

FINAL CONFERENCE ONLINE
**WATER REUSE FOR A
SUSTAINABLE WORLD**
JUNE 29—30, 2020

REWATER
sustainable and safe water
management in agriculture

BOOK OF
ABSTRACTS

Organizing committee

Cristina Delerue-Matos

Henri Nouws

Manuela Correia

Sónia Figueiredo

ORGANIZATION



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FINAL CONFERENCE ONLINE

WATER REUSE FOR A SUSTAINABLE WORLD

JUNE 29—30, 2020



JUNE 29, 2020

9:45	OPENING SESSION			
10:00	PLENARY LECTURES			
PL 1	Water Reuse in Portugal: Trends & Approaches	Anabela Rebelo	Portuguese Environmental Agency, Portugal	
PL 2	Wastewater reuse on irrigation	Vicente Seixas e Sousa	Universidade de Trás-os-Montes e Alto Douro, Portugal	
PL 3	REWATER - Sustainable and Safe Water Management in Agriculture	Cristina Delerue-Matos	REQUIMTE/LAQV ISEP, Portugal	
11:00	INTERVAL			
11:15	KEYNOTE LECTURES			
KN 1	Environmental monitoring of pesticides - river, influents and effluents from WWTP	Valentina Domingues	REQUIMTE/LAQV ISEP, Portugal	
KN 2	Pharmaceuticals and their transformation products in the Lis river: impacts from treated wastewater	Manuela Correia	REQUIMTE/LAQV ISEP, Portugal	
KN 3	Low-cost electroanalytical devices for the on-site determination of emerging contaminants	Maria Teresa Abedul	Universidad de Oviedo, Spain	
KN 4	Molecularly Imprinted Polymers as innovative and cheap analytical tools	João Pacheco	REQUIMTE/LAQV ISEP, Portugal	
12:45	INTERVAL			
14:00	KEYNOTE LECTURES			
KN 5	Wastewater Treatments - Electro-Fenton	Verónica Poza-Nogueiras	Universidad de Vigo, Spain	
KN 6	The use of mycoremediation for wastewater treatment	Malin Hultberg	Swedish University of Agricultural Sciences, Sweden	
KN 7	Fluoxetine Contaminated Real Wastewater Treated by Phycoremediation	Sónia Figueiredo	REQUIMTE/LAQV ISEP, Portugal	
KN 8	Effect-based evaluation for treated wastewater	Laura Guimarães	Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Portugal	
15:30	INTERVAL			
15:45	FLASH PRESENTATIONS			

JUNE 30, 2020

9:30	PRESENTATIONS FROM WWTP MANAGERS AND WWT COMPANIES			
OC 1	Treated wastewater reuse at Águas do Centro Litoral: the REWATER project	Joana Vieira Sandra Jorge	ADCL - Águas do Centro Litoral, SA, Portugal	
OC 2	Treated wastewater reuse: difficulties and opportunities	Jaime Gabriel Silva	ISEP & Águas de Portugal Group, Portugal	
OC 3	Water reuse outlook	João Vilaça	SIMPOURO, SA, Portugal	
OC 4	Advanced technologies for industrial wastewater treatment and reuse	Carlos Oliveira	VentilAQUA, Portugal	
OC 5	Water reuse and applications on reuse technology for non-potable use	Luís Marinheiro	AST/Aquasmart, Portugal	
11:10	INTERVAL			
11:40	PRESENTATIONS OF OTHER JPI PROJECTS			
OC 6	Antibiotic Resistance in the Environment: Abundance is not proportional to Risk	Célia M. Manaia	Universidade Católica Portuguesa, Portugal	
OC 7	Granular sludge technology for valorization of water use - from high to low strength effluents in aquaculture	Paula Castro	Universidade Católica Portuguesa, Portugal	
OC 8	Monitoring and control of water, nutrients and plant protection products towards a sustainable agricultural sector	José Boaventura-Cunha	INESC TEC & Universidade de Trás-os-Montes e Alto Douro, Portugal	
12:40	CLOSING SESSION			
13:00	INTERVAL			
14:30	REWATER FINAL MEETING (ONLY FOR REWATER TEAM MEMBERS)			

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WATER REUSE IN PORTUGAL: TRENDS & APPROACHES

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Abstract

The increasing demand for water supply for multiple purposes along with climate change is putting significant strain on water resources. The use of reclaimed waters has been highlighted as an alternative water source to face water shortages, since reclaimed waters can be used for a wide variety of purposes depending upon its quality. This is significant in assessing and preventing risks and drawbacks of water reuse for health and the environment.

To promote safe water reuse practices in Portugal, last year, was approved a new policy, the Law-Decree n.º 119/2019, 21st August, for the production of reclaimed water from several sources to be used in multiple non-potable purposes [1]. The legislation follows the recent developments at European Level and is mainly supported on the international guidelines developed by the International Organization for Standardization (ISO), namely for irrigation, urban uses and health risk assessment [2-4].

The new policy proposes the application of quality standards developed according to a fit-for-purpose approach, which entails the production of reclaimed water quality that meets the needs of intended end-uses, without compromising the human health and environment. Since water reuse can pose some risks to human health, namely due to presence of pathogenic microorganisms, the new Law-decree requires that all reuse projects shall follow a risk assessment procedure. This appraisal will allow the definition of the quality standards applicable to each reuse project and it will also allow to select the risk management conditions that should be followed to ensure an associated minimum risk value [1,4].

The Portuguese Environment Agency developed a guideline which provides advice on the several aspects of the permitting procedures and technical support for risk assessment for health and environment. In this guideline is proposed an innovative semi-quantitative methodology for the risk characterization that helps the definition and validation of the water quality requirements [5].

The new policy for water reuse in Portugal intends to promote a flexible management approach without compromising the health and environmental safety.

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WASTEWATER REUSE ON IRRIGATION

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Abstract

In the XXI century, hunger is still an huge problem for mankind. In fact, around 9 million people die every year of hunger or related diseases [1]. That's more than malaria, tuberculosis and AIDS all together.

Nowadays, more than 800 thousand people around the world is sufering from food unsecurity [2]. Hunger is associated with water scarcity. Physical water scarcity occurs when there is not enough water to meet all demands (including the environment) According to Mekonnen and Hoekstra [3], 4 billion people in the world suffer from water scarcity, at least one month per year.

The UN agenda for sustainable development identified 17 sustainable development goals (SDGs) divided by 169 targets [5]. One of the goals is dedicated to water and sanitation (SDG 6) that includes a target (6.4) for water scarcity with two indicators: the water-use efficiency (6.4.1) and water stress (6.4.2)

Water use for food production largely compete with other users, namely industry, municipalities and the ecosystems integrity maintenance. At the global level, around 70% of the water withdrawal is used on irrigation, 95% on developing countries.

The techonologie development and energy dessimination during the second half of the XXth century, allowed the increasing exploitation of aquifers, particularly in water scarce regions. It is estimated that by 2010, the share of groundwater in the total water abstraction was 26%, of wich 70% used for irrigation [6]. Overexploitation and contamination of aquifers is threatening this important freshwater resource

As irrigation is by far the largest global water user, strategies to deal with water scarcity include, on one hand, a more efficient use of water and, on the other hand, the use as an alternative to fresh water. treated wastewater both on irrigation and on aquifer recharge.

Improvement of water use efficiency on irrigation, includes not only the adoption of more efficient methods, but also, a better design and management of irrigation schemes.

The use of treated wastewater on irrigation is higly recomendado because it saves fresh water for other uses and is a sustainable way of food production in regions with great water scarcity. However, care should be taken with the possible public health implications due to the risk of contamination with microorganisms, parasites, heavy metals, etc., especially when the watering is intended for products that will be eaten raw, such as lettuce or tomatoes.

Irrigation methods must also take into account the origin and quality of the water, giving priority to those who do not wet the vegetation

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REWATER- SUSTAINABLE AND SAFE WATER MANAGEMENT IN AGRICULTURE

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Abstract

Water is a natural resource vital for social wellbeing and agriculture economy. Wastewater treatment plants (WWTPs) are crucial sources for water reuse for agriculture, since they promote the removal of unwanted substances, thus increasing the water supply in areas in which its demand has exceeded the available natural resources. Yet, reuse of these waters has certain associated risks. In fact, evaluation of water quality for irrigation is focused on conventional pollution parameters such as pH, salinity, heavy metals, nitrates, total suspended solids and microbiological load. However, according to the Watch List of Substances established under the Water Framework Directive (Decision EU 2018/840), emerging contaminants (ECs) such as pharmaceuticals and specific pesticides should also be monitored, especially because of the reduced removal of these and other micropollutants by conventional treatments. This may lead to contamination of receiving surface waters, which could be extended to soils, crops and, in a last instance, to human beings, thus limiting the application of treated WW in agriculture.

In the REWATER project [1] technologies able to produce a final integrated solution for reuse of WW for agricultural purposes are developed. The novelty of REWATER can be summarized as follows: (i) Design of electrochemical (bio)sensors for specific ECs with increased sensitivity, lower costs and easier to use; (ii) Improvement of treatment technologies (biological and electrochemical) to remove ECs, providing new insights into environmental and economic approaches; (iii) generation of ecotoxicological tools that may also be employed in the monitoring of ECs in natural aquatic ecosystems, and strategic follow-up monitoring of WW treatment.

Interaction among consortium partners, allied to stakeholders of water industry, will enhance collaborative research and innovation, as well as national and international cooperation in the water sector, beyond the REWATER lifespan.

Acknowledgements

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[1] <http://www2.isep.ipp.pt/rewater/index.php>

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KN 1

ENVIRONMENTAL MONITORING OF PESTICIDES - RIVER, INFLUENTS AND EFFLUENTS FROM WWTP

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Abstract

Pesticides and their transformation products, when transported to the aquatic environment, can affect water bodies compromising the quality of water intended for human and animal consumption [1]. Runoff and wastewater effluents are important routes for the entry of pesticides into aquatic environments [2]. The monitoring of 50 pesticides belonging to different families and groups were analysed in Lis river waters from the source to the mouth and in influents and effluents from two WWTPs planted along the same river.

Most of the detected pesticides belong to insecticide types and organochlorine group. Concentrations were between 1.29 (pyrimethanil) to 1,153 ng/L (aldrin) in river water; 2.27 (isoproturon) to 2,134 ng/L (γ -HCH) in WWTP effluent, and 6.34 (isoproturon) to 181 ng/L (diuron) in WWTP influent. Aldrin, γ -HCH, and cypermethrin were detected in some samples with concentration of μ g/L. γ -HCH was the pesticide more often detected in μ g/L. The pesticides with the highest detection frequency were cypermethrin, HCB, methoxychlor, and ζ -HCH in river water, isoproturon, cypermethrin, methoxychlor, pyrimethanil, γ -HCH, dieldrin, diuron, α -HCH, α -endosulfan in WWTP effluent, and diuron and isoproturon in WWTP influent.

