

1 **Original Research**

2 **Plant Bioindicators of Pollution in Sadat City, Western Nile Delta, Egypt**

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7 **Abstract**

8 This study investigated using plants growing in industrial and residential areas as bioindicators and biomarkers of industrial pollution by
9 analyzing flavonoids and metals in *Bougainvillea glabra* leaves by HPLC-MS, neutron activation analysis, and atomic absorption
10 spectrophotometry. There were significantly higher levels of flavonoids and phenolic compounds in plants growing in industrial areas compared
11 to those growing in residential zones ($P < 0.05$). Metal accumulation in leaves was also significantly higher in the industrial zone than the
12 residential zone: iron, lead, zinc, nickel, and manganese were present at significantly higher levels in plants growing near steel factories
13 compared to those growing in the residential zone ($P < 0.05$). Air, water, and soil samples associated with the studied plants in both areas were
14 analyzed for metals. In general, heavy metal concentrations in the industrial zone were significantly higher than those in the residential zone, but
15 the concentrations of the heavy metals in air, water, and soil were under legal environmental limits.

16 **Keywords:** Flavonoids, plant biomarkers, Sadat City, heavy metals, environmental pollution

17 **1. INTRODUCTION**

18 Air- and water-borne pollution is an undesirable consequence of industrialization that is having a growing impact on productivity, health, and
 19 climate change [1-5]. Pollution affects plants and animals via a number of routes including through pollutants dissolved in the rain (e.g., sulfur
 20 dioxide producing sulfuric acid), chemical discharge into water courses, and particulate matter (PM) in the air including dust, dirt, and smoke.
 21 Pollution impairs plant photosystem II [1] and is associated with respiratory and cardiovascular diseases [3]. Monitoring and assessing pollution
 22 in the air, water, and soil from industrial sources is an important aspect of managing the impact of industry and pollution on our environment [4].
 23 Bioindicators are living organisms such as plants, planktons, animals, and microbes with the capacity to monitor the health of the environment.
 24 They are therefore a useful means to measure the negative impacts of industrial activity on the environment [6]. Similarly, environmental
 25 biomarkers are identifiable measures (e.g., chemicals or genes) of environmental processes [7] and a useful tool not only to monitor and evaluate
 26 the environmental state but also develop our knowledge of molecular toxicity mechanisms in different animal and plant species in the ecosystem
 27 [8].
 28 Therefore, plants can be used to assess whether certain ecophysiological responses may be useful biomarkers of urban pollution. Some plant
 29 biomarkers are specific to only one pollutant or group of pollutants, while others respond to a wide range of pollutants and/or stressors [9]. For
 30 example, flavonoids and phenolic compounds used to indicate stress in plants [10,11] . These secondary metabolites, in particular polyphenols,
 31 are of particular importance in plant-environment relationships [12]

32 *Bougainvillea glabra* (family: Nyctaginaceae) is a common ornamental plant grown in tropical and subtropical gardens and grown as a shrub or
33 climber [13]. The aim of the present study was to investigate using *B. glabra* as a bioindicator and assess flavonoid, phenolic compound, and
34 metal accumulation in their leaves as biomarkers of environmental pollution.

35 **2. MATERIALS AND METHODS**

36 **2.1 Study area**

37 Sadat City is located about 100 km north of Cairo, Egypt. The city is bounded 30°19'30"-30°40'27" E longitude and 30°15'50"-30°34'00"N
38 latitude (**Fig. 1**), from the east by Kafer Dawoud and El Khatataba, from the west by El Birigat, and from the north by Nubariya Canal and El
39 Tahrir. Industrial zones are located in a separate spine along the south-eastern edge of the city to ensure that industrial pollution travels
40 downwind. To protect the city from wind and storms, a green shelterbelt of trees about 35,000 feddans (1 feddan = about 175 m²) in area was
41 planted around the city, containing about 2,000 feddans of vegetables and fruit.

42 The residential districts and industrial zones of Sadat cover about 50 km² of the study area. With a future planned capacity of 750,000
43 inhabitants, the city currently hosts a population of about 200,000 inhabitants and about 100,000 seasonal residents working in industry and
44 agriculture. The city has one waste disposal site at the southern side of the city behind the industrial zone about 700 feddans in area.

45 **Fig.1.** Map showing the sites of sample collection in the study area.

46 **2.2 Sampling**

47 The study area was sampled between January 2018 and December 2018 at the sites shown in **Fig. 1**: six in the residential area and three at three
48 industrial sites (El Dawlia Oil, El-Ezz Steel, and Prema Ceramics). PM₁₀ (particulate matter <10 micrometers) and heavy metals were sampled
49 along with the physical and chemical properties and heavy metal content of soil, water, industrial chimneys, and inside the factories. Plants were
50 studied morphologically. Flavonoids and phenolic compounds were investigated in *B. glabra* leaves by HPLC-MS, while metals were measured
51 in leaves using neutron activation analysis and atomic absorption spectrophotometry.

52 **2.3 Climatic parameters**

53 The Tropical Rain Measurement Mission (TRMM) records three hourly rainfall measurements and is freely available online including from the
54 TOVAS website (<https://serc.carleton.edu/resources/22961.html>). Climate was also estimated using Egyptian Meteorological Authority data
55 between January and December 2018.

56 **2.4 Identification of the collected plants in the study area**

57 Plants in industrial and residential areas were identified using well identified herbarium specimens of the Environmental Studies and Research
58 Institute (ESRI) and the Herbarium Faculty of Science Botany Department, Mansoura University. A voucher specimen was deposited at the
59 herbarium of the ESRI, University of Sadat City. The morphological features of plants growing in both locations were compared.

60 **2.5 Biomarker analysis**

61 HPLC-MS Ultimate 3000/Amazon SL ion trap mass spectrometry was undertaken using Acclaim 2.2 µm 120 Å 2.1-150 mm columns (Thermo
62 Fisher Scientific, Waltham, MA). The HPLC-MS device was used to detect flavonoids and phenolic compounds in plant leaves.

63 **2.6 Chemicals and reagents**

64 Methanol, acetonitrile, and deionized water were of HPLC-MS grade and were from Sigma Aldrich (St Louis, MO).

65 **2.7 Plant materials and sample preparation**

66 Leaves were collected from plants growing in the study areas. Methanol extraction was performed with a Soxhlet apparatus [14]. Extraction
67 efficiency was enhanced by the application of the methods of [15, 16]. Leaves were dried and ground into powder, and 0.1 g of powdered leaves
68 was mixed with 10 ml 70% methanol and placed on a rotating shaker at 200 rpm at 40°C for 16 h. The filtrate was collected and filtered through
69 a 0.45 µm nylon filter.

70 A volume of plant extract was injected into the HPLC-MS column for analysis. All sample solutions were stored at 4°C prior to use. The
71 compound at each peak was recognized according to its molecular weight and retention time.

