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Wood treatments with siloxane materials and metal complexes for preservation purposes

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1. Introduction

1.1 Preface: wood degradation and preservatives

Wood has been conveniently used as material for a large variety of human artifacts throughout the course of history: architectural woodworks (especially beams, flooring, roofs, ceiling), frames, outdoor or indoor furniture, ship building, railway sleepers and siding, exterior joinery, carpentry, pilings, panelling, tool handles and cutlery, musical instruments or sport equipments. It has also had great importance in the past in the artistic field and it has long been used for paintings on board, sculptures, carvings and church furniture such as altars and choirs. (Italy in particular has got great wooden artistic and historical heritage).

Wood is also widely used nowadays especially in the building and construction industries, both thanks to its many technical advantages such as high tensile strength, high elastic modulus, low density and insulation properties, and thanks to its revolving nature, low cost and aesthetic features.

Its principal disadvantageous characteristics are due to its organic constitution: it is slowly destroyed by the long-term impact of oxygen, light and water under atmospheric conditions; unprotected wood will eventually warp, split, rot and decay from water absorption, and also grey by UV exposure. Moreover, wood is a source of nutrients for micro-organisms, fungi and insects, leading to its complete biological destruction (Eaton, 1993).

Therefore, a lot of treatments have been essayed on wood in the past trying to overcome these drawbacks and to enhance wood durability, hydrophobicity, dimensional stability, strength and hardness, weathering performance and flame resistance. Among them, a large variety of compounds has been tested for wood modification, including anhydrides, carboxylic acids, isocyanates, aldehydes, alkyl chlorides, lactones, nitriles, and epoxides (Rowell 1983; Militz et al. 1997; Norimoto 2001).

As regards the improvement of durability against biological (biotic decay) agents, most of the preservative products developed in the chemical industry in the19th century have been recently banned by European Union because of their high toxicity to human. They were mainly polychlorine and phenolic compounds or arsenic or chrome salts and/or

copper salts, easily leachable and diffusible in the environment.
Research has been focused on developing new non-toxic and eco-friendly wood preservatives, eventually endowed of additional properties for increasing wood mechanical characteristics, dimensional stability, flame or UV resistance.

This thesis deals with the development of new wood treatments for increasing fungal and termite decay resistance.
The treatments performed are based on the well known antifungal and insect-deterrent activities of Cu, Zn and Ag, with the aim of reducing their environmental impact by means of an adequate fixation to wood, reached through two different ways:

1. impregnation of wood with Cu and Zn metal complexes soluble in water or organic solvents;
2. modification of wood with inorganic or inorganic-organic hybrid siloxane materials incorporating the metals or anchoring them through coordinative linkages.

Treatments based on the combined action of the above mentioned compounds with boric acid, used since the 1930's for protection of timber from biological attack, have also been performed, to improve preservation efficacy of the new formulations and to study their capability to increase boron fixation to wood.

Various methods of treatment, ranging from immersion to impregnation under vacuum, have been performed. For each treatment interaction and diffusion into the wood structure, preservative efficacy against fungi and/or termites and improvement of other chemical or physical properties have been evaluated.

1.2 Wood structure and properties

Wood is a natural organic composite material, that used for human artefacts being almost all obtained from the stem of plants belonging to the Spermatophyta subdivisions of gymnosperms (known as conifers) and angiosperms (deciduous trees). The term softwood is used to describe wood from conifers, and it is opposed to hardwood, which is the wood from angiosperm trees (Nardi, 2006).

1.2.1 Wood structure

Wood structure can be examined at different levels (Figure 1):

1) Stem macro structure
2) Timber microscopic structure
3) Cellular structure
4) Wood cell ultrastructure and wall chemistry

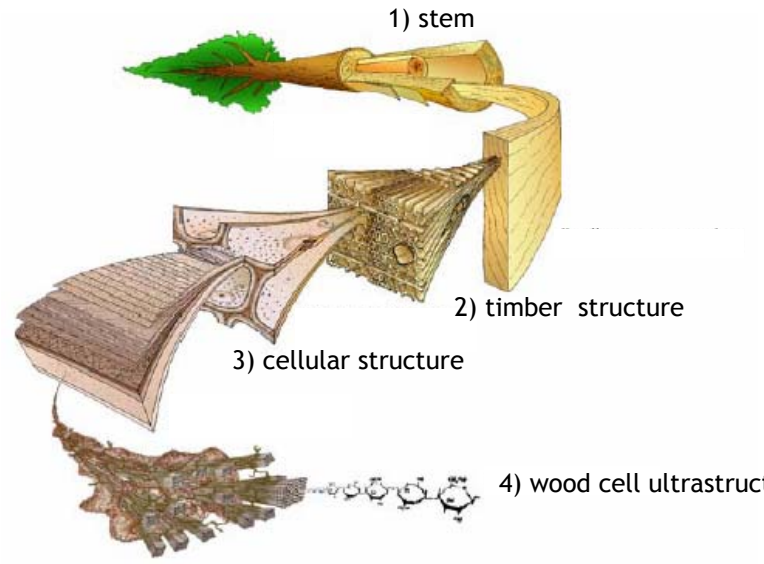

Figure 1 Different levels of examination of the wood structure

1) Stem macro structure

Looking at the cross section of a tree stem, several distinctive parts are visible (Figure 2). From the outside to pith, we can distinguish:

- the cork, i.e. the outermost physiologically dead layer of a woody stem, whose main functions are the protection against damage from parasites, herbivorous animals and diseases (dehydration and fire) and the control gas exchange.

- the phloem, i.e. the live vascular tissue with the role of conduction of sugars and other dissolved foods (lymph) produced by the leaves. It is discarded in the production of wooden items;

- the vascular cambium, i.e. the tissue responsible for the secondary growth of stems and roots (secondary growth occurs after the first season and results in increase in thickness); it produces phloem cells toward the outside and xylem cells toward the central axis of the stem.

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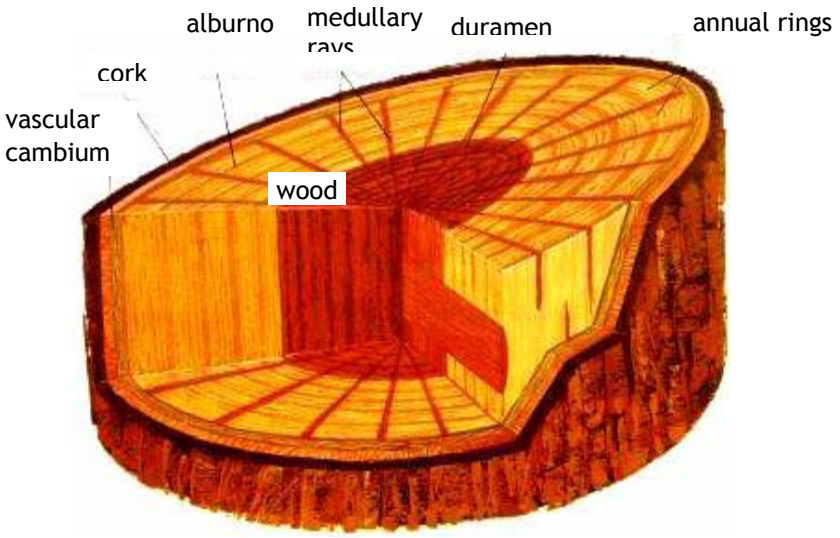


Figure 2 Scheme of the stransversal section of a stem, showing the distinctive parts of its macro structure. All the tissues comprised between the cambium and the stem central axis are called "wood".

All the tissues comprised between the cambium and the stem central axe are called “wood”. In a plant cross-section wood shows growth rings, that in the temperate species correspond to the annual activity of the cambium and are due to the alternate production of cells with large lumen (“spring wood”) and cells shorter with thicker walls (“summer wood" or "latewood"). Medullary rays, perpendicular to the growth rings and allowing the radial transmission of sap and storing up the reserves of carbohydrates and mineral salts, are also visible in a lot of tree species.

As the tree stem increases in circumference, the central part begins to lose water and stored food substances. The central zone of the stem becomes infiltrated with oils, gums, resins, tannins and aromatic compounds and when the transformation is complete these cells eventually die.

So we can distinguish:

- sapwood (alburno): it is the younger, outermost wood and it is its functional and active region: in the growing tree its principal functions are to conduct water from the roots to the leaves and to store up the reserves;

- heartwood (duramen): it’s the stem central area, it is formed by dead cells whose function is no longer of conduction but only of mechanical support to the stem.

The genetically programmed process of sapwood transformation in heartwood is known as“duramification”. In many species, heartwood exhibits an intensification of colour because of oxidation of phenolic compounds in the deposited substances. Thus this region often appears darker, but in some species e.g. aspen, beech and sycamore, the colour difference between sapwood and heartwood is negligibile. Heartwood has the same strenght as sapwood but has greater natural resistance to biological attack because of toxic compounds mostly deposited in the cells during its formation and also because of its lower permeabiliy to water and oxygen.

Chemical preservative treatments are performed on alburno rather than on duramen because of its lower natural durability and higher impregnation properties.

2) Timber structure

Wood has a porous, heterogeneous and anisotropic structure. For this reason, three different wood sections, called "reference planes of wood" (transverse (Tr), tangential (Tg) and radial (Ra) section), are necessary for a complete characterization (Figure 3). They correspond to cuts made in the three different directions of wood anisotropy (longitudinal (L), radial (R) and tangential (T)).

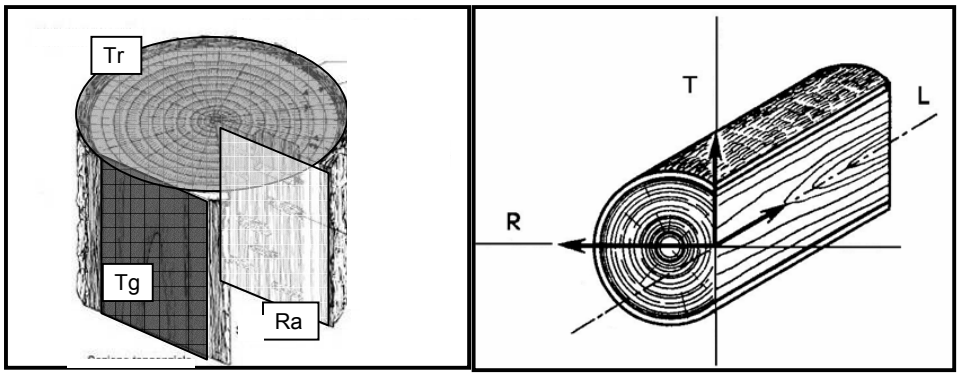


Figure 3 Wood reference planes and directions of anisotropy. Tr = transverse section; Tg= tangential section; Ra=radial section; L=longitudinal axis; R = radial axis; T=tangential axis.

All commercially relevant tree species (excluding monocotyledonous species such as palms) are either hardwoods or softwoods.Softwood, called omoxilo, consists for the 90 to 95% by volume of tracheids, having both the functions of raw sap conduction and support: they consist of dead cells with tangential diameter of about 70 μm and radial diameter variable depending on their position in the annual ring. Softwood also consists of axial and ray parenchyma cells, storing carboyhidrates and mineral salts.

Hardwood is called *eteroxilo* because of the difference, even qualitative, between vessels, with the functions of raw sap cpnduction, and fibers, responsible for mechanical resistance. Vessels are formed by dead cylindrical cells with diameter ranging from 25 to 500 μm or even more, arranged in axial columns without any wall separation; fibers are formed by elongated, thick-walled, lignified dead cells. Parenchyma and epithelial cells are also present.

Differences between hardwood and softwood are mainly visible in transversal section. Resinous canals in softwood are often noticeable at naked eye. Pores corresponding to the vessels in hardwood are sometimes even visible. Microscopic observations of the three wood reference sections enable to distinguish among different ligneous species, taking attention to some features such as:

- presence of resinous canals and their morphology (lumen diameter, cell-wall thickness) (softwood)
- vessels dimension and distribution (hardwood)
- axial parenchyma distribution
- more ore less continuity between latewood and spring wood in annual rings
- presence of radial tracheids (softwood)
- characteristics of pits, that connect adjacent ray cells and contiguous axial and ray cells
- ray parenchyma dimensions and distribution and ray parenchyma cells morphology (Figure 4).

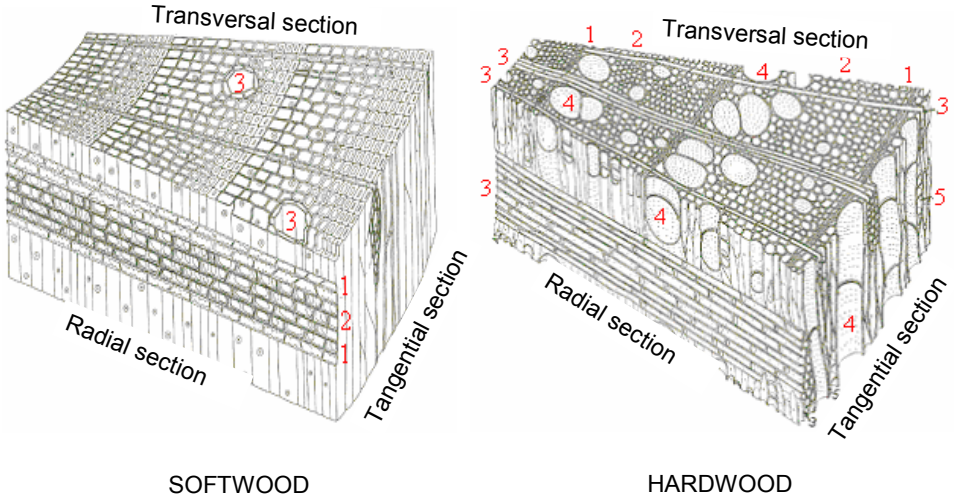


Figure 4 Schematic representation of wood. Softwood: 1. tracheids, 2. ray parenchyma, 3. resinous canals. Hardwood: 1. latewood: 2. spring wood, 3. ray parenchyma, 4. vessels, 5 axial parenchyma (from www.sfera-group.it).

3) The cell wall Structure

Every wood tissue is composed by cells, each formed by the cell wall and by the internal cell lumen. Whereas the lumen is a cavity, the cell wall has a complex structure. It is composed of several layers synthesized at different times in the cell differentiation process. Moving from the lumen (Figure 5), we can distinguish:

- the secondary wall
- the primary wall
- the middle lamella.

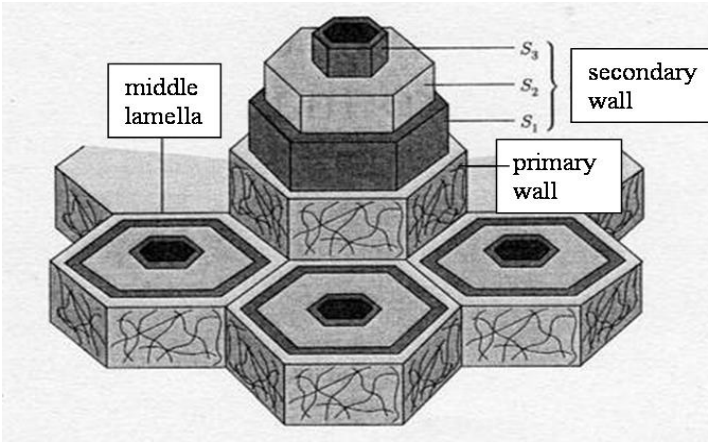


Figure 5 Schematic representation of the cell wall structure, showing the primary wall and the secondary wall with its different sub-layers (S1, S2, S3). Middle lamella is also represented among adjacent cells (from Nardi, 2006).

The secondary wall has a thickness of about 0,1mm and it is mainly composed of cellulose. Cellulose microfibrils are aligned parallel to each other and arranged in three sub-layers (S1, S2 and S3) with different microfibril orientation. These layers can also contain lignin, hemicellulose and pectic substances. The primary wall, with thickness of 0.5 to 1.5 μm, is mainly composed of pectine and lignin. The middle lamella constitutes the cementing layer between adjacent cells, is amorphous in character, is more or less devoid of cellulose but is rich in pectin compounds and especially in lignin (70%).

4) Wood cell ultrastructure and wall chemistry

The major chemical components in wood cell walls are: cellulose (between 40 to 55% of the total cell wall mass), hemicellulose (approximately 25-40%) and lignin (18-33%). Proteins (<10 %) and compounds of lower molecular weight (extractable, mineral salts) (<1 %) can also be present.

Cellulose exists in the form of microfibrils whose function is to impart tensile strength to the cell wall; hemicellulose plays the role of a matrix material; lignin is present as an encrusting substance and contributes to impart compressive strength.

Cellulose

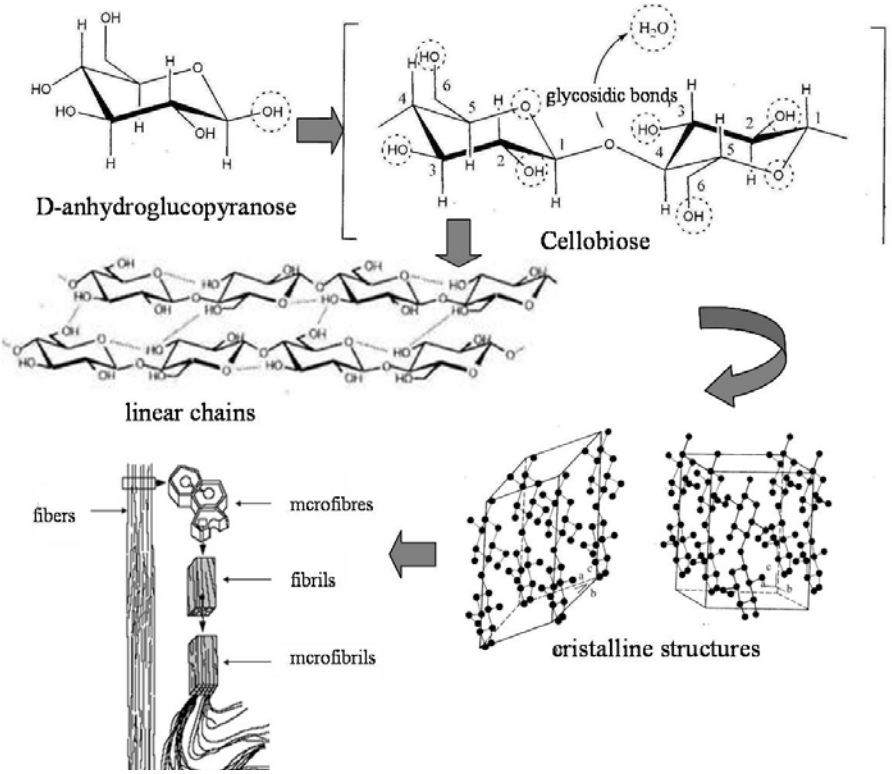


Figure 6 Scheme of cellulose structure.

Among the polysaccharides, cellulose is a linear polymer formed by the sequence of monosaccharide units of D-anhydroglucopyranose linked by ß 1-O-4 glycosidic bonds

(Figure 6). As a polymer, Cellulose can be described as the repetition of the basic structure of the disaccharide cellobiose (1.03 nm in length). The degree of polymerization (the number of glucose units per cellulose molecule) in wood ranges between 8000 and 10000, depending on the tree specie (Campanella, 2007).
The glycosidic bonds are formed through the reaction between the hydroxyl group of a glucuose molecole and the carbonylic group (aldehydic or chetonic) of another glucose molecule. The C(1)-OH group is oriented over the median plane of the pyranosic ring (ß-glycosidic link), conferring linearity to the cellulose chain and promoting the formation of intrachain hydrogen bonds among the singular glycosidic units.
Interchain hydrogen bonds between the oxyidrilic groups of adjacent chains cause the highly ordered arrangement of molecular chains in microfibrils and fibrils. Cellulose I and cellulose II differ for the parallel (cellulose I) or anti-parallel (cellulose II) orientation between adjacent chains (Nardi,2006).
As stated by the current model of microfibril organization, crystalline regions take place, characterized by monocline symmetry (in the case of cellulose I). They take turns with amorphous regions.
The higher is the order degree, the lower is water penetration. The non-crystalline zones also absorb more water than the crystalline ones because of the presence of more –OH groups free from intermolecular linkages.

Hemicellulose

Hemicellulose represent a class of non-cellulosic polysaccharides. They are relatively short, branched heteropolymers, whose monosaccharide components are D-glucose, D-xylose (often acetylated in position 2 or 3), L-ramnose, D-mannose, L-arabinose, D-galactose as well as the uronic acid of glucose and galactose, depending mainly on the tree specie, the wood tissue and cell wall layer. For example, the middle lamella exhibits a relatively even balance of monosaccharide components in both hardwoods and softwoods, whereas the composition of the secondary wall layers shows a preponderance of xylan components in hardwood and glucomannan components in softwood.
The monosaccharide units are linked by 1-3, 1-6 and 1-4 glycosidic bonds, to form polymers having a degree of polymerization usually lower than 200 (Figure 7).

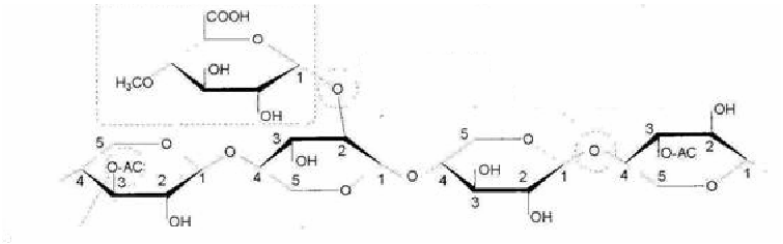


Figure 7 Schematic representation of hemicellulose 1-4 glycosidic bonds between adjacent monosachharides (from Campanella, 2007).

Due to the chain length, to the presence of ramifications and to the non-crystalline structure, hemicelluloses are more soluble in water than cellulose.
In addition, they are enough soluble in alkali because of the presence of carboxylic groups; they are more reactive than cellulose and more easily attacked by micro-organisms.

Lignin

Lignin is a complex aromatic three-dimensional polymer of phenylpropenic alcohols and is completely amorphous. It presents different structure depending on the vegetal specie and kind of wood. There are three types of phenylpropenic alcohols from which all lignins are constructed: p-coumaryl alcohol [4-(3-hydroxy-1-propenyl)phenol], coniferyl alcohol [4-(3-hydroxypropenyl)-2-methoxyphenol] and sinapyl alcohol [4-(3-hydroxypropenyl)- 2,6-dimethoxy-phenol]. The major difference in the chemical structure of these monomers is the presence or absence of methoxyl (-OCH_3) groups at the 3 and 5 positions of the aromatic ring (Figure 8).

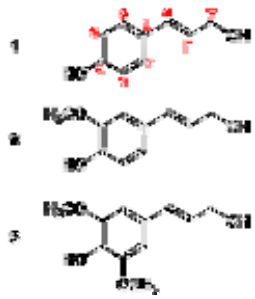


Figure 8 Phenylpropenic alcohols of lignins: (1) paracoumaryl alcohol, (2) coniferyl alcohol (3) sinapyl alcohol. Upon incorporation into lignin, they are referred to as syringyl (S), guaiacyl (G), or hydroxyphenyl (H) units, respectively.

The dehydrogenation of these alcohols, due to the laccase and peroxidase enzymes of the plants, leads to the formation of the mesomeric forms of the phenoxy radicals, that polymerize by radical coupling forming lignin. In this way the phenylpropenic units result linked through C-O-C or C-C linkages, forming polymers with high molecular weight (from 2000 to 15000 a.m.u.) (Figure 9).

Several models have been proposed to explain the structure of hardwood and softwood lignin. It is now generally accepted that softwood lignin is formed mainly from the dehydrogenative polymerization of conyferyl alchol, that hardwood lignin is derived from the dehyrogenative polymerization of coniferyl and sinapyl alcohols and that grass lignin is derived from the dehydrogenative polymerization of coniferyl, sinapyl and p-coumaril alcohols (Higuchi, 1985).

Over 70% of the total lignin occurs in the secondary wall of early wood cells and over 80% in latewood cells. The lignin encrustation of the cellulose microfibrils in the cell wall confers rigidity, and because of lignin less hydrophilic properties, it also influences the swelling characteristics of wood.

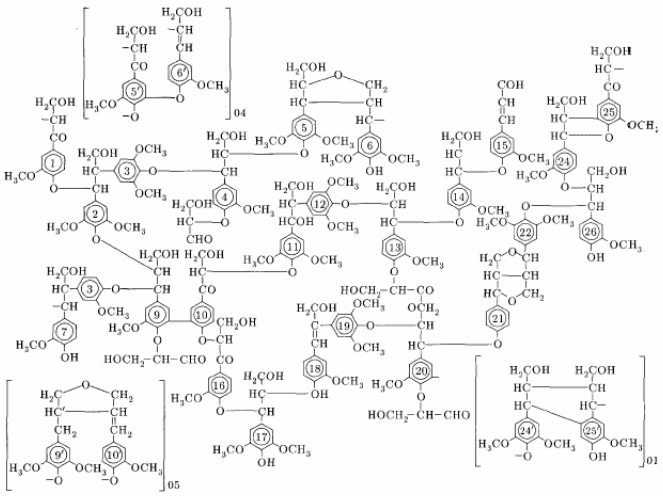


Figure 9 Scheme representation of possible bonds in lignin polymer.

The association among cellulose, hemicellulose and lignin needs to be considered. It is generally accepted that the less-ordered cellulose is linked to hemicellulose molecules through hydrogen bonds; hemicellulose is then linked to lignin molecules through

covalent bonds, acting like a sort of "glue" between cellulose and lignin (Figure 10). Thanks to the presence of a lot of hydroxyl groups, hemicellulose molecules are the wood most hydrophilic compounds and represent the cell wall areas through which polar solvents can diffuse.

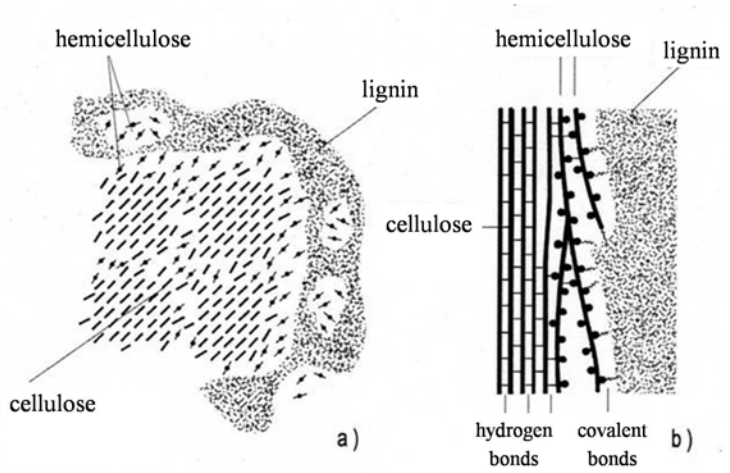


Figure 10 Scheme of the association between cellulose, hemicellulose and lignin. a) Transversal section; b) longitudinal section (from Nardi, 2006).

Substances with low molecular weight

The so called *extractives* are low molecular weight organic substances that can be extracted from wood using polar (e.g. acetone, water, ethanol) or apolar (e.g. toluene) solvents. Although if they represent only in a small percentage of wood cell walls (1.2% of dry mass for hardwood, from 3.5% to 10% for softwood, the extractives influence colour, hygroscopicity, smell and resistance. In some species, they are toxic and play a role against the attack by bacteria, fungi and termites.
The extractives of conifers can be classified into three categories (Frengel, 1989):

1. terpenes and terpenoids (molecules are composed of units of isoprene, up to six grounds in the same molecule)
2. aliphatic saturated and unsaturated fatty acids, alkanes and fatty alcohols (e.g. triglyceride, palmitic acid, stearic acid).

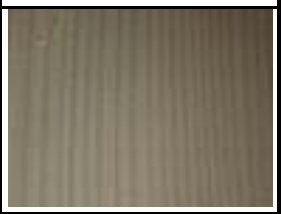
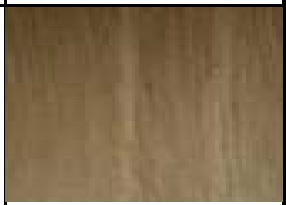
3. phenolic compounds (e.g.vaniline), lignans (e.g. pinoresinol, hinokiresinol) and stilbenes.

The mineral compounds of wood are entirely contained in the ash after combustion of organic matter. They represent the 0.1% dry weight for trees located in the temperate regions. The most abundant minerals are phosphorus, calcium, potassium and magnesium.

1.2.2 Wood properties

There is a strong relationship between the properties of wood and the properties of the particular tree that yielded it. For every tree species there is a range of density for the wood it yields. There is also a rough correlation between density of a wood and its strength (mechanical properties). For example, while mahogany is a medium-dense hardwood which is excellent for fine furniture crafting, balsa is light, making it useful for model building. The main features of some species of commercial importance are reported in Table 1.

Table 1 Features of some wood species in use for human artefacts (data from ottimari.agr.unifi.it; www.ivalsa.cnr.it)

WOOD SPECIE		COLOUR	DENSITY (for UL=12%) [g/cm3]	SHRINKAGE[%] Ax=axial; Rad=radial; Tan=tangential; Vol=volumetric	AXIAL COMPRESSION STRENGHT (for UL=12%) [MPa]	FLEXION RESISTANCE (for UL=12%) [MPa]	ELASTIC MODULUS [MPa]	IMPREGNABILITY (1=very high; 5=very low)
Abies alba Mil.		White	0,44	Ax:0,3 Rad:3,5 Tan:7,2 Vol:11	38	70	14500	Alburno: 2 Duramen: 2-3
Pinus sylvestris L.		Alburno: white-yellowish Duramen rosate	0,55	Ax 0,4 Rad 4,1 Tan 8,3 Vol: 13	46	98	14000	Alburno: 1 Duramen: 3-4
Larix decidua Mill.		Alburno white-yellowish Duramen: red-brown	0,65	Rad: 3,4 Tan: 8,3 Vol:13,8	52	95	14300	Alburno: 2 Duramen: 4
Castanea sativa Mil.		Alburno white-yellowish Duramen: brown	0,57	Rad:0,3 Tan:6,6 Vol:11,2	52	110	11600	Alburno: 2 Duramen: 4
Populus alba L.		Alburno white-yellowish Duramen: brown	0,82	Ax:0,4 Rad:4,4 Tan:8,3 Vol:13,2	62	110	12800	Alburno: 1 Duramen: 4
Quercus robur L.		White-yellowish	0,34	Rad:4,4 Tan:8.36 Vol:13,2	32	56		Alburno: 1 Duramen: 3

1.3 Wood degradation

When untreated wood is exposed to adverse conditions, it can degrade back to carbon dioxide and water through a series of chemical pathways. Fungi, termites, heat, humidity, ultraviolet energy, and chemicals can modify the performance properties of wood. Generally, it is common practice to distinguish between abiotic and botic degradation.

1.3.1 Abiotic degradation

When wood is exposed to outdoor climate, a complex combination of chemical, mechanical and energy factors contribute to what is described as a weathering. We tend to study each of these degradation chemistries as individual events but, in fact, they are all connected by five basic chemical processes: hydrolysis, oxidation, dehydration, reduction, and free radical cleavage (Comstock, 1984).
The chemical reactions involved lead to the degradation of both cellulose (and hemicellulose) and lignin. Cellulose degrades primarily by hydrolisis of glycosidic bonds and oxidation of the polymeric chains extremities; lignin is subjected to substitution reactions, oxidation and hydrolysis, and its reactivity is due to the benzylic, hydroxyl, carbonyl and carboxyl groups on its propanoidic chains and to the phenolic function and aromatic nucleus.

Water is one of the main factors of wood degradation.
Especially when wood is used outdoors, temperatuee and humidity variations cause changes of wood moisture content until new equilibrium conditions (EMC) are reached (Figure 11).
Wood hydroxyl groups, and especially those of hemicellulose, confer a strongly hydrophilic character to the cell walls. When wood is exposed to high relative humidity (RH), water molecules diffuse in the cell walls through formation of hydrogen bonds, resulting in wood swelling. When the cell wall saturation point is passed, water flows in the cell lumens as free water. This process is reversible and wood exposed to variable weather conditions will undergo cycles of shrinking and swelling that can cause cracks in the material.

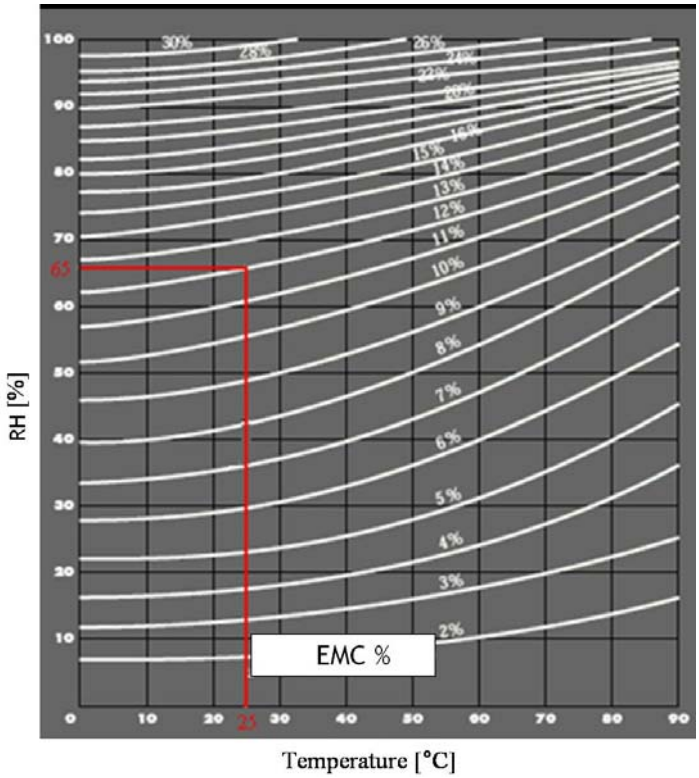


Figure 11 Wood equilibrium moisture content [EMC%] related to the temperature and humidity [RH%] of the surrounding air (www.cas.purdue).

Sun radiations can even cause wood degradation. Wood is a good light absorber due to the presence of several chromophores, e.g. phenolic hydroxyl groups, aromatic skeleton, double bonds and carbonyl groups. The UV light component of sunlight, which acts in combination with moisture, temperature, and oxidative agents such as oxygen and/or ozone, depolymerise both lignin and cellulose (Hon, 1994, Evans, 1992).The oxidation of wood causes discoloration, followed by loss of gloss, roughening and checking, attributed to the formation of carbonyl, α-ß-unsaturated systems in the cellulose and to the modification of the chromophores of lignin, capable of absorbing UV light in the range of 300–400 nm. It also causes an increase of susceptibility to other degradation phenomena such as acid and alkaline hydrolysis, both favored because influenced by the electronic effect of oxidized groups on C (6), C (3) and C (2).

Wood is also susceptible to acid or base attack (Campanella, 2007). Cellulose glycosidic bonds may be splitted by acid hydrolysis, which takes place even at relatively low

temperatures (homogeneous hydrolysis by H_3P0_4 is usually carried out at 25°C) and proceeds through the protonation of the hemiacetal oxygen, the exit of the nucleophilic unit and the entry of water (Figure 12). Even a highly alkaline environment under high temperature conditions (above 150°C) can cause hydrolysis and degradation of the cellulosic structure. In this case, the hydroxyl deprotonation at C (2) creates a reactive nucleophilic specie (alkoxide ion) that attacks the anomeric carbon, thus causing the separation of the alcoholic fragment of the glycoside (Figure 13).

This hydrolytic cleavage can occur randomly, i.e. at every point in the chain. In alkaline environment, further degrading hydrolysis may occur even at temperatures below 100°C, known as *peeling off*: the reducing end (terminal glycose) of the cellulose molecule isomerizes, through the ene-diolic form, in the corresponding ketose, on which a 3-elimination reaction occurs; if it involves the C (4) position, the remaining part of the chain is removed (Figure 14), the residual ketose undergoes further transformation to isosaccarinic acid. The reaction is repeated on the new reducing ends of the chain and then degradation takes place with the detachment of a unit at a time, hence the name of the process (Campanella, 2007).

The hydrolytic processes cause a decrease of the cellulosic degree of polymerization and a consequent reduction of wood mechanical properties.

Lignin can be also subjected to basic attack due to its carboxylic groups and phenolic groups, that may participate in different reactions creating reactive radicalic, quinonic and anionic intermediates.

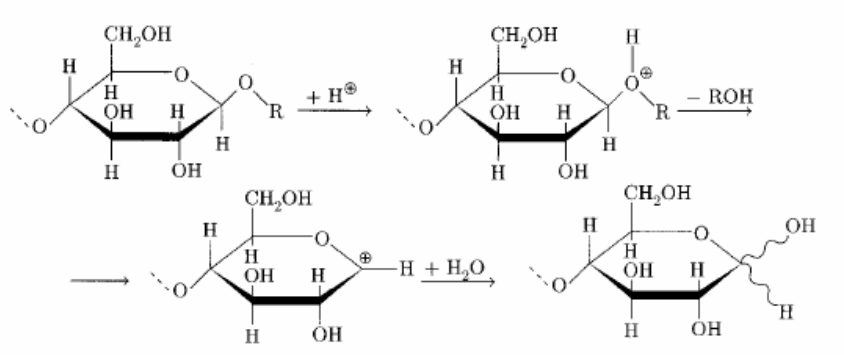


Figure 12 Acid hydrolisis of cellulose (Campanella, 2007).

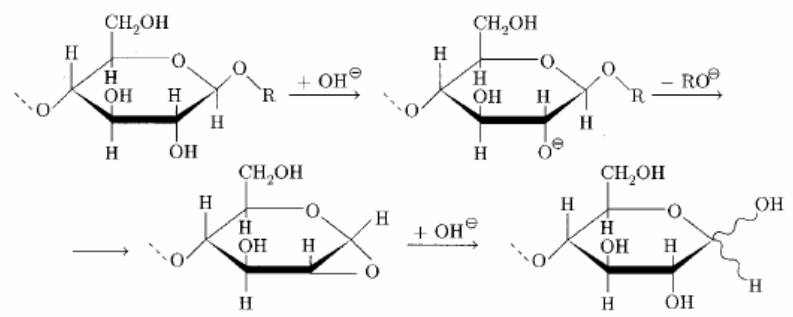


Figure 13 Alkali hydrolisis of cellulose (from Campanella, 2007).

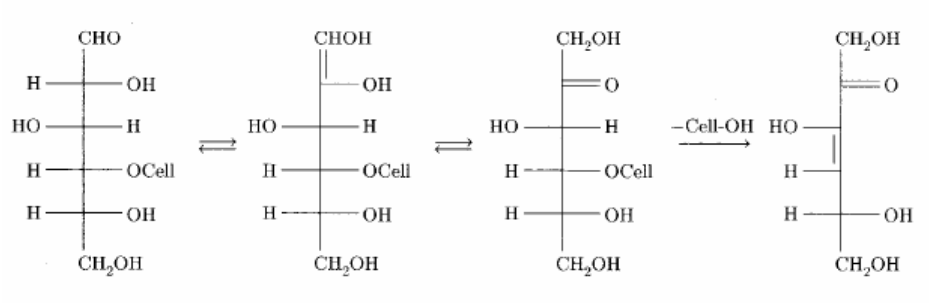


Figure 14 Cellulose dagradation by *peeling off* (from Campanella, 2007).

1.3.2 Biotic degradation

Wood can be degraded by a variety of organisms, causing aesthetic and/or mechanical decay.

The main organisms causing wood biological degradation are reported in Table 2.

Table 2 Main organisms causing wood biological decay (Shmidt, 2006).

Organism	Mechanical damage	Aesthetic damage
Coleoptera insects	yes	yes
Isoptera insects (thermites)	yes	yes
Basidiomycetes fungi	yes	yes
Chromogenic fungi	no	yes (non removable)
Soft rot fungi	yes (superficial)	yes
Molds	no	yes (removable)
Marine borers	yes	yes

1.3.2.1 Fungi

Fungi are eukaryotic and carbon-heterotrophic (free from chlorophyll) organisms with chitin in the cell wall, reproduce asexually and/or sexually by non-flagellate spores; they can be unicellular or pluricellular, but the unicellular forms have a minor part to play in the colonization and decay of wood. Pluricellular fungi are filamentous: they are formed by hyphal filaments and colonize wood through the apical growth of hyphae.
They develop in wood if some environmental conditions are satisfied: depending on fungi and wood species, wood moisture content of about 20% or more and atmospheric temperature ranging from about 10°C to 40°C make wood susceptible to fungal attack. Wood-stain fungi are highly pH sensitive, with pH values of highest growth depending on the specie; they usually grow best in slightly acidic substrates but can also do well in higher pH levels. Basidiomycetes, for example, have an optimum range of 4-6 (Schmidt, 2006) Also light is known to influence enzyme regulation in fungi and there are examples of increased activity of some enzymes and inhibition of others (Eaton, 1993).

Fungal chemistry of decay mainly consists of cell wall enzymatic degradation. More than 3000 wood degrading enzymes are described comprising both polysaccharide degraders (cellulases and hemicellulases) and lignin modifying enzymes.
Cellulose is depolymerized by three major classes of hydrolytic enzymes: endocellulases (Cx), exocellulases (C1) and ß-glucosidase, generally acting synergically in a multi-step process. Endocellulases attack by hydrolysing non-cristalline regions and regions of less ordered crystalline cellulose thus opening up cellulose chains. The cellobiohydrolase (exocellulase) binds to the edges of the cellulose crystallite and cleave off cellobiose units from the non-reducing ends. In addition, it opens up various random points of the crystalline regions, instantaneously attacked by endocellulases. ß-glucosidase converts cellobiose to glucose.
Oxidative enzymes (cellobiose oxidases and glucose oxidases) and oxidoreductive enzymes (cellobiose dehydrogenase) also participate in cellulose degradation. Cellobiose oxidases enzymes act on crystalline cellulose producing gluconic and cellobionic acids. Action on the cellulose reducing-end groups would result in the production of acid groups and would open up the crystallite allowing endocellulase action.
Hemcellulose is degraded by hydrolytic enzymes (both exo- and endo-enzyme types); the

most diffuses among fungi are: xylanases (1,4-ß-D) (that may be divided in those which cleave at L-arabinose branch points or those that cleave throughout the xylan polymer leaving xylobiosyl and xyloriosyl residues attached to the L-arabinofuranose), mannanases (1,4-ß-D) and galactanases (1,3-ß-D). The preferential ability of certain wood-decay fungi to attack different susceptible wood species may be related to hemicellulose composition (Schmidt, 2006).
Lignin degradation by white rot fungi is acheived by an oxidative radical mechanism involving a lignin peroxidase (ligninase) and hydrogen peroxide (generated during cellulose degradation by glucose oxidase) and by other phenol-oxidizing fungal activities.
The lignin-degrading activity of white rot fungi is characterized by the production of phenoloxidases, in particular the copper-containing enzyme called laccase. Some of the pathways attributed to the oxidation reactions of laccase on phenolic end-groups of lignin (Shimada, 1991) are reported.
Since the enzymes are too large to diffuse freely in the unaltered wall, it is now commonly accepted that non-enzymatic, low molecular weight metabolites (oxidative free-radicals) are also involved as precursors and/or co-agents with enzymatic degradation. The main non-enzymatic systems proposed involve Fe^{2+}, H_2O_2, oxalic acid and the production of hydroxyl radicals (Schmidt, 1981).

Taxonomically, the fungal species responsible for wood decay belong to the subdivisions of Ascomycotina, Basidiomycotina and Deuteromycotina; as concerns decay modalities, wood decay fungi have been categorized, since 1874 in species causing brown rot and species causing white rot.

Brown rot is caused by Basidiomycetes which metabolize the carbohydrates cellulose and hemicelluloses of the woody cell wall by non-enzymatic and enzymatic action but leave the lignin almost unaltered. In fact, brown rot fungi don't produce phenoloxidases: lignin degradation is limited to extensive demethoxylation of the aromatic portion with the loss of the propane side chain and the incorporation of molecular oxygen.
Brown rot fungi colonize wood via the rays and spread in the longitudinal tissue through pits and by means of microhyphae. They grow inside the cell lumina and there in close contact with the tertiary wall. The low-molecular agents and/or the cellulolytic enzymes penetrate through the relatively resistant tertiary wall (high lignin content) and diffuse

into the secondary wall, where they degrade the carbohydrates completely. Due to the rapid cellulose depolymerization, the dimensional stability and mechanical properties particularly decreases. During *Serpula lacrymans* attack, for example, a decreasing of the compression strength by 45% at only 10% mass loss is reported. Brown-rotted wood is reddish brown to dark brown in appearance due to the increase of relative lignin content.

At late stage of decay, especially in drying conditions, wood commonly shows deep cross-cracking due to shrinkage caused by the loss of carbohydrates in the wood cell wall. The residue of heavily decayed wood (mainly modified lignin) easily crumbles between the fingers to a fine powder.

Many brown rot fungi have a tendency to be potent acid producers and can cause a drop in pH of the growth medium. The acid substances produced are a variety of organic acids, notably oxalic acid. Particularly in later stages of decay, the highly lignified middle lamella/primary walls were observed to undergo attack. Also, the penetration of the wood cell wall by bore holes removes lignin in the process, all suggesting that low molecular weight lignin degrading agents and potentially even lignin degrading enzymes max occur in some brown-rot fungi, at least with localized activity (Goodell 2003).

Most brown-rot fungi affect conifers (Ryvarden 1993) and are usually uniformly distributed over the substrate.

The characteristics of the brown rot fungus Coniophora puteana are reported in Table 3.

White rot is caused by basidiomycetes and some genera of ascomycetes which not only metabolize cellulose and hemicelluloses but are also able to degrade lignin extensively due to the production of phenol-oxidases. They often cause the decay of lignin, hemicellulose and cellulose at the same time and similar rate ("simultaneous white rot fungi"), but some species degrade lignin and hemicellulose before the cellulose in the cell wall is attacked ("preferential white rot fungi"). They colonize the wood via ray parenchyma and vessels or resin canals (in softwoods) and grow principally in the wood cell lumina. Hyphal colonization from cell to cell in early decay may happen through the pit pores but, latterly, bore holes are produced and a gradual thinning of the wood cell wall around fungal hyphae is seen. The middle lamella is only finally dissolved in later stages of decay leading to a complete breakdown of the wood cell wall structure (Schmidt, 2006).

Table 3 Main features of the brown rot fungus *Coniophora puteana* (Shum.:Fr) P. Karsten.

Coniophora puteana (Shum.:Fr) P. Karsten	
	**Occurrence** Domestic (buildings) and external. Image from www. societe-mycologique-poitou.org
Growth and decay conditions Temperature: optimum: 23°C; range:3-35°C; survives in kilns at 50-65°C for several weeks. Moisture content: optimum: 50-60% (minimum in spruce sapwood:24%)	**Wood species attacked** Mainly softwoods, sometimes hardwoods (e.g. oak)
Rate decay of wood 60% weight loss can be achieved in 12 weeks in soil block test on Scots pine sapwood	**Notes** The characterization of the cellulolytic enzyme system and of some mechanisms of wood degradation are reported in literature (Hyde; Lee et al. 2004).

Table 4 Main characteristics of the white rot fungus *Trametes versicolor* (L.:Fr.) Pilat.

Trametes versicolor (L.:Fr.) Pilat.	
	**Occurrence** Wounded or dead standing trees, logs and stumps, rarely in buildings. Image from Schmidt, 2006
Growth and decay conditions Temperature: optimum: 30°C; maximum: 36°C; moisture content: optimum: 40-45% or higher. (minimum in spruce sapwood:24%).	**Wood species attacked** Mainly hardwoods, occasionally softwoods
Rate decay of wood 38% weight loss can be achieved in 16 weeks in agar block tests on beech (*Fagus sylvatica L.*)	**Notes** The decay by a simultaneous type of attack is described in literature (Schmidt, 2006).