The physical and chemical properties of the studied pesticides and the climatic conditions were crossed with the obtained results. Pesticides and possible routes were identified. Continuous monitoring of pesticides is crucial, as exposure leads to hazardous effects.

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Manuela Correia

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KN 2

PHARMACEUTICALS AND THEIR TRANSFORMATION PRODUCTS IN THE LIS RIVER: IMPACTS FROM TREATED WASTEWATER

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Abstract

Pharmaceuticals play a very important role in modern medicine. However their use is not exempt from negative impacts, as they may enter the environment, resulting in chronic exposure to these compounds and/or to their transformation products [1].

In recent decades the presence of pharmaceuticals in the environment has been demonstrated at levels that may vary between few ng/L to µg/L, in most of the cases, but that may reach up to mg/L. Wastewater treatment plants (WWTPs) are one of the main sources of pharmaceuticals entrance in surface waters. Conventional treatments do not generally attain complete removal for pharmaceuticals, as these facilities were not designed to remove micropollutants, and also due to the different physicochemical properties of these analytes that allow them to resist to biological, physical, and chemical processes.

Within the framework of REWATER project a special attention has been given to the monitoring of pharmaceuticals and their transformation products in the Lis River and to the contributions of the WWTPs located along the river [1], which had been studied previously to the start of the project [2]. In this presentation, the challenges associated to analyte's extraction and analysis by LC-MS/MS at trace levels will be discussed. The results of two different sampling campaigns (2018 and 2019), comprising surface waters collected along the river and influent and effluent samples of two WWTPs that discharge their effluents into the Lis river will be presented.

Pharmaceuticals were detected in all extension of the watercourse, including in the source of the river, though usually the highest concentrations were found in the lower part of the river. Carbamazepine, caffeine, ibuprofen, hydroxyibuprofen, and salicylic acid showed 100% of detection frequency in the Lis river. In the case of WWTP samples, antibiotics concentrations were between <MDL (trimethoprim) and 2195 ng/L (clarithromycin) in WWTP influent and between <MDL (trimethoprim) to 5984 ng/L (oxytetracycline) in WWTP effluent. Concentrations in the µg/L range were reached for carbamazepine, azithromycin, clarithromycin, ofloxacin, atenolol, caffeine, oxytetracycline, diclofenac, and hydroxyibuprofen in WWTP effluent. Generally, higher levels were found in the 2018 campaign when compared to 2019.

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LOW-COST ELECTROANALYTICAL DEVICES FOR THE ON-SITE DETERMINATION OF EMERGING CONTAMINANTS

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Abstract

In recent years, pharmaceuticals have become important environmental contaminants due to their massive use, their high persistence in the environment, and the low capability of the conventional wastewater treatment plants to remove them completely. Thus, their determination in the environmental waters are of increasing concern since their presence, even at very low concentrations, has been associated with harmful effects on animal and human health. In the case of the antibiotics, their presence in the environment also lead to the rapid expansion of antibiotic resistance, an increasing threat to global public health.

The analysis of pharmaceuticals, and contaminants in general, is a great challenge because of their low concentrations, their variability in terms of physical and chemical properties and the spatial-temporal variations in their distribution due to seasonality, inter- and intra-day variations and/or occasional events. Hence, contaminant determination requires, not only highly-sensitive analytical methods, but also sampling plans that allows to obtain a representative spatial-temporal picture of environmental quality. Bearing this in mind, the development of miniaturized and portable analytical devices for on-site contaminant analysis is an interesting challenge since these tools could provide real-time information about the presence and spatial distribution of contaminants in the environment. Therefore, we developed a small and very low-cost paper-based electroanalytical device that was applied to the determination of diclofenac (an anti-inflammatory drug) [1] and macrolide antibiotics. The use of paper as substrate allows to reduce costs of fabrication and to create electrodes in a simple way due to its easy modification and printing. Moreover, we take advantage of its porosity and high surface area to perform a preconcentration step before the measurement that improves the sensitivity of the methodology in a very substantial way: the LOD decreases ca. 800-fold in the case of diclofenac (from 55 μ M to 70 nM). Moreover, the high versatility of the developed design allows to construct multiplexed platforms to perform up to 8 simultaneous measurements.

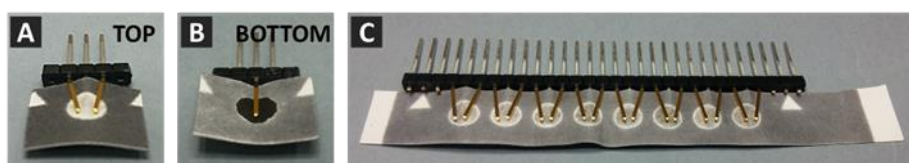


Fig.1. Pictures of the (A,B) single- and (C) multiplex paper-based electroanalytical devices.

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KN 4

MOLECULARLY IMPRINTED POLYMERS AS INNOVATIVE AND CHEAP ANALYTICAL TOOLS

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Abstract

Molecularly imprinted polymers (MIPs) are one of the most promising tools for the design and construction of synthetic biomimetic recognition systems. They are analogues of the natural antibody–antigen systems. Generally, MIPs are obtained by bulk (3-D) polymerization, a process by which selected functional monomers (either organic or inorganic materials) are polymerized around a target analyte (template) in the presence of a crosslinking agent. After polymerization, the template molecule is extracted and a polymer matrix, with sites complementary in shape, size and functionality to the imprinted molecule, is obtained. They show favored affinity to the template molecule compared to other molecules. Although natural systems can produce antibodies against a range of foreign bodies, which are excellent to use as recognition elements, the use of such receptors in chemical sensors has several drawbacks, such as cost and sensitivity to environmental conditions (temperature, pH). The advantages of using MIPs are that they are robust, resistant to changes of chemical and thermal conditions, easy to prepare, reusable and cheap. In analytical chemistry they are widely used in separation science, sample preparation and chemical sensors. Application in several analytical areas, such as, food, environmental and health analysis have been reported. MIPs for the selective recognition of pharmaceuticals have been widely reported. Despite all the advantages, their application and commercialization has been a slow process and few the available commercial applications of MIPs are found and related to materials for separation purposes [1,2].

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WASTEWATER TREATMENTS – ELECTRO-FENTON

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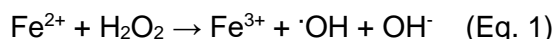
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Abstract

Emerging contaminants represent a potential risk to human health, aquatic life and the environment. Since conventional water and wastewater treatment plants are not able to achieve an adequate elimination of those pollutants, it is necessary to develop more efficient technologies [1]. In this context, electrochemistry is a discipline that can play an important role, both in the detection and the treatment of contaminants [2].

On the one hand, electrochemistry can be applied with analytical purposes. In this sense, differential pulse voltammetry (DPV) is among the most widely used techniques. Thanks to its sensitivity, it is especially interesting for trace analysis [3]. On the other hand, regarding the remediation of pollutants, electrochemical advanced oxidation processes are attracting increasing attention for being highly efficient, versatile and safe. They are based on the generation of highly reactive species, mainly the hydroxyl radical. In particular, in the electro-Fenton (EF) treatment, the radical formation follows the Fenton reaction (Eq. (1)). However, iron used as catalyst in this process is difficult to recover and a subsequent step for eliminating it is required. To overcome this issue, heterogeneous EF (HEF) can be applied [4].



On this basis, the investigations carried out were focused on assessing the effectiveness of HEF, using iron alginate beads as the catalyst, for water remediation. Several organic compounds, such as ionic liquids and pharmaceuticals, were used as target pollutants. Additionally, DPV analyses on screen-printed carbon electrodes were performed in tandem with the HEF treatments, in order to monitor the pollutant degradation and intermediates generation almost in real-time. Recently, to scale up HEF for a possible future industrial application, some experiments were conducted in a bench-scale reactor, achieving promising results.

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Malin Hultberg

Malin Hultberg (Assoc. Prof) is an applied microbiologist working at the Swedish University of Agricultural Sciences, Alnarp, Sweden. She did her PhD on biocontrol and the use of microbial antagonists in the rhizosphere in 1999. Since then she has been involved in research, teaching and extension focusing on sustainable bio-based technologies, with application both in horticulture and for wastewater treatment. Her current research focus on edible white-rot fungi such as oyster mushrooms and shiitake and their potential use in circular systems.



THE USE OF MYCOREMEDIATION FOR WASTEWATER TREATMENT

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Abstract

There is an interest in developing wastewater treatment to allow recirculation of resources such as water and plant nutrients. To reach this goal increased knowledge on the presence of organic micropollutants (OMPs) and efficient techniques for their removal are needed. Within the project REWATER aspects of mycoremediation, the use of fungi for bioremediation, has been explored. Using the microorganisms *Chlorella vulgaris* and *Aspergillus niger*, the effect of fungal-assisted algal harvest [1] on OMPs removal, nutrient removal and lipid composition of the harvested biomass were studied. Major reductions in concentrations of nitrogen and phosphorus were observed and, because of fungal biomass production, the amount of lipids produced per litre of wastewater increased from 5.6 ± 0.9 mg on day 5 (microalgae only) to 20.6 ± 4.9 mg on day 8 in the pellets composed of fungi and microalgae [2]. Removal of different pesticides was tested in this system and of total pesticide load, approximately 15% was removed by the microalgal-fungal pellet [3].

The dominating fungal genus used for algal harvest is *Aspergillus* [1], considering a future large-scale use this can be a drawback [4]. Thus, strains of white-rot fungi with a long record of safe use were evaluated for their potential to form pellets with microalgae. However, none of the tested strains were as efficient as *Aspergillus niger* for algal harvest [5]. Considering relatively low removal of OMPs, safety aspects of large-scale use and that algal harvest was less efficient in large volumes for *Aspergillus niger*, a second scenario for mycoremediation, based on the white-rot species *Pleurotus ostreatus*, was explored. Laccase exudation in lignocellulosic substrate colonized by *P. ostreatus* was studied over time and the substrate was used for treatment of water contaminated with the OMPs diclofenac, bicalutamide, lamotrigine, and metformin using a laccase activity of 50 U L^{-1} . High removal of all four OMPs were observed after only a short period of treatment (submitted manuscript). Further work is needed, e.g. formation of transformation products, however from an applied perspective this approach is promising considering costs and safety as well as the technique for production of the laccase-producing substrate.

