67mg/g increase in total chlorophyll content was obtained when 15 mg/L ^{14}C was used; chlorophyll *a* alone was amounted to 70 percent of this value. Chlorophyll *a*/*c* ratio remained almost constant (with the various concentrations used) and equal to the control, which indicated that stable metabolism was attended in presence of ^{14}C in *Sargassum*. In *Dunaliella* chl *a*/*b* ratio in ^{14}C-treated living cells was almost constant, with a little decrease than the control but still in the range of C_3 plants (±3). A steady increase in chl *a* and *b* of *Dunaliella* with various ^{14}C concentrations was obtained.

Table (3): Effect of different concentrations of ^{14}C, Co and Cr on chlorophyll contents in both *Sargassum linifolium* and *Dunaliella salina* . Values are mg / g fresh wt.

Algal species	Pigment	Control	^{14}C			Co			Cr		
			5	10	15	5	10	15	5	10	15
S. liunifolium	Chl *a*	2.71	5.14	7.38	8.73	2.25	2.10	1.68	1.93	1.84	1.53
	Chl *c*	1.23	2.34	3.32	3.88	1.07	1.02	0.97	1.00	0.93	0.86
	Total	3.94	7.48	10.70	12.61	3.32	3.12	2.65	2.93	2.77	2.39
	a / *c*	2.20	2.19	2.22	2.25	2.10	2.06	1.73	1.93	1.98	1.78
D. salina	Chl *a*	6.14	11.32	12.56	12.71	5.43	5.53	4.79	5.80	5.39	5.12
	Chl *b*	1.75	4.00	4.80	5.02	1.31	1.51	1.04	1.21	1.43	1.30
	Total	8.89	15.32	17.36	17.73	6.74	7.04	5.83	7.01	6.82	6.42
	a / *b*	3.51	2.83	2.62	2.53	4.14	3.66	4.61	4.79	3.77	3.93

In *Sargassum* (Table 3) there was a little decrease in both chl *a* and *c* with 5 and 10 mg/L cobalt; a sharp decrease was attended with 15mg of cobalt. Treatment of algal cells with 15 mg cobalt (*in vitro*) decreased the total chlorophyll contents by 33%; the chl *a*/*c* ratio was decreased to 79 percent of the control value. In *Dunaliella*, a steady decrease in chl *a* with the increase in cobalt concentration was obtained. In spite of that chl *b* content remained almost constant and very close to the control; the *a*/*b* ratio of the cobalt-treated *Dunaliella* was increased by 0.18-fold the control value.

Chromium showed adverse effect on cell vitality even at low concentrations. High concentrations of chromium (10 and 15mg/L) caused a

disorder in chl *a*/*b* ratios. In *Sargassum* (Table 3), the decrease was amounted to 26%, 30% and 40% in the total chlorophyll content with 5, 10 and 15mg/L chromium respectively, the ratio of chl *a*/*c* was 1.9. In *Dunaliella*, although there was a little decrease in both chl *a* and *b* values, the *a*/*b* ratio showed a little increase (1.36 fold the control value) with 5 mg Cr and decreased again with 10 and 15 mg to reach the control ratio (Amorim *et al*., 2003).

Although the effect of ^{14}C on cell vitality was insignificant, it is the most hazardous material when present in lakes or sea water because it enters the metabolic pool of cell constituents of the biological individuals (Bassham and Calvin, 1957). The dangerous effects come from using living cells for ^{14}C removal, because no availability for desorption process for this radionuclide. In case of radio contamination of water sources with ^{14}C, dried *Sargassum* cells is preferred to be used as biosorbant.

Table 4 shows the results obtained for the calculation desorption mechanism by the two investigated algae. Q_{ads} and Q_{des} differed considerably for the three tested elements. Q_{ads} for *Sargassum* was less than the corresponding value for *Dunaliella*, while Q_{des} for *Sargassum* exceeded that of *Dunaliella*. Volesky and Kuyucak (1988) concluded that the theoretical calculations of desorption coefficient did not fit well with the true experimental results obtained so, another desorption parameters will be tried, depending on surface area calculation (Table 5).

Table (4): Desorption kinetics of ^{14}C, Co and Cr from *Sargassum* and *Dunaliella*. Eluents were NaOH (0.1) for ^{14}C; HCl (0.05M) for Co and H_2SO_4 (0.6M) for Cr. Elution periods 3 and 6 hours.

