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Abstract

Wastewater treatment represents a continuous challenge for engineers, environmental scientists and regulators. At the beginning it was the biological load to be reduced as much as possible to prevent infections and eutrophication phenomena. Afterwards, besides domestic discharges, industrial wastewater required specific technologies and treatment facilities to allow the removal of targeted pollutants. Today, an increasing number of new contaminants are rising up such as pharmaceuticals, personal care products and nanomaterials just to cite the most recent categories. The current goal in wastewater management is to move towards the (near-) zero approach or, ideally, the zero-discharge requiring to recover and reuse treated wastewater as much as possible. About that, ecotoxicological tools may offer a great support in the defining the rate of contamination removal during the treatment processes and allow further assessment about effluent second life. An overview of ecotoxicological approaches to assess wastewater environmental impacts to saltwater is presented and discussed considering toxicity tests as essential guidelines.

Keywords:

Wastewater toxicity, recovery and reuse, effluent assessment, wastewater treatment technology

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1. INTRODUCTION

Wastewater management has been receiving a great deal of attention with various methods proposed for discharge hazard estimation. The today aims in the wastewater treatment and industrial sector consist in particular of preventing, reducing and as far as possible eliminating pollution by giving priority to intervention at source and ensuring prudent management of natural resources, in compliance with the 'polluter pays' belief and the principle of pollution prevention (IPPC, 2008). Various authors have suggested more or less user-friendly tools to control and manage wastewater toxicity both as toxicity scores and indexes with a variety of statistical approaches, generally integrated with expert judgments (Libralato el al., 2010a, 2010b). Anyhow, wastewater treatment plants data on toxicity removal efficiencies are generally scarce, especially in the case of the application to specific technological facilities. For example, Activated Sludge Sequencing Batch Reactor (AS-SBR) data about wastewater toxicity removal are still limited (Helmereich *et al.*, 2000; Libralato el al., 2010b), and slight and incomplete information is available about toxicity removal efficiencies and rates for Ultra-Filtration Membrane Biological Reactor (UF-MBR) facilities (Zha and Wang, 2005; Urtiaga *et al.*, 2006), like as for some other treatment techniques such as Activated Carbon (AC), Nano-Filtration (NF) and Reverse Osmosis (RO).

In the case of activated sludge treatments, it should be very important to perform preliminary ecotoxicological tests on incoming wastewater. Firstly, this is to check the effect of toxic substances on the microbial changes and, thereafter to assess the whole performance of the treatment process via monitoring the discharge in order to optimise the treatment efficiency and minimise the hazards for the receiving water bodies (Nyholm, 1996; Paxéus, 1996; Chen *et al.*, 1997; Grau and Da-Rin, 1997; Jenning *et al.*, 2001; La Farré *et al.*, 2001). Indeed, the harm caused especially by toxic industrial effluents to sewage treatment works has been recognised for many years (Paxéus, 1996; Grau and Da-Rin, 1997; Spincer Davis and Murdoch, 2001; Ellouze *et al.*, 2007). High toxicity levels of raw wastewater may result in the need for prolonged treatment times or in the washout of the activated sludge (Chen *et al.*, 1997), while residual toxicity in the discharge might state the inadequacy of the wastewater treatment technology used, poor management of the Wastewater Treatment Plant (WWTP), being a serious harm for the downstream ecosystems.

Many gaps in the knowledge are still remaining in the management of toxicity from WWTPs, especially about the formation of degradation by-products and their relative effects (*e.g.* pharmaceutical and personal care products, engineered nanomaterials, …) (STOWA, 2005). Internationally, the role of toxicity management in treatment works is now receiving more attention by plant managers, like for the Best Available Technologies (BATs) implementation, mainly due to recent environmental requirements (Grau and Da-Rin, 1997; Spincer Davis and Murdoch, 2001; IPPC, 2003; OSPAR, 2005).

2. AN ECOTOXICOLOGICAL OVERVIEW OF WASTEWATER MANAGEMENT

According to the European Inventory of Commercial Chemical Substances (EINECS), wastewater might potentially contain 100,204 substances (EINECS, 2011) as well as their relative by-products. Therefore, the whole characterisation of a wastewater sample could be uneasy and costly just on a chemical basis. As a result of the precautionary principle (Harremoës, 2000), all substances should be analysed, but the limited knowledge on wastewater constituents, due to biotic and abiotic degradation, would start a never-ending process.

Nevertheless the chemical approach is still playing a leading role in wastewater quality monitoring, the properties of bioassays are more and more recognised by the time the Hazardous Waste Directive (91/689/EEC) (EEC, 1991) introduced, officially, the term "ecotoxic" (Annex III – H14) in the EU.

The limited assessment abilities of chemical analyses with complex mixtures has required, increasingly, the intervention of bioassays, because:

- Mixtures contain many substances which cannot be identified and/or detected;
- There is a lack of data about cause-effect relationships for both pure substances and environmental samples presenting a general high level of complexity;
- Micro-pollutants (*e.g.* endocrine disruptors, pharmaceuticals, nano-materials, …) are undefined and cannot be taken into account;
- Combined effects of mixture contaminants on bioavailability can be evidenced only using bioassays (OSPAR, 2000, 2005, 2007);
- Spatial and temporal definition and comparability are not attainable in other ways;
- Large areas to be monitored;
- Hot spots to be identified (Whitehouse *et al.*, 2004).

Most countries seemed to have followed a similar historical trend in their approach to effluent bioassays. Firstly, they started with a chemical hazard and source based approach, followed by the acute lethal bioassays to assess complex environmental mixtures and determine pollution bioavailability (Power and Boumphrey, 2004).

This process was updated with the consideration of sub-chronic and chronic effluent bioassays in order to improve the ability of tracking the changes of effluent quality over time and predicting their potential long-term impacts on the receiving environment (USEPA, 2002a, 2002b; OSPAR, 2005, 2007). Moreover, also *in situ* testing (*e.g.* fish and bivalves) is getting more attention to verify that the proposed regulatory methods are truly protective to the receiving environments, requiring for example the assessment of the receiving water and its bottom sediments (Allen *et al.*, 1999; Matthiessen, 2000).

However, a first barrier that might limit the use of bioassays for wastewater regulatory purposes, like in the *Whole Effluent Toxicity* (WET) current way, is related to the historical infrastructure differences between the USA sewerage system and the European one. In fact, whilst in USA the industrial wastewater is not generally sent to sewage treatment work (STW) alongside with domestic wastewater, in Europe this is a common practice. Because of this, European STW influents are much more variable and companies are potentially much more vulnerable to regulatory non-compliance (Burgess *et al.*, 2000, 2000a). Another problem is related to the different systems of law between countries using effluent bioassays. They could be characterised by statutory requirements with specific detailed documentation such as methods and risk assessment procedures or statutory technical guidance leaving a more wider choice, interpretation and discretion about technical protocols (Power and Boumphrey, 2004). Other troubles about effluent bioassays are in relation to the range of their potential application. Indeed, most of them are suitable for freshwater media, so that marine and estuarine environments are under represented. About that, some difficulties concerning effluent salinity adjustment prior to testing for marine and estuarine discharges should be overcome (USEPA, 1995).