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FLUOXETINE CONTAMINATED REAL WASTEWATER TREATED BY PHYCOREMEDIATION

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Abstract

The reuse of treated wastewater is an alternative water source, which contributes to the sustainable use of water resources, in line with the principles of Circular Economy and preservation of the use of water for the future [1]. Treated municipal wastewater is a good alternative, since the discharges from wastewater treatment plants (WWTPs) are subject to regulation. However, the release of pharmaceuticals (PhCs) into aquatic environment is not yet subject to regulation and WWTPs were not designed for efficient removal of PhCs, which makes them an important pathway of environmental contamination [2]. Several tertiary treatments have been tested, such as membrane technologies and advanced oxidation processes. These technologies are efficient but usually require a high technical knowledge, high operational costs, the use of chemicals and generally lead to the formation of by-products [3]. Aware of this problem, REQUIMTE/LAQV/ISEP team, through the REWATER project developed under the Water Challenges for Changing World Joint Programming Initiative (Water JPI), has been working to find efficient tertiary treatment methods for PhCs removal, in order to allow the reuse of wastewater treated as irrigation water. The use of microalgae in wastewater treatment is well established for the removal of nutrients and contaminants. This technology has low requirements in terms of energy and operational inputs, is eco-friendly, allows the mitigation of atmospheric carbon dioxide and the production of high-value products and value biomass to produce biofuel and animal feed [4]. In this context, the potential of the green microalgae *Chlorella vulgaris* to remove fluoxetine, an antidepressant, and nutrients (nitrogen and phosphorus) from a treated municipal wastewater was assessed. The assay was performed in a closed system under permanent illumination and stirring, at room temperature, for 3 days. The removal efficiencies achieved for fluoxetine and nitrogen were, respectively, 77% and 67%. The results obtained suggest the potential application of this sustainable tertiary treatment in domestic WWTPs.

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EFFECT-BASED EVALUATION FOR TREATED WASTEWATER

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Abstract

Mixtures of pesticides and pharmaceutical drugs are commonly detected in natural waters and can severely impact the receiving ecosystems. These come both from intensive use of those chemicals and their incomplete removal from water by regular treatment technologies available in sewage plants. A wealth of wastewater treatment technologies is thus under refinement to protect the environment and increase water reuse. These technologies aim at reducing the levels of micropollutants in the final effluents. Yet, due to the complex mixture of contaminants found in wastewater, not always reduction of the chemical concentration is reflected on a relevant toxicity reduction. Complementary evaluation of toxicity decrease is thus required. Such evaluation has mostly been based on screening or standard ecotoxicological assays and endpoints, either sensitive but lacking ecological relevance or with ecological relevance but lacking sensitivity and usefulness for early diagnosis of toxic effects. Within the REWATER project, our team is developing and testing integrated endpoints, that can be both sensitive and ecologically relevant, and linking them to early-warning biomarkers. We are taking advantage of different animal models, such as the pond snail [1] and zebrafish embryo/larvae [2]. The work focused on behavioural endpoints, RAMAN vibrational spectroscopy (RS) and molecular biomarkers, all useful to investigate antagonistic and synergistic effects of mixtures of contaminants in aquatic animals. Behavioural measures, such as sensorymotor tests in zebrafish larvae, or video-tracked locomotion with artificial neural networks analysis [3], are non-invasive, ecologically relevant endpoints that can be much more sensitive to pollutants than classical mortality or reproduction endpoints. The RS techniques can provide rapid assessment of integrated physiological responses elicited by toxicant exposure, easy to combine with measurement of gene expression or/and biochemical activity linked to the modes of action of the target micropollutants. From the knowledge gained so far, we are selecting the most sensitive endpoints and early-warning biomarkers are employing them to assess the performance of treatment technologies in decreasing the toxicity of mixtures of micropollutants, such as fluoxetine and pirimicarb [4].

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OC 1

TREATED WASTEWATER REUSE AT ÁGUAS DO CENTRO LITORAL: THE REWATER PROJECT

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Abstract

The potential of treated wastewater reuse as an alternative source of water supply is an important goal for the development of a circular economy in the water sector. On the past few years, in order to boost water reuse, considering health and environment safety, new legislative instruments and new policies have been set.

Águas do Centro Litoral (AdCL) is responsible for the collection, treatment and disposal of domestic and urban effluents from an area of about 5,500 km², including the Lis river basin.

Leiria is a city and a municipality in the Centre Region of Portugal, near the coastline. Lis River is one of Leiria's most important resources. Almost 40 km long, the river mouth is located in Vieira beach, near Leiria, after crossing the Lis fields, a wide farming area watered by its abundant flow (Vieira et al., 2012). Along the course of the river, there is a discharge of treated effluent from 3 AdCL Waste Water Treatment Plants (WWTPs), the only ones in the whole river course.

Although it is not legally required in Europe, the control of micropollutants in surface waters is crucial, because it may affect water quality and potentially impact drinking water supplies, ecosystems, and human health (EU_L78/40, 2015).

In a partnership with Instituto Superior de Engenharia do Porto (ISEP), from August 2013 to June 2014, the occurrence of 33 pharmaceuticals and metabolites was evaluated along the Lis river and in the influents and effluents of the two more important WWTPs located along the river (Paíga et al., 2016). Afterwards, the REWATER project extended the number of pharmaceutical compounds analyzed, using a new sampling campaign. Samples of Coimbrão WWTP (Leiria, Portugal) influent and effluent were collected hourly, for 24 h. (Paíga et al., 2019).

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Águas de Portugal (AdP) Group, since 1998. Between 2009 and 2016, he integrated the board of Simlis, Águas do Mondego and Simria, currently merged in Águas do Centro Litoral. In 2016, he integrated the board of Águas de Santo André.

He started in AdP Group at Águas do Douro e Paiva, since 1998, where he was Director of Engineering. Before, he worked at FASE, at CICCOPN Lab, at IBM and at Comissão de Coordenação Regional Norte. Since 1990, he teaches at ISEP.



OC 2

TREATED WASTEWATER REUSE: DIFFICULTIES AND OPPORTUNITIES

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Abstract

A SWOT analysis on water reuse is presented, focusing specially the REWATER Project case, where the possibility of reusing wastewater treated at a WWTP located in Leiria Region, for non-potable use has been analyzed. The Strengths, Weaknesses, Opportunities and Threats are presented and discussed, while underlining the interactions needed between water sector operators and potential users, namely agriculture but also urban services, pointing out difficulties to face in water reuse approaches and chances to address, on the non-potable use perspective of reusing treated wastewater. The main issues assessed are as follows:

Strengths:

- Knowledge (R&D) on water and wastewater treatment, to find feasible solutions of reuse and lower costs (treatment & monitoring)
- Environment benefits achievable - reducing emerging pollutants accumulation, yet to fully assess, but measurable in some time
- Potential partners master the problem: they are in the field for centuries

Opportunities:

- Help agriculture & urban water needs under climate change effects
- Alignment with European approaches to face climate changes
- Alignment with Portuguese legislation on water reuse
- Reduce water losses (supply, instead of river transference)

Weaknesses:

- Whatever the process used to enable safe water reuse, it's certain to add costs to existing treatment processes
- Risks on water safety / quality, in water reuse implementations, if monitoring water quality isn't fully addressed and assessed (involving more costs)

Threats:

- Lack of interest from potential users due to additional costs
- Costs (capex or opex) to adapt the way water is transported to final users
- Founding difficulties as Europe shows little capacity to solve economy problems (already in the past, even before COVID 19)

These aspects can lead to promote a wide discussion, about the search for a model to improve convergence in the water reuse, between potential partners in the field: wastewater treatment utilities, agriculture, town's urban services and others.

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Main fields of work and interest includes treatment of water and wastewater, anaerobic digestion, energy efficiency and industrial processes management.



OC 3

WATER REUSE OUTLOOK

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Abstract

Water reuse from treated wastewater is a trending topic nowadays in Portugal. 2 main forces are in place pulling for an increase in water reuse:

- a) Fresh water scarcity.
- b) Political and legislation pressure.

Almost a year has passed since Portuguese water reuse legislation was published and no license requirements were submitted until now (to our knowledge). It's important to try to understand why. In my opinion, there are two factors explaining the lack of activity:

- a) The complexity of the law.
- b) Unmatched supply and demand.

The quality standards for reuse of the Portuguese law is in many cases stricter than the quality standards for WWTP discharge. But still this is not the main reason for reuse low number of projects. Where there is water scarcity, there aren't treatment plants nearby. The biggest plants are located on urban areas where there aren't agricultural needs for water. These and others cases of unmatched demand and supply will be presented.

Carlos Oliveira

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OC 4

**ADVANCED TECHNOLOGIES FOR INDUSTRIAL WASTEWATER
TREATMENT AND REUSE****Carlos Oliveira***, António Rodrigues

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Abstract

Industry faces the challenge of improving its productivity and simultaneously reducing its environmental footprint. When it comes to water usage, reducing footprint means reducing consumption, improving treatment efficiencies, increasing reuse and reduce reject. All that reducing also costs.

Costs come from chemicals usage, from power supply and consumption, from fees and taxes, from space requirements, from maintenance operations. All of them need to be reduced to allow productivity to raise.

Current or conventional technologies no longer allow industries to keep the pace with these new requirements, these new challenges, thus new approaches need to be addressed to trigger revampings and retrofittings in existing treatment plants and allow companies to meet all the mentioned goals at once and very shortly.

Community and society pressure, government pressure, either local or central, other stakeholders pressure and shareholder need to show their investments are environmental friendly and complaint urge CEOs, production and plant managers, quality and environment managers to look for alternative tools (technologies) to meet this and their goals.

Water reuse, as a growing business and opportunity, requires ever more efficient upstream treatment procedures, which can only be achieved with new advanced oxidation technologies, to remove recalcitrant molecules, micro-pollutants, heavy metals, pharma residues, chemical hard contaminants, emulsified greases. All these cause great pain to downstream filtration, ultra-filtration and reverse osmosis systems, reducing their efficiencies, increasing reuse costs and affecting economical balances.

There is no longer any doubt where to act, but that action requires technologies like electro-coagulation, electro-oxidation, dissolved ozonation, perozone, electro-fenton, photo-fenton and more. These are the technologies we'll be addressing in our lecture with in depth analysis, examples, case studies and proven results.

