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**Mycological and ecotoxicological characterisation of landfill leachate before and after traditional treatments**

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1 **TITLE:** Mycological and ecotoxicological characterisation of landfill leachate before and after traditional  
2 treatments

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9

## 10 **Abstract**

11 Pollution caused by landfill leachates is one of the main problems of urbanised areas, on account of their  
12 chemical composition, which turn in an ineffective treatment. A characterisation of leachates, which takes in to  
13 account chemical, ecotoxicological and mycological aspects, is basilar for the evaluation of environmental  
14 impact of leachate and the development of suitable treatment techniques. In this study, the toxicity of a raw  
15 leachate and an effluent coming from traditional wastewater treatment plant was assessed by means of 4  
16 ecotoxicological assays. Both the samples exceed the legal threshold value according to all the tested organisms,  
17 indicating the ineffectiveness of activated sludge treatment in the reduction of toxicity. The autochthonous  
18 mycoflora of the two samples was evaluated by filtration. The fungal load was 73 CFU for leachate and 102  
19 CFU for the effluent. Ascomycetes were the dominant fraction (81 % and 61 %, for leachate and effluent  
20 respectively), followed by basidiomycetes (19 % and 39 %, respectively). Most of them were potential emerging  
21 pathogens. A decolourisation screening with autochthonous fungi was set up towards both samples in the  
22 presence or absence of glucose. Eleven fungi (basidiomycetes and ascomycetes) achieved up to 38%  
23 decolourisation yields, showing to be promising fungi for the bioremediation of leachates. Further experiment  
24 will be aimed to the study of decolourisation mechanism and toxicity reduction.  
25

26 **Key Words:** Landfill leachate, autochthonous mycoflora, ecotoxicity, bioremediation.

## 27 **1 Introduction**

28 Municipal landfills are polluted sites rapidly increased with urbanization and economic development.  
29 The percolation of rainwater during the decomposition of wastes generates leachates, which are among the most

30 polluting wastewaters. The concern about landfill leachates is threefold on account of their difficulty in  
31 treatment, their toxic potential and the microbiological composition.

32 The main difficulties observed during the treatment of leachates under conventional processes are  
33 associated with the high concentration of organic matter, the high concentration of nitrogen as ammonia  
34 originating from the decomposition of proteins, the presence of toxic organic compounds and heavy metals, and  
35 the variable pH values (Vedrenne et al., 2012; Zhao et al., 2013). This is particularly true for mature leachate in  
36 methanogenic phase (at least 10 years old), in which the organic matter is principally constituted by recalcitrant  
37 and toxic xenobiotics, indicated by a low BOD/COD ratio ( $<0.5$  mg/l), that represents a limit for the growth and  
38 the metabolic activity of heterotrophic bacteria in activated sludge (Gotvajn and Zagorc-Koncan, 2009; Renou et  
39 al., 2008; Schiopu and Gavrilesu, 2010). The ineffectiveness of traditional treatments is particularly evident in  
40 the persistence of the dark colour in effluents coming out from wastewater treatment plants (Kurniawan, et al,  
41 2010; Primo et al., 2012).

42 Moreover, some recalcitrant compounds are hardly traceable (even in untreated leachate); nevertheless  
43 their impact on the environment is potentially devastating on account of their toxicity. The presence of toxic  
44 substances can be indirectly detected thanks to ecotoxicity bioassays (Thomas et al., 2009; Tigini et al., 2010).  
45 Innovative treatment techniques, effective towards both micro and macro-pollutants, are required.

46 The biological pollution caused by leachates is another crucial aspect: the microorganisms load due to  
47 the presence of high amount of organic matter can pollute both surface and underground waters, with enormous  
48 damage to fresh water reserves (Schiopu and Gavrilesu, 2010). Nevertheless, the presence of fungi in leachates  
49 has mostly been overlooked (Bareither et al., 2013; Matejczyk et al., 2011; McDonald et al., 2010). A possible  
50 reason for this is that the consumption of fungal contaminated water usually does not lead to acute disease,  
51 contrarily to pathogenic bacteria, viruses, and parasites contamination (Pereira et al., 2009). Only in the last  
52 decade fungi have received increased attention as water contaminants but still few countries have introduced  
53 specific law on this aspect (Hageskal et al., 2009).

54 Thus, a characterisation of leachates, which takes into account chemical, ecotoxicological and  
55 microbiological aspects, is basilar for the evaluation of the environmental impact of leachates. In addition, it  
56 could be a key element for the assessment of wastewater treatment and, eventually, the development of  
57 innovative and more effective techniques. For instance, saprotrophic fungi that access leachates from the landfill  
58 waste are potentially suitable microorganisms for the bioremediation of leachate itself. Actually, they are already

59 adapted to the wastewater toxicity and extreme physical parameters (high ammonium and salt concentration and  
60 high pH).

