**Green job bio-aerosol exposure during anaerobic digestion for biomass energetic valorisation**

**TITLE:**

Green job bio-aerosol exposure during anaerobic digestion for biomass energetic valorisation

**AUTHORS:**

Traversi Deborah[1], Ilaria Gorrasi[1], Sara Bonetta[1], Riccardo Leinardi [1], Biancamaria Pietrangeli[2], Elisabetta Carraro [1], Giorgio Gilli[1]

**AFFILIATIONS:**

[1] Department of Public Health and Pediatrics, University of Torino, piazza Polonia 94, 10126 Torino, Italy

[2] Department Productive Plants & Environment Interaction (DIPIA), National Institute of Occupational Safety & Prevention INAIL, I-00184 Rome, Italy

***CORRESPONDING AUTHOR:**

Deborah Traversi

Tel: +390116705822 Fax: +390116705874

Department of Public Health and Pediatrics, University of Torino, piazza Polonia 94, 10126 Torino, Italy

e-mail: deborah.traversi@unito.it

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**ABBREVIATIONS:**

AD: Anaerobic Digestion

CAFOs: Confined Animal Feeding Operations

GIMC: Global Index of Microbial Contamination

MBC: Mesophilic Bacteria Contamination

PM: particulate matter

PM10: PM in which 50% of particles have an aerodynamic diameter of less than 10 µm

PM3: PM in which particles have an aerodynamic diameter of less than 3 µm

PM0.49: PM in which particles have an aerodynamic diameter of less than 0.49 µm

$PM_{10.0\text{-}7.2}$: PM in which particles have an aerodynamic diameter from 10 to 7.2 µm

$PM_{7.2\text{-}3.0}$: PM in which particles have an aerodynamic diameter from 7.2 to 3.0 µm

$PM_{3.0\text{-}1.5}$: PM in which particles have an aerodynamic diameter from 3.0 to 1.5 µm

$PM_{1.5\text{-}0.95}$: PM in which particles have an aerodynamic diameter from 1.5 to 0.95 µm

$PM_{0.95\text{-}0.49}$: PM in which particles have an aerodynamic diameter ranged 0.95 to 0.49 µm

EU: endotoxin unit

**ABSTRACT**

Green economy expansion implies that the risk profile for green occupational jobs also increases. One of the broadest green sectors in terms of growth is the anaerobic digestion of biomasses, and in recent years, this development has also interested Italian regions. The management of biomass includes biological risk and the risk of particulate and endotoxin exposure. In the present work, we evaluated airborne exposure for anaerobic digestion workers in two real-scale plants. Digested biomass has different origins, ranging from cattle sludge and manure to poultry manure to agricultural harvesting or processing residues, especially from maize and fruits. Two sampling points were chosen: at the first, the input biomasses were stored and the hopper was loaded, and at the second, the digested sludge exited the digester. The microbiological parameters, assessed using an active sampler and cultural method, were the total bacteria counts at 22, 37, and 55°C, including yeasts, fungi, Pseudomonaceae, *Clostridia spp.*, Enterobacteriaceae and Actinomycetes. Moreover, at the same sampling points, we evaluated six PM10 fraction levels (10.0-7.2, 7.2-3.0, 3.0-1.5, 1.5-0.95, 0.95-0.49, and <0.49 µm) and the endotoxin content of each fraction. In this investigation, the microbe contamination of the air varied from low to high levels, while the PM10 and endotoxin levels were limited, reaching rural environmental levels (61.40 µg/$m^3$ and 18.88 EU/$m^3$, respectively). However, contamination and occupational risk must be evaluated individually for each plant because numerous variables influence risk magnitude, with particular regard to digested sludge treatments, such as input biomass nature, storage, movement conditions, building configuration and technological processes.

## 1. INTRODUCTION

Currently, there is a worldwide incentive to make different aspects of the economy and job market "green" (WHO, 2014); energy and raw material prices are increasing, producing an increasing pressure to adopt more ecological production methods in order to limit global warming and avoid irreversible climate change (Neira, 2010). A "green revolution" of the economy represents a huge opportunity to start new businesses, develop new lower energy consumption markets, incentivise the activities and investments of companies in local communities, and decrease disparities caused by poor access to energy sources (WHO, 2011). In Canada and North America, these considerations have led to an enormous development in "green" industry, which has generated significant economic growth(UNEP, 2008). Europe arrived later but is now undergoing full development; estimates say that in 2009, approximately 3.4 million persons worked in the "green-jobs" branch, and, when the combination of related activities is considered, that number expands to 8.5 million (ILO, 2012).

