

Bacterial Aerosol, Particulate Matter And Microclimatic Parameters In The Horse Stables In Poland

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Abstract

Keeping horses in good condition requires providing them with living conditions that meet welfare requirements. These animals should be accommodated with suitable space, access to high nutritional fodder and water as well as a suitable microclimate in their shelters. When it comes to the environment in the stables, a serious problem is created by particulate matter (PM), that consists bacteria. High PM concentration may be responsible for developing multiple upper respiratory tract diseases in horses, including allergies and recurrent airway obstruction (RAO). In turn, these ailments may lead to decreasing equine physical and mental fitness. Additionally, people who spend time in the stables are exposed to the same harmful factors.

The study was conducted in Udórz Stud Farm located in the southern region of Poland. The study was carried out in 2 different types of stables: 3 runners and 2 box stables. The research continued for 2 years and the samples were collected in each season. The bioaerosol samples were collected using a six-stage Andersen-Graseby cascade impactor to assess size distribution and concentrations of airborne bacteria. PM concentration was analyzed using the DustTrak™ II Aerosol Monitor 8530, while microclimate parameters were measured using the Kestrel 5000 Weather Meter.

There are almost no studies concerning size distribution of airborne bacteria, individual PM fractions and the impact of everyday handling on the changes in the bioaerosol and PM concentration. This preliminary study provided basic information on this subject. We have revealed a strong correlation between high PM and bacterial aerosol concentrations. Higher contamination levels were recorded in runners, as compared to box stables. The highest bacterial aerosol level was detected in the spring. The analysis of the fractions of the bacterial aerosol in the stables indicated the highest share of ultra-fine fraction (0.65–2.1), while respirable fraction (below 4.7 µm) exceeded 75%. It was established that the concentration of the bacterial aerosol inside the stables was many times higher than outside. It depended significantly on everyday activities undertaken in the stables, like feeding or cleaning. Taking the above into account, a different cleaning system should be developed (a wet cleaning system) and excrement should be removed more frequently.

Introduction

Living conditions created for horses should meet welfare requirements, what ensures keeping horses in good health. It requires implementing guidelines on animal hygiene and environmental sanitation, in particular with regard to maintenance, feeding and equipping stables. The requirements established for horses were defined in the Regulation of the Minister of Agriculture and Rural Development (J.L. of 2018, item 116). This document provides detailed guidelines on horse maintenance systems, including requirements that must be met with regard to stables. Two types of equine management systems (among 4 available, that is box stables, runners, stationary stables and free-range stables) are used in the research facility: box stables and runners. A box system is usually adopted for mares, stallions, sports horses and riding horses. The size of the box should enable free movement and the possibility of lying down. As the horses are herd animals, it is more natural to apply a free stall housing system – in stables called runners. Runners are used for young horses, infertile mares and mares with foals. According to the above-mentioned Regulation, horses in the stables should be provided with bedding. Bedding inside the stable should absorb moisture and protect horse's leg from the contact with a hard surface. Good-quality bedding enables reaching a suitable hygiene level, affects stables microclimate and facilitates keeping horses clean (Kołacz and Dobrzański 2006, Waran 2007, Łojek et al. 2009).

A stable is also a place where many people spend quite a lot time – either as employees involved in taking care and training horses or enthusiasts devoting their free time. We must be aware that all people staying in the stables as well as horses themselves are exposed to inhaling particulate matter. Particulate matter consists of organic components, such as saprophytes and pathogenic bacteria, spores, mites remains, plant remains and inorganic dust. PM concentration in the air inside the stable depends on the type of bedding, fodder provided to animals, animal-associated microbes and their feces (Siegers et al. 2018). PM concentration is closely related to the bioaerosol concentration, as PM represents a major factor responsible for transferring biological particles in the air (Wolny-Koładka 2018).

Particulate matter components found in the stable air may cause respiratory tract inflammation in horses through triggering allergy, infection or indirectly via overloading the pulmonary defense mechanism and a disease called recurrent airway obstruction (RAO) (Witkowska et al. 2012).

As far as the aerodynamic diameter of particulate matter (PM) particles is concerned, PM can be divided into 2 fractions: particles with the diameter exceeding 4.7 μm , that are capable of depositing in the upper respiratory tract (inhalable) and respirable, including particles with the diameter lower than 4.7 μm . In the case of humans, particles classified as respirable fraction penetrate into the lower respiratory tract, what causes inflammation and irritation. When it comes to horses, the exact size of bioaerosol particles that are capable of migrating into particular respiratory tract compartments is not known. It is assumed that respirable fractions play a major role in the pathogenesis of asthma and RAO in horses (Fleming et al. 2008, Ivester et al. 2014, Pirie et al. 2016). Additionally, it is believed that respirable fractions of both particulate matter and bioaerosol, that is with the aerodynamic diameter below 5 μm , reach the same locations in the airways of both horses and other animals (Clements and Pirie 2007, Hessel et al. 2009, Auger and Moore-Colyer 2017).

Employees responsible for handling horses may also experience health problems associated with inhaling PM. An increased incidence of asthma and decreased respiratory capacity was observed in the case of grooms (Wälinder et al. 2011).

Admissible microorganism concentrations in the air inside livestock facilities have not been established yet – only the recommendations were issued (Gołofit-Szymczak and Górny 2010). Many factors contribute to generating high PM concentrations inside the stables, and thus bacterial aerosol. Major ones include: age and type of the building, type of ventilation, facility size, livestock density, type of bedding, type of feed and microclimate conditions (Witkowska et al. 2012).

The study was aimed at establishing how different horse management systems affect the concentration of PM, bacteria and microclimate conditions inside the stables. It will allow for determining potential health risks for horses, grooms and equine enthusiasts.

Materials And Methods

The study was conducted in Udórz Stud Farm. The horse stud is located in the southern region of Poland, in the Silesian Province, in Zawiercie County. Four stables are located within the stud farm area. The first building includes 2 runners (R1 and R2), the second one runner (R3) and next two buildings include box stables (B1 and B2). The control site was located outside the stables, within the stud farm area in the distance of at least 50 m from the closest stable. During conducting the study, 84 horses were maintained on the stud farm. Particular horse groups and their age are presented in Table 1.

