Original Research

Microbiological Air Monitoring around Municipal Wastewater Treatment Plants

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Abstract

This article presents microbiological air pollution around wastewater treatment plants in Słupca, Kostrzyń, and Września, and the Complex Wastewater Treatment and Wastes Composting Plant in Grodzisk Mazowiecki, Poland. The samples of air were collected using the sedimentation and aspiration methods. The largest group of microorganisms in the monitored air were psychrophilic and mesophilic bacteria and microscopic fungi. The number of psychrophilic bacteria ranged from 78 to 225,000 CFU/m^3, the number of mesophilic bacteria varied in the range from 0 to 195,000 CFU/m^3 and the fungi – from 0 to 65,700 CFU/m^3 of air. The amount of other bacteria (*Staphylococcus*, *Pseudomonas fluorescens*, and coliforms) ranged from 0 to 87,500 CFU/m^3.

A great number of bacteria and fungi has been detected near sewage aeration tanks, places of sludge disposal, and grit chambers. The interpretation of the results has been conducted according to the Polish Standards, which classify the degree of air pollution in reference to the number of microflora in the air. A change in air pollution has been noted depending on the season and climatic conditions (wind strength in particular). Research has shown that coliform bacteria, although not included in the Polish Standard, constitutes a good indicator of microorganisms' emission from sewage into the air.

Keywords: atmospheric air, bioaerosols, microorganisms, monitoring, WWTP (wastewater treatment plant)

Introduction

Free air, which is a mixture of different gases, is polluted by dust and bioaerosols that pose a threat to living organisms. The results of atmospheric pollution are visible in the form of plant and animal atrophy, as well as numerous diseases that also affect humans. In many cases the direct working environment of humans, and the harmful factors it contains, may appear to be a place of great health risk. Numerous scientific studies and surveys have shown a connection between the concentration of tiny particles constituting air pollution and bioaerosols, and the condition and mortality of the population exposed to breathing such air [1-8].

Micro- and macroorganisms as well as the structures and substances they produce constitute biological factors detrimental to health [3, 9-14]. They have a negative influence on people at work and can lead to numerous occupational ailments and diseases. Workers involved in gathering or processing wastes and sewage treatment run the potential risk of exposure to hazardous factors. In those people's

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Table 1. Evaluation of the degree of the air pollution in relation to the number of bacteria in 1 m³ of air.

Total number of mesophilic bacteria in 1 m³ of free air	Number				Degree of air pollution
	Actinomycetes	*Pseudomonas fluorescens*	*Staphylococcus* hemolysis		
			type α	type β	
less than 1,000	less than 10	lack	lack	lack	no pollution
from 1,000 to 3,000	from 10 to 100	50 and less	25 and less	50 and less	average pollution
more than 3,000	more than 100	more than 50	more than 25	more than 50	strong pollution

working environment, the source of the biological factors detrimental to their health is most often: human and animal excretion, sewage, wastes, soil, plant and animal products, and dust. These factors are transmitted through the aerial-droplet and aerial-dust route, the skin and mucous membranes, and, rarely, through the digestive tract [3, 15, 16]. Viruses, bacteria, fungi spores, and plant pollen, which can be found in the form of so-called biological aerosols, are the main elements of biological air pollution. They may lead to allergies, infectious, diseases and epidemics. A great number of human, animal, and plant illnesses are caused by microorganisms present in free air. A particular source of microbiological air pollution are municipal facilities, primarily sewage treatment plants. Wastewater treatment plants (WWTP) are considered potential sources of airborne pathogenic microorganisms. Bioaerosol emissions from wastewater treatment plants have been evaluated in several studies due to concern of exposure to surrounding neighborhood and WWTP workers [7, 17-25].

The aim of the research was to determine the degree of microbiological pollution of the air on the premises of wastewater treatment plants and to draw attention to the potential negative effects of these plants on the surroundings.

Material and Methods

Quantitative and qualitative research of microbes are conducted in accordance with the Polish Standards to evaluate microbiological pollution of the air. The present Polish air protection act does not take note of microbiological indicators of air pollution [26-27].