Luís Marinheiro

CEO of AQUASMART – Water and Wastewater Treatment Solutions, Lda, and Global Market Director of AST – Environmental Solutions and Services, Lda. Holds an Environmental Engineering degree, and has over 20 years' experience in project management of environmental technologies for the water and waste management sectors, both nationally and internationally.



WATER REUSE AND APPLICATIONS ON REUSE TECHNOLOGY FOR NON-POTABLE USE

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Abstract

Currently, water scarcity is a worldwide problem with growing severity. Forecasts suggest that in 2030 this problem may reach around 50% of Europe's river basins, so measures are needed to promote a more rational use, and alternative sources need to be exploited [1;2]. More than 40 billion m³ of wastewater is treated annually in Europe, however only 2.4% of this volume of water is reused while there is a potential for six times greater reuse [3]. New EU and national regulations have recently been introduced to ensure water reuse quality and safety for non-potable applications.

Membrane technology has an important role in the treatment of wastewaters from different industries and landfill leachates, and production of potable water through desalination or surface water treatment [4]. The physical separation principle of this technology allows the continuous production of high-quality water, and by tailoring the membrane pore size, water with different quality levels can be produced to suit reuse applications. Different applications of membrane technology processes, including ultrafiltration and reverse osmosis, will be presented within the wastewater and waste management sectors, for wastewater treatment and production of water for reuse.

The reality of contaminants of emerging concern present in wastewaters also imposes new challenges in the field of wastewater treatment, as well as in the detection and quantification for monitoring these pollutants in wastewater and surface water. The FARMASENSE project addresses these two research areas, including the development of an advanced oxidation process (AOP) by non-thermal plasma - an emerging technology in the field of water treatment [5;6]. This AOP has potential to form a wide spectrum of physical and chemical phenomena that, without the usage of chemical reagents, allows a more diversified and effective action on the various contaminants to be treated. Preliminary developments on this project will also be presented.

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OC 6

ANTIBIOTIC RESISTANCE IN THE ENVIRONMENT: ABUNDANCE IS NOT PROPORTIONAL TO RISK**Célia M. Manaia**

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Abstract

Acquired antibiotic resistance emerged with the first therapeutic uses of antibacterial drugs, more than 80 years ago. Over decades antibiotic resistant bacteria were reported in clinical settings and considered a major problem spreading in hospitals, mainly among immunocompromised and debilitated patients. Gradually, the situation changed: today it is known that antibiotic resistant infections can affect anyone, no matter the age, health condition or the country where one lives. Moreover, antibiotic resistance is no longer an exclusive clinical setting issue, it is spread over humans, animals, plants and the environment. The One-Health vision is therefore necessary to better understand how does antibiotic resistance spread from major sources, humans and animals. The impact of different emission sources on the accumulation and spread of antibiotic resistant bacteria and antibiotic resistance genes in the environment has been mainly assessed based on the abundance and prevalence of these self-replicative biological contaminants. However, there are different reasons to believe that this approach may be of limited value to assess risks. These evidences highlight the uncertainty that hampers the establishment of solid recommendations to control antibiotic resistance dissemination and major challenges to regulate practices such as manure soil fertilization or water reuse.

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Paula ML Castro obtained a degree in Food Engineering from Escola Superior de Biotecnologia (ESB-UCP) and holds a PhD degree in Biochemical Engineering from University College London. Main research areas focuses on developing bioprocesses for water and soil sustainability, including pollutants biodegradation and wastewater treatment, concentrating on biofilm technologies such as aerobic granular sludge, with more than 200 international indexed papers published. She is currently Full Professor at ESB-UCP and is the head of the Group Environment & Resources of the Research Center, CBQF.



GRANULAR SLUDGE TECHNOLOGY FOR VALORIZATION OF WATER USE – FROM HIGH TO LOW STRENGTH EFFLUENTS IN AQUACULTURE

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Abstract

Aerobic granular sludge (AGS) is a relatively new compact and energy-efficient technology for wastewater treatment, with over 60 full-scale plants in operation worldwide. AGS is a special case of biofilms in which microorganisms are embedded in a self-produced extracellular polymeric substances (EPS) matrix, forming round-shape microbial aggregates with excellent sedimentation capacity [1]. Due to AGS richness in EPS, surplus biomass is a valuable source for resource recovery, in line with a circular economy approach [2]. AGS applicability for the treatment of high-strength wastewater has been largely reported, demonstrating its robustness to withstand with pollutants commonly found in wastewater e.g. pharmaceuticals [3, 4]. More recently, AGS performance with low-strength wastewater ($< 200 \text{ mg COD L}^{-1}$) has been researched [5, 6]. However, water streams from freshwater aquaculture are characterised by extremely low concentrations of carbon and nutrients and high flows which represent a major challenge for the treatment.

Two different granular based technologies, one operated in sequencing batch and other in continuous mode, were applied for the treatment of extremely low-strength wastewater mimicking aquaculture trout farm effluents. Operation in sequencing mode allowed for a high ammonium removal efficiency which was close to 100 %, whereas the continuous flow reactor allowed for 10-20 % removal. However, the ammonium removal rate was ca. $90 \text{ mg NH}_4^+\text{-N}/(\text{L}\cdot\text{d})$ in the continuous reactor compared to ca. $15 \text{ mg NH}_4^+\text{-N}/(\text{L}\cdot\text{d})$ in the sequencing batch one, due to the different hydraulic retention time. Overall, the sequencing batch reactor generated an effluent with high quality while the continuous flow reactor was able to treat larger flows, and applications may be directed to different stages of the trout production process.

Acknowledgements

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MONITORING AND CONTROL OF WATER, NUTRIENTS AND PLANT PROTECTION PRODUCTS TOWARDS A SUSTAINABLE AGRICULTURAL SECTOR

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Abstract

Better management requires reliable decision-making systems (DSS) based on water quality feedback making use of cost-effective, robust, low-maintenance and accurate sensors for nutrients and pesticides. So far, available sensor technology does not meet the challenges for on-site monitoring and feedback control. AGRINUPES, an ERA-NET / Co-fund WaterWorks2015, started in 2017, intends to develop NPK sensors and integrate them into fertigation equipment, as well as low cost biosensors for plant protection products (PPP) detection, with demonstration of their use for practical management purpose at several European demo-sites. AGRINUPES builds on the extensive experience, competence and early work conducted on optical fibre-based sensors, biosensors, water policy models, plant nutrition, smart irrigation scheduling and robust control. It is implemented by a trans-disciplinary team of experts involving multi-actors. Expected results of AGRINUPES include R&D of an integrated and sustainable monitoring system with innovative ion selective sensors for nutrients (NPK) and bio-based sensing of PPP (imidacloprid and pirimicarb), to be used for optimal water and nutrient supply and reuse, minimizing the effects on the environment; an easy-to-use, robust and fault-tolerant fertigation controller, to meet both crop needs and grower yield/costs expectations; validation and demonstration the applicability of developed technologies at different sites covering several types of crop production systems (recycled or cascaded water system) from greenhouses to open-field agriculture in various climatic regions; and monitoring and control products available for the market. This work describes the AGRINUPES project and its current developments regarding both smart optical sensors for NPK and biosensors for imidacloprid and pirimicarb (PPP) [1,2]. With these sensors, growers will have information about the input and output water quality and can evidence-based decide on how and when to irrigate and fertigate, and on whether the costly task of cleaning is advisable before disposal. Governmental organizations (water authorities) may also use sensors for checking water quality in ground and surface waters. The new sensors will lead to worldwide new markets for European water technology sector, thus strengthening the competitiveness and growth of SMEs and related companies. With this, it is expected a significant increase of water and fertilizer use efficiency in the agricultural/horticultural sector, a longer and economic reuse cycle for the drainage water, and a substantial reduction in pollution of surface and ground waters by fertilizers and PPP.

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PRESENCE OF PESTICIDES IN LEIRIA REGION: SAMPLES COLLECTED IN LIS RIVER

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Abstract

Pesticides are chemical compounds that are applied to eliminate plant and animal pests [1]. However, the intensive use of these compounds can affect human health [2] and cause serious environmental pollution since pesticides are distributed among water, soil, air, and biota, affecting the biodiversity of the ecosystem [1].

The presence of 50 pesticides (different types and groups) was monitor in Lis river. Two sample campaigns (SC) in 2018 and one SC in 2019 were carried out. Five samples in each SC were collected in Lis river. Sampling points comprise the source of the river Lis (R1) and upstream (R2 and R4) and the downstream (R3 and R5) of the two Wastewater Treatment Plants that discharge their effluents to the study river. Solid phase extraction and gas and liquid chromatography with mass spectrometry were the methodologies used for the extraction and analysis.

A total of 20 pesticides were detected in at least one of the 15 analysed samples. The most detected pesticides were cypermethrin (67%), HCB (67%), methoxychlor (60%), and ζ -HCH (60%) and the lowest detection frequency was verified for the pesticides α -HCH, p,p'-DDD, atrazine, aldrin, and o,p'-DDT. As the river receives new inputs on its route, in general, an increase in the concentration of the detected pesticides along the Lis river was observed in each SC. Quantified concentrations ranged from 1.29 (pyrimethanil, R1, first SC) to 1,153 ng/L (aldrin, R5, second SC). Concentration in the $\mu\text{g/L}$ range was found for aldrin (second SC) and γ -HCH (first SC) in sampling point R5. Higher number of pesticides as also higher sum of concentration were found for insecticides type and organochloride group.

This study aims to identify which of the studied pesticides are detected, to know their concentration and variation along the river course and to identify possible sources.

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ASSESSMENT OF PESTICIDES IN THE WASTEWATERS OF TWO WASTEWATER TREATMENT PLANTS, LEIRIA REGION

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Abstract

Pesticides may enter into sewage mainly due to runoff or leaching from treated agricultural fields, disposal of unused products, accidental spills, washing sprayers and formulations bottles, grass management activities in urban areas, and from control of pests in private homes [1-3].

Two Wastewater Treatment Plants (Olhalvas (WWTP-1) and Coimbrão (WWTP-2)) that discharge their effluents to Lis river (Leiria, Portugal) were monitored. For this purpose solid phase extraction and gas and liquid chromatography, both with mass spectrometry were used. The applied methodologies were validated in terms of linearity, method detection and quantification limits, recovery, matrix effects, and successive injections of the standard solution.

Three sampling campaigns (SC) were performed between 2018 and 2019 and samples from the WWTP effluents and influents were collected. The wastewater samples were composite samples collected with the same frequency and obtained by mixing grab samples taken every hour during 24h. WWTP-1 receives domestic and hospital wastewaters. WWTP-2 receives domestic, hospital effluents, livestock production effluents, and landfill leachate.

