1. Contestant profile

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2. Project overview

Title:	**Effect of limestone dust on eco-physiological behavior and biodiversity of natural and semi-natural vegetation in Safi's quarry**
Contest: (Research/Community)	Research
Quarry name:	Safi

Abstract

Cement industries and quarrying operations generate huge amounts of dust and are progressively affect the basic resources; land and vegetation. Dust may have direct physical and/or chemical effects on the surrounding vegetation. In this project, the effect of limestone dust on three species (*Nicotiana glauca*, *Onopordum macracanthum* and *Drimia maritima*) in Safi's quarry was investigated. Study zone was divided into three sites according to a gradient of limestone dust content. Physiological and biochemical analysis were performed on leaves collected from the selected species. Our results showed that the leaf relative water content does not change significantly in the three species among the three sites. However, the stomatal conductance and the efficiency of photosynthetic apparatus decreased significantly with increasing limestone dust deposition in both *N. glauca*, *O. macracanthum*, while these both traits were not change in *D. maritima*. Furthermore, the high limestone dust rate enhanced significantly the leaf concentration of inorganic ions Mg^{2+}, K^{+} and Ca^{2+} in the three species. Regarding the biochemical parameters, significant accumulation of polyphenols and soluble sugar in leaves of the three species was recorded into the site having the highest limestone dust deposition. Thus, the three species exhibited a significant induction of superoxide dismutase and catalase activities, indicating an efficient antioxidative defense system in the three species, with interspecific differences. Therefore, based on these findings, it turned out that limestone dust deposition on leaves can disrupt the physiological activities of natural and semi-natural vegetation in Safi's quarry. However, a significant activation of some biochemical mechanisms of protection was demonstrated in the studied species as effective way of ensuring tolerance to such conditions. These three different types of vegetation can be considered as an elite resource of limestone-tolerance and may prove effective in future biodiversity conservation programs in Safi's quarry. The eco-physiological traits studied in these species provide a good starting point for selecting limestone-tolerant trees and planting them as natural barriers to reduce limestone dust spread from the quarrying zone.

1. Introduction

Quarrying is an economically essential land use that can affect the vegetation in a variety of ways. However, some quarries are of considerable value for conservation. The relevance of rare and endemic plants, in assessing the conservation value of quarries, would be enhanced if it could be demonstrated that the vegetation associated with such sites has features in common with ancient plant communities (Hodgson, 1982). In time, quarry ecotypes might be evolved and the species diversity may be augmented by the invasion of very mobile species from a greater distance and by the introduction of plants by quarry traffic (Hodgson, 1982). The conservation of vegetation in a quarry will almost always mean management; and management requires and involves environmental protection programmes to mitigate the impact of quarry activities. Dust emission is one of the major features of quarrying operations. This dust can be considered as a devastating factor for the environment threatening all animal and plant species (Moradi et al., 2017). The few pieces research that have been undertaken on the effect of dust pollution on plant communities suggest that the effects of dust may be important and are worthy of greater research attention. Dust can have both a physical and a chemical impact on a range of natural and semi-natural vegetation types. Its chemical effects are varied; some dusts are relatively inert, e.g. those from hard acidic rock quarries; however, limestone quarry dust and cement dust are highly alkaline (Farmer, 1993). Limestone dust effects on plants mainly change according to the species, emission and deposition rate and meteorological conditions (Czaja, 1961; Manning, 1971; Brandt and Rhoades, 1972; Gilbert, 1976; Etherington, 1977; 1978; Farmer, 1993; Vardaka et al., 1995).

Effects of limestone dust on the structure and composition of a forest community has been reported by Brandt and Rhoades (1972). The research reviewed by these authors showed that limestone dust accumulation favours some species and limits others. At plant level, Moradi et al. (2017) reported that dust deposits on leaves of three oak species disrupt their physiological activities. Similar results were obtained in *Astragalus jaegerianus*, a perennial herbaceous legume (Wijayratne et al., 2009) and in *Quercus coccifera*, a schlerophyllous shrub (Vardaka et al., 1995). Taking into account both rate of dust deposition and size of dust particles, the leaves receive less light for photosynthesis and the stomata appear occluded by fine dust particles, which in turn affects the leaf stomatal conductance performance, rate of photosynthesis and assimilation of carbon and plant biomass (Farmer 1991; Vardaka et al., 1995; Wijayratne et al., 2009; Moradi et al., 2017). However, some authors demonstrated that the surface applied limestone can improve soil chemical characteristics, soil fertility and nutritional status and yield of several plant species, such as corn (Claassen, 1971), soybean (Carvalho and Nascente, 2014) and spruce (*Picea excelsa* Link) and pine (*Pinus nigra nigricans* Host) (Le Tacon, 1978)

Two types of quarry may be recognized: calcareous (associated with the chalk and limestone) and acidic (gritstone, slate, granite etc.) (Hodgson, 1982). Safi's quarry is classified among the calcareous quarries in Morocco. In this quarry, the spoil is one of the most important habitats of the vegetation. As becomes clear from all this, this, this study was conducted in Safi's quarry with the following objectives, (i) to examine and characterize the effect of limestone dust released continuously, during quarrying operations, on some eco-physiological parameters in the existing vegetation and (ii) to discriminate, insofar as possible, the tolerant species to the local conditions of the quarry, in particular the deposition of limestone dust, for any future plantation and conservation programs.

