Mycorrhizal mushroom biodiversity in PAH-polluted areas

Case Somerharju, Finland

Marina Yemelyanova

Degree Thesis for Bachelor of Natural Resources

The Degree Programme in Sustainable Coastal Management

Raseborg 2016

BACHELOR'S THESIS

Author: Marina Yemelyanova
Degree program: Sustainable Coastal Management
Supervisors: Patrik Byholm, Lu-Min Vaario

Title: Mycorrhizal mushroom biodiversity in PAH-polluted areas: case Somerharju, Finland

---

Date: May 4, 2016 Number of pages: 40 Appendices: 9

Summary

Presence of mycorrhizal fungi affects the phytoremediation efficiency on PAH-polluted soils: fungi participate in the soil bioremediation *per se* and, as symbionts with living trees, help the trees to survive under the pollution and other harsh environmental conditions.

This thesis initiates a study of a mycorrhizal mushroom community on the Somerharju phytoremediation project site in Finland. An inventory of the mycorrhizal mushrooms concerning mushroom abundance, species richness and biodiversity was done during the season of 2015. The distribution of the mushrooms over the site was analyzed in relation to PAH-pollution levels and tree cuttings on the site.

A significant dependence of fungal species richness and biodiversity on a cutting type was detected. The dependence of mushroom abundance, species richness and biodiversity on a PAH-pollution level was not statistically significant. Tolerance of Somerharju's mycorrhizal mushroom community to PAHs allows expecting even emergence of the mushrooms over the site in future and opens perspectives for an intentional inoculation of such mushrooms in the areas with suitable tree coverage on the entire site for enhancing the bioremediation effect.

Language: English Key words: abundance, biodiversity, bioremediation, fungi, mushrooms, mycoremediation, mycorrhiza, mycorrhizal fungi, PAH, polycyclic aromatic hydrocarbons, phytoremediation, species richness

---

Acknowledgements

First of all, I am grateful to Dr. Pertti Pulkkinen for the opportunity to join the Somerharju research team and to apply my interest in fungi within such an exciting project, and for our discussions that helped me to find a direction for my thesis.

I also thank both my supervisors: Dr. Lu-Min Vaario, for her valuable scientific advice and patience in working with me in spite of complicated circumstances in my life and thousands kilometers between us, and Dr. Patrik Byholm, for new knowledge I gained in our meetings and right words inspiring me any time when the work went well.

I thank Raimo Jaatinen very much for his super attentive attitude to all interns, high-class guidance on the sampling site, and providing visual materials on Somerharju that I used in this paper.

My special thanks go to the entire team of Luke's Haapastensyrjä unit – all people who let me feel like home during my work at Luke, those employees and trainees especially who regularly organized my research travels, drove me to Somerharju or helped in fungal sampling there: Jyrki Airaksinen, Julia Auzeral, Camille D'Hervilly, Marta Cortina Escribano, Clotilde Hubert, Raimo Jaatinen, Aulis Leppänen, Pertti Pulkkinen, Veli Suihkonen, Satu Teivonen, Maria Viirros, Raija Viirros, Ruhui Weng, and Meng Xue.

I am grateful to Zinaida Kosinska, Yegor Mukin, Salavat Arslanov and the other active members of Russian professional and amateur mycological communities for our fruitful online discussions on the species identification.

And, finally, I thank warmly my close friend Dr. Aleksandr Lenshin for his daily support, valuable writing advice and scientific life hacks he shared with me: he helped me to walk this uneasy way smiling even in the harshest time.

Acronyms and abbreviations

BTEX	– benzene, toluene, ethyl benzene, and xylene
CHC	– chlorinated hydrocarbon
EC	– European Commission
EEA	– European Environment Agency
EPA	– Environmental Protection Agency
EU	– European Union
Luke	– Luonnonvarakeskus (in Finnish), Natural Resources Institute Finland
Metla	– Metsäntutkimuslaitos (in Finnish), Finnish Forest Research Institute
MiCC	– Microfungi Collections Consortium
MTBE	– methyl tertiary butyl ether
NSO	– hydrocarbons containing nitrogen, sulfur, or oxygen
PAH	– polycyclic aromatic hydrocarbon
PCB	– polychlorinated biphenyl
PHCs	– polyhalogenated compounds
PIMA	– pilaantuneiden maa-alueiden arviointi (in Finnish), assessment of contaminated lands
sp.	– species (in singular)
spp.	– species (in plural)
std.	– standard
TAME	– tertiary amyl methyl ether
TCE	– trichloroethylene
TNT	– trinitrotoluene
VOC	– volatile organic compounds

Table of contents

List of appendices

1 Introduction

1.1 Thesis in the project context

This thesis on mycorrhizal mushroom ecology in the PAH-polluted areas is a part of a phytoremediation research project being implemented in Somerharju, Finland, by Luke (Natural Resources Institute Finland) since 2013. The long-term purpose of the project is to determine the efficiency of plantations of aspens *Populus tremula* and hybrid aspens *Populus tremula* x *Populus tremuloides* for purifying the soil polluted with PAHs (creosote). Simultaneously, the researches on the aspen adaptation and survival under local conditions are being conducted. This thesis on the mycorrhizal mushrooms initiates a side investigation within the project, aimed at providing data that are perspective in a context of both long-term and short-term purposes of the main study.

1.2 Research significance

This thesis is the first investigation on the mycorrhizal mushroom occurrence in Somerharju, and, thus, explores a new issue within the project. Due to mycorrhizal ability to affect both phytoremediation efficiency (Šašek, 2003; Joner, Leyval & Colpaert, 2006; Norton, 2012; Chibuike, 2013; Winquist *et.al*, 2014) and tree survival (Marschner & Dell, 1994; Clark & Zeto, 2000; Dell, 2002; Bandou *et.al*, 2006; Finlay, 2008; Brundrett, 2009; Pringle, Wolfe & Vellinga, 2011) which corresponds to the Somerharju phytoremediation project scope, results of this side study may complement the main research with the valuable data: it may help to consider fungal occurrence as an extra ecological factor in a complex analysis of the phytoremediation and the aspen adaptation in Somerharju.

1.3 Object of research and subject of inquiry

Within this thesis, mycorrhizal mushrooms – fruit bodies of higher fungi with the proven ability to form symbiotic relationships with living trees – occurring in the Somerharju phytoremediation project area are an object of research.

As this thesis initiates the study on the mycorrhizal mushroom community on the Somerharju project site, carrying out a mushroom inventory in order to understand the mushroom distribution over the site was considered as a priority goal of the side project. To fulfil that, the following issues were chosen as a subject of inquiry:

- mycorrhizal mushroom species present on the site over a season;
- mushroom distribution in areas with different PAH-pollution levels;
- mushroom distribution in clear cut and non-cut areas.

1.4 Research questions and tasks

The following research questions were investigated in this thesis:

1. What mycorrhizal mushroom species are present in the Somerharju phytoremediation project area during a mushroom season?

2. Do mycorrhizal mushroom abundance, species richness and biodiversity depend on different PAH-pollution levels on the site?

3. Can clear cutting be an extra factor affecting the mycorrhizal mushroom distribution in the polluted areas?

The following tasks were put and implemented to answer the above listed questions:

- regular monitoring the mushrooms on the site;
- identifying mushroom species;
- calculating mushroom abundance, species richness and biodiversity;
- conducting a statistical analysis considering a PAH-pollution level and a cutting type as fixed factors.

1.5 Previous research

Although the idea of using fungi in remediation of the PAH-polluted soils is being actively discussed (Šašek, 2003; Joner, Leyval & Colpaert, 2006; Norton, 2012; Chibuike, 2013; Winquist *et.al*, 2014), the research questions put in this thesis are quite new: most current researches in this field are carried out *in vitro* and usually deal with separate microfungal species (Leyval, Joner, de Val & Haselwandter, 2002; Khade & Adholeya, 2007; Korade & Fulekar, 2009; Wu, Yu, Wu & Wong, 2014; Lu & Lu, 2015).

The literature on fungal communities in the PAH-polluted areas *in situ* is very scarce. Generally, researches in the issue are also done on the microfungal communities participating in the soil and sediment remediation (Roberts, 2006; Mukherjee, 2014; Ling *et.al*, 2015). As for studies on mushroom producing fungi, a paper on fungal diversity in creosote-treated wastes and resistance to PAHs (Kim *et.al*, 2010) was detected as the only one done *in situ* on the community level, but it is focused on non-mycorrhizal fungi. Scientific papers on the mycorrhizal mushroom occurrence in the PAH-polluted areas were not found.

However, many other aspects of the soil remediation with use of plants and fungi have been studied by today. Serving as a theoretical base of this thesis, they will be described in chapters 2 and 3.

2 Phytoremediation in PAH-polluted areas

2.1 Phytoremediation definition and applications

Phytoremediation (a term coming from Ancient Greek *φυτο* - "plant", and Latin *remedium* - "restoring balance") can be defined as use of plants for purification of contaminated sites based on the ability of plants to metabolize elements and compounds accumulated from the environment. In this thesis, the term "phytoremediation" will refer only to the soil restoration although the method can also be applied to air (Morikawa & Ozgür, 2003), water (Sytar *et.al*, 2016, 361), and sediments (Vervaeke, 2003; Pilipovic, 2015).

Phytoremediation was proved to be an effective way to reduce concentrations of heavy metals, hydrocarbons (in particular, petroleum hydrocarbons) and polychlorobiphenyls in the soil (Macci *et.al*, 2016, 378). There are also practices of phytoremediation for removal of radionuclides (Dushenkov, 2003), explosives (Hanninka, Rossera & Brucea, 2002) and pesticides (Susarla, Medina & McCutcheon, 2002).

2.2 Plant species selection for phytoremediation

Plant species in use for the soil phytoremediation vary a lot depending on project purposes and duration, on pollutant type, microclimate in the contaminated area, physical and chemical properties of the soil, and financing available.

Heavy metals are best removed with hyperaccumulator species – those able to absorb and to store extremely high concentrations of the pollutant in their tissues. In this list, there are numerous *Asteraceae*, *Brassicaceae*, *Caryophyllaceae*, *Cunouniaceae*, *Cyperaceae*, *Euphobiaceae*, *Fabaceae*, *Flacourtiaceae*, *Lamiaceae*, *Poaceae*, and *Violaceae* plant species (Sarma, 2011, 122). Cook and Hesterberg (2013), in their review of species potential for reduction of organic pollutants, mention *Poaceae* and *Fabaceae* grasses and crops, and tree species (e.g. birch *Betula pendula*, red mulberry *Morus rubra*, aspen/poplar *Populus spp.*) as good for PAH reduction, with grasses tall fescue *Festuca arundinacea*, perennial rye-grass *Lolium perenne*, and trees willow *Salix spp.* and aspen / poplar *Populus spp.* as species that are in most common use for that.

Aspens *Populus tremula* and *Populus tremuloides* that are in a focus of this study perfectly match many plant selection criteria for phytoremediation because of their large biomass and absorption surface, deep roots and high water uptake rate, fast growth and easy propagation, ability to adapt to various soils and temperature and moisture conditions and tolerance to pollutants (Mukherjee, 2014, 5).

2.3 Phytoremediation strategies for removing PAHs

Strategies for soil phytoremediation have been summarized by Mukherjee (2014, 3) who grouped them by a mechanism of pollutant reduction. She lists the following phytoremediation strategies:

- phytovolatilization when plants absorb the pollutant from the soil, transform it into a volatile compound and release it into the atmosphere;
- phytoaccumulation, or phytoextraction when plants accumulate pollutants in their harvestable parts;
- phytotransformation, or phytodegradation when plant enzymes and/or plant-associated microbes degrade organic pollutants;
- rhizoremediation when microbes living in the rhizosphere degrade the pollutants.

In the other reviews, some of these strategies are named differently, *e.g.* rhizoremediation is referred to as rhizodegradation or phytostimulation (Etim, 2012, 134), and phytotransformation is to as phytostabilization (Sarma, 2011, 121).

As for the phytoremediation strategies that *Populus spp.* use for reduction of organic pollutants including PAHs, Mukherjee's review of the scientific literature on the topic includes a number of papers demonstrating the aspens ability for phytovolatilization, phytodegradation and rhizoremediation (Mukherjee, 2014, 5).

At that, Mukherjee (2014, 3) underlines that these mechanisms may function simultaneously. So, one can see that, in some cases, phytoremediation mechanisms imply a participation of plant associated organisms (microbes and fungi) that can either be crucial for a phytoremediation process or enhance it in a combination with the other strategies. This statement is important for the current study because the combination of the aspens used for phytoremediation with symbiotic mycorrhizal fungi may have a similar enhancing effect as the aspen combination with microbial fungi has.

2.4 Phytoremediation advantages and constraints

Phytoremediation can be considered as a very promising method of the soil purification due to its various advantages: ease to implement and to maintain (Gerhardt, Huang, Glick & Greenberg, 2009, 24), realization directly *in situ*, without the soil excavation and transferring to the other place, environmental safety with no need to use chemicals (Ndimele, 2010, 716), comparatively lower costs (Sarma, 2011, 121), improving soil quality by adding organics into it (Gerhardt *et.al*, 2009, 24), reduction of the soil erosion and increasing a biomass, creating an aesthetical environment (Cook & Hesterberg, 2013, 845), and high public acceptance (Macek, Mackova & Kas, 2000, 24).

However, the method has some disadvantages and constraints. Phytoremediation is a slow process requiring up to several years to be completed (Ndimele, 2010, 716; Sarma, 2011, 121) and it is not applicable for cleaning up the soil in a short period. The other constraint may be too high toxicity of the pollutants which will be able to kill the plants meant for phytoremediation (Ndimele, 2010, 716). So, a plant survival rate may become an acute problem and should be investigated for certain phytoremediation projects. In case of dealing with the pollutants that are accumulated but not transformed, plants with high concentrations of the pollutants in their tissues should be harvested and destroyed which increases costs; otherwise, the plants full of toxins may be used as food by animals and spread, thus, further through the food web (Macek, Mackova & Kas, 2000, 31).

Furthermore, phytoremediation implies a degree of uncertainty: an uneven distribution of the pollutant in the soil may cause difficulties in planting design; the roots may grow too short and unable to reach the pollutants if they are distributed lower than the rhizosphere is

(Gerhardt *et.al*, 2009, 25); a possibility of the pollutants dissolution may cause their migrating (Macek, Mackova & Kas, 2000, 24); finally, the phytoremediation efficiency depends a lot both on abiotic and biotic factors – Mukherjee (2014, 2) marks that the remediation success is affected by temperature, pH, oxygen, moisture, nutrient availability, toxicity and bioavailability of contaminants. So, an entire complex of factors makes phytoremediation very site-dependent, and a specific phytoremediation project should be based on a solid research of a site.

2.5 Phytoremediation practice and perspectives for removing PAHs in Finland

Organic contaminants (a group including PCBs, PAHs, TCE, TNT, MTBE, pesticides, petroleum hydrocarbons and others) in the soil are an actual issue in modern Europe. About 1.170.000 potentially contaminated sites have been detected in 33 European countries by 2013 (Panagos, Van Liedekerke, Yigini & Montanarella, 2011). Large shares of all soil contaminants are organic (figure 1).

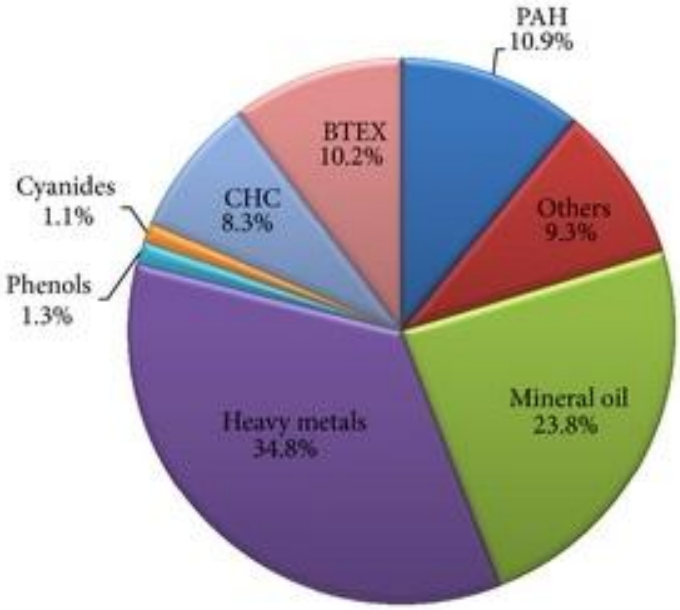


Figure 1. Distribution of contaminants affecting soil in Europe (Panagos et.al, 2013).

In Finland, 23850 soil-contaminated sites and ones suspected in contamination were detected in 2013 (Pyy, Haavisto, Niskala & Silvola, 2013, 49) which include 70%, 10-20% and 20-10% of a total soil volume contaminated with organic compounds, metals and a mixture of metals and organics, respectively (Pyy *et.al*, 2013, 50). Some of these sites have already been remediated, the outcomes of the other ones are still to be decided on. Based on former decisions on remediation, one can conclude that, in Finland, a number of positive decisions on a remediation implementation is proportional to volumes of contaminants of different types, and the largest share falls at the sites polluted with organic contaminants including PAHs (figure 2).

At that, remediation in Finland is usually implemented *ex situ* (EEA, 2014) (figure 3). A share of *in situ* bioremediation techniques is minimal, with no phytoremediation applications: the first big Finnish phytoremediation project was Somerharju one (Pertti Pulkkinen, personal communication, April 2015) which started in 2013 and which is in the

focus of this thesis. Several experiments have been carried out before that (Sillanpää, 2007; Vallinkoski, Hassinen & Servomaa, 2007) but their real results were rather limited because of a too short time span.

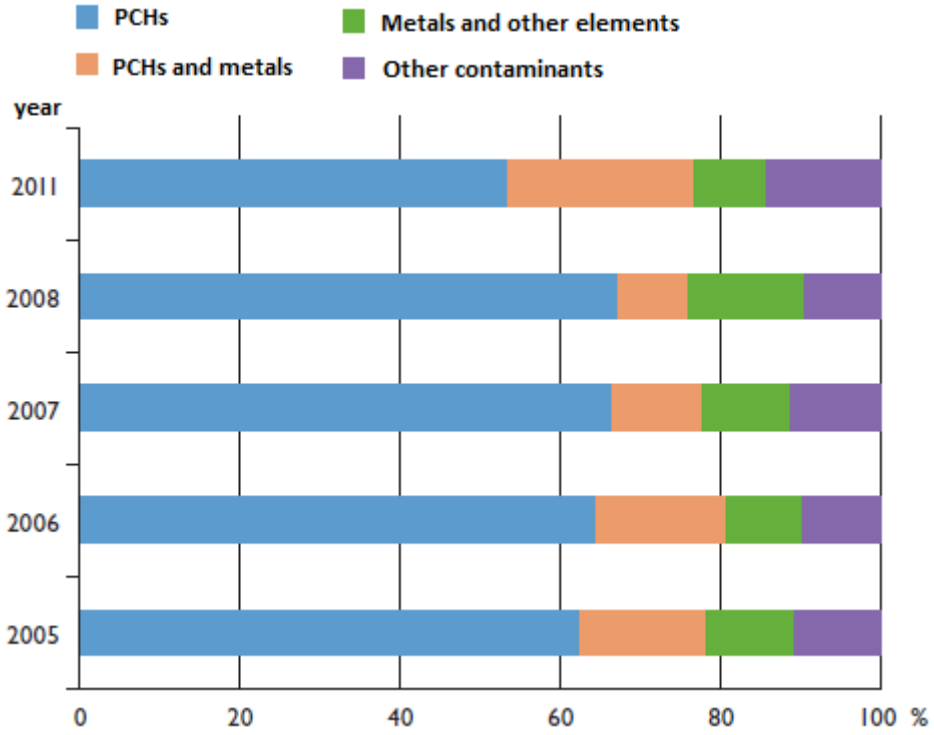


Figure 2. Decisions on remediation of contaminated sites in Finland grouped by a pollutant type: PCHs include petroleum fractions, PAHs, BTEX, petroleum additives MTBE and TAME, and VOC (Adapted from: Pyy et.al, 2013, 32).

