

InteGRated systems for Effective
ENvironmEntal Remediation



greener

NEWSLETTER

Issue 2, June 2020

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GREENER CONCEPT



GREENER aims to develop innovative, low-cost, efficient and sustainable solutions for effective environmental remediation. A combination of the most promising technologies will be up-scaled and tested in the field. Research will be carried out in partnership between EU and China.

Over the last decades, soils/sediments and, consequently, surface waters and groundwater have been increasingly contaminated with harmful chemicals from agricultural (fertilisers), industrial (harmful material dumping, sludge, fly ash) and urban activities (hydrocarbons and metals from vehicles, urban waste dumping, pharmaceuticals). Pollution can adversely affect the quality, and thereby functionality, of soils/sediments and waters (both surface and groundwater), causing disorders of various kinds to the environment and public health and hindering commercial exploitation.

Across Europe only, more than 2.5 million sites

have been estimated to be potentially contaminated, with the consequence that otherwise valuable lands became unusable for habitation, agricultural activities and ecological habitats. Currently approximately 342,000 sites in European countries require urgent remediation and if current trends continue, the number of sites needing remediation is expected to increase by 50% by 2025.

Soil contamination is also a matter of high concern in China, where it has been reported that the quality of soil or sediments is deteriorating at a rapid rate. This pollution has resulted in crop contamination to levels that could threaten human health and ecosystem functions.

Worldwide, groundwater pollution by hydrocarbons (including total petroleum hydrocarbons -TPHs; polycyclic aromatic hydrocarbons, PAHs; and halogenated hydrocarbons), pesticides and toxic metals and metalloids is a widespread environmental threat with consequent impact on public health. The presence of antibiotics in water raises increasing concerns associated with the generation of antibiotic-resistant bacteria and genes. Similarly, synthetic dyes, widely used in the textile, paper, food, cosmetics and pharmaceutical industries, are another source of concern. Among these, azo dyes, considered carcinogenic, account for up to 70%, with 10–15% lost in effluent during the dyeing process. The discharge of azo dyes into surface water

GREENER CONCEPT



reduces light penetration and inhibits photosynthesis, thereby disturbing the natural growth of aquatic life. Finally, industrial activities, such as mining, metal surface treatment (e.g. electroplating, anodising), production of pigments and metal processing, are responsible for pollution by potentially toxic metals and metalloids, which accumulate in living organisms and can act progressively over long periods of time through the food chain.

Conventional technologies for cleaning-up contaminated soils and waters are generally energy intensive, expensive, time consuming, waste-producing and might imply the use of hazardous chemicals.

To this aim, GREENER exploits innovative methodologies, such as bioaugmentation and biostimulation combined to lead to biopiling, which can be employed for the simultaneous removal of soils contaminated by mixtures of hydrocar-

bons (TPHs and PAHs) together with potentially toxic metals and metalloids (mainly Cu, Zn, Hg, among others). Biostimulation, bioaugmentation and phytoremediation will be integrated in the ecopile technique. Phytoremediation is being used for the removal of potentially toxic metals and metalloids from water and soils, whilst phycoremediation, a microalgae based technology, is used for the removal, for the removal of potentially toxic metals and metalloids in wastewater. Among various bio-electrochemical systems (BES) technologies, GREENER also uses plant fuel cells (PFCs), and constructed wetland microbial fuel cells (CW-MFCs) for the treatment of polluted soil and water contaminated with PAHs, TPHs, pesticides and toxic metals, aiming to faster and enhanced bioremediation. GREENER also exploits the use of MFCs for simultaneous removal of potentially toxic metals present in wastewater and production of nanoparticles.

As a result, GREENER follows the need to develop innovative low-cost, eco-friendly and chemical-free technologies to remove toxic compounds from the environment and redevelop contaminated areas. The implementation of bioremediation technologies can instead be considered a cheaper and more sustainable alternative to physico-chemical treatments. Bioremediation and phytoremediation have shown great potential to complement conventional methods for restoring polluted soils and waters contaminated by large environmental catastrophes.

ADVANCEMENTS



GREENER project consortium has selected different contaminated sites to be used during innovation activities. The contaminated sites were finalised and are summarised below:

- ▶ Soil with TPH, PAH and heavy metals
- ▶ Former Irish Sugar Site
- ▶ Saale River Sediment
- ▶ Hospital
- ▶ Industrial Facility
- ▶ Coastal aquifer
- ▶ Chemical industry
- ▶ 2 Major non-ferrous melters
- ▶ Cu and Al wire and cable producer
- ▶ Metal Refining Industry
- ▶ Site contaminated with pesticides
- ▶ GUDAO

Regarding "Characterisation of selected contaminated sites and identification of best available bioremediation techniques", firstly, the physico-chemical parameters of the selected sites are analysed. All parameters provide critical information for design-

ing the optimal conditions to apply in situ remediation technologies testing.