2. Materials and methods

2.1. Plant material

In this project, we chose three contrasting plant species with different structure and taxonomy that have widespread distribution in study site. *Nicotiana glauca* Graham (Solanaceae), *Onopordum macracanthum* Schousb*.* (Asteraceae) and *Drimia maritima* (L.) Stearn (Asparagaceae) represented the plant material of our research in Safi's quarry.

Nicotiana glauca is is indigenous to Bolivia and northern Argentina but is also found throughout North America, Africa, Europe and Australasia, where it thrives in dry arid climates. It is atypical within the genus *Nicotiana*, accumulating predominantly anabasine rather than nicotine and/or nornicotine as the main component of its leaf pyridine alkaloid fraction (DeBoer et al., 2009). *Onopordum macracanthum* Schousb. it is a species native to the Mediterranean regions and introduced as noxious weeds in Australia, California and South America (Susanna and Garcia-Jacas, 2007). It is confined to sandy soils near the Atlantic coastline of north-western Morocco and south-western Spain (Balao et al., 2017). Nevertheless, Morocco, especially the region in the north-western foothills of the Middle Atlas, has been inferred as the ancestral area of many taxa of Asteraceae (Ortiz et al., 2008). *Drimia maritima* is perennial bulbous geophyte, native to the Mediterranean basin and well adapted to its type of climate (Al-Tardeh et al., 2006).This species is used in the production of medicinal products and in homeopathic therapy (Knittel et al., 2015).

2.2. Study zone

The study zone is a part of a limestone quarry located about 30 km north-east away from the coastal city of Safi and about 13 km north of Had Harrara village, on provincial road n°2313 linking Safi city to Oualidia city (Figure 1). Safi's quarry is one of the main quarries of the company of "Ciments du Maroc", a subsidiary of the international HeidelbergCement Group in Morocco. The study zone is considered as protected area to preserve the scenery and local ecosystem. It is located at an elevation of about 85 m above mean sea level. Geographically, the study site lies at 32.53°N latitude and -9.10°W longitude. We subdivided the study zone into three sites (S1, S2 and S3) of about 150 m wide for each site, from the boundary of digging and extraction zone (Figure 1). Each site contains the three species chosen for our study. Thereby, the determination of limestone dust concentration in the upper soil layer (0 - 2 cm depth) in each site showed a decreasing gradient of the limestone concentration when going away from the limit of digging and extraction zone : S1 > S2 > S3. This finding has been confirmed by the determination of limestone dust amount on leaves (upper and lower surfaces) in each site.

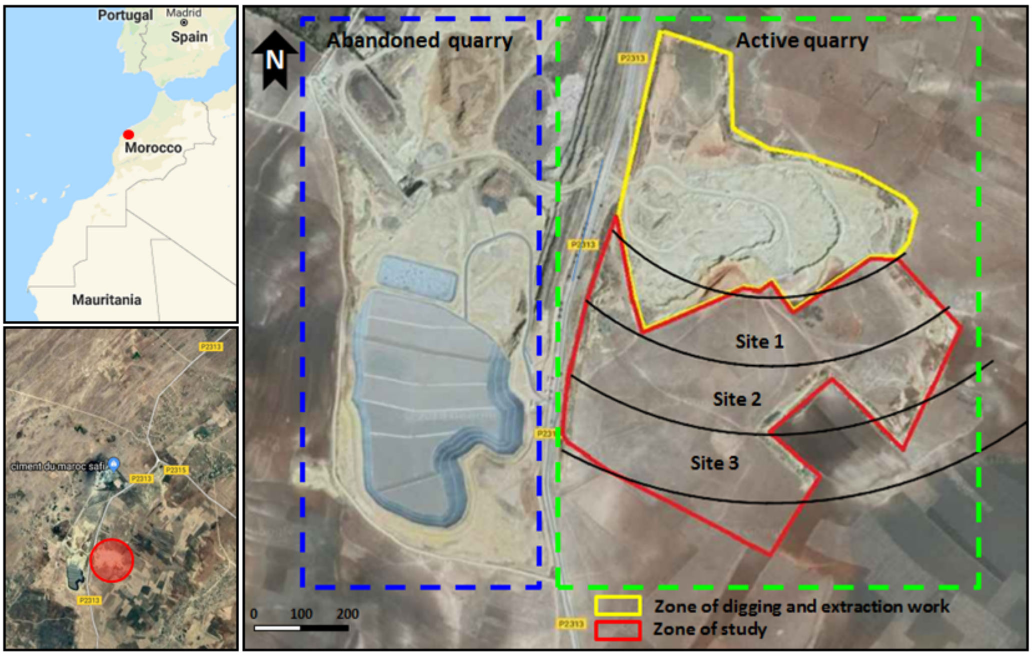

Figure 1. Geographical location of the study zone in Safi's quarry.