Taking air samples for research and marking the number of bacteria and microscopic fungi in the air are performed according to the Polish Standards [28-30]. Two methods of taking air for research have been suggested: sedimentation and aspiration. In the sedimentation method, the exposure duration of the plate with medium was 10 minutes for mesophilic and psychrophilic bacteria and fungi, whereas it was 30 minutes for the remaining microorganisms. In the case of the filter method, 10 L of air were filtered through sterile washers filled with adsorption fluid.

The examination of microbiological pollution of free air was performed at Polish Wastewater Treatment Plants (WWTP) in Słupca (52º18'0"N, 17º52'0"E), Kostrzyń (52º23'40"N, 17º13'19,6"E), and Września (52º20'0"N, 17º35'0"E), and the Complex Wastewater Treatment and Wastes Composting Plant in Grodzisk Mazowiecki (52º6'18"N, 20º37'29"E). Air monitoring studies were conducted in Grodzisk Mazowiecki in 2001-03, and in Słupca, Września, and Kostrzyn in 2004-05.

At present, the average flow of wastewaters into the plant in Kostrzyń Wlkp. is 1,138 m³/d, in Słupca 2,300 m³/d, in Września 5,883 m³/d, and in Grodzisk Mazowiecki 9,360 m³/d.

The research was conducted in all seasons. In order to evaluate the degree of microbiological pollution of free air at those places, the following microorganisms were examined:

- the total number of mesophilic and psychrophilic bacteria (nutrient agar culture)
- the number of mannitol positive and mannitol negative *Staphylococcus* (Chapman medium)
- the number of *Pseudomonas fluorescens* (King B medium and identification of colonies in UV rays)
- the number of microscopic fungi (Czapek-Dox and Waksman medium)
- the number of coliform bacteria (agar Endo medium)

The evaluation of cleanliness of the air in relation to the presence of bacteria in 1 m³ of air is performed according to PN-89Z-04111/02 (Table 1), and the evaluation of air pollution as regards the number of fungi (mold) in 1 m³ of air is performed according to PN-89Z-04111/03 (Table 2).

While examining the air using sedimentation and filter methods, higher values obtained using the two methods are adopted for the interpretation of results. In the examination,

Table 2. Evaluation of the degree of the air pollution in relation to the total number of fungi in 1 m³ of air.

Total number of fungi in 1 m³ of free air	Degree of air pollution
from 3,000 to 5,000	free air on average clean, especially in the late spring and early autumn periods
more than 5,000 to 10,000	pollution that may have negative effects on the natural habitat of humans
more than 10,000	pollution posing threat to the natural habitat of humans

Table 3. The range of the number of examined microorganisms at different measuring points at WWTP in Grodzisk Mazowiecki (CFU/m³) (n=16 samples at each).

Microorganisms	Sampling points							
	Aeration tank	30 m from secondary clarifier tank	Windrow composting system	50-70 m from aeration tanks	150 m from aeration tanks	250 m from aeration tanks	300-400 m from aeration tanks	Control station
Mesophilic bacteria	34,600-195,000	50-310	80-4,400	78-2,650	80-2,280	78-2,300	50-9,500	0-1,050
Psychrophilic bacteria	12,200-225,000	160-470	800-23,600	550-12,000	400-1,900	78-3,300	325-18,900	78-2,280
Staphylococcus mannitol positive	210-262	0-52	13-52	0-183	0-26	0-52	0-52	0-26
Staphylococcus mannitol negative	26-470	0	0-26	0-105	0-26	0-26	0	0-13
Coliform bacteria	250-1,150	0-52	26-43	0-130	0-105	0-43	0-530	0-43
Pseudomonas fluorescens at 4ºC	65-400	0-52	0-52	0-78	0-26	0-52	0-26	0
Pseudomonas fluorescens at 26ºC	625-990	0-52	13-314	26-78	0-105	0-78	0-105	0-52
Fungi (medium Czapek-Doxa)	990-265,000	240-1,890	1,570-4,950	160-14,000	160-17,000	240-12,270	80-13,200	0-3,550
Fungi (medium Waksman)	1,350-188,700	240-3,300	320-5,900	475-17,300	120-34,600	160-18,900	80-12,350	0-5,200

