



RESEARCH ARTICLE

BIO-MONITORING OF RADIONUCLIDES AIR POLLUTION USING PHYSIOLOGICAL RESPONSES OF URBAN TREES

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ABSTRACT

Contamination of environment by radionuclides in territories under urboccosystem conditions is an actual problem. The search for new express methods for radioactivity determination of environment is an important task of research. The present work evaluated the ability to use *Eucalyptus globulus* leaves to act as bio-monitor for environmental radionuclides air pollution. The accumulation features of radionuclides (<sup>226</sup>Ra, <sup>232</sup>Th, <sup>40</sup>K) in the leaves of camphor trees (*Eucalyptus globulus* Labill) growing in areas near Al-Nasr quarry for phosphate production, Upper Egypt during the physical year 2016 have been considered. The activity concentration of <sup>226</sup>Ra, <sup>232</sup>Th and <sup>40</sup>K in samples of soils, plant leaves and deposited dust have been compared. These activities were measured using high resolution gamma- spectroscopy (Hyper Pure Germanium detector) with consideration for the background level. On the basis of radionuclides analysis, leaves of *E. globulus* exhibited different accumulation coefficients according to the distance from the quarry. The relationship between the radionuclides concentrations in leaf deposited dust and soil samples was estimated by Pearson coefficients to resolve two main mechanisms of radionuclide accumulation, atmospheric deposition, and root uptake. Differences in local conditions at the sampling sites were not significant. *E. globulus* physiological responses due to radionuclides stress were determined. The results of investigation recommended the use *E. globulus* leaves as an indicator of radioactivity air pollution.

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INTRODUCTION

Radionuclides, also known as naturally occurring radioactive materials (NORMs) exist naturally in the earth's crust in low concentrations but human activities increase the exposure of people ionizing radiation such as burning coal from power plant, making and using fertilizer (phosphate), Mining and oil gas production. Then, radionuclides are transferred to plants from the soil through their roots and also absorbed into internal and external part of plants through dry and wet atmospheric deposition over long period of time (Karunakara, 2003). Foliar deposition and absorption of radionuclides from air are closely associated with morphological characteristics of leaves and the local climate (e.g., atmospheric humidity, concentrations of dust particles, wind velocity, amount of precipitation). However, the main pathway of long-lived radionuclides (<sup>40</sup>K, <sup>226</sup>Ra and <sup>232</sup>Th) accumulation in leaves is through resuspension from soils (Vandenhove et al., 2009), while atmospheric deposition and foliar uptake are considered the predominant sources of lead (Klaminder et al., 2005; Dean 2007) and <sup>7</sup>Be (Karunakara 2003; Pöschl et al., 2010).

Plants represent an important link in transport and distribution of radionuclides, heavy metals and other pollutants in the environment and are often used as biomonitors of atmospheric pollution (Smith et al., 2004; Djuric and Popović 1994; Aničić et al. 2007). However, little is known regarding bio-monitoring of radionuclides from environment; even less is known about radionuclide contamination and removal by vegetation in Egypt (Abu-khadra and Eissa 2008). As known, higher plants are not as efficient biomonitors of environmental pollution as moss and lichen (Di Lella et al., 2003; Popović et al., 2008). However, in urban areas where moss and lichen are rarely found, higher plants could alternatively be used for biomonitoring purposes (Smith et al 2004; Popović et al., 2009;). Identification of tree species that can biologically monitor air pollution and can endure air pollution is very much important for a sustainable green belt development around any polluted place (El-Khatib et al., 2010; 2011; 2012a, b, c; 2016). As an environmental stress, large doses of radionuclides due to increase of anthropogenic activities are known to induce adverse effects on organisms (Wi et al., 2005; Reisz et al., 2014). Physiological responses as defensive mechanisms are developed by plants to protect themselves under various stress including radiation. Many studies focused on various aspects of the defense mechanisms in plants, such as the activity of

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Map 1. location map showing the study area and the selected sites (I, II, III, V)

antioxidative enzymes, chlorophyll contents, lipid peroxidation, and proline levels under environmental stress including radiation (El-Khatib *et al.*, 2003, 2004; Kim *et al.*, 2004, 2012, 2015; Alikamanoğlu *et al.*, 2007; Aly and El-Beltagi, 2010; Silva *et al.*, 2011; Jan *et al.*, 2012; Marcu *et al.*, 2013; Vanhoudt *et al.*, 2014). Accordingly, the present study aimed to evaluate the suitability of using the tree of *Eucalyptus globulus* as bio-monitor for radionuclides air pollution through determining its physiological responses under such pollution circumstances.