2.3. Physiological traits

Stomatal conductance (g_s)

g_s was determined using a leaf porometer (Decagon Devices, Pullman, WA, USA). Measurements were made on fully exposed leaves between 10.00 and 12.00 h. The data were collected from two leaf samples per plant (five plants per species per site).

Relative water content (RWC)

RWC was measured on five whole leaves per plant (five plants per species per site). It was calculated as RWC = (FW−DW)/(SW−DW) × 100, where FW is the fresh weight, SW is the turgid weight after leaves were soaked in distilled water for 24 h at 4° C in the dark and DW is the dry weight after the leaves were dried for 48 h at 70°C.

Chlorophyll fluorescence traits

Chlorophyll fluorescence emission from the upper surface of the intact leaves of studied plants was measured using a portable Handy PEA fluorimeter (Plant Efficiency Analyser; Hansatech Instruments, King's Lynn, Norfolk, UK). Five independent measurements per species per site were carried out on the adaxial side of the youngest fully expanded leaves. Leaves were adapted to darkness for 20 min, and then exposed to a saturating red light pulse (650 nm, 3000 µmol photons m^{-2} s^{-1}). The maximum quantum efficiency of photosystem II (PSII) photochemistry (F_v/F_m) and the intrinsic efficiency of open PSII reaction centers (F'_v/F'_m) were measured in leaves of studied plant species.

Endogenous content of ions (Mg^{2+}, K^+ and Ca^{2+})

Leaves collected were carefully rinsed with deionized water and the fresh weight of each sample was determined. Then, the leaf material was calcined at 600°C for 6 h and the dry weight of each sample was measured. Each sample was ground into a fine powder and digested with concentrated nitric acid (HNO_3)

overnight at 120°C. Samples were then dissolved in (1:1, v/v) $HNO_3/HClO_4$ (perchloric acid) to 220°C, resuspended in 5% (v/v) HNO3 and analyzed for the determination of Mg^{2+}, K^+ and Ca^{2+} content using inductively coupled argon plasma emission spectrometry (ICP trace analyzer). Ion contents were expressed in mmol/g DW, with three independent measurements per species per site.

2.4. Biochemical traits

Extraction enzyme

The fresh leaf samples from plant species were grounded into a fine powder with liquid nitrogen. Using a potassium phosphate (K2HPO4/ KH2PO4) buffer, the antioxidant enzymes (catalase and supersoxide dismutase) were extracted by homogenizing on ice the powder (0.1 g for each antioxidant enzyme) in 50 mM K2HPO4/KH2PO4 (pH 7.8) containing 1% (w/v) polyvinyl pyrrolidone (PVP), 0.1 mM ethylene-diaminetetra-acetic acid (EDTA), 5 mM 2-mercaptoethanol, 0.2% (v/v) Triton X100 and 0.1 mM, 0.1 mM phenylmethane-sulfonyl fluoride solution (PMSF). After centrifugation at 15,000×g at 4°C for 15 min, the supernatant was used to determine the enzymatic activities and protein content. We opted to five replicates per species per site. According to Bradford (1976), total soluble protein content for the determination of the specific activities of the enzymes was determined using bovine serum albumin as a standard.

All spectrophotometric measurements were taken in a Jenway (6305 UV/VIS, England) spectrophotometer. All reagents used were of analytical grade and were delivered from Sigma (St. Louis, USA), Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany).

Catalase (CAT)

CAT activity was determined according to the method of Aebi (1984). The assay mixture consisted of 50 mM K_2HPO_4/KH_2PO_4 (pH 7.0), 10 mM H_2O_2 and 0.1 mL enzyme extract. An extinction coefficient (E) = 39.4 mM^{-1} cm^{-1} at 240 nm was adopted to express the CAT activity as nmol of H_2O_2 decomposed/min/mg of protein.

Superoxide dismutase (SOD)

Total SOD activity was evaluated by monitoring its ability to inhibit the photochemical reduction of p-nitrobluetetrazolium chloride (NBT) at 560 nm according to the method of Beauchamp and Fridovich (1971). The reaction mixture contained 50 mM K_2HPO_4/KH_2PO_4
buffer (pH 7.8), 0.1 mM EDTA, 33 µM NBT, 10 mM L-Methionine, 4 µM riboflavin and enzyme extract. Reactions were carried out for 8 min at a light intensity of 50 mmol photons m^{-2} s^{-1}. One unit of SOD activity was defined as the amount of enzyme, which causes 50% inhibition of NBT reduction under the assay condition, and the results were expressed as units of enzyme (UE) per mg of protein.

Total polyphenols content

The finely ground powder was ground on ice in 80% methanol (MeOH) and then sonicated for 10 minutes in ultrasonic bath (Elma, Germany) containing 700 ml ultrapure water. The homogenate was centrifuged at 12,000×g for 10 min at 4˚C. The concentration of polyphenols in the supernatants was determined with Folin-Ciocalteu reagent following the colorimetric method and by adopting a differential assay in the presence/absence of 1% (w/v) polyvinylpolypyrrolidine (PVPP) as described in Chakhchar et al. (2015a). Measurements were carried out in five replicates per species per site and calculations were based on a calibration curve obtained with gallic acid. The levels of polyphenols were expressed as mg GAE g^{-1} DW (mg of gallic acid equivalents/g of dry weight).