A wide range of terms is used to describe the wastewater bioassays, but their meaning and way of performance are not always the same. The term *Direct Toxicity Assessment* (DTA) is indistinctly referred to the effluent and the receiving environment, WET is used just regard to effluent testing, but sometimes it might include bioaccumulation, biodegradation and persistence assessment (Power and Boumphrey, 2004). The *Whole Effluent Assessment* (WEA), introduced in Europe by the Oslo and Paris Commission (OSPAR, 2000, 2005), is based on the PBT criteria, including Persistence, Bioaccumulation and Toxicity evaluations. However, several other terms are in use and vary depending on the country. For example, *Integrating Controlling of Effluents* (ICE), *Whole Effluent Environmental Risk* (WEER) and *Environmental Effects Monitoring* (EEM) are the German, Dutch and Canadian way to name the effluent bioassay approach, respectively (OSPAR 2000, 2005; Power and Boumphrey, 2004).

Several papers reviewed the use of effluent bioassays both from the USA (Bergman *et al.*, 1986; Grothe *et al.*, 1996, Environment Canada, 1999) and EU (Tinsley *et al.*, 2004, Wharfe *et al.*, 2004, OSPAR, 2000, 2005, 2007) perspective, focusing particularly on WET (USA) (Chapman, 2000; De Maag, 2000; Sarakinos *et al.*, 2000) that is well studied and fully applied. Under this point of view, it is apparent that there are some differences in the nature of tests, such as the endpoints, and what they really represent. The EU asked for a broader protection of its water resources, so the common USEPA approach to effluent bioassays was implemented with a series of other detections such as bioaccumulation, carcinogenicity, eutrophication, genotoxicity, hormone disruption and indirect biotic effects such as competition (OSPAR, 2000, 2005, 2007).

At the moment, the performance of toxicity assessment is also required to check and validate if a wastewater treatment technology can be classified as BAT for wastewater to be discharged into a surface water body (IPPC, 2003). Indeed, it is seen as a complementary tool with the aim of obtaining more information on the effectiveness of the control measures and/or the hazard assessment for the receiving environment.

2.1 Toxicity as a regulatory parameter

The Power and Boumphrey (2004) review paper indicated the international trends in bioassay use for wastewater management and Whitehouse *et al.* (2004) the regulatory framework for controlling discharges using toxicity testing. Effluent regulations took various forms depending on the focus and the main objectives to achieve, but, in recent times, the legislative trend tended to value the environment more as a resource to be protected rather than exploited.

Whitehouse (2001) evidenced that *the point of contact* standard and the *point of entry* standard are the two main types of situations where bioassays are applied. The *point of contact* standard is designed to protect the receiving environment, based on a site-specific risk assessment, whereas the *point of entry* standard can be seen as the emission limit or load value on a general hazard-based risk assessment (Stortelder and De Guchte, 1995). Occasionally, some regulatory regimes (*e.g.* Canada, EU and USA) require both approaches (Power and Boumphrey, 2004). The *point of entry* standard, also known as the *near-zero approach* (OSPAR, 2000), might also promote the implementation of the best available technologies for wastewater treatment because of its more conservative tendency than the other standard, as requested by the IPPC Directive (96/61/EC) and IPPC (2003, 2008). Anyway, in hazard assessment it must be taken into consideration that effluent bioassays showed that they are not always predictive of the receiving water impacts (Burton *et al.*, 2000) as a consequence of WET testing organisms and field populations experiencing different exposures (USEPA, 1991).

In EU, the Water Framework Directive (WFD) (2000/60/EC) indicated that a *good ecological status* of water resources must be achieved by 2015, offering the opportunities for implementing bioassay-based approaches. The main target of the WFD for the achievement of a sustainable water policy are the prevention of water deterioration protecting and enhancing the status of aquatic ecosystems, the promotion of sustainable water use, the reduction or cessation of discharges, emissions and losses of hazardous substances, the reduction of groundwater pollution preventing its further contamination, and the mitigation of floods and droughts effects.

The WFD proposed a combination of the environmental quality objective (EQO) and emission limit values (ELV) approaches. The ELV approach requires that industry should be responsible for emission reduction as far as possible (Whitehouse *et al.*, 2004), referring to the *polluter pays* and *precautionary* principles (Harremoës, 2000). This way of acting allows for action before demonstrable harm to the environment and put the responsibility on dischargers to reduce environmental risks. But, sometimes, ELV are inadequate, and EQO approach could be appropriate to go beyond BAT scenarios in the perspective of strengthen the efforts to protect the environment. Besides the WFD, the EU defined a wider range of legislative requirements for the European integrated water quality management such as the Bathing Water Quality Directive (2006/7/EC), the Drinking Water Directive (98/83/EC), the IPPC Directive (96/61/EEC) and, more recently, the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Directive (2006/121/EC).

In Belgium, the effluent management framework is based on the implementation of BATs to protect the receiving water on a site-specific condition. Algae, invertebrates and bacteria are used in effluent bioassays (acute and chronic), but they are not routinely applied (OSPAR, 2000).

In Denmark, effluent bioassays are sector and BAT based in order to protect the receiving environment on a risk-based site-specific protocol. The survey covers 23 industries plus a number of specific research studies. Algae, invertebrates, fish, bacteria, plants and a WEA approach (biodegradation, aerobic stabilisation, HPLC screening for bioaccumulation) are considered for effluent monitoring (OSPAR, 2000, 2005; Power and Boumphrey, 2004).

In Finland, effluent bioassays are on the end-of-pipe basis and are occasionally used in compliance monitoring, in particular for pulp and paper mill industries. Fish, water fleas, algae and luminescent bacteria are commonly used for toxicity testing on effluents (OSPAR, 2000, 2005; Power and Boumphrey, 2004).

In France, the effluent management framework is based on BAT to protect the receiving water, with routine monitoring and occasional use in permit licences. Acute *Daphnia* spp. bioassay is used for regular monitoring on industrial effluent. As a consequence of a non-compliance effluent bioassay taxation is increased. Bacterial bioassays, mutagenicity and chronic toxicity approaches are under development (OSPAR, 2000; Power and Boumphrey, 2004).