Table 1
Characteristics of the studied premises

Parameter	Type of stable				
	Runner 1 (R1)	Runner 2 (R2)	Runner 3 (R3)	Box stable (B1)	Box stable (B2)
Year of construction	1955	1955	1935	1976	1995
Type of ventilation	gravity ventilation	gravity ventilation	gravity ventilation	gravity ventilation	gravity ventilation
Type of litter	straw	straw	straw	straw	straw
Group of horses	12 mares. 5 foals	4 mares, 3 young mares (two years old). 5 young mares (one year old)	5 stallions 7 young stallions (two years old) 6 young stallions (one year old)	sport horses: 7 mares. 10 geldings	sport horses: 9 mares. 11 geldings
Number of horses	17	12	18	17	20
Total area [m ²]	283.8	281.6	386.8	395.5	450.3
Height [m]	3.95	3.95	4.0	3.5	3.6
Volume [m ³]	1,121.01	1,112.32	1,545.6	1,384.25	1,621.08
Surface to volume ratio	71.8	71.3	96.7	113.0	125.1
Area per 1 animal [m ²]	16.69	23.47	21.49	23.26	22.52
Mean animal weight [kg]	417.6	387.5	441.66	583.00	575.00
Total animal weight [kg]	7,099.2	4,650.0	7,949.8	9,911.0	11,500.0
Livestock units [LU]*	14.2	9.3	15.9	19.8	23.0
Ratio – kg of animal weight per 1 m ² of area	25	16.5	20.6	25	25.5
Ratio – kg of animal weight per 1 m ³ of volume	6.3	4.2	5.1	7.2	7.1
Legend: *Livestock unit (LU) = standardized to an animal weight of 500 kg					

The measurements were taken throughout the whole 2 calendar years – once each season (spring – April, summer – July, autumn – September, winter – February).

The research facilities were selected based on the following criteria:

- type of the stable (box stable, runner),
- impact of the season.

Air samples were taken using a 6-stage cascade impactor WES-710 model Andersen-Graseby (Westech Instrument, Great Britain). This impactor enables to determine bioaerosol fractions based on the aerodynamic particle size: F1: above 7 µm; F2: 4.7–7 µm; F3: 3.3–4.7 µm; F4: 2.1–3.3 µm; F5: 1.1–2.1 µm and F6: 0.65–1.1 µm. Fractions 3–6 (below 4.7 µm) are classified as respirable (called RF).

The samples were taken about the same time. The first measurement series were taken between 7.00 and 10.00 AM – before feeding and stable cleaning. The second approximately one hour after cleaning the stables, feeding horses and adding fresh bedding (11.00 AM–2.00 PM).

Air samples were collected 1.5 m above the ground, to collect the air from the human and horses breathing zone. Six Petri dishes were used to collect the samples – one for each impactor stage. The time necessary to collect the samples depended on anticipated concentrations of bacteria in a given location. The flow rate through the impactor was constant and amounted to 28.3 dm³/min. The samples were collected within 10 to 30 seconds, and the volume of aspirated air ranged between 4.7 and 14.1 dm³. The measurement in the control site – outside the buildings – took from 120 to 240 seconds (56.6 – 113.2 dm³), depending on the season. The impactor was disinfected using gauze pads moisten in 70% isopropanol before taking each sample.

TSA medium, for the culturing of bacteria, was used in the study (Triticasein Soy Lab Agar, Biomaxima Polska). Media were incubated under the following conditions: 1 day at 37°C, then 3 days at 22°C and 3 days at 4°C under aerobic conditions. Prolonged incubation was aimed at enabling the growth of slow strains at a lower temperature range (Jensen and Schafer 1998). Incubation parameters were selected to enable growth of bacteria in a wide optimal temperature range. After the incubation, the colonies were counted and the results were expressed as colony forming units per 1m³ of air (CFU/m³). The concentrations of bacteria were calculated according to the formula: $L = [Pr \cdot 1000] / v$, where: L – concentration of microorganisms in 1 m³ of air, Pr – probable statistical count according to the impactor manufacturer's table (Pr is read from the table on the basis of the number of colonies), v – volume of air taken by the impactor (dm³), 1000 – converter to 1 m³. The tests were performed in triplicate and the results were presented as the means.

The recorded bioaerosol concentrations, due to the absence of guidelines on the acceptable concentrations of microorganisms in stables, were referred to the proposal of the Team of Experts in Biological Factors (Polish: ZEGB) (Augustyńska and Pośniak 2016) on the recommended concentrations of airborne microorganisms, treating stables as working premises contaminated with organic dust (Table 2).

Table 2
Proposals for acceptable concentrations of bacteria according to the Team of Experts in Biological Factors (ZEGB)

Microbiological agent	Acceptable concentration [CFU/m ³]
Total count of bacteria (TC)	100,000
Respirable fraction of bacteria (RF)	50,000

Particulate matter (PM) concentrations were measured using a DustTrak™ II Aerosol Monitor 8530 (TSI Inc., USA) laser photometer. The device allows to measure 4 fractions of particulate matter: PM₁₀ (i.e. PM particles not larger than 10 µm), PM₄, PM_{2.5} and PM₁ (PM particles with diameters below 4, 2.5 and 1 µm, respectively) using interchangeable heads. The sampling time for each particulate matter fraction was 180 sec, with a sampling time of every 3 sec, which gave a total of 60 independent measurements. Microclimatic parameters (temperature and relative humidity) were measured using the Kestrel 4000 Weather Meter (Nielsen-Kellerman, USA).

Statistical analysis of the data was performed using software Statistica, version 13.1 – 2018 (StatSoft, Inc., Tulsa, OK, USA). After taking into account the fulfillment of the assumptions about the normality of the distribution of variables (Shapiro-Wilk's test) and the homogeneity of variance (Levene's test), the analysis of variance was performed (ANOVA and two-way ANOVA), and the significance of differences between the means was verified with the Tukey's test. The values for which the probability "p" was lower than 0.05 were considered as statistically significant. The impact of microclimatic parameters (air temperature and relative humidity) and particulate matter on the quantitative presence of bacteria in the air was assessed using Pearson's

correlation coefficient for the studied dependencies, assuming statistically significant values at $p < 0.05$ (Bulski et al. 2019, Wolny-Kołodka et al. 2017).

Results

Due to the absence of standards, the results obtained in the study were analyzed against recommendations issued by ZECB presented in Table 2. The stables are treated as “working premises contaminated with organic dust”. On that basis, permissible concentrations were exceeded in the case of 20% of measurements for the total concentration of the bacterial aerosol (TC), while for the respirable fraction (RF) it concerned 28.7% of the measurements taken (Table 3). An allowable bacteria concentration was exceeded most frequently in the spring (35% of the measurements for TC and 40% for RF), three times more frequently within runners as compared to box stables. Significantly more measurements were taken after cleaning the stables (“after bedding”; AB), when permissible concentrations were exceeded, as compared with the measurements made before replacing bedding (“before bedding”; BB): TC 37.5% vs 2.5%, while for RF: 42.5% vs 15%.

