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Study on the Mediterranean coastal seaweed *Ulva linza* exposed to natural and stressed environmental conditions

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ABSTRACT

Objective: To study *Ulva linza* (*U. linza*) as macro-alga exposed to different levels of ionizing radiation from the natural background of radioactivity.

Methods: Samples of *U. linza* were collected from two different habitats at the two terminals of the rocky shore of Abu Qir Bay, Alexandria, Egypt. The western terminal at Abu Qir represented an area of normal background radiation while the eastern terminal at Rosetta represented an area of relatively high background radiation. Distinguishing between the algae grown in areas of relatively high and normal background radiation was investigated by using different indicators. Moreover, the ambient water quality was measured and the concentrations of natural radionuclides (^{238}U, ^{232}Th and ^{40}K) bio-accumulated in the tested macro-alga were detected.

Results: The algae naturally exposed to radionuclides (^{238}U series, ^{232}Th series and ^{40}K) were investigated. Radiation dose rates in *U. linza* inhabiting in Rosetta and Abu Qir were calculated as 70.12 and 42.67 nGy/h, respectively. Chemical analysis of algal samples demonstrated that total pigment contents were 2.21 and 2.19 mg/g on a fresh weight basis for *U. linza* inhabiting in Rosetta and Abu Qir, respectively. Fatty acid compositions showed comparable profiles for both algal samples with saturated fatty acids as major component. The results of protein profiles confirmed slight differential expression in protein bands. Sequence-related randomly amplified polymorphic DNA provided evidence that both samples were strongly similar. By using transmission electron microscopy, no obvious ultra structural changes in the examined cells were observed.

Conclusions: These experimental results demonstrate that radiation doses are not high enough to cause damage or manifest any significant variation in *Ulva* tissues.

1. Introduction

Environmental stress due to exposure to primordial radionuclides is a serious problem worldwide. Radionuclides in the environment occur both naturally and as a result of man-made events following nuclear, military and scientific activities[1]. The primordial and radiogenic radionuclides such as ^{238}U, ^{232}Th series and ^{40}K are the most important radioactivity sources at the Egyptian Mediterranean coast[2]. In marine ecosystems, radionuclides disperse with currents, accumulate in biota, and are absorbed by particles and sediments depending on local conditions and chemical properties of radionuclides. The interaction of dissolved radionuclides with both suspended matter and sediments is dependent on water transport, dispersion due to diffusion and mixing[3].

Radiation hormesis is the stimulatory effect produced by low levels of ionizing radiation[4]. Radiation hormesis is defined as a biopositive effect that results from low-level doses of ionizing radiation[5]. The low radiation doses were demonstrated below 100 mGy and low dose rates were below 2.4 mGy/h[6], while the range of lethal doses of ionizing radiation was 10^2–10^4 Gy[7]. On the other hand, the exposure to radiation comparable to and just above the natural background level of radiation is not harmful, while higher levels of radiation are hazardous[8,9]. The biological effects of ionizing radiation on hormesis have been broadly studied. Most studies have focused on animal models, although some studies have been extended to include land plants, micro-algae and microbes such as bacteria and yeast. Very little work has been conducted on

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the effect of ionizing radiation with respect to macro-algae[10].

Macro-algae or seaweeds are renewable marine resources existing in large quantities along all the world coasts. They are also considered as vital primary producers that form the basis of aquatic food webs. Nowadays, seaweeds are used worldwide for different purposes due to their commercial importance and potential applications. They contain significant quantities of phycocolloids, minerals and vitamins such as thiamine, riboflavin, β-carotene and tocopherol[11]. In addition, they are an important natural source of long-chain polyunsaturated Ω-3 fatty acids such as eicosapentaenoic acid[12]. Moreover, they may be utilized as fodder, as a fertilizer, in human nutrition, waste water treatment and cosmetics[13]. Also, they are widely used as medicinal herbs, in treatment of cancer since various polysaccharides from different brown, green, and red algae constitute a promising source of novel compounds with potential as human therapeutic agents[14].

Pigmentation, lipids and proteins are considered to be important biochemical components in macro-algae. Pigment contents can give fundamental information on the light harvesting capacity of the algae and indirectly provide possible responses to environmental stress[15]. At the same time, chlorophyll is the most vital class of primary compounds as it is the only substance that captures sunlight and makes it available to seaweeds[16]. There is evidence to assume that lipid profile may be useful for taxonomic purposes[17]. In addition, lipids are major sources of metabolic energy during the embryonic and pre-feeding fish larval stages[18]. The use of electrophoresis technique and protein profile for the identification of seaweed species has been described[19]. Moreover, the application of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) for the identification of seaweeds has been tested successfully[20]. Studies on alkaline proteins of *Ulva rigida* and *Ulva rotundata* showed that these two species can be distinguished by their protein patterns using SDS PAGE[21,22]. Also, the study of protein patterns seems to be a useful method for identification of *Ulva* and *Enteromorpha* species although the SDS profiles obtained for these species are different[23]. In previous studies, random amplified polymorphic DNA or arbitrarily primed PCR (RAPD) has developed and successfully used for genetic fingerprinting and molecular typing for many species[24].

