The potential of fungi to improve surface water quality in Amsterdam: a review

Written by Godelieve Franssen (6987842)
Utrecht University Master programme Bio Inspired Innovation
Supervisor: Han Wösten

21/12/2022

Laymen's summary

Dit literatuuronderzoek presenteert een overzicht van de belangrijkste stoffen die het water in de grachten van Amsterdam vervuilen, alsmede onderzoek dat in de afgelopen decennia is gedaan naar het verwijderen van vervuilende stoffen uit water met behulp van schimmels. Amsterdam wordt hier gebruikt als voorbeeldcasus. De hoofdstad is representatief voor de rest van Nederland, dat onderaan de lijst staat van Europese landen op het gebied van waterkwaliteit. In het Nederlandse oppervlaktewater komen veel chemische stoffen voor in hoeveelheden die de Europese normen overschrijden. Om deze hoeveelheden terug te brengen, moeten strenge maatregelen genomen worden in de komende jaren om te zorgen dat de normen vóór 2027 – de Europese deadline – worden gehaald. De belangrijkste vervuilende stoffen in Nederland en in de vaarten van Amsterdam zijn vastgesteld en een overzicht daarvan wordt in dit paper gepresenteerd. Van de Europese stoffenlijst zijn drie soorten metalen, zeven soorten polyaromatische koolwaterstoffen (PAK's), tributyltin-kation en ammoniak en fosfaat de meest problematische stoffen. Vervolgens is gezocht in de literatuur naar wetenschappelijk onderzoek waarin deze chemische stoffen met behulp van schimmels uit water worden verwijderd. Schimmels blijken een veelbelovende oplossing voor de slechte waterkwaliteit, omdat zij zeer effectief verschillende soorten stoffen uit het water kunnen halen. Dit is te danken aan het feit dat schimmels relatief snel groeien en dat zij een hoog percentage aan celwandmateriaal bevatten met verschillende functionele groepen die vervuilende stoffen zoals metalen kunnen binden. Ook scheiden schimmels enzymen uit die vervuilende stoffen kunnen afbreken. Bovendien kunnen schimmels goed groeien in lastige omstandigheden zoals extremen in pH, lage nutriëntengehaltes of extreme temperaturen. Uit het literatuuronderzoek bleek dat bijna alle metalen en PAK's die in de vaarten van Amsterdam voorkomen door schimmels kunnen worden verwijderd, met name door witrotschimmels zoals *Pleurotus ostreatus*. Bij de meeste van de metalen werd zelfs meer dan 95% verwijderd. De andere kritieke stoffen - tributyltin-kation, stikstof en fosfaat - werden niet gevonden in literatuur over schimmels, maar voor de laatste twee stoffen is aangetoond dat andere natuurlijke filtratietechnieken, zoals helofytenfilters, zeer effectief zijn. Andere stoffen die niet in de Europese richtlijn voorkomen, maar wel zijn aangetoond in de Amsterdamse vaarten en problematisch worden geacht, zijn medicijnresten en de darmbacterie *Eschecheria coli*. Ook deze vervuilers kunnen grotendeels door schimmels worden verwijderd, zo toonde onderzoek aan. Voor bijna alle onderzochte stoffen geldt dat deze zowel in lab-omstandigheden als met echt afvalwater zijn getest. In beide soorten omstandigheden hadden de schimmels het gewenste effect. Dit is een belangrijk gegeven, want het betekent dat de implementatie in echte rioolwaterzuiveringsinstallaties binnen handbereik ligt. Rioolwaterzuiveringsinstallaties filteren al een groot deel van de vervuilende stoffen uit rioolwater, maar het uitstromend water van deze installaties is nog steeds de belangrijkste bron van watervervuiling. Als schimmels zouden worden gebruikt als extra filters van het rioolwater, zou dat directe positieve invloed hebben op de kwaliteit van het oppervlaktewater. Al met al toont dit literatuuronderzoek aan dat schimmels een relatief goedkope, gemakkelijke en natuurlijke oplossing zouden kunnen vormen voor de slechte chemische waterkwaliteit in Amsterdam.

Abstract

This review presents the most important pollutants in the surface waters of Amsterdam and an overview of studies that assessed the removal of these pollutants from water by fungi. Amsterdam functions as a case study for the Netherlands in general, a country that is among the lowest in the European ranking on water quality. Chemical pollutants are ubiquitous and present in excessive amounts in Dutch surface water, exceeding the norms set by the 2027 European Water Framework Directive (EWFD) in the majority of the water bodies. To reach the norms, measures have to be taken in the coming years. Mycoremediation seems to be a promising, cost-effective measure, which can be implemented in water treatment plants to prevent pollutants to flush to the surface water. From the EWFD pollutants, three types of metals, seven types of PAHs, the biocide tributyltin cation, and fertilizers ammonium and phosphate were found to be the most problematic pollutants in Amsterdam canals. Nearly all metals and PAHs were shown to be effectively removed by different types of fungi, especially white-rot fungi such as *Pleurotus ostreatus.* The majority of the metals was removed with a removal rate of >95%. Other pollutants that are not included in the EWFD but are regarded as abundant in Amsterdam canals are drug residues and *Eschecheria coli*. Nearly all of these were shown to be effectively removed by fungi. *T. versicolor* showed to be the most effective species to remove drug residues (up to 100% removal rate) and *P. ostreatus* to remove E. coli (up to 99.7%) from wastewater. The majority of these pollutants were studied in mycoremediation research in both lab conditions as well as using real wastewater. The step towards implementation of mycoremediation is therefore relatively small, and could be realised within years. It is highly recommended to start testing these techniques in wastewater treatment plants in Amsterdam, so that pollutant levels in their effluents can be minimized.

Background

As a country that was built on water, the Netherlands are known as the top-of-the-nodge when it comes to water management. Dutch knowledge and technologies on water management are applied, shared and sold worldwide. However, recent assessments of water bodies in the Netherlands show that the quality of the water is one of the lowest in Europe (Figure 1). Dutch water bodies have an acute and chronic chemical risk estimate of 25-50% and 50-75%, respectively, both above the European averages (Figure 1A). Moreover, the Netherlands has the highest percentage of water bodies (60-70%) that do not achieve good chemical status (Figure 1B). In 2027, the aims of the European Water Framework Directive (EWFD) have to be met. This is a Europe-wide assessment framework aiming to protect European waters. Initially, the EWFD stated that waters should be in ‘’good conditions’’ by 2015. This deadline has been postponed by two periods of six years until 2027. The EWFD has formulated specific goals on chemical and ecological water quality. The chemical quality framework is based on 77 ‘priority’ and ‘specifically polluting’ chemical agents that are assessed in all European water bodies. The ecological quality framework consists of the assessment of 1) biological quality, 2) general physical-chemical quality, 3) other relevant pollutants and 4) hydromorphology. The ecological quality is mostly determined by the biological quality. Only when the biological quality is sufficient, the other three assessment categories become relevant. According to the most recent report of the Netherlands Environmental Assessment Agency (PBL), 638 of the 741 water bodies in the Netherlands score badly on biological quality, and 90% score badly on chemical quality (Figure 2).

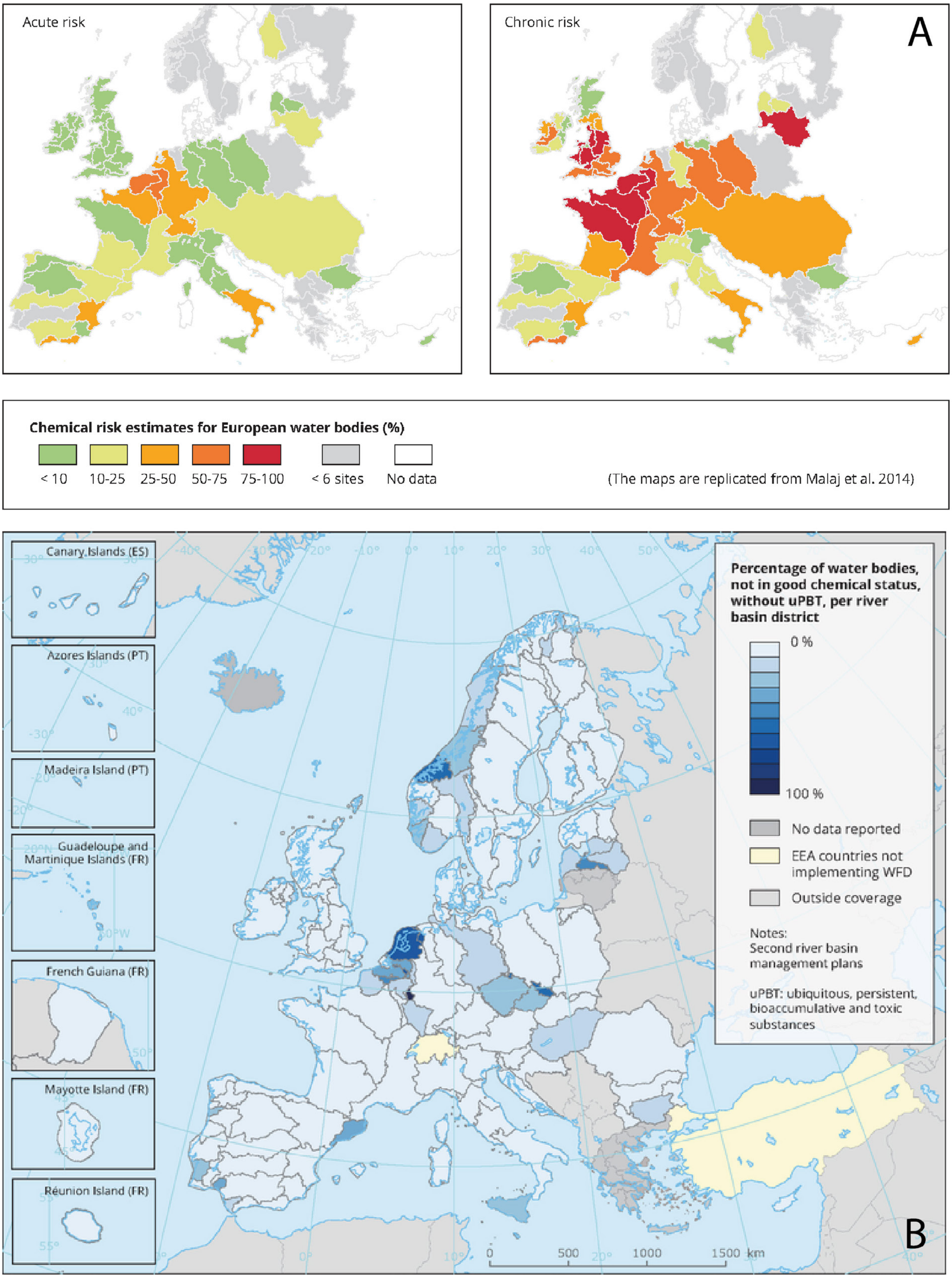


Figure 1: (A) Chemical risk estimates for European water bodies. Fraction of sites where the maximum chemical concentration exceeds the acute risk threshold (left) and the mean chemical concentration exceeding the chronic risk threshold (right) for any organism group. (B) Percentage of water bodies not in good chemical status. Taken from Malaj et al. 2014; European Environment Agency 2019.