61 In this preliminary study, a crude leachate and an effluent coming from nitrification-denitrification and  
62 biological oxidation treatments were characterised by means of ecotoxicological and mycological analyses.  
63 Moreover, the autochthonous fungal stains were tested in a decolourisation screening towards both the raw  
64 leachate and the effluent, in presence or absence of an additional carbon source (glucose).

## 65 2 Materials and Methods

### 66 2.1 Wastewaters

67 The two samples come from a wastewater treatment plant located in the central part of Italy. One was a  
68 crude landfill leachate and the second one was the effluent (consisting of 70 % v/v leachate and 30 % v/v of  
69 other kinds of wastewater) previously treated by biological oxidation with activated sludge and  
70 nitrification/denitrification process. Both the samples were prepared by sampling the leachate and the effluent  
71 daily for a period of 15 days. Both the samples were dark coloured and had high content of ammonium and salts.  
72 Details of their chemical characteristics, provided by the owner of the wastewater treatment plant, are shown in  
73 Table 1.

74

75 **Table 1. Samples chemical characterisation.**

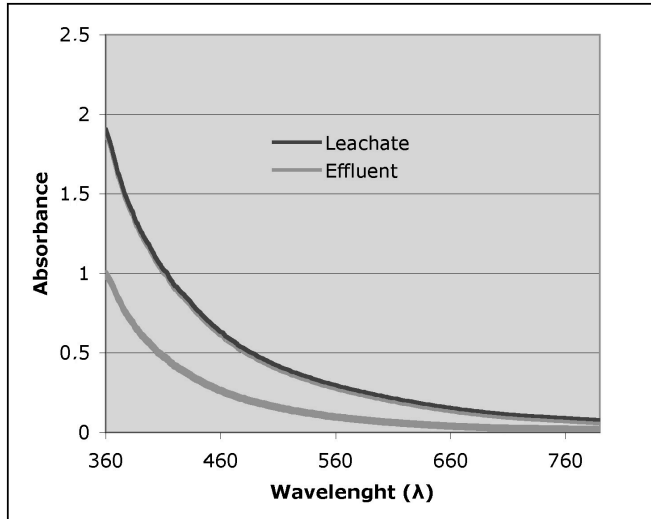
Parameter	Leachate	Effluent	Legal threshold limit
Cadmium (as Cd)	<0.001 mg l <sup>-1</sup>	<0.001 mg l <sup>-1</sup>	0.02 mg l <sup>-1</sup>
Total chromium (as Cr)	1.20 mg l <sup>-1</sup>	0.50 mg l <sup>-1</sup>	2 mg l <sup>-1</sup>
Nichel (as Ni)	0.30 mg l <sup>-1</sup>	0.30 mg l <sup>-1</sup>	2 mg l <sup>-1</sup>
Lead (as Pb)	0.073 mg l <sup>-1</sup>	0.082 mg l <sup>-1</sup>	0.2 mg l <sup>-1</sup>
Cuprum (as Cu)	0.60 mg l <sup>-1</sup>	0.15 mg l <sup>-1</sup>	0.1 mg l <sup>-1</sup>
Zinc (as Zn)	0.66 mg l <sup>-1</sup>	1.38 mg l <sup>-1</sup>	0.5 mg l <sup>-1</sup>
Total hydrocarbons	<100 mg l <sup>-1</sup>	598.7 mg l <sup>-1</sup>	5 mg l <sup>-1</sup>
Ammonium (as NH <sub>4</sub> )	2266.0 mg l <sup>-1</sup>	408.0 mg l <sup>-1</sup>	15 mg l <sup>-1</sup>
Total nitrogen (as N)	2537.9 mg l <sup>-1</sup>	514.1 mg l <sup>-1</sup>	20 mg l <sup>-1</sup>
Sulphates (as SO <sub>4</sub> )	46.9 mg l <sup>-1</sup>	801.7 mg l <sup>-1</sup>	1000 mg l <sup>-1</sup>
Chlorides (as Cl <sup>-</sup> )	3193 mg l <sup>-1</sup>	2550 mg l <sup>-1</sup>	1200 mg l <sup>-1</sup>

