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

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Abstract

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

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

1 Introduction

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

1

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 Gavrilescu, 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 Gavrilescu, 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^{-1}	<0.001 mg l^{-1}	0.02 mg l^{-1}
Total chromium (as Cr)	1.20 mg l^{-1}	0.50 mg l^{-1}	2 mg l^{-1}
Nichel (as Ni)	0.30 mg l^{-1}	0.30 mg l^{-1}	2 mg l^{-1}
Lead (as Pb)	0.073 mg l^{-1}	0.082 mg l^{-1}	0.2 mg l^{-1}
Cuprum (as Cu)	0.60 mg l^{-1}	0.15 mg l^{-1}	0.1 mg l^{-1}
Zinc (as Zn)	0.66 mg l^{-1}	1.38 mg l^{-1}	0.5 mg l^{-1}
Total hydrocarbons	<100 mg l^{-1}	598.7 mg l^{-1}	5 mg l^{-1}
Ammonium (as NH$_4$)	2266.0 mg l^{-1}	408.0 mg l^{-1}	15 mg l^{-1}
Total nitrogen (as N)	2537.9 mg l^{-1}	514.1 mg l^{-1}	20 mg l^{-1}
Sulphates (as SO$_4$)	46.9 mg l^{-1}	801.7 mg l^{-1}	1000 mg l^{-1}
Chlorides (as Cl$^-$)	3193 mg l^{-1}	2550 mg l^{-1}	1200 mg l^{-1}

Anionic surfactants (MBAS)	23.07 mg l^{-1}	37.9 mg l^{-1}	
Non ionic surfactants (PPAS)	23.48 mg l^{-1}	17.2 mg l^{-1}	2 mg l^{-1}
Cationic surfactants	<10 mg l^{-1}	<10 mg l^{-1}	
COD (as O_2)	6166 mg l^{-1}	2099 mg l^{-1}	160 mg l^{-1}
BOD$_5$ (as O_2)	4209 mg l^{-1}	1410 mg l^{-1}	40 mg l^{-1}
pH	8.10	8.40	5.5-9.5
Suspended solids	1064 mg l^{-1}	604 mg l^{-1}	80 mg l^{-1}
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
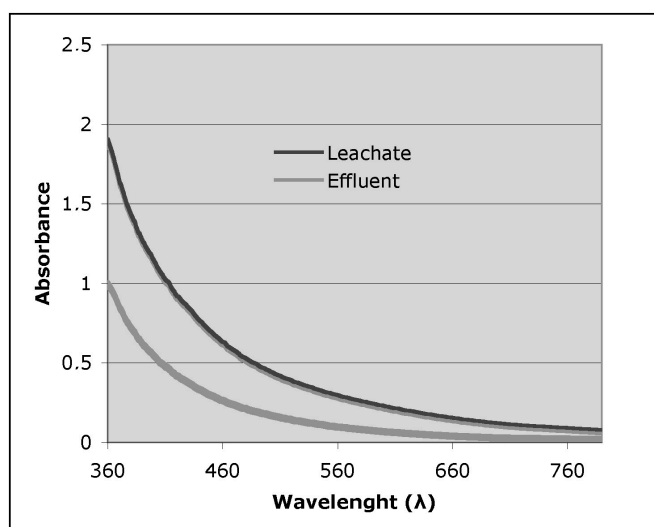
83   **Figure 1. Absorption spectra of 1:4 diluted leachate and effluent.**

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   uM) 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, β-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 (λ = 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_{50},

128   LD_{50} or LC_{50} 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*

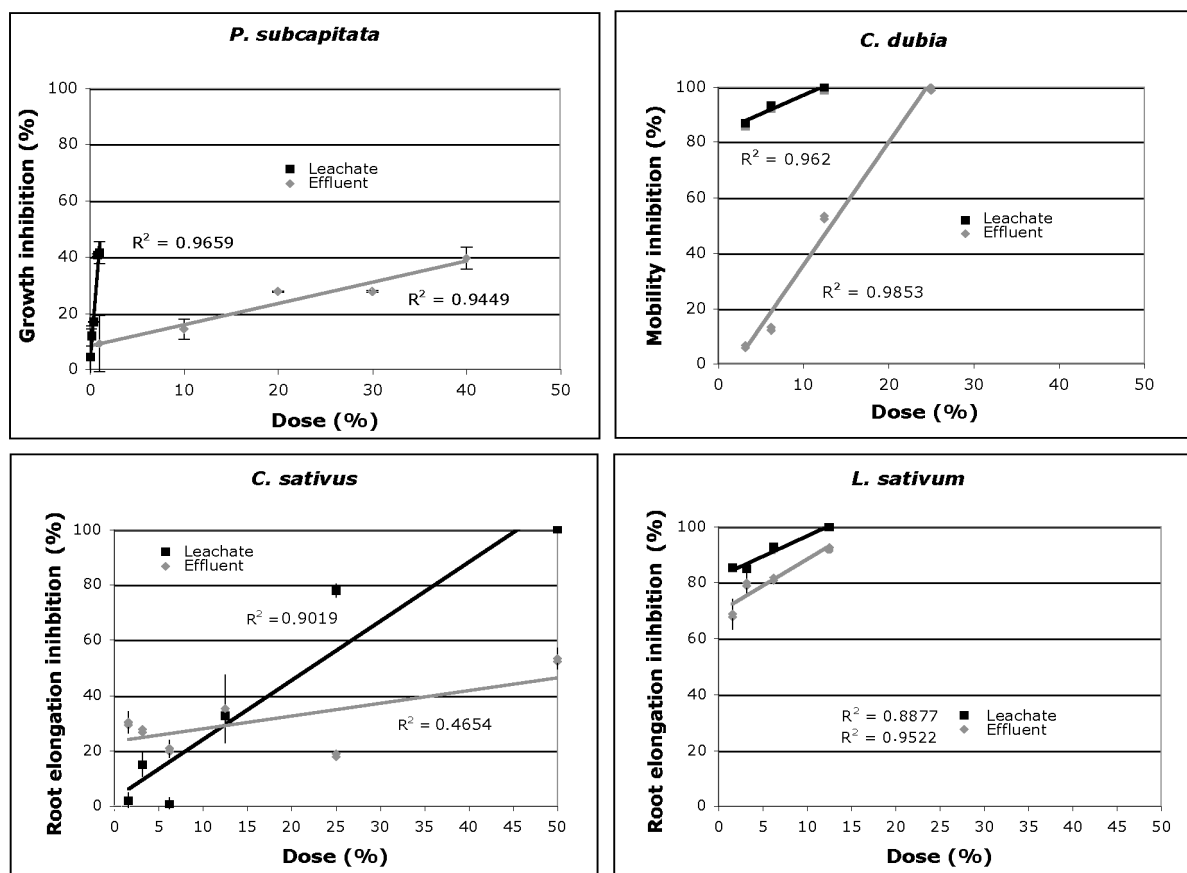134   was the most sensitive one towards the effluent (Figure 2).