For the 20 pesticides detected concentration were quantified between 2.27 ng/L (isoproturon, E2, first SC) and 2,134 ng/L (γ -HCH, E2, second SC). 100% of detection frequency was observed for isoproturon, follow by cypermethrin, methoxychlor, pyrimethanil, γ -HCH, dieldrin, diuron, α -HCH, and α -endosulfan with 67% in WWTP effluent samples, and diuron and isoproturon (50%) in WWTP influent samples. The lowest detection frequency of for endrin and bentazon (17%) for WWTP effluent and for atrazine (25%) in WWTP influent samples.

The detection of the pollutants in wastewaters before (influent) and after (effluent) treatment suggests that this pathway must not be neglected and it is important to include this type of samples in the monitoring studies.

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MONITORING OF PESTICIDES AND PHARMACEUTICALS IN A WWTP

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Abstract

The main scientific and technological objective of the project for the Romanian teams was the monitoring of micropollutants and their corresponding metabolites and/or degradation products (MMD) in different matrices (e.g. WW effluents and sludge) based on chromatographic techniques. With the help of partners from Portugal, the procedures for measuring the concentrations of medicines and pesticides in surface waters were implemented at the Research Centre for the Study of The Quality of Agri-Food Products HORTINVEST at the University of Agricultural Sciences and Veterinary Medicine in Bucharest. Thus, water monitoring was carried out at the entry point and exit point of the Wastewater Treatment Plant in Focsani.

In the first part of 2018, through the collaboration between the members of the project team and those of HORTINVEST, the first experimental procedures were developed, and in the second part and in 2019 the actual measurements were made.

The results showed the predominant presence of NSAIDs/analgesics, diclofenac presenting the highest content in all samples of wastewater, followed by psychiatric drugs and pesticides.

The results obtained for diclofenac, methiocarb and oxadiazon are presented in Table 1. The results obtained for diclofenac were compared with those obtained by Gheorghe et al. [1] who reported values between 3 and 7 ng/mL for analyses performed at three treatment plants in Pitesti, Tg. Mures and Brasov (Romania). The values found in this research are within the same range.

Table 1. Results obtained in the analysis of concentrations of diclofenac, methiocarb and oxadiazon.

Sample Code	Source	Diclofenac concentration (ng/mL)	Methiocarb concentration (ng/mL)	Oxadiazon concentration (ng/mL)
147	Lagoon	2.5723	0.478043	1.866169
147 spike	Lagoon	11.0429	2.01551	8.510995
148	Entry point	4.1030	0.755876	2.111085
148 spike	Entry point	14.2546	2.598454	8.91868
149	Exit point	3.4130	0.630634	0.347174
149 spike	Exit point	10.6582	1.945685	8.549247

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Dr Ferreira (NF) is a young researcher experienced in applying different techniques at different organisational levels (molecular to population) to understand the impacts of pollutants on ecosystems. NF main focus is environmental toxicology working with organisms from aquatic and soil ecosystems, studying the effects of pollutant mixtures and the toxicodynamic of metals, pesticides and even climate changes on non-target organisms. NF is funded by the Marie Skłodowska-Curie European program.



“BELLOW THE EYE”: USING EDNA FOR THE EVALUATION OF ENVIRONMENTAL WATER QUALITY IN A TROPICAL URBAN ESTUARY

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Abstract

Coastal regions are essential areas for the development and reproduction of species. Estuaries play an essential role as areas of high diversity, place of refuge, protection, food and reproduction. It favours the establishment of fishing activities and provides the intense human occupation, as approximately half of the world's population lives in areas coastal areas, including the estuarine areas. Given the importance of these areas, pollution in estuarine regions becomes a matter of concern as anthropic factors, driven by disorderly urban and industrial growth.

Located in Aracaju, the capital of the State of Sergipe (NE- Brazil), the Sergipe River estuary lies within a densely populated area with poor basic sanitation and receives a large quantity of raw and treated domestic sewage. Other activities that contribute to the poor water quality include shrimp's aquaculture and deforestation of mangroves. These activities contributed to increasing the organic matter that may lead to a reduction in dissolved oxygen and increased levels of eutrophication throughout the Sergipe River estuary. There are high levels of anthropogenic activities upon the banks of the Sergipe River. These range from plastic, textile, ceramic and engineering industries.

Studies to this date investigating water quality about the effects on the resident biota are scarce. This research aims to investigate the effect human activity has upon the ecosystems. The study will use water collected from the Sergipe River and a close-by non-contaminated river (Real River) filtered through DNA filter units. The objective was to extract environmental DNA, perform PCR amplification and downstream Illumina sequencing. The PCR amplification targeted algae, bacteria and fish species, allowing species to be identified and to compare biodiversity across the sampling sites. The presence/absence of species was correlated with water physical-chemical parameters and contaminants, and acute toxicity was tested using *Mysidopsis juniae*.

The obtained results show a relationship between different biota communities, the contaminants analysed and the toxicity assays, showing the susceptibility/resistance of some species to species contaminants.

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WATER QUALITY ANALYSIS AND APPLICATION OF GIS IN TRIBUTARIES OF THE LOWER IGUAÇU RIVER BASIN, BRAZIL

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Abstract

Land use and management are fundamental for the natural occurrence of water [1]. In this study we evaluated the effect of water quality variables under different forms of land use of eight streams located in the lower Iguazu River basin (Paraná, Brazil). To determine the Classes of soil use and occupation, the QGIS program and the SCP plugin were used. In order to correlate the data obtained, Redundancy (RDA) and the Pearson's correlation test were used. The results showed that the streams most affected by agriculture had an increase in the parameters of conductivity, turbidity, temperature, and nitrogen. Four streams were negatively affected by the bare soil, increasing turbidity, temperature, orthophosphate, and ammonia. The dynamics of land use and occupation affect the variability of some limnological parameters and soil physical and chemical attributes, especially those that inherit management characteristics [2]. The most preserved stream had a direct influence from forested areas around it, showing a high concentration of dissolved oxygen and the lowest temperatures among all streams. Preserved environments present better physical and chemical conditions, increasing the heterogeneity of streams, which might provide greater environmental resilience (Figure 1). Therefore, the impacted environments suffer more abrupt and prolonged damage, since they do not present a complex environmental and biological structure that could act as a buffer of energy flow allocated to the system. The multiple land use may provide more appropriate forms of management so that the actions are better adjusted for the entire river basin.

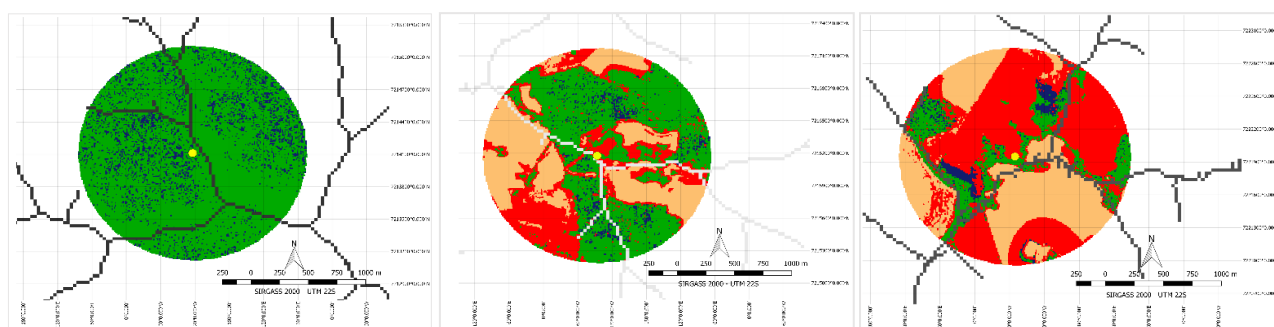


Figure 1. Maps of land use and occupation throughout the study period of 2016 for Manoel Gomes, Arquimedes and Nenê streams, simultaneously classified as "Minimally Impacted", "Intermediate", "Impacted".

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SEASONAL ASSESSMENT OF MACROINVERTEBRATES BIOINDICATORS IN A STREAM ON THE EASTERN EDGE OF THE IGUAÇU NATIONAL PARK, BRAZIL

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Abstract

The Iguaçu National Park (INP), worldwide known by the Iguaçu Waterfalls is one of the last large forested extensions inland Brazil that provides protection to the Atlantic Forest and local hydrographic systems. However, several streams, especially those that flows on the edges of INP, remains subject to different anthropic impacts. Since benthic macroinvertebrates have been widely used for the environmental assessment of streams, seasonal records and quantities of their taxa may provide an additional dataset for biomonitoring of local streams in the face of current agricultural and urban influences on the INP boundaries. In this study we sampled benthic macroinvertebrates on the bottom substrates and provided records covering distinct precipitation/temperature periods along 2016/2017-years, of a stream on the eastern edge of the Iguaçu National Park, Brazil. The records totalize 2,840 individuals distributed in 88 different taxa of 43 families. The most abundant taxa were the Diptera subfamilies, Chironominae (n=1,487) and Tanypodinae (n=256), besides the *Heterelmis* genus (n=152, Elmidae; Coleoptera). Diptera was the richest order in number of families (n=8), while Leptophlebiidae (Ephemeroptera) was the richest taxonomic family in number of genera (n=11). *Aegla* (Crustaceae) and the Insecta genera, *Heterelmis*, *Hexacylloepus*, *Noelmis*, *Phylloicus* and *Thraulodes*, were recorded through all samplings. Twenty-five exclusive genera of EPT (Ephemeroptera, Plecoptera and Trichoptera) and Odonata were recorded during intermediate precipitation/temperature periods. Twenty-one of them were recorded in May 2016, with five genera standing out in abundance (*Hydrosmilodon*, *Anacroneuria*, *Argia*, *Coryphaeschna*, *Americabaetis*) and four (*Needhamella*, *Tikuna*, *Simothraulopsis*, *Neocordulia*) in December 2016. Four general taxa were exclusive of the lower precipitation/temperature period (August 2016), standing out in abundance the *Oxystigma* (Odonata) and *Corydalus* (Megaloptera) genera. In March 2017 (higher precipitation/temperature period), four exclusive taxa were recorded, among them, the *Chimarra* (Trichoptera) genus. Our findings revealed higher number of taxonomic families than other urban/rural streams of the same region [1], besides a varied taxa composition along the year, with the presence of sensitive groups to environmental impacts. It may be considered as an indicative of better environment quality. Nevertheless, seasonal records demonstrated low occurrences and abundance of macroinvertebrates during the higher precipitation/temperature period, another result that indicate surroundings influences on the macroinvertebrate bioindicators.