In Italy in 2010, there were approximately 100,000 workers in green industries, and it is thought that the number will reach 250,000 in 2020, with the majority involved in bioenergy (more than 100,000 workers), followed by aeolian (80,000) and solar (50,000) energy branches (R.E.R., 2012).

Green jobs are, to some extent, activities that predict previously evaluated risks, but with a different scope and exposition in connection to newly applied technology (ILO, 2012). However, it is important to complete an evaluation process regarding new or re-emergent risks with regard to newly applied technologies (Omar et al., 2013).

The renewable energy sources that have been developed recently in Italy include biogas, obtained from the anaerobic digestion of agricultural and livestock biomasses. According to a recent investigation, in the last few years, the number of such plants in Italy has grown by more than 75% and now numbers over 520 plants, with most of them in northern Italy (Fabbri 2011).In the biogas production supply chain, various work-linked risks can be identified, such as explosive, chemical and biological risks. In general, these are not novel risks, but they are minimised in magnitude with respect to similar activities such as concentrated animal feeding, composting or waste water treatment operations (Szadkowska-Stanczyk et al., 2010). In connection with used materials, including vegetable, food production residuals and animal biomasses, and with the properties of fermentation, biological risk deserves particular attention (Pankhurst et al., 2011). Fermentation biomass is rich in microorganisms, including pathogens and opportunistic pathogens, and

anaerobic processes could lead to the selection of microbial flora to promote the presence of anaerobic microorganisms, e.g., clostridia, that are less represented initially (Li et al., 2014).

Italian law on occupational safety (D.Lgs n. 81/2008) identifies a biological agent as "any microorganism, even a genetically modified, cellular culture or human endo-parasitic organism, that could cause infection, allergy or toxicity", while the bioaerosol definition in American Conference of Governmental Industrial Hygienists states explicitly that a microorganism's fragments and microorganism-derived particles are included (ACGIH, 2006). Thus, evaluation of risk for workers linked to bioaerosol exposition includes an evaluation of airborne microorganisms and also of all biological components conveyed as particulates. Breathable particulates (PM10) can settle in different regions of the respiratory tree, depending on particle size; in particular, larger particles settle within the first tract of the tree (10-6 µm), while smaller particles (< 6 µm) can reach the deepest regions, and particles with aerodynamic diameters < 3 µm are able to arrive in alveolar cells (WHO, 2006).

Endotoxins are among the natural components of breathable particulates; they are lipopolysaccharide components of the bacterial cell wall external membranes of gram-negative bacteria. Considering their dimensional features, they can also settle within the respiratory tree, resulting in the development of systemic effects (asthma, ODTS syndrome, etc.) (Liebers et al., 2006). The presence of endotoxins in bioaerosols is not negligible; in fact, it is verifiable that an increase of their concentration is caused by intensive feeding or breeding. Endotoxins are found mostly in the coarse and fine fractions of PM10, contributing strongly to the pathogenicity of these particles (Liebers et al., 2008).

The aim of this work is to evaluate the exposure risk to bioaerosols and particulate matter in biogas production plants. To this end, we have analysed two real-scale plants located in Piedmont, monitoring the activity of airborne microorganisms and fractionated PM10. For each PMx-y, we evaluated the presence of bacterial endotoxins.

## 2. MATERIALS AND METHODS

### 2.1 Anaerobic digestion plants

AD is a natural process where biomasses are broken down by micro-organisms in the absence of air, the operations begin when biomass reaches the AD plant and it is loaded into the hopper or directly into the digester. Then the naturally selected microbiota inside the digester is able to

produce the biogas, mainly composed by methane and carbon dioxide. The remaining material, called digested sludge, can be used as a fertilizer frequently after a de-watering phase.

The biogas plants treating agricultural and livestock biomasses are the most diffused in Italy and more than 70% of these are located in Piedmont (ENAMA, 2011). The evaluation of the general process into this kind of plants showed that most of the process is conducted into closed pipelines and digester but few critical phases for occupation exposure can be pointed, for example, as chosen in the present study: the digester load phase (first sample site) and the sludge exiting phase (second sample site).