Table 3. The degree of exceeding the permissible concentrations of bacteria in relation to the ZECB guidelines [%]

Factor		Fraction	
		TC	RF
Total		20	28.7
Season	Spring	35	40
	Summer	25	30
	Autumn	10	20
	Winter	10	25
Bedding	AB	2.5	15
	BB	37.5	42.5
Stable	Runner	33.3	39.6
	Box stable	9.4	12.5

As shown in Table 4, the highest average concentration of the bacterial aerosol “before bedding” (BB) – within runners was detected in stable R1 (43,782 CFU/m³), while “after bedding” (AB) in R2 (181,132 CFU/m³). The lowest bioaerosol concentration was recorded in box stables: BB in stable B2 (23,997 CFU/m³), AB in stable B1 (45,911 CFU/m³). The assessment of the mean bioaerosol concentration according to the type of stable revealed that higher bacterial contamination occurred in runners (R1-R3), as compared with box stables, both “before bedding” (1.5 times) and “after bedding” (2.25 times). After cleaning the stable the concentration of bacterial aerosol increased 3.5 times for runners, and 2.4 times for box stables (B1 and B2). As compared against the control site (C), the bacterial concentration within runners “before bedding” was 24 times higher on average, while for box stables 16 times higher, and “after bedding”: 85 vs 38 times higher, respectively. The statistical analysis of the TC (total concentration) of the bacterial aerosol (without taking into account the season, Tab. 4) showed significant differences between “before bedding” (BB) and “after bedding” (AB) only in the R2 measuring point (Tukey’s test, $p < 0.05$). There were also statistically significant differences in the concentration of bacteria between the control sample (C) and R1 AB and R2 AB measuring points (Tukey’s test, $p < 0.05$).

Table 4
Average, standard deviation, range and total concentrations (TC) of bacteria in stables [CFU/m³] – before (BB) and after bedding (AB) [CFU/m³]

Stable	Bedding	Average±standard deviation	Range	Total
R1	BB	43,782ab**±47,418	12,425-152,853	350,259
	AB	133,763bc±130,460	11,453-348,350	1,070,106
R2	BB	27,170ab±18,056	4,134-53,449	217,362
	AB	181,132c±171,797	22,578-541,872	1,449,056
R3	BB	41,680ab±22,415	16,117-74,783	333,441
	AB	81,535abc±51,804	1,908-166,670	652,280
all R	BB	37,544a±31,518	4,134-152,853	901,063
	AB	132,143bc±129,251	1,908-541,872	3,171,442
B1	BB	24,730ab±25,308	8,201-85,406	197,838
	AB	45,911ab±30,920	22,957-121,030	367,287
B2	BB	23,997ab±14,447	3,221-44,753	191,977
	AB	71,512abc±50,713	26,251-143,032	572,097
all B	BB	24,363ab±19,911	3,221-85,406	389,815
	AB	58,711ab±42,674	22,957-143,032	939,384
C		1,554a±1,103	612-4,040	12,432
Legend:				
BB – before bedding; AB – after bedding; runners - R; box stable – B, C – control				
** averages marked with the same letters are not significantly different by Tukey's test ($\alpha = 0.05$)				

Table 5 shows the concentrations of bacterial aerosol taking into account the classification based on the aerodynamic diameter of the particles. Depending on the bioaerosol fraction and the type of stables, an increase reached the values from 232% for F6 fraction (0.65–1.1µm) in runners, to as much as 522% for F3 fraction (4.7–3.3 µm) also in runners. The total concentration of bioaerosol (TC) increased more significantly (by 111%) in runners, as compared to box stables. The difference for the respirable fraction (RF) was even higher and amounted to as much as 128%. The highest increase in the bioaerosol concentration after cleaning box stables was recorded for fraction F2 (7.0–4.7µm) – by 346%. A statistical analysis of the variability of the concentration of individual bacterial aerosol fractions (Tab. 5) showed a statistically significant increase in the concentration of bacteria in each tested fraction (F1-F6) at points runners R1-R3 “after bedding”, compared to the concentration of the bacterial aerosol at these points “before bedding” (Tukey's test, $p < 0.05$). At measuring points boxes B1-B2, there was an increase in the concentration of each tested bacterial aerosol fraction (F1-F6) “after bedding” compared to the bacteria concentration “before bedding”, but the increase was not statistically significant (Tukey's test, $p > 0.05$). Analogous results of the statistical analysis were observed for TC (total concentration) and RF (respirable fraction) for points R1-R3 and B1-B2.

Table 5

Average, standard deviation and range of bacteria concentration in stables - split by fraction

Stable	Fraction [µm]	Average±standard deviation	Range (min- max)	Average±standard deviation	Range (min- max)	Change of concentrations - BB vs AB* [%]
Runner (R1 – R3)		BB		AB		
	F1	5,334a***±4,278	867- 21,624	13,970b±13,186	212- 52,286	262%
	F2	3,123a±2,619	106- 12,797	14,493b±14,847	106- 55,668	464%
	F3	3,930a±3,454	212- 12,160	20,516b±24,065	212- 109,180	522%
	F4	5,720a±5,415	497- 21,045	26,973b±29,948	1,166- 126,034	472%
	F5	8,593a±11,071	636- 53,379	31,022b±37,978	0-154,336	361%
	F6	10,845a±11,837	530- 58,540	25,170b±26,181	212- 99,654	232%
	TC	37,544a±31,518	4,134- 152,853	132,143b±129,251	1,908- 541,872	352%
	RF	29,088a±28,392	1,908- 134,189	103,681b±107,707	1,590- 457,602	356%
Box stables (B1 - B2)		BB		AB		
	F1	2,940a±1,909	407- 8,374	7,353a±6,948	1,838- 22,048	250%
	F2	2,199a±2,054	495- 8,268	7,613a±6,757	1,131- 25,800	346%
	F3	2,719a±2,754	213- 10,888	8,824a±8,691	2,121- 36,852	325%
	F4	4,213a±4,025	142- 16,897	9,801a±7,781	1,555- 25,420	233%
	F5	5,574a±6,347	655- 27,290	11,437a±8,463	3,651- 28,408	205%
	F6	6,719a±6,276	655- 25,876	13,683a±11,103	3,690- 45,850	204%
	TC	24,363a±19,911	3,221- 85,406	58,711a±42,674	22,957- 143,032	241%
	RF	19,225a±18,093	2,230- 76,710	43,745a±31,181	17,158- 106,530	228%
Control (C)	F1	278±249	82-817			
	F2	173±178	47-540			

Legend: * - computed in relation to the average; Fractions: F1=11.0-7.0µm, F2=7.0-4.7µm, F3=4.7-3.3µm, F4=3.3-2.1µm, F5=2.2-1.1µm, F6=1.1-0.65µm

*** averages marked with the same letters are not significantly different by Tukey's test ($\alpha = 0.05$), separately for each fraction between before (BB) and after bedding (AB)

