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A RAPID SCREENING METHOD TO DETERMINE THE SUSCEPTIBILITY OF BIO-BASED CONSTRUCTION AND INSULATION PRODUCTS TO MOULD GROWTH

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ABSTRACT

Mathematical models have been developed to evaluate materials' durability and susceptibility to biodeterioration by moulds, however models are material and mould species specific. Ultimately the best way to determine a materials' susceptibility is to expose the material to microorganisms. This study attempted to develop a quick, reliable screening method to evaluate a number of different materials for their susceptibility to moulds at optimal and limiting conditions. This test method was based on modified versions of ASTM 4445-91 and BSEN 846. The water absorption coefficient and Dynamic Vapour Sorption tests were also conducted to determine any correlation between the materials hygric properties and mould growth. The materials used to validate the novel screening method were: MDF, laminated MDF, Chipboard, Laminated chipboard, Wool, Hemp, Wood fibre insulation and pine. It was found chipboard was the most susceptible to mould growth and wool the least when in direct and indirect contact with agar. Primary colonisers (*A. niger*) easily colonised the materials, regardless of the environmental conditions, whereas secondary (*A. alternate*) and tertiary (*T. virens*) colonisers were absent on materials under limiting conditions.

Key Words: Mold, construction materials, insulation, susceptibility, moisture

1. INTRODUCTION

During the service life of buildings, bio based construction materials could be at risk of biodeterioration such as that caused as a result of the biotic processes of microorganisms. In the environment saprophytic organisms such as mould and decay fungi are the main agents responsible for the decomposition and recycling of organic matter. However, in the built environment they are associated with physical and aesthetic damage and human health problems such as allergic and toxic reactions (Airaksinen et al., 2004; Cooley et al., 1998; Jarvis and Miller, 2004; Mensah-Attipoe et al., 2015; Nielsen, 2003). Modern building practices have, in some cases, exaggerated this problem with increased insulation hindering ventilation, resulting in increased areas of condensation and subsequent mould growth (Schmidt, 2006). Moulds will readily colonise lignocellulosic materials but can also attack synthetic floor coverings, airplane fuels, oils, glues, paints and textiles (Pasanen et al., 1992; Schmidt, 2006). This ability to attack a wide variety of

51 materials is enabled by the variety of physiological responses demonstrated by
52 mould fungi in regards to temperature, water activity, relative humidity and pH
53 (Schmidt, 2006).

54 Hygroscopic (water sorption) properties are an inherent characteristic of materials
55 that influence both the application and microbiological resistance (Airaksinen et al.,
56 2004; Xie et al., 2010). Natural fibres are hygroscopic because their cell walls
57 contain high amounts of water sorption sites (hydroxyl groups) and can expand to
58 accommodate the water (Xie et al., 2010). Moulds have been shown to appear in
59 succession on a material as the moisture content of the material fluctuates,
60 according to their minimum moisture demands of the mould, (Pasanen et al., 1992).
61 Therefore, although the need for determining a materials' vulnerability to mould
62 growth is obvious, it is clear that not all materials have equal susceptibility
63 (Johansson et al., 2012; Mensah-Attipoe et al., 2015), which adds complexity when
64 considering composite materials. Isopleths have been used to describe relationships
65 between temperature, moisture and fungal growth on nutrient media and although
66 isopleths can be very useful, they are, however, only suitable for predicting growth of
67 known fungi on one material at a time and are time intensive (Johansson et al.,
68 2013b). There have been a number of mathematical models developed and reported
69 in recent years that can be used to evaluate durability and susceptibility of wood and
70 wood-based materials to biological deterioration (Ojanen et al., 2007; Viitanen et al.,
71 2010). Basic models are used to indicate mould germination conditions, such as the
72 isopleth technique, but these do not account for fluctuations in environmental
73 conditions. More advance models such as the VTT model and the bio-hydrothermal
74 model (Sadovský et al., 2013) can be used but these have also shown significant
75 variations in results due to simplifications and assumptions (Sadovský et al., 2013).
76 There are, however, further disadvantages to using some models to predict
77 microbiological growth, in that most are based on laboratory data, where optimum
78 conditions are used, and are therefore often not comparable to construction
79 materials, which are comprised of less nutrient rich materials (Clarke et al., 1999).
80 Very rarely do they take into account species dominance (Gu and Gu, 2005). One
81 key characteristic in predicting the susceptibility of materials requires a knowledge of
82 the organisms' minimum water requirements, that are specific to the individual mould
83 species (Nielsen et al., 2004). Models also do not consider the materials ability to
84 absorb moisture, in contact or as vapour. It is possible that errors occur, due to a
85 delay in a change in the surface conditions at different relative humidities, when
86 compared to adjacent conditions.

87 These models may therefore not be the most applicable way of determining a
88 materials' susceptibility to mould growth. As stated above, these models are often
89 developed using the moulds optimal growing conditions and therefore, if materials
90 are destined for use outside of these environmental ranges, such as furniture in a
91 bathroom or kitchen, the level of biological attack may be based on false
92 assumptions. Moulds can still colonise materials and grow in sub optimal conditions
93 and it has been shown that even at low humidities, where substantive growth may be
94 retarded or prevented, spores and mycotoxins can still be released (Abbott, 2002;
95 Nielsen et al., 2004). This can be detrimental to both the material, as it may enable
96 degradation by other fungal species and in the case of mycotoxins, to human health.
97 It is highly important to understand how a mould responds to a different substrates
98 and materials susceptibility to microbial attack. Any misunderstanding or poorly
99 informed decisions can have damaging consequences to product industry, economy
100 and human health (Gu, 2016; Gu and Gu, 2005; Mensah-Attipoe et al., 2015).

101 Ultimately the best way to determine a materials' susceptibility to moulds is to
102 physically test the subject material. The aim of this study was the development of a
103 rapid screening method for evaluating the susceptibility of different materials to
104 mould growth under varying conditions and methods of inoculation. The hygric
105 properties of the materials tested were also determined in order to evaluate
106 correlations between mould growth and the materials' hygric properties.