72 **2.8 Determination of phenolic contents**

73 The total phenolic content was determined for plant leaves extract using the Folin–Ciocalteu method [17]. Briefly, 1 mL of extract (100–500
74 g/mL) solution was mixed with 2.5 mL of 10% (w/v) Folin–Ciocalteu reagent. After 5 min, 2.0 mL of Na₂CO₃ (75%) was subsequently added to
75 the mixture and incubated at 50°C for 10 min with intermittent agitation. Afterwards, the sample was cooled and the absorbance was measured
76 utilizing a UV spectrophotometer (Shimazu, UV-1800) at 765 nm against a blank without extract. The outcome data were expressed as mg/g of
77 gallic acid equivalents in milligrams per gram (mg GAE/g) of dry extract.

78 **2.9 Determination of flavonoid contents**

79 The flavonoid contents of plant leaf extracts were measured as per the Dowd method [18]. An aliquot of 1 mL of extract solution (25–200 g/mL)
80 or quercetin (25–200 g/mL) were mixed with 0.2 mL of 10% (w/v) AlCl₃ solution in methanol, 0.2 mL (1 M) potassium acetate, and 5.6 mL
81 distilled water. The mixture was incubated for 30 min at room temperature followed by the measurement of absorbance at 415 nm against the
82 blank. The outcome data were expressed as mg/g of quercetin equivalents in milligrams per gram (mg QE/g) of dry extract.

83 **2.10 Instrumentation**

84 Plant specimens were studied for flavonoids and phenolics in the central laboratory of the ESRI, University of Sadat City, Egypt. The binary
85 mobile phase consisted of solvents A (1% formic acid) and B (acetonitrile with 1% formic acid). A mobile phase gradient was used as follows: 0
86 min, A: B 10–90; 36 min, A: B 70–30; 50 min, A: B 100–0. After each run, the chromatographic system was equilibrated with 20% B for 30
87 min. The injection volume was 20 µl. Flow rate was 0.8 ml/min. The effluent was split in a ratio of 2:3 using a micro-splitter valve before
88 introduction into the mass spectrometer. UV traces were measured at 290, 254, and 350 nm and UV spectra (DAD) were recorded between 190
89 and 900 nm according to [19].

90 **2.11 Gas analysis**

91 NO, NO₂, and NO_x were measured with the Thermo Environmental Instruments 42C NO, NO₂, NO_x Analyzer, EPA reference method RFNA-
92 1289-074, over 10-300 seconds. SO₂ was measured with the Thermo Environmental Instruments 43C SO₂ Analyzer, EPA equivalent method
93 EQSA-0486-060, over 10-300 seconds. CO was measured with the Thermo Environmental Instruments 48C CO Analyzer, EPA reference

94 method RFCA-0981-054, over 10-300 seconds. Outdoor NO₂ concentrations were measured using the method in [20]. Gases were measured in
95 the work environments using Testo Portable Emission Gas Analyzer (Testo Inc., Sparta, NJ; Model 350M/XL).

96 **2.12 Particulate matter (PM)**

97 Particulate matter (PM) was collected using the filtration method [21]. Particles less than 10 µm in diameter (PM₁₀) were trapped by the filter,
98 and a TSP Meter (Kanomax, Andover, NJ) was used to measure TSP in the work environment and PM₁₀ apparatus to measure PM₁₀ according
99 [22].

100 **2.13 Atomic absorption spectrophotometry**

101 Atomic absorption spectrophotometry was performed using a Spectrum 100 (Perkin Elmer, Waltham, MA) to determine heavy metal
102 concentrations in plants.

103 **2.14 Neutron activation analysis**

104 The concentration of sodium (Na), calcium (Ca), magnesium (Mg), chloride (Cl), and manganese (Mn) in plant tissues was assessed by neutron
105 activation analysis (NAA) using the IBR-2 Reactor (IBR2 of the Joint Institute for Nuclear Research, Dubna-Russia (JINR)).

106 **2.15 Groundwater samples**

107 Two water samples were collected from two wells in the study areas (industrial and residential, one sample from each). The water samples were
108 acidified and stored in an icebox before being analyzed by atomic absorption spectroscopy (AAS) at the Water Department, Central Health
109 Laboratories, Ministry of Health and Population, Egypt.

110 **2.16 Soil chemical analysis**

111 Soil samples were collected from the industrial and residential zones (three samples from each), ground, and digested with HF and HClO₄. One
112 gram of each sample was placed in a Teflon beaker and treated with 10 ml HF and 2 ml HClO₄ before being heated to nearly dry the sample.
113 The residual material was dissolved with HCl (12 N) and the filter diluted with 25 ml deionized water [23, 24] . Chemical analysis was
114 performed by atomic absorption spectroscopy (AAS) for chromium (Cr), nickel (Ni), and lead (Pb) [23].

115 **2.17 Statistical analysis**

116 Data are expressed as mean $\pm$ standard deviation (SD). Data were analyzed with t-tests using SPSS ver. 14.0 (IBM Statistics, Chicago, IL). A p-
117 value < 0.05 was considered statistically significant.

118 **3. RESULTS**

119 **3.1 Climatic study**

120 Monthly mean temperatures over the study period varied from a minimum of 12.4°C in January to a maximum of 36.4°C in July over the study
121 period (Table 1). Rainfall ranged from 21.4 mm in January to no rainfall in June, July, and August. Relative humidity ranged from 45.0% in
122 December to 71.0% in August. Wind speed varied from 6.475 m/s in October to 10.36 m/s in March.

123 **Table 1.** Means of meteorological parameters from January to December 2018

124

125 **Morphological changes in plant leaves due to pollution**

126 Overall, the leaves of plants grown in industrial zone had discolored, dusty, and wrinkled leaves compared to their counterparts growing in the
127 residential zone, which had smooth surfaces and edges and were clean with no wrinkles and normal edges (Fig. 2).

128 **Fig. 2.** Illustrative morphological differences in *Bougainvillea glabra* growing in industrial (left) and residential (right) zones.

129

130 **3.2 Metal concentrations in plants growing in industrial and residential zones**

131 Table 2 shows metal concentrations in *Bougainvillea glabra* leaves growing in the industrial and residential zones. Iron, zinc, lead, nickel, and
132 manganese were all present at significantly higher levels in plants growing in the industrial zone than the residential zone ($P < 0.05$), while copper
133 and cadmium were undetectable in both.

134

135 **Table 2.** Heavy metal content in *Bougainvillea glabra* leaves in industrial and residential
136 zones. NS=not significant, ppm=parts per million; values are means $\pm$ SD from three replicates.

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143 3.3 Plant flavonoid analysis

144 HPLC-MS analysis of *B. glabra* (Table 3 and Fig. 3) leaf extracts revealed a set of peaks in plant extracts derived from the industrial zone.

145 Twenty-one compounds were detected in industrial zone leaves; five major peaks were selected on the basis of peaks and retention times (Rt).

