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Final report -

order no. 9218001-A1

This report replaces investigation report no. 9218001 of 28/08/2019, which has been completed and finalised in sections 4.3 to 4.5 and section 6.

Client:	Gütegemeinschaft Paletten e. V. Benrather Schlossallee 2A 40597 Düsseldorf
Order date:	27/02/2018
Project:	Study on the hygienic properties of Euro pallets (load carriers) made from wood, in comparison with Euro pallets made from plastic
Contractor:	Institut für Holztechnologie Dresden gemeinnützige GmbH
Person in charge of project:	Dipl.-Biol. Katharina Plaschkies

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This report consists of 38 pages and an appendix comprising 5 pages. The reproduction of parts of this report and appendix is only permitted with the written consent of IHD. The results published in these documents refer exclusively to the examined materials.

1 Project and purpose

Institut für Holztechnologie Dresden gemeinnützige GmbH (IHD) was commissioned to carry out a study on the hygienic properties of Euro pallets (load carriers) made from wood, in comparison with Euro pallets made from plastic.

For this purpose, IHD first collected and assessed the latest knowledge and technology in the respective fields and examined the hygienic properties of the relevant Euro pallet materials (softwood and plastic) for a comparative study. The results have been compiled in a documentation that contains both general findings regarding the hygienic properties of the examined materials, and specific values determined in experiments and tests of the various pallet materials.

2 Work packages

The study includes a theoretical and a technical part and is divided into 4 work packages:

- Work package 1: Literature survey
- Work package 2: Determination of the microbial load of used pallets
- Work package 3: Laboratory tests for the determination of the anti-bacterial properties of certain pallet materials

3 WP 1: Literature survey

3.1 Objective

The literature survey was carried out to gain an overview of the current state of knowledge on the hygienic properties of wood and plastics with regard microbial colonisation, the survivability of micro-organisms and the test methods for quantitative assessment.

3.1.1 Hygienic properties of wood and plastics

Requirements for pallets used in food industry

The hygiene requirements for the transport and storage of foods are laid down in the German Food Hygiene Ordinance LMHV and the German Food Transport Container Ordinance LMTV.

GS 1 Germany has defined quality classes (A, B and C) for used EPAL pallets. Apart from mechanical properties, this system also defines hygiene characteristics. While class A pallets must not show any discolouration, class B pallets might feature dark stains. In class C, the pallets might be moist on the surface, and even contain contaminants and dirt as long as these cannot be transferred on to the transported goods. Pallets showing signs of mould growth inside the material or on its surface are classified as no longer usable. For lay persons, it is not always easy to determine whether discolouration is caused naturally by light and oxygen, by dirt or by microbial colonisation.

For international trade, the requirements for packaging and transport equipment made from wood are defined in various standards, such as ISPM 15. These standards aim at preventing the spreading of harmful organisms and thus focus on phytosanitary measures rather than hygiene measures in the narrow sense, as they do not specifically deal with human or animal pathogens.

Conditions promoting microbial colonisation in materials

Climate conditions play a key role, whereby moisture availability has been identified as the main limiting factor (Hankammer and Lorenz 2003). Most bacteria only form colonies on surfaces, if the relative air humidity is 98-100 %, corresponding to an Aw value of 0.98-1.00. The Aw value is the measuring value for water activity, i.e. the water freely available to micro-organisms in the substrate. Halophilic and halotolerant[1] species are however showing growth at humidity levels of around 60%. Mould growth starts at approx. 70 % relative air humidity, whereby most species only thrive at an air humidity above 85 % (Mack 2000). Another limiting factor is temperature. Both bacteria and fungi grow within an extremely broad temperature range from a few degrees Celsius above zero to around 40 °C, and some species even tolerate temperatures outside this range. The natural environment contains a huge variety of micro-organisms able to colonise materials, provided the climate conditions are right. Their growth can however be prevented by choosing materials with specific properties or special treatment. Growth is for instance hampered by naturally occurring biocidal substances, pH values outside the range of 2 to 11, protective impregnation or treatment with biocidal products.

'

[1] prefer or tolerate high salt concentrations

When micro-organisms grow on the surface of materials, they normally form biofilms containing various species. Inside these biofilms, the organisms are protected by a polymer matrix against adverse influences such as desiccation, UV light, extreme pH or toxic substances (Hall-Stoodley et al. 2004).

As dirt on the surface of a material as well as air-borne dirt such as dust, grease and sweat provide a rich source of food for micro-organisms, such biofilms even form on inert materials including glass and metal. With regard to pallets, dirt deposits can occur in many industries, and especially in the food-processing sector, so that there is always a risk of organic deposits on pallets, if they have previously been in contact with unpacked goods.

Microbial growth on plastics

The ambient conditions described above also apply to microbial growth on plastic surfaces. Most hygiene pallets are made from polyethylene (PE) or polypropylene (PP). These are organic polymers synthesised from ethylene or propylene respectively, two materials are classified as not readily biodegradable. Low-molecular polyethylene can however be colonised by bacteria (Jen-Hou and Schwartz 1961), and partly degraded by moulds that produce special enzymes (peroxidases) (Iiyoshi et al. 1998). Latest studies on microplastic waste in the oceans show however that plastics are not degraded in nature (Overbeckmann and Labrenz 2019).

Plastics are primarily used for applications where micro-organism growth would have a serious negative impact on health, as is the case in drinking water distribution systems and the field of medicine. In Germany, drinking water pipes must be made from plastics that meet the requirements laid down in the W270 standard of the German Technical and Scientific Association for Gas and Water (DVGW), to ensure that no biofilms can form over time on the surfaces exposed to drinking water (Kötzsch et al. 2016). This does however not mean that the material cannot not be colonised by micro-organisms under different conditions (Kötzsch et al. 2017). There are many plastics that meet the stringent requirements of W270, and polyethylene is for instance widely used for drinking water pipes (https://www.baunetzwissen.de).

In the field of medicine where particularly high hygiene standards must be met, the use of plastics is widespread. To ensure patient safety, devices, consumables and implants are made from top-grade synthetic materials, whereby biocompatibility and easy cleaning are two of the key considerations. Medical-grade plastics often contain anti-microbial components such as metal salts or sliver ions (https://medlexi.de).

Microbial growth on wood

Wood is a natural organic material that can be colonised, and is easily decomposed, by micro-organisms (fungi and bacteria). Whether colonisation or decomposition occurs depends on the factors mentioned above, whereby moisture plays a key role.

For the destruction of wood by wood-decay fungi (brown and white mould, soft rot fungi), it is however not the moisture content of the ambient air, but the moisture contained in the wood that determines the rate of digestion. Wood is only destroyed by micro-organisms, if the wood moisture content is above approx. 30 % (Huckfeldt and Schmidt 2006).

Mould attack is a common problem in wood with a high moisture content. In outdoor areas, moulds cause primarily unsightly discolouration. Indoors, mould attacks pose a hygiene problem, as these fungi can be harmful to human health, for instance by causing allergic reactions. The actual health risk depends however on the type of the mould, the species and the exposure to it. Some people are more disposed to adverse reactions to moulds than others.

If the conditions are right, virtually all solid wood and other timber-based materials can become colonised by moulds, except those with an extremely high pH of > 11, as is found for instance in cement-bonded particle boards. As pallets are normally made from spruce or pine, their surfaces provide an ideal substrate for mould growth, whereby pine is slightly less susceptible to mould attack (Scheiding et al. 2003).

A special type of fungal attack affecting wood is caused by blue-stain fungi which, together with moulds, belong to the phylum of Ascomycota. The dark-brown fungal hyphae extend deep into the wood. The blue colour is the result of optical refraction on these brown filamentous structures. Blue-stain fungi are commonly found in softwood species, while hardwoods such as maple or beech are less susceptible to attack. Blue-stain fungi live off substances contained in the parenchyma cells of sapwood and do digest structural substances such as lignin and cellulose, so that the structural strength of the wood is not affected by an attack. Blue stain also poses no health hazard to humans (UBA 2019), so that the use of the pallets affected by this type of fungi is unproblematic.

In building construction, fungal attack in timber is a major concern, while bacterial attack is a minor issue. Although bacteria also contribute to the digestion of wood, they need a much higher moisture content than moulds to actually cause problems. In humid indoor areas, mould attack therefore always occurs before bacterial growth, and is generally the principal cause of wood decay.

Where the spreading of pathogens and food-spoiling germs must be prevented, bacterial growth on surfaces is obviously a major concern. As a result, there are many studies on the anti-bacterial properties of wood for use in food processing and storage. Other studies look at the use of wood in hospital interiors. The findings of the literature survey are compiled in the next chapter where we examine and compare a number of hygienic properties of wood and plastics.

Comparison of the hygienic properties of wood and plastics

A number of older studies examine whether wood is more hygienic than plastic. In most cases, these comparisons refer to direct (as on chopping boards) or indirect contact with foodstuffs (Ak et al. 1994, Weiker et al. 1997, Gehrig et al. 2002, Prechter et al. 2002, Schönwälder et al. 2002, Mühlbauer and Milenovic 2012, Kleiner and Lampe 2014, Lücke and Skowyrska 2015).