The first plant (Coop. Speranza A.r.l.), hereafter referred to as the S-plant, was situated in Candiolo (≈ 5600 inhabitants) in the vicinity of Turin (<20 km); the second plant (Marco Polo S.p.A.), hereafter referred to as the M-plant, was situated in Vignolo (≈ 2500 inhabitants), located approximately 100 km from Turin near Cuneo city (<10 km). Both plants are on level land and away from population centres. However, the M-plant is located near a provincial town area.

The two plants use different feedstocks: silage, corn cobs, fruit and vegetable waste and cattle manure for the S-plant, and cattle and poultry manure and vegetal biomass from dedicated crops, especially corn, for the M-plant.

In the S-plant, the two sampling points in the same service area so partially overlapping are outdoors, and the digestate is taken directly and spread in a surrounding field. Solid biomasses are stored outdoor in a platform near the loading hopper. The operator loads biomass on the tractor and once arrives near the hopper overturns in it the content, while the sewage reachs directly the digester through pipes which come from the storage tank. The output operations concern the charge in a cistern, linked to a tractor, of the semi-solid digested material, stored in a underground cistern: this operation is repeated several times in a day, depending on the need of fertilizing fields. The operator is in a close cabin with air conditioned and filters. Only sporadically he goes out from the cabin for tractor servicing or for looking at the hopper load level.

In the M-plant, the first sampling site is located in a biomass storage shed, both for solid biomasses and liquid cattle manure tank, where the hopper is loaded, and the second site is in a half-closed shed where the solid digestate product exits separately from the liquid component. The difference between the indoor and outdoor environments for operational activities is partly dictated by the plants' differing distances from highly populated areas moreover the input biomasses has generally more odour release than output sludge, especially if already separated

159 from the liquid fraction. In M-plant the liquid is stored in a tank and reused into the digester while
160 the solid is stored outside a canopy, where it is enlivened by an operator daily, with an excavator.
161 The samplings were performed in the spring of 2013 (May and June) the normal working activities
162 of employees. Typically the bio aerosol and the endotoxin exposure are higher in spring and
163 summer when the temperature is favouring for the microbial growth (Traversi et al., 2010).
164 For both the plants, our microbiological sampling was done during input and output operations
165 while PM sampling lasted 4 hours and was made in correspondence of the sites where input and
166 output operations were conducted and included moments in which operations were effectively
167 done. The duration of the operator exposure in input and output operations is about 2 hours/day.
168 However our sampling can be indeed as areal and not personal samples.
169
170 **2.2 Bioaerosol sampling and analysis**
171 Bioaerosol sampling was performed by a DUO SAS Super 180 sampler (PBI International), which
172 allows microbial monitoring through the use of air contact on apposite Petri plates. For each
173 parameter, various volumes were initially tested to obtain legible plates. Eight microbiologic
174 parameters were chosen as described in Table 1: total bacteria as environmental contamination
175 indicator (sampled volume: 200 air litres outdoor and 50 litres indoor), total bacteria as
176 animal/human contamination indicator (sampled volume: 500 air litres outdoor and 100 litres
177 indoor), total thermophilic bacteria (sampled volume: 500 litres outdoor and 200 litres indoor),
178 fungi and yeasts (sampled volume: 500 litres outdoor and 50 litres indoor), Pseudomonaceae as
179 biofilm formation indicator, *Clostridia spp.* to evaluate the possible anaerobic digestion selection,
180 Enterobacteriaceae as gut contamination indicator (we sampled 1000 air litres both outdoor and
181 indoor), finally, Actinomycetes as another environmental microbiologic component probably
182 linked to such biomasses. All microbiologic indicators were sampled at the selected volume with at
183 least three different plates for total counts; more plates were sampled as previously suggested for
184 this type of sampling (Sanchez-Munoz et al., 2012).
185 At the end of sampling, plates were removed, quickly transported to the lab, and placed in a
186 thermostat controlled environment set at the opportune temperature. Growth conditions are
187 listed in Table 1. To evaluate microbiological contamination levels, concise indicators proposed for
188 work environments have also been used; these allow assessments for indoor (INAIL, 2010) and
189 outdoor (Grisoli et al., 2009; Grover et al., 2006) environments. The results are also expressed as

GIMC (Global Index of Microbial Contamination) and MBC (Mesophilic Bacteria Contaminations) (Dacarro et al., 2000; Dacarro et al., 2005; Grisoli et al., 2012).

