

*Full Length Research Paper*

# Growth and reproductive attributes of radionuclide phytoremediators in the Mediterranean coastal black sands

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**Growth and resource allocation of three black sand plants namely *Cakile maritima*, *Senecio glaucus* and *Rumex pictus* were investigated in a greenhouse experiment. The effect of absorbed radionuclides on the plant growth was monitored at different growth stages including seedling, juvenile, flowering, fruiting and senescing stages. The study reveals that growth attributes including relative growth rate, net assimilation rate, leaf area index and specific leaf area, dry matter allocated to stem and leaves and number of reproductive organs decreased with the increase of radionuclide content of the plant, while the dry matter allocated to root and reproductive organs and root to shoot weight ratio were associated with high level of radionuclides. Because of their tolerance to high levels and accumulation of radionuclides, the species *C. maritima*, *S. glaucus* and *R. pictus* could be potential candidate plants for radio-phytoremediation of soils contaminated with uranium and thorium.**

**Key words:** Uranium, thorium, plant growth, resource allocation.

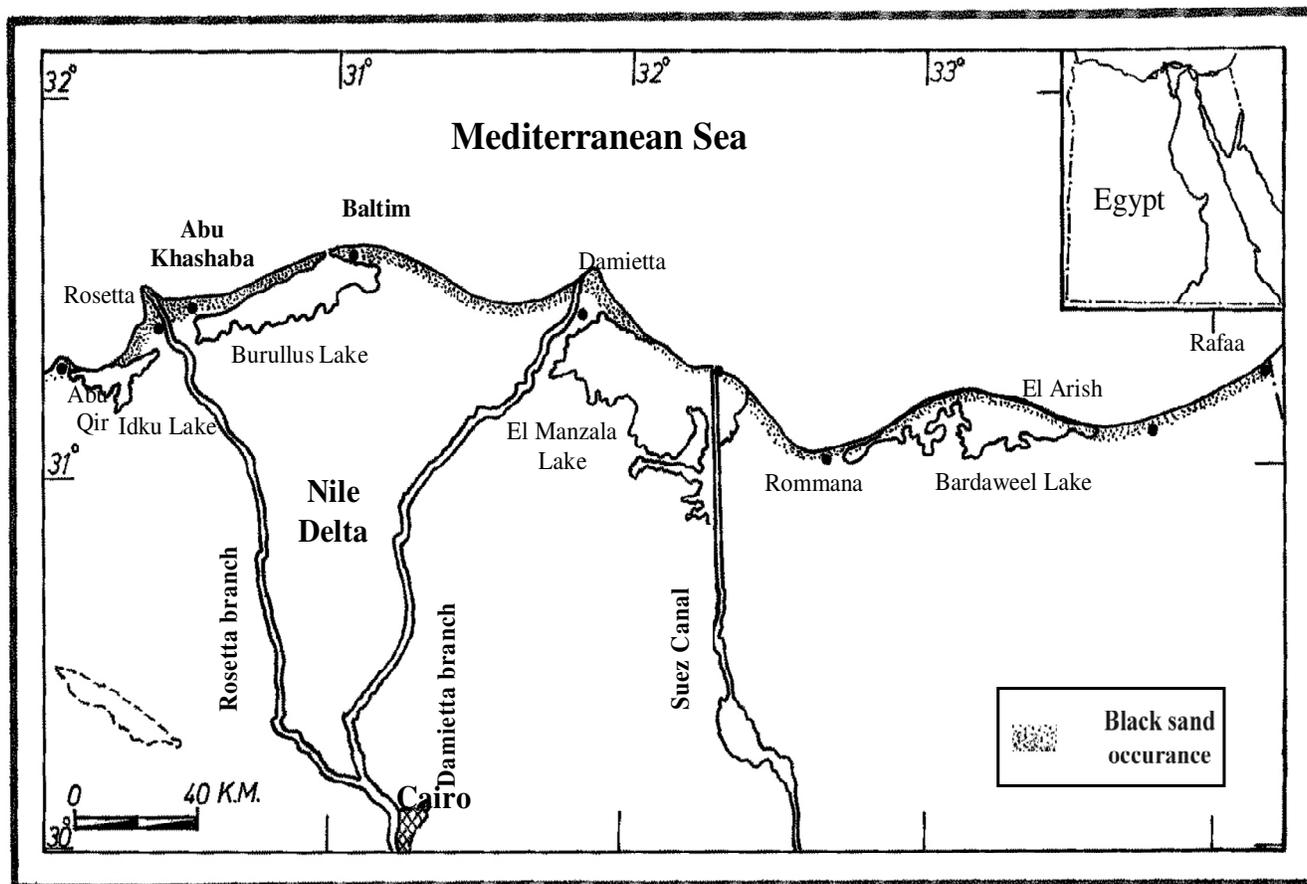
## INTRODUCTION

Black sand accumulations along large stretches of Mediterranean coast in Egypt extend from Abu Qir Bay in the west to Rafaa on the east between longitudes 30 12° and 34 10° E (EL-Hadry, 1998). This part of the Mediterranean coast reaches about 500 km length. Black sands originally derived as erosional products of the crystalline igneous rocks from the mountain ranges in Sudan and Abyssinian and carried down the courses of the River Nile. Rosetta promontory is the area with the highest black sand placers in Egypt (Seddeek et al., 2005; Hegazy and Emam, 2011; Soliman et al., 2011). Although, contaminated soils drastically affect the growth of plants and soil-living microbes, these environments are not totally devoid of all flora and fauna as a number of species have adapted to tolerate the increased radionuclides concentrations (Tomsett and Thurman, 1988). Some naturally occurring radionuclides accumulate in

plants and adversely affect their growth (Murthy et al., 1984). The tolerance of various species for large amounts of uranium, vanadium and selenium in the soil has been studied in plants to determine the absorption of these elements (Gulati et al., 1980; Murthy et al., 1984; Meyer and McLendon, 1997). Plant physiologists Drobkov (1951) and Cannon (1952) have shown that uranium, radium and thorium are necessary nutrient substances for higher plants. The amount needed is infinitesimal, and concentrations above a very low level are retarding or even toxic. Uranium is a normal component in organisms and its concentration in plants and soils has been estimated (Gulati et al., 1980; Soliman et al., 2010). The lack of uranium toxicity data on plants and inconsistencies in the available data are discussed by Sheppard et al. (1992). Much of the literature available on phytotoxicity is anecdotal (Meyer and McLendon, 1997), or focused on agricultural crop species and radiological effects (Murthy et al., 1984; Sheppard et al., 1992).