Chlorophyll content

The contents of photosynthetic pigments were determined spectrophotometrically in 80% acetone (v/v) as the solvent. The extract was centrifuged at 10,000×g for 5 min. The supernatant was collected and measured spectrophotometrically at 663 and 647 nm for chlorophyll a and chlorophyll b, respectively. Their content was calculated according to the equations proposed by Lichtenthaler (1987). The results were expressed in mg per g of leaf dry matter (mg g^{-1} DW) and then the Chl a/b ratio was determined. There were five replicates per species per site (one plant per replicate).

Soluble sugar content

Total soluble sugars concentration was assessed following the anthrone method (van Handel 1968). The finely ground powder (100 mg) was extracted with 1 ml of 80 % ethanol, centrifuged at 15,000×g for 10 min and the pellet was reextracted twice with 90 % ethanol at 80 °C. The extract was then treated with freshly prepared anthrone reagent (2 ml). The absorbance was recorded at 625 nm by spectrophotometer and the concentration of soluble sugars was derived from a standard curve using glucose. The concentration of soluble sugar was presented as µmol g^{-1}DW. There were 5 replicates per species per site.

2.5. Statistical analysis

Results were examined using the analysis of variance (ANOVA) to test the species and site effects on each parameter studied at $P < 0.05$. Means were separated using the Tukey's Post hoc test. All statistical analyzes were established by SPSS 17.0.

3. Results

Regarding the physiological parameters studied, the limestone dust content in the three sites affected the stomatal conductance and F_v/F_m in both *N. glauca* and *O. macracanthum*, while the endogenous content of ions (Mg^{2+}, K^+ and Ca^{2+}) changed in the three species (Table I, and Figures 2a and 3a). However, RWC and F'_v/F'_m did not significantly change in the three species according to the site factor (Figures 2b and 3b). Significant decrease of stomatal conductance and F_v/F_m was recorded in site 1 compared to the other sites in *N. glauca* and *O. macracanthum* species. These both physiological parameters have not been significantly changed in *D. maritima*. The leaf concentration of inorganic ions Mg^{2+}, K^+ and Ca^{2+} has been significantly decreased with the decrease of limestone dust content in site (Table I). The highest contents of these ions in the plant species studied were recorded in the site 1 that has high limestone dust content compared to other sites. Significant statistically difference between species was noted for water status traits (g_s and RWC) and not for chlorophyll fluorescence traits (F_v/F_m and F'_v/F'_m) (P<0.05). *N. glauca* showed the highest g_s values, while both *D. maritima* and *N. glauca* exhibited high RWC (P<0.05). According to chlorophyll fluorescence measurements, we recorded a high values of Fv/Fm (>0.8) which are typical for a well-functioning apparatus. Also the values recorded of F'_v/F'_m can be considered as normal values for plants not stressed (Figure 3b). Likewise, there was no significant difference between sites for Chl a/b ratio in the three species (Figure 3c). Nonetheless, a significant species effect was noted and *D. maritima* showed the lowest Chl a/b ratio compared to the other two species (P<0.05).

Table I. Effect of limestone dust deposition on leaf concentration of K^+, Ca^{2+} and Mg^{2+} (mmol/g DW) in three selected species from Safi's quarry. Values (means ± SD) with different lowercase letters are significantly different at 5% level (Tukey's test). Uppercase letters indicate significant differences between species.

Species	Site	K^+		Ca^{2+}		Mg^{2+}	
	S1	14,27 ± 1,28 a		6,19 ± 0,45 a		8,04 ± 0,42 a	
N. glauca	S2	10,79 ± 1,24 b	*B*	4,73 ± 0,30 b	*A*	6,43 ± 0,42 b	*A*
	S3	11,82 ± 1,34 ab		3,59 ± 0,58 b		3,10 ± 0,14 c	
	S1	12,84 ± 0,85 a		3,92 ± 0,09 a		4,52 ± 0,46 a	
O. macracanthum	S2	10,09 ± 0,62 b	*B*	2,23 ± 0,46 b	*B*	2,79 ± 0,30 b	*B*
	S3	8,67 ± 0,99 b		1,91 ± 0,41 b		2,15 ± 0,30 b	
	S1	17,19 ± 0,51 a		7,78 ± 0,60 a		9,49 ± 0,55 a	
D. maritima	S2	16,16 ± 1,36 a	*A*	5,61 ± 0,50 b	*A*	8,86 ± 0,43 a	*A*
	S3	12,90 ± 0,69 b		5,08 ± 0,28 b		4,42 ± 0,35 b	

