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# AEROMYCOLOGY – MAIN RESEARCH FIELDS OF INTEREST DURING THE LAST 25 YEARS

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**Abstract:** Fungal spores occur very numerously in the air and, on account of their dimensions (several micrometers), are classed as bioaerosols. They are always observed in natural air and their concentration changes depending on environmental conditions. Aeromycology investigates their occurrence in the air of the indoor-outdoor environment. The methods of sampling can be divided into the gravimetric method when the spores fall onto a catching surface by force of gravity, and the volumetric method consisting of analysis of spores contained in a given air unit. The content of fungal spores in air is characterized by a specific seasonal and diurnal cycle. Among other things, these cycles depend on climate and weather conditions, on the accessibility of fresh substrates for the development of the fungus, circadian cycle of light and darkness, and other environmental hardly definable factors. Many fungi undesirably affect human health, cause immunotoxic diseases, and are a frequent cause of allergic diseases. Knowledge of concentrations of airborne fungal spores is especially important for agricultural and occupational medicine. Aeromycology has its application in agrobiology, particularly with respect to pathogenic fungi, and in the conservation of the artistic heritage.

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## INTRODUCTION

Numerous airborne organisms, their fragments as well as particles of biological origin passively float in the atmosphere. Small insects, bacteria; viruses; plant pollen; diaspores; fragments of tissues and talli, and such organic compounds as mycotoxins or allergens can be found in the air. Fungal spores are in this group of particles which are very numerous in the air and, on account of their dimensions (several micrometers), are classed as a bioaerosol [6]. They are always observed in natural air and their concentration changes depending on environmental conditions. Aerobiology, and especially its branch aeromycology, investigates their occurrence in the air of the indoor-outdoor environment.

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Fungi produce varied forms of spores which are differently, i.e. actively or passively released (blown away, rinsed-off or shaken out); however, their further fate usually depends on the wind [19, 31, 35, 50]. The wind is a blind vector, hence an abundant production of spores is necessary for the fulfillment of their biological function [19]. Because of their size, fungal spores can cover enormous distances with air currents [32]. The distant transport is unimportant for the development of the fungus unless its spores are viable [19, 35]; however, even a dead spore can contain an allergen dangerous for humans.

Airborne spores occur detached from the spawn which produced them. This is one of many reasons which make their precise identification difficult or even impossible. Rare spores only can be easily identified in spite of their abundance. Usually, the spores are identified as to species and the result is often debatable, hence in numerous cases the identification is limited to the type, for example *Drechslera*, *Penicillium/Aspergillus* or even to groups: "coloured basidiospores" or "hyaline ascospores" [1]. The problem is more difficult because the taxonomy of fungi is not complete [29]. Hence, Southworth [63] advises begining aeromycological investigations by concentrating on a few to several well discernible spores.

This paper is a revision of the different types of the aeromycological researches published during the last 25 years. The articles concern the seasonal and diurnal occurrence of fungal spores in outdoor and indoor environments, the influence of weather conditions on the fungal spore counts in air, the duration and course of fungal spore seasons. The second part of the references refers to the possibility of practical applications, mainly in medicine, agriculture, followed by heritage. This revision concerns mainly a European study.

