



**Ozone generation by BioZone Scientific air purifiers
and their action on microbial indoor-air quality**

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We certify that the work reported herein was carried out by us, the undersigned, on behalf of Air Health and Safety Solutions Ltd, Cloghran, Co. Dublin, Ireland.

Signed:



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Disclaimer

This Report does not imply endorsement in any form by University College Dublin of any of the products referred herein.

SUMMARY

This Report sets out the results of the investigations carried out concerning the generation of ozone and the action on microbial air quality of air purifiers manufactured by BioZone Scientific International.

1. Generation of ozone by BioZone air purifiers

- 1.1. During eight-hour operation of the BioZone 1000 air purifier with an air-circulation fan running to mix the air in a passively-ventilated seminar room, the un-purified background ozone concentration (0.032 ppm) increased to a maximum 0.043 ppm, a net increment of 0.011 ppm. The maximum concentration was well within the 'Target' level of 0.060 ppm prescribed by the Irish air-quality regulations. The room air-volume was 175 m³, about 50% larger than the recommended minimum room volume (113 m³) for the air purifier operating at maximum setting.

During eight-hour operation of the air purifier at maximum setting without use of an air-circulation fan, the maximum ozone concentration attained was 0.055 ppm, within the 'Target' level; the net increment was 0.022 ppm.

During eight-hour operation of the air purifier at maximum setting, without use of an air-circulation fan and with the ozone sensor on the floor (to detect any ozone layering — ozone is heavier than air), the maximum ozone concentration measured was 0.032 ppm. This concentration was well within the 'Target' level; the net increment was 0.012 ppm.

- 1.2. Operation of the BioZone AC20 air purifier in a passively-ventilated toilet increased the un-purified background ozone concentration from 0.034 ppm, to stabilise at 0.045 ppm; the net increment was 0.011 ppm. The maximum concentration was well within the 'Target' level of 0.060 ppm prescribed by Irish air-quality regulations (SI 53 2004). The room air volume was 40.7 m³, slightly lower than the recommended minimum room volume (45.3 m³).

2. Outcome of the action of BioZone air purifiers on microbial air quality

- 2.1. The large microbial room-contents in a small, hermetically-sealed room, used for incubating and storing microbial cultures, were reduced greatly by the PowerZone-II air purifier run for 18 hours with the ioniser switched off. Use of the air-circulation fan after the PowerZone run increased the room contents only very slightly. The room contents were very low two days after the PowerZone run.
- 2.2. The reduced room content produced when the BioZone 1000 was run with the air-circulation fan for three hours in a seminar room, was increased about three-fold after a lecture had been held in the room. Following a 41-hour BioZone 1000 run with air-circulation fan, started after the lecture, the room content had reduced by about 70% (average of five measurements made towards the end of the run). Six hours after the run (the air purifier and the air-circulation fan were

off), the room content had increased only very slightly (average of five measurements).

- 2.3. Because of its similarity in design to the BioZone 1000, it is inferred that the BioZone AC20 air purifier functions as effectively as the BioZone 1000.

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1. BACKGROUND

Air Health and Safety Solutions Ltd (AHS) wishes to distribute air purification devices supplied by BIOZONE SCIENTIFIC INTERNATIONAL (BSI), and is concerned about the performance and safety of the devices. AHS wished to commission experimental investigations relevant (a) to the risk of unsafe exposure to ozone by users of the device and, (b) to whether the performance of the devices meets claims made by BSI. AHS invited University College Dublin (UCD) to undertake the investigations on foot of a Service Agreement between AHS and UCD. An extension of the Agreement was made by AHS with UCD in early August 2006, in connection with preliminary work on the evaluation of the performance of BSI devices in reducing microorganisms on surfaces; the results of this work were not conclusive.

The experimental work was carried out over ten weeks, in the period June to August 2006, at the School of Biomolecular and Biomedical Sciences, University College Dublin, by Jennifer Croke (honours undergraduate) supervised by Professor Wim Meijer as Principal Investigator.

The Service Agreement entailed specified experimental investigations (2. Objectives), the results of which are set out in this Report.

2. OBJECTIVES

- 2.1. Observe the ozone concentrations produced by normal operation of three selected BSI devices in typical conditions of use.
- 2.2. Evaluate the performance of three selected BSI devices in typical conditions of use in reducing the general microbial burden of the air being treated.

The Agreement also entailed definition of the concentrations in the zone at short distances from the point of treated air emission by the devices (the 'immediate zone'), and the formulation of proposals to cater for further investigations seen to be desirable in the light of the results of the investigations reported here. These are the subjects of a separate Report.

