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Response of free amino acids in four legumes plants to air pollution

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ABSTRACT

The study was intended to investigate the effect of ambient air pollution on four legumes species. The investigated legumes species were *Pisum sativum* L. (green pea), *Vicia faba* L. (broad bean), Glycine *max* (soya bean) and *Vigna sinensis* (cow pea). The four types of legumes were grown during the summer season, in four sites affected by the high traffic intensity and industrial activities in Riyadh, the capital of Saudi Arabia. The results depict an increase in air pollution (O_3, NO_2 and SO_2); as traffic density and industrial activities increased. Mostly free amino acids contents gradually increased in the plant's leaves as pollutants increased. This study concludes that the changes in free amino acids contents indicate the high levels of air pollution in three sites. Accumulation of free amino acids may act as a protective mechanism to air pollution stress.

Key words: Air pollution, Amino acid, Proline, Legumes

1. INTRODUCTION

Recently air pollution has been signified as a worldwide environmental health problem. It can be defined as the fluctuation in any atmospheric constituent from the value that would be existed without human activity (1). The air pollution problem arises mainly from industrialization (2). The primary origins of air pollution are automobiles, aircraft, industrial plants, power generation systems, construction projects and solid wastes. These sources added pollutants like NO_2, SO_2, dust, metals and other chemicals (3). Plants are an integral basis for whole ecosystems, largely affected by air borne pollution, which are named as the organisms with the most likely to experience impacts from ambient air contamination. Air pollutants are responsible for vegetation injury and crop yield losses (4). When plants were exposed to airborne pollutants, most of them experienced physiological changes before exhibiting visible damage to leaves (5). Plants that are constantly exposed to environmental pollutants absorb, gather and integrate these pollutants into their system. It reported that depending on their sensitivity level, plants show visible changes which would include modifications in the biochemical processes or accumulation of certain metabolites (6). Agbaire and Esiefarienrhe (7) reported that pollutants can cause leaf injury, decrease photosynthetic activity, disturb membrane permeability and reduce growth and yield in sensitive plant species (7). The sound effects of the pollutants are caused by the production of reactive oxygen species of plants, which cause peroxidative destruction of cellular constituents (8). It has been reported that proline act as a free radical scavenger to protect plants away from damage by oxidative stress (9). Ozone one (O_3) is considered to be the most important air pollutant affecting the plant productivity in all parts of the world (10, 11). The resulting economic losses and threat to food security has become an issue of concern in several regions of the globe where the expanding economy has led to an increased emission of air pollutants in general and O_3 precursors in particular (12). O_3 is aphytotoxic, a secondary air pollutant formed as a result of catalytic reactions of nitrogen oxides with carbon monoxide, methane and non-methane compounds (volatile organic compounds) in the presence of sunlight (13). O_3 is known to directly oxidize the individual amino acid and thus affect the metabolism of protein synthesis (14). Sulfur dioxide (SO_2) and nitrogen dioxide (NO_2) are the most phytotoxic pollutants which enter leaves through stomata, following the same diffusion pathway as CO_2 (15). Nitrogen oxides result in growth stimulation in low concentration and growth reduction at higher concentration. Proline accumulation often occurs in a variety of plants in the presence of different stresses. For instance, it accumulates in leaves of plants exposed to SO_2 fumigation (16), heavy metals (9) and salt stress (17). The amino acids have numerous roles in plants, for instance they act as osmolytes, detoxify heavy metals, regulate ion transport,

stomatal opening, affect the synthesis and activity of enzymes (18). The information about the effects of air pollution on plants is generally based on experiments where plants have been exposed to high concentrations of air pollutants for short periods under experimental conditions. However, less may know about responses of plants to air pollutants for long durations in field conditions. The present work was proposed to increase the understanding of change in amino acid content of plants under ambient air pollution in sites. To measure their response to such condition and uses these plants as indicators of air pollution in the study sites affected high traffic and industrial activities.

2. MATERIALS AND METHODS

2.1. Sampling sites

The survey was conducted in four sites in Riyadh, the capital of Saudi Arabia. The metrological data revealed that the city characterized by high temperature and low relative humidity and rainfall during the summer season. Likewise, low temperature and moderate relative humidity during the winter season (Figure 1). Sampling sites were placed to represent gradual sources of air pollution, such as, traffic intensity per day, industrial activity, and site without a direct source of air pollution further away from the city center, to represent the control (Table 1).