In Germany, the discharge toxicity levels depend on the type of wastewater and BAT and are the result of a combination of a set of chemicals and bioassays (fish, water flea, algae and luminescent bacteria). Germany routinely uses acute and genotoxicity standards for effluents of several different industrial sectors and there is also a WEA under development about fish egg tests (IPPC, 2003). The overriding principle is to reduce the emission to the receiving environment, with load conditions based on the implementation of BAT, so that the risk assessment of receiving water is not routinely applied. Effluent bioassays are performed according to standardised protocols on fish, invertebrates, bacteria, algae and plants (acute, chronic, genotoxicity, mutagenicity) and new endpoints are going to be considered such as endocrine disruptors, immunotoxicity and bioaccumulation. Like in France, effluent bioassays contribute to determine taxation (OSPAR, 2000; Power and Boumphrey, 2004).

In Iceland and Luxembourg, regulations or experiences about effluent bioassays were not reported (OSPAR, 2000; Power and Boumphrey, 2004).

In Ireland, there is a mandatory emission limit value in terms of TU (from 5 to 10) for effluents discharged to waters from industries that applied to Integrated Pollution Control for various sectors. In addition, industries allowed to discharge to a municipal WWTP may be required to carry out a combination of bioassays and respirometric tests (IPPC, 2003, 2008).

In Italy, the requirements for wastewater discharges are mainly on an end-of-pipe basis, although the WFD called for increasing the level of protection of the receiving water environment with specific Environmental Quality Objectives (EQOs). Since 1973, more than 30 regulations about wastewater management and water quality have been issued, but the role of toxicity tests for the management and safeguarding of water resources was systematically underestimated. Since toxicity tests (*Salmo gairdneri* (*Osteichthyes: Salmonidae*) and *Carassius auratus* (*Osteichthyes: Cyprinidae*) mortality toxicity tests) were firstly introduced in 1976 by the Regulatory Decree (RD) 319/1976, the general approach has not substantially changed. Although some relatively new toxicity tests and endpoints were introduced within the RD 152/1999 and RD 258/2000, their role obtained scarce and inadequate attention. In fact, nevertheless they are compulsory, a positive toxicity result does not determine the application of administrative actions (*e.g.* fine), but just the need to deepen the analytical assessments, in order to identify and remove the source of toxicity. In the last RD 152/2006, besides the acute toxicity test with *D. magna* for freshwater wastewaters, some other toxicity tests can be performed like as with *Ceriodaphnia dubia* (*Crustacea: Daphniidae*), *Selenastrum capricornutum* (recently renamed *Pseudokirchneriella subcapitata*, unicellular algae, *Chlorophyta: Scenedesmaceae*) and bioluminescent bacteria still for freshwater samples or *Artemia salina* for saline wastewater samples.

In Netherlands, a dual approach is supported for the reduction of pollutants at source and the use of environmental quality criteria for the protection of specific receiving water, but effluent bioassays are not compulsory. Fish, invertebrates, algae, plants and bacteria are considered with acute, chronic endpoint and mutagenicity, genotoxicity, enzymatic and estrogenic effects (OSPAR, 2000, 2005; Power and Boumphrey, 2004).

In Northern Ireland, there are regulatory requirements about effluent bioassays in terms of emission limit value as Toxic Units (TU) for Integrated Pollution Control industries with specific limits about Best Available Technologies Not Entailing Excessive Costs (BATNEECs) implemented in various sectors. Bacteria, fish, algae and plants are considered for acute and chronic toxicity tests for source control on an end-of-pipe basis (OSPAR, 2000; Power and Boumphrey, 2004).

In Norway, the effluent management framework presents emission limits and site-specific conditions to protect the receiving water environment (OSPAR, 2000; Power and Boumphrey, 2004).

In Portugal, there are no regulatory requirements concerning effluent bioassays, but toxicity tests are commonly used for water quality monitoring; the pulp and paper mill sector voluntarily performs them on an end-of-pipe basis. Invertebrates, algae and bacteria with acute, chronic and genotoxic endpoints are taken into consideration (OSPAR, 2000; Power and Boumphrey, 2004).

In Spain, there is no regulation at national level requiring the use of effluent bioassays. The effluent management is mainly based on chemical source emission limits for wastewater discharged to surface waters and sewers. On a local basis (Community of Andalucía, Madrid and Murcia) some effluent bioassays are performed, but they are not mandatory. In some regions (Galicia, Valencia and Catalonia), they are used for taxation according to statutory requirements. Invertebrates and bacteria are mainly used, less frequently algae (Power and Boumphrey, 2004).

In Slovenia, effluent bioassays are requested once chemical specific measures failed (acute test with water flea). Generally, wastewater discharged to the sewer is tested for biodegradability (OSPAR, 2000, 2005).

In Switzerland, there are no regulatory requirements about effluent bioassays, but a risk-based approach on the specific receiving water is considered. There is some research experience about effluent toxicity, especially about genotoxicity (OSPAR, 2000, 2005, 2007).

In Sweden, the bioassays are predominantly used to predict the potential effects of effluents to the receiving waters. The *Toxicity Emission Factor* (TEF) is the tool chosen to accept/refuse the industrial wastewater at WWTPs with restriction determined on a municipality basis (Swedish EPA, 1997). Bioassays are used in effluent licensing, but only for larger industries with specific regulatory requirements. In particular, WEA is used to assess if the effluent treatment is adequate for a particular operation; this is usually done as a check on new production units that have been on-line for some time as part of the permitting process (IPPC, 2003). Acute, chronic, mutagenicity, physiological and morphological bioassays and WEA approaches (*e.g.* biodegradation and bioaccumulation) are performed on fish, invertebrates, algae/plants and bacteria (OSPAR, 2000).

In United Kingdom, the existing effluent management framework is based on a risk approach to prevent potential receiving water effects. There are no national regulatory requirements, but there are local and sector regulations, for examples for industries using BAT under IPPC (2003), even if bioassays were used in national programmes for receiving water monitoring of coastal waters. The main target is that of poor biological quality areas. A project to reduce by 80% the point-source discharges from industrial effluent plant causing acute toxicity was undertaken (Power and Boumphrey, 2004). Acute and chronic bioassays with fish, invertebrates, algae/plants and bacteria are performed according to specific laboratory regulatory quality controls (OSPAR, 2000).

Outside Europe, in Australia, there are effluent regulations on a state and territory responsibilities under national general guidance. Water quality guidelines are not mandatory and requirements are defined on a case-by-case basis. The risk-based approach is related to an end-of-pipe basis, but receiving environment monitoring is sometimes required. Algae, invertebrates and fish are used in toxicity testing with both acute and chronic endpoints, but bioaccumulation tests and biomarkers could be taken into consideration too (OSPAR, 2000; Power and Boumphrey, 2004).