3. EQUIPMENT AND METHODS

3.1. Generation of ozone by BioZone air purifiers

3.1.1. Equipment

BioZone 1000 air purifier
BioZone AC 20 air purifier (modified with ioniser on-off switch)
BioZone PowerZone-II air purifier (modified with ioniser on-off switch)
Air-circulation fan (Philips hot air fan 3000)
Aeroqual ozone monitor (series 500; low sensor head)
Aeroqual Ozone Software for Windows
Sony notebook computer with MS Windows 98
Two Kingshield T35 24-hour timers

3.1.2. Methods

The air purifiers were set up and run in various rooms, as detailed below; the BioZone 1000 and the BioZone AC20 were run with the ionisers switched off.

3.1.2.1. Seminar room

Ozone generation by the BioZone 1000 was evaluated in a seminar room located in the Microbiology Annex Building on the University Campus. The room dimensions were 7.76 x 7.50 x 3.00 m, giving the air volume 175 m³. The minimum room-volume specified by the Manufacturers (1) for the BioZone 1000, operating at maximum setting, is 1600 ft³ (200 ft² x 8 ft), i.e. 113 m³. The passive ventilation design of the room was in accordance with the Irish Building Regulations 2002 (2); the windows and doors of the room were closed during the evaluation runs.

As requested by AHS, the Biozone air purifier was positioned 0.5 m out from the middle of a short wall at 2 m height from the floor. The ozone monitor was placed forward from the air purifier at 2/3 the distance to the other facing short wall, and at a height of 1.3 m to simulate a sitting person. The air-circulation fan was positioned at 1 m height from the floor, 2.6 m (1/3 of room width) from the wall at which the air purifier was placed, and 1 m out from a long wall (equivalent to the distance of the air purifier from the ceiling).

The air purifier was run for various time periods at the minimum or the maximum setting, as indicated in the run-protocol below. In accordance with the Health and Safety Assessment for the work, which required that the operator should not enter the room until the ozone generated by the device had decayed to the un-purified background level, a 24-hour timer was used to switch the air purifier on and off at the times required by the run-protocols.

The ozone concentrations were monitored at one minute frequency using the ozone monitor system, according to the Manufacturer's instructions (3); the concentrations were recorded as parts per million (ppm) on the computer using the monitor software. The measured data was transferred and processed in a specially customised MS EXCEL workbook used in the production of this Report.

The air-circulation fan was used at the cold-air setting as described in the following run-protocols.

Run-protocol A

- (i) Switch ozone monitoring system and air-circulation fan on, and exit the room.
- (ii) Allowing time for the ozone sensor to stabilise, switch on the air purifier (at minimum setting) automatically, using a 24-hour timer.
- (iii) After eight hours of operation, switch off the air purifier automatically.
- (iv) Having allowed time for the ozone concentration to decay to the un-purified background level, enter the room and switch off the air-circulation and the ozone monitoring system.

Run-protocol B

- (i) Switch ozone monitoring system on and exit the room.
- (ii) Allowing time for the ozone sensor to stabilise, switch on the air purifier (at maximum setting) automatically, using a 24-hour timer.
- (iii) After eight hours of operation, switch off the air purifier automatically.
- (iv) Having allowed time for the ozone concentration to decay to the un-purified background level, enter the room and switch off the ozone monitoring system.

Run-protocol C

- (i) With the sensor on the floor, switch the ozone monitoring system on and exit the room.
- (ii) Allowing time for the ozone sensor to stabilise, switch on the air purifier (at minimum setting) automatically, using a 24-hour timer.
- (iii) After eight hours of operation, switch off the air purifier automatically.
- (iv) Having allowed time for the ozone concentration to decay to the un-purified background level, enter the room and switch off the ozone monitoring system.

3.1.2.2. Toilet

Ozone generation by the BioZone AC20 was evaluated in a toilet located in the Microbiology Annex Building. The room dimensions were 3.38 x 4.85 x 2.70-1.02 x 1.30 x 2.70 m³ allowing for irregular shape, giving the air volume 40.7 m³. The minimum room-volume specified by the Suppliers (4) is 1600 ft³ (200 ft² x 8 ft), i.e. 45.3 m³. The windows and door of the room were closed during the evaluation run minimising the passive ventilation of the room.

The air purifier was positioned 0.5 m out from the middle of the long wall at 2.0 m height from the floor. The ozone monitor was placed at same height, 0.5 m out from the middle of the opposite long wall. The air-circulation fan was positioned at 1.0 m height from the floor, and 0.5 m out from the centre of a long wall.

The air purifier was run as indicated in the run-protocol D below. In accordance with the Health and Safety Assessment for the work, which required that the operator should not enter the room until the ozone generated by the device had decayed to unpurified background level, a 24-hour timer was used to switch the air purifier on and off at the times required by the run-protocol.

