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Ecotoxicological characterization of aquatic systems within closed municipal solid waste landfill areas

Mariana Sofia Canelas de Oliveira

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Abstract

The issue of waste has become a big worldwide problem due to the high consumerism of our society which increases in the production of Municipal Solid Waste (MSW). This behaviour has serious consequences as our planet is being turned into a wide garbage dump causing the degradation of the environment and affecting the health of the population. In Portugal, a revolution in the waste sector occurred with the approval, in 1997, of the Strategic Plan for Municipal Solid Wastes I and the closure of all dumps. Several municipal systems of waste management were created, including the construction of sanitary infra-structures of MSW containment, the landfills. The present study is part of a broader research project intending to design a framework for long-term ecotoxicological/ecological monitoring of closed landfills. As such, the aim of the present study was to evaluate the risk of a closed landfill, sealed in 2006 and with an area of 19 ha, towards adjacent aquatic systems by conducting the ecotoxicological characterization of potentially leachate-contaminated matrices to which aquatic organisms might be exposed while intending to contribute for long-term monitoring programs. A battery of short-term sub-lethal laboratory toxicity tests, covering species representative of different key taxonomic (bacteria, algae, rotifers, cnidarians, crustaceans, and insects) and functional groups (primary producers, primary and secondary consumers, benthic and epibenthic decomposers) and environmental compartments (water, sediment and soil) was selected to test the various types of matrices within and outside the landfill area: two samples of leachate itself, collected at different seasons, groundwater, water from a puddle, eluates from soil samples, water and sediment from five locations in adjacent aquatic systems. Based on the results obtained, both leachates are toxic, although the leachate sample collected in Spring-Summer was less toxic than the one collected in Autumn-Winter. It was observed toxicity for some matrices of groundwater, however it is not suggested that is related with the leachate. Importantly, the results of the present study of the toxicity tests on the matrices not included in the landfill monitoring programme did not reveal toxicity, at least due

to leachate. The obtained results either demonstrated that further intervention should be taken, since there is no clear information about the source of the contamination of groundwaters and surface waters.

Keywords: Ecological risk, Test battery, Leachate, Groundwater, Soil eluates, River water and sediment

Resumo

A questão dos resíduos tornou-se num grande problema em todo o mundo devido ao alto consumo da nossa sociedade, que aumenta a produção de resíduos sólidos urbanos (RSU). Este comportamento tem consequências graves no nosso planeta, que está a transformado num amplo depósito de lixo, causando a degradação do meio ambiente e afectando a saúde da população. Em Portugal, a revolução no sector dos resíduos ocorreu, com a aprovação, em 1997, do Plano Estratégico de Resíduos Sólidos Municipal I e o encerramento de todas as lixeiras. Vários sistemas municipais de gestão de resíduos foram criados, incluindo a construção de infra-estruturas sanitárias para conter resíduos sólidos urbanos, os aterros sanitários. O presente estudo é parte de um projeto de pesquisa mais amplo com a intenção de criar um quadro para a monitorização ecotoxicológica / ecológica, a longo prazo, dos aterros encerrados. Como tal, o objetivo do presente estudo foi avaliar o risco de um aterro sanitário fechado, selado em 2006 e com uma área de 19 ha, para os sistemas aquáticos adjacentes, através da realização da caracterização ecotoxicológica de amostras de lixiviados, potencialmente contaminados para os organismos aquáticos, que pode contribuir para programas de monitorização a longo prazo. A bateria de testes de ecotoxicidade sub-letais de curto prazo, é representantiva de diferentes grupos taxonómicos (bactérias, algas, rotíferos, cnidários, crustáceos e insetos); grupos funcionais (produtores primários, consumidores primários e secundários, decompositores bênticos e epibênticos) assim como diferentes compartimentos ambientais (água, sedimento e solo) e foi selecionada para testar os vários tipos de amostras recolhidas dentro e fora da área do aterro: duas amostras de lixiviado, colhidas em diferentes estações do ano, as águas subterrâneas, a água de uma poça, eluatos de amostras de solo, água e sedimentos de cinco locais de sistemas aquáticos adjacentes. Com base nos resultados obtidos, ambos são lixiviados tóxicos, embora a amostra de lixiviado recolhido na Primavera-Verão foi menos tóxica do que a recolhida no Outono-Inverno. Foi observada toxicidade para algumas amostras de água subterrânea, no entanto, pode não estar

relacionada com o lixiviado. É importante sublinhar, que os resultados do presente estudo relativamente aos testes de toxicidade sobre as amostras que não fazem parte no programa de monitoramento do aterro, não revelaram toxicidade, pelo menos devido ao lixiviado. Os resultados obtidos demonstraram também que deve ser continuada a investigação visto que não existe informação clara sobre a fonte da contaminação das águas subterrâneas e superficiais.

Palavras-chave: Risco ecológico, bateria de testes, lixiviado, águas subterrâneas, eluatos de solo, água e sedimentos de rio

Chapter 1

Introduction

1.1 GENERAL INTRODUCTION

Ecosystems integrate countless numbers of organisms from micro-organisms to plants and animals that interact dynamically in complex ways among themselves and with their physical environment. Many factors affect the dynamics of an ecosystem and hence the balance within it (light, temperature, energy and nutrient supplies, competition, predation, pollution and disease). The most important property of an ecosystem is its robustness; for instance the way it can withstand or adapt to changes which affect its dynamics. Included in this robustness is its ability to return to "normal" status after the cessation of the stimulus to which it is sensitive. (Aldridge, 1996). The possibility of irreversible change which will reduce biological diversity is most worrying (Aldridge, 1996).