Nuclides	Elution time (h)	*S. linifolium*				*D. salina*			
		C_i-C_f	Q_{ads}	C_{des}	Q_{des}	C_i-C_f	Q_{ads}	C_{des}	Q_{des}
^{14}C	3	124.0	28.5	12.80	6.90	210.00	48.27	10.30	3.12
	6	400.00	20.00	13.58	7.20	213.00	10.65	10.93	4.50
Co	3	133.00	47.9	12.49	5.90	262.00	94.36	8.31	2.05
	6	135.00	82.4	12.42	5.60	246.00	150.15	9.07	2.70
Cr	3	304.00	23.7	11.36	4.28	284.00	115.56	7.35	2.14
	6	1.47	62.85	12.85	5.19	299.00	127.13	8.84	3.23

Table 5: Total surface area (S.A) of *Sargassum linifolium* and *Dunaliella salina*.

	Sargassum linifolium	*Dunaliella salina*
Water contents %	74.60	85.08
Total S.A mm^2/mg fresh wt.	------	48.50
Total S.A mm^2/mg dry wt	2.83	7.24

The results in Table 6 indicated that the desorption mechanism was faster with *Sargassum* alga than with *Dunaliella*. The desorption efficiency (% C_2/C_1) exceeded 90% for the three tested nuclides. Maximum C_2 / C_1 ratio was attended with three hours of elution, and remained almost constant till 6 hours with the nuclide adsorbed by *Sargassum.* With *Dunaliella* a slow removal mechanism was obtained, since there was an increase in the efficiency through 6 hours of elution. On the average, only 82%, 70% and 67% desorption efficiency were obtained for ^{14}C, Co and Cr adsorbed by *Dunaliella*, respectively.

The results in Table 4 indicated that the adsorbed materials on *Sargassum* surface were independent on the valency, this means equal distribution of exchanged anions on the surface of this alga. This is not the case with *Dunaliella*; least efficiency was obtained with chromium after 3 hours elution, with H_2SO_4, followed by cobalt (after 3 hour) and the maximum was obtained with ^{14}C after 6 hours elution period.

In Table 7, the maximum number obtained for elution of radionuclide from algal cell walls were five cycles for dried *Sargassum* cells and only three cycles for the *Dunaliella*.

With *Sargassum linifolium*, desorption efficiency was decreased to about 61% of the first cycle value after five cycles of cobalt elution. With chromium and ^{14}C, the least values obtained were about 47% and 54%, respectively, at the end of the fifth elution cycle. In *Dunaliella*, the amounts obtained after the second and third cycles were close to that of *Sargassum* with Co and Cr. ^{14}C amounted to 26% desorption efficiency. No availability for a fourth elution cycle with *Dunaliella*, supposing to be surface characteristics.

It is clear from the results in Table 8 that the adsorptive power of the living algae used was increased when grown on iron – deficient medium. With *Sargassum linifolium*, the increase was amounted to 5.02%, 3.90% and 3.10% for ^{14}C, Cr and Co, respectively. With *Dunaliella*, these amounts were smaller, compared to *Sargassum*. The high adsorption occurred in presence of siderophore in *Dunaliella* was for chromium (3.3%). These results indicated that siderophore formation was greater in macroalgal cell surfaces than in microalgae; siderophores possessed greater affinity for ^{14}C and Cr than Co. These results indicated that: since siderophore formation was high in macroalgae than in microalgae, iron-deficient media for the growth of macroalgae, especially the brown, must be used as biosorbant to increase their adsorptive power for ^{14}C and Cr III.

Macroalgae are good biosorbant, as a result of their metal binding properties (Phillips, 1990), they have a high capacity to bind trace metals and radionuclide. Their cell wall is rich in sulphated polysaccharides hydroxyl sulphate. Hydroxyl sulphate and carboxyl groups of the polysaccharides are strong ion-exchangers, due to having complexation sites for hard and/or transition metal cations (Manley, 1984; Teresa *et al*., 2001; Liu *et al*., 2002; Tien, 2002).

Table 6: Desorption of the three radionuclides ^{14}C, Co and Cr by *Sargassum* and *Dunaliella* in relation to total surface area. Initial concentration of nuclide was 15 mg/L.

