

(RESEARCH ARTICLE)



## Pollinosis monitoring at middle Albania and public health impact through allergic diseases

Admir Jançe <sup>1,\*</sup>, Panajot Papa <sup>2</sup>, Adea Bajri <sup>3</sup> and Anila Jançe <sup>4</sup>

<sup>1</sup> *Imaging Department, Technical Medical Sciences Faculty, "European University of Tirana", Tirana, Albania.*

<sup>2</sup> *Nursing-Physiotherapy Department, Medical Sciences Faculty, "Albanian University", Tirana, Albania.*

<sup>3</sup> *"Turgut Ozal" High School, Tirana, Albania.*

<sup>4</sup> *Nursing - Physiotherapy Department, Medical Sciences Faculty, "Barleti University", Tirana, Albania.*

GSC Biological and Pharmaceutical Sciences, 2024, 28(02), 184–192

Publication history: Received on 07 July 2024; revised on 16 August 2024; accepted on 19 August 2024

Article DOI: <https://doi.org/10.30574/gscbps.2024.28.2.0301>

### Abstract

We have worked with the co-authors to present the impact of plant allergenic pollen-induced allergic diseases on public health in this scientific paper.

We have provided an overview of the current state of allergic disease spread for the months of March through June 2024, focusing primarily on individuals sensitive to allergic factors residing in the Tirana region.

We conducted a prick test on 328 patients who exhibited symptoms of plant pollen allergy to accomplish this goal, among other allergy tests.

In individuals susceptible to the allergenic factor, allergic pollen primarily irritates the respiratory tract and produces an iliac load.

Additionally, a study was conducted on the variability of allergic diseases according to age, gender, place of residence, and season of analysis.

Based on the collected data, the following conclusions are drawn: Most seasonal pollinosis patients (201 cases) are male and reside in rural area (216 cases); the majority of those with allergic diseases are in the 25–35 age range; the majority of those with pollen-related allergic reactions occur in the spring; and the predominant allergenic pollen species in the Gramineae family (155 cases) is the primary cause of allergic reactions.

**Keywords:** Pollinosis; Public Health; Allergenic Pollen; Allergic Diseases; Gramineae Family; Tirana

### 1. Introduction

Since the beginning of this century, the incidence of various allergic diseases has increased fourfold, especially in the last 20 years. Even though allergic disorders have been known for millennia, some allergies are now recognized as diseases that significantly affect public health worldwide.

This is a worrying trend that may represent a new kind of pandemic. Pollinosis can occasionally be fatal [1-4].

\* Corresponding author: Admir Jançe

Upon reviewing numerous studies on individuals with allergies, it is found that a considerable segment of the populace, approximately 20% of children and 12% of adults, exhibit a range of symptoms related to allergies and asthma. There is no doubt that those who have pollinosis will suffer consequences in their lifestyle, work, or educational pursuits.

Modern medicine has advanced greatly because of these cutting-edge techniques, but allergists still need the technical assistance they need to differentiate between different allergy disorders and recommend the right course of treatment for their patients [4, 5].

The main factors influencing the onset and severity of allergy disorders are certain foods, plant pollens, and air pollution, including suspended particles [7, 8].

Of course, the previously mentioned factors of environmental and air pollution brought on by unwarranted urban growth in a bullish natural environment also contribute to the increase in allergic individuals displaying symptoms from contact with plant pollen [8, 9, 10].

As our study subject, we looked at pollinosis, one of the main allergy illnesses caused by plant pollen, a major aeroallergen. We have concluded that pollinosis represents a major risk to the public health of Tirana residents based on the data we have collected.

By contributing to the identification of allergic variables that serve as risk factors in various pollinosis, we help allergists diagnose pertinent cases of pollinosis quickly and accurately, and we also provide relevant patient advice. Therefore, why not try to educate the pertinent directorates and different businesses about which plants are appropriate for ornamental purposes and which do not cause allergies in the public? [11].