### Study area

The study area represents the areas adjacent to the Al-Nasr quarry for phosphate production at the area of Al-Mahamid, Upper Egypt (25° 6'18.81"N, 32°49'15.53"E and 25° 6'4.11"N, 32°49'31.91"E), where the rocks containing the phosphate ore are collected, milled and processed for processing and phosphate production. During these operations, the dust spreads in varying degrees to cover neighboring areas, including agricultural and other populated areas (Abd El-Gabar *et al.* 2002). For the purposes of this study, four geographically defined locations (12 sampling points) that covers a distance of 10 km in radius to the North (N), North-East (NE) and North-West (NW) were selected. Control site was located away 40 Km distance from the affected area. The area is generally hot and dry along the year with temperatures in the range from 28°C to 40°C. The boundary of the study area is shown in Map 1.

## MATERIALS AND METHODS

### Tree species

*Eucalyptus globulus* Labill. is a member of family of Myrtaceae, which is a large tree attains a height of 60 m or more. It is one of the world's most widespread hardwood trees. Due to its social, economic and environmental impacts,

Camphor (*Eucalyptus globulus*) has been the object of several genetic, ecological and physiological studies. It is the common tree species at the study area due to its using as green fence around the quarry and decorative plant in the streets and public parks.

### Radioactive sampling and sample preparation

Four sampling sites were in definite positions relative to emission sources one of them as control as shown on Map1. Samples of soil, fine dust and plant leaves were collected in two seasons throughout year 2016 at different distance from the El-Nasr quarry for phosphate production. All samples (leaves, soil, and fine dust) were collected in triplicates. Samples of soil were collected from down 25cm depth in the rhizosphere of *E. globulus* trees. Samples with large grain size were crashed to small pieces, dried at 105°C, sieved to a fine grain size powder, weighted and carefully sealed in a tight container, and stored for at least 4 weeks to reach equilibrium before radionuclides counting. Leaves of *E. globulus* were detached from each tree at 1.5-2.0 m above the ground by pruning shears from the outer and inner parts of the canopies in the four sides of the tree (N, S, E, W; 9 leaves per each space direction). Some of them were preserved in ice boxes and transport into the laboratory, where they subjected to physiological investigation. Other collected leaf samples were used to collect the deposited dust on their surfaces according to the method used by El-Khatib *et al.*, (2004, 2007) and Smith *et al.* (2004). The collected dust and cleaned leaves were treated separately as mentioned with soil samples to be ready for radionuclides counting.

### Activity determination

The activity of  $^{226}\text{Ra}$ ,  $^{232}\text{Th}$  and  $^{40}\text{K}$  in the collected samples were determined using the High Purity Germanium Detector (HPGD) system consists of an N-type HPGD (CANBERRA) coupled to a computer based multi-channel analyzer (MCA)

mounted in a cylindrical lead shield High Purity Germanium Detector (HPGD). (100 mm thick) and cooled in liquid nitrogen. For the efficiency calibration, a multi-element standard of known activities was used. Assuming secular equilibrium in the uranium and thorium decay series, the  $^{238}\text{U}$  and the  $^{232}\text{Th}$  activities were determined indirectly via activities of their daughter. The nuclides chosen were  $^{214}\text{Bi}$  (609.3, 1120.3 and 1764 keV) and  $^{214}\text{Pb}$  (351 keV) for  $^{238}\text{U}$ ,  $^{208}\text{Tl}$  (2614 keV),  $^{212}\text{Pb}$  (238 keV) and  $^{228}\text{Ac}$  (911 Kev) for  $^{232}\text{Th}$ . The specific activity of  $^{40}\text{K}$  was determined directly by 1461 keV photo peak. The counting time for each sample was 24 hour to achieve statistically smaller error levels. The background was measured frequently and subtracted from the net count for all measured samples. The activity concentrations of the natural radionuclides in the measured samples were computed using the following relation:

$$A_s = \frac{N}{\varepsilon P_r M} \text{ (Bq/kg)}$$

Where A is the activity concentration expressed in Becquerel per kilogram dry weight (Bq/kg), N is the net counting rate of  $\gamma$ -ray (counts per second) corrected for background,  $\varepsilon$  the detector efficiency of the specific  $\gamma$ -ray,  $P_r$  the absolute transition probability of  $\gamma$ -decay and M the mass of the sample (kg).

### Physiological analysis

Suitable spectrophotometric methods using PerkinElmer UV/VIS Spectrometer Lambda 35 were used to determine the selected parameters. Chlorophyll content (Arnon 1949, Agbaire and Esiefarienrhe, 2010), and they expressed as mg/g fresh weight (fW); Free proline content (Bates *et al.*, 1973), and expressed as mg/g dry weight (dw). Total soluble protein content (Lowry *et al.*, 1951) and expressed as mag/g dry weight (dw); The total carbohydrates (Fales 1951, Schlegel 1956, Badour 959, and expressed as mg/g dry weight (dw).

### Measurement of antioxidative enzyme activity

For enzyme extracts, fresh leaf samples (1 g) from control and treated plants were ground with 5 ml pre-cooled 50 mM Na-phosphate buffer (pH 7.8), 0.1 mM EDTA- $\text{Na}_2$  and 1% (w/w) polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 15,000 rpm for 15 min at 4°C and the resulting supernatants were used for enzyme assay. All enzyme activity data were related to plant fresh weight (FW). The following methods were adopted for: Superoxide dismutase (EC 1.15.1.1) (Beauchamp and Fridovich., 1971); Ascorbate peroxidase (EC 1.11.1.11) Cakmak and Marschner, 1992, and Çakmak *et al.*, (1994); Guaiacol peroxidase (EC 1.11.1.7) (Ekmekci and Terzioglu 2005) and Catalase (EC 1.11.1.6) (Zhao *et al.*, 2007).

### Data analysis

Software package of MINTAB 17.0 for Windows was applied to perform the statistical analyses of the results including: mean, S.D., Pearson correlation coefficients, etc. for the measured data (n= 3). One-way analysis of variance (ANOVA) for all parameters was carried out where significance was measured at levels  $p < 0.05$  and 0.01). Respond of biological

parameters to exposure of radiations were analyzed by using  $Y=A+\beta x$  regression model.