107 **2. MATERIALS AND METHODS**

109 The method described below, is a further development of the study conducted by
110 Stefanowski *et al* (2015) which was derived from BS EN ISO 846: 1997 Plastics –
111 Evaluation of the action of microorganisms and ASTM D 4445-91 1991 Standard
112 Method for Testing Fungicides for Controlling Sapstain and Mould on Unseasoned
113 Lumber (Laboratory Method).

115 **2.1 Materials**

116 The materials tested include three commercial grade construction medium density
117 fibre board (MDF), laminated MDF, chipboard and laminated chipboard and three
118 commercial insulation materials sheep's wool, hemp and wood fibre insulation with
119 solid pine wood (*Pinus sylvestris*) used as a control. The construction panel
120 specimens were prepared to give an upper surface area of 30 mm² with the
121 thickness being that of the test material. As the insulation materials were 50 to
122 60mm thick, a subsample of 5mm thickness was removed from the top surface of
123 the material for use as the test specimen.

125 **2.2 Preconditioning**

126 All specimens were conditioned in conditions of 23 ±1 °C and 60 ±3 % RH and once
127 constant mass was reached the specimens were weighed. The specimens to be
128 inoculated with moulds were sterilised with ethanol and water 70:30 (BSI 1997).

130 **2.3 Hygric**

131 The sorption dynamics of natural fibres are complex partly due to fibre internal
132 structure and partly due to continuous nano-structural changes, associated with
133 dynamic behaviour of cell walls (Xie *et al.*, 2010). Two methods were used to
134 determine the material's sorption properties; Dynamic Vapour Sorption (DVS) and
135 water absorption coefficient (BSI 2002). Pine (*Pinus sylvestris*) was excluded from
136 the hygric tests.

138 **2.3.1 Water absorption coefficient**

139 This was conducted following the standard BS EN ISO 15148 : 2002, Hygro-thermal
140 Performance of Building Materials and Products – Determination of water absorption
141 coefficient by partial immersion.

143 **2.3.2 Dynamic Vapour Sorption (DVS)**

144 DVS is designed to accurately measure weight changes of a sample (less than
145 10mg), as it absorbs and desorbs moisture at differing relative humidities and
146 temperatures. The sample was suspended in a microbalance within a sealed
147 thermostatically controlled chamber, where a constant flow of dry nitrogen gas was
148 passed over the sample at a flow rate of 200 cm³s⁻¹ and a temperature of 21 ± 0.2 °C
149 (Popescu *et al.*, 2013). The inert gas carried a controlled quantity of water,
150 maintaining a set RH. The schedule for the DVS was set to start at 0% RH and then

151 increase in 5% steps up to 95% for the adsorption phase and the reverse for the
152 desorption phase (Popescu et al., 2013). The DVS maintained a given RH until the
153 weight change of the sample was less than 0.002 \% min^{-1} . Mass change data were
154 acquired every 20 s. Sorption and desorption isotherms were produced for each
155 material by plotting mass change against relative humidity (RH).

156

157 **2.4 Mould tests**

158

159 **2.4.1 Moulds**

160 The mould species chosen for this experiment are based on standards used,
161 however they are also consistently found in indoor environments (Cooley et al.,
162 1998). The mould species were acquired from Fungal Biodiversity Centre, Institute of
163 the Royal Netherlands Academy of Arts and Science (KNAW). The species selected
164 were: *Aspergillus versicolor* (Vuill) CBS 117286, *Cladosporium sphaerospermum*
165 (Penz) CBS 122.63, *Chaetomium globosum* (Kunze ex Fr.) CBS 107.14, *Penicillium*
166 *rubens* (Biourge) CBS 401.92, *Alternaria alternata* ((Fr.) Keissl) CBS 120829,
167 *Paecilomyces variotti* (Bainier) CBS 108945, *Trichoderma virens* (J.H. Mill, Giddens
168 & A.A. Foster) CBS 100946 and *Aureobasidium pullulans* (var.*pullulans*) CBS
169 101160.

170 On the basis of the minimal requirement of available water for fungal growth on
171 material surfaces, indoor fungi and moulds can be divided into primary ($<0.80\text{ a}_w$),
172 secondary ($0.80 - 0.09\text{ a}_w$) and tertiary colonisers ($>0.90\text{ a}_w$) (WHO, 2009). Using
173 this definition the aforementioned moulds are divided into the appropriate colonisers.
174 Primary colonisers; *Aspergillus versicolor* (Górny, 2004), *Paecilomyces variotti*
175 (Górny, 2004) and *Penicillium rubens* (WHO, 2009). Secondary colonisers;
176 *Cladosporium sphaerospermum* (Górny, 2004) and *Alternaria alternata* (Klarić et al.,
177 2007). Tertiary colonisers; *Chaetomium globosum* (Klarić et al., 2007) and
178 *Trichoderma virens* (Górny, 2004).

179

180 **2.4.2 Preparation of spores**

181 A mixed spore suspension, containing all of the selected mould species, was
182 produced following the method described in BS EN 846 Plastics – Evaluation of the
183 action of microorganisms 1997

184

185 **2.4.3 Inoculation and exposure**

186 The three mould tests were conducted using sterile 600ml vessels with aluminium
187 lids.

188 For direct/indirect contact test, a mineral salt solution agar was used (BSI, 1997),
189 which was autoclaved at 121°C for 15 minutes, cooled and 60ml was poured into
190 each vessel.

191 To expose samples and moulds to a limited RH, a saturated salt solution was used,
192 mixed with sterilised deionised water, generating a 60% RH within the vessels at $20\pm2\text{ }^\circ\text{C}$. Sterilised supports were added to each vessel to hold the sample in the
193 centre of vessel. Figure 1 shows a diagram of how the samples were positioned in
194 each of the tests. Two of the 6 replicate samples of each material were placed in one
195 vessel.