In 2012, costs of 370 remediation projects implemented in Finland were approximately 68 million euros and the costs for a rehabilitation of the contaminated sites left are expected to increase in future (Pyy *et.al*, 2013, 51). Considering high remediation costs and a large number of contaminated sites, it looks predictable that prioritizing is and will remain an important issue for the Finnish environmental authorities responsible for choice of the sites to remediate. It looks like that, over years, one of those issues affecting the decisions of the authorities was mitigating health risks for people living in and by contaminated sites: in the period of 1986-2012, about 60% of decisions on remediation of a certain site were carried out in residential areas or in their immediate vicinity, and 40% - in classified groundwater areas (Pyy *et.al*, 2013, 49-50).

To summarize the above stated, one can conclude that phytoremediation is considered to be a new soil rehabilitation method in Finland which requires thorough investigations to outline its perspectives in the country more clearly. However, already now one can presume that taking into account abundance of PAH-polluted grounds in Finland, if the results of current and prospective phytoremediation projects turn out satisfactory phytoremediation may become a suitable comparatively cheap rehabilitation strategy for remote places, out of immediate vicinity to residential sites where the urgent soil purification is not crucial.

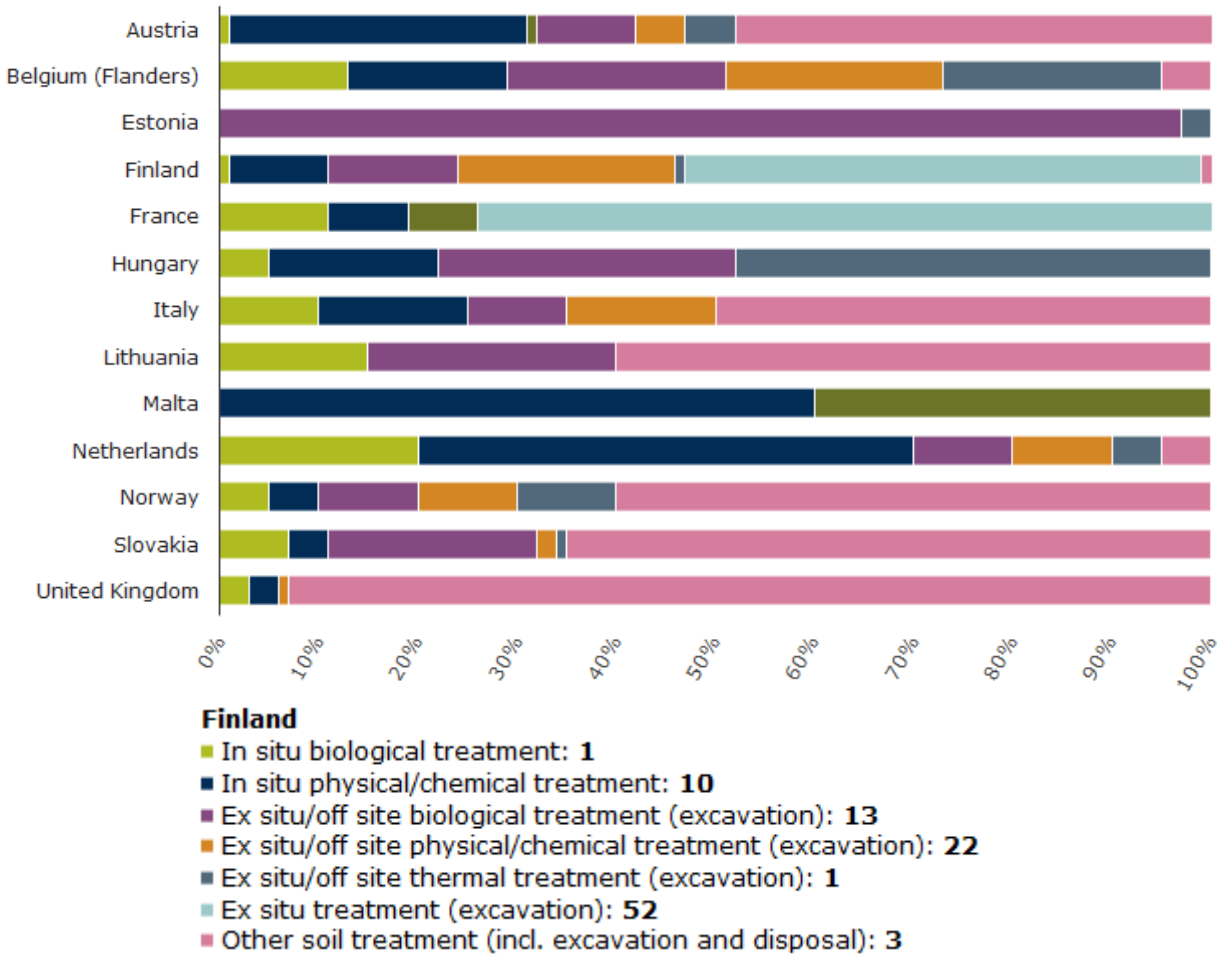


Figure 3. Most frequently applied remediation techniques for contaminated soil in Europe in 2011, below the data for Finland, % (EEA, 2014)

3 Macrofungi in remediation of polluted soils

3.1 Fungal classifications

Fungi are an extremely diverted kingdom of organisms including, according to Blackwell (2011), more than 5 million species of mushrooms, yeasts, rusts, mildews, and molds. Due to such diversity, fungal properties, roles in the ecosystems, and possible applications (including ones in the soil remediation) vary a lot depending on certain fungal groups or species. That is why, before talking about the soil remediation with fungal participation, it is important to put a focus on target fungal groups and, thus, to classify fungi in an appropriate way.

Fungi may be classified in many ways, for example, by their phylogeny, morphological features (characteristics of spores, a hyphal texture, *etc*), reproduction type, origin of spores, trophic guild, functions in the ecosystem, utility for a human, pathogenicity, or even by size. Choice of a classification to use depends on the purposes of every certain case.

In order to put a focus on certain groups of fungi in this study, first, it is important to outline the entire fungal kingdom. For investigating mycorrhizal mushrooms within this thesis, the classifying principles will be considered as follows:

- by phylogeny to provide a framework for the other classifications;
- by a trophic guild to distinguish mycorrhizal and non-mycorrhizal fungi;
- by presence of highly developed fruit bodies to distinguish micro- and macrofungi.

3.1.1 Phylogenetic classification of fungi

According to modern phylogenetic systematics, fungi are divided into 5 phyla: *Basidiomycota*, *Ascomycota*, *Glomeromycota*, *Zygomycota*, and *Chytridiomycota* (Bear *et.al*, 2016, 398) (figure 4).

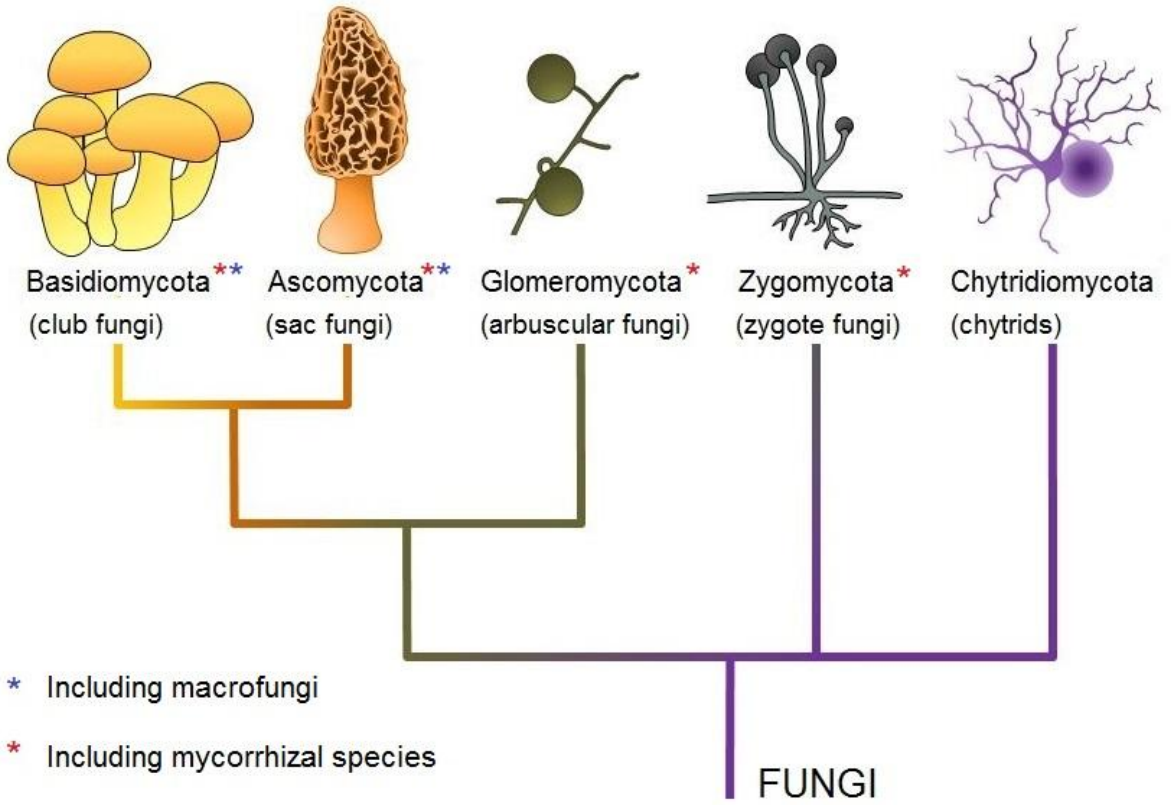


Figure 4. Phylogenetic relations of fungal phyla (Adapted from: Bear et.al, 2016).

Phylum *Basidiomycota* is, first of all, fungi that produce mushrooms – basically club-shaped fruit bodies which, however, can be of the other shapes, for example, spherical as puffballs *Calvatia spp.* and *Lycoperdon spp.*, bracket-shaped as *Polyporus spp.*, star-shaped as earth stars of *Geastraceae*, or shapeless as jelly fungi of *Auriculariales*, *Sebacinales* and the other orders. The mushrooms are easy to see with an unaided eye: their height and width vary from several millimeters to tens of centimeters. However, a large group of *Basidiomycota* is microscopic fungi: some rusts, smuts and bunts, and some yeasts. *Basidiomycota* fungi produce and keep their spores in basidia – microscopic pedestal-like or club-like structures placed on the mushroom gills or inside of the fungal body.

Phylum *Ascomycota* includes fungi that produce their spores in asci – sac-like cells. Some mushrooms belong to *Ascomycota*, for example, morels *Morchella spp.*, false morels *Gyromitra spp.*, cup fungi *Pezizaceae*, truffles *Tuber spp.*, deadman's fingers *Xylaria spp.*. Microscopic *Ascomycota* fungi are some yeasts, mildews, molds, and fungi living in lichens as symbionts with algae.

Fungi representing three other phyla and their relation to *Basidiomycota* and *Ascomycota* can be considered in the context of the fungal division into macrofungi and microfungi.

3.1.2 Division into macrofungi and microfungi

Only *Basidiomycota* and *Ascomycota* (marked with a blue asterisk on figure 4) include both microscopic fungi and mushroom producing ones. The latter are also called higher fungi, or macrofungi. Distinctive features of the mushrooms were described in section 3.1.1.

Among three other fungal phyla: *Glomeromycota*, *Zygomycota*, and *Chytridiomycota*, there are only lower fungi, or microfungi, such as molds, rusts and mildews that are, according to a definition of Microfungi Collections Consortium, "difficult or impossible to see with the unaided eye" (MiCC, 2016).

Although this division into macro- and microfungi (or into higher and lower fungi) is artificial, it may be functional in some everyday or research situations. Particularly, based on this division, one can conclude that *Glomeromycota*, *Zygomycota*, and *Chytridiomycota* should be left out from the current research focusing on the mushrooms only.

3.1.3 Classification by trophic guild

Fungi get nutrients from different sources. This difference served as a base for division of the fungi into 3 large ecological groups, or trophic guilds: saprobic, parasitic, and mycorrhizal fungi. Classifying the fungi by the trophic guilds can be very perspective for ecological investigations including the current one: differentiating the nutrient sources for the fungi, the trophic guilds also demarcate fungal interactions with the other species and fungal roles in the ecosystem.

Saprobic fungi, also called, according to Kuo (2006), sabprobic, saprotrophic, saprophytic fungi, or just saprobes, get nutrients by decomposing dead or decaying organic material: wood, dead plants and animals, and excrements. It is a very diverse guild including macro- and microscopic species of all 5 fungal phyla. Saprobic fungi are essential decomposers in any ecosystems: they are found even in Arctic and Antarctic zones (Robinson, 2001; Hassett & Gradinger, 2016). As saprobic fungi are able to decompose any dead matter they interact with a wide range of the other species. However, they stay specialized; certain fungal species decompose certain matter or certain compounds only. For example, brown rot fungi are good in digesting cellulose, hemicellulose, animal hair, claws and horns but they are ineffective for recycling lignin and waxes which can be decomposed by white rot fungi (Scrimshaw, 2016). In the process of decomposition fungi change the soil chemistry: they convert complex molecules of the organic matter into simple inorganic compounds

and as a result, participate in the soil mineralization providing nitrogen, phosphorus, potassium and the other elements that plants use for their growth (McConnell, 2016, 206).

Parasitic fungi, or parasites, are micro- and macrofungi that survive by consuming nutrients from the other living organisms which are disadvantaged or killed over time. All fungal phyla, excluding *Glomeromycota*, include parasitic species. These fungi are pathogens that infect plants, animals, humans, or the other fungal species. As any other parasites, parasitic fungi play a large part in the ecosystem functioning – they "shape community structure through effects on trophic interactions, food webs, competition, biodiversity, and keystone species" (Preston & Johnson, 2010, 47).

Mycorrhizal fungi that are in a focus of this study live in a symbiosis with a plant when a fungal mycelium penetrates into or between the root cells of the plant. This joint structure, called a mycorrhiza (term coming from Greek μυκός – "fungus" and ριζα – "roots"), makes a nutrient exchange between two symbionts possible. Although different kinds of plants: trees, shrubs, herbs and vegetables – are able to establish mycorrhizal associations (Bonfante & Anca, 2009, 364), in this thesis, a focus will be put on the mycorrhizal fungi interacting with the trees. In the scientific literature, the most commonly mentioned benefits that a tree and a mycorrhizal fungus get as a result of their symbiosis are nutrients that the parties cannot obtain independently: the fungus acquires carbon compounds – products of the tree photosynthesis, while the tree receives minerals, particularly phosphorus and nitrogen (Marschner & Dell, 1994; Clark & Zeto, 2000; Finlay, 2008, 1117; Brundrett, 2009, 172; Pringle, Wolfe & Vellinga, 2011, 468).

However, a range of the tree-fungus symbiotic benefits is not limited with nutrition issues only. There are the other mycorrhizal properties that help the trees survive as well: the ability to improve the tree resistance to drought through increasing water flow by bridging the gap between the soil and the root (Dell, 2002, Finlay, 2008); anti-pathogenicity providing some protection against root invading primary pathogens when fungal hyphae twined round the root act as a physical barrier to pathogens, or when fungi produce antibiotic or antifungal compounds (Dell, 2002); and the ability for amelioration of adverse soil conditions (Dell, 2002; Finlay, 2008).

The amelioration can occur in several ways that have been summarized by Dell (2002). Enhancing the tree resistance to excessive heavy metals in the soil is possible due to more intensive metal immobilization in fungi than in the tree shoot. Unfavourable pH conditions may be handled by the tree with the mycorrhiza due to fungal access to phosphorus, zinc and other limiting nutrients in the alkaline soil, capacity of some fungi to produce oxalic acids dissolving soluble calcium phosphates, or their ability to release siderophores – iron binding secrets for a better iron uptake. There are also data that some macrofungi can alleviate salt stress in trees (Bandou *et.al*, 2006).

An important peculiarity of mycorrhizal relations is a host-fungus compatibility: some mycorrhizal fungi have a wide range of possible hosts, for example, *Laccaria spp.* and *Pisolithus tinctorius* acting as generalists (Molina & Horton, 2015, 17) while some function as specialists, for example, *Suillus spp.* are able to host on pines only (Pringle, Wolfe & Vellinga, 2011, 469). Aspens *Populus tremula* and *P. tremuloides* that are of special interest in this thesis are compatible with a wide range of mycorrhizal fungi. *Populus tremula* can host hundreds of mycorrhizal species (Bahram, Põlme, Kõljalg & Tedersoo, 2011). Some fungal genera hosted by *Populus tremuloides* were listed by Cripps (2003): *Amanita spp., Cortinarius spp., Hebeloma spp., Inocybe spp., Laccaria spp.,*

Lactarius spp., Leccinum spp., Paxillus spp., Russula spp., Scleroderma spp., Thelephora spp., Tricholoma spp., and *Xerocomus spp.*.

In respect to phylogeny, species belonging to three phyla: *Basidiomycota*, *Ascomycota* and *Glomeromycota* – are able to form mycorrhizal associations. Out of them, only *Basidiomycota* and *Ascomycota* may include mycorrhizal macrofungal species (figure 4). So, the focus of this study should be put on these two phyla only.

However, one should mention that although the classification by trophic guilds is good for ecological studies because it describes fungi in the view of their ecological functions, in some cases it is unable to differentiate the fungal roles based on the species name only. There is a problem of "gray" trophic guilds due to the ability of some fungi for performing different ecological roles under different conditions. For example, *Armillaria mellea* can function as saprobic or parasitic (McConnell, 2014, 206), *Paxillus involutus* that is normally mycorrhizal may occur as a saprobe on wood (Kuo, 2008), and saprobic *Morchella spp.* sometimes establish mycorrhiza-like interactions with trees (Dahlstrom, Smith & Weber, 2000). That is why dealing with such species may require especial approaches.

3.2 Fungi in mycoremediation and phytoremediation

3.2.1 Mycoremediation: definition and characteristics

Fungi were proved to be applicable in the process of mycoremediation (a term coming from Ancient Greek μυκός - "fungus", and Latin *remedium* - "restoring balance") – decontamination of soil and water from pollutants (Rhodes, 2014).