White-rotted wood takes on a lightened bleached appearance; it may become rather fibrous along the grain due to the separation of wood cells along the middle lamellae and the surface becomes softened. The wood strength properties are reduced to a lesser extent than in brown-rotten wood, since at the same mass loss, lesser cellulose is consumed, and it does not come to cracking or cubical rot. In a very late stage of attack, a wood mass loss of 97% has been measured. Hardwoods are generally more susceptible to white rot than softwoods. The characteristics of the white rot fungus *Trametes versicolor*, whose type of attack has been object of many studies on the micromorphology of white rot, are reported in Table 4.

1.3.2.2 Insects

Wood-decay insects causing damage to timber and wooden artifacts can be referred to the taxonomic orders of Coleoptera (Anobiidae, Lyctidae, Bostrychidae, Cerambycidae, Curculionidae), Hymenoptera (Siricidae) and Isoptera (Rhinotermitidae and Kalotermitidae) (Chiappini, 2001).
Adults of Coleoptera and Hymenoptera generally depose their eggs on the wood surface; the eggs open and, in the stage of larvae, penetrate the wood structure excavating galleries; inside wood larvae develop in pupae and finally in adults, that fly outside wood.
They attack mainly wood with moisture content of 7-15% (indoor structures) or >15% (timber). As regards buildings, the attack is normally extended to the whole length of the wood structural elements and cause mechanical resistance diminution proportional to the section reduction.

Isoptera comprise Termites, social insects living in colonies. A termite colony is a complex community whose individuals have particular roles and functions concerning with either defence and protection of the nest against predators (“soldiers”), labour including foraging for food (“workers”) or reproduction to expand the existing colony or establish a new colony (“reproductives”). They usually make their nests in soils and extend into the wood by excavating tunnels and galleries, inflicting destruction

especially on timber used for constructional purpose both outdoors and inside buildings. Their ability to destroy wood is due to the population of symbiotic bacteria, protozoa or enzymes in their gut which assist in the breakdown of ingested lignocellulosic food material (Goodell, 2003). The species of termites more diffused in the italian geographical region are the drywood termites *Kalotermes flavicollis* (Fabricius) and the subterranean *Reticulitermes lucifugus* (Rossi). Their main characteristics are reported in Table 5.

Table 5 Main features of the termites Kalotermes Reticulitermes (from Chiappini,2001).

Name	Kalotermes (Drywood termites)	Reticulitermes (Subterranean termites)
Image	  *Kalotermes flavicollis* (Fabricius)	  *Reticulitermes lucifugus* (Rossi).
Occurrence	Wounded or dead standing trees, buildings, ceilings, roofs, wooden artifacts, beams, frames.	Buildings (churches, libraries, archives) also in urban areas, ceilings, roofs, wooden artifacts, beams, frames, paper.
Wood attacked	Springwood, especially with low humidity (drywood)	Wood characterized by high moisture content.
Growth and decay conditions	Colonies, usually not larger than 1000-2000 individuals, make their nest inside wood.	Colonies, usually numerous (larger than 100000 individuals), make their nest on the soil or, sometimes, in empty spaces inside walls and door-frames. The system of unneling is employed to move upward without being exposed to the sunlight.

1.3.2.3 Wood durability against fungi and insects

Timber species differ markedly in performance when exposed to decay risks: the term "natural durability" is used to express a wood species intrinsic resistance to defined xilofagous organisms. It is generally referred to the duramen (alburno is often easily degradable) and depends on chemical and physical characteristics of wood, such as density, nitrogen and starch content, lignin quantity and type and especially the presence of extractives (cyclic, aromatic or phenolic, produced as secondary metabolites via the shikimic acid pathway (hydrolysable tannins, lignans, coumarins, alkaloids), via mevalonicacid (terpenoids, steroids) or by combinations of these two pathways (stilbenes, flavonoids, condensed tannins).
The main characteristics of durability of some wood species used for human artefacts are reported in Table 6.

Table 6 Natural durability of some wood species. Scale of durability against fungi basidiomycetes (UNI EN 350/1): 1=Very durable, 2=Durable, 3=Moderately durable, 4=scarcely durable, 5=Non-durable; Scale of durability against insects (UNI EN 350/1): D=Durable, MD=Moderately durable, ND=Non-durable.

Wood specie	Fungi basidiomycetes	*Hylotrupes bajulus*	*Anobium punctatum*	Termites	notes
Abies alba Mill.	4	ND	ND	ND	
Pinus sylvestris	4	//	ND	ND	
Larix decidua Mill.	3-4	ND	ND	ND	
Castanea sativa Mill.	2	//	ND	ND	Alburno *Hesp.*
Fagus sylvatica L.	5	//	ND	ND	*Hesp.*
Populus canescens sm	5	//	ND	ND	*Hesp.*
Juglans regia L	3	//	ND	ND	*Hesp,*
Quercus robur L.	2	//	ND	MD	Alburno *Lyctus*, *Hesp.*

1.4 Treatments for the improvement of wood properties

Treatments for wood protection and enhancing of properties have been known for almost as long as it has been in use (Mahltig, 2008). Treatments aim especially at the improvement of mechanical resistance, water resistance (dimensional stability of wood), fire resistance and UV protection.

1.4.1 *Surface Coatings and penetrating treatments*

The most common method of protecting wood from weathering and photodegradation is the use of a wide range of coatings such as paints, varnishes, stains and water repellents (Williams, 1999).

In fact, surface coatings, e.g. varnishes, waxes or natural oils such as olive oil, have been applied since ancient times.

Wood coatings are generally classed as either film-forming or penetrating. Film-forming treatments, such as paints, contain pigments that screen wood from solar radiation and, because they form a barrier over the wood surface, they also prevent surface wetting and erosion. Nevertheless, their main drawback is the loss of adhesion during weathering due to failure of the underlying wood (Adebahr, 2000; Fauchadour, 2005). They are also scarcely effective in controlling wood dimensional changes.

Penetrating treatments typically contain a hydrophobic component such as waxes, natural oils or resins.

The formation of a hydrophobic coating raises the contact angle of the treated wood preventing water from being readily taken up by the surface or sub-surface capillaries. This reduces moisture absorption and imparts a certain degree of dimensional stability to wood (Evans, 2009).

Additives are often added to coating products to enhance wood stability to solar radiation. For examples, stains containing pigments partially obscure the wood surface, and hence reduce the amount of light reaching the wood. A more efficient protection of wood can be achieved by reflecting or harmlessly absorbing UV radiations, the main responsible for photodegradation. Stains containing UV reflectors (from 0.01 to 0.15 μm particles, able to scatter UV light while having little effect on visible light, such as micronised titanium dioxide or micronised zinc and cerium oxides) or UV absorbers (a

wide range of organic molecules, including benzophenone, benzotriazole and triazine, whose UV absorbance is determined by the chromoforic group, whereas the volatility, polarity and resistance to leaching are controlled by molecular weight and nature of substituent groups) have also been used.
Flame retardant additives used in the past are: chlorinated and brominated biphenyls and diphenyls, carrying high environmental and health risks; boric acid, soluble zinc, phosphorous and metal salts, whose main disadvantage is the low leaching resistance when in contact with water. (Mahltig, 2008).

1.4.2 *Modification of wood*

Since the abiotic and biotic decays are related to the molecular properties of the constituents of wood, it is possible to eliminate, or at least limit them by chemically modifying the structure of lignocellulosic polymers. Wood properties can be improved by chemical modifications, generally obtained by covalently bonding chemicals to the polymeric constituents of wood and, in certain cases, by bulking the cell wall (Mahltig, 2008). During recent years the numbers of methods and procedures developed to modify wood have increased and a wide variety of compounds have been employed for wood modification including anhydrides, acid chlorides, carboxylic acid, isocyanates, aldehydes, alkyl chlorides, lactones, nitriles, and epoxides (Militz, 1997; Norimoto, 2001), aiming at enhancing wood durability, dimensional stability, strength and hardness, weathering performance and flame resistance (Donath, 2004). In this way, new wood products can be made which differ absolutely in their technological properties from the original wood.
The most effective technologies used are heat treatment, acetylation, furfurylation, treatments with silica, silicones and silanes and treatments with chitosans. There are still many questions concerning the active principles, effectiveness, costs and optimization of these single technologies (Mahltig, 2008).

Several processes to thermally treat wood have been commercialised in Europe in the past decade and are currently used in many indoor and outdoor applications. Various thermal processes have been developed, consisting of wood treatments at elevated temperatures (160-240°C) in conditions of low wood moisture content (by means of

drying steps) and low oxygen present (under-vacuum or nitrogen atmosphere conditions). Linseed oil is used in the OHT (oil heat treatment) process (Germany) as a drying medium and to improve heat flow into the timber; at the same time, the oxygen level in the vessel is low due to the oil (Shultz, 2008). Chemical changes due to thermal treatments to the wood structural polymers, such as increase of the relative amount of crystalline cellulose and lignin bond rearrangements with subsequent formation of a more condensed structure, lead to dimensional stability improvement, decrease hygroscopicity and physical/strength alteration.

Acetylation consists of the substitution of wood hydroxyl groups, responsible for the interaction of wood with the environmental water, with acetyl hydrophobic groups, reducing in this way the moisture content exchange (EMC) and consequently the swelling and shrinkage of wood to a great extent.

Modification of wood with furfuryl alcohol-resins is called furfurylation. Furfuryl alcohol polymerize in situ in presence of an acid catalyst (cyclic carboxylic anhydrides are often used) and reacts with the cell wall components (especially with lignin) leading to an improvement of dimensional stability, anti-shrinkage efficiency and resistance to alkali and acids.

Polyethylene glycol or wood plastic composites were also employed, however these treatments often cause high gain of weight and related drawbacks to the advantages of wood (Okawa, 2002).

Applications of nanotechnology to wood had been performed in recent years, also for the conservation and restoration of the world's cultural heritage: the use of nanoparticles of calcium and magnesium hydroxide and carbonate to restore and protect wall paints and to de-acidify paper and wood is reported (Baglioni, 2006). In particular, wood treatments based on nanoparticulate silica sols ("nanosols") have been studied in the last decades. Nanosols consists of transparent, nano-sized (usually les than 50nm) dispersions of inorganic particles in either water or mixtures of organic liquids, applied as coatings of the wood surface or for impregnation of the whole wood body.
Among the range of wood modifiers, also many types of silicon compounds have been

used (Kartal, 2007; Mai, 2004; Saka, 2001), obtaining improvements in certain physical properties. The silification of wood has been inspired from nature: in the Petrified Forest National Park, Arizona, wood became fossilised and its organic matter was penetrated by minerals (mostly silicate) yet the original structure is still retained after more than 200million years (Mahltig, 2008). Since the 1800s sodium silicate-based treatments were applied trying to mimic the natural process of silification of wood.
A review about the modification of wood with silicon compounds has been recently published (Mai, 2004).
Silica modifications of wood recently studied are mainly based on:

- silica nanosols prepared from aqueous sodium silicate
- commercially available products containing colloidal SiO_2
- silicon alkoxide precursors

Due to their high surface-to-volume ratio, inorganic nanosol particles are metastable. Thus, during the application to wood and subsequent drying, these materials condense into an inorganic three-dimensional xerogel network, modifying the substrate properties. Inorganic nanosols have been applied to wood in order to improve its properties and for consolidation purposes. In fact, cracks can appear on wood due to swelling and shrinking processes caused by changing temperature and climate and by the natural movement of the wood material itself: silica treatments can improve wood mechanical characteristics (Sèbe, 2004; Mai, 2004) as demonstrated by increased Brinell hardness. The introduction of organic additives with an affinity to wood but which don't hinder penetration into the wooden structure makes it possible to achieve a maximum degree of consolidation (Böttcher, 2000); recent applications for restoring and preserving wooden cultural artefacts are reported (Mahltig, 2008).
Resistance of wood treated with silica compounds to liquid water sorption (Temiz, 2006) to photochemical degradation (Temiz, 2004; Temiz, 2006), to combustion (Saka, 1997; Yamaguchi, 2003), especially if in combination with boron (Furuno, 1992; Furuno, 1993) and to swelling (Ogiso, 1994) have been reported. In a recent report, many ways in which the properties of wood might be improved by application of silica and other inorganic nanosols have been reviewed (Mahltig, 2008).
The presence of silica in the cell walls, rather than in the lumina, depends on wood moisture content. This can influence the material final properties: in general, the

increase of durability, dimensional stability and UV-VIS radiation resistance are due to silica compounds deposited onto the cell wall, whereas the presence of chemical compounds into the cell lumen can influence hardness and flame resistance, but don't make any contribution to decreased water absorption (Saka, 1992; Mai, 2004). The addiction of hydrophobic, fire resistant, coloured or UV-protective_additives is reported to improve other important properties of practical interest (Table 7).

Table 7 Additives to silica treatments for the improvement of wood physical/chemical properties (Malthing, 2008).

Wood property	Additive
Hydrophobic	Long-chained alkylsilanes
	Polisiloxanes
	Fluorinated compounds
Colored	Inorganic color pigments
	Natural or synthetic organic dyes
UV-protective	Organic UV absorbers
	Inorganic pigments like TiO_2, ZnO
Photocatalitic	TiO_2

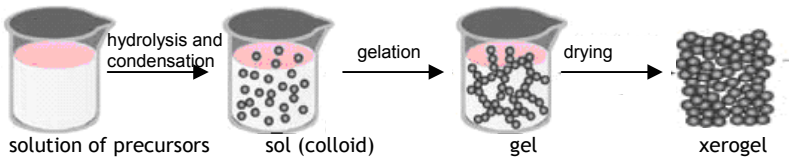
1.4.2.1 Silica modification with alkoxysilanes

The most convenient method for modify wood is to impregnate the cell wall with alkoxy monomers which are then allowed to polymerise *in situ*.

Polymerisation of the alkoxysilanes monomers can be achieved by the sol-gel process, i.e. via condensation of the silanol Si-OH groups to form siloxane bonds after hydrolysis of the alkoxy groups. (Brinker, 1990; Brebner, 1985). (Figure 15).

The so formed siloxane polymers can penetrate into the wood texture and interact, mainly *via* hydrogen bonds, with the wood biopolymers of the cell walls (Schneider, 1985). The possible formation of Si-O-C covalent bonds, by reaction between the silanol groups and the hydroxyl groups of cell wall polymers (transesterification), has been also suggested (Sébe, 2001); nevertheless, this is considered less favoured with respect to the formation of H-bonds by another research group (Ogiso, 1994). The siloxane materials can polymerize both onto the cell walls and inside the lumina. In the last case they behave as simple filling materials exhibiting a sort of barrier effect. In the first case, the presence of silica in the cell walls, rather than in the lumina, strongly influences the resistence of the wood by modifying its physico-chemical characteristics.

SOL-GEL PROCESS – ALKOXYSILANES METHOD



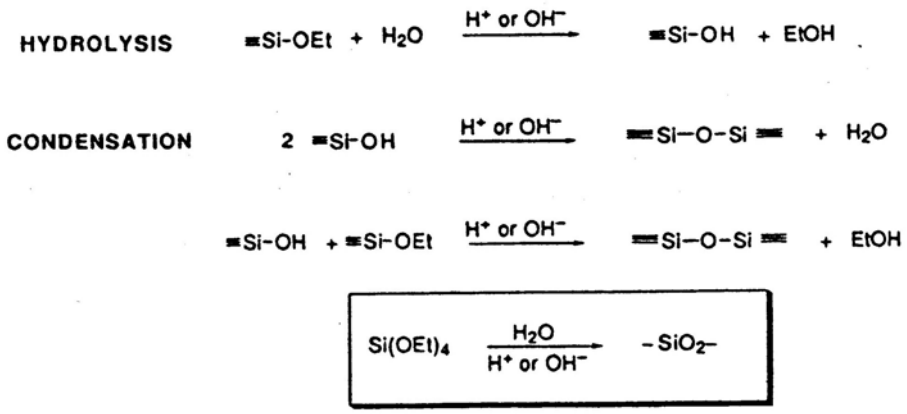


Figure 15 Scheme of the sol-gel process with its hydrolysis and condensation reactions.

In recent reports, many ways in which the properties of wood might be improved by application of silica and other inorganic nanosols have been reviewed: various methods of treatment, from superficial coating, to impregnation under vacuum, and various silane precursors have been tested and compared (Mahltig, 2009, Tingaut, 2005; Tingaut, 2006; Sèbe, 2004)).

Improvements of physical properties of wood through modification with alkoxysilanes are reported. They span from water repellency (De Vetter, 2010), resistance to liquid water sorption (Brebner, 1985), to photochemical degradation and artificial weathering (Tshabalala, 2003; Donath, 2007), to combustion (Saka, 1997) especially if in combination with boron compounds (Marney, 2008) and to shrinkage (Saka, 1992; Donath, 2004).

1.5 Preservation of wood from biotic decay

Wood preservation includes physical or chemical treatments aiming at hinibiting the activity and viability of wood-degrading organisms (remedial treatments) or applied on wood in order to prevent biological attack (preventive treatments).
The landscape of both remedial and preventive preservative treatments has changed drastically in the last few years (Manning, 1997; Hughes, 2004; Lack, 2008).
In 1998 the European Directive on the Biocidal Products (98/8/EC) listed different biocidal product types including, among them, wood preservatives (Product Type 8). According to Article 24 (1) of Directive, Member States have to take the necessary arrangements to monitor whether biocidal products placed on the market comply with some enounced requirements: the Biocidal Products Directive BPD 98/8/EC (1998) came into force in September 2006 and approximately 50% of biocides for wood preservation were removed from the market due to their toxicity for human body and for the environment.
The list of banned biocidal products is reported in the Official Journal of the European Union, Commission Regulation (EC) No 1451/2007 of 4 December 2007.

1.5.1 Remedial treatments

Since the last few years the main chemical remedial treatments against wood biotic degradation have consisted of the application on wood of preservative formulations whose main active ingredients were: mercury, copper, arsenic or other metal and organometal compounds, boron compounds phenolic and trisubstituted tin compounds, thiazoles, polyoxins, petroleum and tar oils, alkylammonium compounds and N-trichloromethythio or phithalimide biocides (Schultz, 2008).
Their modes of action on fungi include inhibition of respiration (copper and arsenic compounds, phenolic compounds, thiazoles such as isothiazolones), inhibition of biosynthetic reactions in cells such as chitin synthesis (polyoxins) and lipid synthesis (imidazoles, pyrimidines, triazoles), inhibition of cell division (benzimidazole derivatives) and non-specific inhibition of several enzymatic processes at the same time (quaternary ammonium compounds, mercury-based compounds, N-trichloromethylthio or phithalimide compounds).

The main active ingredients of preservative formulations to control attack by insects are included in the categories of pyretroids, organophosphorous compounds, carbamates cyclodienes and gamma-HCH. Pyrethroids are mainly used as insecticidal components in many organic-solvent wood preservative formulations and as soil treatments against termite infestations. All these insecticides exert an effect on the insect nervous system by interfering with axonal transmission (pyrethroids), inhibiting acetylcholinesterase activity (organophosphorous compounds, carbamates) or causing excessive release of acetylcholine (gamma-HCH).

Most of the active principles reported above have been banned by the European Community because of their persistence in the environment and high toxicity for human body. Nowadays some copper compounds, quaternary ammonium compounds and boron compounds are admitted, under specific concentration levels. Most of these products have been also used for preventive purposes and will be discussed in the following section.

Copper compounds have been used as fungicides over a century and copper sulphate or other salts have been applied against fungi causing diseases in agricultural crops as well as decay in wood but they are scarcely resistant to water leaching (Shultz, 2008). Other compounds have also been used in the past (e.g. copper oxide, copper naphthenate, copper-8-hydroxyquinoline complex, copper chelates with dialkyldithiocarbammates). The mode of action proposed are the non-specific denaturation of proteins and enzymes, due to the Cu^{2+} affinity for thiol groups in the fungal cell, and the inhibition of respiration due to Cu2+ interference with the activity of conversion of pyruvate to acetyl coenzyme A.

Alkylammonium compounds, especially quaternary and tertiary amine salts, are general biocides that inhibit the cell respiratory activity and affect the semi-permeable membrane causing leakage of cell constituents. Tertiary amines and tertiary amine salts are thought to inhibit extra-cellular hydrolytic enzymes. They are also used as bactericides and surfactants because they are cheap; the efficacy of the addiction of inorganic copper to quaternary ammonium compounds against wood decay fungi and termites has been proved (Tsunoda, 1987; Hwang, 2007).

Boric acid and borates are toxic to all cells - fungi and insects included - forming stable complexes with vitamins and coenzymes, inhibiting enzyme function and with *cis*-adjacent hydroxyls and alpha- hydroxyls carboxylic acids, causing the depletion of available extracellular and intracellular substrates and affecting the biosynthetic activity of the fungal cell (Goodell, 2003). Borate complexes associated with membranes also affect cell transport mechanisms. In presence of borates fungi are often free to continue metabolic activity and growth, so borates can be generally considered as biostatic rather than biocidal agents.

Chemical treatments can be applied on wood by differing techniques as spraying, painting, dipping or impregnating in the presence of vacuum and/or pressure. With painting or spraying, only the wood surface is interested by the treatment, whereas dipping allows an increasing penetration depth; with under-vacuum and/or pressure treatments the whole material can be impregnated.

1.5.2 Preventive treatments

Chemical preventive treatments have been classified in some major groups:

a. tar oil preservatives
b. water-borne preservatives
c. organic solvent-based preservatives

a. The first category comprises creosote and coal tar creosote, which have been used as preservatives throughout the world since the 18th century, mainly to preserve ship's timbers. In Europe it has been restricted to some extent by Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations because of its demonstrated hazardous properties.
So water-borne preservatives and organic solvent-based preservatives remain the more used systems nowadays.

b. Water-borne preservatives are acqueous solutions of salts having the advantages to be odourless, to leave the wood clean for handling and paintable after treatment and to be combined with fire-retardant chemicals. The main disadvantages are that wood may

swell and that many salt treatments are not chemically fixed in wood and will leach.
The formulation of water based preservatives usually consists of a main biocide, sometimes a secondary biocide and other additives (fixatives, water repellents).
Mercury, chrome and arsenic salts have been used in the past as biocides or fixatives but have been now removed from the market because of their toxicity. Copper, sometimes zinc and silver are the main metals used in preventive formulations at present. Ammonium compounds and boric acid/borates are also used as active principles.
The ammonium, boron, copper, silver an zinc active substances in biocides listed by the European Biocidal Products Directive 2007 reported in par. 1.5.4.

c. Organic solvent preservatives consist of biocidal compounds dissolved in a volatile or non-volatile non polar organic solvent.
The main solvent carriers used in the past and now banned were heavy fuel oil, kerosene, petrol and white spirit. Waxes, varnishes and vegetal oils, largely diffused as wood finiture treatments in the past, are now still in use (Svensson, 1987).
Linseed oil, for example, has an intrinsic moderate action against biological colonization because of its hydrorepellent characteristic that prevents wood from reaching humidity higher than 20-30%. It has been used since ancient times (it is also reported by Vitruvio – 23-79 d.C. – in his Naturalis Historia; Scamozzi, famous architetc of the XVII century, suggested the use of linseed oil against insects). The practice of wood treatment with linseed oil is also accepted nowadays, despite its relative efficacy, as a means of surface protection. However, the hydrorepellence effect is easily lost for wooden artefact preserved outside, continously exposed to atmospheric agents (Rapp, 2001).
Pentachlorophenol (PCP) naphtenates, chlorinated naphthalenes, copper-8-quinolinolate, organotin compounds, cyclodienes and expecially organophosphorous compounds (mainly synthetic pyrethroids such as permethrin) have been added in the past to organic solvents such as oils and waxes, but their toxicity for human health is known nowadays.
The addition to hydrorepellent solutions of primary, secondary or tertiary branched chain carboxylic acids combined with copper or zinc to form “soaps” is also reported, together with the addition of CCA (copper-chromium-arsenic preservative, the most diffuse copper compound in wood preservation field until the last few years, now banned) (Treu, 2003).

1.5.3 Modification of wood by sol-gel process for preservative purpose

In the last decade, wood modification techniques allowing the improvement of wood durability without the use of biocides have developed quite rapidly as an alternative to the chemical preventive treatments listed above (par.1.5.2).
Treatments of wood modification based on the sol-gel process, whose application on wood for the improvement of physical-mechanical properties has been reported in par. 1.4.2, have been also considered.
An overview of several sol treatments for the antimicrobial protection of wood is reported by Mahltig (Mahltig, 2008).
Mahltig identifies two types of sol-gel treatments being useful for protecting wood from biodecomposition:
a) formulations without additional biocides;
b) formulations with added biocides.

If the siloxane materials polymerize onto the cell walls they can strongly influence the biotic resistance of the wood by modifying its physico-chemical characteristics. In fact, wood results less recognizable by biodeteriogens as a substrate adequate for their development. In addition these treatments could reduce the moisture sorption properties hence limiting biological attacks and enhancing natural durability (Terziev, 2009).
Several groups reported some enhancement of the stability of wood against the attack by fungi or termites after treatments with pure silica sols (Donath, 2004; Temiz, 2006).
The rate and the uptake of moisture by the modified wood are both reduced as a function of the hydrophobic nature of polymer in the cell wall. Finally, the uptake of fungal metabolites capable of degrading the wood cell components is reduced or prevented, since the impregnated polymer is able to block the cell wall microporous network through which these agents diffuse (Hill, 2004; Eaton, 1993).
The potential use of a wide range of organosilicons as protective agents against basidiomycetes attack of wood used in outdoor applications was investigated by De Vetter and good efficacy results were obtained especially when a sample weight gain above 20-30% was achieved with the treatment (De Vetter, 2009; De Vetter, 2009b).
Polymeric siloxane materials can be obtained by sol-gel processing both

tetraalkoxyslanes, like $Si(OEt)_4$, and trialkoxysilanes, like $RSi(OEt)_3$, the last bearing organic functional groups with Si-C bonds which are stable against hydrolysis. The use of trialkoxysilane leads to the formation of inorganic-organic hybrid materials containing organic functions with desired properties (Brinker, 1990; Loy, 1995; Shea, 2001; Sanchez, 1994). By using TEOS as starting materials for the siloxane polymers, a significant resistance to fungal (Tanno, 1997) and to insect attack has been observed (Cookson, 2007, Reinsch, 2002, Terziev, 2009).

Inorganic sols may be applied not only in their pure form but also with addiction of a broad range of organic or inorganic additives. The preparation of SiO_2 inorganic matrices added of metal complexes for general purposes is reported in literature (Breitscheidel, 1991). Silicones and organosilicones seem not to interfere with the efficacy of the biocides (De Vetter, 2009) and have some potential to limit their release, increasing their lasting effectiveness and reducing their potential threat to the environment; for, example, the presence of silica xerogels can reduce the leaching of metals or boric acid and boron compounds (Saka, 1997; Terziev, 2009), thus increasing biological resistance against wood degrading fungi and insects (Kartal, 2007): nevertheless, if some of these active ingredients and compounds are not fixed into wood by chemical reactions, they seem to be easily leached out by water (Kartal, 2007).

Some efficacy tests on silica formulations with addiction of silver, copper, zinc or boron for general purposes (Trapalis, 2003) and in the field of wood properties improvement (Mahltig, 2008; Kartal 2007, Yamaguchi, 2005) are reported in literature.

1.5.4 Elements involved as active principles in inorganic biocides permitted till now

COPPER

Despite the fact that copper-based preservatives have been used for more than 200 years, they remain one of the most important active ingredients for wood preservation (Eaton, 1993). Their main advantages are: favourable efficacy/cost ratio, broad spectrum of activity, formulation ease and availability. Their two major disadvantages are a notable weakness against copper tolerant insects and fungi and an insufficient fixation (Humar, 2007).

The first disadvantage was overcome in the past by the addiction of co-biocides in the

formulation (e.g. arsenic and later boron compounds); the second one by using combinations with chromium compounds.

Chromated-copper-arsenate (CCA) and chromated-copper-borate (CCB) were used for wood preservation from the fifties and for more than 50 years. They belong to the so-called "first generation of copper preservatves".

Drastical changes in the wood preservation markets happened in the last 20 years: arsenic compounds were banned in the European Union; CCA was removed from residential applications in the USA and Canada; finally, chromium compounds were classified as biocides by a European Commission and removed from the market (Biocidal Products Directive BPD 98/8/EC (1998)).

All these changes forced to search for new copper preservative formulations, called "second generation of copper preservatives".

At present Conventional chemical wood protection is based on a broad spectrum of biocide formulations such as copper / organic biocides, copper organometallics and metal free preservatives (Hughes 2004).The main products against wood decay are copper salts with other "co-formulating" substances such as boron, azoles and organic compounds. The role of these compounds is to increase the protective effect and the fixation of the copper derivatives to the wood reducing their dispersion in the environment. The main commercial copper-based products in use, especially for wood objects exposed outside not in contact with the soil, are: TANALITH E (boric acid 5%; copper(II)hydroxycarbonate 22%, tebuconazole 0.5%); KEMWOOD ACQ 1900 (copper tetramine 23%; n-alkylbenzyldimethilammoniumchloride 4.8%); WOLMANIT CX-8 (bis(n-cyclohexylldiazeniumdioxy)-copper 2.8%, copper(II)hydroxycarbonate 13%; boric acid 4%); WOLMANIT CX-10 (bis (n-cyclohexylldiazeniumdioxy)–copper 3.5%, copper(II)hydroxycarbonate 16.3%, boric acid 5%).

Some copper complexes have been also considered, for examples with amines (Zhang, 2000; Mazela, 2005); among them, ethanolamine was tested (Cao, 2004) owing to its copper fixative properties (Humar, 2007). A disadvantage of the use of copper-ethanolamine preservatives is the fact that their fixation is not as effective as the fixation of copper–chromium-based ones (Zhang, 2000, Temiz, 2006), and therefore, secondary biocides (boron, quaternary ammonium compounds, water-soluble azoles or water repellent additives such as wax emulsion) are necessary. It was proposed that they could even stimulate fungal growth because they contribute significant amounts of

nitrogen to the wood, which is required for wood-decay fungi for the synthesis of proteins and other cell constituents: however, some ethanolamine retardant effects on fungal growth have been demonstrated (Humar, 2007; Humar, 2008).

A theoretical-industrial correlation and perspective on copper-based wood preservatives, with particular attention to thermodynamic and kinetic aspects on copper-wood fixation mechanism has been recently proposed by Craciun (Craciun, 2009).

Table 8 shows the list of copper substances indicated as existing biocides by the European Biocidal Products Directive 2007 (Table 8), most of which are now banned because of their high toxicity to human or to the environment due to their low resistance to leaching and/or to the toxic or mammalian effect of added co-fungicides and fixatives.

Table 9 shows the most diffuse wood preservatives based on copper of the first (no longer on the market) and of the second generation and some recent formulations proposed in literature (Table 9).

Table 8 Active substances indicated as existing by The European Biocidal Products Directive (2007). PPP = Plant protection product.

Copper biocides	
Name (EINECS and/or others)	**EC number**
Copper thiocyanate	214-183-1
Copper oxide	215-269-1
Dicopper oxide	215-270-7
Copper(II) chloride	215-704-5
Copper dinitrate	221-838-5
Copper	231-159-6
Copper chloride	231-842-9
Copper sulphate	231-847-6
Copper sulphate pentahydrate	231-847-6
Oxine-copper	233-841-9
Copper(II) carbonate-copper(II) hydroxide (1:1)	235-113-6
Bis(1-hydroxy-1H-pyridine-2-thionato-O,S)copper	238-984-0
Copper dihydroxide	243-815-9
bis(tetraamminecopper) carbonatedihydroxide	272-415-7
Copper, EDTA-complexes	290-989-7
Bis(2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzofuran dionato-O2,O3)copper	304-146-9
Bis[1-cyclohexyl-1,2-di(hydroxy-.kappa.O)diazeniumato(2-)]-copper	312600-89-8
Aluminium sodium silicate-silver copper complex/Silver Copper Zeolite	PPP
Acypetacs copper Mixture	Mixture
Mixture of chromium trioxide (34,2 %), diarsenic pentoxide (24,1 %), copper(II)oxide (13,7 %) = CCA	Mixture
Naphthenic acids, copper salts	215-657-0

Table 9 Copper first and second generation wood preservatives and recent formulations proposed in literature

Copper substance/mixture	Preservative generation
$Na_2Cr_2O_7$+$CuSO_4$	first
CCA= copper-chromium-arsenic (As_2O_5*$2H_2O$ + $CuSO_4$*$5H_2O$ + $K_2Cr_2O_7$)	first
CCB= copper-chromium-boron preservatives	first
CCF = copper-chromium-phosphorus preservatives	first
ACA = ammoniacal copper arsenate	first
ACZA = ammoniacal copper zinc arsenate	first
ACB= ammoniacal copper borate	second
Ammoniacal copper carbonate	second
ACQ=ammoniacal copper quaternary ammonium compounds	second
AmCC= ammoniacal copper carboxylates	second
ammoniacal copper citrate	second
ammoniacal copper dithiocarbamates	second
Lignin-copper treatments	second
Salts of polyhydroxycarboxylic acids (sodium gluconate, sodium glucoheptonate)	second

BORON

Boron compounds have been used for wood treatments for more than 30 years, mainly with the aim to improve wood fire resistance Treating wood with a boron compound also confers biological stability (Hashim, 1994; Drysdale, 1994). In fact, boron and borates have a broad spectrum of fungicidal action (Humar, 2007) and result also useful for the preservation against insects such as termites (Nunes, 2007).

They have been largely used as co-biocides to improbe efficacy of copper-based preservatives and, after the banishment of the most diffuse copper formulations (Biocidal Products Directive BPD 98/8/EC (1998)), the number of boron formulations on the market has massively increased.

The main advantages are the high efficacy and broad spectrum of action (the minimum efficacy concentration values for different fungal and insect species are reported by Kartal (Kartal, 2007) and the solubility and mobility of borates that allows to treat wood species that are difficult to treat with copper-based preservatives; this is because even when not applied on the whole cross section, they redistribute by diffusion if sufficient moisture is available in wood.

Unfortunately, the majority of boron is released from impregnated wood during its service life (Peylo, 2009): the high leachability of such substances makes the treatment ineffective for wood that is in contact with the ground or exposed to weather, and

current standards (AWPA, 2007) allow for the use of borates in above-ground environments protected from wetting rain.

To achieve extended efficacy of boron preservatives, a chemical treatment to reduce leaching is required.

The strategies employed to reduce boron leaching have been summarized by Obanda (Obanda, 2008). The methods tested include:

- surface coating of boron-treated wood with layers of varnish, resin or hydrophobic wax (Peylo, 2009) or impregnation with an acqueous liquid dispersions or emulsions which contain borates and rosin/resin derivatives simultaneously;
- organo boron compounds (OBC), especially aromatic acids such as phenyl boronic acid (PBA), whose leach resistance is supposed to be due to their possibility to interact with the aromatic subunits of lignin and to the restricting access of water to the boron;
- the chemical complexation of a borate compound with an agent capable of forming a water-insoluble complex in wood
- inorganic metal and borate combinations (zinc borate, copper borate complexes.). E.g. Zinc and copper borates are effective against termites, but impregnation is difficult because of their low solubility in water and addiction of zinc-borate powder to the adhesive used for board manifacture is preferred (Manning, 2008)
- stabilized boron esters and other complexes, for example trialkyl amine borates or trialkanolamine borates.

The formation of insoluble metaborates by impregnating wood samples with a saturated borax solution and then dipping them in Zn^{2+} oc Cu^{2+} solutions was investigated and resulted enhancing the decay and termite resistance (Furuno, 2006). Ammoniacal and amine metallo-borates were also tested (Obanda, 2008).

Finally, boron compounds added to silicon emulsions are already known to be well fixed into wood (Yamaguchi, 2003, Kartal, 2007).

Table 10 shows the list of boron substances indicated as biocides by the European Biocidal Products Directive 2007 (Table 10). Among them, Disodium tetraborate (Borax) has been admitted by the EU (2009/91/EC) together with Disodium octaborate tetrahydrate (2009/96/EC).

Table 10 Active substances indicated as existing by The European Biocidal Products Directive (2007).

Boron-based preservatives	
Name (EINECS and/or others)	EC number
Diboron trioxide	215-125-8
Boron	231-151-2
Tetraboron disodium heptaoxide, hydrate	235-541-3
Hexaboron dizinc undecaoxide/Zinc borate	235-804-2
Barium diboron tetraoxide	237-222-4
Silver-zinc-aluminium-boronphosphate glass/Glass oxide, silver- and zinc containing	Not yet allocated
Silver sodium borosilicate	
Boric acid	233-139-2
Boric oxide	215-125-8
Natural boric acid	234-343-4
Perboric acid, sodium salt	234-390-0
Orthoboric acid, sodium salt	237-560-2
Disodium octaborate tetrahydrate	234-541-0
Disodium tetraborate (Borax)	215-540-4

ZINC

Zinc mainly has an antibacterial activity but it is also known to inhibit fungal growth at adequate concentration. In fact, at low concentrations Zinc is an essential nutrient for fungal growth and participates in many diverse cellular processes (Ross, 1994) (for example, zinc functions as a cofactor and in the structure of many enzymes involved in intermediary metabolism) but, at higher concentrations, it becomes toxic, since heavy metals in general are potent inhibitors of enzymatic reactions and e.g. the addiction of 1mM Zn is known to decrease the activity of laccase (Baldrian, 2002; Baldrian, 2006). An example reported in literature deals with the *Neocosmospora vasinfect*, which grows optimally at zinc concentrations of 25 and 1OOpM, but is inhibited (90% reduction of growth occurs) if subjected to 1OOmM zinc (Paton, 1972).

Zinc preservatives don't represent commercially active products at the moment, with the only exception of zinc borates (actually used against fungi and termites) (Schultz, 2008) but a wide range of zinc-based products have been used in the past, among which Chromate and Zinc chloride formulation (CZC: Zn 80% as ZnO and Cr^{6+} 20% as CrO_3, zinc chloride ($ZnCl_2$, first half of the XX^{th} century) and zinc naphtenate are well known.
The main problems related to these compounds were their leachability and/or their high toxicity.

Zinc nanoparticles have also been studied in the last years against mould fungi, decay fungi, and Eastern subterranean termites. Nano preparations of zinc showed low leaching of the nanometals compared with the rate of leaching for zinc sulphate (changes in charge and Van der Waals forces may account for the low leaching of nanometals) (Kartal, 2009b).

Recently Zinc oxide (ZnO) nanoparticles were synthesized and deposited on the surface of cotton fabrics using ultrasound irradiation (Li, 2007) and both their significant bactericidal effect against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) cultures even in a 0.75% coated fabric (wt %) and their resistance to leaching have been reported (Perelshtein, 2009).

Wood impregnation with carbon-shell nanoparticles with a zinc core were proposed to inhibit fungal decay or destruction of the wood by insects, also for living woody plants (Liu, 2006).

Table 11 shows the list of zinc substances indicated as existing biocides by the European Biocidal Products Directive 2007 (Table 11). Most of them, such as Hexaboron dizinc undecaoxide/zinc borate and zinc oxide, have been banned.

Table 11 Active substances indicated as existing by The European Biocidal Products Directive (2007). PPP=Plant protection product.

Zinc-based preservatives	
Name (EINECS and/or others)	EC number
Zinc bis(2-ethylhexanoate)	205-251-1
Zinc dibenzoate	209-047-3
Zinc oxide	215-222-5
Trizinc diphosphide	215-244-5
Zinc sulphide	215-251-3
Zinc	231-175-3
Zinc sulphate heptahydrate	231-793-3
Hexaboron dizinc undecaoxide/Zinc borate	235-804-2
Pyrithione zinc	236-671-3
Zinc bis(diethyldithiocarbamate)	238-270-9
Zinc neodecanoate	248-370-4
bis(2-ethylhexanoato-O)-.mu.-oxodizinc	259-049-3
Zinc, isodecanoate isononanoate complexes, basic	282-786-7
Silver-zinc-aluminium-boronphosphate glass/Glass oxide, silver- and zinc containing	Not yet allocated
((1,2-Ethanediylbis(carbamodithioato))(2-))manganese mixture with ((1,2-ethandiylbis(carbamodithioate))(2-))zinc/Mancozeb	PPP
Aluminium sodium silicate-silver zinc complex/Silver-Zinc-Zeolite	PPP
Acypetacs zinc Mixture	

SILVER

After the recent restriction on copper based preservatives, the use of wood preservatives based upon silver (Ag) instead of copper was encouraged. Silver has been shown to have potential as a viable, safe, and cost-effective preservative. It has a long history of use in medicine as an antimicrobial agent. Several studies have demonstrated that silver ions are selectively toxic for prokaryotic microorganisms, with little effect on eukaryotic cells (Bae, 2010). The anti-bacterial activity of silver is dependent on the silver cation (Ag^+), which binds strongly to electron donor groups on biological molecules containing sulfur, oxygen or nitrogen. The silver ions act by displacing other essential metal ions such as Ca^{2+} (Dowling, 2001, 2003). The mechanism of action of silver is especially linked with its interaction with thiol group compounds found in the respiratory enzymes of bacterial cells (Klasen, 2000). A synthesis of silver mode of action is reported by Dorau (Dorau, 2004).

Silver can be used as colloidal, ionic or true nano-sized particles (Green, 2007). In the last few years the study of possible applications of silver nanoparticles has been developed. Silver nanoparticles have been used as bactericides in coatings for medical articles and domestic materials, optionally adsorbed on fillers or pigments; toothbrushes with bristles are uniformly impregnated with antibacterial/antifungal Si oxide-coated Ag (prepn. given; at 5 ppm) nanoparticles have been created; Ag NP have been deposited on the surfaces of TiO_2 particles to synthesize antifungal powders (Zhang, 2009). The silver nanoparticles show efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms (Rai, 2009).

If silver is well known for its antimicrobial activity, it has been recently studied also for inhibition of brown-rot fungi, termite damage, and mold contamination. To date, some silver formulations (~ 1% Ag) have been shown to be moderately resistant to brown-rot decay, to inhibit termite damage with 100 percent mortality of termites, and to resist colonization by three common mold species (Dorau, 2004). Silver ions in solution seem to inhibit the activity of their cellulase enzymes in wood decay fungi; the concentration for fungicidal activity resulted 1.9 µg/L.

Table 12 shows the list of zinc substances indicated as existing biocides by the European Biocidal Products Directive 2007 (Table 12).

Table 12 Active substances indicated as existing by the European Biocidal Products Directive (2007). PPP= Plant protection product.

Silver-based preservatives	
Name (EINECS and/or others)	EC number
Silver nitrate	231-853-9
Silver chloride	232-033-3
Disilver(1+) sulphate	233-653-7
Disilver oxide	243-957-1
Silver-zinc-aluminium-boronphosphate glass/Glass oxide, silver- and zinccontaining	Not yet allocated
Silver sodium hydrogen zirconium phosphate	Not yet allocated
Silver zeolite A	
Silver sodium borosilicate	
Aluminium sodium silicate-silver complex/Silver zeolite	PPP
Aluminium sodium silicate-silver copper complex/Silver Copper Zeolite	PPP
Aluminium sodium silicate-silver zinc complex/Silver-Zinc-Zeolite	PPP

A list of commercial silver-containing formulations is reported by Green, together with some efficacy results against termites (Green, 2007).

Attempts to incorporate silver nanoparticles in alkylsilane systems using the sol–gel method are also reported in literature. For example, Bryaskova tested PVA/TEOS/Ag-Np thin films against E. coli, S. aureus and P. Aeruginosa (Bryaskova, 2010); formulations for textiles with nanosized crystalline silver particles, TEOS and amine compounds were also tested in which amines reduce $AgNO_3$ and the SiO_2 particles prevented the aggregation and further growth of silver particles (Mahltig, 2009).

1.6 Aim of the work

The present work arises from the need to achieve novel preventive treatments for the preservation of wood against fungi and termites with broad spectrum of action, low efficacy concentration, low environmental impact and good fixation into the wood.

Two ways were selected to reach this aim, both exploiting the deterrent properties of Ag, B, Cu, or Zn and having the purpose to reduce their leachability.

A. Modification of wood with inorganic or hybrid inorganic-organic systems based on silica sol-gel materials (starting from alkoxysilanes) incorporating the metals or anchoring them through coordinative linkages.

For this purpose, modification of wood with siloxane materials bearing amino groups was performed. Tetraethyl orthosilicate (TEOS) and triethoxysilanes functionalized with amino groups, such as 3-aminopropyltriethoxysilane (APTES), were used as precursors; their hydrolysis and co-condensation (the sol-gel process) was allowed to take place *in situ*. This process generates hybrid inorganic-organic silica xerogels particles penetrating the wood cell walls.

The TEOS/APTES molar ratio has been varied in order to obtain different concentrations of amino-groups in the hybrid material (Palanti, 2010).

The improvement of wood biological resistance due to the presence of amino-groups was already reported in literature: Ghosh et al. (2008) reported on the efficacy of silicones with quaternary ammonium, primary amino and alkyl groups against blue stain and mould fungi in wood whereas Donath et al (2006) studied amino-functional silanes affecting fungal growth.

The TEOS/APTES treatments were performed not only alone but also with addiction of metal species (Feci, 2009), that were encapsulated in the silica xerogel and/or grafted to the amino functions through coordinative interactions (Klonkowsky, 1999), avoiding or minimizing leaching (Palanti, 2010; Palanti, 2011).

A pictorial view of amino-functionalized silica anchoring copper (II) cations is shown in Figure 16.

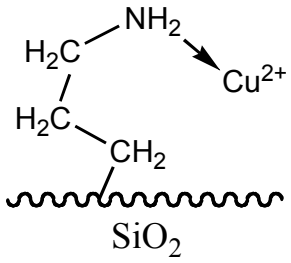


Figure 16 Sketches of the copper grafted to the hybrid inorganic-organic gel. (Palanti, 2008).

B. Production of systems based on metal chelates.

The employment of metal chelates has been studied and applied in the last decades in many fields, such in animal nutrition (Kratzer,1986; Predieri, 2003; Predieri, 2005). The stability of metal chelates complexed e.g. by aminoacydes (methionine, glycine) can result an advantaegous characteristic also in the field of wood preservatives, since it could ensure very low metal leaching and low toxicity of those complexes may represent an eco-compatible alternative to the previous copper based preservatives.

- Water based preservative solutions of copper aminoacid derivatives chelates were tested as fungal deterrents (Palanti, 2008). These species are rather stable, in some cases even in acidic environment, and hence could ensure low copper leaching. Treatments based on the combined action of copper chelates and boric acid were also performed to test both the possibility to improve the preservative efficacy of the new formulations and their capability to increase boron fixation to wood.

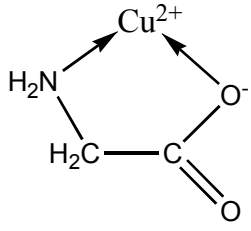


Figure 17 Amino acid (glicine) chelation of copper.

- Organic based preservative solution of copper and zinc chelates were tested as preservatives against termites. Linseed oil, often used for wood surface protective and aesthetic treatments, was chosen as solvent, in order to add to an already diffuse practice also the property of durability enhancement.

Various methods of treatment, ranging from immersion to impregnation under vacuum, were performed and for each treatment the interaction and diffusion into the wood structure, the preservative efficacy on fungi and/or termites and the improvement of other chemical or physical properties were evaluated.