In Canada, the effluent management framework is essentially based on the end-of-pipe approach, but sometimes, on a local basis, receiving environment bioassays might be required. Effluent bioassays are regulated under various acts according to the specific industrial sector. Nationally, the Fishery Act provided the main guidelines about (OSPAR, 2000). Toxicity Identification Evaluation (TIE) and Toxicity Reduction Evaluation (TRE) are not legally mandated, but they are supported as a voluntary measure. Algae, macrophyte, invertebrate and fish with acute and sub-lethal endpoints are considered (Environment Canada, 1999).

In Hong Kong SAR (China), effluent bioassays are not compulsory, but they could be required on a site-specific basis.

In New Zealand, effluent regulations are under national legislation, with regional responsibilities, but there are no compulsory requirements, except on a site-specific basis for biomonitoring programs. Standard as well as native species are used (algae, fish and invertebrates) with acute, sub-lethal and chronic endpoints.

In USA, the use of bioassays for effluent management is a routine practice. The effluent management framework according to the regulatory requirement under the Clean Water Act is primarily focused on the control of effluent source, but in some situations and states the receiving environment might also be considered. WET and TRE/TIE are the main expression of the established management organization under the National Pollutant Discharge Elimination System (NPDES) (USEPA, 1989, 1991, 1992, 1993, 1993a, 1994, 1995, 2000, 2000a, 2004). Several bioassays concerning different fresh and seawater organisms and endpoints are proposed in order to cover most part of situations and requirements (USEPA, 2002, 2002a, 2002b).

2.2 Whole Effluent Toxicity (WET)

Wastewaters have been assessed and regulated to a greater extent on the basis of some background physical and chemical parameters (*i.e.* Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), suspended solids, pH and concentrations of specific hazardous substances) that guaranteed the relatively quick solution of short- and medium-term human health problems. However, it is very difficult to understand the potential environmental significance of complex wastewaters with just that sound basis knowledge.

To go forward the single-substance oriented approach and check the potential effects of whole wastewater samples to ecosystems, WET testing was started up and, now, it is fully applied in the US National Pollutant Discharge Elimination System (NPDES) (USEPA, 2004). WET testing is an important component of USEPA integrated approach for assessing the potential for water toxicity, even though not all point sources are regulated and monitored (USEPA, 1989, 1991, 1992, 1993, 1993a, 1994, 1995, 2000, 2000a, 2004; SETAC, 2004a, 2004b).

The primary objective of WET testing is to ensure that the treated effluent released from industrial and municipal facilities into surface water does not produce adverse effects to aquatic life, compromising its general quality and health status. The WET approach aims to identify, characterise and eliminate toxic effects on aquatic resources (Ausley, 2000).

To determine if an effluent has the potential to be toxic, WET testing is performed on various aquatic organisms considering acute, sub-chronic and chronic endpoints depending on the final goal to be achieved. WET can be performed on a variety of commonly used test species according to various exposure times and dilution regimes. Species-specific threshold limit values for quality assessment are defined mainly based on the Lowest Observed Effect Concentration (LOEC) or No Observed Effect Concentration (NOEC) data, but real final data integration is still missing (*e.g.* toxicity score and indices). Specifically, in the case of wastewater rather that “concentration” it would be better to refer to “percentage of dilution”.

Anyway, it must be kept in mind that the true toxicity of an effluent cannot be really measured, because the true toxicity of an effluent is a relative and not an absolute term. It is relative because it depends on the sensitivities of test species and life stages to the wastewater chemicals, the test method used, type and concentration of compounds, test conditions and the reliability of the benchmark used to gauge toxicity (Warren-Hicks *et al.*, 2000).

Chapman (2000) reviewed pros and cons of the general application of WET testing to several wastewaters with various toxicity tests. The review highlighted that WET testing is clearly a useful tool, but it is not a perfect one, having some imperfections:

- Variability - a range of values is normally provided;
- Species differences due to sensitivities, ecological relevance, sequestration of contaminants (*i.e.* bioaccumulation), depuration, acclimation, adaptation and general lifestyle;
- Laboratory-based experience avoiding all differences in diet, temperature and water quality of *in situ* exposures;
- Over- or under-protective;
- Uncertain level of protection (*i.e.* biotic and abiotic factors, intermittent doses, environmental mixtures with various effects, biostimulation and hormesis);
- Hazard assessment rather than receiving water risk assessment;
- Holistic, but by themselves not diagnostic;

- Potentially simple and cost-effective, but not necessarily environmentally realistic;
- Effect-based, but not suitable for every wastewater specimen.

Toxicity test protocols and method changes could represent further variables that have the potential to affect the performance and interpretation of outcomes. At a minimum, each of the variables may be viewed as choices or steps to optimise the methodological performances, but, unfortunately, most of variables for the greater extent of toxicity bioassays have not yet been evaluated. Therefore, the selection of toxicity testing protocols is not so easy without codified conditions, being, generally, only intuitive or theoretical (Markle *et al.*, 2000).

The conclusion is that WET testing cannot be used alone without consideration of site-specific conditions, even though a properly designed toxicity test can be not only reactive, but also predictive (Chapman, 2000). Moreover, if the direct applicability of WET to the receiving system hazards is to be judged, carefully validated tests that account for all types of impacts, including eutrophic effects, sediment toxicity, phytotoxicity, bioaccumulation, and genotoxic responses, should be designed (De Maag, 2000; Hernando *et al.*, 2005).

Anyhow, the benefit in terms of environmental protection is limited if there is no clear understanding of how the ecotoxicity of effluents of concern can be reduced (Hutchings *et al.*, 2004). A Toxicity Reduction Evaluation (TRE) incorporating a Toxicity Identification Evaluation (TIE) could be undertaken to assess the nature of toxic components and their source (USEPA, 1991, 1993, 1993a). TRE protocols were originally developed and designed on site-specific basis for a stepwise search for effective effluent toxicity control measures. The first TRE stage is to identify the test to be used for toxicity assessment to undertake toxicity tracking and TIE. Once a discharge has been selected for a TRE the causative agents need to be found and the sources of ecotoxicity to be isolated. Chemical analysis of known toxicants may identify an obvious cause of toxicity, while TIE allows the characterisation of toxic effluent components.

2.3 Whole Effluent Assessment

The Whole Effluent Assessment is a methodological approach to assess the possible hazardous character of effluents, which would be insufficiently controlled by chemical indications such as the sum of parameters or the limits set on individual substances (OSPAR, 2000, 2005, 2007; Libralato et al., 2010a, 2010b). WEA as the main tool of OSPAR strategy (2005, 2007) provides a range of information on effluents using Persistent, Toxic and liable to Bioaccumulate-criteria (PBT-criteria) considering at the same time chemical, physical and biological methods to check for potential adverse effects. *OSPAR's objective with regard to hazardous substances is to prevent pollution of the maritime area by continuously reducing discharges, emissions and losses of hazardous substances, with the ultimate aim of achieving concentrations in the marine environment near background values of naturally occurring substances and close to zero for man-made synthetic substances. In achieving this objective OSPAR selects and prioritises substances on the basis of criteria for Persistence, Liability to Bioaccumulate and Toxicity (P, B and T); criteria that reflect the intrinsic hazardous properties of substances* (OSPAR, 2000, 2005, 2007).