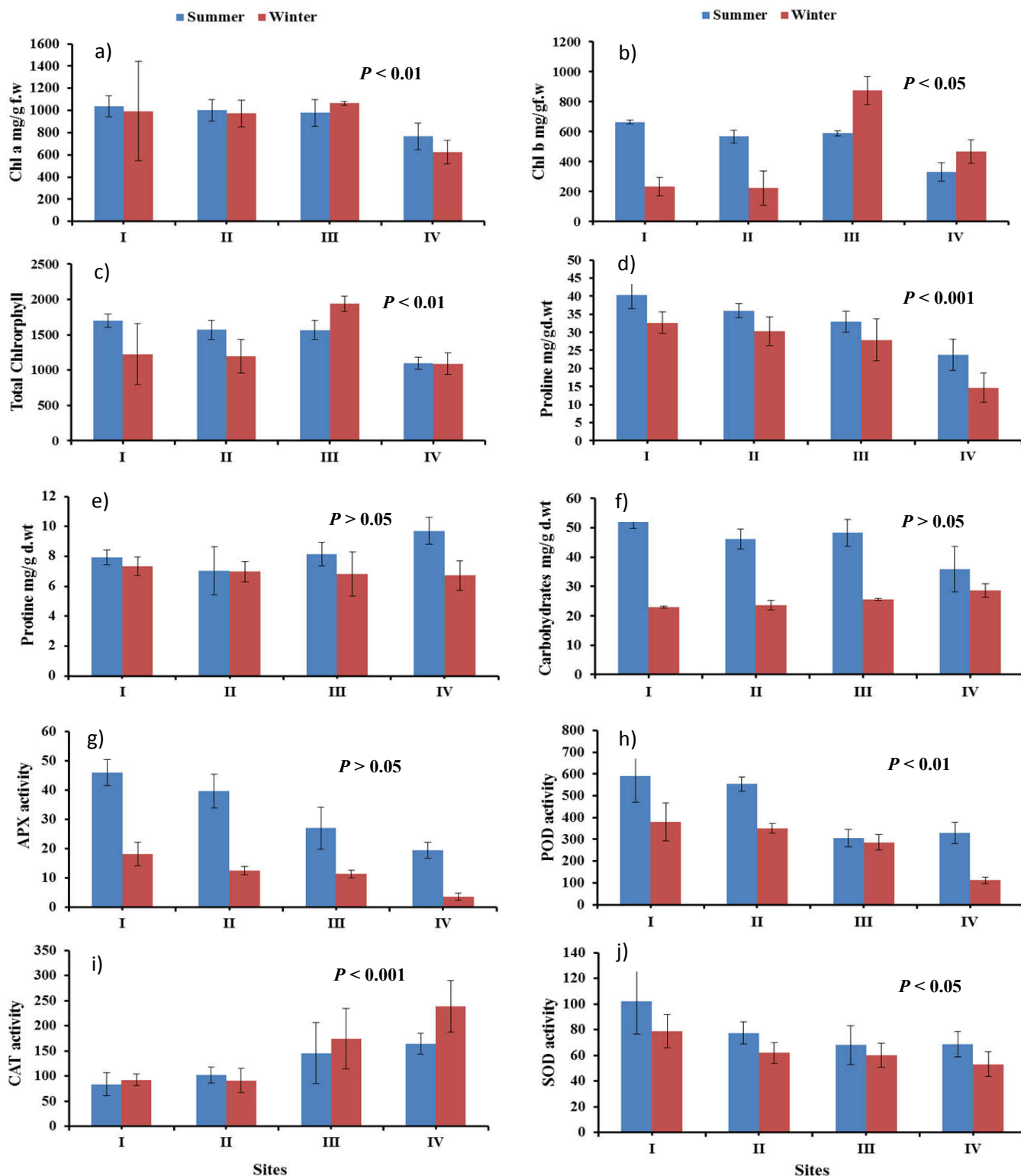
## RESULTS AND DISCUSSION

The radionuclides concentration ( $^{40}\text{K}$ ,  $^{232}\text{Th}$ , and  $^{226}\text{Ra}$ ) in soil samples of present study are presented in Table 1. It was found that the activity concentration of these radionuclides varies significantly ( $P < 0.05$ ) according to the variations of season and sites. Samples of site IV showed lower  $^{226}\text{Ra}$  and  $^{232}\text{Th}$  concentrations than other sites, especially during summer, being 6.80 Bq/ kg and 10.58 Bq/ kg, respectively. This can be attributed to site IV exists between farms and beyond the source of pollution.  $^{40}\text{K}$  activity concentration recorded different maxima during different seasons, reaching 264.75 Bq/ kg at site II. Non significance differences ( $P > 0.05$ ) between sites in their  $^{40}\text{K}$  activity concentration were recorded. However, The results showed that  $^{40}\text{K}$  is the major soil contaminant. Zare *et al.*, (2016) attributed the increase of  $^{40}\text{K}$  in the soil to the heavy use of NPK fertilizers by farmers. Otherwise, such amounts of  $^{40}\text{K}$  resulted from the natural concentration in the Earth's crust (Atwood, 2013). In terms of  $^{226}\text{Ra}$  activity concentration, site I that close to the source of pollution showed the highest values 84.07 and 68.0 Bq/ kg during the winter and summer season, respectively, and hence differed significantly from other sites, especially the control site (IV). Concerning the composition of deposited dust on the surface of plant leaves,  $^{226}\text{Ra}$  radionuclide showed a higher activity concentration at site I (439.03 and 453.32 Bq/ kg during winter and summer season, respectively) than samples of other sites (Table 2).