Algal species		*S. linifolium*								*D. salina*							
Sorbed metal	Elution time (h)	C mg/L	SA/DW $mm^2.mg^{-1}$ dry wt	C_1 mg/L	C_2 mg/L	C_2/C_1 %	C_3 $mg.mm^{-2}$	C_4 $mg.mm^{-2}$	C_{3-4} $mg.mm^{-2}$	C mg/L	SA/DW $mm^2.mg^{-1}$ dry wt	C_1 mg/L	C_2 mg/L	C_2/C_1 %	C_3 $mg.mm^{-2}$	C_4 $mg.mm^{-2}$	C_{3-4} $mg.mm^{-2}$
			2.83								7.24						
^{14}C	3	1.24		13.76	12.80	93.02	4.86	4.52	0.34	2.1		12.90	10.30	79.84	1.78	1.42	0.36
	6	0.4		14.60	13.58	93.01	5.16	4.80	0.36	2.13		12.87	10.93	84.93	1.78	1.51	0.27
Co	3	1.33		13.67	12.49	91.37	4.83	4.41	0.42	2.62		12.38	8.31	67.12	1.71	1.15	0.56
	6	1.35		13.65	12.42	90.99	4.82	4.39	0.43	2.46		12.54	9.07	72.33	1.73	1.25	0.48
Cr	3	3.04		11.96	11.36	94.98	4.23	4.01	0.22	2.54		12.46	7.35	58.99	1.72	1.02	0.70
	6	1.47		13.53	12.85	94.97	4.78	4.54	0.24	2.99		12.01	8.84	73.61	1.66	1.22	0.44

C = metal concentration remaining in the medium; C_1= metal concentration sorbed (mg/L); C_2= metal concentration desorbed (mg/L)
C_3 = metal concentration sorbed (mg/mm^2); C_4 = metal concentration desorbed (mg/mm^2);
C_3 - C_4= non-desorbed portion from sorbed nuclide per unit area.

Table 7: Cycles of desorption processes carried out for *S. linifolium* and *D. salina* dry algal cells. Values obtained after 6 hours elution.

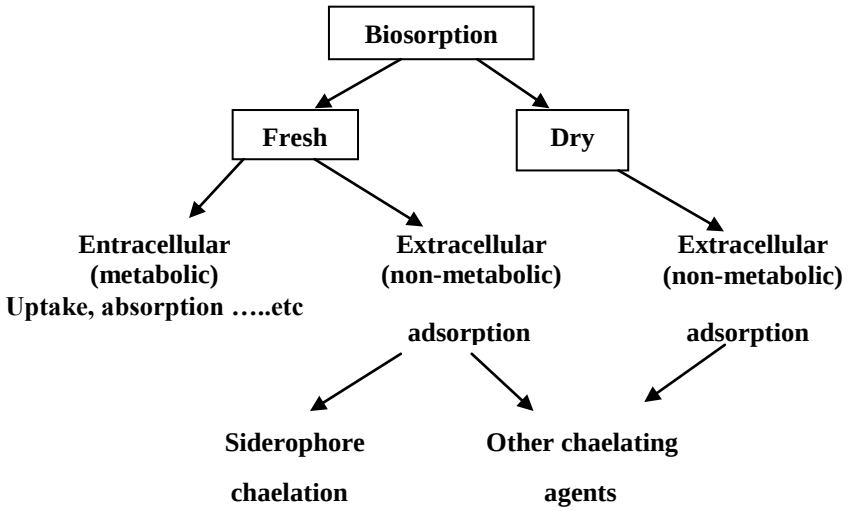
Algal species	*S. linifolium*					*D. salina*		
Cycles / Nuclide	1st	2nd	3rd	4th	5th	1st	2nd	3rd
^{14}C	93.01	67.18	53.37	51.46	39.50	84.93	65.78	58.75
Co	90.99	62.56	50.45	45.32	30.36	72.33	54.49	23.07
Cr	94.97	63.88	52.80	50.21	48.02	73.61	48.13	35.56

Table 8: Siderophore effect on the adsorption of ^{14}C, Co and Cr by *S. linifolium* and *D. salina* living algal material, after 36 hours exposure to the nuclide.

Algal species	^{14}C			Co			Cr		
	t_1	t_2	$t_2 - t_1$	t_1	t_2	$t_2 - t_1$	t_1	t_2	$t_2 - t_1$
S. linifolium	89.80	94.82	5.02	82.14	85.24	3.10	80.40	84.30	3.90
D. salina	91.00	93.70	2.70	87.70	89.77	2.07	87.50	90.80	3.30

t_1 = total sorbed nuclide in absence of siderophore (%)
t_2 = total sorbed nuclide in presence of siderophore (%)
$t_2 - t_1$ = % increase in sorption power due to siderophore formation

A proposal for overall biosorption process in this investigation for the two algal species used is presented in scheme 1.



Scheme 1: A suggested proposal for the biosorption process in this investigation for the two algae used, either fresh or dry.