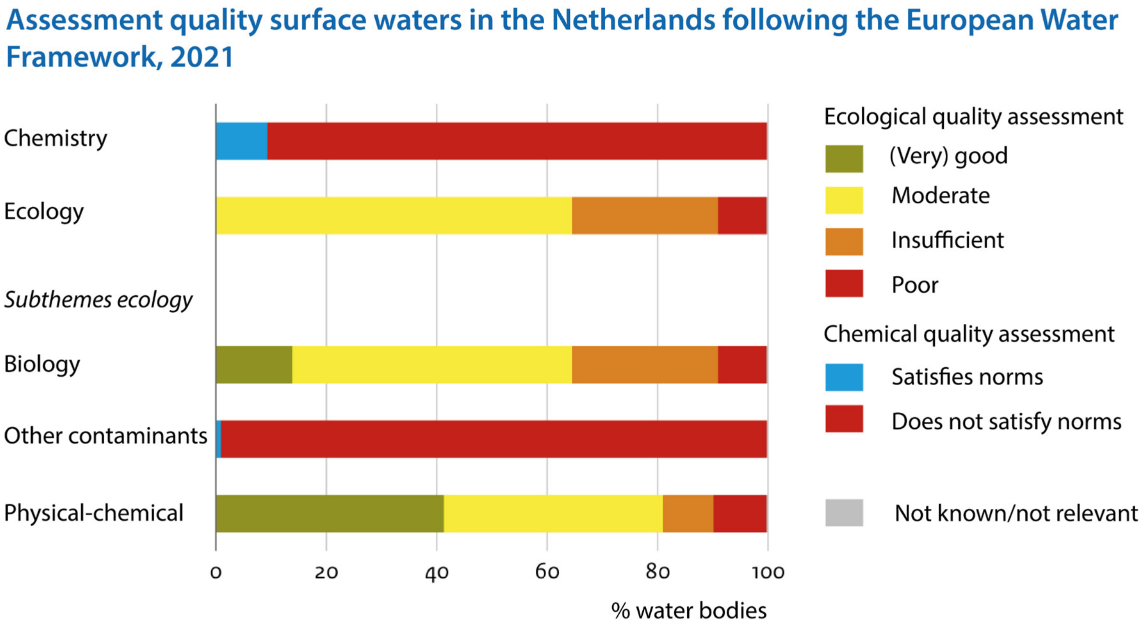


Figure 2: 2021 assessment on quality of surface waters in the Netherlands according to Water Framework Directive (CBS et al 2022).

For the assessment, the principle of ‘’one out, all out’’ is applied: if a single pollutant or a single biological parameter does not meet the norm, the water body is qualified as insufficient. For the assessment, waters are divided into the natural, heavily modified, and artificial categories. For instance, the Waddenzee, Naardermeer, coastal waters and several rivers and streams are classified as natural in the Netherlands; while the canals and ditches are classified as superficial; while the rest (e.g. Rhine-East and Meuse rivers) as heavily modified (CBS et al. 2020). For each type of water body, the EWFD has formulated aims for the water quality (Table 1). The general definitions for each of these classes as formulated by the EWFD can be found in Appendix I. These general aims have been specified and quantified for the Netherlands by the Ministry of Infrastructure and Water. For each type of water body, thresholds have been defined using the natural status as reference (Evers et al. 2018; Altenburg et al. 2018). According to Galen et al. (2020), the most important causes for the poor biological quality of the Dutch water bodies are (Galen et al. 2020; CBS et al. 2022) 1) Fertilisation with nitrogen and phosphorus, contributing to eutrophication of still waters; 2) unnatural design of water systems (most rivers and brooks have been straightened and have vertical and stoney banks, resulting in little natural habitats for flora and fauna; water levels are kept constant, limiting the natural dynamics of the water); 3) limitation of migration possibilities for fish due to pumping stations and weirs; 4) use of pesticides and herbicides that lead to death of macrofauna.

Water body type	**EWFD aim**	**Clarification**
Natural	Good Ecological Conditions (GEC)	Reference is the natural situation
Heavily modified	Good Ecological Conditions (GEC) / Good Ecological Potential (GEP)	Generally GEC; but in individual cases a lower aim (GEP) can be chosen, depending on the degree of modification of the water system
Artificial	Good Ecological Potential (GEP)	For artificial waters, the natural reference is not used, instead the maximal ecological potential is used as reference

Table 1: Aims for water quality as determined by the EWFD for 2027 (EWFD 2000, derived from https://eur-lex.europa.eu/legal-content/EN/LSU/?uri=CELEX:32000L0060).

The most common chemical agents that exceed the norms in more than 10% of the water bodies (see Table 2) originate from fertilisers, metals, fungicides, biocides, insecticides, drug residues, and polycyclic aromatic hydrocarbons (PAH). As in the rest of the country, the water quality is far below the norms in the capital city Amsterdam. The municipality of Amsterdam and the water board Amstel, Gooi en Vecht (AGV) wish to improve the quality of the iconic canals of Amsterdam, in order to meet the 2027 norms of the Water Framework Directive. The canals of Amsterdam (Figure 3) are classified by Stichting Toegepast Onderzoek Waterbeheer (STOWA) as artificial waters. Within the group of artificial waters, these canals are classified as ''M6b - Large shallow canals''. For the norm determination, the natural type M14 (Shallow medium-sized buffered pools) is used as reference. The water quality status in and around Amsterdam (Table 2) is monitored and assessed by AGV. None of the pollutants meet the norms in the canals of Amsterdam. Reasons for this pollution are wastewater overflows from the sewage system that end up in the surface water, large volumes of (salty) inlet water from the sluice of IJmuiden, and nutrient losses from agricultural plots (Waterbeheerprogramma AGV 2022-2027). Table 2 also clearly shows that overall the water quality has decreased over the years. For a substantial part of the pollutants the concentrations still met the EWFD norms during the last monitoring round in 2015, whereas they exceeded those norms in 2021. Reasons for this are increased pressures on the water due to climate change and more intensive usage of the water system (Waterbeheerprogramma AGV 2022-2027).

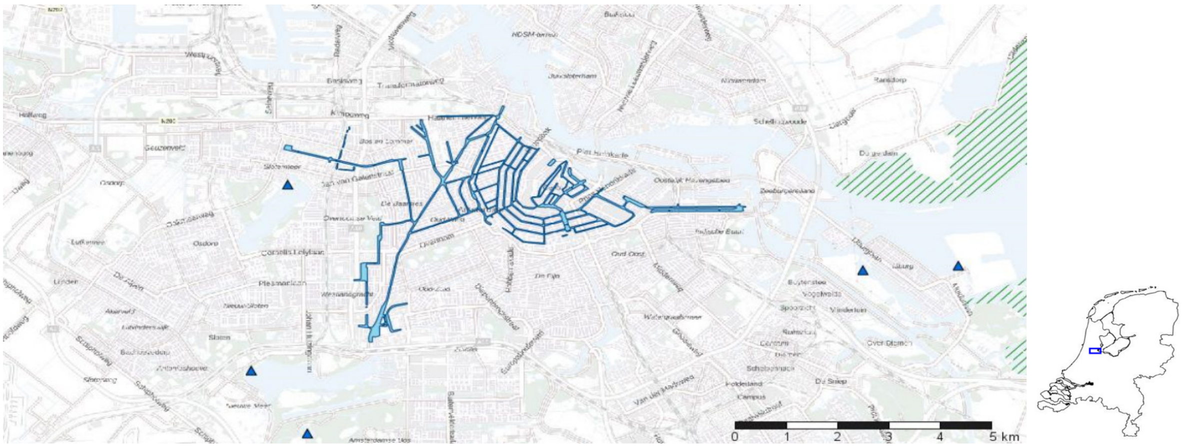


Figure 3: The system of the canals of Amsterdam (taken from https://waterkwaliteitsportaal.overheidsbestanden.nl/factsheets/Factsheets%202021%20December/Oppervlaktewater/factsheet_OW_11_Waterschap_Amstel_Gooi_en_Vecht_2022-05-12.pdf).

Table 2: Most important pollutants in Dutch water bodies. Pollutants that exceed norms in less than 10% of the Dutch water bodies were left out. 1) Chemical water quality: Blue = satisfies norms; Red = does not satisfy norms; 2) Ecological water quality: Yellow = moderate; Green = good. ''na" = not available.

Pollutants	**Type/Origin**	**Norm - yearly average (µg/l) (1, 2)**	**Norm - maximal acceptable risk (µg/l) (1, 2)**	**% of water bodies in NL that meet norm (2021) (3)**	**Amsterdam 2009 (4)**	**Amsterdam 2015 (4)**	**Amsterdam 2021 (4)**
Ammonia	Fertilizers	*0,304*	*0,608*	35	🟦	🟦	🟥
Arsenic	Metal	*0,5*	*8*	68	🟦	🟦	🟥
Azithromycin**	Drug residues	*na*	*na*	*na*	*na*	*na*	*na*
Barium	Metal	*93*	*1100*	83	*na*	*na*	*na*
Benzo[a]antracene	PAH	*0,00064*	*0,28*	58	🟦	🟥	🟥
Benzo[a]pyrene	PAH	*1,7 × 10-4*	*0,27*	68	🟦	🟥	*na*
Benzo[b]fluoranthene	PAH	*1,7 × 10-4*	*0,27*	67	*na*	🟥	🟥
Benzo[ghi]perylene	PAH	*1,7 × 10-4*	*0,27*	66	*na*	🟦	🟥
Benzo[k]fluoranthene	PAH	*1,7 × 10-4*	*0,27*	84	*na*	🟦	🟥
Carbamazepine**	Drug residues	*na*	*na*	*na*	*na*	*na*	*na*
Carbendazim*	Fungicides	*0,6*	*0,6*	88	*na*	*na*	*na*
Chrysene	PAH	*0,0029*	*0,17*	61	🟦	🟦	🟥
Clarithromycin**	Drug residues	*na*	*na*	*na*	*na*	*na*	*na*
Cobalt*	Metal	*0,2*	*1,36*	30	*na*	*na*	*na*
Dichlorvos*	Insecticides	6 × 10-4	7 × 10-4	95	*na*	*na*	*na*
Diclofenac**	Drug residues	*na*	*na*	*na*	*na*	*na*	*na*
Dipyridamole**	Drug residues	*na*	*na*	*na*	*na*	*na*	*na*
Fluoranthene	PAH	0,1	1	50	🟦	🟦	🟥
Heptachlor and heptachlor epoxide *	Insecticides	2 × 10-7	3 × 10-4	93	*na*	*na*	*na*
Imidacloprid*	Insecticides	*0,0083*	*0,2*	94	*na*	*na*	*na*
Irgarol*	Biocides	*na*	*na*	82	*na*	*na*	*na*
Mercury*	Metals	0,00007	0,07	52	🟦	🟥	*na*
Methylazinfos*	Insecticides	*0,0065*	*0,014*	88	*na*	*na*	*na*
Methylpirimiphos*	Insecticides	*0,0005*	*0,0016*	84	*na*	*na*	*na*
Nickel*	Metal	20	*na*	84	*na*	*na*	*na*
Nitrogen (total)	Fertilizers	≤ 3800	*na*	53	🟩	🟩	🟨
Oxazepam**	Drug residues	*na*	*na*	*na*	*na*	*na*	*na*
Perfluorooctane sulphonic acid (PFOS) *	Fire extinguishers, glue	6,5 × 10-4	36	56	*na*	*na*	*na*
Phosporus (total)	Fertilizers	≤ 120	*na*	53	🟨	🟨	🟨
Phosphate	Fertilizers	*na*	*na*	*na*	*na*	🟥	🟥
Selenium	Metals	*0,052*	*24,6*	30	*na*	🟥	🟥
Silver	Metals	*0,01*	*0,01*	88	🟦	🟦	🟥
Sulfamethoxazole**	Drug residues	*na*	*na*	*na*	*na*	*na*	*na*
Tributyltin cation	Biocides	0,0002	0,0015	79	*na*	🟦	🟥
Uranium*	Metals	*0,17*	*8,6*	45	🟦	🟥	*na*
Zinc*	Metals	*7,8*	*15,6*	61	*na*	*na*	*na*
*E. coli****	Pathogens	*na*	*900 kve /100mL (5)*	*na*	*na*	*na*	*na*