Studies carried out indicate that Tirana is one of the cities in our country where cases of allergic illnesses are most prevalent. This phenomenon which is mainly dedicated to the significant pollution with microorganisms and heavy metals of the environment in three directions land-air-water [12-17].

We contend that the main factor contributing to the allergy patient data that is readily apparent in Tirana is the continuous atmospheric pollution brought on by an increase in airborne pollutants and other gases that are extremely harmful to human health. Tirana's air and soil pollution has been worsening lately, and the main reasons for this are the city's expanding population, the disappearance of natural parks, heavy traffic, the age of cars on the road, the capital's manufacturing activities, etc. [12-17].

The primary objectives of our study were to accurately determine which plants function as allergic factors, as well as to describe the identification of allergies based on the residents' age and gender. Additionally, we aimed to determine the potential impact of atmospheric air pollution on the occurrence of various allergic diseases. These tasks included both quantitative and qualitative assessments of the presence of allergenic pollen in the air for the Tirana area.

---

## 2. Materials and Methods

There are two methods that we use to reach the exact determination of the plants that cause pollinosis, and this is dedicated to their pollen that is precisely identified as the responsible allergen that causes a specific pollinosis [18, 19, 20].

One is a skin prick test, and the other is a blood test for immunological conditions [21].

By telling the patient about the plant to which they are sensitive and when the plant is pollinated, it is possible to properly protect them. This is the advantage of identifying the pollen that has acted as an allergenic factor for a specific person.

Eight plant families that are the subject of the general allergy tests are represented by twelve plant species that have been designated as plant allergens (Tab. 1) [21].

**Table 1** Plant allergens associated with the flowering period and the Family they belong to, used in allergy testing

No.	Family	Allergenic plants	Blooming season
1	Betulaceae	<i>Alnus glutinosa</i>	February - March
2		<i>Betula pendula</i>	March - April
3	Compositae	<i>Ambrosia artemisifolia</i>	July - September
4		<i>Artemisia vulgaris</i>	July - October
5	Corylaceae	<i>Corylus avellana</i>	February - March
6	Fagaceae	<i>Quercus sp.</i>	Aprile - May
7	Graminaceae	<i>Phleum pratense</i>	May - August
8		<i>Secale cereale</i>	May - July
9	Platanaceae	<i>Platanus orientalis</i>	April - May
10	Poaceae	<i>Avena sativa</i>	May - June
11		<i>Triticum aestivum</i>	June - July
12	Urticaceae	<i>Parietaria officinalis</i>	May - October

The immunological analysis of blood performed on allergic patients, which entails precisely measuring the concentration of antibodies, helps identify the allergenic component. This method calculates the concentration of immunoglobulin E (IgE) in the blood using six categories (Tab. 2) [21]. For every category, the IgE concentration is given as a kg unit per liter, or kU/l. [21].

**Table 2** Antibody attendance (expressed as kilogrammes per litre, or kU/l)

< 0,35	No specific antibodies present
0,35 - 0,7	Negligible presence
0,7 - 3,5	Little presence
3,5 - 17,5	Clear presence
17,5 - 50	Good presence
50 - 100	Very high presence
> 100	Extremely high presence

### 2.1. Identification by pollen of classified allergic plants

We began by using native plants from the Tirana region, which are the origin of pollinosis because skin prick testing uses their pollen to identify allergies.

For the best pollen grain separation, laboratory analyses were performed using Erdtman's (1960) acetolysis method [22]. Using an acetolysis mixture made up of 9:1 concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) and glacial acetic acid (CH<sub>3</sub>COOH), the material is processed using this method. Following that, glycerin is added to the precipitate, and it is viewed under an optical microscope [22-27].

The Motic BA310 microscope, which has an integrated CMOS 1/2, 3MP digital camera with 2048 x 1536 pixels and a USB 2.0 output, was used to take the pictures.

### 2.2. Determination of pollinosis based on information about the patient

The study ran from March to June of 2024. To accurately determine the allergic condition that had manifested in each of the 328 patients, initially we examined the data given by the Institute of Public Health (IPH) regarding the allergenic patients to a specific plant's pollen.