146 Rosmarinic acid had an Rt of 48.9 min and peak area percentage of 12.01%; chlorogenic acid had an Rt of 6.7 min and peak area of 3.9%,

147 quinic acid had an Rt of 32.0 min and peak area of 3.8%, R-adrenaline had an Rt of 11.0 min and peak area of 2.9%, while caffeine had an Rt

148 of 16.8 min and peak area of 2.5%.

149 **Fig. 3.** HPLC-MS analysis of methanolic extracts of *Bougainvillea glabra* leaves growing in the industrial zone.

150 **Table 3.** HPLC-MS analysis of methanolic extracts of *Bougainvillea glabra* leaves
151 growing in the industrial and residential zones.

The HPLC-MS analysis of *Bougainvillea glabra* from the residential area (**Table 3, Fig. 4**) revealed seventeen compounds all identified as flavonoids, four of which were major peaks with retention times of 5 min and over: hesperidin (Rt 12.5 min), peak area percentage 3.7%; daidzein (Rt 48.9 min), peak area 2.5%; genistein (Rt 48.7 min), peak area 2.2%, and phlorizin (Rt 2.5 min), peak area 1.9%.

Fig. 4. HPLC-MS analysis of methanolic extracts of *Bougainvillea glabra* leaves growing in the residential zone

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Table 4. Determination of total phenolic and flavonoid contents in *Bougainvillea glabra* leaf extracts. Values are means $\pm$ SD from three replicates.

Taken together, the total phenolics and flavonoids of *B. glabra* were significantly higher in the industrial zone than the residential zone ($P < 0.05$; **Table 4**).

3.4 Total suspended particulates (TSP, PM₁₀) and gases in the industrial and residential zones

Table 5. Means of TSP-PM₁₀ and gases in the industrial and residential zones during March and April 2017/2018 (mg/m³). Values are means $\pm$ SD from three replicates.

Table 5 shows that there were significantly higher concentrations of TSPs, PM₁₀, and gases at the industrial site compared to the residential zone.

3.5 Groundwater analysis

Table 6. Water analysis from wells in the residential and industrial zones. ND=not detected, NTU=nephelometric turbidity unit, NS=not significant, TDS=total dissolved solids. Values are means $\pm$ SD from three replicates.

Table 6 shows that turbidity, total dissolved solids (TDS), pH, chlorides, fluorides, sulfates,

hardness, calcium, magnesium, silicon dioxide, zinc, and selenium were all significantly higher in water from the industrial zone than the residential zone (all $P < 0.05$) and potassium and barium were significantly lower in water from the industrial zone than the residential zone (all $P < 0.05$).

3.6 Chemical analysis of soil samples

Table 7. Means of heavy metals concentrations in soil samples (ppm) collected from industrial and residential zones. Values are means $\pm$ SD from three replicates.

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Heavy metal concentrations in the soil samples from industrial and residential zones are listed in Table 7. All metals apart from cadmium were present at significantly higher levels in the samples obtained from the industrial area than the residential area (all $P < 0.05$).

4. DISCUSSION

Sadat City, located north of Cairo, is characterized by extreme aridity, a long hot summer, and short warm winter, which greatly influences the hydrological properties of the drainage basins in the area [25, 26]. Rapid industrialization is causing health hazards, and the effect of industrialization on human health is a major public health concern [27].

Morphological examination of plants growing in industrial and residential zones revealed marked differences in the leaves in different areas. [28] Reported that environmental stresses negatively influence growth, productivity, and trigger a series of morphological, physiological, biochemical and molecular changes in plants. Dust deposition on leaf surfaces may also reduce chlorophyll synthesis due to a shading effect [29]. The resulting changes, either morphological or physiological, can be used as bioindicators of the environmental state [30].

Flavonoids function as stress indicators because they accumulate at high levels in many plant tissues in response to a wide range of biotic and abiotic signals [2]. Flavonoids comprise the large and common group of plant phenolics with more than 5000 different described flavonoids in six major subclasses [31]. Plants may alter their secondary metabolite synthesis, production, secretion and storage when subjected to the abiotic stress factors [32]. The results obtained in the present study revealed that pollution in the industrial area was associated with elevations in total phenolics and flavonoids compared with samples of plants from non-polluted, residential sites. The elevations in total phenolics and flavonoids may have acted as a stress defense mechanism in plants against these environmental pollutants.

HPLC-MS profiles of the studied plant samples from the industrial zone revealed the presence of 21 compounds, five of which were present as major peaks belonging to flavonoid and phenolic compounds. R-adrenaline was detected in plants growing in the industrial zone, which may due to environmental stress; indeed, [33] reported that adrenaline is known to protect against oxidation by flavonoids. [32] Reported that flavonoids comprise a large and common group of plant phenolics, and flavonoids commonly increases in response to unfavorable conditions. By contrast, residential zone plant extracts showed the presence of 17 compounds, four of which were major flavonoid and phenolic peaks. Therefore, total phenolics and flavonoids highly represented in plants in the industrial zone may be a result of stress-inducing pollution, and reported previously [34]. Phenolic compounds (flavonoids) function as stress indicators because they accumulate at high levels in many plant tissues in response to a wide range of biotic and abiotic signals [35 -37] , enhancing the scavenging of free radicals [38]. Our results are also consistent with [39], who observed higher quercetin (phenolic flavonoid) content in samples from a polluted site, suggesting that the increase could be associated with the defensive role of flavonoids under conditions of environmental

stress. Flavonoids are a good indicator of environmental contamination, especially of O₃ pollution [40].

The total concentrations of TSPs in water in the industrial zone were higher than the US Environmental Protection Agency's prescribed standards for air quality, presumably as a result of emissions from local industrial activity such as steel factories in this area. Generally, the outlet values of TSPs in samples were less than legally permitted values (EEAA

(Egyptian Ministry for the Environment) Law 4/1994; 9/2009), 10 mg/m³ in the work environment. Similarly, the PM₁₀ were higher in the industrial zone and only present at trace

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levels in the residential zone, but the outlet PM₁₀ values in all samples were generally lower than required by law (4/1994; 9/2009). The higher SO₂ values at the industrial site may have environmental consequences; NO_x dissolves in cells and gives rise to nitrite ions (NO₂ which is toxic at high concentrations) and nitrate ions (NO₃) that enter into nitrogen metabolism as if they had been absorbed through the roots. In some cases, exposure to pollutant gases, particularly SO₂, causes stomata closure, which protects the leaf against the further entry of the pollutant but also curtails photosynthesis [1].

The high level of contamination with iron and other heavy metals in plants and soil in the industrial zone could have originated from metal-containing waste from the steel industry. In general, the heavy metals concentrations were lower than allowed by law (4/1994; 9/2009). Cadmium and copper were not detected in either zone, perhaps because they were not used as raw materials in any of the industries. The U.S. Environmental Protection Agency [41] regulates nine trace elements for land-applied sewage sludge (As, Cd, Cu, Pb, Hg, Mo, Ni, Se, and Zn), with six of these elements (Cu, Ni, Zn, Cd, Pb, and Se) considered to be phytotoxic (The quality of all groundwater samples was compatible with the Egyptian standards for drinking water and hosing.