**These pollutants are included in the WFD and are regarded as problematic in the Netherlands, but were not assessed in Amsterdam. **Pollutants originating from drug residues are not included in the norms of the WFD and were not assessed in Amsterdam. However, they are problematic pollutants in the Netherlands and in Amsterdam (Vissers et al. 2017). ***E. coli is not included in the WFD but is measured in Amsterdam and is regarded as problematic. (Ishii and Sadowsky 2008). Sources:* (1) *EU norms* - Besluit kwaliteitseisen en monitoring water 2009 (https://wetten.overheid.nl/BWBR0027061/2017-01-01#Bijlage1); (2) *NL norms (when deviate from EU norms)* - Regeling monitoring kaderrichtlijn water (https://wetten.overheid.nl/BWBR0027502/2022-04-01); (3) National assessment water quality (Galen et al. 2020) (*NB Percentages were retrieved from graphs);* (4) Factsheet OW 11 Waterschap Amstel, Vecht en Gooi (https://www.waterkwaliteitsportaal.nl/krw-factsheets) (5) EU Directive for swimming water (https://eur-lex.europa.eu/legal-content/NL/TXT/PDF/?uri=CELEX:32006L0007&from=hr)

Only a subset of the pollutants that are taken up in the national analysis was measured in Amsterdam. The reasoning for this is not known. However, the pollutants that were measured and that do not meet de norms in Amsterdam (i.e. all of them), also show to be problematic on a national scale, implying that the canals of Amsterdam are representative for the surface waters in the Netherlands. As one can see in Table 2, pollutants originating from drug residues were not quantified in the assessment report from AGV, but a report from STOWA shows that the sewage treatment plant in Amstelveen – from which the effluent ends up in the Amstel river that flows to the canals of Amsterdam – contributes substantially to the concentration of drug residues in surface waters (Vissers et al. 2017). According to the National Analysis Water Quality report, seven of these pollutants are of substantial risk for the water system: azithromycin, carbamazepine, clarithromycin, diclofenac, dipyridamole, oxazepam and sulfamethoxazole (Figure 4). Therefore, I have chosen to include these pollutants in Table 2. Another pollutant which is highly problematic in Amsterdam, but which is not included in the EWFD norms, is the bacterium *Escherichia coli.* According to Waternet, E. *coli* concentration often exceeds the norms, and is therefore a serious threat for the water quality (Ishii and Sadowsky 2008). Therefore I also have chosen to include this pollutant.

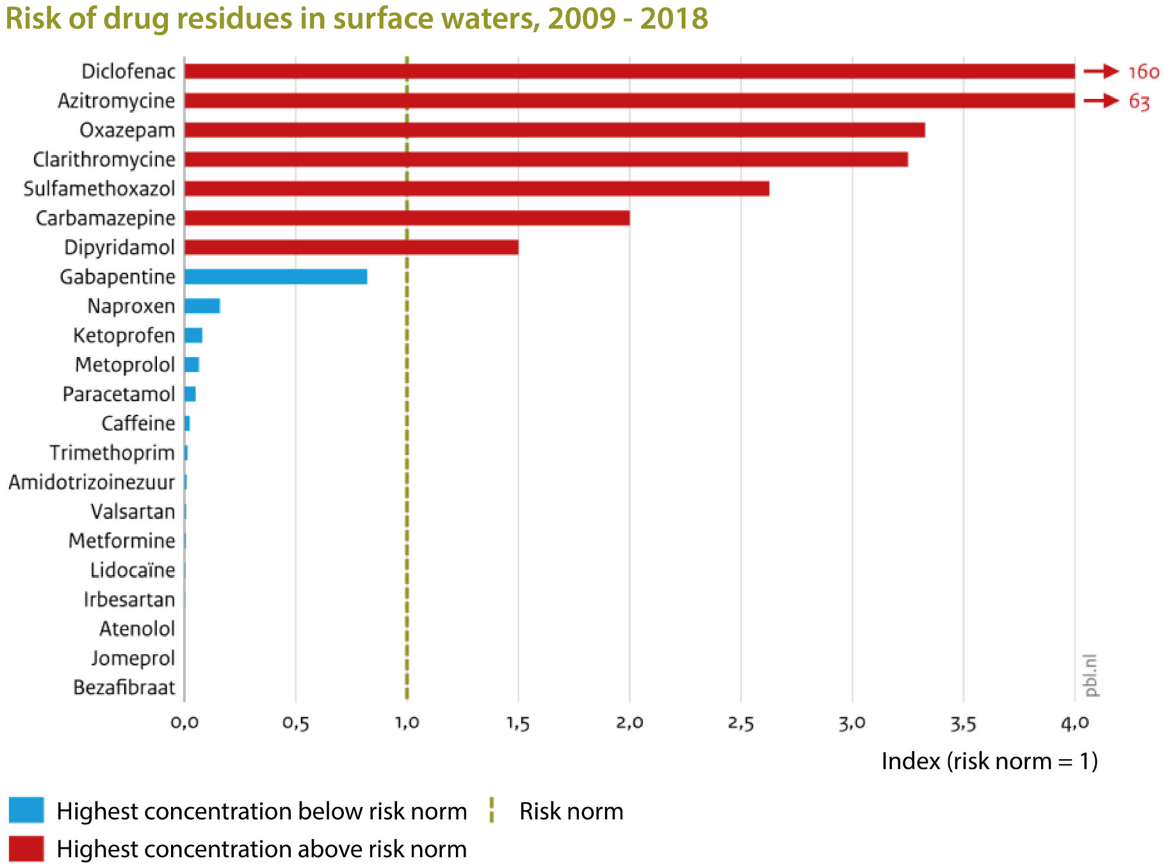


Figure 4: Pollutants from drug residues and their risk quota. Risk quotas are calculated by dividing the highest measured concentration by a risk norm. If the risk quota is higher than 1, the risk norm is thus being exceeded.

Clearly, something needs to change in the way our waters are managed in order to meet the aims of the EWFD in 2027. Existing measures that are currently being implemented to improve the water quality are nature friendly design and management of waterways, better management of sewage overflows, connecting houseboats to the sewerage system, stimulating better agricultural practices and agricultural water management and enhancing the growth of mussels (in e.g. the Sloterplas). However, regardless of the implementations of these measures, the assessment states that for each of the pollutants it is still unsure whether the EWFD norms will be met in 2027. Additional measures are needed to ensure that the European aims will be reached.

Many techniques are available to control and maintain the abiotic factors in the water bodies. Besides physical and chemical techniques, biological technologies for filtration and degradation of toxic pollutants and bacterial load are commonly used. Microorganisms such as bacteria and fungi have the ability to adsorb selected substances and to degrade different contaminants (Lu et al. 2016). Among the available technologies, mycoremediation is one of particular interest. In this method, fungi are used to reduce the toxic load of the environment by either filtration or degradation. In the case of filtration, functional groups on the fungal cell wall (such as carboxyl, hydroxyl, amino, sulfonate and phosphonate) sorb the specific pollutants (Lu et al. 2016). In the case of degradation, fungal mycelium degrades the pollutants through extracellular enzymes (Kulshreshtha et al., 2010). Fungi are advantageous over other types of microorganisms, because of the high amounts of degrading enzymes produced by fungi, their high adsorption capacity, easy solid-liquid separation, broad degradation ability, and their ability to withstand unstable and harsh conditions such as fluctuating pollutant loads, low nutrient concentrations and low pH (Espinosa-Ortiz et al. 2016) (Lu et al. 2016).

The main objective of this review is to identify the hurdles and knowledge gaps that need to be overcome to come to successful implementation and application of mycoremediation with the aim to improve the quality of the surface waters in Amsterdam. To this end, the following questions are addressed:

- *What is the status quo of scientific knowledge on mycofiltration (degradation or absorption of pollutants) of water?*
- *Which of the identified pollutants could be targeted by mycofiltration?*
- *What ''best practices'' in mycofiltration exist and what is known about their effectiveness in laboratory conditions and in water treatment plants?*
- *What obstacles hamper experiments with mycofiltration?*

The idea behind this study is to identify mycofiltration methods that could potentially be implemented in wastewater treatment plants in Amsterdam. Not only are wastewater treatment plants still the most important sources of surface water pollution, they could also - in potential - be the solution to the polluted surface water. The amount of water present in the canal system of Amsterdam is approximately 7,3 billion litres (Figure 3), calculated using an average canal depth of 2,6 metre[1]. The total wastewater treatment plant of the AGV water system treats 343 million litres per day.[2] So theoretically, the existing wastewater treatment installations could rinse the complete volume of the surface water system of Amsterdam in 22 days. If the city of Amsterdam wants to meet the norms for 2027, it is important to study the possibilities for extra measures to improve the water quality and mycoremediation seems to be a promising and innovative direction.