2. Experimental

2.1. Materials and analytical instruments

2.1.1. Starting chemicals and analytical instruments

All starting chemicals used for the preparation of wood preservative formulations were commercial products (Sigma-Aldrich, Carlo Erba, Fulka, Akzo Nobel, Novus), used without further purification. All commercial and synthesized products were analytically characterized. Elemental analyses were performed on a Carlo Erba CHNS-O EA1108 automatic equipment. FT-IR spectra (MIR range: wavenumber 4000 - 400 cm^{-1}) were recorded on a Thermo-Nicolet Nexus spectrophotometer by means of the Thermo Smart Orbit ATR 2mm diamond accessory. A Jobin-Yvon Horiba LabRam linearly polarized He-Ne laser (632.8 nm) Raman spectrometer equipped with an Olympus microscope (10x, 50x, 100x) and laser power on the sample of about 3 mW was employed for μ-Raman analyses. Morphology and compositional data were obtained using a Jeol 6400 Scanning Electron Microscope (SEM) equipped with a Oxford INCA Energy Dispersive System (EDS) microanalysis (15-20 kV, 1.2 nA, electron beam about 1 mm in diameter, 60 s counting time; standards comprise pure elements, simple oxides or simple silicate compositions). An Environmental Scanning Electron Microscopy (ESEM) Quanta 200 (Fei Corporation, Eindhoven, The Netherlands) equipped with a EDAX EDS spectrometer was also employed. ^{29}Si and ^{13}C CP/MAS NMR spectra were recorded with a Bruker 400 WB spectrometer with carrier frequency of 400.13 MHz (1H) equipped with a double resonance probe. Samples were packed in 4 mm zirconia rotors and spun at 9.5 kHz for silicon and 11 kHz for carbon. ^{29}Si CPMAS spectra were obtained with pulse length 4.3 μs, scan delay 10 s; 6k scans with 2 contact time of 5ms. SP sequence was used for quantitative analysis. Q8M8 was used as external secondary reference. ^{13}C CPMAS spectra were acquired with 90° pulse length of 3.36 μs, contact time of 2 ms and scan delay of 5 s. Adamantane was used as external secondary reference.

Thermogravimetric analyses (under nitrogen, 10°/min) were performed with a Perkin-Elmer Delta series TGA 7 equipment. Electron Spin Resonance (EPR) spectra of products containing copper were obtained with a Bruker 200D SRC X-band spectrometer.

Microwave frequencies were measured with an XL Microwave model 3120 counter (Jagmar, Krakow, Poland). The spectrometer was interfaced with a PS/2 Technical Instruments Hardware computer and the data acquired using the EPR data system CS-EPR produced by Stelar Inc., Mede, Italy. A Jobin Yivon Ultima2 equipment was employed for Inductively Coupled Plasma Atomic Emssion Spectroscopy (ICP-AES) elemental analyses; the operating conditions were: power generator (normal condition 1000 W, plasma gas flow rate 12 L/min, nebulization pressure 3 bar, nebulization flow rate 0.50 L/min and pump speed of 20 rpm. An LTQ XL FT Orbitrap Mass spectrometer coupled with HPLC Dionex Ultimate 3000 accessorized with Electrospray (ES) source was used for qualitative analyses of silica fragments released from treated samples to water solutions. The system is based on Ion Trap technology of new generation (Linear Trap).

2.1.2. Wood samples features

Sets of *Pinus sylvestris* L. wood samples were used as base-materials on which preservative treatments were developed and tested. The employment of the *Pinus sylvestris* L. sapwood is usually recommended for the evaluation of preservative effectiveness by The European Standards because of its high impregnability and low durability especially to fungal attack. Each set was cut from untreated boards without any defect in samples with their longitudinal faces parallel to the grain. Samples dimensions and replicates number were chosen depending on the European Standards recommendations for each kind of test or on test necessities. *Picea abies* L. sapwood samples were also employed for physical characterizations (Figure 18).

- *Pinus sylvestris* L. mini-block samples (0,5 cm_{rad} x 1 cm_{tang} x 3 cm_{long}, where rad = radial, tang = tangential, long = longitudinal directions along the grain) were employed for the accelerated efficacy tests against fungi basidiomycetes (EN 113, 1996) and for most of the analyses for the characterization of the wood treatments; they were divided in sets of samples having the same number of rings (2 or 3) in their transversal face;
- *Pinus sylvestris* L. 1 cm_{rad} x 1 cm_{tang} x 3 cm_{long} ("t blocks") samples and 1,5 cm_{rad} x 2,5 cm_{tang} x 5 cm_{long} ("T blocks") samples were employed for the evaluation of impregnation treatments preservative effectiveness against termites *Reticulitermes Grassei* (Accelerated test as in Nunes (1997) and EN117 (2005) respectively);
- *Pinus sylvestris* L. mini-tablets (0,3 cm_{rad} x 3 cm_{tang} x 4 cm_{long}) samples were employed

for the evaluation of immersion treatments preservative effectiveness against termites (Isoptera) *Reticulitermes lucifugus* (Fabr) and *Kalotermes flavicollis* (Rossi).Sly thickness was chosen to make it possible to observe termites behaviour in wood during the test;

- *Picea abies* L. tables (0,5 cm_{rad} x 7 cm_{tang} x 20 cm_{long}) and block samples (2 cm_{rad} x 2 cm_{tang} x 4 cm_{long}) were employed for the evaluation of physical properties of treated wood (sorption (Allegretti, 2008) and dimensional stability (Allegretti, 2007) respectively). A maximum tolerance of ± 0.5 mm was accepted for each sample dimension.

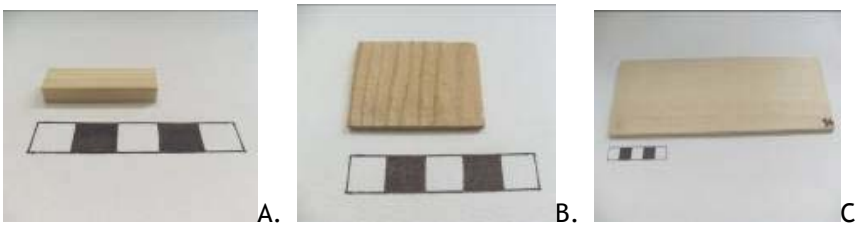

A. B. C.

Figure 18 Some examples of wood samples. A. *Pinus sylvestris* **L. mini-block sample; B.** *Pinus sylvestris* **L mini-tablet sample; C.***Picea abies* **tablet sample.**

Pinus sylvestris L FTIR-HATR bands (cm^{-1}): 3331s (OH str.), 2920-2854m (C-H str.), 1734-1738m (C=O str. Xylanes), 1650m-1593m-1506m (str. aromatic rings of lignin), 1370m (OH bend. rings of polysaccharides), 1459m-1423m-1322m-1232m (CH bend. cellulose), 1159s-1023vs (C-O str.), (Figure 19). SEM images on the wood transversal section were collected to verify the wooden structure conditions (absence of crushes or cleavages). EDX microanalysis revealed an approximately homogeneous Ca distribution towards the whole section (Ca/O atomic ratio about 0.02).

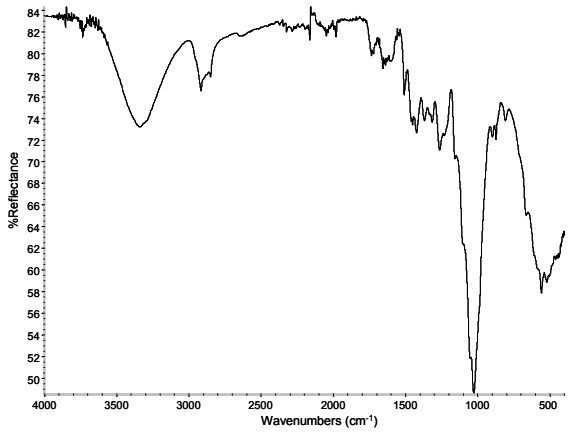


Figure 19 FTIR-ATR spectrum of the longitudinal surface of an untreated pinewood sample.

2.2. Preparations of the preservative formulations

2.2.1. TEOS-APTES formulations

Hybrid organic-inorganic silica polymers were synthesized by sol-gel process. Tetraethoxysilane (TEOS) and 3-aminopropyltriethoxysilane (APTES) were employed as precursors (Figure 20), whose hydrolysis and co-condensation into the wood could lead to generate silica xerogel particles bearing amino groups interpenetrating the wood cell walls.

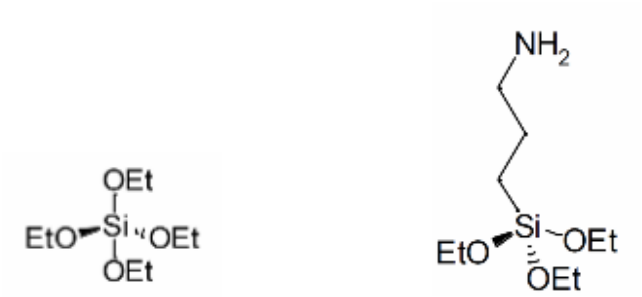


Figure 20 Tetreaethoxysilane (TEOS) (a) and Aminopropyltriethoxysilane (APTES) (b), employed as alkoxy precursors.

The TEOS/APTES molar ratio was varied obtaining different concentrations of amino-groups in the hybrid material. Adequate volumes of TEOS and APTES were poured in a schlenk container. Ethanol was added to dilute the solutions and facilitate their penetration into the wood structure.

1. Formulation 1: TEOS/ APTES 1:1 (v/v). TEOS (10 ml, 44,8 mmol), APTES (10 ml, 42.8 mmol), ethanol (20 ml).
2. Formulation 2: TEOS/ APTES 4:1 (v/v). TEOS (40.8 ml,182.7mmol), APTES (7.2 ml, 30.5 mmol), ethanol (12 ml).
3. Formulation 3: TEOS/ APTES 10:1 (v/v). TEOS (19.1 ml, 85.5 mmol), APTES, (1.9 ml, 8.1 mmol), ethanol (21 ml).

The prepared solutions were kept under nitrogen atmosphere (1atm) until utilization. They appeared fluid and uncoloured. Then, they were put in contact with the wooden

samples (see par.2.3.2). The amount of solution that was not absorbed into the wooden structure was kept on air in a watch glass until gelation and aging. The white opaque homogeneous xerogels obtained were characterized by FTIR-HATR analysis (peaks (cm^{-1}): 2920-2872, mw, 1560 mw,1480 mw, 1385 mw, 1324 mw, 1005 vs, 767 m,689 m, 581 m). Therefore, an amount of xerogel from formulation 1 was powdered and analyzed by means of MAS-NMR spectroscopy.

2.2.2. TEOS-APTES-COPPER formulations

Hybrid organic-inorganic silica polymers as described in par.2.1.1 were prepared with addition of copper. In fact, these systems are able to graft copper ions through coordinative interactions with the amino groups, avoiding or minimizing leaching. The TEOS/APTES ratio was varied in the different formulations. The APTES/Cu 5:1 molar ratio was chosen because supposed adequate to guarantee the strong copper fixation through multiple N-coordination on a single cation. Two different methods of wood treatment were tested. In the "one step" method (see par. 2.3.2.2) TEOS, APTES and ethanol and copper chloride ($CuCl_2*2H_2O$) were stirred in a schlenk container until complete solubilization of the copper salt. In the "two step" method, only TEOS, APTES and ethanol were employed to prepare the impregnation solution. Impregnated wood samples were dried on air and subsequently dipped in a 0,1M acqueous solution of copper sulphate pentahydrate ($CuSO_4*5H_2O$).

Formulations for the one-step method:

4. Formulation 4: TEOS/ APTES 1:1 (v/v) + Cu. TEOS (10 ml, 44.8 mmol), APTES (10 ml, 42.8 mmol), copper (II) chloride (1.454 g, 8.53 mmol), ethanol (20 ml).
5. Formulation 5: TEOS/ APTES 10:1 (v/v) + Cu. TEOS (19,1ml, 85.5 mmol), APTES (1.9 ml, 8.1 mmol), copper (II) chloride (0.290 g, 1.7 mmol), ethanol (21 ml)
6. Formulation 6: TEOS/ APTES 20:1 (v/v) + Cu. TEOS (19.05 ml), APTES (0.95 ml), copper (II) chloride (0.145 g), ethanol (20 ml).

Formulations for the two-step method:

7. Formulation 7: TEOS/ APTES 4:1 (molar ratio) + Cu. (First step): TEOS (6.25 ml, 28

mmol), APTES (1.64 ml, 7 mmol), ethanol (2 ml). (Second step): 0,1M acqueous solution of copper (II) sulphate ($CuSO_4*5H_2O$).

8. Formulation 8: TEOS/ APTES 6:1 (molar ratio) + Cu. (first step): TEOS (40.8 ml, 180 mmol), APTES (7.2 ml, 30 mmol), ethanol (12 ml). (second step): 0,1M acqueous solution of copper (II) sulphate ($CuSO_4*5H_2O$).

The one-step prepared solutions were kept under nitrogen atmosphere (1 atm) until utilization. They appeared fluid and blue coloured. Then, they were poured to immerse the wood samples (see par. 2.3.2.2). The amount of solution that was not absorbed into the wooden structure was kept on air in a watch glass until gelation and drying. Blue opaque homogeneous xerogels were obtained. FTIR-HATR characterization of the residual xerogel: peaks at 2920-2872 cm^{-1} mw, 1560 cm^{-1} mw,1480 mw, 1385 mw, 1324 cm^{-1} mw, 1005 cm^{-1} vs, 767 cm^{-1} m,689 cm^{-1} m, 581 cm^{-1} m). μ-Raman characterization of the residual xerogel: main peaks at about 1800cm^{-1}, 1600 cm^{-1},480cm^{-1}. Further characterizations: EPR analysis of copper coordinative state; evaluation of Si/Cu and Cl/Cu atomic ratios into the xerogel from formulation 4 by means of SEM-EDX (Si/Cu = 14 ± 2 Cl/Cu= 4±0.4).

2.2.3. SODIUM METASILICATE-COPPER formulation

Silica gels were obtained from sodium metasilicate pentahydrate ($Na_2SiO_3*5H_2O$) as silica precursor instead of TEOS. The synthesis of silica xerogels from sodium metasilicate is well known (Zawrah, 2009). APTES was added to SiO_4^{4-} acqueous solution prepared from sodium metasilicate; their co-condensation was supposed to give rise to a hybrid organic-inorganic silica polymer functionalized with amino-groups such as in formulations 1-8. Copper ions grafted to the APTES amino groups were also obtained by adding a copper salt copper to the starting solution.

9. Formulation 9 : $Na_2SiO_3*5H_2O$/ APTES 1:1 (molar ratio) + Cu(Cu/APTES molar ratio 1 :5). Sodium metasilicate (3.88 g, 0.018 mol) was dissolved in distilled water (30 ml) (pH=12). Therefore, $CuCl_2*2H_2O$ (0.614g, 0.0036 mol) was dissolved in APTES (4.2 ml, 0.018 mol), under stirring. The APTES solution was added to the silicate solution and the system was acidified with HCl 37% (0.6ml) until pH 8-9 was

reached. The obtained solution was employed for the treatment of wood samples (see par.2.2.3). The amount of solution that was not absorbed into the wooden structure was kept on air in a watch glass until gelation (about 1 day) and drying. Pale blue opaque xerogels were obtained, FT-IR xerogel characterization: bands at (cm^{-1}): 3400m, 2920mw, 1457m, 1408m, 1052s, 953ms, 794m.

2.2.4. TEOS-COPPER formulations

TEOS-copper formulations with different Si-Cu molar ratio but without any addiction of APTES were performed on wood samples to evaluate TEOS capability to reduce copper leaching by physically retaining certain amounts of copper into the silica reticulation. The chosen TEOS-Cu molar ratios are listed below.

10. Formulation 10: TEOS/ Cu 10:1 (molar ratio). Tetraethoxysilane (TEOS, 11 ml, 49 mmol), copper (II) chloride (0.840g, 4.9mmol) and ethanol (11ml).
11. Formulation 11: TEOS/ Cu 25:1 (molar ratio). Tetraethoxysilane (TEOS, 11 ml, 49 mmol), copper (II) chloride (0.336 g, 1.97 mmol) and ethanol (11ml).
12. Formulation 12: TEOS/ Cu 50:1 (molar ratio). Tetraethoxysilane (TEOS,11 ml, 49 mmol), copper (II) chloride (0.1679 g, 0.98 mmol) and ethanol (11 ml)

After exposition to wood, they were kept on air in a watch glass until gelation and drying. Transparent homogeneus xerogels were obtained, with green colour weakening from Formulation 10 to formulation 12 (Figure 21).

Figure 21 Xerogels from TEOS-Cu formulations. From left to right: formulation 10 (TEOS/ Cu 10:1molar ratio); formulation 11 (TEOS/ Cu 25:1molar ratio); formulation 12(TEOS/ Cu 50:1molar ratio).

2.2.5. TEOS-APTES-ZINC formulations

Hybrid organic-inorganic silica polymers as described in par.2.2.1 were prepared with addiction of zinc. As for copper (Formulation 4-8), the system was supposed to able to graft zinc(II) cations through coordinative interactions with the amino groups, avoiding or minimizing leaching. Formulation 7, containing copper, was used by replacing copper (II) sulphate with zinc (II) sulphate. The two step method was followed (see par.2.3.2.2). As regards the one-step method, a ternary sol was prepared based on TEOS/APTES 1:1 (v/v) with addiction of zinc(II) chloride. White opaque xerogels were obtained from the residual sol after exposure to the pinewood samples.

13. Formulation 13: TEOS/ APTES 4:1 (molar ratio) + Zn. Solution 1 (first step): TEOS (6.25 ml, 28 mmol), APTES (1.64 ml, 7 mmol), ethanol (2 ml). Solution 2 (second step): 0,1M acqueous solution of zinc (II) sulphate ($ZnSO_4$*$7H_2O$).
14. Formulation 14: TEOS/ APTES 1:1 (v/v) + Cu. TEOS (10 ml, 44.8 mmol), APTES (10ml, 42.8 mmol), zinc(II) chloride (2.453 g, 8.53 mmol), ethanol (20 ml).

2.2.5. TEOS-APTES-BORON formulations

Siloxane polymers containing boron were prepared starting from TEOS, APTES and Boric Acid. Both the APTES/Boron and the TEOS/APTES ratios were varied in the different formulations and both the impregnation “one step” and “two step” methods (see par.2.3.2.2) were tested. Colourless sols were obtained.

Formulations for the one-step method:
In formulations 15-17 APTES/Boric Acid molar ratio was varied whereas the TEOS/ APTES volume ratio 1:1 was maintained.

15. Formulation 15: TEOS/ APTES 1:1 (v/v) + B (APTES/Boric Acid molar ratio 10:1). TEOS (10ml, 44.8 mmol), APTES (10ml, 42.8 mmol), boric acid (0.2641 g, 0.438 mmol), ethanol (20 ml).
16. Formulation 16: TEOS/ APTES 1:1 (v/v) + B (APTES/Boric Acid molar ratio 5:1). TEOS (10ml, 44.8 mmol), APTES (10ml, 42.8 mmol), boric acid (0.528 g, 8.540 mmol),

ethanol (21 ml).

17. Formulation 17: TEOS/ APTES 1:1 (v/v) + B (APTES/Boric Acid molar ratio 2:1). TEOS (10ml, 44.8 mmol), APTES (10ml, 42.8 mmol), boric acid (1.320 g, 0.0214 mmol), ethanol (21 ml).

In formulations 16-18-19 the TEOS/ APTES volume ratio was varied whereas the APTES/Boric Acid molar ratio 1:5 was maintained.

18. Formulation 18: TEOS/ APTES 5:1 (v/v) + B (APTES/boric acid molar ratio 5: 1). TEOS (10 ml, 44.8 mmol), APTES (2 ml, 8.56 mmol), boric acid (0.1057 g, 1.171 mmol), ethanol (21 ml).
19. Formulation 19: TEOS/ APTES 10:1 (v/v) + B (APTES/boric acid molar ratio 5:1). TEOS (10ml, 44.8 mmol), APTES (1ml, 4.28 mmol), boric acid (0.0528 g, 0.854 mmol), ethanol (21 ml).

Formulation for the two-step method:

20. Formulation 20: TEOS/ APTES 4:1 (molar ratio) + B. (first step): TEOS (6.25 ml, 28 mmol), APTES (1.64 ml, 7 mmol), ethanol (2 ml). (second step): 0,1M acqueous solution of boric acid (H_3BO_3).

The one-step sol appeared fluid and colorless. After exposure to wood (see par.2.2.3), the amount of solution that wasn't absorbed into the wooden structure was kept on air in a watch glass until gelation and drying. White opaque homogeneous xerogels were obtained. FTIR-HATR characterization of the residual xerogel: peaks at 2928-2867 cm^{-1}mw,1562 cm^{-1}mw, 1480 cm^{-1} mw, 1005 cm^{-1} s, 763 cm^{-1}m, 670 cm^{-1}m, 577 cm^{-1}m.

2.2.6. *Commercial colloidal SiO_2-boron formulations*

Colloidal SiO_2 acqueous solutions were used as precursors instead of silica alkoxydes in a formulation containing boric acid tested against termites.

21. Formulation 21: 100 ml of a commercial colloidal SiO_2 (BindzilCC30, Akzo Nobel; SiO_2 30%; pH 9.7; 3.6cP at 20°C) were mixed to a solution of H_3BO_3 (4 g, 65 mmol) diluted in 50 ml H_2O.

The obtained uncoloured solution was applied on wood by autoclave impregnation (see par. 2.3.2.2).

2.2.7. TEOS formulations with silver nanoparticles

Silver nanoparticles, obtained from $AgNO_3$ by Ag reduction with $NaBH_4$

$$AgNO_3 + NaBH_4 \rightarrow Ag + 1/2H_2 + 1/2B_2H_6 + NaNO_3$$

were added to TEOS and TEOS-APTES mixtures with the aim to obtain silica xerogel embedding Ag nanoparticles and avoiding aggregation.

22. *Formulation 22:* TEOS/ APTES 1:1 (v/v) + Ag (0,034M Ag final concentration). TEOS (7 ml, 31 mmol), APTES (7 ml, 30 mmol), ethanol (14 ml), $AgNO_3$ (1.008 g, 1.48 mmol), $NaBH_4$ (5 ml of a 0.0045M $NaBH_4$ ethanol solution, corresponding to 0.067 mmol). The product was obtained through the following procedure. The $NaBH_4$ solution of was freshly prepared in a flask by solving $NaBH_4$ (17 mg) in 100 ml EtOH. After preparation, it was kept in a cooling bath. Meanwhile, a shlenk container was cooled in a cooling bath for 20 minutes. TEOS and APTES were poured in the cooled shlenk together with $AgNO_3$ and ethanol. The system was maintained under stirring for some minutes. Then, the $NaBH_4$ solution was slowly dropped in the shlenk, under stirring. After having dropped 5ml, the system assumed a pale yellow appearance, due to the formation of silver nanoparticles. The obtained colloidal solution was immediately used for the impregnation of wood (see par.2.3.2.2). The amount of solution that was not absorbed into the wooden structure was kept on air in a watch glass until gelation and drying.

2.2.8 Polyaminoamide(PAA) formulations

Hybrid organic-inorganic polymers were obtained starting from a diacrylamide (MBA, N-N'-metilenbisacrilammide), a secondary diamine (C6) and a primary amine (APTES) (Cauzzi, 2004) (Figure 22) and tested as vehicle for Cu^{2+} ions and Ag nanoparticles into the wooden structure.

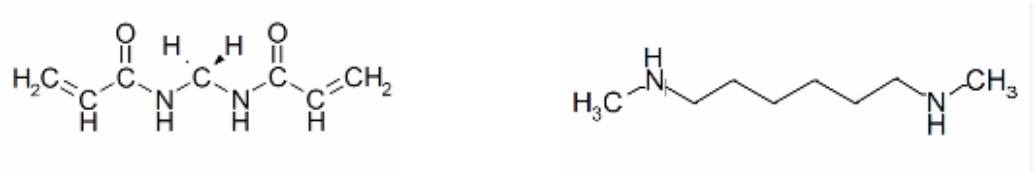


Figure 22 N-N'-metilenbisacrilammide (left) and C6 diamine (right) employed in the preparation of the Polyaminoamide polymer.

Organic polymerization is due to the addiction of the aminic nirogen to the acrylic group trough a 1,4 attack, followed by rearrangement (Figure 23).

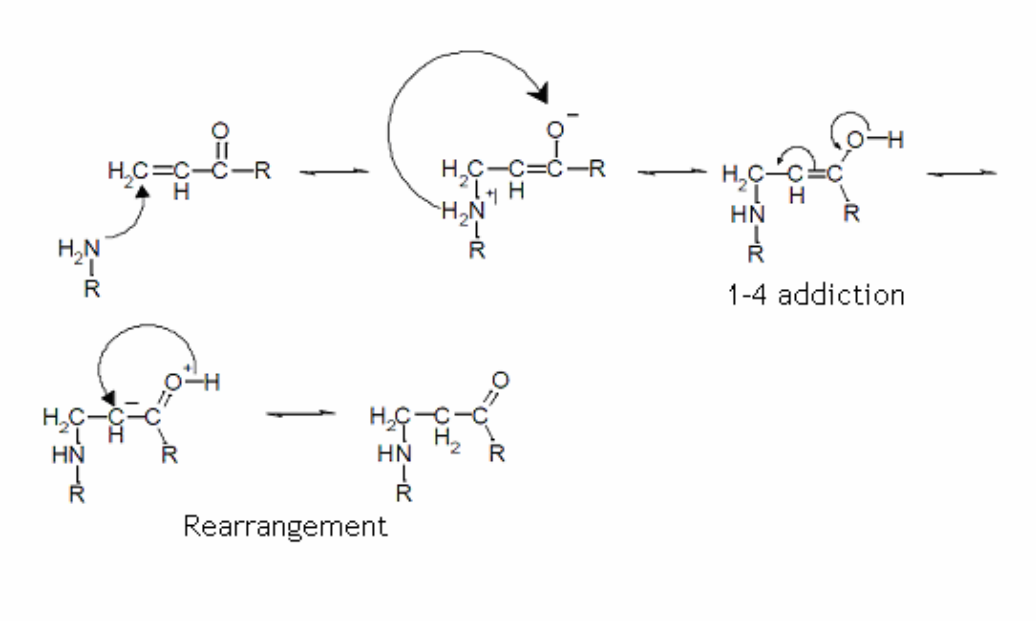


Figure 23 Reaction scheme of the organic polymerization of polyaminoamide (PAA).

The syloxanic functions introduced with APTES also allow an inorganic polymerization and ramification. The molar ratio MBA/ APTES/C6 1: 0,5: 0,5 was chosen. Cu^{2+} ions or Ag nanoparticles were added to the base formulation.

23. Formulation 23: SiPAA (hybrid syloxane-polyaminoamide).
24. Formulation 24: Cu-SiPAA (hybrid syloxane-polyaminoamide +$CuCl_2$).
25. Formulation 25: AgNP-SiPAA (hybrid syloxane-polyaminoamide + Ag nanoparticles).

The formulations were obtained through the procedures described below.

Synthesis of SiPAA

MBA powder (2 g, 12.96 mmol) were suspended in water (1 ml) in a glass flask and kept under stirring at room temperature for 15 minutes. Afterwards, an APTES solution (1.48 ml, 6.48 mmol) and a C6 solution (1.1 ml, 6.48 mmol) were added, together with 1 ml EtOH. After the addiction of the amines, the solution acquired a yellowish colour. The system was sonicated till complete solubility; after stirring at room temperature for about 2 hours, the mixture became available for wood impregnations. If kept in the flask and not used for any wood treatment, the system reached gelation until 1 day. Complete drying in the air at room temperature was achieved in about two weeks (constant weight). If the polymer was oven dried at 180°C for 4 h, it became orange transparent and hard, and its weight was reduced of about 70% of its weight immediately after gelation.

FTIR-HATR spectrum (cm^{-1}): 3274m, 3060m, 2934m, 2860m, 1632s, 1534s, 1459m, 1381m, 1221m, 1113s (Figure 24).

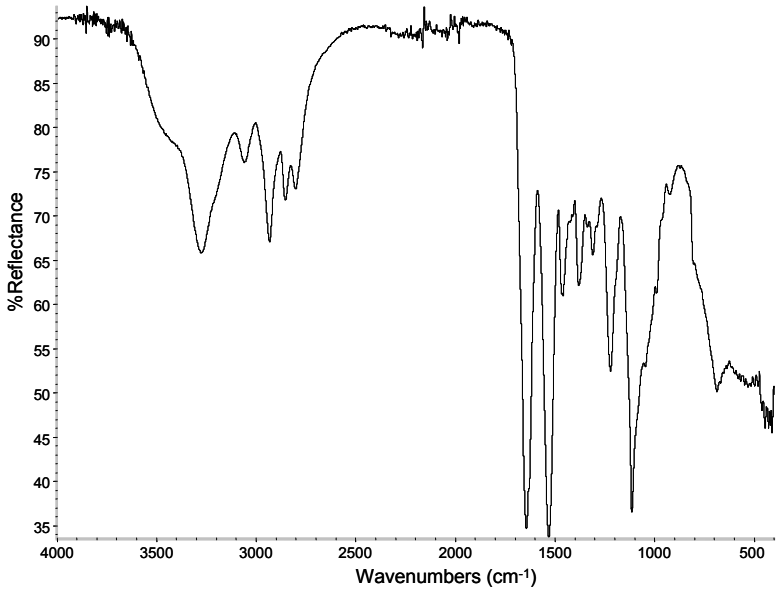


Figure 24 FTIR-HATR spectrum of the hybrid inorganic-organic polyaminoamide polymer functionalized with siloxane groups.

Synthesis of Cu-SiPAA

The same procedure and quantities of formulation 22 were followed. After having kept the system under stirring at room temperature for about 2 hours, copper(II) chloride (0.221g, 1.296 mmol) was slowly added under vigorous stirring. A pale blue solution was obtained and used for the treatment of wood (par.2.3.2.2). If kept in the flask and don't used for wood treatment, the system reached gelation until 1 day.

FTIR-HATR spectrum of the dried polymer (cm^{-1}): 3274m, 3060m, 2934m, 2853m, 1643s, 1530s, 1459m, 1381m, 1221m, 1113s.

Synthesis of AgNP-SiPAA

As for formulations 22, a MBA-APTES-C6 mixture was prepared. After having kept the system under stirring at room temperature for about 2 hours, 7.4 ml of a 0.4M $AgNO_3$ acqueous solution (corresponding to 0.296 mmol of Ag) were slowly dropped under vigorous stirring. After the addition, the system maintained a yellowish colour and was used for the treatment of wood (par.2.3.2.2). Inside the flask, gelation was obtained in about 1 day. The gel appeared yellow, transparent and homogeneous. If kept to dry on air in the open flask, it started to become grey and opaque in about 5 days for the formation of silver agglomerates.

FTIR-HATR spectrum of the dried polymer (cm-1): 3274m, 3060m, 2934m, 2853m, 1643s, 1530s, 1459m, 1381m, 1221m, 1113s.

2.2.9 Copper glycinate in acqueous solutions

Acqueous solutions of cis-copper(II)-bisglycinate (Figure 25) monohydrate and aqueous solutions of copper sulphate and glycine were prepared, also with addition of boric acid.

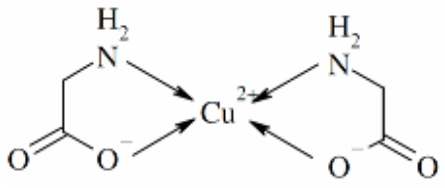


Figure 25 Cis-copper(II)-bisglycinate.

Different concentrations were tested by means of screening efficacy tests against fungi (see par.2.5.3), among which the following formulations were chosen for wood treatments.

26. Formulation 26 Acqueous solution of cis-copper (II)-bisglycinate (0,023M)
27. Formulation 27 Acqueous solution of cis-copper (II)-bisglycinate (0,023M) and boric acid (0,023M)
28. Formulation 28 Acqueous solution of cis-copper (II)-bisglycinate (0,004M)
29. Formulation 29 Acqueous solution of copper sulphate (0.05 M) and glycine (0.1 M).
30. Formulation 30 Acqueous solution of copper sulphate (0.1 M) and glycine (0.2 M).
31. Formulation 31 Acqueous solution of copper sulphate (0.05M), glycine (0.1M) and boric acid (0.05M).
32. Formulation 33 Acqueous solution of copper sulphate (0.1M), glycine (0.2M) and boric acid (0.1M).

Cis-copper (II)-bisglycinate was prepared from copper(II)acetate monohydrate ($Cu(CH_3COO)_2*H_2O$) and glycine (NH_2CH_2COOH). Copper acetate (2.08 g, 0,01 mol) has been solved in distilled water (30 ml) in a hot water bath (60°C). Ethyl alcohol (20 ml) has been added. In a beacker, glycine (1.48 g, 0.02 mol) has been solved in distilled water (15ml). The glycine solution has been added to the copper acetate solution, at 60°C under gentle stirring. The flask was cooled at room temperature and finally in a cooling bath until complete precipitation a blue solid. Crystals were filtered with a Buchner funnel. Recrystallization was performed by solving the solid product in water (100 ml) and reprecipitate at room temperature by adding ethyl alcohol (100ml). The solid has been filtred with a Buchner funnel, washed with ethyl alcohol and acetone and left to dry on air on filter paper. 1.8 g of cis-$[Cu(Gly)_2]H_2O$ were obtained, corresponding to a yield of 80%.

The cis nature of the obtained copper chelate was confirmed by 419.6 cm^{-1} peak in its FT-IR spectrum, due to the Cu-N asymmetric stretching. Copper glycinate was powdered and solved in distilled water in flasks to obtain solutions of adequate concentration. For formulation 26, 0.528 g of $Cu(Gly)_2]H_2O$ were required for a total volume of 100ml. Boric acid powder (0.142g for 100ml) was also added to the solution of formulation 27. Sonication at 25°C for 30 minutes was applied to the 0.04 M $Cu(Gly)_2]H_2O$ solution

(0.919g in 100ml) to facilitate complete solubilization. Higher concentrations resulted not stable for more than 30 minutes after sonication and the reprecipitation of copper glycinate was observed.

Therefore, copper sulphate-glycine 1:2 molar ratio water solutions (100ml) were prepared (Formulation 29: 1.2484 g $CuSO_4*5H_2O$ and 0.7507g glycine; Formulation 30: 2.4968 g $CuSO_4*5H_2O$ and 1.5014 g glycine) to achieve further copper concentrations. Boric acid powder was also added to the 100ml solutions of formulation 31 (0.309 g H_3BO_3) and 32 (0.618 g H_3BO_3).

2.2.10 Metal salicylates in linseed oil solutions

Zinc and copper salicylates in linseed oil solutions were synthesized as follows.

Zinc salicylate was prepared starting from salicylic acid and basic Zn carbonate (titre: ZnO 71.5±1.5%) 2:1 molar ratio.

In a becker, salicylic acid (3g, 21.7mmol) was solved in distilled water (500ml) at 50°C, under stirring. When complete solution was achieved (in about 1 hour), 1.2375 g of basic zinc carbonate (10.85 mmol) were added. The system was maintained under vigorous stirring at 60°C for about 30 minutes. A pale pink solution was obtained, then transferred in a large glass container in order to facilitate water evaporation and subsequent formation of white crystals. Yield: 90% (Figure 26).

Figure 26 Crystals of zinc salicylate obtained from salicylic acid and zinc carbonate basic.

Suitable crystals for the X-ray analysis were obtained by slow evaporation. The X ray diffraction analysis confirmed that the obtained product is Zinc bis(salicylate) bi-hydated $[Zn(Hsal)_2(H_2O)_2]$, MW= 375.4 g/mol, whose structure has already been reported in the literature (Rif. Rissanen,1987). An image of the structure is reported in Figure 27. The two salicylate ligands asymmetrically chelate the zinc atom (Figure 27), which exhibits an irregular coordination ("kernel coordination") of the six oxygens, with four Zn-O 2 Å bonds and two Zn-O 2.5 Å bonds. Elemental analysis revealed the presence of about 45% C and 3,33% H, corresponding to the bi-hydrated molecule (theoretical C and H percentages 44% and 3,7% respectively). TGA profile: weight loss 11.95% at 120°C (theoretical bi-hydrated molecule: 9.6%). FTIR-HATR spectrum (cm^{-1}): 3274ms-3063ms (str.OH), 1621ms- 1589s-1460s-1382s (str.C-C, str. C=O carboxyl group), 1153s (bend. C-H-C), 872m-817m (sciss. carboxyl group, 758s (bend. C-H aromatic rings), 703vs, 675s-501s (bend. benzenic rings).

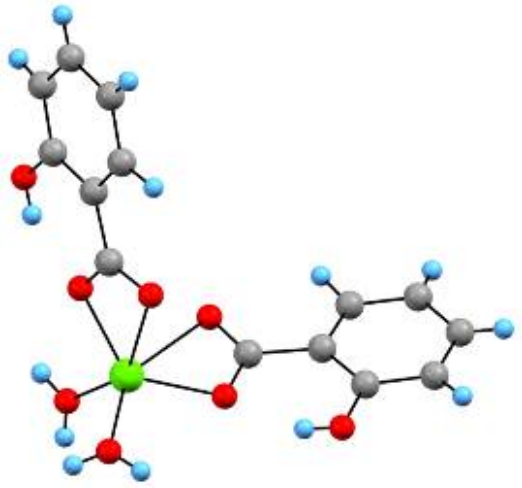


Figure 27 Crystal structure of $[Zn(Hsal)_2(H_2O)_2]$. Zinc=green, Oxygen=red, Carbon=gray, Hydrogen=blue.

Copper complexes starting from salicylic acid were obtained by the same way.
In a becker, salicylic acid (4.792 g, 34.7 mmol) was solved in distilled water (500 ml) at 50°C, under stirring. When complete solution was acheived (in about 1 hour), 1.062 g of copper oxide ($Cu(OH)_2$) were added. The system was maintained under vigorous stirring at 60°C for about 30 minutes. A green solution was obtained, then transferred in a large glass container in order to facilitate water evaporation and subsequent formation of dark-green crystals (Figure 28). Yield:90%.

Figure 28 Crystals of copper salicylate obtained from salicylic acid and copper oxid.

The single crystal X-rays diffraction analysis confirmed that the product is Copper bis(salicylate) tetra-hydated $[Cu(Hsal)_2(H_2O)_4]$, MW=373.5g/mol, whose structure has been reported in literature (Jagner, 1976). The compound is polymeric, containing chains, in which one of the salicylate ligands acts as a bridge between two copper atoms. The copper atom is squared pyramidally coordinated by oxygen atoms from the salicylate ligands and water molecules (Figure 29).

TGA profile:17.39% weight loss at 80°C (theoretical tetra-hydrated molecule: 17.9%). FTIR-HATR spectrum bands (cm^{-1}): 3174 m, 1618 s, 1584 s, 1558 s, 1549 s, 1470 s, 1385 vs, 1350 vs, 1303 s, 1247 vs, 1143 m, 1035 m, 871 m, 814 m, 750 s, 672 vs, 525 s.

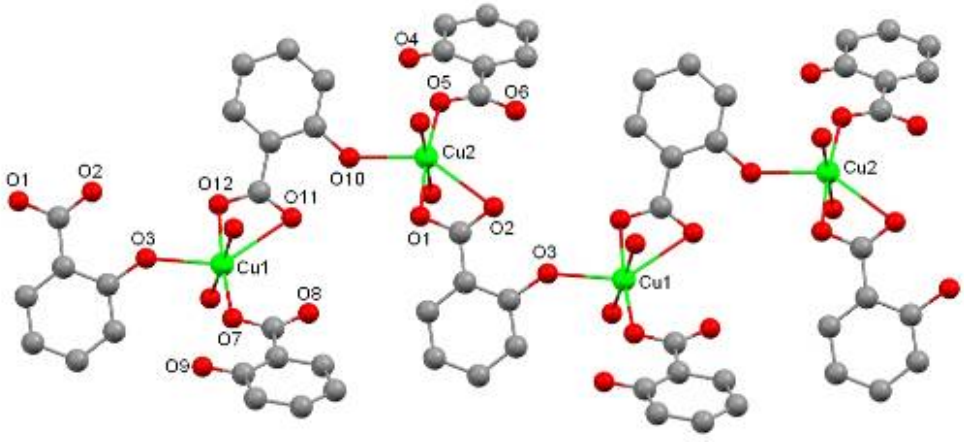


Figure 29 Crystal structure of $[Cu(Hsal)_2(H_2O)_4]$. Copper=green, Oxygen=red, Carbon=gray.

Linseed oil solutions can be obtained by solving the powdered metal complexes in oil at 60°C under stirring for 1 hour.

A Zn-salicylate 0.065M (1.952 g in 80 ml) solution in linseed oil was prepared under stirring at room temperature; it appears pale orange. Cu-salicylate was solved in linseed oil to form a 0.043M (1.285 g in 80 ml) dark green solution. A 0.065M (1.942 g in 80 ml) solution was also obtained at 60°C under stirring for 6 hours, but it wasn't employed for wood treatments since the presence of copper facilitated the oil polymerization as the system becomes non-reversibly solid after about 24 hours.

The formulations employed to evaluate the preservative effectiveness against termites are listed below:

33. Formulation 33 Zn-salicylate 0.065M solution in linseed oil (Figure 30)
34. Formulation 34 Cu-salicylate 0.043M solution in linseed oil (Figure 30)

The same solutions were also obtained by solving the salicylic acid in linseed oil and allowing to react with zinc or copper subsequently added in the form of oxides or salts directly in the oil solution.

The Zn-salicylate 0.043M (Formulation 32) was obtained in the following way.

In a becker container, Zinc(II)sulphate heptahydrate ($ZnSO_4*7H_2O$, 5.613g, 0.0195 mol) was solved in about 150 ml of distilled water, at room temperature under stirring. Meanwhile, NaOH granules (1.56 g, 0.039 mol) were solved in 100 ml of distilled water. Under stirring, the NaOH solution was poured into the $ZnSO_4$ solution. The immediate precipitation of $Zn(OH)_2$ as a white precipitate was observed. It was filtered with a Buchner funnel and dried on air on filter paper for about 60 minutes. Meanwhile, the salicylic acid (5.392g, 0.039mol) was put in a becker containing 300 ml of pure linseed oil and solved by stirring with a magnetic stirrer at 50°C for about 60 minutes. Then, $Zn(OH)_2$ was added and the system temperature was raised to 60-65°C. Vigorous stirring was applied for at least 4 hours in such a way that the mixture appear firstly like an homogeneous dense cream and finally like a transparent orange solution. FTIR-HATR spectrum (cm^{-1}): 3006 mw, 2919-2854 vs, 1739 vs, 1597 w, 1463 m, 1234 m, 1160 s, 1095 m, 724 m.

Figure 30 From left to right: pure linseed oil, zinc salicylate in linseed oil, copper salicylate in linseed oil.

The Cu-salicylate 0.043 M solution in linseed oil was prepared by solving salicylic acid (3.75g, 0.027 mol) in 300 ml of pure linseed oil under stirring at 50°C for 60 minutes.
Copper hydroxide $Cu(OH)_2$ was added (1.3125 g, 0.0135 mol) and the system was vigorously stirred at 65°C for about 10 hours, until the formation of a bright green solution.
FTIR-HATR spectrum (cm^{-1}): 3006 mw, 2919-2854 vs, 1739 vs, 1597 w, 1463 m, 1234 m, 1160 s, 1095 m, 724 m.

2.1.11 Metal salicylates in ethylene glycol

Zinc salicylate and Copper salicylate are more soluble in eyhylene glycol than in linseed oil, so they were also solved in ethylene glycole to obtain higher concentrations than the corresponding linseed oil solutions.

35. Formulation 35 Zn-salicylate 0,2M solution in ethylene glycol
36. Formulation 36 Cu-salicylate 0,1M solution in ethylene glycol

2.3. Wood samples treatment

2.3.1 Samples preparation

Wood samples were conditioned at 20°C and 65% relative humidity until constant mass and weighed with analytical balance to determine their mass at 12% wood humidity. Therefore, they were oven dried (103 ± 2)°C for 18 hr, kept in a desiccator for 0.5hrs, weighed to determine their anhydrous mass (m_0); finally, samples were conditioned at 20°C and 65% and kept in a sealed plastic bag until utilization. Each sample was weighed before any preservative treatment (m_1) and wood initial humidity (H%) was calculated as follows: $H\ \% = [(m_1 - m_0)/m_0]*100$.
Samples dimensions were measured with a calibre and their volume was calculated. If tolerance on each dimension was lower than ±0.2 mm, the geometrical volume was considered (EN 113/prA1 2003-06).

2.3.2 Methods of wood treatment

The synthesized formulations were utilized for the treatment of wood samples.
The applications on wood by dipping or impregnating were tested depending on the preservative solution.

2.3.2.1 Application by immersion

The immersion procedure was developed as follows: wood specimens were pencil signed on the longitudinal face; transverse faces were wax sealed with exception for *Pinus* mini-tablets for tests against termites; conditioned samples were bathed into a volume of preservative solution corresponding to 10/1 of the sum of wood sample volumes. Immersion lasted 8 hrs; it was followed by 24 hrs of air dry at room temperature. In all cases wood samples were maintained bathed by means of tweezers (Figure 31).
Sample weights were measured with analytical balance immediately after immersion and after 24 hrs of air dry; they were also oven dried at 103 ±2°C for 18 hrs to determine the dry weight. The immersion procedure was employed for the following formulations:

- alkoxysilane preparations containing $CuCl_2$ (employed for the evaluation of gel penetration into the wooden structure)
- inseed oil formulations containing Cu or Zn chelates (10 samples employed for efficacy tests against termites, further samples for characterization) both freshly prepared and aged solutions were tested in order to evaluate possible differences.
- ethylene glycol formulations containing Cu or Zn chelates (10 samples employed for efficacy tests against termites, further samples for characterization)
- formulation with copper glycinate 0,04M (employed for the evaluation of gel penetration into the wooden structure).

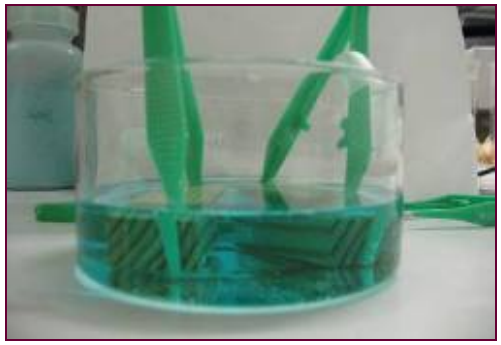


Figure 31 Image of the immersion treatment of wood samples. Tweezers were employed to maintain the samples bathed with all their faces exposed to the preservative solution.

