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Contribution to the quantitative and qualitative study of atmospheric pollens (Guelma, Northeast of Algeria)

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ABSTRACT

Three sites were chosen at the province of Guelma (Northeast Algeria) to carry out a first palynological study of its atmosphere and to highlight the pollen content, among other things, the diversity and the presence of allergenic pollen. The gravimetric method was chosen to ensure the trapping of pollen in suspension in the air, during eitht months of follow-up. In order to carry out a checklist, anemophilous, entomophilous and anemo-entomophilous pollen identification and counting were carried out as well as that the allergenic and non-allergenic families. Thus, 3,348 pollen grains, belonging to 33 families, were captured, of which 2,186 are anemophilous, 756 entomophilous and 406 are anemo-entomophilous. In addition, 35 species and 31 genera were identified in the atmosphere of the three studied sites. As for the allergenic potential, 65% of the identified families are allergenic, mainly in March and April, while 35% have no risk of allergy. This diversity opens up an important field for the disciplines of palynology and allergology in the studied area, because the continued presence of pollen, especially the one with an allergenic potential, as well as the extended pollination seasons, may present risks to public health.

Key words: *Pollen, Anemophila, Entomophil, Allergenic, Pollinosis, Guelma, Algeria.*

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INTRODUCTION

Numerous authors have studied the problem of determining the pollen calendar (Saumande et al. 1980) by identifying atmospheric pollens qualitatively and quantitatively (Negrini et al. 1987). The presence of these gametophytes in the atmosphere can be regarded as natural pollution possibly having an effect on public health, among other things, leading to allergic manifestations. Thus, pollen calendar is intended to guide allergologists in their research and therapeutics in the context of pollen allergies (Saumande et al. 1980). The latter, also called pollinosis, represents seasonal affections related to the presence of pollen grains of anemophilous species in the air (Thibaudon & Olivier 2007). Epidemiological studies have shown that the prevalence of allergic diseases and rhinitis of pollen allergy is increasing (Kopferschmirt-Kubler et al. 2003). In the Mediterranean region, allergic reactions induced by pollen upset more than 10% of the population (D'Amato 1998). The region of Guelma (northeastern Algeria) is characterized by a natural basin surrounded by mountains; this denotes a very important plant diversity, in particular allergenic species. Because no study has been carried out regarding this region, it is necessary to establish a calendar or a list of the various pollens, especially allergens, suspended in the atmosphere.

MATERIALS AND METHODS

The province (Wilaya) of Guelma is situated in the north-east of Algeria (36°27′43″ N, 007°25′33″ E) (Figure 1), It constitutes, from a geographical point of view, a crossroads between the northern poles (Annaba and Skikda) and southern trade centers in the south (Oum-El-Bouaghi and Tebessa), besides the proximity of the Tunisian territory to the East. It is situated at the heart of a large farming area at an altitude of 290 m.

pollens, especially allergens, suspended in the atmosphere.

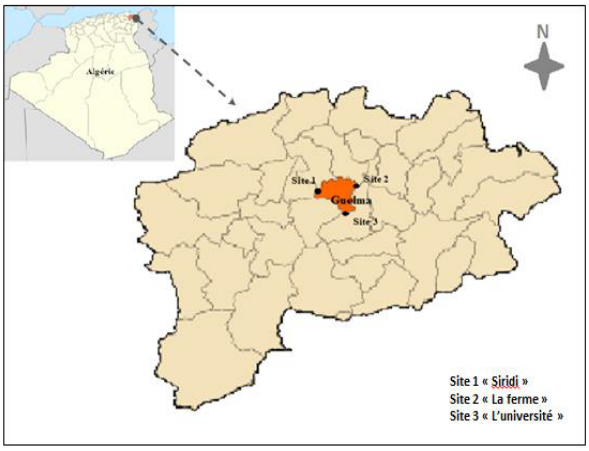

Figure 1: Geographical location of the province of Guelma and the representation of sampling sites (Site1: Siridi; Site 2: the farm; Site3: The university)

In order to carry out the systematic accounts of atmospheric pollens in the Guelma's region, monthly sampling was made for eight months, from January to August of 2013. Thus, three points of deposition were selected; site 1: Siridi (36°26′35.29″ N, 007°24′45.23″ E), site 2: The farm neighborhood (36°26′31.90″ N, 007°26′44.23″ E) and site 3: The University campus, Guelma (36°26′′58.25″ N, 007°24′15.64″ E). Trapping of airborne pollen grains was

carried out using the gravimetric method (Thibaudon & Olivier 2007). The latter involves exposure to air of a slide coated with glycerin gelatin for 24 hours. The slides were then transported in Petri dishes to the laboratory in order to count and identify all the collected pollen grains.