---

[1] Gemeente Amsterdam, Doorvaartprofielen (Programma Varen)

[2] https://www.agv.nl/onze-taken/schoon-water/schoonmaken-van-vuil-water

Application of mycoremediation in wastewater treatment

An overview of the 39 articles that were reviewed is given in Tables 3-6, each dealing with one of the four categories of pollutants (drug residues, heavy metals, polycyclic aromatic hydrocarbons (PAHs) and E. coli). From each article, I identified the pollutant that was addressed, the fungus/fungi that was/were used, the experimental conditions (controlled vs. not controlled) and the degradation rate. Sterilized water was used in the case of controlled experimental conditions, while this was not the case for non-controlled experimental conditions.

Drug residues

Table 3: Overview of removal of drug residues from water using mycoremediation. Only pollutants that are problematic in the surface waters of Amsterdam are displayed. C = controlled conditions (spiked water, sterilized), NC = not controlled (non-sterilized wastewater).

Pollutants	Fungal specie(s)	Conditions	Removal	Sources
Azithromycin	*Trametes versicolor*	NC	80 - 100%	Cruz-Morató et al. 2013; 2014
Carbamazepine	*Phanerochaete chrysosporium*	NC	60 - 80%	Zhang and Geißen 2012; Li et al. 2015; Li, de Toledo et al. 2015
Carbamazepine	*Trametes versicolor, Ganoderma lucidum; Phanerochaete chrysosporium*	C	58 – 96%	Marco-Urrea et al. 2009; Jelic et al. 2012; Tran et al. 2010; Rodarte-Morales et al. 2012
Clarithromycin	*Trametes versicolor*	NC	80%	Cruz-Morató et al. 2014
Diclofenac	*Trametes versicolor*	NC	100%	Cruz-Morató et al. 2013
Diclofenac	*Trametes versicolor; Phanerochaete chrysosporium*	C	100%	Tran et al. 2010; Rodarte-Morales et al. 2012
Sulfamethoxazole	*Trametes versicolor*	NC	93%	Cruz-Morató et al. 2014
Sulfamethoxazole	*Trametes versicolor*	C	66%	Rahmani et al. 2015

Yearly, more than 190 tonnes of pharmaceutical residues end up in Dutch surface waters. Only a certain degree of medicines are metabolised in the human body; the rest ends up in the sewage system and is from there emitted to surface water. Drug residues cause negative effects on animals and plants that live in surface water, such as hormone disruption in fish (Moermond et al. 2020). In the past decade, the interest in studying the removal of drug residues by means of fungi has increased. Compared to other types of pollutants in wastewater, drug residues are studied extensively. Especially diclofenac - used widely to treat pain and inflammatory diseases - and carbamazepine - an antiepileptic - are recurring in many studies. From these studies, it can be concluded that pharmaceuticals are good candidates for mycoremediation. Removal rates are high in all studies (Table 3). The fungus that was mostly used in these studies is the white-rot fungus (WRF) *Trametes versicolor*. *T. versicolor* can remove pharmaceuticals from real bioslurry systems and urban wastewater under non-sterile conditions and without extra nutrients in the media (Cruz-Morató et al. 2013). However, in some cases, degradation can be enhanced by the addition of external nutrients (Svobodova and Novotny 2018). *Phanerochaete chrysosporium* is another WRF species that has been used repeatedly in mycoremedation research. Zhang and Geißen (2012) and Li et al. (2015) showed that this fungus can successfully remove carbamazepine from water under non-sterile conditions. WRF produce extracellular enzymes which degrade lignin. Due to the irregular structure of lignin, these enzymes have extremely reduced substrate specificity, which makes these fungi able to also degrade and mineralize various organopollutants, such as pharmaceuticals. From the pollutants that are identified as problematic in the Netherlands, only dipyridamole and oxazepam were not addressed in mycoremediation experiments. Azithromycin, carbamazepine, clarithromycin, diclofenac and sulfamethoxazole have all shown to be removed to a high extent in non-sterile conditions, i.e. using non-sterilized wastewater. This is an important step towards implementation into wastewater treatment plants, as the removal by the fungi might be stimulated by other microflora present in the water.

Heavy metals

Table 4: Overview of removal of heavy metals from water using mycoremediation. Only pollutants that are problematic in the surface waters of Amsterdam are displayed. C = controlled conditions (spiked water, sterilized), NC = not controlled (real non-sterilized wastewater).

Pollutants	**Fungal specie(s)**	**Conditions**	**Removal**	**Source**
Arsenic	*Penicillium sp; Inonotus hispidus*	C	30 – 91%	Visoottiviseth and Panviroj (2001); Sarı and Tuzen 2009
Cobalt	*Aspergillus niger; Aspergillus tamarii (NRC 3); Mortierella SPS 403; Paecilomyces sp.; Penicillium sp.*	C	50 – 100%	Cárdenas González et al. 2019; Pal et al. 2006; Saad et al. 2019
Cobalt	*Pleurotus ostreatus*	NC	99.3%	Vaseem et al. 2017
Mercury	*Aspergillus flavus* strain KRP1	C	97.5%	Kurniati et al. 2014;
Nickel	*Aspergillus versicolor; Beauveria bassiana; Saccharomyces cerevisiae;*	C	30.1 – 89.0%	Gola et al. 2016; Machado et al. 2010; Tastan et al. 2010
Nickel	*Pleurotus ostreatus; Saccharomyces cerevisiae*	NC	89.0 - 98.0%	Machado et al. 2010; Vaseem et al. 2017; Vaverková et al. 2018
Selenium	*Aspergillus niger*	C	86.0 – 94.3%	Negi et al. 2020; Sabuda et al. 2020
Selenium	*Alternaria sp; Alternaria alternata*	NC	22 – 53%	Sabuda et al. 2020
Uranium	*Penicillium piscarium; Pleurotus ostreatus*	C	90.8 - 97.5%	Coelho et al. 2020; Zhao et al. 2016
Zinc	*Beauveria bassiana; Cunninghamella elegans; Pleurotus djamor; Pleurotis ostreatur; Streptomyces ciscaucasicus;*	C	67.8 – 69.3	Chandra et al. 2022; Gola et al. 2016; Li et al. 2010; Tayel et al. 2016
Zinc	*Aspergillus flavus; Pleurotus ostreatus*	NC	30.8 – 82.6 %	Anupong et al. 2022; Vaseem et al. 2017; Vaverková et al. 2018;

Heavy metals are among the types of pollutants of wastewater that are studied most broadly worldwide. The reason for this is that metal pollution is a worldwide problem. In the Netherlands, heavy metals in surface water mostly originate from historical pollution from the metal industry (nickel and cadmium); from pyrite oxidation through nitrate leakage from ground water (arsenic and nickel); from animal and artificial fertilizers and through atmospheric deposition (Galen et al. 2020). High concentrations of toxic metals negatively affect water ecosystems due to their non-biodegradable and long persistence in nature, e.g. by manipulating fish physiology (Shahjahan et al. 2022). Relatively much research has been done using microorganisms as filtration method, because microorganisms (bacteria, yeast, algae and filamentous fungi) are relatively good at accumulating heavy metals compared to non-biological industrial methods. Fungi can remove heavy metals under a large range of pH (1.0 to 9) and temperature (Ayele et al. 2021). They also have a higher sensitivity and capacity to accumulate heavy metals compared to bacteria (Dusengemungu et al. 2020) and in certain circumstances they can even outperform activated carbon (Rangabhashiyam et al. 2014). Compared with other microorganisms, fungi have a high percentage of cell wall material, consisting of a significant volume of polysaccharides, proteins, and metal-binding groups such as amines, phosphates, carboxyls, and hydroxyls (Kanamarlapudi et al. 2018; Ayele et al. 2021). The main pathway used by microorganisms to accumulate heavy metals is physical adsorption (extracellular), in which metal ions adhere to the cell surface of the fungal cell, from where they are absorbed (intracellular) into the cell. Adsorption does not require energy from metabolism. Other mechanisms by which filamentous fungi immobilise, complex or remove metals from solutions are ion exchange, electrostatic interactions, precipitation, accumulation, mineralization, transformation, complex formation and redox-reactions (Ayele et al. 2021; Dusengemungu et al. 2020). The fungal species that were used in the reviewed articles (Table 4) are especially economically interesting, as they are abundantly available and low cost in mass production (Lu et al. 2016).

The study done by Sabuda et al. (2020) compared industrial wastewater to municipal wastewater. This study shows that the pollutant concentration has significant impact on the removal rate. *Alternaria alternata* had much higher removal capacity with industrial wastewater (2000 µg/L Se) than municipal wastewater (25 µg/L Se) with 75% vs 53%, respectively. From the metals that were identified as problematic in the Netherlands, the ones that were not covered in the studied literature are barium and silver. Reasoning for this is not known. As barium and silver exceed the norms in Dutch water bodies, further research on bioremediation of these metals from wastewater would therefore be valuable.

PAH's

Table 5: Overview of removal of PAHs from water using mycoremediation. Only pollutants that are problematic in the surface waters of Amsterdam are displayed. C = controlled conditions (spiked water, sterilized), NC = not controlled (real non-sterilized wastewater).

Pollutants	**Fungal specie(s)**	**Conditions**	**Removal**	**Source**
Benzo[a]anthracene	*Phanerochaete chrysosporium; Trametes versicolor;*	C	10 – 89%	Bogan and Lamar 1995; Majcherczyk et al. 1998
Benzo[a]anthracene	*Pleurotus ostreatus*	NC	89.4%	Kumar et al. 2022
Benzo[a]pyrene	*Aspergillus caesiellus; Cadophora sp.; Phanerochaete chrysosporium; Pleurotus ostreatus; Pseudogymnoascus sp. TS12; Trametes versicolor; Tramates hirsuta*	C	19.0 - 100%	Bogan and Lamar 1995; Majcherczyk et al. 1998; Deng et al. 2022; Batista-García et al. 2017
Benzo[a]pyrene	*Pleurotus ostreatus*	NC	99.8%	Kumar et al. 2022
Benzo[b]fluoranthene	*Trametes versicolor; Pleurotus ostreatus*	C	10 – 47%	Majcherczyk et al. 1998; Bogan and Lamar 1995
Benzo[b]fluoranthene	*Pleurotus ostreatus*	NC	67.5%	Kumar et al. 2022
Benzo[ghi]perylene	*Pleurotus ostreatus*	C	54%	Bogan and Lamar 1995
Benzo[k]fluoranthene	*Pleurotus ostreatus*	C	62%	Bogan and Lamar 1995
Benzo[k]fluoranthene	*Pleurotus ostreatus*	NC	26.4%	Kumar et al. 2022
Chrysene	*Trametes versicolor;*	C	10%	Majcherczyk et al. 1998
Chrysene	*Pleurotus ostreatus*	NC	89.69%	Kumar et al. 2022
Fluoranthene	*Absidia cylindrospora CS; Aspergillus niger CS; Cladosporium sphaerospermum CS; Mucor genevensis CS; Trichoderma harzianum CS; Ulocladium chartarum CS &NCS*	C	39 - 79%	Bogan and Lamar 1995; Giraud et al. 2001
Fluoranthene	*Pleurotus ostreatus*	NC	100%	Kumar et al. 2022

PAH is the umbrella term for a wide range of organic compounds (more than 100) with two or more fused benzene rings. Their origin can be either natural or anthropogenic (oil and carbon deposits), and their effects include toxicity, mutagenicity and carcinogenicity. Because of their properties (semi-volatile, thermodynamically stable and low aqueous solubility) they persist over long periods of time in ecosystems (Batista-García et al. 2017) and are ubiquitous and widely distributed in the environment. Like heavy metals, PAHs can be transformed by different processes and mechanisms, of which microbial transformation is the main process of their mineralization in nature. Compared to bacteria, fungi are advantageous for degradation of polyaromatic hydrocarbons because of their capability to grow on a large spectrum of substrates and their ability to produce extracellular hydrolytic enzymes (Kadri et al. 2017). Table 5 shows that the majority of studies used *P. ostreatus*, which, like *P. chrysosporium* and *T. versicolor*, is a WRF and thus suitable for degrading various organopollutants thanks to its delignification properties. Initial oxidation of PAHs is executed by extracellular peroxidases (Kadri et al. 2017). Other fungal lignolytic enzymes that catalyse radical formation by oxidation are laccase, and manganese peroxidase. Oxidation then causes bonds in the molecule to destabilize (Haritash and Kaushik 2009). PAHs are then transformed through mineralization into inorganic materials, H_2O, CO_2 (aerobic) or CH_4 (anaerobic).