Other studies assess the suitability of wood as an interior building material where high hygiene standards need to be met, such as in hospitals and clinics (Strehlein et al. 2004, Schuster et al. 2006). There are also some studies that look at the hygienic properties of packaging materials Steinkamp and Wilms 2000, HPE 2014, Milling 2005-1).

Using a range of different methods, nearly all studies conclude that wood has certain antibacterial properties, be it due to its structure or its chemical composition. Wood is a porous material with a very large inside surface area. Wood is hygroscopic, which reduces the availability of water for bacteria. Depending on the actual tree species, wood contains antimicrobial substances (Stingl and Hansmann 2006).

A comprehensive overview of the hygienic properties of wood was compiled by Aviat et al. (2016) who reviewed 86 publications in order to determine whether direct contact between wood and foodstuffs is safe. The authors discuss the antimicrobial properties of wood as well as the testing methods used in the reviewed studies. They conclude that wood is suitable for direct food contact, as its rough surface and porous structure often generate unfavourable conditions for micro-organisms and/or bind or trap them in the material. These physical characteristics of wood, rather than any potentially antimicrobial constituents, have been found to be the main reason for the antibacterial properties of wood (Lukowsky 1994). Compounds found in wood that have potentially antimicrobial properties belong to the various groups including phenols, lignans, tannins, stilbenes, flavonoids and terpenoids (Pearce 1996, Mourey and Canillac 2002).

Of special interest here are hygiene pallets made from pine heartwood. Steinkamp (2004) performed laboratory and field tests with relevant germs from meat and animal production, and from hospital environments. The results show that hygiene pallets made from pine heartwood had clear antibacterial properties and thus outperform plastic pallets. Further proof of the antibacterial activity of pine heartwood can be found in Milling et al. (2005-2) and Ripolles-Avila et al. (2019) who examined its use in the transport of fresh fish.

Detection methods

There is currently no standardised method to determine which bacteria survive under specific conditions. Agar diffusion plate tests according to DIN EN ISO 846[2] or DIN EN 1104[3] are not useful, as they only determine whether the wood excretes antimicrobial constituents. In the context of food safety, such excretions are however problematic, as article 3 of Regulation (EC) No. 1935/2004 prohibits the transfer of antimicrobial constituents from the packaging to foodstuffs, as these substances can adulterate the food.

For practice-oriented research projects, methods where the material is inoculated with bacteria, which are then transferred to a culture medium for incubation have been found more useful to determine the survival of bacteria on certain materials.

[2] DIN EN ISO 846:2019: Plastics - Evaluation of the action of micro-organisms

[3] DIN EN 1104:2019: Paper and board intended to come into contact with foodstuffs - Determination of the transfer of antimicrobial constituents

With such methods, samples taken from the surface of the material under investigation are transferred with a punch under pressure to a solid culture substrate (Gehrig et al. 2002, Fürst 2007, Kavian-Jahromi 2015), which however means that bacteria inside the material are not comprised in the samples. More suitable for most purposes rinsing methods where the survival rate of the bacteria is determined by rinsing them off the material followed by transfer to the culture medium. With rinsing methods, there is however some uncertainty, as it is not clear whether all bacteria are removed from the material in the process. While simple rinsing is sufficient to detach all bacteria from smooth plastic surfaces, the recovery rate from porous or rough wood surfaces is relatively small (Carpentier 1997), but can be increased by ultrasonic treatment (Le Bayon et al. 2010) or brushing (Mariani et al. 2007). The highest recovery rate is achieved with scraping, which shows that bacteria penetrate the wood and become firmly attached to it (Ismail et al. 2014).

The method developed to determine the antibacterial activity on plastics and other non-porous surfaces (ISO CD 22196) can be applied without modifications to the examination of plastic pallets. It is however not suitable to test untreated wood, which is by its nature porous, so that the method always returns a much lower bacteria count as only a fraction of the micro-organisms are rinsed from the material.

For wood products, there is currently no standardised method for the determination of its antibacterial properties. The following methods can however be adapted for use on and in wood:

- DIN EN ISO 846: Plastics - Evaluation of the action of micro-organisms
- ASTM G-22-76: Standard practice for determining resistance of plastics to bacteria
- DIN EN ISO 20645: Textile fabrics - Determination of antibacterial activity - Agar diffusion plate test
- EN 1104: Paper and board intended to come into contact with foodstuffs - Determination of the transfer of antimicrobial constituents.
- ISO 22196: Measurement of antibacterial activity on plastics and other non-porous surfaces
- DIN EN ISO 20743: Textiles - Determination of antibacterial activity of textile products
- DIN 54379: Testing of paper and board - Determination of the total colony count

For the development of a method, factors such as the age and history of the wood (new or used product, ambient conditions (temperature, air humidity and wood moisture content), as well as the transfer of nutrients must be taken into account.

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- Regulation (EC) No 1935/2004: Materials and articles intended to come into contact with food
- Regulation (EC) No. 852/2004: Hygiene of foodstuffs
- Regulation (EC) No 853/2004: Specific hygiene rules for food of animal origin
- German Food Hygiene Ordinance LMHV
- German Food Transport Container Ordinance LMTV
- ISPM 15-2017: International standard for phytosanitary measures (International Plant Protection Convention)

4 WP 2: Determination of the microbial load on used pallets

4.1 Objective

We determined the microbial load on pallets in order to assess its the relevance with regard to their use in hygiene-sensitive areas.

4.2 Wood pallets for examination

According to the objectives of this study, pallets from various manufacturers, users and processors in a range of industries (e.g. food processing, animal feed production, agriculture) were microbiologically examined and assessed. The client was responsible for the selection, procurement and provision of the material for examination.

The following wood pallets were delivered by Collico Verpackungslogistik und Service GmbH on 29/11/2018 to the lab:

- 3 packs numbered 1.1 to 1.3, containing 10 Euro pallets each (E1 to E30)

The supplied pallets had been in use. The stamps (if applied) indicate their origin; the contractor had no further information regarding their previous use or ownership. The stamp details are listed in table 1.

All pallets conform to the EPA standard and consist of 13 boards and 9 blocks each. The determination of the wood species was not part of the scope of this study. A preliminary assessment of some random samples indicates however that most of the pallet elements are made from softwood, with few hardwood components. The blocks are solid wood or woodchip blocks (Presspan); some pallets contain a combination of these two block types. For details, see section 4.4, table 2.

Table 1: Pallets and stamp data

No.	IPPC stamp	Country code	Reg. no. of PS authority	Licence no./month/year	Seal
Pack 1.1					
E 1	x	DE-SN1	497027	021-3-03	EPAL
E 2		illegible			EPAL
E 3	x	illegible	illegible	...-6-03	UIC
E 4	x	illegible	illegible		EPAL
E 5	x	DK	8015	GC-148-5-0	EPAL
E 6	x	illegible	illegible	024-4-07	EPAL
E 7	x	DE-RP	illegible	illegible	EPAL
E 8		illegible			EPAL
E 9	x	BY	009	010-2-02	EPAL
E 10	x	H	illegible	604-2-05	MAV
Pack 1.2					
E 11	x	SK	2291	2156 A27-7-11	UIC
E 12	x	SK	2291	2156 A27-7-08	UIC
E 13	x	PI	18153	152-7-01	EPAL
E 14	x	LV	012	008-4-03	EPAL
E 15	x	SK-3231	2291	2156 A27-8-01	UIC
E 16	x	DE-HE	illegible	illegible	EPAL
E 17	x	RO-CV	illegible	illegible	EPAL
E 18	x	BE	99012	012-3-09	EPAL
E 19	x	GB	FC-035	05-3	EPAL
E 20	x	BA	illegible	003-6-05	EPAL

Pack 1.3					
E 21	x	DE-HE	493027	003-0-12	EPAL
E 22	x	DE-SN1	497027	021-3-07	EPAL
E 23	x	SK	2291	2156 A27-5-09	UIC
E 24	x	illegible	illegible	004-5-11	EPAL
E 25	x	SK	2291	2156 A27-7-08	UIC
E 26	x	SK	2291	2156 A27-7-95	UIC
E 27	x	PL	02-387	181-8-01	EPAL
E 28	x	DE-SN1	497027	021-5-05	EPAL
E 29	x	RO CV	illegible	004-7-07	EPAL
E 30	x	DE-SN1	497027	021-6-12	EPAL

4.3 Plastic pallets for examination

A delivery of five plastic pallets was received on 28/11/2019. According to the client, these had been previously used by meat-processing companies. The contractor had no further information regarding their previous history. The pallets bore no labels or stamps and were numbered by the contractor K1 to K5.

4.4 Examination methods

Quantitative and qualitative determination of the microbial surface contamination with the contact method

The wood pallets were assessed by examining samples taken from three randomly chosen areas as follows:

- on the top side of a deck board
- on the bottom side of a deck board
- on a pallet block

The samples were taken by pressing special contact Petri dishes with a solid culture medium onto the material in order to collect micro-organisms from the surface of the material. The following culture mediums were used: DG18 agar for xerophilic moulds and malt extract agar (MEA) as a universal medium for moulds and bacteria. The Petri dishes were then incubated for one week in an incubator at 25 °C. Subsequently, the micro-organism colonies were counted and the species were determined based on micromorphological and macromorphological characteristics.