2.3.2.2 Application by impregnation

Impregnation consists of wood treatment under vacuum followed by exposition to the preservative solution, at a certain pressure. Wood samples were pencil signed on the longitudinal face; transverse faces were not sealed; after determination of the anhydrous weight, conditioned samples were exposed to vacuum conditions (55 mbar) at R.T. for 45 minutes. Furthermore, they were dipped in the preservative mixture and maintained under vacuum for 15 min. Subsequently the mixture was gently stirred under nitrogen for 30 min at 1 atm. Then the samples were recovered, weighted and dried at room temperature and atmospheric pressure. Final oven dry at 103 ±2°C for 18 hrs consented to determine the Weight Percent Gain WPG (see 2.3.1). The equipment used for the impregnation procedure is shown in Figure 32. The treatment solution was

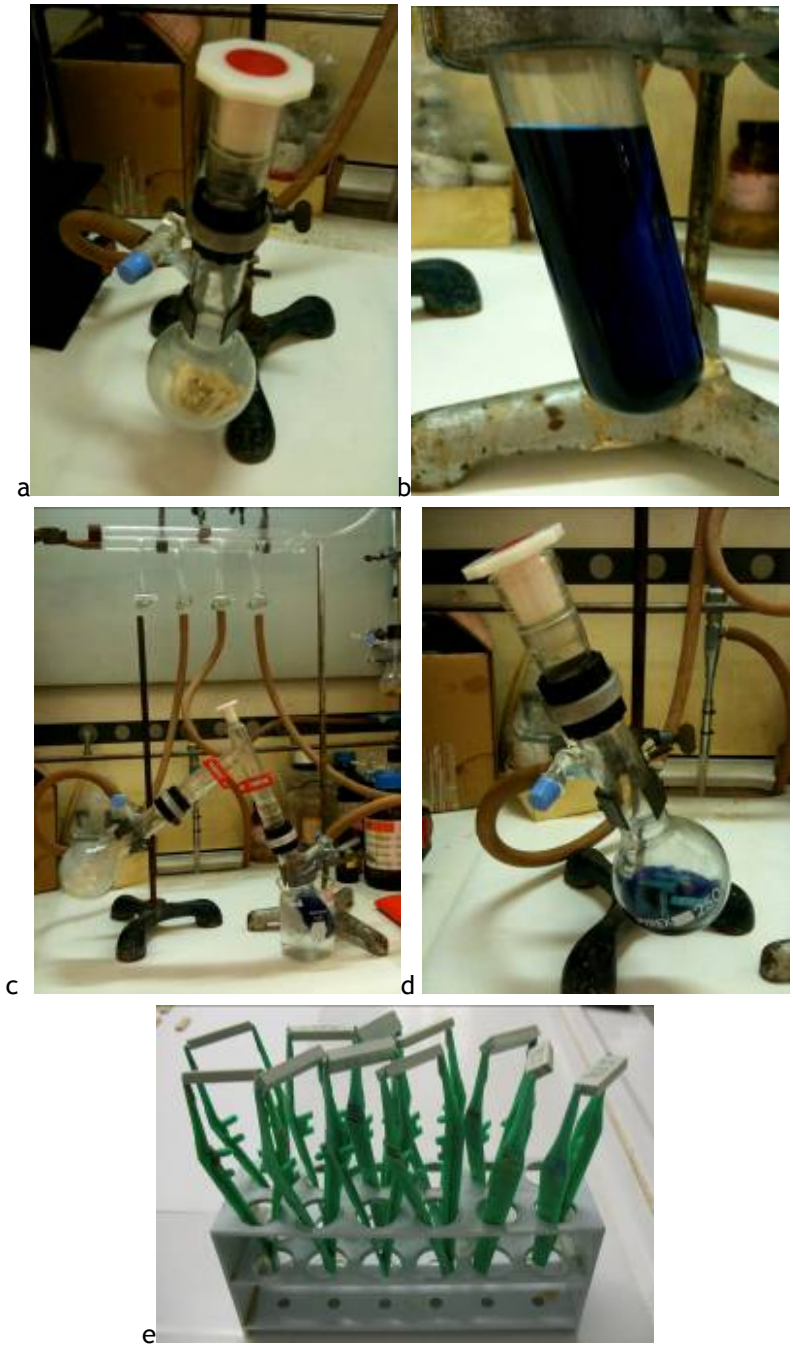


Figure 32 Impregnation procedure. (a) Wood samples were kept under vacuum for 1 hour in a flask; (b) The treatment solution was prepared in a shlenk container; (c) the schlenk was linked with a Claisen connector to the glass flask containing the wood samples and the system was kept under vacuum; (d) by rotation of the system, the solution was poured into the flask containing the wood samples, maintained under vacuum and finally hand stirred under nitrogen at 1 atm; (e) samples were dried on air on tweezers.

prepared into a schlenk; the schlenk was linked with a Claisen connector to a glass flask containing from 4 (blocks) to 10 (mini-blocks) wood samples. The flask-schlenk system was connected to an oil pump and put under vacuum. The schlenk was cooled by a cooling bath to avoid solvent evaporation. Furthermore, by rotation of the system, the solution was poured into the flask with wood samples, maintained under vacuum and finally hand stirred under nitrogen at 1 atm.

Impregnation treatment was tested for all kinds of formulations, from organic or acqueous solutions of metal complexes to alkoxysilane solutions with or without metal additives. The impregnation method described above is a one-step process, where the preservative solution is applied all at once. A two-step process was also tested for the formulation based on alkoxysilanes. In this case, wood blocks were treated by impregnation under vacuum with the sol-gel mixture of alkoxysilanes as for the one-step process; then the samples were recovered and dried at room temperature (R.T.) and atmospheric pressure for 24 h. Afterwards they were dipped for 2 h into a acqueous copper solution and finally recovered and dried at R.T. and atmospheric pressure for 24 h. Autoclave impregnation was performed as follows (Yamaguchi 2005): samples were initially placed in a vacuum of 60 mmHg for 30 minutes, then the pressure was increased by 2 bars every 5 minutes for three times (6 bars). Next, a pressure of 7.7 bars was reached and maintained for 30 minutes. Finally pressure was reduced to normal and the impregnated blocks were dried at 60°C for 48 hours. Autoclave impregnation was performed on 1.5 x 2.5 x 5 cm samples, which were impregnated with formulation 21 (silicic acid + boric acid).

The following table summarises the performed treatments and the kind of wood samples employed (Table 13).

Table 13 Kinds of treatment performed and wood samples employed.

N	Formulation	Treatment	samples
TEOS-APTES formulations			
1	TEOS/ APTES 1:1(v/v).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
		Impregnation	Picea abies block samples 2x 2 x 4 cm
		Immersion	Picea abies tablet samples 0,5 x 7 x 20 cm
2	TEOS/ APTES 4:1 (v/v).	Impregnation	Pinus sylvestris L. "t block" samples 1 x 1 x 3 cm
		Impregnation	Pinus sylvestris L. "T block" samples 1,5 x 2,5 x 5 cm
3	TEOS/ APTES 10:1 (v/v).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
TEOS-APTES-COPPER formulations			
4	TEOS/ APTES 1:1 (v/v) + Cu (Cu/APTES molar ratio 1:5).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
		Immersion	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
		Immersion	Picea abies tablet samples 0,5 x 7 x 20 cm

N	Formulation	Treatment	samples
5	TEOS/ APTES 10:1 (v/v) + Cu	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
6	TEOS/ APTES 20:1 (v/v) + Cu	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
7	TEOS/ APTES 6:1 (molar ratio) + Cu (0,1M solution)	Impregnation (two step).	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
			Pinus sylvestris L. "t block" samples 1 x 1 x 3 cm
			Pinus sylvestris L. "T block" samples 1,5 x 2,5 x 5 cm
8	TEOS/ APTES 4:1 (molar ratio) + Cu (0,1M solution)	Impregnation (two step).	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
9	$Na_2SiO_3*5H_2O$/ APTES 1:1 (molar ratio) + Cu	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
10	TEOS/ Cu 10:1 (molar ratio).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
11	TEOS/ Cu 25:1 (molar ratio).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
12	TEOS/ Cu 50:1 (molar ratio).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
TEOS-APTES-ZINC formulations			
13	TEOS/ APTES 1:1 (v/v) + Zn (0,1M solution)	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
14	TEOS/ APTES 1:1 (v/v) + Zn	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
TEOS-APTES-BORON formulations			
15	TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:10).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
16	TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:5).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
17	TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:2).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
18	TEOS/ APTES 5:1 (v/v) + B (B/APTES molar ratio 1:5).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
19	TEOS/ APTES 10:1 (v/v) + B (B/APTES molar ratio 1:5).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
20	TEOS/ APTES 6:1 (moar ratio) + B (0,1M solution)	Impregnation + immersion (two step process).	Pinus sylvestris L. "t block" samples 1 x 1 x 3 cm
			Pinus sylvestris L. "T block" samples 1,5 x 2,5 x 5 cm
Commercial colloidal SiO_2-BORON formulations			
21	commercial colloidal SiO_2 +H_3BO_3	Autoclave impregn.	Pinus sylvestris L. "T block" samples 1,5 x 2,5 x 5 cm
TEOS-APTES-SILVER formulations			
22	TEOS/ APTES 1:1 (v/v) + Ag np	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
Poliacrylamide(PAA) formulations			
23	SiPAA	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
24	Cu-SiPAA	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
25	AgNP-SiPAA	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
Copper glycinate in acqueous solution			
26	$Cu(GLY)_2$ (0,023M)	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
27	$Cu(GLY)_2$ (0,023M) +H_3BO_3 (0.010)	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
28	$Cu(GLY)_2$ (0,004M)	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
		Impregnation	Pinus sylvestris L. "t block" samples1 x 1 x 3 cm
		Immersion	Pinus sylvestris L mini-tablet samples 0,3 x 3 x 4 cm
		Immersion	Pinus sylvestris L. "t block" samples 1 x 1 x 3 cm
29	$CuSO_4$ (0.1 M) + GLY(0.2 M).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
30	$CuSO_4$ (0.05 M) + GLY(0.1 M).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
31	copper sulfate (0.05M),glycine (0.1M) and boric acid (0.05M).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
32	copper sulfate (0.1M), glycine (0.2M) and boric acid (0.1M).	Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
Metal salicylates solutions in linseed oil			
33	Zinc salicylate in linseed oil (0,065M)	Immersion	Pinus sylvestris L mini-tablet samples 0,3 x 3 x 4 cm
		Impregnation	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
		Immersion	Picea abies block samples 2x 2 x 4 cm
		Immersion	Picea abies tablet samples 0,5 x 7 x 20 cm
34	Copper salicylate in linseed oil (0,043M)	Immersion	Pinus sylvestris L mini-tablet samples 0,3 x 3 x 4 cm
		Immersion	Pinus sylvestris L. mini-block samples 0,5 x 1 x 3 cm
Metal chelate solutions inethylene glycol			
35	Zinc chelate in ethyl glycol (0,2M)	Immersion	*Pinus sylvestris* L mini-tablet samples 0,3 x 3 x 4 cm
		Immersion	*Pinus sylvestris* L. "t block" samples 1 x 1 x 3 cm
		Impregnation	*Pinus sylvestris* L. "t block" samples 1 x 1 x 3 cm
		Impregnation	*Pinus sylvestris* L mini-tablet samples 0,3 x 3 x 4 cm
35	Copper chelate in ethyl glycol (0,1M)	Immersion	*Pinus sylvestris* L mini-tablet samples 0,3 x 3 x 4 cm

2.4. Characterization of the treated wood

2.4.1 Weight characterization

Characterization of the impregnation processes was performed through the determination of the retention (R) of the preservative solution in wood and through the calculation of the final weight percent gain (WPG).

- Samples were weighed before and immediately after the preservative treatment and the retention for each treatment solution was calculated as follows:

$$R=[(M / V] \times 10 \text{ kg m}^3$$

where $M = m_t - m_i$; M is the Absorption value, i.e. the grams of treating solution absorbed by the sample (initial mass m_i subtracted from the initial mass plus the treating solution m_t) and V is the volume of sample in cubic centimetres.

- The final gain of weight was evaluated by the determination of Weight Percent Gain (WPG) calculated as follows:

$$WPG\ \% = [(m_{at} - m_{ai}) / m_{ai}] \times 100$$

where m_{ai} is the anhydrous mass of untreated wood and m_{at} is the anhydrous mass of treated samples.

2.4.2 Analytical characterization

Analytical evaluation of the presence, distribution and interaction of the preservative solutions in wood was performed, depending on the kind of treatment and formulation, by means of FT-IR, μ-Raman and MAS-NMR spectroscopic investigations, SEM-EDX, ICP-AES and EPR analysis.

FT-IR analyses were performed on the whole kinds of sample and formulations. Sly chips were cut with a bistoury from the transversal and longitudinal sample sections and analyzed in ATR mode (for FT-IR apparatus see par.2.1.1). The resulting spectra were

compared both to those obtained from untreated wood samples and to those collected from the same formulations without wood.

Powder from samples impregnated with TEOS APTES 1:1 (formulation 1) and TEOS APTES 1:1 + Cu (Formulation 4) were subjected to μ-Raman analyses (for Raman apparatus see par.2.1.1); the spectra were compared to those performed on the corresponding xerogel powder.

CP/MAS solid NMR spectroscopy was employed for the evaluation of possible interactions between the wood cell walls and the TEOS-APTES material employed in the sol-gel formulations (for MAS-NMR apparatus see par.2.1.1). The resulting spectra were compared to those obtained from the same xerogel formulations without wood, are reported.

The penetration depth of silicon, copper, zinc and silver into the wood was determined by X-ray microanalysis carried out on the cross sections of samples representative of the different kinds of treatment. Treated specimens were cut along the cross section to obtain two parts of the same length. The cut was performed using a CO_2 Laser (power max 2 kW El. En), with emission wavelength 10.6 μm and power 500 W, laser beam diameter width 3 mm, air flow pressure 0.5 bar. During the cutting the samples were positioned at a distance of 10 mm from the lens focus of laser source to obtain two equal cross sections. Samples were coated with carbon (20μm thick) and analysed. Areas at different depth from the surface were selected and the atomic ratios for each area were determined. X-ray maps were also performed all over the cross section.

EDX analyses were also performed on wooden blocks directly coated with carbon without any cut to obtain information about the element distribution on the external surface.

Samples representative of each kind of formulation containing Si, Cu, Zn or Ag were analysed.

Total Cu content inside the treated wood specimens were determined by means of ICP-AES analysis after acid extraction. The determination was performed on *Pinus sylvestris* L. mini-block samples. They were dipped in a 20% H_2SO_4 bath (10 ml H_2SO_4, 40 ml bidistilled water for each sample) for 40 hours, at 60°C, under stirring. Therefore, solutions were filtered, made up to volume in a 100 ml flask and analyzed by ICP-AES (wavelengths used: 324.754 nm (Cu)). Concentration results, in mg/L, were referred to the 100 ml solution and to the sample volume, and expressed in kg/m^3.

The determination of copper in *Pinus sylvestris* L. mini-block samples treated with

TEOS/APTES/COPPER formulations by means of two-step (Formulation 7: TEOS/ APTES 6:1 (molar ratio) + Cu (0,1M solution)) or one-step (Formulation 4: TEOS/ APTES 1:1 (v/v) + Cu (Cu/APTES molar ratio 1:5)) impregnation was also performed by atomic absorption (Perkin-Elmer Analyst 100), at a wavelength of 324.8 nm, after mineralization of the samples with concentrated nitric acid in a microwave-oven for 45 minutes.

EPR spectra were recorded for wood samples treated with solutions containing copper to evaluate the characteristics of the coordinative sphere of copper ions. The EPR spectra of wooden blocks treated TEOS/APTES/Cu two step (Formulation 7) and one step (Formulation 4) impregnation were compared. The corresponding copper xerogel were used as reference. EPR spectra of wooden blocks dipped in a linseed oil solution of copper salicylate (Formulation 34) were compared to the corresponding chelate solution without wood. As exposition of these samples to determine their efficacy against termites had lead to their darkening, probably due to the high humidity conditions, so the EPR spectrum of treated pine wood after termite exposition was also recorded.

The following table summarizes the analytical methods used for the characterization of wood samples for each tested formulation (Table 14).

Table 14 Summary of analytical characterizations for each formulation.

N	Formulation	Characterization
TEOS-APTES formulations		
1	TEOS/ APTES 1:1 (v/v).	FT-IR, Raman, SEM-EDX, MAS-NMR, ESEM
2	TEOS/ APTES 6:1 (molar ratio).	FT-IR, Raman,SEM-EDX, EPR
3	TEOS/ APTES 10:1 (v/v).	FT-IR , SEM-EDX, ICP-AES
TEOS-APTES-COPPER formulations		
4	TEOS/ APTES 1:1 (v/v) + Cu (Cu/APTES molar ratio 1:5).	FT-IR, Raman, SEM-EDX, EPR, ICP-AES
5	TEOS/ APTES 10:1 (v/v) + Cu (Cu/APTES molar ratio 1 :5).	FT-IR, SEM-EDX, ICP-AES
6	TEOS/ APTES 20:1 (v/v) + Cu (Cu/APTES molar ratio 1 :5).	ICP-AES
7	TEOS/ APTES 6:1 (v/v) + Cu (0,1M)	EPR
9	Na_2SiO_3*5H_2O/ APTES 1:1 (molar ratio) + Cu	FT-IR, SEM-EDX
10	TEOS/ Cu 10:1 (molar ratio).	ICP-AES
11	TEOS/ Cu 25:1 (molar ratio).	ICP-AES
12	TEOS/ Cu 50:1 (molar ratio).	ICP-AES
TEOS-APTES-ZINC formulations		
13	TEOS/ APTES 6:1 (v/v) + Zn (0,1M)	
14	TEOS/ APTES 1:1 (v/v) + Zn (Zn/APTES molar ratio 1:5).	SEM-EDX
	TEOS-APTES-BORON formulations	
15	TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:10).	ICP-AES
16	TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:5).	FT-IR, ICP-AES
17	TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:2).	FT-IR, ICP-AES
18	TEOS/ APTES 5:1 (v/v) + B (B/APTES molar ratio 1:5).	ICP-AES
19	TEOS/ APTES 10:1 (v/v) + B (B/APTES molar ratio 1:5).	ICP-AES
20	TEOS/ APTES 6:1 (moar ratio) + B (0,1M solution)	
Commercial colloidal SiO_2-BORON formulations		
21	commercial colloidal SiO_2 + Boric acid	
TEOS formulations with Silver nanoparticles		
22	TEOS + Ag nanoparticles	
	Poliacrylamide(PAA) formulations	
23	SiPAA	FT-IR
24	Cu-SiPAA	ICP-AES
25	AgNP-SiPAA	FT-IR, SEM-EDX
Copper glycinate in acqueous solution		
26	$Cu(GLY)_2$ (0,023M)	FT-IR
27	$Cu(GLY)_2$ (0,023M) +H_3BO_3 (0.010)	ICP-AES
28	$Cu(GLY)_2$ (0,04M)	ICP-AES, SEM-EDX
29	$CuSO_4$ (0.1M) + GLY(0.2M).	
30	$CuSO_4$ (0.05 M) + GLY(0.1 M).	
31	$CuSO_4$ (0.05M), GLY(0.1M) H_3BO_3 (0.05M)	
32	$CuSO_4$ (0.1M), GLY(0.2M) H_3BO_3 (0.1M)	
Metal salicylates solutions in linseed oil		
33	Copper chelate in linseed oil (0,045M)	FT-IR, EPR, ICP-AES
34	Zinc chelate in linseed oil (0,065M)	FT-IR, SEM-EDX
Metal salicylates solutions in ethylene glycol		
35	Zinc chelate in ethylene glycol (0,043M)	SEM-EDX
36	Copper chelate in ethylene glycol (0,065M)	ICP-AES

2.4.3 Leaching tests

The leaching process was conducted according to Japanese Industrial Standard (JIS) K 1571 (2004) as reported by Kartal (Kartal, 2007); 0.5 x 1 x 3 cm treated pinewood samples were employed on this purpose, whose real volumes (0.1 mm precision) were measured by means of a calibre. The process consists in bathing wood specimens in distilled water, stirring with a magnetic stirrer (400-450 rpm) at 27°C for 8 h followed by drying at 60°C for 16 h. This cycle was repeated 10 times. After each leaching cycle, the water was renewed with fresh distilled water (15 ml i.e. a ratio of 10 volumes of water to 1 volume of wood). The employed water for each leaching day was diluited to 100ml in a volumetric flask and analysed with an ICP-AES spectrometer (see par. 2.1.1) to determine copper (λ=324.800 nm), boron (λ=249.773 nm) and silicon (λ=251.611 nm) amounts. The percent of copper, boron and silicon in the wood specimens was calculated based on the initial amount in the specimens. Copper initial amount was determined by dipping the leached samples in a 20% H_2SO_4 bath as described in par. 2.4.2. Total boron and silicon were approximately evaluated from the samples weight percent gain due to the preservative treatment, respecting the initial atomic ratios among elements. The list of the formulations on which the leaching test was performed is reported on Table 15.

Table 15 List of the formulations on which the leaching test was performed and indication of the elements searched in the leaching waters by means of ICP-AES analyses.

Formulation	Element analysed
4 TEOS/ APTES 1:1 (v/v) + Cu (Cu/APTES molar ratio 1:5).	Cu, Si
5 TEOS/ APTES 10:1 (v/v) + Cu (Cu/APTES molar ratio 1:5).	Cu, Si
10 TEOS/ Cu 10:1 (molar ratio).	Cu, Si
11 TEOS/ Cu 25:1 (molar ratio).	Cu, Si
12 TEOS/ Cu 50:1 (molar ratio).	Cu, Si
15 TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:10).	B, Si
16 TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:5).	B, Si
17 TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:2).	B, Si
18 TEOS/ APTES 5:1 (v/v) + B (B/APTES molar ratio 1:5).	B, Si
19 TEOS/ APTES 10:1 (v/v) + B (B/APTES molar ratio 1:5).	B, Si
24 Cu-SiPAA	Cu, Si
27 cis-copper (II)-bisglycinate (0,023M) and boric acid(0.010)	B, Cu
28 cis-copper(II)-bisglycinate(0,004M)	Cu
33 Copper chelate in linseed oil (0,045M)	Cu
36 Copper chelate in ethyl glycol (0,1M)	Cu

2.5. Efficacy tests against biological attack

2.5.1 Efficacy of sol-gel treatments against brown rot and white rot fungi

The efficacy of TEOS/APTES treatments, with or without addiction of boron, zinc or copper, against fungi basidiomycetes in wood was evaluated.

The accelerated methodology proposed by Bravery (1979) was employed with some modifications.

After preservative treatment (impregnation), conditioning (20 °C e 65 % RH) and sterilization (flame sterilization), *Pinus sylvestris* L. mini-block samples (4 replicates for formulation) were exposed to the brown rot fungus *Coniophora puteana* (Schumacher ex Fries) Karsten (strain BAM Ebw. 15) or to the white rot fungus *Trametes versicolor*(L.:Fr.) Pilat.

Each treated sample was placed side by side with an untreated reference sample (control) of sapwood of *Pinus sylvestris* L. in a Petri dish, measuring 90 mm of diameter and containing the brown rot fungus grown on 20 ml of 4% malt, 2.5 % agar medium (Figure 33). At the same time, the strain virulence was assessed by placing six pairs of untreated wood blocks on Petri dishes inoculated with the same fungus.

The wood blocks were incubated with the fungus for six weeks at 22°C and 75% RH. According to Bravery (1979), toxic values obtained after such incubation time were comparable with those obtained after 12 weeks using sample sizes described in EN 113 (EN 113/prA1 2003-06). Furthermore, two wood blocks for each treatment were put in contact with culture medium without fungal strains. These specimens were utilized for the determination of the correction coefficient. The correction coefficient permits calculation of the mass variation due to factors that are different from fungal decay. The resistance against *C. puteana* was evaluated through the measurement of the wood mass loss (due to the fungal attack under controlled environmental conditions) which was calculated for each individual block as the difference between the dry mass before the impregnation process and after the fungal exposure, corrected by the WPG due to the impregnated treatment and by the correction coefficient. The test was considered valid if virulence control samples (untreated samples exposed to the fungal strain) showed a weight loss not lower than 20% and if samples final humidity was comprised between 20% and 80%.

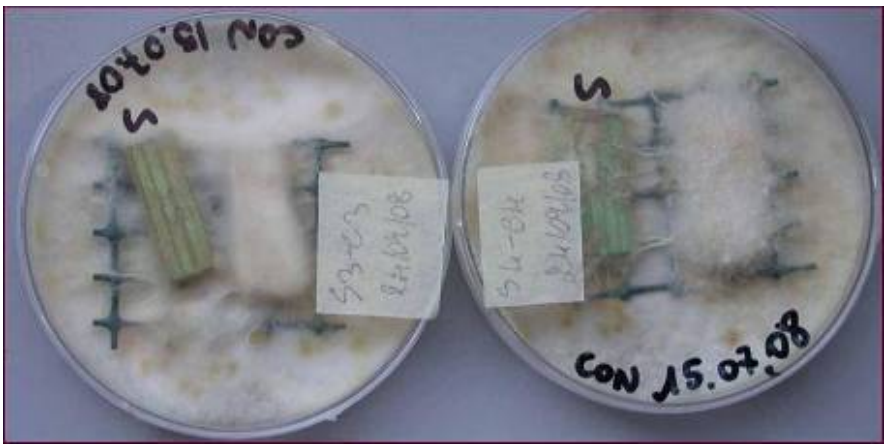


Figure 33 Accelerated efficacy test against the fungus *C.puteana*. Pine wood samples impregnated with TEOS-APTES-Cu (formulation 4) (left in each Petri dish) were not attacked by the fungus whereas the corresponding control samples (untreated, right in each Petri dish) are covered with the fungal hyphae.

Accelerated tests were applied to the following formulations:

- Formulation 1 TEOS/ APTES 1:1 (v/v).
- Formulation 3 TEOS/ APTES 10:1 (v/v).
- Formulation 4 TEOS/ APTES 1:1 (v/v) + Cu (Cu/APTES molar ratio 1:5).
- Formulation 5 TEOS/ APTES 10:1 (v/v) + Cu (Cu/APTES molar ratio 1 :5).
- Formulation 6 TEOS/ APTES 20:1 (v/v) + Cu (Cu/APTES molar ratio 1 :5).
- Formulation 7 TEOS/ APTES 6:1 (molar ratio) + Cu (0,1M)
- Formuation 8 TEOS/ APTES 4:1 (molar ratio) + Cu (0,1M)
- Formulation 9 $Na_2SiO_3*5H_2O$/ APTES 1:1 + Cu (Cu/APTES molar ratio 1 :5).
- Formuation 14 TEOS/ APTES 6:1 (molar ratio) + Zn (Zn/APTES molar ratio 1:5).
- Formuation 16 TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:5).
- Formuation 17 TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:2)
- Formulation 22 TEOS/APTES 1:1 + Ag nanoparticles.

Further investigations were performed on sets of 20 replicates for formulation. Each set was divided in groups of 10 samples: one of the two groups of impregnated samples was subjected to the leaching procedure according to EN84 (1997) before to be exposed to the fungal stain.

Wood specimens were placed in a glass beaker filled with deionised water conforming to

EN ISO 3696 (1996). Wood specimens were prevented from floating by the use of weights. The beaker was put in a desiccator and vacuum was applied corresponding to a residual pressure of 4 kPa. Vacuum was maintained for 20 minutes, and then released to return to normal pressure. Wood specimens were kept in water (ratio of water to wood 5:1) for 14 days with 9 water changes, and then conditioned to constant mass.

As regards the exposition to the fungal strain, groups of 5 treated samples were placed together with two untreated reference samples in a Kolle flask containing the brown rot fungus grown on 20 ml of 4% malt and 2.5 % agar medium and the procedure of efficacy evaluation was followed as for the tests with 4 replicates.

The test on leached/unleached samples was applied to the following formulations with different TEOS/APTES ratio and with or without the presence of copper (Figure 34)

- Formulation 1 TEOS/ APTES 1:1 (v/v).
- Formulation 3 TEOS/ APTES 10:1 (v/v).
- Formulation 4 TEOS/ APTES 1:1 (v/v) + Cu (Cu/APTES molar ratio 1:5)
- Formulation 5 TEOS/ APTES 10:1 (v/v) + Cu (Cu/APTES molar ratio 1 :5)

and to the following formulation with addiction of boric acid

- Formuation 16 TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:5).
- Formuation 17 TEOS/ APTES 1:1 (v/v) + B (B/APTES molar ratio 1:2).

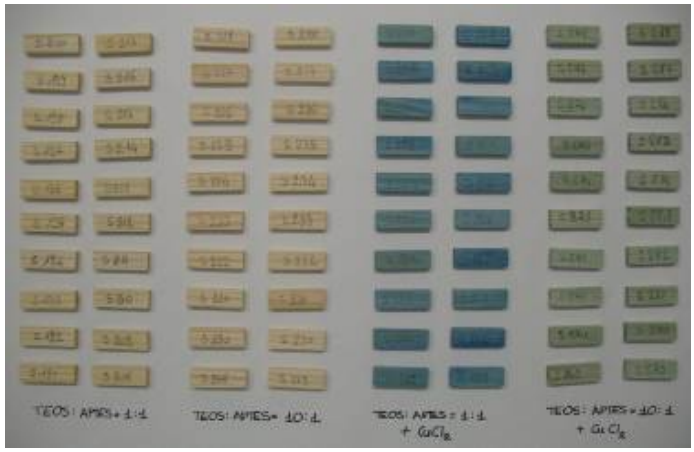


Figure 34 Sets of samples prepared to be employed for the efficacy test against *C.puteana.* From left to right: TEOS-APTES 1/1 (formulation 1), TEOS-APTES 10/1 formulation 3), TEOS- APTES 1/1 + Cu (formulation 4) TEOS- APTES 10/1 + Cu (formulation 5)

2.5.2 Efficacy of sol-gel treatments against termites - no-choice test

To assess the resistance of the sol-gel modified wood against subterranean termites both the European Standard EN 117 (2005) and an accelerated method as in Nunes (1997) were used. Termites belonging to the species *Reticulitermes grassei* Clément were collected from broken branches and stubs in a mixed forest of *Pinus pinaster* Ait. and *Eucalyptus* spp situated near Moitas (39º 43' 85 N; 7º° 53' 08 W, alt. 423 m asl) in Portugal. In the accelerated test adapted from EN 117, colonies of 150 workers together with 0-3 soldiers and nymphs, depending on the presence in the colony of origin, were established in 200 ml glass jars with moisturised sand (Fontainebleau sand and water, 4:1 v/v) as substrate. Treated *Pynus sylvestris* small samples ("t"blocks 1,5 cm_{rad} x 2,5 cm_{tang} x 5 cm_{long}, six replicates per treatment) were exposed to the colonies inside the containers and then placed in a climatic room at (24 ± 2)°C and (80 ± 5)% relative humidity for four weeks. Untreated specimens were also included as reference and virulence controls. Three formulations were selected to test their efficacy against termites: formulation 2, formulation 7, formulation 20. They are sol-gel TEOS/APTES 6:1 formulations differing in the absence (formulation 2) or presence of a second step of treatment consisting in the immersion in a 0,1M CuSO4 solution (formulation 7) o in a 0,1M H_3BO_3 solution (formulation 20) (Figure 35).

Figure 35 Sets of samples prepared to be employed for the efficacy test against *Reticulitermes grassei*. The two-step treatment was performed on these specimens. From left to right: TEOS-APTES 6/1 (formulation 2), TEOS- APTES 6/1 + copper (formulation 7) TEOS- APTES 10/1 + boron (formulation 20).

Formulation 21 was also tested, consisting in commercial colloidal SiO_2 + H_3BO_3 diluted in 50 ml H_2O. In addition, a silicic acid and a boric acid experimental formulation, whose efficacy against termites is reported in literature (Yamaguchi 2003), was selected for comparison with the previous ones. The same formulations were also tested according to the EN 117. Four wood samples ("T" blocks, 1,5 cm_{rad} x 2,5 cm_{tang} x 5 cm_{long}) for each formulation were placed in contact with colonies of 250 workers and some nymph/soldier inside a glass jar on the same substrate of moisturised Fontainebleau sand as previously described. The containers were then maintained at the same climatic conditions as the previous test for 8 weeks (Figures 36-37). After exposure, samples were dried at (103 $\pm$ 2)°C and the mass loss (ML) of the tested samples, was calculated by difference with the two dry masses before and after the test. Test results were expressed in terms of grade of attack, according to the visual scale in EN 117 and mean percentage of survival. The test was considered valid when at least 50% of the colony survived on the virulence control samples.

Figure 36- Figure 37 No-choice test against termites *Reticulitermes grassei* as adapted from the EN 117.

2.5.3 Efficacy of copper glycinate formulations against the brown rot fungus Coniophora puteana

Screening agar tests in Petri dishes were performed to evaluate the efficacy of copper glicinate formulations against fungal attack before to test them into wood. The screening tests also aimed at selecting the lower efficacy concentration values.
Amounts of copper glycinate and of copper glycinate combined to boric acid were added to 20 ml of sterilyzed agar-malt substrates (malt: 4%, agar:2.5%) in order to obtain the

final concentration values showed in Table 16. Pure malt-agar substrates, without any additive, were used as control.

Table 16 Screening test in Petri dishes: tested formulations and concentrations.

$CuGly_2$[M]	$CuGly_2$+ H_3BO_3[M]	H_3BO_3[M]
0.010	0.010 + 0.010	0.010
0.015	0.010 + 0.015	0.015
0.020	0.010 + 0.020	0.020
	0.010 + 0.040	0.040
	0.015 + 0.010	
	0.015 + 0.015	
	0.015 + 0.020	
	0.015 + 0.040	

The substrate mixtures were placed in Petri dishes (diameter 90mm), then inoculated in the centre of each dish (8mm diameter) with the brown rot fungus *Coniophora puteana* (Schum.: Fr. Karst. BAM Ebw.15). Inoculate Petri dishes were placed at 22 ± 2 °C and 70 ± 5 % RH climatic chamber for 10 days. Three replicates for each concentration and formulation were prepared. Fungal activity was calculated by the ratio between the maximum diameter of fungal growth at different concentrations and formulations and control and expressed as percentage of growth (the value 100 – growth percentage is the growth inhibition %).

Bravery accelerated tests (see par. 2.5.1) on impregnated wood mini-block samples were performed against the brown-rot fungus *Coniophora puteana*. (Schum.:Fries) P.Karsten or to the white rot fungus *Trametes versicolor*(L.:Fr.) Pilat.

Tree formulations were selected:

- Formulation 25 cis-copper (II)-bisglycinate (0,023M)
- Formulation 26 cis-copper (II)-bisglycinate (0,023M) and boric acid (0.023)
- Formulation 27 cis-copper(II)-bisglycinate(0,004M)

The choice was based on the results of the previous screening tests and on evaluations on cis-copper (II)-bisglycinate solubility in water. Further formulations were also tested, characterized by higher copper and glycine concentrations, obtained by solving separately copper(II)sulphate and glycine in water:

- Formulation 28 copper sulfate (0.1 M) + glycine (0.2 M).
- Formulation 29 copper sulfate (0.05M) and glycine (0.1M)
- Formulation 30 copper sulfate (0.05M),glycine (0.1M) and boric acid (0.05M).
- Formulation 31 copper sulfate (0.1M), glycine (0.2M) and boric acid (0.1M).

2.5.4 Efficacy of copper and zinc salicylate formulations in linseed oil against termites

The species *Kalotermes flavicollis* (Fabricius) and *Reticulitermes Lucifugus* (Rossi) were chosen for the tests of effectiveness of copper and zinc salicilate against termites. *Pinus sylvestris* L. mini-tablet samples (0,3 cm_{rad} x 3 cm_{tang} x 4 cm_{long}) employed, cut from the same wooden block (about 10 x 15 x 15 cm) previously oven dried at 80°C. Four theses were considered, with 5 (test against *R.Lucifugus*) or 10 (test against *K.Flavicollis*) replicates for thesis:

- Thesis 1: not treated samples (control)
- Thesis 2: samples treated with pure linseed oil
- Thesis 3: samples treated with zinc salicylate in linseed oil (formulation 34)
- Thesis 4: samples treated with copper salicylate in linseed oil (formulation 33)

Samples were pencil signed and weighed before (M_a) and after (M_{at}) oven dry treatment at 80°C for 15 hours. Therefore, they were subjected to immersion in zinc or copper salicylate solutions. They were dipped 10 at a time in 80 ml solution and were prevented from floating by the use of tweezers. Immersion lasted 12 hours. Then, samples were dried on air for 24 hrs, rolled on filter paper for 20 seconds and weighed before and after oven dry treatment (Figure 38).

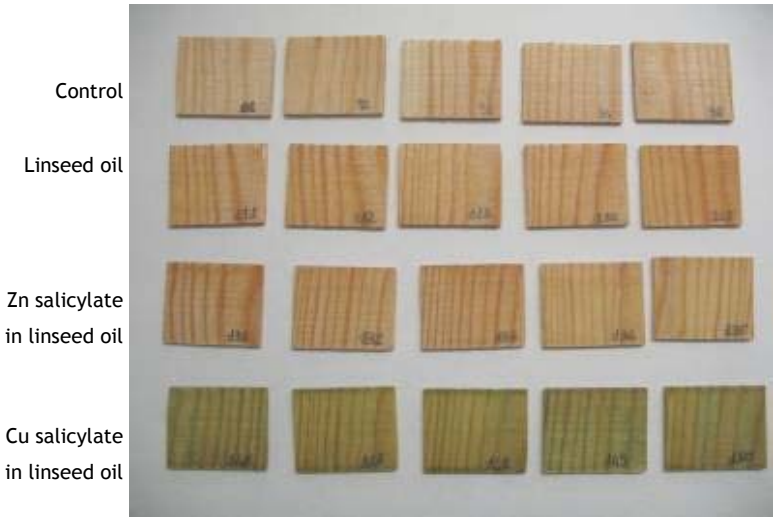


Figure 38 Mini-tablet samples prepared by immersion in linseed oil solutions to be employed for the efficacy test against termites.

Effectiveness tests against *R. Lucifugus (Rossi)* and *K. flavicollis (Fabricius)* were performed as follows.

R.Lucifugus were extracted from infested trunks coming from S. Rossore park (Pisa) immediately before the beginning of the test and maintained in a dark room, at room temperature. The number of workers and soldiers consisted of 50 workers and 1 soldier for each replicate. Plastic cylindrical containers endowed with caps were employed. Holes were performed on the caps and grids were applied on the holes. Containers were sterilized (UV exposure for 30 minutes) and filled with sand (15 g) and distilled water. Wood samples were inserted one for container, vertically disposed in order to facilitate the count of died individuals and the observation of termites behavior during the test (Figure 39). A further sterilization was performed after the wood sample positioning. Termites were inserted; finally, containers were placed in sterilized plastic boxes with caps previously equipped with absorbing paper soaked with water in order to maintain high UR%. A control of the termite behavior and mortality was performed after 10 days (Figure 40). Cylindrical containers were not open in order to keep the systemconditions unaltered. The test lasted 20 days, corresponding to the time when all the individuals (with exception of two workers) in the control samples were died. Samples were removed, softly brushed and kept in plastic bags until final anhydrous weight was determined (oven drying, 80°C, 15 h).

Tests against *K.flavicollis* were performed in closed Petri dishes each filled with sand (35g) and one wood sample. Sand was sieved under running water and microwave sterilized for some minutes before to be inserted in glass Petri capsules (diameter 11 cm). The Petri dishes filled with sand were autoclave sterilized for 20 minutes at 120°C. Therefore, filtered distilled water (3 drops) was added for each capsule. Samples were inserted with tweezers and disposed with the larger face on the sand. *K.flavicollis* termites were extracted from infested trunks immediately before the beginning of the test. Individuals were microscope observed to verify their health and 28 workers and 1 soldier were inserted in each Petri dish. A control of the termite's behavior and mortality was carried out after 10 days and 20 days. Containers were not open in order to maintain system conditions unaltered. The test lasted 30 days; samples were finally removed, softly brushed and kept in plastic bags until final anhydrous weight was determined (oven drying, 80°C, 15 h). Final mortality was determined by counting died individuals for each replicate and determining wood mass loss (ML%).

Figure 39 Efficacy test against termites R. lucifugus. Plastic cylindrical containers were employed, filled with sand and covered with holed caps. Wood samples were vertically disposed inside each container in order to facilitate the counting of died termite individuals and the observation of termite's behaviour without altering the system.

Figure 40 Efficacy test against termites R. Lucifugus. Observation of a sample treated with copper salicylate after 10 days from the beginning of the test. Termites are mainly disposed in the sand.

2.5.5 Efficacy of copper and zinc salicylate in ethylene glycol against termites

Tests were performed to evaluate the effect of copper and zinc salicylate formulations in ethylene glycol against termites *Kalotermes flavicollis (Fabricius)* and *Reticulitermes Lucifugus (Rossi)*. The procedure described in par.2.5.4 was followed, with the following thesis (5 replicates):

- Thesis 1: not treated samples (control)
- Thesis 2: samples treated with pure ethylene glycol
- Thesis 3: samples treated with zinc salicylate in ethylene glycol (formulation 35)
- Thesis 4: samples treated with copper salicylate in ethyl glycol (formulation 36).

Effectiveness tests are still in progress.

2.6 Physical-mechanical characterization of treated specimens

2.6.1 Colorimetric measurements

Preservative treatments can cause colour changes in the wood specimens depending on the formulation and concentration applied. Treated wood samples were characterized from a colorimetric point of view to evaluate and define surface colour changes.

Pinus sylvestris L. mini-blocks were chosen, whose light natural colour avoids to evidence possible differences in value, brightess or lightness. Colorimetric characterization was performed by means of a multi-spectral scanner (Antonioli 2004). The instrument was calibrated by recording the spectral image of a white sheet of paper considered as referring sample. Therefore, tree replicates for each formulation were placed under the scanner system with their not signed main surface upward. After focusing, samples were scanned (3mm/sec, 500 scans). A scan area of 3cm^2, corresponding to the whole main surface, was considered for each sample. Average values of light reflection intensity, as function of wavelength, were obtained for each area, then normalized basing on the spectral reflection factor of the white reference in order to obtain Reflection factor vs wavenumber curves (spectral characterization). CIE L*a*b* (1976) colorimetric coordinates (L=lightness; a=red-green coefficient; b=yellow-blue coefficient) in the Chromaticity Diagram or Psychometric Colour Diagram (Figure 41) were mathematically deduced, representing the colorimetric characterization of each sample surface.

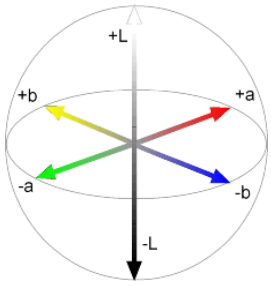


Figure 41 Chromaticity Diagram. L, a and b represent the three dimensions of the diagram. L= lightness coordinate, a = red-green coordinate; b=blue-yellow coordinate.

Scan measurements of the same samples before any preservative treatment had been previously obtained in the same way, so that the wood samples color differences before

and after any treatment could be deduced. They were expressed as ΔE values, representing the geometrical distance between two colour points in the CIE L*a*b* colour-space.

$$\Delta E = \sqrt{(L_2-L_1)^2+(a_2-a_1)^2+(b_2-b_1)^2}$$

where

L_2 and L_1= lightness coordinates before and after treatment, respectively

a_1 and a_2= red-green coordinates before and after treatment, respectively

b_1 and b_2= yellow-blue coordinates before and after treatment, respectively.

Reflection factor vs wavenumber curves, CIE L*a*b* colorimetric coordinates and ΔE values are reported.

2.6.2 Sorption test

Treatments performed on wood may influence its sorption properties. Wood is, in fact, a hygroscopic material: whenever the hygro-thermal conditions of the environment change, wood tends to reach a new Equilibrium Moisture Content (EMC), adsorbing or desorbing water. Surface treatments, for example, may alter the rate exchange of water vapour between wood and air by creating a barrier effect (Allegretti 2008); this leads to a reduction of the shrinkage and swelling of the wood structure related to the variations of moisture content, and avoids deformations, internal stresses or cracks as consequence of environmental changes. Changes in sorption properties due to linseed oil or sol-gel treatments, with or without metal addiction, were studied.

Spruce *(Picea abies)* L. tablets (20 x 7 x 0.5 cm) were used for the this purpose. Spruce wood was selected because it is more studied than other wood species, with well-known and documented physical and mechanical features, and because it is easy to find defect-free (knots, spiral grain, reaction wood etc.) and it has homogeneous characteristics (density, rings pattern etc.) (Allegretti 2008). Tablets were cut from the same wooden board. They were divided into sets of 7 replicates depending on their mass weight and each set was treated with a different formulation.

Linseed oil treatments: one set of samples was dipped in a zinc-salicylate solution in linseed oil for 22 hours. Samples were prevented from floating by the use of weights and were rotated periodically in order to ensure homogeneous contact conditions between the oil solution and the wooden surfaces. After immersion, the residual solution was removed from the sample surfaces by means of a spatula and samples were dried on air until constant weight was reached (7 days).

A set of samples dipped into pure linseed oil without any addiction of chelates was also prepared.

Sol-gel treatments: formulations with or without copper were tested. Tablet samples were dipped, two at a time, in solutions prepared with 75ml TEOS (0.040 mol), 75 ml APTES (0.042 mol) and 150 ml EtOH (TEOS/APTES 1:1 without copper, Formulation 1) or with 75 ml TEOS (0.040 mol), 75 ml APTES (0.042 mol), 10,875 g $CuCl_2$ (0.00085 mol) and 150 ml EtOH (TEOS/APTES 1:1 and APTES/Cu 5:1, Formulation 4). During immersion, the system was maintained sealed in order to avoid the sol gelation. After any treatment, samples were dried at room temperature on tweezers.

The following step consisted in the waterproofing of five of the six samples faces in order that only one of the 20 x 7 cm radial faces was able to exchange water with the environment. Waterproofing was obtained by covering the wooden surface with aluminium sheets pasted with glue. All the treated samples and 7 untreated samples (used as reference for the evaluation of the sorption properties of untreated wood) were inserted altogether into a climatic chamber Figure 42). The quality of the data from a sorption test mainly depends on the performance of the climatic chamber: it must ensure very stable climatic conditions over time and uniformity throughout the volume of the chamber; the relative humidity (RH%) change must be very fast and without any significant variation of temperature (T). For this purpose a special dew-point climatic chamber was designed and built (Allegretti, 2007). The volume of the chamber is small (0.15 m^3) suitable for small specimens. The analytical balance (resolution 0.01g) is in the interior of the chamber, protected against vibrations and air fluxes. Two holes with rubber gloves permit manipulation of the samples during weighing operations without the need to open the door. The cell parameters T (°C), and RH (%) can be regulated by means of a PID controller connected to a computer system. The sorption test method as in Allegretti (Allegretti, 2008) was adopted. T and RH were varied as shown in Table 5, in order to induce four "jumps" (Table 17). Each temperature-humidity condition was

maintained until the samples reached a constant weight (corresponding to the Equilibrium Moisture Content condition, EMC).

Figure 42 Sorption test: samples disposition in the climatic chamber used to create controlled environmental (temperature, relative humidity) condition changes.

Table 17 Temperature-humidity conditions in the climatic chamber during the sorption test. The mass variation of the wood samples as a response to the changing environmental condition was evaluated. Each temperature-humidity condition was maintained until samples reached a constant weight.

	Initial Equilibrium Condition	Jump 1	Jump 2	Jump 3	Jump 4
RH [%]	58	35	28	73	87
T [°C]	27	27	27	20	20
H_{H2O} [%]	17	9	5	15	18

Normalized Sorption curves (i.e. the fractional weight change *E* versus the time square root) showing the sample mass variation as function of time were obtained for each "jump". From the slope of the normalized sorption curve the apparent diffusion coefficient *D** can eventually be calculated through the following equation representing an analytical solution of Fick's second law:

$$D^* = \frac{\pi x^2}{4}\left(\frac{dE}{d\sqrt{t}}\right)^2$$

where *E* is the normalized weight change = $\Delta M_t/\Delta m_e$, and ΔM_t and Δm_e are the weight changes at time *t* and at final equilibrium respectively, and *x* is the thickness of the sample [26]. The slope $dE/d\sqrt{t}$ is usually calculated in the part of the curve from 0 to *E*=0.5 corresponding to the half sorption time ($t_{0.5}$).