At what time the whole effluent sample is tested, WEA increases the understanding of the combined effects of all known and unknown substances within the discharge, especially in complex mixtures. WEA has an added value respect to the single substance oriented approach when it comes to the task of identifying effluents of concerns to the marine environment, to recognize priorities for action, trigger further site specific investigations and chart progress in reducing whole effluent persistence, liability to bioaccumulate and toxicity (OSPAR, 2005). The potential applications of WEA include the regulation and guidance of effluent toxicity, the ranking of the environmental risk of discharges to aquatic ecosystems, the appliance of TRE/TIE procedures, the prioritisation of waste treatment measures, the assessment of treatment technology efficiency and improvements, the observation of backtracking effects and the definition of site-specific hazard/risk assessment.

The WEA approach takes into consideration wastewater compounds regardless to their origin that, generally, do no necessitate to be identified, assessing at the same time by-products and metabolites effects directly on aquatic organisms. In this way, combined effects can be also considered, delivering proactive information to operators and regulators. Moreover, in many cases backtracking procedures inside complex industrial plants are possible in order to check the presence of hot spots as sources of hazardous effluents (IPPC, 2003, 2008). This means that WEA has also the potential of contributing to the WFD targets in order to achieve a *good chemical status* and a *good ecological status* for surface water. However, further agreement and standardisation at international level are required to improve WEA testing and its application as a tool within an Environmental Management Systems (EMS).

For example, WEA is suitable for effluents with a complex composition (*i.e.* industrial wastewaters), like for side-products that end-up in the discharge. In this case, WEA may help to identify priorities, contributing to prevent the discharge of effluents of concern. Indeed, *the added value of WEA was evaluated by comparing WEA results with permit requirements and with an extended chemical specific approach. It was concluded that in many effluents, testing toxicity has an added value, since toxicity was found that could not be explained with the (extended) chemical specific approach. The added value is most apparent in effluents that have a complex constitution* (OSPAR, 2005). Generally, WEA approach is considered as more suitable for complex wastewater, where unknown substances could be present. Also the IPPC (2003) stated the importance of WEA application for BAT identification, suggesting that the learning-by-doing WEA approach, in the next future, could be performed in advance to chemical analyses, which would be reduced in number and, potentially, subjected directly to WEA results.

2.4 Bioassays: bioindicator species, sensitivity and variability

The first assumption to keep in mind is that there is no perfect effluent bioassay, no holy grail, no ideal endpoint or related statistics, but the best practise should be defined on a case-by-case basis by each jurisdiction according to a more general scientific and regulatory agreement (Libralato et al., 2010a, 20120b).

The main recommendation in the ecotoxicological literature is, generally, about the choice of *good* bioindicator species, taking into consideration that all of them are characterised by some advantages and disadvantages. USEPA (1991) suggested using a minimum of three test species to span a range of species sensitivities, instead of selecting the most sensitive one. Some authors indicated that the use of a *test battery approach* should be preferred rather than a *single species approach* (Environment Canada, 1999; Tonkes *et al.*, 1999; Power and Boumphrey, 2004). Generally, it is recommended to consider a vertebrate, an invertebrate and a photosynthetic organism, but due to practical reasons, lack of information and ecological relevance it is not always possible to satisfy this requirement (USEPA, 1991).

The main question to answer about effluent bioassays is: how ecologically relevant should be an effluent bioassay? Power and Boumphrey (2004) suggested that some whole organism-based bioassays were selected for regulatory purposes because at the decision time they were the most well known and developed, offering the perception to be ecologically relevant. In particular, the simplest forms of life are preferred for ecotoxicity testing in order to minimise the number of unknowns and avoid the restrictions adopted in some European jurisdictions about *in vivo* testing, for example invertebrates should be used in preference to vertebrates (Tinsley *et al.*, 2004).

Chapman (2000) evidenced that whole organism-based effluent bioassays might be over- or under-protective, although the considered species are ecologically relevant. So that new methods such as biomarkers, genotoxic microbial tests and micro-scale tests (Environment Canada, 1999) started to be considered for regulatory requirements and standardisation. As a consequence effluent bioassays can be performed both as *in vivo* or *in vitro* testing.

Effluent bioassays do not provide a single value, but a range of values because any biological system has a degree of innate variability. This variability could be intra-test, intra-laboratory and/or inter-laboratory such a consequence of the analyst experience and test organisms general conditions and health (Chapman, 2000). Generally, it is recognised that a variability of a factor or two is not unreasonable (WSBSAB, 1994), so that the statistical weight of variability in decision-making processes, inter-laboratory ring-tests and schemes for ensuring the quality of regulatory data are of main importance (Power and Boumphrey, 2004). However, it should be accepted that variability could not be reduced until a certain point so that an estimate of uncertainty should be included in regulatory compliances, according to the considered effluent bioassays (Gully *et al.*, 2000).

Another point of debate about bioassay test variability is the endpoint to be measured. There are two main approaches: the derivation of the No Observed Effect Concentration and the calculation through regression models corrected with a specific reduction for the control response of the Effect Concentration (EC) at a specific level of effect (*e.g.* EC50, concentration producing an effect in 50% of the treated population) (USEPA, 2000, 2002, 2002a, 2004). Although the NOEC has been extensively used in WET monitoring programmes according to USEPA WET testing manuals, some studies criticised its application evidencing that the NOEC is not a good indicator of the No Effect Concentration (NEC), is highly variable between tests leading to contradictory results and it was shown that relatively subtle differences in the way an analysis is carried out could produce quite different results. On the other hand, EC50s or other similar endpoints (*e.g.* LC50, concentration producing a lethal effect in 50% of the treated population, or EC20) estimates are more reliable, less variable and comparable between tests (Chapman *et al.*, 1996).

The OSPAR (2000, 2005, 2007) provided a general European overview of wastewater ecotoxicity testing considering various organisms, endpoints and sensitivities both for fresh and saltwater environments, but the information about organisms to be used for marine and estuarine WET testing was incomplete. In Table 1,

Table 2, Table 3 and Table 4, the most common species used for marine and estuarine wastewater toxicity testing have been summarised, providing test temperature, salinity and life stage of interest. The taxonomic classification was in accordance with Myers *et al.* (2006). Organisms have been ranked as algae, bacteria, invertebrates and vertebrates to respect the general recommendation about the definition of a test battery, that is to consider at least three organisms taking into account a plant (or algae), an invertebrate and a vertebrate (USEPA, 1991; Farré and Barceló, 2003).