The ozone concentrations were monitored at one minute frequency, using the ozone monitor system according to the Manufacturer's instructions (3); the concentrations were recorded as parts per million (ppm) on the computer using the monitor software. The measured data was transferred and processed in a specially customised MS EXCEL workbook used in the production of this Report.

The air-circulation fan was used intermittently at the cold-air setting as described in run-protocol D.

Run-protocol D

- (i) Switch ozone monitoring system on and exit the room.
- (ii) Allowing time for the ozone sensor to stabilise, switch on the air purifier automatically, using a 24-hour timer. Also, switch on the air-circulation fan automatically for three one-hour periods during the operation of the air purifier.
- (iii) After eight hours of operation, switch off the air purifier automatically.
- (iv) Having allowed time for the ozone concentration to decay to the unpurified background level, enter the room and switch off the ozone monitoring system.

3.2. Outcome of the action of BioZone air purifiers on microbial air quality

3.2.1. Equipment

Millipore M Air T portable air tester

Millipore M Air T agar cassettes pre-filled with w/TSA media

Incubator (37 °C)

Incubator (17 °C)

Other equipment used is already described in 3.1.1. above.

3.2.2. Methods

Air samples were taken, using the portable air tester according to the Manufacturer's instructions (5), for a variety of air-volumes stated in the protocols below. Cassettes were taken singly for incubation at 37 °C for 48 hours, or in pairs to provide for incubation additionally at 17 °C for six days — variously before, during or after air purifier runs. Following incubation, colony-forming unit (cfu) counts were carried out by visual inspection of the cassettes, and the counts were recorded and processed in a specially customised MS EXCEL workbook used in the production of this Report. The results were calculated as room contents, i.e. as the total number of microorganisms in the air in a room.

3.2.2.1. Sealed room

The impact of the PowerZone-II air purifier on microbial air quality was evaluated in a small, hermetically-sealed room located in the Microbiology Annex Building. The room dimensions were 2.04 x 3.88 x 2.21 m, giving the air volume 17.5 m³. The PowerZone-II is designed particularly for purifying heavily-contaminated rooms; the Manufacturers (6) do not specify a minimum room volume. The room served normally as a sealed warm-room for incubating and storing microbial cultures, as a result of which the concentration of microorganisms in the air in the room was persistently high. A uniform internal air-temperature was provided by a fan air-heater with thermostat control. The room had plastic-coated surfaces, and about 30 linear meters of empty wooden-lath shelving fitted around the walls.

The air purifier was positioned on a shelf about 0.5 m from a short wall, at 1.5 m height from the floor; the ozone monitor was placed at the same height on the shelf at the other facing short wall. The air-circulation fan was positioned at 1.5 m height from the floor, and 0.5 m out from the centre of a long wall.

The air purifier was run as described in the following run-protocol E. In accordance with the Health and Safety Assessment for the work, which required that the operator should not enter the room until the ozone generated by the device had decayed at least to the un-purified background level, a 24-hour timer was used to switch the air purifier on and off at the time required by the run-protocol.

The air-circulation fan was used at the cold-air setting, as described in run-protocol E.

Run-protocol E

- (i) Switch on the air purifier automatically, using a 24-hour timer.
- (ii) After eighteen hours of operation, switch off the air purifier automatically.
- (iii) Having allowed time for the ozone concentration to decay to the un-purified background level, enter the room and to take air samples.

3.2.2.2. Seminar room

The impact of the BioZone 1000 air purifier on microbial air quality was evaluated in the seminar room arranged as described in Methods 3.1.2.1. It had already been established that the BioZone 1000 did not produce ozone concentrations in the seminar room that exceed the 'Target' limit set in the Irish Regulations (Results 4.1.1.). So it was possible to take air samples during and shortly after use of the air purifier in this room; details of the run-protocols used are given in Results 4.2.2. in tandem with the particular sets of results obtained.

4. RESULTS

4.1. Generation of ozone by BioZone air purifiers

4.1.1. Seminar room

A BioZone 1000 was run in a seminar room as described in Methods 3.1.2.1, using the three different run-protocols A, B and C. Run-protocol A was in accordance with that requested by AHS. For run-protocol B, the air-circulation fan was not used with a view to determining any difference in apparent performance due to reduced air mixing, and in run protocol C, the air-circulation fan was not used and the ozone sensor was placed on the floor with a view to determining whether the ozone generated by the air purifier accumulated at floor level.

The ozone concentrations measured during these runs are shown in Figures 1-3.