The marine green macro-alga *Ulva linza* (*U. linza*) (Linnaeus) J. Agardh., which is widely distributed along the Egyptian Mediterranean coast was used as experimental material in this study[25-27]. This macro-alga exposed to different levels of ionizing radiation from the natural background of radioactivity is the main objective of the present investigation. Distinguishing between the algae grown in areas of relatively high and normal background radiation was investigated by using different indicators. These indicators are pigment contents, fatty acid composition, protein profiles, and RAPD as well as ultrastructures. Moreover, the ambient water quality was measured and the concentrations of natural radionuclides (^{238}U, ^{232}Th and ^{40}K) bio-accumulated in the tested macro-alga have been detected as ^{226}Ra, ^{228}Ra and ^{40}K, respectively and its derived absorbed dose rate was also determined.

2. Materials and methods

2.1. Site description

Abu Qir Bay is a semi-circular shallow basin lying longitude between 30°4' E and 30°20' E, and latitude between 31°16' N and 31°28' N as shown in Figure 1. The average depth of the bay is less than 10 m and it has a gentle slope with a maximum depth of 22 m. The bathymetric study of Abu Qir Bay showed the contrasting variations between the sea floor characteristics of the eastern and western sides. The western side has a very gentle slope and a wide extension area. On the other hand, the eastern side has a very steep slope and narrow extension area. Rosetta area is considered as one of the relatively high natural background radiation areas due to the occurrence of beach placer, well known as the black sand deposits[28]. The black sand has uranium and thorium in its heavy mineral contents (zircon and monazite)[2].

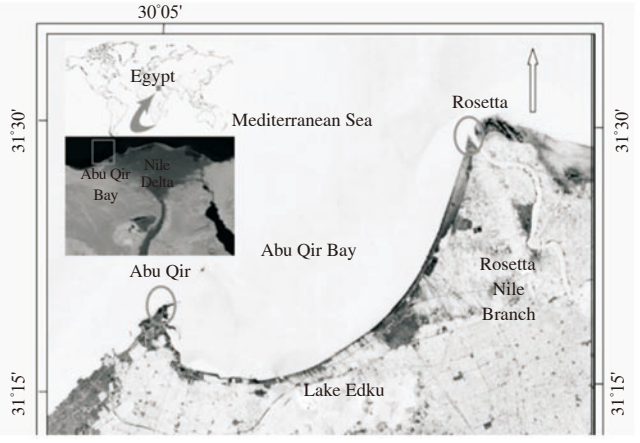


Figure 1. Map of sampling locations for Abu Qir and Rosetta.

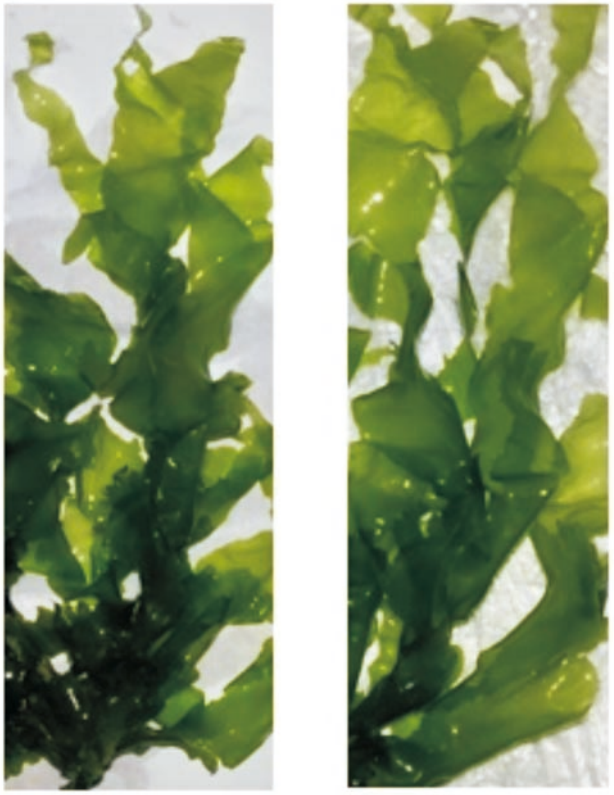
Rosetta Promontory is one of the most important areas for monitoring beach erosion and suffers from long-shore and cross-shore sediment transport. This erosion is mitigated by the construction of a series of coastal engineering structures at the rapidly eroding promontories[29]. Two dolos seawalls were constructed between 1989 and 1991 on both sides of the Rosetta Nile branch mouth and extended alongshore 1.50 km to the west and 3.35 km to the east. The seawall toe extends seaward to about 30.0 m with a depth of 2.5 m. Actually, the erosion did not stop but continues vertically with rates from 0.1 to 0.5 m per year in front of the seawall[30]. As a consequence, the original erosion/ accretion patterns along the Nile delta promontories has been reshaped as a result of these protective structures.