Apart from administering the allergy test, we gathered data from the patient's medical file and had conversations regarding his age, gender, birthplace, workplace, and living situation to provide the most accurate diagnosis and precisely evaluate the range of allergy symptoms.

### 3. Results and Discussion

According to laboratory analysis of pollen and statistical and analytical processing of data gathered in the Tirana region between March and June 2024 by the Institute of Public Health, a sizable fraction of the population is afflicted with pollinosis, a disease mainly brought on by pollen from Cereal plants (*Phleum pratense* - timothy and *Secale cereale* - rye).

The scientific data from March to June, spanning all four months, are merged into a single group.

**Table 3** Allergic patients to the pertinent plant

No.	Plant that triggers allergies	Cases found
1	<i>Alnus glutinosa</i>	44
2	<i>Betula pendula</i>	31
3	<i>Ambrosia artemisifolia</i>	8
4	<i>Artemisia vulgaris</i>	12
5	<i>Corylus avellana</i>	6
6	<i>Quercus sp.</i>	28
7	<i>Phleum pratense</i>	69
8	<i>Secale cereale</i>	86
9	<i>Platanus orientalis</i>	5
10	<i>Avena sativa</i>	15
11	<i>Triticum aestivum</i>	14
12	<i>Parietaria officinalis</i>	10
<i>Total allergic cases</i>		328

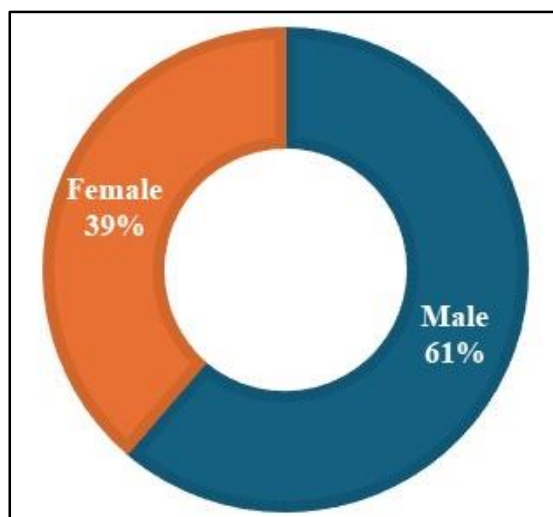
Table 3 lists the number of people who have allergies to plant pollen, based on information provided by individual families. The allergy testing was carried out using the two previously described procedures, yielding the intended results.

The *Secale cereale* plant allergen is clearly the main cause of pollinosis in patients who have had allergy testing done; in fact, it has been identified in 86 cases.

Next, we look at the plants that are important allergenic factors: *Phleum Pratense*, whose pollen causes allergic reactions in 69 patients; *Alnus glutinosa*, which causes allergic reactions in 44 people; and *Betula pendula*, which causes allergic reactions in 31 patients. Certain plants can be classified as having fewer common allergens.

We have taken into consideration the variables of gender, age, and year period that have been found in individuals suffering from pollinosis, bearing in mind that the nature and manifestations of allergies depend on the allergenic agent that causes them, as well as making this the aim of our investigation. For this reason, we have made the matching graphs for the relevant data.

Figure 1 demonstrates that of the 328 patients who were examined and evaluated, 201 were male and 127 were female, or 39% of the total.