## METHODS IN AEROMYCOLOGY

Various investigative techniques are used in aeromycological studies. The method of sampling can be divided in the gravimetric method, when the spores fall down on a catching surface by force of gravity, and the volumetric method consisting in an analysis of spores contained in a given air unit [35]. When the method of analysis is considered, we can speak about the method of culturing on media (e.g., the Koch sedimentation method) and microscopic analysis (the Burkard or Lanzoni apparatus). The Andersen impactor is a facility that combines the volumetric method with the culture on media. It is constructed of six segments with filters of diminishing diameters and with Petri dishes. The apparatus sucks in a definite volume of air and biological particles of specific diameter are caught on respective segments. They are then cultured on standard agar media in Petri dishes. The content of spores in air is estimated on the basis of the obtained colonies. In the Hirst spore trap (Burkard or Lanzoni apparatuses), biological particles are sampled together with sucked in air (10 l/min) and are stuck to a sticky band on a cylinder moving at a constant speed. The material is microscopically analysed and the obtained result expressed as a daily average of fungal spores/1 $m^3$ of air [6]. The assessment of the concentration of spores in a 24 h/cycle is also possible; however, in this case the viability of the spores cannot be established [35]. The different methods have their drawbacks and merits and the results cannot be directly compared, as depending on the method applied the list of spores occurring in air and reliably identification is different [31, 57, 64, 61]. Thus, if we use the culture on media we can precisely assess the occurrence of conidia of numerous species of the genera *Aspergillus* spp. and *Penicilium* spp. [24, 34, 70], but it would be very difficult to identify them using the volumetric method owing to their great morphological similarity. These fungi frequently occur in the indoor environment, produce mycotoxins noxious for living organisms, and often cause allergic, immunotoxic diseases [5, 22, 27, 28, 34]. Conidia of the genera *Alternaria* and *Cladosporium* are most frequently identified using the volumetric method because they occur at high concentrations in air at almost all geographical latitudes [1, 35, 52, 56, 68] and are important both in medicine and agrobiology [10, 11, 22, 27, 64]. Among other genera of fungi whose spores can be identified on the basis of their morphology are *Botrytis*, *Chaetomium*, *Coprinus*, *Didymella*, *Entomophtora*, *Epiccocum*, *Erysiphe*, *Ganoderma*, *Nigrospora*, *Pithomyces*, *Polythrincium*, *Stemphyllium*, *Torula* and *Ustilago* [8, 12, 16, 22, 28, 52, 57].

## FUNGAL SPORES IN AIR

The content of fungal spores of every taxon in air is characterized by a specific seasonal and diurnal cycle [7, 37, 38, 39, 51, 56, 70]. Among other things, these cycles depend on climate and weather conditions [29, 32, 41, 41, 66], on the accessibility of fresh substrates for the development of the fungus, circadian cycle of light and darkness, and other environmental hardly definable factors [27, 63]. The effect of all these factors is fairly complicated and difficult to investigate.

Airborne fungal spores occur throughout almost the whole year, but the seasonal rhythm in the occurrence of airborne spores and their spectrum depends on the type of climate [1, 7, 9, 16, 52, 61]. In the conditions of a moderate climate the maximum concentration of most spores occurs in summer or early autumn (Fig. 1) [10, 38, 57]. In Europe, differences in the pattern of these phenomena are fairly inconspicuous [12]. In tropical and subtropical regions the greatest abundance of spores is noted in cold months (November-February) and the smallest in the warm ones (May-September) [2, 30, 46]. Daily concentrations may differ significantly in the following seasons and in different habitats. The differences may concern seasonal sums of total spores, the concentrations, duration of seasons, as well as time of maximum occurrence (Fig. 1) [38, 39, 57]. Calderon *et al.* [8] stress the role of the microclimate. In highly urbanized areas the phenomenon of an urban heat island can affect seasonal and circadian changes in the concentration of airborne spores.

Weather conditions affect the sporulation, dispersal and deposition of spores and their elements correlate with each other. Conditions that prevailed a few days earlier frequently influenced the current concentrations [32, 41, 49, 61]. In spite of these complicated dependences, and the effect of weather conditions being taken into consideration, most authors divide fungal spores into the wet and dry weather. The first group consists of spores whose concentration increases in wet and warm weather. *Ganoderma*, *Leptosphaeria* and *Didymella* are classed in this group. In the circadian cycle the greatest concentrations occur at night and in early morning in conditions of the highest air