2.2. Sampling

Water quality parameters were measured in the field by using a water quality analyzer (CTD-model Turo T-611) at different strata of the water column. Transparency was tested by using a Secchi Disc.

U. linza is a common green seaweed in intertidal to shallow subtidal areas which attaches to any available substrate and grows abundantly in Abu Qir Bay. The abundance of *Ulva* was similar in both Abu Qir and Rosetta and any differences occurred could be attributed to the seasonal variation. Sample No. 1 was collected from the intertidal zone rocks at the western terminal of Abu Qir Bay while sample No. 2 was collected from dolos blocks from the intertidal zone of the eastern terminal of Abu Qir Bay and the western site of the estuary of Rosetta (Rashid) Nile branch during spring of 2010. The sediment depths were 3.5–4.0 m and 0.5 m apart from the collected algae in Rosetta and Abu Qir Bay, respectively.

Algal samples were collected by hand from the rocks, placed in plastic bags and brought back to the laboratory in an icebox. The algal materials were washed with filtered sea water many times to remove epiphytes, salts and sands then air dried and stored at -20 ˚C. Figure 2 illustrates that *U. linza* is composed of clusters of long, unbranched, bright green ribbons of 15 mm wide with undulate edges for upper portions. It can be observed that thalli are tubular, especially near the base. The thalli taper into small distinct tubular stipes which then clusters into a common holdfast. The blade surfaces are smooth, and often described as silky.



Sample 1 Sample 2

Figure 2. Photos of the studied samples.
Sample 1: *U. linza* inhabiting in Abu Qir; Sample 2: *U. linza* inhabiting in Rosetta.

2.3. Radioactivity measurements

For radioactivity measurements[31,32], algal samples were dried in the oven at 90 ˚C. A total of 2.25 g of dried, ground and homogenized algae were stored in cylindrical plastic containers that fited into a well detector. These containers were sealed by epoxy to isolate them from air and to ensure that the radon gas was confined within the volume. Each sealed sample was carefully stored for four weeks to reach secular equilibrium between ^{226}Ra and its short-lived daughter products. Radioactivity measurements were performed by using a shielded well-type high-resolution γ-ray spectrometer with hyperpure germanium detector. The accumulated live time counting (real time) of sample was about 72 h. For algal samples, ^{226}Ra activities indicated that ^{238}U activities were estimated from γ transition lines of ^{214}Pb (295.0 and 351.9 keV) and ^{214}Bi (609.3 keV). The γ ray energies of ^{228}Ac (338.4 and 911.2 keV) were used to estimate the concentration of ^{228}Ra which indicated ^{232}Th activities. The activity concentrations of ^{40}K were measured directly by its own γ ray (1 460.8 keV).

The radiation dose rate at shore sediment due to natural radionuclides (^{226}Ra series, ^{232}Th series and ^{40}K) was calculated according to United Nations Scientific Committee on the Effect of Atomic Radiation by using the following equation[33]:

$$D\ (nGy/h) = 0.462C_U + 0.604C_{Th} + 0.042C_K$$

Where D is the radiation dose rate, C_U, C_{Th} and C_K are the concentrations (Bq/kg dry weight) of ^{238}U, ^{232}Th and ^{40}K, respectively.

2.4. Chemical analysis of algae

2.4.1. Estimation of moisture and ash contents

The moisture content of 100 g homogenized seaweed samples was determined by drying them in oven at 80 ˚C until constant weight. Moisture was considered as the loss in the mass. The residual mass in these samples was heated in a muffle furnace (Hobersal HD-230, maximum temperature 1 200 ˚C) at 550 ˚C for about 3 h until a white ash was obtained according to the Association of Official Analytical Chemists[34].

2.4.2. Pigments analysis

Chlorophyll content was estimated by using the procedure of Holden[35]. Weighed samples (1 g) were homogenized in a mortar in the presence of an excess of 90% acetone until the color was released. Samples were kept in the dark at 4 ˚C for 12 h and then centrifuged at 5 000 r/min for 10 min. For pigment estimation, the clear supernatant was collected and made up to a known volume (10 mL). The test tubes were wrapped with black paper to prevent chlorophyll from degradation. The 90% acetone was used as blank,

sample absorbance was measured at 664 nm for chlorophyll a, 647 nm for chlorophyll b and 450 nm for carotenoids via UV-vis spectroscopy spectrophotometer Perkin-Elmer Lambda 1. The amounts of chlorophylls and carotenoids pigments were calculated according to the formulas of Jaspers[36], and Jeffrey and Humphrey[37].

2.4.3. Lipid extraction and gas-liquid chromatography analysis

Lipids were extracted with a chloroform-methanol mixture (2:1 v/v) according to the method of Bligh and Dyer[38]. The mixture was agitated for 60 min. The extraction procedure was repeated twice and the extracted lipid was collected by using a rotary evaporator. These lipids were methylated through base-catalyzed transmethylation according to the method of Christie by using methanolic potassium hydroxide and *n*-hexane[39].

