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Section 5 VVVVVV

Bibliographical study: Decontamination of wood and soils polluted by pesticides used in wood preservation: *focus on Bioremediation methods using microbes.*

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ABSTRACT: The purpose of this bibliographical study was to seek out the existence of projects (past, present, or future) on soil decontamination in wood treatment plants. Indeed, such polluted soils may contain dioxins and various types of furans. The aim was to find out whether there existed one or more methods bringing into play biodegradation techniques using, for example, species of fungi, yeasts, bacteria or enzymes enabling decontamination. Knowledge about the feasibility, costs, toxicity and implementation of such projects is also a key point. Likewise, the bibliographical study needed to focus on methods enabling such decontamination pathways and on whether it is possible to consider them using microbes.

Key words: decontamination, pesticides, wood preservation, bioremediation, wood inhabiting fungi, microbes.

1. INTRODUCTION

Soil clean-up primarily consists in making the soil and subsoil of a zone suitable for new industrial use, or residential use, or even in some extreme cases suitable for a return to the wild or to agricultural use, after being polluted by an industrial activity or accident.

Some sites that are more polluted, or suspected of being so, are listed in all EU countries with a view to cleaning them up or avoiding their use for certain activities. This graph shows for Europe the number of cleaned up soils, polluted soils, or soils that are probably polluted or supporting a polluting activity, and their status (as of 2006). Compared to the number of those remaining, few polluted sites seem to have been already cleaned up (Bodénan 2005) Indeed, the existence of pollutants in the soil raises problems of toxicity as soon as the pollutants migrate (under the effect of water flow, soil handling, planting) and are found in the food chain or come into contact with humans via their food. In addition, even when the

environmental danger linked to the pollution of a site does not appear to be immediate, there may be a wish to clean it up and make use of it (e.g. in a buildable area), by reducing the risks to future users.
There exist various methods for extracting pollutants present in soil, and sometimes for destroying them in the soil (where degradable pollutants are involved). They depend on the type of pollutant (hydrocarbons, heavy metals, various chemicals, etc.) and the nature of the land (permeable or not, granular, presence of water, pH, etc.).
Also, soil decontamination cannot be considered without dealing with the question of possible purification of water stored in it or circulating within it, be it on the surface or underground (water tables).

2. SCIENTIFIC CONTEXT AND LATEST PUBLICATIONS SINCE 2000

2.1 Presentation

Firstly, the biographical study shows that treating soils polluted by dioxins and furans has been a major line of research for many scientists since the beginning of the 2000s. A great deal of research work undertaken at several universities (theses, scientific publications, seminars, etc.), along with various reports from the Ministry of Ecology (Bodénan 2004-2005) or from the call for projects issued by ADEME (ADEME 2004) bear witness to the keen interest of the public authorities in the search for new decontamination methods. In addition, other countries, such as Togo in 2004 (Moctar 2004), have assessed and expressed their soil decontamination needs, particularly with regard to dioxins/furans.

2.2. State-of-the-art

Certain bacteria are able to break down complex molecules and draw from them the energy they need to live.
They have been used for years to treat soils polluted by chlorinated solvents. However, it was found that this microbiological treatment technique using biological degradation of certain pollutants *in situ* (possibly encouraged by the control of parameters such as oxygenation, relative humidity and temperature) can generate degradation products (metabolites) that are more toxic and/or more mobile than the initial products. These metabolites differ depending on whether the conditions of microbial activity are aerobic or anaerobic. In order to control production of the most dangerous metabolites, it is possible to switch to one or other of the biodegradation modes as suitable.

At the moment, research is focusing on selecting species able to break down each type of pollutant. The research is being undertaken jointly by research laboratories and eco-industrialists. It is beginning to give results for certain types of polycyclic aromatic hydrocarbons (comprising more than three benzene nuclei), using certain fungus strains with the particularity of attacking pollutants by extracellular enzymes. Examples of a correspondence exist between pollutants and pollutant-removing bacteria, such as: Pesticides: *Enterobacte*r and Dioxins: *Brevibacterium.*