Despite the scale and the serious health related risks of the environmental pollution of PAHs, research into biodegradation of PAHs has only recently gained increased attention. Studies are still limited, but show promising results. Especially benzo[a]anthracene, benzo[a]pyrene, chrysene and fluoranthene have notably high remediation rates (Table 5). Giraud et al. (2001) did a screening of 40 fungal species that were present in soil samples from a contaminated wetland and a control wetland. Fluoranthene, one of the most commonly present PAHs, was degraded efficiently by 33 of them, originating from both contaminated soils and non-contaminated soils. The fungal species with the highest removal rates all originated from the contaminated soil samples, and 19 species that were capable of removing fluoranthene were only isolated from contaminated soil and were not present in the control wetlands. This suggests that proliferation of these species was favoured by the presence of fluoranthene. Kumar et al (2022) found that laccase produced by *P. ostreatus* is able to remediate 91% of the 15 PAHs that were present in biorefinery wastewater. Using cassava waste, the maximum yield of laccase enzymes was produced. Compared to earlier studies, such as the one by Majcherczyk et al. that used *T. versicolor* (1998), the removal rate was much higher. This might imply that the laccase-assisted approach is more efficient than the direct approach. Another possible explanation is that *T. versicolor* is not the most efficient fungus to use for PAH removal. Batista-García et al. (2017) showed high removal rates (93.4 – 100%) of benzo[a]pyrene by five different fungal species, not including *T. versicolor*. Factors influencing PAH degradation rate are pH, oxygen, temperature, microbial population, nutrients and chemical structure of the compound (Haritash and Kaushik 2009). Moreover, the concentration of the contaminant represents a key point, which influences the degradation of PAHs. Fungal growth is inhibited in highly contaminated soils. Overall mycoremediation is slow, needing many days or even more than a month (Kadri et al. 2017).

E. coli

Table 6: Overview of removal of E. coli from water using mycoremediation. C = controlled conditions (spiked water, sterilized), NC = not controlled (real non-sterilized wastewater).

Pollutants	**Fungal specie(s)**	**Conditions**	**Removal**	**Source**
E. coli	*Stropharia rugoso-annulata*	C - spiked water	20%	Taylor et al. 2015
E. coli	*Pleurotus ostreatus*	C - spiked water	19.5 - 99.25%	Rogers 2012; Pini and Geddes 2020
E. coli	*Pleurotus ostreatus; Pleurotus ulmarius; Stropharia rugosoannulata*	NC - real wastewater used	90 - 99.74%	Thomas et al. 2009; Pini and Geddes 2020

Compared to chemical pollutants, the pathogen *E. coli* is far less studied in mycoremediation experiments. This is despite the fact that *E. coli* is a definitive indicator of fecal contamination in surface waters, which poses numerous threats on human health and habitat quality (Taylor et al. 2015; Pini and Geddes 2020). The most recent study by Pini and Geddes (2020) shows that *P. ostreatus* has high potential for removing *E. coli* in both lab and field conditions. In this study, spiked water and real river water were tested. The water was processed through organic wheat straw with mycelia of *P. ostreatus.* The removal rate was 99 - 100% after 96h in both settings. The outcomes of Taylor et al. (2015) were much lower with only 20% removal. This could be explained by the fact that they cultivated the fungus *Stropharia rugoso-annulata* under non-sterile conditions, which might have led to bacterial contamination. Pini and Geddes (2020) followed their recommendation to use sterile methods for the growing of fungi - which does not exclude the possibility of using real wastewater! - and showed that when the fungus was pre-grown in sterile conditions mycoremediation was extremely effective. The mechanism behind degradation of *E. coli* by *Pleurotes* spp. Is known: mycelia of these species release antimicrobial exoenzymes that are able to stun and digest bacteria (Pini and Geddes 2020). To my knowledge, however, the mechanism by which *S. rugoso-annulata* degrades *E. coli* was not explicitly revealed in literature yet.

Conclusions & Discussion

As a solution to the high amounts of pollutants in the surface waters of Amsterdam, mycoremediation seems to be a promising possibility. An important note to make here is that ultimately, the most important measure to reduce the amount of pollutants is to tackle the source of these pollutants. Unfortunately, most of the sources are currently unknown – the only norm-exceeding pollutant of which the source is known is zinc (Galen et al. 2020). Therefore, other types of measures are needed to reach the EWFD norms in 2027. In the past years, research on mycoremediation to remove pollutants from water has increased. Fungi have shown to be successful in removing a broad range of pollutants. They are favorable over other water filtration techniques (chemical, mechanical and other microbial techniques) as they are relatively easy, fast and cost-effective to grow; they are able to withstand a wide range of pH (from 1.0 to 9) and other harsh conditions such as fluctuating pollutant loads and low nutrient concentrations, and they are more effective in adsorption and degradation than other microorganisms (Dusengemungu et al. 2020; Espinosa-Ortiz et al. 2016; Lu et al. 2016). Moreover, mycoremediation does not leave sludge by-products and requires no further disinfection or sterilization, unlike wastewater treatment processes using bacteria (Pini and Geddes 2020). The most promising fungal species are whit-rot fungi from the class Basidiomycetes, because of their high growth rate and their lignin biodegradation activity (Kadri et al. 2017). The ligninolytic activity not only enables to grow the fungi on organic waste streams such as straw but also releases enzymes that can degrade PAHs and pharmaceuticals.

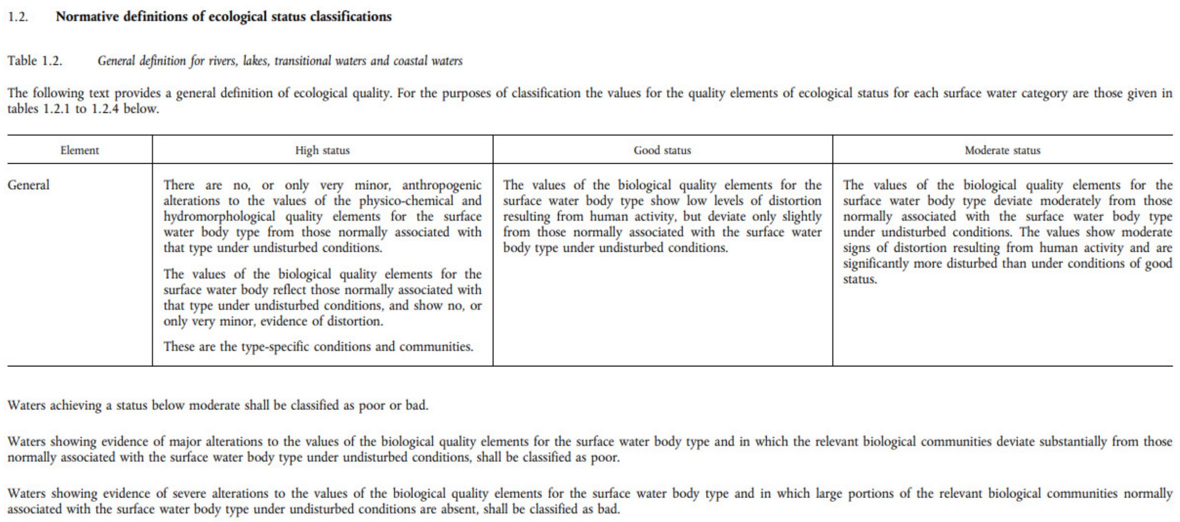
The most problematic pollutants in the waters of Amsterdam are metals (arsenic, selenium and silver), PAHs (benzo[a]anthracene, benzo[b]fluoranthene, benzo[ghi]perylene, benzo[k]fluoranthene, chrysene and fluoranthene), tributyltin cation and ammonium and phosphate. The last three were not found in mycoremediation studies, but it is known that other bioremediation methods such as helophyte filters can effectively remove nitrogen and phosphorus (Gacia et al. 2019; Ribot et al. 2019). Tributyltin cation has shown to be effectively removed by alginate filters and aquatic plants (Luan et al. 2006; Xiao et al. 2020). Of all metal pollutants, silver was the only one not found in literature on mycoremediation. However, it was found that certain bacteria are excellent at removing silver from water (Ahmad et al. 2019). The other abovementioned pollutants were all found to be promising candidates for mycoremediation. Their maximum removal rates were: arsenic 91% (*I. hispidus*), selenium 94.3% (*A. niger*), benzo[a]anthracene 89.4% (*P. ostreatus*), benzo[b]fluoranthene 67.5% (*P. ostreatus*), benzo[ghi]perylene 54% (*P. ostreatus*), benzo[k]fluoranthene 62% (*P. ostreatus*), chrysene 89.7% (*P. ostreatus*) and fluoranthene 100% (*P. ostreatus*). Other metals and PAHs that were not measured in Amsterdam, but are problematic on the national scale and are thus a potential threat to Amsterdam surface waters are cobalt, mercury, zinc, uranium, nickel and benzo[a]pyrene. These were found to have removal rates of 100% (*Penicillium spp.*), 97.5% (*A. flavus*), 82.6% (*P. ostreatus*), 97.5% (*P. piscarium*), 98% (*P. ostreatus*) and 100% (*T. hirsuta* and *A. caesiellus*) respectively. Pollutants that are not included in the European Water Framework Directive but that are identified as problematic in the Netherlands and in Amsterdam (Vissers et al. 2017; Waternet) are drug residues (azithromycin, carbamazepine, clarithromycin, diclofenac, dipyridamole, oxazepam, sulfamethoxazole) and the pathogenic bacterium E. coli. The majority of these were found to have high removal rates by fungi: 100% (azithromycin; *T. versicolor*), 96% (carbamazepine; *T. versicolor*), 80% (clarithromycin; *T. versicolor*), 100% (diclofenac; *P. chrysosporium* and *T. versicolor*), 93% (sulfamethoxazole; *T. versicolor*) and 99.7% (E. coli; *P. ostreatus*). Lastly, the reviewed studies show that even other pollutants, which are currently not problematic in the Netherlands but might be in the future, such as anthracene, pyrene (Bogan and Lamar 1995; Kumar et al. 2022), phenantrene (Batista-García et al. 2017), cadmium (Gola et al. 2016), chromium, copper (Vaseem et al. 2017), ibuprofen (Marco-Urrea et al. 2009) and different types of microplastics (Sangeetha Devi et al. 2015; Yuan et al. 2020) can also be removed by fungi.