Ecosystems have been largely impacted by increasing human population growth and changing global processes. Various habitats have lost a significant proportion of their species and might be in greater danger of further losses from intense anthropogenic impacts, such as dams, pollution, overfishing and other threats (McAllister *et al*., 1997). In particular, the contamination of the aquatic environment with high amounts of chemicals posing most of the times unintended effects on ecosystems has reached regional and global scales (Schwarzenbach *et al*., 2006), with their high complexity influencing the fate and bioavailability of chemicals which will directly or indirectly affect the biota (Schmitt-Jansen *et al*., 2007).

Nowadays, toxicological studies of the environment can still be mostly characterized as environmental toxicology studies, which are conducted independently from ecological considerations, though perhaps subsequently compared to ecological studies in a burden-of-evidence approach (Ingersoll *et al*., 1997). The ecological/biological component of

environmental assessment can be realized by monitoring the status of communities in real ecosystems and/or through the use of ecotoxicity tests conducted at different levels of biological organization, being the most common single-species laboratory toxicity tests employing organisms that can readily be obtained, cultured and tested (Burton *et al.*, 2002).

The elemental assumption of the ecotoxicological approach is that toxicants impact molecular, cellular and physiological processes, and therefore have the potential to adversely affect the health of individual organisms (Baird *et al*., 2007); effects that might propagate and impair ecosystem function through effects on populations (Loreau *et al*., 2002). Ecotoxicity tests allow establishing causal relationships between contaminants and biological effects, which, at least at the laboratory scale, are typically based on measures of survival, individual growth and reproduction (Aldridge, 1996). Although such an approach is a considerable oversimplification of real ecological conditions, the establishment of such cause-effect relationships has been proven to be very useful to address the impacts of various types of environmental wastes, including impacts on municipal solid waste containment areas (Keuter *et al.,* 2011).

1.2 MUNICIPAL SOLID WASTE

The high consumerism of our society has led to critical increases in the production of waste, which accumulates and eventually is deposited in public areas often unsuitable for this purpose (dumps), though at present most dumps have been turned into closed landfills and new landfills have been constructed. The issue of waste has become a big worldwide problem, with consequences that go beyond the concerns on our environment; there is a lack of sustainability across the linear cycle of production, consumption and disposal of materials, which in addition to depleting natural reserves has turned our planet into a wide garbage dump causing the degradation of the environment and affecting the health of the population (Costa, 2005). Although nature has a certain capacity to absorb negative impacts that occur on

the earth, this capability has been proved insufficient to absorb all impacts caused by the generation of waste arising from human activities (Morejon, 2011).

Municipal Solid Waste (MSW), commonly called “trash” or “garbage,” includes wastes such as durable goods (e.g. tires, furniture), nondurable goods (e.g. newspapers, plastic plates/cups), containers and packaging (e.g. milk cartons, plastic wrap), and other wastes (e.g. yard waste, food). This category of waste generally refers to common household waste, as well as office and retail wastes, but excludes industrial, hazardous and construction wastes (Center for Sustainable Systems, 2014). The handling and disposal of MSW is a growing concern as the volume of waste generated continues to increase worldwide. Moreover, for instance, in the U.S.A., over the past several years, there is still significant controversy concerning the proper management of the residues from MSW and their regulatory classification as hazardous or non-hazardous waste. This controversy and other factors (e.g. lack of legislative guidance, contaminants content, etc.) have resulted in inconsistent management requirements and uncertainty about beneficial utilization of the residues (Wiles, 1996).

MSW generation continues to grow both per capita and in overall terms. Waste production increased by 3 and 4.5% per year between 1992 and 1996, in Norway and in the U.S.A., respectively (Renou *et al.*, 2008). In 2002, French population produced 24 million tons of MSW, namely 391 kg per person (Renou *et al*,. 2008). During the latter part of the 1990s, annual waste production ranged from 300 to 800 kg per person in the most developed countries to less than 200 kg in other countries (Renou *et al.,* 2008). In 2009, Americans generated about 243 million tons of garbage and recycled and composted 82 million tons of this material (EPA, 2010). Since 1990, the total amount of MSW going to landfills dropped by more than 13 million tons, from 145.3 million to 131.9 million tons in 2009 (EPA, 2010).

The adverse effects of MSW on the environment and public health have been widely reported by several authors (Anjos *et al*., 1995; Cantanhede, 1997; Hammer, 2003; Mashi, 2014; Accurio

et al., 1998), overall pointing to deficiencies in the collection and disposal systems. Besides potential health hazards, the concerns about a landfill include fires and explosions, vegetation damage, unpleasant odours, landfill settlement, groundwater and surface water pollution, air pollution and global warming (El-Fadel *et al*., 1997).

MSW comprises 10% of the total waste generated in the European Union and has been an important focus of European legislation, due to its complex and variable composition and its distribution among waste generators (Blumenthal, 2011). Council Directive 99/31/EC (EC, 1999) was created to prevent or reduce as far as possible negative effects on the environment (in particular on surface water, groundwater, soil, air, and human heath) caused by the landfilling of waste, by introducing stringent technical requirements for waste and landfills; a policy for all members of European Union based on which each member has to create their own legislation in order to comply with this Directive.

In Portugal, a revolution in the waste sector occurred due to the approval, in 1997, of the Strategic Plan for Municipal Solid Wastes I and the definitive closure of all the 314 active dumps (Russo, 2005). Several municipal systems of waste management were created, including the construction of sanitary infra-structures of MSW containment, the landfills. In fact, landfill disposal constitutes the simplest and most cost-effective method of MSW management, despite the European directives addressing the sustainable waste management through their reduction, recycling, composting, and energetic valorisation (EC, 1999). Much national legislation and programs that recommend/obligate that landfills must be monitoring and controlled prior to and after their establishment has been issued; a summary of Portuguese legislation is presented in Table 1. According to Decree-Law no. 183/2009 (Decreto-Lei n.º 183/2009), concentration limits for several physico-chemical parameters (including contaminants such as metals and organic compounds) in leachates, groundwater and gas emissions must be respected. In particular, to minimize the impacts of a landfill in the

environment, the landfill operator shall submit to the competent authority, once a year, a report synthesis on the state of the landfill after its closure, specifying the maintenance operations and the results of checks carried out during the previous year (Decreto-Lei n.º 152/2002; Annex IV, Part II, point 10.6). However, there is no control of the ecological/biological information. Particularly, none of the policies regulates the ecotoxicological characterization of the various matrices towards the ecosystems within and surrounding closed landfills (see also below).

