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Use of *Phragmites australis* for phytoremediation of organic compounds in municipal waste water treatment plants

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Abstract

Phytoremediation as a means of cleaning up polluted soils has gained popularity during the last decade due to its convenience and low costs of installation and maintenance. When the target pollutant is biodegradable, this technology exploits the stimulating effect that roots have on microbial processes and physical/chemical modifications in the

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rhizosphere. Among the microorganisms that affect rhizosphere processes, symbiotic fungi forming mycorrhizas induce a series of changes in plant physiology, nutrient availability and microbial composition that may determine the outcome of a phytoremediation attempt. Beyond the rhizosphere, mycorrhizal hyphae act as the roots of the roots, and may thus extend the rhizosphere into the bulk soil by creating a new interface of soil-plant interactions: the hyphosphere.

Introduction

Despite the fact that the resource drinking water is among the most precious goods on earth, we have to acknowledge that some bodies of European surface waters and ground water reserves are polluted by organic and inorganic substances. The same accounts for numerous terrestrial ecosystems, above all by organic pollutants from industrial processes, dumping or accidents. Many of the pollutants have high stability and recalcitrance. In Germany more than 70,000 problem sites have been identified (Franzius 1994).

How dangerous a material can become for ground water reserves depends above all on its toxic properties, its chemistry and its mobility in the system. In Germany, compounds hazardous to aqueous ecosystems are classified in danger classes from 1 (weakly hazardous to waters) to 3 (very hazardous to waters). Most chlorinated hydrocarbons are recalcitrant, partially very toxic in the environment, mobile in the subsoil and very hazardous to waters, hence they belong to class 3. They even easily penetrate meter-thick concrete and water-impervious layers in the underground soil. Thanks to EU restrictions the occurrence of hazardous substrate to waters could be reduced in many areas. However, problems exist in brownfields as well as after acute accidents which can lead to heavy pollution of the immediate surroundings.

In the 19-th century the environmental damages clearly increased with growing industrialization. Nevertheless, the majority of our current brownfields has developed after 1950. At that time industrial waste and household garbage were not disposed of on carefully sealed garbage dumps yet. Only from 1972 the messy municipal garbage tips were closed and converted into central house garbage dumps. Today these fulfil high environmental standards and prevent pollution of ground water (Bayerisches Landesamt für Wasserwirtschaft. 2004b). As the concern about environmental pollution had risen steadily in the 1990s, the first political reactions were issued by the European Union (EU). Today the European water framework guideline (WRRL) strives for an uniform and sustainable water pollution control in European dimension. Here for the first time the states of Europe take over a common international responsibility for all their bodies of water, for the ground water, the lakes, rivers and the sea. Of course, this novel feeling of responsibility will also have to include the input of water into the water reserves, especially from waste water treatment facilities. Green and environmentally sound technologies are urgently sought to solve the European water safety problem in a sustainable manner.

Example: The waste water treatment unit of Mörlbach, Bavaria

Numerous studies have underpinned that significant concentrations of pollutants from households or human excretions would be able to pass through municipal waste water treatment facilities into adjacent water bodies, where they expand potential risk for the members of the food web (ATV-DVWK 2003). Innovative technologies and specific water treatment facilities like membrane filtration or UV-radiation can diminish this load of micropollutants from the effluent water, but some residues will still persist. Furthermore, the use of these expensive technologies is usually mostly restricted to large technical water treatment facilities of bigger cities (Spengler 2001). Smaller waste water treatment units in rural areas do usually not have any chance to participate in the use of elaborate technological solutions for their pollution problem.