Proteins, lipids and nucleic acids may also exist on the surface of the macroalgae cellular wall. The amine, carboxyl, imidazole, thiol, thioester and the nitrogen and oxygen of the peptidic bindings are thought to be responsible for the metal ions co-ordination in the cell body (Majidi *et al.*, 1990).

The results of this investigation indicated the suitability of *Sargassum* and *Dunaliella* as biosorbant, suggesting that they can be used for the treatment of water containing chromium, cobalt and ^{14}C radionuclide in both one and two sorption systems. In spite of that, living microalga as *Dunaliella* outperforms non-living microalga in its biosorbant capacity; the dry-non-toxic alga could also be used successfully as a safe biofilter for radioactive chromium and cobalt removal from lakes, but insufficient for ^{14}C. Periodic harvesting of biosorbant microalgae could sustain the growth rate for a self-regenerating, remediation system (Terry and Stone, 2002). Desorption mechanism would then be applied for the recycling of biosorbant alga.

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تنقية مياه البحار والبحيرات من المواد المشعة و المعادن باستخدام الطحالب

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يطرح هذا البحث حلا بيولوجيا لتخليص مياه البحار والبحيرات من العناصر المشعة، وذلك إما بامتصاصها داخل الخلايا الحية للطحالب، أو بادمصاصها على أسطح مسحوق مجفف من الطحالب ثم استعادتها من على السطح. واختصت هذه الدراسة بثلاثة من العناصر وهي الكربون المشع ك^{14} من يوريا ك^{14} و العنصرين الكروم والكوبالت ، أما الطحالب التي اختيرت فهي: طحلب سارجاسم البحري شائع التواجد على سواحل الإسكندرية وطحلب الدونالييلا من طحالب البحيرات. ولقد ثبت أن النقل النشط هو الميكانيكية المستخدمة لنقل عناصرالكروم و الكوبالت والكربون المشع في كلا من مستخلص خلايا الطحالب، والعينات المجففة من الطحالب. ولقد وجد أن معدلات التراكم للعناصر المستخدمة تزيد في الخلايا الحية للدونالييلا عنها في الخلايا الميتة، إلا أن العكس كان صحيحا بالنسبة للطحلب البحري سرجاسم. وعند استخدام مخاليط من الكربون والكوبالت والكربون مع الكروم.....الخ تبين أن العلاقة بين امتصاص الكربون والعنصرين الآخرين تكون علاقة تضاد بينما تكون علاقة مساعدة حينما يجتمع الكروم والكوبالت ويزيد امتصاص كل عنصر في وجود الآخر، وذلك في حالة الخلايا الحية للطحلبين، أما في حالة الخلايا الميتة للطحلبين المستخدمين فقد كانت العلاقة بين الثلاث عناصر لا تفاعلية (لا يؤثر أحد على الآخر) حيث مال توزيع العناصر على سطح الطحالب الميتة الى التساوي. ولقد وجد أن لمساحة السطح للطحلبين دور رئيسي في الكميات الممتصة من المواد المشعة و غير المشعه. حيث فاقت كمية العناصر الممتصة على سطح خلايا السارجسم المجففة عدة مرات الكميات الممتصة على خلايا السارجسم الحي. اما في حالة الدونالييلا فقد فاقت مساحة السطح في الخلايا الحية نظيرتها المجففة وبالتالي قلت كمية العناصر المتجمعة على سطح الخلايا المجففة. ولقد تبين من النتائج ان الكربون المشع لا يؤثر على حيوية الخلايا الحية للطحالب، بينما يؤثر كل من الكوبالت والكروم تأثيرا سلبيا على حيوية الخلايا ممثلة في المحتوى الصبغي للطحلب، أما تأثير الكروم فقد كان شديدا حتى مع التركيزات المنخفضة منه، وقد تسبب وجود كل من الكوبالت و الكروم بتركيزات عالية في حدوث خلل في نسب الأصباغ كذلك كان تأثيرهما شديد على تفاعل هيل بينما لم يحدث أي تثبيط للـ ك^{14} . عند استرجاع المواد الممتصة من على أسطح الطحالب وجد أن هذه العملية تكون سريعة في طحلب سرجاسم ، بينما تكون معدلات الاسترجاع بطيئة في حالة الدونالييلا. توصي الدراسة باستخدام طحلب السرجاسم المجفف للتخلص بكفاءة من كل من الكوبلت ، الكروم والكربون المشع في حالة وجودها في مياه البحار، خاصة إذا أمكن تجويعه من الحديد وزيادة مساحة سطحه.