For the examination of the plastic pallets, swab samples were taken at five points from an area of 20 cm^2 each. These samples were taken from both visibly contaminated and from inconspicuous areas. The swab samples were eluded in saline solution (0.9 %) containing Tween 80, and then transferred with a spiral plater to various culture media for incubation. Here, malt extract agar, DG18 agar and plate count agar were used. The incubated colonies were counted and the species were determined based on morphological and biochemical characteristics.

Quantitative and qualitative determination of the microbial surface contamination of wood pallets with the suspension method, using wood cross-sections

For this examination, two bore cores were taken from each pallet. The bore cores had a diameter of 10 mm and were taken from horizontal deck boards in areas where there was no visible dirt or mould. Sampling was performed according to DIN ISO 16000-21:21014-05[4]. The bore cores were transferred instantly into a 10 ml phosphate buffer solution. To bring all germs into solution, the samples were processed on an orbital shaker for 15 minutes. The eluate dilution stages were then transferred with a spiral plater to the culture medium for incubation and subsequent germ count.

4.5 Findings

4.5.1 Wood pallets

The pallets vary greatly in appearance, as is shown in tables 2 to 4.

Most pallets show some greyed areas and blue-staining of the wood, which are however not deemed quality defects. 18 pallets were contaminated to some degree, often by footprints or black traces from forklift arms. The pallets of pack 1.1 were contaminated more than the others, showing visible mould attack in the form of coloured films at various points of the pallets. In total, 14 pallets showed mould attack to a degree that rendered them no longer suitable for use according to the EPAL quality criteria.

A large number of different fungi could be identified in the contact samples on the culture media. The predominant mould species belonged to the genus of *Penicillium, Cladosporium, Aspergillus* and *Trichoderma.* The most common blue-stain species was *Aureobasidium pullulans*. All species of fungi identified in this study are also commonly found on wood used in other areas, in particular building construction. In addition, some of the samples also contained bacteria and yeasts.

The microscopic examination of the bore cores revealed significant differences with regard to colonisation (see table 5). In the majority of the samples (20 pallets), the micro-organism concentration per volume was below the detection limit, reflecting a normal microbial load. The concentrations determined from pallets of pack 1.1. were considerably higher than those from other pallets. Here, five pallets topped the table with > 100,000 CFU/cm^3.

For the interpretation of these findings, one must take into account that the pallets were delivered in packs where they were in close contact with each other, facilitating the transfer of fungal spores. The fungi cultivated from contact samples are therefore not necessarily the species predominant on the respective pallets. On the other hand, the findings show that there were viable mould structures on all materials, ready to germinate and spread quickly, if the conditions were right.

[4] DIN ISO 16000-21:2014-05: Indoor air - Part 21: Detection and enumeration of moulds - Sampling of materials

Table 2: Description of pallets E1 to E10 (pack 1.1)

Top boards	Bottom boards	Blocks
E1 • slightly greyed • very dirty • green deposits 	• heavily greyed • green and white deposits 	• Presspan • partly damaged • grey deposits 
• visible mould attack • Germination: *Cladosporium* spp., *Penicillium* spp., *Aspergillus glaucus* group, *A. niger complex, A. westerdijkiae, Alternaria* sp., zygomycetes		
E2 • locally heavily greyed/blue-stained • very dirty • partly damaged • green deposits 	• heavily greyed • grey-green deposits 	• solid wood and Presspan • green and white deposits 
• visible mould attack • Germination: *Cladosporium* spp., *Penicillium* spp., *Aspergillus glaucus* group, *Trichoderma* sp., bacteria		
E3 • partially blue-stained • little dirt 	• no major defects 	• solid wood • no major defects 
• visible mould attack • Germination: *Cladosporium* spp., *Penicillium* spp., bacteria *[Bacillus* sp.)		
E4 • little blue stain • little dirt • spotty, black deposits 	• green deposits 	• Presspan • no major defects 
• visible mould attack • Germination: *Cladosporium* spp., *Penicillium* spp., *Aspergillus flavus, Aureobasidium pullulans, Trichoderma* sp., yeast *(Rhodotorula mucilaginosa)*		

Top boards	Bottom boards	Blocks
E5 • partially greyed • moderately dirty • black deposits	• heavily greyed/blue-stained • dark deposits	• Presspan • grey deposits
		
• visible mould attack • Germination: *Cladosporium* spp., *Penicillium* spp., *Aspergillus ochraceus, Trichoderma* sp., *Alternaria* sp.		
E6 • partially greyed • moderately dirty • dark discolouration in spots	• large areas of dark discolouration • white and dark deposits	• Presspan • grey deposits
		
• visible mould attack • Germination: *Cladosporium* spp., *Penicillium* spp., *Aureobasidium pullulans, Aspergillus niger*complex, *Aspergillus glaucus* group, *Trichoderma* sp., yeast *(Rhodotorula mucilaginosa),* bacteria		
E7 • little dirt • grey deposits	• partly greyed/ blue-stained	• Presspan • moderately dirty
		
• visible mould attack • Germination: *Cladosporium* spp., *Penicillium* spp., *Aureobasidium pullulans, Aspergillus niger* complex, *Aspergillus glaucus* group, *Trichoderma* sp., *Alternaria* sp.		
E8 • large greyed areas • little dirt • few black deposits	• slightly blue-stained	• solid wood + 1x Presspan • dark
		
• mould attack not clearly identifiable by the naked eye • Germination: *Cladosporium* spp., *Penicillium* spp., *Aspergillus niger* complex, *Aspergillus glaucus* group, *Trichoderma* sp., *Alternaria* sp., *Mucor* sp., *Fusarium* sp., bacteria		

	Top boards	Bottom boards	Blocks
E9	• partially greyed • moderately dirty	• large areas of dark discolouration • very dirty	• solid wood + Presspan • no major defects
			
	• visible mould attack • Germination: *Cladosporium* spp., *Penicillium* spp., *Aureobasidium pullulans, Aspergillus glaucus* group, *Trichoderma* sp., various bacteria		
E10	• slightly greyed • moderately dirty • grey-black deposits	• blue-stained in certain areas	• solid wood • grey-green
			
	• visible mould attack • Germination: *Penicillium* spp., *Cladosporium* spp., *Aspergillus glaucus* group, zygomycetes, *Trichoderma* sp.,		

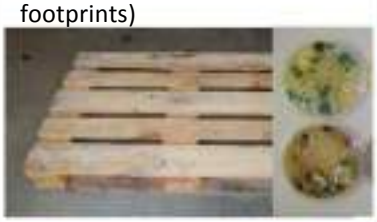
Table 3: Description of pallets E11 to E20 (pack 1.2)

	Top boards	Bottom boards	Blocks
E11	• moderately dirty • grey deposits	• few grey-green deposits	• solid wood • sapwood blue-stained
			
	• visible mould attack • Germination: *Cladosporium* spp., *Penicillium* spp., *Aspergillus glaucus* group, *Aureobasidium pullulans, Trichoderma* sp., zygomycetes, bacteria		
E12	• partially blue-stained • clean	• partially blue-stained • black deposits	• solid wood • sapwood blue-stained
			
	• visible mould attack • Germination: *Cladosporium* spp., *Penicillium* spp., *Aspergillus glaucus* group, *Aspergillus niger* complex, *Alternaria sp., zygomycetes*		

	Top boards	Bottom boards	Blocks
E13	• slightly greyed • clean • grey-black deposits 	• slightly greyed 	• solid wood • no major defects 
	• no visible mould attack • Germination: *Cladosporium* spp., *Penicillium* spp., *Aspergillus niger* complex, bacteria		
E14	• slightly greyed, partially blue-stained • moderately dirty • grey deposits 	• clean 	• Presspan • no major defects 
	• mould attack not clearly identifiable by the naked eye • Germination: *Cladosporium* spp., *Penicillium* spp., *Aspergillus glaucus* group, *Mucor* sp., bacteria *(Bacillus* sp.) sp.]		
E15	• little dirt 	• locally heavily greyed/blue-stained • grey deposits 	• solid wood • sapwood blue-stained 
	• mould attack not clearly identifiable by the naked eye • Germination: *Cladosporium* spp., *Penicillium* spp., *Aspergillus glaucus* group, *Aspergillus niger* complex, zygomycetes		
E16	large greyed/blue-stained areas moderately dirty • grey-green deposits 	• clean 	• Presspan • grey-green deposit 
	• no visible mould attack • Germination: *Cladosporium* spp., *Penicillium* spp., *Aspergillus glaucus* group, zygomycetes, bacteria *(Bacillus* sp.)		