Table 1. Summary of Portuguese legislation on landfills and its objectives

Legislation (Decree Law)	**Objective**
DL no. 239/1997 from 9th September (Decreto-Lei n.º 239/1997)	Regulates the disposal of MSW in landfills.
DL no. 321/1999 from 11th August (Decreto-Lei n.º 321/1999)	Legal regime applicable to ordinary landfill waste industrials that regulates the installation and operation.
DL no. 152/2002 from 23th May (Decreto-Lei n.º 152/2002)	Regulates the installation, operation, closure and post-closure maintenance of landfills for MSW.
DL no. 183/2009 from 10th August (Decreto-Lei nº. 183/2009)	Establishes the policy of disposal of the MSW in a landfill and regulates the conception, building, exploration, closure and post closure of landfills; and the scientific characteristics for each class of a landfill.

Landfills are today the most widespread medium and lower cost for the storage of MSW, but the fact that they are stocked does not mean they are inactive. Storage conditions as well as the influence of natural agents (rain and microorganisms) activate physical, chemical and biological processes transformation. The elements are dissolved in water, fine particles are detached and the bioconversion of the soluble organic material into gaseous forms occurs; all this leads to the formation of biogas and leachate (Castilhos *et al*, 2003). For leachates, the percolation process in landfills is defined as the amount of water that exceeds the water

retention capacity of a residue. Leachate comes from three main sources: moisture natural waste, the water by product during the decomposition process and the liquid expelled from organic materials by bacteria in the form of enzymes (Castilhos *et al*., 2003).

Leachate occurrence is by far the most significant threat to groundwater and ultimately also do surface water. Once it reaches the bottom of the landfill or an impermeable layer within the landfill, if not properly collected in wells, leachate either travels laterally to a point where it discharges to the ground's surface as a percolate, or it will move through the base of the landfill and into the subsurface formations (El-Fadel *et al*., 1997). For instance, a study by Mattos (2006) found high levels of metals, in aquatic ecosystems near a landfill.

Pollution caused by landfill leachates is one of the main problems of urbanized areas, on account of their chemical composition, and major concerns about landfill leachates are threefold: their difficulty in treatment, their toxic potential and their microbiological composition (Kjeldsen *et al*., 2002). Potential contaminants in landfill leachates can be categorized into four major groups: dissolved organic matter, inorganic macro components, metals, and organic compounds; with existing data showing high concentrations of all leachate components in the early acid phase due to strong decomposition and leaching (Kjeldsen *et al*., 2002).

The presence of the potential toxic substances can be directly detected through chemical analysis. However, chemical analyses do not indicate which proportion of the chemical is bioavailable to organisms neither the associated potential antagonistic and/or synergistic effects of the existing mixture contaminants. Thus, the inclusion of the biological/ecological component in the current monitoring programs of closed landfills is essential to obtain a more realistic view of the potential impacts over time. Ecotoxicity tests will allow establishing the causal relationships between chemical concentrations and the biological effects observed (Calow and Forbes, 2003). Pablos *et al*. (2011) studied the correlation between the physico-

chemical and ecotoxicological characterization in order to facilitate the eco-management of the leachates. The latter authors used 21 samples comprising untreated leachates or compost leachates from MSW landfills collected at different seasons and with highly variable composition, depending on several factors such as waste composition and age, precipitation rate, and landfill management. *Daphnia magna* lethal toxicity tests showed toxicity for all 21 samples, suggesting that the toxicity of leachate was maintained in time within the same landfill.

1.3 STUDY OBJECTIVES

The present study is part of a broader research project intending to design a framework for long-term ecotoxicological/ecological monitoring of closed landfills (MSW containment structures). It covers the work focused on the aquatic compartment and has as main objective to determine the risk of a closed landfill, sealed in 2006 and with an area of 19 ha, towards adjacent aquatic systems by conducting the ecotoxicological characterization of potentially contaminated matrices to which aquatic organisms might be exposed.

To fully investigate whether the landfill leachate – the main source of contamination of a closed landfill – is a potential source of contamination for aquatic systems in the area and the extent of such potential contamination, various types of matrices were ecotoxicologically characterized, namely, the leachate itself, groundwater collected from piezometers at the fringe of the landfill, water from a puddle within the landfill, soil extracts from sites within and adjacent to the landfill area, and water and sediment samples from adjacent aquatic systems. Soil extracts, i.e., eluates, were used as a measure of the soil retention capacity, which is the capacity of a soil to retain contaminants precluding them to be leached through the soil-water pathway to aquatic systems (ISO, 2003).

To fully evaluate the risks posed by the landfill to aquatic systems and, thus, the ecological receptors at most risk via exposure to this type of contamination, while intending to contribute for long-term monitoring programs, a battery of short-term sub-lethal laboratory toxicity tests was selected to test the various types of matrices. The battery of tests included species both originated from standard laboratory cultures and naturally occurring, being all indigenous in Portuguese habitats to minimize uncertainty. Also, species were selected to cover a range of organisms representative of different key taxonomy (bacteria, algae, rotifers, cnidarians, crustaceans, and insects) and functional groups (primary producers, primary and secondary consumers, benthic and epibenthic decomposers). For samples shown to be toxic their toxicity was quantified in the form of a point estimate of the effective concentration toward a certain percentage of test organisms (e.g., the median avoidance effective concentration [EC50]) to allow sensitivity comparisons across the various ecological receptors.