higher values have been most often obtained through the sedimentation method. While examining the air around wastewater treatment plants, it has been suggested that the study should also encompass the coliform bacteria not included in the Polish Standards, since the bacteria together with aerosol from sewage emerge into the air. They are also the indicator of pathogenic bacteria air pollution. Bacteria colonies and microscopic fungi, which grew on particular grounds and were counted, and the result was given as the number of cells or cell aggregates capable of developing in the form of colonies (CFU – Colony Forming Units) present in 1 m³ of air. In order to specify in detail the degree of atmospheric air pollution in WWTP in Kostrzyń, Słupca, and Września, identification of isolated microorganisms was conducted. The identification included, among other things, morphological analysis of colonies and microscopic examination of cells stained according to Gram's Method. After that, dominant strains were identified as belonging to a particular genus or species on the basis of their metabolic properties using API biochemical tests by the BioMerieux Company. Microscopic fungi were identified on the basis of taxonomical writing.

The controls stations were located windward, at the borders of the examined plants. The remaining stations were situated leeward, near those facilities thought to emit pollution, and at different distances from the examined facility (according to the wind rose). Additionally, in the course of every study, at every station the air temperature (ºC), relative humidity (%), and speed and direction of the wind were measured.

Results

Table 3 presents the results of the research gained in Grodzisk Mazowiecki. The table shows the range (minimum-maximum) of the detected microorganisms (CFU/m³) throughout the research (years 2001-03) at different measuring points.

Microbiological analysis of the biological aerosols revealed large numbers of all the examined groups of microorganisms. Heterotrophic bacteria were dominated by psychrophilic forms (from 78 to 225,000 CFU/m³). On the basis of the achieved results, strong microbiological air pollution has been identified in the direct neighborhood of sewage aeration tanks and next to compost piles coming from the city dumping ground. A high emission of microbes into the free air has been observed, both in the process of sewage aeration in active sludge radial tanks and during periodic mixing of compost piles by a compost mixer (PK-24 type). While analyzing levels of microbiological air pollution in that area depending on climatic conditions, a greater number of microbes was usually observed in the months of lower temperatures (autumn-spring) and stronger winds.

Tables 4, 5, and 6 illustrate the results of the research in Słupca, Września, and Kostrzyń Wlkp. These tables show the range (minimum-maximum) of the detected microorganisms (CFU/m³) throughout the research (in 2004) at different measuring points.

On the basis of the results of the research conducted on the premises of the WWTP in Słupca, it can be noted that

Table 4. The range of the number of examined microorganisms at different measuring points at WWTP in Słupca (CFU/m^3) (n=8 samples at each).

Microorganisms	Sampling points						
	Grit chamber	Grit separator	Aeration tank	Secondary sedimentation tank	Conventional sand drying beds	Island station roof of dray sludge	Control station
Mesophilic bacteria	160-34,000	390-3,100	320-1,100	390-630	470-6,450	710-2,650	160-430
Psychrophilic bacteria	3,000-84,200	1,900-9,900	710-6,600	1,810-5,050	2,600-37,600	1,890-11,500	200-1,340
Staphylococcus mannitol positive	0-157	0-210	0-603	0-157	26-288	0-577	0-52
Staphylococcus mannitol negative	0-315	0-79	0-26	0	0-26	26	0-26
Coliform bacteria	130-87,500	0-79	0-210	0-26	52-131	26-79	0
Pseudomonas fluorescens at 4ºC	0	0	0	0	0	0	0
Pseudomonas fluorescens at 26ºC	0-3,150	0-183	0-26	0	0	0	0
Fungi (medium Czapek-Doxa)	2,520-13,600	4,800-13,400	940-8,550	1,500-8,200	1,420-50,200	1,200-9,800	100-7,540
Fungi (medium Waksman)	240-50,000	870-23,300	710-10,700	5,350-7,900	2,650-65,700	160-16,000	260-3,790

the air at many of the research stations is highly polluted. As far as the total number of mesophilic bacteria, is concerned, no clear tendency to pollution connected with the seasons of the year was reported. While analyzing indicator bacteria it was noted that in most cases the air pollution was lower in the summer or winter and higher in autumn. A reverse situation was in the case of microscopic fungi that could be observed in large numbers in the summer. At that time it was clear that according to the Polish Standards the air pollution posed a threat to the natural habitat of humans. The highest air pollution in relation to the presence of fungi was in the vicinity of the grit chamber and sludge drying beds. As far as indicator bacteria are concerned, the place of the highest air pollution was also near the grit chamber. At all the stations, the presence of coliforms bacteria was identified, which confirms the hypothesis that they are good indicators of sewage aerosols emission into the atmosphere.