Nearly 50% of the treatment plants in Bavaria are lagoons. They fulfil the legal frame of water treatment (Fig. 1). The units consist of a mechanical pretreatment, followed by one or more lagoons (specific area: 12 - 15 $m^{-2}p.e.^{-1}$) for the biological treatment of the waste water. This biological treatment step includes microorganisms and small multicellular organisms (i.e. ciliates) that are found suspended in the water body, in biofilms, or the upper levels of the sediments in the lagoon. In contact with air, the organic compounds in the mechanically pretreated waste water are converted to inorganic end products like

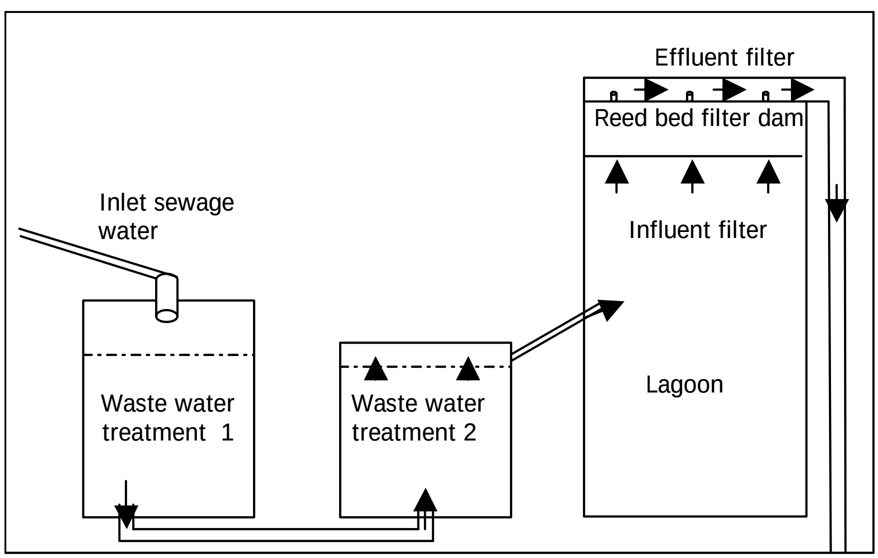

Figure 1. Schematic outline of a typical municipal waste water treatment lagoon, in this case equipped with a phytoremediation filter.

CO_2 and water. Nitrification and denitrification lead to metabolism of inorganic and organic nitrogen compounds. Phosphorus compounds are mainly physically adsorbed to the sediment.

The microorganisms in such a system form a symbiosis in equilibrium with its environment and the source of nutrients, i.e. the waste water. Besides, physico-chemical processes like adsorption or ion exchange in the sludge play a major role in the removal of pollutants. The interactions of nutrients, light, and temperature in the water lead to algal blooms especially in autumn and spring, which are a secondary pollution to the biologically treated wastewater, as algal biomass is being metabolized by the inhabiting bacteria, leading to anaerobic conditions in the receiving waters, lethal to small organisms and fish.

Because of their simplicity in construction and performance accompanied by stable treatment results, such systems are found in many rural regions in Germany, especially in Bavaria and Mecklenburg-Vorpommern. One example is the lagoon in the community of Berg, Mörlbach close to Lake Starnberg in upper Bavaria. This area is drained by several aquifers and sensible to groundwater pollution. A little village with 300 inhabitants sends its domestic wastewater to the plant. It consists of a mechanical pretreatment and a lagoon.

Although small, this plant has found al lot of attention due to the fact that a planted soil filter has been constructed in the effluent in order to remove all secondary load like algae. Furthermore special attention has been focused on the removal of organic micropollutants. These substances can, in case they percolate into the adjacent water bodies, exert negative influence on ecosystems and pollute the ground water to an extent that makes further action necessary. Besides well known organic solvents, pharmaceuticals and their metabolites were frequently found in the effluent of the plant.

In 1997, the WWTP had been provided with a planted soil filter (constructed of loose, medium size gravel) of 40 m length and 50 cm depth to filter the effluent water using phytoremediation technology by planting it with cattail and reed plants to improve the effluent quality.

In its present form, the lagoon delivers effluent water from which organic load has been removed to 75 %, algal biomass is retained by 95 % and with stable pH values throughout the year (Gschlößl et al. 1998). From the successful application of the phytoremediation filter it becomes clear that effluent filter attached to the lagoon can improve the effluent water quality significantly by removing organic compounds, as well as accumulating metal ions, i.e. Fe, Cu. The success of the system is obviously in part based on the longer residence time in the system caused by its percolation through the dense root mat of the plants, and due to rhizosphere microorganisms capable of attacking difficultly degradable pollutants. The plant itself contributes significantly by providing valuable root exsudates, by transpiring water, and by taking up and degrading organic pollutants as well as accumulating heavy metals throughout the year.