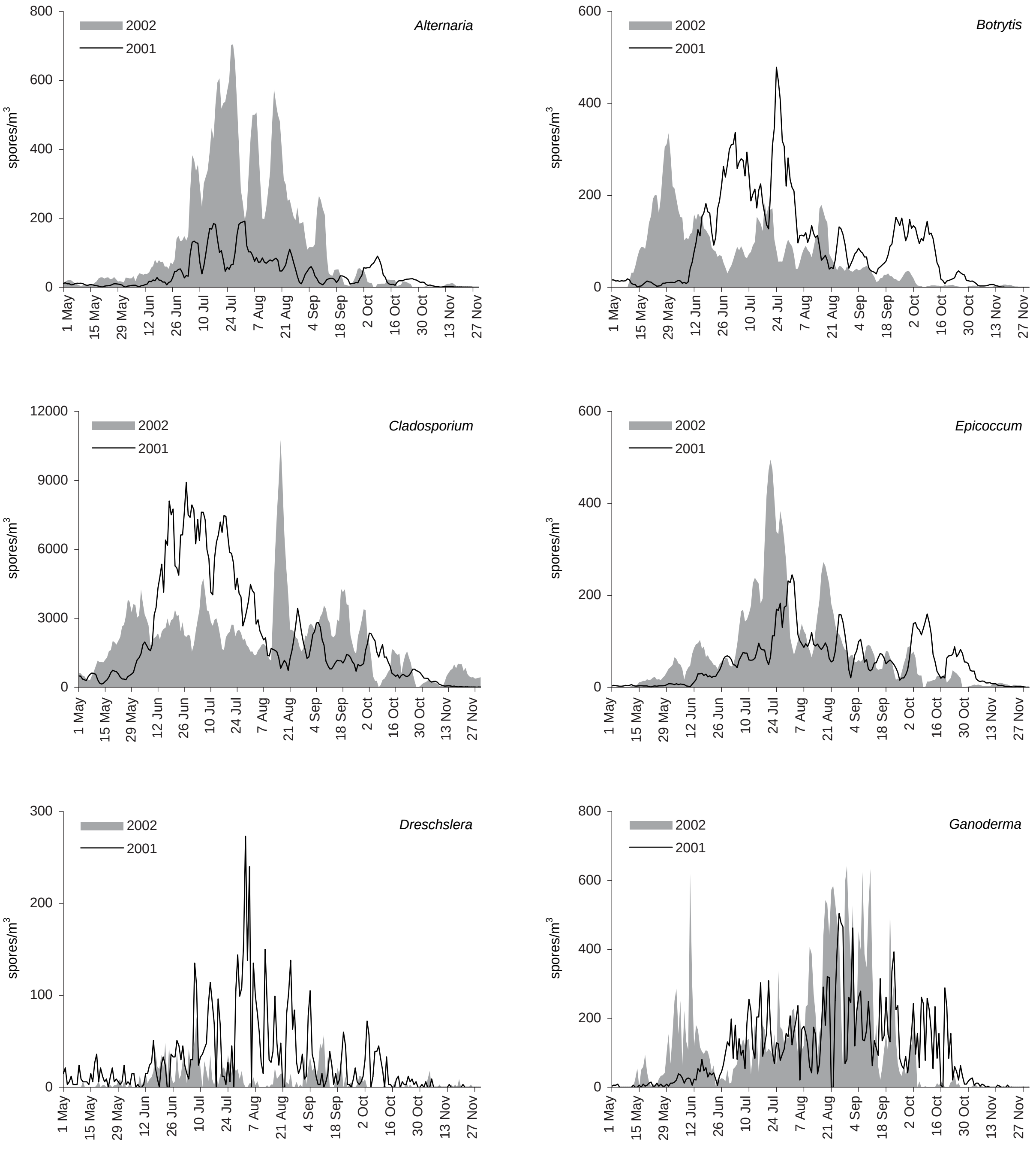


**Figure 1.** Daily fungal spore concentrations of chosen types in the atmosphere of a rural area (Krasne, SE Poland) in 2001–2002 years.

humidity [1, 7, 30, 37]. Warm and dry weather favours the development, sporulation and dispersal of *Cladosporium*, *Epicoccum* and *Alternaria* conidia (Fig. 2) [12, 33]. The greatest daily concentration of conidia of the above-given genera usually occurs at noon and in the afternoon hours, when the highest temperature prevails and the lowest values of humidity are recorded [1, 4, 37, 63, 64].