Anionic surfactants (MBAS)	23.07 mg l <sup>-1</sup>	37.9 mg l <sup>-1</sup>	2 mg l <sup>-1</sup>
Non ionic surfactants (PPAS)	23.48 mg l <sup>-1</sup>	17.2 mg l <sup>-1</sup>	
Cationic surfactants	<10 mg l <sup>-1</sup>	<10 mg l <sup>-1</sup>	
COD (as O <sub>2</sub> )	6166 mg l <sup>-1</sup>	2099 mg l <sup>-1</sup>	160 mg l <sup>-1</sup>
BOD <sub>5</sub> (as O <sub>2</sub> )	4209 mg l <sup>-1</sup>	1410 mg l <sup>-1</sup>	40 mg l <sup>-1</sup>
pH	8.10	8.40	5.5-9.5
Suspended solids	1064 mg l <sup>-1</sup>	604 mg l <sup>-1</sup>	80 mg l <sup>-1</sup>
Colour	visible at 1:20 dilution	visible at 1:20 dilution	not visible at 1:20 dilution

76

77 Both samples exceeded the legal limit for almost 13 parameters, which variation could be due also to  
78 the mix of leachate with other influent in wastewater treatment plant, in addition to the performed treatments.

79 Among these, the colour is the most evident. Despite the Italian legal limit is not objectively fixed (the legal  
80 threshold is based on the perception of colour at dilution higher that 20:1), the samples colour is very dark and  
81 clearly visible even when diluted. The absorption spectra of 1:4 diluted wastewaters are reported in Figure 1.



82

83 **Figure 1. Absorption spectra of 1:4 diluted leachate and effluent.**

84

## 85 2.2 Ecotoxicity tests

86 A battery of four acute ecotoxicity tests was performed in order to evaluate the environmental impact of  
87 the leachate and the effluent. The target organisms were selected among different trophic levels: a unicellular  
88 green alga (*Pseudokirchneriella subcapitata* (Korshikov) Hindak), two dicotyledonous plants (*Cucumis sativus*

89 L. and *Lepidium sativum* L.), and a fresh water crustaceous (*Ceriodaphnia dubia* Richard). The endpoints  
90 considered were respectively: the algal growth inhibition, the root elongation inhibition, the mobility inhibition.

91 The tests were performed according to the standard methods: UNI EN ISO 8692:2005, EN ISO 20079:2005,  
92 UNICHIM N. 1651 (2003), and Ceriodaphtoxkit F (Ecotox, Cornaredo - MI) adhering to the USEPA test  
93 guideline, respectively.

94 After 48 h of incubation at 23 °C and at 8000 lux, the algae concentration was measured with a  
95 spectrophotometer. The two dicotyledonous plants, were purchased from Ingegnoli S.p.A. (Milano). For both  
96 species, ten seeds were placed in 9 cm Petri dishes, containing 5 ml of sample solution and a paper filter  
97 (Whatman No.1). The control was performed in four replicates, using distilled water. The seeds were incubated  
98 for 72 h in the dark at 25 °C.

99 Each dose–response curve consisted of at least 6 dilutions in at least three replicates. To test the  
100 sensitivity of each species, acute toxicity tests with  $K_2Cr_2O_7$  were performed at regular intervals.

101

## 102 2.5 Isolation and identification of the autoctonous mycoflora

103 Ten aliquots of 100 ml of each wastewater were filtered on sterile membrane filters (pore size of 0.45  
104  $\mu$ M) by means of a vacuum pump. The membranes were placed in Petri dishes (9 cm diameter) containing MEA  
105 with antibiotics (15 mg  $l^{-1}$  streptomycin and 50 mg  $l^{-1}$  chloramphenicol). The plates were incubated at 25 °C in  
106 the dark. At regular time intervals, the colony forming units (CFU) were counted and the different fungal  
107 morphotypes were isolated in pure culture. Fungi were identified conventionally according to their macroscopic  
108 and microscopic features. After determination of their genera (Domsch et al., 1980; Kiffer and Morelet, 1997;  
109 von Arx 1981), they were transferred to the media recommended by the authors of selected genus monographs  
110 for species identification. Molecular identification of all fungi was performed by amplification and sequencing  
111 of the internal transcribed spacers (ITS), D1/D2 region,  $\beta$ -tubulin and actin genes (Carbone and Kohn, 1999;  
112 Gardes et al., 1993; Glass and Donaldson, 1995; White et al., 1990).

113 The nonparametric Spearman test was run to assess the significance ( $p \leq 0.05$ ) of the quantitative and  
114 qualitative differences between the mycoflora of the two samples.

