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Research article

AIR POLLUTION INDUCED CHANGES IN SOME FATTY ACIDS IN FOUR SPECIES

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ABSTRACT: the study was aimed to investigate the change of some fatty acids to ambient air pollution in four legumes species *Pisum sativum* L. and (*Vicia faba* L.m and during summer season were *Glycine max* and *Vigna sinensis* grown in five sites mostly affected by high traffic intensity and or industrial activities in Riyadh city capital of Saudi Arabia. The results showed increasing of air pollution (O_3, NO_2 and SO_2) as traffic density and industrial activities increased, and all saturated fatty acids relative contents increased significantly, while the unsaturated fatty acids decreased significantly as pollution levels increased. The study conclude that the changes in fatty acids percentage either increasing of saturated and or decreasing of unsaturated fatty acids in tested plants at four polluted sites as compared to control.
Key wards*:* Air pollution, Fatty acids, Nitrogen dioxide, Ozone, Sulphur dioxide

INTRODUCTION

The most important environmental problems world facing is air pollution, which is restricted mostly to the urban areas. In general, human activities, population growth in cities together with the rising industrialization and energy consumption, and the extensive transportation, increase air pollution. Recently world is facing severe air pollutants which is prevalent in cities. several pollutants such as nitrogen dioxide (NO_2), sulphur dioxide (SO_2), particulate matter and heavy metals mainly arise from anthropogenic activities such as urbanization, transportation and industrialization [1]. SO_2 and NO_2 concentrations are higher at urban sites [2], in which transportation is main sources of air pollution which contaminate air everywhere [3]. Road traffic emissions have emerged as the major cause of poor air quality [4]. Diesel and petrol fuelled vehicles are responsible for the generation of a wide range of pollutants, with concentrations and relative proportions of pollutants depending on vehicle technology and operating conditions [5]. During combustion, other pollutants, including sulphur dioxide (SO_2) and volatile organic compounds (VOCs), are emitted, together with carbonaceous particles from incompletely burnt fuel droplets [5]. High concentrations in exhaust emissions, nitric oxide (NO) and nitrogen dioxide (NO_2) are the most important phytotoxic pollutants associated with road transport [6]. Previous research has shown that at high concentrations, many of the pollutants present in exhaust gases can be damaging to plants [7]. Pollutants have the potential to disrupt plant-biochemical processes after absorption through stomata or the cuticle [8]. They dissolve in the extracellular fluid and disrupt the cellular homeostasis, leading to an enhanced production of reactive oxygen species (ROS) [9]. ROS are highly toxic for plants and can cause, for example, peroxidation of poly-unsaturated fatty acids of the cell membrane [10]. The capability of the free radical NO_2 to initiate peroxidation processes within lipid membranes has been known for a considerable time [11]. In nature, plants play a major role in monitoring and maintaining the ecological balance. It is actively involved in the cycling of nutrients and gases like carbon dioxide, oxygen and also provides enormous leaf area for adsorption, absorption and accumulation of air pollutants to reduce the pollution level in the atmospheric environment [12]. Many researchers reported that significant negative and positive correlation between air pollutants and biochemical characteristics [13,14,15,16]. Lipids, fatty acids play an important role in maintaining normal physiological cell function under environmental stress, including temperature, salt, chemicals, ions, pressure, and oxidative stress [17]. Many researchers had, looked solely at the individual components of exhaust emissions and there is little information on the impacts of the particular mix of pollutants characteristic of urban areas. This study aimed to investigate the different levels of pollutants (mainly O_3, NO_3 and SO_3) in Riyadh under different sources of air pollution, responses of fatty acids percentage in four species exposed to such pollutants and uses of these species as biomarker to air pollution.

MATERIAL AND METHODS

Metrological data

The study was carried out in Riyadh city(latitude 19º30 , 7 º 30 N, longitude 42 º and 48 º E altitude 600 m above mean sea level).The monthly average temperature varied from a minimum of 14.7 º C to a maximum of 37.5 ºC, average relative humidity from 11.1 to 46.3% and rainfall was 0.00 to 17.30 mm (Table 1).