In the further sections, the role of bacteria, crustaceans and bivalve molluscs in toxicity testing have been analysed paying specific attention to estuarine and marine environments.

Table 1 Most common species for marine and estuarine wastewater ecotoxicity testing as bacteria.

Test organism		Taxonomy	T (°C)	Salinity (‰)	Life stage	References
MARINE AND ESTUARINE BACTERIA						
Vibrio fischeri	bacterium	*Proteobacteria, Gammaproteobacteria, Vibrionales, Vibrionaceae*	15	20		Azur Env., 1998

Table 2 Most common species for marine and estuarine wastewater ecotoxicity testing as algae.

Test organism		Taxonomy	T (°C)	Salinity (‰)	Life stage	References
MARINE AND ESTUARINE ALGAE						
Phaeodactylum tricornutum	diatom	*Ochrophyta, Bacillariophyceae, Naviculales, Phaeodactylaceae*	20	25-35	adult	ISO, 1991
Skeletonema costatum	diatom	*Ochrophyta, Coscinodiscophyceae, Thalassiosirales, Skeletonemataceae*	20	25-35	adult	ISO, 1991
Dunaliella tertiolecta	green alga	*Chlorophyta, Chlorophyceae, Volvocales, Dunaliellaceae*	20-22	26-35	adult	Hall and Golding, 1998
Champia parvula	red alga	*Rhodophyta, Rhodophyceae, Rhodymeniales, Champiaceae*	20	26-34	adult	USEPA, 1994a
Macrocystis pyrifera	giant kelp	*Heterokontophyta, Phaeophyceae, Laminariales, Lessoniaceae*	15	34	spore	USEPA, 1995

Table 3 Most common species for marine and estuarine wastewater ecotoxicity testing as invertebrates.

Test organism		Taxonomy	T (°C)	Salinity (‰)	Life stage	References
MARINE AND ESTUARINE SPECIES: INVERTEBRATES						
Haliotis refuscens	red abalone	*Mollusca, Gastropoda, Vetigastropoda, Haliotidae*		31-35	Embryos	USEPA, 1995
Mytilus spp.	bivalve mussel	*Mollusca, Bivalvia, Mytiloid, Mytilidae*	18	32-34	Embryos	USEPA, 1995
Crassostrea gigas	pacific oyster	*Mollusca, Bivalvia, Ostreoida, Ostreidae*	20-25	25-32	Embryos	USEPA, 2002
Crassostrea virginica	American oyster	*Mollusca, Bivalvia, Ostreoida, Ostreidae*	20-25	20-32	Embryos	USEPA, 2002
Artemia salina	brine shrimp	*Arthropoda, Branchiopoda, Anostraca, Artemiidae*			Survival test	USEPA, 2002
Callinectes sapidus	blue crab	*Arthropoda, Malacostraca, Decapoda, Portunidae*	20-25	25-30	Juvenile	USEPA, 2002
Cancer magister	dungeness crab	*Arthropoda, Malacostraca, Decapoda, Cancridae*	12	32-34	Juvenile	USEPA, 2002
Crangon septemspinosa	sand shrimp	*Arthropoda, Malacostraca, Decapoda, Crangonidae*	20-25	25-32	Post-larval	USEPA, 2002
Palaemonetes aztecus	brown shrimp	*Arthropoda, Malacostraca, Decapoda, Palaemonidae*	20-25	20-32	Post-larval	USEPA, 2002
Palaemonetes duorarum	pink shrimp	*Arthropoda, Malacostraca, Decapoda, Palaemonidae*	20-25	20-32	Post-larval	USEPA, 2002
Palaemonetes intermedius	brackish grass shrimp	*Arthropoda, Malacostraca, Decapoda, Palaemonidae*	20-25	20-32	1-10 days	USEPA, 2002
Palaemonetes pugio	grass shrimp	*Arthropoda, Malacostraca, Decapoda, Palaemonidae*	20-25	20-32	1-10 days	USEPA, 2002
Palaemonetes vulgari	grass shrimp	*Arthropoda, Malacostraca, Decapoda, Palaemonidae*	20-25	20-32	1-10 days	USEPA, 2002
Pandalus jordani	oceanic shrimp	*Arthropoda, Malacostraca, Decapoda, Pandalidae*	12	25-32	Juvenile	USEPA, 2002
Penaeus setiferus	white shrimp	*Arthropoda, Malacostraca, Decapoda, Penaeidae*	20-25	20-32	Post-larval	USEPA, 2002
Holmesimysis costata	mysid shrimp	*Arthropoda, Malacostraca, Mysida, Mysidae*	12	32-34	1-5 days	USEPA, 2002
Metamysidopsis elongata	mysid shrimp	*Arthropoda, Malacostraca, Mysida, Mysidae*	20-25	20-32	1-5 days	USEPA, 2002
Mysidopsis almyra	mysid shrimp	*Arthropoda, Malacostraca, Mysida, Mysidae*	20-25	20-32	1-5 days	USEPA, 2002
Mysidopsis bahia	mysid shrimp	*Arthropoda, Malacostraca, Mysida, Mysidae*	20-25		1-5 days	USEPA, 2002
Mysidopsis bigelowi	mysid shrimp	*Arthropoda, Malacostraca, Mysida, Mysidae*	20-25		1-5 days	USEPA, 2002
Neomysis americana	mysid shrimp	*Arthropoda, Malacostraca, Mysida, Mysidae*	20-25	20-32	1-5 days	USEPA, 2002
Arbacia punctulata	sea urchin	*Echinodermata, Echinoidea, Arbacioida, Arbaciidae*	20-25	32-34	Gametes - embryos	USEPA, 2002
Strongylocentrotus drobachiensis	green sea urchin	*Echinodermata, Echinoidea, Echinoida, Strongylocentrotidae*	12	32-34	Gametes - embryos	USEPA, 2002
Strongylocentrotus purpuratus	purple sea urchin	*Echinodermata, Echinoidea, Echinoida, Strongylocentrotidae*	12	32-34	Gametes - embryos	USEPA, 2002
Dendraster excentricus	sand dollar	*Echinodermata, Echinoidea, Clypeasteroida, Dendrasteridae*	12	32-34	Gametes - embryos	USEPA, 2002

Table 4 Most common species for marine and estuarine wastewater ecotoxicity testing as vertebrates.