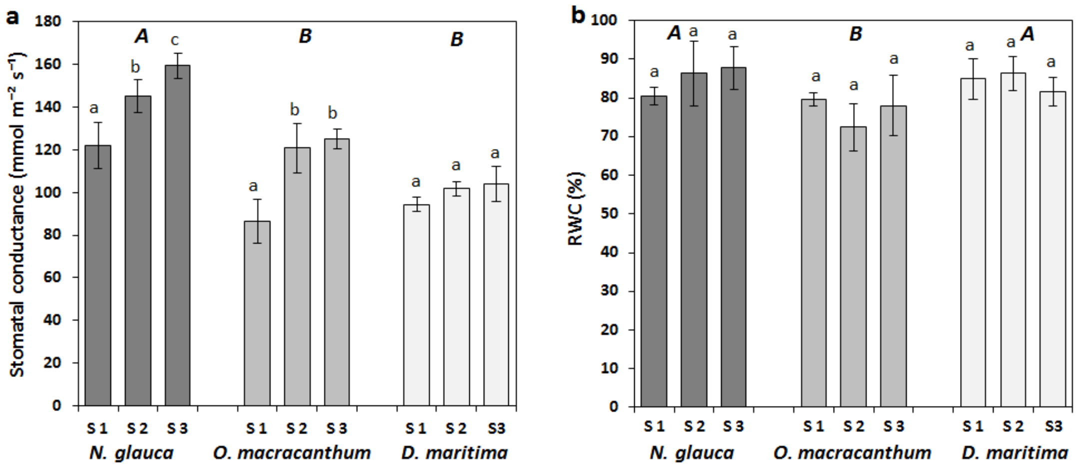

Figure 2. Effect of limestone dust deposition on stomatal conductance (a) and RWC (b) in three selected species from Safi's quarry. Values (means ± SD) with different lowercase letters are significantly different at 5% level (Tukey's test). Uppercase letters indicate significant differences between species.

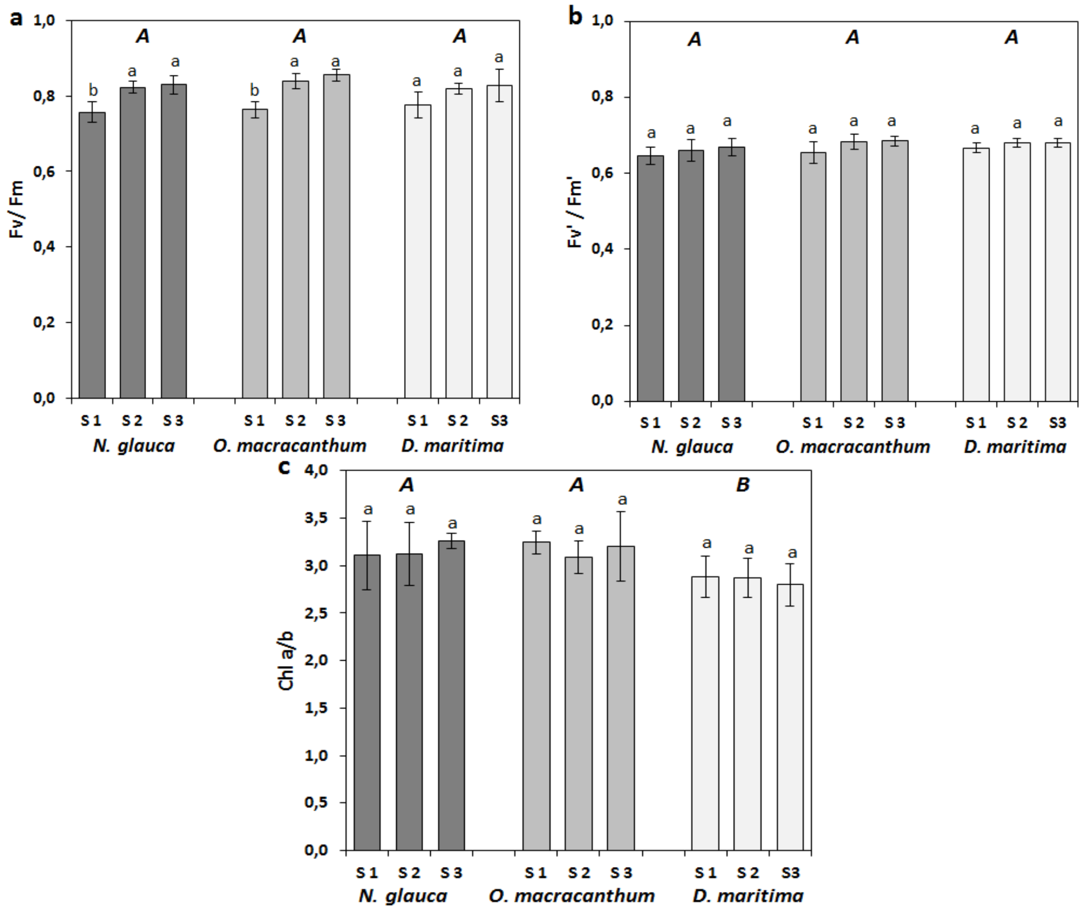

Figure 3. Effect of limestone dust deposition on F_v/F_m (a), F_v'/F_m' (b) and Chl a/b (c) in three selected species from Safi's quarry. Values (means ± SD) with different lowercase letters are significantly different at 5% level (Tukey's test). Uppercase letters indicate significant differences between species.