196 Specimens were introduced to the vessel one at a time and placed according to test
197 specifications. Samples for the relative humidity experiment were securely sealed to
198 ensure no moisture loss or gain. Each sample for all three experiments was

200 inoculated with 1ml of the mould spore solution. The vessels and sample were then
201 stored in a dark chamber at 20 ±2 °C at 70 ±3 % RH for three weeks.

202 Each mould underwent a viability test on sterilised 4% malt agar plates (40g malt
203 extract and agar 20g in 1000ml) apart from, for *Chaetomium* and *Trichoderma*
204 moulds, where a 4% oatmeal agar culture was used. 1ml of the spore solution was
205 pipetted onto the agar and spread using a sterile glass rod. The agar plates were
206 then sealed with a wax film and incubated at 20 ±2 °C at 70 ±3 % RH.

207

208 **2.3.4. Assessment**

209 After exposure to mould, the samples were removed from the vessel and visually
210 evaluated for mould growth (Table 1). Where possible, primary, secondary and
211 tertiary colonisers were identified and recorded. A rating of 1 (present) or 0 (absent)
212 was given to the presence or absence of primary, secondary and tertiary colonisers.
213 The occurrence of the colonisers was given as percentage across the replicates.

214

215 **3. RESULTS AND DISCUSSION**

216

217 **3.1 Wide Range of Moisture Properties**

218 The principle of the water sorption coefficient test was to measure the water
219 absorption by partial immersion in water, by measuring the change in mass over
220 time. Table 2 shows the results for water absorption coefficient. For this test, water
221 absorption relies on capillary action (uptake) and in natural fibres this can cause the
222 swelling of the material. As wood fibres can expand to accommodate additional
223 water (Xie et al., 2010), for wood-based construction materials, the results should
224 therefore be taken as indicative only.

225 Figure 2 shows the mass change over time. Chipboard had almost become
226 saturated by the end of the 24 hour period, as the rate of water uptake decreased. In
227 comparison, MDF and laminated MDF showed a slower, steady rate of water
228 absorption. This could be due to a combination of variables between materials such
229 as particle and resin distribution and the density difference between MDF and
230 chipboard, 700 kg m⁻³ and 600 kg m⁻³ respectively. The laminated chipboard showed
231 a slower rate of absorption due to its less permeable melamine coating. Wool and
232 hemp materials became saturated within the first hour as these materials have a low
233 density of 22.64 kg m⁻³ and 43.72 kg m⁻³ and therefore can hold less water
234 proportionally within matrix before becoming saturated. Wood fibre insulation has a
235 density of 205.03 kg m⁻³, therefore absorption was slower but the sample still
236 became saturated within 5 hours. The test for all three insulation materials, ceased
237 after four hours, as water was absorbed through to the top surface of the sample.

238

239 The data (figure 3) derived from the DVS shows that all the materials exhibited
240 varying levels of hysteresis (the difference in EMC (equilibrium moisture content)
241 dependent on sorption or desorption) with them all exhibiting IUPAC type 2 sorption
242 and desorption isotherms (Hill et al., 2009). The construction materials all exhibited
243 significant hysteresis effect, though in contrast hemp and wool showed only a small
244 hysteresis effect. This could be related to the materials densities and higher lignin
245 content of the wood-based materials (Hill et al., 2009).

246 Table 2 show the maximum moisture content (EMC) of the material when exposed to
247 humidity of 95%. Hemp absorbed the most moisture at 95% RH with 20% of dry
248 weight and laminated MDF the least at 14.18%. Laminated materials have lower

249 EMC values than un-laminated materials due to the presence of the less permeable
250 melamine coating.

251

252 **3.2 Material Specific Mould Ecology**

253 Observations were made at the end of a three week period to evaluate the mould
254 coverage over the sample (%). Note was taken of the presence of primary,
255 secondary and tertiary species, to give an indication of colonising mould succession
256 and competition. The mineral salt agar was present to act as a moisture source and
257 would not act as a carbon source for growth; therefore the mould fungi had to use
258 carbon derived from the samples i.e. utilisation of the sample material (Gu, 2016).
259 Although moulds had successfully grown in all vessels there were differences
260 dependent on the material and exposure method.

261 For contact samples, the chipboard had the highest intensity of growth, (5 rating).
262 Laminated chipboard, hemp and pine had the second highest, (4 rating). Figure 5
263 shows that in the vessels containing chipboard and dense wood fibre, all moulds
264 were present, as primary, secondary and tertiary colonisers were observed. This
265 corresponds to previous work where the greater availability of free water in the
266 structure of the chipboard (Górny, 2004) enhanced its susceptibility to moulds. The
267 data derived from the DVS studies also shows that the chipboard and dense wood
268 fibre had higher moisture contents than the other materials at similar relative
269 humidity levels. However, greater intensity of growth was observed on chipboard,
270 showing a greater susceptibility to moulds than the other materials tested which may
271 be due to availability of nutrients and a preference of the moulds.

272 In contrast the least intensity of growth was observed on MDF and wool – with MDF
273 exhibiting lower nutrient availability than chipboard, because it is processed wood
274 fibre (Johansson et al., 2013a). This shows that moulds attack materials suited to
275 their chemical and physical capabilities and material composition (Gu, 2003).

276 For indirect contact, chipboard (both un-laminated and laminated) had the most
277 intense growth, with a rating of 5 and 4 respectively. All materials showed a reduced
278 intensity of growth when not in direct contact with the agar, except wood fibre
279 insulation and laminated chipboard (Table 3). This may be indicative that the
280 sample's hygric properties enhance mould growth through the sorption of moisture
281 from the agar. However, the presence of primary, secondary and tertiary colonising
282 moulds are similar in both set of samples.