The hexane layer was taken for gas chromatography (GC) analysis. GC of fatty acid methyl esters (FAMEs) was performed by using a Shimadzu gas-liquid chromatography equipped with a flame ionization detector with column packing material Hp-5. The carrier gas was nitrogen and the short speed was 5 mm/min. Peaks were identified according to retention time based on available FAME mix standard. FAMEs were identified and quantified by comparison with the retention time based on available FAME mix standard. The GC analyses were performed in three replicates and the values of fatty acids were expressed as percentage of total fatty acids mass as a mean value.

2.4.4. Gel electrophoresis and protein profile

To separate proteins from relative molecular mass of no smaller than 10 kDa, freshly frozen algal materials were ground in a mortar by using quartz sand until homogeneous. The homogenate was extracted several times with small volumes of 0.5 mol/L Tris-HCl buffer (pH 7.2). The suspensions were centrifuged for 10 min at 5 000 r/min, and the supernatants were concentrated in pre-activated dialysis tubing over a sucrose bed. Concentrated samples were subjected to SDS PAGE analysis by using 8% polyacrylamide gel stained with Coomassie blue staining[40].

2.4.5. RAPD analysis

A total of 500 mg of the algae stored at -20 ˚C were subjected to DNA extraction by using method of Neilan[41]. DNA samples were quantified based on spectrophotometric measurements. Seven primers were used for the genetic characterization of the algae collected from the two different terminals (Table 1). PCR was performed in a reaction volume of 25 μL by using 25 ng genomic DNA of each sample, 25 pmol of each primer, 10× *Taq* DNA polymerase buffer including $MgCl_2$, 0.2 mmol/L deoxynucleotide triphosphates and 5 IU/μL *Taq* DNA polymerase (Promega Co.). Thermal cycling (Perkin Elmer 9700) was carried out by initial denaturation at 95 ˚C for 5 min and 40 cycles at 95 ˚C for 1 min, 30 ˚C for 1 min and 72 ˚C for 1 min, followed by extension cycle at 72 ˚C for 10 min. The PCR product was separated on 2% agarose gel (GibcoBRL), stained with ethidium bromide, visualized on a ultraviolet transilluminator and photographed by gel documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

Table 1
Information of the seven primers used for PCR analysis.

Primer	Primer code	Length (m)	Sequence	Marker size (bp)
18Sf	P1	19	GTA GTC ATA TGC TTG TCT C	2 000
18Sr	P2	21	GGC TGC TGG CAC CAG ACT TGC	1 500
NAR46	P3	10	GTC GTT CCT G	1 000
NAR47	P4	10	CGG CAG CGC C	700
NAR48	P5	10	CCT TTC CCT C	500
NAR49	P6	15	GAC GAC GAC GAC GAC	400
NAR50	P7	15	ACG GAG TTG GAG GTC	300
				200
				75

2.4.6. Transmission electron microscopy

Algal samples were pre-fixed for 3 h by using 2.5% glutaraldehyde solution (pH 7.3) and re-fixed for 1 h in 1% osmium tetroxide at 20 ˚C[42], and subsequently they were dehydrated for 30 min by using absolute alcohol and embedded in Spurr resin[43]. A microtome was utilized to slice the prepared samples, and the slices were stained with uranyl acetate[44], followed by lead citrate[45]. Ultrastructures of algal materials were observed by a transmission electron microscope (Jeol JEM-100CX).

2.5. Statistical analyses

Experiments were performed in three replicates. Data were statistically analyzed by using SPSS software to determine whether there were any significant differences between the average values obtained. Statistical analyses on Two-way ANOVA were performed.

3. Results

3.1. Characteristics of seawater

Table 2 illustrates the characteristics of seawater in the two studied sites. No significant differences between the measured values of pH, water temperature, and dissolved oxygen were detected. The electrical conductivity of seawater and its derivative salinity at Abu Qir [(35.43 ± 0.02) ppt] were higher than those at Rosetta [(24.33 ± 0.02) ppt]. The value of oxidation-reduction potential of seawater at Abu Qir was higher than that at Rosetta. Furthermore, the turbidity

of Abu Qir seawater was observed to be higher than that of Rosetta with turbidity values of (104.56 ± 0.73) and (32.52 ± 1.86) ntu, respectively. Secchi disc measurements reflected a dominating low transparency in Abu Qir area.

Table 2
Characteristics of coastal water in Abu Qir and Rosetta.

Parameter	Abu Qir	Rosetta
Temperature (℃)	20.38	22.46
Conductivity (ms/cm)	35.43	24.33
Salinity (ppt)	36.09	23.95
Dissolved oxygen (mg/L)	3.97	4.02
pH	7.64	8.76
Oxidation-reduction potential (mV)	407.22	319.51
Turbidity (ntu)	104.56	32.52
Transparency (cm)	19.9	37.7

3.2. Radioactivity measurements

Figure 3 shows the differences in concentrations of the radioactive nuclides ^{226}Ra (^{238}U) and ^{228}Ra (^{232}Th) series accumulated in *Ulva* inhabiting in Rosetta and Abu Qir. The average concentration values of ^{226}Ra (^{238}U) in Rosetta and Abu Qir were found to be close to 16.28 and 16.45 Bq/kg, respectively. Concerning algae collected from Abu Qir, the average concentration values of ^{228}Ra (^{232}Th) were relatively higher (9.17 Bq/kg) than those in Rosetta (3.56 Bq/kg). The data of the average concentrations of ^{40}K revealed that ^{40}K level was higher in Rosetta (1 449.5 Bq/kg) than that in Abu Qir (708.2 Bq/kg).