Table 1: Sampling sites number, location and description

Sampling site number	Location and description of sampling site
S1	Located at 40 km from city with very low traffic density
S2	in center of the city, characterized by high traffic density
S3	Southern Ring Rood near cement factory
S4	Southeastern of the city near industrial city

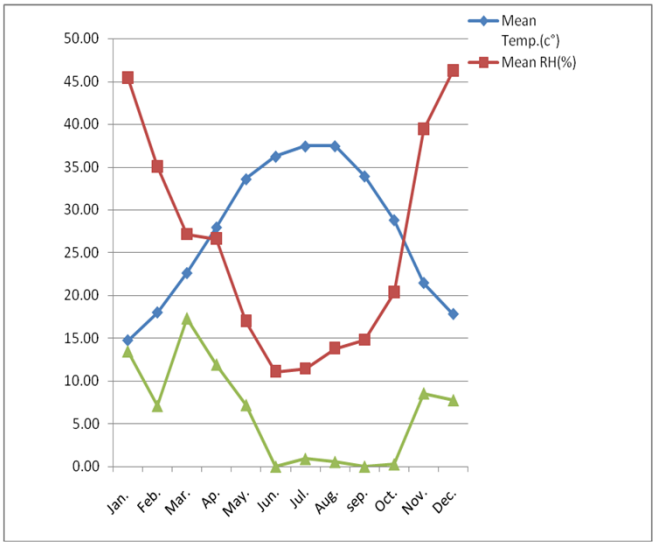

Figure 1: Monthly mean maximum, relative humidity and total rainfall in Riyadh

2.2. Plants

Plants tested during winter seasons were *Pisum sativum* L. and *Vicia faba* L. and during summer season were *Glycine max* and *Vigna sinensis*. 15 seeds were sown in plastic pots (40 cm) with 50% clay, 50% sand and pH was adjusted to 8.4. After germination, five uniform plants per pot were selected and 10 pots from each species were transferred to each sampling sites. Peters, NPK: 4/25/35 plus micro metals was added to prevent nutritional deficiencies. Winter plants and summer plants were exposed to ambient air for three months on their respective season.

2.3. Gases monitoring

The gas concentrations of ozone, sulfur dioxide and nitrogen dioxide were measured per day for each sampling site period from December to march for winter experiment and from April to September for summer experiment. O_3 concentration was monitored using O_3 analyzer Model UV -100 Serial # 111. Eco sensors, Inc. USA; NO_2 and SO_2 were measured by using Aeroqual Series Monitor with multi head. Then average of data were calculated and recorded.

2.4. Free amino acids

Free amino acids were extracted from dry plant materials (oven dry at 105°C for 24 h) using ethyl alcohol (80%, v/v) according to Malik and Singh. The qualitative and quantitative determinations of amino acids were carried out using LKB 415 alpha plus amino acid analyzer according to Christias *et al*. (19). Standard amino acids were used as reference.

2.5. Statistical analysis

ANOVA was used to test the effect of sampling sites and LSD was used for mean separation. In addition, the generalized linear model (GLM) was used to test the interaction between species and sampling sites. All statistical analyses were carried out using SAS statistical package.

3. RESULTS AND DISCUSSION

3.1. Air pollution gases

During winter season, the average monthly values of O_3, NO_2 and SO_2 concentration - 110.4, 29.3 and 29.1 ppb respectively. In sampling site 4 it was higher during February and March compared to all other sampling sites. Minimum pollutants concentrations were measured in the control sire (S1). During the summer season, as in August the pollutants increased in the site near industrial area (S4), which was characterized by high ozone (150.1ppb). Higher NO_2 and SO_2 - 36.4 and 28.2 ppb respectively during the month of September. But overall highest NO_2 concentration (30.4 ppb) was measured in S2 (site with high density of traffic) compared to other sites and control (Figure 2, Figure 3, Figure 4, Figure 5). The studied air pollution gases (O_3, NO_2 and SO_2) concentration was