Inoculating fungi on contaminated sites for decomposition or accumulation of the pollutants is a cheap (figure 5), environmental friendly bioremediation method: along with phytoremediation, it requires neither transporting of the polluted soil for the further treatment, nor use of chemicals although, according to Chibuike (2013, 1684), chemical methods may be combined with mycoremediation for enhancing the remediation effect.

Chibuike (2013, 1684) also marks the other advantages of mycoremediation: possibility to be applied to a wide range of pollutants; a prolonged action since fungal spores remaining in the soil for a long time "colonize any introduced plant and continue the remediation process"; and improving vegetation after clean up in case of using mycorrhizal fungi due to the soil properties modification and symbiotic actions.

Mycoremediation disadvantages include its slowness (it may require months to achieve the desired effect), necessity for thorough selection of matching fungal and plant species in case of dealing with a mycorrhiza, and dependency of its effectiveness on the pollutant (Chibuike, 2013, 1684). Šašek (2003) also marks the insufficiency of current knowledge in mycoremediation behaviour outside of laboratory conditions when many unknown environmental factors may influence on the remediation process. In some cases, it leads to unpredictable and unexpected results: for example, it has been reported that attempts of mycoremediation with use of mycorrhizal fungi gave a contrary effect and impeded the PAH-polluted soil restoration (Joner, Leyval & Colpaert, 2006).

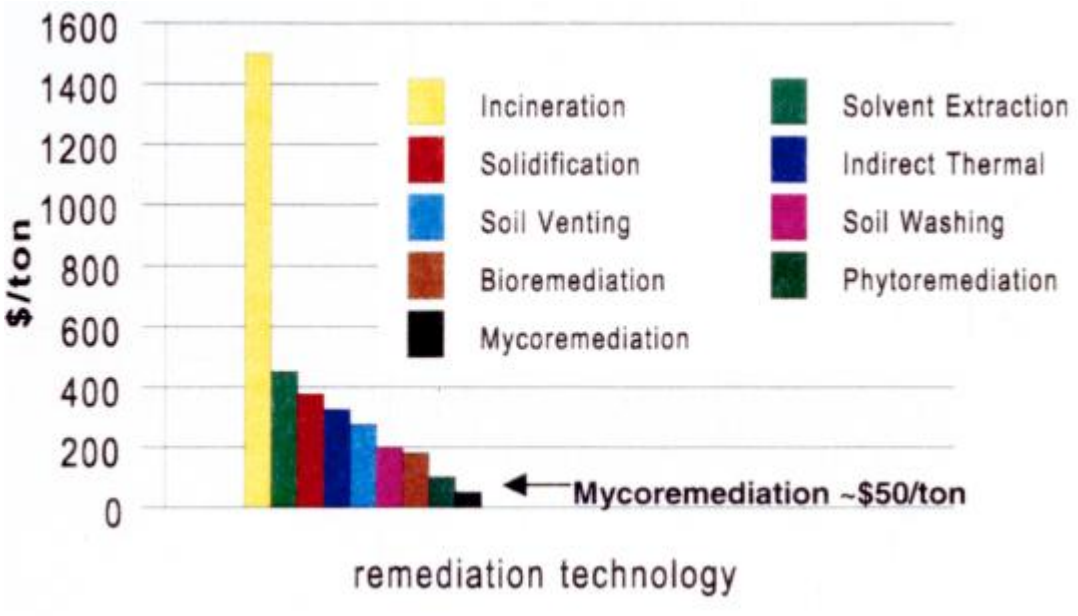


Figure 5. Costs comparison of remediation methods of PAHs (Adapted from: Stamets, 2012).

3.2.2 Mycoremediation: applications and studies

A range of substances can be treated with fungi: pesticides (Maloney, 2001; Fragoeiro, 2005; Singh, 2006), PAHs (Cabello, 2001; Serniglia & Sutherland, 2001; Singh, 2006; Norton, 2012; Winquist *et.al*, 2014), heavy metals (Gadd, 2001; Tobin, 2001; Singh, 2006; Turnau *et.al*, 2006), chlorinated hydrocarbons and BTEX (Soares, Albergaria, Domingues, De Marco & Delerue-Matos, 2012), energetic compounds (Singh, 2006) and other.

However, today mycoremediation is not commonly used, mainly due to a lack of relevant information on it (Šašek, 2003) and a problem of unpredictability mentioned in section 3.2.1. But in many countries, mycoremediation projects are implemented experimentally, and there are evidences on mycoremediation trials involving contaminated public grounds that have been quite successful, for example, in the USA (Norton, 2012, 20; Stamets, 2012).

At the same time, a scientific interest in remediation of polluted soils with fungi is proven with a number of papers on the topic. A brief review of the scientific literature on the fungal remediation of the PAH-contaminated soils will be presented here.

Mainly saprobic fungi, lignin decomposing white rot ones especially, are in the focus of current researches. Since 1985 when white rot fungi were proposed for bioremediation (Bumpus, Tien, Wright & Aust, 1985) a lot of researches on their remediation capacity have been done, usually by the example of *Phanerochaete spp.*, *Pleurotus spp.* and *Stropharia spp.* (Reddy & Mathew, 2001; Isikhuemhen, Anoliefo & Oghale, 2003; Polcaro *et.al*, 2008; Singh *et.al*, 2013; Winquist, 2014; Winquist *et.al*, 2014; Sadiq *et.al*, 2015). Generally, the researches in the issue are carried out *in vitro*, and a smaller part of these studies has been done *in situ*, for example, in the papers of Andersson *et.al* (1997), Baldrian (2003), Kim *et.al* (2010), Winquist (2014), Winquist *et.al* (2014). The paper by Kim *et.al* (2010) is of special interest – it represents a very uncommon investigation of the fungal community *in situ* and its resistance to PAHs.

The studies on the soil remediation with mycorrhizal fungi are rarer. General reviews of mycorrhizal fungi use in bioremediation were done, for example, by Meharg (2001), Joner and Leyval (2003), Singh (2006) and Chibuike (2013).

As for more specific investigations on the mycorrhizal fungi potential for bioremediation, arbuscular mycorrhizal fungi are commonly studied, for example, be Leyval, Joner, de Val and Haselwandter (2002), Khade and Adholeya (2007), Korade and Fulekar (2009), Wu, Yu, Wu and Wong (2014), Lu and Lu (2015). These researches can be applied rather to agriculture and grass farming than to forestry because the arbuscular mycorrhiza (one that is formed with a fungal mycelium which enters into a plant root cells and branches intensively inside of the cells) is typical for microfungi interacting mainly with herbs.

The scarcest information is provided on the remediation potential and properties of ectomycorrhizal fungi (with the mycelium covering the plant root with a mantle and penetrating only partially into the cells). As these are macrofungi interacting with trees, this fungal group is in the focus of the current paper. However, most studies on the issue are conducted on the capacity of ectomycorrhizal fungi for the heavy metal sorption and surviving on the heavy metal contaminated sites, for example, papers by Sell *et.al* (2005) on fungi symbiotic with poplars and willows, Krpata *et.al* (2008) on the aspen-associated ectomycorrhizal community, Luo *et.al* (2014) on ectomycorrhizal interactions with metals.

PAH-polluted soil remediation by the ectomycorrhizal fungi is insufficiently explored, so, there is a lack in the data for general conclusions on their effectiveness under different conditions. The information about the effect of ectomycorrhizal fungi is contradictory (Meharg & Cairney, 2000). It has been reported on the experiments ending with both success (Meharg, Cairney & Maguire, 1997; Gramms, Voight & Kirsche, 1999; Dittmann, Heyser & Bucking, 2002; Gunderson, Knight & Van Rees, 2007) and failure (Koivula, Salkinoja-Salonen, Peltola & Romantschuk, 2004; Joner, Leyval & Colpaert, 2006) in effective use of ectomycorrhizal fungi for removing PAHs. In addition, all listed papers contribute to knowledge in the bioremediation efficiency of certain fungal or tree species. However, no studies on the fungal community level have been detected.

One can conclude that today there is room for further investigations on bioremediation capacity of ectomycorrhizal fungi in many directions, for example, in view of certain contaminants and their combinations, the soil properties, fungal species, plant species, and plant-bacterial-fungal-worm communities.

3.2.3 Mycoremediation: mechanisms and affecting factors

The ability of fungi for hyperaccumulating and decomposing makes transformation of hazardous substances into innocuous ones possible. In the process of remediation, fungi result in the full or partial mineralization of pollutants through their transformation to CO_2, H_2O, N_2, HCl, *etc*, and non degradable heavy metals and radioactive ions can be rendered into lowly soluble forms that are less harmful in the ground (Rhodes, 2014). In latter case, as heavy metals are removed from the soil by their accumulation in the fungal tissues, used fungi should be harvested as hazardous waste on the analogy of trees applied to phytoremediation of heavy metals.

When dealing with degradable compounds, decomposition occurs as a result of fungal nutrition. For nutrition, fungi excrete enzymes into the environment: the enzymes degrade different environmental compounds transforming them to nutrients appropriate for fungi. Then these nutrients are absorbed by fungi. During decomposition, the enzymes are able to cleave off certain atoms, for example, chlorine from chlorinated pesticides, and then to break bonds between carbon and hydrogen (Johnston & Mosco, 2010).

Many biotic and abiotic factors can interfere in the mycoremediation effectiveness. Knowledge in these factors, acting *in situ* especially, is uneven conformably to different fungal groups. However, common factors with a proven impact on mycoremediation will be briefly described here.

An ecological community structure (including species richness, diversity, abundance, biomass, presence of different trophic guilds and other factors) plays a large part in mycoremediation success *in situ*.

Trophic guilds of fungi and the fungal species composition itself are important mycoremediation drivers. Saprobes living on different substrates and fungi associated with host plants interact with the environment in different ways and have different capacity for removing contaminants. For example, in the experiment with organic-rich sterile soils (Gramms, Voigt & Kirsche, 1999), terricolous saprobic fungi demonstrated the best efficiency in removing PAHs in comparison with saprobic wood- and straw-degrading and ectomycorrhizal fungi.

One should also understand that, within a certain trophic guild, most fungi are contaminant specific – actually, only few fungal species act as universal degraders able to deal with a wide range of contaminants at the same time (Chibuike, 2013, 1684). So, it is logical to assume that the fungal community with the higher species richness will be able to degrade more different contaminants. As applied to a certain contaminant, various species also demonstrate different accumulation or degrading capacity that was shown, for example, by Iram *et.al* (2009) for heavy metals and by Steffen, Hatakka and Hofrichter (2002) for PAHs. So, a ratio of certain species in the community will impact on the remediation success as well.

Fungal relations with other degraders: bacteria and worms - should also be taken into account as factors affecting mycoremediation. Interactions between microbes (fungi and bacteria from the rhizosphere) and the plant roots have a huge soil remediation potential and even serve as a base for a separate bioremediation strategy called rhizoremediation (term coming from Greek ριζα – “roots” and Latin *remedium* - "restoring balance") which is currently a subject of many studies (Wenzel, 2009; Shukla *et.al*, 2013; Mukherjee, 2014; Sahsavari, Adetutu, Taha & Ball, 2015). It means that microfungi and bacteria may complement each other for successful remediation. On the other hand, there are evidences that activity of macrofungi may enhance or decrease degrading abilities of the bacteria (Gramms, Voigt & Kirsche, 1999). Earthworms as degraders may also influence on the overall efficiency of bioremediation on the site. The experiments combining earthworms with the arbuscular mycorrhiza and herbaceous plants for remediation of PAH-polluted soil were done by Lu and Lu (2015).

In some cases, mycoremediation will depend on presence of fungus compatible plants on the site. For example, mycorrhizal fungi need certain plant species for surviving and, thus, functioning as it was shown in section 3.1.3. In case of the soil remediation with ectomycorrhizal fungi which are associated with tree species, presence of trees of an

appropriate age is important: as it was indicated by Smith (1970) by the example of sugar maple *Acer saccharum*, roots of younger trees excrete less exudates that help the mycorrhizal fungi colonize the tree root. This is a reason why ectomycorrhizal fungi host better on the roots of older trees. In addition, mycorrhizal fungi associated with younger trees grow more slowly and produce smaller mushrooms (Ortega-Martínez, Agueda, Fernández-Toirán & Martínez-Peña, 2011). So, less fungal biomass is produced in this case.

However, based on phytoremediation experience where large biomass is considered as advantage when choosing a species for phytoremediation (Mukherjee, 2014, 5), one can assume that biomass may be another important factor affecting the mycoremediation efficiency. Considering that larger organisms and the community with larger biomass require more resources, the nutrition and the other metabolic processes will run more intensively in the communities with larger biomass. So, biomass may be crucial for successful degradation, accumulation and the other remediation strategies. It may be applicable not only for the fungal biomass on the site but for the biomass of all species capable to remediation on the site – right selection of species for phytoremediation and mycoremediation combined together seems to be able to enhance the remediation efficiency.

As both aspens and fungi associated with them are able to remove PAHs, it seems that phytoremediation and mycoremediation can act as good complementary bioremediation methods *in situ* in case of finding and maintaining the optimum combination of the above listed biotic factors on the site.

Although some (for example, mycorrhizal) fungi are able to adapt to harsh soil or environmental conditions: high or low phosphorus levels, soil micronutrient levels, toxic levels of metals, aridity and water stress, low or high temperatures, different soil pH (Brundrett, 2009, 187), abiotic factors have an influence on mycoremediation as well. There are no sufficient data on how abiotic factors impact on mycoremediation efficiency of ectomycorrhizal macrofungi directly. However, considering knowledge in fungal physiology and ecology and the data on fungi participating in rhizoremediation, there is room for extrapolation.

Generally, abiotic factors affecting the purification of the PAH-polluted soils are concerned with the bioavailability of PAHs: soil type, texture, particle size, nutrients and organic matter content (Wenzel, 2009). Based on rhizoremediation cases one can say that better degradation occurs in presence of low molecular weight PAHs on the sites with recent PAH emissions at moderate soil pH (Shukla *et.al,* 2013). As PAHs dissolve poorly in water, microbial degradation of PAHs may depend on the amounts dissolved in the water phase (Johnsen, Wick & Harms, 2005).

Presence of specific chemicals in the soil can also stimulate fungal activity. As it was reported by Norton (2012), adding nitrogen, phosphorus, potassium or manganese into the soil enhanced remediation efficiency of macrofungi.

Another aspect of successful mycoremediation is factors which are important for fungal colonization of the site, such as nutrient availability, temperature, pH, oxygen, moisture, and toxicity of the contaminant (Mukherjee, 2014, 2). Considering fungal fruiting periods and necessity in the sufficient soil moisture for producing mushrooms, the fungal biomass production and thus remediation efficiency will depend on a season and a microclimate and depositions on the site.

One can conclude that because of all listed factors mycoremediation (as well as mycoremediaction combined with phytoremediation) *in situ* is a very complicated process that can be hard to manage due to a range of variables.

4 Somerharju phytoremediation project set up

4.1 General description of the project site

Somerharju is an area in the municipality of Luumäki, Eastern Finland (the UTM coordinates of the site in WGS84 are zone 35V E: 523173.77 N: 6753348.8) (figure 6).

Figure 6. Somerharju, location in Finland. Source: maps.google.com

The area outline is close to a half-oval shape (figure 7). According to Metla's description (2012, 8), the area is mostly flat, with the North edge rising steeply. The soil is predominantly sandy, including silt-like, clay-like and sand/moraine layers though.

Figure 7. Aerial picture of Somerharju in 2011, with the area outlined, 1:8000. (Adapted from: Metla, 2012; Clavel, 2013).

The local groundwater is suitable for water supply and is in use by a local co-operative company and some domestic users. The water is taken from the water supply point that is about 1,3 km far from the site (Metla, 2012, 9). The water bodies on the site surface are absent. The residential area, the village of Arvola, with its houses, holiday houses and wells, is situated nearby, on the South of the site (Metla, 2012, 8).

Currently, the site is meant for the phytoremediation experiment and fenced to prevent both an access of people to the polluted area and possible damage to experimental trees by wild animals (Metla, 2012, 19). For the experiment, the territory was divided into 200 squares with the side length of 20 meters; each square got its coordinates (coded as a combination of a letter and a numeral) on the map (Appendix 1).

Before the experiment started, trees had been growing on the site. In 2013 clear cutting was done on the entire territory for planting experimental aspens (excluding a few of squares where non-cut trees were left) (Appendix 1). Numerous root suckers and wild seedlings that appear on the territory yearly are considered as weeds and being regularly destroyed excluding some squares where wild aspens were left. More information on the left wild aspens is given in section 4.4 and Appendix 4.

As the presence of trees is crucial when dealing with mycorrhizal mushrooms, a monitoring of tree and shrub species that remained non-cut or that grow as root suckers and wild seedlings has been done for this thesis especially. Except for young aspens *Populus tremula* and hybrid aspens *P. tremula* x *P. tremuloides* planted intentionally for the experiment, the following wild trees and shrubs were detected on the site: birches *Betula pendula* and *B. pubescens*, pines *Pinus sylvestris*, and aspens *Populus tremula*. Norway spruces *Picea abies*, willows *Salix sp.*, and raspberry bushes *Rubus idaeus* are rarer. Young rowans *Sorbus sp.* and oaks *Quercus sp*. occur sporadically.

4.2 Somerharju's contamination history

According to Metla (2012, 7), in 1947-1958, a wood impregnation plant covering 7 hectares was situated here, with storages of impregnated and non-impregnated logs in most of the area (figure 8). Although the plant buildings have been dismantled, the remnants of logs, transportation rails, and reinforcement bar can still be found on the site.