283 From figure 5 it can be observed that primary, secondary and tertiary colonisers
284 were present in all vessels on all samples. It should be noted that where tertiary
285 colonisers occurred >80% of replicates, *Trichoderma virens* and *Chaetomium*
286 *globosum* were the prevalent moulds. *Trichoderma* sp. where present, had excellent
287 growth, which in other studies has been attributed to its production of antifungal
288 products (Šegvić Klarić et al., 2007) that enable it to outcompete other fungal
289 species (Ghisalberti and Sivasithamparam, 1991; Wiest et al., 2002).

290 Figure 4 graphically shows the difference in mould growth between contact and
291 indirect contact samples. All growth on all materials in contact with agar had a higher
292 intensity of mould growth compared to indirect contact samples, although not
293 statistically different, except for chipboard. This shows the influence of the presence
294 of the moisture in the agar on a sample's MC and subsequently, mould growth.
295 However, materials with higher water absorption coefficient and MC values, did not
296 necessarily have the highest intensity of growth.

297 There were no clear correlations between water absorption and mould growth as
298 those materials with high levels of water sorption did not always show the highest

299 levels of colonisation. Hemp had the highest water absorption coefficient but showed
300 lower growth intensity than laminated chipboard. This reduced intensity of growth
301 could be a result of the presence of *T. virens*, which may have colonised quicker on
302 hemp than chipboard and thus prevented other mould growth. Also, wool samples
303 had high hygric values of sorption but the lowest levels of growth. This is due to the
304 limits of the moulds themselves, as they are not capable of decaying such materials.
305 Using hygric data, materials can be assigned a 'critical moisture level' where moulds
306 can develop on the materials. These results show that moisture environment alone is
307 not enough to model the likelihood of mould growth, as other factors such as
308 material composition and mould species capabilities and preference, have a major
309 influence on comparative growth between materials. The hygric data does illustrate
310 an important point that although some materials may not sustain heavy mould
311 growth, they can easily absorb water. This factor may influence fungal growth on
312 other materials if they are used in conjunction (Curling et al., 2015).

313 When analysing these results, it was observed that there were differences in the
314 presence of primary, secondary and tertiary colonisers (Figure 5). A value of 1
315 represents the presence of primary, secondary or tertiary colonisers across all
316 replicates. All samples and replicates had primary species colonising the samples in
317 contact, indirect contact and at 60% RH. No secondary or tertiary colonisers were
318 observed on any material when cultivated at 60% RH. This shows that building
319 materials can form small niches in indoor environments for different organisms
320 (Mensah-Attipoe et al., 2015).

321 Figure 5A and 5B shows the succession of growth on MDF and laminated MDF
322 respectively. The frequency of growth of secondary and tertiary colonisers was
323 significantly reduced when samples were out of contact with agar and at 60% RH.
324 However, on MDF, secondary colonisers were still present even when out of contact
325 with the agar, whereas on Laminated MDF the frequency of growth of secondary
326 colonisers was reduced. This shows that a change in the surface of a material can
327 alter the susceptibility to different mould species. Laminated chipboard and
328 chipboard revealed a similar pattern.

329 Figure 6 shows the combined data of intensity of growth and frequency of colonisers
330 for materials in direct contact with agar. It illustrates that different materials, exposed
331 to the same moulds under the same conditions, experience differing colonisation
332 patterns. Chipboard showed that with a high intensity of growth, there is a full
333 succession of colonisers, whereas laminated chipboard, while exhibiting a similar
334 intensity of growth, had reduced incidence of tertiary colonisers. Wool samples,
335 showed a high, almost full succession of growth but the intensity of growth was the
336 lowest of all materials tested. This is important as it demonstrates the usefulness of
337 the test method in identifying the differing response of the the organisms to the
338 different materials. In the case of wool, moulds can colonise and survive on the wool,
339 likely surviving off contaminants in the insulation (Górny, 2004; Johansson et al.,
340 2013a) which, again is highly important when considering real life scenarios where
341 different materials are used in conjunction.

342 Many mould growth models rely on known optimum conditions for specific moulds
343 and assume uniform susceptibility of different species of mould spores (Viitanen et
344 al., 2010). However, in real life, environmental conditions in buildings are rarely
345 optimal and fluctuate (Johansson et al., 2013a; Pasanen et al., 2000) so it is crucial
346 to know the extreme limits for mould growth, as different mould species may be
347 either actively growing or just surviving on a material.

349 From the DVS isotherms, it can be observed that different materials absorb vapour
350 at different rates at different RH. It is considered that the limit value of relative
351 humidity is between 70-90% for fungal growth on building materials (Pasanen et al.,
352 2000). However, as mould species have differing limiting conditions, a study was
353 made to determine the effects on colonisation, growth and competition at a lower
354 limit of 60% RH. This showed that only primary colonising moulds were observed
355 growing on construction materials at 60% RH (Figure 5).

356 Table 3 shows the differences in intensity of growth at 60% on different materials.
357 The highest moisture content observed was that of chipboard, at just under 10% MC
358 and this is generally considered too low for mould growth, although in this study
359 mould growth was obvious on all samples. This could be a result from the initial
360 equilibration period, following inoculation, where water availability was slightly
361 higher. After which time the mould growth rate reduced but primary colonisers were
362 largely established. It was observed that the most intense growth was on the wood-
363 based materials, with chipboard showing the greatest growth (2 rating). This is highly
364 important as there is evidence that *Penicillium* species are strong indoor
365 contaminants and contribute significantly to SBS (Abbott, 2002; Cooley et al., 1998).
366 No secondary or tertiary growth was observed in any vessels. This is due to the MC
367 requirements of the mould species, as only xerophytic moulds (dry loving) such as
368 *Aspergillus* and *Penicillium* species were observed, which require $<0.80 \text{ a}_w$ (Pasanen
369 et al., 2000a). The same was observed by Pasanen et al., 2000 were xerophytic
370 moulds have great prevalence at low water activity. Secondary and tertiary
371 colonisers are more hydrophytic moulds and require higher levels of moisture to
372 successfully grow and colonise a material.