Lead, a toxic heavy metal [42] was recorded in plants in the industrial zone but not the residential zone. Possible sources of lead are batteries, chemical substances from photograph processing, lead-based paints, and lead pipes deposited in the landfill. The high levels of Zn in the plants, water, and soil in the industrial zone compared to the residential zone could be attributable to metal-containing waste in the industrial zone, which might have leached into the underlying soil to be absorbed by plants [43].

5. CONCLUSIONS

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This study compares the presence and levels of pollutants between residential and industrial areas of a city and their possible effects on plants. Pollution is becoming an ever-growing global environmental problem. Industrialization, population growth, and the associated increase in energy demands have resulted in a profound deterioration of the environmental quality in developing countries like Egypt. Pollution comes from many different sources such as factories, automobiles, and even wind-blown dust. Pollution is not harmful only to plants but to all living organisms. Plants are very good bioindicators of pollution and may be used as pollution biomarkers.

CONFLICTS OF INTEREST

None

AUTHOR CONTRIBUTIONS

MFA designed the study, conducted the experiments, and wrote the manuscript.

REFERENCES

- [1] Rahul J, Jain MK. An investigation in to the impact of particulate matter on vegetation along the national highway: a review. *Research Journal of Environmental Sciences* 2014; 8: 356.
- [2] Rezanejad F. Air pollution effects on flavonoids in pollen grains of some ornamental plants. *Turkish Journal of Botany* 2012; 36: 49-54.

- [3] Sengupta S, Venkatachalam P. Health hazard assessment in an industrial town with the help of GRAM-GIS. *Environmental Monitoring and Assessment* 1994; 32: 155-160.
- [4] UNEPDO,. Making tourism more sustainable: A guide for policy makers, World Tourism Organization Publications 2005.
- [5] Woo S, Lee D, Lee Y. Net photosynthetic rate, ascorbate peroxidase and glutathione reductase activities of *Erythrina orientalis* in polluted and non-polluted areas. *Photosynthetica* 2007; 45: 293-295.
- [6] Holt EA, Miller SW. Bioindicators: using organisms to measure environmental impacts. *Nature Education Knowledge* 2011; 3: 8.
- [7] Peakall DB. The role of biomarkers in environmental assessment (1). Introduction. *Ecotoxicology* 1994; 3: 157-160.
- [8] Nighat F, Iqbal M. Stomatal conductance, photosynthetic rate, and pigment content in *Ruellia tuberosa* leaves as affected by coal-smoke pollution. *Biologia Plantarum* 2000; 43: 263-267.
- [9] Petersen IL, Christensen JH, Ravn SSHW, Hansen HCB. Biomarkers in herbicide exposed plants. University of Copenhagen, Faculty of Life Sciences 2009.
- [10] Qayoom Mir A, Yazdani T, Ahmad S, Yunus M. Total flavonoids and phenolics in *Catharanthus roseus* L. and *Ocimum sanctum* L. as biomarkers of urban auto pollution. *Caspian Journal of Environmental Sciences* 2009; 7: 9-16.
- [11] Seyyednejad S, Niknejad M, Yusefi M. The effect of air pollution on some morphological and biochemical factors of *Callistemon citrinus* in petrochemical zone in South of Iran. *Asian Journal of Plant Sciences* 2009; 8: 562.
- [12] Rhodes M. Physiological roles for secondary metabolites in plants: some progress, many outstanding problems. *Plant Molecular Biology* 1994; 24: 1-20.

- 300 [13] Ahmed AH. New flavone from the aerial parts of Bougainvillea glabra. *IJCER Online*
 301 2014; 73.
- 302 [14] Soxhlet FV. Die gewichtsanalytische bestimmung des milchfettes. *Polytechnisches J*
 303 1879; 232: 461-465.
- 304 [15] Herrera M, De Castro ML. Ultrasound-assisted extraction for the analysis of phenolic
 305 compounds in strawberries. *Analytical and Bioanalytical Chemistry* 2004; 379: 1106-
 306 1112.
- 307 [16] Rostagno MA, Palma M, Barroso CG. Ultrasound-assisted extraction of soy isoflavones.
 308 *Journal of Chromatography* 2003; 1012: 119-128.
- 309 [17] Lee YH, Choo C, Watawana MI, Jayawardena N, Waisundara VY. An appraisal of
 310 eighteen commonly consumed edible plants as functional food based on their
 311 antioxidant and starch hydrolase inhibitory activities. *Journal of the Science of Food*
 312 *and Agriculture* 2015; 95: 2956-2964.
- 313 [18] Arvouet-Grand A, Vennat B, Pourrat A, Legret P. Standardization of propolis extract
 314 and identification of principal constituents. *Journal de Pharmacie de Belgique* 1994;
 315 49: 462-468.
- 316 [19] El-Naggar E-MB, Azazi M, Svajdlenka E, Zemlicka M. Artemisinin from minor to
 317 major ingredient in Artemisia annua cultivated in Egypt. *Journal of Applied*
 318 *Pharmaceutical Science* 2013; 3: 116.
- 319 [20] Harrison RM. Handbook of air pollution analysis, Springer Science & Business Media;
 320 2012.
- 321 [21] Shakour A, El-Taieb N. Effect of atmospheric lead exposure on urban children. *Egyptian*
 322 *J Occup Med* 1994; 18: 37-47.
- 323 [22] Breuer D, Bower J,. Monitoring ambient air quality for health impact assessment, WHO
 324 Regional Office Europe; 1999.

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325 [23] Esakku S, Selvam A, Joseph K, Palanivelu K. Assessment of heavy metal species in
326 decomposed municipal solid waste. *Chemical Speciation & Bioavailability* 2005; 17:
327 95-102.

328 [24] Sims J, Cunningham S, Sumner M. Assessing soil quality for environmental purposes:
329 roles and challenges for soil scientists. *Journal of Environmental Quality* 1997; 26:
330 20-25.

331 [25] El Abd E. The Geological impact on the water bearing formations in the area south west
332 Nile Delta, Egypt. *Unpublished PhD Thesis, Fac. Sci., Menufiya Univ., Egypt*; 2005.
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333 [26] Zahran M, Ayyad S, El-Khatib A. Ecopalynological studies in the extreme arid part of
334 Egypt. 2nd Symposium on African Palynology, Tervuren, Belgium, CIFEg 1995;
335 57-72.

336 [27] Azzazy M. Environmental impacts of industrial pollution on pollen morphology of
337 Eucalyptus globulus Labill.(Myrtaceae). *J. App. Biology Biotech* 2016; 4: 057-062.

338 [28] Mahfoozi S, Limin AE, Ahakpaz F, Fowler DB. Phenological development and
339 expression of freezing resistance in spring and winter wheat under field conditions in
340 north-west Iran. *Field Crops Research* 2006; 97: 182-187.