Information gathered on plant uptake of radionuclides

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**Figure 1.** Map of northern part of Egypt showing the black sand deposits (dotted coastal strip) and the study sites location in Abu Khashaba and Baltim.

has primarily been used for radiological assessment and radioecological research. This kind of information has now become more important for the purpose of phytoremediation of sites contaminated with high levels of radionuclides (Zhu and Shaw, 2000; Meagher et al., 2000). The present study was conducted to assess the radionuclides uptake by plants naturally growing in the black sand habitats along Mediterranean coast of Egypt and to determine their effects on the plant growth.

## MATERIALS AND METHODS

### Study area

The present study was conducted in two localities; Abu Khashaba (Rosetta) and Baltim (Figure 1). Two sites were selected in Abu Khashaba; coastal sand plain, sand mounds, and one site in Baltim called Al Narreges coastal sand dunes. Abu Khashaba area is situated about 5 km to the north of Rosetta and lies on the eastern side of Rosetta branch of the River Nile. Abu Khashaba area is bounded to the west by Rosetta branch of the River Nile and to the

east by the Mediterranean Sea and occupies about 8 km<sup>2</sup> (Said, 1990). The topography of the area is almost flat where the coastal plain occupies most of the surface except the southern part where accumulations from eolian sands occur as small dune belts and sand bars (Hassan, 1993). Baltim is located in the north of Kafr El-Sheikh Governorate facing the Mediterranean Sea, El Burullus Lake from the south, Al Hammul town to the east, and El-Sheikh Mabrouk and Al-Raba villages to the west.

The climatic records of two meteorological stations, Dilingat station (El-Behairah) and Sidi-Salim station (Kafr El-Sheikh), represent the study sites during the period of 2002 to 2006. Dilingat station represents the climate of Abu Khashaba area and Sidi-Salim station represents the climate of Baltim. January is the coldest month at Dilingat and Sidi-Salim stations (14.7 and 13.2°C respectively) while July and August are the warmest months (28.0 and 27.8°C, respectively at Dilingat station but 26.7 and 27.3°C, respectively at Sidi-Salim station). The annual mean maximum temperatures at Dilingat and Sidi-Salim weather stations are approximately 27.17 and 26.1°C respectively while the minimum of 16.5 and 15.4°C respectively. Total annual rainfall is 125 mm at Dilingat station and 142.8 mm at Sidi-Salim station with the majority of rain falling in the winter months of January/February. The highest mean relative humidity at Dilingat station is 63.8% as recorded in August while at Sidi-Salim station is 72.8% as recorded

**Table 1.** Radionuclide content (ppm) in phytomass of *C. maritima*, *S. glaucus* and *R. pictus* grown in soils from the study black sand habitats (Al Narreges coastal sand dune in Baltim and coastal sand plain and sand mounds in Abu Khashaba) and in a control soil. Values are mean ( $\pm$  S.D.). Different superscript letters indicate a significant difference at  $P = 0.05$ .

Site	Plant species	Uranium		Thorium	
		Plant (ppm)	Soil (ppm)	Plant (ppm)	Soil (ppm)
Coastal sand dunes of Baltim	<i>C. maritima</i>	0.21 $\pm$ 0.00 <sup>a</sup>		0.86 $\pm$ 0.05 <sup>b</sup>	
	<i>S. glaucus</i>	3.69 $\pm$ 0.04 <sup>c</sup>	51.33 $\pm$ 1.15 <sup>d</sup>	3.52 $\pm$ 0.16 <sup>c</sup>	43.67 $\pm$ 1.15 <sup>c</sup>
	<i>R. pictus</i>	0.87 $\pm$ 0.03 <sup>b</sup>		1.80 $\pm$ 0.02 <sup>c</sup>	
Sand mounds of Abu Khashaba	<i>C. maritima</i>	0.41 $\pm$ 0.00 <sup>c</sup>		0.84 $\pm$ 0.07 <sup>b</sup>	
	<i>S. glaucus</i>	0.27 $\pm$ 0.00 <sup>a</sup>	40.50 $\pm$ 1.32 <sup>c</sup>	1.32 $\pm$ 0.01 <sup>b</sup>	61.67 $\pm$ 1.53 <sup>d</sup>
	<i>R. pictus</i>	0.51 $\pm$ 0.02 <sup>a</sup>		0.59 $\pm$ 0.01 <sup>b</sup>	
Coastal sand plain of Abu Khashaba	<i>C. maritima</i>	0.58 $\pm$ 0.03 <sup>d</sup>		0.74 $\pm$ 0.01 <sup>a</sup>	
	<i>S. glaucus</i>	1.20 $\pm$ 0.05 <sup>b</sup>	31.00 $\pm$ 1.73 <sup>b</sup>	1.18 $\pm$ 0.04 <sup>b</sup>	40.00 $\pm$ 1.00 <sup>b</sup>
Control soil	<i>C. maritima</i>	0.26 $\pm$ 0.0 <sup>b</sup>		1.21 $\pm$ 0.03 <sup>c</sup>	
	<i>S. glaucus</i>	0.30 $\pm$ 0.01 <sup>a</sup>	19.67 $\pm$ 1.53 <sup>a</sup>	0.91 $\pm$ 0.03 <sup>a</sup>	29.67 $\pm$ 0.58 <sup>a</sup>
	<i>R. pictus</i>	1.40 $\pm$ 0.2 <sup>c</sup>		0.40 $\pm$ 0.01 <sup>a</sup>	

in December and the lowest mean values are 49.5 and 59.3% as recorded in May at Dilingat and Sidi-Salim stations respectively.

### Sample collection

Seeds of three monocarpic plant species *Cakile maritima* Scop., *Senecio glaucus* L. and *Rumex pictus* Forssk. were collected from the naturally growing plant populations recorded in the study sites. The soil collected from the plants' natural habitat shortly before the start of the experiment was excavated between depths 5 and 30 cm. Control soil was collected from Cairo Alexandria desert road near Wadi El Natron. The soil samples were air dried and passed through 2 mm sieve to separate litter and gravel. For estimation of the soil radionuclide content, the concentration of uranium and thorium was chemically determined using spectrophotometric technique (Marczenko, 1986) at Uranium and Thorium Laboratory, Central Labs, Nuclear Materials Authority, Anshas, Egypt.

### Plant growth

The plants were raised in open greenhouse in plastic pots, 18 cm in diameter and 25 cm depth. Ten seeds were sown at 1 cm depth. After seedling establishment, plants were thinned to 5 individuals per pot. Plants were irrigated regularly with tap water until maturation. The experiment started in 22 February and terminated in 12 June 2005. The maximum temperature during the experimental period was 20.73°C in February and was 34.54°C in June while the minimum temperature ranged between 7.14°C during February and 21.47°C during June. Relative humidity ranged between minimum values of 24.86% in February to a maximum value of 77.33% in June. Plant sampling and measurements were taken at seedling, juvenile, flowering, fruiting and senescing stages. Plants were harvested at every growth stage from 5 replicate pots. At the flowering / fruiting stage uranium and thorium content of the plant samples was determined by inductively coupled plasma mass

spectrometer (ICP-MS technique) model JEOL JMS-Plasma X2 high resolution ICP-MS.