The majority of studies were done in laboratory conditions, using an isolated fungus strain grown under sterile conditions, and distilled water with an added pollutant. However, in the recent years studies to pollutant removal from real wastewater have gained more popularity. This is an important step towards the end goal: implementation. Especially those pollutants that were already proved to be efficiently removed by fungi from real environmental effluents, should be tested in wastewater treatment plants in and around Amsterdam, as they will have a high chance on succeeding. Water treatment plants are to date the most important sources for surface water pollution, so they would be the logical place for implementation. Also, theoretically, the volume of water in the canals of Amsterdam could be pumped through and filtered by one wastewater treatment plant in 22 days, although enabling this would be a technological challenge. As Pini and Geddes (2020) point out, mycoremediation is likely to be most effective when the fungus is grown under sterile conditions. This should be considered in further research and implementation of mycoremediation techniques. Something else that should be taken into account are the facts that, in general, mycoremediation is extremely slow, needing many days or even more than a month (Kadri et al. 2017) and that the pH should be optimal, as this is the most important parameter influencing the sorption capacity (Ayele et al. 2021). These factors could have consequences for the timing of mycoremediation within the process flow of wastewater treatment, and are therefore important to take into account in future process engineering. Altogether, the advantages of mycoremediation make it a viable addition to, or alternative for, traditional water treatment techniques. Future research should be focused on implementing mycoremediation in wastewater treatment plants, and pollutants such as microplastics, tributyltin cation, silver, PFOS, dipyridamole and oxazepam should be included in these experiments as they are currently underexposed in this research field.

Appendices

Appendix I - Definitions EWFD

Screenshots taken from: https://eur-lex.europa.eu/resource.html?uri=cellar:5c835afb-2ec6-4577-bdf8-756d3d694eeb.0004.02/DOC_1&format=PDF – Appendix V



1.2. **Normative definitions of ecological status classifications**

Table 1.2. *General definition for rivers, lakes, transitional waters and coastal waters*

The following text provides a general definition of ecological quality. For the purposes of classification the values for the quality elements of ecological status for each surface water category are those given in tables 1.2.1 to 1.2.4 below.

Element	High status	Good status	Moderate status
General	There are no, or only very minor, anthropogenic alterations to the values of the physico-chemical and hydromorphological quality elements for the surface water body type from those normally associated with that type under undisturbed conditions. The values of the biological quality elements for the surface water body reflect those normally associated with that type under undisturbed conditions, and show no, or only very minor, evidence of distortion. These are the type-specific conditions and communities.	The values of the biological quality elements for the surface water body type show low levels of distortion resulting from human activity, but deviate only slightly from those normally associated with the surface water body type under undisturbed conditions.	The values of the biological quality elements for the surface water body type deviate moderately from those normally associated with the surface water body type under undisturbed conditions. The values show moderate signs of distortion resulting from human activity and are significantly more disturbed than under conditions of good status.

Waters achieving a status below moderate shall be classified as poor or bad.

Waters showing evidence of major alterations to the values of the biological quality elements for the surface water body type and in which the relevant biological communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions, shall be classified as poor.

Waters showing evidence of severe alterations to the values of the biological quality elements for the surface water body type and in which large portions of the relevant biological communities normally associated with the surface water body type under undisturbed conditions are absent, shall be classified as bad.

1.2.1. Definitions for high, good and moderate ecological status in rivers

Biological quality elements

Element	High status	Good status	Moderate status
Phytoplankton	The taxonomic composition of phytoplankton corresponds totally or nearly totally to undisturbed conditions. The average phytoplankton abundance is wholly consistent with the type-specific physico-chemical conditions and is not such as to significantly alter the type-specific transparency conditions. Planktonic blooms occur at a frequency and intensity which is consistent with the type-specific physico-chemical conditions.	There are slight changes in the composition and abundance of planktonic taxa compared to the type-specific communities. Such changes do not indicate any accelerated growth of algae resulting in undesirable disturbances to the balance of organisms present in the water body or to the physico-chemical quality of the water or sediment. A slight increase in the frequency and intensity of the type-specific planktonic blooms may occur.	The composition of planktonic taxa differs moderately from the type-specific communities. Abundance is moderately disturbed and may be such as to produce a significant undesirable disturbance in the values of other biological and physico-chemical quality elements. A moderate increase in the frequency and intensity of planktonic blooms may occur. Persistent blooms may occur during summer months.
Macrophytes and phytobenthos	The taxonomic composition corresponds totally or nearly totally to undisturbed conditions. There are no detectable changes in the average macrophytic and the average phytobenthic abundance.	There are slight changes in the composition and abundance of macrophytic and phytobenthic taxa compared to the type-specific communities. Such changes do not indicate any accelerated growth of phytobenthos or higher forms of plant life resulting in undesirable disturbances to the balance of organisms present in the water body or to the physico-chemical quality of the water or sediment. The phytobenthic community is not adversely affected by bacterial tufts and coats present due to anthropogenic activity.	The composition of macrophytic and phytobenthic taxa differs moderately from the type-specific community and is significantly more distorted than at good status. Moderate changes in the average macrophytic and the average phytobenthic abundance are evident. The phytobenthic community may be interfered with and, in some areas, displaced by bacterial tufts and coats present as a result of anthropogenic activities.
Benthic invertebrate fauna	The taxonomic composition and abundance correspond totally or nearly totally to undisturbed conditions. The ratio of disturbance sensitive taxa to insensitive taxa shows no signs of alteration from undisturbed levels. The level of diversity of invertebrate taxa shows no sign of alteration from undisturbed levels.	There are slight changes in the composition and abundance of invertebrate taxa from the type-specific communities. The ratio of disturbance-sensitive taxa to insensitive taxa shows slight alteration from type-specific levels. The level of diversity of invertebrate taxa shows slight signs of alteration from type-specific levels.	The composition and abundance of invertebrate taxa differ moderately from the type-specific communities. Major taxonomic groups of the type-specific community are absent. The ratio of disturbance-sensitive taxa to insensitive taxa, and the level of diversity, are substantially lower than the type-specific level and significantly lower than for good status.
Fish fauna	Species composition and abundance correspond totally or nearly totally to undisturbed conditions. All the type-specific disturbance-sensitive species are present. The age structures of the fish communities show little sign of anthropogenic disturbance and are not indicative of a failure in the reproduction or development of any particular species.	There are slight changes in species composition and abundance from the type-specific communities attributable to anthropogenic impacts on physico-chemical and hydromorphological quality elements. The age structures of the fish communities show signs of disturbance attributable to anthropogenic impacts on physico-chemical or hydromorphological quality elements, and, in a few instances, are indicative of a failure in the reproduction or development of a particular species, to the extent that some age classes may be missing.	The composition and abundance of fish species differ moderately from the type-specific communities attributable to anthropogenic impacts on physico-chemical or hydromorphological quality elements. The age structure of the fish communities shows major signs of anthropogenic disturbance, to the extent that a moderate proportion of the type specific species are absent or of very low abundance.

Physico-chemical quality elements (1)

Element	High status	Good status	Moderate status
General conditions	The values of the physico-chemical elements correspond totally or nearly totally to undisturbed conditions. Nutrient concentrations remain within the range normally associated with undisturbed conditions. Levels of salinity, pH, oxygen balance, acid neutralising capacity and temperature do not show signs of anthropogenic disturbance and remain within the range normally associated with undisturbed conditions.	Temperature, oxygen balance, pH, acid neutralising capacity and salinity do not reach levels outside the range established so as to ensure the functioning of the type specific ecosystem and the achievement of the values specified above for the biological quality elements. Nutrient concentrations do not exceed the levels established so as to ensure the functioning of the ecosystem and the achievement of the values specified above for the biological quality elements.	Conditions consistent with the achievement of the values specified above for the biological quality elements.
Specific synthetic pollutants	Concentrations close to zero and at least below the limits of detection of the most advanced analytical techniques in general use.	Concentrations not in excess of the standards set in accordance with the procedure detailed in section 1.2.6 without prejudice to Directive 91/414/EC and Directive 98/8/EC. (<EQS)	Conditions consistent with the achievement of the values specified above for the biological quality elements.
Specific non-synthetic pollutants	Concentrations remain within the range normally associated with undisturbed conditions (background levels = bgl).	Concentrations not in excess of the standards set in accordance with the procedure detailed in section 1.2.6 (2) without prejudice to Directive 91/414/EC and Directive 98/8/EC. (<EQS)	Conditions consistent with the achievement of the values specified above for the biological quality elements.

(1) The following abbreviations are used: bgl = background level, EQS = environmental quality standard.
(2) Application of the standards derived under this protocol shall not require reduction of pollutant concentrations below background levels: (EQS >bgl).

Bibliography

Ahmad, Fiaz, Noreen Ashraf, Ren-Bin Zhou, Jing Jie Chen, Ya-Li Liu, Xiangbin Zeng, Feng-Zhu Zhao, and Da-Chuan Yin. 2019. "Optimization for Silver Remediation from Aqueous Solution by Novel Bacterial Isolates using Response Surface Methodology: Recovery and Characterization of Biogenic AgNPs." *Journal of Hazardous Materials* 380: 120906. doi:10.1016/j.jhazmat.2019.120906. https://dx.doi.org/10.1016/j.jhazmat.2019.120906.

Altenburg, W., G. Arts, J. G. Baretta-Bekker, van den Berg M.S, van den Broek Broek T, R. Buskens, R. Bijkerk, et al. 2018*. Referenties En Maatlatten Voor Natuurlijke Watertypen Voor De Kaderrichtlijn Water 2021-2027*: Stowa.

Anupong, Wongchai, Khumchai Jutamas, Ruangwong On-uma, Maha Alshiekheid, Amal Sabour, Ramakrishnan Krishnan, Nguyen Thuy Lan Chi, Arivalagan Pugazhendhi, and Kathirvel Brindhadevi. 2022. "Bioremediation Competence of Aspergillus Flavus DDN on Pond Water Contaminated by Mining Activities." *Chemosphere (Oxford)* 304: 135250. doi:10.1016/j.chemosphere.2022.135250. https://dx.doi.org/10.1016/j.chemosphere.2022.135250.