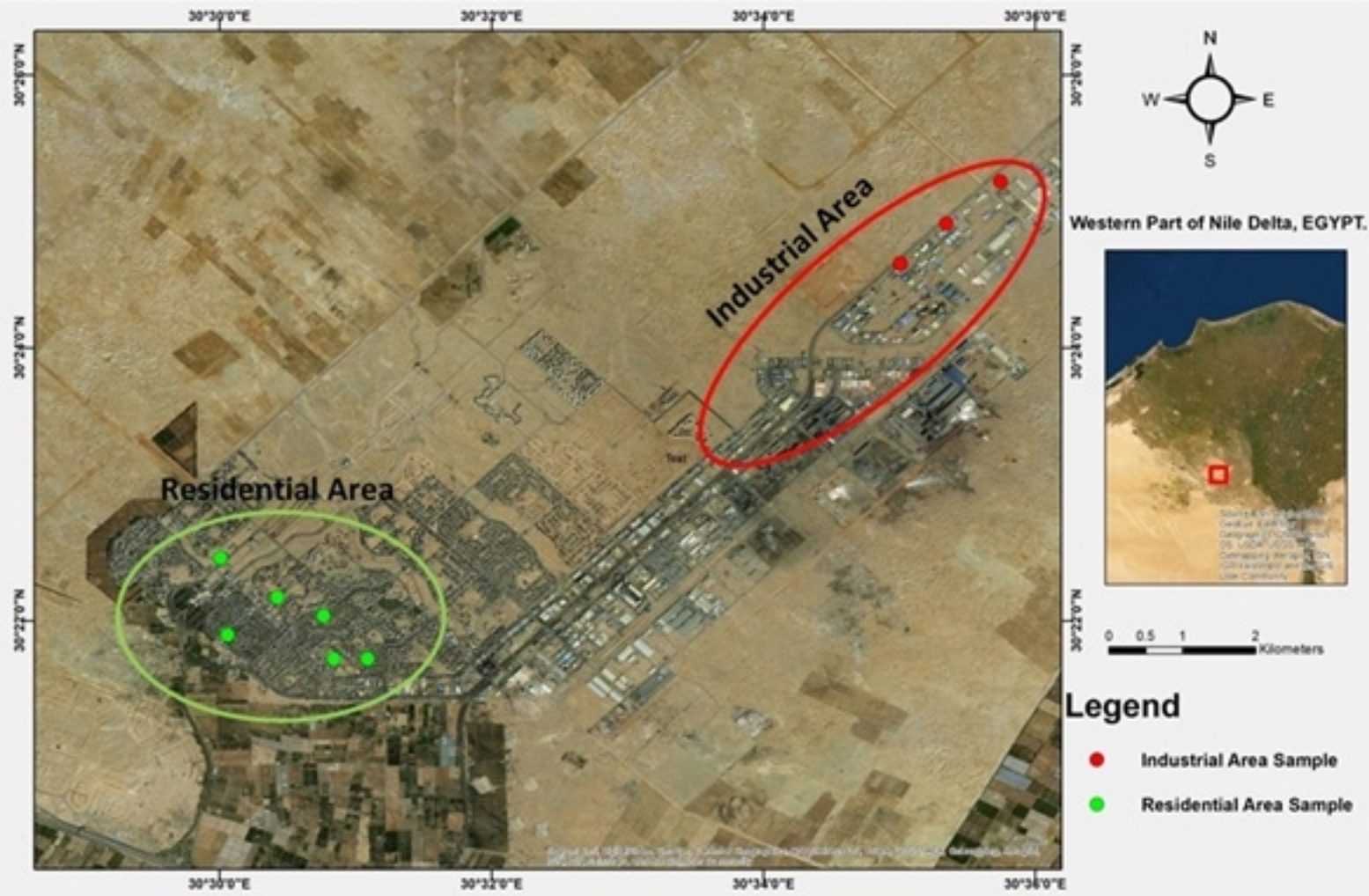
341 [29] Singh R, Das U, Prasad B, Jha S. Monitoring of dust pollution by leaves. *Pollution*
342 *Research* 2002; 21: 13-16.

343 [30] Pidek IA, Svitavská-Svobodová H, Van Der Knaap WO, Noryśkiewicz AM, Filbrandt-
344 Czaja A, Noryśkiewicz B, Latałowa M, Zimny M, Święta-Musznicka J, Bozilova E.
345 Variation in annual pollen accumulation rates of Fagus along a N–S transect in
346 Europe based on pollen traps. *Vegetation History and Archaeobotany* 2010; 19: 259-
347 270.

348 [31] Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety.
349 *Annu Rev Nutr* 2002; 22: 19-34.

- [32] Cetinkaya H, Koc M, Kulak M. Monitoring of mineral and polyphenol content in olive leaves under drought conditions: Application chemometric techniques. *Industrial Crops and Products* 2016; 88: 78-84.
- [33] Hughes R, Wilson H. 6 Flavonoids: Some Physiological and Nutritional Considerations. Progress in medicinal chemistry. Elsevier; 1977.
- [34] Yang Y, He F, Yu L, Chen X, Lei J, Ji J. Influence of drought on oxidative stress and flavonoid production in cell suspension culture of *Glycyrrhiza inflata* Batal. *Zeitschrift für Naturforschung* 2007; 62: 410-416. bioRxiv preprint doi: <https://doi.org/10.1101/856229>; this version posted November 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.
- [35] Ennajeh M, Vadel AM, Khemira H. Osmoregulation and osmoprotection in the leaf cells of two olive cultivars subjected to severe water deficit. *Acta Physiologiae Plantarum* 2009; 31: 711-721.
- [36] Swigonska S, Amarowicz R, Król A, Mostek A, Badowiec A, Weidner S. Influence of abiotic stress during soybean germination followed by recovery on the phenolic compounds of radicles and their antioxidant capacity. *Acta Societatis Botanicorum Poloniae* 2014; 83.
- [37] Upadhyaya H, Panda SK, Dutta BK. Variation of physiological and antioxidative responses in tea cultivars subjected to elevated water stress followed by rehydration recovery. *Acta Physiologiae Plantarum* 2008; 30: 457-468.
- [38] Verma K, Shrivastava D, Kumar G. Antioxidant activity and DNA damage inhibition in vitro by a methanolic extract of *Carissa carandas* (Apocynaceae) leaves. *Journal of Taibah University for Science* 2015; 9: 34-40.
- [39] Robles C, Greff S, Pasqualini V, Garzino S, Bousquet-Mélou A, Fernandez C, Korboulewsky N, Bonin G. Phenols and flavonoids in Aleppo pine needles as bioindicators of air pollution. *Journal of Environmental Quality* 2003; 32: 2265-2271.