373 Figure 6 graphically depicts the differences between intensity of growth of mould on
374 sample materials in contact and indirect contact, at optimal conditions and at 60%
375 RH. It highlights the differences between growth at optimal and less than optimal
376 conditions, which is important to understand these relationships as moulds can still
377 produce metabolites and mycotoxins at low RH and temperatures (Nielsen et al.,
378 2004). Statistically there is a difference between intensity of growth between test
379 conditions on materials tested, except for MDF and laminated MDF. This indicates
380 that MDF may have a lower MC requirement to support mould growth.

381 The results also show that moulds will grow on all materials even at limiting
382 conditions, albeit with reduced growth. This suggests that testing specific material
383 characteristics on a small scale may not be representative of the full product, due to
384 bulk effects, especially when considering composite materials.

385

386 **3.3 Fast and Versatile Method**

387 This rapid screening method took only 3 weeks to obtain data on a materials'
388 susceptibility to mould growth. However, consideration must be given to time as an
389 influencing factor for mould growth (Vereecken and Roels, 2012). If a mould species
390 is known to have a slow growth rate, extra time should be provided. It has an
391 advantage over BS EN 846 in that it can be used for a range of materials and not
392 just plastics. The method uses the principle from ASTM D 4445 to use a support,
393 which can be used to evaluate the vulnerability of the material as a "carrier" of
394 moulds. This may be important when considering wall constructs. As with wool for
395 example, although all moulds were present, the intensity of growth on the wool itself
396 was minimal in both situations but the mould growth on wool implies it will not act as
397 barrier for more vulnerable materials such as MDF. This can result in the spread of
398 moulds and may increase the materials and adjacent materials vulnerability to

399 degradation fungi. Testing materials at lower RH can show which, if any species can
400 survive and continue to utilise the material. This is especially important when
401 considering drying materials, particularly after water damage (A.-L. Pasanen et al.,
402 2000).

403 Although in this study a mixed spore suspension of commonly used test strains was
404 used, the method is equally adaptable for use with specific single mould types or a
405 mix of test or naturally isolated fungal strains. The method is also adaptable for study
406 of any construction or insulation material and the test environmental conditions can
407 be altered to simulate particular environmental conditions a material is intended for.
408 Using the 600 ml vessel over thinner agar plates, enables the testing of whole
409 thickness samples rather than thinner sample. This is highly beneficial especially
410 when testing composite materials. At full thickness, the sample is more
411 representative of the product and the interactions with moisture and subsequent
412 mould growth are more comparable with the product in service. It is also beneficial
413 as it tests any bulk effects of the product.

414 **4. Conclusion**

415 Identification of susceptible materials and mould growth patterns is highly important,
416 especially when considering toxic moulds. This study developed a rapid screening
417 method to enable the determination of the susceptibility of different materials to
418 different mould species. The method provides data enabling identification of more
419 vulnerable materials, materials that may have synergistic with other materials,
420 material responses to varying moisture environments and consequential mould
421 growth dynamics under varying environmental conditions: data which is unlikely to
422 be obtained by modelling alone.

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Table 1: Visual assessment of mould growth (BSI, 1997)

Intensity of growth	Evaluation
0	No growth apparent under the microscope
1	No visible growth to the naked eye but visible under a microscope
2	Visible growth, up to 25% coverage
3	Visible growth up to 50% coverage
4	Visible growth up to 75% coverage
5	Heavy growth, covering more than 75% of sample surface

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Table 2: Water absorption coefficient and EMC of test materials at 95% and 60% RH

Material	Water absorption coefficient (Kg/(m ² hr ⁻¹))	EMC at 95% RH (%)	EMC at 60% RH (%)
MDF	3.25	17.46	7.92
Laminated MDF	3.38	14.18	7.55
Chipboard	4.65	18.01	9.39
Laminated chipboard	4.25	16.58	9.28
Hemp	5.28	20.92	4.02
Wood fibre insulation	2.50	21.77	4.21
Wool	4.50	19.17	11.78

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Table 3: Intensity of mould growth on sample in contact and indirect contact with agar and suboptimal conditions at 60% RH

Material	Intensity of growth		
	Contact	Indirect contact	60% RH
MDF	3	2	1.8
Laminated MDF	3	2	1.6
Chipboard	5	4	2
Laminated chipboard	4	4	1.6
Wool	2	1	0.4
Hemp	4	3	1.6
Wood fibre insulation	3	3	1.2
Pine	4	3	1.6