Stable	Fraction [µm]	Average±standard deviation	Range (min- max)	Average±standard deviation	Range (min- max)	Change of concentrations - BB vs AB* [%]
	F3	170±175	35-582			
	F4	197±196	53-667			
	F5	341±236	47-696			
	F6	395±202	106-781			
	TC	1,554±1,103	612- 4,040			
	RF	1,103±722	458- 2,684			
Legend: * - computed in relation to the average; Fractions: F1=11.0-7.0µm, F2=7.0-4.7µm, F3=4.7-3.3µm, F4=3.3-2.1µm, F5=2.2-1.1µm, F6=1.1-0.65µm						
*** averages marked with the same letters are not significantly different by Tukey's test (α = 0.05), separately for each fraction between before (BB) and after bedding (AB)						

The comparison of the mean concentration for individual bioaerosol fractions (Table 5) and percentage share of individual fractions (Table 6) revealed that, irrespective of the type of stables, the highest bacterial concentrations were detected for fine fractions, mainly fraction F6. Percentage share for that fraction before cleaning runners and box stables amounted to: 28.9% and 28.5%, and after the cleaning to: 19.0% and 20.0%, respectively (Table 6). A decrease in the proportion of the finest fraction AB means that particles that float into the air include bacteria mainly from the range: 2.1–4.7 µm (fractions F3-F4). The lowest bacteria concentrations and percentage shares were recorded before cleaning for the fraction F2 and amounted to 8.3% and 8.5% in runners and box stables, while after the cleaning for fraction F1: 10.6% and 11.0%, respectively.

Table 6. The share of the bacterial aerosol fraction [%] in stables: before (BB) and after bedding (AB)

Stable	Bedding	Bioaerosol fraction (RF = F3÷F6)						
		F1	F2	F3	F4	F5	F6	RF
R1	BB	13.1	8.6	10.6	15.2	23.6	28.8	78,3
	AB	9.7	15.4	16.9	16.5	19.1	22.4	74,9
R2	BB	13.4	8.5	10.1	14.4	24.4	29.2	78,1
	AB	10.2	12.3	15.8	18.6	23.4	19.7	77,5
R3	BB	13.9	8.4	10.4	14.9	23.5	28.8	77,6
	AB	10.5	11.0	15.5	20.0	24.2	18.9	78,5
all R	BB	14.2	8.3	10.5	15.2	22.9	28.9	77,5
	AB	10.6	11.0	15.5	20.4	23.5	19.0	78,5
B1	BB	13.8	8.5	10.6	15.7	22.9	28.6	77,7
	AB	10.5	11.0	15.4	20.3	23.3	19.4	78,5
B2	BB	13.6	8.5	10.7	15.8	22.9	28.5	77,9
	AB	10.8	11.2	15.4	19.8	22.9	19.9	78,0
all B	BB	13.6	8.5	10.7	15.9	22.9	28.5	77,9
	AB	11.0	11.4	15.4	19.6	22.6	20.0	77,6
C		13.5	8.6	11.2	16.0	22.5	28.2	77.9

Legend: as to table 4

The statistical analysis of the TC (total concentration) of the bacterial aerosol showed significant differences in the concentration at measuring points between the seasons (Fig. 1, Fig. 2). In the case of runners (R1-R3), statistically significant differences in the concentration of bacteria between the seasons were noted at points R1 BB, R1 AB, R2 AB, and R3 AB (Tukey's test, $p < 0.05$). At points R2 BB and R3 BB, no significant differences in the concentration of bacterial aerosol between the seasons were found (Tukey's test, $p > 0.05$). The highest significant statistical differences in the concentration of bacteria between the seasons were noted at point R2 AB (summer – autumn). In the case of box stables, statistically significant differences in the concentration of bacteria between the seasons were noted at all measuring points (B1 BB, B1 AB, B2 AB, B2 BB) (Tukey's test, $p < 0.05$). The highest significant statistical differences in the concentration of bacteria between the seasons were detected in point B2 BB (spring – autumn).

The analysis of the proportion of the respirable fraction (RF) in the total concentration of bacterial aerosol (TC) depending on the season (table 7) indicated that the highest proportion of RF occurred in the autumn in all runners (82–86.8%) and box stable no. 2 (84.1%). In the stable B the highest proportion of RF in TC was detected in the spring (88.0%). The lowest content proportion for RF was obtained for most research facilities in the summer (R1, R3, B1), and for the remaining ones (R2 and B2) in the winter.

Table 7. Bacterial aerosol: RF share in TC depending on the season [%]

Stable	Season			
	Spring	Summer	Autumn	Winter
R1	83.3	63.8	86.8	76.2
R2	80.8	81.9	83.4	72.8
R3	75.3	72.8	82.0	80.1
all R	79.8	72.8	84.0	76.4
B1	88.0	63.9	79.0	70.7
B2	73.1	70.1	84.1	72.4
all B	80.6	67.0	81.5	71.5
C	72.7	59.2	88.0	66.1

Legend: as to table 4

The assessment of the air quality inside the stables covers the occurrence of bacterial intoxication. This phenomenon happens when the concentration of bacteria indoors (I) is higher than outdoors (O) (Table 8). Especially high intoxication level was observed in the stable R2 in the summer AB; the bacterial concentration inside this facility was 380-fold higher compared to the control site. The lowest intoxication level was recorded in the winter AB in the stable B2 (1.5x). The lowest average intoxication occurred in the stable B2, while the highest in the stable R2. The average intoxication for all measurements “before bedding” (BB) amounted to 22.8, while in the case of measurements taken “after bedding” (AB) the intoxication level increased more than 3-fold and reached the value of 76.5. The average intoxication level for runners amounted to 62 and was almost 2-fold higher than in box stables.

Table 8
Inside/outside ratio (I/O) depending on the season and the horse care system

Stable	Season								Average
	Spring		Summer		Autumn		Winter		
	BB	AB	BB	AB	BB	AB	BB	AB	
R1	55.2	107.8	30.5	150.2	11.1	9.7	16.4	82.5	57.9
R2	30.1	118.5	21.2	379.5	19.5	30.2	5.9	51.4	82.0
R3	15.0	66.3	32.9	79.0	54.3	87.0	19.1	14.7	46.0
all R	33.4	97.6	28.2	202.9	28.3	42.3	13.8	49.5	62.0
B1	31.8	48.8	22.2	36.1	7.0	30.7	6.5	12.5	24.5
B2	18.8	57.3	32.8	130.2	24.6	22.8	1.5	15.3	37.9
all B	25.3	53.1	27.5	83.2	15.8	26.7	4.0	13.9	31.2

Table 9
Particulate matter divided into fractions [$\mu\text{g}/\text{m}^3$]