115

## 116 2.6 Bioremediation screening

117 Mycelium discs (3 mm diameter) were taken from the marginal portion of colonies in active growth  
118 onto MEA and inoculated in multiwell containing 2.5 ml of wastewater samples. The addition of 1 g  $l^{-1}$  glucose

119 as carbon source was also considered, in order to evaluate a possible co-metabolism. The tests were carried out  
120 in duplicate. The multiwell plates were incubated for 20 days at 25 °C, in stirred condition (150 rpm), in the  
121 dark. At regular intervals (10-15-20 days) 200 µl were taken from each well, centrifuged at 14000 rpm for 10  
122 minutes, and absorption spectra in the visible ( $\lambda = 360-790$  nm) were acquired. The decolourisation percentage  
123 was determined as the decrease of the spectrum area with respect of the abiotic control.

124

### 125 **3 Results and discussion**

#### 126 *3.1 Leachate ecotoxicity before and after traditional treatments*

127 A classical elaboration of ecotoxicity results was not always possible. Actually, the calculation of EC<sub>50</sub>,  
128 LD<sub>50</sub> or LC<sub>50</sub> was not possible, on account of the sample dark colour that affect the instrumental reading  
129 (spectrophotometer). Actually, doses higher than 40% was not testable with the algal test. In other cases, there  
130 was a sort of all-nothing effect (*C. dubia* and *L. sativum*), which did not allow to determine a complete dose-  
131 effect regression line.

132 However, all the organisms of the ecotoxicological battery showed clearly a high sensitiveness to the  
133 toxicity of the samples. *C. dubia* was the most sensitive organism towards the crude leachate, whereas *L. sativum*  
134 was the most sensitive one towards the effluent (Figure 2).



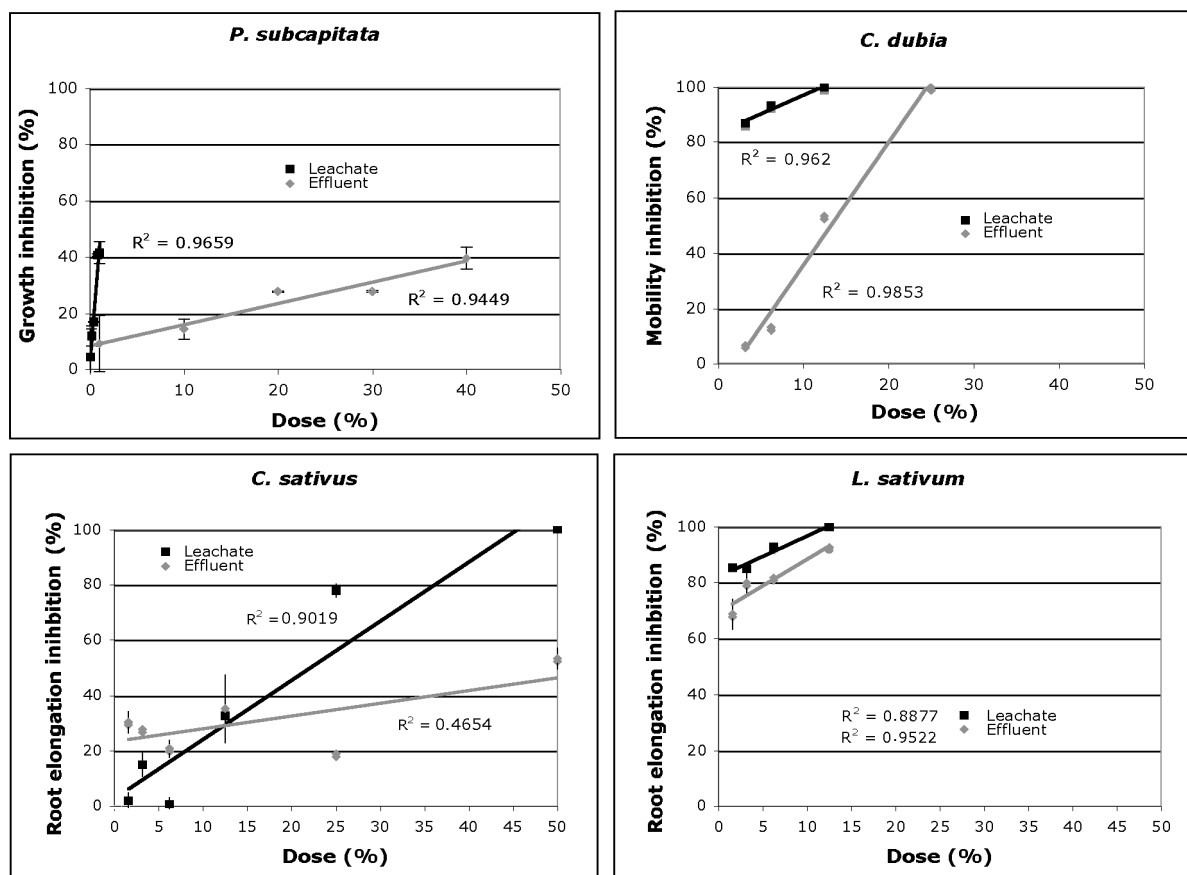


Figure 2. Toxicity of leachate and effluent vs different test organisms.