The generic term “dioxins” covers no fewer than 210 dioxin (PCDD) and furan (PCDF and PCB) molecules possessing similar chemical structures, derived from that of benzene (Bodénan 2004-2005). Their toxicity and lifespan (between 1 and 10 years) varies depending on their molecular structure. Many degradation pathways exist for these pollutants (chemical, biotic). However, numerous studies are now focusing on methods based on <u>biodegradation and biotransformation.</u> These techniques involve bacterial and fungal microorganisms. These various techniques are known as "<u>bioremediation techniques</u>" (Cadiere 2006). Other techniques use the properties of certain plant species (herbaceous strata, plants, bushes, trees, algae) to interact with organic or mineral chemical compounds to clean up contaminate land *in situ*. These are phytoremediation techniques (Campanella 2002). Whatever the method, the difficulty lies in the way certain parameters are controlled, and the precautions to be taken to prevent the diffusion of microbes in the neighbouring environment (water tables). These techniques can also be used to treat pollutants other than dioxins and offer numerous advantages:

- Economic merits: low treatment costs;
- Technical merits: treatment of a diversified range of pollutants (organic, mineral), possibility of preparing specialized microorganisms, ability of microorganisms to live under extreme conditions (pH, oxygenation, high pollutant concentrations, etc.), numerous identified and characterized microorganisms;
- Conclusive laboratory tests.

The weak points are: More complex application in the field: high pollutant concentrations can slow down the process, effects of land composition and properties, climatic conditions (seasonal fluctuations affect the metabolism of microorganisms), difficulty in finding pilot sites to validate laboratory work.

As regards current research, numerous bibliographical sources are available (Cadiere 2006). We found over 150 patents for bioremediation techniques, be it in France or abroad. Most of the patents covering the subject are American and Japanese.
Lines of research are now mostly focusing on these new techniques. We can cite "ectomycrorrhizal symbiosis". This technique involves fungi that live in symbiosis with tree roots. Their virtues would seem to apply to various pollutants: dioxins but also furans and PCB-DLs. (Carlier, 2010). The budget for this study is 500,000 euros.
Other lines of research are being pursued by enzymatic pathways (Sakaki 2010), but also in the search for new fungi (All 2010).

3. CLEAN-UP ECONOMICS

In terms of the competition and economic context, soil decontamination and remediation are proving to be a fast developing emerging activity. It is necessary to have a very good command of soil clean-up procedures, of the different pollutant transfer mechanisms and a sound financial base in order to gain a foothold in this market. In recent years, competition has developed much faster than the market has grown. However, according to some experts, the rate of site decontamination is set to increase, partly due to the restrictions imposed by the public authorities: it is a regulatory market.

Competition and economic context
The decontamination and remediation of soils are proving to be a fast developing emerging field of activity. Gaining a foothold on this market calls for a good command of soil clean-up processes, of the different pollutant transfer mechanisms and a sound financial base. In recent years, competition has strongly developed quicker than the market has grown. However, according to some experts, the site clean-up rate should increase, partly due to the constraints imposed by the public authorities: it is a regulatory market.

Functions fulfilled
The different soil decontamination procedures consist in treating contaminated soils to remove or greatly reduce their contaminant nature. As for the various remediation operations, these include both site clean-up and remediation operations with a view to enabling fresh use.

The ultimate objective is to remove the nuisances and eliminate or minimize risks to the environment, people and goods.
Restrictions for the re-use of polluted sites are becoming the driving force of clean-up requirements; in addition, regulatory restrictions make clean-up compulsory at the end of site occupation. These factors have led to the creation of a veritable clean-up market, with the creation of companies specialized in this activity, be it for the detection and analysis of soil pollution, or for actual clean-up. Some companies whose activities are, by their very nature, polluting have adapted and set up ad hoc subsidiaries.

Technological developments and research programme
Research and development work are mainly organized around knowledge and understanding of the environmental impacts of polluted sites, of polluted site inventory methods, intervention by prioritizing risks, and analysis of clean-up objectives, and also around the development of new technologies for the remediation of polluted sites.
Many European Eureka programmes are underway such as:

- RAUMATREDI (development of an extraction technique for organic pollutants using supercritical CO_2).
- RESCOPP (remediation of sites polluted by petrochemical products).