Plant growth measurements were taken following Evans (1972) and Hunt (1981). The relative growth rate (RGR) was estimated as the difference in dry weight over time interval ( $\text{mg g}^{-1} \text{day}^{-1}$ ) as:  $\text{RGR} = (\ln W_2 - \ln W_1) / (t_2 - t_1)$ , where  $W_1$  and  $W_2$  are weights at time  $t_1$  and  $t_2$  respectively. The net assimilation rate (NAR) was estimated as the difference in the dry weight over time interval in relation to leaf area ( $\text{mg cm}^{-2} \text{day}^{-1}$ ) as:  $\text{NAR} = \{(W_2 - W_1) / (t_2 - t_1)\} \times \{(\ln A_2 - \ln A_1) / (A_2 - A_1)\}$ , where  $A_1$  and  $A_2$  are leaf areas at time  $t_1$  and  $t_2$  respectively. The leaf area index (LAI) was measured as total leaf area per pot surface area ( $\text{cm}^2 \text{cm}^{-2}$ ) and the specific leaf area (SLA) was calculated as the total leaf area per total leaf weight ( $\text{mm}^2 \text{mg}^{-1}$ ).

For dry matter allocation, individual plants were excavated and separately sorted into component organs and the oven dry weight at 80°C of each component was expressed as a percentage of the total dry weight of each individual plant. The root-shoot ratio was estimated as based on dry weight, and calculated as shoot dry weight divided by root dry weight. For sampling, five replications ( $n = 5$ ) were taken for every measurements. The data were analyzed by ANOVA test to determine the significant differences among the mean values at  $P < 0.05$  probability level.

## RESULTS

### Radionuclides content

Uranium and thorium contents in the soil and study species are summarized in Table 1. The radionuclide content of the soil in the study sites is varied. Uranium and thorium concentrations attained the lowest values of 19.67 ppm and 29.67 ppm, respectively in the control soil. The maximum concentration of uranium reached 51.33 ppm in soil of the coastal sand dunes in which

thorium concentration attained 43.67 ppm. The highest concentration of thorium was 61.67 ppm in soil of the sand mounds in Abu Khashaba, where as uranium concentration reached 40.5 ppm. Uranium and thorium concentrations in the soil of Abu Khashaba coastal sand plain exhibited the values 31.0 ppm and 40.0 ppm, respectively. In general the levels of thorium concentrations are slightly above the uranium concentrations in the soils of the three study sites.

With respect to radionuclide content in the study species as shown in Table 1, the level of uranium in *Cakile maritima* attained the minimum values of 0.21 ppm in the plants grown in the soil of Al Narreges coastal sand dunes. The highest concentration of uranium (0.58 ppm) was detected in *C. maritima* grown in the soil of the coastal sand plain of Abu Khashaba. With respect to thorium concentration in *C. maritima*, it attained the highest concentration of 1.21 ppm when the plant was grown in the control soil but thorium was collected with the lowest concentration of 0.74 ppm when the plant was grown in the soil from the coastal sand plain of Abu Khashaba. With respect to *S. glaucus*, the plants grown in the soil of the coastal sand dunes contain the highest concentrations of both uranium and thorium (3.69 ppm and 3.52 ppm respectively) while the lowest concentrations of uranium and thorium (0.3 ppm and 0.9 ppm respectively) were observed when grown in the control soil. In the case of *R. pictus*, the concentrations of both uranium and thorium attained significantly higher values (0.87 and 1.82 ppm respectively) in the plants grown in soil from the coastal sand dunes than that grown in the soil from the sand mounds of Abu Khashaba where the concentration of uranium was 0.51 ppm and thorium was 0.59 ppm.

### Plant growth

Growth attributes of the three test species including relative growth rate (RGR), net assimilation rate (NAR), leaf area index (LAI) and specific leaf area (SLA) are illustrated in Figure 2.

### Relative growth rate

The relative growth rate (RGR) of the three test species generally decreased with time. RGR attained the highest value in the plants of the three species grown in the soil from the sand mounds of Abu Khashaba and the lowest value when grown in the soil of Al Narreges coastal sand dune during the most growth intervals. As shown in Figure 2, the RGR is generally lower in plants which have accumulated more radionuclides especially at flowering-fruiting growth interval. In *C. maritima*, RGR of plants grown in the soil of Al Narreges coastal sand dune and