The pollen morphology is characteristic of each species. The identification rests on the size, the shape of the grains, the number and the form of apertures and the extremely varied architecture of its external membrane (exine).
In spite of a form and apertures diversity of pollen grains, the pollinic determination, seldom, allows a species determination. Commonly, the grains of pollen are more identified on the level of the genus or the family (Reille 1990; Calleja et al. 2005).

The identification and pollen counting were made under the photonic microscope to the enlargement (x400), while referring to identification keys from where we found the principal identification characters of each pollinic type. Pollens identification was carried out according to Reille (1992), Trigo et al. (2008), Laine et al. (2008) and the key of the national network of aerobiologic monitoring of Spain (RNSA). Doubtful pollens were identified according to the reference slides containing each one only one pollinic type corresponds to only one taxon, provided by Vegetable Biology and Environment Laboratory (University Badji Mohktar, Annaba -Algeria) and the Institute of Olivier (Tunis - Tunisia).

RESULTS

Our study allowed us to highlight a set of 33 families (Table 1), after capturing a total of 3,348 grains of pollen (GP), by the gravimetric method, of which 2,186 are anemophilous, 756 entomophilous and 406 GP are anemo-entomophilous. According to the graph of abundance illustrated in Figure 2, a remarkable peak was found in March with 1,229 GP and maximum pollen levels both anemophilous (782 GP) and entomophilous (372 GP). However, the month of April also shows a plenty of anemophilous pollen. On the other hand, those of the anemo-entomophilous type were noted during the month of May (Figure 2).

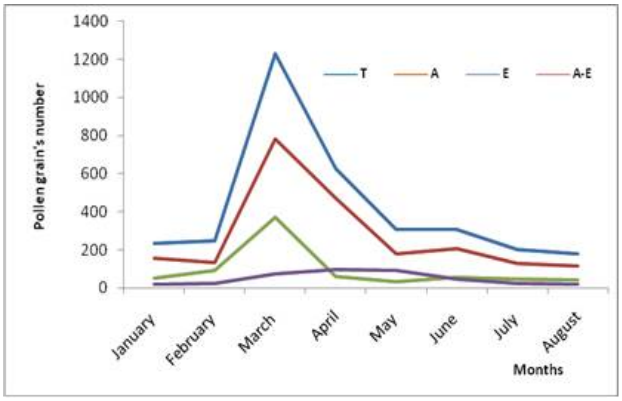

Figure 2: The abundance of total pollinated (T), anemophilous (A), entomophilous (ET) and anemo-entomophilous (A-E).

Pollen diversity from one month to another is based on the number of families that are in the period of pollination. In this study, it was found that the month of May is characterized by the pollination of 22 families, whereas in July, only 17 families were found (Figure 3).

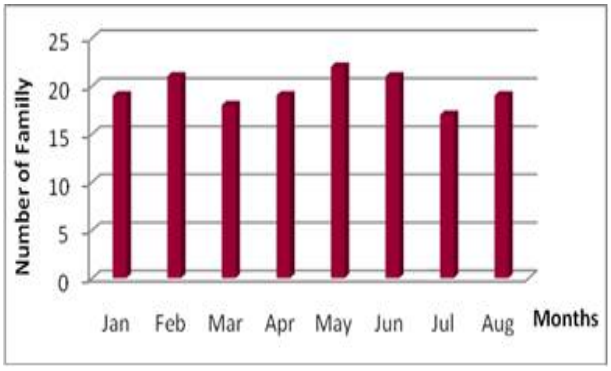

Figure 3: The monthly pollen quantity in the province of Guelma.

From an allergenic point of view, 65% of the identified families are allergenic, while 35% have no risk of allergy (Figure 4a). Taking into account the time factor, it can be seen that the abundance of allergenic pollen is higher during the months of March and April with 559 and 537 GP, respectively (Figure 4b).