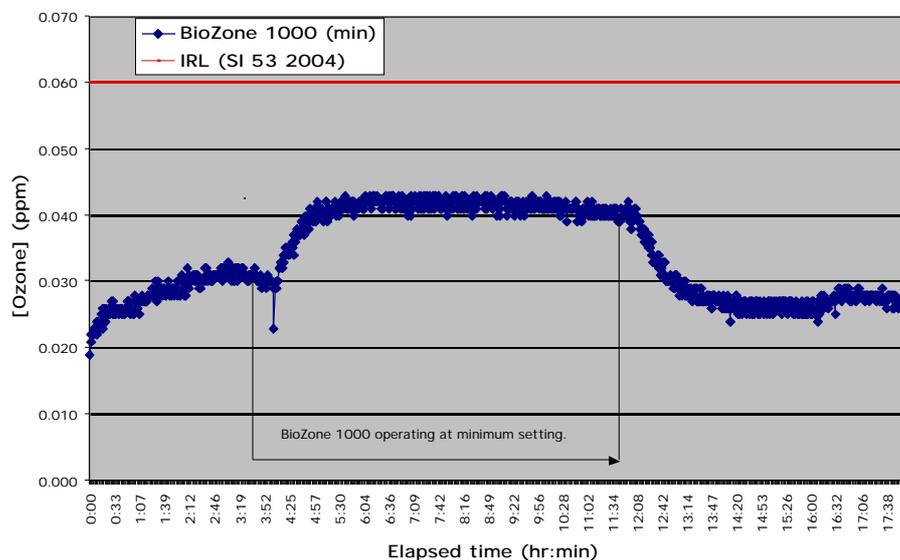


Figure 1. Ozone concentrations measured in a passively-ventilated seminar room before, during and after operation of a BioZone 1000 air purifier at minimum setting. The operational details are given in Methods 3.1.2.1. (run-protocol A).

Prior to operation of the air purifier the ozone concentration rose from 0.019 ppm, to stabilise at 0.032 ppm (Figure 1); this apparent rise is most likely associated with the stabilisation of the ozone sensor. During the eight-hour operation of the air purifier at minimum setting, the maximum ozone concentration attained was 0.043 ppm. This concentration was well within the ‘Target’ level of 0.060 ppm prescribed by Irish air-quality regulations, SI 53 2004 (7). The net increment (the increase on the un-purified background concentration) was 0.011 ppm. The air-circulation fan was operated throughout the run.

When operation of the air purifier had ceased, the ozone concentration reduced to values in the range 0.024–0.029 ppm.

With run-protocol B, which was without use of the air-circulation fan, prior to operation of the air purifier the ozone concentration rose from 0.023 ppm, to stabilise at 0.033 ppm (Figure 2). This apparent rise is most likely associated with the stabilisation of the ozone sensor. During the eight-hour operation of the air purifier at maximum setting, the maximum ozone concentration attained was 0.055 ppm; the net increment (the increase on the background concentration) was 0.022 ppm. The maximum concentration was within the ‘Target’ level of 0.060 ppm prescribed by Irish air-quality regulations.

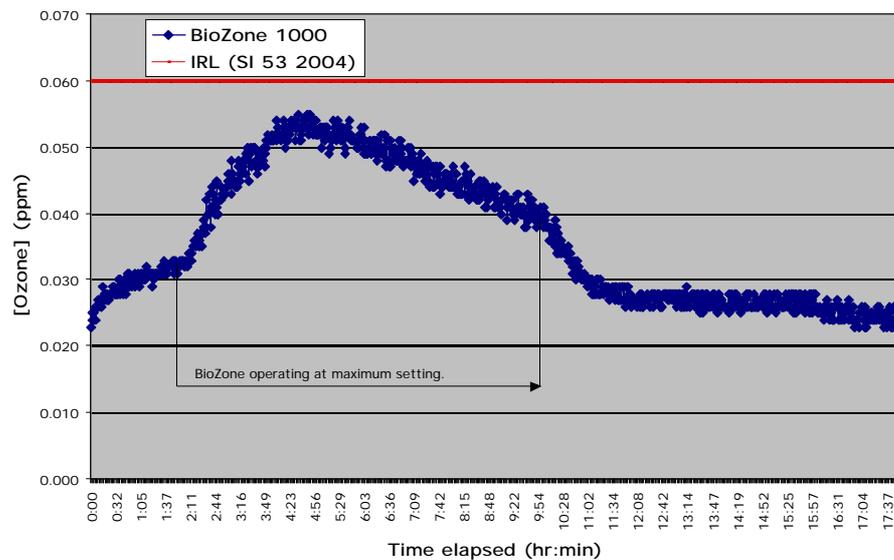


Figure 2. Ozone concentrations measured in a passively-ventilated seminar room before, during and after operation of a BioZone 1000 air purifier at maximum setting. The operational details are given in Methods 3.1.2.1. (run-protocol B).

Following operation of the air purifier, the ozone concentrations reduced to 0.023 ppm.