Acknowledgements

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AZITHROMYCIN ELECTROCHEMICAL DETECTION USING A MOLECULARLY IMPRINTED POLYMER ON A DISPOSABLE SENSOR

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Abstract

In recent years, the spread of pharmaceutical compounds into the aquatic environment has led to a great global concern [1]. Due to its wide utilisation and misuse, significant concentrations of antibiotics have been discharged to the environment through many different routes [2]. Azithromycin (AZY) is among the broad-spectrum antibiotics with higher concentrations in aqueous matrices [3], which explains its inclusion to the EU Watch list of emerging aquatic contaminants (Decision 2018/840/ EU). Besides its low concentrations, its health effects on humans and the environment are not yet well understood. Therefore, the development of sensitive and selective analytical methods for AZY determination is very important. In this work, an electrochemical molecularly imprinted polymer (MIP) sensor for the quantification of AZY was developed. Quantum mechanics calculations were performed to select the optimum functional monomer. The MIP was prepared by electropolymerization in the surface of screen-printed carbon electrodes (SPCEs), using a solution containing 4-aminobenzoic acid (4-ABA) in the presence of AZY as the template. The characterization of the sensor was carried out using scanning electron microscopy (SEM), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Electrochemical detection studies were performed by differential pulse voltammetry (DPV). Under optimized conditions, the present MIP sensor exhibited a limit of detection (LOD) of 0.08 μM (S/N=3) and was successfully applied in the analysis of tap water and water samples collected upstream of a wastewater treatment plant (WWTP) output in a Ave river. The proposed sensor presents an environmentally friendly strategy for AZY determination in environmental water.

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MOLECULARLY IMPRINTED POLYMER FOR SELECTIVE ELECTROCHEMICAL DETERMINATION OF DICLOFENAC IN WATER

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Abstract

Although essential for people's well-being, pharmaceuticals and their metabolites are released in the environment since wastewater treatment plants are not able to remove them completely from contaminated waters [1]. Diclofenac is a non-steroidal anti-inflammatory drug widely used classified as an emerging contaminant. Its occurrence in surface waters and potential impact on humans, animals and microorganisms even at low concentrations attracted special attention to its control [2]. There are some well-established analytical methods for diclofenac quantification in the environment, however they usually are time consuming and expensive. Electrochemical methods offer simple, fast and cost-effective analysis.

Molecularly imprinted polymers are tailor-made materials with recognition sites capable of selectively binding to a target molecule [3]. They find several applications, from drug delivery systems, chromatographic separation, solid-phase extraction.

In this work a molecularly imprinted polymer was used for the selective detection of diclofenac. Diclofenac sodium salt, acrylamide (functional monomer), ammonium persulfate (initiator) and sodium nitrate were used for MIP building. The surface of a screen-printed carbon electrode was previously modified with nano-diamonds for ultrasensitive detection. Detection of DCF was carried out by differential pulse voltammetry. The sensor detected DCF in concentrations as low as 1 nM.

Acknowledgements

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CARBONACEOUS-FE ELECTROCATALYST FOR THE DEGRADATION OF PERSONAL CARE PRODUCTS BY ELECTRO-FENTON PROCESS

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Abstract

Pharmaceutical and personal care products (PPCPs) consumption has increased in the last years. Accordingly, the release of these products into the environment has become a problem due to they are not efficiently removed in the traditional wastewater treatment plants, and they cause a significant impact on the environment including human beings [1]. Thus, new treatments are required to overcome these problems.

Electrochemical advanced oxidation process (EAOPs) have demonstrated their efficiency in the treatment of a wide variety of pollutants. These processes are based on the effective generation of the hydroxyl radicals, which are powerful oxidants. Among them, electro-Fenton has arisen as a greener, suitable alternative for the abatement of these pollutants. However, there are several drawbacks to overcome in order to achieve more efficient and effective process, which includes the development of new electrodes/catalysts.

In this study, the preparation of new electrocatalysts was developed. For this purpose, graphite felt was selected as a substrate and the catalyst (Fe) was fixed to its surface by different methods (adsorption, precipitation). These electrocatalysts were evaluated for the remotion of methylparaben, a PPCPs, attaining total removal of the pollutant in a short period of time (< 1 h). Moreover, the reduction of the total organic carbon was monitored. The best produced catalyst was selected for further improvement of the treatment and its characterisation by SEM, EDS and cyclic voltammetry was carried out. Several key variables affecting the process were optimised, including intensity, pH and the reusability as a crucial factor in the development of a suitable electrocatalyst. Finally, intermediate products including carboxylic acids, generated during the degradation process, were identified and a plausible degradation pathway was elucidated.

The developed electrocatalyst demonstrated to be a feasible alternative for the degradation of the selected paraben via *in situ* generation of hydroxyl radicals. This developed electrocatalyst enhanced the efficiency in the treatment of the PPCPs also allowing an extension on the pH operational range and activity of the catalyst in several runs.

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USE OF GREEN ZERO-VALENT IRON NANOPARTICLES FOR OXYTETRACYCLINE REMOVAL FROM AQUEOUS MEDIA

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Abstract

Antibiotics reaching water systems is becoming a worldwide environmental issue, since they can cause serious negative impacts on the human and the ecological health. Because conventional wastewater treatment processes do not effectively eliminate these compounds, new technologies need to be developed for their removal. In this sense, the use of green zero-valent iron nanoparticles (gnZVIs) has recently emerged as a low-cost and ecofriendly technology for water remediation. gnZVIs are synthesized using plant extracts containing antioxidant compounds which have high reducing capacities and assure the reduction of Fe^{3+} or Fe^{2+} to Fe^0 , thus producing nZVIs [1]. In the present work the effectiveness of gnZVI synthesized using vineyard pruning residues extracts for oxytetracycline (OTC) removal from aqueous media was studied.

The gnZVIs were synthesized following the procedure described by Machado et al. [2]. The removal tests were carried out in the darkness, using an initial OTC concentration of 50 $\mu\text{mol/L}$ and a OTC/gnZVI molar ratio of 1:2.

Figure 1 shows the OTC removal kinetics in the absence and in the presence of Reactive and Unreactive gnZVIs. In the absence of gnZVIs no OTC degradation took place, discarding hydrolysis as a short-term mechanism for OTC removal. However, the addition of Unreactive gnZVIs led to OTC dissipation, obtaining removal percentages of 29 and 53% after 5 and 240 min, respectively. This OTC removal is attributed to adsorption, because once synthesized the gnZVIs were stirred in contact with the air prior to start the removal experiments, guaranteeing the total oxidation of Fe^0 to iron oxides and hydroxides. On the other hand, the addition of Reactive gnZVI increased the removal percentages to 51 and 65% after 5 and 240 min, respectively. This OTC removal in presence of Reactive gnZVIs can be attributed to adsorption plus reductive degradation, since the Reactive gnZVIs are produced in the bulk of the contaminated solution, thus limiting the Fe^0 oxidation and avoiding loss of reactivity.

In conclusion, the results indicate that gnZVIs were able to remove up to 65% of OTC using a very low OTC/gnZVI molar ratio (1:2), demonstrating that the use of gnZVIs constitutes a potential alternative technology for water remediation, reducing environmental impact and operational costs.

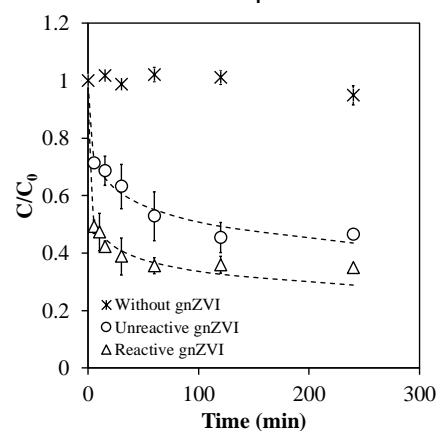


Figure 1. OTC removal kinetics in absence and in presence of Reactive and Unreactive gnZVIs.

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These studies, and his experience working in various companies and institutions made him develop a great interest in environmental pollution and its treatment. His main research interest and study is focused in the remediation of contaminated spaces.



BIOCHAR ADSORPTION AS A POSSIBLE SOLUTION FOR FREQUENT ANTIDEPRESSANTS REMOVAL IN WASTEWATERS

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Abstract

Pharmaceuticals have been detected in surface waters and wastewater across the world. These compounds can enter the environment via various routes, such as by manufacture process, human consumption and excretion, agricultural or veterinary sources. Although at low concentrations (so-called micro-contaminants), these compounds may cause harm to the aquatic environment. The majority of pharmaceuticals detected in aquatic environments are antibiotics, sex hormones, antidiabetics, antidepressants, anticonvulsants. Specifically, the use of antidepressants by the population has been increasing in the last years [1]. The current wastewater treatment plants (WWTPs) are inefficient in the management of these micro-contaminants as they are not designed for this purpose. Therefore, research efforts are focused on technologies able to eliminate these contaminants efficiently. Among them, it is adsorption, which is considered an efficient, easy and fast process to remove these micro-contaminants. In this process, one of the main issues is the selection of an appropriate and sustainable sorbent based on the pollutant properties.

In this study, five biochars obtained from forest and agricultural residues were prepared and analyzed: eucalyptus biochar (EB), quince biochar (QB), walnut biochar (WB), ilex biochar (IB) and vine biochar (VB). These biochars were studied for the removal of venlafaxine (VLX), trazodone (TRZ) and fluoxetine (FLX), commonly used antidepressants. After a preliminary assay, the three most efficient biochars were selected: EB, IB and VB. Then, an in-depth adsorption study was performed involving kinetics, isotherms and also operational parameters, such as the pH effect, on the adsorption of VLX and TRZ, separately, for a 2 mg/L concentration. The adsorption mechanisms, studied through D-R isotherm, showed physisorption for VLX and chemisorption for TRZ, in agreement with the results of the kinetics models. After studying the adsorption process of each pharmaceutical individually, the adsorption of the tri-component mixture (VLX, TRZ and FLX) was also investigated, namely equilibrium and pH effect studies. Using the biochar that attained the best performance, EB, a fixed-bed column experiments were performed and models such as Thomas model were fitted to the breakthrough curves in order to understand its behaviour. The results were promising, and carrying this study even further towards a possible real execution, EB was tested in a fixed-bed column using a real matrix from the WWTP outlet. The high removal levels achieved with the biochar EB confirm that the proposed biosorbents are a sustainable alternative for the effective elimination of antidepressants from wastewaters.