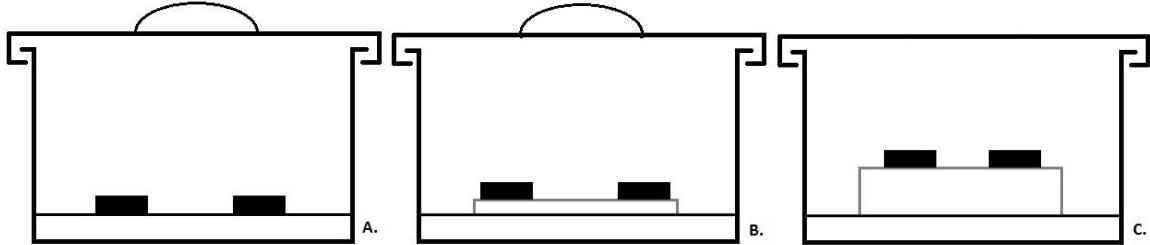
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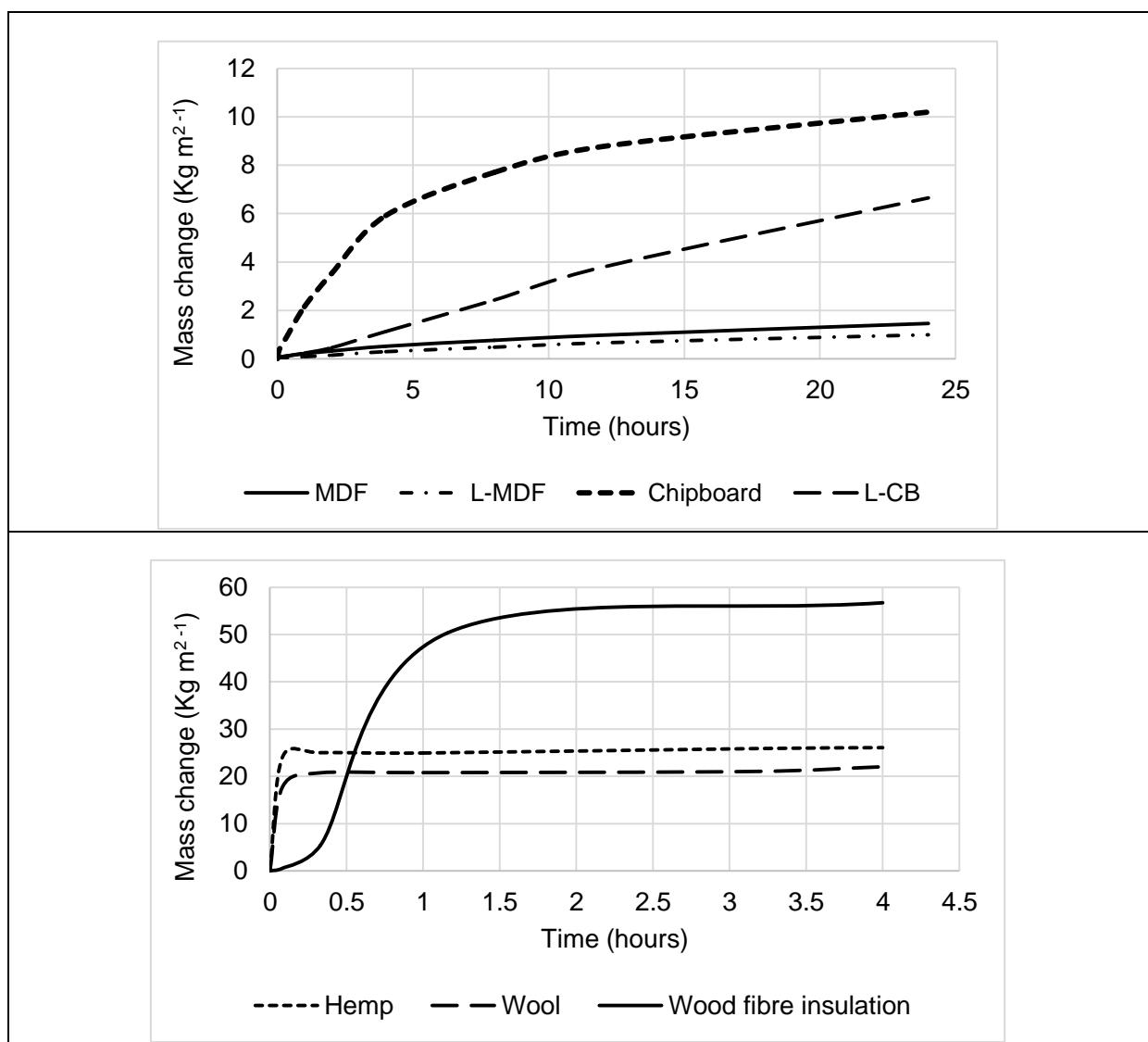
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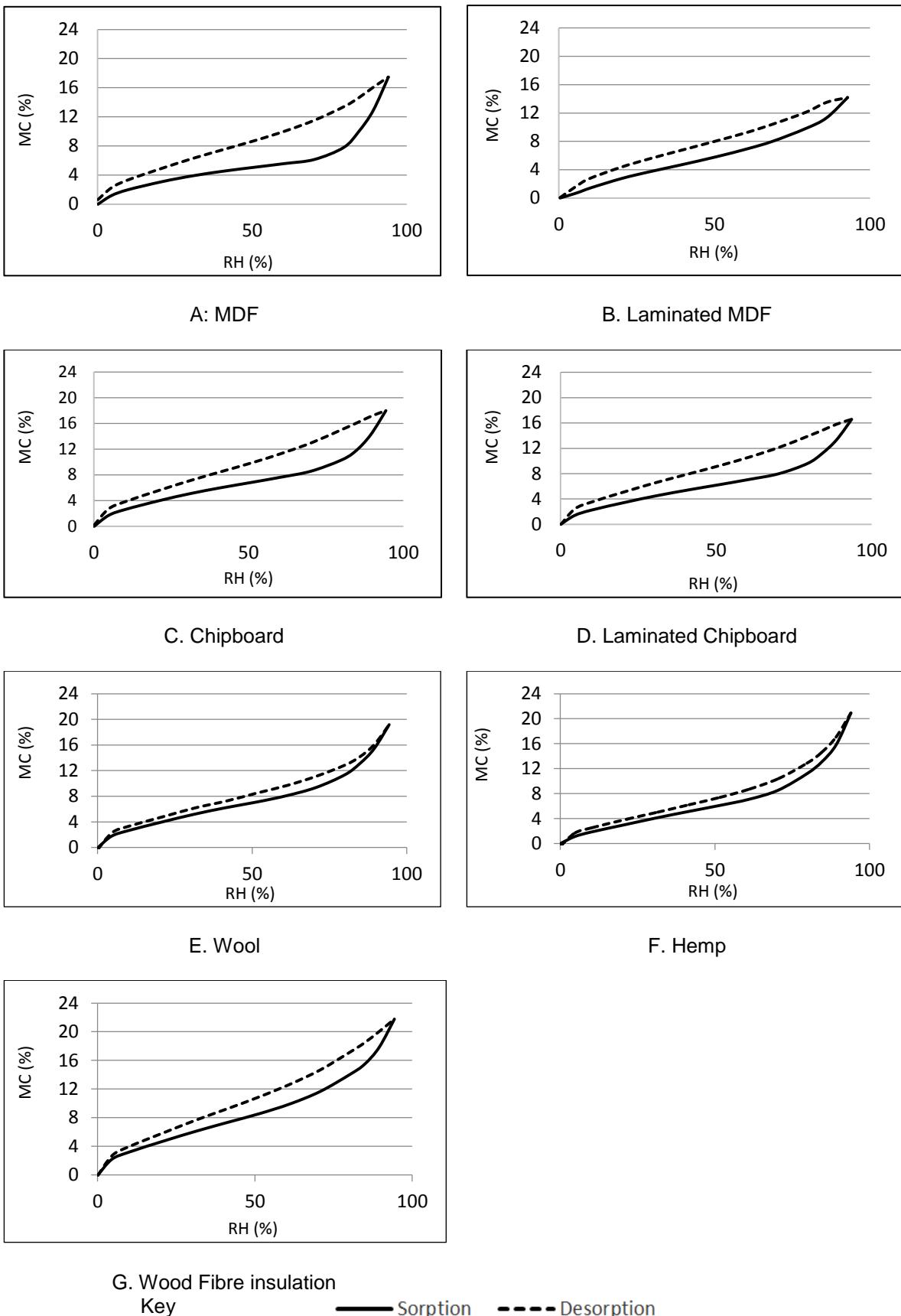
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560 Fig 1: A; Sample in direct contact with agar, B; Sample indirect contact and C; Sample raised
561 to the centre of the vessel RH test