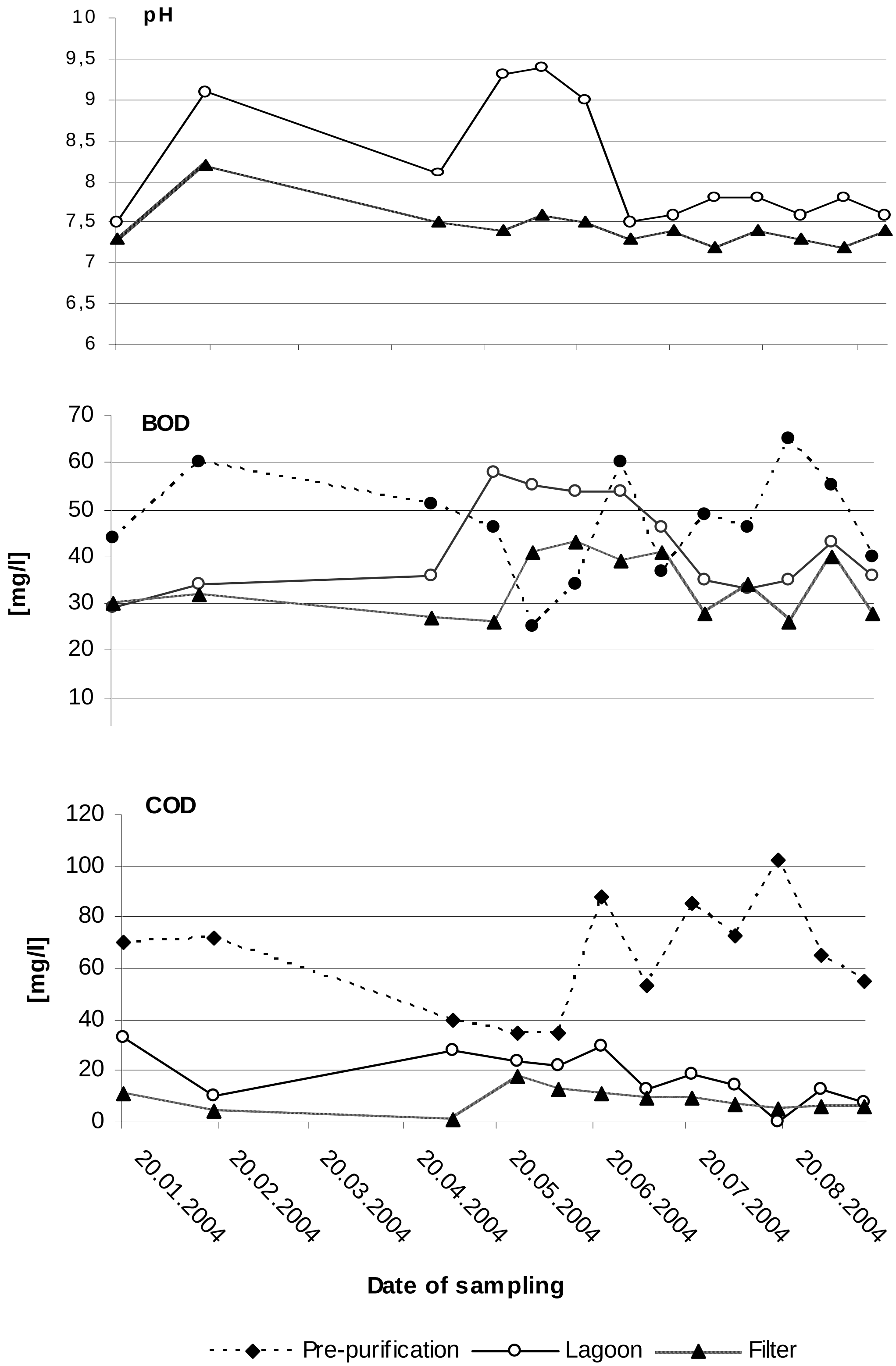

Figure 2. pH, BOD, COD, and pH of the water in the Mörlbach lagoon, as determined in spring and summer 2004.