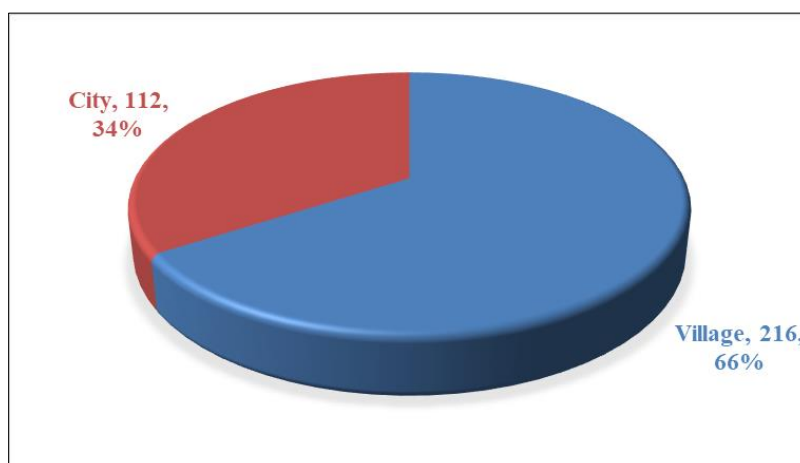


**Figure 1** Allergen distribution according to the patient's gender

According to Figure 2, approximately 216 people with allergy diseases live in villages, while about 34% of allergy sufferers live in cities.

*We think this phenomenon happens for two main reasons:*

- The plant is more common among the people living in the villages surrounding Tirana.
- There are very few green areas and high levels of air pollution in Tirana, a true metropolis [12-17].



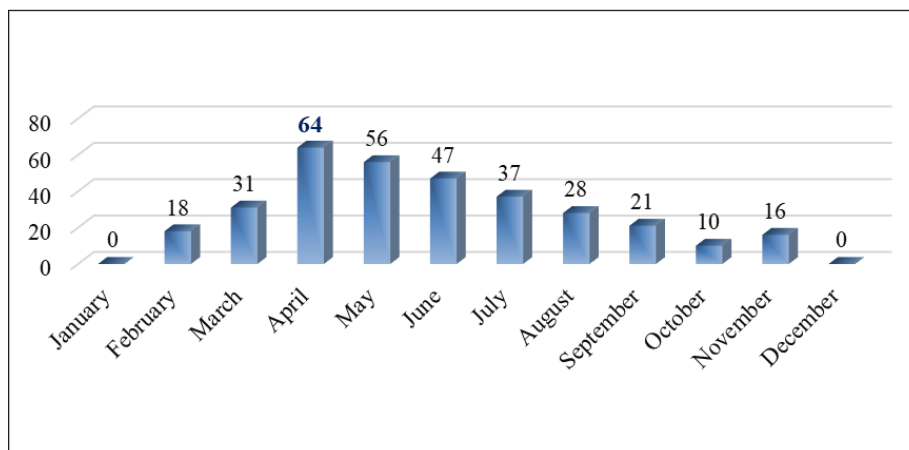
**Figure 2** Allergy distribution according to the patient's residence

The allergy patient cases that have been identified between July 2023 and June 2024 are shown in Figure 3. There is a noticeable difference between the months: May has the second-highest number of allergic individuals, while April has the greatest number of patients (64 cases) with a specific pollinosis-related diagnosis.

From our point of view, the phenomenon that has been observed is directly linked to the fact that most plants flower during these months. This corresponds with springtime, when most trees and flowers bloom.

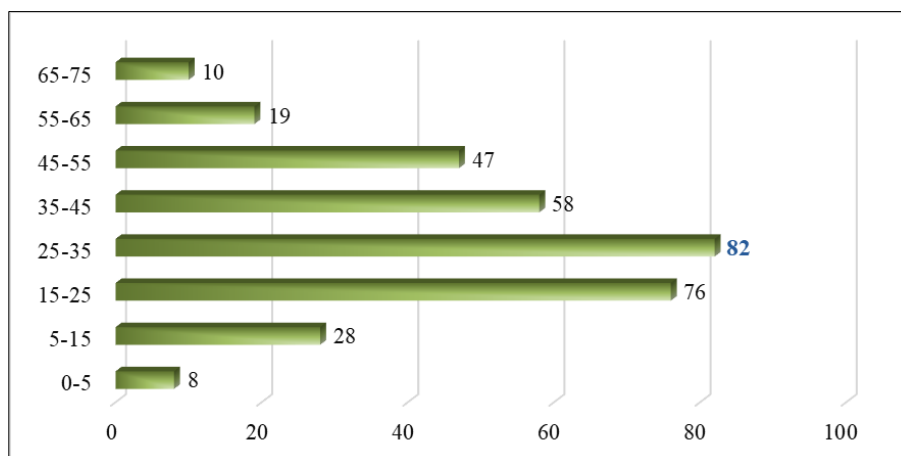
A week of flowering results in about 10 plant pollen grains/m<sup>3</sup> of air being collected every day, according to the literature review. This amount of pollen, which can reach over 100 pollen grains/m<sup>3</sup> of air over the course of a week, may be the cause of some allergic diseases in humans [28].