higher during summer season, which characterized by high temperature and low relative humidity (Figure 1). The finding of this study was matched with previous results. Ozone is strongly correlated with temperature (20). Camalier et al. (21) found that as much as 80% of the variance in the maximum daily 8-h average ozone in the eastern U.S. can be explained by a generalized linear model with temperature and relative humidity as the two most important variables. Sulfate concentrations increase in temperature (20), nitrate and organic semi-volatile compound change from the particle phase to the gas phase with increasing temperature (22). As found in the outcome of this study O_3, NO_2 and SO_2 concentration (ppb) in sampling sites was increased as traffic density and industrial activities increased. The site S4 and S3 were characterized by high concentrations of O_3, NO_2 and SO_2 respectively compared to control S1. The higher level of ozone in these sites which were characterized by high air pollution sources (such as industrial activities) was explained by increasing emission of ozone precursor gases such as CO, NO_x, and volatile organic compounds as a result of industrial activities. The transport sector is responsible for high amounts of ozone precursors emitted to the atmosphere from the urban area. Traffic rush hours clearly behave as a sink of ozone due to the emissions of NOx (23). Air pollution is arising mainly from industrialization.

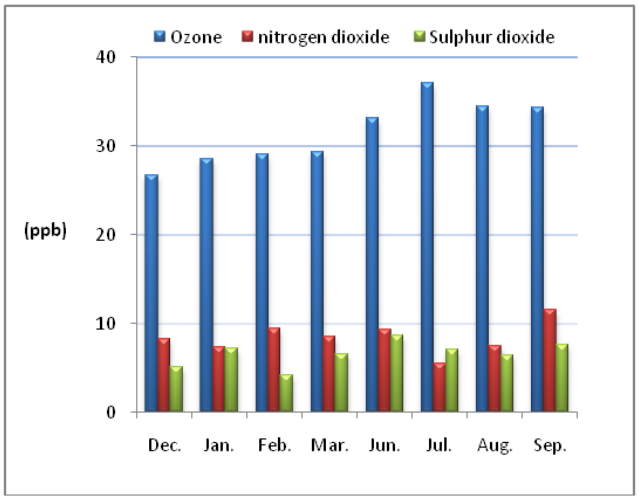

Figure 2: pollutants concentration in S1

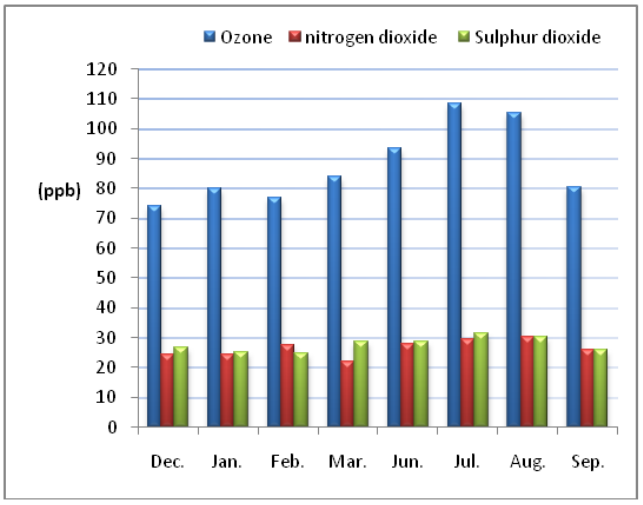

Figure 3: Pollutants concentration in S2

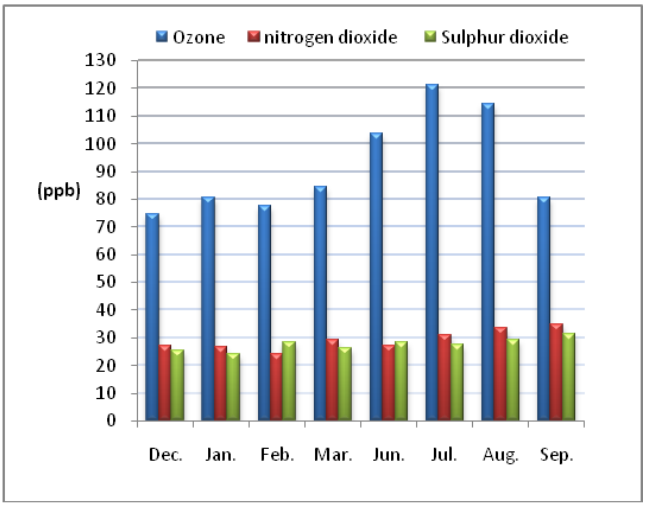

Figure 4: pollutants concentration in S3

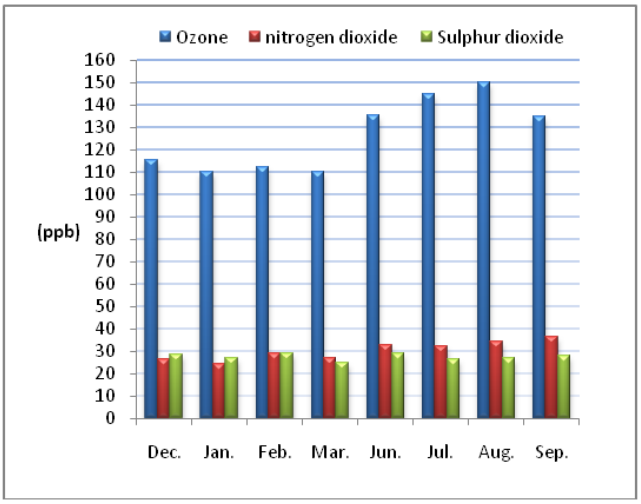

Figure 5: pollutants concentration in S4

3.2. Free amino acids

Table 2 showed highly significant differences between plants in their response to air pollution to accumulate essential free amino acids accept threonine ($p>0.9128$). Alanine content in three plants not significantly affected by air pollution in all sites, but *V. sinensis* accumulated high content (1.227 mg g^{-1}) in S2 compared to other sites.

Pollutants increased arginine content in all plants leaves significantly. Nevertheless, the high content (0.845, 0.840 and 0.790 mg g^{-1}) found in leaves of V.faba, *G.max*, *V. sinensis* respectively in S4 compared to control plants (table 2). Cystine content decreased significantly ($p>0.001$) as air pollution increased in all species leaves. However, the reduction in two species *V. faba* and *G. max* was higher compared to other species. *V. faba* and *G. max* accumulated more content of glutamic acid when air pollution, increased under the effect of S4. Increased trend of glycine was observed in all species, but *V.faba* and *G.max* grown in S2, S3 and S4 accumulated more content (i.e. 0.913 ,1.067, 1.148, in *G.max* leaves and 0.980 1.064 and 1.154 mg g^{-1}). Isoleucine increased as pollutants increased. The leaves of *V. sinensis* accumulated 0.735 mg g^{-1}as high isoleucine content under effect of S2 compared to other species (table 2). Phenylalanine content was high (1.747 and 1.545 mg g^{-1}) in the leaves of *V. sinensis* under effect of pollutants in the S3 and S4 respectively and 1.559 mg g^{-1}in *P. sativum* leaves in S4. In all species, Proline contents increased significantly, as pollution increased. In *P. sativum* the increase was 135 % in S3 and 153 % in S4. In *V. sinensis* proline increased approximately by 96, 109 and 138% in S2, S3 and S4 respectively. In both species *V. faba and G. max* proline increased approximately by 16% in S4 compared to control (Table 2). Serine content