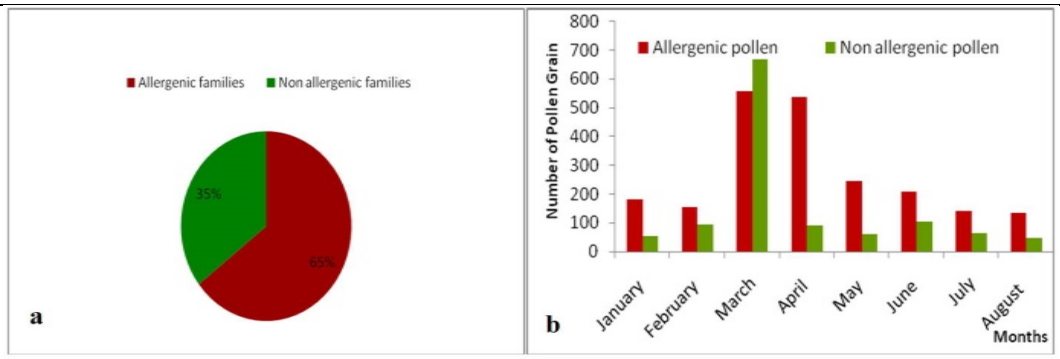

Figure 4: Allergenic and non-allergenic pollen
(a) Percentage; (b) pollen count

However, the rate of *Plataginaceae, Uticaceae, Mimosaceae* and *Fagaceae* does not even reach 50 grains of pollen (Figure 5).

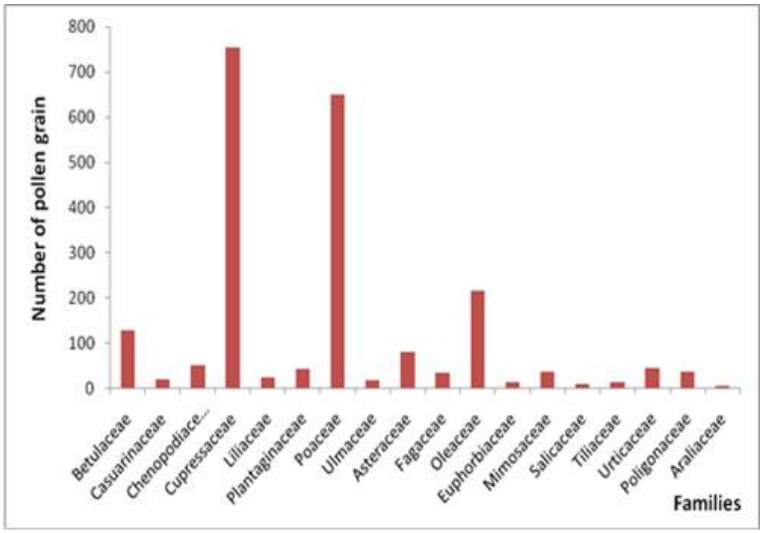

Figure 5: The Allergenic families in the province of Guelma.

For non-allergenic pollen, the *Pinaceae* family was the most common with 438 GP, followed by *Ericaceae* (258 GP), *Brassicaceae* (78 GP) and *Boraginaceae* (66 GP). The remaining non-allergenic families, their pollen levels did not exceed 50 grains (Figure 6).

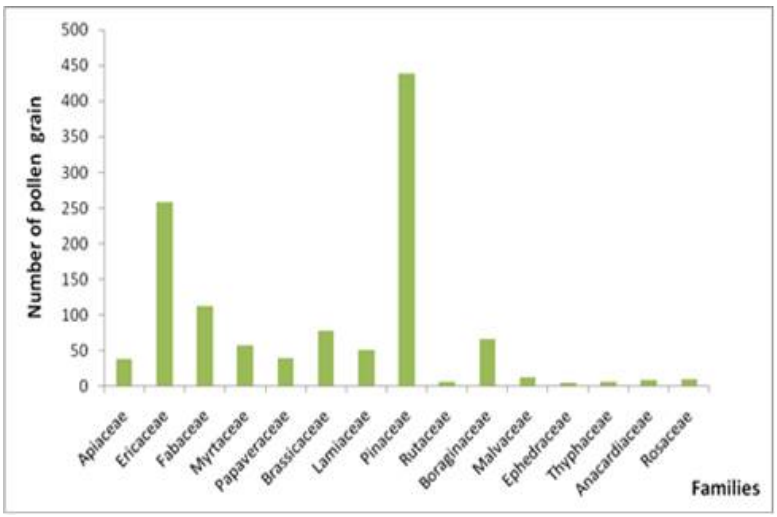

Figure 6: The main non-allergenic families in the province of Guelma.