The effect of limestone dust deposition on the activity of the main antioxidant enzymes (SOD and CAT) that prevent ROS damage by scavenging free radicals is shown in figure 4. A significant statistically difference in constitutive and/or induced activity of SOD and CAT was found among sites, and between species (*P*<0.05). *N. glauca* showed the highest SOD activity, whereas the high CAT activity was measured in *O. macracanthum* (Figure 4). In the three species, a high activity of both SOD and CAT was observed in Site 1 in comparison to other sites (S2 and S3) (*P*<0.05).

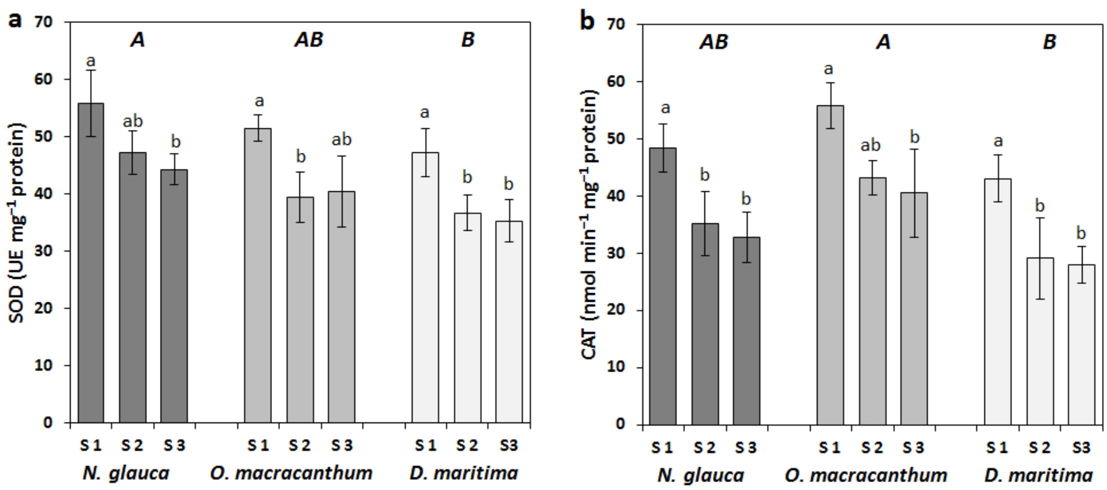

Figure 4. Effect of limestone dust deposition on SOD (a) and CAT (b) activities in three selected species from Safi's quarry. Values (means ± SD) with different lowercase letters are significantly different at 5% level (Tukey's test). Uppercase letters indicate significant differences between species.

There was significant difference in total polyphenols among sites in the three species (Figure 5a). However, the total soluble sugars content was significantly changed only in *N. glauca* and *D. maritima* species. Limestone dust deposition did not result in any change in sugar content in *O. macracanthum* (Figure 5b). In all species, the highest total sugar and polyphenols content was found in site1 (P<0.05). Moreover, there was significant difference in these both biochemical compounds, between the plants species studied (P<0.05), that could be explained by the interspecific variation in gene expression level and adaptive function. In terms of comparison between species, the highest content of total polyphenols and sugar was measured in *N. glauca*, while the lowest was found in *D. maritima* (Figure 5).

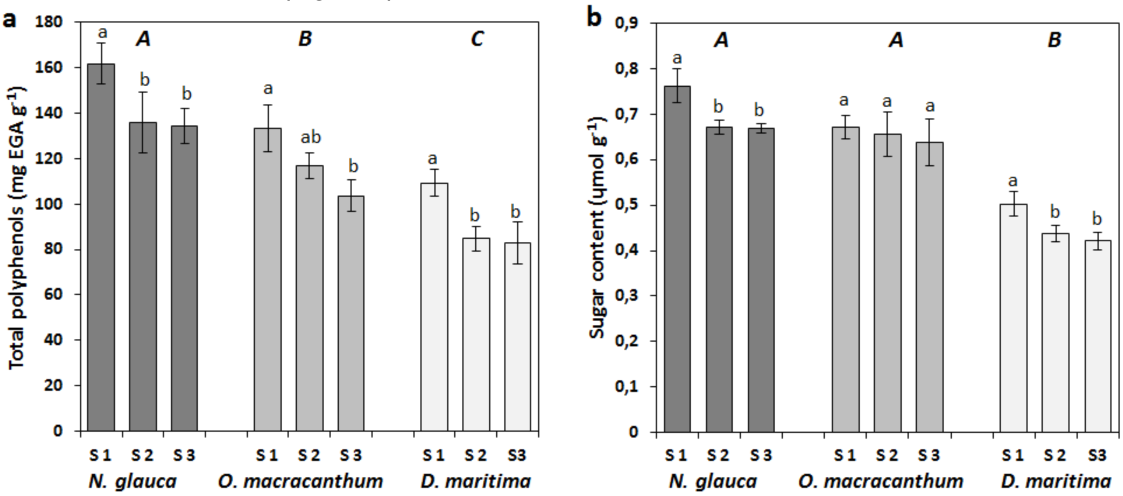

Figure 5. Effect of limestone dust deposition on total polyphenols (a) and soluble sugar (b) contents in three selected species from Safi's quarry. Values (means ± SD) with different lowercase letters are significantly different at 5% level (Tukey's test). Uppercase letters indicate significant differences between species.