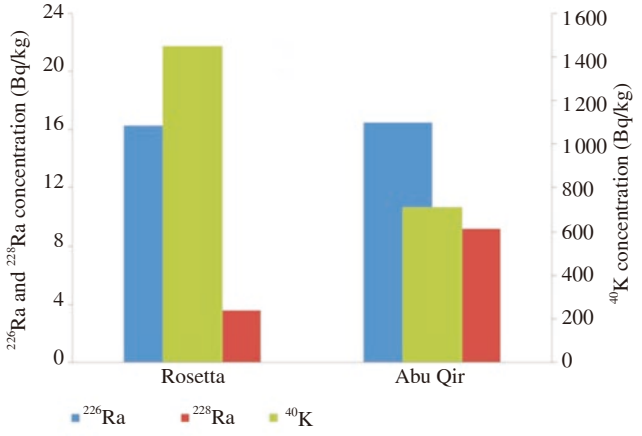


Figure 3. Concentrations of naturally occurring radionuclides in *U. linza* collected from Rosetta and Abu Qir coasts.

Figure 4 shows the internal dose rate of radiation accumulated in the investigated algae, the external radiation dose rates of sediments and ambient seawater, and total radiation dose rates from environmental γ emitters (^{238}U series, ^{232}Th series and ^{40}K). Internal radiation dose rates were calculated as 70.12 and 42.67 nGy/h for *U. linza* inhabiting in Rosetta and Abu Qir, respectively. However, total radiation dose rate at the coastal zone of Rosetta (255.31 nGy/h) was relatively higher than that in Abu Qir (82.06 nGy/h).

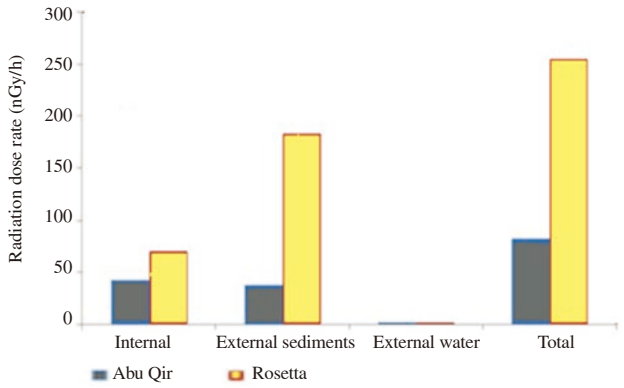


Figure 4. The internal dose rate accumulated in the investigated algae, the external dose rates of sediments and ambient seawater as well as total radiation dose rates from environmental gamma emitters (^{226}Ra series, ^{232}Th series and ^{40}K) at the two studied sites.

3.3. Biochemical composition of algae

3.3.1. Moisture and ash contents

The contents of moisture and ash were recorded in Table 3. Moisture percent of *Ulva* collected from Abu-Qir (48.35%) was considerably higher than that collected from Rosetta (35.60%). Additionally, the ash content observed in *Ulva* inhabiting in Abu Qir (12.20%) was higher than that in *Ulva* inhabiting in Rosetta (8.51%).

Table 3
Total moisture (% of fresh weight), total ash (% of dry weight) and pigment concentrations (mg/g fresh weight) of *U. linza* inhabiting at the two different sites.

Sample	Moisture	Ash	Chlorophyll a	Chlorophyll b	Carotenoids
No. 1	48.35 ± 0.51	12.20 ± 0.28	0.71 ± 0.02	0.87 ± 0.03	0.61 ± 0.03
No. 2	35.60 ±0.22	8.51 ± 0.34	0.73 ± 0.03	0.89 ± 0.03	0.59 ± 0.03

Each value is presented as mean of triplet treatments ± SE.
No. 1: *U. linza* inhabiting in Abu-Qir; No. 2: *U. linza* inhabiting in Rosetta.

3.3.2. Pigments analysis

The data in Table 3 show no significant changes in the concentrations of pigment fractions of the tested algae. Concerning *Ulva* inhabiting in Abu Qir and Rosetta, chlorophyll a contents were (0.71 ± 0.02) and (0.73 ± 0.03) mg/g fresh weight, chlorophyll b contents were (0.87 ± 0.03) and (0.89 ± 0.03) mg/g fresh weight, while carotenoid contents were found to be (0.61 ± 0.03) and (0.59 ± 0.03) mg/g fresh weight, respectively. Whereas, total sum of pigments were 2.19 and 2.21 mg/g fresh weight for *Ulva* inhabiting in Abu Qir and Rosetta correspondingly.