A set of 35 species and 31 genera, belonging to 33 families, were identified in the atmosphere of the three studied sites. In general, it can be said that the studied sites reveal a diversified monthly pollen frequency, shown in figure (7) and table (1). It has been observed a considerable presence of different families with low pollen levels between January and February. During the month of March, the number of pollen increased with a dominance of the *Cupressaceae* and the *Ericaceae* as well as an appearance of the *Oleaceae*, the *Fabaceae* and the *Poaceae*. The latter characterize mainly the period that extends between the months of April and July. However, this diversity diminishes during the month of August.

Table 1: Annotated list of identified specimens, pollen number per sites, pollination type and allergenic potential.

Familie / Species / Sites	January			February			March			April			May			June			July			August			Pollination type	Allergenicity
	S1	*S2*	*S3*	*S1*	*S2*	*S3*	*S1*	*S2*	*S3*	*S1*	*S2*	*S3*	*S1*	*S2*	*S3*	*S1*	*S2*	*S3*	*S1*	*S2*	*S3*	*S1*	*S2*	*S3*		
Apiaceae *-Daucus carota* L. *-Angelica vulgaris* L.	4 -	7 -	- -	12 -	4 -	- -	- -	- -	4 -	- -	- -	- -	- -	- -	- -	- -	- 3	- 4	- -	- -	- -	- -	- -	- -	**E**	**-**
Betulaceae *-Alnus glutinosa* L. Gaertn. *-Betula* sp.	3 1	9 5	- -	30 1	10 -	13 -	1 3	8 4	- 2	3 1	3 -	2 2	2 -	- -	- -	- -	- -	- -	- -	13 -	7 1	- -	- 2	- 1	**A**	**+**
Casuarinaceae *-Casuarina* sp.	4	5	-	3	-	-	-	3	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	**A**	**+**
Chenopodiaceae *-Chenopodium album* L.	4	2	2	-	-	-	-	3	1	-	-	-	-	-	1	-	-	6	-	-	-	11	7	13	**A**	**+**
Cupressaceae *-Cupressus sempervirens* L. *-Juniperus* sp.	26 -	8 -	3 2	2 10	5 1	7 -	80 57	113 24	89 21	39 -	187 -	11 -	11 -	6 1	- -	3 -	13 -	7 -	- -	2 -	4 4	6 -	4 1	8 -	**A**	**+**
Ericaceae *-Erica arborea* L. *-Arbutus* sp.	2 -	- -	4 -	18	- -	- -	929	71488	719	2 -	- -	3 -	2 -	- -	- -	13 -	- -	3 -	- -	- -	1 -	- -	- -	- -	**E**	**-**
Fabaceae *-Robinia pseudoacacia* L. *-Melilotus* sp. *-Trifolium* sp. *-Vicia* sp. *-Ceratonia siliqua* L.	- 26 - - -	- 10 52 - -	- - - - -	- - 3 - - -	- - 5 - 3 -	4 - - - - -	- 873 - -	- 12 27 - - -	- - 5 - - -	- - - - - -	- - 2 - 3	- - - - - -	4 - - - - -	- - - - - -	- - - - - -	- - - - - -	- - - - - -	- - - - - -	- - - - - -	- - - - - -	- - - - - -	- - - - - -	- - - - - -	1 - - - - -	**E**	**-**
Liliaceae *-Lilium* sp.	1	-	5	12	-	-	-	-	-	-	-	2	-	-	-	-	-	2	-	-	-	-	-	-	**E**	**+**
Myrtaceae *-Eucalyptus rudis* Endl. *-Myrtus communis* L.	4 -	1 -	5 -	- -	- -	11 -	- -	7 -	5 -	5 -	- -	- -	1 -	2 -	- -	- -	- -	- -	3 -	- 3	5 -	2 -	- -	3 -	**A-E**	**-**
Plataginaceae *-Plantago lanceolata* L	3	7	1	-	-	-	-	-	-	3	2	-	-	3	2	-	5	-	-	-	-	9	-	7	**A**	**+**
Poaceae : *Phragmites australis* (Cav.) Trin.	-	-	3	-	-	-	-	-	-	-	-	-	15	-	16	37	-	5	19	3	17	3	7	5	**A**	**+**

