Biomonitoring airborne pollution: a case study of “Urginea maritima” species in Bentael natural reserve – Lebanon

Yara Khairallah a, Tarek Houri b, Bilal Osta b, Dany Romanos c and Georges Haddad d

a Faculty of Science, Beirut Arab University, Debbieh, Lebanon; b Faculty of Science, Department of Biology, Beirut Arab University, Tripoli, Lebanon; c Department of Soil, Plants and Fertilizers, Lebanese Agricultural Research Institute, Fanar, Lebanon; d Faculty of Science, Department of Biology, Lebanese University, Fanar, Lebanon

ABSTRACT

Biomonitoring airborne pollution has been a widespread practice. Its advantages make it an alternative to costly technological techniques. In this context, a rare species “Urginea maritima” was tested to measure the changes surrounding Bentael natural reserve in Lebanon, triggered by the inauguration of a new road on its south side, and revealed alarming results through two years study (2015–2016). Three environmental indicators were evaluated: Air Pollution Tolerance Index, Total Antioxidant Capacity and leaf Relative Water Content. These factors showed the escalated evolution of the air pollution during the studied months. In addition, six parameters, separated between pollution markers and scavengers, were studied: hydrogen peroxide, pheophytin, proline, ascorbic acid, carotenoids and total phenolic compounds showed a gradual and sharp increase. The passive biomonitoring with plants confirmed to be a reliable method to evaluate airborne pollution effects which will expose new extent to establish a routine monitoring program in Lebanon.

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1. Introduction

In 2015, in the caza of Byblos-Lebanon, a new road work began at the south side of the Bentael natural reserve, breaching the resolution number 1/25, article 4, of the law of “the creation of Bentael’ nature reserve” and the item 8 in the “Law of forests’ protection” number 558 in Lebanon.

A new approach of assessing oxidative stress generated from anthropogenic activities is the use of biomonitorors. Biomonitoring is a widely used technique in detecting stress responses in a suspected or presumed endangered environment [1]. This practice was performed with the use of a rare plant species named “Urginea maritima”, which is dedicated for the depiction of oxidative stress from road activities in the Bentael natural reserve, Lebanon [2].

After launching pollution alarms, we chose to study an exceptional plant in the reserve: “Urginea maritima”. This plant’s characteristics are numerous: it was declared rare by the United Nations Environment Programme [3], has a succulent root system, has rodenticide effects and its bulb highly accumulates heavy metals [4].

First-line analysis was related to describing the environment conditions: Air Pollution Tolerance Index (APTI), total antioxidant capacity (TAC) and leaf relative water content (RWC). APTI values give consistent information about the sensitivity/tolerance level of the studied plant [5], which will determine the suitability of the plant as a biomonitor. TAC determines the antioxidant activity of the plant and thus the motion defence system [6] and RWC helps the plant to maintain its balance under stressful conditions [5]. These indexes define the probability of the presence of oxidative stress.

Next-level analyses went deeper and were split between oxidative stress scavengers and markers.

Markers were the reactive oxygen species (ROS) and hydrogen peroxide (hydro), which are normally produced by the plant but their excessive presence indicates oxidative stress [7], and the chlorophyll degradation product, pheophytin (pheo) [8]. The studied oxidative stress scavengers were the most common stress-associated amino acids such as proline (pro) [9], ascorbic acid (AA) – which work as a modulator of key enzymatic reactions [10], carotenoids (caro) – considered as antioxidant protective molecules [11] and the most powerful antioxidant compounds, polyphenols (poly) [12].
2. Materials and methods

2.1. Sampling area

The area of study is on the south side of the “Bentaël” natural reserve (coordinates 34°8′26″N 35°42′14″E) [13]. Three sites were selected to collect samples for the airborne-pollution-related parametric study. The roadside of the reserve was chosen as the polluted area for the investigation (P1 – as “Very Polluted Area”), a second spot facing site 1 laterally (P2 – as “Medium Polluted Area”) and a third spot relatively far from the road, situated in the middle of the reserve was indicated as the control site (Ctrl – “Control Area”) (Figure 1).

This study was conducted for two years (2015 and 2016) in May–June and December months, when the plant (*Urginea maritima*) completes its sprout phase of the growth cycle and enters the blooming phase [14].

2.2. APTI determination

Following the method of Singh and Rao [15], APTI is an index that can be determined by four parameters: total chlorophyll content of the leaf, leaf pH, RWC and ascorbic acid content.

2.3. TAC determination

TAC was determined by the phosphomolybdate method after soxhlet extraction for two hours [16].

2.4. RWC determination

Singh and Rao’s method was followed in estimating RWC [17]. RWC was determined by the gravimetric method in estimating leaves’ weight in fresh, turgid and dried conditions.