Test organism		Taxonomy	T (°C)	Salinity (‰)	Life stage	References
MARINE AND ESTUARINE SPECIES: VERTEBRATES						
Atherinops affinis	topsmelt	*Chordata, Osteichthyes, Atheriniformes, Atherinidae*	21	25-30	7-15 days	USEPA, 2002
Menidia beryllina	fish, inland silverside	*Chordata, Osteichthyes, Atheriniformes, Atherinidae*	20-25	in situ	1-14 days	USEPA, 2002
Menidia menidia	fish, Atlantic silverside	*Chordata, Osteichthyes, Atheriniformes, Atherinidae*	20-25	in situ	1-14 days	USEPA, 2002
Menidia peninsulae	tidewater silverside	*Chordata, Osteichthyes, Atheriniformes, Atherinidae*	20-25	in situ	1-14 days	USEPA, 2002
Fundulus heteroclitus	mummichog	*Chordata, Osteichthyes, Cyprinodontiformes, Fundulidae*	20-25	25-32	1-30 days	USEPA, 2002
Fundulus similis	killfish	*Chordata, Osteichthyes, Cyprinodontiformes, Fundulidae*	20-25	20-32	1-30 days	USEPA, 2002
Poecilia reticulata	guppy	*Chordata, Osteichthyes, Cyprinodontiformes, Poeciliidae*	20-30	max 35	96 h	Sbrilli et al., 1995
Gasterosteus aculeatus	threespine stickleback	*Chordata, Osteichthyes, Gasterosteiformes, Gasterosteidae*	20-25	20-32	1-30 days	USEPA, 2002
Orthopristis chrysoptera	pigfish	*Chordata, Osteichthyes, Perciformes, Haemulidae*	20-25	15-30	1-90 days	USEPA, 2002
Lagodon rhomboides	pinfish	*Chordata, Osteichthyes, Perciformes, Sparidae*	20-25	20-32	1-90 days	USEPA, 2002
Leiostomus xanthurus	spot	*Chordata, Osteichthyes, Perciformes, Sciaenidae*	20-25	25-32	1-90 days	USEPA, 2002
Citharichthys stigmaeus	sanddab	*Chordata, Osteichthyes, Pleuronectiformes, Paralichthyidae*	12	32-34	1-90 days	USEPA, 2002
Paralichthys dentatus	flounder	*Chordata, Osteichthyes, Pleuronectiformes, Paralichthyidae*	20-25	32-34	1-90 days	USEPA, 2002
Paralichthys lethostigma	flounder	*Chordata, Osteichthyes, Pleuronectiformes, Paralichthyidae*	20-25	32-34	1-90 days	USEPA, 2002
Parophrys vetulus	English sole	*Chordata, Osteichthyes, Pleuronectiformes, Pleuronectidae*	12	32-34	1-90 days	USEPA, 2002
Pseudopleuronectes americanus	winter flounder	*Chordata, Osteichthyes, Pleuronectiformes, Pleuronectidae*	12	32-34	Post metamorphosis	USEPA, 2002

2.4.1 Bacteria

Toxicity and inhibitory compounds are usually found in industrial wastewater and their negative impacts on biomass activity require a specific treatment cycle that is quite often a combination of chemical and biological stages, such as in big factories, but also in small and seasonal industrial activities (Gutiérrez *et al.*, 2002). In order to evaluate the impact of toxic and bio-refractory compounds on activated sludge, some screening procedures have been proposed along time based on the detection of variables indicative of the bacterial activity such as the growth rate (variability of cells, substrate uptake, etc) like as the Ames assay (Stahl *et al.*, 1991; Houk, 1992; Hubbard *et al.*, 1994) and umuC-assay (Oda *et al.*, 1995) with genetically modified *Salmonella typhimurium* (*Gammaproteobacteria: Enterobacteriaceae*), the enzymatic activity (dehydrogenase, adenosine triphosphate, etc), the bacterial luminescence, the metabolic heat production and the respiration rate (Ricco *et al.*, 2004). Anyhow, bacterial toxicity tests are applied not only to assess indirect discharges with the objective to protect the biological treatment plants, but also on a WET testing basis. Actually, bacterial toxicity is also measured as a routine parameter to assess impacts of specific direct discharges such as cooling water on the aquatic environment (OSPAR, 2000).

Two of the most widespread bacterial tests are the inhibition of respiration (Oxygen Uptake Rate, OUR) and nitrification (Nitrate Uptake Rate, NUR) tests. The OUR is used to determine the toxicity of substances and effluents by measuring the microorganisms respiration rate (OECD, 1987), generally for environmental hazard and risk assessment of industrial effluents; and the NUR test is used to check the toxicity effects of nitrification processes in aerobic microbial treatment plants (ISO, 2006). About the bacterial luminescence bioassay, the acute test with *V. fischeri* is one of the most commonly used bio-test methods, and is well recognised and established at international level (ISO, 1998, 1998a, 1998b) and a huge literature is available (Kaiser and Devillers, 1994; Nohava *et al.*,1995; Gutiérrez *et al.*, 2002; Ricco *et al.*, 2004). This method measures the inhibition of the light emission by the marine bacterium *V. fischeri* as endpoint. The test is performed with a specifically designed apparatus to store, carry out and photometrically measure the light emission of the bacteria suspension exposed to the wastewater sample. The test duration varies from 5 minutes to 30 minutes and the final toxicity is generally expressed as IC50/EC50. In addition to acute toxicity test with *V. fischeri*, an inhibition growth test has been developed to determine chronic toxicity effects. Bacteria are incubated for 7 h with the test item and the inhibition of growth is determined. In Germany a national standard guideline is available for the chronic bioassay (OSPAR, 2000).

2.4.2 Bivalve molluscs

Bivalve molluscs are considered good bioindicators because they have most of the characteristics that a good bioindicator should have. They are substantially widespread in all fresh, brackish and saltwater environments, having a key ecological role both as filter and deposit feeders according to various species and habitats (Dame, 1996). All stages of their life cycles (gametes, embryos, larvae and adults) have been used to define an endpoint for a specific monitoring purpose (acute, sub-chronic or chronic toxicity) (His *et al.*, 1997). Bivalve molluscs toxicity tests are easy to perform, highly sensitive, cost-effective and not time consuming ranging from 24 h to 48 h to obtain a first meaningful result. The main disadvantage is related to the reproductive period, which could be quite short for some species (*e.g. C. gigas*) sampled from the wild for the performance of embryotoxicity tests. However, conditioning techniques there exists and they may help to provide reproductive organisms during all the year (His *et al.*, 1999).

Bivalve toxicity tests are available both as short- and log-term bioassays, varying from exposures lasting hours, days or weeks. The short-term test is quick and sensitive and provides immediate results, while the long-term test (more than 2 days or weeks), although very sensitive, requires phytoplankton cultures for larvae feeding (less cost-effective and more time consuming). The most frequent short-term test is the embryotoxicity test that can provide results both on the basis of the ratio of normal/abnormal larvae and on larvae morphometric parameters. The long-term test main endpoint is, generally, the mortality of embryos during the veliger-pediveliger development stage or subsequent to that, but morphometric parameters can be considered too.