However, foliar deposition and absorption of radionuclides from air are closely associated with morphological characteristics of leaves and the local climate (e.g., atmospheric humidity, concentrations of dust particles, wind velocity, amount of precipitation). As shown in Table 3, the variations in the radionuclides content in pant leaves were lowest for  $^{40}\text{K}$  which is generally uptaken from soil through roots (Popović *et al.*, 2008; Popović 2009).  $^{226}\text{Ra}$  is of relatively lower mobility compared to  $^{232}\text{Th}$ , which resulted in higher plant uptake and accumulation (Vandenhove *et al.*, 2009). Table 4 shows Pearson Correlation Coefficients matrix for radionuclides activities in plant leaves samples with their corresponding in both soil and deposited dust samples. Except  $^{226}\text{R}$ , radionuclides in leaves have large positive correlation coefficients with those of deposited dust coefficients ( $r$  for  $^{232}\text{Th} = 0.109$ ,  $r$  for  $^{40}\text{K} = 0.60$ ) than that of soil ( $r$  for  $^{232}\text{Th} = -0.056$ ,  $r$  for  $^{40}\text{K} = 0.222$ ), reflecting the role of atmospheric deposition and foliar uptake in the observed accumulation. Meanwhile,  $^{226}\text{R}$  in plant leaves showed positive correlation coefficients with their corresponding in both soil ( $r = 0.534$ ) and deposited dust ( $r = 0.494$ ), indicating to the accumulation of this radionuclide in leaves may be also due to resuspension from soil. However, these results reflect the role of deposited dust in the observed radionuclides plant accumulation. This finding of prime importance, when considering the use of *E. globulus* leaves for the purpose of monitoring radionuclides air pollution. Gamma radiation exerts different effects on photosynthetic pigments depending on the plant species and the radiation dose (Kim *et al.*, 2015). In present work, the contents of chlorophyll *a*, chlorophyll *b* and the total chlorophyll showed variations with the decrease of distance from the source of pollution (Figure 1, a, b, c).

Table 1. Activity of radionuclides (mean  $\pm$  standard deviation) in soils samples collected from different sites during the study period

Seasons	Sites	Radionuclides activities in Becquerel per kilogram dry weight soil (Bq/kg)		
		$^{226}\text{R}$	$^{232}\text{Th}$	$^{40}\text{K}$
Winter	I	84.07 $\pm$ 11.2	15.17 $\pm$ 1.45	225.68 $\pm$ 11.3
	II	19.70 $\pm$ 3.21	11.23 $\pm$ 2.1	205.27 $\pm$ 15.9
	III	44.61 $\pm$ 12.32	9.20 $\pm$ 1.2	176.51 $\pm$ 18.4
	IV	14.45 $\pm$ 4.3	15.30 $\pm$ 1.4	224.77 $\pm$ 22.9
Summer	I	68.00 $\pm$ 13.2	10.67 $\pm$ 0.91	210.31 $\pm$ 23.4
	II	53.56 $\pm$ 4.21	20.12 $\pm$ 1.32	264.75 $\pm$ 30.1
	III	42.98 $\pm$ 5.2	22.35 $\pm$ 1.11	233.56 $\pm$ 34.3
	IV	6.80 $\pm$ 0.54	10.58 $\pm$ 0.89	183.34 $\pm$ 17.3

Figure 1. Average annual concentration (Mean  $\pm$ SD) of a) chlorophyll a, b) chlorophyll b, c) total chlorophyll, d) proline, e) protein, f) carbohydrates, g) APX, h) POD, i) CAT, j) SOD in leaves of *Eucalyptus globulus* at the studied sites during the study period

**Table 2. Activity of radionuclides (mean ± standard deviation) in deposited dust samples collected from the leaves surface of plants growing at different sites during the study period**

Seasons	Sites	Radionuclides activities in Becquerel per kilogram dry weight soil (Bq/kg)		
		<sup>226</sup> Ra	<sup>232</sup> Th	<sup>40</sup> K
Winter	I	439.03±45.9	10.97±1.8	123.13±13.8
	II	168.53±21.5	8.14±0.98	171.64±21.9
	III	63.04±13.1	5.38±0.56	153.52±25.9
	IV	14.45±3.20	15.30±1.50	224.77±27.9
Summer	I	453.32±87.9	11.21±0.98	94.19±17.9
	II	230.96±32.8	9.50±0.67	135.41±15.8
	III	28.63±0.78	15.79±1.7	185.25±17.54
	IV	6.80±0.34	10.58±1.65	183.34±19.65