2.6.3 Dimensional stability

Among the physical-mechanical properties of treated wood, the effect of sol-gel treatments on wood dimensional stability was evaluated. *Picea Abies L.* sol-gel treated (Formulation 2) block samples (4 x 2 x 2 cm^3) were employed on this purpose. Untreated samples of the same type (25 replicates) were also employed as reference. Treated samples were prepared by impregnation procedure, using 176 ml of sol-gel ethanol-precursor solutions for groups of 5 samples.

Impregnated and not impregnated samples were measured in their three dimensional directions by means of a centesimal mechanical comparator and placed in a microclimatic chamber (see par. 2.6.2). Temperature/humidity-jump perturbations were applied as in Table 17; samples dimensions were measured after any jump.

Further tests were performed on the same samples once extracted from the microclimatic chamber. The first one consisted in the immersion in distilled water for 1 week in a desiccator; during the test, vacuum (80 mbar) - air cycles were applied in order to favor wood imbibition and sample dimensions were recorded immediately after the test, representing the major dimensional values, in air saturation conditions (UR=100%). The second one consisted in a thermal treatment of 60°C in a thermostatic oven for 24 hours followed by temperature enhancing to 103°C. Lowest dimensional values were obtained in this way, corresponding to anhydrous conditions. Finally, all the samples were placed again in the microclimalic chamber to reach the equilibrium conditions at 87% RH and subsequently at 37% RH. The time necessary for treated and untreated samples to reach again a constant weight (hygroscopic equilibrium) at 37% RH and 27°C (that means the initial environmental conditions of the dimensional stability test) was evaluated, the cycle of temperature/humidity-jump perturbations was repeated and the respective hysteresis curves for treated and untreated specimens were constructed.

3. Results and Discussion

Preface

As pointed out in the Introduction, interest in using alternative wood preservative formulations, less dangerous as possible for the environment and for the human health, has recently increased due to the banishing (Biocidal Products Directive, 1998) of most of the previous preservative products, characterized by high toxicity and easy diffusion in air, water or soil.

Copper, zinc, boron and silver represent the main used inorganic active principles in formulations against biological attack (Schultz, 2008). Copper(II) is largely used as fungicide as it is supposed to denaturate proteins and enzymes due to its affinity for thiol groups in the fungal cells and to inhibit fungal respiration due to its interference with the activity of conversion of pyruvate to acetyl coenzyme A (Eaton, 1993); zinc and silver are meanly known for their anti-bacterial activity, but, as in the case of copper and many heavy metals, at adequate concentrations they become potent inhibitors of fungal enzymatic reactions (Baldrian, 2002; Dorau, 2004); boric acid and borates act as biostatic or biocide agents against fungi and insects since they form stable complexes with vitamins and coenzymes inhibiting the enzyme functions; copper, boron, zinc and silver also act against termites since they inferfere with the population of symbiotic bacteria, protozoa or enzymes present in the termite's gut and responsible for the breakdown of lignocellulosic material.

Preliminary studies have been performed with the aim of avoiding or minimizing the release of active principles into the environment in the framework two different research programs named A and B.

A. Modification of wood with inorganic or hybrid inorganic-organic systems based on silica sol-gel materials (starting from alkoxysilanes) with the possibility of incorporating active elements or anchoring them through coordinative linkages;

B. treatment of wood with stable metal chelates (instead of the corresponding metal salts) dissolved in water or organic solvents.

PART A - TREATMENTS WITH SILOXANE SOL-GEL MATERIALS

3.1. Characterization of treated wood

Treatments with sol-gel siloxane materials have been performed on *Pinus sylvestris* L. wooden samples, starting from tetraethoxysilane (TEOS) and 3-aminopropyltriethoxysilane (APTES), as described in the experimental part.
Hydrolysis and co-condensation of the two precursors make it possible to realize hybrid inorganic-organic materials in which a silica network is functionalized with aminopropyl groups (Figures 43-44).

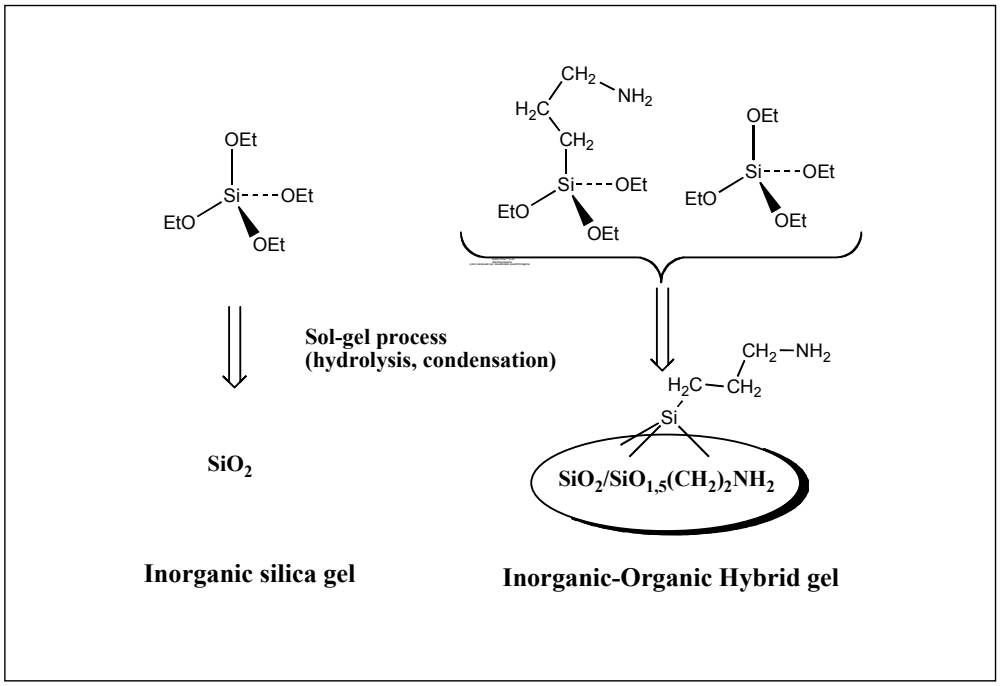


Figure 43 Hybrid aminosil gels from TEOS and APTES.

$$\underset{\textbf{TEOS}}{n\mathrm{Si(OEt)_4}} + \underset{\textbf{APTES}}{\mathrm{H_2N(CH_2)_3Si(OEt)_3}} \xrightarrow[-(4n+3)\mathrm{EtOH}]{(2n+3/2)\mathrm{H_2O}} n\mathrm{SiO_2 \cdot SiO_{3/2}(CH_2)_3NH_2}$$

Figure 44 Scheme of reaction between TEOS and APTES by means of sol-gel process.

The improvement of biological resistance conferred by means of wood modification with silica xerogels is reported in literature: it seems to be due both to a lower recognition of modified wood as a substrate adequate for biological development and to a reduction of its moisture sorption properties hence limiting biological attacks (Terziev, 2009; Hill, 2004; see par. 1.5.3). Further enhance of durability should be due to the presence of amino-groups (Donath, 2006; Ghosh, 2008).

Wood samples were treated with TEOS-APTES-Ethanol sols without any addiction of water. For the hydrolysis reactions, water bonded to the wood cell walls was used (Saka, 2001), consenting to selectively favour the *in situ* polimerization inside the cell walls rather than in the lumen, those enhancing wood biological resistance (see par. 1.4.2).

Wood moisture content was calculated before any treatment from the following formula:

$$\text{Humidity } \% = \frac{M_I - M_O}{M_O} \times 100$$

where

M_I = sample mass before any treatment, at environmental conditions of about 20°C temperature and 65% RH;

M_0 = sample dry mass (oven drying 103 ± 5 °C, 18 hours)

A wood humidity among 11% and 13% was detected, i.e. near to the Equlibrium Moisture Content (EMC) of 12% reported for wood in equilibrium with "normal" 20°C T - 65% RH environmental conditions.

A preliminary comparison between simple immersion treatment and impregnation under vacuum (see par. 2.3.2) was carried out on samples treated with ethanol solutions with TEOS-APTES 1/1 molar ratio. The superior efficacy of the impregnation method (right image) against simple immersion (left image) is evidenced by the micrographs reported in Figure 45. Both samples gave almost the same microanalytical data on the surface, nevertheless the impregnated one exhibits a more homogeneous surface texture without formation of crusts that could cause significant leaching of material.

IMMERSION IMPREGNATION

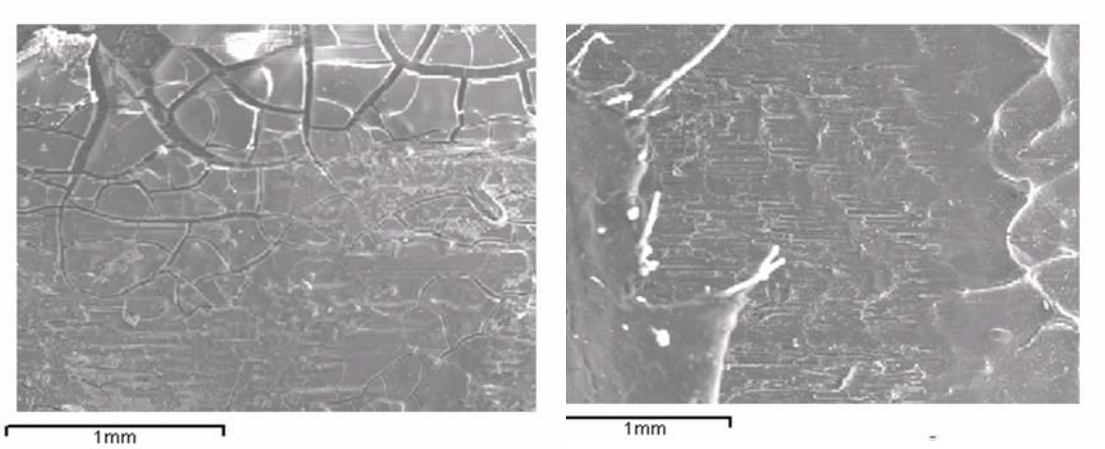


Figure 45 SEM Secondary Electron images of the wood surface after immersion in alkoxysilanes mixture (left) and after impregnation under vacuum with the same mixture (right).

In the case of impregnation, the presence of the silica xerogel into the wooden structure was confirmed by the SEM image of the cross section of wood after treatment, showing silica flakes especially near and inside the cell walls. In Figure 46, SEM images of untreated (left) and treated (right) wood cross sections are reported (Figure 46).

UNTREATED TREATED

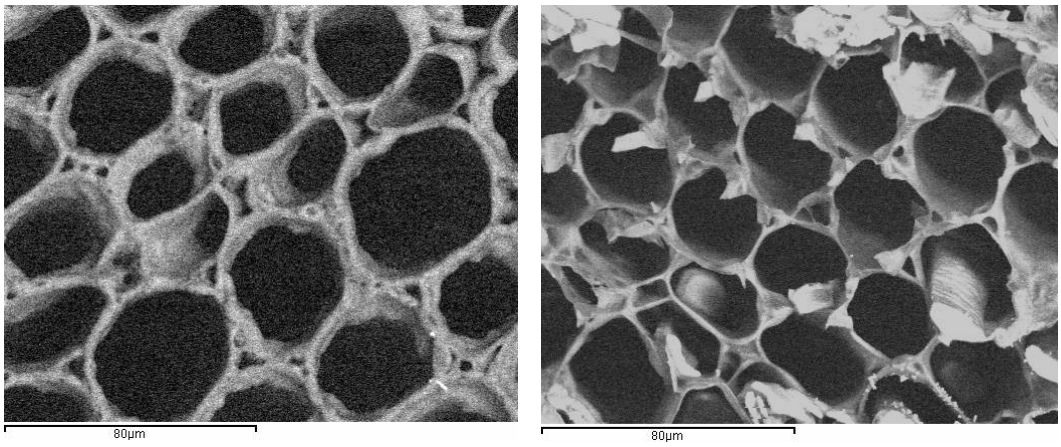


Figure 46 SEM Secondary Electron images of the transversal cross section of untreated (left) and sol-gel TEOS-APTES impregnated (right) wood.

From the ESEM image of the cross section of an impregnated wood and corresponding EDX microanalyses, a deeper sol penetration inside the wood sample achieved by the impregnation procedure was demonstrated by observing the presence of silicon in deeper areas from the wood surface (Figure 47).

Therefore, all sol-gel treatments, based on the modification of wood with siloxane materials bearing amino groups (TEOS-APTES formulations) and described in this thesis, were carried out by impregnation.

Besides microscopic characterization, solid state NMR investigations were performed to evaluate the degree of texture of the siloxane xerogel inside the wood compared with that obtained in absence of wood substrates. ^{29}Si CP/MAS solid NMR spectra of the pure TEOS-APTES 1/1 xerogel and the same impregnated into the wood are reported in Figure 48. The similarity among the two spectra suggests that siloxane reticulation of the two materials is analogous.

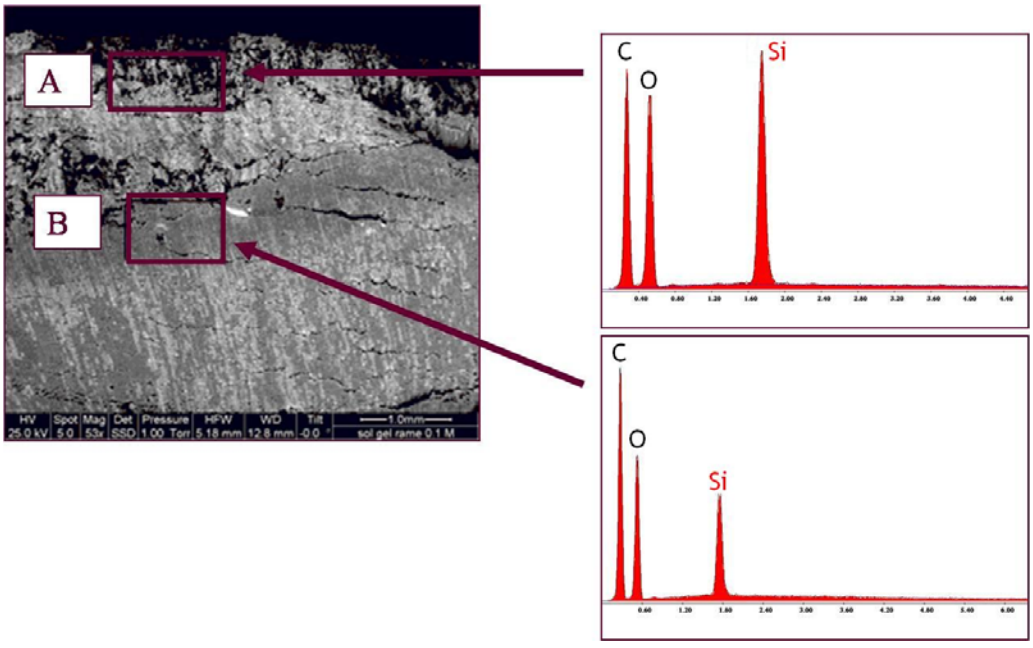


Figure 47 ESEM image of the transversal cross section of wood treated by sol-gel process (impregnation) with relative EDX microanalyses showing the presence of silicon in areas at different depth from the wood upper surface.

Two intense groups of signals are visible in a ratio of 41:59 and 46:54, respectively. This is in agreement with the nominal concentrations of the two reagents. They are related to condensed TEOS (T) (Q units at around -100ppm) and APTES (A) (T units at around -60ppm). The molecular structures of the binary matrix both in the xerogel and in the treated wood samples are similar. The structural units are Q4 [$Si(OSi)_4$], Q3 [$RO\text{-}Si(OSi)_3$], Q2 [$(RO)_2\text{-}Si(OSi)_2$] belonging to TEOS-based gel and T3 [$RNSi(OSi)_3$], T2 [$RNSi(OSi)_2OR$], T1 [$RNSi(OSi)(OR)_2$] belonging to APTES-based gel; R could be H or OEt, RN refers to aminopropyl group (Rahman, 2009). Both samples show a high degree of condensation, DOC, indicating that the matrix is quite stable and will not change significantly upon ageing. Considering the different rate of hydrolysis and condensation of the two silanes, the higher amount of fully condensed species T3 with respect to Q4 is expected (Table 18).

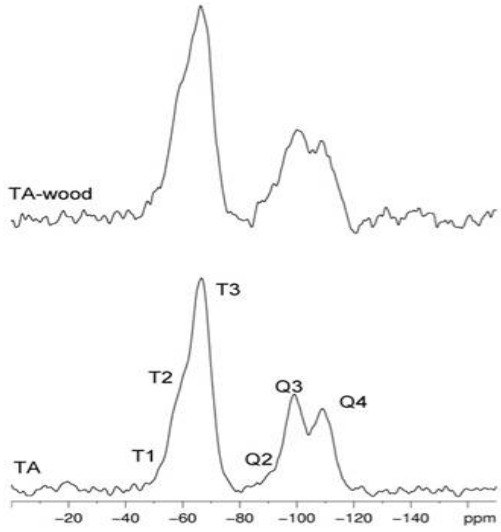


Figure 48. ^{29}Si CPMAS spectrum of TA xerogel and TA-impregnated wood. T=TEOS, A= APTES.

Table 18. ^{29}Si NMR chemical shifts and related assignments. T=TEOS, A= APTES. DOC=degree of condensation.

	T:A 1:1			T:A on wood		
Unit type	**δ (ppm)**	**Rel. Area (%)**	**DOC**	**δ (ppm)**	**Rel. Area (%)**	**DOC**
Q4	-108.4	22.0	87.5	108.8	22.3	89.2
Q3	-100.0	15.6		-98.9	20.9	
Q2	-90.7	3.2		-90.2	2.7	
T3	-66.1	41.8		-65.9	43.9	
T2	-58.9	13.8		-58.2	7.8	
T1	-52.7	3.6		-53.2	2.4	

^{13}C CP/MAS NMR measurements were performed to determine the existence of organic group R^N and possible residual OEt (Figure 49), due to a non-complete hydrolysis, in modified silica xerogel and wood coating. The results are shown in Figure 50. The spectrum of the xerogel TA exhibits three peaks at 9.8 ppm (1), 20.9 ppm (2), and 41.7 ppm (3) assigned to the three methylene carbons. Minor peaks at 56.3 and 17.5 ppm refer to residual OEt groups. There are also two unexpected peaks at 163.2 and 24.0 ppm. (probably acetone contamination?).

The sample of the impregnated wood gives rise to a spectrum that is the superimposition of the spectra of the main wood components, i.e. cellulose, hemicelluloses and lignin (Bardet, 2009) and the organic part of APTES. Main peaks are identified (Figure 51) and listed in Table 19.

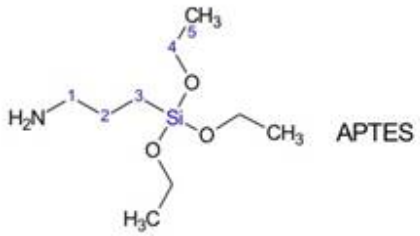


Figure 49 Structures and numbering scheme of carbon atoms of silanes used for impregnation of wood.

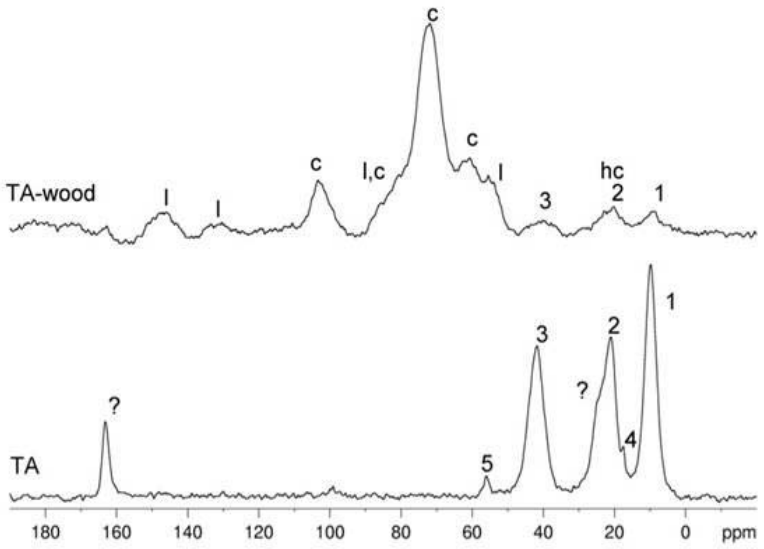


Figure 50. ^{13}C CPMAS NMR spectrum of TA xerogel and TA-impregnated wood (l = lignin, c=cellulose, hc= hemicellulose).

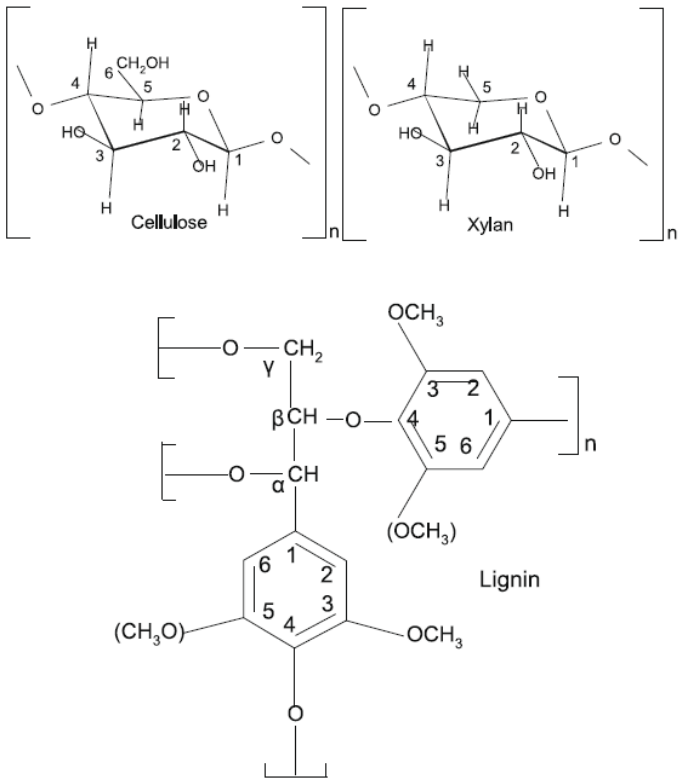


Figure 51 Structures and numbering scheme of the main components of the wood.

δ (ppm)	lignin	cellulose	Hemicelluloses	APTES
9.2				1
20.6			**C**H_3-COO-	2
40.2				3
55.5	-O**C**H_3			
60.8	Cγ	6		
71.9	Cα	2, 3, 5		
84.4	Cβ, 4	4		
103.4	S2, S6	1		
131.3	S1, S4			
146.9	S3, S5			
164.0			-**C**OO-	

Table 19. ^{13}C NMR chemical shifts and related assignments referring to scheme 1 and 2 (S refers to the syringyl unit of lignine, i.e. aromatic ring with two methoxy groups, in non-etherified arylglycerol ß-aryl ethers) (Bardet, 2004).

3.1.1 Sol-gel treatments with addiction of copper

The TEOS/APTES sol-gel treatment is not only suitable for the enhancement of wood durability in virtue of the silica network and of the presence of amino-groups. The specific important feature of the amino-groups is the capability to anchor copper ions through coordinative interactions, avoiding or minimizing copper leaching so making the preservative system more efficace, durable and environmentally-friendly than copper-based preservatives used in the past.

Two different procedures were performed to add copper ions to the base formulation. A two-step process consisted of the vacuum impregnation of wood specimens with the sol-gel mixture followed by dipping into a solution of copper sulphate. The process occurs in the wood producing the hybrid gel, where copper may be subsequently grafted by amino-groups into the net. A one-step process consisted of the vacuum impregnation of wood with a homogeneous sol mixture containing TEOS, APTES and copper (II) chloride in a well-defined molar ratio. Also in this case the sol-gel process occurs in the wood after the impregnation. Copper chloride was chosen instead of sulphate for its higher solubility in the sol mixture. This process should lead copper to penetrate deeply into wood, driven by the sol.

Both sol-gel impregnation processes caused a high increase in mass. In the two-step process, the average retention (expression of the solution quantity retained in the wood sample immediately after impregnation, considering that each wood block had the same nominal volume of 1.5 10^{-6} m^3) was 350 kg/m^3 and the average Weight Percent Gain (WPG, expression of the sample increasing of mass, due to the presence of the silica xerogel) was 28%. In the one-step process, the average retention was 404 kg/m^3 and the WPG was 19% as shown in Table 20 and Table 21.

Table 20 Absorption and Retention values of 0.5 x 1 x 3 cm pinewood samples impregnated with TEOS-APTES-Cu formulations through a two or one-step process.

Impregnation	Absorption [g]	Retention [kg/m^3]
Two-step process (n=8)	0.524 ± 0.08	350 ± 52
One-step process (n=9)	0.607 ± 0.08	404 ± 53

Table 21 Weight Percent Gain values of *Pinus sylvestris* L. wood samples impregnated with TEOS-APTES-Cu formulations through a two or one-step process.

Impregnation	Dry weight before impregnation [g]	Dry weight after impregnation [g]	WPG [%]
Two-step process (n=8)	0.626 ± 0.04	0.805 ± 0.05	28.58 ± 5.47
One-step process (n=9)	0.634 ± 0.10	0.832 ± 0.10	19.96 ± 6.71

Average copper retentions, determined by atomic absorption following EN 84 (1997) procedure, with four replicates for each thesis, were 1.57± 0.22 kg/m^3 in the two-step process and 4.61± 0.86 kg/m^3 in the one-step process: higher efficacy in retaining copper in case of the one-step process results from this data. However, the increase of mass (WPG) is mainly due to the mass of silicate, independent of the copper concentration into wood. The American Wood Preserver's Association currently distinguish the wood preservatives that have copper in their formulation as first generation, such as CCA and second generation, such as ACQ (alkaline copper quat) and CA (copper azole). Effective retentions of copper (II) are 1.18 kg/m^3 in the CCA and around 4.00 kg/m^3 in ACQ and CA (Laks 2008). It is possibile to compare the two-step and one-step processes copper amount to the two different generations of preservatives respectively (Table 22).

Table 22 Absorption and Retention values of 0.5 x 1 x 3 cm *Pinus sylvestris* L. wood samples impregnated with TEOS-APTES-copper formulations through a two-step or a one-step process.

Treatment	Cu (kg/m^3) mean ± sd
Two- step	1.57 ± 0.22
One- step	4.61± 0.86
First generation preservatives (CCA) (,Schultz, 2008)	1.18
Second generation preservatives (ACQ, CA) (Schultz,2008)	4.00

In the two-step process, although silica gel penetrated effectively into the wood as shown in X-ray spectrum in Figure 52, it appears that copper ions were not able to penetrate into the whole cross section, probably because the silica gel formed a barrier in the first layer of the wood surface. In fact, copper was not found in the cross section inner area. In conclusion, it appears that in the two-step process copper is grafted to silica gel mainly on the wood surface. On the other hand, in the case of the one-step process, copper penetrates effectively into the wood drawn by the coordinative interactions with the amino groups of APTES. This is well evidenced by the X-ray spectrum in Figure 53 obtained for 1 mm^2 inner cross section, showing the presence of silicon, copper and chlorine in the Si/Cu 12:1 and Cl/Cu 1:2 atomic ratios.

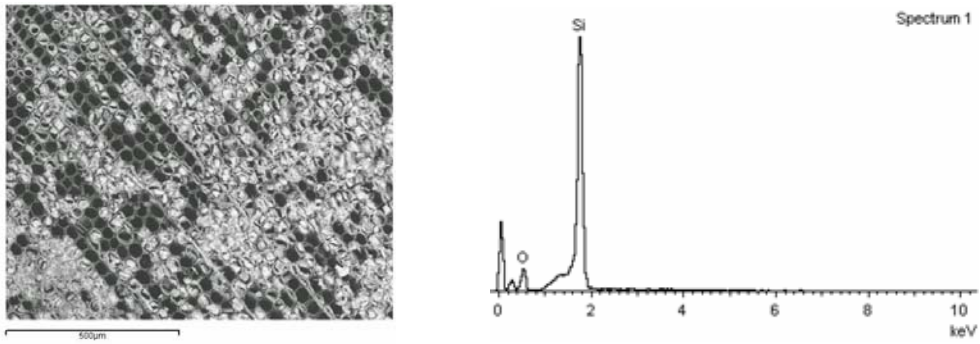


Figure 52 X-ray microanalysis from the inner area of the transversal section (surface ca 1 mm^2) of a wood sample subjected to the two-step process. Only silicon is present, whereas copper is not revealed.

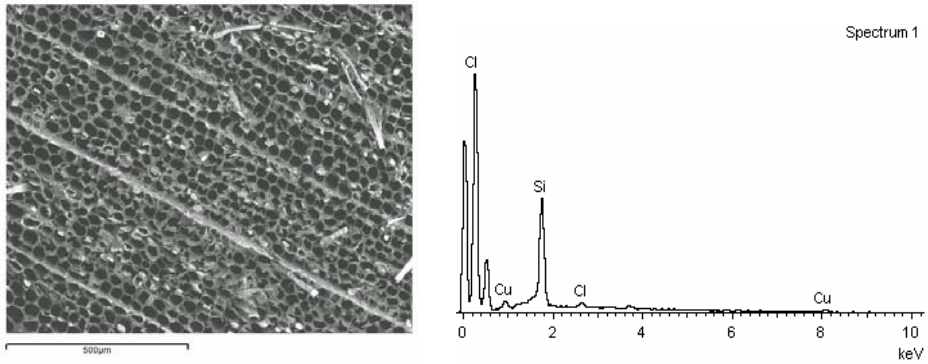
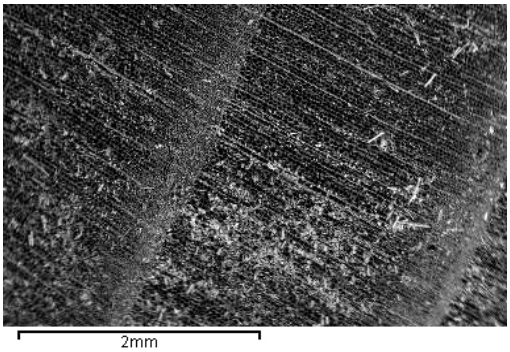


Figure 53 X-ray microanalysis from the inner area of the transversal section (surface ca 1 mm^2) of a wood sample subjected to the one-step process.

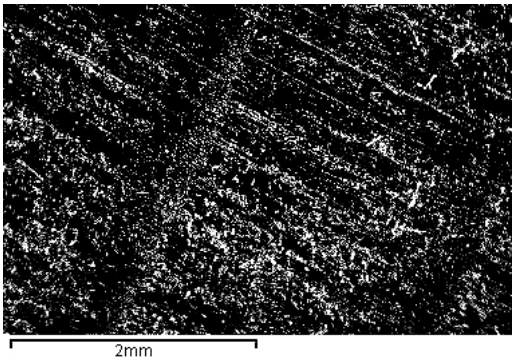
Silicon and copper maps performed with SEM on one-step treated wood transversal sections confirm that the two elements are distributed all over the sample thickness (Figure 54) and that are also present into the cell wall (Figure 55).

In order to gain insight about copper coordination in the composite material, EPR investigations have been performed.

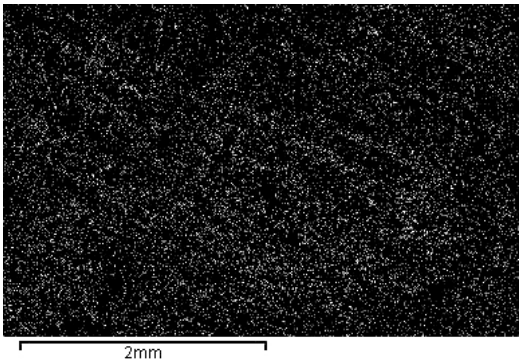
Figure 56 shows the low temperature EPR spectra paired with the best fit simulations of the one-step treated wood sample spectrum (a), of the corresponding xerogel spectrum without wood (b) and of the two-step treated wood sample (c). The anisotropic magnetic parameters for the experimental spectra refined with computer simulations are reported in Table 23. All spectra are characteristic of an axial geometry around the metal coordination site and computed spectra suggest that two and three nitrogen atoms



a.

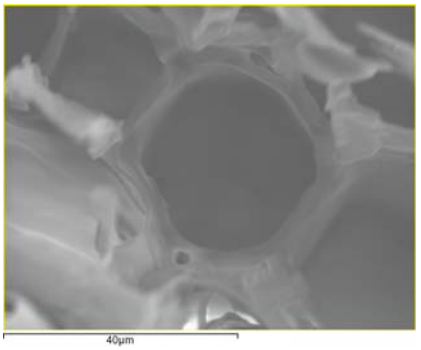


b.

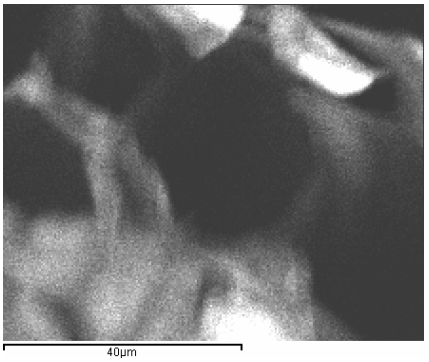


c.

Figure 54 SEM image of area map into a one-step treated wood cross section (a), X -ray maps of Si (b) and Cu (c).



a.



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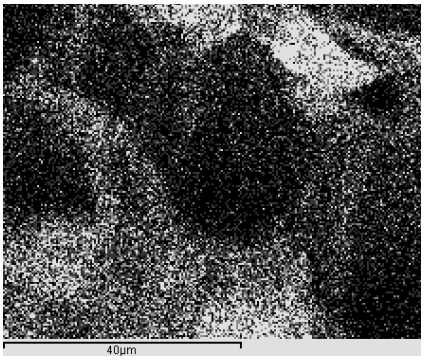


c.

Figure 55 SEM image of area map into the treated wood cross section (a), X -ray maps of Si (b) and Cu (c).

coordinate in the one-step, xerogel and two-step samples, respectively. The assignment of coordination site is also in agreement with Peisach-Blumberg plots that relate the parallel magnetic parameters towards the number of nitrogen and/or oxygen atoms present in the coordination sphere of the copper ion (Peisach, 1974; Pogni, 2000; D'Amelio, 2004; Fragoso, 2004).

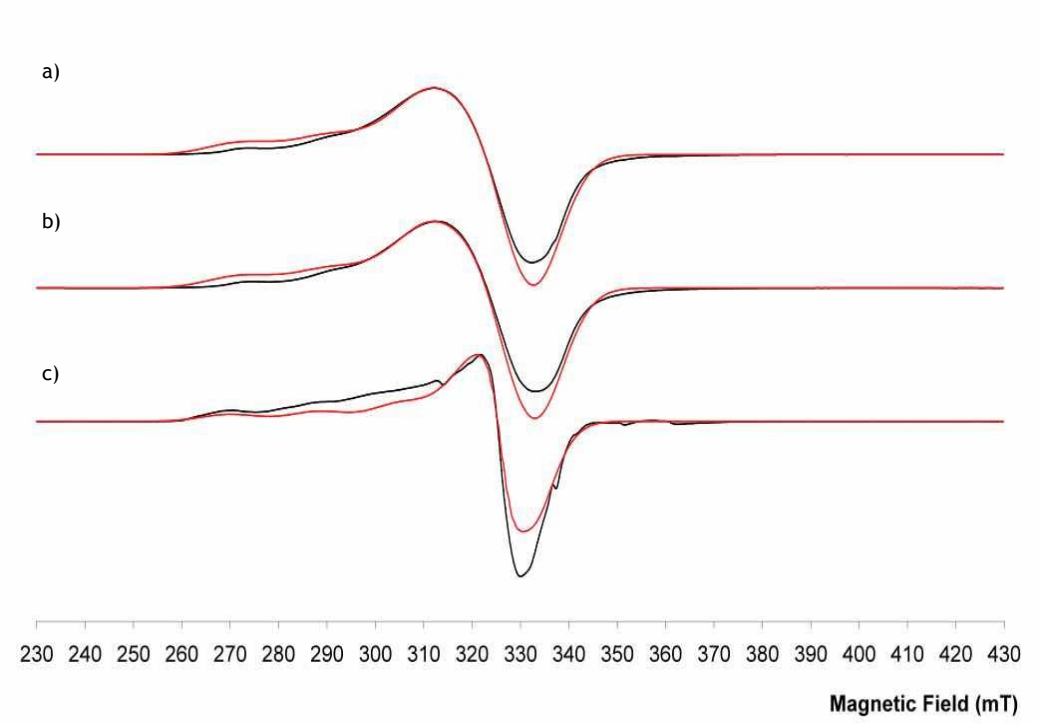


Figure 56 120K X-band EPR spectra of a) pine wood treated with the TEOS-APTES-Cu formulation (formulation 4: TEOS/ APTES 1:1 (v/v) + $CuCl_2$) by means of the one-step treatment b) xerogel from the same formulation without wood c) pine wood treated by means of the two-step treatment with the TEOS-APTES-Cu formulation (formulation 7: TEOS/ APTES 6:1 (v/v) and further immersion in 0,1M $CuSO_4$ aqueous solution) (black line) paired to the simulations that gave the best fit (red line)

From the comparison of spectra (a) and (b) in Figure 56, it appears that the copper environment in the one-step treated sample is identical to that of the xerogel blank, as confirmed by the corresponding magnetic parameters reported in Table 23, suggesting that coordination of nitrogen atoms to copper is not affected by the wood substrate. On the other hand, for the two-step sample a completely different lineshape of the EPR signal, with different magnetic parameters, is observed being due to different coordination to copper. In the two-step sample copper is added after the formation of

the xerogel and therefore it is strongly adsorbed onto the surface by strong coordinative interactions, exhibiting a mean coordination number of three nitrogen atoms. These interactions with the rigid xerogel prevent copper to penetrate beyond the first layer of the composite material.

Anyway, from an accurate inspection of the parallel region of the c) spectrum the presence of more than one species can not be ruled out; therefore the magnetic parameters reported in Table 23 for that specimen refer to the most abundant species formed which has been spectroscopically characterised.

Table 23. Anisotropic magnetic parameters obtained for one-step treated wood sample, corresponding residual xerogel and two-step treated wood sample.

	$g_{\|\|}$ [a]	$g_{\perp}$ [a]	$A_{\|\|}$ [b]	$A_{\perp}$ [b]	n° N [c]	A_N [b]
One-step treated wood sample	2.265	2.086	17	2.4	**2**	1.1
Xerogel	2.265	2.085	17	2.4	**2**	1.1
Two step Treated wood sample	2.285	2.076	17	0.9	**3**	1.0

[a] Estimated error 0.001
[b] Coupling constant are given in mT.
Estimated error for A_{Cu} = 0.1mT and for A_N =0.05mT
[c] Number of equatorial nitrogen donor atoms

In conclusion, the Cu coordination by means of two equatorial nitrogen donor atoms both in case of the one-step treated sample and in the corresponding xerogel outside wood suggests that this kind of coordination should be present also in the starting sol, and in case of wood treatment it could have facilitated Cu vehicolation deep inside wood. In the two-step treated sample Cu coordination occurs by means of three equatorial nitrogen donor atoms that probably could contribute to anchor copper ions in correspondence to the wooden surface and prevent them from penetrating deeper in the wood sample, as stated above.

Copper determination after the leaching procedure (EN 84) proved that fixation of copper to the silica gel was adequately strong, in fact its average content determined by

atomic spectroscopy resulted nearly the same for both leached and not leached samples in the two-step as in the one-step process (Table 24).

The one-step treatment seems to be more effective to fix copper, with a percentage of Cu leached of only 3.04% as compared to 7.1% in the two-step treatment. Both treatments seem to improve copper retention if compared to conventional copper treatment (Temiz, 2006b). This is in accordance with literature data (Kartal, 2007b, Kartal, 2009a) showing that silicon compounds play a role in retaining compounds into the treated wood during the leaching processes.

Table 24 Cu determination before and after leaching procedure. Number of replicates = 4; sd = standard deviation. Comparison with conventional copper preservative formulations.

Sol-gel process	Samples not leached	Samples leached
	Cu (kg/m^3) mean ± sd	Cu (kg/m^3) mean ± sd
Two- step	1.57 ± 0.22	1.49 ± 0.093
One- step	4.61± 0.86	4.72 ± 0.42

The satisfactory degree of impregnation achieved by the two-step and one-step impregnation processes and the improvement of copper retention made both methods worthy to be used for sol-gel modification of wood samples to be tested with regard to their resistance to biological attack (see par.3.2).

Nevertheless, further investigations were carried out on the one-step kind of impegnation, since it showed higher potentiality of copper penetration.

Treatments with different APTES concentration were considered, with and without the addiction of copper (Table 25); for each sample absorption, retention and Weight Percent Gain parameters were evaluated to verify the success of impregnation.

Table 25 Absorption, retention and weight percent gain values of samples impregnated by means of the one-step treatment with formulations having different APTES concentration, with and without copper. n=number of samples.

Formulation	Absorption [g] Average (n=20) ± st.dev.	Retention [kg/m3] Average (n=20) ± st.dev.	Weight Percent Gain [%] Average (n=20) ± st.dev
TEOS-APTES 1:1	0.5 ± 0.14	330.85 ± 90.68	22.78 ± 6.33
TEOS-APTES 1:1, APTES-Cu 5:1	0.56 ± 0.19	372.67 ± 125.06	27.06 ± 5.39
TEOS-APTES 10:1	0.52 ± 0.11	345.62 ± 70.11	26.09 ± 9.69
TEOS-APTES 10:1, APTES-Cu=5:1	0.59 ± 0.05	393.35 ± 31.71	20.5 ± 3.54

Vibrational spectroscopy characterizations were also performed. The infrared spectrum of impregnated pinewood was compared to the separate spectra of untreated wood and of xerogel without wood (Figure 57). Infrared spectra of the xerogels show the strong Si-O stretching band centred at 1029 cm^{-1}, the bands at 940 cm^{-1} and at 790 cm^{-1}, attributable to the Si-OH groups (Temiz,2006) and to the Si-O vibrational modes respectively, and the C-H stretching (3500-2800 cm-1) and bending (1600-1300 cm^{-1}) of the APTES chain.

The presence of the silica gel in the impregnated wood samples is shown by the enlargement of the very strong band of C-O bonds of wood in the region of 1462-1158 cm^{-1} owing to the contribution of the Si-O-Si linkages of the silica backbone.

Moreover, a shoulder appeared for impregnated wood at 940 cm^{-1} , which can be assigned to external Si-OH groups (Klonkowsky, 1999). Peaks around 790 can be also attributed to Si-O vibrational modes. No significant differences were observed in the O-H, N-H and C-H stretching (3500-2800 cm^{-1}) and bending regions (1600-1300 cm^{-1}), where contributions of the wood functional groups prevail.

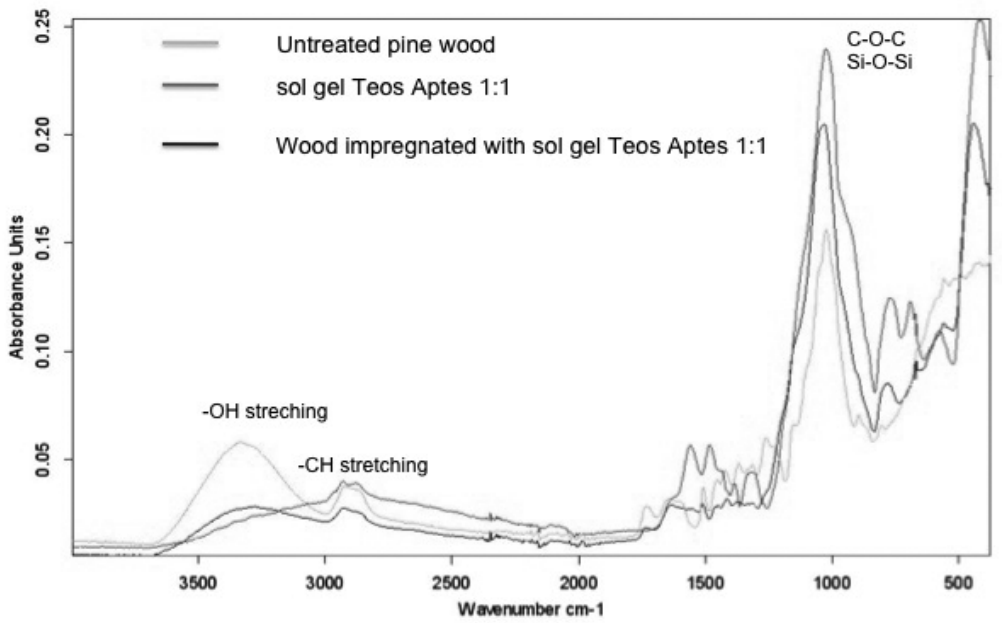


Figure 57 FT-IR spectra of pine wood before and after sol-gel TEOS/APTES 1:1 treatment and of the residual xerogel.

In case of TEOS-APTES 1/1 + Cu formulation, IR did not permit to detect possible Si-O-Cu linkages (vide infra), owing to the low copper concentration; in fact pure copper metasilicate shows only a very weak band at 668 cm^{-1} assignable to that group, which is undetectable in the spectra of impregnated wood. The Raman spectrum of pinewood impregnated with the formulation TEOS-APTES 1/1 v/v + Cu was compared to the xerogel obtained from external gelation of the same solution (Figure 58). The C-H stretching (1800cm^{-1}), the OH-NH bending (1600 cm^{-1}) and the Si-O bending (480 cm^{-1}) peaks are distinguishable. Apart from the high signal/noise ratio of the impregnated wood spectrum, the spectral pattern of the pure xerogel is reproduced in the fingerprint region of the impregnated wood spectrum: this could suggest the formation, in wood, of an analogous polymeric structure, but any possible interaction between wood, silica gel and copper shouldn't be extractable because of the low resolution of the typical silica gel bands.

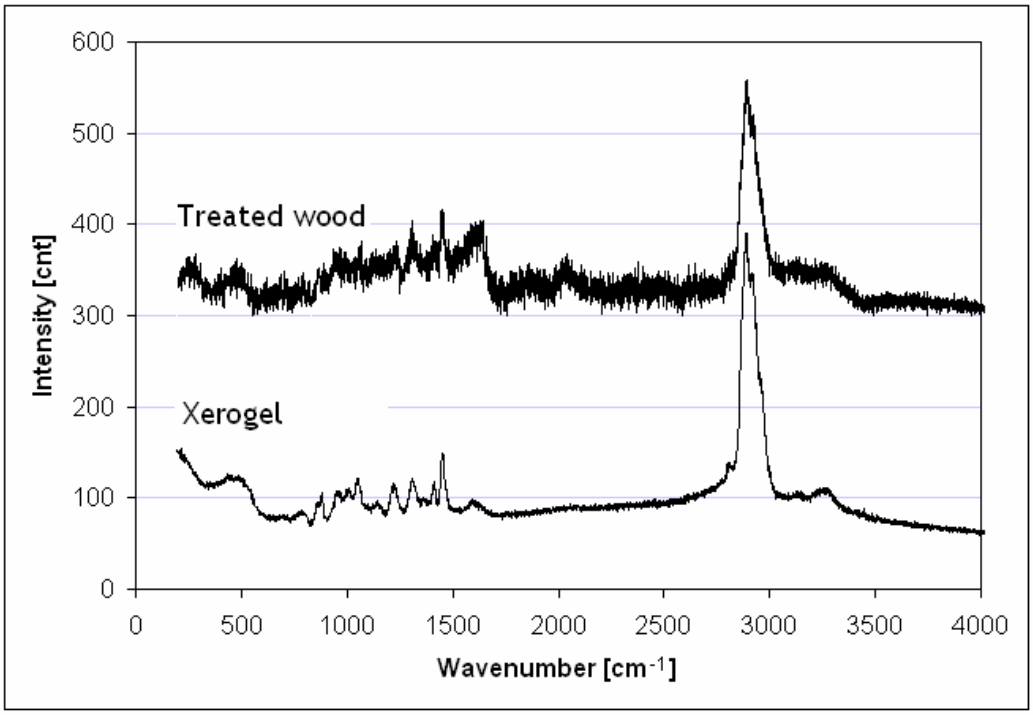


Figure 58 Raman spectra of pinewood impregnated with TEOS-APTES + Cu solution and of the residual xerogel obtained from external gelation of the same solution.