Top boards	Bottom boards	Blocks
E17 • no greying/blue stain • little dirt • 	• clean (few forklift arm traces) 	• Presspan • no major defects 
• no visible mould attack • Germination: *Penicillium* spp., *Aspergillus glaucus* group, *Aspergillus niger* complex, *Aspergillus flavus, Rhizopus stolonifer*		
E18 • completely greyed/blue-stained • little dirt (forklift arm traces) 	• grey deposits in areas 	• Presspan • no major defects 
• mould attack not clearly identifiable by the naked eye • Germination: *Penicillium* spp., *Aspergillus glaucus* group, *Aspergillus niger* complex, *Aspergillus flavus,* zygomycetes*, Trichoderma* sp., bacteria *[Bacillus* sp.)		
E19 ▪ slightly greyed/blue-stained ▪ little dirt ▪ reddish wood (larch/Douglas fir) 	▪ clean (fork forklift arm traces 	• Solid wood • grey deposits in spots 
• mould attack not clearly identifiable by the naked eye • Germination: *Cladosporium* spp., *Penicillium* spp., *Aspergillus glaucus* group, *Aureobasidium pullulans, Rhizopus stolonifer*		
E20 • little dirt (footprints) 	• clean (few forklift arm traces) • grey deposits in spots 	• solid wood • mouldy deposits 
• visible mould attack (on blocks) • Germination: *Penicillium* spp., *Aspergillus glaucus* group, *Aspergillus niger* complex, *Aspergillus ochraceus, Rhizopus stolonifer,* yeasts		

Table 4: Description of pallets E21 to E30 (pack 1.3)

Top Boards	Bottom boards	Blocks
E21 • little dirt (few footprints) • white spots along edges 	• few forklift arm traces 	• Presspan • no major defects 
• no visible mould attack • Germination: *Penicillium* spp., *Aspergillus glaucus* group, *Aspergillus niger* complex, *Aureobasidium pullulans, Rhizopus* sp., *Achaetomium* sp., yeasts, bacteria *(Bacillus* sp.)		
E22 ▪ slightly greyed/blue-stained ▪ little dirt (few forklift arm traces) 	• locally greyed/blue-stained 	• solid wood • partially blue-stained • discoloured core 
• no visible mould attack • Germination: *Penicillium* spp., *Cladosporium* spp., Aspergillus *glaucus* group, *Rhizopus stolonifer, Trichoderma* sp., yeasts		
• E23 • v. slightly greyed/blue-stained • little dirt 	• locally greyed/blue-stained • clean 	• solid wood • no major defects 
• no visible mould attack • Germination: *Aspergillus glaucus* group, *Penicillium* spp., *Cladosporium* spp., *Aspergillus niger* complex, *Rhizopus sp.,* bacteria *(Bacillus* sp.)		
E24 • little dirt (few footprints) 	• black and green deposits in spots 	• solid wood • no major defects 
• visible mould attack (at bottom) • Germination: *Penicillium* spp., *Aspergillus glaucus* group, *Aspergillus niger* complex, *Aureobasidium pullulans, Rhizopus* sp., *Trichoderma* sp., yeasts, bacteria *(Bacillus* sp.)		

Top boards	Bottom boards	Blocks
E25		
▪ slightly greyed/locally blue-stained ▪ little dirt (footprints)	▪ locally greyed/blue-stained ▪ little dirt (forklift arm traces) ▪ grey deposits in spots	▪ solid wood ▪ minor grey deposits
		
▪ mould attack not clearly identifiable by the naked eye ▪ Germination: Penicillium spp., Cladosporium spp., Aspergillus glaucus group, Aureobasidium pullulans, Rhizopus stolonifer, bacteria (Bacillus)		
E26		
▪ partly blue-stained ▪ little dirt (footprints)	▪ partly blue-stained ▪ clean ▪ grey deposits in spots	▪ solid wood ▪ no major defects
		
▪ mould attack not clearly identifiable by the naked eye ▪ Germination: Aspergillus glaucus group, Penicillium spp., Cladosporium spp., Aspergillus niger complex, Rhizopus sp., yeasts (Rhodotorula sp.), bacteria (Bacillus)		
E27		
▪ partially blue-stained ▪ little dirt (footprints)	▪ partially blue-stained	▪ solid wood and Presspan ▪ no major defects
		
▪ visible mould attack (at bottom) ▪ Germination: Penicillium spp., Cladosporium spp., Aspergillus glaucus group, Rizopus sp.		
E28		
▪ clean	▪ clean	▪ Presspan ▪ no major defects
		
▪ no visible mould attack ▪ Germination: Penicillium spp., Cladosporium spp., Aspergillus versicolor, Aspergillus ochraceus, Aureobasidium pullulans, Rhizopus stolonifer, bacteria (Bacillus)		

Top boards	Bottom boards	Blocks
E29 ▪ clean 	• clean 	• solid wood • end grain: dark discolouration 
• no visible mould attack • Germination: *Penicillium* spp., *Aspergillus glaucus* group, *Aspergillus versicolor, Aspergillus niger* complex, *Rhizopus sp.*		
E30 • little blue stain • clean 	• no blue stain 	• Presspan • grey deposits 
• no visible mould attack • Germination: *Penicillium* spp., *Cladosporium* spp., *Aspergillus flavus, Aspergillus versicolor, Aspergillus ochraceus, Aureobasidium pullulans, Rhizopus stolonifer,* bacteria *(Bacillus)*		

Table 5: Comparison of microbial load of wood pallets determined with contact sampling and suspension method (see appendix for representative photographs)

Pallet no.	Live germ count on surface, determined with contact samples [CPU/cm^2]			Live germ count in cross-section, determined in drill cores with suspension method [CPU/cm^3] (lower detection limit = 400)
	Top side	Bottom side	Block	
Pack 1.1				
E 1	> 20	> 20	>20	1×10^4
E 2	> 20	> 20	>20	9×10^3
E 3	> 10	< 10	< 10	2×10^2
E 4	> 20	> 20	< 10	1×10^3
E 5	> 20	> 20	> 30	4×10^4
E 6	> 30	> 30	> 30	4×10^5
E 7	> 30	< 10	< 10	2×10^4
E 8	> 30	>30	>30	1×10^4
E 9	> 30	>30	>30	4×10^5
E 10	> 30	>30	>30	5×10^5
Pack 1.2				
E 11	> 30	< 10	< 10	6×10^2
E 12	> 30	< 10	< 10	2×10^2
E 13	> 30	< 10	< 10	2×10^2
E 14	< 10	< 10	< 10	4×10^2
E 15	> 10	< 10	< 10	2×10^2
E 16	> 10	< 10	< 10	2×10^2
E 17	> 10	> 10	> 10	2×10^2
E 18	> 30	< 10	< 10	2×10^2
E 19	> 30	< 10	< 10	2×10^2
E 20	> 10	> 10	> 10	2×10^2
Pack 1.3				
E 21	> 30	< 10	< 10	2×10^2
E 22	> 30	< 10	> 10	2×10^2
E 23	> 10	< 10	< 10	2×10^2
E 24	2	< 10	< 10	2×10^2
E 25	> 10	< 10	< 10	2×10^2
E 26	> 30	< 10	< 10	2×10^2
E 27	> 10	< 10	< 10	2×10^2
E 28	> 10	< 10	< 10	2×10^2
E 29	> 30	> 10	> 10	2×10^2
E 30	> 10	< 10	< 10	2×10^2

4.5.2 Plastic pallets

All plastic pallets showed signs of wear in the form of scratches on their surfaces. Some of them were clearly contaminated, for instance by dark stains, or with blood and meat residue. The germ counts of the plastic surfaces determined by swab sampling are compiled in table 6. Pictures of the incubated samples on culture media are included in the section below.

Table 6: Microbial load of plastic surfaces

Pallet no.	Sampling point no.	Live germ count [CPU/cm^2]		
		PCA	MEA	DG 10
K1	1.1	2×10^4	1×10^4	2×10^3
	1.2	4×10^4	2×10^3	n/a[5]
	1.3	4×10^3	3×10^3	2×10^1
	1.4	3×10^3	2×10^3	3×10^3
	1.5	5×10^1	2×10^2	4×10^1
K2	2.1	2×10^1	2×10^1	3×10^1
	2.2	8×10^3	8×10^3	1×10^1
	2.3	6×10^4	7×10^2	1×10^2
	2.4	< 10	< 10	< 10
	2.5	< 10	< 10	< 10
K3	3.1	1×10^5	2×10^4	2×10^3
	3.2	2×10^4	4×10^3	2×10^3
	3.3	1×10^3	3×10^2	2×10^3
	3.4	4×10^2	3×10^3	2×10^3
	3.5	< 10	1×10^2	3×10^1
K4	4.1	5×10^4	n/a	1×10^4
	4.2	2×10^4	n/a	1×10^4
	4.3	8×10^4	2×10^4	2×10^4
	4.4	8×10^1	3×10^2	2×10^2
	4.5	5×10^1	1×10^2	1×10^2
K5	5.1	4×10^4	n/a	2×10^4
	5.2	< 10	2×10^1	2×10^1
	5.3	2×10^1	2×10^1	2×10^1
	5.4	< 10	2×10^1	2×10^1
	5.5	4×10^1	1×10^1	< 10

Pallet K1

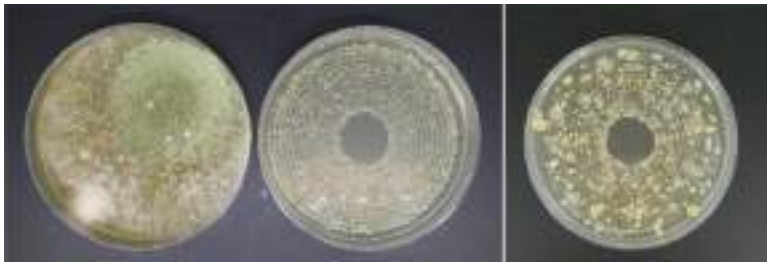
This pallet was quite heavily contaminated by dried meat residue and blood stains. There was no clearly identifiable microbial attack, and the microbial load became only apparent after incubation. The predominant micro-organisms were yeasts (including *Rhodotorula mucilaginosa)* and bacteria. In samples containing dried meat residue, primarily coliform bacteria, other enterobacteria including *Escherichia coli,* as well as gram-positive cocci were identified. Samples taken from areas free of visible residue showed low germ counts (K1.5).