Particulate matter fractions	Runners (R1 – R3)				Change in relation to the average particulate matter fraction concentrations [%]
	BB		AB		
	Average \pm standard deviation	Range	Average \pm standard deviation	Range	
PM10	177 \pm 115	39-636	368 \pm 234	64-1,110	208.0
PM4	155 \pm 73	36-298	276 \pm 143	54-592	178.4
PM2.5	143 \pm 73	33-314	230 \pm 116	49-454	160.4
PM1	135 \pm 66	28-258	225 \pm 162	39-847	166.3
Box stables (B1 – B2)					
	Average \pm standard deviation	Range	Average \pm standard deviation	Range	
PM10	169 \pm 85	49-370	305 \pm 153	140-740	180.4
PM4	156 \pm 76	43-318	254 \pm 88	120-412	162.4
PM2.5	148 \pm 71	38-282	216 \pm 78	110-387	146.2
PM1	134 \pm 65	32-244	182 \pm 68	66-321	135.6
Control (C)					
	Average \pm standard deviation	Range			
PM10	117 \pm 53	41-191			
PM4	115 \pm 56	43-189			
PM2.5	117 \pm 61	42-196			
PM1	107 \pm 61	31-198			

The study also involved monitoring particulate matter contamination inside the stables (Table 10). The highest concentration was recorded for PM₁₀ fraction and in runners amounted to 177 $\mu\text{g}/\text{m}^3$ before cleaning and to 368 $\mu\text{g}/\text{m}^3$ after cleaning, in box stables dustiness was slightly lower. In both types of stables, BB dustiness increased: from 166.3–208% depending on the fraction for runners, and for boxing stables from 135.6–180.4%. The dustiness of the air in the stables in relation to the atmospheric air at the control site was at most several times higher.

Table 10. Average particulate matter concentrations (average calculated from all particulate matter fractions) depending on the season, the horse care system - before (BB) and after bedding (AB) [$\mu\text{g}/\text{m}^3$]

Stable	Season							
	Spring		Summer		Autumn		Winter	
	BB	AB	BB	AB	BB	AB	BB	AB
R1	146	171	216	244	151	205	123	350
R2	139	234	162	243	160	282	121	263
R3	160	365	154	148	174	357	124	434
all R	148	257	177	211	162	281	123	349
B1	134	170	158	248	168	248	127	227
B2	128	174	178	188	187	271	132	388
all B	131	172	168	218	178	259	130	308
C	120		56		141		140	

The calculations of the average particulate matter concentrations in the stables shown in Table 11 were made assuming that the average PM concentration at the control site is constant irrespective of the season and amounts to 100 µg/m³. Taking into account this criterion, the lowest PM level for all studied stables BB was recorded in the winter. It was found that the air in the winter in all stables contains less particles than the control site. The lowest PM concentration was observed in the spring after cleaning (AB) in almost all stables. The heaviest air contamination with particulate matter was recorded in the summer. The lowest PM increase caused by cleaning was observed in the summer – by 19% in runners and by 30% in box stables, on average. Contradictory results were obtained for the measurements taken in the winter. In that case, an increase in PM concentration amounted to 185% and 137%, respectively.

Table 11

Relative average particulate matter concentration in the stables (assuming that at the control it is constant in all seasons and amounts to 100 µg/m³) and the change in concentration between BB and AB

Stable	Season											
	Spring			Summer			Autumn			Winter		
	BB	AB	Ch	BB	AB	Ch	BB	AB	Ch	BB	AB	Ch
	[µg/m ³]	[µg/m ³]	[%]	[µg/m ³]	[µg/m ³]	[%]	[µg/m ³]	[µg/m ³]	[%]	[µg/m ³]	[µg/m ³]	[%]
R1	122	142	17	389	438	13	107	145	36	88	250	183
R2	116	195	68	292	437	50	114	200	76	86	188	118
R3	133	304	129	277	267	-3	123	253	105	88	310	251
all R	124	214	73	319	381	19	115	200	74	88	249	185
B1	112	141	26	284	446	57	119	176	47	91	162	79
B2	107	145	35	320	338	6	133	192	45	95	277	193
all B	109	143	31	302	392	30	126	184	46	93	220	137
C	100											
Legend: Ch - change of particulate matter concentration between measurements: BB and AB												

The lowest average temperatures were recorded in the winter, while the highest in the summer (Table 12). The difference between the temperatures measured within runners and box stables did not exceed 1.3°C. Higher temperatures were observed

in runners in the spring, summer and autumn. Temperature variations inside studied stables were usually minor and reached up to 5°C only in the spring. The values obtained for relative humidity in the spring and winter were comparable and fell within the range of 43.9–74.7%. The highest relative humidity was recorded for autumn measurements. No significant differences between relative humidity in two types of stables were detected. Relative humidity increased by few percent, as compared with the control site in three seasons – spring, autumn and winter. As a counterbalance – in the summer humidity inside the stables was several times higher. However, statistical analysis failed to indicate any significant effect of microclimate parameters (temperature and humidity) on the bacterial aerosol concentration (Pearson correlation, $p>0.05$).

Table 12
Microclimatic parameters depending on the season and the horse care system

Season	Type of research point	Microclimatic parameters			
		Temperature [°C]		Relative humidity (RH) [%]	
		Average±standard deviation	Range	Average±standard deviation	Range
Spring	all R	14.8±4.8	8.9-23.0	57.5±6.7	43.9-68.5
	all B	15.3±4.9	10.6-21.0	56.3±6.3	46.3-64.7
	C	15.3±5.3	11.5-19.0	63.5±8.5	57.5-69.5
Summer	all R	25.1±2.0	23.2-29.6	72.8±7.1	57.8-83.7
	all B	26.4±1.7	24.2-29.3	69.9±6.1	57.5-76.4
	C	28.4±2.3	26.8-30.0	58.9±12.9	49.7-68.0
Autumn	all R	11.2±1.1	9.9-12.7	72.7±2.6	68.3-75.7
	all B	12.2±1.3	11.0-13.4	72.0±7.4	61.2-77.3
	C	10.1±1.6	8.9-11.2	83.3±4.2	80.3-86.2
Winter	all R	5.9±1.5	3.1-7.5	57.2±7.8	46.4-74.7
	all B	5.5±1.4	3.1-7.0	57.9±4.0	51.6-62.4
	C	-3.5±2.1	-5.0- -2.0	64.9±21.7	49.5-80.2

Positive correlations were found between individual bioaerosol and particulate matter fractions (Table 13). It is common knowledge that bacteria is transmitted through the air not itself but thanks to particles. The results presented here confirm this fact, as we have established a positive correlation between bioaerosol and particulate matter fractions (Pearson correlation, $p<0.05$). It means that the majority of particulate matter inside studied livestock facilities constituted biological particles, not dust particles (grain dust). An interesting fact is that the study documented a negative correlation between the temperature and all PM fractions.