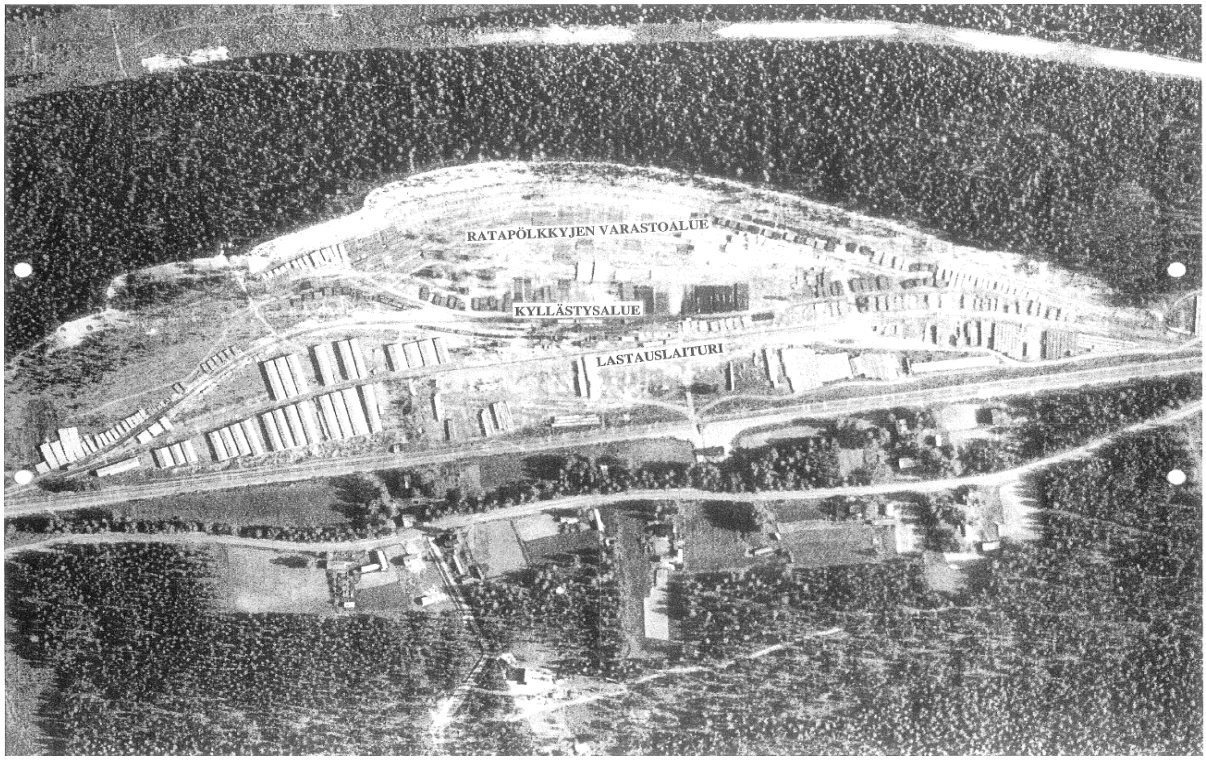


Figure 8. Aerial picture of Somerharju in 1953, when the industrial activities were still running (Metla, 2012).

The plant was a source of the soil and ground water contamination with creosote – substance used for the impregnation. On the territory, there were at least 2 creosote containers. The contamination occurred in two ways: as a result of creosote oil depositions into the soil during the impregnation process, and as a consequence of a single large leakage when about 10 thousand liters of creosote oil were spilt into the soil, and 1,3 hectares out of 7 hectares in total were contaminated (Metla, 2012, 7).

The monitoring and the environmental analysis of the site were held by Golder Associates Oy in 2003-2010 (Metla, 2012, 5, 9). In 2012, based on the results of the research, the Regional Administrative Agency of Southern Finland issued the decision 176/2012/1 on the site rehabilitation to "Senaatti-kiinteistöt", the state company owning the area (Etelä-Suomen aluehallintoviraston päätös 176/2012/1). In 2012, the rehabilitation plan through phytoremediation was developed by Metla (also referred to as Luke, after Metla's reorganization and renaming in 2016) in order to stop possible spreading of contaminants and to decrease risks for humans caused by touching and eating local wild mushrooms and berries and using local groundwater (Metla, 2012, 11). The implementation of the project started since 2013, under the guidance of Metla (Luke since 2016).

4.3 Creosote as pollutant in Somerharju

Creosote (the term coming from Greek κρέας – "meat" and σωτήρ – "preserver"), so called due to its possible application as a food preservative, is a carbonaceous oily by-product of coal- or wood tar distillation (Metla, 2012, 10; Columbia electronic encyclopedia, 2015). It contains hundreds of different compounds, most of which are PAHs (75-85%). The rest are phenols (5-10%), NSO heterocyclic compounds (about 5%), and BTEX in a small quantity (less than 1%) (Metla, 2012, 10).

Wood tar based creosote is in use in medicine, coal tar based one is highly toxic and can be applied for protection of wood materials against fungi and other pests (Columbia electronic encyclopedia, 2015). However, nowadays, in the EU, the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation prohibits applying creosote for wood treatment and restricts its industrial applications, for example, for railway sleepers and telegraph poles (EC 1907/2006, annex XVI, 31), because of its potential carcinogenicity.

A possible carcinogenic effect of creosote was first detected in 1950s (Kammer & Poel, 1957; Boutwell & Bosch, 1958). The further studies on the issue that had been done till the 70s were summarized by the USA Environmental Protection Agency (EPA, 1988). Modern investigations also provide evidences of negative effects of creosote-polluted sites on animal organisms and ecosystems, in particular, fish (Vines, Robbins, Griffin & Cherr, 2000), plankton communities (Sibley *et.al*, 2004), oyster populations (Smith, 2008), and on human health (Holme, Refsnes & Dybing, 1999; Brender, Pichette, Suarez, Hendricks & Holt, 2003; Yang, Yoon, Ryu, Chung, Nam & Nam, 2013).

A health risk is possible through a skin contact with the contaminated material, swallowing contaminated soil, or eating mushrooms and berries grown on the contaminated site (Metla, 2012, 11). One can assume that a potential harm caused by creosote may depend on its concentrations (EC, 1999) and a chemical composition of the contaminant on the certain site because a ratio of different compounds in creosote may vary (Metla, 2012, 10).

According to Metla (2012, 10), in Somerharju, a monitoring of pollutants and an analysis of their concentrations on the site was done by Golder Associates Oy in 2011, and 16 PAH-compounds were detected in the soil and the groundwater: naphtalene, acenaphthene, acenaphthylene, anthracene, benz(a)anthracene, dibenz(a,h)-anthracene, fluorene, fluoranthene, benzo(b)fluoranthene, benzo(k)fluoranthene, pyrene, benzo(a)pyrene, indeno(1,2,3-cd)-pyrene, benzo(ghi)-perylene, chrysene, phenanthrene. Their distribution over the site is not homogenous, and the areas were detected where naphthalene, fluoranthene, phenanthrene or total 16 PAHs concentrations exceed base and threshold reference values stated in the Finnish government decree on soil contamination and remediation needs (214/2007) – so called PIMA-regulation, with the largest toxic substance deposits in depth of about 10-11 meters.

Metla developed a 4-leveled scale for total PAH concentrations according to PIMA-reference values and applied it to the site mapping. Based on PIMA-values, total PAH concentrations up to 15 mg/kg are considered safe, and the squares with these concentrations can be referred as clean. For this thesis, the scale was supplemented with numbers from 0 to 3 standing for the pollution levels (Appendix 2). These numbers are meant for further use in a statistical analysis within the side project on the mushrooms.

4.4 Arrangement of aspens for the phytoremediation project

As Somerharju phytoremediation experiment is quite new and neither results, nor information on the aspen arrangement on the site have been published yet, all information provided in this section is based on the personal communication with the phytoremediation team members Raimo Jaatinen and Pertti Pulkkinen in April 2015 and on personal field observations in Somerharju.

The phytoremediation efficiency and the tree survival in Somerharju are being studied by the example of common aspens *Populus tremula* and hybrid aspens - a mix of common aspen *P. tremula* and American aspen *P. tremuloides*.

Aspen clones selected and produced by Metla and grown in Metla's nursery were used for planting. Ten different genotypes: three of common aspens and seven of hybrid ones - were replicated and planted in total. Individual codes were allocated to each genotype: R2, R3, R4 for common aspens, and 14, 27, 34, 134, 172, 191, and 291 for hybrid ones.

In 2013 the seedlings were planted on the so called "intensive area" where the pollution levels are the highest, in four repetitions, with ten entries inside of each repetition (Appendix 3). Each entry corresponds to a separate genotype (table 1). The entries were randomly attributed to the squares. Entries 11 and 12 are control squares without planted aspens: entry 11 has never been cut; in entry 12, clear cutting has been done once before the experiment; currently the wild trees grow naturally in both these squares.

Table 1. Correspondence of entries to aspen genotypes.

Entry on the map	**Aspen genotype**	**Aspen species**
1	R2	*P. tremula*
2	R3	*P. tremula*
3	R4	*P. tremula*
4	14	*P. tremula* x *P. tremuloides*
5	27	*P. tremula* x *P. tremuloides*
6	34	*P. tremula* x *P. tremuloides*
7	134	*P. tremula* x *P. tremuloides*
8	172	*P. tremula* x *P. tremuloides*
9	191	*P. tremula* x *P. tremuloides*
10	291	*P. tremula* x *P. tremuloides*
11	-	-
12	-	-

Each square is planted with 400 aspen trees with the planting density 1 tree per 1 square meter. Young wild aspens grown as root suckers also occur on the site. As an investigation on planted and wild aspens adaptation is also planned to be done within the project, the wild aspens were counted. The wild aspen growing density and distribution over the site is shown in Appendix 4.

5 Occurrence of mycorrhizal mushrooms in Somerharju: project set up

5.1 Sampling grounds selection

According to measurements by Golder Associates Oy (Metla, 2012, 10), zones with 4 different pollution levels have been detected on the project site of Somerharju (Appendix 2).

The map with the aspen clone numbers (Appendix 3) was used for selection of sampling grounds. First, several clone numbers corresponding to entries 2, 4, 5 and 9 on the map were randomly selected. All squares containing these clones were chosen as sampling grounds, excluding the squares I22, J22 and K22 for which the pollution data are unavailable. As a result, 13 squares (coordinates G12, G13, H11, H14, H16, H17, I13, I15, I16, I19, J11, J15, J18) were selected (Appendix 5).

After that it was checked whether the selected squares cover zones with all 4 pollution levels (Appendix 2). It was found out that, in the process of the random selection, zone with pollution level 1 did not fall within the list of the selected sampling grounds. So, the squares of zone 1 (coordinates H18, I12, J17 – all squares from the intensive area) were deliberately added into a list of sampling grounds (Appendix 5).

Outside the intensive area, 2 additional squares were deliberately chosen as control ones: square I9 represents a non-polluted and non-cut area; and square K11 represents a non-polluted but clear-cut area.

So, in total, 18 squares were selected as sampling grounds, including 2 control squares outside the intensive area. Their distribution on the site is shown in Appendix 5.

5.2 Mushroom samples selection

The mycorrhizal mushroom species were considered as target ones and, thus, sampled. So, genera and/or species identified *in situ* and recognized as saprobic (e.g. morels *Morchella*, false morels *Gyromitra*, puff-balls *Bovista, Lycoperdon* and the like, *Coprinellus, Galerina etc*.) were ignored. Mushrooms species that were not recognized *in situ* but were obviously saprobic (because of having fruit bodies on the trees, trunks and dead wood like *Flammulina sp., Polyporus sp., Tricholomopsis sp.*) were also ignored.

However, the ability of some mycorrhizal macrofungi to develop fruit bodies on the trees and wood (Misra, Tewari, Deshmukh & Vágvölgyi, 2014, 32) was considered. If a saprobic status of a wood-inhabiting mushroom was thrown doubt upon, this mushroom was sampled for further identification.

At that, sampling of morels *Morchella spp.* (Dahlstrom, Smith & Weber, 2000) and common roll-rims *Paxillus involutus* (Kuo, 2008) that are able to live as symbionts or saprobes in some cases had to be decided on separately. It was decided to include *Paxillus involutus* into a sample list based on its most common status: in most cases, *Paxillus*

involutus is reported to act as a mycorrhizal species, for example, in mushroom guides (Laessoe, 2002; Lahti, 2013; Афонкин, 2014) and in the articles on mycorrhizal fungi (Jentschke *et.al*, 2000; Cripps, 2003; Bojarczuk, Karlinski, Hazubska-Przybył & Kieliszewska-Rokicka, 2015). *Morchella spp.* that may be equally likely mycorrhizal or saprobic (Kuo, 2012a; Kuo, 2012b, Kuo, 2012c) were excluded from the sample due to impossibility to define their status *in situ* in every certain case.

The other mushrooms were sampled for further species and trophic guild identification.

5.3 Sampling technique

A shuttle search of mushrooms was done in each sampling square (figure 9). Only a general direction of the search track is shown on the scheme. Practically, the space around aspens and the other trees growing within the square was examined as well.

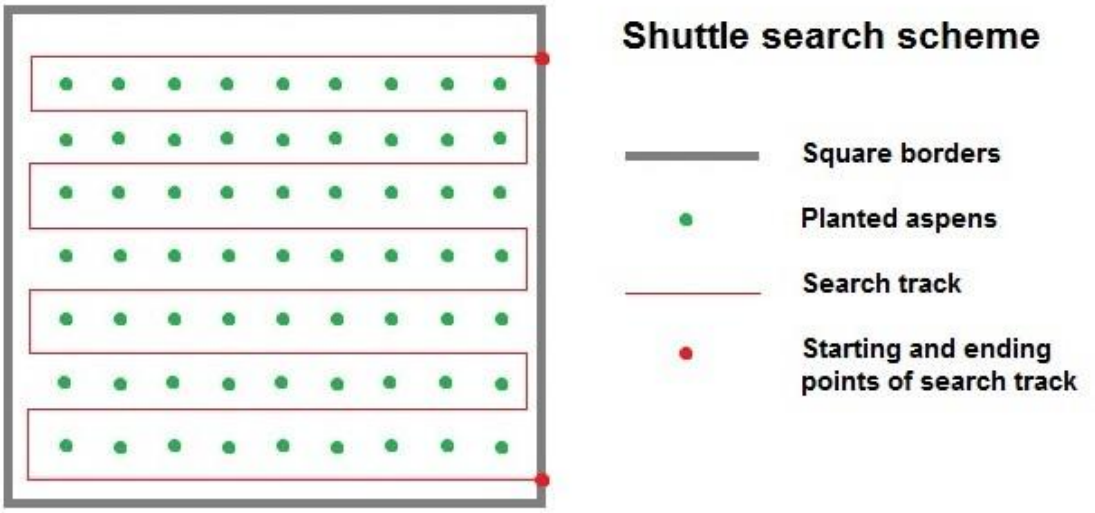


Figure 9. Scheme of a shuttle search within a sampling square.

Mushrooms found in each square and identified *in situ* were recorded with the indication of a sampling date, a square coordinate, a species and a number of mushrooms found. If the species identification was hard to do *in situ*, the mushroom was photographed from various angles (including cuttings in horizontal and vertical profiles) and picked up for further identification. The characteristics of any unknown mushroom described in section 5.5 were recorded. The spore prints were taken *in situ* with use of facilities brought to the site: black and white paper or carton, and plastic or glass vessels covering the mushroom caps in order to keep them not dismissed.

For knowing abundance each target mushroom found was counted. If some fruit bodies were not picked up for species identification, they were marked by setting a stick near each of them in order to exclude counting the same fruit body twice in different sampling days.

5.4 Sampling periodicity

Sampling periodicity was chosen according to the average lifetime of mushrooms and to the expected productivity of the fruiting period under certain conditions.

The mushroom lifetime depends on environmental conditions (such as moisture, temperature, CO_2 levels) (Beyer, 2003, 14). Moreover, the lifetime of different mushroom species also varies: for example, mushrooms of *Coprinus sp.* dissolve themselves in several hours because of presence of degrading enzymes in their gills (Cabrita, 2013) while a polypore *Trametes versicolor* growing on the trees is able to produce perennial mushrooms living for years (Mauseth, 2014, 581). The data from mushroom farms were used to consider a period of 7-10 days as an average lifetime of a mushroom (Beyer, 2003, 14).

Sampling was done in 2015 during the entire mushroom season that may last from May until early November in Finland (Nordic Recipe Archive, 2014). The period from late July until early October was considered as the most productive one (Metsähallitus, 2015). Taking into account an average lifetime of a mushroom and productive and non-productive months, sampling was done every other week during potentially not productive periods (May-June, first week of November), and weekly during the productive fruiting period (July-October). 20 sampling sorties were done in total. Sampling dates are shown in table 2.

Table 2. Mushroom sampling dates in 2015 (green cells stand for a productive fruiting period).

Month	**Days**
May	6, 20
June	4, 16
July	1, 9, 16, 21, 28
August	13, 20, 27
September	3, 17, 25, 30
October	5, 14-16, 26
November	4

5.5 Species and trophic guild identification

Species identification was done based on macroscopic phenotypic features of the mushrooms, such as a mushroom shape, size and proportions of a stem and a cap, hymenophore structure, presence of distinguishing features such as a ring or a veil, colour of mushroom parts, its smell, and flesh discoloration after damaging. Spore prints were also taken for detecting the spore colour. Mushroom species identifiers (Nishida, 1989; Kibbey & Fatto, 2002; Kuo, 2007; Leonard & MacAdam, 2008) and mushroom guides and handbooks (Laessoe, 2002; Lahti, 2013; Афонкин, 2014) were used for identification of most species. The same guides and handbooks were used for identification of a trophic guild.

Additional specific characteristics were also considered for identification of *Russula spp.* and *Lactarius spp.*. *Lactarius spp.* were checked for a milk colour, its changes over time

and for a milk taste (for mushrooms collected in the non-polluted areas only) according to the guidance by Leonard and MacAdam (2008). *Russula spp.* were additionally checked for separability of a cap cuticle and for a taste (for mushrooms collected in the non-polluted areas only) according to Kibbey and Fatto (2002).

In some cases, the information on a month when the mushroom was collected, its substrate, tree species nearby and its occurrence in groups or separately was taken in account.

However, considering a lack of information on microscopic features and on DNA, there is still some degree of uncertainty on some species sampled. First of all, it is applied to *Inocybe spp.* and *Russula spp.* which are very hard to distinguish based on the macroscopic characteristics only. Those species were numbered. In lists of mushrooms detected on the site (Appendices 7-9), these species are presented with indication of their genus, species number and presumed species name in the format: *Russula sp. 1* (presumedly *grisea*).

5.6 Biodiversity calculation

5.6.1 Biodiversity measuring approaches

Biodiversity (or biological diversity) can be defined as "the sum total of all biotic variation from the level of genes to ecosystems" (Purvis & Hector, 2000). According to Convention of Biological Diversity (1992, Article 2), the concept of biodiversity "can be applied within species, between species and of ecosystems". So, biodiversity is a very broad concept which puts a problem of measuring it in a unified way.

When dealing with a community level, such parameters as species richness (a number of species on the site) and species evenness (based on abundance, or a number of individuals) may be reasonable for measuring biodiversity. They are commonly used today (Purvis & Hector, 2000). Species richness and abundance may be applied as biodiversity indicators independently as it was done, for example, by Burgas, Byholm and Parkkima (2014), or different biodiversity indices (such as Shannon index, Simpson index, Berger-Parkson index) based on those parameters may be calculated.

Both approaches were applied to this thesis when processing the dataset statistically. Trying two approaches simultaneously is expected to increase the reliability and accuracy of the results.

5.6.2 Statistical method: variables and factors analyzed

Species richness (a number of mushroom species), mushroom abundance (number of fruit bodies) and diversity index – were calculated for each sampling square within each month and then statistically analyzed as three separate parameters. Shannon index resulted from the formula

$$-\sum_i \left(\frac{n_i}{N} \cdot \log_2\left(\frac{n_i}{N}\right)\right)$$

– was used for the biodiversity indices calculation. The biodiversity indices were calculated with AlYoung Studios' online biodiversity calculator (Biodiversity calculator, w.y.).

Species richness, mushroom abundance and a diversity index were analyzed for their dependency on a pollution level and a cutting type as fixed factors. It was decided to consider the cutting type as a fixed factor because its influence on the results had been expected: as presence of mature trees is crucial for a successful colonization of the site by mycorrhizal fungi (Smith, 1970), it was logical to presume a high difference in mushroom occurrence between clear cut and non-cut squares. A distance of clear cut sampling squares from the non-cut ones was also considered: according to Dickie and Reich (2005), the distance from established vegetation impacts on the presence, abundance, diversity and community composition of ectomycorrhizal fungi and, thus, may affect the results.