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STUDY OF A THREE-DIMENSIONAL ELECTROCHEMICAL PROCESS FOR THE REMOVAL OF CARBAMAZEPINE FROM WASTEWATER

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Abstract

The primary source of entry of drugs and their metabolites into the environment is through excretion. The evolution of analytical methodologies has enabled their detection in the environment, making the scientific community more concerned regarding their effects on aquatic organisms and human health. There is evidence that conventional procedures used in wastewater treatment plants (WWTPs) do not efficiently remove the majority of these compounds. Therefore, more effective and sustainable treatments are necessary.

The three-dimensional electrochemical process (3D-EP) is efficient in removing substances resistant to biological treatments. This method differs from the two-dimensional electrochemical process (2D-EP) because of the introduction of a (third) particulate electrode, in which the particles act as individual microelectrodes, increasing the removal efficiency due to the simultaneous contribution of different phenomena, such as adsorption/electrosorption, electrocoagulation, oxidation, and catalytic degradation. Carbamazepine (CBZ), an emergent pollutant, has been proposed as an anthropogenic marker to assess water quality due to its persistence in conventional treatment plants and widespread presence in water bodies. In this work, the 3D-EP was used to remove CBZ from water. Tests were performed with carbamazepine (10 mg/L initial concentration and a 250-mL volume), in the presence of an electrolyte (0.01 mol/L sodium sulfate), using a carbon felt cathode, a boron-doped diamond (BDD) anode (both 2 cm x 7 cm), a current intensity of 0.30 A, a potential of 20.42 to 31.70 V and an airflow of 1 L/min. The particulate electrodes were commercial activated carbon (MX-C-F from Zhongshan Vmox Environmental Filter Material) and a biochar produced by pyrolysis of vineyard pruning. The results were compared with a 2D-EP using the carbon felt and BDD electrodes and with adsorption studies performed with the activated carbon and the biochar.

These tests show better efficiencies for the 3D-EP system when compared with the 2D-EP and with the corresponding adsorption studies with an increase of more than 50% of CBZ elimination after 30 min for both particulate electrodes. Besides this, the commercial activated carbon provided a better removal efficiency than the biochar (removal efficiencies of 100 and 60%, respectively).

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EVALUATION OF ACUTE AND CHRONIC TOXICITY OF INDUSTRIAL AND URBAN EFFLUENTS USING AQUATIC ORGANISMS

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Abstract

As a result of anthropogenic activities, organic micropollutants (MP) of different origins are released into the environment causing short or long-term adverse effects to exposed organisms and humans. These MP can be of domestic, industrial, or agricultural origin and their occurrence has substantially increased in recent decades. Among MP of environmental relevance are pesticides, hormones, detergents, various industrial products, and pharmaceuticals [1]. These pollutants reach aquatic systems by discharging effluents from wastewater treatment plants (WWTPs) that do not have the capacity to eliminate them completely. Further, the increase of residual water (RA) production due to the growing urbanization and industrialization, as well as the scarcity of water, led effluents to be used for urban irrigation, agriculture, and solid wastes used in soil fertilization. Therefore, residues of MP have been found in food and contributed to soil deterioration, contamination of aquatic systems, as well as adverse effects on exposed organisms [2,3]. Currently, WWTPs can use three types of treatment: primary treatment, secondary or biological treatment that allows the removal of organic micropollutants and nutrients and the removal of solid waste. In some WWTPs, a third treatment can be applied, which consists of disinfecting the effluent to remove pathogenic organisms. Legislation establishes admissible values for the quality of the effluents to be released into the aquatic systems, focusing essentially on physical-chemical (FQ) parameters (Decree-Law No. 152/97 and Decree-Law No. 236/98). However, toxicity to exposed organisms is not evaluated [3]. The aim of this study was to evaluate the toxicity of different types of effluents using ecologically relevant aquatic organisms, namely the crustacean *Daphnia magna* and the protozoan *Tetrahymena thermophila*. Acute and chronic toxicity tests were carried out on three WWTP effluents with different treatments (A, B and C) and FQ parameters determined. WWTP A does not perform tertiary treatment while WWTP B and C perform tertiary treatment with UV light and ozonation, respectively. FQ parameters were within the established values for all WWTPs effluent samples. Considering toxicity assays, all WWTP samples were not toxic to daphnia, however, WWTPs samples inhibited protozoan growth and WWTP C showed the highest toxicity (growth inhibition) to this organism. These data showed low effluent toxicity of the selected effluents. However, toxicity depends on the organism under study and on the WWTPs treatment used.

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NORFLUOXETINE AND VENLAFAXINE IN ZEBRAFISH LARVAE: SINGLE AND COMBINED TOXICITY AND INSIGHTS FOR RISK ASSESSMENT

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Abstract

Antidepressants and their metabolites are constantly found in natural and waste water samples, as well as fish tissues [1,2]. However, investigation of their potential toxic effects on aquatic organisms, by itself or in mixture with other occurring psychoactive drugs, has been unheeded[3]. In this work, effects of 80hpf exposure to norfluoxetine (0.64, 3.2, 16, 80 and 400 ng/L), venlafaxine (16, 80, 400 and 10000 ng/L) or their combination (3.2 ng/L +2000 ng/L, respectively) were investigated in zebrafish embryos and early larvae. Mortality, several embryonic malformations, sensorymotor reflexes and the expression of 34 genes involved in the antidepressants mode-of-action (MoA) and metabolism were evaluated (i.e. monoamine transporters and receptors, nuclear receptors, and detoxification enzymes and transporters). In comparison to control animals, norfluoxetine treatments only caused depigmentation of embryos and larvae. Larvae exposed to venlafaxine, exhibited depigmentation and spinal deformities, diminish sensorymotor reflexes, alterations (mainly downregulation) in the expression of genes belonging to the serotonergic, noradrenergic and dopaminergic systems, as well as nuclear receptors related to lipid and drug metabolism. The mixture showed distinct interaction effects, depending on the level of biological organisation analysed and the neurotransmitter pathways affected; synergism (mortality), no interaction (sensorymotor reflexes), antagonism and inverse agonism (gene expression). The results clearly show the need for investigation of the toxicity of pharmaceutical metabolites single and in mixture, as well as their risk assessment in methodologies accounting for possible interactions with other pharmaceuticals or endocrine-disrupting compounds.

Acknowledgements

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HOW DO ZEBRAFISH EMBRYOS RESPOND TO CAFFEINE EXPOSURE?

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Abstract

Coffee is among the most consumed stimulant beverages in the world. The active compound, caffeine (CAF) has been considered an emerging contaminant and indicator of human contamination due to its widely detection in aquatic systems [1, 2]. The existence of CAF in the environment may pose a risk to human and environmental health. Within this context, the goal of this work was to evaluate the effects of CAF in zebrafish (*Danio rerio*) embryos heartbeat and behaviour.

Locomotor behavioural analysis was performed after exposure to CAF in two scenarios: embryos were exposed from 2 to 120 hours post fertilization (hpf) in the first scenario and from 96 to 120 hpf in the second. Concentrations used were 0; 0.0001; 0.01; 0.1; 1; 10 and 100 mg.L⁻¹. Behaviour was determined using the video tracking system Zebrabox and analysing the following parameters: % distance travelled by embryos near the edges of the well (DE) and % time spent by embryos near the edges of the well (TE). Heartbeat rate was assessed by counting heart beats under the stereomicroscope at 48h (first scenario). CAF exposure from 2 to 120 hpf affected the locomotor behaviour of embryos (increase in % DE and TE), whereas embryos exposed from 96 to 120 hpf showed no significant alterations in behavior. Moreover, in the longer exposure, low concentrations of CAF increased heartrate in 2-day-old embryos. Overall, results showed that heartbeat and locomotor behaviour were sensitive parameters showing the importance of considering sublethal effects of CAF to better estimate their effects in aquatic ecosystems.

Acknowledgements

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PRESENCE OF PESTICIDES AND OF DICLOFENAC IN MUNICIPAL WASTEWATER. A LIFE CYCLE ANALYSIS APPROACH

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Abstract

The massive use of pesticides in agriculture and pharmaceuticals for both human health and veterinary purposes lead to the introduction of these compounds in municipal wastewaters [1]. The presence of pyrimethanil, hexachlorocyclohexanes, cypermethrin, among others [2], as well as of diclofenac [1] was observed in the treated effluent of a Portuguese municipal wastewater treatment plant (WWTP). To assess the environmental impacts associated to these contaminants at the reference plant, an environmental Life Cycle Assessment (LCA) based on ReciPE 2016 H/ End Point methodology was developed in SimaPro software package, by using ecoinvent 3.5 database.

Even though the pesticides and diclofenac detected in wastewater are not responsible for the main environmental impacts associated to the studied municipal wastewater treatment process, their presence affects negatively some endpoint impact categories of Ecosystems and Human Health, namely the Freshwater, Marine, and Terrestrial toxicities, and Human Carcinogenic and Non-Carcinogenic toxicities. Among the different technologies studied during REWATER project, the Anodic Oxidation (AO) and the Heterogeneous Electro-Fenton (HEF) processes have shown promising results in terms of removal efficiency towards both diclofenac and pyrimethanil. The results of a preliminary techno-economic screening, performed to assess the viability of these technologies at full scale, suggested that AO is the most promising option due to its higher operational simplicity and lower associated energy demand. The LCA and the Life Cycle Costing of the upgraded AO at the reference WWTP will be developed in a further study to assess the sustainability and the economic viability of AO at full scale.

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CIRCULAR ECONOMY APPLIED TO WATER MANAGEMENT IN A FOOD PROCESSING INDUSTRY

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Abstract

In view of the constant population growth, the unrestrained economic development and the consequent demand for water resources for different sectors (e.g., industrial, agricultural and domestic), the water stress index became alarming in several countries. The scarcity of water has grown considerably and is a worrying phenomenon. International policies to mitigate water consumption, as well as the reuse of water resources, emerged with the aim of reducing imminent risks for the coming decades.

Facing the needs to create strategies to mitigate water consumption by the most representative sectors in the demand for water resources, the circular economy appears as a model of sustainable development. It allows materials to be returned to the production cycle through treatment and/or reuse/recovery/recycling, ensuring greater efficiency in the use and management of resources [1]. According to Ellen MacArthur Foundation [2], this model is based on three principles: (i) preservation and increase of natural capital through the control of finite stocks; (ii) optimization of yields and resources through the circulation of products and materials in technical and biological cycles; (iii) stimulation of the system's effectiveness through the identification and elimination of negative externalities.