[5] n/a: not assessable

Area K1.1: dried meat residue

MEA DG18 PCA

Area K1.2: brownish contamination of surface (with 20 cm^2 stencil)

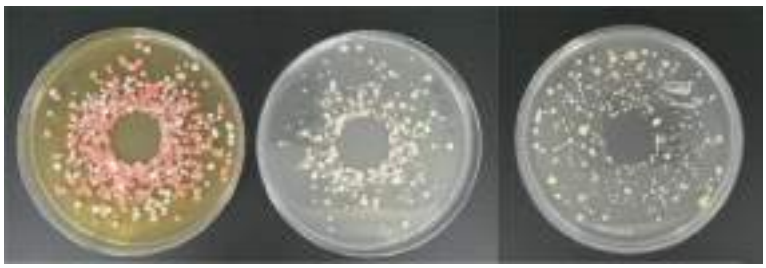


MEA DG18 PCA

Area K1.3: dried meat residue

MEA DG18 PCA

Area K1.4: dried meat residue



MEA DG18 PCA

Area K1.5: no obvious defects, little dirt

MEA DG18 PCA

Pallet K2

This pallet was similar in appearance to pallet K1, showing relatively heavy contamination in the form of dried meat residue and blood stains. The microbial load was however significantly lower than on pallet K1. The predominant germs where yeasts, gram-negative bacteria including enterobacteria, and few moulds. The pictures below show the colonies on culture media produced from the five random samples.

Area K2.1: dried meat juice on top side

MEA DG18 PCA

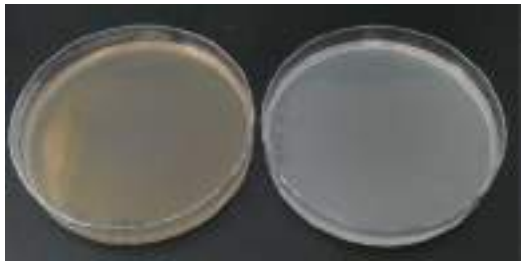
Area K2.2: unobtrusive area on top side

MEA DG18 PCA

Area K2.3: dried meat juice on top side

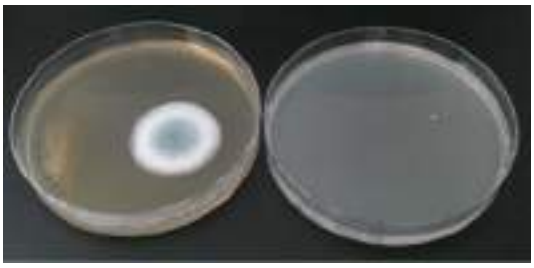
MEA DG18 PCA

Area K2.4: dried meat juice in recess



MEA DG18

Area K2.5: dried meat juice in bottom area



MEA DG18

Pallet K3

This pallet showed signs of normal wear and generally low contamination, apart from some wet, transparent stains. Despite these minor stains, there was considerable microbial contamination, dominated by yeasts and gram-negative as well as gram-positive bacteria. The yeast/bacteria concentration was particularly high in the sampled wet area (K3.1). Samples from unobtrusive areas also contained moulds (Aureobasidium pullulans, Cladosporium sp., Alternaria sp.).

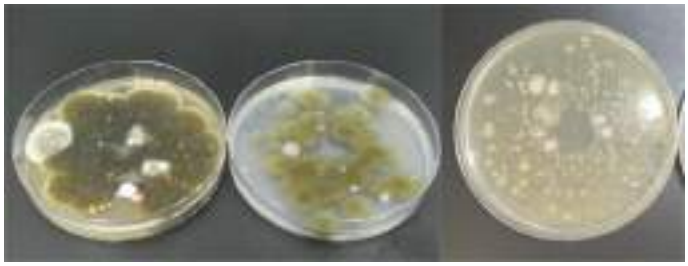
Area K3.1: wet area on top side

MEA DG18 PCA

Area K3.2: dark stain on top side

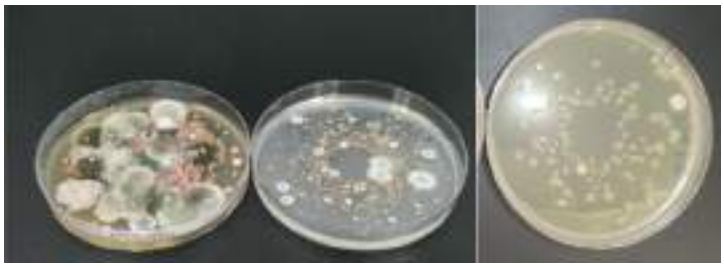
MEA DG18 PCA

Area K3.3: unobtrusive area in recess



MEA DG18 PCA

Area K3.4: slightly dirty area on bottom side



MEA DG18 PCA

Area K3.5: slightly dirty area in recess

MEA DG18 PCA

Pallet K4

This pallet showed signs of normal wear, was generally dirty and partly wet. Again, there was considerable microbial contamination by yeasts and bacteria, with few moulds *(Paecilomyces variotii)*. The yeast/bacteria concentration was particularly high in the sampled wet areas (K4.1 and K4.3).

Area K4.1: wet, dirty area on top side

MEA DG18 PCA

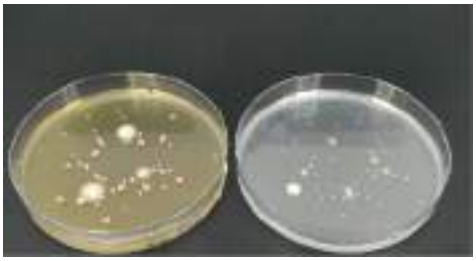
Area K4.2: dark stain on top side

MEA DG18 PCA

Area K4.3: wet, dirty area on top side

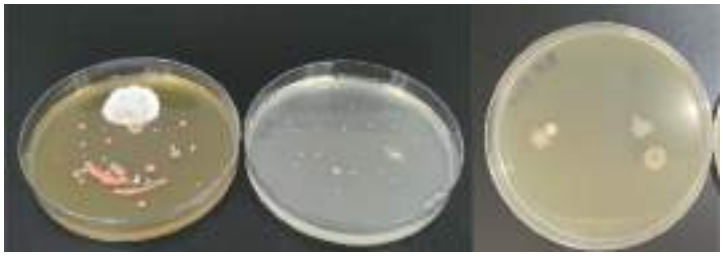
MEA DG18 PCA

Area K4.4: dirty area in recess



MEA DG18

Area K4.5: dirty area in recess



MEA DG18 PCA

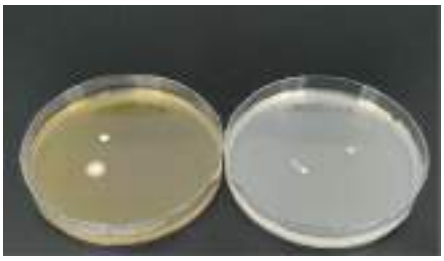
Pallet K5

This pallet was heavily contaminated but showed no stains from meat or blood. Its bottom was covered in large black deposits. However, contamination by bacteria, yeasts and a few moulds was only found on the top side.

Area K5.1: grey, dirty area on top side

MEA DG18 PCA

Area K5.2: grey, dirty area on top side

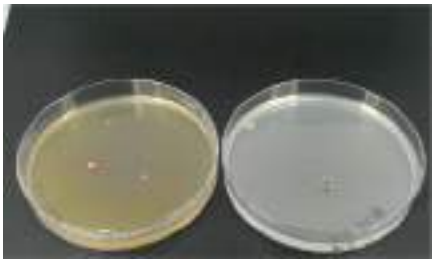


MEA DG18

Area K5.3: grey dirty area in recess

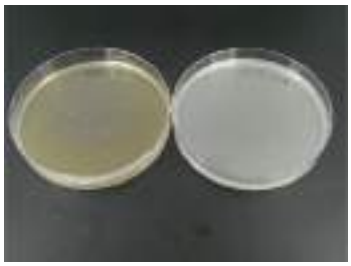
MEA DG18 PCA

Area K5.4: black deposits on bottom side



MEA DG18

Area K5.5: black deposits on bottom side



MEA DG18

5 WP 3: Laboratory investigation to determine the anti-bacterial properties of certain pallet materials

5.1 Objective

In this work package, a number of pallet materials (wood and plastic) were examined to determine the likelihood of microbial colonisation, and the survivability of bacteria.

5.2 Sample material

The client provided a range of materials for examination. The following pallets were delivered on 29/11/2018:

- Hygiene pallets H1 made from HDPE
- Euro pallets made from spruce

An initial investigation based on random samples revealed that not all wood was spruce, and that the pallets also contained pine wood. The Euro pallets were therefore not included in the subsequent examination.