Another significant element of seasonal changes in the concentration of spores is the accessibility of substrates where they can grow. Fungal spores occur in the air almost all the year round. In winter and early spring, when the substrate for spawn development is scarce, airborne spores are absent or their concentrations are low. Larsen [42] did not record conidia of the genus *Cladosporium* only on days with a fresh snowfall.

The occurrence of fungal spores in air is also associated with ecological dependences of the parasite – host type Thus, fungi of the genus *Alternaria* are frequent parasites and saprophytes of crop plants, in this number of cereals the greatest concentrations of their spores are noted at the harvest time [9, 10, 39, 49]. In regions where the vine is grown great concentrations of grey mould spores (*Botrytis cinerea*) are recorded during the vegetation season [15, 16].

Aeromycological investigations show that the effect of external factors on the growth and development of colonies, sporulation rhythm, and the method of spore release is stronger than that of endogenous factors [35].

It is difficult to decide, which spores occur most frequently and at greatest concentrations in air since no universal investigation method ensuring final and reliable identification is available [12, 63]. Many authors claim that *Cladosporium* conidia are most numerous, recording their percentage in the annual total of spores within 40–90% [1, 3, 12, 16, 42, 52]. The literature also confirms the high frequency of other conidial spores of the genera *Alternaria*, *Epicoccum* and *Botrytis* (Fig. 1) [12, 38, 57]. Basidiospores and ascospores also occur at significant concentrations [1, 3, 8, 29, 46, 52]; however, Levetin *et al.* [43] stresses that they are rarely identified.

## PRACTICAL APPLICATIONS OF AEROMYCOLOGICAL STUDIES

The study of seasonal and diurnal rhythm in the occurrence of airborne spores is a basis for further analyses and generalizations. It also is of a great practical importance. This knowledge can be a useful tool in agrobiology, particularly with respect to pathogenic fungi. The control of airborne spores permits a better understanding of the mechanisms in the dispersal and deposition of spores, epidemiology of fungal plant diseases, and the application of suitable plant protection measures [23, 31]. Among them, the most frequently used are fungicides. Considering the fact that the concentration of spores in air changes in annual, seasonal, weekly and diurnal cycles, the use of such means, which are not neutral to people, is not and has not always been justified. On the basis of aeromycological data, high concentrations of pathogenic spores can be prognosticated and proper plant protection means applied. Such actions can limit an excessive application of chemical plant protection preparations and also result in economical benefits [15, 23]. In the North American Plant Disease Forecast Center at North Caroline State University the results of aeromycological research are implemented in practice. On the basis of continuous monitoring of spore contents in the air, information for tobacco growers about the imminent danger of pathogenic spores appears three times a week on the Internet. Maps with current dispersal ranges are presented and the time of spore transport and risk of infections estimated. Thus, the growers can undertake early protective actions [46]. Recently, the first Polish monitoring system of *Leptosphaeria maculans* and *L. biglobosa* was developed in Poznań [36]. These fungi not only cause a virulent disease of rape – the stem canker of crucifers, but also have allergenic potential [5]. The timely application of fungicides can bring benefits in agriculture and also improve the conditions of work for farmers by decreasing the concentration of allergenic spores and noxious mycotoxins.