Other programmes have been initiated on an international scale and seek to determine the state of knowledge and know-how at international level. They involve:
-preliminary and global methods of investigating sites by geophysical techniques and area measurements
-methods for characterizing polluted soils using sampling and analysis techniques
-physico-chemical treatment techniques
-procedures for securing by isolation-containment
-stabilization-solidification techniques
-biological treatment techniques

4. BIOREMEDIATION

Bioremediation consists in decontaminating polluted soils by techniques derived from chemical degradation or other activities of living organisms.
In the past, organic residues have been stockpiled and humidified, thereby enabling biological decomposition, a process known as organic waste composting. The technology was extended to include the treatment of food waste, agricultural waste and wastewater. More recently, bioremediation has been applied to the treatment of hazardous waste, thereby enabling the remediation of polluted soils and wastewater.

a) Bioremediation techniques consist of monitored natural attenuation:
Principle
Passive tracking of lower levels of pollutants through natural processes:

- Degradation by microfauna
- Dilution
- Absorption
- Evaporation

Method
Leave nature to its own devices! Observation wells are used to keep track of and check developments.

b) Bioaugmentation
Principle

Bioaugmentation is a technique involving microorganisms (usually bacteria, nematodes, protozoa, fungi) in order to treat zones (soil and water) affected by various carbon, nitrogen or phosphorus-based pollutants.
Microorganisms already present in the soils or water are not able to clean up, making the use of external microorganisms necessary. It may be that genetic modifications are made to the inoculated strains in order to improve or enable clean-up. The main example of bioaugmentation use is the treatment of urban wastewater by activated sludges (wastewater treatment plants). The microorganisms in these sludges use the pollutants as sources of energy, along with oxygen, which is why it is necessary to aerate activated sludges to enable the microorganisms to survive and grow.
Method
Direct addition of microorganisms able to break down contaminants and speed up their destruction.

c) Biosparging and Bioventing
Venting is a procedure suited to permeable soils (sand) and mainly for the clean-up of volatile compounds. The soil is depressurized by suction, moist air from the soil is then sucked up and passed through a condensation chamber and then through a biofilter (bacterial support) or over activated charcoal where the pollutants are then degraded. The air is then checked and discharged into the atmosphere.
Bioventing is a variant of venting. In this process, in addition to conventional ventilation, forced ventilation of unsaturated soil is carried out; this circulation of air provides oxygen which promotes the development of microorganisms present in the soil and, thereby, degradation of pollutants. This procedure is mostly used for volatile compounds such as hydrocarbons, and the microorganisms then develop using carbon from those pollutants. This treatment is usually combined with soil amendment to rebalance the carbon-nitrogen-phosphorus ratio.
In addition, the air current sets the volatile compounds in motion and promotes volatilization of the liquid phase. The injected air flow is recovered by suction along the lines of venting. However, the injection and suction rate must be low enough to leave the microorganisms enough time to break down the volatilized compounds circulating in the air stream.
Moreover, the cost price of this technique is relatively low for effective results.

d) Use of bacteria and microorganisms
Bacteria that break down nitrates:
The bacterium *Pseudomonas halodenitrificans* is able to break down nitrates. The latter, which are used in agriculture as fertilizers, become a threat to water quality when released into the environment. The bacterium can breath using nitrates, which it converts by reduction into molecular nitrogen, an inert gaseous element, which returns to the atmosphere and no longer presents any danger of pollution. This ability to reduce nitrates into molecular nitrogen (denitrification) is widespread in bacteria. It is used in wastewater treatment plants to remove nitrogen from wastewater after nitrification of organic nitrogen and ammonia into nitrates.

Microorganisms against bad odours
They are able to eliminate simple gaseous effluents or volatile organic compounds (VOC): solvents, sulphur and nitrogen compounds, aldehydes, ketones, etc. whose main drawback is their unpleasant smell.
To be purified, polluted air passes through a trickling filter continuously fed by a bioreactor containing microorganisms specialized in breaking down these pollutants. Yield exceeds 99% for sulphur derivatives. For nitrogen derivatives, organic acids, aldehydes/ketones and other VOCs, efficiency exceeds 80%. Another advantage: totally organic, the technique produces very little residue.

This technique is intended primarily for agrifood industries, wastewater treatment plants and treatment centres for solid wastes.