A standardized protocol has been established for the isolation of DNA from soil samples and will be applied for all the DNA extractions, while metag-

PROJECT MANAGEMENT: 5 STEPS AND PHASES

For more information on contaminated sites visit: <https://www.greener-h2020.eu>

INITIATION

PLANNING

EXECUTION

MONITORING
& CONTROLLING

CLOSURE



ADVANCEMENTS



enome analysis has been used to determine the biodegradation potential of the soils. Groundwaters contaminated with heavy metals, aliphatic and aromatic hydrocarbons or chlorinated hydrocarbons will be remediated using a bio-electrochemical approach.

Regarding biological characterisation of contaminated soil/sediment and water based on ribosomal RNA gene sequencing, the DNA samples from soils obtained were also used to generate libraries of amplicons for 16S RNA (bacteria) gene and ITS (fungi). Soil samples were used for DNA extraction followed by amplicon libraries construction and bioinformatics analysis, using a standardized protocol and pipeline. Regarding ITS libraries, most of the obtained sequences belonged to fungi, although a minor percentage corresponded to protists and algae. Beta-diversity analysis (Kruskal-Wallis) has also shown that both bacterial and fungal communities from the three examining soil samples are different. Focusing on the microbiome analysis of the water samples, samples of DNA extracted from groundwater and sediment samples were subjected to microbial community analysis.

Under the GREENER project microbial studies and isolation are also taking place. The enrichment of microbial communities for bio-electrochemical treatment of contaminated sites was conducted by using the electrochemical conditions as the selective pressure. Electroactive microbial cultures for anodic degradation of organic compounds were enriched from anaerobic digester sludge and semi-urban pond sediment in two-chamber microbial fuel cells.

Regarding bioaugmentation, consortia from the first two soil samples have been developed by enrichment cultures. Total DNA has been extracted from these consortia and they are ready for microcosms experiments. In order to perform the isolation of metal resistant bacteria and sulfate reducing bacteria, soil samples have also been selected, while protocols for enrichment and isolation have been developed. The tests and analyses to be performed in these tasks include 16S/18S profiling, Metagenomics, Metatranscriptomics, Metaproteomics, Enzyme activities/Biochemical analyses, and Microbial isolation.

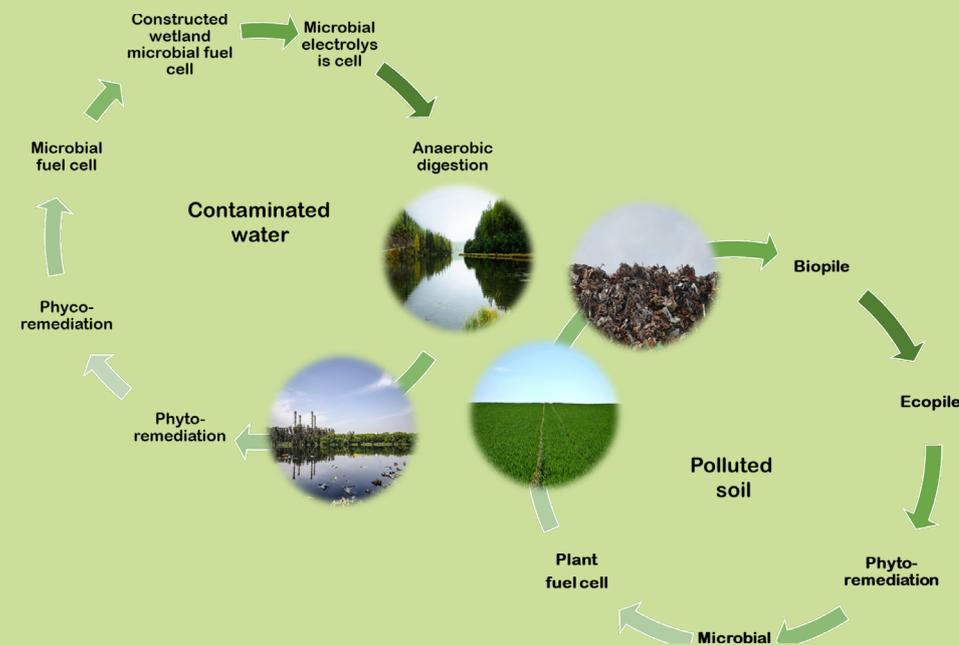
Moreover, the consortium has been working on "Remediation technologies development for surface water and groundwater technologies." Mendel University worked on the definition of operational condition of phytoremediation approach to treat dye-polluted waters using different microalgae strains, setting the pavement for future scaling-up. The development of metal phytoextraction technology, by means of hyperaccumulating plant species grown on microcosms floating systems, has been led by University of Burgos. Related to this task, Carlow Institute of Technology has studied rhizospheric microbial consortia, with the aim of increasing phytoextraction performance. The development of bioelectrochemical systems has been jointly undertaken by several partners. Leitat's activities focused mainly on the study of electroactivity of hydrocarbons-degrading microbial consortia available within Greener and the recovery of metals by abiotic-cath-

