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Evaluating mould colonisation and growth on MDF panels modified to sequester Volatile Organic Compounds

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ABSTRACT

The increased effort to improve energy efficiency, has led to improved "air-tightness" of buildings, therefore leading to a reduction in ventilation. This results in an increase in concentration of indoor air pollutants, namely formaldehyde and volatile organic compounds (VOCs), which are suspected to contribute to "sick building syndrome" (SBS). There has been considerable research into the reduction of emissions via modification of current construction products. One modification is to use solid additives, "scavengers" in wood-based panels. This paper examines the effects of these scavengers on mould growth and the absorption of the VOCs; toluene, limonene and formaldehyde. The effects of the sorption of VOCs on the colonisation and growth of different mould species on modified MDF panels were also studied. It was shown that modified boards absorbed the 3 VOCs tested and this absorption did effect mould growth with differences observed in species present and in succession of mould colonisation.

INTRODUCTION

In recent years, indoor air quality and volatile organic compounds (VOCs) have received increasing attention. Coupled with this, there is an increased effort to improve the energy efficiency of buildings, which has resulted in the improved "air tightness" of buildings. An adverse effect of this, however, is the increase in the concentration of air pollutants, such as VOCs, inside homes where concentrations of many pollutants can be higher than in the outdoor environment (Rong et al., 2002). These VOCs often cause sick building syndrome (SBS) and have a number of symptoms; dizziness, eye and lung irritation, nausea and depression (Zhang and Xu, 2003). Therefore, indoor air quality has become an issue of public health (Kim et al., 2011).

The World Health Organisation defines VOCs as compounds with a boiling point between 50^{o}C to 260^{o}C (WHO, 1989), which encompasses a great variety of compounds including, hydrocarbons, terpenes and aromatic hydrocarbons. Therefore it is an immense challenge to develop a product that will act as a sink to all VOCs.

Historically, there has been considerable research into the reduction of emissions from their original source, such as odourless paints and replacing formaldehyde based resins with bio-based resins during wood-based panel production. More recently, there have been investigations into modifying wood-based construction materials and insulation materials to actively absorb formaldehyde and VOCs from the atmosphere.

One such modification is to use chemical and solid additives, termed "scavengers", in wood-based panels, to bond with free VOCs and formaldehyde. These scavengers are added to panels during production, directly as part of the resin or as a solid additive. These scavengers usually take the form of commercially available chemicals. However, recent research has shown by-products from various industries have shown promise as low cost scavengers such as, waste materials (both inorganic (Kim, 2009) and organic (Pirayesh et al., 2013)). Waste peanut and pistachio nut shells have also shown potential use as a bio-absorbent of pollutants such as heavy metals and dyes in aqueous solutions (Johns et al., 1998; Tavakoli Foroushani et al., 2016; Witek-Krowiak et al., 2011; XU and LIU, 2008). Peanut shell has also shown potential to absorb CO_2 (Deng et al., 2015). Walnut shell waste can be used as absorbent of copper ions (Kim et al., 2001).

The use of these additives, especially organic scavengers, raises the question as to what implication this sequestering of VOCs has on the resistance of modified MDF panels to microbial growth. Moulds will attack lignocellulosic materials, seeds, seedlings, food stuffs and books (Pasanen et al., 1992). Moulds can also attack synthetic floor coverings, airplane fuels, oils, glues, paints and textiles (Schmidt, 2006). Moulds rapidly colonise and grow on surface substrates and conidia develop rapidly. On timber, hyphae of mould fungi are able to penetrate the wood to a depth of a few millimetres and live on parenchyma cells that store sugar, starch and protein (Schmidt, 2006). Most moulds do not attack lignified cell walls, so therefore the wood strength properties remain unchanged (Viitanen, 1994).