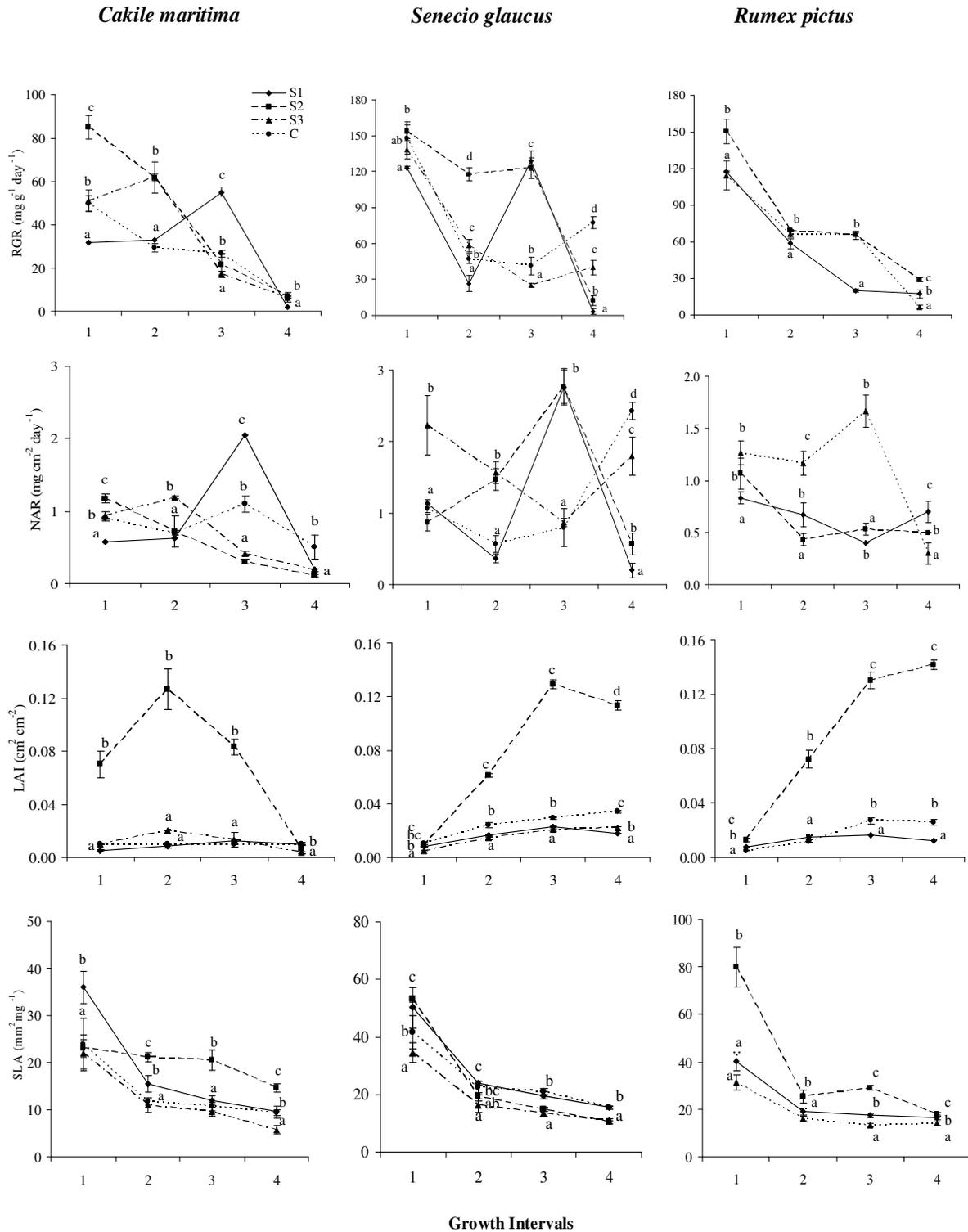
during the flowering-fruiting growth interval showed the value of  $55.05 \text{ mg g}^{-1} \text{ day}^{-1}$ . The RGR reduced to  $21.72 \text{ mg g}^{-1} \text{ day}^{-1}$  when the plants were grown in the soil from Abu Khashaba sand mounds and reduced to  $17.07 \text{ mg g}^{-1} \text{ day}^{-1}$  when the plants were grown in the soil from Abu Khashaba coastal sand plain from which they absorbed more uranium. Also in *S. glaucus* and during the most growth intervals, RGR attained the highest values in plants grown in the soil from the sand mounds of Abu Khashaba where these plants absorbed the lowest amount of uranium (0.27 ppm) when compared to *S. glaucus* grown in the soil of other black sand habitats which caused reduction in the RGR. The same results were observed in *R. pictus* as RGR of plants grown in the soil of Al Narreges coastal sand dune was lower than that in plants grown in the soil from the sand mounds of Abu Khashaba. In most cases, RGRs in plants of the three species grown in soil from their natural habitats was generally higher than RGR in these plants grown in the control soil.

### Net assimilation rate

The net assimilation rate (NAR) of the three species demonstrate irregular pattern of increase or decrease with growth (Figure 2). As for NAR of *C. maritima* and *S. glaucus* grown in the soil from Al Narreges coastal sand dune and during flowering-fruiting growth interval values were higher than in the other growth intervals. The relation between the NAR and radionuclide content of plants is dependant on the plant species and growth stage. In *C. maritima* and *R. pictus* especially during the flowering-fruiting growth interval NAR decreased with increasing the level of radionuclides content in plant tissues, but in *S. glaucus* there was no clear relation between NAR and radionuclide content. NAR was generally higher in the plants grown in the soil from their natural habitats than that in the plants grown in the control soil, especially in *S. glaucus*. This trend of results is observed all over the growth intervals of *C. maritima* and *S. glaucus* except at fruiting-senescing growth interval. For *Rumex pictus* this trend is observed only in fruiting-senescing growth interval only.

### Leaf area index

The study species exhibited the highest values of leaf area index (LAI) when grown in the soil from the sand mounds of Abu Khashaba in contrast to the plants grown in the soil of Al Narreges coastal sand dune which attained the lowest values of LAI (Figure 2). In *C. maritima*, the LAI of plants grown in the soil of Abu Khashaba sand mounds was higher than that in the plants grown in the soil of Al Narreges coastal sand dune



**Figure 2.** Relative growth rate (RGR as  $\text{mg g}^{-1}\text{day}^{-1}$ ), net assimilation rate (NAR as  $\text{mg cm}^{-2}\text{day}^{-1}$ ), leaf area index (LAI as  $\text{cm}^2\text{cm}^{-2}$ ) and Specific leaf area (SLA as  $\text{mm}^2\text{mg}^{-1}$ ) of *Cakile maritima*, *Senecio glaucus* and *Rumex pictus* grown in soil from the study black sand habitats; S1 = Al Narreges coastal sand dune, S2 = sand mounds of Abu Khashaba and S3 = Coastal sand plain of Abu Khashaba (S1 and S2 only in case of *Rumex pictus*) and in a control soil (C) at different growth intervals; 1 = seedling-juvenile, 2 = juvenile-flowering, 3 = flowering-fruiting, 4 = fruiting-senescing. Vertical bar around the mean is the standard deviation and different letters indicate a significant difference at  $P = 0.05$ .

and in control soil. LAI values in plants of *S. glaucus* and *R. pictus* grown in the soil of Abu Khashaba sand mounds also reduced when these plants grown in the soil of the other black sand habitats and in the control soil. The increased radionuclide content of plant caused reduction of the LAI in *S. glaucus* and *R. pictus* while in *C. maritima* LAI increased. Values of LAI are generally higher in the plants grown in the soil of their natural habitats than the plants grown in the control soil throughout the whole growth intervals.

### Specific leaf area

The three species attained the highest specific leaf area (SLA) values in seeding-Juvenile growth interval (Figure 2) then the values decreased in the subsequent growth intervals recording the minimum values in the Fruiting-senescing interval. The SLA in *C. maritima* attained the lowest values nearly throughout the whole life span when grown in the soil from the coastal sand plain of Abu Khashaba. Plants grown in the soil of Al Narreges coastal sand dune and in the control soil attained higher values of SLA with decreasing the uranium content. In case of *R. pictus*, SLA values in the plants grown in the soil from Al Narreges coastal sand dune was lower than those in the plants grown in the soil from Abu Khashaba sand mounds due to high uranium and thorium contents. The SLA values did not show any particular trend with radionuclides content of plants in case of *S. glaucus*. Comparison of the plants grown in the soil of their natural habitats to those in the control soil revealed that, SLA values are generally higher in the plants grown in the soil of their natural habitats than that grown in the control soil especially in *C. maritima* and *R. pictus*.