### 2.3 PMx-y sampling

PM10 samples were collected using a Sierra-Andersen high volume cascade impactor (AirFlow PM10-HVS sampler which a multi-stage cascade impactor, with pre-selector complies with EN-12341 norm by Analitica Strumenti) at a flow electronically controlled at 1.27 $m^3$ $min^{-1}$. Sampling durations of PMx was 4 hours and was repeated 12 times (6 times per plant) in 6 different size ranges. Firstly, the PM10 was selected by a pre-selector, then the multistage impactor determined the division of different particle sizes of the sampled particles by differentiation of the aerodynamic diameter, which is able to identify the type of trajectory that particles take inside the suction flow related to the three main aerodynamic factors of the particles themselves: dimension, shape and density (Analitica Strumenti). Particles having sufficient inertia will impact on that particular stage collection plate, whilst smaller particles will remain entrained in the air stream and pass to the next stage where the process is repeated. The stages are assembled in a stack or row in order of decreasing particle size.

Particle size fractions are: 10.0-7.2, 7.2-3.0, 3.0-1.5, 1.5-0.95, 0.95-0.49, and <0.49 µm. Glass microfiber filters with ten splits (Type A/E, 8”x10”, Gelman Sciences, Michigan, USA) were used to collect particles on each impactor plate; at the end, glass microfiber filters (20.32 x 25.40 cm, Pall Corporation, NY, USA) were present as back-up filters to collect the finest particles (<0.49 µm). All filters (approximately 80) were pre- and post-conditioned by placing them in a dry and dark environment for 48 h, then weighed in a room with controlled temperature and humidity. Each sampling session was carried out for a total of approximately 4 h each day. In each session, when possible, we collected samples at the two different sites. The PMx concentration in the air volume sampled was calculated as previously described (Traversi et al., 2011; Traversi et al., 2010).

### 2.4 Gravimetric and endotoxin analysis

Each filter was treated individually. Different portions of the filters were used for extraction: one half (51.75 $cm^2$) of the impactor plate filters and one-eighth (70 $cm^2$) following a radial portioning, of the back-up filters. Each portion was cut in single strips and placed in a 50 ml sterile polypropylene pyrogen-free tube with 15 ml of RPMI-1640 medium and supplemented with 0.025% Tween-20. The tubes with the filter’s stripes were placed in an ultrasonic water bath for 10

minutes and then vortexed for 30 seconds. This procedure was repeated three times. The samples were then centrifuged at 5000 rpm for 10 minutes to remove the glass fibre, and the supernatant was collected in clean tubes. The suggested standard procedure for storage and determination was followed (Duquenne et al., 2013; Paba et al., 2013). The resulting clear supernatant was assayed for endotoxin evaluation. If not otherwise specified, all chemicals were purchased from Sigma, USA.

### 2.5 Statistics

Statistical analyses were performed using SPSS Package, version 21.0. We applied (1) a log transformation of non-normally distributed data, (2) the Spearman rank-order correlation coefficient to assess relationships between variables, (3) a T-test to compare means, and (4) an ANOVA for multivariate analysis, in which we assumed an equal variance, followed by a Tukey post-hoc test for multiple comparisons. The mean differences and correlations were considered significant if $p<0.05$ and highly significant if $p<0.01$.

## 3. RESULTS AND DISCUSSION

### 3.1 Microbiologic determination

Table 2 showed the microbiological evaluation on the sampled air for the two sites at the M and S plants. As expected, the higher the microbe indicator is when the environmental total bacteria is significantly higher than the others (ANOVA p= 0.023, F=2.482). In decreasing order, we find the total bacteria at 37°C to be fungus and yeast, thermophilic bacteria, Actinomycetes and Clostridia, and finally Pseudomonaceae and Enterobacteriaceae.

Our environmental total bacteria results are in the range of the mesophilic bacteria observed in composting facilities and are equal to $10^2$-$10^8$ UFC/$m^3$. The same evidence is observable for thermophilic Actinomycetes and moulds (Le Goff et al., 2010; Le Goff et al., 2012; Le Goff et al., 2011; Wery, 2014).