However, the presence of moulds in damp buildings, can also contribute to SBS as many mould species' spores are known to cause health problems such as asthma and bronchitis (Fog Nielsen, 2003; Jarvis and Miller, 2004). The presence of moulds in construction materials can also increase a materials susceptibility to more destructive biological activity, such as decay fungi. Therefore it is highly important to study the implications of modifying current products to sequester VOCs on mould growth. It is widely known that the presence of formaldehyde will significantly prevent the growth of fungi on wood-based panels (Curling and Murphy, 1997; Dennis and Gaunt, 1974). However, little is known of the effects of absorbed VOCs on the colonisation and growth of moulds.

The work described below is an initial study developing a method to evaluate mould growth on modified MDF boards, modified with different organic scavengers from organic waste: peanut shell and walnut shell. The sorption of water and VOCs by the modified panels was also evaluated. The modified boards were "flooded" with VOCs, formaldehyde, toluene and limonene and then exposed to five different mould species: *Trichoderma virens, Cladiosporum sphaerospermum, Chaetomium globosum, Aspergillus niger* and *Penicillium rubens.*

MATERIALS AND METHOD

Materials

The three chemical solutions used in this experiment were chosen to represent different chemical groups of VOCs. Formaldehyde (F) represents polar VOCs (37% concentration in water), limonene (L) represents nonpolar VOCs (99% concentration) and toluene (T) represents aromatic VOCs (99% concentration). These chemicals were sourced from Sigma Aldrich without further purification Sterile de-ionised water (W) was also used as a control.

The materials tested were modified MDF construction materials and solid pine wood (*Pinus sylvestris*) as a control. The MDF panels were produced at pilot scale, using a mix of pine, fir and spruce wood chips and a urea-formaldehyde (UF) resin (12%). 6 MDF panels were modified with different VOC scavengers, walnut shells and peanut shells, at three different

loading percentages: 5, 10 and 15% (on a dry weight basis). The peanut shell and walnut shell were milled to a particle size of 5mm. A Control MDF without scavengers was also produced. The boards were produced using a formaldehyde based resin, therefore all the samples were placed into conditioning room at 23^{o}C ±1 and 60 ±3 %, for 6 months to allow for de-gassing of free formaldehyde (Curling and Murphy, 1997).
Six replicates of each material were used for the sorption test of each VOC. As the analysis of mould growth was visual, dimensions of the test specimens were not critical but were approximately 50 x 25 mm (±2 mm) at product thickness of 12mm. A further six replicates were used as sorption control specimens. All the test specimens were conditioned in standard conditions of 23^{o}C ±1 and 60 ±3 % RH until a constant mass was reached.

Moulds

All the mould species were purchased from Fungal Biodiversity Centre, Institute of the Royal Netherlands Academy of Arts and Science (KNAW). The mould species selected for use in testing are representative of species commonly found within buildings and used in standards (BS 1982-3):

1. *Cladiosporum sphaerospermum* (Penz) CBS 122.63
2. *Chaetomium globosum* (Kunze ex Fr.) CBS 107.14
3. *Penicillium rubens* (Biourge) CBS 401.92
4. *Trichoderma virens* (J.H. Mill, Giddens & A.A. Foster) CBS 100946
5. *Aspergillus niger* (M. Frank) CBS 101698

Preparation of spores

Using well sporulated cultures of each of the 6 moulds, a final mixed spore suspension following EN ISO 846 1997, was produced. 5ml of sterilised water were added to the culture and a sterile needle was used to gently scrape the spores from the surface into the water. The spore suspension was decanted off into a sterile tube and agitated using an orbital shaker and then filtered to remove mycelial fragments. The five spore suspensions were combined together and agitated again.