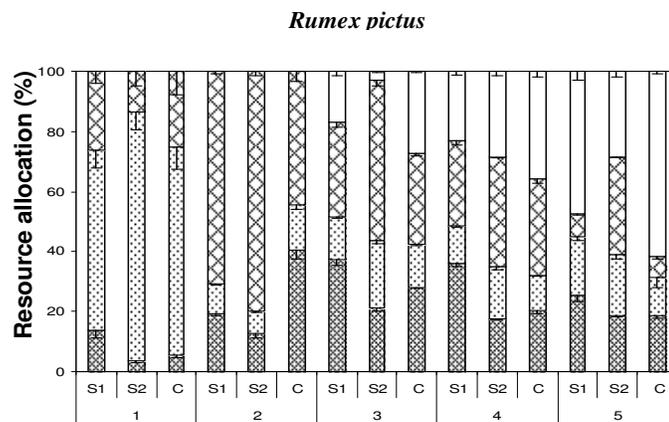
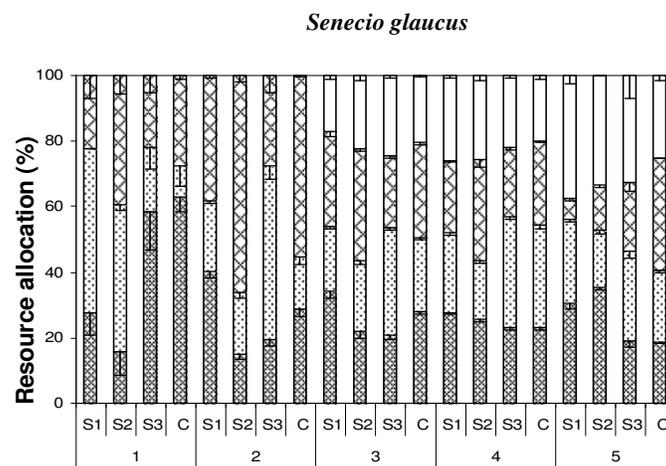
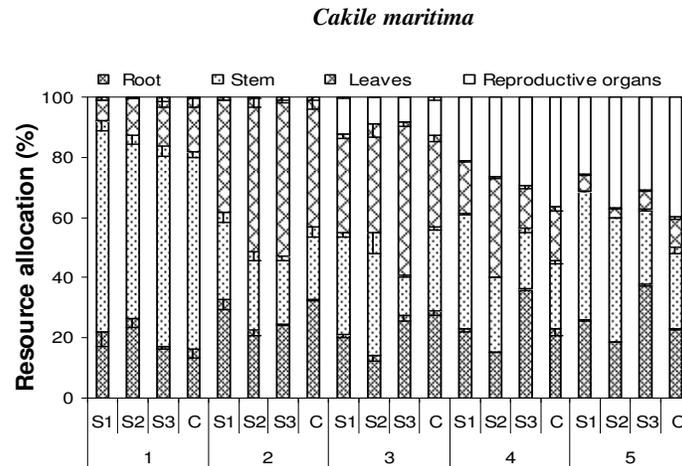
### Resource allocation

Allocation of dry matter among plant organs in the three test species is illustrated in Figure 3. For *C. maritima*, the allocation to leaves was generally higher than to the other plant organs in the intermediate growth stages, where at seedling and senescing stages the proportion of dry mass allocated to stem was higher than that allocated to leaves and other organs. Dry matter allocation to the root, leaves and reproductive organs of *C. maritima* at most growth stages was generally higher in plants grown in the soil of Abu Khashaba than the plants grown in the soil of Al Narreges coastal sand dune and in the control soil. On the other hand, the dry matter allocation to the stem of plants grown in the soil from the two sites of Abu Khashaba was lower than the plants grown in Al Narreges coastal sand dune and control soil. The proportion of dry matter allocated to different plant organs all over the growth stages was generally higher in the plants grown in the soil of their natural habitats than in

the plants grown in the control soil with the exception of reproductive organs.

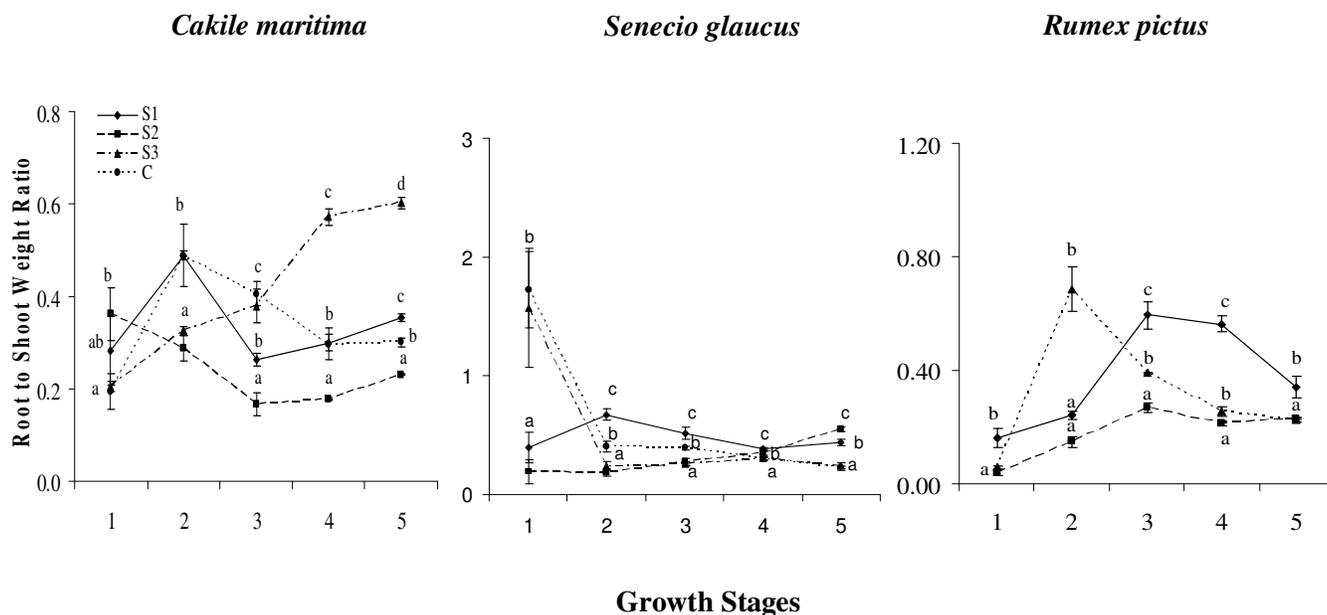
As shown in Figure 3, the dry matter allocation to root of *S. glaucus* was higher than the other plant organs during the most growth stages in plants grown in the soil from Al Narreges coastal sand dune, while plants grown in the soil from Abu Khashaba sand mounds and in the control soil, a highest proportion of their dry mass allocated to leaves. Percent of dry matter allocation to the root was found to be 15.019, 21.77 and 25.79% during Juvenile, flowering and fruiting stages respectively of plants grown in soil from sand mounds of Abu Khashaba, while these values increased to 40.14, 34.09 and 27.74% during the same stages respectively when the plants grown in the soil of Al Narreges coastal sand dune. The similar trend of results was shown with the allocation to the reproductive organs. The proportion of the dry mass allocation to the stem of plants grown in the soil from the coastal sand plain of Abu Khashaba during juvenile, flowering and fruiting stages reduced from 52.73, 33.10 and 33.79% respectively to 21.83, 19.99 and 24.26% during the same growth stages respectively in the plants grown in the soil of Al Narreges coastal sand dune. The same trend of results was observed in the dry matter allocation to leaves. Percent dry matter of leaves during juvenile, flowering and fruiting stages was 66.28, 34.32 and 30.77, respectively in the plants grown in the soil of Abu Khashaba sand mounds and these values are reduced during the same growth stages into 38.03, 28.72 and 22.90%, respectively in the plants grown in the soil of Al Narreges coastal sand dune. As indicated from the values between the brackets in APP. The dry matter allocation to different organs was generally higher in the plants grown in the soil from their natural habitats than in the plants grown in the control soil.