The following null hypotheses being tested were: i) the organisms responses in control exposures were equal to those in leachate exposures, at two seasons; ii) the organism responses in control exposures were equal to those in groundwaters; iii) the organism responses in control exposures were equal to those in water from a puddle within the landfill; iv) the organism responses in control exposures were equal to those in soil extracts from sites within and adjacent to the landfill area; and v) the organism responses in control exposures were equal to those in water and sediment samples from adjacent aquatic systems. Expected results were: i) leachate exposures result in greater ecotoxicity than control exposures, as leachate is still being produced within this recently closed landfill with an extensive area of 19 ha, with pollution levels after a rainy season either highest, due to increased leachate generation by the augmented weight of the top layer impermeable cover, or lowest, due to leachate dilution by groundwater infiltration; ii) groundwater exposures result in greater ecotoxicity than control exposures, due to deficiencies (e.g. leakages) in the system of capture and drainage of leachate at the base of the landfill; exposures of (iii) water puddle and (iv) soil

extracts within the landfill result in greater ecotoxicity than control exposures, due to the occurrence of leachate overflow; exposures of (iv) soil extracts nearby the landfill and of (v) river water and sediment from aquatic systems nearby the landfill, due either to the occurrence of leachate overflow coupled with deficiencies in the drainage of surface water to the rain water drainage system of the landfill, or to the infiltration of contaminated groundwater.

Chapter 2

Materials and Methods

2.1 STUDY CASE

All information here disclosed is the minimum required to understand the study design and is not complemented with bibliographic citations for reasons of confidentiality.

To accomplish the aim of the present study, a landfill constructed in 2006 to close a dump with an area of 19 ha was selected as the study case. The dump was active during 30 years which led to the deposition of 2 500 000 tons of MSW and its closure clearly aimed to solve the adverse environmental impacts, particularly regarding water, soil and air pollution, and also the impact on the co-existing populations. The dump was sealed by covering the pile of MSW with an impermeable coating above which a soil layer of approximately 40-cm depth was placed. Systems installed for environmental protection and monitoring of emissions consisted of capture and drainage of gases and leachate and drainage of surface water to the rain water drainage system of the landfill. Measures to restore the landscape were also implemented, namely, an arboreal curtain in the external limit and a vegetation cover (meadow type) in all the area with shrubs and trees of species similar to those of the surrounding area.

To comply with Portuguese legislation (Decreto-Lei n.º 152/2002; Decreto-Lei n.º 183/2009), not only regarding the final covering and the landscape restoration, there is a post-closure monitoring program in what regards meteorological data, environmental noise, topography, gas and leachate emissions and groundwater quality; being the latter three monitored through a detailed physico-chemical characterization of the respective matrices, at least two times per year. To monitor groundwater quality five piezometers installed along the entire perimeter of the landfill allow the collection of groundwater samples from the aquifer underlying the landfill area, up to depths between 20 and 30 m.

2.2 STUDY DESIGN

To accomplish the aim of the present study it was essential to identify the potential ecological receptors at most risk in the area within and near the landfill due to exposure to the main source of contamination of a closed landfill – the leachate. Aside from the leachate itself, the following types of matrices, those that can most likely be contaminated by the leachate, were selected to be ecotoxicologically characterized (Figure 1): groundwater collected from four of the five piezometers installed at the fringe of the landfill (Pz2, Pz3, Pz4, and Pz5; for logistic reasons of groundwater accessibility Pz1 was not selected), water collected from a puddle within the landfill (Pd; although it was collected after a few days with rainfall it could also represent an overflow of leachate), soil extracts, i.e., eluates, from sites within and adjacent to the landfill area not only to determine the possible occurrence of leachate overflow but also of surface runoff from the top to the bottom of the landfill (S28, S38, S63, S70, S73, and S83), and water and sediment samples from two water courses nearby the landfill (Pw1,Pw2, Pw3, Pw4, Pw5, Ps1,Ps2, Ps3, Ps4, and Ps5,), potentially contaminated via surface runoff or groundwater infiltration.

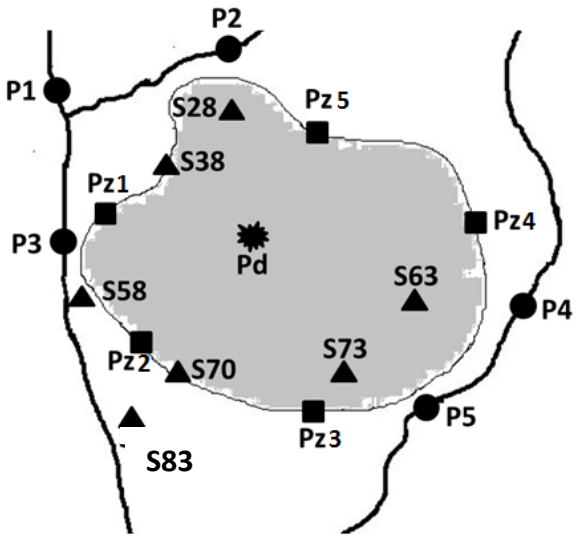

Figure 1. Scheme of the study area within and nearby the landfill with location of the sites were the matrices groundwater (Pz2 to Pz5; for logistic reasons Pz1 was not selected), puddle water (Pd), soil (S28, S38, S63, S70, S73, and S83) and river water and sediment (P1, P2, P3, P4,

and P5) were collected (for confidential reasons this scheme is merely illustrative and no scale is provided).

Although the present study also intended to evaluate possible seasonal differences in the direct and indirect effects of leachate toxicity, by collecting samples twice, in Autumn-Winter and Spring-Summer, a factorial design was not possible for logistic reasons and the only matrice collected in both seasons was the leachate (L1 and L2, respectively). Samples Pd, S28 to S83 and P1 to P5 were only collected in Autumn-Winter, the first season to be sampled, because obtained results pointed to the absence of toxicity (see Results section). Samples Pz2 to Pz5 were only collected in Spring-Summer for logistic reasons.