2.5. Hydrogen peroxide

It was determined according to Karata et al. [18]. Briefly, fresh leaves were homogenized with trichloroacetic acid, centrifuged and the supernatant was collected and added to a reagent of potassium phosphate buffer and potassium iodide.

2.6. Pheophytin and carotenoids

According to Arnon and McKinney, fresh leaves are immersed in methanol until total discolouration and then the solution is read at the appropriate wavelength [19].

2.7. Proline

Bates et al. described the spectrophotometric method of free proline determination: fresh leaves were heated in ethanol and then the colour was developed with the use of ninhydrin solution in acidic conditions [20].

2.8. Ascorbic acid

Ascorbic acid is measured by iodometric titration of an acidic solution of dried leaves with potassium dichromate [21].

Figure 1. Map of Bentaël natural reserve: Sampling area: P1 is considered the polluted side because of his closeness to the roadside, P2 is laterally opposite to P1 and Ctrl site in the middle of the reserve.
2.9. Total polyphenolic compounds

Augusto et al. described the spectrophotometric determination of total phenolic compounds according to Folin Ciocalteu's method [22].

3. Results and discussion

ROS are naturally produced in different plant's organs: chloroplasts' membranes and mitochondria produce approximately the major species such as superoxide, hydrogen peroxide and hydroxical radical. In normal conditions, these ROS are instantly scavenged and recently, scientists have discovered a signalling role of these molecules [23]. However, when a stress factor is revealed, excessive production of ROS is launched [24]. Mean analysis of the recorded values of all studied parameters is presented in the tables. Table 1 presents the indexes mean values, Table 2 summarizes the means values of oxidative stress scavengers and Table 3 shows the results of the oxidative stress markers. All means were compared using ANOVA and their significance levels in month, year and site are listed.

As presented in the three tables, there is a highly significant difference in AA in both years and between sites, followed by Pro. These two scavengers and markers, respectively, are always linked to the establishment of oxidative stress [25]. In addition, APTI presented a significant difference all over both years, which indicates an evident impact of air pollution on the plant's level of tolerance.

To make the results more representative, paired sample t-tests were computed to compare the stress markers and scavengers in the years 2015 and 2016 (Table 4). The first remarkable observation is the increase in the paired association between groups in the following year (2016). Carotenoids showed a significant difference when compared with the other parameters. Proline and hydrogen peroxide and also ascorbic acid with polyphenols recorded a significant difference. According to many authors, proline, ascorbic acid, polyphenols and carotenoids are direct scavengers of oxidative stress [23]. The statistical data presented by the t-test analysis concord with these postulations.

Under stressful conditions, carotenoids are known to protect the photosynthetic system against the impact

Table 1. ANOVA test applied to studied stress indexes: TAC (total antioxidant capacity), APTI (Air Pollution Tolerance Index) and RWC (relative water content).

| Indexes means | Site/Month | May-15 | Jun-15 | Dec-15 | May-16 | Jun-16 | Dec-16 |
|---------------|------------|--------|--------|--------|--------|--------|--------|
| TAC P1        | 42.3 ± 1.39 | 12.9 ± 1.27 | 3.44 ± 0.13 | 0.15 ± 0.009 | 5.59 ± 0.53 | 1.51 ± 0.2 |
| mg/mL P2      | 0.87 ± 0.38 | 0.57 ± 0.17 | 0.87 ± 0.07 | 0.12 ± 0.01 | 5.69 ± 0.55 | 1.36 ± 0.4 |
| Ctrl          | 0.24 ± 0.04 | 0.86 ± 0.012 | 0.13 ± 0.01 | 0.04 ± 0.002 | 0.38 ± 0.09 | 0.92 ± 0.015 |
| Significance level | ** | NS | *** | *** | NS | ** | NS |
| APTI P1       | 11.92 ± 0.61 | 15.61 ± 0.94 | 17.32 ± 2.08 | 19.57 ± 0.77 | 19.24 ± 2.51 | 21.55 ± 2.08 |
| % P2          | 11.45 ± 0.34 | 8.29 ± 0.66 | 10.53 ± 0.31 | 13.4 ± 0.24 | 9.69 ± 2.15 | 10.58 ± 1.42 |
| Ctrl          | 9.34 ± 0.32 | 12.45 ± 0.25 | 8.84 ± 0.5 | 11.86 ± 0.35 | 10.98 ± 1.78 | 9.26 ± 0.43 |
| Significance level | *** | *** | *** | *** | *** | *** |
| RWC P1        | 58.29 ± 5.4 | 0.63 ± 0.56 | 23.97 ± 5.4 | 77.26 ± 5.8 | 17.87 ± 7.8 | 53.26 ± 5.5 |
| % P2          | 59.48 ± 0.01 | 0.16 ± 0.001 | 82.09 ± 5.1 | 99.72 ± 0.1 | 41.18 ± 16.6 | 23.88 ± 2.5 |
| Ctrl          | 50.48 ± 0.01 | 63.04 ± 2.4 | 70.65 ± 6.1 | 98.04 ± 2.1 | 71.31 ± 21.9 | 56.12 ± 5.2 |
| Significance level | * | *** | NS | *** | ** | NS |