With run-protocol C, the ozone sensor was placed on the floor and the air-circulation fan was not used. Prior to operation of the air purifier the ozone concentration was in the range 0.020-0.023 ppm (Figure 3). During the eight-hour operation of the air purifier at maximum setting, the maximum ozone concentration attained was 0.032 ppm; the net increment (the increase on the background concentration) was 0.012 ppm. The maximum concentration was well within the ‘Target’ level of 0.060 ppm prescribed by Irish air-quality regulations.

When operation of the air purifier had ceased, the ozone concentration reduced to 0.019 ppm.

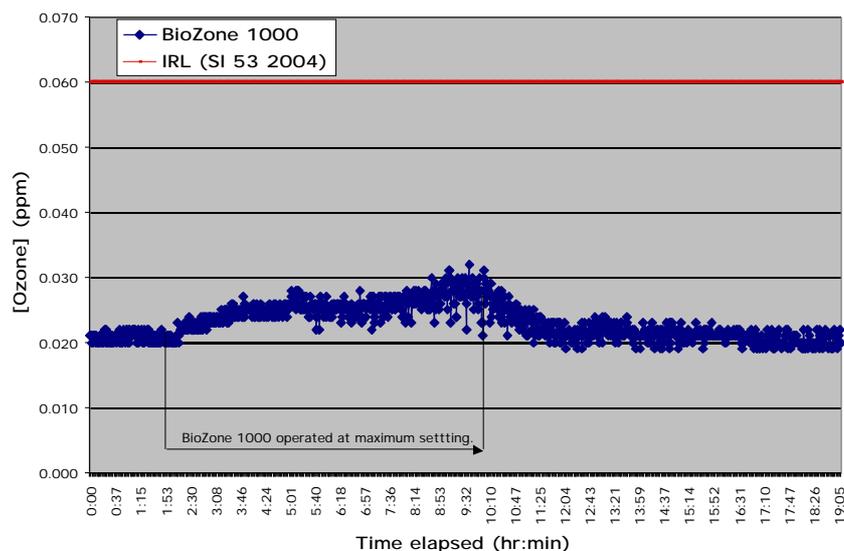


Figure 3. Ozone concentrations measured in a passively-ventilated seminar room before, during and after operation of a BioZone 1000 air purifier at maximum setting. The operational details are given in Methods 3.1.2.1. (run-protocol C).

4.1.2. Toilet

A BioZone AC20 was run in a toilet as described in Methods 3.1.2.2. The ozone concentrations measured during the run are shown in Figure 4.

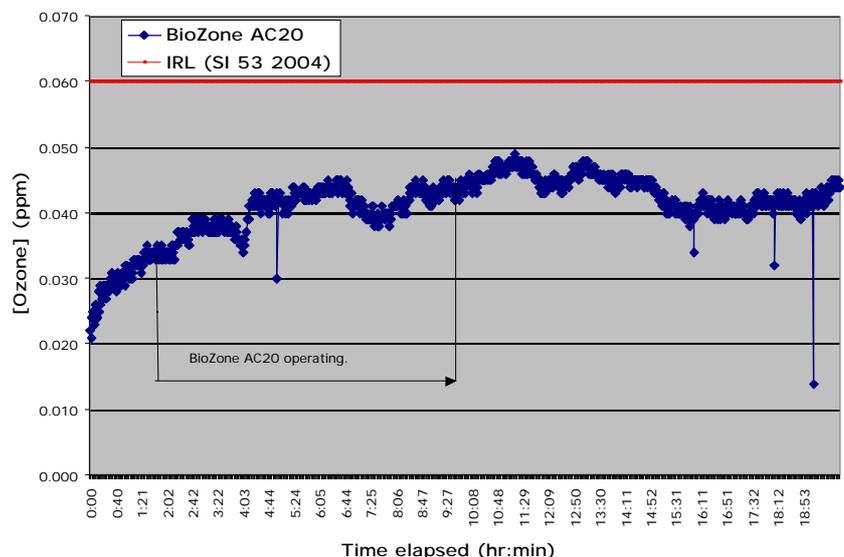


Figure 4. Ozone concentrations measured in a passively-ventilated toilet before, during and after operation of a BioZone AC20 air purifier. The operational details are given in Methods 3.1.2.2.

Prior to operation of the air purifier, the ozone concentration rose from 0.022 ppm, to stabilise at 0.034 ppm; this apparent rise is most likely associated with the stabilisation of the ozone sensor. During the eight-hour operation of the air purifier, with mixing of the air by the air-circulation fan for three one-hour periods, the maximum ozone concentration attained was 0.045 ppm. This concentration was well within the 'Target' level of 0.060 ppm prescribed by Irish air-quality regulations. The net increment (the increase on the un-purified background concentration) was 0.011 ppm.