To fully distinguish the ecological receptors at most risk, the following extensive battery of toxicity tests with species representative of various aquatic system compartments, taxonomic groups and ecosystem functions was selected: 5-minutes luminescence of the marine bacteria *Vibrio fischeri* Lehmann and Neumann (decomposer) for both water-phase and solid-phase (sediments, in the case of the present study) matrices, 72-hours growth of the green planktonic microalgae *Pseudokirchneriella subcapitata* (Koršhikov) Hindak (primary producer), 72-hours growth of the floating macrophyte *Spirodela polyrhiza* (L.) Schleid (primary producer), 48-hours population growth of the rotifer *Brachionus calyciflorus* Pallas (primary consumer), 48-hours postexposure feeding of the cnidarian *Hydra attenuata* (Pallas) (secondary consumer), 24-hours feeding of the planktonic cladoceran *Daphnia magna* Straus (primary consumer), 6-days growth of the epibenthic omnivorous ostracod *Heterocypris incongruens* (Ramdohr) (primary consumer), and 48-hours postexposure feeding of the benthic midge deposit feeder *Chrinomus riparius* Meigen (decomposer). All toxicity tests selected for the present study have largely been used in other ecotoxicity studies. Besides, most of them have already been standardized and guidelines/standard operational procedures for recommended test procedures have been published (see section 2.5).

Given that species sensitivity differences within the same sample and across samples were expected, preliminary toxicity tests were performed with wide ranges of samples concentrations to establish the definitive range of dilutions allowing to reach precise estimates of toxicity parameters (e.g. EC50; median effective concentration) or for samples with low toxicity the lowest concentration causing a significant organisms response inhibition (see section 2.4). Table 2 outlines the group of toxicity tests performed with each type of collected matrice with the respective range of tested concentrations.

Table 2. Toxicity tests performed with *Vibrio fischeri* (5-minutes luminescence), *Pseudokirchneriella subcapitata* (72-hours growth), *Spirodela polyrhiza* (72-hours growth), *Brachionus calyciflorus* (48-hours population growth), *Hydra attenuata* (48-hours postexposure feeding), *Daphnia magna* (24-hours feeding), *Heterocypris incongruens* (6-days growth), and *Chrinomus riparius* (48-hours postexposure feeding) on the matrices leachate (L1 and L2), groundwater (Pz), water puddle (Pd), soil extracts (S), and river (Pw) and sediment (Ps) water, collected in Autumn-Winter (L1, Pd, S, Pw, and Ps) and Spring-Summer (L2 and Pz) within and nearby the landfill and respective range of tested concentrations (in %) (values within parenthesis are dilution factor).

Toxicity test	L1	L2	Pz2	Pz3	Pz4	Pz5	Pd	S	Pw	Ps
V. ficheri	81.9 - 10.2 (2)	81.9 - 10.2 (2)	81.9 - 10.2 (2)	81.9 - 10.2 (2)	81.9 - 10.2 (2)	81.9 - 10.2 (2)	100	×	100	100
P. subcapitata	100 - 3.13 (2)	100 - 3.13 (2)	100 - 6,25 (2)	100	100	100	100	100	100	×
S. poyrhiza	100 - 6.25 (2)	100 - 6.25 (2)	100 - 6.25 (2)	100 - 6.25 (2)	100	100 - 6.25 (2)	100	100	100	×
B. calyciflorus	15.0 - 0.469 (2)	30 - 3.95 (2)	15 - 0.469 (2)	100 - 6.09 (1.75)	100 - 41 (1.5)	100 - 6.09 (1.75)	100	100	100	×
H. attenuata	6.00 - 0.567 (1.3)	10 - 0.484 (1.4)	10 - 0.678 (1.4)	100 - 6.25 (2)	100 - 6.25 (2)	100 - 3.13 (2)	100	100	100	×
D. magna	11.5 - 3.01 (1.25)	70 - 4,10 (1.5)	80 - 10,5 (1.5)	100 - 41 (1.5)	100 - 41 (1.5)	100 - 41 (1.5)	100	100	100	×
H. incongruens	30.0 - 9.83 (1.25)	100 - 19.8 (1.5x)	100 - 6.25 (2)	100	100	100	100	100	100	100
C. riparius	35.0 - 8.75 (1.3)	45 - 12.1 (1.3)	70 - 18.9 (1.3)	100	100	100	100	100	100	100

X - no toxicity test was performed

2.3 COLLECTION AND CHARACTERIZATION OF WATER, SOIL AND SEDIMENT SAMPLES

As stated above, samples were collected twice, once in Autumn-Spring and another in Spring-Summer. The L1 sample, water from Pd and all water (Pd, Pw1, Pw2, Pw3, Pw4 and Pw5) and sediment samples (Ps1, Ps2, Ps3, Ps4 and Ps5) from the rivers nearby the landfill and the soil

samples to prepare soil eluates were collected in Autumn-Winter. In Spring-Summer only the L2 and all Pz samples were collected. Samples L1 and L2 were collected by landfill operators and corresponded to a sub-sample of the L that the landfill sends periodically to a wastewater treatment plant. Samples Pz were also collected by landfill operators. All liquid samples (L1, L2, Pz, Pw1-Pw5) were collected into acid washed 5-L polyethylene bottles and transported to the laboratory in thermally insulated boxes. Soil and sediment samples were collected with a scoop, placed in airtight black plastic bags and transported as the water samples. All water and sediment samples were stored immediately upon arrival to the laboratory, either at 4 °C in darkness to be used within less than two months or at -20 °C in darkness to be used within less than 12 months.