Some examples

Some microorganisms capable of clean-up:

- Nitrates: *Comamonas, Hyphomicrobium*;
- Phosphates: *Acinetobacter, Moraxella*;
- Pesticides: *Enterobacter;*
- Dioxins: *Brevibacterium;*
- Cyanides: *Thiobacillus, Rhizoctonia;*
- Sulphur compounds: *Thiobacillus;*
- Rubber: *Sulfolobus, Rhodococcus, Thiobacillus;*
- Oils, fats: *Pseudomonas, Xanthomonas, Bacillus;*
- Hydrocarbons: *Acinetobacter, Flavobacterium, Bacillus, Pseudomonas, Achromobacter, Arthrobacter;*
- Heavy metals: *Saccharomyces, Rhizopus, Chlorella, Thiobacillus, Zoogloea*

e) Phytoremediation

The major principles of phytoremediation:

Phytoremediation is based on the use of plants to clean up a soil. In fact, some plants are able to bind pollutants in contaminated soils to their cells. They are able to accumulate and tolerate extremely high levels of metals, for example, in their tissues, shoots and leaves.

Principle

There are several strategies in phytoremediation:

e.1) Phytostabilization

Phytostabilization consists in halting pollution and is therefore a way of controlling pollutants. This involves establishing a plant cover with pollutant-tolerant species. The roots of the plant, combined with additives able to immobilize metals, precipitate, absorb or trap pollutants contained in the soil. The presence of such plants helps reduce erosion, and the runoff and deep penetration of pollutant-bearing particles. However, through this technique, metals are only trapped in the plant so, ultimately, the soil is not really cleaned up.

e.2) Phytoextraction

This is what hyper-accumulating plants do; they are able to accumulate over 1% of metals in their tissues. There are around 400 such species. This technique enables the use of plants to treat soils contaminated by metals in particular. Pollution by metals is one of the most difficult to treat as they are not biodegradable. The pollutants are taken up by the roots, but are conveyed to the shoots and leaves where they are accumulated. Consequently, the plants are mowed and stored in a place provided for the purpose, then incinerated. The ashes of the plants can then be used in the metal industry or recycled. There are two types of phytoextraction: continuous phytoextraction (which occurs naturally) and induced phytoextraction (which occurs in the presence of chelating agents). For this technique to be effective, the plants have to produce a great deal of biomass.

Examples:

- *Thlaspi caerulescens*, preferentially a zinc hyperaccumulator, but also lead and cadmium in the foliage;
- *Niemeyera acuminata*, nickel accumulator in the sap (over 20%);
- Zinc violet (*Viola calaminaria*), a plant growing on land rich in lead and zinc and which therefore stores up these metals).

e.3) Phytodegradation

Phytodegradation involves speeding up the degradation of organic pollutants using plants. It therefore involves organic compounds and hydrocarbons. Pollutants are converted into non-toxic substances by enzymatic reactions in the soil or in the plant.

Example: stimulation of microorganisms in the soil by the combined action of hydrocarbons and maize rhizosphere exudates.

e.4) Phytovolatilization

Phytovolatilization consists in cleaning up metals. Unlike phytoextraction, it only includes the clean-up of a few metals such as mercury, selenium and hypothetically arsenic. Metals are taken up by the roots and transferred to the shoots and leaves where they are stored pending conversion into volatile compounds for evapotranspiration by the plant into the atmosphere in methylated form. The released compounds are usually less toxic than the compounds taken up from the soil by the plant's roots. Decontamination occurs continually throughout the life of the plant. Soil pollution becomes air pollution as the pollutants are merely evaporated.

f) Conclusion

Phytoremediation can be seen as a way of treating polluted soils that is inexpensive and more respectful of the natural balance of a biotope. It can be used in place of physico-chemical techniques or in addition to them at sites with acute pollution. This use of plants reveals numerous beneficial effects, such as preventing erosion through the root system, decomposition of organic matter by the associated microorganisms, biogeochemical cycling of elements, restoration of the water cycle, but above all the concentration/degradation of pollutants. Phytoremediation paves the way for the colonization of polluted urban areas by plants, making the landscape more attractive and pleasant to live in.