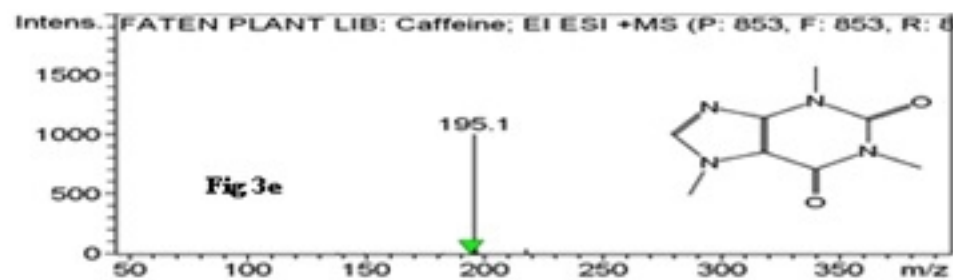
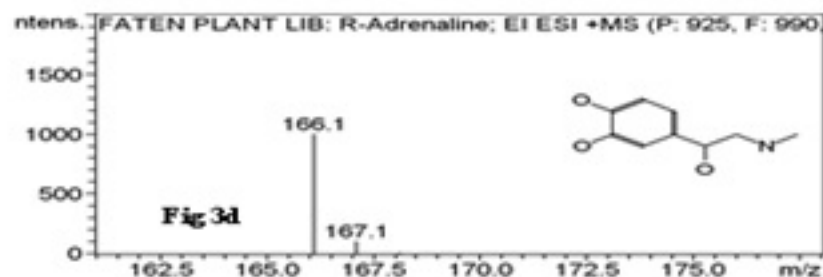
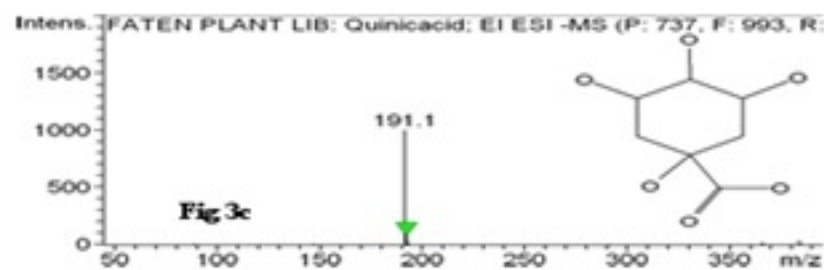
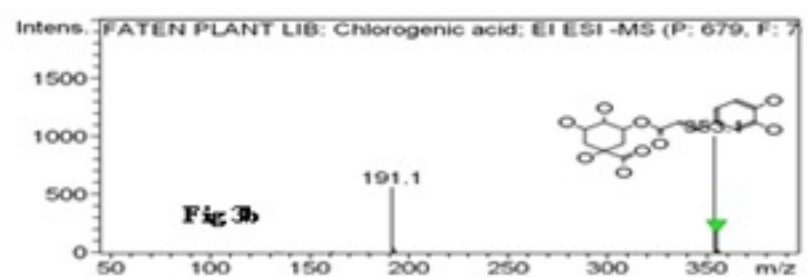
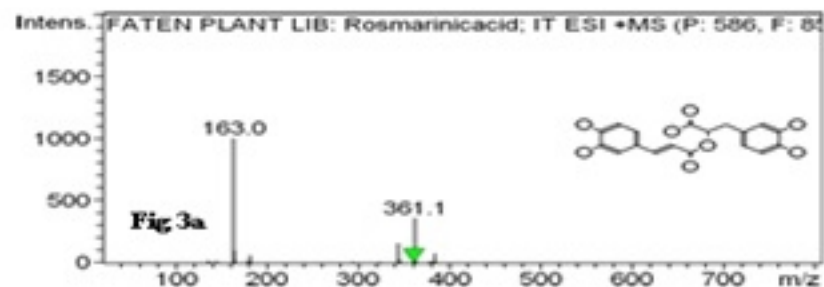
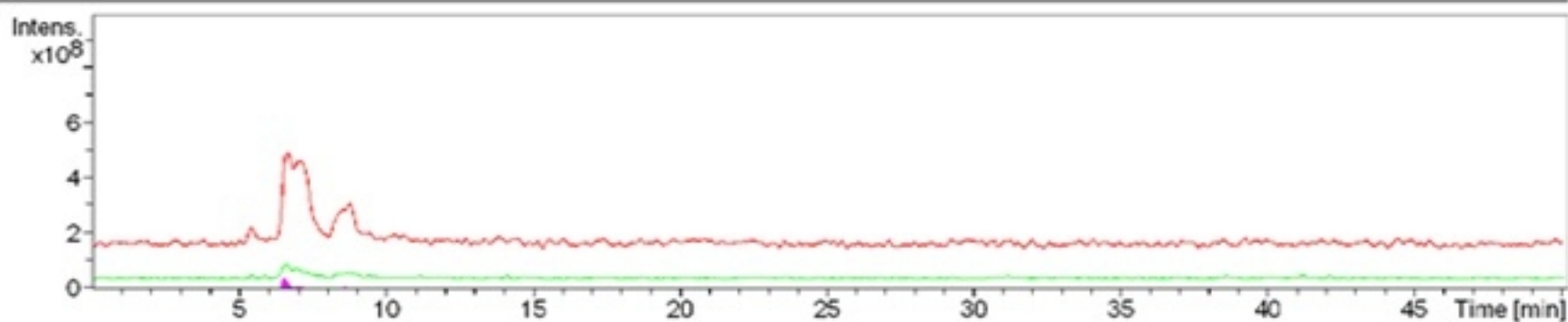
- 374 [40] Rezende, F.M. Rezende, C.M. Furlan Anthocyanins and tannins in ozone-fumigated
 375 guava trees Chemosphere 2009; 76, 1445-1450
- 376 [41] Weber CI. Methods for measuring the acute toxicity of effluents and receiving waters to
 377 freshwater and marine organisms, Environmental Monitoring Systems Laboratory,
 378 Office of Research and Development, US Environmental Protection Agency;1991.
- 379 [42] Rai UN, Sinha S. Distribution of metals in aquatic edible plants: *Trapa natans* (Roxb.)
 380 Makino and *Ipomoea aquatica* Forsk. *Environmental Monitoring and Assessment*
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 381 2001; 70: 241-252.
- 382 [43] Olayiwola H, Abudulawal L, Adewuyi G, Azeez M. Heavy metal contents in soil and
 383 plants at dumpsites: A case study of Awotan and Ajakanga dumpsite Ibadan, Oyo
 384 State, Nigeria. *Journal of Environment and Earth Science* 2017; 7: 11-24.