3.3.3. Fatty acid composition

The fatty acid composition of the investigated *U. linza* is listed in Table 4. Saturated fatty acids were major components accounting for 50.57% for *Ulva* inhabiting in Abu Qir and 62.15% for *Ulva* inhabiting in Rosetta, while the percentage of unsaturated fatty acids were recorded as 10.29% and 14.39%, in the same order. Additionally, the polyunsaturated fatty acids were 7.48% and 5.30%, whereas the monounsaturated fatty acids were 2.81% and 9.09% of total fatty acids, correspondingly. Palmitic acid (C16:0) was the most

abundant fatty acid. It accounted for about a half of the total fatty acid content in both algal samples (46.80% for alga inhabiting in Abu Qir and 59.13% for alga inhabiting in Rosetta).

Table 4

Fatty acid composition of *U. linza* inhabiting at the two different sites.

Fatty acids	Methyl ester (% of total fatty acids)	
	Abu-Qir	Rosetta
C14:1	1.12	5.87
C14:0	1.18	1.07
C15:1	1.23	0.82
C15:0	0.43	0.41
C16:1	0.21	0.11
C16:0	46.80	59.13
C17:1	0.17	0.37
C17:0	0.06	0.19
C18:3	0.29	0.14
C18:2	0.19	0.79
C18:1	0.08	1.11
C18:0	1.83	1.23
C20:5	0.73	0.69
C20:1	-	0.33
C20:0	0.27	0.12
C22:2	-	0.14
C22:1	-	0.48
C22:6	6.27	3.54

3.3.4. Protein profile

The Coomassie-stained gel loaded with protein of examined samples revealed a degree of homogeneity along the protein pattern to some extent (Figure 5). It comprised several bands with different molecular weights. Bands with 222, 210, 192, 138, 55 and 41 kDa were common among the examined samples. Whereas, bands with 79 kDa for *Ulva* inhabiting in Abu Qir and bands with 180, 92 and 46 kDa for *Ulva* inhabiting in Rosetta were categorized as dissimilar bands. However, any adaptive response to environmental conditions will be reflected by alterations in protein activity, location and concentration[40].

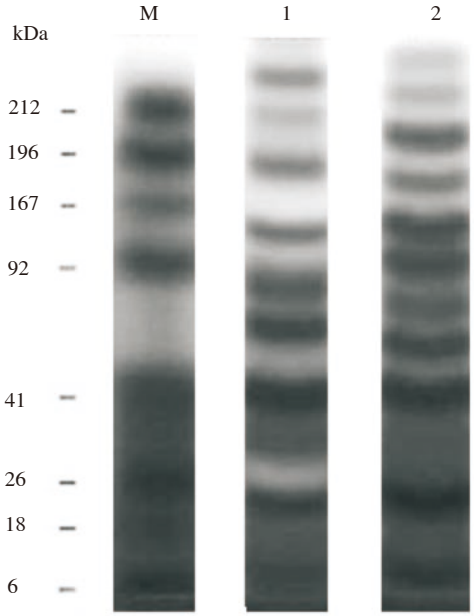


Figure 5. Representative photographs of Coomassie-stained PAGE pattern of separated marine algal proteins.

Lane M: SDS-PAGE molecular weight markers in kDa; Lane 1: *Ulva* inhabiting in Abu Qir; Lane 2: *Ulva* inhabiting in Rosetta.

3.3.5. RAPD profiling

RAPD technique was performed to detect the differences among the two green algae studied through seven arbitrary primers (Table 5 and Figure 6). Random primers were used with the genomic DNA of the tested *Ulva*, primer 1 reflected seventeen genomic bands. The number of polymorphic bands was six with 35% of polymorphism. Primer 2 showed three bands as polymorphic bands with 25% polymorphism. Three polymorphic bands were recorded with 43% of polymorphism by using primer 3. Concerning primer 4, only one band was recorded as a polymorphic band with 14% polymorphism. When primer 5 was applied, the number of polymorphic bands were five with 45% of polymorphism. For primer 6, one polymorphic band reflected 20% of polymorphism. Fourteen genomic bands were recorded with three polymorphic bands for the seventh random primer performing 21% of polymorphism. Based on percentages of polymorphism, the fourth primer highly reflected the similarity between the two algae studied with 14% of polymorphism. On the other hand, the fifth primer showed significant dissimilarity between the two algae with 45% of polymorphism.

Table 5

Number of total bands, polymorphic bands, monomorphic bands and percentages of polymorphism (%) of tested *Ulva* generalized from seven primers of random sequences.