Advantages and disadvantages

Techniques	Advantages	Disadvantages
Monitored natural attenuation	• No site disturbance • Inexpensive	• Not a remediation technique in itself • Often too slow to be acceptable to the administration • Regular and prolonged monitoring
Bioaugmentation	• *In situ* remediation of contaminants • Minimum impact on the ecosystem surface • Minimum waste production after treatment • Inexpensive compared to *ex situ* techniques	• Fairly slow process
Biosparging and Bioventing	• Aerobic/anaerobic • Organic contaminants • Limited costs • Effective against residual volatile pollutants and	• Not applicable to high concentrations of contaminants • Uniform and

	petroleum oils and lubricants	permeable lithology needed • Fairly slow • Less effective at low temperatures
Use of bacteria and microorganisms	• No soil excavation or transportation needed • Inexpensive • Natural process	• Treatment duration
Phytoremediation	• 100 to 10,000 times cheaper than traditional treatments • Plants can be easily monitored • The least destructive method • Recovery and re-use of valuable metals (companies specializing in “phytomining”)	• Slowness of this method • Extra cost of storing biomass containing hazardous materials. • Quite heavy installation

g) Fungi

Since 1985, numerous studies relate to bioremediation of soils by fungi especially by rot fungi are well known in the field of wood to release certain enzymes such as lignin and manganese peroxidases, dehalogenases, or cytochrome P450, which are capable of degrading dioxins (Harjanto *et al*. 2000, Sakaki and Munetsuna 2010).

Team Gold in 1992 offers a degradation of polychlorodibenzodioxine by the Basidiomycete *Phanerochaete* chrysporium. These authors were able to determine the degradation mechanism by the white rot fungus characterizing metabolites and oxidation products generated from lignin involving in particular manganese peroxidases.

Other work also focuses on this type of fungus (Hiratsukaa *et al*. 2005).

Fungi can also be used for biodegradation. There are two categories: lignolytic filamentous fungi (such as *Phanerochaete chrysosporium*), non-lignolytic fungi (*Aspergillus, Cunninghamella, Penicillium*, etc.) or yeasts (*Candida, Saccharomyces*, etc.).

Fungi can act in two different ways:

- Via mono-oxygenases which enable epoxide formation: (Wittich 1998);

PAH + ½ O_2 gives an arene oxide that undergoes numerous reactions up to energy production. This enzyme is a set of membrane-bound P450 cytochromes.

- Via lignolytic enzymes (e.g. *Phanerochaete chrysosporium* and *Pleurotus ostreatus*).

These are fungi which, in addition to mono-oxygenases, have the ability to synthesize extra-cellular enzymes such as lignin peroxidases (or ligninases) and manganese-dependent peroxidases.

An article wrote on the feasibility of bioremediation methods involving different white rot fungi (Pointing 2001).

Another review, from 2008 confirms that the degradation of chlorinated dioxins requires the use of white rot fungi, also known to be players in the degradation of lignin (Field and Sierra-Alvarez 2008).

Many other white rot fungi were studied:

- *Phlebia Lindtneri* (Mori and Kondo, 2002a, 2002b, Kamei and Kondo 2005) ;
- *Ceriporia* sp.MZ-340 (Suhara *et al*. 2003);
- *Phanerochaete sordida* YK-624 (Sato *et al.* 2003);
- *Bjerkandera* sp.strain BOS55 (Manji and Ishihara 2004);
- *Cordyceps sinensis* strain A (Nakayama *et al*.2005);
- *Coriolus hirsutus* (Orihara *et al.* 2005);
- Strains BMC 3014, BMC9152 and BMC9160 (Kamei *et al*. 2005b);
- *Phlebia radiata* (Kamei *et al*. 2005a)
- *Phlebia brevispora* (Kamei *et al*. 2009);
- *Pseudallescheria boydii* (Ishii *et al.* 2009);
- *Coprinellus* species (Suhara *et al.* 2011).

A study of remediation for benzofurans was made from another type of fungus: *Paecilomyces lilacinus* (Gesell et al. 2004).

It may be noted that fungi are also used for bio-remediation salts of chromium, copper and arsenic (Sierra-Alvarez 2007, 2009). In this case it is brown rot fungi.