To replace these pallets, the client contracted Treyer Paletten GmbH in Peterstal to send us boards made from spruce and pine, which were delivered on 31/05/2019. The pine wood had been separated by the client into sapwood and heartwood.

The client also provided the polyethylene foil serving as the reference material. All examined materials are listed in table 7.

Table 7: Examined materials (fig. 1)

No.	Material
1	H1 plastic hygiene pallet
1.1	smooth surface
1.2	rough surface (roughened with sandpaper to simulate normal wear)
2	Spruce
3	Pine sapwood
4	Pine heartwood
5	Polyethylene foil (reference material)

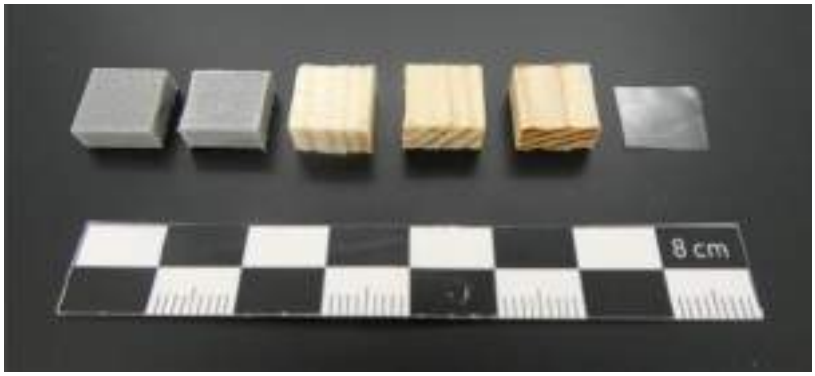


Fig. 1: Examined materials (from left: no. 1.1 to 5)

5.3 Method of examination

As there is no standard method for the determination of the antimicrobial properties of wood, the following two methods were used in a modified form:

- ISO 22196[6]: The test organisms proposed by this standard were used, and the bacteria suspensions were produced as prescribed by the standard.
- DIN EN ISO 20743[7]: The survivability was determined using the luminescence method described in the standard.

Test principle

The test samples were inoculated with a known bacterial suspension, and the cultures were incubated for a period between 18 to 24 hours at 36 °C in a humid chamber. Subsequently, the survival rate was determined with the luminescence method. In order to determine the effect of cleaning, some of the cultures were cleaned, using various methods, after incubation and prior to determining the survival rate.

Test versions

Test version 1:	Determination of colonisability by bacteria under optimised conditions for bacterial growth: inoculation of moist material samples
Test version 2:	Determination of colonisability by bacteria under suboptimal conditions for bacterial growth: inoculation of dry material samples
Test version 3:	Determination of colonisability by bacteria under modified conditions: inoculation of moist material samples and addition of nutrient solution
Test version 4:	Determination of effect of cleaning on the survival rate of bacteria

Test bacteria

- *Escherichia coli* DSM 1576
- *Staphylococcus aureus* DSM 799

Test samples

The tests were performed on rectangular blocks (volume: 0.5 cm^3) with a test surface of 10 mm x 10 mm and a thickness of 5 mm (see fig. 1).

Preparation of test samples

The wood samples were sterilised with steam. The plastic samples were sterilised by gamma irradiation.

Subsequently, the samples were prepared in line with the respective test version:

Test version 1:	Application of 1 ml of demineralised water, followed by a dwell time of several hours until all water had been adsorbed
Test version 2:	No preliminary treatment
Test versions 3 and 4:	Application of 1 ml of malt solution (5 %) followed by a dwell time of several hours until the entire nutrient solution had been absorbed

[6] ISO 22196:2011: Measurement of antibacterial activity on plastics and other non-porous surfaces
[7] DIN EN ISO 20743:2013: Textiles - Determination of antibacterial activity of textile products

Inoculation

The bacteria suspension with a concentration of 5 x 10^5 bacteria/ml was applied by means of a pipette in batches of 500 µl to the centre of the test samples.

Incubation

The inoculated samples were incubated for 24 hours at 36 °C and a relative air humidity > 95 ± 4 %.

Cleaning of samples after incubation (test version 4 only)

The samples were cleaned, using two different methods:

A) Cleaning with water: shaking for 1 minute in 10 ml of water in a vortexer
B) Cleaning with water and washing-up liquid: shaking for 1 minute in 10 ml of water containing washing-up liquid, followed by shaking for 1 minute in 10 ml of water in a vortexer

Removal of bacteria by rinsing after incubation

The samples were placed on glass beads in cell culture plates, with the inoculated side down. After addition of 6 ml of phosphate-buffered saline solution, the plates were processed on an orbital shaker at 250 rpm for 15 minutes (fig. 2 and 3.). Subsequently, the survivability of the bacteria in the eluate was determined with the luminescence method.

Fig. 2: Orbital shaker with test cultures

Fig. 3: Cell culture plates with samples on glass beads for shaking

Method to determine the survivability of bacteria (luminescence method)

The luminescence method makes use of the fact that all living cells have the capacity to produce adenosine triphosphate (ATP) in order to store and transfer energy within the cell. The presence of ATP can be detected with the luciferin-luciferase reaction, where ATP provides the energy to convert the luciferin into light, using the luciferase enzyme. The amount of light emitted in the process is proportional to the available ATP, so that the luminescence can be used to determine the survivability of micro-organisms and thus the live germ count. The method is therefore also known as ATP luminescence test.

For our test, we used the "bactiter glo" test system from Promega. For testing, 50 µl of eluate was mixed with 50 µl of the detection reagent in 96-well micro-titration plates. After an incubation period of 5 minutes, the luminescence was measured.

5.4 Findings

The measured luminescence signal is an indicator of bacterial activity. The examination of the bacteria suspensions used for inoculation showed a linear relationship between the luminescence signal (RLU) and the number of live cells (fig. 4), so that the RLU could be used to calculate the actual bacterial count.

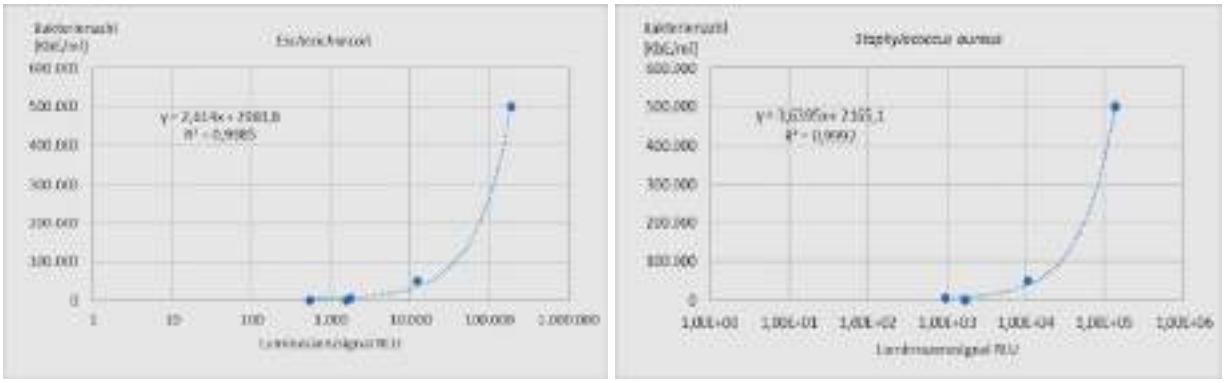


Fig. 4: Calibration curve: Relationship between bacterial count and luminescence signal

The results of the luminescence measurements are compiled in table 8 and the charts in fig. 5.

After incubation over 24 hours, all wood samples showed similar results. The activity of *Escherichia coli* was significantly reduced in all woods, irrespective of the actual test version, while it increased on nearly all plastic pallets. The only exceptions here were the results of test version 3 (addition of nutrient solution), which showed a reduction in activity.

The results for *Staphylococcus aureus* were similar, with the exception of the pine heartwood samples where no significant change in activity could be measured after incubation. In contrast, the luminescence signals measured with the plastic pallet samples were significantly higher after incubation than before. On the rough plastic surface, *Staphylococcus aureus* grew much faster than on the smooth surface.

The initial moistening of the test version 1 samples had only an insignificant effect on the results. The addition of a nutrient solution in test version 3 generally reduced the activity, which might be due to the slightly sticky nature of the sugar solution that might held back the bacteria during rinsing.

In all samples, cleaning with water or with water and washing-up liquid resulted in a further reduction of the activity.

Table 8: Luminescence signals (RLU) of the eluates before and after incubation (mean of n=4 tests, minus blank value; in pine heartwood: minus correction value)

Material	Before incubation	RLU after incubation period of 24 h				
		Test version 1	Test version 2	Test version 3		
		without cleaning	without cleaning	without cleaning	cleaning with water	cleaning with washing-up liquid
Escherichia coli						
1.1 H1 pallet, smooth	1,220	3,044	2,357	963	<250[8]	< 1
1.2 H1 pallet, rough	1,312	3,211	2,104	942	<250	< 1
2 Spruce	1,000	< 250	< 250	< 250	< 250	< 250
3 Pine sapwood	1,179	< 250	< 1	< 1	n.d.[9]	n.d.
4 Pine heartwood	1,138	< 250	< 250	< 1	< 250	< 250
5 Polyethylene	1,021	< 250	1,951	1,520	< 250	< 1
Staphylococcus aureus						
1.1 H1 pallet, smooth	305	2,551	2,593	538	n.d.	n.d.
1.2 H1 pallet, rough	423	3,291	4,107	2,795	n.d.	n.d.
2 Spruce	471	< 250	< 250	< 250	n.d.	n.d.
3 Pine sapwood	606	< 250	< 250	< 250	n.d.	n.d.
4 Pine heartwood	252	311	408	311	n.d.	n.d.
5 Polyethylene	246	1,400	932	690	n.d.	n.d.