The data designed for the statistical analysis are presented in Appendix 6. The following variables were considered in the table: Square, Pollution level, Cutting type, Month, Fruit bodies.

Squares were marked with its coordinate according to the site project map (Appendix 1). Fruit bodies stand for a number of mushrooms found. Pollution levels, cutting type, and month were coded with numbers.

Pollution levels were coded with the numbers from 0 to 3, from 0 standing for non-polluted squares to 3 marking the highest pollution level as shown in Appendix 2. Cutting types were coded with the numbers from 0 to 2 where 0 stands for non-cut squares, 1 for cut squares that are next to non-cut ones (those having a common square side or a vertical angle with the non-cut square), and 2 for cut squares. Only months when the mushrooms were found on the site were considered in the table and, thus, coded: so, 1 stands for June, 2 for July, 3 for August, 4 for September, 5 for October.

For calculations of the mushroom distribution over the site, a generalized linear mixed model (GLMM) from R software 3.2.4 Revised (R Development Core Team, 2016) was applied.

6 Results

6.1 Mushroom species richness, abundance and biodiversity

Both saprobic and mycorrhizal macrofungi were present on the Somerharju project site during a mushroom season. In total, 28 mycorrhizal species of 12 genera were found in the project area during the entire mushroom season. The largest number of the mycorrhizal mushroom species detected belongs to three genera: *Russula* (10 species), *Inocybe* (6 species), and, to a lesser degree, *Lactarius* (3 species). A full list of the mycorrhizal mushrooms detected on the site includes the following species:

Amanita rubescens
Cantharellula umbonata
Coltricia perennis
Cortinarius mucosus
Inocybe sp. 1 (presumedly *bongardii*)
Inocybe sp. 2 (presumedly *napipes*)
Inocybe sp. 3 (presumedly *geophylla*)
Inocybe sp. 4 (presumedly *flocculosa*)
Inocybe sp. 5 (presumedly *calospora*)
Inocybe sp. 6 (presumedly *lacera*)
Laccaria laccata
Lactarius flexuosus
Lactarius rufus
Lactarius torminosus
Leccinum scabrum
Paxillus involutus
Russula sp. 1 (presumedly *grisea*)
Russula sp. 2 (presumedly *badia*)
Russula sp. 3 (presumedly *consobrina*)
Russula sp. 4 (presumedly *mustelina*)
Russula sp. 5 (presumedly *exalbicans*)
Russula sp. 6 (presumedly *atropurpurea*)
Russula sp. 7 (presumedly *betularum*)
Russula sp. 8 (presumedly *versicolor*)
Russula sp. 9 (presumedly *vinosa*)
Russula sp. 10 (presumedly *aeruginea*)
Suillus luteus
Xerocomus subtomentosus

In addition, the saprobic mushrooms of 17 genera were found on the site. The list of identified saprobes includes the following genera/species:

Agrocybe spp.
Clitocybe agrestis
Collybia dryophila
Cystoderma carcharias
Discina sp.
Flammulina sp.
Galerina spp.
Gymnopilus penetrans
Hygrocybe spp.
Lycoperdon spp.
Morchella sp.
Omphalina sp.
Pholiota alnicola
Pholiota destruens
Pluteus leoninus
Psathyrella sp.
Polyporus spp.
Tricholomopsis rutilans

This list of saprobic species is not full: it ignores unknown mushrooms that, however, were recognized as saprobic *in situ* and, thus, were not sampled for further identification as non-target species.

The mushroom season lasted for 8 months from late April until early November. The mushroom season for mycorrhizal species lasted for 5 months from June until late October. The composition of both saprobic and mycorrhizal species changed over time on the site because of different fruiting periods of each fungal species. The changes in the saprobic species composition were not recorded as the saprobes are out of focus of this thesis. Different mycorrhizal species produced their fruit bodies within a period of 1-5 months: monthly changes in the mycorrhizal species composition are presented in Appendix 7.

The highest species richness in mycorrhizal fungi on the entire site was observed in September (24 species), the lowest one was in June (1 species) (table 3, figure 10).

Table 3. Quantitative changes in mycorrhizal mushroom species richness over a mushroom season in Somerharju.

	May	**June**	**July**	**Aug**	**Sep**	**Oct**	**Nov**
Species richness	0	1	7	8	24	10	0

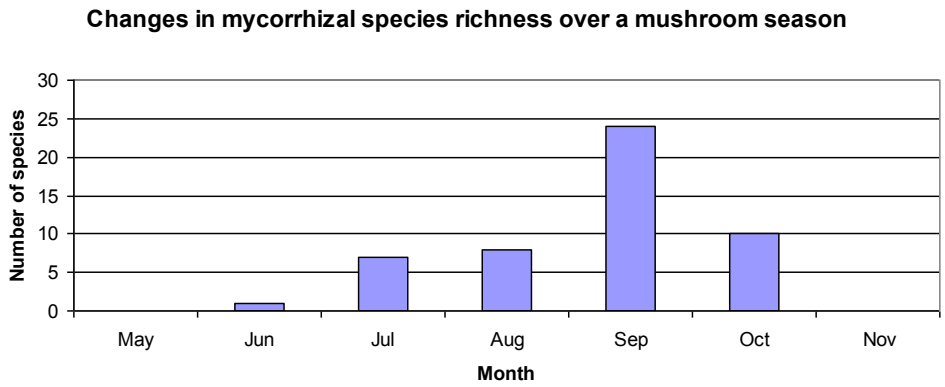


Figure 10. Changes in mycorrhizal mushroom species richness over a mushroom season in Somerharju.

Changes in mycorrhizal mushroom species richness do not depend on a cutting type (table 4, figure 11): generally, curves on the figure follow the same way reaching their peak in September.

Table 4. Quantitative changes in mycorrhizal mushroom species richness in the areas with different cutting types.

Cutting type	May	Jun	Jul	Aug	Sep	Oct	Nov
Non-cut	0	0	6	5	18	3	0
Next to non-cut	0	1	2	3	12	8	0
Cut	0	1	3	3	12	7	0

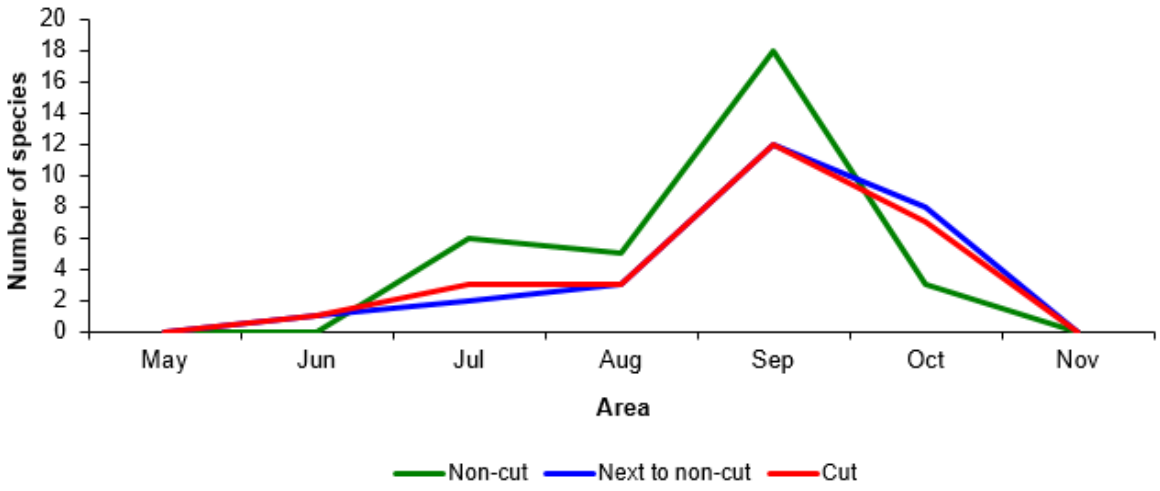


Figure 11. Changes in species richness in the areas with different cutting types.

Changes in species richness in the areas with different pollution levels do not depend significantly on a PAH-pollution level (table 5, figure 12): generally, the curves follow the same way reaching their peak in September (excluding pollution level 1 with the peak in October).

Table 5. Quantitative changes in mycorrhizal species richness in the areas with different pollution levels.

	May	Jun	Jul	Aug	Sep	Oct	Nov
Pollution level 0	0	1	2	1	10	6	0
Pollution level 1	0	0	2	1	4	6	0
Pollution level 2	0	0	1	1	4	2	0
Pollution level 3	0	0	2	2	12	7	0
Control square I9	0	0	6	5	18	3	0
Control square K11	0	0	1	1	4	2	0

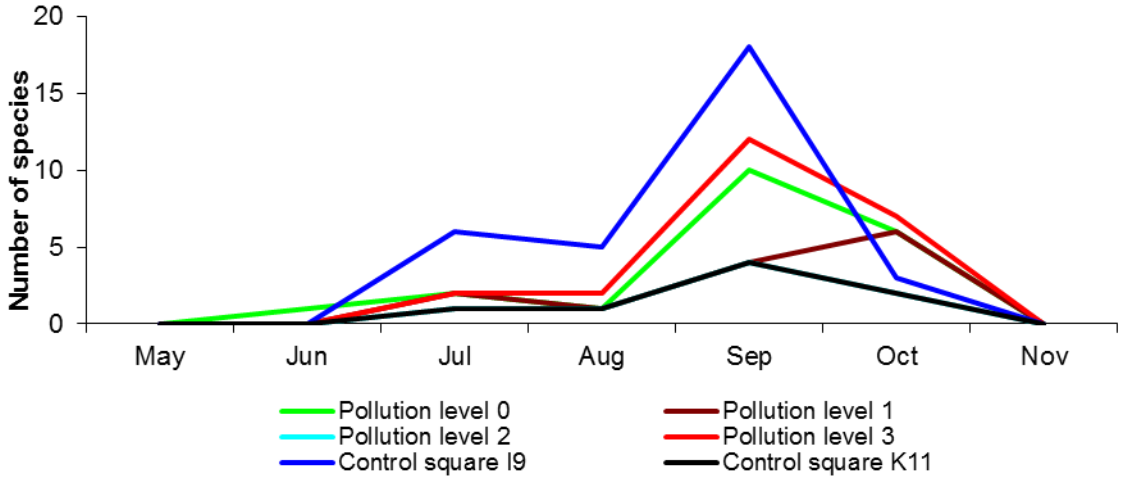


Figure 12. Changes in species richness in the areas with different pollution levels over a season.

The highest mycorrhizal mushroom abundance was observed in September at all pollution levels (Appendix 8) and at all cutting types (Appendix 9). During the entire mushroom season, *Laccaria laccata* and *Inocybe sp. 6* (presumedly *lacera*) were the most abundant mycorrhizal species at all pollution levels and at all cutting types (Appendices 8-9).

Shannon biodiversity indices calculated for each sampling square in each month varied from 0 to 2,423 (Appendix 6).

6.2 Mushroom distribution affected by PAH-pollution level and cutting type

The collected data (Appendix 6) on mushroom abundance, species richness and biodiversity indices are distributed according to Poisson distribution. In order to check dependency of mushroom abundance, species richness and biodiversity indices on a pollution level and a cutting type, a generalized linear mixed model (GLMM) fit by maximum likelihood was applied with use of R-software function glmer.nb {lme4}. Pollution level and Cutting type were, thus, taken as fixed factors. Square was taken as a random factor.

Dependence of variables on the fixed factors was calculated by formulas:

Fruit.bodies ~ Pollution.level * Cutting.type + (1 | Square)
Species.richness ~ Pollution.level * Cutting.type + (1 | Square)
Diversity.index ~ Pollution.level * Cutting.type + (1 | Square)

- for mushroom abundance, species richness and diversity indices, respectively.

It was found out that a pollution level bound with a cutting type does not have any statistically significant effect on mushroom abundance (*Pr* = 0.6) (table 6), species richness (*Pr* = 0.45) (table 7) and biodiversity indices (*Pr* = 0.48) (table 8).

Table 6. Dependence of mushroom abundance on a pollution level and a cutting type.

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
Pollution.level	0.01316	0.49343	0.027	0.979
Cutting.type	-0.61005	0.42675	-1.430	0.153
Pollution.level:Cutting.type	-0.15495	0.30163	-0.514	0.607

Table 7. Dependence of mushroom species richness on a pollution level and a cutting type.

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
Pollution.level	0.08084	0.25444	0.318	0.75069
Cutting.type	-0.66470	0.21786	-3.051	0.00228 **
Pollution.level:Cutting.type	-0.11861	0.15912	-0.745	0.45603

Table 8. Dependence of mushroom biodiversity on a pollution level and a cutting type.

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
Pollution.level	0.19370	0.30929	0.626	0.5311
Cutting.type	-0.55908	0.27977	-1.998	0.0457 *
Pollution.level:Cutting.type	-0.14666	0.20931	-0.701	0.4835

However, analyzed as two independent factors, Pollution level and Cutting type show different impacts on the mushroom occurrence. Based on statistics, Pollution level still does not have any significant impact on the mushroom abundance (*Pr* = 0.97) (table 6), species richness (*Pr* = 0.75) (table 7), and biodiversity indices (*Pr* = 0.53) (table 8). On the contrary, a cutting type demonstrated a statistically significant effect on two variables: species richness (*Pr* = 0.002) (table 7) and biodiversity indices (*Pr* = 0.04) (table 8). Dependence of mushroom abundance on Cutting type is still insignificant (*Pr* = 0.15) (table 6).

At that, negative correlation between each of three variables and Cutting type was detected: species richness and biodiversity are the largest in the control square with the original forest, decrease on the edge of the cut and non-cut areas and is the lowest in the cut squares.

7 Discussion and conclusions

Based on the species monitoring results, one can conclude that the mushroom community in the Somerharju project area is diverted and represents both saprobic and mycorrhizal species.

Mycorrhizal mushroom occurrence concerning species composition, richness and mushroom abundance on the site varies during a mushroom season. Species richness and mushroom abundance reach their maximum levels in September. It corresponds to a general tendency of a mushroom season all over Finland where September is considered as one of the most productive months (Metsähallitus, 2015). Generally, seasonal increase and decrease in species richness go in parallel at all pollution levels and cutting types.

PAH-pollution levels and cutting types considered as two bound factors do not affect the mycorrhizal mushroom abundance, species richness and biodiversity on the site. However, analyzing a pollution level and a cutting type as separate factors leaves more room for interpretations.

Mushroom abundance, species richness and biodiversity do not depend on the PAH-pollution level on the site. So, mycorrhizal fungi in Somerharju act as organisms that are tolerant to different PAH concentrations, and, thus, have a potential to colonize the entire site evenly, in spite of small or large amounts of creosote in the soil.

However, presence of suitable tree coverage can be a limiting factor in the colonization process: mycorrhizal mushroom species richness and biodiversity depend significantly on a cutting type: the largest species richness and biodiversity are observed in the areas with a natural forest; on the edge of the cut and non-cut areas, species richness and diversity decrease and are the lowest in the cut squares with very young aspens. Such a result was expected because of well-known symbiotic relationships between the trees and mycorrhizal fungi and, thus, their crucial role in each other's survival (Marschner & Dell, 1994; Clark & Zeto, 2000; Dell, 2002; Bandou *et.al*, 2006; Finlay, 2008; Brundrett, 2009; Pringle, Wolfe & Vellinga, 2011).

Despite expectations, no significant impact of the cutting type on the mushroom abundance was found. It contradicts to findings by Dickie and Reich (2005) who reported on that both mushroom species richness and abundance were affected by the distance to the non-cut area.

In Somerharju case, this result could be explained by possible influence of some other unaccounted factors. The Somerharju project site is not a homogenous territory, with a large variation in heights, tree coverage, tree age, and a tree species composition. Those factors might affect the distribution of the fruit bodies over the territory. Among the other affecting factors, there could be a precipitation level during a mushroom season, or induced simplicity of the mushroom investigation set up: for example, wild aspens growing intensively in some squares were not considered in the analysis, the data could be biased (the amount of squares with different cutting types differed a lot), or some mushrooms were hidden in vegetation so that they were extremely hard to detect by the researchers in spite of a thorough search. These factors can and should be considered in further researches. Possible variance between different seasons should also be checked in future.

The latter can be applied not only to the mushroom abundance but to all three biodiversity dimensions (species abundance, richness, and biodiversity indices). The difference in the results obtained for mushroom abundance, species richness and biodiversity indices let judge on that a strategy to measure biodiversity as three different values (species richness, abundance and biodiversity indices) is reasonable. As expected, this approach improved the accuracy of the results.

Based on the current findings, one can conclude that as Somerharhu's mycorrhizal mushroom community is tolerant to PAHs but sensitive to presence of trees, in future when the experimental aspens are older and if they show their compatibility with the local mycorrhizal community one can expect even emergence of the mushrooms over the site. Besides, if the significant effect of the mycorrhizal mushrooms on phytoremediation is detected in future, there are perspectives for an intentional inoculation of such mushrooms in the areas with suitable tree coverage on the entire site for enhancing the bioremediation effect.

Works cited

Andersson, E., Henrysson, T., Alleman, B. & Leeson, A. (1997). Bioremediation of PAH-contaminated soils using white-rot fungi. *Bioremediation, 4 (2),* 535-541.

Bahram, M., Põlme, S., Kõljalg, U. & Tedersoo, L. (2011). A single European aspen (*Populus tremula*) tree individual may potentially harbour dozens of *Cenococcum geophilum* ITS genotypes and hundreds of species of ectomycorrhizal fungi. *Federation of European Microbiological Societies Microbiology Ecology, 75 (2),* 313-320.

Baldrian, P. (2003). Microbiological aspects of *in situ* bioremediation using white-rot fungi. 7th international *in situ* and on-site bioremediation symposium, Orlando, June 2-5, 2003.

Bandou, E., Lebailly, F., Muller, F., Dulormne, M., Toribio, A., Chabrol, J., Courtecuisse, R., Plenchette, C., Prin, Y., Duponnois, R., Thiao, M., Sylla, S., Dreyfus, B. & Bâ, A. (2006). The ectomycorrhizal fungus *Scleroderma bermudense* alleviates salt stress in seagrape (*Coccoloba uvifera* L.) seedlings. *Mycorrhiza 16,* 559– 565.

Bear, R., Rintoul, D., Snyder, B., Smith-Caldas, M., Herren, C. & Horne, E. (2016). *Principles of biology*. (w.p.): New Prairie Press, Kansas State University.

Beyer, D. (2003). *Basic procedures for Agaricus mushroom growing.* (w.p.): Pennsylvania State University.

Biodiversity calculator (w.y.). www.alyoung.com/labs/biodiversity_calculator.html?numberOfSpecies=2&speciesName1=&speciesPopulation1=1&speciesName2=&speciesPopulation2=0&acc= (retrieved: 21.04.2016).