Soil samples were stored at 4 °C in darkness for a maximum period of one month until eluate preparation. Eluates were prepared following standard methods (DIN, 1984). The soil was mixed with distilled water (1:10 ratio, w/v, based on the soil dry weight), magnetically stirred during 12 hours, centrifuged at room temperature (20 minutes at 3370 *g*) and the supernatant collected as eluate, which was stored as the other liquid matrices.

The full physico-chemical characterization of all L and Pz samples according to the monitoring program implemented by the landfill is not yet available. However, a summary of the latter relatively to the monitoring of such parameters between 2006 and 2012 is here provided (Table 3), since it is considered relevant information to interpret the results of the present study. Additionally measurements of pH (Wissenschaftlich Technische Werkstätten, 537 pH meter, WTW, Weilheim, Germany), conductivity (WTW Cond315i/SET conductivity meter) and dissolved oxygen (WTW OXI 92 oxygen meter) were taken in the 100% concentration of all liquid samples (L1, L2, Pz, Pw1-Pw5, and S20-S83) every time a toxicity test was performed, as a mean to physico-chemically characterize them while verifying sample stability in time (Table 4).

Table 3. Range (minimum - maximum) of physico-chemical parameters measured according to the landfill implemented monitoring program complying with Portuguese legislation (Decreto-Lei n.º 236/1998; Decreto-Lei n.º 152/2002; Decreto-Lei n.º 183/2009) in the period of 2006 to 2012 based on data provided by the landfill (values within parenthesis are last available measurement of 2012) in samples of leachate (L) and from piezometers (Pz2, Pz3, Pz4, and Pz5).

Parameter	Unit	Leachate	Pz2	Pz3	Pz4	Pz5	LL[a,b]
pH	Sorensen Scale	7.05 – 8.26 (8.14)	5.6 - 6.5 (5.6)	4.6 – 10 (4.8)	4.8 - 6.7 (6.7)	4.6 - 7.2 (5.6)	4.5 - 9.0 [a]
Conductivity	µS/cm	1810 – 25600 (25600)	136 – 3900 (136)	1110 – 2300 (2300)	119 – 618 (137)	48 – 1990 (48)	X
COT (total organic carbon)	mg/L C	220 – 1500 (1100)	32 – 96 (43)	<1.0 – 55 (15)	<1.0 – 6 (1)	6 – 10 (6)	X
Total arsenic	µg/L As	<1.0 – 220 (130)	<5.0 – 11 (<5)	<5.0	<1.0 - <5 (<5)	<1.0 - <5 (<5)	10 [a]
Total cadmium	µg/L Cd	<0.5 - <10 (<10)	<1.0 - 4.0 (<1)	<1.0 - <5.8 (5.8)	<1.0	<1.0 (<1.0)	0.05 [a]
Total copper	µg/L Cu	<5.0 - <50 (<50)	<2.0 - 18.0 (<2,0)	11 – 39 (39)	<2.0 - 4.6 (<2.0)	4.9 – 15 (<5)	5 [a]
Total chromium	µg/L Cr	<0.5 – 980 (420)	<5.0 - 6.0 (6)	<5 – 5 (<5)	<5	<5	20 [a]
Total mercury	µg/L Hg	<1 - <5 (<5.0)	<0,5 – 50 (<0.5)	<0.5 - <1.0 (<0.5)	<0.5 - <1.0 (<0.5)	<0.5 - <1.0 (<0.5)	x
Total nickel	µg/L Ni	<2.0 – 260 (180)	15 – 62 (24)	64 – 171 (67)	<6.0 – 7 (<6)	10 – 138 (10)	2.0 [a]
Total lead	µg/L Pb	<2.0 - <20 (<20)	<7 - 9.5 (<7)	<7 – 14 (<7)	<7	<7 – 10 (<7)	20 [a]
Total zinc	µg/L Zn	<1 – 330 (120)	<50 – 130 (<50)	60 – 160 (160)	<50 – 60 (<50)	<50 – 190 (<50)	10 [a]
Phenols	µg/L C_6H_5OH	<5.38 - <6.3 (<5.4)	1.90 – 34 (34)	<1 – 103 (9.3)	<1- 49 (8,9)	2.6 – 21 (17)	X
Fluorides	-	-	<0.3	<0.3 - 0.49 (0.33)	<0.3	<0.3 - 0.32 (<0.30)	X
Chlorides	mg/L Cl	1400 – 3000 (2000)	<10 – 607 (415)	<10 – 662 (601)	20 – 86 (29)	<10 – 618 (37)	70 [b]
Sulfates	mg/L SO4	<0.5 – 150 (<5.0)	<5.0 – 80 (80)	7.8 – 80 (41)	<5.0 – 60 (28)	11 – 60 (27)	x
Nitrates	mg/L NO_3	<0.5	<10 – 10.7 (<10)	<1.0 – 31 (<10)	<10 - 11.3 (<10)	<10	50 [b]
Cyanides	µg/L CN	-	<5 - <10 (<5)	<5 - <10 (<5)	<5 - <10 (<5)	<5 - <10 (<5)	x
AOX (absorbable organic halogen compounds)	mg/L Cl	<0.030	<0.030 - 0.35 (0.212)	<0.03 - 0.54 (0.308)	<0.01- 0.042 (<0,010)	<0.030 - 0.33 (0.025)	x
Potassium	mg/L K	400 – 1400 (890)	11.1 – 106 (11.1)	6.7 - 21.5 (6.74)	<2.5 – 33 (2.86)	2.28 - 8.59 (2.28)	x
Ammonium	mg/L NH4	490 – 6700 (2800)	2.1 – 290 (212)	1.1 - 31.8 (30.7)	<0.026 - 1.14 (<0.05)	0.83 - 10.6 (2.4)	x
Total iron	µg/L Fe	1500 – 28000 (11000)	0.127 – 30 (30)	0.283 – 26 (11)	<0.04 - 5.9 (1.6)	1.75 – 17 (17)	5.0 [b]
Manganese	µg/L Mn	<0.5 – 21000 (140)	0.495 - 12.7 (12.7)	0.0055 - 69.4 (69,4)	0.008 – 66 (0.064)	1.36 - 12.1 (1.36)	10 [a]
Nitrites	mg/L NO3	<0.5 - 0.5 (<0.50)	<0.01 - <0.05 (<0.01)	<0.01 - <0.05 (<0.01)	<0.01 - <0.05 (<0.01)	<0.01 - 0.34 (<0.01)	x