4. Discussion

Research on the effects of dust pollution on plant communities has never received the same level of attention as that given to phytotoxic pollutants such as SO_2, NO_2 and O_3. However, dust can have both a physical and a chemical impact. The results of this research show that limestone dust accumulation affected some physiological and biochemical parameters in the three plant species studied. Species and sites to some extent determine the variation of these parameters. Indeed, the change in the studied traits did not appear to be affected by other environmental factors other than the limestone dust. The decrease in conductance stomatal and F_v/F_m per site, in *N. glauca* and *O. macracanthum*, as a result of increasing limestone dust deposition can be explained by a blockage of leaf stomata. Dust deposition can cause obstruction of the stomata on both adaxial and abaxial surfaces of the vegetation (Christian et al., 2008; Moradi et al., 2017). It was suggested that the dust may act directly on the guard cells (Farmer, 1993). Vardaka et al. (1995) reported that the reduced stomatal conductance in *Quercus coccifera* would be consistent with the blockage of leaf stomata by limestone dust particles. This reduction in g_s may be a factor responsible for the decline in photosynthesis (Vardaka et al., 1995). Analysis by scanning electron microscope of treated leaves with dust in three species of *Quercus infectoria*, *Q. libni*, and *Q. brantii* showed that the stomatal pores were occluded with dust and thus can limit gas exchange rates through stomata (Moradi et al., 2017). Also, Fv/Fm decreased significantly in site 1 (highest dust level) indicating that the efficiency of the primary photochemistry of PSII is reduced in leaves experiencing stress from limestone dust. A similar result was pointed out by Vardaka et al. (1995) in leaves of *Quercus coccifera*, suggesting that the size of the pool of electron acceptors on the reducing side of PSII was reduced as a result of increasing stress from limestone dust. In terms of comparison between the three species, these both physiological traits were not affected by limestone dust deposition in *D. maritima*. In fact, the plant species differ in their ability to collect dust particles from the air and in their reaction to dust depositions (Moradi et al., 2017). Nonetheless, limestone dust did no show effect on RWC and F'_v/F'_m in all species. As an indicator of plant water status and use, the obtained results of RWC trait exhibited good water conservation in leaves which is probably in part due to the obstruction of the stomata by limestone dust and hence, transpiration decreases. Thereby, the changes in water potential