Table 13
Correlations between the examined parameters

	F1	F2	F3	F4	F5	F6	RF	TC	PM10	PM4	PM2.5	PM1	temp
F2	0.68												
F3	0.77	0.85											
F4	0.77	0.82	0.93										
F5	0.68	0.67	0.80	0.83									
F6	0.57	0.70	0.71	0.70	0.83								
RF	0.75	0.81	0.92	0.93	0.95	0.88							
TC	0.80	0.85	0.94	0.94	0.93	0.87	0.99						
PM10	0.36	0.39	0.32	0.34	0.19	0.23	0.28	0.32					
PM4	0.34	0.31	0.29	0.30	0.18	0.28	0.28	0.30	0.89				
PM2.5	0.24	0.23	0.17	0.19	0.08	0.18	0.16	0.19	0.69	0.88			
PM1	0.17	0.16	0.14	0.14	0.08	0.17	0.14	0.15	0.84	0.87	0.72		
temp	-0.08	-0.03	0.04	-0.01	0.02	0.07	0.03	0.01	-0.33	-0.35	-0.40	-0.34	
RH	-0.17	-0.08	-0.06	-0.04	0.04	0.04	0.00	-0.03	-0.11	-0.03	-0.02	-0.04	0.22

Discussion

The concentration of airborne microorganisms inside the stables depends on many factors, among others, type of horse management system, feeding system and bedding, the number of horses, feeding schedule, horses' health and the surface-area-to-volume ratio (Budzińska et al. 2016). In the case of stables analyzed in this study, the surface-area-to-volume ratio ranged between 71.8 and 96.7 for runners, while for box stables it fell within the range of 113.0–125.1.

In stables, similarly to other livestock facilities, the concentration of bacteria was few to several dozen times higher as compared with residential buildings. It depends on multiple factors and the most important ones include animal density, type of feed and the presence of feces. Despite the fact that admissible concentrations of airborne microbial contamination seem to be high, they were exceeded in the case of 1/5 of TC measurements for the bacterial aerosol and for more than 1/4 of measurements for the bioaerosol RF. In our study, the recommended bioaerosol concentration was exceeded most frequently in the spring, and thus the highest concentrations were recorded in that season too (Table 3). These levels were slightly lower in the summer, what means that warm seasons are distinguished by worse bacteriological conditions. Witkowska et al. (2012) detected the highest bioaerosol concentrations in the summer. The occurrence of the elevated bioaerosol concentrations in warm periods is understandable, as an increased temperature and suitable humidity stimulate bacterial growth and their migration into the air (Perrin 2021). Seasonal variations in bacteria concentration were confirmed by Budzińska et al. (2016). Bacterial concentrations from the spring to autumn fell within the range of 104,000–590,000 CFU/m³, while the concentrations recorded in the winter were one order of magnitude lower. Samadi et al. (2009) detected very low bioaerosol concentrations in the stables. Our results were several dozen times higher as compared with the measurements obtained by these authors. On the other hand, bacterial concentrations measured by Witkowska et al. (2012) covered a lot wider range, with our results falling within that limits. Even higher concentrations were detected by Sowińska et al. (2015) who conducted research in box stables during autumn and winter: 447,000–1,175,000 CFU/m³.

As mentioned above, particulate matter and bioaerosol concentrations depend to a large extent on the type of bedding used in the stable. Straw bedding was used in the studied facilities. A fresh layer of straw was added every day in both stable types. Old straw bedding was replaced at different frequency: on average, once a month in runners and once per week in box stables.

It translates into the obtained results – bioaerosol concentrations in runners were significantly higher than in box stables. The differences reached 250%. Fleming et al. (2008) obtained the lowest bioaerosol concentrations when straw pellet was used as bedding. Irrespective of the material applied as bedding in the stable, the authors revealed a significant increase of the airborne particle concentration during cleaning the stable, especially when the straw was used. As reported by Claußen and Hessel (2017), the lowest concentrations for both bioaerosol and particulate matter were detected when the boxes were thoroughly cleaned from the straw and excrement on a daily basis. Higher concentrations were obtained when additional straw layers were added and feces were not removed. These observations confirm the results obtained in the course of our study.

The effect of the season on the bacterial concentration is unquestionable. Witkowska et al. (2012) suggests that high bacterial contamination of the air in the winter inside the stables results from poor ventilation (closed doors and windows), while higher contamination levels in the summer are probably associated with increased temperatures and humidity that encourage microbial growth.

There is no literature data available regarding bioaerosol fraction and the proportion of respirable fraction to compare with the results obtained in this study. We can only make a comparison with the results delivered in the studies conducted in zoological gardens concerning similar animals, e.g. giraffes or camels. The air in the facilities dedicated for giraffes in the zoological garden in Cracow was characterized by a different grain distribution – higher parentage shares were obtained for larger particle fractions and it amounted to 59% for the respirable fraction, while in the stables after cleaning (AB – these conditions were applied during carrying out research in zoological gardens) it reached the value of 68% (Grzyb and Lenart 2019). Contradictory results were obtained in the facilities in other zoological garden – in Chorzów. In that case, RF share was even higher and amounted to 74% for giraffes and as much as 86% for camels (Grzyb and Pawlak 2021).

As far as the problem of bacterial intoxication inside livestock facilities is concerned, it has not been studied and explained in detail yet. Available literature provides data regarding a vet clinic (Bulski et al. 2019) or facilities for other livestock animals, but not horses. Matković et al. (2007) studied intoxication occurrence in the stable and reported that bioaerosol concentrations indoors were 73-fold to 102-fold higher depending on the time of the day.

Polish legislation provides for the admissible concentration of PM_{10} and $PM_{2.5}$ in the atmospheric air. Direct comparisons can be made only with regard to a limit value for PM_{10} , as the value indicated for fraction $PM_{2.5}$ regards the whole calendar year. The maximum admissible PM_{10} concentration amounts to $50 \mu\text{g}/\text{m}^3$, what means that permissible PM_{10} concentration in the studied stables was exceeded at all times, especially after cleaning. On the other hand, Fiedorowicz (2007) claims, based on animal hygiene handbooks, that the maximum particulate matter concentration inside the stable cannot exceed $3 \text{mg}/\text{m}^3$. In that case, permissible particulate contamination in the studied facilities was not exceeded.

The evaluation of the particulate matter content inside the stables revealed that the highest concentrations AB occur in the summer and autumn, while BB in the winter (Table 10). However, when we standardize particle concentration against the control site (Table 11), particulate content changes – the highest PM concentrations were detected in the summer and were several dozen percent higher than in other seasons. High PM levels generated in a warm season were not compensated by better ventilation supported by opening doors and windows in the stables. Poor ventilation in the winter was reflected in the indicator of the changes in PM concentration BB in relation to AB. Similar patterns were noticed by Elfman et al. (2009).