slightly increased in all species as the air contained more pollutants. *V.faba* leaves showed decreasing manner as the high contents present in control. Although there is an increasing trend in the threonine content of *V.faba* and *G.max* as pollution increased but there is no significant effect on any plants. Tryptophan content was higher in all species as pollution increased. However, the two species V. *sinensis* and *P. sativum* accumulated higher concentration (2.143 and 1.972 mg g^{-1}) respectively under effect of air pollution in S4. Tyrosine contents increased significantly ($p>0.001$) as pollution, increased especially in S4. As general trend all free amino acids contents showing an increasing trend in all species as air pollution, increased compared to control, accept alanine, cysteine (decreased as pollution increased) and threonine (no significant effect). Although in *V. sinensis* accumulated more alanine contents in S2. The most affected species among the studied species was *V. sinensis* in terms of more accumulated free amino acids content under air pollution. The effects of amino acid contents, which showed escalating trend under the issue of increasing air pollution. It may be attributed to defense mechanisms of the tested species to the air pollution. Which can cause deleterious effects of the production of reactive oxygen species (ROS) in plants, which cause peroxidative destruction of cellular constituents (8). The study results agree with many other fields, which reported that proline act as a free radical scavenger to protect plants away from damage by oxidative stress. Although the scavenging reaction of ROS with other amino acids, such as tryptophan, tyrosine and so on are more effective compared with proline. It is of special interest as its extensive accumulation in plants during environmental stress (9). Jahan, and Iqbal (24) observed that plants growing in the urban areas are affected greatly by pollutants such as nitrogen, sulfur oxides, hydrocarbon, ozone, particulate matters. Proline level in the polluted leaves significantly increased, for triggering the protective mechanism in these plants under air pollution, stress. The observed responses are viewed as adaptive and compensating for the adverse effects of aviation contamination (25). Previously Ito *et al*., (26) observed change in amino acid composition of *Vicia faba* after NO_2 fumigation. Plants evolved several enzymes that convert amino acids, amides, keto-acids to be utilized as carbon source under environmental stress conditions when carbon shortage becomes a determining element for development and evolution (27). The regulation of biosynthesis of several minor amino acids was related to the coordination of different metabolic functions. Proline accumulation often occurs in a variety of plants in the presence of different stresses. For instance, proline accumulation in leaves of plants are exposed to SO_2 fumigation (16) , heavy metals (9) and salt (17). It has been found that the synthesis and accumulation of low molecular weight metabolites, such as free amino acids, is an ubiquitous mechanism for reducing various biotic and abiotic stresses in plants (28). Amino acids as well act as an important part in plant stress tolerance via regulating intracellular pH and ion transport, modulating stomatal conductance, and detoxifying ROS (18). The increasing content of glycine in all tested species and glutamic acid in some of them may play role in plants. Some biochemical process as mentioned by Zhang *et al*., (29) in the family of free amino acids, glycine and glutamine are fundamental metabolites involved in the process of chlorophyll synthesis .

