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# The role of bioassays in the evaluation of ecotoxicological aspects within the PEF/OEF protocols: The case of WWTPs



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#### ABSTRACT

The suitability evaluation of any industrial process should rely on economic, technical, social and, in particular, environmental aspects. The Commission Recommendation 2013/179/UE enables the improvement and the harmonization of the conventional evaluation of environmental footprints, such as LCA (Life Cycle Assessment), Carbon and Water Footprint, by suggesting the assessment of life cycle environmental performance of products and organisations (PEF, OEF). Novelty aspects reside in including new impact categories (namely, human toxicity cancer effects, human toxicity not-cancer effects and eco-toxicity). This paper presents an application of PEF/OEF protocol to the example case of an activated sludge wastewater treatment plant. Strengths and criticisms of this approach are discussed, by taking into consideration the possible final goal of the suitability assessment. Valuably, the adoption of bioassays (i.e., the input of their results in the models for calculating the life cycle environmental performance) for a more reliable evaluation of the impact on the ecosystem and human health is proposed.

#### 1. Introduction

Any industrial process should undergo a complete evaluation of its suitability, which might rely on economic, technical and social aspects, the environmental issue playing a crucial role. The Commission Recommendation 2013/179/UE (European Commission, 2013a) on the use of harmonized methods to measure and communicate the life cycle environmental performance of products and organisations (PEF, OEF) allows to improve and complete the conventional evaluation of environmental footprint, carried out by means of LCA (UNI EN ISO 14040 and 14044) (ISO, 2006a, 2006b) and subsequent methodologies (e.g., Organisation Carbon Footprint, UNI ISO 14064, 2006; Product Carbon Footprint, ISO/TS WD 14067-1, 2013; Water Footprint, ISO 14046) (ISO, 2006c, 2013, 2014). In particular, PEF and OEF broaden the environmental impact categories (fifteen, mandatory) by including human toxicity cancer effects, human toxicity not-cancer effects and toxicity towards the freshwater ecosystems. Nowadays, several European pilot projects are under way in different industrial sectors. Actually, Italy pioneers with the very first applications in textile and fertilizer production industries (Alini and Cavallotti, 2015). The Italian Law no. 221/2015 defines the voluntary appellation "Made Green in Italy" in case of adoption of PEF/OEF.

Calculation of the environmental footprint for different impact categories is based on mass flows of pollutants discharged into the environment, which are related to the various activities involved in the studied process, thus including both direct and indirect emissions. Field measurements are preferred, even if literature data and emission factors may be also employed. This huge amount of information is then processed by means of mathematical models which include official database.

Waste Water Treatment Plants (WWTP) have been recognized to be an important source of point pollution since the last decade, as for emerging contaminants related to human and ecotoxicity (Auriol et al., 2006; Stasinakis et al., 2008; Ying et al., 2009; Sánchez-Avila et al., 2009). Applications of the OEF/PEF methodology to WWTPs are still missing in the scientific literature, despite of the availability of detailed information on emission loads for many substances, in particular for the liquid effluent. Indeed, due to the number of chemicals and their abiotic and biotic transformations it is practically impossible to measure in the effluent all those compounds which may be of environmental concern, and which are related, in particular, to eco-toxicological aspects. These issues have been extensively described and discussed in the scientific literature: Escher et al. (2008), Stalter et al. (2010) and Avbersek et al. (2011).

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Based on this, researchers have moved to try measuring the effect of mixtures and whole effluents by means of biological assays, instead of detecting single compounds both on the liquid (Gartiser et al., 2010; Escher and Leusch, 2012; Burgess et al., 2013; Papa et al., 2013; Escher et al., 2014; Papa et al., 2016a, 2016b; USEPA, 2017)) and sludge (Gonzalez-Gil et al., 2016) phases. Innovative methodologies have been also proposed for using these measurements for assessing the damage on human health as an endpoint category (Papa et al., 2013, 2016a, 2016b).

Therefore, this work is aimed at exploring the possibility to improve the robustness and reliability of OEF/PEF results by using precisely the results of bioassays, carried out specifically for the considered situation, for the calculation of the mid-term impact categories, which are of interest for the present case study. In particular, a real scale WWTP is investigated, since it is considered crucial for its dual action of cleaning the water matrix and collecting and concentrating the pollutants (removed to a certain extent, depending on their biodegradability, chemical and physical properties, and process features). Moreover, the authors have successfully experienced the application of bioassays to WWTP effluents.

Strengths and criticisms of eco-toxicological aspects within PEF/ OEF processes as commonly measured are discussed.

#### 2. Materials and methods

Criteria underpinning the followed approach, details of the procedure, as well as the description of the case study and the model input data are described.