Adherence was observable for other microbiologic indicators such as Enterobacteriaceae. Even if the microbiologic parameter is often split into more specific ones such as enterococci and faecal coliforms (Heinonen-Tanski et al., 2009), Pseudomonaceae were less present than in other types of biomass facilities (Liang et al., 2013), especially in semi outdoor or outdoor sampling sites. Clostridia were generally associated with a soil contaminated environment, near municipally

254 landfill sites,in a range comparable to our data (Kalwasinska and Burkowska, 2013); moreover, a
255 marked selection of the anaerobic digestion process was not observed on Clostridia growth (Li et
256 al., 2014).
257 Comparing the two plants, we observed a greater contamination in the M-plant for the bacteria
258 count (T-test $p<0.01$); moreover, this evidence is confirmed also for moulds and Actinomycetes
259 and Pseudomonaceae (T- test $p<0.05$). The comparison between the first steps of the operation in
260 the plant during the hopper loading and the last steps during the digested sludge exiting showed a
261 large amount of contamination in the first steps; however, this is because of the characteristics of
262 the sampling site, an indoor site in the M-plant. Considering separately the two plants, we
263 observed a generally comparable contamination of the input and output operation in the S-plant;
264 only the total bacteria at 37°C is greater in the output operation. Considering only the M-plant, all
265 the parameters were significantly greater in the hopper loading indoor sampling site (T- test,
266 $p<0.05$, bacteria and fungal T test $p>0.01$).
267 Moreover, the microbiologic indicators with a higher level of UFC/$m^3$ are significantly correlated
268 each other (Spearman's rho > 0.650 $p<0.01$) and the thermophilic bacteria, fungal and yeast
269 amounts are significantly correlated with the fraction 7.2-3 µm (Spearman's rho > 0.650 $p<0.05$).
270

271 **3.2 PMx-y**

272 In figure 1, in the PMx-y levels, the particles with an aerodynamic diameter comprised between 10
273 and 7.2 µm and the particles with an aerodynamic diameter less than 0.49 µm are shown to have
274 the highest mass with respect to the other (ANOVA $p<0.01$, $F=6.972$). The PM10 was higher at the
275 M-plant in both the sampling sites and at the digested sludge exiting of the S-plant. In this last
276 operation, there was frequent transit of the sludge spreading vehicle, which could influence the
277 mass of the 10-7.2 µm fraction. The semi-indoor and indoor characteristic of the M-plant sampling
278 sites justified the higher level of the PM10, with PM10 levels often reaching above 50 µg/$m^3$. The
279 mean values are not high (28.9 µg/$m^3$) for such sampling sites; however, the mean values are
280 higher than the range of the mean levels at a rural site, approximately 15 µg/$m^3$(Heal and
281 Hammonds, 2014; ProvinciaTorino, 2014; Querol et al., 2014). Also the PM3 (as a result of the
282 finest fraction sum) is around 60% higher than the PM2.5 rural background level (8 µg/$m^3$)(Heal
283 and Hammonds, 2014; Querol et al., 2014). A statistically significant difference between the plants
284 is observable only for the intermediate PMx-y (T-test $p<0.05$).
285

### 3.3 Endotoxin determination

In this study, the endotoxin expressed in terms of EU/mg ranged from 5 to 3220, with a mean of 428. Figure 2 shows that the endotoxin evaluation amount is very limited especially considering the intermediate PM10 fractions. The endotoxin pollution, for the two plants, is quite low and comparable to levels observed in other studies for waste collection and treatment plants (Duquenne et al., 2013). Moreover the recorded values are comparable to those showed in other studies on inhalable particles sampled in life environment (Fromme et al., 2013) and a rural site in summer (Ferguson et al., 2013). While, very contaminated sites, such as poultry house, showed levels of at least two order of magnitude above (Lawniczek-Walczyk et al., 2013)

On the other hand, the ratio between endotoxin in $PM_3$ (as a sum of the finest fractions) with respect to the endotoxin in the $PM_{3-10}$ (as a sum of the less fine fractions) is equal to 4 because of the higher endotoxin presence in the finest particles (>0.49 μm). This evidence is not comparable to the general literature that observes endotoxins, especially in the coarse fraction (Chang et al., 2014). This incongruity could be justified considering two evidences. Firstly the finest fraction is the most relevant both to the mass and, of course, particle numbers during our sampling activities and the ratio between $PM_3$ and $PM_{3-10}$ is at least 4 with an average, for all sampling, equal to 8. Secondly, other previous studies showed the possible association of the endotoxin with the finest fraction with respect to the coarse, with particular regard to indoor and semi-indoor sampling sites (Paba et al., 2013).

### 3.4 Occupational risk evaluation

Table 3 shows the levels for each evaluated risk factor as a maximum and mean observed values; moreover, the last column reported is a reference guide line for human health suggested in the literature both for occupational and life environments. Of course such reference are not perfectly comparable in terms of averaging time and sampling methods especially for PM guide line value (Krzyzanowski and Cohen, 2008) however the comparison could be useful to place such pollution into the occupational and environmental exposure characterization contest.