Blackwell, M. (2011). The fungi: 1, 2, 3 … 5.1 million species? *American Journal of Botany, 98 (3),* 426-438.

Bojarczuk, K., Karlinski, L., Hazubska-Przybył, T. & Kieliszewska-Rokicka, B. (2015). Influence of mycorrhizal inoculation on growth of micropropagated Populus x canescens lines in metal-contaminated soils. *New Forests, 46,* 195–215.

Bonfante, P. & Anca, I. (2009). Plants, mycorrhizal fungi and bacteria: a network of interactions. *Annual Review of Microbiology, 63,* 363-383.

Boutwell R. & Bosch, D. (1958). The carcinogenicity of creosote oil: its role in the induction of skin tumors in mice. *Cancer Research, 18,* 1171-1175.

Brender, J., Pichette, J., Suarez, L., Hendricks, K. & Holt, M. (2003). Health risks of residential exposure to polycyclic aromatic hydrocarbons. *Archives of Environmental Health, 58 (2),* 111-118.

Brundrett, M. (2009). Mycorrhizas in natural ecosystems. *Advances in Ecological Research, 21,* 171-313.

Bumpus, J., Tien, M., Wright, D. & Aust, S. (1985). Oxidation of persistent environmental pollutants by a white rot fungus. *Science, 224,* 1434–1436.

Burgas, D., Byholm, P. & Parkkima, T. (2014). Raptors as surrogates of biodiversity along a landscape gradient. *Journal of Applied Ecology, 2014,* 1-9.

Cabello, M. (2001). Mycorrhizas and hydrocarbons. In: Gadd, G. (ed.). *Fungi in bioremediation* (456-473). Cambridge: Cambridge University Press.

Cabrita, P. (2013). The shaggy ink cap, *Coprinus comatus*: an unsuspitious predator. (w.p.) www.hubpages.com/education/The-Shaggy-Ink-Cap (retrieved: 09.04.2016).

Chibuike, G. (2013). Use of mycorrhiza in soil remediation: a review. *Academic Journals, 8(35),* 1679-1687.

Clark, R. & Zeto, S. (2000). Mineral acquisition by arbuscular mycorrhizal plants. *Journal of Plant Nutrition, 23,* 867-902.

Clavel, M. (2013). Use of aspen trees for cleaning polluted soil at Luumäki. Unpublished practical training report: Luke.

Columbia electronic encyclopedia, 6th edition (2015). Creosote. (w.p.).

Convention on biological diversity, June 5, 1992.

Cook, R. & Hesterberg, D. (2013). Comparison of trees and grasses for rhizoremediation of petroleum hydrocarbons. *International Journal of Phytoremediation, 15 (9),* 844-860.

Cripps, C. (2003). Native mycorrhizal fungi with aspen on smelter-impacted sites in the Northern Rocky Mountains: occurrence and potential use in reclamations. Report on 2003 National Meeting of the American Society of Mining and Reclamation and the 9th Billings Land Reclamation Symposium. Lexington: ASMR. www.plantsciences.montana.edu/facultyorstaff/faculty/cripps/0193-Cripps.pdf (retrieved: 28.03.2016).

Dahlstrom, J., Smith, J. & Weber, N. (2000). Mycorrhiza-like interaction by *Morchella* with species of the *Pinaceae* in pure culture synthesis. *Mycorrhiza, 9 (5*), 279-285.

Dell, B. (2002). Role of mycorrhizal fungi in ecosystems. *Chiang Mai University Journal of Natural Sciences. 1(1),* 47-60.

Dickie, I. & Reich, P. (2005). Ectomycorrhizal fungal communities at forest edges. *Journal of Ecology, 93,* 244–255.

Dittmann J., Heyser, W. & Bucking, H. (2002). Biodegradation of aromatic compounds by white rot and ectomycorrhizal fungal species and the accumulation of chlorinated benzoic acid in ectomycorrhizal pine seedlings. *Chemosphere, 49,* 297–306.

Dushenkov, S. (2003). Trends in phytoremediation of radionuclides. *Plant and Soil, 249 (1),* 167-175.

EC (1999). Opinion (revised) on cancer risk to consumers from creosote containing less than 50 ppm benzo-[a]-pyrene and/or from wood treated with such creosote and estimation of respective magnitude expressed at the 8th CSTEE plenary meeting, Brussels, 4 March 1999. (w.p.): European Commission. www.ec.europa.eu/health/scientific_committees/environmental_risks/opinions/sctee/sct_out29_en.htm (retrieved: 13.02.2016).

EC regulation concerning registration, evaluation, authorisation and restriction of chemicals (REACH), December 18, 1907/2006.

EEA (2014). Progress in management of contaminated sites. (w.p.): European Environment Agency. www.eea.europa.eu/data-and-maps/indicators/progress-in-management-of-contaminated-sites-3/assessment (retrieved: 06.02.2016).

EPA (1988). Integrated Risk Information System (IRIS): chemical assessment summary. Creosote, CASRN 8001-58-9. (w.p.): National Center for Environmental Assessment. www.cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0360_summary.pdf (retrieved: 13.02.2016).

Etelä-Suomen aluehallintoviraston päätös, 176/2012/1, dnro ESAVI/235/04.08/2011.

Etim, E. (2012). Phytoremediation and its mechanisms: a review. *International Journal of Environment and Bioenergy, 2 (3),* 120-136.

Finlay, R. (2008). Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. *Journal of Experimental Botany, 59 (5),* 1115–1126.

Fragoeiro, S. (2005). *Use of fungi in bioremediation of pesticides. Thesis for a PhD degree*. (w.p.): Cranfield University, Applied Mycology Group Institute of Bioscience and Technology. www.dspace.lib.cranfield.ac.uk/bitstream/1826/906/2/Fragoeiro%20thesis.pdf (retrieved: 17.04.2016).

Gadd. G. (2001). Metal transformations. In: Gadd, G. (ed.). *Fungi in bioremediation* (359-382). Cambridge: Cambridge University Press.

Gerhardt, K., Huang, X., Glick, B. & Greenberg B. (2009). Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. *Plant Science, 176,* 20-30.

Gramms, G., Voight, K. & Kirsche, B. (1999). Degradation of polycyclic aromatic hydrocarbons with three to seven aromatic rings by higher fungi in sterile and unsterile soils. *Biodegradation, 10 (1),* 51-62.

Gunderson, J., Knight, J. & Van Rees, K. (2007). Impact of ectomycorrhizal colonization of hybrid poplar on the remediation of diesel-contaminated soil. *Journal of Environmental Quality, 36(4),* 927-934.

Hanninka, N., Rossera, S. & Brucea, N. (2002). Phytoremediation of explosives. *Critical Reviews in Plant Sciences, 21 (5),* 511-538.

Hassett, B. & Gradinger, R. (2016). Chytrids dominate Arctic marine fungal communities. *Environmental Microbiology, February,* 4-13.

Holme, J., Refsnes, M. & Dybing, E. (1999). Possible carcinogenic risk associated with production and use of creosote-treated wood. *Tidsskrift for den Norske lægeforening: tidsskrift for praktisk medicin, ny række, 119 (18),* 2664-2666.

Iram, S., Ahmad, I., Javed, B., Yaqoob, S., Akhtar, K., Kazmi, M. & Uz-Zaman, B. (2009). Fungal tolerance to heavy metals. *Pakistan Journal of Botany, 41(5),* 2583-2594.

Isikhuemhen, O., Anoliefo, G. & Oghale, O. (2003). Bioremediation of crude oil polluted soil by the white rot fungus, *Pleurotus tuberregium* (Fr.) Sing. *Environmental Science and Pollution Research International, 10 (2),* 108-112.

Jentschke, G., Brandes, B., Kuhn, A., Schröder, W., Becker, J. & Godbold, D. (2000). The mycorrhizal fungus *Paxillus involutus* transports magnesium to Norway spruce seedlings. Evidence from stable isotope labeling. *Plant and Soil, 220 (1),* 243-246.

Johnsen, A., Wick, L. & Harms, H. (2005). Principles of microbial PAH-degradation in soil. *Environmental Pollution, 133(1),* 71-84.

Johnson, P. & Mosco, R. (2010). Mycoremediation. In: *Encyclopedia of Earth*. (w.p.) www.eoearth.org/view/article/154739 (retrieved: 30.03.2016).

Joner E. & Leyval C. (2003). Phytoremediation of organic pollutants using mycorrhizal plants: a new aspect of rhizosphere interactions. *Agronomie, 23,* 495–502.

Joner E., Leyval C. & Colpaert J. (2006). Ectomycorrhizas impede phytoremediation of polycyclic aromatic hydrocarbons (PAHs) both within and beyond the rhizosphere. *Environmental Pollution, 142,* 34-38.

Kammer, A. & Poel, W. (1957). Experimental carcinogenicity of coal-tar fractions: the carcinogenicity of creosote oils. *Journal of the National Cancer Institute, 18(1),* 41-55.

Khade, S. & Adholeya, A. (2007). Feasible bioremediation through arbuscular mycorrhizal fungi imparting heavy metal tolerance: a retrospective. *Bioremediation Journal, 11 (1),* 33-43.

Kibbey, G. & Fatto, R. (2002). On-line synoptic key to the species of *Russula* in North America. (w.p.) www2.muse.it/russulales-news/id_kibby_fatto.asp (retrieved: 08.04.2016).

Kim, M., Lee, H., Choi, Y., Kim, G., Huh, N., Lee, S., Lim, Y., Lee, S. & Kim, J. (2010). Diversity of fungi in creosote-treated crosstie wastes and their resistance to polycyclic aromatic hydrocarbons. *Antonie van Leeuwenhoek, 97 (4)),* 377-387.

Koivula, T., Salkinoja-Salonen, M., Peltola, R. & Romantschuk, M. (2004). Pyrene degradation in forest humus microcosms with or without pine and its mycorrhizal fungus. *Journal of Environmental Quality, 33 (1),* 45-53.

Korade, D. & Fulekar, M. (2009). Development and evaluation of mycorrhiza for rhizosphere bioremediation. *Journal of Applied Biosciences, 17,* 922 – 929.

Krpata, D., Peintner, U., Langer, I., Fitz, W. & Schweiger, P. (2008). Ectomycorrhizal communities associated with *Populus tremula* growing on a heavy metal contaminated site. *Mycological Research, 112 (9),* 1069-1079.

Kuo, M. (2006). *Glossary of mycological terms.* (w.p.) www.mushroomexpert.com/glossary.html (retrieved: 23.02.2016).

Kuo, M. (2007). *Key to major groups of mushrooms.* (w.p.) www.mushroomexpert.com/major_groups.html (retrieved: 10.04.2016).

Kuo, M. (2008). *Key to major groups of mushrooms: Paxillus involutus.* (w.p.) www.mushroomexpert.com/paxillus_involutus.html (retrieved: 12.09.2015).

Kuo, M. (2012a). *Key to major groups of mushrooms: Morchella angusticeps.* (w.p.) www.mushroomexpert.com/morchella_angusticeps.html (retrieved: 16.04.2016).

Kuo, M. (2012b). *Key to major groups of mushrooms: Morchella esculentoides.* (w.p.) www.mushroomexpert.com/morchella_esculentoides.html (retrieved: 16.04.2016).

Kuo, M. (2012c). Key *to major groups of mushrooms: Morchella punctipes.* (w.p.) www.mushroomexpert.com/morchella_punctipes.html (retrieved: 16.04.2016).

Laessoe, T. (ed.) (2002). *Smithsonian handbooks: mushrooms.* New York: Dotling Kindersley.

Lahti, M. (ed.) (toim.) (2013). *Sieniopas*. Porvoo: Bookwel Oy.

Leonard, P. & MacAdam, A. (2008). *Lactarius synoptic keys to British species of Lactarius*.(w.p.) www.britmycolsoc.org.uk/files/7112/8294/3516/Lactarius%20Synoptic%20Key%20by%20Patrick%20Leonard.pdf (retrieved: 08.04.2016).

Leyval, C., Joner, E., de Val, C. & Haselwandter, K. (2002). Potential of arbuscular mycorrhizal fungi for bioremediation. In: Gianinazzi, S., Schüepp, H., Barea, J. & Haselwandter, K. (eds.). *Mycorrhizal technology in agriculture: from genes to bioproducts* (175-186). Basel: Springer Basel AG.

Ling, J., Zhang, Y., Wu, M., Wang, Y., Dong, J., Jiang, Y., Yang, Q. & Zeng, S. (2015). Fungal community successions in rhizosphere sediment of seagrasses *Enhalus acoroides* under PAHs stress. *International Journal of Molecular Sciences, 16 (6),* 14039-14055.

Lu, Y. & Lu, M. (2015). Remediation of PAH-contaminated soil by the combination of tall fescue, arbuscular mycorrhizal fungus and epigeic earthworms. *Journal of Hazardous Materials, 285,* 535-541.

Luo, Z., Wu, C., Zhang, C., Li, H., Lipka, U. & Polle, A. (2014). The role of ectomycorrhizas in heavy metal stress tolerance of host plants. *Environmental and Experimental Botany, 108,* 47-62.

Macci, C., Peruzzi, E., Doni, S., Poggio, G. & Masciandaro, G. (2016). The phytoremediation of an organic and inorganic polluted soil: A real scale experience. *International Journal of Phytoremediation, 18 (4),* 378-386.

Macek, T., Mackova, M. & Kas, J. (2000). Exploitation of plants for the removal of organics in environmental remediation. *Biotechnology Advances, 18,* 23-34.

Maloney, S. (2001). Pesticide degradation. In: Gadd, G. (ed.). *Fungi in bioremediation* (188-223). Cambridge: Cambridge University Press.

Marschner, H. & Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil, 159,* 89-102.

Mauseth, J. (2014). *Botany: an introduction to plant biology*. Burlington: Jones & Bartlett Learning.

McConnell, O. (2014). *Unicoi unity: a natural history of the Unicoi and Snowbird mountains and their plants, fungi and animals*. Bloomington: Author House.

Meharg, A. (2001). The potential of utilizing mycorrhizal associations in soil bioremediation. In: Gadd, G. (ed.). *Fungi in bioremediation* (445-455). Cambridge: Cambridge University Press.

Meharg A. & Cairney J. (2000). Ectomycorrhizas – extending the capacities of rhizosphere remediation? *Soil Biology and Biochemistry, 32,* 1475–1484.

Meharg A., Cairney J. & Maguire N. (1997). Mineralization of 2,4-dichlorophenol by ectomycorrhizal fungi in axenic culture and in symbiosis with pine. *Chemosphere, 34,* 2495–2504.

Metla (2012). General plan of rehabilitation. (w.p.)

Metsähallitus (2015). *Wild berries and mushrooms*. (w.p.): Metsähallitus. www.nationalparks.fi/hikinginfinland/whattosee/treesandplants/wildberriesandmushrooms (retrieved: 09.04.2016).

MiCC (2016). Microfungi. (w.p.): Microfungi Collections Consortium. www.microfungi.org (retrieved: 23.02.2016).

Misra, J., Tewari, J., Deshmukh, S. & Vágvölgyi C. (eds.) (2014). *Fungi from different substrates*. Boca Raton: CRC Press, Taylor and Francis group.

Molina, R. & Horton, T. (2015). Mycorrhizal specificity: its role in the development and function of common mycelial networks. In: Horton, T. (ed.). *Ecological studies 224*: *Mycorrhizal networks.* Dordrecht: Springer.

Morikawa, H. & Ozgür C. (2003). Basic processes in phytoremediation and some applications to air pollution control. *Chemosphere, 52 (9),* 1553-1558.

Mukherjee, S. (2014). *Successional and spatial patterns of bacterial communities in hydrocarbon-contaminated soils and Populus rhyzosphere: academic dissertation*. Helsinki: University of Helsinki.

Ndimele, P. (2010). A review of phytoremediation of petroleum hydrocarbons. *Pakistan Journal of Biological Sciences, 13 (15),* 715-722.

Nishida, F. (1989). Key to the species of *Inocybe* in California. *Mycotaxon, 34(1),* 181–196.

Nordic Recipe Archive (2014). *Finnish mushrooms.* (w.p.) www.dlc.fi/~marianna/gourmet/i_mushro.htm (retrieved: 09.04.2016).

Norton, J. (2012). Fungi for bioremediation of hydrocarbon pollutants. *Hohonu, 10,* 18-21.

Ortega-Martínez, P., Agueda, B., Fernández-Toirán, L. & Martínez-Peña, F. (2011). Tree age influences on the development of edible ectomycorrhizal fungi sporocarps in *Pinus sylvestris* stands. *Mycorrhiza, 21 (1),* 65-70.

Panagos, P., Van Liedekerke, M., Yigini, Y. & Montanarella, L. (2013). Contaminated sites in Europe: review of the current situation based on data collected through a European network. *Journal of Environmental and Public Health, 2013,* 1-11.

Pilipovic, A., Orlovic, S., Roncevic, S., Nikolic, N., Zupunski, M. & Spasojevic, J. (2015). *Agriculture & Forestry / Poljoprivreda i Sumarstvo, 61 (4),* 205-211.

Polcaro, C., Brancaleoni, E., Donati, E., Frattoni, M., Galli, E., Migliore, L. & Rapanà, P. (2008). Fungal bioremediation of creosote-treated wood: a laboratory scale study on creosote components degradation by *Pleurotus ostreatus* mycelium. *Bulletin of Environmental Contamination and Toxicology, 81 (2),* 180-184.

Preston, D. & Johnson, P. (2010). Ecological consequences of parasitism. *Nature Education Knowledge, 3 (10),* 47. www.nature.com/scitable/knowledge/library/ecological-consequences-of-parasitism-13255694 (retrieved: 17.04.2016).

Pringle, A., Wolfe, B. & Vellinga, E. (2011). Mycorrhizae. In: Simberloff, D. & Rejmanek, M. (eds.). *Encyclopedia of biological invasions* (468-471), Berkley and Los Angeles: University of California Press.

Purvis, A. & Hector, A. (2000). Getting the measure of biodiversity. *Nature, 405,* 212-219.

Pyy, O., Haavisto, T., Niskala, K. & Silvola, M. (2013) (toim.). Pilaantuneet maa-alueet. Katsaus 2013. Technical report September 2013 (Contaminated land in Finland – report 2013: reports of the Finnish Environment Institute 27/2013). Helsinki: Suomen ympäristökeskus.

R Development Core Team (2016). R: a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria.

Reddy, C. & Mathew, Z. (2001). Bioremediation potential of white rot fungi. In: Gadd, G. (ed.). *Fungi in bioremediation* (52-78). Cambridge: Cambridge University Press.

Rhodes, C. (2014). Mycoremediation (bioremediation with fungi) – growing mushrooms to clean the earth. *Chemical Speciation and Bioavailability, 26 (3),* 196-198.

Roberts, L. (2006). *Molecular analysis of the rhizosphere fungal diversity on a regenerating Louisiana oil brine spill: perspectives in biorestoration. Thesis for a degree of Master of Science*. (w.p.): Louisiana Tech University, College of Applied and Natural Sciences.www.researchgate.net/publication/238088765_Molecular_Analysis_of_the_Rhizosphere_Fungal_Diversity_on_a_Regenerating_Louisiana_Oil_Brine_Spill_Perspectives_in_Biorestoration (retrieved: 17.04.2016).