Table 2: Free amino acids content (mg g^{-1}) in the leaves of the studied species

Amino acid	Sampling sites	Species			
		P. sativum	*V.faba*	*G.max*	*V. sinensis*
Alanine	S1	0.270 ^{b}	0.148 ^{b}	0.226 ^{b}	0.317 ^{b}
	S2	0.272 ^{b}	0.225 ^{b}	0.256 ^{b}	1.227 ^{a}
	S3	0.190 ^{b}	0.313 ^{b}	0.262 ^{b}	0.256 ^{b}
	S4	0.169 ^{b}	0.392 ^{b}	0.305 ^{b}	0.219 ^{b}
	P value > 0.01				
Arginine	S1	0.502 ^{ef}	0.468 ^{f}	0.619 ^{bcde}	0.555 ^{cdef}
	S2	0.553 ^{def}	0.609 ^{bcd}	0.650 ^{bcde}	0.598 ^{bcde}
	S3	0.606 ^{bcde}	0.726 ^{ab}	0.764 ^{ab}	0.661 ^{bcd}
	S4	0.708 ^{bcd}	0.845 ^{a}	0.840 ^{a}	0.790 ^{abc}
	P <.0001				
Cystine	S1	0.144 ^{fg}	0.758 ^{a}	0.702 ^{ab}	0.154 ^{fgh}
	S2	0.067 ^{gh}	0.608 ^{cd}	0.666 ^{bc}	0.123 ^{fg}
	S3	0.028 ^{h}	0.534 ^{d}	0.615 ^{bc}	0.063 ^{gh}
	S4	0.013 ^{h}	0.413 ^{e}	0.523 ^{de}	0.024 ^{h}
	P value > 0.0001				
Glutamic acid	S1	0.227 ^{e}	1.079 ^{d}	1.500 ^{abcd}	0.282 ^{e}
	S2	0.369 ^{e}	1.383 ^{cd}	1.528 ^{abcd}	0.300 ^{e}
	S3	0.294 ^{e}	1.492 ^{abcd}	1.599 ^{abc}	0.329 ^{e}
	S4	0.348 ^{e}	1.643 ^{ab}	1.708 ^{a}	0.391 ^{e}
	P value > 0.0001				
Glycine	S1	0.209 ^{de}	0.593 ^{ab}	0.159 ^{e}	0.265 ^{cd}
	S2	0.244 ^{de}	0.913 ^{ab}	0.978 ^{a}	0.284 ^{cd}
	S3	0.294 ^{cde}	1.067 a^{b}	1.064 ^{a}	0.329 ^{cd}
	S4	0.337 ^{bcd}	1.148 ^{a}	1.154 ^{a}	0.384 ^{bc}

