R&D needs in the field of biomonitoring, evaluation of the ecological risks, and bioremediation of emerging chemical (pharmaceuticals, endocrine disruptors, hormones, viruses, toxins etc) and biological (bacteria, viruses) micropollutants in soils, sediments, groundwater, industrial and municipal wastewaters, aquaculture effluents, and freshwater and marine ecosystems
1. **Emerging pollutants in the environment**

Over the last decades, the world has been experiencing in the adverse consequences from the unbridled development of multiple human activities (in industry, transport, agriculture, domestic space). The increase in the standard of living and higher consumer demand have amplified the pollution of air (with CO$_2$ and other greenhouse gases, NO$_x$, SO$_2$, particulate matter), water (with a variety of chemical, nutrients, leachates, oil spills, etc.) and soil (due to the disposal of hazardous wastes, spreading of pesticides), as well as the use of disposable goods or non-biodegradable materials, and the lack of proper facilities for waste. Furthermore, human activities have resulted in contamination of water resources with biological micropollutants such as viruses and bacteria. Such adventitious agents have generated renewed awareness due to their potential pathogenicity and are referred to as emerging or reemerging pathogens. Biological micropollutants such as e.g. enteric bacteria, mycoplasmas, viruses, and protozoa are the source of many waterborne diseases and remain a major cause of death worldwide (Theron and Cloete 2002). A significant part of these diseases are caused by classical water related pathogens, but also the spectrum of diseases is constantly increasing. Diseases which were thought to be controlled may later reemerge as exemplified by the appearance of *Cryptosporidium*, *Legionella*, rotavirus and hepatitis virus in water (WHO, 2003).

Many chemical and microbial agents that were not traditionally considered contaminants are now present in the environment on a global scale. More and more of these emerging contaminants are derived from municipal, agricultural, and industrial wastewater sources and pathways. These newly recognized contaminants represent a shift in the conventional approach to pollution prevention and control, since many are produced industrially, but are dispersed into the environment as a result of domestic, commercial and industrial activities (Kolpin et al., 2002). Chemical micropollutants are often generated through the degradation of organic compounds resulting in accumulation of persistent metabolites (Sørensen et al., 2007) or they may spread through the disposal of miscellaneous chemical products (such as pharmaceuticals) in the natural environment. The increased appearance of biological pollutants in drinking water production and distribution may be related to several factors including changes in human demographic behavior. Also change in agricultural practice towards intensive farming and spreading of manure or sludge on agricultural fields may cause health problems.

Nowadays, there is an increasing concern regarding many environmental contaminants that produce adverse effects by interfering with endogenous hormone systems, the so-called endocrine disruptor compounds (EDCs). They constitute a class of substances defined not only by their chemical nature, but also by their biological effects.
A lack of information still exists regarding the potential impact associated with the occurrence, fate and ecotoxicological effects of endocrine disruptors, including pharmaceuticals and personal care compounds in the environment, since few compounds were inventoried or regulated worldwide. Personal care products continuously released into the environment can constitute a potential source of endocrine disruption. Other endocrine disruptors include, alongside the natural hormones, several additives used in high amounts in drugs (i.e., parabens and siloxanes), domestic and industrial cleaning agents, fire-retardants, some pesticides and heavy metals such as cadmium, lead or mercury. Concerning pharmaceuticals, apart from the sex hormones, glucocorticoids, veterinary growth hormones and a few non-steroidal pharmaceutical substances, not many drugs were recognized as having an impact on natural endocrine systems.

Relevant studies indicate that widely used industrials chemicals (Bisphenol A) and pharmaceuticals and personal care products (PPCPs) (talonide (AHTN), galaxolide (HHCB), ibuprofen, and estriol) occur frequently in samples from the freshwater environment but toxicity data are not yet available. These compounds enter in the environment from different sources such as:

(i) Effluents and sludge from wastewater treatment plants;
(ii) Leakage from septic tanks or landfill sites;
(iii) Surface water run-off;
(iv) Direct discharge into waters.

Pharmaceuticals, for example, are more concentrated in the wastewater discharged from hospitals, long term care facilities and other medical facilities.