Nevertheless this kind of toxicity tests are widely used, more than 1,000 peer reviewed scientific publications (PAN, 2007), the standardisation of methods is not yet clear, especially at European level. Even if, recently, the England and Wales Environment Agency developed a *C. gigas* embryo larval guideline that has been already ring-tested (OSPAR, 2000).

The most widespread toxicity test with bivalves is the embryotoxicity bioassay. Initially developed by Woelke (1972), some primary modifications were introduced by Thain and Watts (1984). Nowadays, globally, there exist four main protocols to carry out a bivalve embryotoxicity test (USEPA, 1995; His *et al.*, 1999; RIKZ, 1999; ASTM, 2004). The main differences are mainly about the source of testing animals, possible acclimation periods, the use of gametes pool, the test temperature and duration, and the final endpoint, like as the type of dilution water to be considered.

USEPA (1995) and ASTM (2004) guidelines prescribe methods for the evaluation of sub-chronic toxicity of chemicals and mixtures to different bivalves: eastern oyster (*Crassostrea virginica*), quahogs (*Mercenaria mercenaria*, little-neck clam, *Bivalvia: Veneridae*), pacific oyster (*C. gigas*) or bay mussel (*Mytilus edulis*, but also *M. galloprovincialis*). The ASTM guidelines also recommend the use of the test with the appropriate modifications for aqueous effluents, leachates, oils, particulate matter, sediments and surface water. Other species that are, generally, considered for toxicity testing are *Ostrea edulis* and *Mulinia lateralis* (dwarf surf clam, *Bivalvia: Veneridae*) (His *et al.*, 1999).

At the moment, bivalves are included as test organisms for marine-coastal and transitional waters in all international bioassay lists (Chapman and Wang, 2001, Nendza, 2002; ICES, 2003) for a wide range of matrices from the sediment (Thain, 1992; Beiras and His, 1995; Volpi Ghirardini *et al.*, 2005) to the wastewater (OSPAR, 2000, 2005; ICES, 2003; SEPA, 2003), from metals to pesticides and detergents (His *et al.*, 1999).

2.4.3 Crustaceans

Several species of crustaceans were tested along time for WET purposes in relation to marine and estuarine environments, as indicated in Table **3**, measuring both acute and chronic effects. However, the main problem is that most of these crustaceans are not really ecologically representative of the European brackish and saltwater environments, and, at what time they are relatively available, their sensitivity and reliability could be scarce.

OSPAR (2000) highlighted that marine toxicity tests with crustaceans, nevertheless very important to assess the hazard of effluents to the marine environment, are not used as often as freshwater tests (*C. dubia*, *D. magna* and *D. pulex*). Some more efforts to strengthen their role in marine and estuarine toxicity testing and hazard assessment should be done.

One of the more widespread marine and estuarine crustacean toxicity test for wastewater testing is the acute toxicity bioassay based on *A. franciscana*, which is also available as toxkit (Persoone *et al.*, 2003).

2.4.3.1 The case of *Artemia spp*.

Artemia spp. has been greatly used in toxicological testing because of its adaptability to a wide range of salinities (5 – 300 g/L) and temperatures (6 – 40 °C) (USEPA, 2002), considering, mainly, mortality and growth as endpoints (Sarabia *et al.*, 1998). *Artemia spp*. have a short life cycle, high flexibility to adverse environmental conditions, high fecundity, bisexual/parthenogenetic reproduction strategy (nauplii or cysts), small body size and adaptability to varied nutrient resources as it is a non-selective filter feeder (Nunes *et al.*, 2006). Per se, these characteristics should make *Artemia spp*. as a really suitable organism for ecotoxicity testing, but several criticisms about its sensitivity have been presented according to a leaning-by-doing approach. The sensitivity towards a wide range of substances is really lower than that of other species, so that under-estimation may frequently occur. *Artemia spp*. showed to be less sensitive than *Streptocephalus rubricatus* (shrimp, *Crustacea: Streptocephalidae*) and *S. texanus* (Crisinel *et al.*, 1994), *Echinometra lucunter* (rock boring urchin, *Echinoidea: Echinometridae*), *Crassostrea rhizophorae* (eastern oyster, *Bivalvia: Ostreidae*) (Nascimento *et al.*, 2000) and *Crassostrea gigas* (Libralato *et al.*, 2007), *Selenastrum capricornutum* and *Dunaliella tertiolecta* (Gaggi *et al.*, 1994), *V. fischeri*, *D. magna* (water flea, *Branchiopoda: Daphniidae*) and *Brachionus plicatilis* (rotifer, *Monogononta: Brachionidae*) (Guerra, 2001), *Daphnia similis* (Silva *et al.*, 2004), *Paracentrotus lividus* (sea urchin, Echinoidea: Echinidae) (Fichet *et al.*, 1998), *Daphnia pulex* and *Thamnocephalus platyurus* (shrimp, *Branchiopoda: Thamnocephalidae*) (Nunes *et al.*, 2006), and *Spirostomum ambiguum* (ciliate protozoan, *Heterotrichea: Spirostomidae*) and *Tetrahymena thermophila* (ciliated protozoan, *Oligohymenophorea: Tetrahymenidae*) (Nalecz-Jawecki *et al.*, 2003). Other criticisms could be related to the use of cysts (Migliore *et al.*, 1997) and cyst-hatched nauplii (Persoone and Wells, 1987) of *Artemia* for testing purposes that could introduce some variability in test results. Indeed, in the genus *Artemia* there is no cyclic bisexual versus parthenogenetic mechanism like for rotifers or cladocerans, that is generally on a seasonal basis, but cysts production may reflect the occurrence of genetic variation to increase adaptability; it is evident that genetic variability is not a desirable condition for ecotoxicity assays (Nunes *et al.*, 2006).

3. CONCLUSION

The scientific community tends to drive the research on and the application of new bioindicators considering species melting good sensitivity, geographical and yearly availability, ecological relevance, cost-effectiveness and, generally, that are easy to manage. Their application in the wastewater quality control and management produced the so-called Whole Effluent Toxicity and Whole Effluent Assessment, in USA and EU respectively. The general approach of these tools is not so new, but the WEA one has refreshed the whole effluent toxicity on the basis of the PBT criteria and the integrated pollution and prevention control strategies supporting the technological advancement in the wastewater treatment sector. Indeed, they might be able to assess and validate the quality of treated wastewater providing effluent that might be not only suitable for the receiving ecosystem, but also able to be recovered and reused for specific industrial, commercial, agricultural and even potable purposes that can be seen as the extreme scenario of reuse. Thus, environmental sustainability in the water sector will be increased and some contributions to solve water scarcity and drought phenomena might be carried on as an unavoidable option in response also to the potential adverse consequences of a global climate change.

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