The illustrated data in Figure 3 showed that the allocation to the leaves of *R. pictus* was generally higher than the other plant organs in most growth stages. Dry mass allocation to root during juvenile, flowering and fruiting stages amounted to 12.80, 21.08 and 17.51%, respectively when the plants were grown in the soil of Abu Khashaba sand mounds. In plants grown in the soil of Al Narreges coastal sand dune dry matter allocated to the root during the same growth stages increased to 19.40, 37.20 and 36.04%, respectively. The same trend of results observed in reproductive organs during flowering and senescing stages. Plants grown in the soil of Abu Khashaba sand mounds showed allocation to stem and leaves higher than the allocation when the plants grown in the soil of Al Narreges coastal sand dune and in the control soil. The plants of *R. pictus* grown in the soil from their natural habitats exhibited values higher than that exhibited in the plants grown in the control soil with the exception of dry mass of reproductive organs which was generally highest in the plants grown in the control soil.



**Growth stages**

**Figure 3.** Resource allocation of *C. maritima*, *S. glaucus* and *R. pictus* grown in soil from the study black sand habitats; S1 = Al Narreges coastal sand dune, S2 = Sand mounds of Abu Khashaba and S3 = coastal sand plain of Abu Khashaba (S1 and S2 only in case of *R. pictus*) and in a control soil (C) at different growth stages; 1 = seedling stage, 2 = Juvenile stage, 3 = flowering stage, 4 = fruiting stage and 5 = senescing stage. Vertical bar around the mean is the standard deviation.



**Figure 4.** Root to shoot weight ratio of *C. maritima*, *S. glaucus* and *R. pictus* grown in soil from the study black sand habitats; S1 = Al Narreges coastal sand dune, S2 = Sand mounds of Abu Khashaba and S3 = Coastal sand plain of Abu Khashaba (S1 and S2 in case of *Rumex pictus*) and in a control soil (C) at different growth stages; 1 = Seedling stage, 2 = Juvenile stage, 3 = flowering stage, 4 = fruiting stage and 5 = senescing stage. Vertical bar around the mean is the standard deviation and different letters indicate a significant difference at  $P = 0.05$

### Vegetative attributes

The root–shoot (R/S) weight ratio for all test species in the different growth stages was almost lower than unity (Figure 4). In *C. maritima*, the R/S weight ratio was lowest during the most growth stages when the plants were grown in the soil from the sand mounds of Abu Khashaba and attained the highest value when grown in the soil from the coastal sand plain of Abu Khashaba with the increase of uranium content. In *S. glaucus* and *R. pictus*, the highest values of R/S weight ratio were recorded in the plants grown in the soil of Al Narreges coastal sand dune while the lowest values were recorded when grown in the soil from the two sites of Abu Khashaba and in control soil. As detected from the values between brackets, R/S weight ratio was generally higher in the plants grown in the soil from the control soil especially during flowering and fruiting stages than the plants grown in the soil from their natural habitats.