[a,b] LL- Legal Limit for irrigation waters in Portugal (Decreto-Lei n.º 236/1998) applied to the groundwater collected from the piezometers ([a] admissible maximum value; [b] recommended maximum value).

Table 4. Range (minimum - maximum) of pH, conductivity and dissolved oxygen levels measured during the performance of the toxicity tests in the 100% concentrated leachate (L1 and L2), groundwater (Pz), water puddle (Pd), soil extracts (S), and river water (Pw) samples collected within and nearby the landfill.

Sample	pH (Sorensen scale)	Conductivity (µS/cm)	Dissolved ogygen (mg/l)
L1	7.01 - 7.90	8860 - 10920	9.18 - 9.30
L2	7.21 - 8.34	2260 - 10900	6.34 - 8.2
Pz2	7.05 - 8.52	262 - 1149	8.21 - 9.28
Pz3	5.47 - 7.35	1278 - 1412	8.61 - 9.84
Pz4	6.28 - 7.64	296 - 361	8.57 - 9.84
Pz5	5.32 - 8.10	673 - 858	8.86 - 10.07
Pd	5.97 - 7.88	433 - 494	8.08 - 10.41
Pw1	7.03 - 7.45	974 - 1047	8.68 - 8.83
Pw2	6.81 - 7.78	472 - 530	8.77 - 8.85
Pw3	7.01 - 7.85	517 - 525	8.80 - 8,88
Pw4	6.56 - 7.61	426 - 456	8.84 - 9.15
Pw5	6.55 - 7.59	455 - 494	8.74 - 8.80
S28	5.95 - 6.63	32.7 - 59.4	7.83 - 7.86
S38	5.54 - 6.40	72.3 - 73.2	7.93 - 7.98
S58	5.29 - 6.28	71.9 - 73.2	7.69 - 8.09
S63	4.40 - 6.32	71.6 - 73.0	8.63 - 8.65
S70	6.44 - 6.79	170 - 172	8.39 - 8.40
S73	6.49 - 6.83	46.9 - 52.5	8.02 - 8.19
S83	6.21 - 6.51	170 - 176	8.04 - 8.18

In what regards the sediments (Ps1-Ps2) they were characterized based on their content in organic matter and particle size distribution, both estimated in percentage relatively to the ash-free dry weight of the sediment (after ignition at 500 °C for 24 hours). These data were provided by external colleagues and are presented in Table 5. All sediments presented high percentages of particles with a size range equal to or higher than very coarse to coarse sand (82 to 91%) and low percentages of silt and organic matter (≤ 2%).

Table 5. Organic matter content and particle size distribution of the five sediment samples (Ps1 to Ps5) collected in the rivers nearby the landfill in Autumn-Winter (all in % relatively to the sediment ash-free dry weight).

Parameter		Ps1	Ps2	Ps3	Ps4	Ps5
Particle size	Fine to very fine gravel (⩾ 2 mm)	63.0	45.2	43.6	24.2	51.5
	Very coarse to coarse sand (< 2 mm - > 500 µm)	27.8	46.1	43.9	58.7	34.8
	Medium sand (< 500 - > 250 µm)	5.14	6.50	8.41	12.1	7.52
	Fine to very fine sand (< 250 - > 63 µm)	2.44	1.51	2.22	3.07	3.83
	Silt (< 63 µm)	1.62	0.713	1.90	1.97	2.27
Organic matter content		0.934	0.823	0.842	1.79	1.45

2.4 TEST ORGANISMS

The battery of eight laboratory toxicity tests was selected to cover a range of organisms representative of different taxonomic (bacteria, algae, plants, rotifers, cnidarians, crustaceans, and insects) and functional groups (primary producers, primary and secondary consumers, epibenthic and benthic decomposers) and of environmental compartments (water and sediment). From a practical viewpoint, other criteria used to select the test species were availability in sufficient numbers, easily manipulated and species for which data exist on the species sensitivity to a broad range of stressors (environmental/chemical) (Tesfaye, 2013).

Overall, test species originated either from dormant stages (*V. fischeri*, *S. polyrhiza*, *B. calyciflorus*, and *H. incongruens*) or standard laboratory cultures (*P. subcapitata*, *H. attenuata*, *D. magna*, and *C. riparius*).

All dormant stages were purchased from commercial suppliers and animate forms obtained as described in the respective protocols. *V. fischeri* were reconstituted from lyophilized (freeze-dried) bacteria (Azur Environmental, Carlsbad, CA, USA), *S. polyrhiza* turions were germinated for 72 hours (http://www.microbiotests.be/toxkits/Spirodelastp.pdf, last viewed October 2014) and cysts of *B. calyciflorus* and *H. incongruens* were hatched for 18 and 48 hours, respectively, and animated forms fed with artificial food for an additional period of 2 and 4 hours (http://www.microbiotests.be/toxkits/RotoxkitFshort-chronicstp.pdf last viewed

October 2014; http://www.microbiotests.be/toxkits/OstracodtoxkitFstpl.pdf, last viewed October 2014).