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Fig 2: Water absorption of construction (a) and insulation (b) materials



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Fig 3: Mass change (MC) (%) against RH (%) isotherm curves

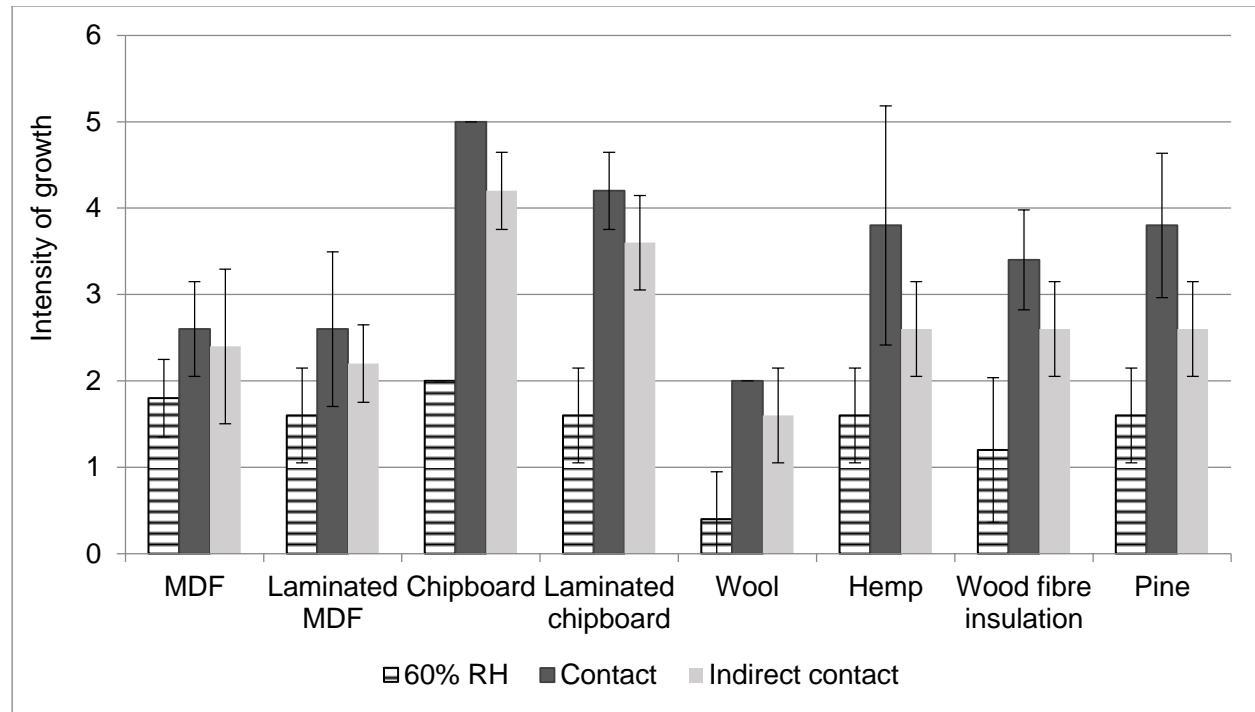
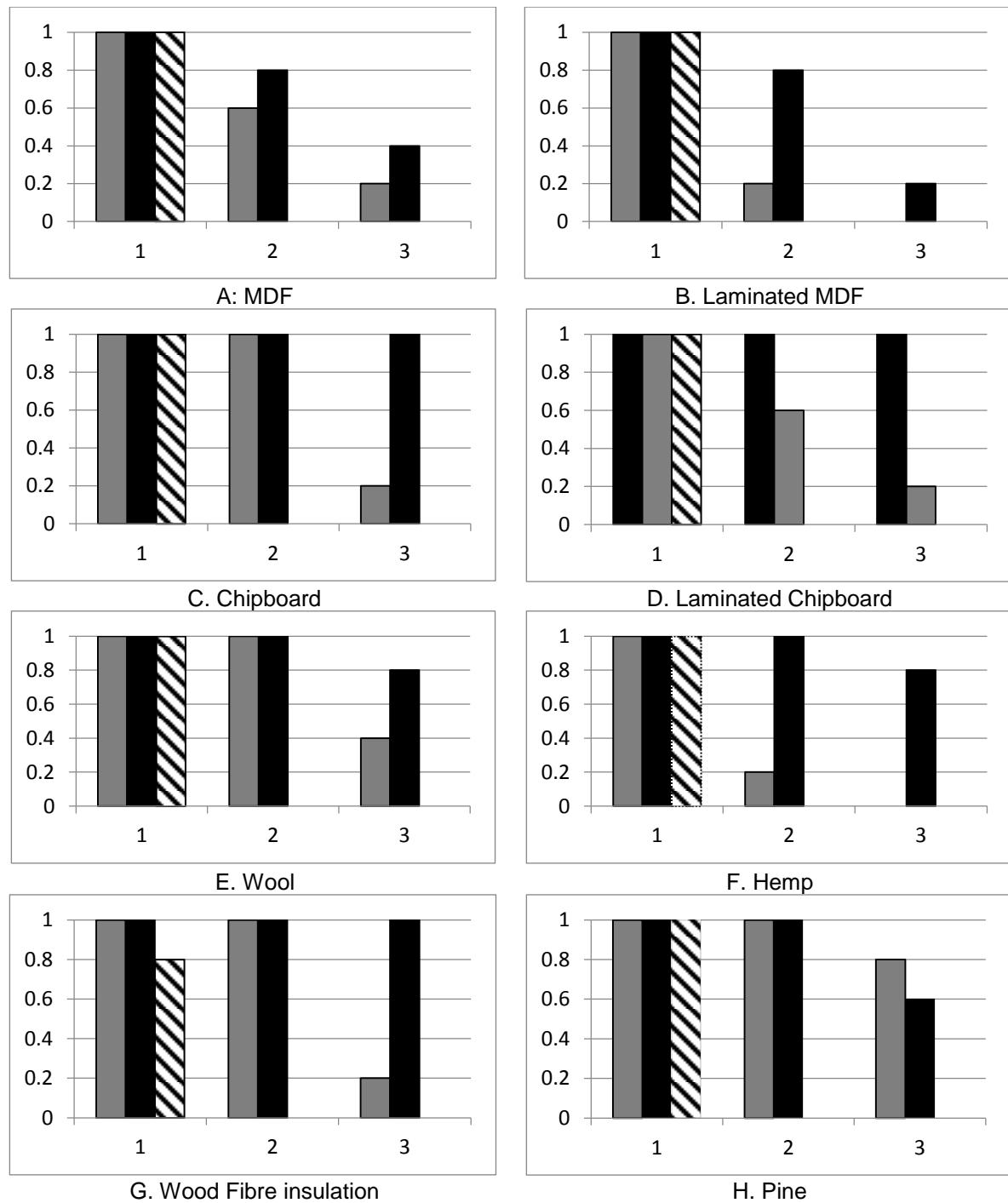