The present work explores the possibility of reusing treated water in the production cycle, in a context of circular economy. Therefore it contributes to the sustainability of water resources in a case study of a food-processing industry that produces frozen pre-fried potatoes and dehydrated potato flakes, with an annual production of 200 thousand tons. This research is characterized as an exploratory method, as it aims at improving mutual knowledge and at understanding the volume of water that can be reused. This is achieved through an analysis of the productive process of the case study and through bibliographic research addressing this subject.

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DEVELOPMENT OF SUSTAINABLE BIOTECHNOLOGICAL SOLUTION FOR TREATMENT OF WASTEWATER FROM GRAPE PROCESSING

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Abstract

Wine production represents an important agro-industrial activity, which has been traditionally practiced in many agricultural and rural regions around the world. Although winemaking is considered as an environmentally friendly process, the wastewater generated during the grape processing into wine has notable negative environmental impact. It is generally accepted that agro-industrial waste management is an important strategy for conservation of natural resource and maintenance of environmental quality. However, the wastewater generated during grape processing in wineries is usually disposed into the environment without adequate treatment because it is more difficult to treat than liquid effluents from other food processing industry due to seasonal nature of wine production. Consequently, the main issue in wine producing countries around the world is the management of variable quantity of wastewater with different concentration of organic and inorganic contaminants [1,2]. Over the past years, biotechnological processes for obtaining market-valuable products, including commercially important bacterial biopolymers like xanthan, have received a considerable attention for reuse of various waste streams and utilization of inorganic and organic compounds [3]. In this study, the xanthan biosynthesis by *Xanthomonas campestris* ATCC 13951 is presented as a sustainable solution for treatment of wastewater from grape processing. To improve proposed biotechnological process, definition of optimal sugar content in medium for xanthan production composed of wastewater generated during washing of the crusher, press and tank after clarification of must by flotation was carried out. The optimization experiments were performed in 3 L laboratory stirred tank bioreactor with a working volume of 2 L. Obtained results suggest that optimal sugar content for the most profitable xanthan production on medium based on wastewater from garpe processing is 30 g/L. Cultivation of producing strain on defined medium under applied experimental conditions in 14 L laboratory stirred tank bioreactor with a working volume of 10 L resulted in 69.13% larger accumulation of good-quality xanthan (20.92 g/L) and achievement of significant purification levels of winery effluents which is confirmed by high values of sugar, nitrogen and phosphorus conversion (88.56%, 53.10% and 80.60%, respectively). The present study provides valuable information about efficacy of treatment of wastewater from grape processing that can be used for bioprocess scale up and design of bioreactors with appropriate constructional characteristics, which is a prerequisite of the industrialization of examined bioprocess.

Acknowledgements

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INFLUENCE OF THE TEMPERATURE ON THE PHENOLIC CONTENT OF KOMBUCHA BEVERAGE PRODUCED USING WINERY WASTEWATER

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Abstract

Kombucha is a symbiotic association of several yeasts and bacteria strains, mainly acetic acid bacteria. Traditional substrate for kombucha fermentation is sweetened black or green tea, while the obtained beverage is non-alcoholic, slightly carbonated and refreshing. Yeasts metabolize sugars and produce ethanol, while bacteria oxidize ethanol into acetic acid. The aim of this research was the investigation of the influence of the temperature on the total phenolic content of the kombucha beverage produced on the floated must.

Wastewater was collected from local winery after the clarification of the grape must from Rhein Riesling production. Typically this wastewater is disposed of in the sewerage. Reducing sugar content (15.33%) was measured using the method described by Miller, 1959 [1], and diluted to 7%. The substrate was sterilized and inoculated with 10% (v/v) of previously fermented beverage. Fermentation was performed on 20 °C, 25 °C, and 30 °C for nine days. Samples were taken periodically, and total phenolic content was measured spectrophotometrically using the method described by Singleton and Rossi, 1965 [2].

Phenolic compounds are produced by kombucha culture over time. For all samples, after nine days of fermentation, the phenolic content was higher than that of the initial substrate. For the maximal phenolic content temperature of 25 °C should be selected as optimal. The difference is particularly significant on day 3 (0.011 mg_{GAE}/L for 25 °C and 0.007 mg_{GAE}/L and 0.006 mg_{GAE}/L for 30 °C and 20 °C, respectively) when the beverage had the most pleasant taste.

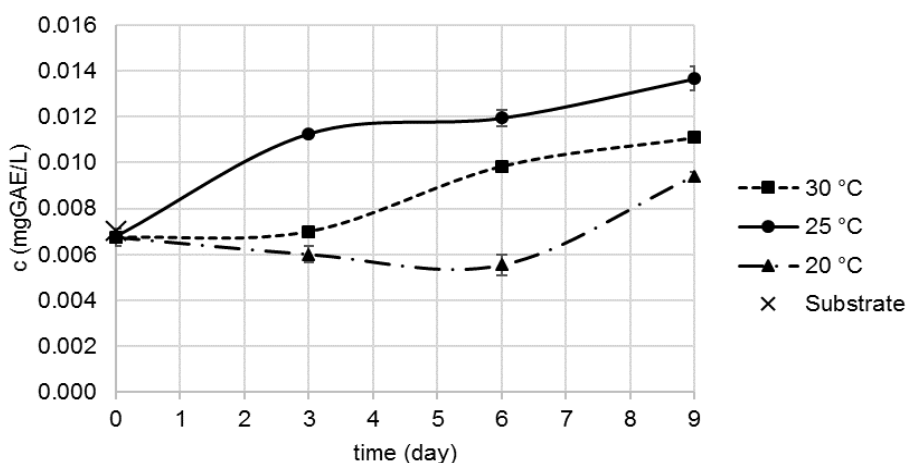


Fig. 1 The total phenolic content depending on the fermentation temperature

Acknowledgements

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UTILIZATION OF WASTE GLYCEROL FOR PRODUCTION OF PLANT PROTECTION AGENTS BY *STREPTOMYCES HYGROSCOPICUS*

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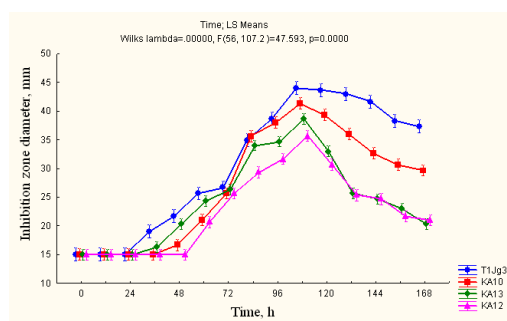
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Abstract

Protection of the environment is one of the most important issues of politics in many countries. Industry generates large amounts of waste, which can be further managed using biotechnological processes. These wastes can then be managed as components of microbial media in the biotechnological production of different active compounds, such as biopesticides [1]. The surplus of waste glycerol, by-product of the biodiesel production process, is available at global market [2]. As the waste glycerol purification process is unprofitable, after biodiesel production it lags behind as a waste. However, some microorganisms have ability to assimilate waste glycerol and convert it into value-added metabolic products. In the present study, ability of *Streptomyces hygroscopicus* to assimilate waste glycerol and convert it into metabolic compounds with antifungal activity against four phytopathogenic fungi obtained from apple fruit samples expressing rot symptoms, was investigated. Production of antifungal metabolites by *S. hygroscopicus* was carried out in 3 L stirred tank bioreactor (Biostat Aplus, Sartorius AG Germany) during 7 days. Fermentation was carried out at 26±1 °C with aeration rate of 1.5 vvm and stirring speed of 100 rpm. The aim of this work was to examine at which stage of bioprocess production of antifungal metabolites occurs. Activity of the cultivation liquid on two isolates of *Alternaria alternata* (T1Jg3 and KA10) and two isolates of *Fusarium avenaceum* (KA13 and KA12) were determined every 12 h using *in vitro* well diffusion method [3]. It was found that the maximum antifungal metabolites production occurred in 108 hour of cultivation. In accordance with the fact that all inhibition zones diameters above 22 mm indicate



that the applied antifungal agent is highly effective [3], it can be concluded that the produced antifungal metabolites have high efficacy on tested phytopathogenic fungi (inhibition zone diameter higher than 35 mm for all test fungi). So, *in vitro* study was confirmed that the bioagents produced on waste glycerol medium can be successfully used for biological control of stored apple fruits from phytopathogenic isolates *A. alternata* T1Jg3 and KA10 and *F. avenaceum* KA13 and KA12.

Acknowledgements

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PESTICIDE DEGRADATION USING GREEN NANO-ZERO-VALENT CATALYSTS

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Abstract

Water scarcity is nowadays an emerging issue that attempts to be solved by the development of new remediation alternatives. Among them, the utilisation of green processes has been encouraged to achieve more sustainable goals avoiding secondary pollution and additional costs. Nano zero-valent particles (NZV) can promote a micro-electrolysis process [1], due to their high reduction potential (they tend to oxidise themselves), which can lead to the direct catalytic destruction of pollutants as well as the generation of oxidative oxygen intermediates [2]. However, those particles are usually synthesised by costly, dangerous and toxic procedures, such as decomposition of $\text{Fe}(\text{CO})_5$ with organic solvents, reduction of iron salts with NaBH_4 [3], etc. In this study, antioxidant extracts obtained from vineyard pruning from *Alvarinho* and *Tinta Roriz* varieties, blueberry pruning, green and brown algae and black tea, are used to reduce metal cations to NZV. This will enable the application of an inexpensive and safe synthesis process and, at the same time, an extra value is added to the referred agro-forestry wastes contributing to circular economy. Chlorpyrifos-methyl is a widely used insecticide to control pests on a range of crops. In this study, chlorpyrifos-methyl was selected as a model pollutant for conducting degradation studies with NZV.

In order to optimise the pesticide degradation, several parameters were studied. Initially, the potential of different transition metals to synthesize active NZV particles was evaluated. Thus, Fe, Ag, Cu, Mn, Mg and Zn NZV were synthesized and deeply characterised (XRD, SEM, XPS, etc.). All of them showed activity for the pesticide degradation both in darkness and under radiation. The combination of NZV particles enhanced the process performance due to the protection against oxidation of one of them (the one acting as a cathode on micro-electrolysis [1]). Regarding the application of the NZV on the effluents, a notable effect of NZV concentration was observed, so this parameter was optimised, avoiding either an excess or lack of catalyst. Under the optimal conditions (0.12 mM Fe + 0.19 mM Ag), it was achieved up to 90% of chlorpyrifos-methyl degradation in 5 min for the treatment of real wastewaters, opening a path for future applications.

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