Primer	Total amplified bands	Polymorphic bands	Monomorphic bands	Polymorphism
1	17	6	11	35
2	12	3	9	25
3	7	3	4	43
4	7	1	6	14
5	11	5	6	45
6	5	1	4	20
7	14	3	11	21

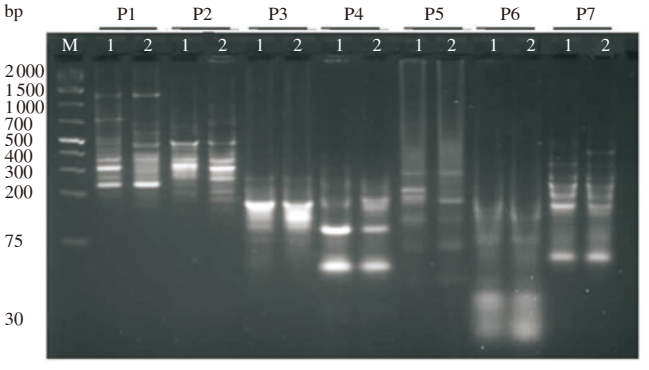


Figure 6. Random amplified polymorphic DNA profile generated by PCR using seven primers.

Lane M: Molecular weight marker; Lane 1: *Ulva* inhabiting in Abu Qir; Lane 2: *Ulva* inhabiting in Rosetta.

3.3.6. Electron microscopy

To investigate changes between *Ulva* inhabiting at the two different sites, the ultrastructure of the cells was examined by using transmission electron microscopy. Sections in Figure 7 show two cell layers of rectangular cells with ordinary cell walls. No alterations of organelles were detected. Normal vacuoles were observed. Existence

of some grains may be attributable to the generation of secondary materials such as flavonoids and alkaloids. The chloroplasts contain a single bright body (pyrenoid) or two if the cell is dividing. Additionally, the stroma in membrane system and photosynthetic lamellae were not changed, suggesting that the photosynthesis function was not affected.

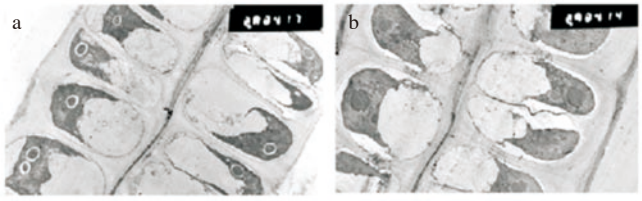


Figure 7. Transmission electron microscopy results of the studied samples. a: *U. linza* inhabiting in Abu Qir; b: *U. linza* inhabiting in Rosetta.

4. Discussion

4.1. Characteristics of seawater

The results of pH, water temperature, and dissolved oxygen were in agreement with the findings of Haslund and his group[41]. They reported that there was no considerable variation in these parameters concerning Egyptian Mediterranean coastal water as well as Abu Qir Bay. On the other hand, the results of electrical conductivity of seawater and its derivative salinity at Abu Qir may be attributed to dilution resulting from the discharging of Nile water into the Rosetta estuary as the finding of Haslund and Emam and their groups[41,42]. Furthermore, the relatively high turbidity and low transparency of Abu Qir seawater were possibly due to the variation in depths between the two sites. The water in front of the Rosetta seawall is deeper compared with the Abu Qir due to the vertical erosion[26].

4.2. Radioactivity measurements

Concerning the differences in concentrations of ^{226}Ra (^{238}U) and ^{228}Ra (^{232}Th) series accumulated in *Ulva* inhabiting in Rosetta and Abu Qir, the results indicated the presence of a relatively high background radiation caused by the black sand placer[43]. On the other hand, the results of ^{40}K, as one out of the characteristic components of the black sand, indicated the contribution of Nile River discharging more K as a result of agricultural wastes to the Rosetta estuary[44]. Several studies proved that *Ulva* sp. has the ability to concentrate particular radionuclides in its tissues even at very low environmental levels[45,46]. They have the ability to accumulate radionuclides both within the cells of the alga, and as physical adsorption to the surface which is not removed by chemical cleaning[47]. The accumulation of such radionuclides depends on salinity, pH, as well as environmental levels of these radionuclides[48]. However, in the study of the natural and anthropogenic radioactivity in *Ulva lactuca* from low and high background radiation areas at the coast of Tamil Nadu, India, the overall mean concentrations of radionuclides in *Ulva lactuca* were 4.88, 42.35, 34.40 and 347.70 Bq/kg for ^{228}Ra, ^{40}K, ^{238}U, ^{228}Th, respectively[49]. Other authors have also reported that the brown alga *Fucus* is a good accumulator of radionuclides, which contributes to its usefulness for removing radionuclides from ecosystems and as bioindicator of radionuclide exposure[50].

The investigation of the internal dose rate of radiation accumulated in the studied algae revealed that these dose rates were still lower than the proposed threshold value of absorbed dose rate for protection of algae against radiation toxicity (0.1 mGy/h) as mentioned by Hinck[51]. However, the radioactive outdoor absorbed dose rate was calculated from ^{238}U, ^{232}Th and ^{40}K at Abu Qir and the Rosetta coasts[2]. The calculated absorbed dose rate has been categorized; the Egyptian coast is categorized as normal area with dose range of 8.39–38.50 nGy/h and Rosetta black sand area as a relatively high radioactivity background area with absorbed dose rate of 0.72 μGy/h. The concentrations of ^{238}U, ^{232}Th and ^{40}K in Abu Qir sediments were previously investigated as 38.60, 28.74 and 60.90 Bq/kg and in Rosetta sediments as 199.28, 147.80 and 49.50 Bq/kg. At the same time, it was measured as 1.8, 0.4 and 14.0 Bq/L in Abu Qir seawater and as 1.98, 0.50 and 13.80 Bq/L in Rosetta seawater, respectively[25]. Nevertheless, data of radioactive analyses in the present study revealed that radiation dose rates were not high enough to damage the *Ulva* tissues. *Ulva* can grow at both examined sites, can resist and tolerate the levels of radioactivity doses from natural sources, which could be related to defense mechanisms in them[52].