VOC and water exposure

The VOCs chosen for this work were formaldehyde (F) (37% concentration), representative of polar compounds, toluene (T) (99% concentration), representing aromatic compounds and limonene (L) (99% concentration), representing non-polar compounds. Samples were also exposed to water (W) as a control.
To expose the modified boards to VOCs and water, 600ml volume vessels were used. Prior to exposing the samples to the VOCs, the jars and samples were sterilised. This was achieved by spraying the inside of the jars, metal stand and the samples with 70% ethanol and allowed to dry in sterile conditions. 60 ml of either water or liquid VOC source was poured into the jars with a sterile supporting metal mesh. The samples were placed on top of the mesh, to ensure that the samples were out of contact with the solvents. Each chamber was sealed with an aluminium lid and wrapped with wax film to ensure that no solvent was lost through evaporation. The chambers were then stored for seven days at a constant temperature and humidity of 20 ^{O}C ±2 at 70% RH ±3.

Inoculation and exposure

The setup of vessels in which the "flooded" VOC samples were exposed to mould growth, was based on the BSEN 12038 2002 standard procedure using 600ml vessels with ventilated

aluminium lids. 80ml of water agar was poured into each vessel and then autoclaved at 121 ^{O}C for 50 minutes. Sterile, inert plastic meshes were added to ensure the samples were not in direct contact with the water agar.
Under sterile conditions, two of the board samples were removed from the VOC chambers and into 600ml vessels on top of the plastic mesh. Each sample was then inoculated with 0.5ml of the spore suspension. The vessels were then quickly sealed with an aluminium lid. These vessels were then stored in a dark chamber at 20 ^{O}C ±2 at 70% RH ±3 for two weeks.

Assessment

For the assessment, the presence or absence of the different mould species was identified and given a score of 1 (present) or 0 (absent) on each of the replicates. A mean value of this was then calculated to show the frequency of growth of all the mould across all replicates i.e. value of 0.5 shows that the mould was present on 3 out of 6 replicates.
The sum of the mean values of frequency of growth was then calculated to show the total frequency of growth of all the moulds collectively. Where possible, the primary, secondary and tertiary colonisers were identified and the dominant mould species identified and recorded.

RESULTS AND DISCUSSION

Water and VOC Sorption

Table 1, shows the results of the sorption of water and the three VOCs by the different modified panels. The boards containing 15% walnut absorbed the most moisture in the water chamber (1.87 g/kg) and 5% walnut, the least (1.28 g/kg). Of the formaldehyde VOC, the blank MDF samples, absorbed the most and solid pine wood the least, 1.56 g/kg and 0.98 g/kg, respectively. Panels containing 10% peanut shall absorbed the most limonene and solid pine the least, 1.17 g/kg and 0.35 g/kg respectively. Of the toluene, solid pine absorbed the most (1.55 g/kg) and 15% walnut boards absorbed the least (0.66 g/kg). Figure 3 graphically shows the results of the water and VOC sorption by the modified panels.
As is obvious, the standard error bars are quite large for the toluene sorption results. This is due to toluene being a volatile compound. Once removed from the chamber, the boards were de-gassing and the free toluene immediately begins to evaporate. It is likely to be the same for limonene sorption, as only some will be bound to the scavenger and MDF (Figure 4).

Table 1: Shows the average sorption of VOCs by modified panels

Panel	**Water (g/kg)**	**Formaldehyde (g/kg)**	**Toluene (g/kg)**	**Limonene (g/kg)**
5 % Walnut	1.28	1.12	0.94	0.49
10 % Walnut	1.53	1.17	0.87	0.74
15 % Walnut	1.87	1.35	0.66	0.59
5 % Peanut	1.47	1.18	1.20	0.78
10 % Peanut	1.37	1.37	1.29	1.17
15 % Peanut	1.52	1.52	0.67	0.48
MDF Blank	1.56	1.56	1.00	0.91
Pine	1.43	0.98	1.55	0.35

Mould growth

Table 2 shows the results for the mean frequency of growth of each mould species across the replicates, exposed to water and the four VOCs. Table 3 shows the total frequency of mould growth observed on the modified MDF panels and pine wood. Unfortunately, *Chaetomium globosum* failed to grow across all the samples. The greatest mould growth was, as expected, observed on the modified boards exposed to water.