The role of plants in phytoremediation

The capability of plants to take up and metabolize nutrients from water and by this inhibit eutrophication has been known for long time. It is utilized in agricultural practise of many southern European countries for the removal of phosphorus and nitrogen from drainage ditches or smaller water bodies in agricultural landscapes. Farmers use predominantly reed (*Phragmites australis*) or cattail (*Typha spec*.) for this purpose, as these plants are also found as natural inhabitants of those sites. The above ground biomass is harvested at the end of the vegetation period and distributed on the fields as green manure. This application of plant species to remove unwanted substances is adopted by modern techniques of phytoremediation to extract nutrients and/or pollutants from water bodies, sediments or soils. The role of plants can be manifold in this context.

Phytoaccumulation is the most frequently used technology wherein plants take up and store pollutants in amounts up to 1000 fold above soil concentration (Schnoor et al. 1995). This is usually found for heavy metal pollution from mine tailings or industrial processes.

Phytoremediation summarizes techniques where plants take up, detoxify and metabolize the pollutant, and finally produce non-toxic end products that are ideally stored in the plant cell wall as hardly degradable bound residues.

Phytostabilization uses plants to transpire water above pollutant plumes and hence reduce the interstitial vertical water flows that would move the xenobiotics towards ground water bodies. The rooting plants would also increase the organic matter content of the soil and enable fungi and bacteria to get in contact with the pollutants.

Phytovolatilization describes the metabolism of organic xenobiotics and (some) metals to volatile compounds in the aerenchmya of the plants under considerations. Here it is not important whether the plant itself or endophytic bacteria would perform the respective chemical reactions.

One of the largest problems of all the mentioned phytoremediation techniques is the limited accessibility of the pollutants in the soils and the restricted uptake and transport in the plant. It is clear that the uptake is determined by physicochemical properties of the pollutant, and the possibilities to come into contact with the living tissue, where detoxification or sequestration takes place.

In case the pollutants are adsorbed to minerals or humic substances deep in soil or sediment, the chosen plant has to be able to grow its roots into their vicinity. Furthermore it is essential for the process that the plant is able to influence the chemistry of the rhizosphere in a way that leads to solubilization of the pollutant and facilitates uptake into the roots. The latter can only be achieved for substances with octanol water coefficients between 0 and 3, and with dissociation constants between 0 and 6 (Briggs et al. 1983, Behrend & Brüggemann 1993, Rigatoni & Briggs 1986, Schröder and Collins, 2002).

Once inside the plant, detoxification of organic xenobiotics, be it industrial chemicals, air pollutants, agrochemicals and herbicides or unwanted molecules of other origin, follows a scheme known as "the green liver concept" (Sandermann 1994).

This expression refers to the analogy of the plant detoxification process with animal liver metabolism. In liver cells, pollutants are recognized and activated, i.e. enzymatically altered to yield hydroxylated, oxidised or reduced primary metabolites. These reactions are predominantly catalyzed by P450 monooxygenases or peroxidases. This first phase of activation is followed by the phase (II) of detoxification in the strict sense of the word. Here conjugation reactions take place that couple biomolecules like sugars, amino acids or peptides like glutathione to the activated foreign compound. Conjugation leads to severe alterations in the chemistry of the product - it is well documented that conjugated xenobiotics have lost their capability to penetrate through biomembranes (Lamoureux and Rusness 1989, Schröder 2001).