4.3. Biochemical composition of algae

4.3.1. Moisture and ash contents

The differences in the moisture content of the dried samples may denote that the crushed samples have different capability to absorb atmospheric moisture throughout processing and storage. At the same time, higher ash content present in *Ulva* collected from Abu Qir compared to that from Rosetta may be as a result of the extraordinary ability of this seaweed to accumulate elements present in the seawater where they live in[14]. This characteristic was attributed to active groups and ion exchangers present in the cellular wall of the alga. However, geographical location and local environmental conditions can influence the composition and ash content of seaweeds[53].

4.3.2. Pigments analysis

Both algae inhabiting in Abu Qir and Rosetta were particularly rich in pigmentation and the response to radiation was insignificantly biological.

In fact, photosynthetic apparatus may probably respond to environmental stress. Low doses of radiation may stimulate changes in the photosynthetic apparatus to optimize light absorption and minimize damage to this system[54]. Different photoacclimation mechanisms have been reported in macro-algae[55]. These mechanisms can possibly support the recovery of photosynthetic parameters of algae from the harmful effects of radiation, even under additional stress.

4.3.3. Fatty acid composition

The results of fatty acid composition of the investigated *U. linza* were in agreement with the referenced work in which it was reported that palmitic acid is predominant[12,56]. In the case of the studied macro-algae, there are no clear differences in their fatty acid composition since both inhabiting regions differ slightly in their seawater characteristics and sediment history. However, these differences in fatty acid composition are considered as habitat-specific adaptations.

The fatty acid composition of algal lipids varies widely with species, habitat and pollution[57]. Accordingly, it appears to be a promising tool to study the relationships of marine environment and algal flora. Variation in fatty acid profiles would allow the organism to control and adapt to the changing environment. Furthermore, various studies have reported that algal fatty acid compositions have been affected by many factors such as temperature, salinity, light, nutrients and water depth[17].

4.3.4. Protein profile

The results of Coomassie-stained gel loaded with protein of examined samples indicated that any variation may support that different geographical location and environmental properties of seawater play an important role and accordingly can affect the protein profile. However, proteins represent the sequential copy of the genetic information in which the language of the genetic code is translated from nucleotide bases into sequences of amino acids. At this point, comparisons of protein explain and facilitate the measurement of similarities between the investigated samples in characteristics such as morphological and physiological properties. Furthermore, the authors recommended that the exposure of algae inhabiting in Abu Qir and Rosetta to low natural doses of radiation, however slight, may affect the magnitude of the protein profile rather than the behavior of the protein pattern. Protein expression is a phenotypic character[20], which is regulated by both genotype and environmental factors, and differences in protein profiles must be a reflection either of underlying differences in the regulation of gene expression or in posttranslational modification of common proteins.

4.3.5. RAPD profiling

The low percentage of polymorphism indicates that the two samples studied are closely similar. They are easy to grow, adaptable to environmental stresses in different habitats with no altered band patterns. Molecular marker is a preferred method for the identification or comparison of plants since it could even distinguish between closely related genotypes[58]. In fact, application of RAPD markers in several investigations has been successful to evaluate the genetic mapping, taxonomy, phylogeny and to detect the various kinds of DNA damage and mutations[59].

4.3.6. Electron microscopy

The examination results of the ultrastructure of the cells by using transmission electron microscopy pointed towards a good adaptation of *Ulva* to natural doses of radiation in Rosetta and Abu Qir. Although many plants could adjust their mechanisms according to the radiation intensity, previous studies had pointed out that a variety of alterations occurred in a naturally stressed environment[60].

The target of this study was to compare the coastal seaweed *U. linza* collected from two natural background radiation areas that differ with respect to their environmental characteristics, Abu Qir and Rosetta coasts at Abu Qir bay, Alexandria, Egypt. Internal levels of radionuclides in marine algae, external levels in sediments and ambient seawater as well as total levels were determined. Although, Rosetta is characterized as high background radiation area than Abu Qir, the data proved that radiation at both sites is not high enough to be outside the tolerance limit of the studied seaweeds. Levels of all radionuclides were generally low to pose serious risk and not significantly biological. The dominant *U. linza* living year-around in these habitats grows successfully and healthily. They are able to tolerate the natural radiation doses with no apparent genetic modification or differences in biochemical composition of algal tissues. For future work, experiments on other dominant seaweed species in these environments are recommended.

Conflict of interest statement

We declare that we have no conflict of interest.

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