When operation of the air purifier had ceased, the ozone concentration remained in the range 0.032-0.049 ppm.

4.2. Outcome of the action of BioZone air purifiers on microbial air quality

4.2.1. Sealed room

The results for air samples taken for an experimental run carried out in a sealed room described in Methods 3.2.2.1. are set out below (Series 1) and are summarised in Table 1. Air samples were taken according to Methods 3.2.2.

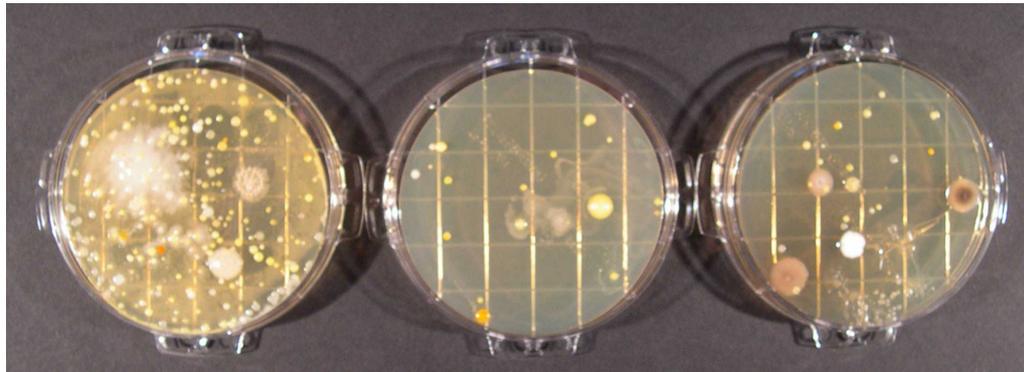
Series 1.

The room content for an air sample incubated at 37 °C for 48 hours, taken prior to a run with the PowerZone-II air purifier, could not be estimated, because the colony-forming units that grew on the cassette was too numerous to count (the cassette is shown in Figure 5a). The room content for a sample incubated at 17 °C for six days was 665 cfu (the cassette is shown in Figure 6a).

An 18-hour PowerZone run was commenced (Methods 3.2.2.1.; run-protocol E) and a pair of air samples was taken three hours after the end of the run for incubation at 37 °C for 48 hours and 17 °C for six days. The room content for the 37 °C incubate was 385 cfu, and was 140 cfu for the 17 °C incubate.

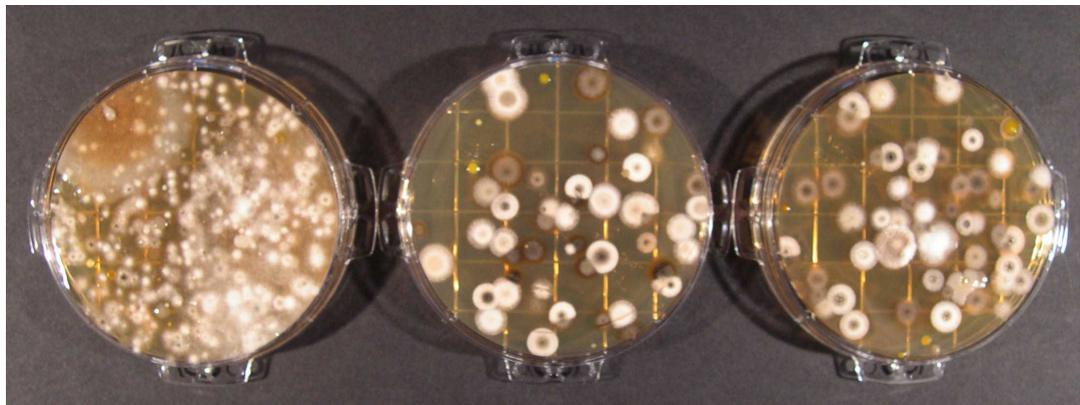
Then, samples were taken following operation of an air-circulation fan for one hour after the PowerZone run, the room content for a 37 °C incubate was 630 cfu (the cassette is shown in Figure 5b), and was 105 cfu for a 17 °C incubate (the cassette is shown in Figure 6b).

Finally, samples were taken two days after the PowerZone run; the room content for a 37 °C incubate was 980 cfu (the cassette is shown in Figure 5c) and was 210 cfu for a 17 °C incubate (the cassette is shown in Figure 6c).



(a) (b) (c)

Figure 5. Cassettes incubated at 37 °C for 48 hours for air samples taken in a sealed room (Series 1): sample taken (a) prior to an 18-hour PowerZone-II run; (b) after a further one-hour operation of an air-circulation fan; (c) two days after the PowerZone run. Experimental details are given in the text.