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

Month	Mean T.(c°)	Mean RH(%)	Mean RF (mm)
January	14.74	45.45	13.45
February	18.00	35.09	7.09
March	22.61	27.18	17.30
April	27.95	26.64	11.91
May	33.60	17.00	7.18
June	36.25	11.09	0.00
July	37.45	11.45	0.91
August	37.46	13.82	0.55
September	33.94	14.82	0.00
October	28.81	20.36	0.27
November	21.45	39.45	8.55
December	17.82	46.33	7.76

T; temperature, RH; relative humidity and RF; rainfall

Sampling sites

Five sampling sites were located gradually to represent density of traffic during most of the day, high buildings that limit wind circulation**,** and industrial activities in Riyadh. An area without a direct source of pollution further away from the city centre, which was used as control (S1), site characterized by moderate traffic density names as S2, site with high traffic density which located in city centre named as S3, site near cement factory and ring road named as S4 and site near industrial city named as S5.

Air pollution monitoring

The concentrations of O_3, NO_2, and SO_2 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. Ecosensors. Inc., USA. NO_2 and SO_2 were measured by using Aeroqual Series Monitor with multihi head. Then average of data were calculated and recorded (Table 2).

Table 2: average gases concentration during experiment period

Sampling site	Gas concentration (ppb)		
	O_3	**NO_2**	**SO_2**
S1	31.58±3.65	9.53±1.44	7.21±2.09
S2	61.25±3.87	14.74±1.80	12.71±3.23
S3	87.81±13.06	26.53±2.78	27.83±2.44
S4	92.11±18.19	29.25±3.67	27.54±2.26
S5	126.84±16.45	30.45±4.20	27.59±1.38

Plants

Plants tested during winter seasons were *Pisum sativum* L. and *Vicia faba* L.m and during summer season were *Glycine max* and *Vigna sinensis*. Seeds were sown in plastic pots (40 cm) with a 50% clay, 50% sand, After germination five uniform plants per pot were selected and 10 pot from each species and transferred to each sampling sites. (Peters, NPK: 4/25/35 plus micrometals) was added to prevent nutritional deficiencies. Winter plants exposed for consecutive periods of sixteen weeks to ambient air and also summer plants exposed to ambient air for the same period during summer season.

Fatty acids analysis.

Total lipids will be extracted from fresh plant materials (air dried) according to Fölsh *et al*. [18]. Fatty acid methyl ester was prepared according to Kates [19]. Ester was analyzed by Gas Liquid Chromatography (GLC) according to Johnson and Stocks [20]. Qualitative and quantitative analysis of peak fatty acid methyl esters were carried out by comparing their retention times with those of an authentic methyl standard of fatty acids methyl esters (Sigma Co., St. Louis, USA).

Statistical analysis

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

RESULTS

Air pollution level

The results showed high levels of average O_3 (87.81, 92.11and 126.84ppb) and NO_2 (26.53, 29.25 and 30.45ppb) in S3, S4 and S5 as compared to control. Also SO_2 increased as air pollution in S2, S3, S4 and five compared to control (table 2).

Table 3 : ANOVA test showing levels of significance for various tested parameters

Parameter	Species ×sampling site
palmitic acid (16:0)	****
Stearic acid (18:0)	*
Arachidic acid (20:0)	***
TSFAs	****
Oleic (C18:1)	**
Linolenic (C18:3)	***
Lignoceric (C24:0)	****
TUSFAs	****

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;**** P> 0.0001

Saturated fatty acids

Table 3 showed that air pollution significantly affect relative contents of saturated fatty acids SFAs(in term of Palmitic, Stearic and arachidic) in all studied species, as air pollution increased as SFAs increased compared to control (S1). Palmitic fatty acids relative content increased, especially in plants grown in S4 and S5 compared to control plants. *G.max* accumulated more content (13.64 %) of Palmitic fatty acid in S5 then, *P. sativum*, *V.faba* and *V. sinensis* respectively in S5.the results also indicated the increasing trend of Stearic acid as air pollution increased, but the increasing trend was clear in *G.max grown in S5 (13.43%) and V. sinensis* grown in S3. In case of arachidic showed the same trend of palmitic but the relative content was less.