Informations about the xerogel distribution inside the wood samples were obtained by means of SEM-EDX analysis performed along the samples cross section of TEOS-APTES-Cu treated wood.

The almost constant atomic ratio Si/C recorded by the X-rays analysis at different penetration depths suggests a homogeneous distribution of the gel inside wood (Figure 59, Table 26).

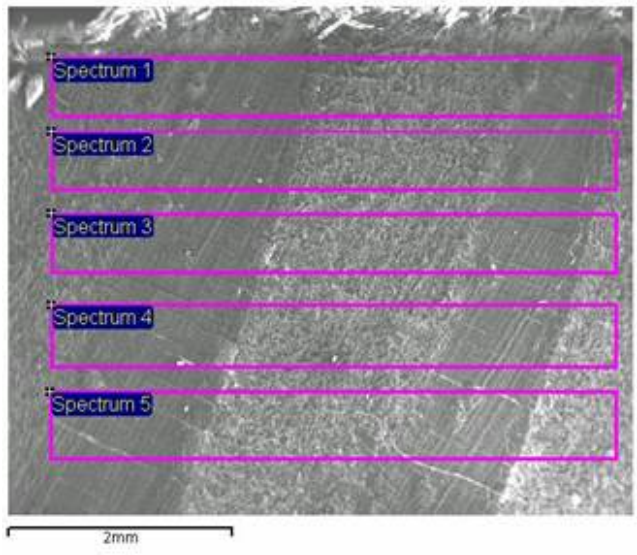


Figure 59 SEM (Secondry Electrons) image of the cross section of a sample impregnated with the TEOS-APTES 1/1 + Cu formulation. The areas on which mycroanalyses were performed are shown (Spectrum 1- Spectrum 5)

	Atomic ratio
	Si/C
Spectrum 1	1.38E-02
Spectrum 2	1.31E-02
Spectrum 3	1.47E-02
Spectrum 4	1.43E-02
Spectrum 5	1.49E-02

Table 26 Si/C microanalysis results corresponding to the areas shown in Figure 59. Errors are ± 2-5% for major elements and ± 5-10% for minor components.

Further details about the functionalized gel distribution in five areas at different depth from the sample surface are shown in Figure 60. The X-ray analysis show the presence of copper and chlorine all over (Figure 60, Table 27), with Si/Cu relative atomic ratios almost constant from the upper 500µ (SP1 and SP5 in figure 60) to the innest areas. (SP3 in figure 60) suggesting that effectively silicon and copper were penetrated into the wood during the sol-gel process.

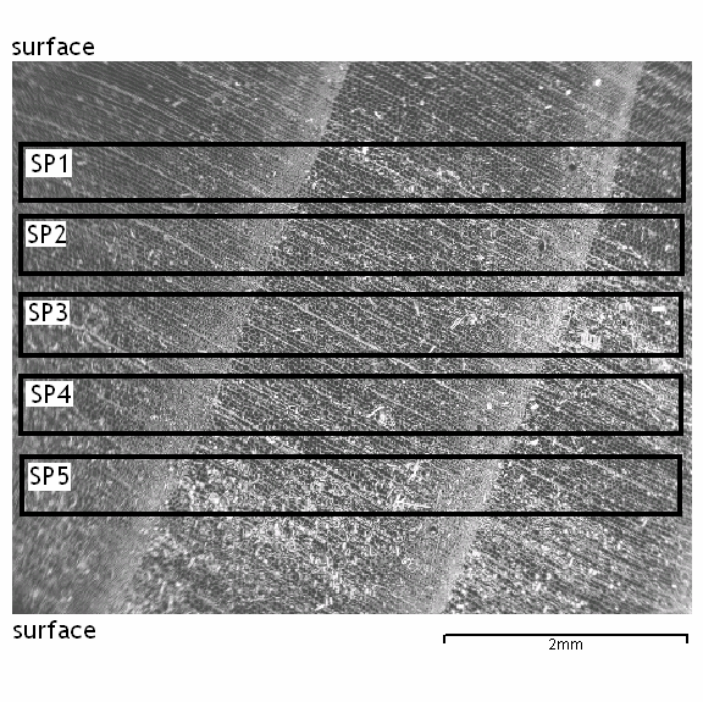


Figure 60 Different areas from surface to bulk) of a TEOS-APTES 1/1 + Cu treated sample cross section where X-rays microanalysis were performed.

Table 27 Si/Cu and Cl/Cu atomic ratios at different penetration depth from the surface (Figure17) of a sample treated with formulation TEOS-APTES 1/1 + Cu. Errors are ± 2-5% for major elements and ± 5-10% for minor components.

	Atomic ratio	
	Si/Cu	Cl/Cu
Spectrum 2	10.5	1.3
Spectrum 3	12.5	1.2
Spectrum 4	12.7	0.9
Spectrum 5	12.3	1.0
Spectrum 5	10.9	1.1

After impregnation into the wood samples, the sol-gel process takes place mainly onto the cell walls leading to the formation of an inorganic-organic hybrid xerogel which should preserve the same Si/Cu and Cl/Cu molar ratio of the starting solution. Our preliminary SEM investigations on this system [Palanti, 2010; Palanti, 2011] showed that

actually copper penetrates into the wood drawn by the coordinative interactions with the amino groups of APTES. In fact, the values found for the Si/Cu molar ratios are close to the nominal ratio 10:1. Nevertheless the Cl/Cu ratio is significantly lower than that of the starting $CuCl_2$ salt. This suggested that, when exposed to the wood samples, at the beginning of the of the sol–gel process, the silanol groups Si–OH could interact with copper cations and substitute chloride anions by forming Si–O–Cu linkages (Figure 61) through a simple exchange reaction:

$$Si\text{-}OH + CuCl_2 + R\text{-}NH_2 \text{ -----> } Si\text{-}O\text{-}Cu\text{-}Cl + R\text{-}NH_3^+Cl^-$$

The hydrochloridric acid formed could be neutralized by the excess of amine functions of the sol giving anchored alkylamonium chloride species. This reaction could take place also in the stock solution before exposition to wood owing to the presence of adventitious water. Furthermore, the chlorine deficit in the inner wood suggests that these amonium species could polymerize more rapidly becoming less able to penetrate into the wood and hence remaining both on the surface of the wood sample and in the external sol mixture.

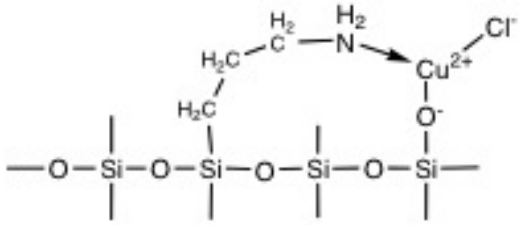


Figure 61 Pictorial view of grafting of copper to the functionalized silica gel in the wood.

Here we present new evidence confirming this unexpected behaviour. In fact, data summarized in Figure 62, for a inner wood sample cross section, show that the starting Si/Cu molar ratio 10:1 is substantially maintained, differently to the Cl/Cu ratio, which again results significantly lower. SEM investigations performed on the surface of impregnated samples show the presence of silicon, copper and chlorine homogeneously distributed over the surface area. As a support of the hypothesis formulated above, the Cl/Cu molar ratio on the surface results significantly higher (3.3) than that of the starting salt.

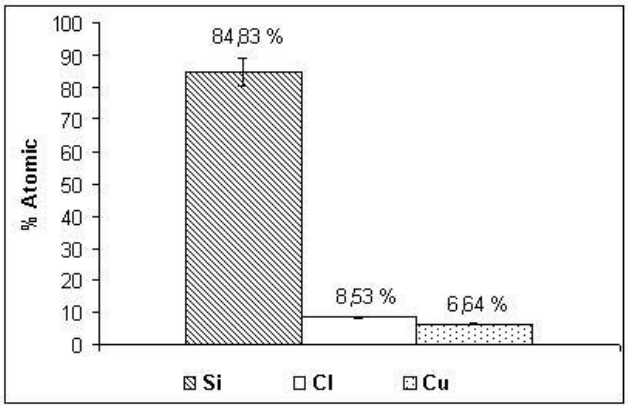


Figure 62 Relative atomic percentages, referred to the X-ray microanalysis on 4 x 4 mm inner cross section areas of samples impregnated with formulation TEOS-APTES 1/1 + Cu.

Further evidence for this hypothesis comes from the data collected for the residual xerogel, i.e. the hybrid material obtained by allowing the residual solution to undergo hydrolysis/condensation reactions in the air. EDX data show a value of the Cl/Cu molar ratio much higher (4.2) than that observed in the inner wood.

Figure 63 summarizes these results, by comparing the Si/Cu and Cl/Cu atomic ratios found in the residual xerogel, on the wood surface and in the inner wood derived by the TEOS-APTES-$CuCl_2$ sol. Whereas the Si/Cu ratio appears near the same on the surface and in the inner, the Cl/Cu ratio displays a clear trend to decrease by passing from the residual xerogel to the surface and to the inner, according to the formation of Si-O-Cu linkages and consequently of amonium chloride species remaining in prevalence out of the inner wood.

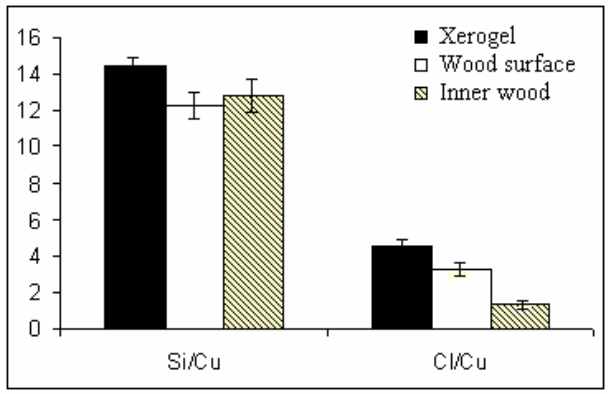


Figure 63 Comparison of the Si/Cu and Cl/Cu atomic ratios in the residual xerogel (obtained from solution **S2** after impregnation), on the wood surface and in the impregnated inner wood (average of 4 data).

As regards the Cu/siloxane micro-distribution into the wood sample, SEM/EDX investigations, summarized in Figures 64-65, have shown that the xerogel is present in the innermost wood mainly by penetrating the texture of cell wall and middle lamella (Figure 64, Table 28). This is a necessary requirements for a preservative formulation to warrant durability and dimensional stability. Figure 65 and Table 29 emphasize that the siloxane xerogel is also present in some lumina as compact flakes. Microanalytical data reported in the figure captions suggest that copper/chlorine distribution is not as uniform as it appears on a larger scale, the Cl/Cu ratio ranging from 0.6 to 2.1 in Fig.65.

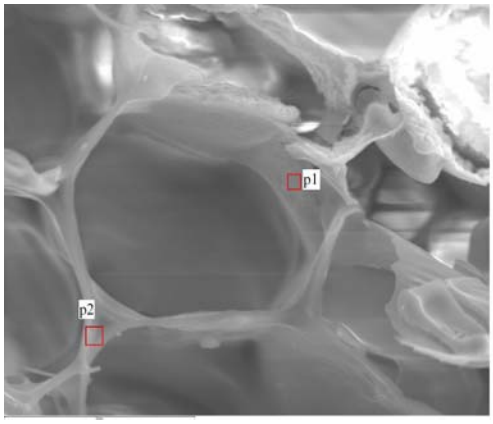


Figure 64 SEM (Secondary Electron) image of the cell of a wood sample impregnated with formulation TEOS-APTES 1/1 + Cu.

	Atomic ratio	
	Si/Cu	Cl/Cu
p1	16.15	2.66
p2	11.65	1.03

Table 28 Si/Cu and Cl/Cu atomic ratios obtained from EDX microanalyses on cell wall (p1) and middle lamella (p2). Errors are ± 2-5% for major elements and ± 5-10% for minor components.

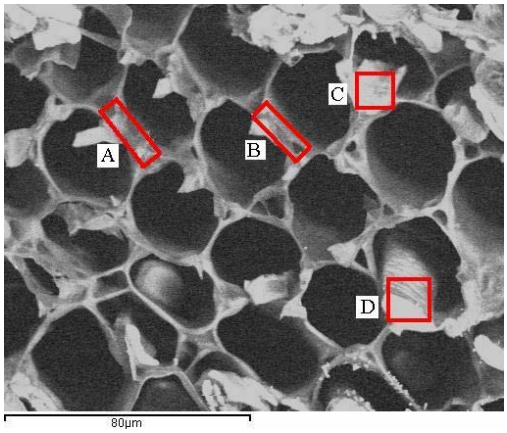


Figure 65 SEM (Secondary Electron) image from the cross section of pine wood impregnated with formulation TEOS-APTES 1/1 + Cu.

		Atomic ratio	
		Sl/Cu	Cl/Cu
A	Cell wall	13.5	0.6
B	Cell wall	10.1	0.6
C	lumen	6.6	0.8
D	lumen	17.2	2.1

Table 29 Si/Cu and Cl/Cu atomic ratios obtained from EDX microanalyses on cell wall (A-B) and lumen (C-D) areas.

The impregnation with analogue sol-gel formulations obtained with sodium metasilicate as precursor, instead of TEOS, resulted in a lower final weight percent gain (Table 30). FT-IR spectra show the presence of condensed Si-O linkages, as in the case of TEOS formulations. It is revealed by the enlargement of the C-O wood band due to the Si-O contribute in the impregnated wood FT-IR spectrum (Figure 66).

Table 30 Absorption and Retention average values (n=10) of 0.5 x 1 x 3 cm *Pinus sylvestris* L. wood samples impregnated with sodium silicate -APTES-copper formulation through a one-step process.

Impregnation	Absorption [g] Average ± st.dev.	Retention [kg/m^3] Average ± st.dev.	WPG [%] Average ± st.dev.
Sodium silicate – APTES 1/1, APTES – Cu 5/1	0.73 ± 0.09	483.28 ± 60.54	14.70 ± 0.71

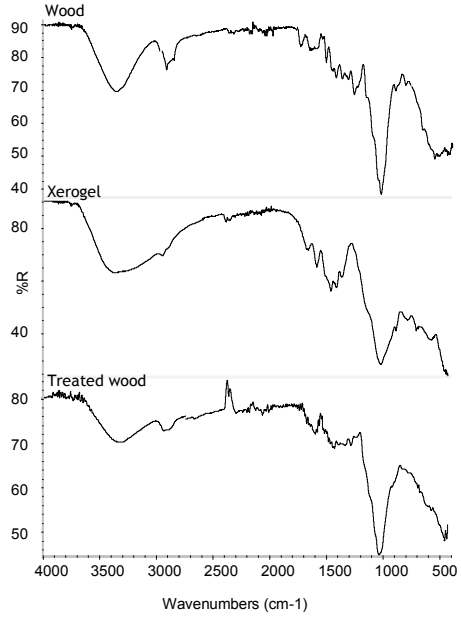


Figure 66 FT-IR spectra of pine wood before and after sol-gel sodium silicate - APTES 1/1 treatment compared to the residual xerogel. The enlargement of the C-O str. wood band (about 1050 cm^{-1}) due to the xerogel Si-O str. contribute in the spectrum of wood.

The cross-section SEM-EDX microanalysis showed higher heterogeneity in the silicon distribution compared to the TEOS-APTES formulation. Despite the presence of silicon in the inner wood area (Figure 67), its concentration, evaluated from the C/Si atomic ratio, is lower than that found on the wood sample surface (Figure 68, Table 31). Copper presence is revealed only within the first 200 μm (Figure 68): compared to the formulation with analogue starting APTES and copper concentration, copper ions are less

easily vehicled. In the TEOS-APTES formulations the nominal copper ratio of 10/1 between formulation (TEOS-APTES 1/1, APTES-Cu 5/1) and formulation (TEOS-APTES 1/1, APTES-Cu 5/1) is reflected in a 10/1 copper content revealed by ICP-AES in impregnated samples (5.4 kg/m^3; 0.54 kg/m^3) but in the formulation with metasilicate, despite the same nominal content ratio of formulation 4, copper content kept inside wood is five times lower (1.51 kg/m^3). This could be related both to the lower xerogel retention inside wood (confirmed by lower WPG) and to the less effective copper penetration (Table 32).

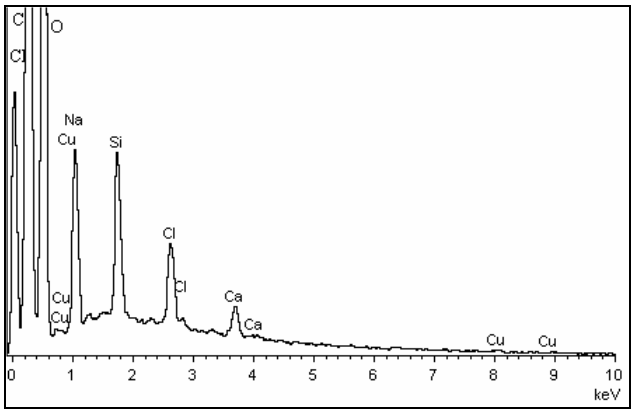


Figure 67 EDX spectrum of the SP1 area in Fig. 24, showing the presence of Si, Na (from sodium metasilicate), Cu and Cl (from copper chloride).

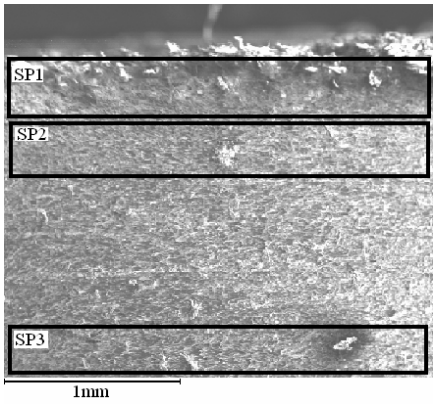


Figure 68 SEM (Secondary Electron) image of the cross section of pine wood treated with formulation based on sodium silicate-APTES 1/1 + copper. The areas of analysis EDX, at three different depth from the surface, are reported.

	Atomic ratio		
	Si/C	Si/Cu	Cl/Cu
SP 1	2.4E-2	13.4	8.6
SP 2	1.7E-2	-	-
SP 3	0.6E-2	-	-

Table 31: Si/C, Si/Cu and Cl/Cu atomic ratios at different penetration depth inside wood (areas of analyses are shown in Figure 68). Errors are ± 2-5% for major elements and ± 5-10% for minor components.

Table 32 Comparison among the copper content of formulations with TEOS and with meta-silicate as starting precursors.

	Copper content	
	mg/L	kg/m^3
TEOS-APTES 1/1 v/v, APTES: Cu 5/1 molar ratio	81	5.40
TEOS-APTES 10/1 v/v, APTES: Cu 5/1 molar ratio	8.1	0.54
Sodium metasilicate-APTES 1/1 v/v, APTES: Cu=5:1 molar ratio	22.7	1.51

The amount of copper released from the treated wood specimens during the leaching process for 10 days was evaluated. In fact, it is supposed that copper could interact with the xerogel both through covalent bonds with ending sylanol groups forming Si-O-Cu linkages, both with the amino groups through coordinative interactions: in all cases, leaching should be reduced by the presence of the TEOS-APTES network (Figure 69).

All samples showed the higher amount of released copper during the first day of leaching. Then it decreases but a further enhancement was always recorded in correspondence of the sixth-eight leaching days. In the last days of the process, no further copper released was detected.

Figure 70 shows the trend of copper leaching from a sample impregnated with the formulation TEOS-APTES 10/1, APTES-Cu 5/1 (Figure 70).

The final amount of copper released from this specimen during the leaching process was 0.004 ± 0.002 kg/m^3, that means about 7% of the 0,054 kg/m^3 total copper content. This is a satisfactory result considering that copper water-soluble salts, such as copper sulphate, reach about the 50% of copper leached out (Humar, 2005).

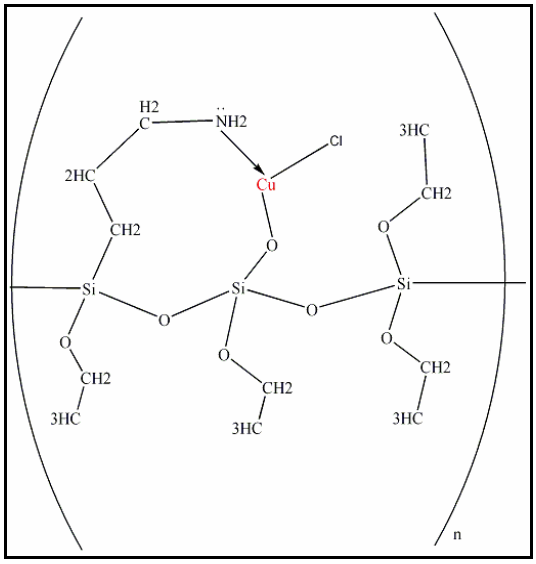


Figure 69 Possible interactions among copper and the silica reticulation, not yet hydrolized in the scheme.

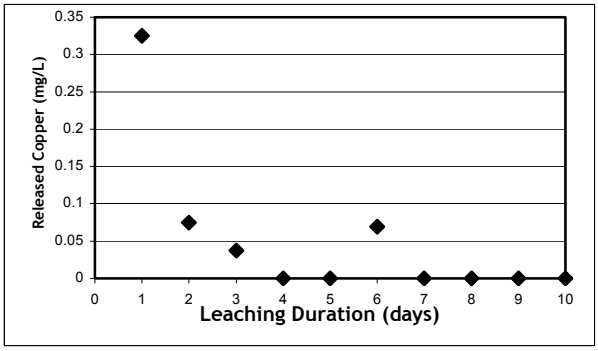


Figure 70 Copper leaching from a sample impregnated with the formulation TEOS-APTES 10-1, APTES-Cu 5/1 (WPG= 19%; total copper content: 0.054 kg/m^3).

In case of the TEOS-APTES-copper treatments, the release during the first leaching days was attributed to weakly interacting copper and/or to copper bonded to siloxane soluble oligomers; the copper leaching observed around the seventh day is reasonably due to the hydrolysis process of part of the siloxane network. This reaches the maximum rate after a week resulting in partial breakdown of the silica network and in the release of covalenltly-linked copper. This hypotesis seems to be confirmed by an analogue trend in the silicon release, with peaks in correspondence of the days of supposed hydrolysis. That is shown in Figure 71 concerning the leaching results of a sample treated with the

same TEOS-APTES 10/1 APTES-Cu 5/1 formulation, but exposed to the leaching test three days after impregnation.

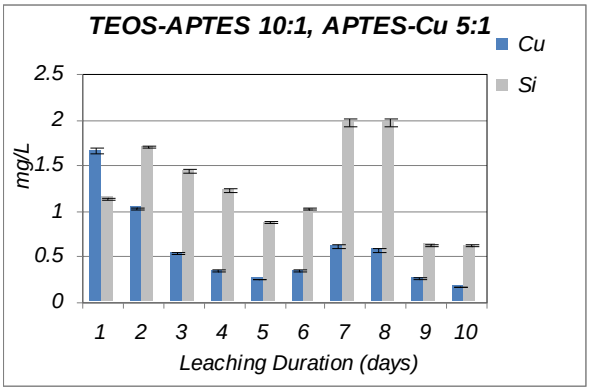


Figure 71 Leaching of copper and silicon from a freshly prepared TEOS-APTES 10-1, APTES-Cu 5/1 treated sample.

The mass spectrum related to the water leaching solution reveals only silica fragments with low molecular weight, mainly not condensed monomers and dimers with and without functionalization (Figure 72).

In the freshly prepared sample, leaching was much higher than in the aged one (exposed to the leaching test two months after impregnation) and copper loss can be easily observed as a decoloration of the sample during the leaching procedure (Figure 72).

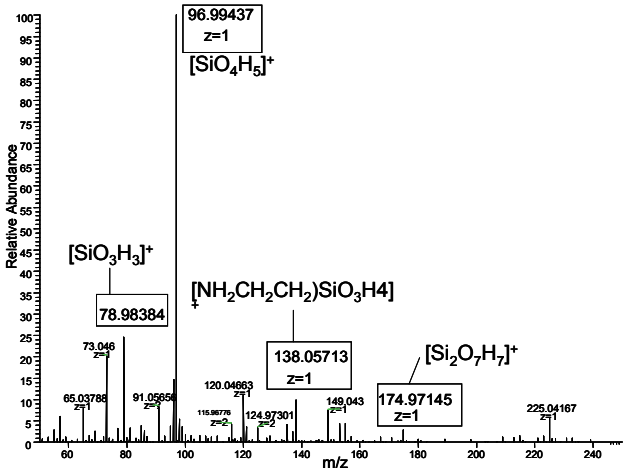


Figure 72 Mass spectrum of the water solution of the leaching test on a TEOS-APTES 10-1, APTES-Cu 5/1 treated sample. Only silica fragments with low molecular weight were releaved.

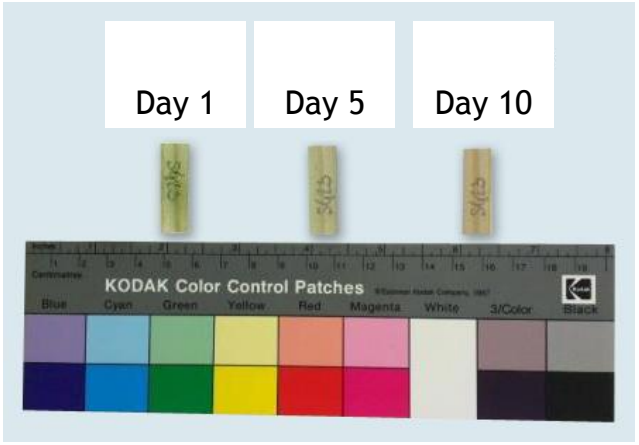


Figure 72 Decoloration of a TEOS-APTES 10-1, APTES-Cu 5:1 freshly treated sample during the leaching test.

The final amount of copper released was 0.346±0.008 kg/m^3 kg/m^3, that means about 27% of the 1.264±0.013 kg/m^3 copper content extracted with sulphuric acid from an analogue sample (Figure 73).

These results demonstrate that xerogel aging is an important factor in determining copper retention and that high retention values are actually due to the presence of the aged xerogel.

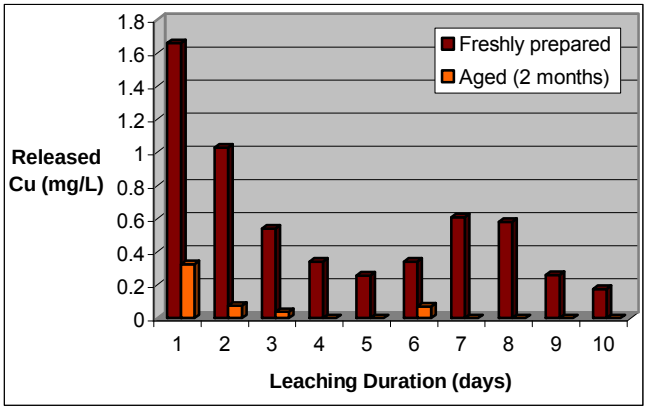


Figure 73 Comparison among copper leaching from TEOS-APTES 10/1 APTES-Cu 5/1 freshly and aged treated samples.

Leaching treatments were also performed on samples previously impregnated with TEOS-ethanol-copper chloride formulations without APTES to evaluate separately the silica network and the amino-groups contribute in the retention of copper. Different copper

concentrations were tested and compared: TEOS/Cu 50:1; TEOS/Cu 25:1 and TEOS/Cu 10:1. In absence of amino-groups, copper is supposed to eventually interact with the xerogel only by covalent Si-O-Cu linkages (Figure 74).

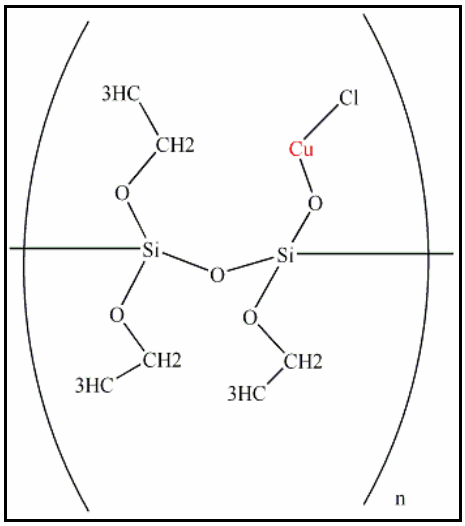


Figure 74 Possible interactions among copper and the silica reticulation, not yet hydrolized in the scheme.

As for the previous tests, all the samples were oven dried after the impregnation procedure in order to determine the dry mass, but only in these cases a browning of the wood samples was observed, possibly due to the formation of CuO, since it is no more protected by amino functions. The darkening increase with increasing amounts of starting copper, probably because a major metal amount is weakly bonded to the polymer.

Freshly prepared (12 hrs) samples also showed solid spots encrusting the wood surface: probably the heating caused the diffusion of the sol from the wood pores to the sample surface with subsequent solidification outside the wooden structure (Figure 75). The so-formed solid crusts, pale green-white in case of the TEOS-Cu 50:1 sample (lower copper content) and brownish in case of the TEOS-Cu 10:1 sample (higher copper content) were recognized as silica-based by means of FT-IR μ-ATR analyses (peaks at 1100 cm^{-1}, 850 cm^{-1}).

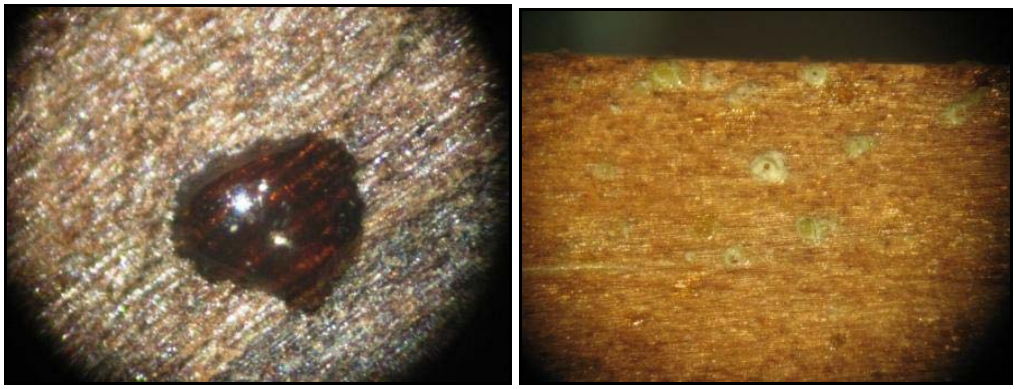


Figure 75 Superficial drops and spots after heating of TEOS-Cu treated wood samples.

The WPG values resulting after the three kinds of treatment decrease with the increase of copper amount (Table 33): the presence of copper could perhaps influence the sol fluidity and reticulation, e.g. facilitating the formation of oligomers that penetrate more difficultly into the wooden structure.

Table 33 Wood samples weight percent gain values due to the TEOS-copper impregnations.

Formulation	Weight Percent Gain %
TEOS/Cu 50:1	43
TEOS/Cu 50:1	49
TEOS/Cu 25:1	39
TEOS/Cu 25:1	22
TEOS/Cu 10:1	21
TEOS/Cu 10:1	28

Copper and silicon leaching for each of the three formulations during the ten-days test are reported in Figures 76-78. Copper leached out from the sample treated with the TEOS-Cu 50:1 formulation was 1.037±0,023kg/m^3; the subsequent treatment with sulphuric acid of the same sample caused the extraction of further 9.07 kg/m^3; assuming 10.104 kg/m^3 the total amount (that means the sum of the two extractions), only about 10% was leached out during the water leaching test.

Copper leached out from the sample treated with the TEOS-Cu 25:1 formulation was 1.917±0.041 kg/m^3; the following treatment with sulphuric acid of the same sample caused the extraction of further 14.07 kg/m^3; following the procedure describes above, the amount of leached copper resulted 12% of the total copper amount.

As concerns the TEOS-Cu 10:1 treatment, copper leached out was 5.963±0.147 kg/m^3, that means 17% of the 34.963 kg/m^3 total copper amount.

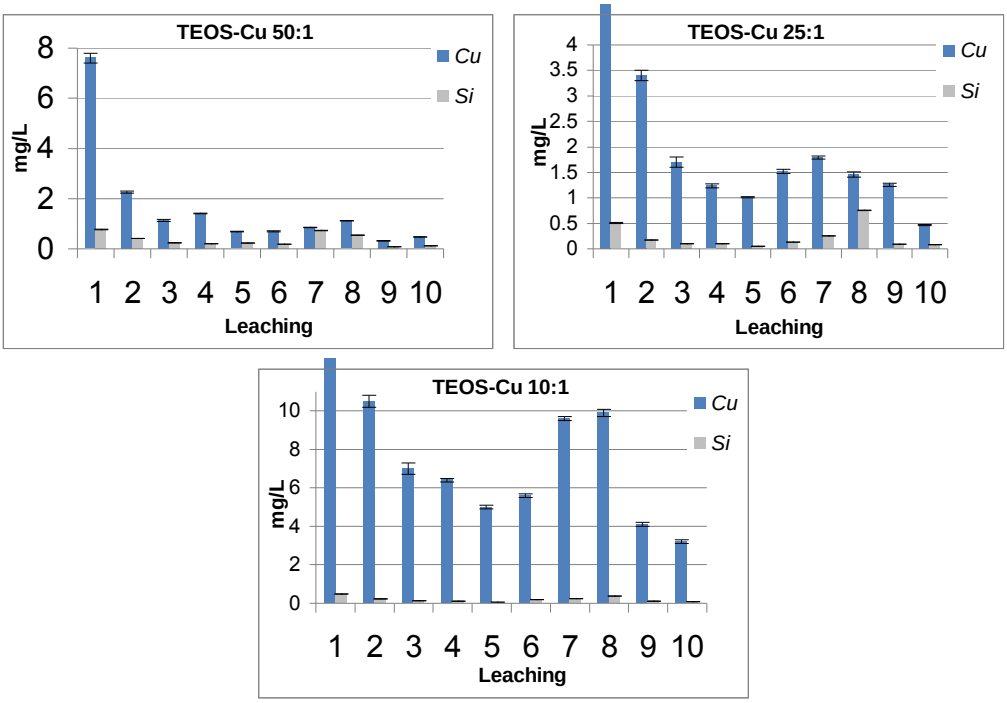


Figure 76-78 Copper leaching of samples treated with TEOS-Cu formulations (TEOS- TEOS-Cu 25:1 release at day 1: 16.3 mg/L; TEOS-Cu 10:1 release at day 1: 39.7 mg/L)

Leached copper results to increase with the increase of starting copper content. This should mean that the major is the starting copper, the minor is the relative amount of silica "sites" giving rise to copper leaching-resistant bonds (Figure 79).

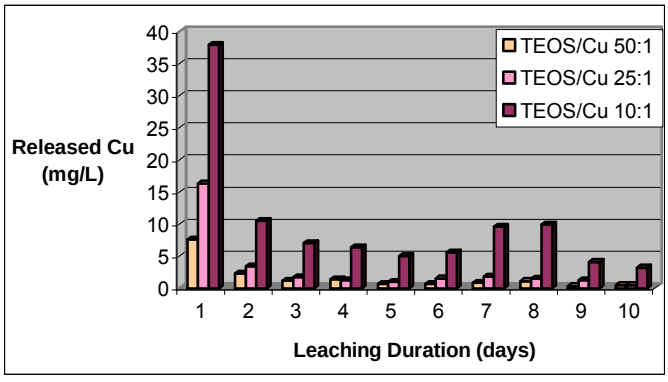


Figure 79 Summary of copper leaching for the TEOS-Cu formulations.

Silicon release was also evaluated. Differently from copper, it diminished with the increase of copper amount: this could be due both to the decreasing amount of starting silica impregnating wood, and to the different silica texture.

The indicative amount of total silicon retained in the wood samples was calculated from the gain of weight after impregnation, mainly due to the silica xerogel corrected by the weight contribute of copper. The corresponding percentages of silicon released during leaching were: 0.28% (TEOS-Cu 50:1), 0.26 (TEOS-Cu 25:1), 0.16 (TEOS-Cu 10:1). They result lower than those observed for the sample treated with the TEOS-APTES formulation (Figure 80), probably due to the ability of APTES to form oligomers such as ($(CH_2)_3NH_2)_8Si_8O_{12}$), called POSS, polyhedral oligomeric silsesquioxanes (Figure 81), more easily leached out from the wood structure.

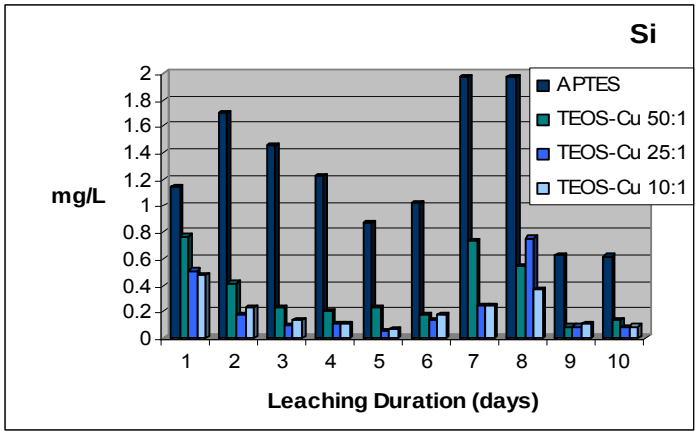


Figure 80 Silicon release from samples treated with TEOS-APTES-Cu and TEOS-Cu formulations. The highers release is obtained from samples treated with the TEOS-APTES formulationprobably due to the ability of APTES to form oligomers such as silsesquioxanes.

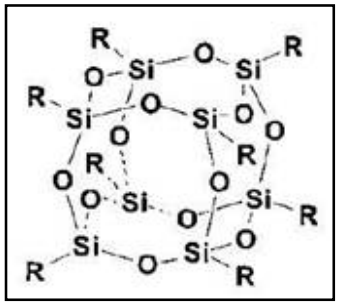


Figure 81 (T8) $R_8Si_8O_{12}$ (silsesquioxane) structure (Li, 2001); with APTES as precursor, R= $(CH_2)_3NH_2$

3.1.2 Sol-gel treatments with addiction of boron

Boric acid and borates are used as "actives" in preservative products against biological attack since they are toxic to all cells (fungi and insects included) (see par. 1.5.5). TEOS-APTES treatments were performed on pinewood samples such as described for

copper formulations (see par. 3.1.1) but with addiction of boric acid instead of copper salts. Boric acid is supposed to interact with the silica xerogel in two ways: by condensation with silanol groups, giving rise to Si-O-B linkages (formation of borosilicates); by formation of ionic interactions with the amino groups (Figure 82).

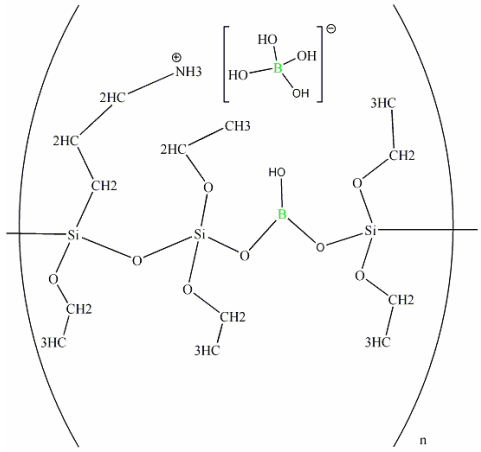


Figure 82 Possible interactions among boron and the silica reticulation, not yet hydrolized in the scheme.

Both the two-step and the one-step treatments were performed (see par. 2.3.2.2). Impregnated wood samples were characterized through the calculation of the weight percent gain after treatment. The FT-IR ATR spectra recorded from the samples surface also attested the presence of Si-O linkage showing an enlargement of the band around 1000 cm^{-1} but no differences were revealed attesting the presence of boric acid or its interaction with the silica reticulate. The indicative amount of total boron retained in the wood samples was also calculated from the gain of weight after impregnation. In case of the one-step treatment, different boric acid concentrations were tested (Table 34) and for each of them sets of samples were exposed to leaching procedure (EN 84: immersion in deionized water under vacuum (0.20 hrs) + atmospheric pressure (336 hrs)) before to be tested against fungi (see par. 3.2.2).

Treatment	Formulation	WPG [%] Average ± sd.dev. (n=7)
Two-step	TEOS-APTES 1/1, H_3BO_3 0.1M	26.13±3.78
One-step	TEOS-APTES 1/1, APTES-B 2/1	30.34±2.81
One- step	TEOS-APTES 1/1, APTES-B 5/1	22.18±3.01

Table 34 Weight percent gain average values of pine wood samples treated with TEOS-APTES-Boric acid formulations.

Analogue pinewood samples were employed for the evaluation of the amount of boron released during a leaching process over 10 days. For this purpose, sets of samples were treated varying both the starting amounts of APTES and boric acid. The formulation chosen as concerns boron variation were TEOS-APTES 1/1, APTES-H_3BO_3 2/1 and TEOS-APTES 1/1, APTES-H_3BO_3 5/1; for each formulation, two different aging rates were considered.

All samples showed the higher amount of released boron during the first day of leaching. Then it decreased but, as for samples treated with copper, a further enhancement was recorded in correspondence of the seven-nine leaching days, probably due to hydrolysis (see par.3.1.1) involving the partial release of covalently-bonded boron of the borosilicate chains.

Boron release seems to be scarcely influenced by the aging time of the impregnated samples if aging is greater than 4 months. The amount of boron leached out was much higher in case of the APTES-H_3BO_3 2/1 formulation compared to the APTES-H_3BO_3 5/1 (Figures 83-84). The chosen amount of boron in the APTES-H_3BO_3 2/1 seems to exceed the fixation "capacity" of the silica network whereas a lower boron amount (APTES-H_3BO_3 5/1) finds more easily adequate fixation.

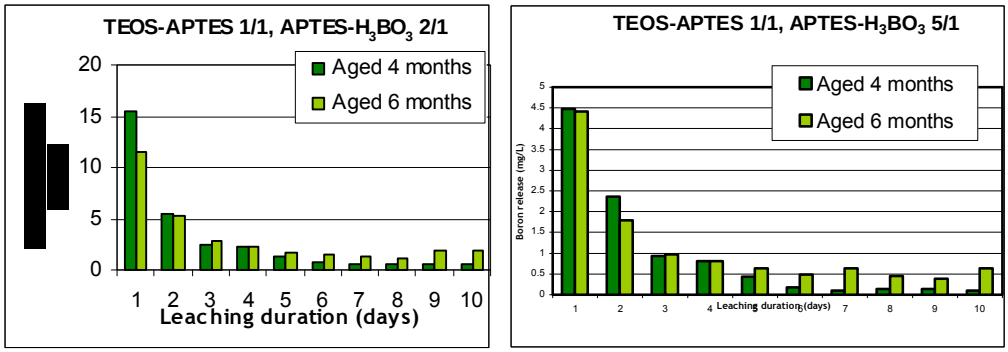
For all the specimens, the indicative total amount of boron was evaluated by calculation from the gain of weight after impregnation, supposing the maintenance of the starting molar ratios among boron and silicon components.

The calculation of total boron theoretical amount for each sample allowed to evaluate the approximate percentages of boron released during leaching.

Boron leached out from the sample treated with the APTES-H_3BO_3 2/1 formulation aged 4 months was 1.470 ± 0.028 kg/m^3; total boron theoretical amount was 6.451 kg/m^3 (corresponding to a WPG of 30%); about 30% was released during the water leaching test. On the contrary, boron leached out from the sample treated with the APTES-H_3BO_3 5/1 formulation aged 4 months was 0.494 ± 0.008 kg/m^3, corresponding to only about 6% of total boron theoretical amount (WPG: 34%).

The same samples, having different starting boron concentration and differing so much in the amount of boron leached out, show no relevant difference among the silicon release during the 10-days test.

This suggests that the amount of boron engaged in strong interactions with the siloxane network cannot exceed a sort of threshold value and, unlike copper-treated samples, silicon release seems to be scarcely influenced by boron amount. Probably boron release is mostly due to intact H_3BO_3 and $[B(OH)_4]^-$ species anchored to protonated amine functions of the siloxane network (Figure 85).



Figures 83-84 Boron release from samples treated with TEOS-APTES-boron formulations differing for aging time and boron starting amount.

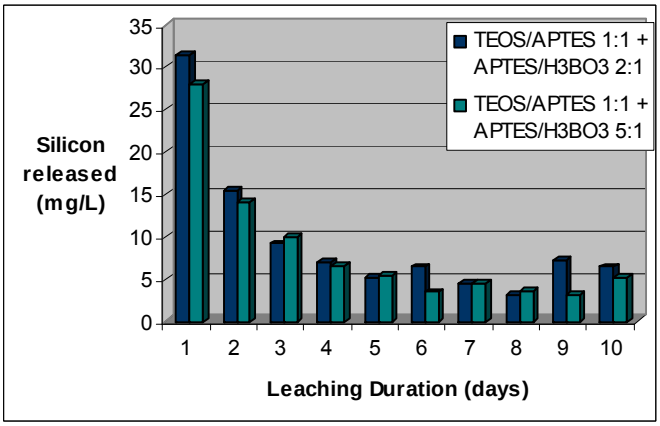


Figure 85 Silicon released from samples treated with TEOS-APTES-boron formulations differing for boron starting amount.

Formulations differing for APTES concentration were also exposed to the leaching test. The comparison among the results of boron leaching from TEOS-APTES 1/1, TEOS-APTES 5/1 and TEOS-APTES 10/1 (APTES-H_3BO_3 5/1) (Figure 86), show the highest amount of

leached boron in case of the highest APTES concentration. It appears that the lowest is the number of amino-groups, linking boron through weak ionic bonds, the highest is the amount of boron involved in covalent bonds and so less easily leached out.

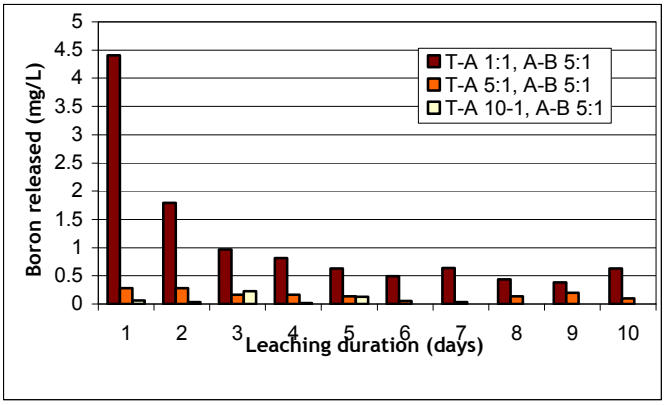
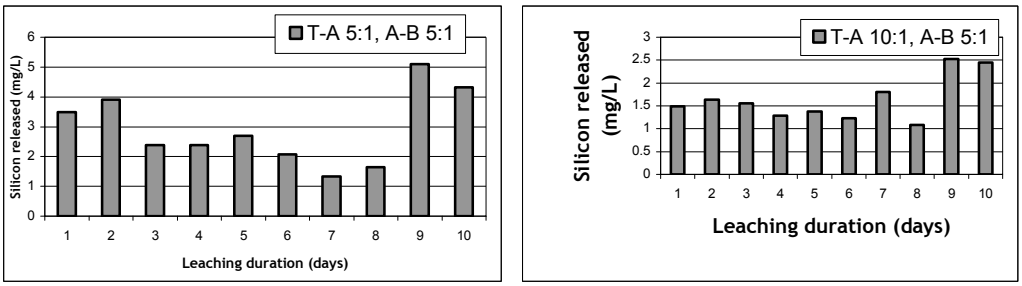


Figure 86 Boron released from samples impregnated with TEOS-APTES-boron formulations differing for the APTES concentration.