ADVANCEMENTS



ode pathways. University of Surrey has isolated promising electroactive consortia from real samples, for their use in metal recovery by cathode associated bioreductive pathways. Nanjing Technical University studied the influence of anchoring metal nanoparticles on

Finally, GREENER focuses on the development of low cost and self-powered "Remediation Technologies Development for Soil/sediment Technologies". The involved partners currently work on different approaches, which will be initially individually opti-



mised and subsequently integrated for a synergistic action. In particular, advanced biostimulation and bioaugmentation methodologies are being investigated to enhance the bioremediation action of microorganisms in soil, for contaminants, such as TPHs and PAHs. Moreover, ecopiles for the bioremediation of soil contaminated by TPH are also on the focus of activities, while the first pilot experiments on a former Irish sugar site in Carlow have already commenced. The most ef-

ficient plants for TPHs removal by phytoremediation are also investigated. Finally, the GREENER partners are exploring the use of bioelectrochemical systems (BES) for the electrochemical oxidation of pollutants, such as TPHs, PAHs, pesticides and pharmaceuticals in soils. Currently, innovative and low-cost design is being tested aiming to easy scale-up. The design and scale-up of the BES technologies the consortium is exploring is supported by mathematical simulation, while advanced analytical methods, based on ultra-performance liquid chromatography coupled with tandem mass spectrometry mass, are used to understand the degradation pathways.

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GREENER MEETINGS



On February 18-19 2020 the GREENER Project 12th month meeting took place at the ITCarlow premises, in Carlow, Ireland. All the partners had the opportunity to present their work and their future plans for the implementation of the project, towards the development of green, sustainable, efficient, and low-cost solutions for soil/sediment and water bioremediation.

On March 2nd 2020 the 12th month review meeting of the GREENER Project was held remotely. All partners took the opportunity to reflect on the significant progress so far and plan for the next phase of the GREENER project. The Project Officer and EU experts gave feedback to the team, recognising the excellent work done so far, highlighting some comments and warnings and providing advice on future improvements.



PUBLISHED WORK



UAM: Daniel Garrido-Sanz, Paula Sansegundo-Lobato, Miguel Redondo-Nieto, Jachym Suman, Tomas Cajthaml, Esther Blanco-Romero, Marta Martín, Ondrej Uhlík, Rafael Rivilla, "Analysis of the biodegradative and adaptive potential of the novel polychlorinated biphenyl degrader *Rhodococcus* sp. WAY2 revealed by its complete genome sequence", *MICROBIAL GENOMICS*, Open Access Journal, 6(4), 2020 (<https://doi.org/10.1099/mgen.0.000363>)

JIAU: Yu-Xuan Chen, Xiang Liu, Zhen Fang, Chun-Lian Zhang, Syed Zaghum Abbas, Yang-Yang Yu, Yang-Chun Yong "Self-assembling of *Shewanella*@Pd bionanohybrid for synergistic bio-abiotic removal of Cr(VI)", *Chemical Technology and Biotechnology*, Open Access Journal, 2020 (<https://doi.org/10.1002/jctb.6409>)

JSI: Rybkin Iaroslav, Gorin Dmitry, Sukhorukov Gleb, Lapanje Aleš, "Thickness of Polyelectrolyte Layers of Separately Confined Bacteria Alters Key Physiological Parameters on a Single Cell Level", *Frontiers in Bioengineering and Biotechnology*, Open Access Journal, 7, 2019, p.378 (<https://doi.org/10.3389/fbioe.2019.00378>)