Mould growth post water exposure

Aspergillus niger had successfully developed on all types of modified boards and was observed in all replicates. *Cladiosporum sphaerospermum* and *Trichoderma virens* were also seen on all types of modified panels, but not of the same frequency as *Aspergillus niger*. *Penicillium rubens* was observed on all types of boards but showed the least frequency of growth. However, as a primary coloniser it may have been out-competed by the other secondary and tertiary colonisers over the two weeks.

The greatest extent of growth was observed on the 10 and 15% walnut boards after exposing to water. This is likely to be a result of the higher moisture content of the boards, due to absorbing more moisture when in the chamber (Figure 1).

Solid pine showed the lowest frequency of mould growth, after exposure to water. This is likely to be result of the lower moisture sorption, inhibiting the mould growth.

Table 2: Frequency of species mould growth on samples exposed to water (W), formaldehyde (F), toluene (T) and limonene (L)

Panel	*Chaetomium globosum*				*Trichoderma virens*				*Cladiosporum sphaerospermum*				*Aspergillus niger*				*Penicillium rubens*			
	W	**F**	**T**	**L**	**W**	**F**	**T**	**L**	**W**	**F**	**T**	**L**	**W**	**F**	**T**	**L**	**W**	**F**	**T**	**L**
5 % Walnut	0	0	0	0	0.83	0	0	0	1	0	0	0	1	0	0	0	0.83	0	0	0.67
10 % Walnut	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0.33	0.33
15 % Walnut	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0.17	0
5 % Peanut	0	0	0	0	0.83	0	0	0	1	0	0	0	1	0	0	0	0.5	0	0	0
10 % Peanut	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0.33	0	0	0	0.33	0
15 % Peanut	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0.33	0	0.67	0	0.33	0
MDF Blank	0	0	0	0	0.83	0	0	0	1	0	0	0	1	0	0	0	0.5	0	0.17	0
Pine	0	0	0	0	0.00	0	0	0	0.50	0	0.17	0	1	0	0	0	0	0	0	0

Table 3: Total frequency of mould growth on modified MDF, MDF and pine

Panel	Water	Formaldehyde	Toluene	Limonene
5 % Walnut	3.67	0	0	0.67
10 % Walnut	4	0	0.33	0.33
15 % Walnut	4	0	0.17	0
5 % Peanut	3.33	0	0	0
10 % Peanut	3.0	0	0.67	0
15 % Peanut	3.67	0	0.83	0
MDF Blank	3.33	0	0.17	0
Pine	1.50	0	0.17	0

Figure 5 shows the differences in the frequency of mould growth of primary, secondary and tertiray colonisers. All primary, secondary and tertiary colonisers were present on all samples tested, except on pine samples. On pine samples, only primary and secondary colonisers were observed. This could be a result of the pine having a lower moisture content than MDF samples. There is little difference between the samples, however there is a difference between the dominating species (Table 4).

Mould growth post formaldehyde exposure

Across all the modified boards and replicates, no mould growth of any mould species was observed (Table 2). Formaldehyde is toxic (Rong et al., 2002; Rosenkranz, 1972) and therefore the lack of mould growth is not surprising. Of the VOCs tested, formaldehyde was absorbed to the greatest extent by the modified boards, see Table 1 and Figure 2. The formaldehyde used was in an aqueous solution at 37%, therefore part of the observed weight gain after absorption is likely to be water as well as formaldehyde. However, there was a total absence of mould growth, compared to samples exposed to water alone. This shows that a sufficient amount of formaldehyde was absorbed to prevent any mould growth. This also shows that the presence of formaldehyde in the resins is not responsible for the lack of mould growth, as all panel samples underwent the same de-gassing period to remove any free formaldehyde.

As mould growth was not observed on solid pine, this indicates that wood can absorb enough formaldehyde to prevent mould growth too. However, this experiment was not continued after two weeks. Therefore it is possible that the formaldehyde was not trapped within the pine wood chemically and would eventually de-gas and mould would colonise and grow on the wood.