Wastewater treatment plants (WWTPs) have been recognized as a key collection point for emerging chemical and biological micropollutants in the water cycle (RECLAIM WATER, UE Project) and potentially a perfect location to treat and remove them, thus diminishing their rate of release into the environment. However, the knowledge about the nature, variability, transport and fate of this class of compounds in typical wastewaters and treatment facilities is still limited. Moreover, not many studies have been performed to monitor or understand the capability of conventional or innovative wastewater treatment processes to eliminate or diminish the concentrations of a wide variety of emerging pollutants at wastewater facilities. These species can be chemically degraded by microorganisms or by UV light action. However, neither basic wastewater treatment, nor basic drinking water treatment will eliminate the estrogens, androgens or detergent breakdown products from water, because of the chemical stability of the structures, as well as their low bioavailability, which affects biodegradation. In addition, municipal sewage sludge is also a repository for these emerging pollutants and only recently has there been an efforts to assess their occurrence and biotreatment potential (Stamatelatou et al., 2011) Bacteria and enteric viruses are abundant in sewage and the later have also been detected in the effluents of
WWTPs (Hovi et al., 2001). Because treated wastewater, and untreated sewage may eventually drain into water resources biological micropollutants threaten public health. The development of new and cost-effective technologies for disinfection of water is therefore needed. Recently it was shown that biologically produced zerovalent silver nanoparticles (bio-Ag\(^0\)) is an very effective disinfectant which may be used at WWTPs, a technology which deserve further attention (De Gusseme et al., 2010).

The consequences manifest particularly in aquatic organisms, as they are subjected to multigenerational exposure. The problem becomes more difficult when these contaminants are detected at trace concentrations in the freshwater environment (namely at low concentrations normally in the nanogram or microgram per liter range) since they may have adverse human health effects, associated with chronic toxicity, endocrine disruption and the development of pathogen resistance. The presence of micropollutants also endangers the reuse of treated wastewater, a generally proposed solution to achieve a sustainable water cycle management. The uptake, mode of action, and biological endpoints of each emerging contaminant must be researched and documented to establish a correlation between contaminant and consequence. A successful approach to solving the problem of emerging contaminants will have to be highly interdisciplinary. Drinking water resources such as groundwater aquifers are often contaminated by extremely low concentrations of pesticides being in the nanogram to microgram per liter range, but still above the EU limit values. Development of new technologies for treatment of drinking water resources to below the EU threshold limit is today urgent.

2. (Bio)monitoring and ecological risk assessment for emerging pollutants

The relevance of emerging and new chemical and biological agents and their impact on soil, water, and ecosystems can be addressed by means of the following future research needs:

- Identification and preparation of a complete European list of emerging contaminants;
- Characterization of interactions and physical/chemical fate of such chemicals/biological emerging pollutants in soil, sediment and water ecosystems;
- Assessment of the functioning of the water/soil system.

The set up of an action plan for emerging and reemerging infectious agents is an addition European need.
There is little toxicological information for the large majority of the chemicals in use, predominantly with regard to long-term, low-level exposure. Current challenges the environment is facing are often hidden so that long-term threats or intermittent exposure can restructure ecosystems and often lead to a decrease in biodiversity and a loss of important functions and services.

In this context, a major challenge is to identify the chemicals and biological agents which are hazardous or will become potentially dangerous in the future, and to clarify if it is sufficient to look for persistent, high flux, toxic, endocrine active compounds. Although the scientific literature about the fate of many of these substances is extensive, limited knowledge is available on their effects at realistic low concentrations in the environment, especially in the case of exposure to complex mixtures of these compounds. Also the development of new technologies for remediation and disinfection of water resources to drinking water standards is a challenge needing attention.

In some cases, there are no legal requirements to assess the impact of long-term exposure to low concentration of these chemicals. This may be because important classes of these compounds have not been studied in detail up to now due to the non-availability of suitable instrumental techniques or analytical standards for low concentration levels.

To understand the full range of potential contaminant effects, it is important to measure and monitor pollutant concentrations at the source, in the environment, and within organisms such as invertebrates and fish. Currently, these processes make use of a variety of chemical/physical separation methods and treatments to remove one or more toxicant classes, coupled with toxicity testing following each manipulation. The time and cost associated with conducting toxicity tests in this manner can be substantial. On-line monitoring can be used to improve the reliability of monitoring data but it requires expensive equipment that needs regular maintenance. An effective alternative currently being considered is passive sampling methods such as Polar Organic Chemical Integrative Samplers (POCIS), which uses devices that can efficiently sample significant volumes of water, can be deployed over extended time periods and provide time weighted average (TWA) concentrations.

Trying to understand the causes of ecosystem harms or effects resulting from chronic exposure to a pollutant is not an easy task and requires innovative approaches. **Innovative tools for toxicity identification and evaluation should allow determining the causal agents in sediment samples found to be toxic in laboratory tests, by differentiating broad classes of toxicants in sediments, but also successfully identifying individual pollutants.** There is a growing understanding that chemical data alone are not enough to assess the potential risks of all emerging pollutants, so that
pollution-induced biological and biochemical effect-analyses are desirable to appraise or predict the impact of chemicals on human health.