Note: Very high significance is observed in December 2015 and May 2016 for all indexes between the studied sites.

8 Each value is a mean of 3 samples.

Table 2. ANOVA test applied to oxidative stress scavengers: AA (ascorbic acid), Poly (total polyphenols) and Caro (carotenoids).

| Oxidative Stress Scavengers | Site/Month | May-15 | Jun-15 | Dec-15 | May-16 | Jun-16 | Dec-16 |
|----------------------------|------------|--------|--------|--------|--------|--------|--------|
| AA P1                      | 12.24 ± 0.55 | 27.49 ± 1.67 | 23 ± 3.02 | 22.81 ± 2.4 | 33.78 ± 3.13 | 25.43 ± 3.5 |
| % P2                       | 10.1 ± 0.69 | 14.06 ± 1.8 | 3.68 ± 0.18 | 7.24 ± 0.5 | 10.71 ± 1.1 | 12.95 ± 0.87 |
| Ctrl                       | 7.52 ± 0.51 | 10.89 ± 0.25 | 3.52 ± 0.7 | 3.99 ± 0.8 | 7.67 ± 0.8 | 5.28 ± 0.07 |
| Significance level | *** | *** | *** | *** | *** | *** |
| Poly P1                    | 1.35 ± 0.09 | 1.49 ± 0.3 | 1.51 ± 0.1 | 1.52 ± 0.5 | 0.65 ± 0.32 | 1.17 ± 0.09 |
| % P2                       | 1.14 ± 0.4 | 1.76 ± 0.1 | 1.11 ± 0.5 | 1.14 ± 0.45 | 0.59 ± 0.131 | 1.11 ± 0.16 |
| Ctrl                       | 0.201 ± 0.05 | 0.278 ± 0.08 | 1.05 ± 0.2 | 0.75 ± 0.05 | 0.36 ± 0.115 | 0.74 ± 0.1 |
| Significance level | ** | *** | NS | NS | NS | * |
| Caro P1                    | 0.3 ± 0.1 | 0.14 ± 0.01 | 0.19 ± 0.01 | 0.14 ± 0.001 | 0.13 ± 0.03 | 0.23 ± 0.081 |
| mg/g P2                    | 0.23 ± 0.07 | 0.15 ± 0.02 | 0.16 ± 0.02 | 0.064 ± 0.007 | 0.04 ± 0.008 | 0.14 ± 0.18 |
| Ctrl                       | 0.16 ± 0.04 | 0.13 ± 0.05 | 0.25 ± 0.01 | 0.14 ± 0.01 | 0.24 ± 0.047 | 0.2 ± 0.01 |
| Significance level | NS | NS | *** | *** | ** | NS |
of ROS [11], which is truly revealed when carotenoids represented significant differences with all the parameters, stressors and scavengers. Moreover, ascorbic acid showed a noteworthy difference with hydrogen peroxide, pheophytin and proline. Ascorbic acid participates in widely important acts in the plant defence system: it is a strong reductant that activates defence mechanisms in plants and its reducing power is proportional to its concentration [26]. But the most remarkable significance is of proline with mostly of the parameters, especially with hydrogen peroxide. Recent studies on plants have revealed multiple functions of proline: it acts primarily as a compatible solute in osmotic adjustments; as a signalling molecule, it is an activator of defence pathways and acts directly on scavenging free radicals and chelating metals [27].