Robinson, C. (2001). Cold adaptation in Arctic and Antarctic fungi. *New Phytologist, 151 (2),* 341-353.

Sadiq, S., ul Haq, M., Ahmad, I., Ahad, K., Rashid, A. & Rafiq, N. (2015). Bioremediation potential of white rot fungi *Pleurotus spp.* against organochlorines. *Journal of Bioremediation and Biodegredation, 6 (5),* 1-6.

Sarma, H. (2011). Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. *Journal of Environmental Science and Technology, 4 (2),* 118-138.

Šašek, V. (2003). Why mycoremediations have not yet come into practice. In: Šašek, V., Glaser, J. & Baveye, P. (eds.). *The utilization of bioremediation to reduce soil contamination: problems and solutions, NATO Science Series, 19,* 247-266. (w.p.): Springer Netherlands.

Scrimshaw, L. (2016). Decomposers - bacteria engines of earth's nutrient cycles. (w.p.) www.the-compost-gardener.com/decomposers.html (retrieved: 24.02.2016).

Sell, J., Kayser, A., Schulin, R. & Brunner, I. (2005). Contribution of ectomycorrhizal fungi to cadmium uptake of poplars and willows from a heavily polluted soil. *Plant and Soil, 277 (1/2),* 245-253.

Serniglia, C. & Sutherland, J. (2001). Bioremediation of polycyclic aromatic hydrocarbons by ligninolytic and non-ligninolytic fungi. In: Gadd, G. (ed.). *Fungi in bioremediation* (136-187). Cambridge: Cambridge University Press.

Shahsavari, E., Adetutu, E., Taha, M. & Ball, A. (2015). Rhizoremediation of phenanthrene and pyrene contaminated soil using wheat. *Journal of Environmental Management, 155,* 171-176.

Shukla, K., Sharma, S., Singh, N., Singh, V., Bisht, S. & Kumar, V. (2013). Rhizoremediation: a promising rhizosphere technology. In: Patil, Y. & Rao, P. (eds.). *Applied bioremediation - active and passive approaches* (333-352). (w.p.): InTech.

Sibley, P., Harris, M., Bestari, K., Steele, T., Robinson, R., Gensemer, R., Day, K. & Solomon, K. (2004). Response of zooplankton and phytoplankton communities to creosote-impregnated Douglas fir pilings in freshwater microcosms. *Archives of Environmental Contamination and Toxicology, 47 (1),* 56-66.

Sillanpää, P. (2007) (toim.). *Öljyhiilivedyillä saastuneen maan puhdistaminen puiden avulla. Suomen ympäristö 2.91*. (w.p.): Pirkanmaan ympäristökeskus.

Singh, H. (2006). *Mycoremediation: fungal bioremediation.* Hoboken: John Wiley and Sons, Inc.

Singh, M., Vishwakarma, S. & Srivastava, A. (2013). Bioremediation of direct blue 14 and extracellular ligninolytic enzyme production by white rot fungi: *Pleurotus spp.*. *BioMed Research International, 2013,* 1-4.

Smith, P. (2008). Risks to human health and estuarine ecology posed by pulling out creosote-treated timber on oyster farms. *Aquatic Toxicology, 86 (2),* 287-298.

Smith, W. (1970). Root exudates of seedling and mature sugar maple. *Phytopathology, 60,* 701-703.

Soares, A., Albergaria, J., Domingues, V., De Marco, P. & Delerue-Matos, C. (2012). Sequential soil vapor extraction and bioremediation processes applied to BTEX-contaminated soils. In: Goltapeh, E., Danesh, Y. & Varma, A. *Fungi as bioremediators: Soil Biology series, 32,* 181-201.

Stamets, L. (2012). *Best mycorestoration practices for habitat restoration of small land parcels. Thesis for a degree of Master of Environmental Studies*. Olympia: Evergreen State College. www.archives.evergreen.edu/masterstheses/Accession86-10MES/Stamets_L-MESthesis2012.pdf (retrieved: 17.04.2016).

Steffen, K., Hatakka A. & Hofrichter, M. (2002). Removal and mineralization of polycyclic aromatic hydrocarbons by litter-decomposing basidiomycetous fungi. *Applied Microbiology Biotechnology, 60,* 212–217.

Susarla, S., Medina, V. & McCutcheon, S. (2002). Phytoremediation: an ecological solution to organic chemical contamination. *Ecological Engineering, 18 (5),* 647-658.

Sytar, O., Brestic, M., Taran, N. & Zivcak, M. (2016). Plants used for biomonitoring and phytoremediation of trace elements in soil and water. In: Ahmad, P. (ed.). *Plant metal interaction. Emerging remediation techniques*. Amsterdam: Elsevier.

Tobin, J. (2001). Fungal metal biosorption. In: Gadd, G. (ed.). *Fungi in bioremediation* (424-444). Cambridge: Cambridge University Press.

Turnau, K., Orlowska, E., Ryszka, P., Zubek, S., Anielska, T., Gawronski, S. & Jurkiewicz, A. (2006). Role of mycorrhizal fungi in phytoremediation and toxicity monitoring of heavy metal rich industrial wastes in Southern Poland. In: Twardowska, I.,

Allen, H., Häggblom, M. & Stefaniak, S. (eds.). *Soil and water pollution monitoring, protection and remediation, 69,* 533-551. Dordrecht: Springer.

Vallinkoski, V.-M., Hassinen, V. & Servomaa, K. (2007) (toim.). *Hybridihaapa metalleilla pilaantuneen alueen kasvikunnostuksessa*. *Pohjois-Savon ympäristökeskuksen raportteja 2/2007*. Kuopio: Pohjois-Savon ympäristökeskus.

Valtioneuvoston asetus maaperän pilaantuneisuuden ja puhdistustarpeen arvioinnista, March 1, 214/2007.

Vervaeke, P. (2003). Phytoremediation prospects of willow stands on contaminated sediment: a field trial. *Environmental pollution, 126 (2),* 275-282.

Vines, C., Robbins, T., Griffin, F. & Cherr, G. (2000). The effects of diffusible creosote-derived compounds on development in Pacific herring (*Clupea pallasi*). *Aquatic Toxicology, 51 (2),* 225-239.

Wenzel, W. (2009). Rhizosphere processes and management in plant assisted bioremediation (phytoremediation) of soils. *Plant and Soil, 321,* 385-408.

Winquist, E. (2014). The potential of ligninolytic fungi in bioremediation of contaminated soil. Aalto University Publication Series: doctoral dissertations 54/2014. Helsinki: Unigrafia Oy.

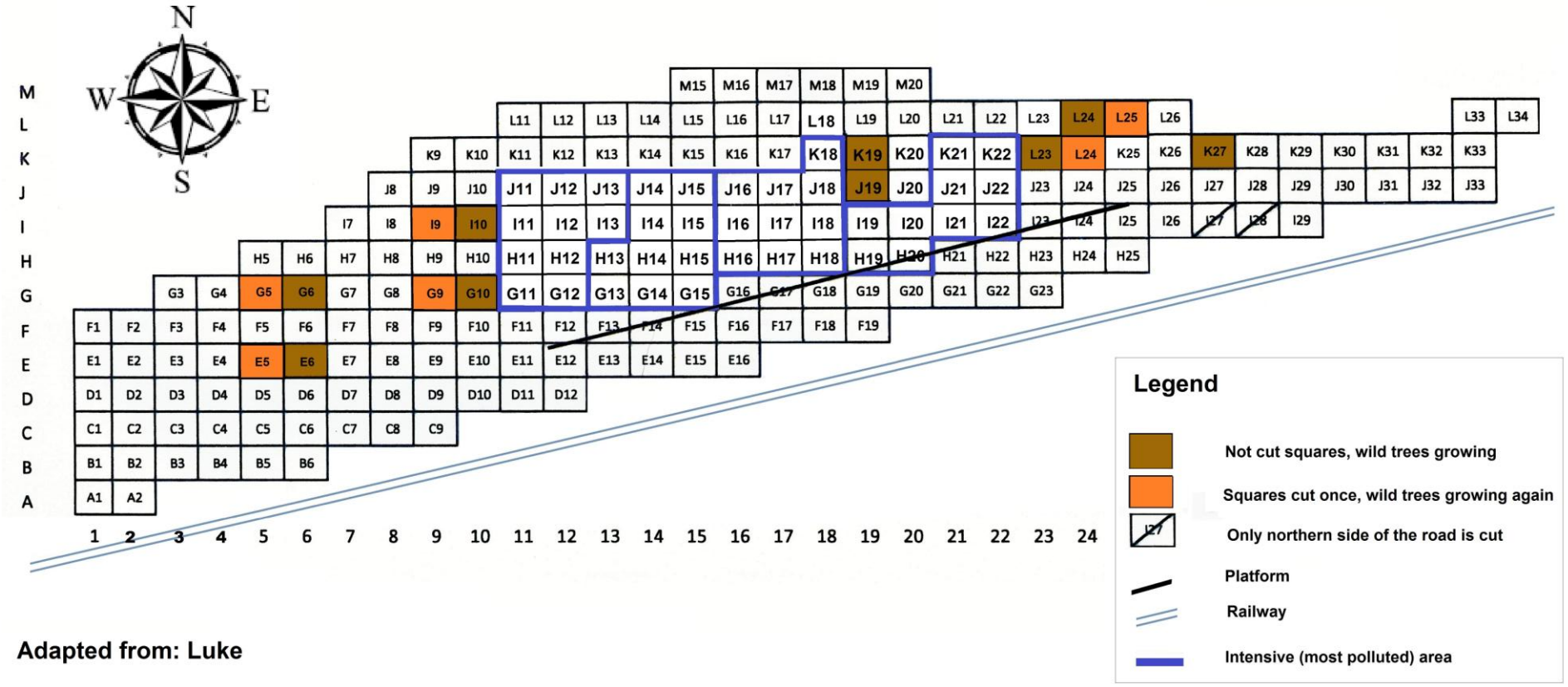
Winquist, E., Björklöf, K., Schultz, E., Räsänen, M., Salonen, K., Anasonye, F., Cajthaml, T., Steffen, K., Jørgensen, K. & Tuomela, M. (2014). Bioremediation of PAH-contaminated soil with fungi – from laboratory to field scale. *International Biodeterioration and Biodegradation, 86, Part C,* 238–247.

Wu, F., Yu, X., Wu, S. & Wong, M. (2014). Effects of inoculation of PAH-degrading bacteria and arbuscular mycorrhizal fungi on responses of ryegrass to phenanthrene and pyrene. *International Journal of Phytoremediation, 16 (2),* 109-122.

Yang, K., Yoon, J., Ryu, H., Chung, D., Nam, T. & Nam, K. (2013). Potential health risk of reused creosote-treated old railway ties at recreational sites in Korea. *Human and Ecological Risk Assessment, 19 (3),* 778-791.

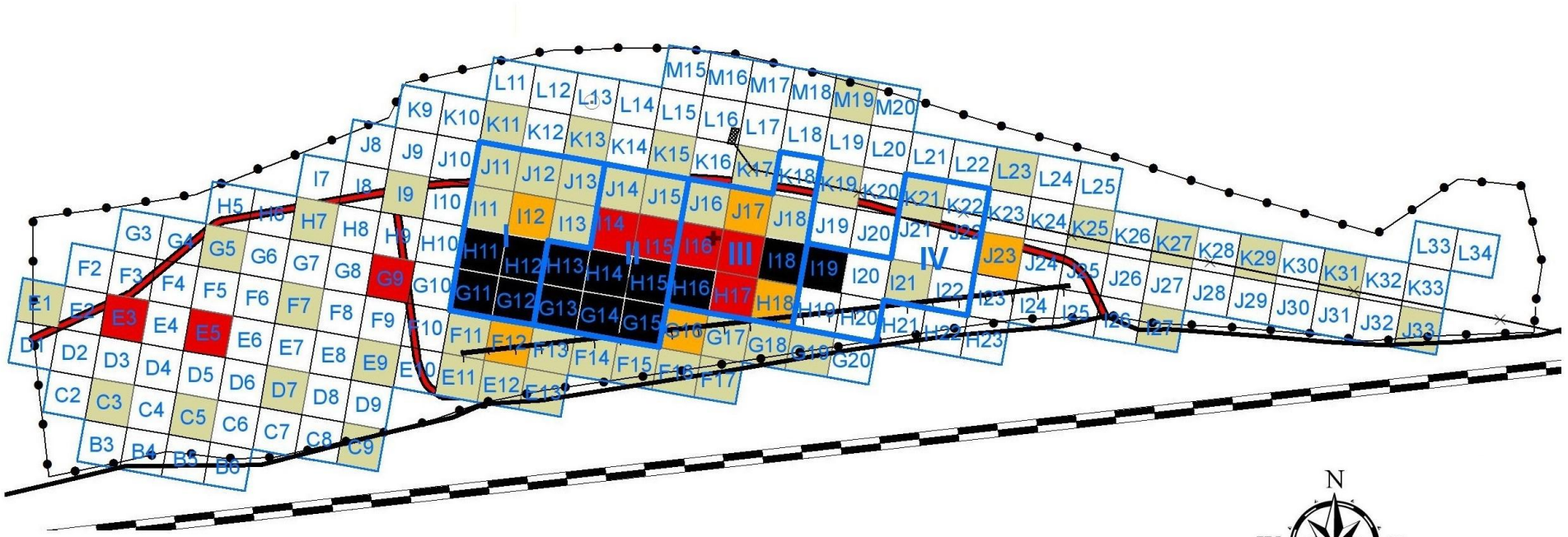
Афонкин, С. (ed.). (2014). Грибы России: большая иллюстрированная энциклопедия. Vilnus: UAB Bestiary.

Appendix 1. Somerharju phytoremediation project site: square coordinates and cuttings



Adapted from: Luke

Appendix 2. PAH-pollution levels on Somerharju project site



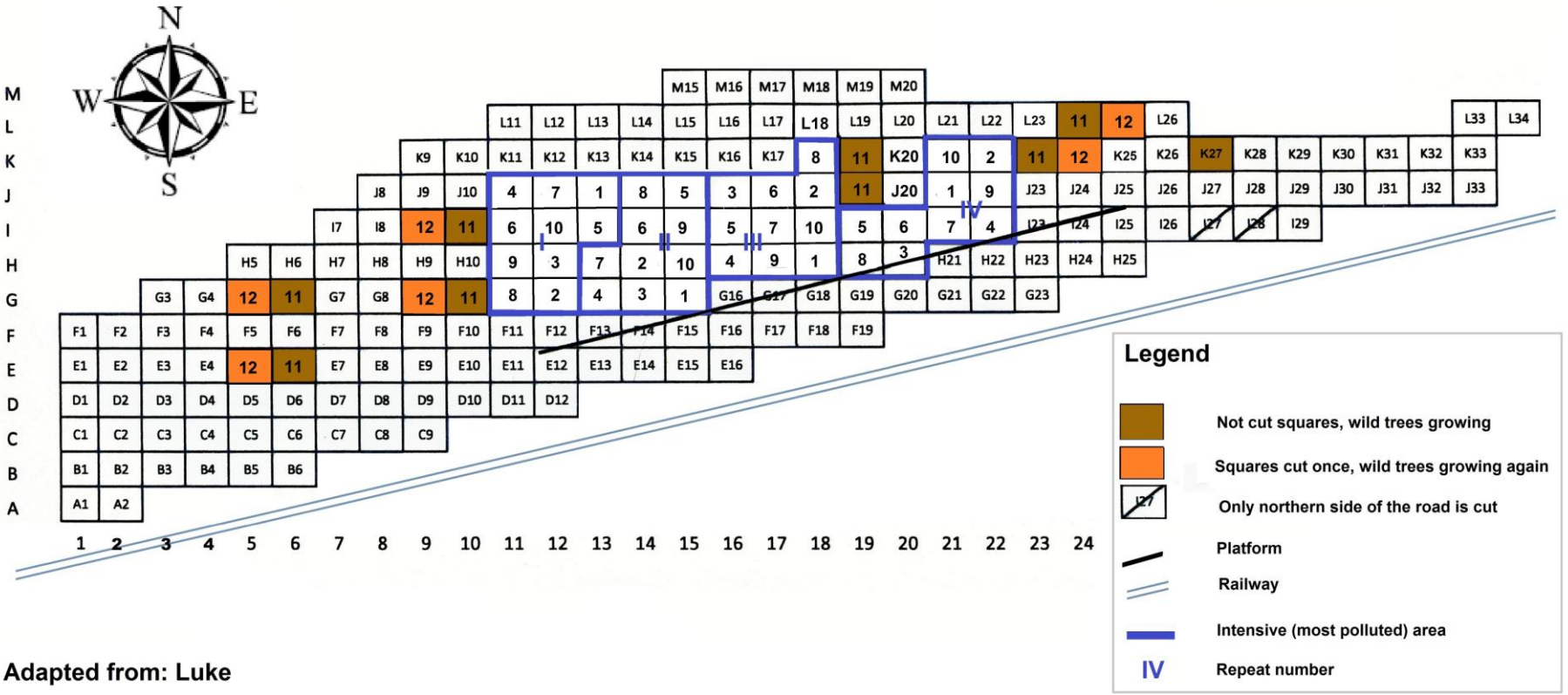
Pollution level	Colour on the map	Total PAH, mg/kg	PIMA-values
0		0,16 – 15	15 referred as threshold value
1		> 15 – 30	30 " as lower reference value
2		> 30 – 100	100 " as upper reference value
3		> 100 – 1000	1000 " as toxic waste limiting value

buffer
repetition 1
repetition 2
repetition 3
repetition 4
base water measuring point
lysimeter
pylon
soil moisture measuring point
fence
electric line
platform
house