and/or soil water content can also cause significant stomatal closure (Chakhchar et al. 2015a). In addition, the results of F'_v/F'_m revealed that the efficiency of the excitation energy interception by PSII centres and/or the effectiveness of light quanta trapping by PSII antennas in leaves did not affected by limestone dust.
Chl a/b ratio was not changed significantly among sites in the three species, suggesting that the chlorophylls content in leaves were not suffering seriously from the shading effects of limestone dust. Corroborating with our results, Vardaka et al. (1995) reported that the total chlorophyll (a+b) concentrations in *Quercus coccifera* leaves did not vary significantly between different sites in a limestone quarry. Chl a/b ratio can be considered as a key trait to judge tolerance to environmental stresses in plants (Chakhchar et al., 2018)
The three species showed the highest leaf content of inorganic ions K^+, Ca^{2+} and Mg^{2+} in site 1. This significant accumulation could be due to the elevated levels of limestone dust in site 1 compared to others sites. Limestone dust deposition on both leaves and soil constitutes an important exogenous contribution of these ions for the plants in Safi's quarry. Ions such as K^+, Ca^{2+} and Mg^{2+} are considered as essential for plant growth and development. They are attributed to enhanced nutrition, physiological process and biochemical metabolism (as cofactors) and to mitigate the negative effects of some environmental stresses (Chakhchar et al., 2017). Castro and Crusciol (2015) reported that a surface application of dolomitic limestone positively influenced Ca and Mg nutrition in soybean cropped under a well-established no-tillage system. Since limestone is mainly composed of calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$), the existence of these species is indicative of their alkaline tolerance.
Dust affects plant physiology both physically and biochemically (Przybysz et al., 2014; Moradi et al., 2017). In the three species studied, the antioxidant activity of SOD and CAT increased significantly with increasing limestone dust in site. This significant change is indicative of the occurrence of oxidative stress in leaves. To maintain homeostasis and prevent oxidative stress, the plants activate a an antioxidant defense system composed of certain enzymes such as catalase and superoxide dismutase (Chakhchar et al., 2015b). The significant increase in enzymatic activity of these enzymes, in the three species, reflects their role as an effective line of antioxidant defense against oxidative damage caused by reactive oxygen species (ROS). SOD has been considered to be the most potent enzymatic antioxidant involved in the tolerance process, providing the first line of defense against ROS. Also, CAT is an extremely effective enzyme that speeds up the breakdown of hydrogen peroxide into water and oxygen in plant tissues (Gill and Tuteja, 2010). According to our results, it appears that limestone dust at high concentration could induce oxidative stress in the plant species present in the study area.
In both species, *N. glauca* and *D. maritima*, sugars content increased in site 1, which is characterized by high limestone dust content compared to other sites. Soluble sugars accumulation is considered to play a major role in osmotic adjustment to maintain metabolic activity in leaves. Nonetheless, soluble sugars may also accumulate in leaves because of a decreased demand as a consequence of growth limitation (Lemoine et al., 2013). *O. macracanthum* did not show any significant difference in sugars content among sites. As a tolerance strategy to high limestone dust deposition in site 1, the high sugar contents in both *N. glauca* and *D. maritima* can be beneficial to the developing leaf as they are essential for fast cell division and expansion.
There was significant difference in total polyphenol contents among species and between species. These compounds are a diverse group of plant secondary metabolites involved in a range of functions in plant growth, development, and defense (Pandino et al., 2015; Bazghaleh et al., 2018). Furthemore, the polyphenol content of a given plant tissue is strongly influenced by the growing environment, but is also genetically determined (Pandino et al., 2015). *N. glauca* showed the highest leaf polyphenols content compared to other species. In fact, limestone dust has induced significant accumulation of polyphenols, suggesting the induction of its biosynthesis in leaves of the three species, especially in site 1. This enhanced level of polyphenols may be beneficial to enhance the degree of limestone tolerance in the species studied. Polyphenol compounds have very diverse structures that make them unique and multifunctional natural products in plants (Quideau et al., 2011).
These interspecific differences in the physiological and biochemical traits studied highlight the biodiversity of natural and semi-natural vegetation in Safi's quarry. The relative contribution of each of these traits to species tolerance to limestone dust makes Safi's quarry a potential location for conservation programs that can offer protection of this biodiversity. So, the safeguard of biodiversity in the studied zone cannot be done without the environmental assessment of the quarry and the evaluation of limestone dust impact on vegetation in order to provide ecological solutions to limit or reduce limestone dust spread. The plantation of large and dense limestone-tolerant trees, such as natural barriers, along the boundary separating the quarrying zone from the study area can reduce the flow of dust and therefore their deposition on vegetation.

5. Conclusion

Our results show that limestone dust does not pose a threat to the examined species and generally to the Flora of Safi's quarry. Deposition of limestone dust on the surface of *N. glauca* and *O. macracanthum* and *D. maritima* leaves, whilst being aesthetically a little offensive, did not significantly affect the water status. However, dust limestone was detrimental for the stomatal conductance and chlorophyll fluorescence traits (photosynthesis). This could be partially explained by blockage or closure of stomata and shading effect of limestone dust. The studied species exhibited an efficient antioxidative defense system, including different types of enzymatic and non-enzymatic systems, against probable oxidative damage caused by limestone dust. Taking into account these eco-physiological responses, *N. glauca* and *O. macracanthum* and *D. maritima* species can be expected as a valuable genetic resource for limestone-tolerance.

Limestone quarries present both a considerable challenge and some encouragement to ecologists. It is important, therefore, that limestone dust effect in Safi's quarry is identified as well as the vegetation types that are likely to be affected on not by such dust pollution, in order to conserve and preserve plant biodiversity. Identification of species tolerant to such conditions can contribute to think of ecological solutions able to reduce the flow of limestone dust and to better manage the functioning of the local biodiversity of the Safi's quarry vegetation.

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To be kept and filled in at the end of your report

Project tags (select all appropriate):

This will be use to classify your project in the project archive (that is also available online)

Project focus:

- ☐Beyond quarry borders
- ☐Biodiversity management
- ☐Cooperation programmes
- ☐Connecting with local communities
- ☐Education and Raising awareness
- ☐Invasive species
- ☐Landscape management
- ☐Pollination
- ☐Rehabilitation & habitat research
- ☒Scientific research
- ☐Soil management
- ☐Species research
- ☐Student class project
- ☐Urban ecology
- ☐Water management

Flora:

- ☒Trees & shrubs
- ☐Ferns
- ☒Flowering plants
- ☐Fungi
- ☐Mosses and liverworts

Fauna:

- ☐Amphibians
- ☐Birds
- ☐Insects
- ☐Fish
- ☐Mammals
- ☐Reptiles
- ☐Other invertebrates
- ☐Other insects
- ☐Other species

Habitat:

- ☐Artificial / cultivated land
- ☐Cave
- ☐Coastal
- ☐Grassland
- ☐Human settlement
- ☐Open areas of rocky grounds
- ☐Recreational areas
- ☐Sandy and rocky habitat
- ☐Screes
- ☐Shrub & groves
- ☒Soil
- ☐Wander biotopes
- ☐Water bodies (flowing, standing)
- ☐Wetland
- ☐Woodland

Stakeholders:

- ☐Authorities
- ☒Local community
- ☐NGOs
- ☐Schools
- ☒Universities