There is evidence, according to Becker et al. (2021), that there will always be hospital stays and medical visits for pollinosis when there are approximately 12 pollen grains/m<sup>3</sup> of air in the atmosphere [29].



**Figure 3** Allergy sufferers from July 2021 to June 2022

Figure 4 shows the distribution of allergy sufferers by corresponding age groups. Based on the gathered data, 82 cases of pollinosis have been found, indicating that a large proportion of patients fall into the 25- to 35-year-old age range. The age group of 15 to 25 years old, which includes 76 cases of allergic reactions, comes after this one.



**Figure 4** Age distribution of patients affected by pollinosis

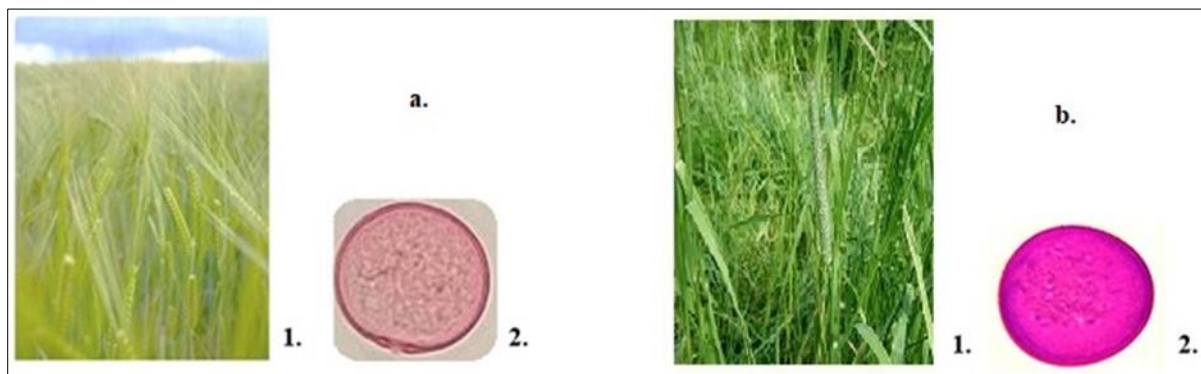
We think that the dynamics of entry and departure, which inevitably lead to the most frequent encounter of these age groups with allergy pollen, are the main cause of this observed behavior. Prolonged stays in enclosed areas and bars where smoking is allowed have also been shown to promote the occurrence of allergy illnesses, making this lifestyle choice a definite factor that significantly influences the emergence of various allergy illnesses.

Regarding the most significant allergenic factor, *Secale Cereale* (Figure 5. a.), we can identify it as an annual herb grown by humans for use as a grain, fodder, and cover crop. It belongs to the Gramineae family. Its primary application stems from its recognition as an essential component to produce bread, flour, alcoholic beverages like beer, whisky, and vodka, as well as its extensive use in animal feed. Research has shown that this plant's pollen grains contribute to a variety of pollinosis by acting as allergenic factors [1, 30, 31, 32, 33].

We can identify as the second allergenic component *Phleum pratense* (Figure 5. b.), as an annual plant that is grown by humans for use as a grain, fodder, and cover crop. It is also a member of the Gramineae family. It's commonly planted for horse hay, especially when used as cow feed. Its fiber content is relatively high, especially when cut later. [4, 34]. It is said to be a coarse, grittier grass that animals do not particularly like if mowed sooner. It provides horses with superior nutrients and is thought to be a standard ingredient in grass hay.