When conjugation capacity was measured in reed plants from the Mörlbach municipal waste water treatment plant, it became clear, that all investigated plant samples contained relatively high glutathione S-transferase activity for the detoxification of organic pollutants. Interestingly, the enzyme activity was always higher in the samples taken from plants at the inlet side of the dam, indicating that the polluted water increased the demand for plant

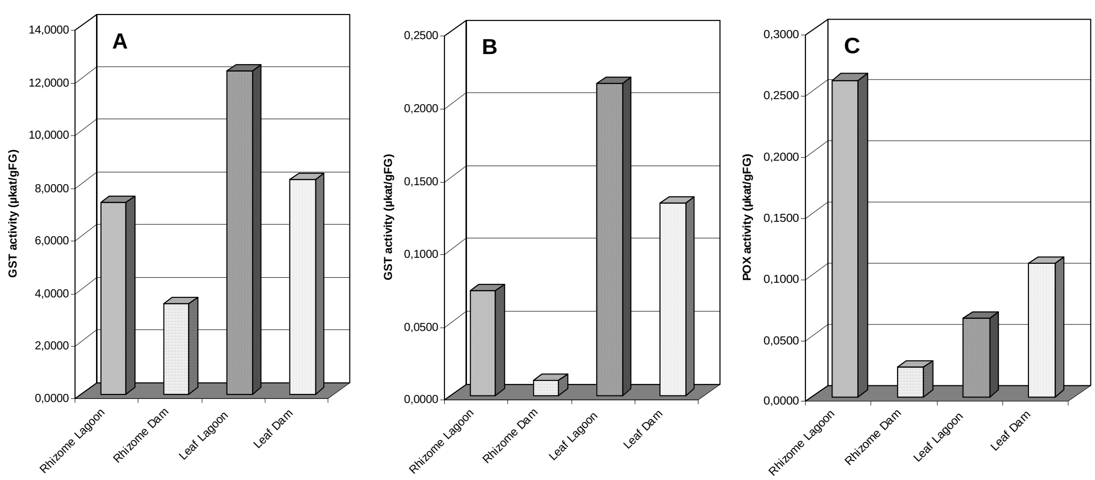

Figure 3. Detoxification enzymes in *Phragmites australis* plants from the Mörlbach lagoon. Plants were sampled on the lagoon and on the efflux side (dam) of the pond. High differences exist between the enzyme activities between the two sites. A: GST activity for the substrate CDNB, B: GST activity for DCNB, C: Peroxidase activity. For methods refer to Schröder et al. 2007.

defense. On the rear side of the dam, where lower pollution occurs, the plant glutathione S-transferases had lower activities (Fig. 3). The same reaction was monitored for an enzyme of general stress response: the peroxidases (POX, Fig. 3C). Here, rhizome activities differ by a factor of 5, indicating high detoxification demand in plants on the polluted side.

In animal metabolism, xenobiotic conjugates are further metabolized and finally excreted in urine or faeces. Plants do not possess excretion organs, and so final plant metabolites are soluble vacuolar metabolites (Wolf et al. 1996, Schröder et al. 2007), or bound residues in the cell wall (Langebartels and Harms 1986, Schröder 1997). Ideally, organic xenobiotics are metabolized to an extent that makes them indistinguishable from natural metabolites of the organism and inaccessible in food and fodder.

Concepts for the future

I. Improving plant performance

Detoxification reactions in plants are very flexible and inducible. Already by minor disturbances by pathogens, predators, UV-radiation or changes in temperature lead to significant alterations in enzyme activities and defence. Of special interest is the influence of chemicals, pesticides and herbicide antidotes on the detoxification capacity of many plants (Schröder et al. 1993, Schröder 2001). The fast reaction of the detoxification enzymes towards safeners is used to induce detoxification in crops, prior to the application of herbicides to the fields. Of course, such an increase in detoxification capacity would be beneficial for phytoremediation, but marketable products are lacking so far. Pollutant safeners would be urgently needed to detoxify and metabolize higher loads of agrochemicals, pollutants and pharmaceuticals in municipal waste water treatment facilities that use the phytoremediation technique.