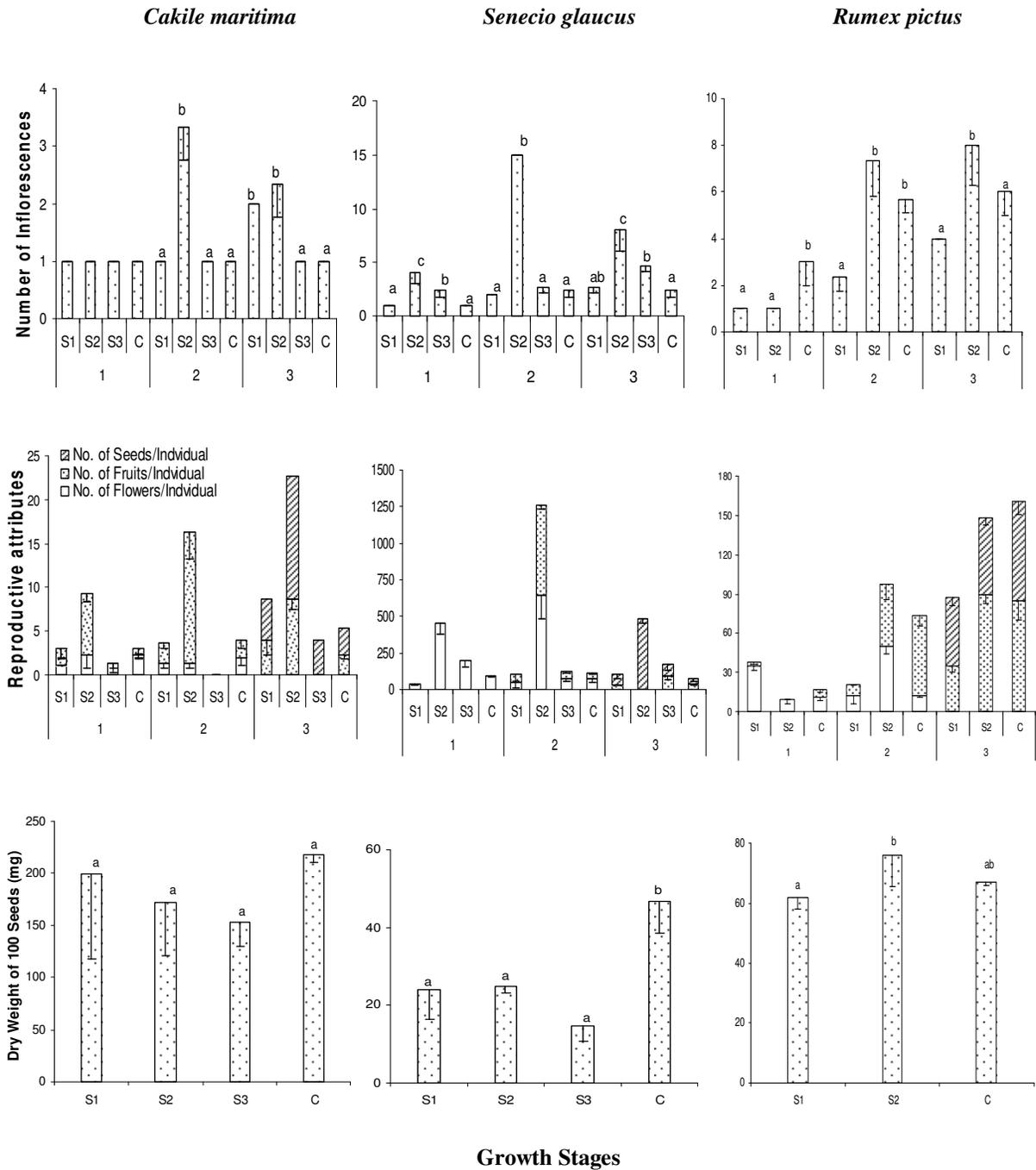
### Reproductive attributes

As shown in Figure 5, reproductive attributes generally attained highest values when the plants of the three species were grown in the soil from the sand mounds of Abu Khashaba especially during the fruiting stage. In *C. maritima*, number of inflorescences in the plants grown in

the soil from Al Narreges coastal sand dune attained the value of 1.00 during fruiting stage and this value increased into 3.33 with increasing uranium content in plants grown in the soil from Abu Khashaba sand mounds. Also number of flowers, number of fruits and number of seeds per individual exhibited higher values in the plants accumulated high level of uranium and thorium than plants containing low uranium and thorium contents. There was no significant difference between the plants in the dry weight of 100 seeds. With respect to *S. glaucus* and *R. pictus*, the trend of values are contrast to that of *C. maritima*, as the number of reproductive organs mostly reduced in the plants which absorbed more radionuclide content. Reproductive attributes showed values higher in the plants grown in the soil from their natural habitats than in the control soil throughout most growth stages of *C. maritima* and *S. glaucus* but in *R. pictus* the contrary results were observed.

### Comparison between the study species

Comparison between the three study species in the study black sand habitat using the ratio between the values in the plants grown in the soil from their natural habitats to that in the plants grown in the soil from the control site revealed that the growth attributes for the plants in the three black sand habitats are generally lower in the plants of *S. glaucus* throughout the most growth intervals than



**Figure 5.** Reproductive attributes in *Cakile maritima*, *Senecio glaucus* and *Rumex pictus* grown in soil from the study black sand habitats; S1 = Al Narreges coastal sand dune, S2 = Sand mounds of Abu Khashaba and S3 = Coastal sand plain of Abu Khashaba (S1 and S2 only in case of *Rumex pictus*) and in a control soil (C) at different growth stages; 1 = Flowering stage, 2 = Fruiting stage and 4 = Senescing stage. Vertical bar around the mean is the standard deviation. Different letters indicate a significant difference at P = 0.05.

the plants of *R. pictus* and *C. maritima*. With relation to the resource allocation to different plant organs, the dry matter allocated to root and reproductive organs was

generally higher in *S. glaucus* than *C. maritima* and *R. pictus* of the three black sand habitats during the most growth stages. In contrast to root and reproductive

organs, the dry mass allocation to stem and leaves attained the lowest values in *S. glaucus*. When the root to shoot weight ratio of the three species was compared, it was indicated that root to shoot weight ratio of *S. glaucus* was higher than that of *C. maritima* and *R. pictus* grown in the soil of Al Narreges coastal sand dune and Abu Khashaba sand mounds. With respect to reproductive attributes, it can be indicated that the number of inflorescences per individual was generally higher in *S. glaucus* when grown in the soil of its natural habitats than *C. maritima* which was mostly higher than *R. pictus*. Comparison between the three study species using different growth parameters revealed that growing of *S. glaucus* in a soil not from its natural habitats will exhibit lower values of RGR, NAR, LAI and dry matter allocation to stem and leaves and will exhibit higher values of dry matter allocation to root, dry matter allocation to reproductive organs and number of inflorescences per individuals than *C. maritima* and *R. pictus* grown in the same soil.

## DISCUSSION

Mineralogical analysis of black sand in Egypt revealed that the most important minerals are zircon and monazite as they exhibit uranium, thorium, zirconium and hafnium (Hassan, 1993). The plants take up the nutrient ions, in accordance to their requirement. They are transported to specific tissues based on the function of the element in plant metabolism and it gets reflected in its higher concentration in a particular part compared to others (Pulhani et al., 2005). Radionuclides can also be picked up along with nutrients and may have similar chemical behavior as the essential nutrient. It has been demonstrated by Cannon (1952) that plants absorb small amounts of uranium and vanadium that can be detected by analyzing a given part of the plant. It's so important to state that literature data on uranium toxicity is scarce (Anders and Nils, 1982). The effect of uranium and radiation from uranium on the germination of seeds has been studied experimentally and concluded that plant growth is most stimulated by addition of uranium nitrate to the nutrient solution in concentrations of 2 ppm uranium. In concentrations containing 47.6 ppm, poisoning effects are noticeable and yellow color develops in the root tips; at 476 ppm the roots are thin and yellow, and the leaves are twisted together. The relation to radionuclide content of the study plant species demonstrated the response of plant growth and resource allocation to high level of radionuclides accumulated in plant tissues.