Timothy hay is a staple that often comprises a significant portion of the diet of domestic pet rabbits, guinea pigs, chinchillas, and degus. It usually needs nutrient-rich soils, but it can also grow in fields that have been abandoned or by roadsides. The allergen in Timothy grass pollen is used to treat hay fever [6, 35, 36, 37].

Studies have demonstrated that the pollen grains of this plant serve as allergens and thus aggravate a range of pollinosis [1, 30, 31, 32].



(Photo 1 – Rye and Timothy in their native environment; Photo 2 – Microscopic view of *Secale cereale* and *Phleum pratense* pollens); (Refer to Jançe, 2024).

**Figure 5** a. *Secale Cereale* and b. *Phleum pratense*

The pollen of plant species that are members of the Gramineae family is the main allergenic factor for many cases of pollinosis, according to a review of numerous studies carried out by both domestic and foreign researchers [30, 31, 38, 39].

In terms of the main categories of allergic diseases brought on by airborne allergen exposure, we can mainly deal with respiratory disorders such as rhino conjunctivitis, bronchial asthma, and allergic rhinitis. The lungs and respiratory system are almost always found to be severely burdened [8, 29, 31, 40, 41, 42].

As a conclusion, it is important to emphasize that the Institute of Public Health provided the data used in this study, so the conclusions are general and shouldn't be taken at face value. It should be kept in mind that the true number of allergy sufferers might be much higher, as many people might have been diagnosed as pollen-allergic after undergoing the required allergy testing at the city's many private clinics.

#### 4. Conclusion

- The results of the study showed that a significant percentage of 328 people had positive results from the allergy tests that were performed.
- Regarding the types of plants with the highest allergen factor, we mention the following (*where it is established that the first two representatives are species belonging to the Gramineae family with a total of 155 cases*):
  - In 86 cases, *Secale cereale* – Rye pollen is the main allergenic factor influencing the development of pollinosis.
  - *Phleum pratense* – Timothy is another significant allergenic factor that has been identified in 69 cases.
  - *Alnus glutinosa*, an ornamental tree, comes next because it has been identified as an allergenic factor in 44 allergic individuals.
- The majority of pollinosis, exactly 216 cases, were discovered in the villages surrounding Tirana, while 112 cases of allergy patients were found in the Tirana metropolitan area.
- About 61% of cases of pollinosis, or 201 cases, are found in men, while 39% of cases, or 127 cases of allergies, are found in women. Whereas the affected age range is 25–35 years old.
- Moreover, research indicates that the months of April through June, which coincide with most plants identified as allergic factors blossoming, are the ones with the highest frequency of allergy cases.

---

## Compliance with ethical standards

### *Disclosure of conflict of interest*

The authors declare that there is no conflict of interest for the presented study.