The results of the examination of air at the WWTP in Września indicate that the lowest air pollution can usually be noted in the spring time or winter and the highest in the summer or autumn. At some stations (e.g. primary sedimentation tank, open sludge digester – OKF and sludge transport), very strong air pollution by indicator bacteria and microscopic fungi was identified. The place of sludge transportation was also severely polluted because sludge was transported by a band conveyor from the sludge dewatering system to a trailer for future use in land reclamation. As a result of such practices, even a breeze led to severe air pollution. The station was also located in the close vicinity of the open sludge digester, which also contributed to high air pollution. These stations and the primary and secondary sedimentation tanks were the places of the highest emission of fungi into the air. Coliform bacteria, which can be emitted into the atmosphere at different stages of sewage treatment, were identified at all the stations.

On the premises of WWTP in Kostrzyń, at all the control stations a large number of bacteria and microscopic fungi were detected. As regards the total number of bacteria and fungi, worse results were recorded in the autumn, which made the air severely polluted at that time. A slightly cleaner air, as far as the analyzed microorganisms are concerned, was in the springtime or winter. Taking into account air pollution at different control stations, it was noted that the worst results were in the vicinity of sludge drying beds, where emission of microscopic fungi was particularly heavy throughout the research. The station of sewage from a septic tank in Kostrzyń was recently rebuilt and a hermetic facility was installed, which is unfortunately broken. The examination has shown that an application of airtight sealing to the process of sewage collection greatly reduces the level of air pollution in comparison to the old method (pouring sewage into the drain with bar racks). The presence of coliform bacteria was recorded at every examined station.

The analysis of bacteria species composition revealed that among the mesophilic microflora of the air, Gram-positive cocci, corynebacterium, spores bacilli in species *Bacillus,* and Gram-negative rod-bacterium were present. The composition of mesophilic microflora at these sewage treatment plants was similar, with the domination of Gram-negative bacteria (*Enterobacter cloacae*, *Escherichia coli*, *Flavobacterium* spp., *Acinetobacter baumanii*, *Citrobacter koseri*, and *Pseudomonas fluorescens*) and saccha-

Table 5. The range of the number of examined microorganisms at different measuring points at WWTP in Września (CFU/m³) (n=8 samples at each).

Microorganisms	Sampling points							
	Station of sewage from septic tank	Grit chamber near bar racks building	Primary sedimentation tank	Aeration tank	Secondary sedimentation tank	Open unheated standard-rate digester	Transport of sludge	Control station
Mesophilic bacteria	160-1,650	800-3,100	2,300-3,000	80-550	470-1,350	250-700	800-5350	0-240
Psychrophilic bacteria	1,550-6,700	1,180-5,030	3,400-15,100	1,200-2,850	3,100-7,200	1,500-6,600	2,200-4,500	650-1,260
Staphylococcus mannitol positive	0-52	0	0-26	0-79	26-79	0	79-131	0
Staphylococcus mannitol negative	0-26	0-79	0	0	0	52-79	26-108	0
Coliform bacteria	0-52	0-210	26-3,000	0-79	0-52	0-26	26-108	0
Pseudomonas fluorescens at 4ºC	0	0	0	0	0	0	0	0
Pseudomonas fluorescens at 26ºC	0	0-108	0-26	0	0-26	0-26	0-131	0
Fungi (medium Czapek-Doxa)	4,650-3,700	2,850-8,200	7,500-16,700	4,500-7,300	6,000-6,900	4,200-13,900	4,000-12,000	120-2,590
Fungi (medium Waksman)	320-7,300	700-8,500	1,500-22,500	160-6,500	6,900-46,600	4,400-29,300	3,500-20,100	280-3,540

Table 6. The range of the number of examined microorganisms at different measuring points at WWTP in Kostrzyń (CFU/m³) (n=8 samples at each).