135  
136

137

138 In general, the leachate was more toxic than the effluent according three out of four tests, as indicated  
139 by the regression lines in dose-effect charts of Figure 2. However the toxicity of both samples was much higher  
140 than the legal threshold value (DL 152/06), since all the assays showed 100% inhibition at the 100% sample  
141 dose (data not shown).

142 The decrease of leachate toxicity after the traditional treatments recorded by *P. subcapitata*, *C. dubia*  
143 and *L. sativum* could be due to the lower content of ammonium and, partially, to the decrease of chloride ions  
144 with respect to the untreated leachate. On the contrary, sulphate and total hydrocarbon increased in effluent with  
145 respect to crude leachate, thus their role in the inhibition of these three organisms may be marginal. In support to  
146 this hypothesis, it is noteworthy that as the pH of the samples is adjusted at acidic value (pH 5) the inhibition  
147 effect drop under 60% for *L. sativum* (Tigini et al., 2013). Actually, in acidic conditions ammonia (NH<sub>3</sub>) turns in  
148 the less toxic ammonium ion (NH<sub>4</sub><sup>+</sup>). On the base of the same experiment with acidified leachate and effluent,  
149 we hypothesized that also heavy metals play a marginal role in samples toxicity. In fact, heavy metals at low pH  
150 should be mobilised becoming bioavailable. Despite this, the toxicity decreased as previously reported.

151 Although for *P. subcapitata*, *C. dubia* and *L. sativum* toxicity could mainly due to the ammonium  
 152 content in the sample, the synergistic effect of xenobiotics under subacute levels concentration present in the  
 153 samples can not be excluded. This could be particularly true for *L. sativum*, which recorded a weak decrease of  
 154 the toxicity after the leachate treatment (Figure 2).

155 On account of these considerations, the major role of ammonium in sample toxicity could explain the  
 156 relatively low sensitiveness showed by *P. subcapitata* with respect of other organisms. In fact, the green alga  
 157 was already reported as a suitable organism for the toxicity assessment of phenyls, aldehydes and alkenes, but  
 158 not ammonia (Thomas et al., 2009). However, in our case, additional analyses should be performed in order to  
 159 confirm this hypothesis.

160 The results obtained by *C. sativus* shows a singular trend, actually crude leachate caused higher  
 161 inhibition than effluent. However, it is noteworthy that the relationship between effluent dose and *C. sativus*  
 162 inhibition is not linear, as indicated by the low R value ( $R < 0.4$ ). Thus, in this case any comments would be  
 163 speculative.

164

### 165 3.2 Characterisation of autochthonous mycoflora of leachate

166 The total fungal load of the crude leachate was very low (7.3 CFU/100 ml) (Table 2).

167

168 **Table 2. Fungal taxa isolated from leachate and effluent, and their fungal load (CFU / 100 ml<sup>-1</sup>).**

Fungal taxa	Leachate (CFU /100 ml)	Effluent (CFU /100 ml)
<i>Arthrimum sphaerospermum</i> Fuckel	0.0	0.5
<i>Aspergillus fumigatus</i> Fresen.	0.1	0.0
<i>Aspergillus tubingensis</i> (Mosseray) Kozakiewicz	0.0	0.1
<i>Aspergillus sydowii</i> (Bainier & Sartory) Thom & Church	0.0	0.4
<i>Bjerkandera adusta</i> (Willd.) P. Karst.	0.0	0.1
<i>Candida parapsilosis</i> (Ashford) Langeron & Talice	0.7	0.4
<i>Ceriporia purpurea</i> (Fr.) Donk	0.0	0.1
<i>Coprinellus micaceus</i> (Bull.) Vilgalys, Hopple & Jacq. Johnson	0.2	0.0
<i>Cladosporium brunei</i> Linder	0.0	1.5
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	0.4	0.9
<i>Cladosporium perangustum</i> Bensch, Crous & U. Braun	0.0	0.1