---

## References

- [1] Lewis WH, & Lewis PF. Medical Botany: Plants Affecting Man's Health. First Edition. J. Wiley and Sons Publ., New York-London, 1982; 544.
- [2] Lewis WH, Dixit AB, Wedner HJ. Aeropollen of weeds of the western United States Gulf Coast. *Ann. Allergy.*, 1991; 67(1): 47-52.
- [3] Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur. Respir. J.*, 2004; 24: 758-764.
- [4] Camacho I. Pollen grains as airborne allergenic particles. *Acta Agrobot*, 2015; 68(4): 281–284.
- [5] Bottelli R, Falagiani P, Galimberti M, Lenzi G, Pacini E, Rolo J. Pollen and Pollinosis. New Bookstore, Piccini Publ., Padova, 1982; 10(13): 15-20.
- [6] Barber D, Rico P, Blanco C, Fernandez-Rivas M, Ibañez MD, Escribese MM. Grazax: a sublingual immunotherapy vaccine for Hay fever treatment: from concept to commercialization. *Human Vaccines & Immunotherapeutics*, 2019; 15(12): 2887-2895.
- [7] Ducker CS, Knox BR. Pollen and people. *Biotechnology and ecology of Pollen*, Springer-Verlag, New York Inc., 1986; 399-404.
- [8] Croner S. Prediction and detection of allergy development: influence of genetic and environmental factors. *J. Pediatric*, 1992; 121(2): 58-63.
- [9] De Swert LF. Risk factors for allergy. *Eur. J. Pediatr.*, 1999; 158(2): 89-94.
- [10] Galli SJ. Allergy. *Curr. Biol.*, 2000; 10(3): 93-95.
- [11] Knox BR. Pollen and Allergy. Edward Arnold Publ. Ltd., London, 1979; 59.
- [12] Jance A, Jance A. Assessment of Chemical and Bacterial Pollution in Soil Samples from Industrial Areas of Elbasan, Albania. *International Journal of Agriculture and Animal Production (IJAAP)*, 2024; 4(03): 26-32.
- [13] Jance A, Jance A, Bogoev V. Underground Distribution of Heavy Metals in Central Albania. *International Journal of Advanced Natural Sciences and Engineering Researches (IJANSER)*, 2024; 8(4): 180-184.
- [14] Jance A, Jance A, Bogoev V. Quantitative Data on Microorganisms and Heavy Metals in Middle Albania Soil. *International Journal of Advanced Natural Sciences and Engineering Researches (IJANSER)*, 2023; 7(6): 432-435.
- [15] Jance A, Jance A, Bogoev V. Nickel Dispersion in Soil and its Effects on Agricultural Culture in Elbasani town, Albania. *Plant Cell Biotechnology and Molecular Biology (PCBMB)*, 2021; 22(1-2): 18-24.
- [16] Jance A, Bogoev V, Jance A. Description of soil impurity for Elbasan city - Albania. *GSC Biological and Pharmaceutical Sciences (GSCBPS)*, 2020; 13(02): 240-244.
- [17] Jance A, Bogoev V, Jance A. Soil pollution caused by heavy metals presence, in Elbasani town, Middle Albania. *Journal of Multidisciplinary Engineering Science and Technology (JMEST)*, 2020; 7(11): 13018-13021.
- [18] Moore PD, Webb JA. An illustrated Guide to Pollen Analysis. Department of Plant Sciences, King's College, London, 1978; 133: 216-217.
- [19] Pacini E, Franchi G. Pollen: Biology and Applications. *Biology Notebooks*, Bologna, 1978; 12: 8-53.
- [20] Faegri K, Iversen J. Textbook of Pollen Analysis. 4<sup>th</sup> ed. Wiley., Chichester, 1989; 328.
- [21] Pablos I, Wildner S, Asam C, Wallner M, Gadermaier G. Pollen allergens for molecular diagnosis. *Current allergy and asthma reports*, 2016; 16: 1-12.
- [22] Erdtman G. The acetolysis method, A revised description. *Svensk Botanisk. Tidskrift*, 1960; 54: 561-564.