Nowadays, people are more and more conscious that the degree of environmental pollution negatively affects not only the comfort but also the quality of our life. Fungal spores belong to naturally occurring particles in air but from the point of view of medicine they can be regarded as biological pollution. Many fungi undesirably affect human health, cause immunotoxic diseases, such as sick building syndrome, and are a frequent cause of allergic diseases [4, 18]. Numerical data concerning the frequency of sensitization to fungal allergens are varied, depending on the kind and methods applied in the investigation and on the geographical region. In Sweden and Switzerland 3–4% of patients with diagnosed asthma positively react to fungal allergens, in the United States – 80% of patients [12]. Numerous authors claim that the number of the sick, particularly of sick children, constantly increases [20, 48]. The most frequent cause of inhalant diseases brought about by fungi is the spores of the genera *Alternaria* (particularly in southern Europe) and *Cladosporium* (northern Europe) [12]. However, the list of spores inducing allergic reactions is fairly long. Of the group of anamorphous (conidial) spores, those of the genera *Botrytis*, *Epicoccum*, *Stemphylium* and *Torula*, i.e. spores, which can be reliably identified by the volumetric method using continuous aerobiological monitoring, are also allergenic [27, 28]. However, fungi of the Basidiomycetes, *e.g. Ganoderma*, *Puccinia*, *Ustilago* and *Coprinus* or Ascomycetes, e.g. *Leptosphaeria*, *Chaetomnium*, and *Erypiphe* [5], frequently cause allergies. In comparison with the allergenic plant pollen correct diagnosis, treatment and particularly immunotherapy, are much more difficult in the case of fungi. No close correlations occur between the highest concentration of spores in air and the intensity of pathological symptoms [12]. In general, standard extracts of allergens are used in immunotherapy; however, Incehour and Levetin [34] claim that the indoor species composition of the genus *Penicillium* spp. differs depending on the geographical region, environmental conditions, and also on the reliability of the identification. That is perhaps why the results of immunotherapy are not always positive. Now it is a challenge to identify all more important fungal allergens and mycotoxins and to establish threshold values inducing allergic reactions for a greater number of allergenic spores. Knowledge of the seasonal occurrence of allergenic airborne spores, their concentrations and diurnal rhythm is particularly important in assessing the etiology of allergic diseases, the more so because the seasons overlap with the seasonal occurrence of allergenic pollen grains in air [54].

Agricultural work is considered to be a major risk factor for occupational allergy diseases caused by microorganisms. Additional hazard factors are noise, vibration, change of temperature, chemical factors and irregular working time [53]. Airborne microflora is a common cause of respiratory

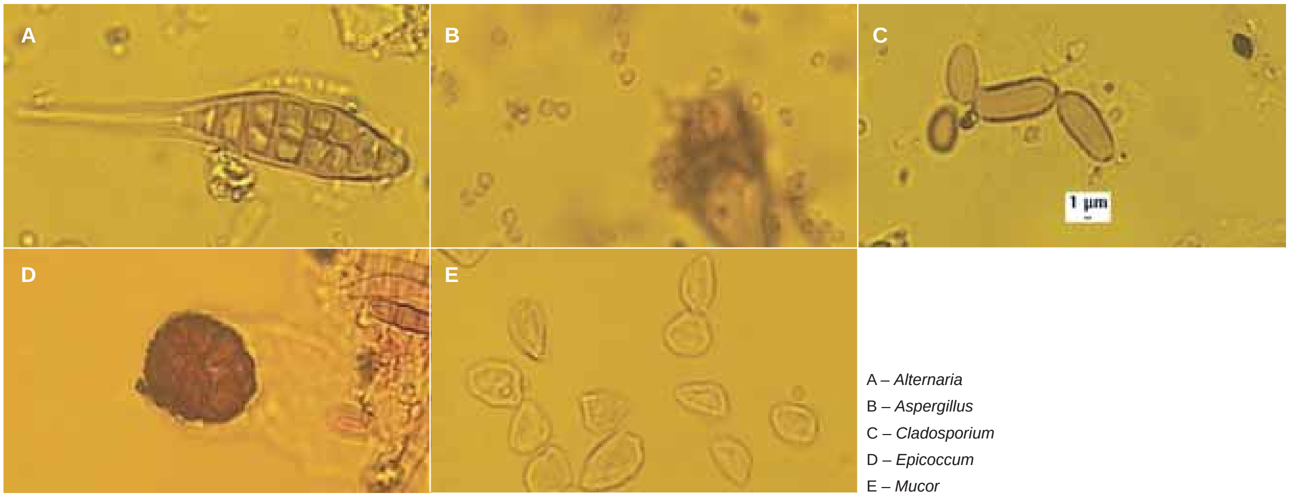


**Figure 2.** Microscopic pictures (400× magnification) of chosen fungal spores characteristic to the ambient air, dwellings and farmer's buildings.