Mould growth post toluene sorption

Little growth was observed on the toluene exposed samples, see Table 2. Only primary colonisers were observed towards the end of the two weeks of the experiment on 10% and 15% Walnut and peanut MDF boards. Figure 6 and 7 shows that there is no correlation between fungal growth and the amount of toluene absorption on all peanut and walnut modified panels respectively. It is possible that due to the off-gassing from the sample of free toluene, toluene gas could have accumulated inside the vessels to toxic level, preventing the growth of mould species. The vessels were all tightly sealed with a plugged hole of non-absorbent cotton wool to allow oxygen ventilation. Therefore, over the two weeks experimental time, the oxygen levels will have increased and the toluene levels decreased inside the vessels. Eventually, this allowed for primary colonisers, *Aspergillus niger* and *Penicillium rubens* to grow and establish on the exposed MDF boards. If allowed more time to grow, it is possible that more growth and further colonisation may occur. This suggests that, although a lot of toluene was not chemically bound within the panel, it can reduce the time for colonisation, growth and succession.

Mould growth post limonene sorption

Table 2 shows the subsequent results of the mould growth on the boards. Minimal growth was observed on the 5% and 10% walnut boards and only of the primary coloniser *Penicillium rubens.* No growth was observed on all other boards. This suggests that peanut shell maybe a better scavenger than walnut shell for non-polar compounds. This is important

when considering what scavengers to use for target VOCs. Figure 6 and 7 shows that there is no correlation between fungal growth and the amount of limonene absorption on peanut and walnut modified panels respectively. However, when compared to the growth on samples exposed to water, there is a marked reduction in growth, so the presence of limonene is preventing mould growth.

Dominating species

Table 4 shows the dominant species found on the different types of modified boards exposed to water, toluene and limonene. Formaldehyde is not included in Table 4, as there was no growth observed for any of the test moulds.

Table 4: Dominant species found modified boards

Panel	**Water**	**Toluene**	**Limonene**
5 % Walnut	*A. niger*	-	*P. rubens*
10 % Walnut	*C.sphaerospermum*	*P. rubens*	*P.rubens*
15 % Walnut	*A. niger*	*P. rubens*	-
5 % Peanut	*C.sphaerospermum* *A. niger*	-	-
10 % Peanut	*T. virens, A. niger*	*P. rubens*	-
15 % Peanut	*A. niger,*	*P. rubens* *A. niger*	-
MDF Blank	*A. niger,* *C. sphaerospermum*	-	-
Pine	*A. niger*	-	-

Key: - no mould growth observed

On samples exposed to water, a different succession of growth was observed. On boards containing 5% walnut, although Table 2 shows primary, secondary and tertiary species present, the dominant mould species was a primary coloniser *Aspergillus niger*. *Aspergillus niger* was also a dominant species on the boards containing 15% walnut. This suggests a slower succession of growth of the mould species grown on boards containing a walnut scavenger.

There was a lower frequency of growth on the boards containing peanut shells, when compared against those boards containing walnut shell. There was also a difference in the specific species growing and their prevalence on the peanut samples. Although primary colonisers are still present, the secondary and tertiary colonisers are more dominant. This suggests a difference in the growth rate of the moulds on boards containing peanut shell, compared to boards containing walnut shell.

The mould growth observed on limonene and toluene exposed samples is by the primary coloniser *Penicillium rubens.* Primary coloniser *Aspergillus niger*, was observed only on boards containing 15% peanut exposed to toluene. This shows that the presence of these two VOCs significantly reducing colonisation, when compared to samples exposed to water.