In addition, the technique of landfarming (application controlled) has been studied at pilot scale for the remediation of contaminants from treatment for wood preservation (including creosote) (Hansen *et al*. 2004) and recent work has focused on bio-detoxification of effluents containing phenols produced by a wood processing plant by another white rot fungus *Lentinula edodes* UEC 2019 (Barreto-Rodrigues *et al*. 2009).

5. AN OUTCOME THAT COMPLIES LITTLE WITH THE “BIOCIDAL PRODUCTS” DIRECTIVE

For the sake of simplicity today, it can be said that there are two major types of formulations frequently used in the construction field to afford the necessary durability to timber species, namely:

- Products based on organic biocide molecules (Pyrethroids, Triazoles, etc.).

These biocides, which are frequently used in the agricultural and/or pharmaceutical fields, are applied in very small quantities and are usually in the form of colourless products. They usually display a low tendency to evaporate (low vapour pressure) and low solubility in water, hence less risk of leaching. These treatments are usually applied on wood by surface treatment procedures such as daubing, soaking and spraying.

- Copper-based products: These products are primarily intended for the treatment of woods in use category 4. These formulations give a green to brown colouring to treated timbers. The copper contents in wood preservation products have been very precisely defined, to obtain the best effectiveness/use ratio.

An overview of the characteristics of the chemical products described above and used as biocides shows that their main drawback is their toxicity.
Creosotes are carcinogenic and mutagenic, and the metals present in CCAs contaminate soils and water tables.

Consequently, the majority of wood preservation products currently used, such as CCAs, PCP (extremely phytotoxic) and creosotes barely comply with the *"Biocidal Products"* directive, if at all, and would not currently obtain permission to be released onto the market.
It is therefore essential, in the future, to promote the use of new products that are just as effective but less harmful. Some of the treatments proposed in the literature in recent years tend to meet that requirement.

6. ALTERNATIVE WOOD PRESERVATION TREATMENTS

Some lines of research towards low-pollution or pollution-free products

A novel technique from the textile and plastic sectors has been adapted to wood treatment: hydrophobing with cold plasma, where a Teflon or silicone microfilm is deposited on the surface. Under the action of the plasma, fluorinated or siliconized monomers polymerize, making the wood impermeable to water.

Another process consists in combining boron with egg white. Thus, the combination of boric acid with the proteins seems to delay its leaching. Biological tests on wood treated with this mixture show identical results to those obtained with CCA treatments.

A third way consists in proposing new active molecules seeking to mimic natural biological protection phenomena. It is thus that, after, isolating, purifying and identifying the molecules responsible for the durability of exotic woods, it has been possible to chemically synthesize them. Some new families of molecules are being used: derivatives of methoxyaminophenylacetamide such as thiazole, copper oxinate combined with the phosphite ion, and benzimidazole emulsions. Others are currenly being assessed: these are copper octanoate, proteins combined with boric acid or diazene, and also so-called “natural” biocides such as the tropolones, resin compounds, terpenoids and stilbenes are also being studied.

Genetic modification of some fragile species is based on the same principle. It would enable woody plants themselves to produce molecules capable of protecting against biological degradation. These tests are very laborious to conduct due to the time it takes for a tree to grow. The quantity needed, and the effect and toxicity of these molecules in relation to the environment are not yet known.

The biological pathway mainly concerns protecting wood from fungi. It consists in inoculating a bacterium or another non-pathogenic fungus. These biological control agents can then act:

- either by competing for nutrients and preventing fungi responsible for wood decay,
- or by inhibiting the growth of fungi, notably those responsible for blue stain,
- or by producing antibiotics.

Impregnation with polymers: this technique is used to improve wood properties against living organisms, chemical compounds, and for better dimensional stability. The monomers used include styrene, vinyl acetate, acrylonitrile, and methyl- or butyl methacrylate, etc.

Retification

Retification consists of controlled pyrolysis of wood at a high temperature (270°C) and in an inert atmosphere in a specialized kiln. Several are in operation at the moment, mostly to treat cluster pine and Douglas fir.

Despite some modifications to the wood due to the high treatment temperature, the wood microstructure is apparently conserved, though the changes do give it a hydrophobic nature.