#### 2.1. The OEF/PEF protocols

Product Environmental Footprint (hereinafter PEF) and Organisation Environmental Footprint (hereinafter OEF) are the methodologies established by the European Commission (2013a) to quantify a complete set of relevant environmental performance indicators (namely, 15 EF, Environmental Footprint indicators) using a life cycle approach. A life-cycle approach synthetically described by the expression "from cradle to grave", includes all the stages ranging from the raw material acquisition and the end-of-life, i.e., processing, distribution, use, based on a supply chain perspective. The PEF method is specific for individual goods or services, whereas the OEF method applies to all the activities associated with the goods and/or services provided by an organisation. EF impact categories are quantified using established models and refer to resources use and emissions of substances harmful for human health and environment. In order to achieve consistent, robust and reproducible results, PEF/OEF studies must adhere to specific analytical principles (relevance, completeness, consistency, accuracy, transparency), that must be applied at each phase of the study, from the goals and scope definition, through data collection, environmental impact assessment, reporting and review.

The emissions from a wastewater treatment plant were analysed. The environmental impacts originated from the pollutants detected during a monitoring campaign (case #1) were compared to the ones obtained by using the results of bioassays (cases #2 and #3). Data were processed and modelled in accordance with PEF/OEF protocol. The functional unit was selected as  $1 \text{ m}^3$  of purified water.

The study focused on the evaluation of three environmental indicators: *Human Toxicity Cancer Effects, Human Toxicity Non-Cancer Effects* and *Freshwater Ecotoxicity*. These are, indeed, those items that are mainly affected by the residual pollution of the final effluent, which was addressed by the present study. In compliance with the most recent publications (European Commission, 2016), these three toxicity-related impact categories shall be temporarily excluded from the external communications, when determining the benchmark and identifying the most relevant life cycle stages/processes, since they are not reputed sufficiently robust. Characterization factors (CF) (European

#### Table 1

Effluent standards according to the discharge license in force (the plant is located in a Sensitive Area, according to the definition of the Directive EEC/271/91).

Parameter	Concentration	Measurement unit
Total suspended solids (TSS)	35	mg/L
BOD <sub>5</sub>	25	mg/L
COD	125	mg/L
N <sub>tot</sub>	15	mg/L
P <sub>tot</sub>	2	mg/L
E. coli	5000	UFC/100 mL

Commission, Joint Research Centre, 2012) related to those impact categories are currently involved in a series of studies done in collaboration by the European Commission and the ECHA (The European Chemicals Agency), aimed to develop a new CF-set based on the REACH (Registration, Evaluation, Authorization and Restriction of Chemicals, the EU regulation entered in force on 1 June 2007) data.

The definition and the meaning of the present study is in line with the ongoing work in this field, improving the reliability and the robustness of the results.

#### 2.2. The case study

The studied Waste Water Treatment Plant (WWTP) is an activated sludge facility treating the municipal sewage of some villages and the winery wastewater produced in the surrounding area. The latter causes a significant load increase during September and October.

The effluent standards to be complied with are summarized in Table 1, for the main parameters.

The water line consists of: equalization  $(1300 \text{ m}^3)$ , coarse screening (30 mm), lifting, fine screening, grit and oil removal, intermediate lifting, pre-denitrification and nitrification (2 parallel lines, 2.700 m<sup>3</sup> each + 860 m<sup>3</sup>, the latter used only in case of pick load), final sedimentation (5 parallel tanks), filtration (disk), UV disinfection. Phosphorus co-precipitation is achieved by dosing Alum in the oxidation basins. The surplus sludge is processed by means of dynamic thickening and mechanical dewatering (belt press).

The main operational conditions are as follows (referred to the years 2015 and 2016). The average treated sewage flowrate, under dry weather conditions, is  $25000 \text{ m}^3/\text{d}$ . The COD concentration varies remarkably all along the year due to the seasonal activity of wineries, the median and 90° percentile being 230 mg/L and 460 mg/L, respectively. The BOD<sub>5</sub>/COD ratio is around 0.5. The median and 90° percentile of total nitrogen concentration are 20 mg/L and 27 mg/L, respectively. For total phosphorus, the median and 90° percentile are 2.3 and 4.3 mg/L, respectively.

The organic load ranges between around 50,000 p.e. (population equivalent; 1 p.e. =  $60 \text{ g BOD}_5/d$ , according to EEC/271/91), without the contribution of winery wastewater (this value corresponds to an average flow rate of 25000 m<sup>3</sup>/d and a BOD<sub>5</sub> average concentration of 120 mg/L), and around 90,000 p.e. during the grape harvest time (corresponding to an average flow rate of 25000 m<sup>3</sup>/d and a BOD<sub>5</sub> average concentration of 216 mg/L).

The solids retention time (SRT) is kept between 20 and 30 days and the wastewater temperature ranges between 11 and 25  $^{\circ}$ C.

Average concentrations (2015–2016) of the main pollutants in the discharged wastewater are well below the effluent standards (reported in Table 1): COD = 17 mg/L; BOD<sub>5</sub> = 8 mg/L; TSS = 9 mg/L; Ntot = 5.8 mg/L; Ptot = 0.4 mg/L. Detailed figures adopted for the environmental impact calculation and obtained during a dedicated monitoring campaign (seven months duration) are reported in the following sections.

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