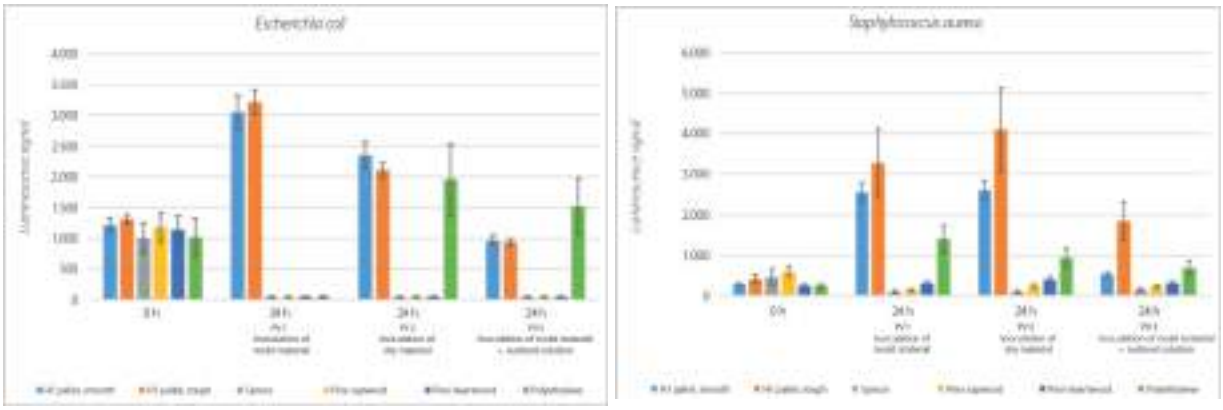


Fig. 5: Luminescence as a measure for activity before and after incubation for 24 hours

By comparing the cell counts after incubation with those of the reference sample, the antibacterial activity can be determined with formula [1]:

[1] $A = F - G$

A: antibacterial activity

F: Increase value on reference material ($F = \lg N_t - \lg N_0$)

G: Increase value on test samples ($G = \lg N_t - \lg N_0$)

N_t: live germ count after incubation N_0: live germ count before incubation

The antibacterial activity measured in the tests are compiled in tables 9 and 10. Positive values indicate antibacterial activity; the higher the value, the stronger the activity.

[8] below detection limit of 250 RLU

[9] n.d.: not determined

Negative values indicate that bacterial growth was greater than on the reference material.

Table 9: Antibacterial activity of pallet materials, test germ: *Escherichia coli*

Material	N_0 before incubation	N_t after incubation period of 24 h				
		Test version 1	Test version 2	Test version 3		
		without cleaning[10]	without cleaning	without cleaning	cleaning with water	cleaning with washing-up liquid
Reference PE	4.8	< 3.7	5.0	4.9	< 1.0	< 1.0
Increase value F			0.2	0.1		
1.1 H1 pallet, smooth	4.9	5.1	5.0	4.8	< 1.0	< 1.0
Increase value G			0.1	-0.1		
Antibact. activity A			**0.1**	**0.2**		
1.2 H1 pallet, rough	4.9	5.1	5.0	4.8	< 1.0	< 1.0
Increase value G			0.1	-0.1		
Antibact. activity A			0.1	0.2		
2 Spruce	4.8	< 3.7	< 3.7	< 3.7	< 1.0	< 1.0
Increase value G			- 1.1	- 1.1		
Antibact. activity A			**1.3**	**1.2**		
3 Pine sapwood	4.9	< 3.7	< 1.0	< 1.0	n.d.	n.d.
Increase value G			-3.9	-3.9		
Antibact. activity A			**4.1**	**4.0**		
4 Pine heartwood	4.9	< 3.7	< 3.7	< 1.0	< 1.0	< 1.0
Increase value G			-1.2	-3.9		
Antibact. activity A			**1.4**	**4.0**		

Table 10: Antibacterial activity of pallet materials, test germ: *Staphylococcus aureus*

Material	N_0 before incubation	N_t after incubation period of 24 h		
		Test version 1	Test version 2	Test version 3
		without cleaning	without cleaning	without cleaning
Reference PE	4.6	4.9	4.8	4.7
Increase value F		0.3	0.2	0.1
1.1 H1 pallet, smooth	4.6	5.1	5.1	4.7
Increase value G		0.5	0.5	0.1
Antibact. activity A		**-0.2**	**-0.3**	**0.0**
1.2 H1 pallet, rough	4.6	5.2	5.3	5.2
Increase value G		0.6	0.7	0.6
Antibact. activity A		**-0.3**	**-0.5**	**-0.5**
2 Spruce	4.7	< 3.7	< 3.7	< 3.7
Increase value G		-1.0	-1.0	-1.0
Antibact. activity A		**1.3**	**1.3**	**1.3**
3 Pine sapwood	4.7	< 3.7	< 3.7	4.5
Increase value G		-1.0	-1.0	-0.2
Antibact. activity A		**1.3**	**1.3**	**0.3**
4 Pine heartwood	4.6	4.6	4.6	4.6
Increase value G		0.0	0.0	0.0
Antibact. activity A		**0.3**	**0.2**	**0.1**

[10] As the bacterial count on the reference material for test version 1 decreased within 24 hours, the antibacterial activity of the tested materials could not be calculated for this test version.

Exploratory study regarding the adhesion of bacteria on the tested materials

Using additional test samples and *Escherichia coli*, a number of tests were performed to determine to which extend the chosen shaking method was able to detach the bacteria from the material. For this purpose, samples taken from pine sapwood and from plastic pallets were dried after inoculation or shaking respectively, and then examined in a scanning electron microscope (SEM). Bacteria were only clearly visible on the inoculated samples. This indicates that the rinsing process used in the above tests was effective, and that only small amounts of bacteria remained attached to the material, especially to the wood. Figures 6 to 9 show representative images of the findings.

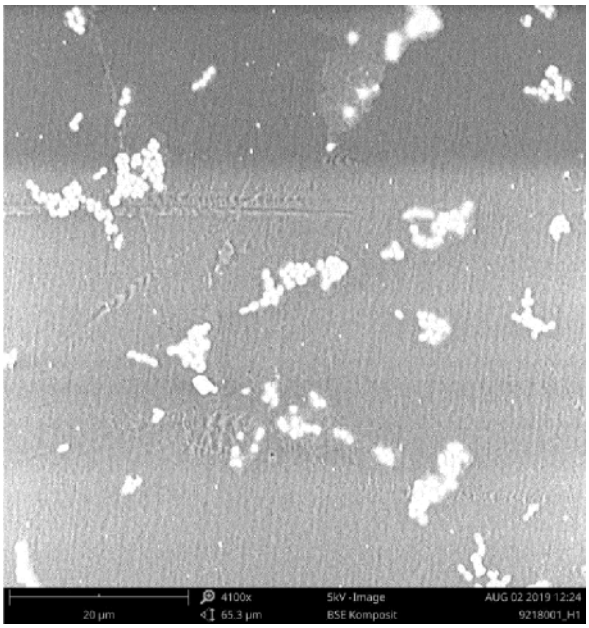


Fig. 6: H1 plastic pallet after inoculation and incubation for 2 hours:
bacteria clearly visible (SEM)

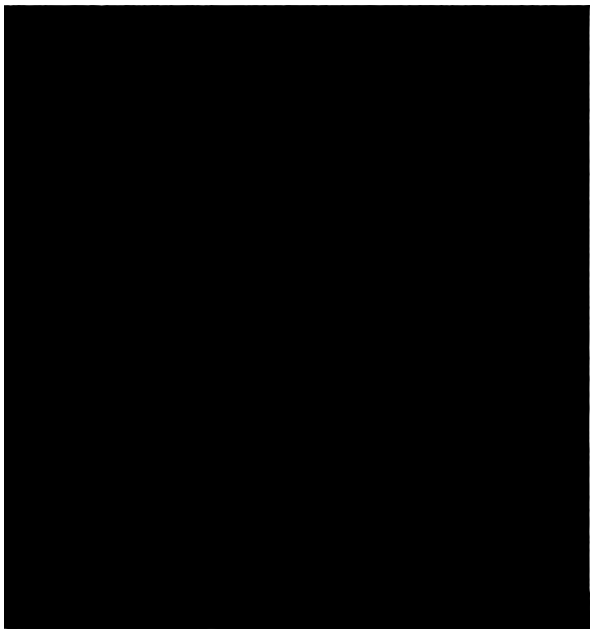


Fig. 7: H1 plastic pallet after inoculation, incubation for 2 hours and shaking: no bacteria visible (SEM)