-*Cynodon dactylon* (L.)Pers. -*Poa* sp. -*Triticum* sp. -*Triticum sativum* L. -*Hordeum vulgare* L. -*Avena sterilis* L. -*Dactylis glomerata* L. -*Secale cereale* L.	- - 30 - - - - -	- - 27 - - - - -	- - 11 - - - - -	9 18 - - - - - -	- 11 - - - - - -	- - 5 - - - - -	8 20 - - - - - -	6 10 - - - - - -	- 5 - 112 3 - - -	- 7 - 41 - - - -	- - 13 - 29 - - -	- 34 - 51 - - - -	- - - 137 4 - - -	- - - - - - - -	- - 38 - 11 - - -	- - - 7 - 2 - -	- - - - 11 - - -	- - 11 - - - - -	- - - - - 412 - -	- - - 17 - - - -	- - 19 - 2 - - -	- - - 5 - - - -	- - - - 5 - - -	- - - - - - - -	- - - - - - - -		
Ulmaceae -*Ulmus* sp.	4	-	-	5	-	-	5	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**A**	+
Asteraceae -*Artemisia vulgaris* L. -*Hilianthus annus* L. -*Ambrosia* sp. -*Taraxacum* sp. -*Centauria* sp.	- - - - -	2 - - - -	4 - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- 1 - - -	- 35 37 -	- 26 -	- 5 - 2	- - - - -	1 - - - -	- - - - -	- 5 - - -	- 2 - - -	- - - - -	- - - - -	- 3 - - -	2 - - - -	- - - - -	- - - - -	**A-E A E A E E E**	+
Fagaceae -*Quercus* sp. -*Castanea* sp.	- -	2 -	2 -	- -	- -	1 -	3 -	- -	5 -	6 -	9 -	- -	- -	1 -	- -	- -	- -	1 -	- -	- -	- 1	- -	- -	- 2	**A-E A A E-E**	+
Oleaceae -*Olea europaea* L. -*Phillyrea* sp. -*Ligustrum* sp. -*Fraxinus* sp.	- - - -	1 - - -	1 - - -	3 - - -	3 - - -	- 2 - -	- - - -	38 - - -	17 - - -	17 - - -	- 2 -	- 5 -	18 - - -	363 - 2	113 - 3	5 - 5	6 - 3	11 - -	- - 3	1 - -	4 - -	32 - -	- 3 -	22 - -	**A-E A-E A E A**	+
Papaveraceae -*Papaver rhoeas* L.	-	1	-	-	-	-	8	-	-	-	5	-	-	5	-	8	-	3	6	4	-	-	-	-	**E**	-
Euphorbiaceae -*Mercurialis annua* L. -*Euphorbia* sp.	- -	- -	1	- -	- -	1 -	- -	- -	- -	- -	- -	- -	5 -	- -	- -	- -	4	- -	- -	- -	- 2	- -	- -	- -	**A-E A E**	+
Mimosaceae -*Acacia* sp.	-	-	3	-	-	-	-	15	-	-	-	2	-	2	-	-	1	-	2	11	-	-	-	-	**E**	+
Brassicaceae -*Sinapus arvensis* L. -*Brassica napus* L.	- -	- -	2 -	816	- -	- 3	37	4 -	- -	- -	- 9	93	- -	- 2	5	- -	- 3	- -	- -	- 3	- -	- 1	- -	- -	**E**	-
Lamiaceae : *Lavandula angustifolia* Miller.	-	-	-	-	1	-	3	32	11	-	2	1	1	-	-	-	-	-	-	-	-	-	-	-	**E**	-
Pinaceae -*Pinus maritima* Lamk -*Pinus* sp. -*Cedrus* sp.	- - -	- - -	- - -	- - -	1 - -	- - 1	153111-	7446-	536-	84-	13 - -	116-	-6-	3 - -	-17-	-17-	175-	9 - -	11 - 1	1 - -	2 - -	-4-	- - -	- - -	**A**	-
Rutaceae -*Citrus* sp.	-	-	-	-	1	1	-	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**E**	-
Boraginaceae -*Borago officinalis* L. -*Echium vulgare* L.	- -	- -	- -	- -	2 -	25	- -	- -	- -	2 -	- -	- -	3 -	- 7	- -	- -	4 -	-11	-5	- -	- -	- -	95	-11	**E**	-
Malvaceae -*Malva sylvestris* L.	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	5	**E**	-
Ephedraceae -*Ephedra* sp.	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-	-	-	-	**A**	-
Salicaceae																									**A-**	+