(a) (b) (c)

Figure 6. Cassettes incubated at 17 °C for six days for air samples taken in a sealed room (Series 1): sample taken (a) prior to an 18-hour PowerZone-II run; (b) after a further one-hour operation of an air-circulation fan; (c) two days after the PowerZone run. Experimental details are given in the text.

Table 1. Numbers of microorganisms as colony-forming units (cfu) in a sealed room (room contents) from air samples incubated for 48 hours at 37 °C and for six days at 17 °C. For replicate samples, the number of replicates, the average room content, and the relative standard deviation (RSD) are given also. The experimental conditions are detailed more fully in this section (4.2.1.; Series 1).

Timing w.r.t. air purifier run	Run protocol	Air fan	Room content (\pm RSD)	
			cfu (37 °C)	cfu (17 °C)
Before run	-	off	TNTC	665
3 hr after run	E	off	385	140
After 1 hr fan	-	on	630	105
2 days after run	-	off	980	210

TNTC: too numerous to count.

4.2.2. Seminar room

The results for air samples taken for three experimental runs carried out in a seminar room described in Methods 3.1.2.1. are set out below (Series 2–4) and are summarised in Table 2. Air samples were taken according to Methods 3.2.2.

Series 2.

The room content for an air sample incubated at 37 °C for 48 hours, taken prior to a run with the BioZone-1000 air purifier, was 33136 cfu. In line with Methods 3.2.2.2., the air purifier and an air-circulation fan were switched on for a three-hour period. Air samples were taken at the end of the 1st, 2nd and 3rd hour; the room contents were 19184, 13603, and 6627 cfu respectively. Then, a sample was taken at one and at two hours after the run; the room contents were 6976 and 12208 cfu respectively. The air-circulation fan was left on throughout. Typical cassettes are shown in Figure 7a-c.

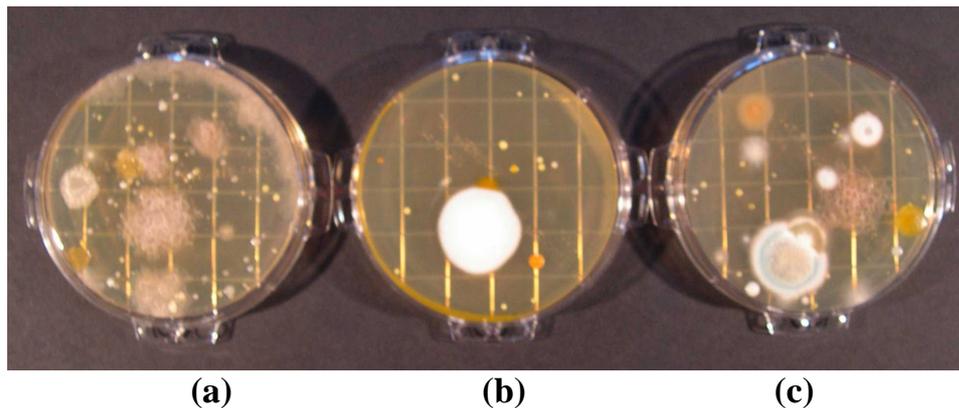


Figure 7. Cassettes incubated at 37 °C for 48 hours for air samples taken in a seminar room (Series 2): sample taken (a) prior to a three-hour BioZone 1000 run; (b) after three hours, i.e. at the end of the run; (c) two hours after the run. Experimental details are given in the text.

Series 3.

An air sample was taken at the end of a 21-hour, overnight run (Methods 3.2.2.2.), with the BioZone 1000 (air-circulation fan on), and incubated at 37 °C for 48 hours. The room content for the sample was 11162 cfu. The run was terminated to allow a lecture to take place in the room. A typical cassette is shown in Figure 8a.

Shortly after the lecture (air-circulation fan off), triplicate air samples were taken and incubated at 37 °C for 48 hours; the average room content was 33485 cfu (RSD $\pm 5.5\%$). A typical cassette is shown in Figure 8b.

Then a 41-hour run with the BioZone 1000 with air-circulation fan on was commenced. Air samples were taken during the run and incubated at 37 °C for 48 hours. The room content for the samples taken at the end of the 16th, 17th, 18th, 20th and 22nd hour were 10697, 9999, 11859, 8371 and 4651 cfu respectively; the average was 9115 cfu (RSD $\pm 31\%$). A typical cassette is shown in Figure 8c.