<i>Cladosporium pseudocladosporioides</i> Bensch, Crous & U. Braun	2.4	0.0
<i>Cladosporium xylophilum</i> Bensch, Shabunin, Crous & U. Braun	0.5	0.0
<i>Doratomyces microsporus</i> (Sacc.) F.J. Morton & G. Sm.	0.0	0.1
<i>Epicoccum nigrum</i> Link	0.1	0.3
<i>Eurotium amstelodami</i> L. Mangin	0.1	0.0
<i>Flammulina velutipes</i> (Curtis) Singer	0.1	0.0
<i>Galactomyces geotrichum</i> (E.E. Butler & L.J. Petersen) Redhead & Malloch	0.0	0.1
<i>Hamigera striata</i> (Raper & Fennell) Stolk & Samson	0.0	0.1
<i>Hypholoma sublateritium</i> (Fr.) Quél.	0.1	0.3
<i>Leptospheria</i> sp.	0.1	0.0
<i>Penicillium brevicompactum</i> Dierckx	0.5	0.5
<i>Penicillium corylophilus</i> Dierckx	0.0	0.1
<i>Penicillium glandicola</i> (Oudem.) Seifert & Samson	0.3	0.5
<i>Penicillium olsonii</i> Bainier & Sartory	0.1	0.0
<i>Phanerochaete sanguinea</i> (Fr.) Pouzar	0.0	0.1
<i>Phlebia acerina</i> Peck	1.0	0.0
<i>Phlebia tremellosa</i> (Schrad.) Nakasone & Burds.	0.0	0.4
<i>Pseudallescheria boydii</i> (Shear) McGinnis, A.A. Padhye & Ajello	0.6	0.3
<i>Rhodotorula minuta</i> (Saito) F.C. Harrison	0.0	3.0
<i>Talaromyces assiutensis</i> Samson & Abdel-Fattah	0.0	0.1
<i>Talaromyces helicus</i> (Raper & Fennell) C.R. Benj.	0.0	0.2
<b>Total</b>	<b>7.3</b>	<b>10.2</b>

169

170           Despite the high concentration of organic matter in this sample, it is surprising that the total fungal load  
171 is similar to that of drinking water (2-9 CFU/100 ml), and even lower than that of ground water (4450 CFU/100  
172 ml) recorded with the same techniques by other authors (Hageskal et al., 2007; Kanzler et al., 2008). This could  
173 be due to the high toxicity that affects the sample. In particular, the inhibitory effect of ammonia on fungal  
174 growth and enzymes secretion by allochthonous fungi in landfill leachate has been already demonstrated  
175 (Ellouze et al., 2009).

176           The leachate mycoflora consisted of 16 taxa ascribable to 11 ascomycetes, of which 10 filamentous  
177 fungi (71 % of the fungal load) and 1 yeast (10 % of total CFU), and 5 basidiomycetes (19 % of total CFU).

178 Most of the ascomycetes were in their anamorphic (asexual) form. They can be considered adaptive  
179 extremophiles (Cantrell et al., 2011). Many extremophilic fungal species have a mainly mitotic life style,  
180 although even in species with sexual cycles, small isolated populations can diversify independently (i.e.  
181 *Eurotium*). This is partially due to the progressing genetic drift rapidly fix alleles in small populations that have  
182 managed to adapt to extreme habitats, thanks to the absence of sexuality and/or gene flow (Gostin car et al,  
183 2010).

184 In addition, some of these fungal species are potentially pathogen (in particular *Candida parapsilosis*  
185 which is an H2 organism). The pathogenicity of fungi has been associated with moderate osmotolerance at the  
186 order level. Actually, warm-blooded animal body and skin represent an extreme environment for  
187 microorganisms, with elevated temperatures and salt concentration, as well as mechanic stress (de Hoog et al.,  
188 2005). Thus, generalist fungi with suitable pre-adaptation, as that ones in leachates, represent a pool of potential  
189 medically important fungi, which might readily switch from environmental niches to human bodies (Gostin car et  
190 al., 2010).

191 Opportunistic pathogenic fungi, such as *Aspergillus fumigatus*, *Candida* spp., *Geomyces pannorum*,  
192 *Geotrichum candidum*, *Microsporium canis* and *Scedosporium* spp. were often isolates from both polluted water  
193 and soil (Tigini et al., 2009; Ulfig, 1994). These fungi are emerging pathogens with an incredibly high ecological  
194 success in the last decades. Their rapid spread is particularly alarming on account of their increasing impact on  
195 plants, animals and humans (Picco et al., 2011). Many of these species are able to use hydrocarbons, aromatic  
196 compounds and natural gas as carbon source; they can growth at very low oxygen partial pressure and in  
197 presence of high ammonium and salt concentrations (Kaltseis et al., 2009; Mooner et al., 2013).