Table 3. Results of stress markers (hydrogen peroxide, pheophytin and proline).

| Site/Month | May-15 | Jun-15 | Dec-15 | May-16 | Jun-16 | Dec-16 |
|------------|--------|--------|--------|--------|--------|--------|
| Hydro ppm  | Ctrl   | 3.79   | 0.08   | 4.36   | 10.68  | 19.13  |
| Hydro ppm  | P2     | 0.22   | 0.03   | 0.89   | 28.9   | 118.93 |
| Hydro ppm  | P1     | 4.04   | 0.45   | 50.86  | 139.28 | 151.53 |
| Significance level | NS | * | *** | *** | *** | *** |

Table 4. Paired t-test calculated for the different parameters.

| 2015 | 2016 |
|------|------|
| Paired t-Test | Paired t-Test |

| Estimation for paired difference | t-Value | p-Value |
|----------------------------------|---------|---------|
| Caro1-Pheo1                      | -4.38   | 0.000174|
| Caro1-Pro1                       | -2.15   | 0.041   |
| Caro1-Poly1                      | -0.54   | 0.608   |
| Caro1-Hydro1                     | -4.35   | 0.000174|
| Caro1-RWC1                       | -8.12   | 0.000051|
| Caro1-TAC1                       | -8.44   | 0.000051|
| Caro1-AA1                        | -8.1    | 0.000051|
| Caro1-APTI1                      | -20.02  | 0.000051|
| Pheo1-Hydro1                     | 3.27    | 0.000051|
| Pheo1-APTI1                      | -3.55   | 0.000051|
| Pheo1-RWC1                       | -7.6    | 0.000051|
| Pheo1-TAC1                       | -8.44   | 0.000051|
| Pheo1-AA1                        | -8.44   | 0.000051|
| Pheo1-APTI1                      | -7.83   | 0.000051|
| Pheo1-TAC1                       | -2.54   | 0.000051|
| Pheo1-AA1                        | -5.44   | 0.000051|
| Hydro1-Hydro1                    | -4.2    | 0.000051|
| Hydro1-RWC1                      | -7.87   | 0.000051|
| Hydro1-TAC1                      | -4.87   | 0.000051|
| Hydro1-AA1                       | -7.73   | 0.000051|
| Hydro1-APTI1                     | -18.78  | 0.000051|
| Hydro1-RWC1                      | -2.19   | 0.000051|
| Hydro1-TAC1                      | -4.76   | 0.000051|
| Hydro1-AA1                       | 2.29    | 0.000051|
| Hydro1-APTI1                     | 2.28    | 0.000051|
| Hydro1-APTI1                     | -3.67   | 0.000051|
| Hydro1-APTI1                     | 4.8     | 0.000051|
| Hydro1-APTI1                     | 5.81    | 0.000051|
| TAC1-AA1                         | 4.81    | 0.000051|
| TAC1-APTI1                       | 4.81    | 0.000051|

Note: Nearly 90% of the studied parameters recorded significant difference. Correlation significant at the level 0.05.
It is not new that phenolic compounds possess antioxidant properties [28]. Accumulation of polyphenols is observed in plants under oxidative stress as they support the ascorbate detoxification system as a backup defence mechanism [29].

Recent literature denotes the central role of stress scavengers in combatting stress in plants. Figure 2 shows the variation of APTI and TAC among the three sites: in P1, APTI is highly surpassing the other sites, which indicate that the plant is trying to tolerate the pollution. As for the TAC, as shown in P1, the antioxidant power is extremely higher than the other sites, signalling the presence of an oxidative stress.

Another statistical test was performed: paired sample correlations analyses between sites P1, P2 and Ctrl (Table 5). This test revealed significant positive and negative correlations between the evaluated parameters. TAC, between P1 and Ctrl, showed negative correlations, likewise pheophytin in P1 and P2 in 2016. Hydrogen peroxide, which is the main ROS indicators of oxidative stress, is closely correlated in P1 and Ctrl from both years. The high negative correlation coefficient was found in TAC between P1 and Ctrl in 2015.

To identify the occurrence and the significance of the oxidative stress, a bar graph of markers–scavengers means with SD was drawn (Figure 3). Comparison between the studied sites is obvious: P1 is the area most exposed to pollution, followed by P2 and finally by Ctrl. It is interesting to note that even control site, supposed to be unpolluted, may have encountered the effects of airborne pollution, as indicated in Figure 3.

### 4. Conclusion

The excessive production of free radicals and toxic oxygen derivatives implicates the establishment of oxidative stress. Plants have developed a wide range of defence mechanisms against this condition. The
production of scavengers is very common and necessary for limiting these toxic compounds. Many plant species can be used as biomonitors to evaluate stress responses especially related to airborne pollution situation. "Urginea maritima" has revealed to be a suitable biomonitor of air pollution; nonetheless more studies must be done to establish a biomonitor programme for natural reserves in Lebanon.

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ORCID

Yara Khairallah (http://orcid.org/0000-0002-9581-0780)
Tarek Houri (http://orcid.org/0000-0003-4297-1000)
Bilal Osta (http://orcid.org/0000-0001-9943-5423)
Dany Romanos (http://orcid.org/0000-0002-6819-998X)
Georges Haddad (http://orcid.org/0000-0001-6325-0523)

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