**Table 3. Activity of radionuclides (mean ± standard deviation) in leaves of plants growing at different sites during the study period**

Seasons	Sites	Radionuclides activities in Becquerel per kilogram dry weight soil (Bq/kg)		
		<sup>226</sup> Ra	<sup>232</sup> Th	<sup>40</sup> K
Winter	I	1.82±0.03	0.45±0.01	143.23±12.45
	II	1.51±0.04	1.16±0.14	160.83±43.78
	III	1.50±0.02	1.81±0.32	144.38±23.98
	IV	0.13±0.01	0.87±0.11	155.07±13.87
Summer	I	5.19±0.03	3.93±0.21	107.37±9.79
	II	4.97±0.43	3.06±0.15	162.72±12.87
	III	3.63±0.12	2.55±0.23	143.88±23.43
	IV	1.78±0.03	4.35±0.54	151.19±12.98

**Table 4. Pearson correlation coefficients for radionuclides in the different samples**

		Leaves			Soil			Dust		
		<sup>226</sup> Ra	<sup>232</sup> Th	<sup>40</sup> K	<sup>226</sup> Ra	<sup>232</sup> Th	<sup>40</sup> K	<sup>226</sup> Ra	<sup>232</sup> Th	<sup>40</sup> K
Leaves	<sup>226</sup> Ra	1.00								
	<sup>232</sup> Th	0.44 *								
	<sup>40</sup> K	-0.45*	-0.31							
Soil	<sup>226</sup> Ra	0.53 **	-0.07	-0.49 *						
	<sup>232</sup> Th	0.33	-0.06	0.30	0.22					
	<sup>40</sup> K	0.48 *	-0.04	0.22	0.36	0.83**				
Dust	<sup>226</sup> Ra	0.49 *	0.01	-0.55 **	0.83 **	-0.11	0.25			
	<sup>232</sup> Th	0.01	0.11	-0.10	-0.09	0.56 **	0.45 *	-0.15		
	<sup>40</sup> K	-0.69**	-0.19	0.60 **	-0.83**	0.16	-0.10	-0.88**	0.40	1.00

\*Significant at the 0.05 level., \*\*Significant at the 0.01 level

**Table 5. Regression analysis of the studied physiological parameters under the effects of radioactivity series <sup>226</sup>Ra, <sup>232</sup>Th- and <sup>40</sup>K in plant**

Parameters	Radionuclides species		
	<sup>226</sup> Ra	<sup>232</sup> Th	<sup>40</sup> K
Chlorophyll a	y=804.0+49.87x P> 0.05, r= 0.39	y=995.3-25.93x P>0.05, r= -0.17	y=817.9+0.832x P>0.05, r= 0.12
Chlorophyll b	y= 384.2+43.07x p< 0.05, r= 0.33	y=421.4+28.38x P>0.05, r= 0.18	y=113.3+2.819x P>0.05, r= 0.38
Total chlorophylls	y= 1188.2+92.94x p< 0.05, r= 0.46	y=1417+2.45x P>0.05,r=0.01	y=931.2+3.651x P>0.05, r= 0.32
Proline	y= 20.09+3.843x p<0.001, r= 0.79	y=28.66+0.448x P>0.05, r= 0.07	y =30.84-0.0078x P>0.05, r= -0.03
Protein	y= 7.34+0.097x P> 0.05, r= 0.13	y=6.274+0.52x p< 0.01, r= 0.57	y =7.325+0.0019x P>0.05, r= 0.05
Carbohydrates	y= 20.66+5.87x p< 0.001, r= 0.83	y =23.71+4.669x p< 0.01, r= 0.54	y=30.87+0.034x p< 0.0001, r= 0.09
APX	y= 1.910+8.048x p< 0.001, r= 0.96	y= 8.73+5.35x p< 0.01, r= 0.52	y=23.04-0.006x P>0.05, r= -0.01
POD	y= 172.0+75.92x P < 0.001, r= 0.84	y=269.2+37.47x P>0.05, r= 0.34	y= 366.2-0.019x P>0.05, r= 0.004
CAT	y=185.9-19.56x P< 0.01, r= -0.54	y=144.0-2.97x P>0.05, r= -0.07	y=102.3+0.25x P>0.05, r= -0.13
SOD	y= 54.08+6.831x P< 0.01, r= 0.63	y=59.73+4.59x P>0.05, r= 0.35	y=95.75-0.18x P>0.05, r= 0.3