*Biomonitoring tools* (e.g. bioassays, biomarkers, microbial community analyses) have enormous potential for reducing uncertainty in the risk assessment of both regulated and emerging chemical pollutants. **Development of sensors capable of determining several analytes simultaneously may constitute a useful tool in environmental monitoring and screening.**

*Biosensor* is a broad term that refers to any system that detects the presence of a substrate by use of a biological component which then provides a signal that can be quantified. Research on biosensing techniques and devices for the environment, as well as in genetic engineering for sensor cell development has recently expanded. For example, many endocrine disruptors are also believed to bind to the estrogen receptor (ER) as agonists or antagonists. Thus, the binding ability of the chemicals toward the ER would be a crucial factor for screening or testing their potential environmental toxicity. Taking advantage of this feature, *endocrine effect biosensors* have been developed.

Current methods for the detection of pathogenic viruses, bacteria, protozoa, and helminths tend to be inaccurate, time-consuming, and expensive. Inspired by nature, molecular self-assembly has been proposed for the synthesis of nanostructures capable of performing unique functions. Self-assembly is the formation of organized, patterned structures without external direction. Biomaterials, such as proteins, lipids, and nucleic acids, can self-assemble. Such nanostructures are applied for the development of amperometric immunosensors. **The development of large-scale biosensor arrays composed of highly miniaturized signal transducer elements, for example, enables the real-time parallel monitoring of multiple species and is an important driving force in biosensor research.**

Genomics is a new tool to understand the molecular pathways affected by emerging pollutants and relate them to whole organism or population level effect. Ecotoxicogenomics is an emerging field in ecotoxicology that applies genomic technologies such as DNA microarrays to understanding the effects of pollution at the molecular level. **Developing molecular biomarkers as detection tools for emerging contaminants can help to understand the bioavailability of nanoparticles in environmental matrices.**

Since the quantification of organic contaminant bioaccessibility in soils and sediments is essential for the risk assessment and remediation of contaminated
land, techniques which can directly predict microbial availability and degradation in the environment should be developed.

3. Innovative approaches in bioremediation of emerging pollutants

Environmental hazards and risks that occur as a result of accumulated toxic chemicals or biological micropollutants could be reduced or eliminated through the application of (bio)technology in the form of treatment/remediation of historic pollutions as well as (bio) technologies for disinfection of water resources addressing chemical and biological agents resulting from changed human demographic behavior, break down of public health measures and current industrial/agricultural practices through pollution prevention and control practices.

Studies and researches demonstrated that some of these pollutants can be readily degraded or removed thanks to biotechnological solutions, which involve the action of microbes, plants, animals under certain conditions that envisage abiotic and biotic factors, leading to non-aggressive products through compound mineralization, transformation or immobilization. Biological processes, combined with adsorption of the solids, may lead to 45-99% removal of EDCs from influent wastewater. Monitoring and managing the biological aspects of bioremediation require the characterization of the fate of the compounds of interest in the environment to update the choice of the bioremediation strategy to employ. However, insufficient information is available at this time to suggest that the overall concentration of trace chemicals or the associated environmental impacts would subsequently be reduced or eliminated.

Activated sludge treatment remains a workhorse technology for controlling pollution of aquatic environment. However, biological processes performed in conventional (activated sludge) treatment plants demonstrated reduced efficiency in removing EDCs from wastewater, even in state of the art WWTPs with multiple biological treatment units.

Membrane bioreactors (MBRs) are considered an alternative to WWTPs, effective in the elimination of very recalcitrant compounds for conventional activated sludge. Studies reported that the removal of EDCs within a membrane bioreactor prior to disinfection led to 96% removal of cholesterol, coprostanol and stigmastanol from municipal wastewaters compared to around 85% removal achieved in a conventional treatment plant receiving the same influent. However, in some cases, an MBR reactor operating at prolonged Sludge Retention Time (SRT) proved to be detrimental for the removal of several compounds.

Isolation of bacteria from activated sludge capable of degrading endocrine disruptors appeared to give the possibility of efficiently removing such compounds that show a low rate of hydrolysis and photocatalysis. White rot fungi and their
oxidative enzymes are also attractive candidates to be applied to decontaminate waters containing EDCs such as the ubiquitous plasticizer bisphenol A. The development of innovative/advanced packed or fluidized bed bioreactors or MBR is also necessary for a more effective exploitation of such specialized microbes or enzymes.