### Plant growth

The RGRs of most test species of the present work

generally decreased with plant's age. This in agreement with slow RGR that was observed by Hegazy and Ismail (1992) as a result of decreased age-specific LAR and slow NAR which reflect the decreased amount of leaf production with age resulting in slower growth. The RGR may increase in the seedling-juvenile growth interval and then decrease in the proceeding growth intervals as shown in *C. maritima* and *R. pictus*. This may be explained by the fact that growth rate in the seedling-juvenile growth interval is normally the most rapid in the life of desert plants compared to the subsequent growth intervals (Burdon and Harper, 1980; Hegazy and Ismail, 1992). The study of growth attributes in the three species revealed that RGR, LAI and SLA are generally decreased as uranium and thorium content of the plant increases. The lower RGR of plants with high radionuclide content as compared to plants containing low radionuclide content may be explained by the increment of dry matter allocated to the reproductive organs. Similarly, Sayed and Hegazy (1994), found that the pattern of RGR increment followed that of dry matter allocated into vegetative parts (stem and leaves) and a decrease in RGR resulted from an increased dry matter allocated to sexual structures (flowers and fruits) at the expense of vegetative parts. The wide variation in RGR among species was explained mainly by the variation in the plant morphological variables, such as LAI and in particular the SLA. This finding is in agreement with many other studies supporting SLA as a major factor associated with variation in the RGR (Qian et al., 1999; Antúnez et al., 2001). The variation of NAR between the test species and among plants grown in control and in black sand soils of the same test species was very dynamic with age. Variability of NAR values among different species was parallel to the fluctuations in the RGR values of most species.

### Resource allocation

The increase of radionuclide content in the plants is associated with the dry matter allocation to certain organs and reduced the percent of allocation to other plant organs as found in the allocation of dry matter to root and reproductive organs which mostly increased while the dry matter allocation to stem and leaves decreased with the rise of radionuclide content of plants. The dry matter allocated to stem and leaves was generally higher than that allocated to root and reproductive organs in the three species. The total plant biomass generally decreased as the radionuclide content of plant increase this result is in agreement with the results of Sheppard et al. (2005) who indicated that there was decrease in total plant biomass at 300 mg Ukg<sup>-1</sup> dry soil compared to control. Similarly, Meyer and McLendon (1997) mentioned that the above ground biomass was affected by the highest uranium

level and all studied species demonstrated a decrease in production of biomass as uranium content in the plant increase. The study of Gulati et al. (1980) on the assimilation of uranium by wheat and tomato crops revealed that wheat yield increased with the increase of uranium to a certain level and then decreased with further increase in uranium concentration. Decline of dry mass of plant with increase of radionuclide content in the present investigation may be due to the reduction of RGR. In most studies, dry matter production is measured as the relative growth rate (RGR) of young isolated individuals (de Caluwe, 1995).

The reduction of dry matter allocated to stem and leaves with high level of radionuclide content in plants can be explained by the fact that uranium forms dissociable complexes with certain active groups on the cell surface (Murthy et al., 1984). The active complexing groups may be carboxyl, hydroxyl or phosphate. Uranium precipitation and complexing causes disfunction of xylem and phloem tissues of roots. The translocation to above ground organs especially stem and leaves may be reduced due to the disfunction of xylem and phloem. This might have been the major causes of the decrease in the proportion of dry matter allocated to stem and leaves. The rise of dry mass allocated to reproductive organs can be explained by the results of study by Drobkov (1951) who has shown by radiophotographs that uranium in the green portion of the plant migrates to those parts of the plant where development is most intensive; the growing tips, young leaves, seeds and so forth; flowering, fruiting and ripening are stimulated; and carbohydrate content is increased. The study of mortality and reproduction of *Limonium delicatulum* by Hegazy (1992) in similar sites revealed that flowering and fruiting are associated with the decreased cushion plant leafiness and leaf appearance rate, that is high resource allocation to reproductive organs.

### Vegetative and reproductive attributes

Root to shoot weight ratio was found to be increased with the increase of radionuclide content of plants. This can be explained by the increase of the percent of dry matter allocated to the root at the expense of stem and leaves. Tolerance indices calculated from root growth data showed that plants grow better in the presence of metals than in the control treatment as indicated by Archambault and Winterhalder (1994). Accordingly, plants may have responded in a compensatory way by increasing the root/shoot ratio, and maximizing nutrient intake more by reduction of shoot growth than by increasing root absorption capacity (Nilsson et al., 1993).

The response of reproductive organs to radionuclide content is plant species dependant. The increase of radionuclide content is associated with the formation of

reproductive organs in *C. maritima* but the contrary occurred in *S. glaucus* and *R. pictus*. The reduction in the number of reproductive organs in *S. glaucus* and *R. pictus* by high radionuclide content is in accordance with the results of Meyer and McLendon (1997) who mentioned that there's a noticeable decreased trend of inflorescences number. Also, Koul et al. (1983) reported that fruits in uranium treated plants were few with non viable seeds. Murthy et al. (1984) demonstrated the reduction of leaf chlorophyll content due to the absorbed uranium by soybean plants. Reduction of chlorophyll content may affect the net photosynthetic rate of plant which in turn affects the reproductive opportunity (Inderjit and Dakshini, 1995) and as a result, suppression in reproductive organs was shown by plants containing high level of radionuclide content.

The effect of increased radionuclide content of plants on their growth revealed that RGR, NAR, LAI and SLA generally decrease with the increase of plant's radionuclide content. Also, high level of uranium and thorium caused reduction of dry matter allocated to leaves and stem. Meanwhile, the percent of dry mass allocated to root and reproductive organ and root to shoot weight ratio increase with the increase of plant's radionuclide content. Number of reproductive organs was found to decrease with the plant's radionuclide content.

Comparison between plants grown in black sand and control soils indicated the better growth in the soil of its natural habitats (black sand) than in the control soil, especially *S. glaucus* as all studied growth criteria were generally higher in the plants grown in the soil from black sand habitats than the plants grown in the control soil, even though the plants may absorb more radioactive elements when grown in the soil from their natural habitats. *S. glaucus* is the most tolerant species to radionuclides compared to *C. maritima* and *R. pictus*.

### Conclusion

The response of plant growth and resource allocation to high level of radionuclides accumulated in plant tissues demonstrated that the three species can be used in phytoremediation and phytostabilization of radionuclide contaminated soils because of their tolerance and ability to accumulate radioactive elements. Species of plants that are best for phytoremediation have a well developed root system and tolerate high levels of the target radionuclide contaminants (Tang et al., 2001). Also, selection of the appropriate plant species should be based on element content in plant tissues if element recovery from harvested plant materials is an important goal of phytoremediation process (Qian et al., 1999; Hegazy and Emam, 2011). *S. glaucus* which show similar features may be considered as the most suitable test species for phytoremediation of contaminated soil

followed by *Cakile maritima* and *Rumex pictus*. From phytoremediation stand point, the actual plant removal efficiency of radionuclides is the product of plant density and the rate of element accumulation in harvestable plant parts. The determination of plant tissue radionuclide content will establish the correlation between metal concentration in soil and the optimum bioaccumulation of these metals in plant tissue. To explore these possibilities, further studies are required.

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