Microorganisms	Sampling points				
	Activated sludge reactor and secondary clarifier	Disinfection and dewatering system	Conventional sand drying beds	Station of sewage from septic tank	Control station
Mesophilic bacteria	80-1,700	470-4,300	1,400-8,350	80-6,300	52-540
Psychrophilic bacteria	320-5,050	1,200-7,650	10,900-28,600	1,650-29,300	300-780
Staphylococcus mannitol positive	0-26	0-52	0-105	0-26	0
Staphylococcus mannitol negative	0	0-52	26	0	0
Coliform bacteria	0-52	0-650	0-450	0-52	0
Pseudomonas fluorescens at 4ºC	0	0	0	0	0
Pseudomonas fluorescens at 26ºC	0-26	0-52	0-1,200	0-26	0
Fungi (medium Czapek-Doxa)	1,950-6,900	5,300-8,200	9,300-33,700	1,650-4,900	210-1,610
Fungi (medium Waksman)	1,750-11,000	800-7,100	4,600-44,100	390-24,500	200-5,380

romycetes in genus *Candida* and *Zygomycetes*. The following, potentially pathogenic infectious bacteria, were detected in the microflora of the air bioaerosol: *Bacillus* spp., *Staphylococcus aureus*, *Escherichia coli*, *Citrobacter koseri*, *Streptococcus* spp., *Micrococcus* spp., *Pseudomonas aeruginosa* and fungi represented by *Candida albicans*. Those and other microorganisms present in the examined sewage treatment plant areas have been classified as Category II human pathogenic organisms [31]. According to the Health Minister's regulation dated April 22, 2005 [32] concerning biological hazardous factors in work environment and health protection of employees exposed to those factors, most of the microorganisms were classified as belonging to the 2nd class of danger, and it was declared that "... these factors may cause diseases among humans and may be hazardous for employees...".

Discussion

On the basis of the results achieved from free air examination, it can be claimed that the emission of bioaerosols into the atmosphere takes place at almost every stage of sewage treatment. Bacteria and microscopic fungi emitted at that time can cause lighter or heavier pollution, depending on the season. Comparing the achieved results with the Polish Standards, one can say that the degree of free air pollution can often be high enough to threaten the natural habitat of humans. If one takes into consideration the fact that there are employees on the premises of such wastewater treatment plants who are totally unprotected against the impact of the polluted air, it can be said that those people run a relatively high risk of falling ill with dangerous diseases (often infectious ones). Analyzing the size of the researched WWTP and their influence on the emission of bioaerosols, one can say that those plants have a diverse flow capacity (1,138-9,360 m^3/d). This is confirmed by observations of other authors, who found out that the quantity of emitted bioaerosol is related to type of sewage and type of microorganisms present in sewage, the type of aeration system and aeration method (large or small air bubbles).

The research of Wlazło et al. [33] identifying the risk of exposure to bacterial aerosol of the employees of a small WWTP in Myszkow (Poland) showed that the highest concentration of bacteria was around the sewage aeration tanks. The number and composition of the microflora of the air on the premises of sewage treatment plants depend on such factors as: the area of sewage exposed to atmospheric factors, the type and degree of sewage contamination, means of sewage management, and atmospheric and climatic conditions. Mixing and transporting sewage, gathering and lengthy storing of unstable biomass, as well as elevated temperature, is conducive to bioaerosols formation. A total number of bacteria, over 3,000 CFU/m^3 were identified there. However, in the vicinity of the wastewater pumping station there were only 450 CFU/m^3 of them. The obtained data were compared with plants in other countries. In Finland, for example, from $2.5 \cdot 10^3$ CFU/m^3 to $1.6 \cdot 10^4$ CFU/m^3 [34] were recorded near aeration tanks, while in Norway it was $5 \cdot 10^5$ CFU/m^3 [35]. Similarly high values of the studied microorganisms were found in the vicinity of the aeration tank in Grodzisk Mazowiecki.

In a study by Kruczalak and Olańczuk-Neyman [16] in two mechanical-biological wastewater treatment plants in northern Poland, total number of mesophilic bacteria fluctuated from 1 to 1,324 CFU/m^3 (impaction method – IM) and from several tens to 9,430 CFU/m^3 (sedimentation method – SM). The number of psychrotrophic bacteria varied in a wide range, from 14 to 5,255 CFU/m^3 (IM) and from 157 to 18,250 CFU/m^3 (SM).