Table 4: Saturated fatty acids percentage

Fatty acid	Sampling sites	Species			
		P. sativum	*V.faba*	*G.max*	*V. sinensis*
palmitic (16:0)	**S1**	3.17 ^{ef}	8.46 ^{bcd}	3.36 ^{ef}	4.84 ^{e}
	S2	4.46 ^{e}	9.95 ^{b}	2.15 ^{f}	4.59 ^{e}
	S3	6.79 ^{de}	7.07 ^{cde}	5.15 ^{e}	4.04 ^{ef}
	S4	6.39 ^{de}	9.30 ^{bcd}	10.49 ^{abc}	8.58 ^{bcde}
	S5	11.56 ^{ab}	11.48 ^{ab}	13.64 ^{a}	10.34 ^{ab}
Stearic (18:0)	**S1**	5.24 ^{bcdef}	3.17 ^{ef}	2.46 ^{f}	2.98 ^{f}
	S2	3.74 ^{def}	5.35 ^{bcdef}	3.72 ^{e}	5.32 ^{bcdef}
	S3	5.197 ^{bcdef}	3.96 ^{cde}	5.31 ^{bcde}	4.28 ^{bcdef}
	S4	4.77 ^{bcdef}	3.45 ^{ef}	4.55 ^{bcdef}	8.54 ^{ab}
	S5	7.89 ^{abcd}	4.39 ^{bcdef}	11.20 ^{a}	7.64 ^{abcd}
Arachidic (20:0)	**S1**	3.92 ^{f}	4.42 ^{ef}	3.91 ^{f}	8.30 ^{bcde}
	S2	7.45 ^{bcde}	6.07 ^{def}	5.36 ^{de}	7.19 ^{bcde}
	S3	6.83 ^{bcde}	8.27 ^{bcd}	6.24 ^{cd}	10.15 ^{ab}
	S4	6.30 ^{cde}	8.622 ^{bcd}	7.56 ^{bcd}	9.71 ^{bc}
	S5	7.03 ^{bcde}	5.27 ^{de}	13.43 ^{a}	8.21 ^{bc}

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

The variation of the total saturated fatty acids (TSFAs) content in the tested species leaves subjected to increasing air pollution is reported in Fig. 1., the trend of TSFAs was increasing as air pollution increased in all species but the effect was great in *G.max* which accumulated high percentage (38.27 %) of TSFAs in their leaves in S5 (table 4 and fig. 1).

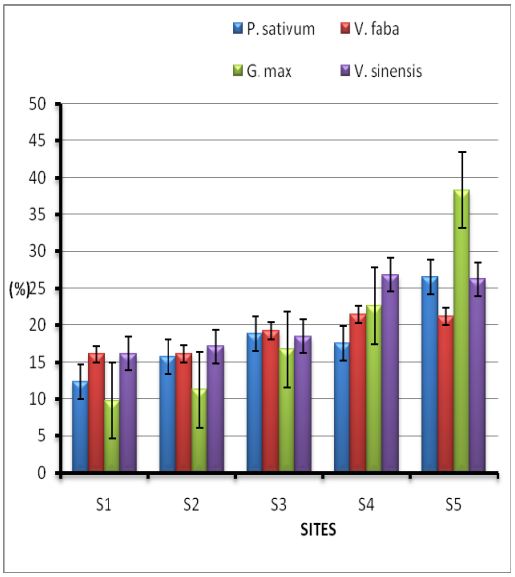

Fig. 1: comparison Total saturated fatty acids percentage in tested plants under effect of air pollution in five sampling sites

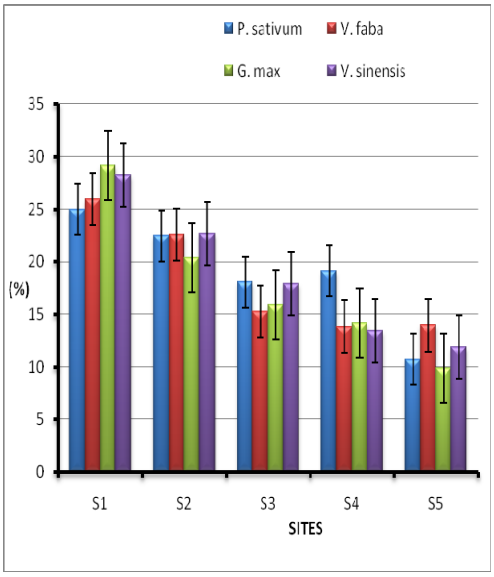

Fig. 2: comparison Total unsaturated fatty acids percentage in tested plants under effect of air pollution in five sampling sites