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

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

The decrease of leachate toxicity after the traditional treatments recorded by *P. subcapitata*, *C. dubia* and *L. sativum* could be due to the lower content of ammonium and, partially, to the decrease of chloride ions with respect to the untreated leachate. On the contrary, sulphate and total hydrocarbon increased in effluent with respect to crude leachate, thus their role in the inhibition of these three organisms may be marginal. In support to this hypothesis, it is noteworthy that as the pH of the samples is adjusted at acidic value (pH 5) the inhibition effect drop under 60% for *L. sativum* (Tigini et al., 2013). Actually, in acidic conditions ammonia (NH_3) turns in the less toxic ammonium ion (NH_4^+). On the base of the same experiment with acidified leachate and effluent, we hypothesized that also heavy metals play a marginal role in samples toxicity. In fact, heavy metals at low pH should be mobilised becoming bioavailable. Despite this, the toxicity decreased as previously reported.

7

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^{-1}).**

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

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

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 (Gostinĉar 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 (Gostinĉar et

190   al., 2010).

191   Opportunistic pathogenic fungi, such as *Aspergillus fumigatus*, *Candida* spp., *Geomyces pannorum*,

192   *Geotrichum candidum*, *Microsporum 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 notheworty. 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 cytocrome 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 (microciclic

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^{-1} 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. sphaerospemum* 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

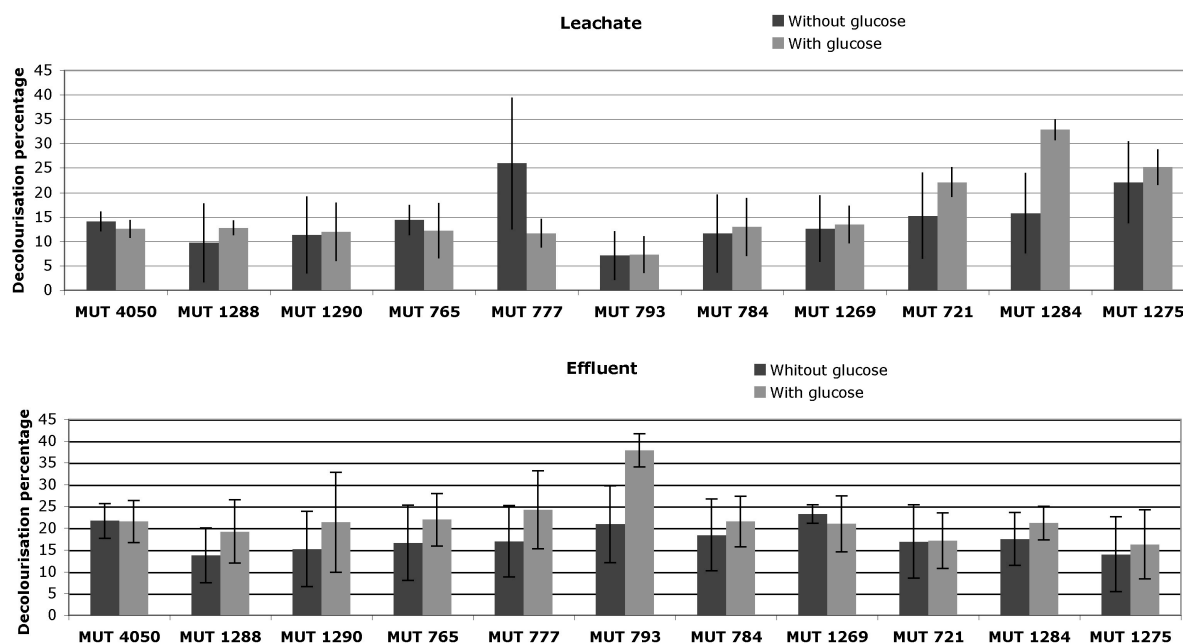250   towards leachate and 38% towards effluent, respectively).

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.

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