Ayele, Abate, Setegn Haile, Digafe Alemu, and M. Kamaraj. 2021. "Comparative Utilization of Dead and Live Fungal Biomass for the Removal of Heavy Metal: A Concise Review." *TheScientificWorld* 2021: 5588111-10. doi:10.1155/2021/5588111. https://dx.doi.org/10.1155/2021/5588111.

Bogan, B. W. and Lamar, R. T. 1995. "One-Electron Oxidation in the Degradation of Creosote Polycyclic Aromatic Hydrocarbons by Phanerochaete Chrysosporium." *Applied and Environmental Microbiology* 61 (7): 2631-2635. doi:10.1128/AEM.61.7.2631-2635.1995. http://aem.asm.org/content/61/7/2631.abstract.

CBS, PBL, RIVM, WUR (2022). Biologische waterkwaliteit KRW, 2021 (indicator 1420, versie 05 , 18 augustus 2022). www.clo.nl. Centraal Bureau voor de Statistiek (CBS), Den Haag; PBL Planbureau voor de Leefomgeving, Den Haag; RIVM Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven; en Wageningen University and Research, Wageningen.

CBS, PBL, RIVM, WUR (2020). Europese Kaderrichtlijn Water (indicator 1412, versie 05 , 30 juli 2020). www.clo.nl. Centraal Bureau voor de Statistiek (CBS), Den Haag; PBL Planbureau voor de Leefomgeving, Den Haag; RIVM Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven; en Wageningen University and Research, Wageningen.

CBS, PBL, RIVM, WUR (2022). Waterkwaliteit KRW, 2022 (indicator 1438, versie 09 , 18 augustus 2022). www.clo.nl. Centraal Bureau voor de Statistiek (CBS), Den Haag; PBL Planbureau voor de Leefomgeving, Den Haag; RIVM Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven; en Wageningen University and Research, Wageningen.Chandra, Deepshikha, Ashutosh Mishra, M. Das Trakroo, Rikhi Singh Chauhan, and Sarvesh Kumar Mishra. 2022. "Impact of Mycofiltration on Water Quality." *Environmental Quality Management* 31 (3): 253-266. doi:10.1002/tqem.21782. https://onlinelibrary.wiley.com/doi/abs/10.1002/tqem.21782.

Cruz-Morató, Carles, Laura Ferrando-Climent, Sara Rodriguez-Mozaz, Damià Barceló, Ernest Marco-Urrea, Teresa Vicent, and Montserrat Sarrà. 2013. "Degradation of

Pharmaceuticals in Non-Sterile Urban Wastewater by Trametes Versicolor in a Fluidized Bed Bioreactor.” *Water Research (Oxford)* 47 (14): 5200-5210. doi:10.1016/j.watres.2013.06.007. https://dx.doi.org/10.1016/j.watres.2013.06.007.

Cruz-Morató, Carles, Daniel Lucas, Marta Llorca, Sara Rodriguez-Mozaz, Marina Gorga, Mira Petrovic, Damià Barceló, Teresa Vicent, Montserrat Sarrà, and Ernest Marco-Urrea. 2014. “Hospital Wastewater Treatment by Fungal Bioreactor: Removal Efficiency for Pharmaceuticals and Endocrine Disruptor Compounds.” *The Science of the Total Environment* 493: 365-376. doi:10.1016/j.scitotenv.2014.05.117. https://dx.doi.org/10.1016/j.scitotenv.2014.05.117.

Deng, Jibao, Hefei Wang, Haisheng Zhan, Chenxi Wu, Yi Huang, Bing Yang, Ahmed Mosa, and Wanting Ling. 2022. “Catalyzed Degradation of Polycyclic Aromatic Hydrocarbons by Recoverable Magnetic Chitosan Immobilized Laccase from Trametes Versicolor.” *Chemosphere (Oxford)* 301: 134753. doi:10.1016/j.chemosphere.2022.134753. https://dx.doi.org/10.1016/j.chemosphere.2022.134753.

Dusengemungu, Leonce, George Kasali, Cousins Gwanama, and Kennedy Ochieng Ouma. 2020. “Recent Advances in Biosorption of Copper and Cobalt by Filamentous Fungi.” *Frontiers in Microbiology* 11: 582016. doi:10.3389/fmicb.2020.582016. https://www.ncbi.nlm.nih.gov/pubmed/33408701.

Espinosa-Ortiz, Erika J., Eldon R. Rene, Kannan Pakshirajan, Eric D. van Hullebusch, and Piet N. L. Lens. 2016. “Fungal Pelleted Reactors in Wastewater Treatment: Applications and Perspectives.” *Chemical Engineering Journal (Lausanne, Switzerland : 1996)* 283: 553-571. doi:10.1016/j.cej.2015.07.068. https://dx.doi.org/10.1016/j.cej.2015.07.068.

European Environment Agency, The European environment : state and outlook 2020 : executive summary, Publications Office of the European Union, 2019, https://data.europa.eu/doi/10.2800/48006

Evers, C. H. E., Broek, A. J. M. v. d., Buskens, R., Leerdam, A. v., Knoben, R. A. E., Herpen, F. C. J. v. and Pot, R. “Omschrijving MEP En Maatlatten Voor Sloten En Kanalen Voor De Kaderrichtlijn Water 2021-2027.”, https://library.wur.nl/WebQuery/groenekennis/2249667.

Galen, Frank v., Osté, Leonard and Boekel, Erwin v. “Nationale Analyse Waterkwaliteit : Onderdeel Van De Delta-Aanpak Waterkwaliteit : Eindrapport.”, https://library.wur.nl/WebQuery/groenekennis/2291177.

Gola, Deepak, Priyadarshini Dey, Arghya Bhattacharya, Abhishek Mishra, Anushree Malik, Maneesh Namburath, and Shaikh Ziauddin Ahammad. 2016. “Multiple Heavy Metal Removal using an Entomopathogenic Fungi Beauveria Bassiana.” *Bioresource Technology* 218: 388-396. doi:10.1016/j.biortech.2016.06.096. https://dx.doi.org/10.1016/j.biortech.2016.06.096.

Haritash, A. K. and C. P. Kaushik. 2009. “Biodegradation Aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A Review.” *Journal of Hazardous Materials* 169 (1): 1-15. doi:10.1016/j.jhazmat.2009.03.137. https://dx.doi.org/10.1016/j.jhazmat.2009.03.137.

Ishii, Satoshi and Michael J. Sadowsky. 2008. “Escherichia Coli in the Environment: Implications for Water Quality and Human Health.” *Microbes and Environments* 23 (2): 101-108. doi:10.1264/jsme2.23.101. https://www.jstage.jst.go.jp/article/jsme2/23/2/23_2_101/_

article/-char/en.

Jelic, Aleksandra, Carles Cruz-Morató, Ernest Marco-Urrea, Monserrat Sarrà, Sandra Perez, Teresa Vicent, Mira Petrović, and Damia Barcelo. 2012. "Degradation of Carbamazepine by Trametes Versicolor in an Air Pulsed Fluidized Bed Bioreactor and Identification of Intermediates." *Water Research (Oxford)* 46 (4): 955-964. doi:10.1016/j.watres.2011.11.063. https://dx.doi.org/10.1016/j.watres.2011.11.063.

Kadri, Tayssir, Tarek Rouissi, Satinder Kaur Brar, Maximiliano Cledon, Saurabhjyoti Sarma, and Mausam Verma. 2017. "Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) by Fungal Enzymes: A Review." *Journal of Environmental Sciences (China)* 51: 52-74. doi:10.1016/j.jes.2016.08.023. https://dx.doi.org/10.1016/j.jes.2016.08.023.

Kanamarlapudi, Sri Lakshmi Ramya Krishna, Vinay Kumar Chintalpudi, and Sudhamani Muddada. 2018. *Application of Biosorption for Removal of Heavy Metals from Wastewater* IntechOpen.

Kumar, Vinay and Shiv Kumar Dwivedi. 2021. "Mycoremediation of Heavy Metals: Processes, Mechanisms, and Affecting Factors." *Environmental Science and Pollution Research International* 28 (9): 10375-10412. doi:10.1007/s11356-020-11491-8. https://link.springer.com/article/10.1007/s11356-020-11491-8.

Kurniati, Evi, Novi Arfarita, Tsuyoshi Imai, Takaya Higuchi, Ariyo Kanno, Koichi Yamamoto, and Masahiko Sekine. 2014. "Potential Bioremediation of Mercury-Contaminated Substrate using Filamentous Fungi Isolated from Forest Soil." *Journal of Environmental Sciences (China)* 26 (6): 1223-1231. doi:10.1016/S1001-0742(13)60592-6. http://lib.cqvip.com/qk/85265X/201406/49943480.html.

Li, Huifen, Yanbing Lin, Wumeng Guan, Jiali Chang, Lin Xu, Junkang Guo, and Gehong Wei. 2010. "Biosorption of Zn(II) by Live and Dead Cells of Streptomyces Ciscaucasicus Strain CCNWHX 72-14." *Journal of Hazardous Materials* 179 (1): 151-159. doi:10.1016/j.jhazmat.2010.02.072. https://dx.doi.org/10.1016/j.jhazmat.2010.02.072.

Li, Xueqing, Renata Alves de Toledo, Shengpeng Wang, and Hojae Shim. 2015. "Removal of Carbamazepine and Naproxen by Immobilized Phanerochaete Chrysosporium Under Non-Sterile Condition." *New Biotechnology* 32 (2): 282-289. doi:10.1016/j.nbt.2015.01.003. https://dx.doi.org/10.1016/j.nbt.2015.01.003.

Li, Xueqing, Jiaming Xu, Renata Alves de Toledo, and Hojae Shim. 2015a. "Enhanced Removal of Naproxen and Carbamazepine from Wastewater using a Novel Countercurrent Seepage Bioreactor Immobilized with Phanerochaete Chrysosporium Under Non-Sterile Conditions." *Bioresource Technology* 197: 465-474. doi:10.1016/j.biortech.2015.08.118. https://dx.doi.org/10.1016/j.biortech.2015.08.118.

Lu, Tao, Qi-Lei Zhang, and Shan-Jing Yao. 2016. "Application of Biosorption and Biodegradation Functions of Fungi in Wastewater and Sludge Treatment." In *Fungal Applications in Sustainable Environmental Biotechnology*, 65-90. Cham: Springer International Publishing.

Luan, T. G., Jing Jin, Sidney M. N. Chan, Y. S. Wong, and Nora F. Y. Tam. 2006. "Biosorption and Biodegradation of Tributyltin (TBT) by Alginate Immobilized Chlorella Vulgaris Beads in several Treatment Cycles." *Process Biochemistry* 41 (7): 1560-1565. doi:10.1016/j.procbio.2006.02.020. https://dx.doi.org/10.1016/j.procbio.2006.02.020.