Riihimäki et al. (2008) reported approximately 3-4 higher PM concentrations in box stables for racehorses in Sweden, as compared with the results obtained in our study. Millerick-May et al. (2013) reported that in high stables in the breathing zone both for groomers and horses the concentration of particulate matter is lower – both for PM_{10} and $PM_{2.5}$ fractions. It results from more efficient ventilation and greater air volume inside the stable.

Claußen and Hessel (2017) suggest that using wet cleaning systems with opening doors and windows has a significant impact on the particulate level in box stables.

The results delivered by Wålinder et al. (2011), who measured two PM fractions: total particulate matter (PM₁₀) and respirable particulate matter (PM₄), are consistent with the data obtained in our study for the runner BB. The measurements made by Wålinder et al. (2011) fell within the following ranges – for PM₁₀: 100–790 µg/m³ (mean: 210 µg/m³), and for PM₄: 40–410 µg/m³ (mean: 100 µg/m³).

The studies undertaken by Clements and Pirie (2007) on the correlation between particulate matter contamination and the type of bedding and feed produced very interesting results. The lowest concentrations of the respirable fraction (PM₄) were recorded when wood shavings were used as a bedding material and animals were fed with silage (the mean particulate matter concentration amounted to as little as 26 µg/m³). When the straw was used as bedding and the horses were provided with hay as fodder, PM concentration amounted to 87 µg/m³, on average. Siegers et al. (2018) applied the same bedding/fodder pattern in runners and reported that PM₁₀ concentration amounted to 140 µg/m³ for the first setup and 1,100 µg/m³ for the second one.

Our results are in conflict with the ones delivered by Wolny-Koładka (2019). The author conducted research in 2 box stables and reported a significant increase (even 6-fold) in PM concentration in the winter, as compared to other seasons.

Microclimatic conditions, temperature and relative air humidity, exert considerable influence on the horses health and wellbeing. Exceeding optimum values for these parameters may result in deteriorating both physical and mental condition of the horses (Budzińska et al. 2016). Adult horses in comparison with foals display higher tolerance to low temperatures (Kończ and Dobrzański 2006, Kośla and Porowska 2013). According to Kośla (2011), the minimum temperature inside the stable for adult horses should not fall below 4-6°C. In our study, the minimum temperature was slightly lower and amounted to 3.1°C, while the maximum temperature recorded in the summer reached almost 30°C. The temperatures measured by Kośla and Porowska (2013) were comparable; however, the minimum temperature was moderately lower (reached 2.5°C), while the maximum was almost the same. Bombik et al. (2011), who conducted research in box stables in Mazury in the spring, recorded temperatures covering the following range: 8.5–14.4°C; a temperature range in our study was significantly broader (8.9–21.0°C).

As far as our study is concerned, the average temperature was higher in box stables – in the period from the spring to autumn (0.5–1.3°C), while in the winter higher temperatures were detected in runners (by 0.4°C). Slightly different results were obtained in the study carried out by Kwiatkowska-Stenzel (2011), who recorded lower temperatures in runners than in box stables throughout the entire year. Bombik et al. (2009) reported a significantly higher temperature difference in the winter between different types of stables: the temperature in a stationary stable was lower by 4°C as compared to a box stable.

Relative humidity recorded in our study in the stables fell within the following range: 43.9–83.7%. The measurements of relative humidity made by Bombik et al. (2009) covered similar range (50.7–89.1%), with few percent higher upper limit. Pursuant to the Regulation of the Minister of Agriculture and Rural Development of 2017, relative humidity shall not exceed 80%. When it comes to our study, this threshold was only slightly exceeded. Higher maximum relative humidity was recorded by Sowińska et al. (2015) – it reached as much as 92.26%. Relative humidity inside the stables in relation to the control site in the summer was higher by several percent. However, Budzińska et al. (2016) demonstrated a similar correlation in the winter. The author suggests that it depends on the shape of the stables and the quality of the building insulation. To sum up, most microclimate measurements meet animal hygiene standards.

Conclusions

Based on the results obtained in the course of a 2-year study, we can confirm that both airborne bacterial and particulate contamination is higher in runners than in box stables. A statistically significant correlation between all studied particulate matter and bioaerosol fractions was found. Recommended bioaerosol concentrations were exceeded three times more frequently in runners. High bioaerosol concentrations were recorded mostly in the spring. The activities performed by the staff in the stables, especially cleaning and feeding horses, have a significant impact on the level of the studied particles in the air.

Bioaerosol concentrations correlated with microclimatic conditions (temperature and humidity) and were subject to seasonal fluctuations. It is worth highlighting that temperature and humidity in most cases fell within the limits that ensure good animal welfare. We must be aware that exposure of animals, groomers and other people having contact with horses to increased particulate matter and bacterial contamination may affect their health. Thus, more frequent bedding replacement, especially in the runners, and the application of wet cleaning systems should be taken into consideration.

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no competing interests.

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Authors' contributions J. Grzyb and Z. Podstawski conceived, designed, and conducted the study. J. Grzyb conducted the literature search and drafted the manuscript. K. Bulski was involved in the analysis interpretation of data. All authors read and approved the final manuscript.