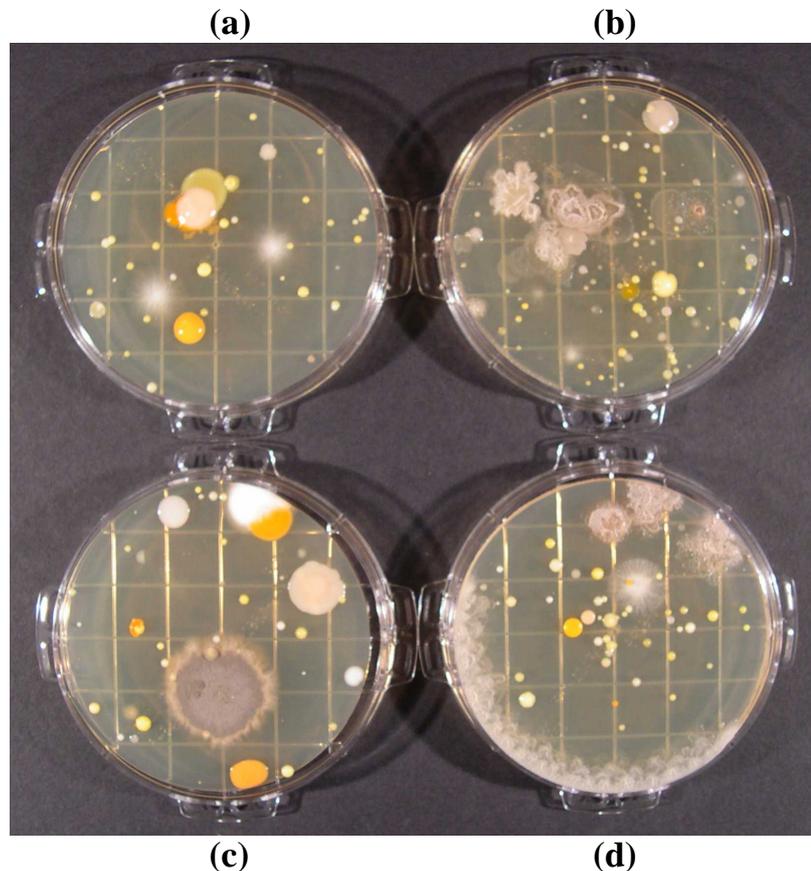


Figure 8. Cassettes incubated at 37 °C for 48 hours for air samples taken in a seminar room sample taken (a) at the end of a 21-hour BioZone 1000 run, prior to a lecture held in the room, (b) after the lecture, and (c) 17 hours after the start of a further 41-hour run (Series 3); taken (d) after the end of the run (Series 4). Experimental details are given in the text.

Series 4.

Air samples were taken after the 41-hour run with the BioZone 1000 had been terminated and the air-circulation fan switched off, and were incubated at 37 °C for 48 hours. The room content for the samples taken immediately afterwards, and 1.25, 3.50, 5.00, and 6.00 hours after the run were 3255, 16742, 12092, 6278 and 8604 cfu respectively; the average was 9394 cfu (RSD \pm 56%). A typical cassette is shown in Figure 8d.

Table 2. Numbers of microorganisms as colony-forming units (cfu) in a seminar room (room contents) from air samples incubated for 48 hours at 37 °C. For replicate samples, the number of replicates, the average room content, and the relative standard deviation (RSD) are given also. The experimental conditions for the six series are indicated and are detailed more fully in the text in this section (4.2.2.).

Series no.	Timing w.r.t. air purifier run	Air fan	Replicate samples	Room content cfu (\pm RSD%)
2	Before run	off		33136
	1 hr after start	on		19184
	2 hr after start	on		13603
	3 hr after start	on		6627
	1 hr after run	on		6976
	2 hr after run	on		12208
	3	After 21 hr run (after lecture)	on off	3
16 hr after start		on	10697	
17 hr after start		on	9999	
18 hr after start		on	11859	
20 hr after start		on	837	
22 hr after start		on	4651	
Average (5):			9115 (31)	
4		0.00 hr after run	off	
	1.25 hr after run	off		16742
	3.50 hr after run	off		12092
	5.00 hr after run	off		6278
	6.00 hr after run	off		8604
	Average (5):			9394 (56)

5. REFERENCES

1. Owner's Guide (models 500, 1000, 2000, 3000, 4000, 5000) Biozone Scientific, Inc.
2. Department of the Environment, Heritage and Local Government. Building Regulations 2002. Technical Guidance Document F: ventilation. Stationery Office, Dublin.
3. Aeroqual Ozone Monitors: series 300 & 500. User Guide.
4. Personal communication from Air Health and Safety Solutions Ltd.
5. M Air T Millipore Air Tester. Operation and Maintenance Instructions.
6. Owner's Guide (PowerZone™ I, II, III, IV) Biozone Scientific, Inc.
7. Department of the Environment, Heritage and Local Government. Statutory Instrument No. 53 of 2004. Ozone in Ambient Air Regulations 2004. Stationery Office, Dublin.

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