Unsaturated fatty acids

At S5 *all tested species* exhibited significant (table 3) reduction in all unsaturated fatty acids percentage, but (C18-1) in *G. max* relative content was less (3.03 %) compared to other species grown in the same site and control plants. Also the plant of this species grown in S5 contained fewer percentage (3.09 ,3.75, 9.89 %) of Linolenic (C18:3) , Lignoceric (C24:0) and TUSFAs respectively as compared to other species in the same site and control plant (S1 plants) although *P. sativum* contained fewer relative content (2.51%) of Linolenic (C18:3) under effect of S5. At S4 *V. sinensis* leaves contained less percentage 4.62 % of Linolenic (C18:3) and 13.48% of TUSFAs as compared to other species grown in the same site and control plants (table 5 and fig. 2). All the plants grown in polluted sites showed significant reduction in individual and TUSFAs percentage compared to control (S1), but a maximum reduction was exhibited at S5 followed by S4 , S3 and S2.but the reduction was more severe in *G. max* followed by *V. sinensis* especially under effect of S5 compared to other species(table 5 and fig. 2)

Table 5: Unsaturated fatty acids Percentage

Fatty acid	Sampling sites	Species			
		P. sativum	*V.faba*	*G.max*	*V. sinensis*
Oleic (C18:1)	**S1**	11.66 ^{ab}	8.82 ^{bc}	13.58 ^{a}	9.27 ^{b}
	S2	7.03 ^{cd}	7.21 ^{cd}	10.32 ^{ab}	6.95 ^{c}
	S3	7.46 ^{cd}	5.01 ^{de}	8.06 ^{bc}	6.31 ^{c}
	S4	8.72 ^{bc}	5..30 ^{de}	5.79 ^{cd}	4.62 ^{de}
	S5	2.51 ^{e}	4.07 ^{de}	3.09 ^{e}	4.47 ^{de}
Linolenic (C18:3)	**S1**	6.02 ^{abc}	9.67 ^{a}	6.93 ^{abc}	6.27 ^{abc}
	S2	7.13 ^{abc}	8.26 ^{ab}	4.89 ^{c}	9.19 ^{ab}
	S3	4.40 ^{c}	4.78 ^{c}	4.44 ^{c}	7.34 ^{abc}
	S4	6.33 ^{abc}	3.94 ^{c}	3.13 ^{c}	5.25 ^{b}
	S5	3.27 ^{c}	5.36 ^{b}	3.06 ^{c}	3.41 ^{c}
Lignoceric (C24:0)	**S1**	7.28 ^{bc}	7.73 ^{bc}	9.73 ^{ab}	12.73 ^{a}
	S2	8.29 ^{b}	5.52 ^{cde}	5.14 ^{cde}	6.56 ^{bcd}
	S3	6.23 ^{bcde}	5.20 ^{cde}	3.39 ^{e}	4.25 ^{d}
	S4	4.10 ^{de}	4.59 ^{cde}	5.22 ^{cde}	3.60 ^{d}
	S5	4.96 ^{cde}	4.55 ^{cde}	3.75 ^{e}	4.06 ^{d}

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

DISCUSSION

The results indicated that the site 5 which located near industrial city has more pollutants which agreed with pervious finding numerous pollutants such as nitrogen dioxide (NO_2), sulphur dioxide (SO_2), particulate matter and heavy metals mainly arise from anthropogenic activities such as urbanization, transportation and industrialization [1]. SO_2 and NO_2 concentrations are higher at urban sites (Tiwari et al., [2], in which transportation is main sources of air pollution which contaminate air everywhere [22]. As table (2) showed high ozone level was found in the site5 which has high level of NO_2. High level of ozone in this site which were characterized by high air pollution sources (such as industrial activities) may be explained by increasing emission of ozone precursor gases such as CO, NO_x, and volatile organic compounds as a result of industrial activities. Forster et al., [21] mentioned such compounds are converted to ozone via photochemical reactions.