JSI: Rijavec, T., Zrimec, J., van Spanning, R., Lapanje, A., "Natural Microbial Communities Can Be Manipulated by Artificially Constructed Biofilms", *Advanced Science*, Open Access Journal, 6 (22), 2019 (<https://doi.org/10.1002/advs.201901408>)

JSI: Rinke J. van Tatenhove-Pel, Tomaž Rijavec, Aleš Lapanje, Iris van Swam, Emile Zwering, Jhonatan A. Hernandez-Valdes, Oscar P. Kuipers, Cristian Picioreanu, Bas Teusink, Herwig Bachmann. "Microbial competition reduces interaction distances to the low μm -range" *bioRxiv* 2020.01.22.915835; (<https://doi.org/10.1101/2020.01.22.915835>)

NTU: Xiayuan Wu, Chunrui Lia, Zuopeng Lvb, Xiaowei Zhouc, Zixuan Chena, Honghua Jiaa, Jun Zhoua, Xiaoyu Yonga, Ping Weia and Yan Li, "Positive effects of concomitant heavy metals and their redox states on hexavalent chromium removal in microbial fuel cells", *RSC Adv*, Open Access Journal, 10(26), 2020, p. 15107-15115 (<https://doi.org/10.1039/D0RA01471K>)

NTU: Xiayuan Wu, Zhenzhen Tian, Zuopeng Lv, Zixuan Chen, Yongdi Liu, Xiaoyu Yong, Jun Zhou, Xinxin Xie, Honghua Jia, Ping Wei, "Effects of copper salts on performance, antibiotic resistance genes, and microbial community during thermophilic anaerobic digestion of swine manure", *Bioresour Technol*, 300, 2020, p. 122728 (<https://doi.org/10.1016/j.biortech.2019.122728>)

UAM: Daniel Garrido-Sanz, Miguel Redondo-Nieto, Marta Martín and Rafael Rivilla, "Comparative Genomics of the *Rhodococcus* Genus Shows Wide Distribution of Biodegradation Traits", *Microorganisms*, Open Access Journal, 8, 2020, p. 774 (<https://doi.org/10.3390/microorganisms8050774>)

GREENER project has already published their work in peer reviewed international journals. 12 Publications have already been accepted, out of which 8 in open access journals. For more information kindly visit zenodo under GREENERPprojectH2020

zenodo

DISSEMINATION EVENTS



On January 22-23 2020 University of Surrey, and Dr. Mira Sulonen, participated with a poster presentation in the EBNet Research colloquium 2020. The event took place at Edinburgh, UK. Programme themes from the main focus areas of the Network included: i. Pollutants and Media, ii. Biology and Engineering and ii. Society and Environment and Technoeconomics



The Research Center Iccram from Universidad de Burgos participated in the initiative "February 11st", International Day of Women and Girls in Science. ICCRAM scientists performed different lectures, highlighting Greener Project objectives, in several schools, with the aiming for students to be able to get an easier and funnier approach to Science.



On Tuesday, February 11, UBU representatives participated in the television show "Magazine" at La8 Burgos. CyLTV presented the work developed in the projects of the research center, also highlighting the research lines of the Greener Project .

On the 6th of March our consortium partner Materia Nova has presented the advances of their work under Greener Project at the University of Mons.



UPCOMING EVENTS



IE EXPO CHINA 2021

20-21 April, 2021
Shanghai, China

TRADE FAIR FOR
ENVIRONMENTAL
TECHNOLOGY
SOLUTIONS: WATER,
WASTE, AIR
AND SOIL

EuroNanoForum 2021

June, 2021
TBD

2021
NANOTECHNOLOGY
AND ADVANCED
MATERIALS
PROGRESS UNDER
HORIZON2020
AND BEYOND

BIO-EUROPE SPRING 2020

22-24 March, 2020
Munich, Germany

PARTNERSHIPS
THAT DRIVE
LIFE SCIENCE
DEALMAKING

CONDEGRES 2020

30 September -
2 October 2020

9TH NATIONAL
SYMPOSIUM ON
CONTROL OF SOIL
DEGRADATION AND
RECOVERY



* FOR MORE UPCOMING MAJOR EVENT VISIT THE PROJECT'S DEDICATED WEBSITE SECTION



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The GREENER team Project Coordination team: University of Burgos - ICCRAM



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MATERIALS FOR ADVANCED INDUSTRIAL TECHNOLOGIES



WEBSITE: www.greener-h2020.eu



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