Fig 4: Intensity of growth, in contact, indirect contact and 60% RH conditions



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Fig 5: Frequency of growth by primary (1), secondary (2) and tertiary (3) colonisers on in contact (black), indirect contact (grey) and 60% RH (stripe)

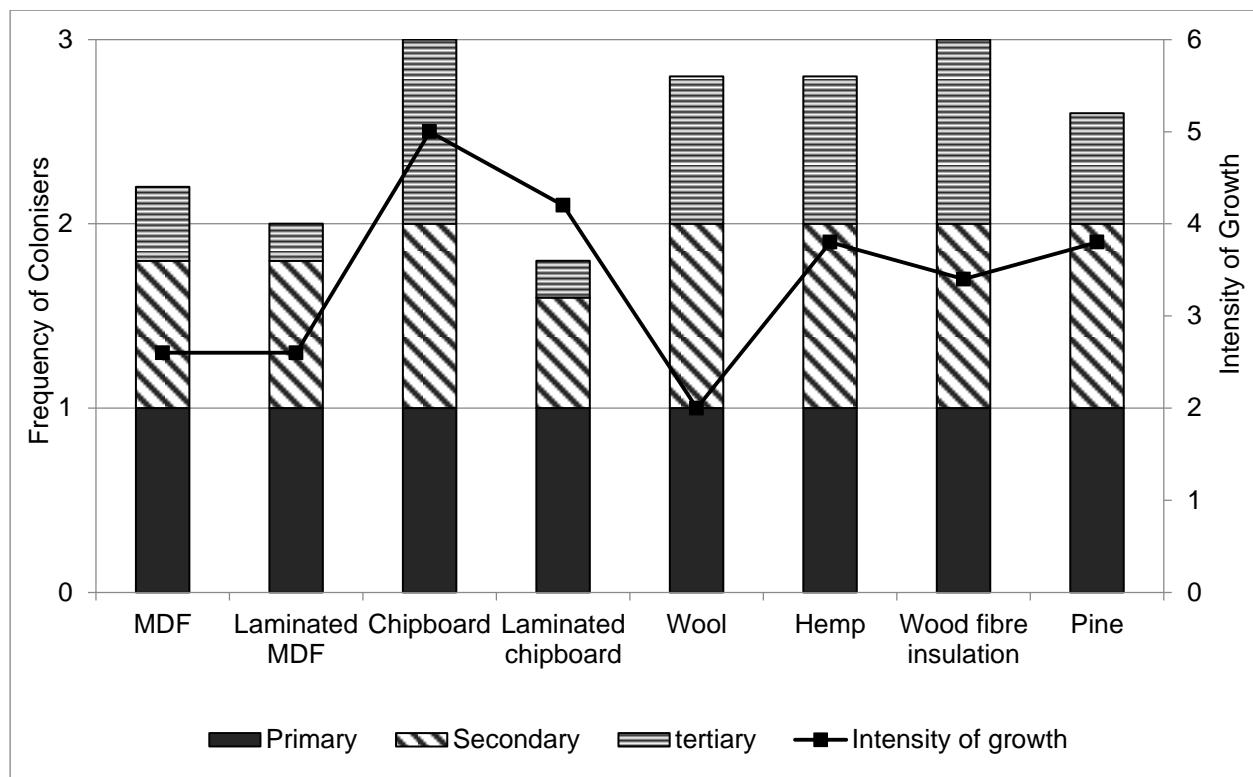


Fig 6: Intensity of growth and frequency of primary, secondary and tertiary colonisers on in contact samples