CONCLUSION

The aim of this paper was to evaluate the addition of organic scavengers on absorption of VOCs and the effect this has on mould growth. The study conducted suggests that the addition of the walnut shell increases the boards' susceptibility to mould colonisation and growth. This is possibly due to an increased moisture uptake by the walnut. The presence of peanut scavenger also seems to increase vulnerability but to a lesser extent. It can also be concluded that the boards modified by the addition of walnut shell or peanut shell do absorb formaldehyde, toluene and limonene. The absorption of formaldehyde from the atmosphere by the scavengers prevents colonisation and initial growth. This sorption of VOCs does effect mould growth on boards, reducing frequency as well as causing a variation in the presence of different mould species.

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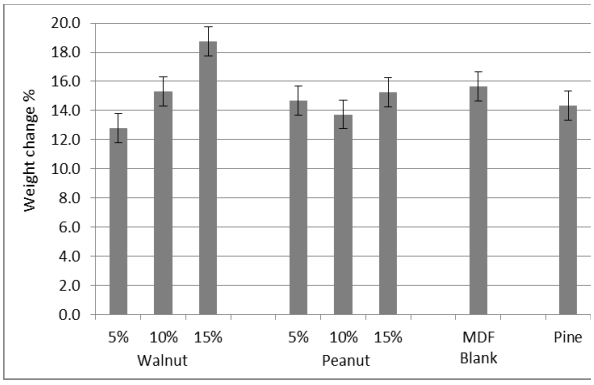