The modern state of molecular biological tools would also enable us to improve the detoxification capacity of plants under consideration by gene manipulation. Here, plant detoxification enzymes themselves could be over expressed and kept at high levels, or bacterial of fungal foreign genes could be introduced that code for enzymes that would lack in plants. Hence a whole array of pharmaceuticals could be degraded in phytoremediation plants. Presently, European laws do not allow the construction or use of such GMO "super plants" but the question has to be asked whether future decision makers would still exclude the use of beneficial organisms for the removal of pollutants from water. One could think of specifically constructed plants that would degrade high pollutant loads to non toxic metabolites, and then die off under the influence of the self-induced pollutant deprivation. With view of the steadily degrading water quality such an idea should not be excluded, especially if it would be accompanied by state of art security research.

II. Introducing microbial assisted rhizoremediation

In the future, this approach could be even widened by designing rhizospheres. We do know virtually nothing about the interplay between bacteria, fungi and plant roots in sediments or phytoremediation plants. Information is scarce about the stimulation of bacterial activity under the influence of specific plant root exsudation products that would enable bacteria to do the job. Presently new concepts for productive rhizospheres that might be produced through inoculation with plant growth promoting bacteria (PGPR) are being discussed in agriculture. The most common bacteria in the mycorrhizosphere are *Pseudomonas* (Vosátka and Gryndler, 1999), while different bacterial species exist in the hyphosphere.

On the other hand, inoculation of soil or plant saplings with mycorrhiza is an upcoming technology with many advantages concerning plant nutrient supply and pathogen defense.

It is well established that universal and ubiquitous symbiotic arbuscular mycorrhizal (AM) fungi, belonging to *Glomales*, form symbiotic relationships with roots of 80%~90% land plants in natural and agricultural ecosystems (Brundrett, 2002). Mycorrhizal fungi are known to benefit plant nutrition, growth and survival, due to their greater exploitation of soil for nutrients (Smith and Read, 1997). These associations represent a key factor in the below ground networks which influence diversity and plant community structure (van der Heijden et al., 1998; Burrows and Pfleger, 2002; O'Connor et al., 2002; Chaudhry et al., 2005.

AM can help phytoremediation activities, particularly in phytostabilization (Gonçalves et al., 1997; Leyval et al., 1997, 2002; Orlowska et al., 2002; Regvar et al., 2003; Turnau et al., 2005). Among possible mechanisms by which AM fungi improve the resistance of plants to HMs is the ability of the AM fungi to sequester HMs through the production of chelates or by absorption. AM plants typically translocate less HM to their shoots than the corresponding non-AM controls. The role of AM fungi in phytoextraction is thought to be less significant. However, the involvement of AM is being investigated now because of the recent interest in plants able to hyper-accumulate HMs (Turnau et al., 2006). The AMF can be screened for their ability to produce maximum levels of extra-radical mycelium in polluted soils (Joner et al., 2000), and to utilize adapted AMF to help accumulate heavy metal (HM) both within the plant roots (phytoaccumulation) and the extracellular fungal mycelium. While some workers observed that the external mycelium of AMF was the main site for trace element localization (Kaldorf et al., 1999; Turnau, 1998), others reported selective exclusion of toxic and non-toxic elements by adsorption onto chitinous cell walls (Zhou, 1999), onto extra-cellular glycoprotein, glomalin (Wright and Upadhyaya, 1998), or intracellular precipitation. All these mechanisms have implications in reducing a plant's exposure to potentially toxic elements. However, the nature of accumulation and mechanisms involved require further studies in order to better understand the participation of AMF in plant tolerance and its ecological significance in polluted soils. Their potential role in phytoremediation of HM contaminated soils and water is becoming evident although there is need to completely understand the ecological complexities of the plant-microbe-soil interactions and their better exploitation as consortia in remediation strategies employed for contaminated soils. These multitrophic root microbial associations deserve multi-disciplinary investigations using molecular, biochemical, and physiological techniques. Ecosystem restoration of contaminated soils practices need to incorporate microbial biotechnology research and development. Such microbial communities, selected plant species with desired root characteristics, and specific inoculates of potent degraders could open new perspectives for waste water management without genetic modifications. Applied biological research, as it is also stimulated through the EU COST Actions, is presently on the way to produce interdisciplinary concepts for cleaning the environment and sustainable management of our most precious resource, water.

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