The percentage of released boron was indicatively evaluated as reported above: it was 11% in case of the TEOS-APTES 5/1 (APTES-H_3BO_3 5/1) formulation (leached boron: 0.094±0.001 kg/m^3; theoretical total amount: 0.883) and 5% for the TEOS-APTES 10/1(APTES-H_3BO_3 5/1) formulation, confirming the higher boron retention due to the less functionalized silica reticulation. The leaching duration related to the last formulation didn't show any boron amount released from the sixth to the tenth day. It is possible that the whole scarcely bonded boron was released in the first days and that the amount of retained boron was almost all constituted by the Si-O-B bonded fraction.

The presence of low APTES amounts also gave rise to low silicon leaching (Figures 87-88), confirming that APTES easily form soluble oligomers that contribute significantly to silicon release. In the case of TEOS-APTES 5/1 (APTES-H_3BO_3 5/1) formulation, the leached Si value was 1.956±0.031 kg/m^3, the 3% of the calculated silicon amount, and the leaching trend followed that of boron, whereas in case of the TEOS-APTES 10/1(APTES-H_3BO_3 5/1) formulation Si leached out was 1.097±0.011 Kg/m^3, that means only the 1% of theoretical silicon amount.



Figures 87-88 Silicon released from samples treated with formulations based on TEOS (T), APTES (A) and boron (B). Two formulations were considered differing for APTES starting amount. (ratios among components: T-A 5:1, A-B 5:1 for the formulation whose results are reported in the left figure and T-A 10:1, A-B 5:1 for the formulation whose results are reported in the right figure).

Whatever was the starting amount of APTES and boric acid, the silica reticulation contributed to high boron retention values, especially if compared to data reported for boric acid alone (Kartal, 2009) (Table 35).

Table 35 Retained boron amount of tested formulations compared to boric acid (*Kartal, 2009).

Formulation	Retained boron amount (%)
TEOS/APTES 1:1 + APTES/H_3BO_3 2:1	70%
TEOS/APTES 1:1 + APTES/ H_3BO_3 5:1	91%
TEOS/APTES 5:1 + APTES/ H_3BO_3 5:1	89%
TEOS/APTES 10:1 + APTES/ H_3BO_3 5:1	95%
H_3BO_3 *	<5%

3.1.3 Further hybrid inhorganic-organic treatments with addition of zinc or silver

Zinc and silver, known for their antibacterial activity, but also toxic for fungi and insects at high concentration levels (see par.1.5.5), were also employed in place of copper or boron for alternative formulations. Zinc was expected to be anchored to the functionalized silica xerogels such as copper (Figure 89). The one-step procedure, consisting in the TEOS-APTES-$ZnCl_2$ impregnation (TEOS-APTES 1/1 v/v, APTES-Zn 5/1 molar ratio) was applied to samples then tested against fungal attack (see par.3.2.2).

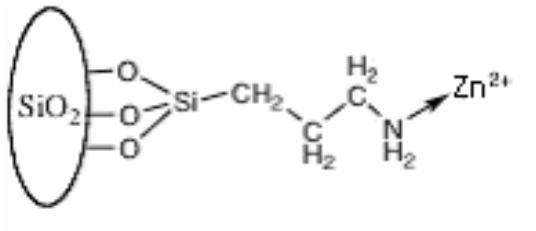


Figure 89 Scheme of possible interaction throug coordinative linkeage among functionalised silica xerogel and zinc(II) ion.

Silver nanoparticles were also tested as further inorganic biocides (see par. 2.2.7). Impregnation was verified for each treated sample through the calculation of the WPG, always higher than 20%. Table 36 summarizes the weight variation of one-step treated samples with addiction of zinc or silver compared to the copper and boron formulation previously described; examples of impregnated samples are reported in Figure 90.
The possibility to vehicle silver through a hybrid organic-inorganic polymer differing from the TEOS-APTES formulation for the higher organic fraction (see par. 2.2.8) was also tested. The satisfactory penetration inside wood was confirmed by SEM-EDX analyses, attesting the presence of silver deep inside the wood samples (Figures 91-92). Brownishing of silver samples was observed after wood exposure to termites.

Table 36 Weight percent gain values of *Pinus sylvestris* L. samples treated with sol-gel one-step processes.

Impregnation	Dry weight before impregnation [g]	Dry weight after impregnation [g]	WPG [%]
sol-gel (n=5)	0.660 ± 0.034	0.845 ± 0.039	27.86 ± 2.07
sol-gel + Cu (n=10)	0.630 ± 0.024	0.777 ± 0.04	23.27 ± 2.29
sol-gel + B (n=5)	0.657 ± 0.026	0.809 ± 0.026	23.11 ± 2.30
sol-gel + Zn (n=5)	0.631 ± 0.033	0.814 ± 0.047	28.99 ± 2.13
sol-gel + AgNP (n=5)	0.681 ± 0.055	0.883 ± 0.065	29.70 ± 1.27

Even if the nominal Si-Ag ratio 5/1 is maintained on large scale, some points of unhomogeneity are revealed at microscopic level (Figure 93, Table 37).

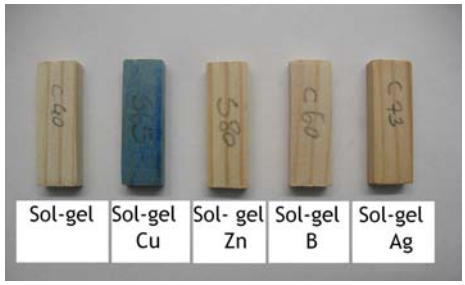
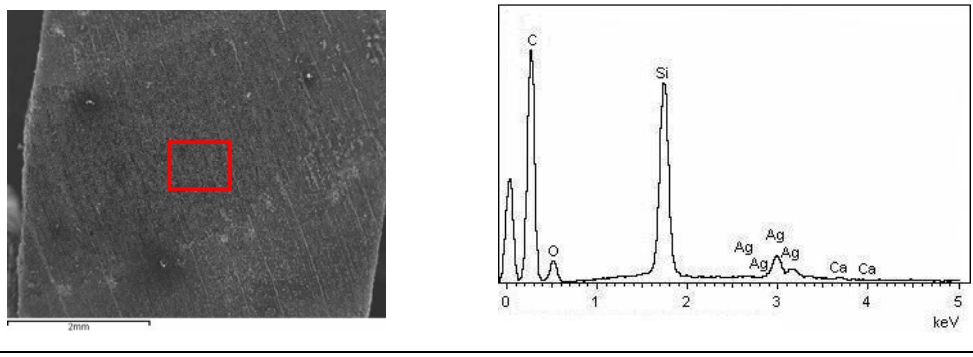


Figure 90 Samples after one-step sol-gel treatments



Figures 91-92 SEM (Secondary Electron) image of the cross section of pine wood treated with PAM-Ag formulation and microanlysis of the central (red) area.

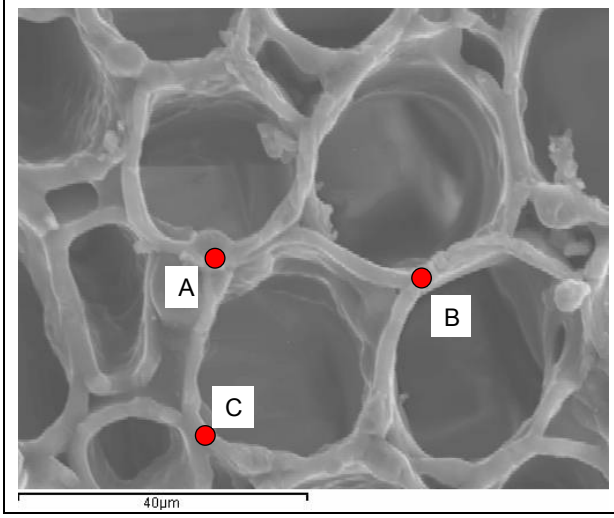


Figure 93 SEM (Secondary Electron) image of the cross section of pine wood treated with PAM-Ag formulation.

	Si/Ag Atomic ratio
A	13.3
B	7.3
C	10.2

Table 37 Si/Ag atomic ratio from EDX analysis on areas A, B,C. Errors are ± 2-5% for major elements and ± 5-10% for minor components.

3.2. Evaluation of effectiveness as biocides

3.2.1 Effectiveness of sol-gel treatments coupled with copper and boron against subterranean termites

The European Standard EN117 (2005) test (8 weeks) and the accelerated method as in Nunes (1997) (4 weeks) were performed to evaluate the efficacy of sol-gel treatments coupled with copper and boron against the subterranean termites *Reticulitermes grassei* Clément.

The following two-step treatments, with eventual addiction of copper sulphate or boric acid, were considered. A formulation based on a colloidal silica emulsion with addiction of boric acid was also tested (see par.2.2.6). Impregnation data of treated pinewood samples are reported in Table 38.

Table 38 Characteristics of the impregnated samples series 1. TEOS = tetraethoxysilane, APTES = aminopropyltriethoxysilane, EtOH = ethanol. $CuSO_4$ = copper sulphate, H_3BO_3 = boric acid; n= number of replicates

Treatment	Anhydrous mass m_a (n=7) [g]		Calculated impregnated anhydrous mass m_{at} [g]		Theoretical WPG [%]	
	mean	*sd*	mean	*sd*	mean	*sd*
1. sol-gel (TEOS - APTES – EtOH)	1.20	0.03	1.51	0.04	25.86	2.94
2. sol-gel + 0.1 M $CuSO_4$	1.18	0.04	1.68	0.08	42.53	3.27
3. sol-gel + 0.1 M H_3BO_3	1.18	0.04	1.49	0.04	26.13	3.78
4. silicic acid - H_3BO_3	1.18	0.02	1.73	0.12	46.95	9.72
5. None (controls)	1.21	0.05	-	-	-	-

Both the 4-weeks and the 8-weeks tests were considered valid because the survival of workers in the controls was highly over 50% (average value 79.1% and 84.6% respectively). Results reported in Table 39 show a different behaviour of termites on the copper and boron sol-gel treatments. The survival of termites on the sol-gel and copper treated wood is better: they do not avoid it (Figure 94) but they eventually die of hunger since a low mass loss and grade of attack were registered (Figure 95). This non-feeding behaviour might explain the complete mortality occurred in the standard test, which is 4 weeks longer than the accelerated one.

Table 39 Results of the no-choice tests. Av = average, sd = standard deviation

Treatment	Accelerated test 4 weeks				EN 117 8 weeks		
	Sample code	Survival [%]	Attack	Mass Loss [%]	Sample code	Survival [%]	Attack
Sol-gel	t1	86.7	4	8.5	T1	0.0	4
	t2	81.3	4	8.1	T2	0.0	4
	t3	83.3	4	7.6	T3	0.0	4
	t4	0.0	4	7.3	T4	0.0	4
	t5	76.0	4	9.0	-	-	-
	t7	80.7	4	5.6	-	-	-
	Av (sd)	**68.0 (33.5)**	**4**	**7.7 (1.2)**		**0.0**	**4**
Sol-gel and $CuSO_4$	t8	53.3	2	0.6	T9	0.0	2
	t9	81.3	3	0.8	T10	0.0	2
	t10	0.0	1	0.6	T11	0.0	1
	t11	71.3	0	0.5	T12	0.0	3
	t12	26.0	3	0.7	-	-	-
	t13	0.0	1	0.6	-	-	-
	Av (sd)	**38.6 (0.6)**	**1.7 (1.2)**	**0.6 (0.1)**		**0.0**	**2.0 (0.8)**
Sol-gel and H_3BO_3	t15	0.0	4	2.2	T5	0.0	4
	t16	0.0	4	1.8	T6	0.0	3
	t17	2.0	4	6.3	T7	0.0	3
	t18	0.0	4	4.3	T8	0.0	2
	t19	31.3	4	4.0	-	-	-
	t20	0.0	4	2.9	-	-	-
	Av (sd)	**5.6 (12.6)**	**4**	**3.6 (1.6)**		**0.0**	**3.0 (0.8)**
Silicic acid and H_3BO_3	t22	0.0	0	0	T13	0.0	1
	t23	0.0	0	0	T14	0.0	2
	t24	0.0	1	0	T15	0.0	1
	t25	0.0	1	0	T16	0.0	1
	t26	0.0	1	0	-	-	-
	t27	0.0	1	0	-	-	-
	Av (sd)	**0.0**	**0.7 (0.5)**	**0.0**		**0.0**	**1.2 (0.5)**
Untreated	c1	96.0	4	14.1	C1	78.8	4
	c2	72.0	4	12.6	C2	78.0	4
	c3	88.0	4	6.9	C3	78.0	4
	c4	84.7	4	16.2	C4	81.6	4
	c5	80.7	4	18.7	-	-	-
	c6	86.0	4	11.2	-	-	-
	Av (sd)	**84.6 (8.0)**	**4**	**13.3 (4.1)**		**79.1 (1.7)**	**4**

Conversely, on the sol-gel and boric acid treated wood termites feed avidly, thus causing their own rapid death. Samples showed a grade of attack of 4 (strongly attacked, small specimens) and an average mass loss of 3.6%.

The toxic action of boron is also confirmed by the mortality that occurred in the silicic acid and boric acid treatments, where termites died within the first four weeks and just caused some attempts of attack (average values: 0.7 and 1.2).

Results obtained on the sol-gel treatment alone are different. It seems that this treatment produced a toxic delayed effect: after four weeks of the accelerated test, termites have a high survival percentage (average 68.0%) and strongly attack the wood, but the standard test resulted in the same strong attack but a complete mortality of workers. This phenomenon could be explained in terms of chronic toxicity and sublethal dose of silicon that only in a longer period can determine the death of the termites.

In conclusion, wood modification with the solution of TEOS, APTES and ethanol coupled with both copper and boron seem to be effective against subterranean termites, although the mode of action is very different. While sol-gel and copper seems to act more like as a repellent, boron has a real toxic action and termites die after ingestion of the active ingredient. The sol-gel alone seems to act in another way, hypothetically with a delayed effect or with a chronic toxicity produced by a sublethal dose of silicon, whose effects are evident a long time after the ingestion.

The total mortality occurred in the longer test suggested that active ingredients may be added in lower quantity; in this case, a test to determine the toxicity threshold should be necessary. It can be considered that these tests should be repeated after exposure of wood to a leaching test, in order to assess the fixation of all compounds into the wood. However, if the wood preservatives are intended for indoor use, they do not need such a procedure.

Figure 94 Termites tunnels on a sol-gel and copper treated sample.

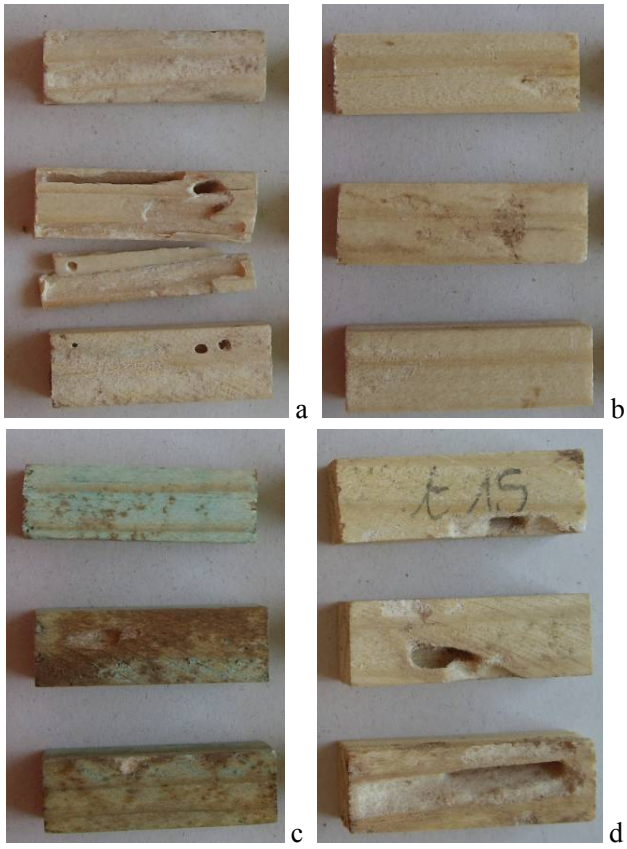


Figure 95 Small samples after the no-choice test. a. controls, b. sol-gel and boric acid, c. sol-gel and copper, d. silicic acid and boric acid treatments.

3.2.2 Effectiveness of sol-gel treatments against fungi basidiomycetes

The sol-gel formulations with copper were tested against the brown rot fungus *Coniophora puteana* L. (Shum.:Fr) P.Karsten Efficacy was evaluated by comparison of the mass loss percentage of the untreated samples (control) with that of the treated ones after exposure to the fungus for six weeks (Accelerated Bravery efficacy test).
The two-step and one-step treatments were compared. The tests were considered valid since untreated samples were attacked. Samples used to control the fungal virulence gave an average mass loss on six replicates of 25.8% and confirmed the vitality of the fungal strain. Both tests gave good efficacy results. All the samples treated by means of the two-step process had a mass loss below 6%, while the control wood blocks mean mass loss was 48.6%. With the one-step process, samples mean mass loss was 3.4% despite the 38% of the controls (Table 40). Among them, one specimen lost 10% and the remaining less than 3% of the initial weight.
These results are comparable to those obtained by De Vetter et al. (De Vetter, 2009) in a similar mini block test, where it was evidenced that by increasing WPG, due to different concentrations of five commercial organosilicon emulsions, always corresponded to a mass loss decrease. In particular, a WPG on Scots pine samples around 30% caused a median mass loss after 8 weeks exposure to *C. puteana* around 3%.

Table 40 Results of accelerated test against fungal decay (fungus *Coniophora puteana*). n = number of samples.

Efficacy test against Coniophora puteana		
Two-step process	**WPG % after impregnation**	**Final Mass loss %**
Sol-gel + Cu (n=4)	28.5	3.81 ± 1.43
Control untreated (n=4)	-	44.34 ± 6.10
One-step process	**WPG % after impregnation**	**Final Mass loss %**
Sol-gel + Cu (n=4)	27.1	3.36 ± 5.08
Control untreated (n=4)	-	38.38 ± 2.94

Tests against *C.puteana* on TEOS-APTES 1:1 and 10:1 formulations, with and without copper, were performed both for leached and not leached samples. The results expressed in terms of average mass loss are shown in Table 41.

It is possible to observe a significant protective activity of TEOS and APTES components, with copper and also without copper when their ratio is 1:1. At this relative concentration the leaching treatment did not influence their fungicidal activity.

When the ratio between TEOS and APTES is 10:1 the treatment appears ineffective in absence of copper. The presence of copper in 5:1 APTES/Cu ratio seems to provide biocide efficacy to the treatment, as the mass loss results rather scarce. However, the leached samples do not exhibit the same resistance. This is probably due to the removal of copper from the surface where the metal cation is present in higher concentration than in the bulk (as stated above), resulting in the fall of a sort of barrier effect.

Actually, the Cu loss due to leaching (found by ICP analysis) is 12%.of the total Cu content of the samples (0.6 kg/m^3) whereas silicon loss is only 0.7% (total content 37.8 kg/m^3). Otherwise, in case of TEOS/APTES 1:1 samples with $CuCl_2$ (APTES/Cu 5:1) the Cu loss was 0.5% (total copper content 5.4 kg/m^3).

These results demonstrate both the contribute of APTES and of copper to determine the preservative effectiveness against the fungus.

Table 41 Average mass loss (ML) and standard deviation (sd) of wood samples treated with different sol-gel processes, leached (Y) or not leached (N).

Efficacy test against Coniophora puteana				
Sol-gel treatment	**Leaching (Y/N)**	**ML [%]**	**sd**	**Effectiveness result**
TEOS/APTES 1:1, $CuCl_2$ (n=4)	Y	-1.5	0.6	Effective
TEOS/APTES 1:1. $CuCl_2$(n=4)	N	1.1	0.27	Effective
TEOS/APTES 1:1(n=4)	Y	-3.93	0.46	Effective
TEOS/APTES 1:1 (n=4)	N	0.4	1.1	Effective
TEOS/APTES 10:1, $CuCl_2$ (n=4)	Y	23.5	2.57	Not effective
TEOS/APTES 10:1, $CuCl_2$ (n=3)	N	0.26	0.08	Effective
TEOS/APTES 10:1 (n=3)	Y	28.6	23.8	Not effective
TEOS/APTES 10:1 (n=4)	N	21.28	11.1	Not effective

The negative weight loss values of the impregnated samples is probably due to the effect of the treatment that, as reposted in par. 3.1.1, affords high weight percent gains.
The formulation with sodium metasilicate as main precursor instead of TEOS is effective (weight Loss 4.07 ± 0.23 % compared to the 27.28 ± 6.07 % of control samples, Table 42), being only slightly lower than the TEOS-APTES combination with the same 1:1 molar ratios (weight loss 1.10 ±0.27 %). The major efficacy of the TEOS formulation could be probably due to the highest sol penetration and copper retention. The leaching procedure on samples treated with the sodium-metasilicate formulation didn't seem to influence the effectiveness of the treatment. The weight loss due to the *C.puteana* is similar to that obtained from samples treated with a TEOS-APTES 20/1 APTES-$CuCl_2$ 5/1 formulation. The partial efficacy of this last (weight loss 5.84 %, significantly lower than the value 35.91 ±11.84 % of controls) demonstrated the significant copper contribute in affording preservative efficacy. Actually, this had been already supposed in the case of the effectiveness of TEOS-APTES 10/1 with addition of copper compared to the unefficacy of the same formulation without copper or after copper leaching.

Table 42 Average mass loss (ML) and standard deviation (sd) of wood samples treated with different sol-gel processes, leached (Y) or not leached (N).

Sol-gel treatment	Leaching (Y/N)	ML [%]	sd
Sodium silicate/APTES 1:1, $CuCl_2$ (n=4)	Y	5.9	3.4
Sodium silicate/APTES 1:1, $CuCl_2$ (n=4)	N	4.1	0.2
TEOS/APTES 20:1, $CuCl_2$ (n=4)	N	5.84	4.8

The addition of boric acid to the TEOS/APTES formulations affords satisactory efficacy not only against the brown rot fungus *C.puteana* (whose attack was prevented just with the TEOS/APTES formulation without any co-preservative addiction) (Table 43), but also against the white rot fungus *T.versicolor* (Table 44). The two formulations containing different boron amounts resulted both effective, suggesting that the formulation with the lower boron concentration is just applicable against these fungal species.

Table 43 Boric acid test results against the brown rot fungus *Coniophora puteana*.
The table reports Average mass loss (ML) and standard deviation (sd) of wood samples treated with the different sol-gel processes, leached (Y) or not leached (N).

Efficacy test against *Coniophora puteana*			
Sol-gel treatment	**Leaching (Y/N)**	**ML [%]**	**sd**
TEOS/APTES 1:1, APTES/B 5:1 (n=4)	Y	5.45	1.42
TEOS/APTES 1:1, APTES/B 5:1 (n=4)	N	-1.48	1.02
TEOS/APTES 1:1, APTES/B 2:1 (n=4)	Y	7.83	0.86
TEOS/APTES 1:1, APTES/B 2:1 (n=4)	N	3.47	0.62

Table 44 Boric acid test results against the white rot fungus *Trametes versicolor*. Average mass loss (ML) and standard deviation (sd) of wood samples treated with the different sol-gel processes, leached (Y) or not leached (N).

Efficacy test against *Trametes versicolor*		
Formulation	**Mass Loss %**	**Mass Loss % Control**
TEOS-APTES 1/1, APTES-B 5/1	Average -1.91	Average 15.24
	SD.DEV. 1.31	SD.DEV. 3.52
TEOS-APTES 1/1, APTES-B 2/1	Average 3.06	Average 59.72
	SD.DEV. 1.20	SD.DEV.2.52

Promising efficacy results against *C.puteana* were also obtained for samples treated with zinc (mass loss: -1.29% against 40.97% of control samples) and silver formulations (Tables 45); however the exiguous number of replicates makes data to be considered only as indicative.

Samples treated with sol-gel and silver were not attacked, as the weight loss percentage was only 2.2%, much lower than the 44.6 % mass loss of the control sample. In these cases the presence of TEOS and APTES in 1/1 the molar ratio gave just a great contribute to the final positive result, but the silver contribute to efficacy is suggested by the fact that by changing the vehiculating solution (PAM), the same positive results (1.98% against the 40.32% of control samples) have been obtained.

Table 45 Average mass loss (ML) of wood samples treated with zinc or silver formulations.

EN113 - Efficacy test against Coniophora puteana		
Formulation	**ML %**	**ML % Control**
Sol-gel + Zn (n=2)	-1,29	40.97
Sol-gel + Ag (n=4)	2.19	44.57
PAM + Ag (n=1)	1.98	40.32

PART B
STUDIES ON PRESERVATIVES BASED ON METAL CHELATES

3.3. Copper glycinate formulations: evaluation of preservative properties against the brown rot fungus *C.puteana*

The possible use of copper glycinate, $Cu(Gly)_2$, eventually added of boric acid, as water-borne preservatives against fungi was investigated through efficacy tests against the brown rot fungus *C.puteana*.

Preliminary tests in agar were carried out to establish toxic thresholds for copper glycinate, boric acid and copper glycinate plus boric acid. Results, expressed as growth inhibition, are shown in Table 46. In the case of copper glycinate alone (Figure 96), the higher growth inhibition was observed when the concentration was 0.02M; boric acid 0.02M also gave 100% growth inhibition, and its threshold value was estimated to be between 0.02M and 0.015M (Figure 97). In case of combination of both (Figure 98), when the boric acid concentration was higher than 0.02M the 100% growth inhibition was obtained for whatever concentrations of copper glycinate, as expected. Synergic effects between the two components were not observed: in fact, the combination 0.01M copper glycinate – 0.01M boric acid gave a growth inhibition lower than 0.01M copper glycinate alone and the combination 0.01M copper glycinate – 0.015M boric acid had a growth inhibition lower than 0.015 M boric acid alone. The evaluation of synergic effect in the combinations 0.015M copper glycinate and boric acid at the two concentrations 0.01M and 0.015M boric acid gave similar results: at the lowest concentration of boric acid, growth inhibition was lower than that

Table 46 Percentage growth inhibition of *C.puteana* on copper glycinate and boric acid different concentrations (n.t.: not tested). Preliminary tests in petri dishes, without wood.

	H_3BO_3 [M]				
$CuGly_2$ [M]	**0.000**	**0.010**	**0.015**	**0.020**	**0.040**
0.000	0 (*control*)	26.5	75.0	100.0	100.0
0.010	37.5	29.9	67.7	100.0	100.0
0.015	85.7	75.6	68.9	100.0.	100.0
0.020	94.6	n.t.	n.t.	100.0	100.0

Figure 96 Preliminary tests against C.puteana. Copper glycinate formuations. High fungal growth can be observed in the control petri dishes. Growth is inhibited by the presence of $Cu(GLY)_2$ dependng on the concentration.

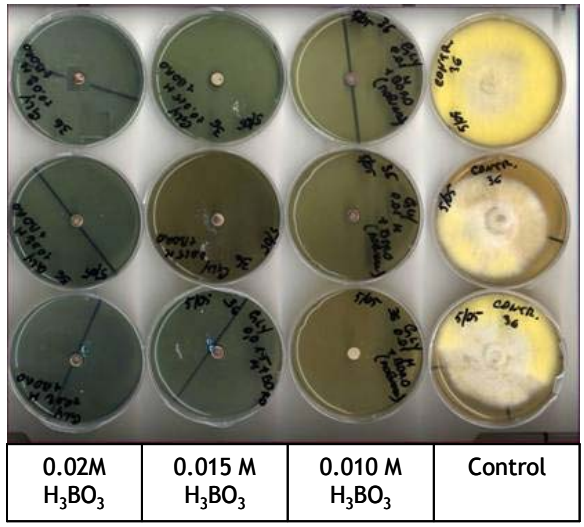
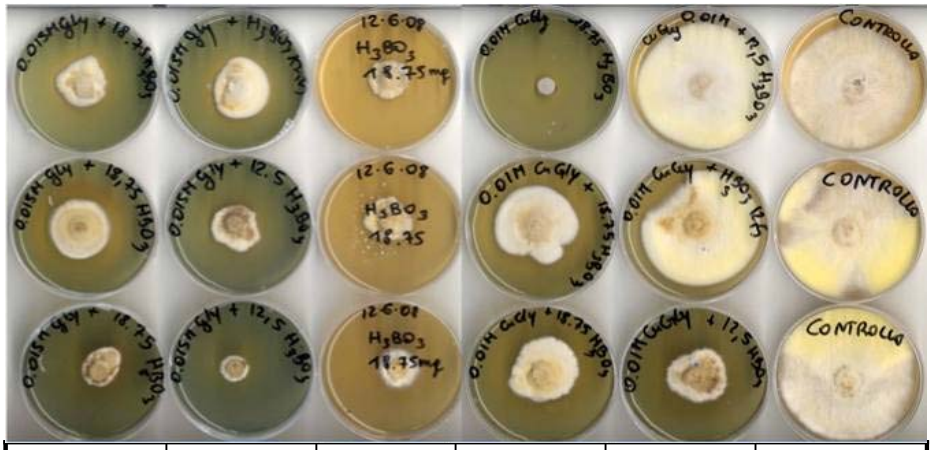


Figure 97 Preliminary tests against C.puteana. Boric acid formuations. High fungal growth can be observed in the control petri dishes. Growth is inhibited by the presence of boric acid.



0.015 M $(CuGLY)_2$ + 0.015M H_3BO_3	0.015 M $(CuGLY)_2$ + 0.010M H_3BO_3	0.015M H_3BO_3	0.010 M $(CuGLY)_2$ + 0.015M H_3BO_3	0.010 M $(CuGLY)_2$ + 0.010M H_3BO_3	Control

Figure 98 Preliminary tests against C.puteana. Copper glycinate + boric acid formuations. High fungal growth can be observed in the control petri dishes. Growth is inhibited by the presence of $Cu(GLY)_2$ and boric acid, dependng on the concentration.

with copper glycinate alone, while at the highest concentration growth inhibition was lower than with boric acid alone.

The formulations and concentrations for the efficacy tests on wood were selected on the basis of the agar tests results. Being the $Cu(Gly)_2$ 0.02M solution able to cause a 94.6%, growth inhibition, the few higher concentration 0.023M was chosen in order to try to achieve 100% growth inhibition. The same $Cu(Gly)_2$ concentration was also tested with the addition of boric acid 0.01M to evaluate possible synergic effects in wood. The highest $Cu(Gly)_2$ concentration (0.04M, i.e. the limit of solubility of $Cu(Gly)_2$ in water) was also tested.

Formulations characterized by higher concentration were also considered: they consisted in solutions of copper sulphate and glycine (1/2 molar ratio) added to water separately, with eventual further addition of boric acid. It is known that, under these conditions, copper sulfate and glycine form soluble cationic complexes of the type $[Cu(Gly)(HGly)]^+$ HSO_4^- . In these cases the tests take 16 weeks instead of the 6 weeks of the accelerated tests. The choice of the wood treatment procedure (8-hours immersion or impregnation under vacuum) was based on the results of SEM-EDX analysis on the cross sections of two 1 x 1 x 3 cm samples after exposure to 0.04M $CuGly_2$ acqueous solution by means of the

two kinds of procedure respectively. The sample for the immersion test was previously wax-sealed with exception for the two opposite longitudinal faces. Impregnation resulted to provide a deeper copper diffusion and more homogeneous copper distribution inside the wood sample, as copper was found also in the central area (5 mm depth from the longitudinal surface) with a low fluctuation of the Cu/O atomic ratio (Figure 99). On the other hand, in the bathed sample copper was almost entirely restrained in the first 0.2 mm layer under the surface and its presence was detected only till 0.8 mm depth.
On the basis of these data, impregnation was chosen for the efficacy tests, instead of simple immersion.
Each *Pinus sylvestris* mini-block sample was weighed before use in the tests, in order to determine the retention of solution, i.e. the impregnation efficacy. It was found that the average retentions were similar for each kind of treatment ranging from 660 to 750 kg/m^3 (Table 47).

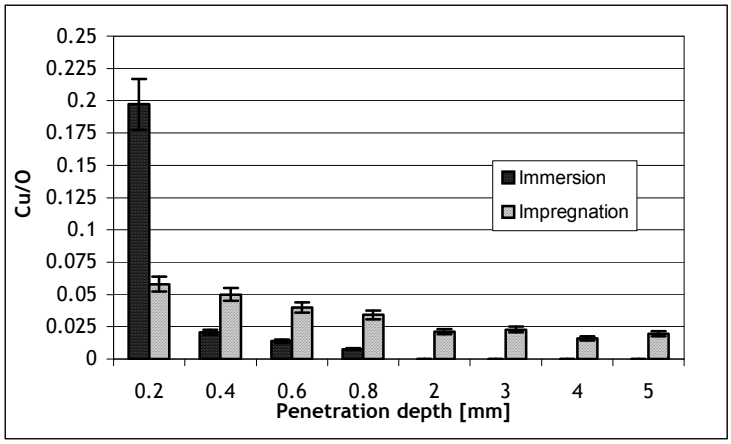


Figure 99 Copper penetration depth inside wooden blocks treated with copper glycinate. Penetration is shown as Cu/O atomic ratio, revealed by EDX microanalysis, vs distance from the surface.

Table 47 Retention average values for different glicinate formulations tested in wood.

Formulation	Retention [kg/m^3]
0.023M $Cu(Gly)_2$	682.2± 84.9 (n=6)
0.023$Cu(Gly)_2$+0.01H_3BO_3	716.59±105.06 (n=6)
0.04M $Cu(Gly)_2$	663.26±135.19 (n=6)
Copper sulphate (0.1 M) and glycine (0.2 M)	745 ± 56 (n=4)
Copper sulphate (0.1 M), glycine (0.2 M), boric acid (0.1M)	725 ± 59 (n=4)

Results concerning the effectiveness of copper glycinate (formulation 26, formulation 28) and copper glycinate formulation with addiction of boric acid (formulation 27) are not avaliable at the moment since tests are still in progress.

Formulations with copper sulphate (0.1M) and glycine (0.2M) seemed to provide an excellent resistance against *Coniophora puteana* (Table 48), as 0% mass loss was achieved, compared to the 48.6% and 27.6% of control blocks. Moreover, also the few data collected from the copper sulphate (0.05M)-glycine (0.1M) formulations (some samples had to be rejected since the corresponding control samples did not reach the 20% ML required for the 16-weeks-test) were promising (0% mass loss was achieved, compared to the 31.7% and 46.2% of control blocks). Despite this, in all formulations where glycine was added separately in water a final humidity percentage higher than the value required by EN 113 (i.e. 80%) was observed, so results cannot be considered valid.

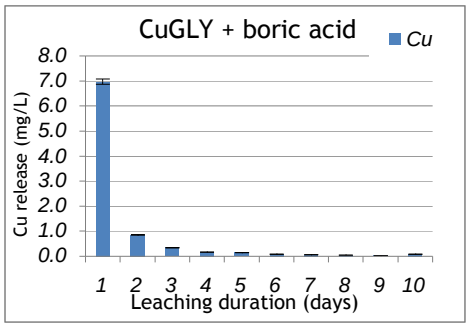
Table 48 Copper sulphate and glycine formulations: results of fungal decay tests (n=number of samples).

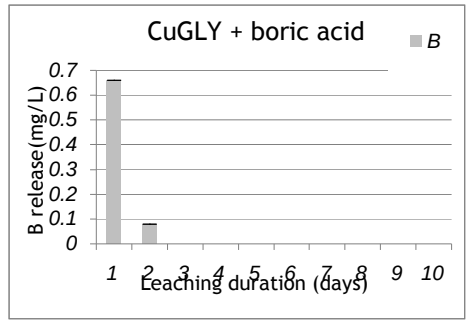
Efficacy against Coniophora puteana		
Formulation (n)	**Treated blocks mass loss average (%)**	**Control blocks mass loss average (%)**
copper sulfate (0.1 M) and glycine (0.2 M)	0	48,6
copper sulfate (0.1 M) glycine (0.2 M) boric acid (0.1M)	0	27.6
copper sulfate (0.05 M) and glycine (0.1 M).	0	31.7
copper sulfate (0.05 M) glycine (0.1 M) and boric acid (0.05M)	0	46.2
Mass loss wood blocks virulence control (6)	25,8	

The amounts of copper and boron released during a ten-days leaching test from samples treated with the $Cu(Gly)_2$- Boric acid formulations are reported in Figures 100-102. Copper leached out from the sample treated with the $Cu(GLY)_2$ 0.023M formulation was 0.585±0.023 kg/m^3; the subsequent treatment of the same sample with sulphuric acid caused the extraction of further 0.707 kg/m^3; assuming 1.292 kg/m^3 (that means the sum of the two extractions), about 45% was leached out during the water leaching test. Leaching tests performed on samples treated with the 0.04M $Cu(GLY)_2$ formulation gave similar results (0.547 kg/m^3 of leached copper, i.e. 45% of the 1.201 kg/m^3 total copper amount). It appears that the amount of glycinate retained into the wood samples is

independent on the starting concentration of copper glycinate, but leaching of glycinate don't appear significantly lower than the about 50% reported in literature for copper sulphate (Humar, 2005).

All samples showed the higher amount of released copper during the first day of leaching and a subsequent decrease. As concerns boron, some release was recorded only within the first two days. No further boron leaching was evidenced during the following days neither during a further treatment with sulphuric acid.





Figures 100-102 Copper (left) and Boron (right) water leaching from a pine wood sample impregnated with a 0.023M $Cu(Gly)_2$-0.010M H_3BO_3 acqueous solution.

3.4. Wood treatment with metal complexes in linseed oil and ethylene glycol

3.4.1 Zinc-salicylate formulation

The possible addition of zinc to linseed oil, as a tool to improve its preservative efficacy, was investigated. For this purpose, a chelate form of zinc derivative was chosen, in order to ensure satisfactory resistance to leaching; however, chelation should not be so strong to limit zinc interaction with bacteria or fungal enzymes, so compromising its preservative effectiveness. Zinc salicylate resulted suitable for this purpose, both because of weakly chelation of zinc, and because of its solubility in oil.

The FT-IR ATR spectrum of the solution of zinc salicylate in oil showed the linseed oil bands (3010 cm^{-1} (str. C=C-H), 2923 cm^{-1} (str. CH_2), 2854 cm^{-1} (str. CH_2), 1744 cm^{-1} (str.

C=O ester), 1458 cm^{-1} (bend. C-H), 1372 cm^{-1} (bend. C-H), 1238 cm^{-1} (str. C-O), 1156 - 1095 cm^{-1} (str. C-O), 719 cm^{-1} (rock. C-H) with addition of a peak at 1600 cm^{-1} attributable to salicylate (str. C-O of the carboxyl group) (Figure 103).

Pinus sylvestris wooden tablets (0.2 x 3 x 4 cm) dipped in the zinc-salicylate oil solution (see par.2.2.10) showed, in their FT-IR ATR spectra, the bands at 3010, 2923 and 1744 cm^{-1} confirming the linseed oil presence (Figure 104). Linseed oil capability to vehicle the metal deep inside wood was verified by dipping 2 cm thick samples and analysing their cross section through SEM-EDX investigations. A satisfactory penetration was achieved (independent on the drying pre-treatment of the samples) and zinc presence (reported as Zn/Ca molar ratio, assuming the Ca amount constant, Figure 105) was revealed till 6 mm under the surface (Figure 106).

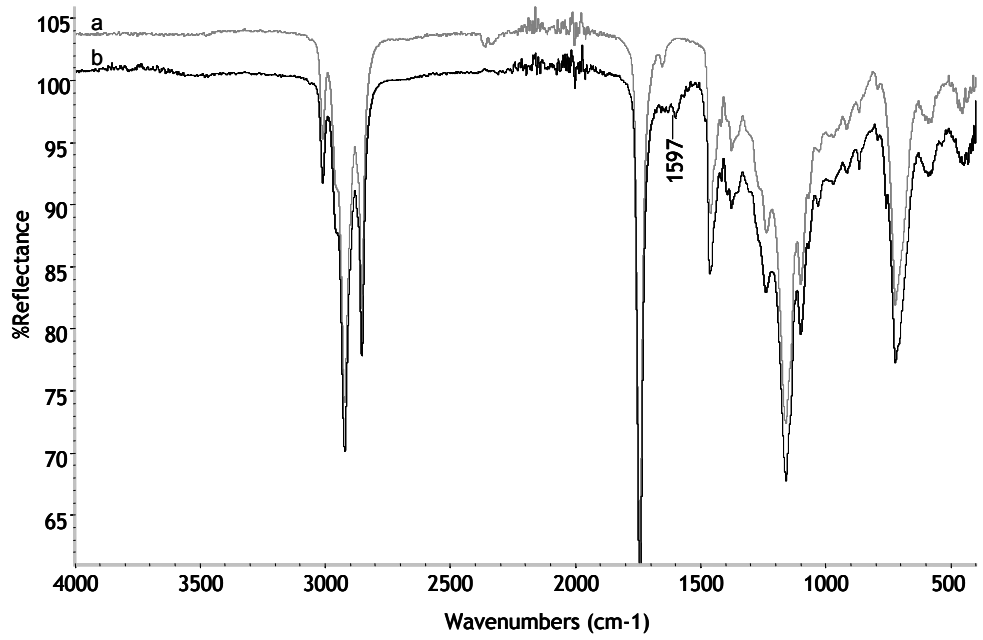


Figure 103 Comparison among the ATR-FTIR spectra of (a) Linseed oil (b) Zn-salicylate linseed oil. The peak at 1597 cm^{-1} is attributable to salicylate, as C-O stretching of the carboxyl group.

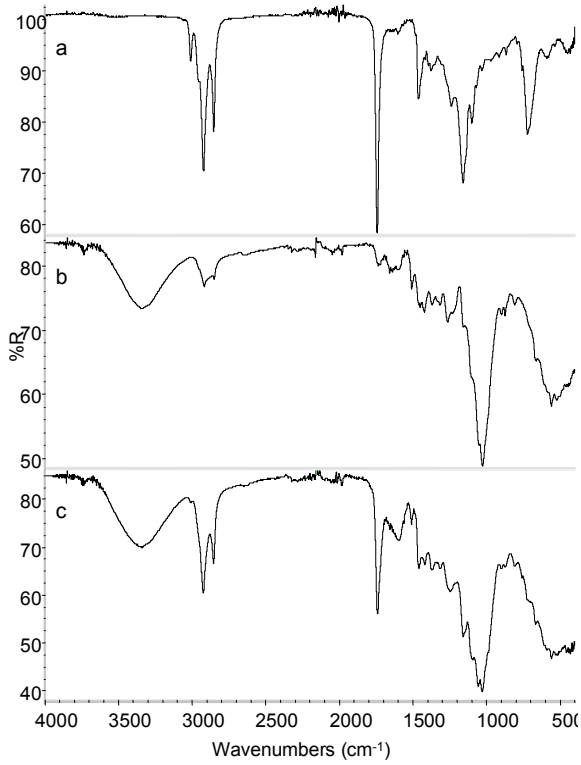


Figure 104 Comparison among the ATR-FTIR spectra of (a) pure linseed oil; (b) pine wood; (b) Zn salicylate in linseed oil (c) wood after immersion in Zn-salicylate linseed oil solution.

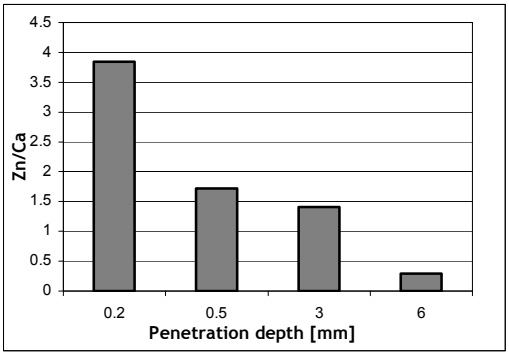


Figure 105 Zn penetration depth, expressed as Zn/Ca (molar ratio) into a wood sample immerged in zn-salicylate linseed oil solution.

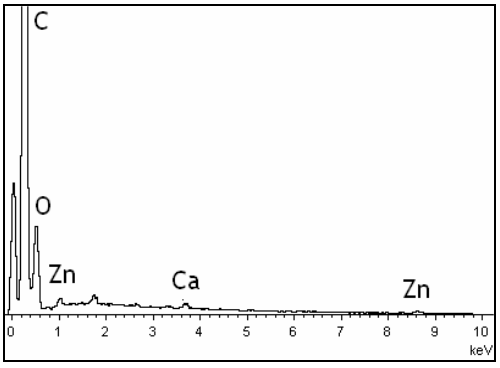


Figure 106 EDX spectrum of wood treated with Zn-salicylate in linseed oil, showing the presence of zinc.

Ethylene glycol was also tested as solvent for the Zinc-salicylate diffusion into the wooden structure: the higher chelate solubility made it possible to reach concentration levels higher than in linseed oil (up to 0.2M compared to the 0.065M of linseed oil).
In the FT-IR spectrum of the surface of treated samples, the bands around 2950 cm $^{-1}$ (str. C-H) and at 870 cm $^{-1}$ attested the presence of glycol in both cases of simple immersion and impregnation under vacuum (Figure 107). As regards bathed samples, relatively large amounts of zinc were found in the first millimetre under the surface (SEM-EDX analysis on the sample cross-section, Figure 108) but its penetration occurred till 4 mm depth making immersion procedure suitable for the treatment of the 2 mm pinewood samples required for the efficacy tests against termites (see par. 3.4.3).

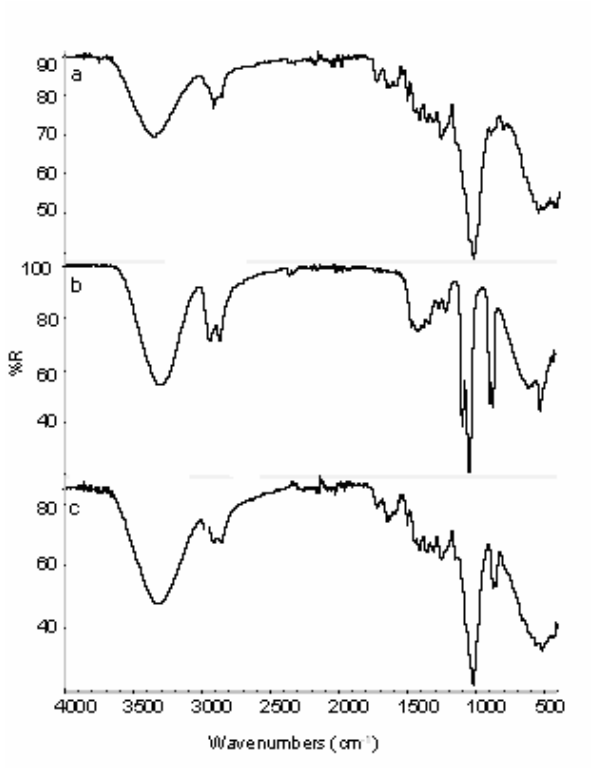


Figure 107 Comparison among the ATR-FTIR spectra of (a) wood, (b) zinc salicylate in ethylene glycol (c) wood immerged in the same ethylene glycole solution.