Chlorophyll *a* and total chlorophylls appeared to be more affected by <sup>226</sup>Ra ( $\beta = 49.87$  and  $92.94$ , respectively) than <sup>232</sup>Th ( $\beta = 25.23$  and  $2.45$ , respectively) and <sup>40</sup>K ( $\beta = 0.83$ ,  $3.65$ , respectively) (Table 5). In comparison to control (site IV), significant increase ( $P < 0.01$ ) in the chlorophyll *a* contents were recorded in the leaves samples taken from other sites

during the two seasons, while chlorophyll *b* showed a different trend during winter season. However, the highest chlorophyll *a* and *b* contents ( $1037.75$  and  $873.73$  mg/g fw, respectively) were recorded at site I that close to the source of pollution (Figure 1 a, b). As an environmental stress, radionuclide pollution may enhance the production of chlorophyll

molecules in plants to resist the unfavorable conditions posed by such pollution, and consequently maintain their photosynthetic activity. In this concern, the higher chlorophyll concentrations might have been due to the efficacious activity of the chlorophyll a/b binding (Cab) protein gene, which codes for chlorophyll protein (Arulbalachandran *et al.*, 2007). However, many authors reported the increase of chlorophylls in their tested plants with increasing of dose of gamma radiation (Borzouei *et al.*, 2010; Desai and Rao, 2014). Concerning proline, its content showed high significantly regularly increase ( $P < 0.001$ ) at the different studied sites during the different seasons, reaching its maximum value 40.32 mg/g dw at site I (Figure 1, d). Regression analysis of proline data (Table 5) exerted higher sensitivity of  $^{226}\text{Ra}$  ( $\beta = 3.84$ ,  $r = 0.79$ ) as compared to those of  $^{232}\text{Th}$  ( $\beta = 0.448$ ,  $r = 0.07$ ) and  $^{40}\text{K}$  ( $\beta = 0.0078$ ,  $r = 0.03$ ). Anyhow, the increase in the proline content under radionuclides pollution were stated by many researcher (Jan *et al.*, 2012; Desai and Rao, 2014; Kebeish *et al.*, 2015). As reported, radiation induces formation of ROS, which is highly toxic to plant cells, therefore the accumulation of proline may act as a scavenger of ROS and stabilize the structure and function of macromolecules such as DNA, protein and membranes (Akshatha *et al.*, 2013). However, the molecular mechanism of proline accumulation is still unclear and needs to be investigated under radiation stress (Qi *et al.*, 2014).