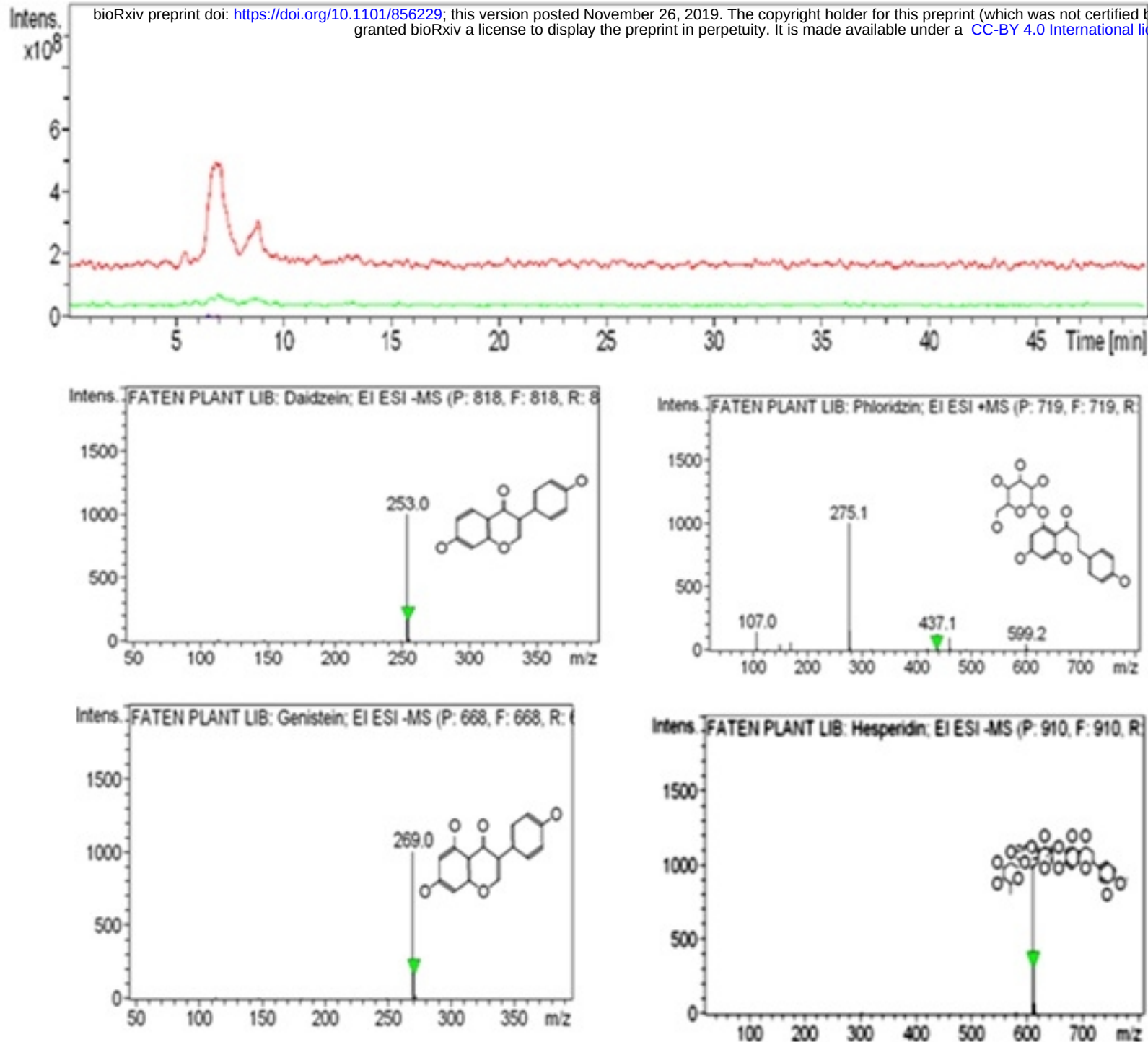
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Figure



Figure

Table 1

Month	Air temperature (°C)	Highest of rainfall mm/month	Relative humidity (RH) %	Wind speed (km/h)
January	12.4	21.4	56.0%	9.435
February	13.5	15.2	57.0%%	9.99
March	15.7	16.7	60.0%	10.36
April	19.3	16.8	62.0%	9.99
May	22.7	15.9	66.0%	7.955
June	25.8	Trace	68.0%	8.325
July	36.4	Trace	70.0%	7.215
August	37.8	Trace	71.0%	6.66
September	25.0	2.6	70.0%	7.955
October	22.1	7.1	67.0%	6.475
November	17.8	18.3	55.0%	7.03
December	13.8	16.0	45.0%	7.77

Table 2

Item	Industrial Zone	Residential Zone	Sign.
Fe (ppm)	494.00 ± 1.63	0.0 ± 1.63	P<0.05
Zn (ppm)	445.00 ± 2.41	33.28 ± 2.41	P<0.05
Pb (ppm)	0.0066 ± 0.001	0.0 ± 0.001	P<0.05
Ni (ppm)	24.11 ± 0.10	99.91 ± 1.7	P<0.05
Mn (ppm)	452.47 ± 3.9	211.00 ± 6.3	P<0.05
Cu (ppm)	0.00	0.00	NS
Cd (ppm)	0.00	0.00	NS

Table 3

Industrial Zone								
#	Rt (min)	Area	Chemical Formula	Compound Name	Intensity	Range (min)	Area %	Int. %
264	3.0	128922.3	C15H22N2O4	(L) Tyrosyl-L-leucine	10091	2.8 - 3.1	0.1	0
472	5.5	6944	C11H21NO4	Isobutyryl carnitine	2303	5.0 - 5.9	0.6	0
498	5.6	173466.7	C6H6O3	Phloroglucinol	36603	5.6 - 5.7	0.2	1
598	6.6	2520797	C10H19NO4	BOC valine	115237	6.3 - 7.1	2.2	19
603	6.7	25	C16H18O9	Chlorogenic acid	35	6.5 - 6.8	3.9	0.4
638	6.9	27714	C6H10O3	2-Oxohexanoic acid	101243	6.7 - 7.5	2.5	2
3770	46.3	280901.1	C18H39NO3	Phytosphingosine	10576	45.9 - 46.5	0.3	0
3990	48.9	32388.1	C18H16O8	Rosmarinic acid	5120	48.8 - 49.0	12.01	10
665	7.1	21737	C8H10O2	4-Hydroxyphenylethanol	13513	7.0 - 7.3	2.0	2
682	7.5	584315.0	C17H33NO4	Decanoyl-L-carnitine	20242	7.3 - 7.8	0.5	0
1114	17.0	1751314.7	C15H10O4	Daidzin	60869	16.8 - 17.2	0.4	0
1102	16.8	2302295	C8H10N4O2	Caffeine	5761	17.1	2.5	3.2
1713	20.8	111325.2	C13H25NO4	Hexanoyl-L-carnitine	15612	22.5 - 22.8	0.1	2
1683	20.5	299060.8	C5H12O5	Ribitol	46576	20.2 - 20.7	0.3	1
1963	24.2	136154.4	C6H5NO2	Isonicotinic acid	13267	24.1 - 24.3	0.1	0
925	11.0	1189877.7	C9H13NO3	R-Adrenaline	58886	10.7 - 11.2	2.9	2
2426	29.9	148877.8	C10H15NO	Hordenine	11543	29.7 - 30.8	0.1	0
2533	24.8	1386306.8	C15H10O5	Genistein	11352	24.5 - 24.9	1.3	2
2635	32.3	641082	C12H23NO4	Isovalerylcarnitine	45205	32.2 - 32.5	0.6	1
2757	33.8	82761.4	C21H24O10	Phlorizin	7046	33.7 - 33.9	0.1	0
2286	32.0	1366521.9	C7H12O6	Quinic acid	71701	31.6 - 32.3	3.8	1
Residential Zone								
240	2.5	1017309.7	C21H24O10	Phlorizin	59274	6.4 - 7.3	1.9	2
659	7.3	2659727.5	C10H13NO	Phenacetin	125701	7.2 - 7.5	1.0	2
669	7.4	165148.5	C16H18O9	Chlorogenic acid	22810	12.2 - 12.7	0.3	1
1041	12.5	1424751.3	C28H34O15	Hesperidin	96603	14.0 - 14.4	3.7	3
1157	14.2	543080.9	C5H12NO	Betaine aldehyde	36220	16.3 - 16.8	1.0	1
1349	16.6	48288.9	C6H7N5	6-Methyladenine	3681	17.1 - 17.6	0.1	0
3286	42.4	1119499.3	C13H25NO4	Hexanoyl-L-carnitine	67359	41.6 - 41.9	0.8	2
3293	41.7	3293 41.7	C8H8O4	2-Hydroxy-5-methoxybenzoic	6503	42.4 - 42.7	0.1	2
3301	42.6	2181244.8	C18H16O8	Rosmarinic acid	100251	48.6 - 48.9	1.1	1
3819	48.7	114496.2	C15H10O5	Genistein	10172	48.6 - 49.1	2.2	2