system diseases. Farmers suffer more frequently from allergic skin disease by contact during farming activities [68]. Respiratory symptoms generally depend on the hours spent in farm buildings and surroundings [13, 60]. Farm workers are exposed to dust containing mycotoxins and spores from fungi growing on plants, herbs, grains, grain elevators, and greenhouses [25, 26, 49, 59, 62]. Spores can become airborne due to agricultural activities, from stables, cowshed, poultry farms, sheep's sheds. In animals houses the air is polluted by bacteria, fungi, endotoxins, and allergens of plants and animals. This results in increase biological health hazards to farms workers and to people living in the surroundings [3]. Concentrations of fungal spores increased inside stables when the farmers' activities are being undertaken and are positive correlated with the number of animals and their activities [55, 59, 60]. Fungi are connected with specific habitas. For example, in stables we can find especially spores of *Aspergillus*, *Penicilium*, *Wallemia sebi* (Fig. 2) [55, 60], in greenhouses *Cladosporium* and *Botrytis*, an on poultry farms *Eurotium* and therophilic fungi [59]. Farmers cultivating thyme are exposed to allergenic spores of *Aspergillus fumigatus* [25]. In rural environments there are more organic material where fungi can grow as parasites or saprophytes. This is probably the main reason why their airborne spores are more common then in the city, particularly for the taxa which are known as plant pathogens, such as *Alternaria solani*, *A. tenuis* and *Botrytis cinerea*, *B. alli*, *B. byssoidea* [39].

In is also important to recognize mycoflora inside the buildings. There are no indoor environments without fungi. Nowadays, we spend most of our time indoors and the quality of air is therefore highly important. Indoor fungal spores are strongly connected with respiratory diseases [69]. Numerous works show considerable concentrations of spores inside buildings, which can be responsible for allergy symptoms among inhabitants [24, 34, 40, 44]. We can find fungi connected with biodeterioration of materials (*Stachybotrys charatum* on dump materials). In dwellings, fungi can especially colonize walls, widows frames, damp furniture, carpets, and wallpapers. Air-conditioning systems can additionally provide sources of fungi. Indoor concentrations of fungal spores change during seasons because some fungal spores may enter from outside (like allergic *Ganoderma*), especially during summer and autumn [21, 67]. The spectrum of indoor taxa varies considerable between geographical regions, habitats and type of building, e.g. in offices there are less spore concentrations then in dwelling houses. The most frequent indoor fungal spores are *Cladosporium* (*C. herbarum*, *C. cladosporoides*), *Mucor*, *Alternaria* (*A. alternata*), *Penicillium* (*P. glaucum*, *P. notatum*), *Aspergillus* (*A. glaucus*, *A. niger*, *A. vesicolor*), *Rhizopus* (Fig. 2) [21, 40, 45].

Another interesting implementation of this type of studies is the conservation of the artistic heritage. Fungi can colonize almost everything, finding particularly favourable conditions of growth and spawn development in museums, historic buildings or libraries. Their endospores can remain viable for hundreds of years. A precise quantitative and qualitative analysis of the content of fungal spores in this type of space and their description of collected materials permit the estimation of the degree of imminent biodegradation, and the undertaking of proper protection measures [14, 17, 58].

## SUMMARY

Despite their occurrence at high concentrations in air, fungal spores have not been the objects of detailed and frequent studies. In Europe, and also in Poland, only a few centres conduct aeropalynological and aeromycological analyses. Owing to the many difficulties in identification of spores and to varied investigation methods our knowledge of their occurrence in air is still incomplete. Aeromycology is one of the ecological sciences and draws on the achievements of mycology, taxonomy, climatology and allergology. As in the case of aeropalynology, a constant development of sciences and research methods is observed and interdisciplinary studies seem to be a necessity and opportunity for interesting experiments and discoveries.

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