-*Salix* sp.	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	**E**	
Rosaceae																										
-*Rosa* sp.	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**E**	**-**
-*Rubus* sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-		
-*Prunus* sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-		
Tiliaceae																										
-*Tilia* sp.	-	-	-	-	-	-	-	-	-	-	1	11	-	-	-	-	-	-	-	-	-	-	-	-	**E**	+
Urticaceae																										
-*Urtica dioïqua* L.	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	13	-	21	-	-	-	-	-	7	**A**	+
Polygonaceae																										
- *Rumex acetosa* L.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	7	-	-	-	-	-	-	11	5	**A**	+
Anacardiaceae																										
-*Pistacia lentiscus* L.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	6	-	-	**E**	**-**
Typhaceae																										
-*Typha angustifolia* L.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	4	-	-	-	1	-	**A**	**-**
Araliaceae																										
-*Hedera helix* L.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	**E**	**+**

A: Anemophilous; E: Entomophilous; A-E: Anemo-entomophilous; S1: site 1 (Siridi Region); S2: Site 2 (The farm); S3: site 3 (University campus, Guelma); (-): absence / non-allergenic; (+): presence / allergenic

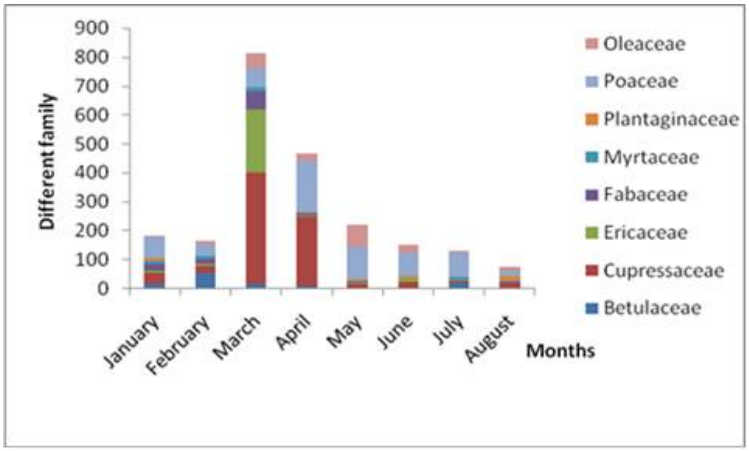

Figure 7: Monthly frequency of pollen grain in the province of Guelma.

We illustrate in figure 8 examples of identified pollen of which:

***Artemisia* sp.,** tricolpate and has a spherical form (subtriangular in polar sight and spherical in equatorial sight), average size varies between 17 and 28 µm and the exine is ornamented by small granulous elements.

***Betula* sp.,** triporate pollen with an average size located between 18 and 28 µm.

Alnus glutinosa, stephanopate pollen (4 to 5 oval pores distributed on the edges of the grain) with oblate form and average size of 19x25 µm.

***Arbutus* sp.,** tetrad tetrahedral pollen, each tetrad spore presents three complex apertures (the endoapertures are equatorial furrows). The average size varies between 30 and 65 µm and the exine is thick.

Erica arborea, tetrad tetrahedral pollen, thick exine, but the sculpture elements are often closer tending to constitute a pseudo-reticulum.

***Lavandula* sp.,** subspheric pollen (oblate), stephanocolpate, the exine is reticulate and the average size of the grain is 29 µm.

***Eucalyptus* sp.,** breviaxe grain, the equatorial contour is triangular, the exine is strongly thickened close to the apertures and the average size of the grain is 18x23 µm.

***Olea* europaea,** the pollen is tricolpate (subtriangulaire in polar sight) with a circular meridian contour. The average size is 25 µm.