3892	48.9	656974.6	C15H10O4	Daidzein	45629	48.6 - 49.1	2.5	2
1683	20.5	299060.8	C5H12O5	Ribitol	46576	20.2 - 20.7	0.2	1
1713	20.8	111325.2	C13H25NO4	Hexanoyl-L-carnitine	15612	22.5 - 22.8	0.2	2
598	6.6	2520797	C10H19NO4	BOC Valine	115237	6.3 - 7.1	1.3	1
2635	32.3	641082	C12H23NO4	Isovalerylcarnitine	45205	32.2 - 32.5	0.5	1
498	5.6	173466.7	C6H6O3	Phloroglucinol	36603	5.6 - 5.7	0.2	1
3770	46.3	280901.1	C18H39NO3	Phytosphingosine	10576	45.9 - 46.5	0.4	0

Table 4

	Industrial Zone	Residential Zone	Sign.
Total phenolics	61.72 ± 0.70 GAE mg/100g	45.82 ± 0.50 GAE mg/100g	P<0.05
Total flavonoids	233.53 ± 16.10 QE mg/100g	212.23 ± 9.05 QE mg/100g	P<0.05

Table 5

Item	Study Zone and Value		Sign.
TSP	Industrial	7.06 ± 2.3	P<0.05
	Residential	0.40 ± 0	
PM ₁₀	Industrial	15.72 ± 6.5	P<0.05
	Residential	0.0 ± 0.0	
NO	Industrial	9.66 ± 5.0	P<0.05
	Residential	0.0 ± 0.0	
NO ₂	Industrial	0.12 ± 0.04	P<0.05
	Residential	0.04 ± 0.0	
NO _x	Industrial	68.43 ± 1.66	P<0.05
	Residential	0.0 ± 0.0	
SO ₂	Industrial	16.76 ± 12.74	P<0.05
	Residential	0.05 ± 0.0	
CO	Industrial	42.16 ± 24.1	P<0.05
	Residential	0.0 ± 0.0	
CO ₂	Industrial	473.00 ± 35.5	P<0.05
	Residential	0.01 ± 0.0	

Table 6

Parameters	Industrial zone well	Residential zone well	Sign.
Turbidity (NTU)	0.3500 ± 0.15000	0.20000 ± 0.00	P<0.05
TDS (ppm)	426.500 ± 1.5000	248.000 ± 0.00	P<0.05
pH	8.100 ± 0.1000	7.6000 ± 0.00	P<0.05
Ammonia (ppm)	ND	ND	NS
Nitrite (ppm)	ND	ND	NS
Nitrate (ppm)	ND	ND	NS
Chlorides (ppm)	93.000 ± 1.0000	20.000 ± 1.000	P<0.05
Fluorides (ppm)	0.11500 ± 0.0050	0.01400 ± 0.0050	P<0.05
Sulfates (ppm)	39.000 ± 1.0000	17.000 ± 0.70	P<0.05
Total hardness AS CaCO ₃	173.750 ± 0.25000	121.000 ± 0.00	P<0.05
Temporary hardness AS CaCO ₃	170.000 ± 1.0000	119.000 ± 0.00	P<0.05
Permanent hardness AS CaCO ₃	2.4000 ± 0.1000	0.000	P<0.05
Ca hardness AS CaCO ₃	93.000 ± 0.5000	69.000 ± 0.00	P<0.05
Mg hardness AS CaCO ₃	8.1000 ± 1.000	40.000 ± 0.00	P<0.05
Na (ppm)	75.500 ± 0.500	41.000 ± 0.00	P<0.05
K (ppm)	1.500 ± 0.500	2.000 ± 0.00	P<0.05
Ca (ppm)	38.200 ± 0.115	27.000 ± 0.577	P<0.05
Mg (ppm)	20.166 ± 0.611	12.000 ± 0.577	P<0.05
Fe (ppm)	ND	ND	NS
Mn (ppm)	ND	ND	NS
SiO ₂ (ppm)	29.000 ± 0.577	18.000 ± 0.577	P<0.05
Cd (ppm)	ND	ND	NS
Cr (ppm)	ND	ND	NS
Cu (ppm)	ND	ND	NS
Ba (ppm)	0.006 ± 0.00005	0.013 ± 0.003	P<0.05
Pb (ppm)	ND	ND	NS
Zn (ppm)	0.00690 ± 0.00005	0.00343 ± 0.00014	P<0.05
Se (ppm)	0.00113 ± 0.000088	ND	NS

Table 7

Metal	Industrial Zone	Residential Zone	Sign.
Fe	8750.00 ± 21.6	5559.0 ± 0.57	P<0.05
Cu	2.08 ± 0.20	1.37 ± 0.008	P<0.05
Cd	0	0	NS
Zn	12.50 ± 0.0	0.28 ± 0.005	P<0.05
Mn	33.33 ± 4.16	19.71 ± 0.005	P<0.05
Pb	4.57 ± 0.15	0.48 ± 0.005	P<0.05
Ni	20.48 ± 2.02	0.500 ± 0.005	P<0.05
Cr	30.29 ± 2.2	23.50 ± 0.005	P<0.05
Co	0.39 ± 0.013	1.25 ± 0.005	P<0.05