Data of present work showed no significant effect for the variation of site and season on the content of total protein, but mathematically site IV which far from the source of pollution still retains the maximum value (Figure 1 e). The radiation induces the splitting of peptide bonds that form free radicals (Aziz *et al.*, 2006) and the total amount of protein content is decreased due to metabolic activities and hydrolyzing enzyme activities as the gamma radiations are increased (Maity *et al.*, 2005). As shown in Table 5, gamma  $^{232}\text{Th}$  ( $\beta = 0.52$ ,  $r = 0.57$ ) appeared to be more affect protein content than both  $^{226}\text{Ra}$  ( $\beta = 0.097$ ,  $r = 0.13$ ) and  $^{40}\text{K}$  ( $\beta = 0.0019$ ,  $r = 0.05$ ). On the other hand, the total carbohydrate content showed a significant difference ( $P < 0.05$ ) between the different sites and not significantly ( $P > 0.05$ ) between the different seasons, reaching its maximum value (52.95 mg/g d.wt) during summer at site I (Figure 1, f). It is important to mention the role of microclimate variations in the observed maximum value during summer, where the temperature is high and may act as additional stress factor beside the radionuclides pollution. Table 5 shows that total carbohydrate contents have correlation coefficient with  $^{226}\text{Ra}$  ( $r = 0.82$ ) higher than that of  $^{232}\text{Th}$  ( $r = 0.54$ ) and  $^{40}\text{K}$  ( $r = 0.09$ ). In agreement with our results, Maity *et al.* (2009) reported this role of radiation on the carbohydrate content. In present work, the antioxidant enzyme activities in *Eucalyptus globules* differentially responded to gamma radiation. Except CAT, all antioxidant enzyme activities positively correlated with radionuclide series  $^{226}\text{Ra}$ ,  $^{232}\text{Th}$  and  $^{40}\text{K}$  (Table 5). Some authors (Wada *et al.*, 1998; Al-Rumaih and Al-Rumaih, 2008; Moussa *et al.*, 2011; Vanhoudt *et al.*, 2014; Jan *et al.*, 2012; Fan *et al.*, 2014;) showed different CAT activity change patterns under radiation stress in different dose ranges, where a significant decrease in the activity of this enzyme was observed in their tested plant species. Meanwhile, other authors (Aly and El-Beltagi, 2010; Silva *et al.*, 2011) reported increased tis enzyme activity with increased doses of gamma irradiation. Others antioxidant enzymes (APX, POD and SOD) in present work exhibited the same trend of increase in their activities with the decrease distance from the pollution

source (Figure 1, g, h, j). Significant differences ( $P < 0.05$ ,  $P < 0.01$ ) between sites were recorded, especially from control one (Site IV). In comparison,  $^{226}\text{Ra}$  and  $^{232}\text{Th}$  were more correlated with these enzymes activates than  $^{40}\text{K}$  (Table 3). Such increase in the activities of these enzymes were reported by several studies (Kim *et al.*, 2005, 2015; Selvia *et al.*, 2011; Fans *et al.*, 2014). They reported an ineffectiveness of APX in improving photosynthesis in response to gamma radiation. Sun *et al.* (1998) and Kim *et al.*, (2012) clear up the role of SOD in converting superoxide radicals into hydrogen peroxides and hence protect cells against the ROS injury during ionizing radiation exposure. The highest POD activity was observed at site I (591.23  $\mu\text{mol/g fw}$ ) which equal to 179.7% of that of the control (IV). Anyhow, Aly and El-Beltagi (2010) reported the increase of the antioxidant enzyme activities may be one of the mechanisms of the hermetic effects of gamma radiation.

## Conclusion

Radionuclide activities was studied on Physiological responses of *Eucalyptus globules* and corresponding soil and dust samples. The distribution of  $^{226}\text{Ra}$ ,  $^{232}\text{Th}$  and  $^{40}\text{K}$  activity concentrations in plant is observed to be decreasing in the pattern of  $^{40}\text{K} > ^{226}\text{Ra} > ^{232}\text{Th}$ , whereas, high concentration for  $^{226}\text{Ra}$  recorded in fine dust collecting from the near sites of the El-Naser Mining. The result shows that the activity concentration of plant incorporated  $^{226}\text{Ra}$  have a positive correlation (0.534) with those of soil more than those of dust (0.494), reflecting the role of soil in the observed  $^{226}\text{Ra}$  plant accumulation. Our study has shown that radionuclide activities in *Eucalyptus globules* results in changes in a number of physiological parameters. Radiation stress causes an increase in the chlorophyll content, proline content, activities of antioxidative enzyme, such as SOD, APX and POD, and a decrease in the total protein, carbohydrate content, and CAT activity. In addition, this study enabled us to propose a basic mechanism of response of the plant to radiation and oxidative stress produced by ROS. It has provided basic information for future studies of the gamma-irradiated mutagenesis in *Eucalyptus globules*.

## Recommendation

Due to the cumulative effect exhibited in the accumulation of radionuclides, mature *E. globulus* leaves could be used as biomonitors of gamma air pollution. A more detailed analysis, investigating behavior of the radionuclides activities in leaves, deposited dust and aerosols during a growing season, is needed to further assess the reliability of *E. globulus* as a biomonitor.

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