Figure 1: Water sorption

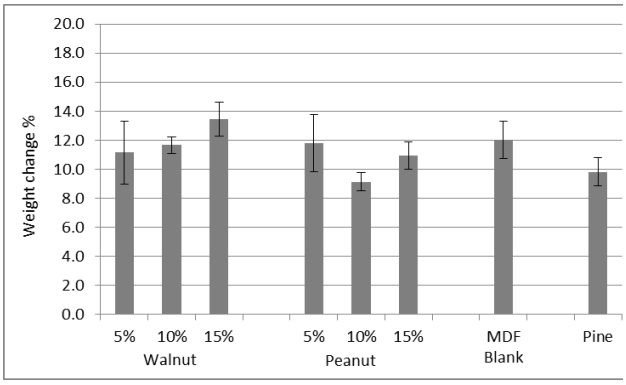

Figure 2: Formaldehyde sorption

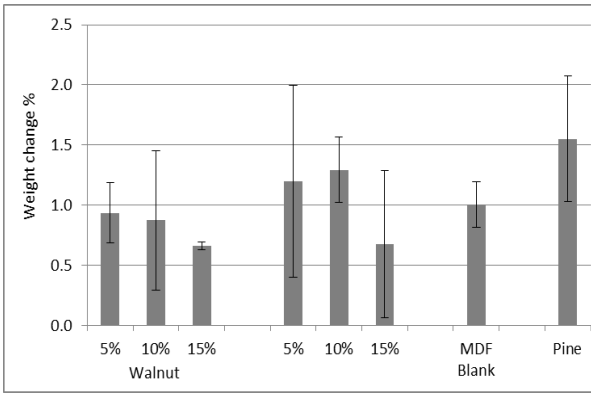

Figure 3: Toluene sorption

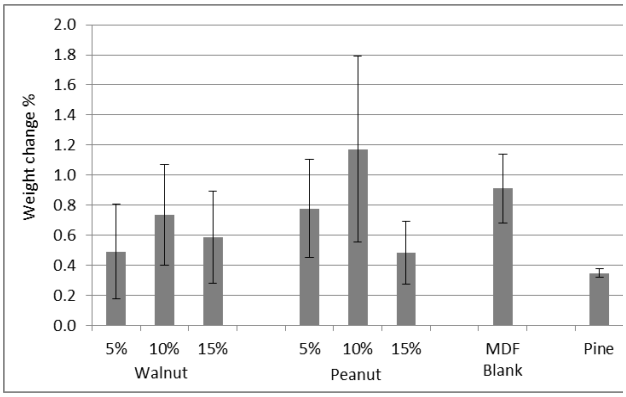

Figure 4: Limonene sorption

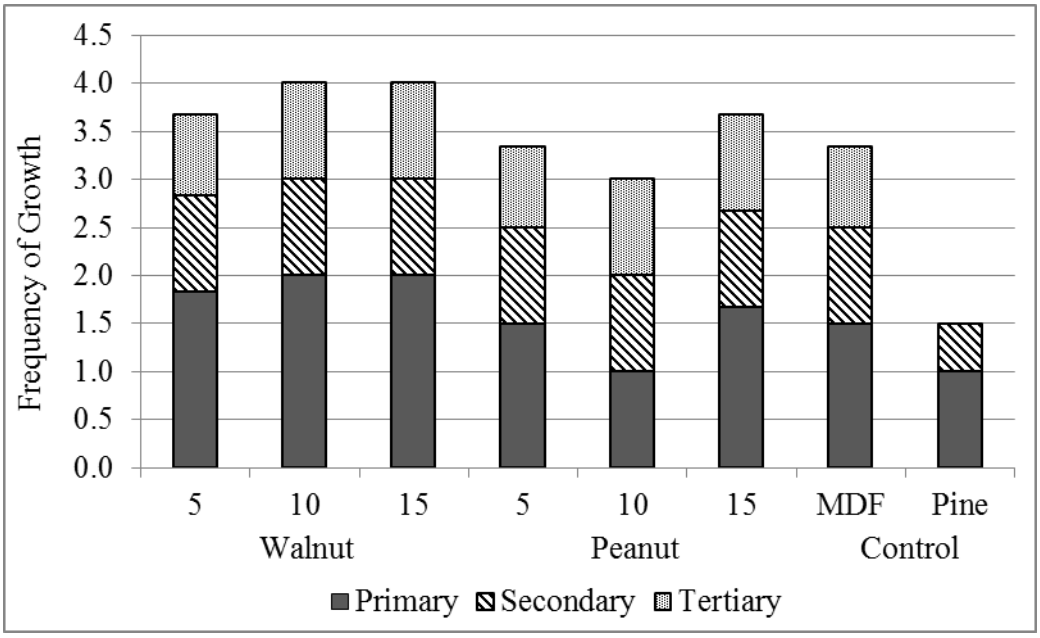

Figure 5: Total frequency of colonising mould specie's growth and the different colonising species on modifed boards after water exposure