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Figures

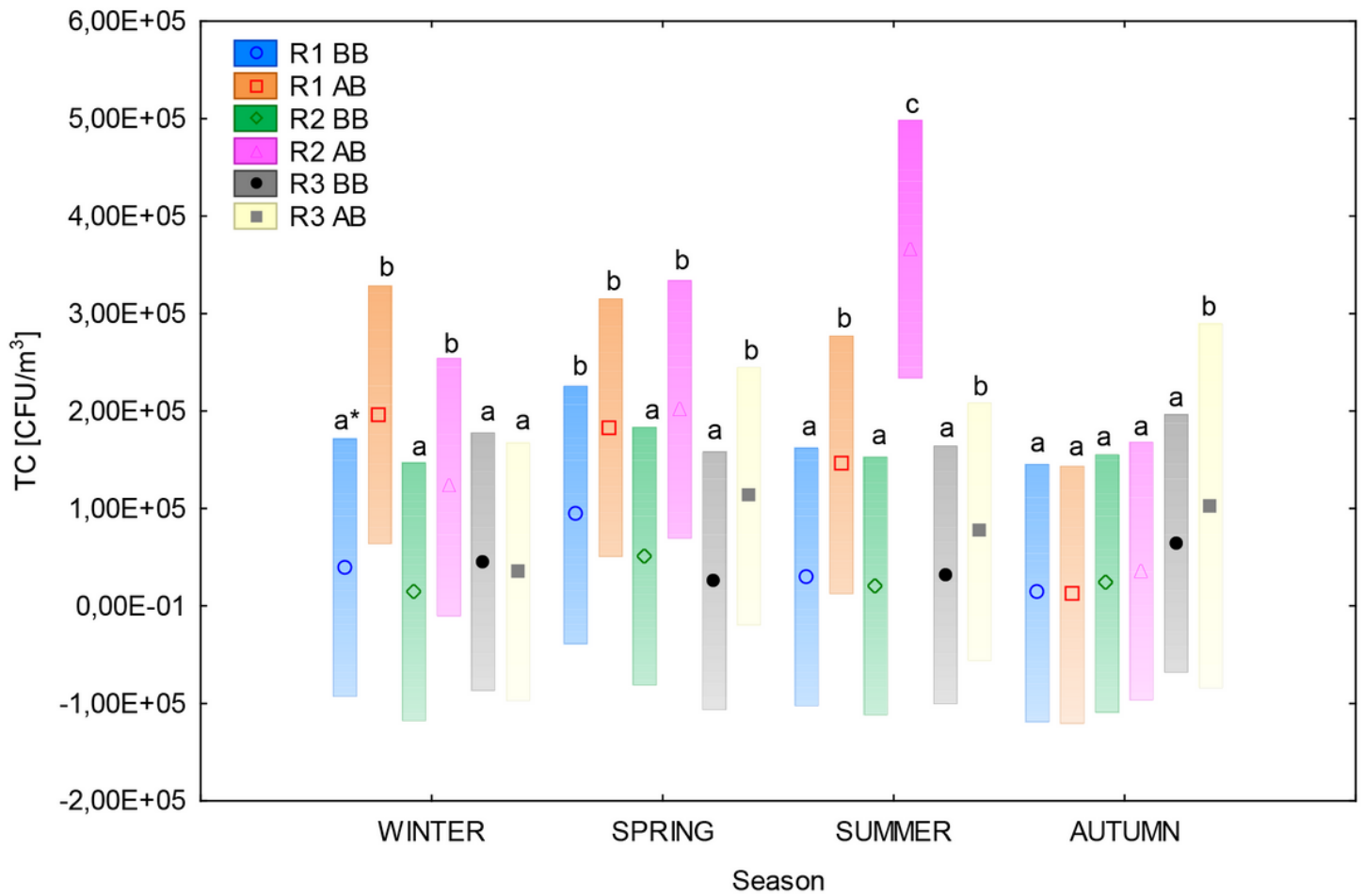


Figure 1

The significance of differences in average total bacterial aerosol concentration (TC) [CFU/m³] in runners (R1-R3) – before (BB) and after bedding (AB) between the seasons (two-way ANOVA, vertical bars represent confidence intervals .95) * averages marked with the same letters are not significantly different by Tukey's test ($\alpha = 0.05$)

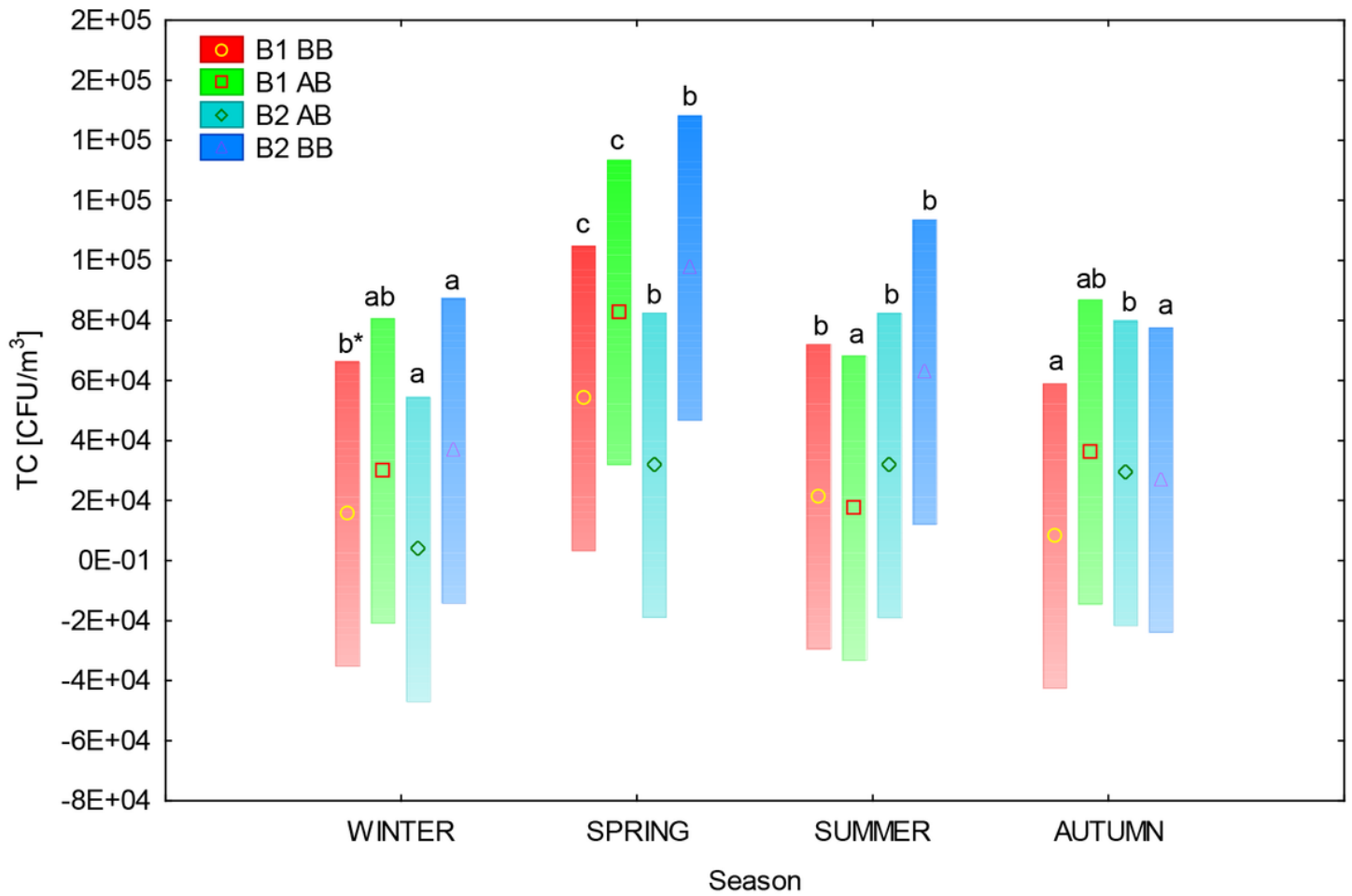


Figure 2

The significance of differences in average total bacterial aerosol concentration (TC) [CFU/m³] in box stables (B1-B2) – before (BB) and after bedding (AB) between the seasons (two-way ANOVA, vertical bars represent confidence intervals .95) * averages marked with the same letters are not significantly different by Tukey's test ($\alpha = 0.05$)