The growing outbreaks of infectious water-borne diseases are a challenge to both the water and public health sector. The development of new (bio) technologies for water disinfection and monitoring biological micropollutants is therefore urgent. Novel concepts for the removal of such agents and of potential usefulness for the biotreatment of water and wastewater are starting to emerge (Forrez et al., 2011): the recently demonstrated co-metabolism of estrogenic compounds during nitrification (including the action of ammonia oxidizing archaea) might be also applicable to the removal of other micropollutants such as PPCPs, while recruitment of other heterotrophic bacteria seems to be necessary to further degrade the intermediate metabolites of these micropollutants produced by the action of aerobic nitrifiers. The growing outbreaks of infectious water-borne diseases are a challenge to both the water and public health sector. Scientifically validated and innovative processes and tools are further necessary to address these issues and the demands of decision makers and the public to understand the impact of chemicals and pathogens on human health and the environment.

4. Acknowledgements

The colleagues of the EFB Environmental Biotechnology section Maria Gavrilescu, Kateřina Demnerová, Jens Aamand and Spyros Agathos are kindly acknowledged for the key contribution provided to the preparation of the present position paper.

The present paper is approved by all members of the Experts Group of the EFB Environmental Biotechnology section, i.e., Fabio Fava (University of Bologna, Italy & Chair of the Experts Group and of the EFB section on Environmental Biotechnology), Spyros Agathos (University of Louvain, Belgium and Vice-Chair of the Experts Group and of the EFB section on Environmental Biotechnology), Valter Tandoi (CNR, Italy and Secretariat of the Experts Group and of the EFB section on Environmental Biotechnology), Mauro Majone (University of Rome La Sapienza, Italy and Delegate of SusChem Italy), Alessandro Sidoli (Italian Association of Biotech Industry – Assobiotec-, Italy), Ludo Diels (VITO, Mol, Belgium), Willy Verstraete (Ghent University, Belgium), Nicolas Kalogerakis (Technical University of Crete, Greece), Daniel Mamais (National Technical University of Athens, Greece), Philippe Corvini (University of Applied Sciences NS Muttenz, Switzerland), Hans Peter Kohler (EAWAG, Dübendorf, Switzerland), Steven A. Banwart (University of Sheffield, United Kingdom), Piet Lens (UNESCO-IHE, Delft TheNetherlands), Victor de Lorenzo (CSIC, Madrid,
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Spain), Katarina Demnerova (ICT, University of Prague, Czech Republic), Vladimir Brenner (AECOM, Czech Republic), Venko Beschkov (Bulgarian Academy of Sciences, Sofia, Bulgaria), Katarina Dercova (Slovak University of Technology, Slovak Republic), Katalin Belafi-Bako (University of Pannonia, Veszprem, Hungary), Maria Reis (University Nova de Lisboa, Portugal), Bruno Sommer Ferreira (APBIO, Portuguese Association of Biotech Industries, Portugal), Andreas Loibner (BOKU, Vienna, Austria), Eric Trably (INRA - Laboratoire de Biotechnologie de l’Environnement, Narbonne, France), Kornelius Miksch (The Silesian University of Technology, Gliwice, Poland), Jan W Dobrowolski (AGH University Science & Technology Krakow, Poland), Maria Gavrilescu (Gheorghe Asachi Technical University of Iasi, Romania), Thomas Schafer (Novozymes, Denmark), Jens Aamand (Geus, Denmark), Rainer Meckenstock (Universität Tübingen, Tübingen, Germany), Manfred Kircher (CLIB2021, Germany), Sara Sjöling (Sodertorn University, Sweden), George O’Malley (Biorefinery Ireland, Newport, Ireland), Vladimir Popov (Russian Academy of Sciences, Moscow, Russia), Fazilet Vardar Sukan (Ege University, Izmir, Turkey), Vladimir Elisashvili (Durmishidze Inst. Biochem. Biotechnol., Tbilisi, Georgia), Hector Poggi-Varaldo (CINVESTAV-IPN, Mexico), Jose Osvaldo Beserra Carioca (Federal University of Ceará – UFC, Brasil), Hanan Malkawi (Yarmouk University, Jordan), Yasser R Abdel-Fattah (Mubarack City for Scientific Research and Technology Applications, Egypt), Ameur Cherif (Univesity of Tunis, Tunisia), Pedro J. Alvarez (Rice University, Houston, TX, USA), Jose Duarte (President of International Association Mediterranean Agroindustrial Waste, IAMAW), Hisao Ohtake (President of the Asian Federation of Biotechnology -AFOB- Japan), Eliora Ron (General Secretary of European Academy of Microbiology -EAM- & Chair of BAM section of International Union of Microbiological Societies –IUMS), James Philp (General Secretariat of Task force on Industrial and Environmental Biotechnology at OECD) and Joanna Dupont (Director at EUROPABIO & Delegate ETP-SUSCHEM).

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RECLAIM WATER: http://www.reclaim-water.org/


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