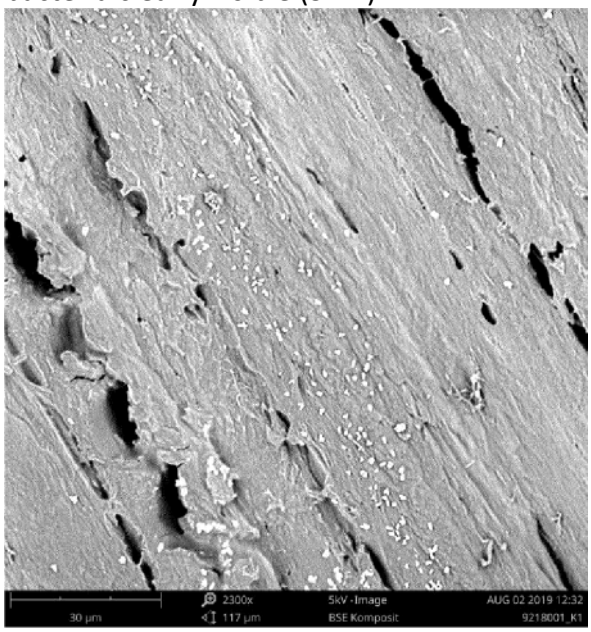


Fig. 8: Pine sapwood after inoculation and incubation for 2 hours:
bacteria clearly visible (SEM)

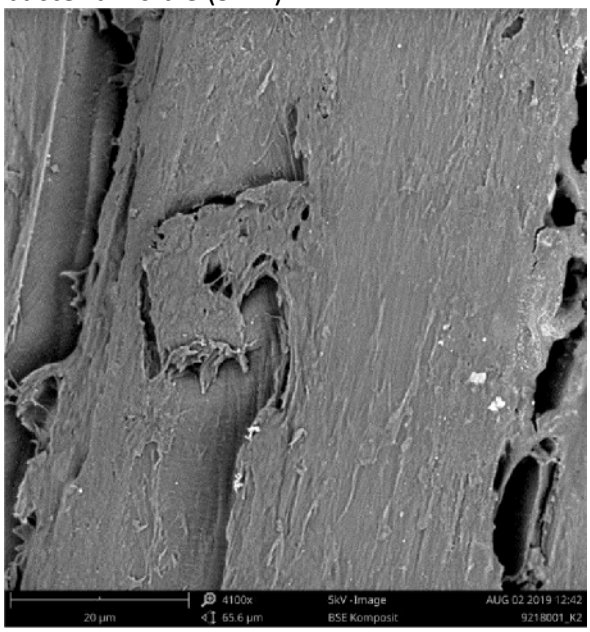


Fig. 9: Pine sapwood after inoculation, incubation for 2 hours and shaking: no bacteria visible (SEM)

6 Summary and discussion

Based on the literature survey and the results of our tests, it is safe to conclude that pallets made from wood are no less hygienic than those made from plastics, and that wood pallets even have a number of favourable hygiene-relevant properties.

Survivability of bacteria on material surfaces

In the various tests, less bacteria survived on wood than on plastic. A distinct antibacterial activity of pine heartwood, as suggested in the surveyed literature, could not be confirmed, as the activity in some samples was lower than that of spruce and pine sapwood. It is however possible that the tested samples were not actually of heartwood.

Wood has some disadvantages with regard to cleaning. As it is a porous material, it cannot be cleaned as easily as the smooth plastic surfaces. It must be assumed that contaminants (and in particular organic substances) become firmly attached to the wood surface, providing a potential feeding ground for bacteria and moulds. The tests showed however that simple cleaning with water is highly effective and significantly reduces the bacterial count on both plastic and wood surfaces.

The antimicrobial properties of plastic pallets are greatly affected by scratches and rough areas on the surface, which are probably unavoidable when pallets are re-used multiple times. The bacterial counts on rough plastic surfaces were higher than those on smooth surfaces.

Microbial growth on used material

The wood and plastic pallets examined in work package 2 showed significant signs of wear, as well as high microbial loads in certain areas. On the wood pallets, moulds were the predominant micro-organisms, with few bacteria and yeasts. On the plastic pallets, significantly more yeasts and bacteria prone to spoil food were found. The live germ count on the surface of the wood pallets was between < 10 CFU/cm^2 and > 30 CPU/cm^2. In cross-sections, the live germ count was between 2 x 10^2 CFU/cm^3 and 5 x 10^5 CFU/cm^3. On plastic pallets, the live germ count on the surface was between < 10 CPU/cm^2 and 10^5 CFU/cm^2.

A comparison of the absolute values of the two groups of materials is not possible, due to the differences in the methods used for the examination of the two types of pallets, and the fact that the history of the individual pallets was not known. The findings show however that microbial attack is a common and serious issue in the practical use of pallets. On the wood pallets, microbial attack, in particular by moulds, was often clearly visible, so that affected pallets tend to be separated and disposed of. In contrast, the microbial growths on the plastic pallets we examined were not identifiable with the naked eye, although our tests showed that there were significant germ concentrations.

An issue of concern is the fact that some of the pallets sent to us for examination were moist or even wet, thus providing ideal conditions for the spreading of micro-organisms.

Survivability of bacteria on wood and plastic surfaces

Laboratory tests show that, in general, the survivability of bacteria on wood is lower than on plastic. It is therefore safe to conclude that wood pallets are suitable for use in food processing and transport where hygiene is of great importance. However, such use requires strict adherence to the hygiene regulations and standards that apply to the production, transport and storage of foodstuff, including continuous control of the pallet quality and regular cleaning, which are requirements that apply of course also to plastic pallets. To prevent microbial attack, the material must be protected against humidity and dirt, and regularly cleaned.

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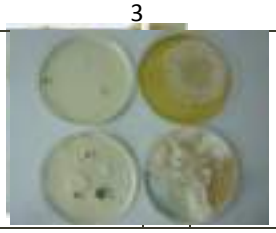
Author of report

Appendix

Comparison of contact samples and microbial load of used wood pallets determined with suspension method

Pallet no.	Live germ count on surface, determined in contact samples			Live germ count in cross-section, determined in drill cores and with suspension method [CPU/g]
	Top side	Bottom side	Block	
E 1	4	4	4	4
				
E 2	4	4	4	4
				
E 3	2	1	2	1
				
E 4	3	3	2	3
				
E 5	4	3	4	4
				
E 6	4	4	4	5
				
E 7	4	2	2	

CPU/cm^2	Category
< 1	1
> 1 ... ≤ 10	2
> 10 ... < 20	3
> 20	4

CPU/cm^3	Category
≤ 400 (detection limit)	1
> 400 ... ≤ 1000	2
> 1000... ≤ 5000	3
> 5000	4

Pallet no.	Live germ count on surface, determined in contact samples			Live germ count in cross-section, determined in drill cores and with suspension method [CPU/g]	
	Top side	Bottom side	Block	CPU/cm³	Category
				≤ 400 (detection limit)	1
				> 400 ... ≤ 1000	2
				> 1000... ≤ 5000	3
				> 5000	4
E 8	4	4	4	5	
E 9	4	4	4	5	
E 10	4	4	4	5	
E 11	4	2	2	3	
E 12	4	1	1	1	
E 13	4	1	1	1	

CPU/cm²	Category
< 1	1
> 1 ... ≤ 10	2
> 10 ... < 20	3
> 20	4

Pallet no.	Live germ count on surface, determined in contact samples			Live germ count in cross-section, determined in drill cores and with suspension method [CPU/g]	
	Top side	Bottom side	Block	CPU/cm³	Category
				≤ 400 (detection limit)	1
				> 400 ... ≤ 1000	2
				> 1000... ≤ 5000	3
				> 5000	4
E 14	 2	2	2	 1	
E 15	 2	1	1	 1	
E 16	 2	1	2	 1	
E 17	 2	2	2	 1	
E 18	 4	2	2	 1	
E 19	 4	2	1	 1	

Pallet no.	Live germ count on surface, determined in contact samples			Live germ count in cross-section, determined in drill cores and with suspension method [CPU/g]
	Top side	Bottom side	Block	

CPU/cm²	Category
< 1	1
> 1 ... ≤ 10	2
> 10 ... < 20	3
> 20	4

CPU/cm³	Category
≤ 400 (detection limit)	1
> 400 ... ≤ 1000	2
> 1000... ≤ 5000	3
> 5000	4

				
E 20	2	2	2	1
21				
E	4	1	1	1
22				
E	4	1	2	1
23				
E	2	1	1	1
24				
E	2	2	1	1
25				
E	2	1	2	1

Pallet no.	Live germ count on surface, determined in contact samples			Live germ count in cross-section, determined in drill cores and with suspension method [CPU/g]
	Top side	Bottom side	Block	
E 26	4	1	1	1
E 27	2	2	1	1
E 28	2	2	2	1
E 29	4	2	2	1
E 30	2	1	2	1

CPU/cm²	Category
< 1	1
> 1 ... ≤ 10	2
> 10 ... < 20	3
> 20	4

CPU/cm³	Category
≤ 400 (detection limit)	1
> 400 ... ≤ 1000	2
> 1000... ≤ 5000	3
> 5000	4