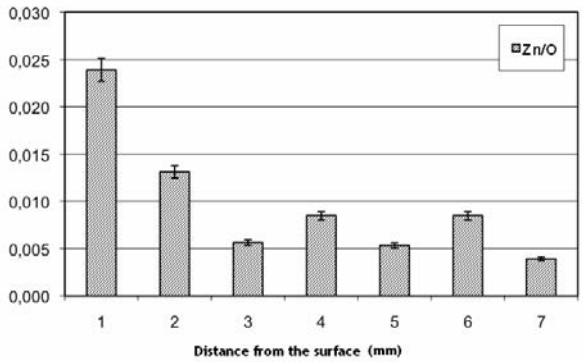


Figure 108 Zn penetration depth, expressed as Zn/O (molar ratio) into a wood sample bathed in Zn-salicylate ethylene glycol solution.

3.4.2 Copper-salicylate formulation

As for zinc salicylate, copper salicylate formulation in linseed oil was prepared to be tested against termites. Unlike zinc, copper salicylate crystals are characterized by a polymeric structure in the solid state (see par. 2.2.10). It can be dissolved in warm oil, but its solubility is lower than that of Zn-salicylate. Electron Paramagnetic Resonance (EPR) analyses were performed on Cu-salicylate in linseed oil and on wood treated with the same solution in order to investigate copper coordination environment.

The spectrum of the Cu-salicylate in linseed oil solution revealed the presence of more Cu complex species. The same is observed in the EPR spectra of treated wood, both before and after exposure to termites (Figure 109). This is indicative for the weakness of the coordinative interactions, which is also suggested by the differences among the spectra of freshly prepared and aged solutions (Figure 110) indicating a modification of the Cu coordinative environment by aging.

Assuming that the treatment with sulphuric acid was able to extract the whole copper content that had been reatained into the wood samples with the immersion procedure (see par. 2.3.2.1), the total copper amount resulted 0.681 kg/m^3 in case of ethylene glycol solution and 0.209 kg/m^3 in case of the linseed oil solution. These data appear in agreement with the starting concentrations (0.2M and 0.065M respectively).

Copper leaching reduction resulted higher in case of linseed oil (0.00734 kg/m^3 leached out i.e. 3.5% of the total amount) rather than with ethylene glycol (0.348 kg/m^3 leached out i.e. 51.1% of the total amount) (Figures 111-113).

So, despite higher solubility of copper salicylate in ethylene glycol, linseed oil appears more suitable to provide high leaching resistance.

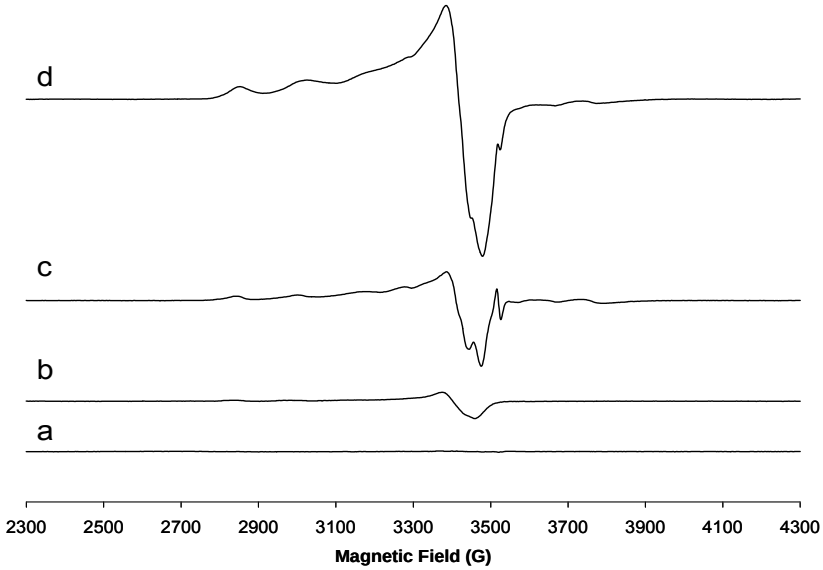


Figure 109. EPR spectra of (a) linseed oil (b) Cu-Salicylate in linseed oil (c) wood bathed in Cu-salicylate in linseed oil solution; (d) wood bathed in Cu-salicylate in linseed oil solution after exposure to termites.

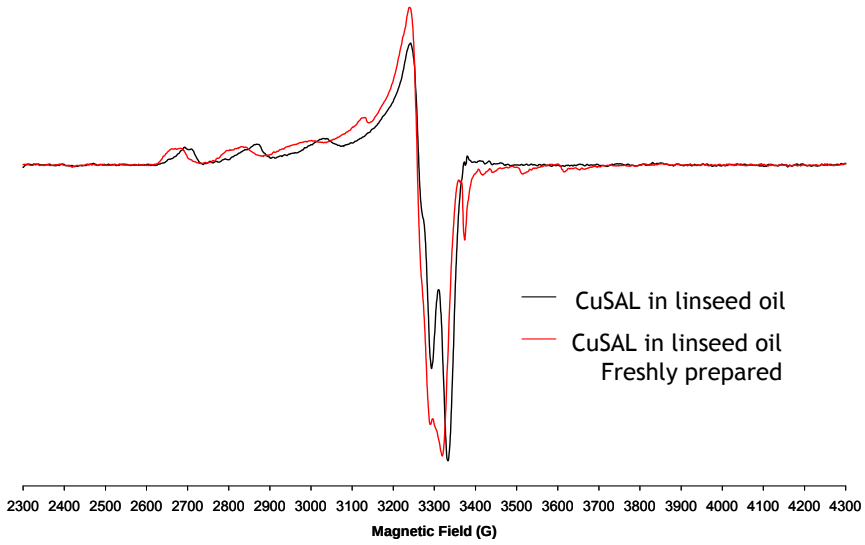
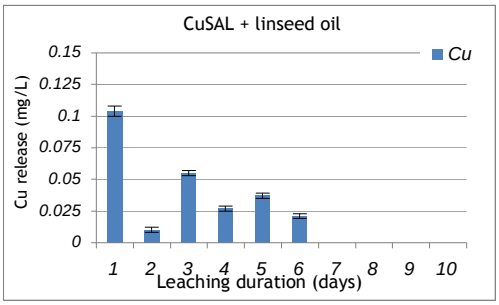
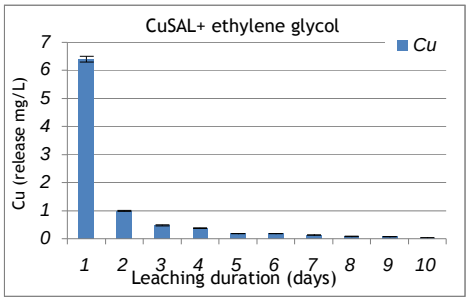


Figure 110 Comparison among EPR (low temperature) spectra of Cu-Salicylate in linseed oil freshly prepared and aged (4 months).





Figures 111-113 Copper leaching from a pine wood sample bathed in Cu Salicylate linseed oil solution (left) Cu Salicylate ethylene glycol solution (right).

3.4.3 Salicylates effiectiveness against termites

Tests to evaluate the effect of salicylates against termites were performed against the species *Reticulitermes lucifugus* (Rossi) *and K.flavicollis* (Fabricius).

As concerns *R.lucifugus,* mortality of individuals exposed to treated wood samples (5 repicates for each thesis) was the first parameter considered. Data obtained from the four theses (Control i.e. water, linseed oil, Zn-salicylate in linseed oil, Cu-salicylate in linseed oil) were statistically analyzed as dead termite percentage on the total inserted in each container (50). As variances were not homogeneous, ANOVA could not be applied. The results of non-parametrical analysis (Kruskal –Wallis), reported in the box-plot (Figure 114), show an extreme variability in the control samples, that could contribute to influence the esit of non-significative differences among theses. The 100% mortality was observed for temites exposed to Cu-salicylate in linseed oil treated samples, but mortality is not a suitable parameter to stress possible differences among the theses since total mortality was reached by some replicates of each thesis, with a wide variability especially in case of the control samples.

The observation of the behaviour of individuals and of their interaction with wood resulted much more interesting.

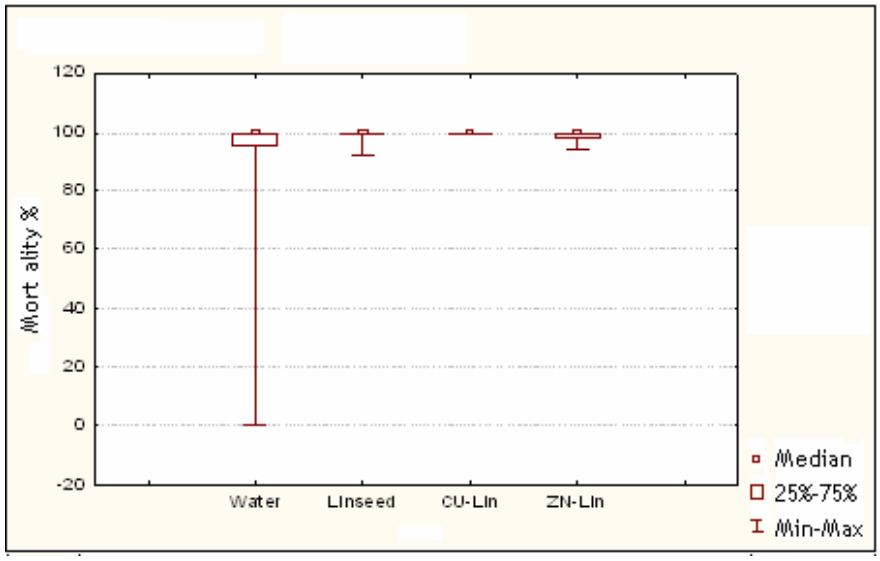


Figure 114 Mortality percentage results of efficacy tests against *R.lucifugus.* Water=control samples; Linseed= wood treated by immersion with linseed oil solution; Cu-Lin= wood treated with copper salicylate in linseed oil solution; Zn-Lin= wood treated with zinc salicylate in linseed oil solution.

Table 49 shows the distribution of termites within each container at the tenth day. Termites exposed to treated wood were less attracted from the samples. This was evidenced especially in samples treated with copper salycilate where almost all the individuals were found in the surrounding sand rather than on wood. The Cu-salicylate in linseed oil formulation seems to have repellent properties against the termitical specie. This behaviour was also evidenced by Zn-salicylate treated samples, but in this case a high mortality was also noticed, accompainied by bad health of the remaining indiviuals (cracked abdomen): as concerns Zn-salicylate formulation, the biocidal effect seems to prevail.

Finally, the mass loss data of wood samples are reported in Table 50, Figure 115. Compared to the control samples, linseed oil samples showed a mass loss reduction, especially with the addition of copper or zinc salicylate.

Table 49 Behaviour of termites after ten days from exposition to the wood samples. Distribtion among wood and the surrounding sand. C= control; L= linseed oil; Zn= Zinc salicylate in linseed oil; Cu= Copper salicylate in linseed oil
***= cracked abdomen; O = almost all dead.**

		R. lucifugus behaviour (day 10)		
Sample	**Treatment**	**Termites on sand**	**Termites in wood**	**mortality**
C96	Control (untreated)	7	43	
C97	Control (untreated)	28	22	
C98	Control (untreated)	17	33	
C99	Control (untreated)	30	20	
C100	Control (untreated)	37	13	
Li116	Linseed oil	50	0	
Li117	Linseed oil	48	2	*
Li118	Linseed oil	47	3	
Li119	Linseed oil	45	5	
Li120	Linseed oil	37	13	
Zn131	ZnSAL in linseed oil	43	7	*
Zn132	ZnSAL in linseed oil	42	8	*
Zn133	ZnSAL in linseed oil		0	O
Zn134	ZnSAL in linseed oil		1	O
Zn135	ZnSAL in linseed oil		0	O
Cu146	CuSAL in linseed oil	50	0	
Cu147	CuSAL in linseed oil	36	4	
Cu148	CuSAL in linseed oil	33	7	
Cu149	CuSAL in linseed oil	50	0	*
Cu150	CuSAL in linseed oil	49	1	*
Temperature		26,6 C		

Data are in agreement with the observations of termite behaviour, since the scarce permanence of termites in treated samples caused a reduction of eaten wood. Being the variance homogeneity violated, the non-parametric analysis (Kruskal–Wallis test) was performed on weight loss data (Figure 116).

From the multiple comparison analysis significant differences only emerge between Water and Cu-Lin ($z= 3.34$, $p= 0.005$).

Table 50 Wood samples Mass loss percentage (ML%) after exposure to the termites *R.lucifugus*. Water= control; Linseed= linseed oil Cu-Lin= Copper salicylate in linseed oil; Zn-Lin= Zinc salicylate in linseed oil.

Thesis (number of replicates)		Average ML % (st.dev.)
1	Water (4)	2.59±0.79
2	Linseed (5)	1.10±0.06
3	Zn-Lin (5)	0.70±0.35
4	Cu-Lin (5)	0.31±0.09

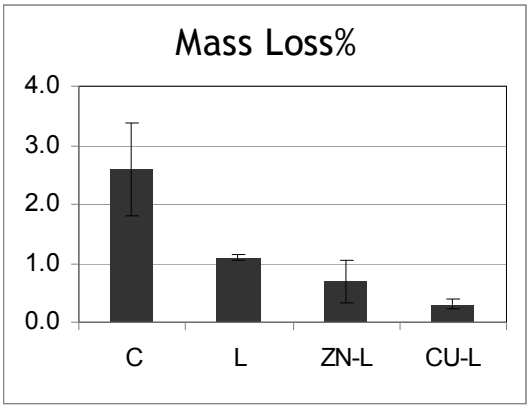


Figure 115 Histogram showing the wood samples mass loss % caused by termites *R.lucifugus*. C= control; L= linseed oil; Zn-L= Zinc salicylate in linseed oil; Cu-L= Copper salicylate in linseed oil.

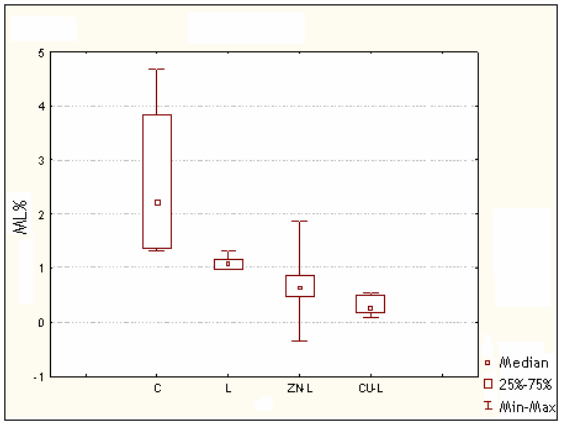


Figure 116 Kruskal-Wallis test results histogram showing the wood samples mass loss % caused by termites *R.lucifugus*. C= control; L= linseed oil; Zn-L= Zinc salicylate in linseed oil; Cu-L= copper salicylate in linseed oil.

The tests with *K.flavicollis* shown gave worse results: the tested treatments didn't produce significant mortality: two termites are dead in the case of samples impregnated with zinc salicylate, in copper salicylate tests too, and one dead in the test control, but in all cases the cause of death is not attributable to the action of the biocide chemicals, infact these termites were damaged at the beginning of the test.

As concernes termites behaviour, table 51 shows the distribution of termites within each container at day 15. No differences were noticed between untreated and treated samples, despite a slightly lower termites feeding activity on wood treated with copper salycilate, not sufficient to indicate preferential feeding.

Average Mass loss % data (Table 52) and histogram (Figure 117) show that termites exposed to treated wood feed the samples; this is less evident for the samples with copper salycilate**.** Infact, compared to the control samples, linseed oil samples didn't show a great mass loss reduction, only the sample with the addiction of copper salicylate presents some differences. However, from the sum of mortality, behaviour and mass loss data, neither copper salicylate in linseed oil can be considered as having repellent or biocide activity against *K.flavicollis* at the tested concentration: the Cu-salicylate in linseed oil formulation seems morepromising than the corresponding formulation with zinc. In both cases, higher concentration values should be evaluated.

Table 51 Behaviour of termites after 15 days from exposition to the wood samples. Distribution among wood and the surrounding sand. C= control; L= linseed oil; Zn= Zinc salicylate in linseed oil; Cu= Copper salicylate in linseed oil

		K. flavicollis **behaviour (day 15)**	
Sample	**Treatment**	**Termites on sand**	**Termites in wood**
C81	Control (untreated)	2	26
C82	Control (untreated)	4	24
C83	Control (untreated)	5	23
C84	Control (untreated)	0	28
C85	Control (untreated)	2	26
C86	Control (untreated)	7	21
C87	Control (untreated)	3	25
C88	Control (untreated)	4	24
C89	Control (untreated)	4	24
C90	Control (untreated)	5	23

		K. flavicollis behaviour (day 15)	
Sample	**Treatment**	**Termites on sand**	**Termites in wood**
Li101	Linseed oil	10	18
Li102	Linseed oil	4	24
Li103	Linseed oil	6	22
Li104	Linseed oil	1	27
Li105	Linseed oil	11	17
Li106	Linseed oil	6	22
Li107	Linseed oil	9	19
Li108	Linseed oil	2	26
Li109	Linseed oil	7	21
Li110	Linseed oil	2	26
Zn121	ZnSAL in linseed oil	3	25
Zn122	ZnSAL in linseed oil	12	16
Zn123	ZnSAL in linseed oil	7	21
Zn124	ZnSAL in linseed oil	5	23
Zn125	ZnSAL in linseed oil	6	22
Zn126	ZnSAL in linseed oil	8	20
Zn127	ZnSAL in linseed oil	13	15
Zn128	ZnSAL in linseed oil	9	19
Zn129	ZnSAL in linseed oil	5	23
Zn130	ZnSAL in linseed oil	9	19
Cu141	CuSAL in linseed oil	20	8
Cu142	CuSAL in linseed oil	5	23
Cu143	CuSAL in linseed oil	12	16
Cu144	CuSAL in linseed oil	11	17
Cu145	CuSAL in linseed oil	2	26
Temperature		27°C	
Relative Humidity		65%	

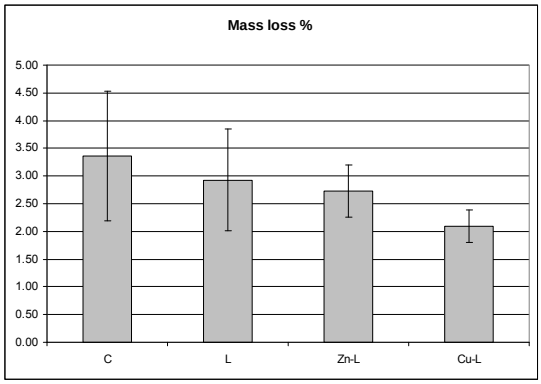


Figure 117 Histogram showing the wood samples mass loss % caused by termites *K.flavicollis*. C= control; L= linseed oil; Zn-L= Zinc salicylate in linseed oil; Cu-L= Copper salicylate in linseed oil.

Thesis (number of replicates)		Average Mass loss % (standard deviation)
1	Water (10)	3,36 ± 1,17
2	Linseed (10)	2,93 ± 0,92
3	ZN-Lin (10)	2,73 ± 0,47
4	CU-Lin (5)	2,10 ± 0,29

Table 53 Wood samples Mass loss percentage after exposure to the termites *K.flavicollis*. Water= control; Linseed= linseed oil Cu-Lin= Copper salicylate in linseed oil; Zn-Lin= Zinc salicylate in linseed oil.

The comparison among results obtained against *K.flavicollis* and *R.lucifugus* seem to indicate that the efficacy of tested shell treatments in preventing termite attack is a function of the termite species. The tested formulations show repellent/biocidal activities more evident against the drywood termite *R.lucifugus* than against the subterranean termite *K.flavicollis*.

PART C

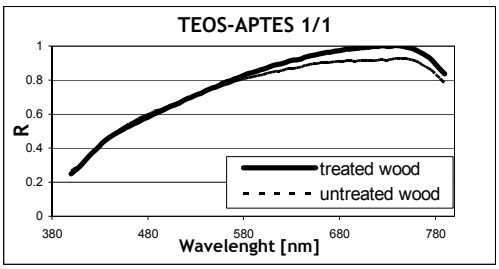
PHYSICAL-MECHANICAL CHARACTERIZATIONS

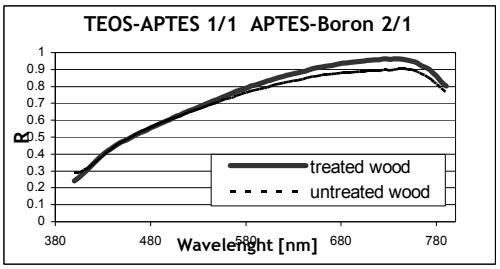
3.5. Physical and mechanical properties of wood treated samples

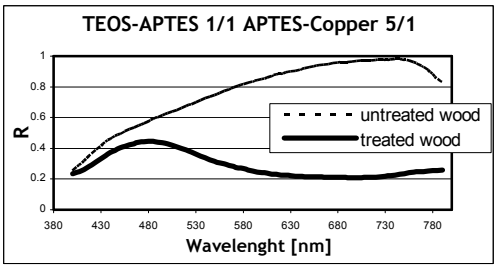
3.5.1 Colorimetric characterization

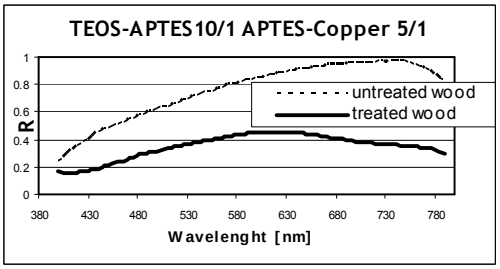
The Spectral Reflectance Factor R of treated samples in the visibile range of the electromagnetic spectrum is shown in the curves in Figures 118-126. Table 54 shows the colour difference of the wood samples before and after treatment, expressed as ΔE data. TEOS-APTES and TEOS-APTES-BORON treatments only show a small increase of R in the range between 500 and 700 nm (yellow-red): the yellowish tone is probably due to the natural wood aging (Temiz, 2004). As for both treatments the value of the average ΔE is lower than 3, TEOS-APTES impregnations with or without the addition of boron does not seem to alter wood original colour to an extent perceptible to human eyes.
Both linseed oil and zinc salicylate in linseed oil afford a variation of the *b* parameter towards the yellow tint; the ΔE measured for the two kinds of treatment are similar, suggesting that zinc-salicylate does not influence the colour change if compared to the common oil treatment. Also zinc-salicylate in ethylene glycol does not alter wood original colour significantly for human eyes perception. The introduction of Cu^{2+} cations to all formulations causes a whiteness decrease (variation of the L parameter) combined to a chromatic change (*a* parameter) towards the blue-green range. The sodium silicate formulation was expected to create a colour variation similar to that obtained with the TEOS-APTES 1/1 having an equal copper theoretical amount but ΔE was comparable to the TEOS-APTES 10/1 formulation. Further scanner acquisition on all the samples with copper formulations after aging could provide some information on the time stability of the acquired colour.

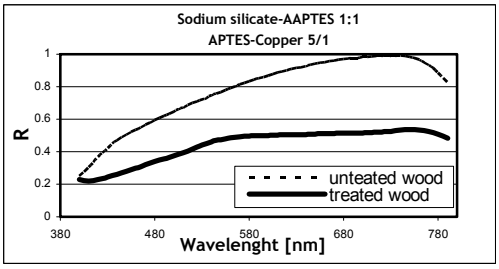
Figures 118-126 (following page) Comparison among the Spectral Reflectance curves in the visible range of the electromagnetic spectrum before and after treatments on pinewood.

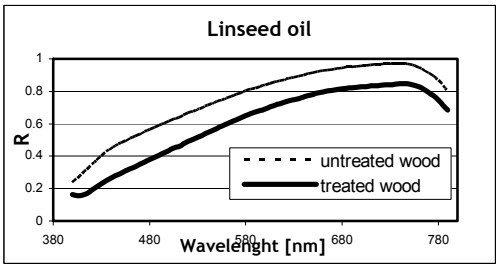

TEOS-APTES 1/1
R
Wavelenght [nm]
treated wood
untreated wood

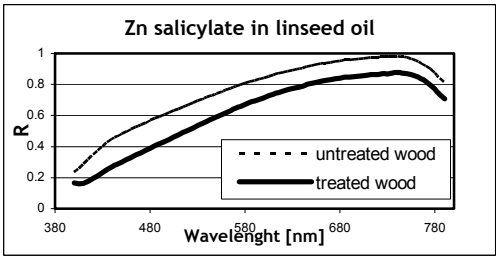

TEOS-APTES 1/1 APTES-Boron 2/1
R
Wavelenght [nm]
treated wood
untreated wood

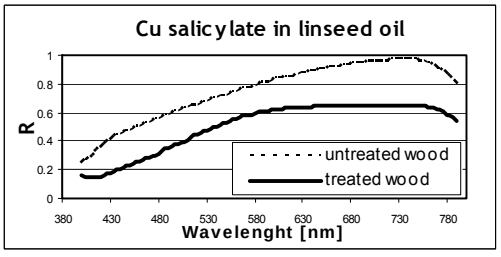

TEOS-APTES 1/1 APTES-Copper 5/1
R
Wavelenght [nm]
untreated wood
treated wood


TEOS-APTES10/1 APTES-Copper 5/1
R
Wavelenght [nm]
untreated wood
treated wood


Sodium silicate-AAPTES 1:1
APTES-Copper 5/1
R
Wavelenght [nm]
unteated wood
treated wood


Linseed oil
R
Wavelenght [nm]
untreated wood
treated wood


Zn salicylate in linseed oil
R
Wavelenght [nm]
untreated wood
treated wood


Cu salicylate in linseed oil
R
Wavelenght [nm]
untreated wood
treated wood

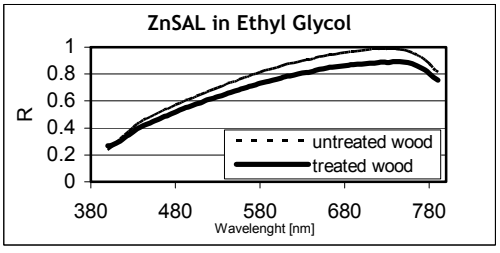

ZnSAL in Ethyl Glycol
R
Wavelenght [nm]
untreated wood
treated wood

Table 54 Colour differences of pinewood samples before and after treatment, expressed as ΔE values. L=lightness, a=red-green coefficient, b=yellow-blue coefficient

Formulation		L.a.b. coordinates before treatment Average (n=3)	L.a.b. coordinates after treatment Average (n=3)	ΔE
TEOS-APTES 1:1	*L*	89.45	89.87	**1.94**
	a	0.80	2.24	
	b	21.35	23.93	
TEOS-APTES 1:1 + Boric acid	*L*	87.4	89.04	**1.52**
	a	1.31	2.23	
	b	23.08	23.28	
TEOS-APTES 1:1 + Copper chloride	*L*	89.93	65.17	**30.44**
	a	2.28	-12.33	
	b	23.44	-6.43	
TEOS-APTES 10:1 + Copper chloride	*L*	89.59	69.14	**14.52**
	a	2.18	-0.51	
	b	23.74	24.71	
Sodium silicate-APTES 1:1+ Copper chloride	*L*	73.57	90.53	**12.11**
	a	-1.88	1.73	
	b	21.72	23.60	
Linseed oil	*L*	89.09	80.89	**6.63**
	a	2.26	4.20	
	b	23.73	32.05	
Zn-salicylate in linseed oil	*L*	89.09	81.96	**6.31**
	a	2.21	4.15	
	b	23.91	32.85	
Cu-salicylate in linseed oil	*L*	88.55	77.64	**9.41**
	a	2.38	0.49	
	b	23.20	36.23	
Zn-salicylate in ethylene glycol	*L*	89.57	86.04	**2.36**
	a	2.11	1.88	
	b	24.2	22.8	

3.5.2 Sorption and dimensional stability tests

The evaluation of possible changes in permeability due to wood treatments was performed by means of sorption tests on wood samples belonging to the following categories: sol gel TEOS-APTES, sol-gel TEOS-APTES with copper, linseed oil, linseed oil with addiction of zinc salicylate. The results are displayed in Figure 127 in terms of values Mx-Mi in function of time resulting after four temperature/humidity jumps (see par. 2.6.2). Among the four temperature-humidity jumps performed (Figure 127), the third one (from 28% RH- 27°C to 73% RH – 20°C), over 2 days, reached values nearest to the asymptotic ones (corresponding to the hygroscopic equilibrium): so, at first, permeability was evaluated from the normalized sorption curves of the third jump (Figure 128), as a comparison among the "half-times" $t_{0.5}$ (i.e. square root of the time necessary to reach a new moisture content equilibrium). Samples treated with linseed oil, with or without the addition of the zinc chelate, showed the highest $t_{0.5}$; this means the slowest vapour exchange conditions: the performed oil treatments gave to the wood samples a vapour barrier effect. It does not seem significantly influenced by the presence or absence of zinc salicylate as resulted from analysis of variance (ANOVA) on the $t_{0.5}$ obtained from the normalized curves of all the four jumps. For each treatment the average $t_{0.5,}$ expressed as percentage relative of untreated wood values, is reported (Table 55). The box-plot in Figure 129 shows a summary of statistical results (Figure 129). All the four treatments afforded a significant permeability reduction if compared to untreated wood.

The weight variations caused by the temperature-humidity jumps are expression of the hygroscopicity of the material. Results from the variance analysis (ANOVA) on the average weight variation data of all the "jumps" are reported in the box-plot graphic in Figure 130. Hygroscopicity differs significantly from untreated samples in case of both sol-gel and sol-gel+Cu categories. Further investigations on hygroscopicity can be performed by studying the isotherms of water sorption and desorption due to a specific hygrometric condition. Wood moisture content variation of samples reaching 35% RH starting from anhydrous conditions (absorption curve) or from 58% RH conditions (first jump, desorption curve) was evaluated for sol-gel treated and untreated samples. As reported in literature, sorption and desorption curves do not coincide but enclose an hysteresis area (Figure 131).

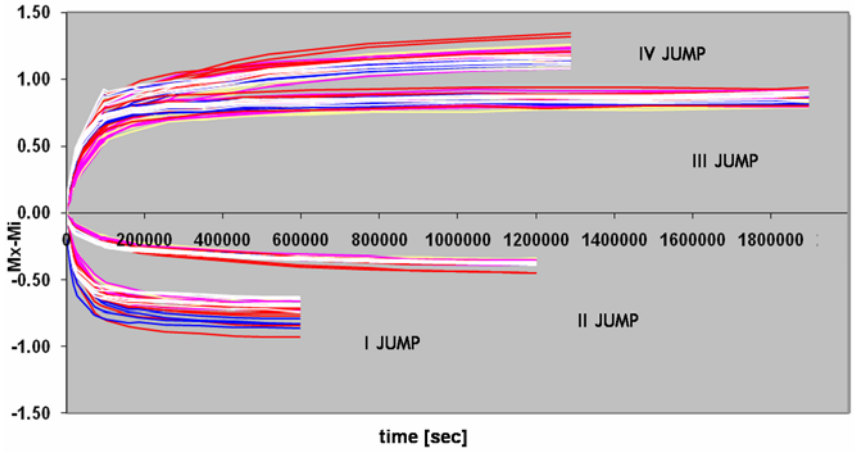


Figure 127 Sorption curves of untreated (white line) and treated samples related to four temperature-humidity jumps.

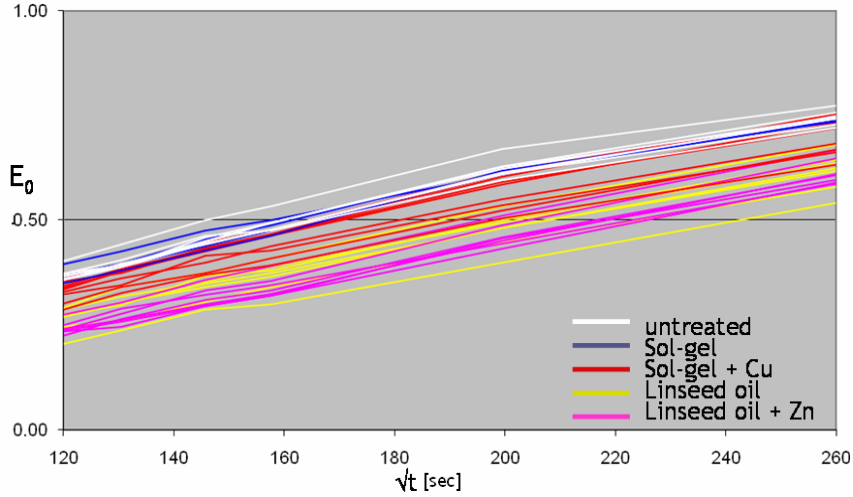


Figure 128 Magnification of the sorption curves of the third jump showing the $t_{0.5}$ values of untreated and treated samples. E0 = ΔMt/ΔMe with ΔMt=mass variation at time t and ΔMe=mass variation at the final equilibrium time.

Table 55 Half-time values for treated samples (expressed ad increase % of untreated samples).

Treatment	Relative $T_{0.5}$ (%)
Linseed oil	30.80
Zn-salicylate in linseed oil	33.47
Sol-gel TEOS-APTES	17.18
Sol-gel TEOS-APTES +Cu	7.00

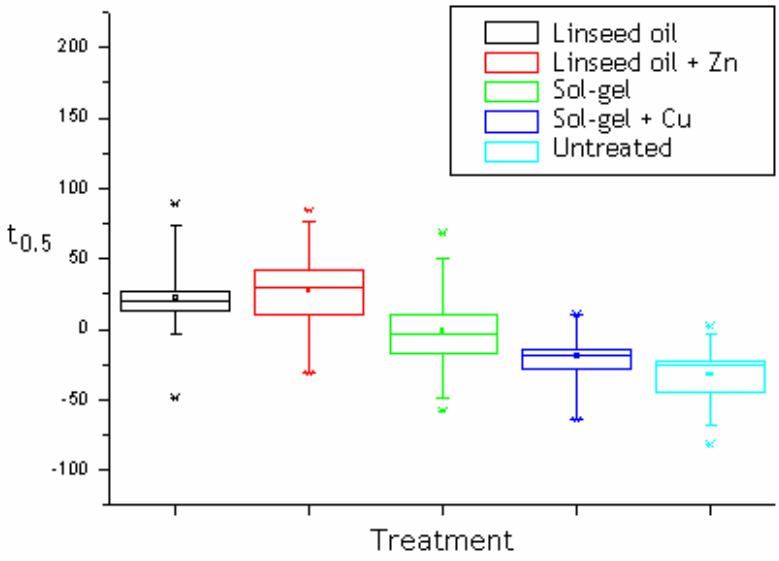


Figure 129 Sorption tests results: comparison (ANOVA) among $t_{0.5}$ values of samples undergone to different treatments.

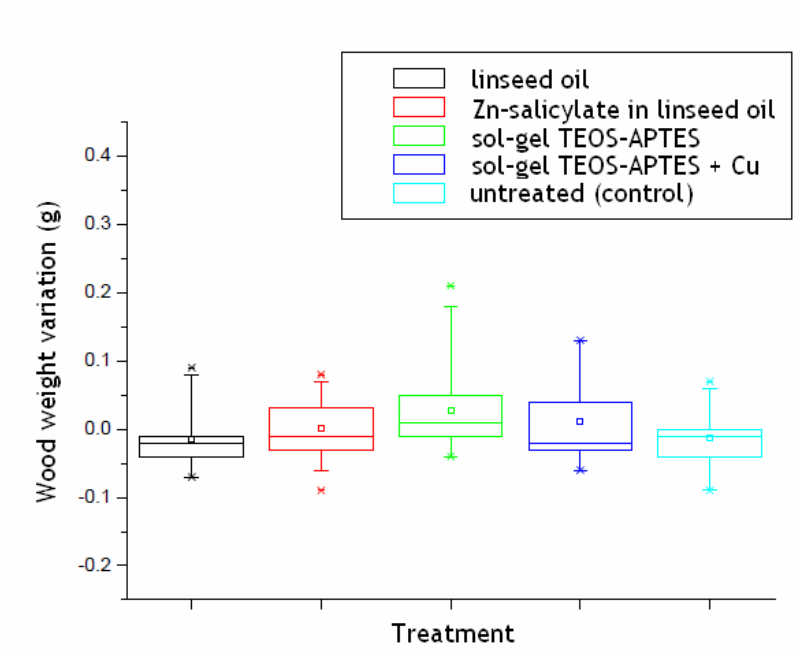


Figure 130 Sorption tests results: comparison (ANOVA) among average weight variation data of samples undergone to different treatments.

The average differences among absorption and desorption values of the hysteresis cycle are reported in Table 56. In both cases the lowering of MC% value from desorption to absorption confirms the hysteresis phenomenon. The average percentage wood humidity difference was 1.4% for sol-gel treated samples and 1.2% for untreated samples. Generally, this value can vary from 2.5% to 8% if referred to the whole hygroscopic field i.e. from 0% to 30% MC; the lower data obtained are probably related to the reduced hygroscopic field considered (from 0% to 11.4% MC). Variance analysis (ANOVA) verified that results from treated and untreated samples do not differ significantly.

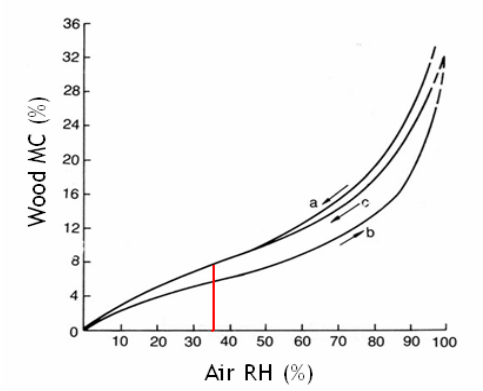


Figure 131 Hygroscopic hysteresis: a: desorption; b: absorption; c: further desorption (Tsoumis, 1991). RH: air relative humidity; MC: moisture content.

Table 56 Average differences among absorption and desorption values of the hygroscopic hysteresis cycle. Ranges: 58%- 35% RH (desorption); 0% - 35% RH (absorption).

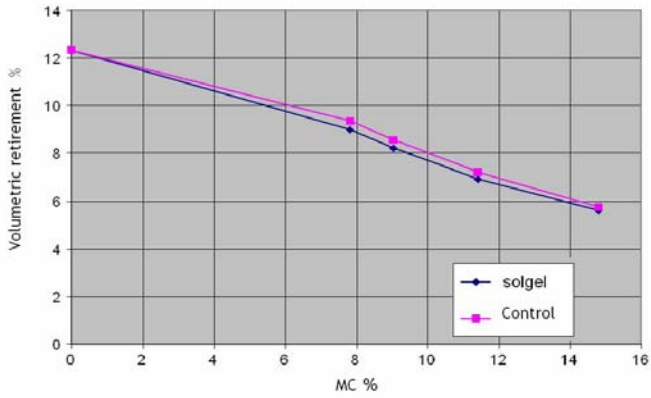
	MC% desorption	MC% absorption	ΔMC
Sol-gel	9.04	7.60	**-1.4**
Control	9.03	7.87	**-1.2**

Wood shrinkage and swelling are related to variations of its moisture content (MC). Due to wood anisotropy, its dimensional variation coefficients vary depending on the anatomical direction (radial, tangential, longitudinal). Dimensional retirements are usually expressed as percentages of the dimensions of "fresh" wood, i.e. having a moisture content higher than the Fiber Saturaton Point (FSP); usually, they are lowest in the longitudinal direction β_L (0.3% = Picea abies retirement) (Giordano, 1981), highest in

the tangential direction B_T (8.5% = *Picea abies* L.retirement) (Giordano, 1981) and middle in the radial direction B_R (3.8% = *Picea abies* L. retirement) (Giordano, 1981).
Some wood treatments can eventually increase the dimensional variation coefficients reducing wood dimensional stability and facilitating cracks in the wooden structures. The measurement of the radial, tangential and longitudinal dimensions of 4 x 2 x 2 *Picea abies* L. samples (25 replicates), before and after each jump i.e. with the variation of MC, made it possible to study the dimensional stability of sol-gel treated wood compared to the untreated one of the same specie.
The following properties were considered:

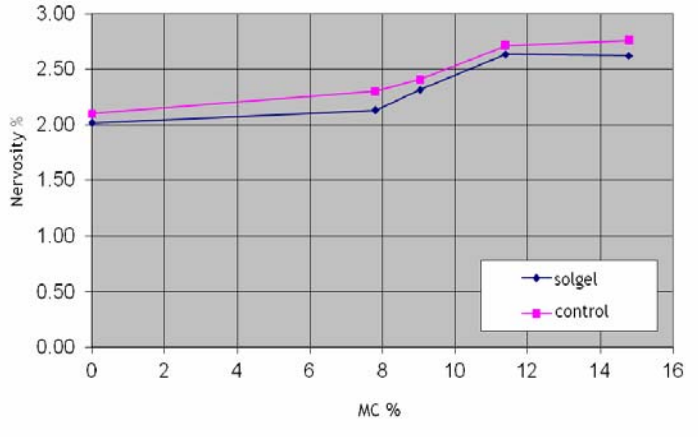
- volumetric retirement : $(B_V = B_L + B_T + B_R) * 100$
- nervosity : B_T / B_R
- angular retirement : $B_T - B_R$

The trend of each property as a function of MC is reported below together with the related statistical elaboration (ANOVA) (Tables 57-59; Figures 132-135). The performed TEOS-APTES sol-gel impregnation has not significantly influenced wood dimensional stability and don't contribute in any way to the worsening of this property. The unaltered wood behaviour can be considered as a positive property of the tested wood treatment.



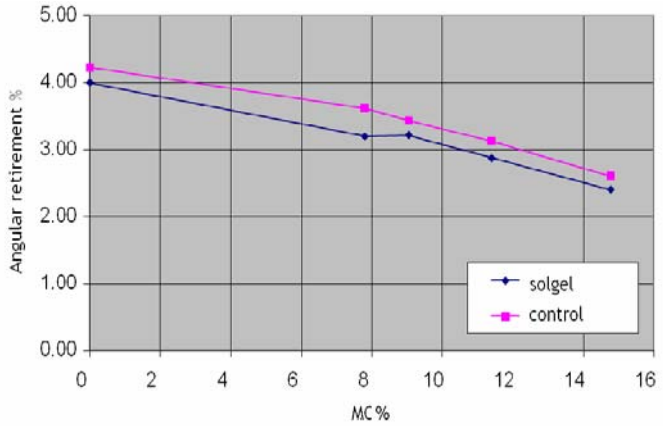
MC (%)	0.0	7.8	9.0	11.4	14.8
VR solgel (%)	12	9.0	8.2	6.9	5.6
VR control (%)	12	9.3	8.6	7.2	5.8

Figure 132- Table 57 Comparison among the volumetric retirement (VR) of sol-gel treated and untreated samples for different wood moisture content values (MC%).



MC (%)	0.0	7.8	9.0	11.4	14.8
N solgel (%)	2.02	2.13	2.32	2.64	2.62
N control (%)	2.10	2.30	2.41	2.71	2.76

Figure 133- Table 58 Comparison among the nervosity (N) of sol-gel treated and untreated samples for different wood moisture content values (MC%).



MC (%)	0.0	7.8	9.0	11.4	14.8
AR solgel (%)	4.0	3.2	3.2	2.9	2.4
AR control (%)	4.2	3.6	3.4	3.1	2.6

Figure 134- Table 59 Comparison among the Angular retirement (AR) of sol-gel treated and untreated samples for different wood moisture content values (MC%).

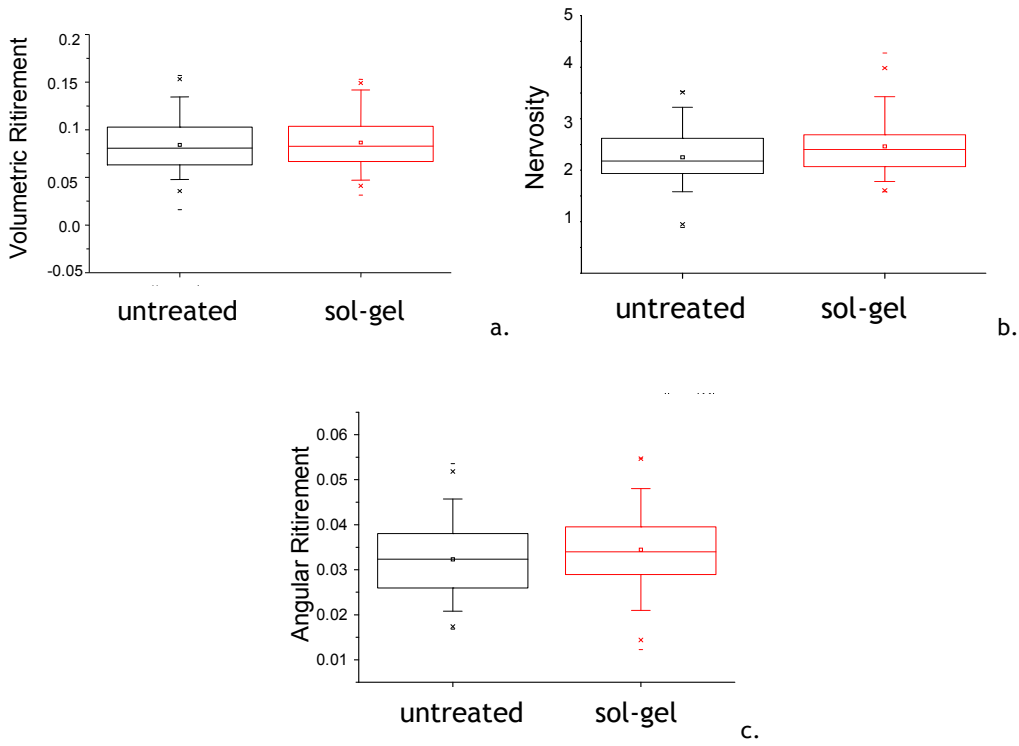


Figure 135 Box-plot graphics showing ANOVA results for (a) volumetric retirement (b) nervosity (c) angular retirement of sol-gel treated and untreated wood samples.

4. Conclusions

The present work has allowed to reach the following goals.

A. The preservative effectiveness of wood treatments based on tetraethoxysilane-aminopropyltriethoxysilane formulations against fungi and termites, especially with addiction of copper, other heavy metals such as silver or zinc or boric acid was demonstrated.

- Satisfying wood impregnation was achieved; metals were well vehicoled by the sol inside the wooden structure as verified by means of spectroscopic and microanalytical methods.
- Characterizations of the hybrid inorganic-organic xerogel interpenetrating wood were performed.
- The presence of the xerogel inside wood increased leaching resistance.

B. The preservative effectiveness of wood treatments based on Cu and Zn metal chelates in water and organic solutions, palticularly in linseed oil, was demonstrated.

- In particular, zinc and copper salicylate formulations in linseed oil were developed showing preservative effects against termites.
- Good leaching resistance results were obtained.

C. The improvement or stabilization of wood physical-mechanical properties such as sorption and dimensional stability were identified as further characteristics of the developed treatments.

The followed study provides promises for the development of new classes of eco-friendly wood preservatives.

5. References

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