The concentrations of total mesophilic bacteria were within a range of 240-7,070 CFU/m^3 in a medium-size municipal WWTP in eastern Poland [25]. The highest concentrations of airborne bacteria in the bioaerosol from municipal wastewater treatment plants of Chania (Crete, Greece) were 4-933 CFU/m^3 [12]. The highest concentrations of airborne microorganisms were observed at the aerated grit removal of wastewater at the pretreatment stage.

In a study by Brandi et al. [36], aeration basins yielded few significant concentrations of aerosols, even though they were believed to be significant sources of aerosols. Multiple meteorological factors were found to directly impact the viability of microbes found within aerosols.

An assessment of sewage workers' exposure to airborne cultivable bacteria, fungi and inhaled endotoxins was performed at 11 WWTP [6]. Results show that only fungi are present in significantly higher concentrations in summer than in winter (2,331±858 versus 329±95 CFU/m^3). It was also found that there are significantly more bacteria in the enclosed area, near the particle grids for incoming water, than in the unenclosed area near the aeration basins (9,455±2,661 versus 2,435±985 CFU/m^3 in summer and 11,081±2,299 versus 2,002±839 CFU/m^3 in winter). All bioaerosols were frequently above the recommended values of occupational exposure. The species composition and concentration of airborne Gram-negative bacteria were also studied. A broad spectrum of different species within the *Pseudomonadaceae* and the *Enterobacteriaceae* families were predominant in nearly all plants investigated. The dominance of *Enterobacteriaceae* is also indicated in our results.

Pascual et al. [5] observed that the highest concentration of heterotrophic bacteria corresponds to the pretreatment point with a median of 5,600 CFU/m^3 (min 978, max 89,750 CFU/m^3). In this way, median values of 2,200 CFU/m^3 (min 450, max 13,280 CFU/m^3) at the primary clarifier and of 513 CFU/m^3 (min 100, max 6,317 CFU/m^3) at the aeration basin are obtained.

The coliform test requirement is indicated by other authors. According to Piekarska and Traczewska [37], who examined WWTP in Bolesławiec, Kamienna Góra and Świętoszów (Poland), at a distance of 100-200 m from the source of emission (an aeration tank), a stabilization process of the concentrations of cells of higher resistance to external factors takes place. The research on the one hand also stresses the usefulness of coliform bacteria examination, which is a good indicator of air pollution by sewage, while on the other hand it criticizes accepting the total number of microorganisms as a proper indicator of air pollution. The reason for that was the elimination of the possibility of identifying pathogenic hazards. The number of microbes detected at the examined sewage treatment plants was relatively small, which suggested a lack of negative effects of these facilities on the surroundings. The presence of bioaerosols in the air can be detrimental to human health. A great number of fungi spores in the air are of great importance, since it influences the incidence of respiratory system diseases. Besides being allergens, fungi spores can also cause allergy symptoms [18, 23, 38]. A high concentration of bioaerosols can periodically occur even in cities [1, 18, 39, 40] and around city dumps [15].

On the basis of the conducted research, it can be said that bacteria and fungi aerosols emerge into the air at different stages of sewage treatment. They can constitute a potential source of hazards to people staying nearby.

Climatic conditions (mainly winds) and land form can contribute to spreading microbes, even over a long distance. In order to prevent negative effects of such facilities on the surroundings, areas of protective zones around sewage treatment plants should be defined and properly developed.

Conclusions

- The aim of our research was to determine the degree of microbiological pollution of air on the premises of Polish wastewater treatment plants (in Słupca, Kostrzyń, Września, Grodzisk Mazowiecki) and to draw attention to the potential negative effects of these plants on their surroundings.
- In the course of that examination, particular attention was paid to specific facilities that could be the source of microbe emissions.
- At some stations (e.g. primary sedimentation tank, grit chamber, sewage aeration tanks, biological reactor, open sludge digester, and sludge transport) very strong air pollution by indicator bacteria and microscopic fungi was identified.
- Coliform bacteria, which can be emitted into the atmosphere at different stages of sewage treatment, were identified at many stations.
- On the basis of the results achieved from free air examination, it can be claimed that the emission of bioaerosols into the atmosphere takes place at almost every stage of sewage treatment.
- The presence of bioaerosols in the air can be detrimental to human health and the treatment of workers.

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