On non-durable or low-durability species such as the cluster pine, poplar, fir and spruce, retification would apparently lead to excellent dimensional stability and house longhorn beetle resistance. Retified wood is also more resistant than natural wood to the fungi responsible for white, brown and soft rots. Its durability after treatment is apparently between categories 2 and 3. Moreover, a conventional finish can also be applied due to good adherence and excellent stability of paints and adhesives. Retification is therefore recommended for weather-boarding.

However, several reservations are expressed as regards its industrial development. Indeed, mechanical properties are greatly decreased. A 45% loss in tensile strength and a 40% loss in impact bending strength has been found. In addition, it would seem not to be very reproducible, releases carcinogens, and requires the use of wood without defects or knots or otherwise an end-product with cracks and splits is obtained. This restriction therefore means working with expensive wood at a high cost for the end-product. This technique is also criticized for the dark colour and characteristic odour given to the wood.

Chemical wood grafting

Grafting is a chemical modification of wood that is primarily intended to reduce its hydrophilous nature that is partly responsible for biological attacks and dimensional instability. The reactions bring into play the cellulose, hemicellulose and lignin hydroxyl groups. It has been found that most of the work is geared towards better dimensional stabilization of the material. Biological resistance or mechanical properties have been less studied. All the treatments proposed use toxic reagents or solvents that are difficult to eliminate. They also cause a reduction in the mechanical properties of wood.

The treatment gives good resistance to brown and white rots, termites, marine molluscs, though without achieving the effectiveness of CCAs and creosotes. However, it does not protect wood from lower fungi such as moulds. In addition, the formation of acetic acid during synthesis gives wood an unpleasant vinegary smell and reduces certain mechanical properties in treated wood. Its use in wood protection does not seem very likely due to the implementation difficulties.

7. CONCLUSION

Fungicide and biocide treatments

As already explained, wood is a material that can be highly susceptible to its environment. Its natural hardness therefore needs to be enhanced by chemical or natural processes. Up to the 1980s, first generation biocides were used. This class of products includes arsenic-, copper-, boron- or fluor-based metal salts, along with synthetic organic biocides (pyrethrinoids, azoles, the imidazolium family, etc.) and polychlorophenols (PCP) formulated in petroleum solvents. Different wood impregnation procedures exist, including autoclaving for good penetration of the product. The oldest methods include immersion, daubing or spraying. Consequently, first generation biocide products are easily washed away into the environment, polluting soils and run-off water.

In fact, today, third generation biocides are used, such as the Propiconazole/Tebuconazole combination, or other different combinations of fungicides depending on the uses. There thus arises the question of recycling wood treated with 1st and 2nd generation products, and with particular acuteness given the changes in regulations that now compel network owners to manage poles that have reached the end of their useful life. Such poles are likely to pollute soils when stored pending their recycling and destruction. In France, they are incinerated in cement works to fire the kilns. In the case of CCA, they may also be distilled into charcoal by a specific process known as CHARTHERM™. In fact, unlike combustion, this new process does not give off smoke, mineral elements or heavy metals. Moreover, this process makes it possible to re-use treated woods.

Using fungi as biodegradation and bioremediation agents for easier processing of such woods is an alternative that has yet to be widely assessed.

Bioremediation microorganisms

Among the eukaryotic microorganisms living in forest soils, fungi, and more particularly the Basidiomycetes, are in the majority. They are in the majority both in terms of their presence and in their high activity detected by the presence of their RNAs. They play an essential role in the life and fertility of soils, as they are primary decomposers of organic matter. Certain human pressures durably disrupt soil functioning (e.g. the dumping of biocides). Nevertheless, some Basidiomycetes are able to develop under polluted conditions and help to depollute the soil through bioremediation.

Bioremediation experiments

In virtually all the studies on bioremediation and its ecological aspects, soil has only been considered as a sterilized medium prior to being seeded with the bioremediation agent, selected empirically (e.g. *Trametes versicolor, Phaerochaete chrysosporium*). The soil was thus prepared externally for seeding, leading to the loss of almost 83% of fungal biomass.

It should thus be noted that little work has been undertaken *in situ*, as that calls for preliminary studies that are sometimes too expensive and restrictive. Most of these authors have therefore not studied the fungal species present but rather the ability of a precise species to resist the physic-chemical constraints being tested. It has thus been shown that simple addition of substrates sometimes gives equally good results as treatment with Basidiomycetes.

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