In summary saturated fatty acids increased , while unsaturated ones (in terms of individual and total) decreased significantly as air pollution increased in all tested species .the results of this study were agreed with many others studies. The effects of pollutants on plants include pigment destruction, depletion of cellular lipids and peroxidation of polyunsaturated fatty acid [2].Wang et al. [22] found The proportions of saturated fatty acids(i.e., palmitic acid and stearic acid) increased and unsaturated fatty acids decreased in two cultivars of *Camellia sinensis* under Fluoride treatments.A reduction in unsaturated fatty acid content in *Pinus halepensis* has also been reported as a result of air pollution by ozone, through radical mechanisms [23,24]. In cell membrane, polyunsaturated fatty acids represent the primary target for ozone, stimulating lipid peroxidation and impairing membrane fluidity [25]. Navari-Izzo *et al.* [26] reported that the degradation of membrane lipid components possibly by de-esterification rather than peroxidation with SO_2.Pollutants have the potential to disrupt plant-biochemical processes after absorption through stomata or the cuticle [8]. They dissolve in the extracellular fluid and disrupt the cellular homeostasis, leading to an enhanced production of reactive oxygen species (ROS) [9]. ROS are highly toxic for plants and can cause, for example, peroxidation of poly-unsaturated fatty acids of the cell membrane [10]. The capability of the free radical NO_2 to initiate peroxidation processes within lipid membranes has been known for a considerable time [11]. Also Chen et al. [27] observed an increase in lipid peroxidation of one-year-old Cinnamomum camphora L. seedlings exposed to high NO_2 concentrations. Linoleic and a-linolenic acids, the two main polyunsaturated fatty acids in plant lipids, play key roles in plant metabolism as structural components of membrane lipids, as precursors of signaling molecules involved in plant development and stress response, and as storage compounds in the form of triacylglycerols [28] . The results showed decreased of linoleic acid as compared to oleic acid ,this findings consist with many authors findings. Decreases in polyunsaturated fatty acid (especially linoleic acid) were consistent with increases in the proportion of oleic acid [29, 30,31] .This decrease should result in decreased fluidity of the membrane lipid bilayer [32] Under high polluted sites (i.e S3,S4and S5) plants UFAs relative content was decreased which may be due to high exposure to air pollutants which forces chloroplasts into an excessive excitation energy level, which in turn increases the generation of ROS and induces oxidative stress [33]. The deleterious effects of the pollutants are caused by the production of reactive oxygen species (ROS) in plants, which cause peroxidative destruction of cellular constituents [2]. this study showed clear changes of SFAs and USFAs in the leaves of the tested species under air pollution stress which indicated the oxidative effect of air pollutant on cell as previously, Price *et al*. [34] reported the decomposition product of polyunsaturated fatty acids (PUFA) of biomembranes, is a good indicator of the severity of cell injury during oxidative stress. Lipids and fatty acids play an important role in maintaining normal physiological cell function under environmental stress, including temperature, salt, chemicals, ions, pressure, and oxidative stress [17]. From this results we found summer species accumulated more relative content of saturated fatty acids under effect of S3, S4 and S5 compared to winter species. All the plants grown in polluted sites showed significant reduction in individual and TUSFAs percentage compared to control (S1), but a maximum reduction was exhibited at S5 followed by S4, S3 and S2.but the reduction was more severe in *G. max* followed by *V. sinensis* especially under effect of S5 compared to other species. The differences between species in response to air pollution may be attributed to different sensitivity of tested plants to air pollution as mentioned previously he sensitivity to different pollutants differs between plants and even between clones of the same species [35]. Several factors can alter the results of such studies. For example, determination being tolerance or sensitive for one tested plant species could be changed during different seasons [36].

CONCLUSION

In conclusion *G.max* had high relative content of saturated fatty acids in S5 as compared to all sites and other studied species followed by *P. sativum* and the lower total relative content of saturated fatty acids was found in *G.max* followed by *P. sativum* under control effect. Also both summer species accumulated more relative content of saturated fatty acids under effect of S3, S4 and S5 compared to winter species.

All the plants grown in polluted sites showed significant reduction in individual and TUSFAs percentage compared to control (S1), but a maximum reduction was exhibited at S5 followed by S4, S3 and S2.but the reduction was more severe in *G. max* followed by *V. sinensis* especially under effect of S5 compared to other species.

The decomposition product of polyunsaturated fatty acids (PUFA) of biomembranes, is a good indicator of the severity of cell injury during air pollution stress.

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