Cupressus sempervirens, the pollen is inaperturate with spherical form and average size of 35 µm.

***Plantago* sp.,** periporate and spherical pollen and the diameter of the grains varies between 20 and 30 µm.

***Pinus* sp.,** the grain is flanked of two small balloons facilitating the wind transport of the pollen. The size average is 50 µm diameters.

Quercus* sp.,** the pollen is tricolpate and longiaxe, with an average diameter of 30 µm. The exine is ornamented by very many low and tightened warts. ***Poaceae, the pollen is monoporate and spherical. The average diameter, for the majority of *Poaceae*, varies between 30 and 50 µm.

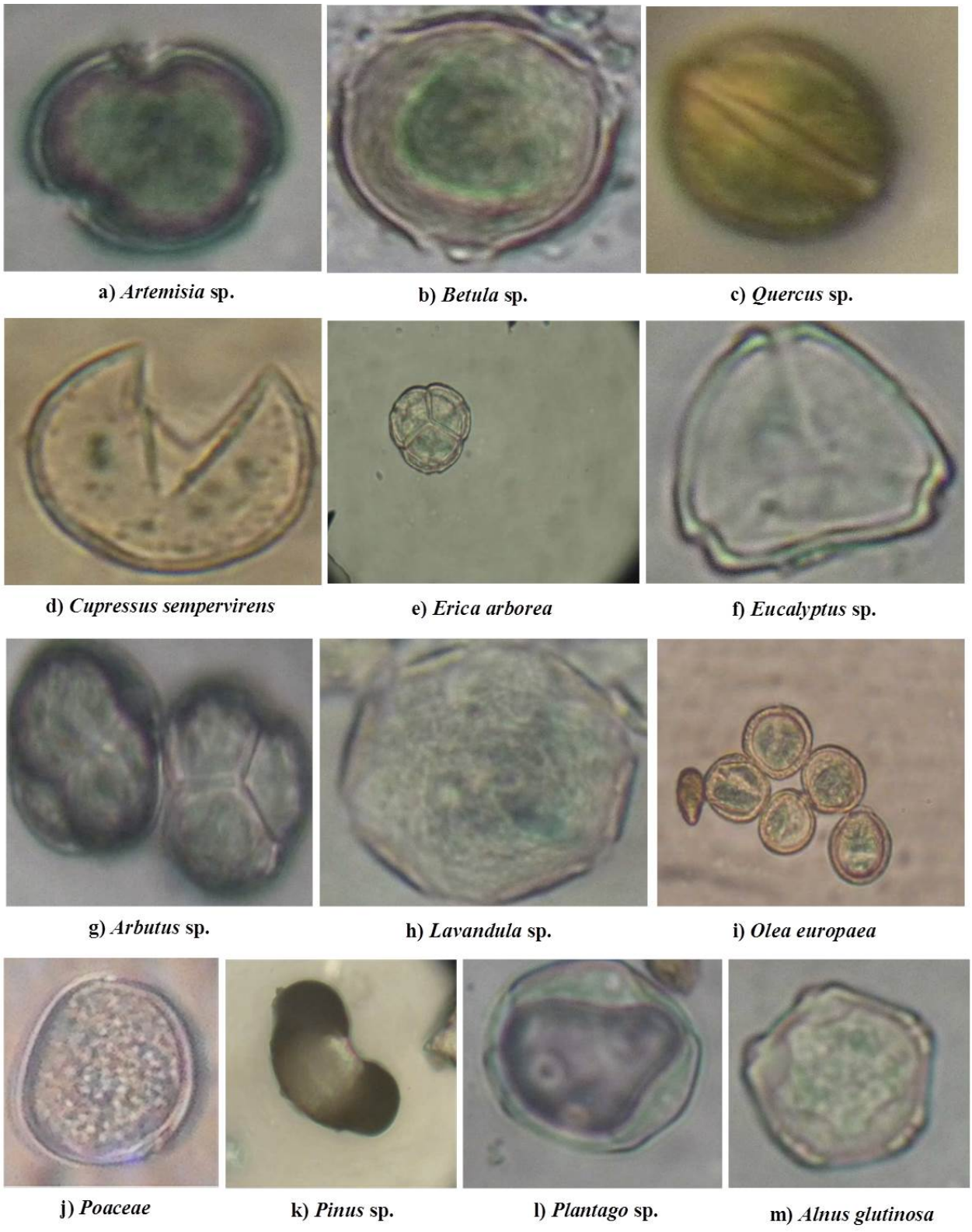

Figure 8: Pollens photographies (x 400)

(g): February 2013; (b, d, k): March 2013; (c, e, m): April 2013; (f, I, h, l): May 2013; (j): June 2013; (a): August 2013.