Machado, Manuela D., Eduardo V. Soares, and Helena M. V. M. Soares. 2010. "Removal of Heavy Metals using a Brewer's Yeast Strain of Saccharomyces Cerevisiae: Chemical Speciation as a Tool in the Prediction and Improving of Treatment Efficiency of Real Electroplating Effluents." *Journal of Hazardous Materials* 180 (1): 347-353. doi:10.1016/j.jhazmat.2010.04.037. https://dx.doi.org/10.1016/j.jhazmat.2010.04.037.

Majcherczyk, Andrzej, Christian Johannes, and Aloys Hüttermann. 1998. "Oxidation of Polycyclic Aromatic Hydrocarbons (PAH) by Laccase of Trametes Versicolor." *Enzyme and Microbial Technology* 22 (5): 335-341. doi:10.1016/S0141-0229(97)00199-3. https://dx.doi.org/10.1016/S0141-0229(97)00199-3.

Malaj, Egina, Peter C. von der Ohe, Matthias Grote, Ralph Kuhne, Cedric P. Mondy, Philippe Usseglio-Polatera, Werner Brack, and Ralf B. Schafer. 2014. "Organic Chemicals Jeopardize the Health of Freshwater Ecosystems on the Continental Scale." *Proceedings of the National Academy of Sciences - PNAS* 111 (26): 9549-9554. doi:10.1073/pnas.1321082111. https://agris.fao.org/agris-search/search.do?recordID=US201600144769.

Marco-Urrea, Ernest, Miriam Pérez-Trujillo, Teresa Vicent, and Gloria Caminal. 2009. "Ability of White-Rot Fungi to Remove Selected Pharmaceuticals and Identification of Degradation Products of Ibuprofen by Trametes Versicolor." *Chemosphere (Oxford)* 74 (6): 765-772. doi:10.1016/j.chemosphere.2008.10.040. https://dx.doi.org/10.1016/j.chemosphere.2008.10.040.

Moermond, CTA, MHMM Montforts, EWM Roex, and B. J. Venhuis. 2020. *Medicijnresten En Waterkwaliteit: Een Update*: Rijksinstituut voor Volksgezondheid en Milieu RIVM.

Negi, Bharat Bhushan, Arindam Sinharoy, and Kannan Pakshirajan. 2020. "Selenite Removal from Wastewater using Fungal Pelleted Airlift Bioreactor." *Environmental Science and Pollution Research International* 27 (1): 992-1003. doi:10.1007/s11356-019-06946-6. https://link.springer.com/article/10.1007/s11356-019-06946-6.

Pini, Andrea K. and Pamela Geddes. 2020. "Fungi are Capable of Mycoremediation of River Water Contaminated by E. Coli." *Water, Air, and Soil Pollution* 231 (2). doi:10.1007/s11270-020-4464-7. https://link.springer.com/article/10.1007/s11270-020-4464-7.

Rahmani, Kourosh, Mohammad Ali Faramarzi, Amir Hossain Mahvi, Mitra Gholami, Ali Esrafili, Hamid Forootanfar, and Mahdi Farzadkia. 2015. "Elimination and Detoxification of Sulfathiazole and Sulfamethoxazole Assisted by Laccase Immobilized on Porous Silica Beads." *International Biodeterioration & Biodegradation* 97: 107-114. doi:10.1016/j.ibiod.2014.10.018. https://dx.doi.org/10.1016/j.ibiod.2014.10.018.

Rodarte-Morales, A. I., G. Feijoo, M. T. Moreira, and J. M. Lema. 2012a. "Biotransformation of Three Pharmaceutical Active Compounds by the Fungus Phanerochaete Chrysosporium in a Fed Batch Stirred Reactor Under Air and Oxygen Supply." *Biodegradation (Dordrecht)* 23 (1): 145-156. doi:10.1007/s10532-011-9494-9. https://link.springer.com/article/10.1007/s10532-011-9494-9.

Rodarte-Morales, A. I., G. Feijoo, M. T. Moreira, and J. M. Lema. 2012b. "Operation of Stirred Tank Reactors (STRs) and Fixed-Bed Reactors (FBRs) with Free and Immobilized Phanerochaete Chrysosporium for the Continuous Removal of Pharmaceutical Compounds." *Biochemical Engineering Journal* 66: 38-45. doi:10.1016/j.bej.2012.04.011. https://dx.doi.org/10.1016/j.bej.2012.04.011.

Sabuda, Mary C., Carla E. Rosenfeld, Todd D. DeJournett, Katie Schroeder, Karl Wuolo-Journey, and Cara M. Santelli. 2020. “Fungal Bioremediation of Selenium-Contaminated Industrial and Municipal Wastewaters.” *Frontiers in Microbiology* 11: 2105. doi:10.3389/fmicb.2020.02105. https://search.proquest.com/docview/2448640021.

Sangeetha Devi, Rajendran, Velu Rajesh Kannan, Duraisamy Nivas, Kanthaiah Kannan, Sekar Chandru, and Arokiaswamy Robert Antony. 2015. “Biodegradation of HDPE by Aspergillus Spp. from Marine Ecosystem of Gulf of Mannar, India.” *Marine Pollution Bulletin* 96 (1-2): 32-40. doi:10.1016/j.marpolbul.2015.05.050. https://dx.doi.org/10.1016/j.marpolbul.2015.05.050.

Sarı, Ahmet and Mustafa Tuzen. 2009. “Biosorption of as(III) and as(V) from Aqueous Solution by Macrofungus (Inonotus Hispidus) Biomass: Equilibrium and Kinetic Studies.” *Journal of Hazardous Materials* 164 (2): 1372-1378. doi:10.1016/j.jhazmat.2008.09.047. https://dx.doi.org/10.1016/j.jhazmat.2008.09.047.

Shahjahan, Md, Khanam Taslima, Mohammad Shadiqur Rahman, Md Al-Emran, Shanon Iffat Alam, and Caterina Faggio. 2022. “Effects of Heavy Metals on Fish Physiology – A Review.” *Chemosphere (Oxford)* 300: 134519. doi:10.1016/j.chemosphere.2022.134519. https://dx.doi.org/10.1016/j.chemosphere.2022.134519.

Svobodova, Katerina and Cenek Novotny. 2018. “Bioreactors Based on Immobilized Fungi: Bioremediation Under Non-Sterile Conditions.” *Applied Microbiology and Biotechnology* 102 (1): 39-46. doi:10.1007/s00253-017-8575-z. https://link.springer.com/article/10.1007/s00253-017-8575-z.

Tastan, Burcu Ertit, Sevgi Ertugrul, and Gönül Dönmez. 2010. “Effective Bioremoval of Reactive Dye and Heavy Metals by Aspergillus Versicolor.” *Bioresource Technology* 101 (3): 870-876. doi:10.1016/j.biortech.2009.08.099. https://dx.doi.org/10.1016/j.biortech.2009.08.099.

Tayel, Ahmed A., Mohamed M. Gharieb, Hanaa R. Zaki, and Nihal M. Elguindy. 2016a. “Bio-Clarification of Water from Heavy Metals and Microbial Effluence using Fungal Chitosan.” *International Journal of Biological Macromolecules* 83: 277-281. doi:10.1016/j.ijbiomac.2015.11.072. https://dx.doi.org/10.1016/j.ijbiomac.2015.11.072.

Taylor, Alex, Alicia Flatt, Marc Beutel, Morgan Wolff, Katherine Brownson, and Paul Stamets. 2015. “Removal of Escherichia Coli from Synthetic Stormwater using Mycofiltration.” *Ecological Engineering* 78: 79-86. doi:10.1016/j.ecoleng.2014.05.016. https://dx.doi.org/10.1016/j.ecoleng.2014.05.016.

Tran, Ngoc Han, Taro Urase, and Osamu Kusakabe. 2010. “Biodegradation Characteristics of Pharmaceutical Substances by Whole Fungal Culture Trametes Versicolor and its Laccase.” *Journal of Water and Environment Technology* 8 (2): 125-140. doi:10.2965/jwet.2010.125. https://www.jstage.jst.go.jp/article/jwet/8/2/8_2_125/_article/-char/en.

Visoottiviseth, P. and Panviroj, N. 2001. “Selection of Fungi Capable of Removing Toxic Arsenic Compounds from Liquid Medium.” ScienceAsia 27 (2001): 083-092. doi:10.2306/scienceasia1513-1874.2001.27.083.

Vaseem, Huma, V. K. Singh, and M. P. Singh. 2017. “Heavy Metal Pollution due to Coal Washery Effluent and its Decontamination using a Macrofungus, Pleurotus Ostreatus.” *Ecotoxicology and Environmental Safety* 145: 42-49. doi:10.1016/j.

ecoenv.2017.07.001. https://dx.doi.org/10.1016/j.ecoenv.2017.07.001.

Vissers, Marc, Vergouwen, Lideke, Witteveen, Stefan, Derksen, Anja and Raadgever, Tom. “Landelijke Hotspotanalyse Geneesmiddelen RWZI’S.”, https://library.wur.nl/WebQuery/groenekennis/2228653.

Xiao, Xiaoyu, Shuhui Zhu, Xiaoming Zou, Yongsheng Song, Jing Jiang, and G. Daniel Sheng. 2020. “Uptake and Elimination of Butyl- and Phenyltins by Ceratophyllum Demersum L.” *Chemosphere (Oxford)* 249: 126113. doi:10.1016/j.chemosphere.2020.126113. https://dx.doi.org/10.1016/j.chemosphere.2020.126113.

Yuan, Jianhua, Jie Ma, Yiran Sun, Tao Zhou, Youcai Zhao, and Fei Yu. 2020. “Microbial Degradation and Other Environmental Aspects of Microplastics/Plastics.” *The Science of the Total Environment* 715: 136968. doi:10.1016/j.scitotenv.2020.136968. https://dx.doi.org/10.1016/j.scitotenv.2020.136968.

Zhang, Yongjun and Sven-Uwe Geißen. 2012. “Elimination of Carbamazepine in a Non-Sterile Fungal Bioreactor.” *Bioresource Technology* 112: 221-227. doi:10.1016/j.biortech.2012.02.073. https://dx.doi.org/10.1016/j.biortech.2012.02.073.

Zhao, Changsong, Jun Liu, Hong Tu, Feize Li, Xiyang Li, Jijun Yang, Jiali Liao, Yuanyou Yang, Ning Liu, and Qun Sun. 2016. “Characteristics of Uranium Biosorption from Aqueous Solutions on Fungus Pleurotus Ostreatus.” *Environmental Science and Pollution Research International* 23 (24): 24846-24856. doi:10.1007/s11356-016-7722-x. https://link.springer.com/article/10.1007/s11356-016-7722-x.