- [23] Erdtman G. Handbook of palynology. An introduction to the study of pollen grains and spores. Hafner Publishing Company, New York, 1969; 486.
- [24] Jance A, Jance A. Paleopalynological Analysis of Primulaceae Family Evolution during the New Holocene Period in Elbasan, Albania. Journal of Environmental Impact and Management Policy (JEIMP), 2024; 4(03): 1-7.
- [25] Jance A, Jance A, Kapidani G. Holocene Distribution of Boraginaceae Plants in Central Albania. International Journal of Advanced Natural Sciences and Engineering Researches (IJANSER), 2024; 8(4): 175-179.
- [26] Jance A, Jance A, Kapidani G. Holocene Data on Fossil Pollen of Dipsacaceae Plants, Central Albania. International Journal of Advanced Natural Sciences and Engineering Researches (IJANSER), 2023; 7(6): 428-431.
- [27] Jance A, Jance A, Kapidani G. Pteridophyta landscape through Holocene epoch in Elbasan, Albania. Plant Cell Biotechnology and Molecular Biology (PCBMB), 2021; 22(15-16): 34-40.
- [28] Pfaar O, Bastl K, Berger U, Buters J, Calderon A, Clot B, Darsow U, Demoly P, Durham SR, Galan C, Gehrig R, van Wijk RG, Jacobsen L, Klimek L, Sofiev M, Thibaudon M, Bergman KC. Defining pollen exposure times for clinical trials of allergen immunotherapy for pollen-induced rhinoconjunctivitis – an EAACI position paper. Allergy, 2017; 72(5): 713-722.
- [29] Becker J, Steckling-Muschack N, Mittermeier I, Bergmann KC, Bose-O'Reilly S, Buters J, Damialis A, Heigl K, Heinrich J, Kabesch M, Mertes H, Nowak D, Schutzmeier P, Walser-Reichenbach S, Weinberger A, Korbely Ch, Herr C, Heinze S, Kutzora S. Threshold values of grass pollen (Poaceae) concentrations and increase in emergency department visits, hospital admissions, drug consumption and allergic symptoms in patients with allergic rhinitis: a systematic review. Aerobiologia, 2021; 37(4): 633-662.
- [30] Laffer S, Vrtala S, Kraft D, Scheiner O. cDNA cloning of a major allergen of rye (*Secale cereale*) timothy grass (*Phleum pratense*). Allergy, 1992; 47: 1-25.
- [31] D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, Liccardi G, Popov T, van Cauwenberge P. Allergenic pollen and pollen allergy in Europe. Allergy, 2007; 62(9): 976–990.
- [32] Damialis A, Konstantinou GN. Cereal pollen sensitisation in pollen allergic patients: to treat or not to treat? Eur. Ann. Allergy. Clin. Immunol., 2011; 43(2): 3-44.
- [33] Gyulai F. Archaeobotanical overview of rye (*Secale Cereale L.*) in the Carpathian-basin I. from the beginning until the Roman age. Journal of Agricultural and Environmental Science, 2014; 1(2): 25-35.
- [34] Galan C, Emberlin J, Dominguez E, Bryant RH, Villamandos F. A comparative analysis of daily variations in the Gramineae pollen counts at Cordoba, Spain and London, UK. Grana, 1995; 34: 189-198.
- [35] Kay AB. An extract of Timothy-grass pollen used as sublingual immunotherapy for summer hay fever. Drugs of Today, 2007; 43(12): 841–8.
- [36] Nelson HS. Oral/sublingual Phleum pratense grass tablet (Grazax/Grastek) to treat allergic rhinitis in the USA. Expert Review of Clinical Immunology, 2014; 10(11): 1437–51.
- [37] Scaparrotta A, Attanasi M, Petrosino MI, Di Filippo P, Di Pillo S, Chiarelli F. Critical appraisal of Timothy grass pollen extract Grazax in the management of allergic rhinitis. Drug Design, Development and Therapy, 2015; 9: 5897–909.
- [38] Prieto-Baena JC, Hidalgo PJ, Dominguez E, Galan C. Pollen production in the Poaceae family. Grana, 2003; 42: 153-160.
- [39] Pietzak M. Celiac Disease, Wheat Allergy, and Gluten Sensitivity. Journal of Parenteral and Enteral Nutrition, 2012; 36(1): 68-75.
- [40] D'Amato G, Spieksma FM, Liccardi G, Jager S, Russo M, Kontou-Fili K, Nikkels H, Wuthrich B, Bonini S. Pollen-related allergy in Europe. Allergy, 1998; 53: 567-578.
- [41] Chinn S, Burney P, Sunyer J, Jarvis D, Luczynska C. Sensitization to individual allergens and bronchial responsiveness in the ECRHS. Eur.Respir. J., 1999; 14: 876-884.
- [42] Riedinger F, Kuehr J, Strauch E, Schulz H, Ihorst G, Forster J. Natural history of hay fever and pollen sensitization, and doctor's diagnosis of hay fever and pollen asthma in German schoolchildren. Allergy, 2002; 57: 488-492.