It is important to highlight that the operators exposed are normally limited (three for each plants during our samplings) and the duration of their exposure is globally limited in routinely conditions, at least a couple of hours day. Following the risk evaluation for the two plants are discussed.

Considering the microbiologic health risk assessment as GIMC and MBC ratio for the S-plant workers, we have to note that the sampling site could be considered to have generally low

contamination. It is possible that the contamination can reach an intermediate level but without a worsening due to the MBC ratio. Moreover, no particular relevance can be observed for microbiologic parameters both linked to biofilm formation (as risk factor for respiratory diseases and contact dermatitis) (Sethi, 2013; Williams et al., 2010) and to gastroenteritis (Latasa et al., 2005). No occupational hazard can be individuated for PMx or endotoxin exposure. The particulate is near environmental levels recorded into the north Italy (Pianura Padana)(Traversi et al., 2008) and no hazard evidence is clear for finest fraction near the background levels (WHO-Europe, 2013). The endotoxins associated with the inhalable particles are widely below the occupational safe guide lines (DECOS, 2010; Duquenne et al., 2013). Thus, the risk control in such outdoor sites can be obtained by applying a good work practice and for the biologic risk using protective measures provided by the equipment such as filtering the cabin and using individual protective devices.

For the M-plant the microbiologic risk is significant and great attention to risk management is necessary, especially in the hopper loading indoor operations (table 1). Moreover, the GIMC showed a very high contamination level (table 3). However, this risk can be managed using occupational safety measures, including limited and protected access to the hangar, the use of the appropriated control measures and avoiding access during hopper mixing. This last operation is also characterised by ammonia and hydrogen sulphide liberation from the mixed biomasses that represents an additional chemical hazard for the workers (Malhautier et al., 2003). In this study such chemicals were not objected of samplings but their presence were smelling recognised. However, the MBC level is not an aggravating factor. The PM levels, even if far from reaching the occupational limit of exposure (Forstater, 2004; Lacey et al., 2006), are quite above the environmental guide line values, especially in the hangar for biomass storage and hopper loading, where the levels are double the outdoor ones. However this can't be considered a not respect of an occupational limit exposure but only a caution call in the occupational risk manage. Finally the endotoxin content is largely below occupational limits (Duquenne et al., 2013).

These are two biomasses plants operating into our territory. They can be considered examples of the current practices. The transformation of such kind of plant, from only agricultural and livestock to also energy production, introduced a different risk profile for the operators. This aspect is generally underestimated for the occupational health and safety manage and the large number of plants are maintaining for the operators the same individual risk profile evaluation than before the plant transformation.

## 4. CONCLUSION

Our findings highlighted the necessity of an occupational risk re-evaluation for anaerobic digestion workers. This evaluation has to be focussed on the biological and chemical risks linked to the biomass movement; of course other risks such as explosive, electric, motor vehicle transit, and so on can be identified and evaluated as well. Moreover, the comparison of the two plants showed different contamination levels in relation to the involved biomasses and to the technological and building characteristics, so a single plant approach has to be adopted. In general, we can assume, after this work, that PM10 and its associated endotoxin exposure are not a relevant risk for anaerobic digestion workers, while, the biologic risk has to be carefully quantified and managed especially in indoor environment Moreover additional specific assessment could be necessary for emerging pathogens such as virus.

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## FIGURE LEGENDS

**Figure 1 -** PM10 and its fraction concentrations observed during the sampling in the two different anaerobic digestion plants (S and M) divided by sample point: one for biomass storage and loading into the hopper and one for digestate output.

**Figure 2 -** Endotoxin concentrations assessed in PMx observed during the sampling in the two different anaerobic digestion plants (S and M) divided by sample point: one for biomass storage and loading into the hopper and one for digestate output.

## TABLE LEGENDS

**Table 1 -** Selected parameters for bioaerosol sampling and the cultural method adopted for each one.

381 **Table 2 -** Microbiologic contamination level assessed during sampling in the two different
382 anaerobic digestion plants (S and M) divided by sample point, one for biomass storage and loading
383 into the hopper and one for digestate output.
384 **Table 3 -** Risk evaluation comparing the data from the sampling as maximum and mean value
385 observed for each plant to a reference guide value for human health protection for the assessed
386 parameters from bioaerosol, PMx-y and its endotoxin content. The GIMC and the MBC was
387 assessed as previously proposed (Dacarro et al., 2000).
388

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