	P value > 0.001				
Isoleucine	S1	0.147 ^{e}	0.482 ^{d}	0.565 ^{bc}	0.491 ^{d}
	S2	0.208 ^{e}	0.561 ^{bc}	0.609 ^{b}	0.735 ^{a}
	S3	0.413 ^{d}	0.630 ^{b}	0.651 ^{ab}	0.607 ^{b}
	S4	0.547 ^{c}	0.661 ^{ab}	0.710 ^{a}	0.522 ^{bc}
	P <.0001				
Phenylalanine	S1	1.317 ^{b}	0.478 ^{g}	0.735 ^{def}	1.338 ^{b}
	S2	1.387 ^{b}	0.711 ^{defg}	0.799	1.449 ^{b}
	S3	1.450 ^{b}	0.778 ^{cdef}	0.848 ^{cdef}	1.545 ^{a}
	S4	1.559 ^{a}	0.845 ^{cdef}	0.970 ^{cd}	1.747 ^{a}
	P <.0001				
Proline	S1	0.617 ^{d}	0.845 ^{b}	0.835 ^{c}	0.738 ^{c}
	S2	1.387 ^{a}	0.711 ^{c}	0.799 ^{bc}	1.449 ^{a}
	S3	1.450 ^{a}	0.778 ^{c}	0.848 ^{bc}	1.545 ^{a}
	S4	1.559 ^{a}	0.978 ^{b}	0.970 ^{b}	1.747 ^{a}
Serine	S1	0.054 ^{d}	1.046 ^{a}	0.610 ^{bc}	0.136 ^{d}
	S2	0.035 ^{d}	0.515 ^{bc}	0.566 ^{bc}	0.029 ^{d}
	S3	0.201 ^{d}	0.623 ^{bc}	0.648 ^{bc}	0.270 ^{d}
	S4	0.348 ^{cd}	0.682 ^{bc}	0.730 ^{bc}	0.459 ^{bc}
	P value > 0.001				
Threonine	S1	0.438 ^{a}	0.541 ^{a}	0.542 ^{a}	0.575 ^{a}
	S2	0.389 ^{a}	0.573 ^{a}	0.602 ^{a}	0.448 ^{a}
	S3	0.441 ^{a}	0.616 ^{a}	0.667 ^{a}	0.484 ^{a}
	S4	0.492 ^{a}	0.784 ^{a}	0.806 ^{a}	0.537 ^{a}
	P>0.9128				
Tryptophan	S1	1.578 ^{cd}	0.934 ^{f}	1.249 ^{def}	1.679 ^{ab}
	S2	1.664 ^{ab}	1.230 ^{def}	1.325 ^{cde}	1.229 ^{def}
	S3	1.816 ^{ab}	1.300 ^{cdef}	1.372c ^{de}	1.929 ^{a}
	S4	1.972 ^{a}	1.358	1.441 ^{c}	2.143 ^{a}
	P <.0001				
Tyrosine	S1	0.625 ^{bc}	0.629 ^{bc}	0.431 ^{d}	0.683 ^{ab}
	S2	0.665 ^{ab}	0.425 ^{d}	0.468 ^{cd}	0.696 ^{ab}
	S3	0.712 ^{ab}	0.484 ^{cd}	0.536 ^{bc}	0.776 ^{ab}
	S4	0.781 ^{ab}	0.551 ^{bc}	0.612 ^{bc}	0.802 ^{a}

Mean values on each column and row followed by the same letter do not differ significantly (P<0.05).

4. CONCLUSION

The present investigation clearly shows the levels of air pollution gases in the sampling sites and pollution sources such as traffic and industrial activities. Although all the species showed significant variation in all the amino acids contents, the extent up to which plant species were affected varies from species to species and site to site. Nevertheless, almost all the species showed maximum accumulation of amino acids, in severe air pollution site (S4). In addition, the results demonstrate a clear relation between changes in the tested plants free amino acid contents and urban ambient air pollution. Substantial variation occurred which varied from species to species and site-to-site. It can be due to species tolerance or sensitivity to air pollution, stress by increasing amino acids content to cope with pollution stress. The obtained results may used to monitor the air contamination levels in Riyadh using such types of plants. However, investigations may need to study the effect of air pollution on many plant biochemical parameters to screen the sensitivity, suitability and ability of using these species as to air contamination.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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