1 : 3 500

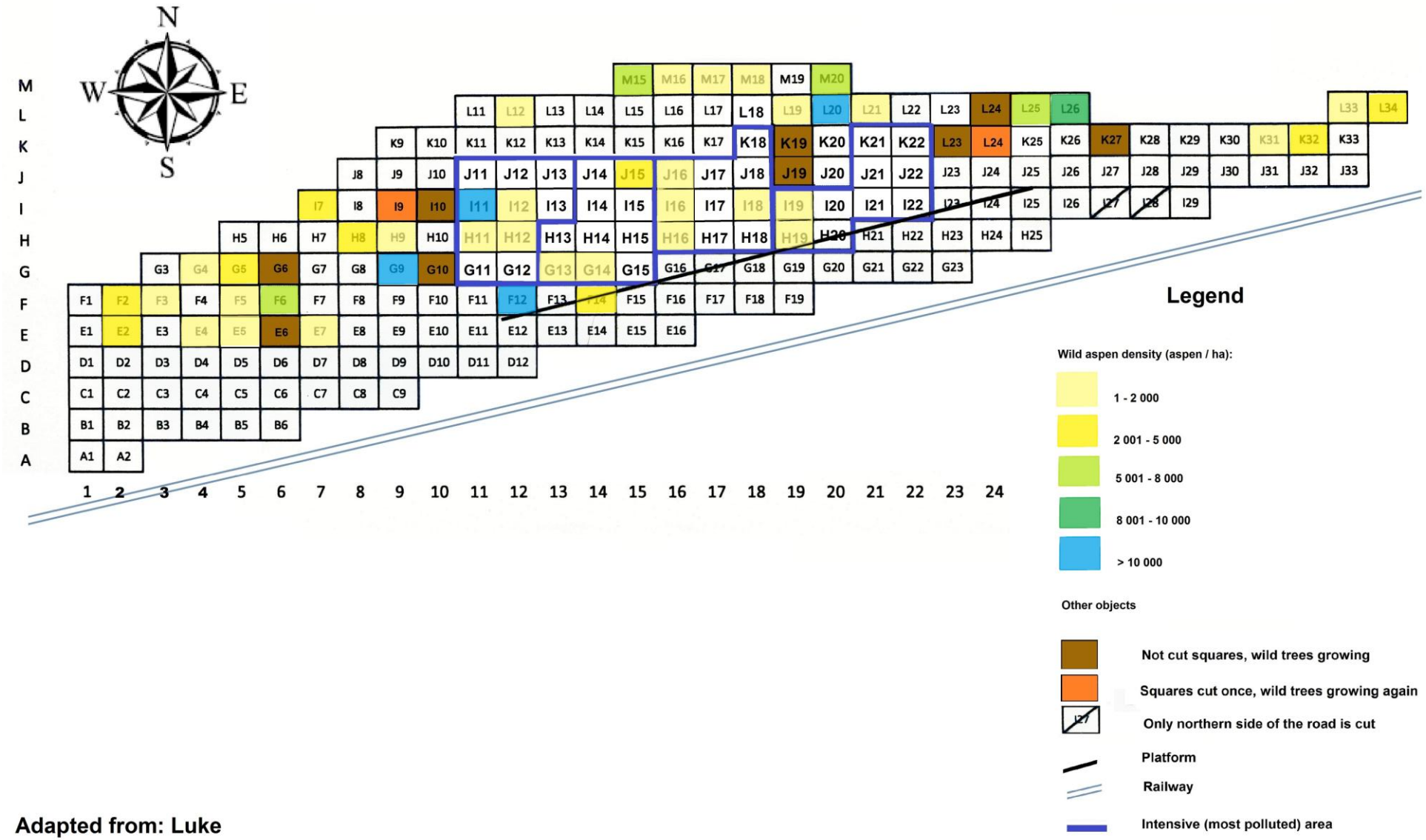
Adapted from: Luke

Appendix 3. Aspen clone arrangement in Somerharju: entries in the intensive and non-cut areas



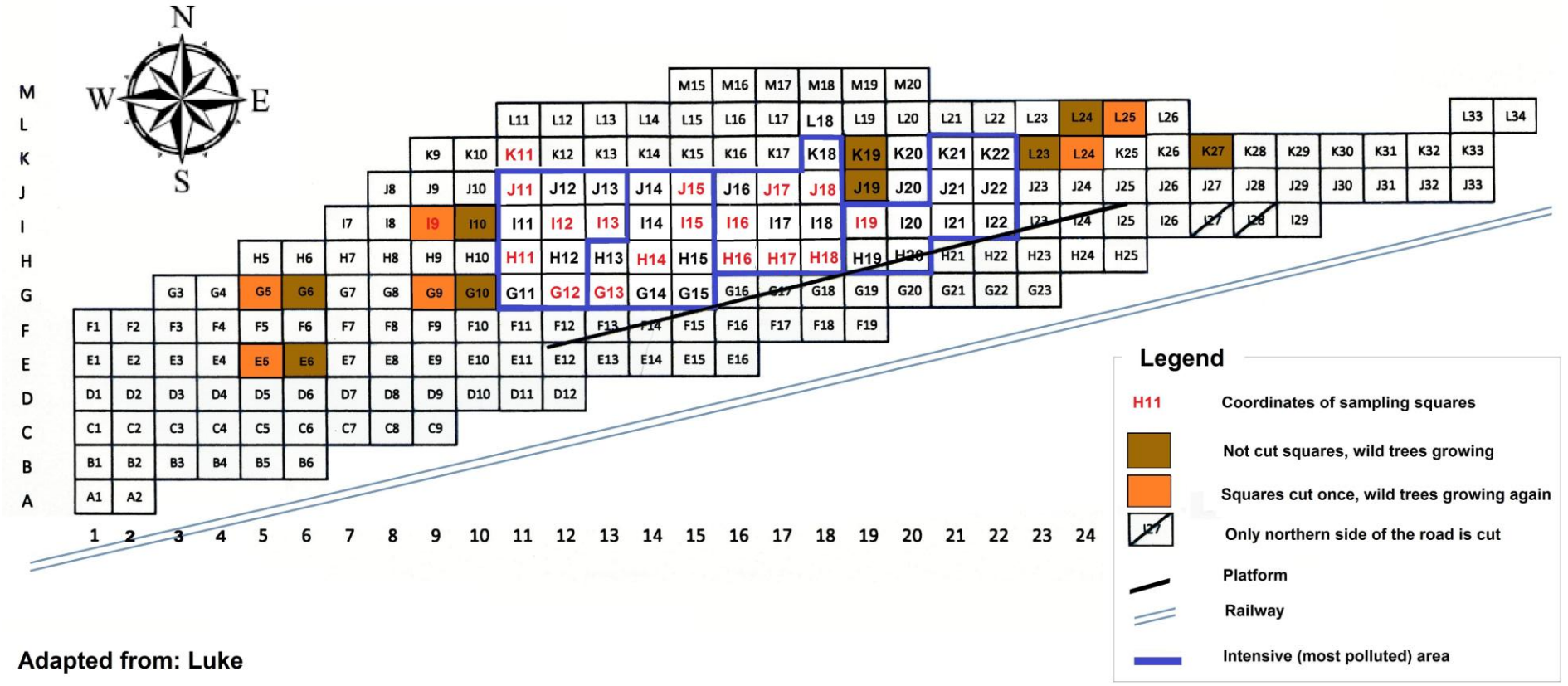
Adapted from: Luke

Appendix 4. Wild aspens density on Somerharju project site



Adapted from: Luke

Appendix 5. Mushroom sampling squares



Adapted from: Luke

Appendix 6. Data designed for a statistical analysis

Square	Pollution level	Cutting type	Month	Fruit bodies	Species richness	Diversity index
G12	3	2	1	0	0	0
G12	3	2	2	40	1	0
G12	3	2	3	0	0	0
G12	3	2	4	6	1	0
G12	3	2	5	1	1	0
G13	3	2	1	0	0	0
G13	3	2	2	0	0	0
G13	3	2	3	0	0	0
G13	3	2	4	2	1	0
G13	3	2	5	1	1	0
H11	3	1	1	0	0	0
H11	3	1	2	24	1	0
H11	3	1	3	2	2	0.6931
H11	3	1	4	107	6	1.641
H11	3	1	5	4	2	0.5623
H14	3	2	1	0	0	0
H14	3	2	2	3	1	0
H14	3	2	3	0	0	0
H14	3	2	4	22	3	1.544
H14	3	2	5	13	3	1.335
H16	3	2	1	0	0	0
H16	3	2	2	29	2	0.7355
H16	3	2	3	0	0	0
H16	3	2	4	9	3	1.224
H16	3	2	5	2	2	0.6931
H17	2	2	1	0	0	0
H17	2	2	2	87	1	0
H17	2	2	3	0	0	0
H17	2	2	4	30	1	0
H17	2	2	5	1	1	0
H18	1	2	1	0	0	0
H18	1	2	2	8	1	0
H18	1	2	3	0	0	0
H18	1	2	4	131	4	0.5689
H18	1	2	5	3	1	0
I12	1	2	1	0	0	0
I12	1	2	2	50	2	0.1414
I12	1	2	3	1	1	0
I12	1	2	4	0	0	0
I12	1	2	5	15	3	0.6998
I13	0	2	1	0	0	0
I13	0	2	2	15	1	0
I13	0	2	3	0	0	0
I13	0	2	4	42	2	0.65
I13	0	2	5	0	0	0

Table continued on the next page

Continuation of table. See beginning on previous page.

Square	Pollution level	Cutting type	Month	Fruit bodies	Species richness	Diversity index
I15	2	2	1	0	0	0
I15	2	2	2	4	1	0
I15	2	2	3	0	0	0
I15	2	2	4	3	2	0.9183
I15	2	2	5	3	2	0.9183
I16	2	2	1	0	0	0
I16	2	2	2	4	1	0
I16	2	2	3	0	0	0
I16	2	2	4	22	3	1.352
I16	2	2	5	95	2	0.7004
I19	3	1	1	0	0	0
I19	3	1	2	80	1	0
I19	3	1	3	1	1	0
I19	3	1	4	34	8	2.423
I19	3	1	5	12	4	1.626
I9	0	0	1	0	0	0
I9	0	0	2	92	6	1.539
I9	0	0	3	29	5	1.871
I9	0	0	4	278	18	1.926
I9	0	0	5	92	3	0.5807
J11	0	1	1	2	1	0
J11	0	1	2	8	1	0
J11	0	1	3	0	0	0
J11	0	1	4	33	4	1.329
J11	0	1	5	12	3	0.8167
J15	0	2	1	1	1	0
J15	0	2	2	18	1	0
J15	0	2	3	0	0	0
J15	0	2	4	57	8	2.206
J15	0	2	5	9	3	1.436
J17	1	2	1	0	0	0
J17	1	2	2	27	1	0
J17	1	2	3	0	0	0
J17	1	2	4	89	2	0.8567
J17	1	2	5	21	4	1.863
J18	0	1	1	0	0	0
J18	0	1	2	16	2	0.5436
J18	0	1	3	7	1	0
J18	0	1	4	176	7	1.310
J18	0	1	5	20	3	0.7476
K11	0	2	1	0	0	0
K11	0	2	2	21	1	0
K11	0	2	3	1	1	0
K11	0	2	4	65	4	1.357
K11	0	2	5	6	2	0.65

End of table

Appendix 7. Changes in a mycorrhizal species composition over a season

Species	May	Jun	Jul	Aug	Sep	Oct	Nov
Amanita rubescens			x				
Cantharellula umbonata					x	x	
Coltricia perennis			x	x	x		
Cortinarius mucosus					x	x	
Inocybe sp. 1 (presumedly *bongardii*)			x		x		
Inocybe sp. 2 (presumedly *napipes*)			x				
Inocybe sp. 3 (presumedly *geophylla*)				x			
Inocybe sp. 4 (presumedly *flocculosa*)					x	x	
Inocybe sp. 5 (presumedly *calospora*)					x		
Inocybe sp. 6 (presumedly *lacera*)		x	x	x	x	x	
Laccaria laccata					x	x	
Lactarius flexuosus					x	x	
Lactrarius rufus					x	x	
Lactarius torminosus					x		
Leccinum scabrum				x	x		
Paxillus involutus					x		
Russula sp. 1 (presumedly *grisea*)					x	x	
Russula sp. 2 (presumedly *badia*)					x		
Russula sp. 3 (presumedly *consobrina*)				x	x	x	
Russula sp. 4 (presumedly *mustelina*)					x		
Russula sp. 5 (presumedly *exalbicans*)					x		
Russula sp. 6 (presumedly *atropurpurea*)					x		
Russula sp. 7 (presumedly *betularum*)					x	x	
Russula sp. 8 (presumedly *versicolor*)				x	x		
Russula sp. 9 (presumedly *vinosa*)					x		
Russula sp. 10 (presumedly *aeruginea*)			x	x	x		
Suillus luteus				x	x		
Xerocomus subtomentosus			x				

Appendix 8. Mushroom abundance grouped by species and pollution level

The results for May and November are nulls and, thus, not shown in the table.

Pollution level	**Species**	Jun	Jul	Aug	Sep	Oct
Pollution 0						
	Cortinarius mucosus					2
	Inocybe sp 6 (presumedly *lacera*)	3	55		75	2
	Laccaria laccata				186	41
	Lactarius flexuosus					1
	Lactrarius rufus				7	6
	Paxillus involutus				2	
	Russula sp. 10 (presumedly *aeruginea*)		2			
	Russula sp. 2 (presumedly *badia*)				1	
	Russula sp. 7 (presumedly *betularum*)				5	
	Russula sp. 3 (presumedly *consobrina*)			7		
	Russula sp. 1 (presumedly *grisea*)				11	1
	Russula sp. 8 (presumedly *versicolor*)				2	
	Russula sp. 9 (presumedly *vinosa*)				7	
	Suillus luteus				11	
Number of species		**1**	**2**	**1**	**10**	**6**
Pollution 1						
	Cantharellula umbonata					8
	Coltricia perennis		1			
	Inocybe sp. 4 (presumedly *flocculosa*)					6
	Inocybe sp. 3 (presumedly *geophylla*)					
	Inocybe sp. 6 (presumedly *lacera*)		84	1	213	1
	Laccaria laccata				106	18
	Lactarius flexuosus					2
	Lactrarius rufus					4
	Paxillus involutus				1	
	Russula sp. 10 (presumedly *aeruginea*)				1	
Number of species		**0**	**2**	**1**	**4**	**6**
Pollution 2						
	Inocybe sp. 6 (presumedly *lacera*)		95		43	19
	Inocybe sp. 2 (presumedly *napipes*)					
	Laccaria laccata				8	80
	Lactarius flexuosus					
	Lactrarius rufus				3	
	Paxillus involutus				1	
	Russula sp. 10 (presumedly *aeruginea*)			1		
Number of species		**0**	**1**	**1**	**4**	**2**

Table continued on the next page

Continuation of table. See beginning on previous page.

Pollution level	**Species**	Jun	Jul	Aug	Sep	Oct
Pollution 3						
	Cortinarius mucosus					1
	Inocybe sp. 6 (presumedly *lacera*)		136		67	6
	Inocybe sp. 2 (presumedly *napipes*)		46		2	3
	Laccaria laccata				66	16
	Lactarius flexuosus				1	
	Lactrarius rufus				7	5
	Paxillus involutus				11	
	Russula sp. 10 (presumedly *aeruginea*)				5	
	Russula sp. 7 (presumedly *betularum*)				1	1
	Russula sp. 3 (presumedly *consobrina*)			1	1	3
	Russula sp. 1 (presumedly *grisea*)				6	
	Russula sp. 4 (presumedly *mustelina*)				4	
	Suillus luteus			1	2	
Number of species		**0**	**2**	**2**	**12**	**7**
Control I9						
	Amanita rubescens		3			
	Coltricia perennis		13	4	5	
	Cortinarius mucosus				1	1
	Inocybe sp. 1 (presumedly *bongardii*)		6		2	
	Inocybe sp. 5 (presumedly *calospora*)				1	
	Inocybe sp. 6 (presumedly *lacera*)		63		175	81
	Laccaria laccata				56	10
	Lactrarius rufus				1	
	Lactarius torminosus				11	
	Leccinum scabrum			1	1	
	Russula sp. 10 (presumedly *aeruginea*)		5	13	10	
	Russula sp 6 (presumedly *atropurpurea*)				1	
	Russula sp. 2 (presumedly *badia*)				2	
	Russula sp. 3 (presumedly *consobrina*)			2	2	
	Russula sp. 5 (presumedly *exalbicans*)				1	
	Russula sp. 1 (presumedly *grisea*)				6	
	Russula sp. 4 (presumedly *mustelina*)				1	
	Russula sp. 8 (presumedly *versicolor*)			9	5	
	Suillus luteus				1	
	Xerocomus subtomentosus		2			
Number of species		**0**	**6**	**5**	**18**	**3**
Control K11	*Cantharellula umbonata*				1	
	Inocybe sp. 3 (presumedly *geophylla*)			1		
	Inocybe sp. 6 (presumedly *lacera*)		21		38	5
	Laccaria laccata				21	1
	Lactrarius rufus				5	
Number of species		**0**	**1**	**1**	**4**	**2**

End of table

Appendix 9. Mushroom abundance grouped by species and cutting type

The results for May and November are nulls and, thus, not shown in the table.

Cutting type	**Species**	Jun	Jul	Aug	Sep	Oct
Non-cut areas						
	Amanita rubescens		3			
	Coltricia perennis		13	4	5	
	Cortinarius mucosus				1	1
	Inocybe sp 1 (presumedly *bongardii*)		6		2	
	Inocybe sp 5 (presumedly *calospora*)				1	
	Inocybe sp 6 (presumedly *lacera*)		63		175	81
	Laccaria laccata				54	10
	Lactrarius rufus				1	
	Lactarius torminosus				11	
	Leccinum scabrum			1	1	
	Russula sp. 10 (presumedly *aeruginea*)		5	13	8	
	Russula sp. 6 (presumedly *atropurpurea*)				1	
	Russula sp. 2 (presumedly *badia*)				2	
	Russula sp. 3 (presumedly *consobrina*)			2	2	
	Russula sp. 5 (presumedly *exalbicans*)				1	
	Russula sp. 1 (presumedly *grisea*)				6	
	Russula sp. 4 (presumedly *mustelina*)				1	
	Russula sp. 8 (presumedly *versicolor*)			9	5	
	Suillus luteus				1	
	Xerocomus subtomentosus		2			
Number of species		**0**	**6**	**5**	**18**	**3**
Next to non cut						
	Cortinarius mucosus					3
	Inocybe sp. 4 (presumedly flocculosa)					3
	Inocybe sp. 6 (presumedly *lacera*)	26	102		94	1
	Laccaria laccata				204	33
	Lactarius flexuosus				1	1
	Lactrarius rufus				8	5
	Paxillus involutus				5	
	Russula sp. 10 (presumedly *aeruginea*)		2	1	7	
	Russula sp. 7 (presumedly *betularum*)				1	1
	Russula sp. 3 (presumedly *consobrina*)			8	1	
	Russula sp. 1 (presumedly *grisea*)				12	1
	Russula sp. 4 (presumedly *mustelina*)				4	
	Russula sp. 8 (presumedly *versicolor*)				1	
	Suillus luteus			1	12	
Number of species		**1**	**2**	**3**	**12**	**8**

Table continued on the next page

Continuation of table. See beginning on previous page.

Cutting type	**Species**	Jun	Jul	Aug	Sep	Oct
Cut areas	*Cantharellula umbonata*				1	8
	Coltricia perennis		1			
	Inocybe sp. 4 (presumedly *flocculosa*)				2	6
	Inocybe sp. 3 (presumedly *geophylla*)			1		
	Inocybe sp. 6 (presumedly *lacera*)	1	259	1	277	30
	Inocybe sp. 2 (presumedly *napipes*)		40	6		
	Laccaria laccata				154	110
	Lactrarius rufus				14	10
	Lactarius flexuosus					2
	Paxillus involutus				10	
	Russula sp. 10 (presumedly *aeruginea*)				1	
	Russula sp. 2 (presumedly *badia*)				1	
	Russula sp. 7 (presumedly *betularum*)				5	
	Russula sp. 3 (presumedly *consobrina*)					3
	Russula sp. 1 (presumedly *grisea*)				5	
	Russula sp. 8 (presumedly *versicolor*)				1	
	Russula sp. 9 (presumedly *vinosa*)				7	
Number of species		**1**	**3**	**3**	**12**	**7**

End of table