198 The presence of basidiomycetes in leachate was notheworthy. Actually, they were the second group in  
199 terms of both biodiversity and fungal load. Moreover, it must be kept in mind that their biomass can be  
200 underestimated considering their CFU, on account of the absence of propagules. These fungi play a key role in  
201 the decomposition of organic matter and they represent the last phase of the series that colonise organic matter in  
202 the soil (van der Wal et al., 2013). Since their occurrence in aquatic habitats has been reported in several works  
203 (Richards et al., 2012), they could play the same ecological role also in these environments, such as leachates.  
204 This is supported also by other studies on the composition of fungal community in water contaminated by  
205 leachate coming from over than 30 years old landfill, in which basidiomycota was the most represented phylum  
206 followed by ascomycota (Brad et al., 2008). Neither in this last study nor in our one the presence of zygomycota

207 was recorded. Actually, fungi belonging to this phylum are considered pioneers and exploit easy available  
208 carbon sources (Anastasi et al., 2013).

209

### 210 3.3 Variation of the mycoflora after traditional treatments

211 The fungal load significantly increased after the biological oxidation with activated sludge and  
212 nitrification/denitrification treatments. However, the effluent showed still a low fungal load (10.2 CFU/100 ml)  
213 (Table 2). The biodiversity of the treated leachate consisted of 24 taxa, 16 out of them were not present in the  
214 raw leachate. They were ascribable to 18 ascomycetes, among which 17 filamentous fungi (57 % of total CFU)  
215 and 1 yeast (4 % of total CFU), and 6 basidiomycetes, among which 5 filamentous fungi (10 % of total CFU)  
216 and 1 yeast (29 % of total CFU) (Table 2).

217 Filamentous ascomycetes remained most abundant group, in particular the anamorphic form.

218 Yeasts load significantly increased after the leachate treatments. This was due mainly to *Rhodotorula*  
219 *minuta* (basidiomycetes), which was not present in the untreated leachate, and thus it probably came from the  
220 treatment plant. This genus seems to be very common in different kinds of wastewater treatment plants (Yang et  
221 al., 2011) and represent an emerging halophilic pathogen (Wirth and Goldani, 2012). This yeast can produce  
222 cytochrome P450, which can be at the base of several biotransformation and bioremediation process (Fukuda et al.,  
223 1996). Its physiological and metabolic characteristics could have determine its acclimatisation in the oxidation  
224 tank and, thus, in the effluent.

225 Filamentous basidiomycetes were halved with respect to the untreated leachate. The turbulent aquatic  
226 environment, that generally characterises wastewater treatment plants, is quite unusual for basidiomycetes, on  
227 account of their obligate filamentous *habitus* that can be damaged by the continuous stress due to the mechanical  
228 agitation. Ascomycetes, instead, can implement an adaptative strategy called microconidiation (microcyclic  
229 conidiation without the mycelial phase), which allows to overcome this environmental limit. This factor, jointed  
230 to the competition with the microflora of the treatment plant, could be the cause of filamentous basidiomycetes  
231 decrease. Among the 4 identified basidiomycetes, 3 were exclusively isolated from the treated effluent:  
232 *Bjerkandera adusta*, *Phlebia tremellosa*, and *Phanerochaete sanguinea*. Noteworthy, all of them are well known  
233 xenobiotic degraders (Kim et al., 2008; Medvedeva et al., 1994; Spina et al., 2012) and they are probably active  
234 in the treatment plant.