Discussion

During the month of March, only a single spring pic was recorded. This result differs from the previous one carried out during 2012 in the Oued Zénati region (south-west of the Guelma province), where two peaks were distinguished; the first was in April characterized by a strong anemophilous pollination and the second appeared in June with the highest rate of entomophilous pollen (Bouguenoun 2015).

In the studied sites, *Cupressaceae* ranks first among allergenic families, dominating the pollen spectrum with a total of 755 grains. Their pollens are inaperturate, with a spherical form and exine strewn with round granules (Laine et al. 2008). In France (Toulouse) atmosphere, their pollination was the most abundant and the earliest from January to March (Didier et al. 1988). The *Poaceae* come later with 649 GP where their pollination was mostly recorded between May and July (Dopazo et al. 2000). A study of 7 European countries showed that the seasonal grass pollens differ from one region to another depending on climatic and geographic conditions (Emberlin et al. 2000). Generally, this family is considered one of the most important in the world (Fernandez-Gonzalez et al. 1999) characterized by a spherical pollen, its wall is smooth and presents only one aperture which is a small round pore located at the distal pole (the pollen of Poaceae is heteropolar) (Reille 1990). *Oleaceae* occupy the third place with 215 GP. Its pollination is mainly carried out between May and June and sometimes it can start at the end of April (Dominguez-Vilches et al. 1993). Their pollens have a thick exine slimming towards the furrows, ornamented by columella forming pearls which appear darker (Laine et al. 2008). That pollen is easily transported to the atmosphere for long distances (Fornaciari et al. 2000). Therefore, the presence of these gametophytes in the atmosphere of Guelma is due to the abundance of the oleaster in the mountainous massifs surrounding the region, in addition to the intensive reforestation of the olive tree supported by the forest services in particular during the last decade. In France, *Oleaceae* are responsible on a high percentage of pollinosis, especially the olive tree (*Olea europaea*) (Mattei 2000).
For *Betulacea*e, our study counted an average of 127 GP compared to the large quantities of their pollen collected in the atmosphere of El Hadjar (Annaba) (Chafai-Ketfi et al. 2009). In Spain, it is responsible for 12% of pollinosis (Vega Maray et al. 1999). However, in France, the involvement of *Betulaceae* in pollinosis is also very noticeable (Pauli & Bessot 1999). The previous family is followed by *Asteraceae* with 80 GP and *Chenopodiaceae* (50 GP). The latter is characterized by prolonged pollination, but the highest concentrations of pollen appear in August. This suggests that the species of this family are much more resistant to the drought than the others (Carinanos et al. 2000). With a triangular form in polar sight and elliptic in equatorial sight, the surface of *Betula* sp. exine present of micro-warts (Trigo et al. 2008), around the pore, the entexine and the ectexine are clearly separated (Reille 1990). For *Alnus* sp., the pores are surrounded by rings and arcs of thicker ectexine from one pore to another (Laine et al. 2008).

Generally, it can be said that the sampling of atmospheric pollen for eight months (from January to August) enabled us to establish the first aeropalynological list, both qualitative and quantitative for the province of Guelma. The checklist established showed an almost continuous presence of pollens in the atmosphere, with a remarkable abundance of *Cupressaceae*. It was also found remarkable pollen diversity, with prolonged pollen seasons characterized by richness, especially in pollen allergens which represents a risk to public health.

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Author's contribution
CHAHAT Nora: Preparing thesis, Procurement of data, data analysis and interpretation, preparing, revising and final confirmation of the article.

BOUGUENOUN Imane: Analysis, pollen identification and interpretation of data, article preparing, article revising and confirmation of the adaptation to be published.

BOUGUENOUN Widad: Data analysis and interpretation, preparing, revising and final confirmation of the article.

HOUHAMDI Moussa: Thesis diractor, Analysis and interpretation of data, article preparing, critically revising and confirmation of the adaptation to be published.

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