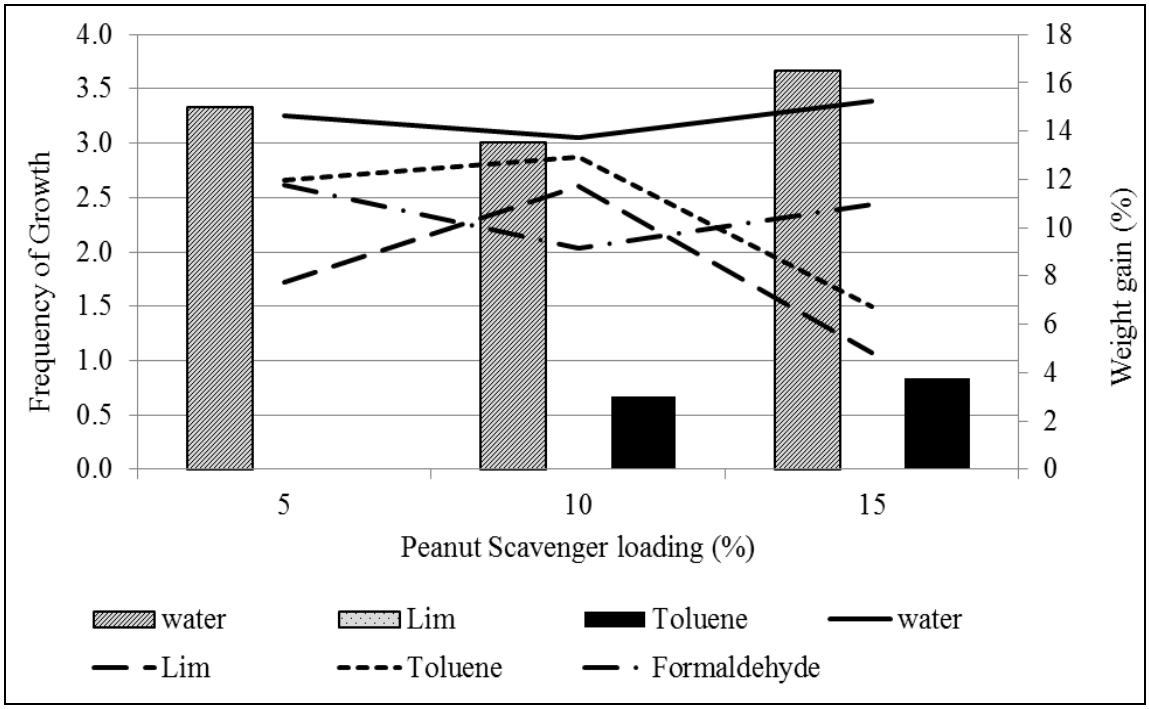

Figure 6: Frequency of mould growth (bars) and sorption weight gain (%) (lines) of MDF panels modified with peanut shell

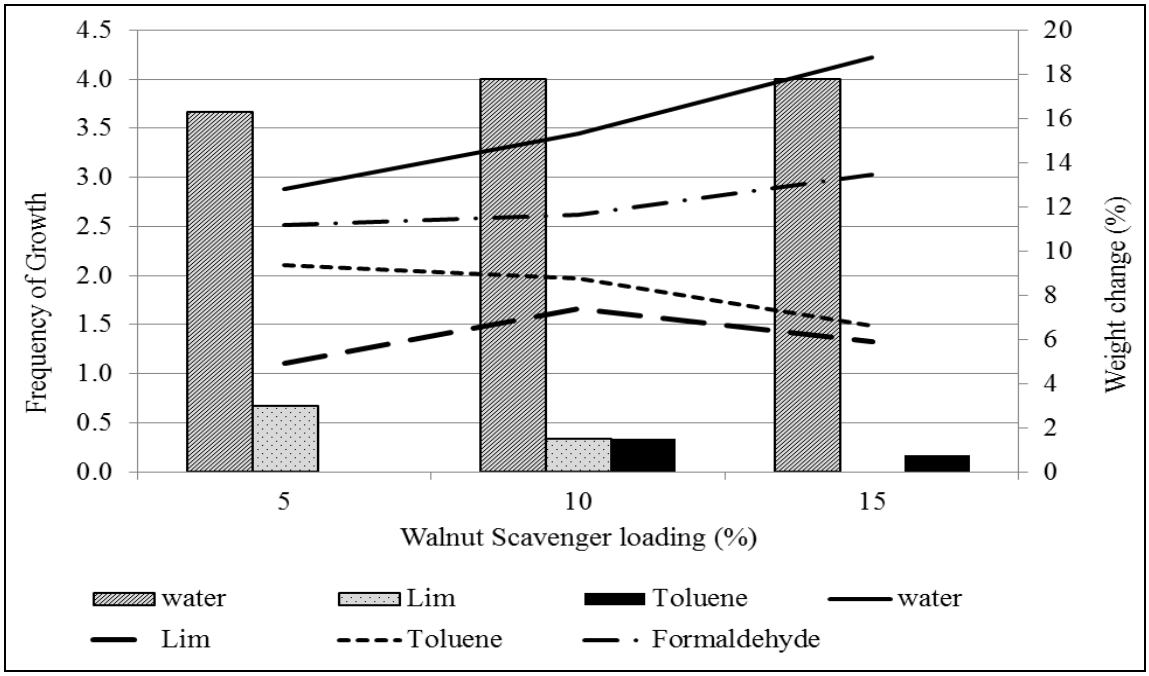

Figure 7: Frequency of mould growth (bars) and sorption weight gain (%) (lines) of MDF panels modified with walnut shell