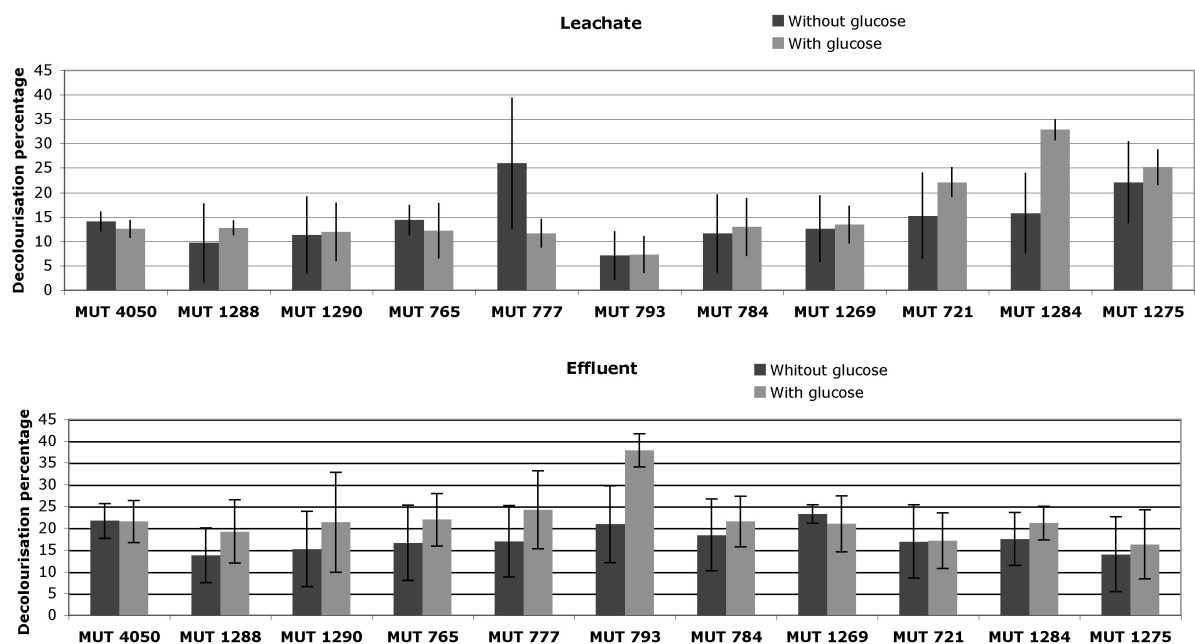
235

### 236 3.4 Decolourisation screening

237 A total of 51 fungi isolated from the raw and treated leachates were deposited in the *Mycotheca*  
 238 *Universitatis Taurinensis* (MUT) collection and screened in a decolourisation experiment with the aim to select  
 239 fungi with high bioremediation potential in terms of decolourisation yields. The addition of 1 g l<sup>-1</sup> glucose as  
 240 carbon source was also considered, in order to evaluate a possible co-metabolism of xenobiotics.

241 Some isolates belonging to the genera *Aspergillus* (*A. fumigatus* MUT 4050, *A. tubingensis* MUT 1288,  
 242 *A. sydowii* MUT 1290), *Arthrinium* (*A. sphaerospermum* MUT 777), *Penicillium* (*P. brevicompactum* MUT 793,  
 243 *P. corylophilum* MUT 784), *Pseudallescheria* (*P. boidii* MUT 1269, MUT 721), and some basidiomycetes (*B.*  
 244 *adusta* MUT 765, *P. sanguinea* MUT 1284, *F. velutipes* MUT 1275) emerged as the most promising strains.  
 245 They determined up to 7-26% decolourisation for the leachate and up to 14-23% for the effluent, after 20 days  
 246 incubation (Figure 3).

247 The addition of glucose had not determined significant difference in decolouration of wastewaters,  
 248 indicating a direct metabolism of pollutants. Only *P. sanguinea* MUT 1284 and *P. brevicompactum* MUT 793  
 249 showed a significant improvement of the decolouration rate in the presence of glucose (up to 33% decolouration  
 250 towards leachate and 38% towards effluent, respectively).



251  
 252 **Figure 3. Decolourisation yields of selected autochthonous fungal strains.**

253  
 254 Autochthonous basidiomycetes are particularly promising. Actually, the exploitation of an  
 255 autochthonous fungus for the bioremediation of wastewaters potentially presents a greater rate of success with  
 256 respect to the allochthonous ones. First of all, strains already adapted to this environment will be more resistant

257 to the extreme conditions of wastewater. In addition, they can resist also to the competition with other  
258 microorganisms present in wastewater (Prigione et al., 2009; Tigini et al., 2009). Thus, the bioaugmentation of  
259 an autochthonous fungus in wastewater could bring direct and indirect benefits: the bioremediation of  
260 wastewater thanks to its action towards pollutants, and the limitation of pathogenic mycoflora fast and massive  
261 consumption of residual carbon source present in it.

262 In this phase of the research, it was not possible to understand the mechanism at the base of the  
263 decolourisation. However, experiments aimed to the discrimination between biosorption and biodegradation  
264 processes will be performed in the next future.

265

#### 266 **4 Conclusion**

267 In our study the high environmental and sanitary risks associated to municipal landfill leachates  
268 emerged thanks to an ecotoxicological and mycological characterisation of both a raw landfill leachate and the  
269 effluent coming from traditional treatments (both activated sludge and nitrification-denitrification). It can be  
270 hypothesised that initial high toxicity of the raw leachate **may be** the cause of the failure of traditional treatments  
271 in the decolourisation. **Actually, both the samples exceed the legal threshold value according to all the tested**  
272 **organisms.**

273 **Despite the relatively low fungal load, both the samples may represent a risk for human health, on**  
274 **account of the presence of some emerging pathogens. We** assessed the possibility to exploit the autochthonous  
275 fungi in the decolourisation of the leachate itself, since they are already acclimatised to this extremely toxic  
276 environment and are probably able to transform the recalcitrant substances present in it. The results of this  
277 preliminary study open interesting perspectives on bioremediation of leachates by means of both biodegradation  
278 and biosorption. **Further experiment will be aimed to the study of decolourisation mechanism and toxicity**  
279 **reduction.**

280

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284

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