Non-axenic stock cultures of the green freshwater microalgae were maintained in 250-ml sterile glass Erlenmeyer flasks, with Woods Hole MBL growth medium (Stein, 1973) supplemented with vitamins (0.1 mg/L B1, 0.5 µg/L B12 and 0.5 µg/L biotin), at 19–21 °C, under continuous cool-white fluorescent illumination (8000 lx). To start new cultures and obtain organisms for the tests, algae were harvested while still in the exponential growth phase (5–7 d old) (Tesfaye, 2013).

Hydra attenuata was maintained at 19-21 °C, under a 14:10-hours light:dark photoperiod (6000 lx), in an artificial medium described in Trottier et al. (1997). The organisms were cultured in crystallizers with 200 ml of medium. Cultures were fed three times a week with *Artemia franciscana nauplii* (24-hours old), times at which the medium was renewed. Hydranths for toxicity tests were organisms without buds not fed for the previous 24 hours.

Organisms for tests with *D. magna* (Clone A originated from IRCHA in France; OECD, 1998) were obtained from cultures maintained at 19-21 °C, under a 14:10-hours light:dark photoperiod, in reconstituted hard water (ASTM, 2002) supplemented with vitamins (7.5 µg/L B1, 1 µg/L B12, and 0.75 µg/L biotin) and Marinure extract (Glenside, Stirling, UK; 7.5 ml/L of a suspension with an absorbance of 620 units at 400 nm). Cultures were fed daily with *P. subcapitata* (3×10^5 cells/ml; 25 and 15 daphnids/L up to the first brood and from there onwards, respectively) and the medium was renewed every other day. To perform the toxicity tests, 4-days old organisms were used.

Cultures of *C. riparius* consisted of crystallizing dishes containing 180 - 200 g of quartz sea sand (0.1-0.4 mm particle size; Merck, Darmstadt, Germany) and 300 ml of reconstituted hard water (ASTM, 2002), fed a suspension of ground Tetramin (Tetrawerk, Melle, Germany) three times per week (0.1 g/dish, with 30 and 15 larvae/dish up to day seven and from there onwards,

respectively), and maintained 19-21 °C, under a 14-hours:10-hours light dark photoperiod with 90-minutes dawn and dusk periods (7000 lx) (for further details see Rosa *et al.*, 2010). Larvae used in tests were from 10 days old cultures.

2.5 ECOTOXICITY TESTS

The 5-minutes *V. fischeri* luminescence test was conducted following the 81.9% basic test protocol for water samples and solid-phase test protocol for sediment samples (Azur Environmental, Carlsbad, CA, USA). According to these protocols, maximum tested concentrations are 81.9% for water and 197400 mg/L for sediment (on a wet weight basis). The Microtox toxicity analyzer was used to measure the light emission (in Lt) of the bacteria after an exposure periods of 5 minutes. A single reading was measured for the samples subjected to a dilution gradient (L1, L2, Pz2-Pz5) whereas for the 100% tested samples two replicates (Pd, Pw1-Pw5) or two replicates with two sub-replicates each (Ps1-Ps5) were read.

The 72-hours growth test with *P. subcapitata* was done following standard guidelines (OECD, 1984; EC, 1992). All waters were tested after being supplemented with nutrients in the same amounts as the control medium to discriminate potential toxic effects from those due to differences in nutrient levels among the treatments; the exception being the soil eluates. The test was conducted in 24-wells plates with each replicate well consisting of 900 µl test solution plus 100 µl of algal inoculum; three and at least six replicates for each test solution and the control (also used as dilution medium; same as stock culture medium slightly diluted to adjust for N/P ratio according to guidelines), respectively, were set up with an initial algal concentration of 10^4 cell/ml; each plate had at least a control replicate to verify for variability in environmental conditions within the tested area. The border line wells of each plate were filled with distilled water to minimize water evaporation during the test duration. The test was carried under the same light and temperature conditions as the stock cultures. After the 72-

hours exposure period, the final cell densities were counted from well-mixed aliquots of each replicate under a microscope (×400 magnification) using a Neubauer chamber to estimate the growth rate as the difference between the final and initial cell density (r; /day).

The 72-hours growth of *S. plyrhiza* was based on the *Spirodela* duckweed microbiotest (http://www.microbiotests.be/toxkits/Spirodelastp.pdf, last viewed October 2014), which is conducted in 48-wells test plates containing 1 ml of test solution per well (i.e., per replicate). The standard control and dilution medium consisted of Steinberg medium (ISO, 2005). At least eight controls were set up per test, with a minimum or four per test plate. For each tested solution four replicates were set up. All tests were incubated at 24-26 °C and at least 6000 lx. The area measurements of the initial and final fronds were made by Image Analysis of the photos of the test plates at the start and end of the test. The estimated test endpoint was the increase in frond area after 72 hours of exposure (mm^2/72 h).

The 48-hours population growth test with *B. calyciflorus* was performed following the Rotoxkit F Chronic test (http://www.microbiotests.be/toxkits/RotoxkitFshort-chronicstp.pdf). This test was also conducted in 48-wells plates and eight replicates (one organism per well per ml) of each tested solution and control were set up; control and dilution medium was reconstituted moderately hard water (ASTM, 2002). The test was incubated at 24-26 °C and food was provided (*P. subcapitat*; $2x10^6$ cells/ml). After the 48-hours exposure the number of live rotifers was counted in each well to estimate specific growth rates based on the number of initial and final organisms per replicate (r; /day).

The 48-hours *H. attenuata* postexposure feeding is a test done with 12 replicates per tested solution and control, each consisting of 2-ml solution and one organism; the medium used in the standard control and for test dilutions was reconstituted moderately hard water (ASTM, 2002). The postexposure feeding was done by providing 10 *A. franciscana nauplii* per well after transferring each hydranth to 2 ml of fresh control medium. After 30 minutes of feeding in

darkness, *nauplii* not consumed were counted to estimate feeding rates (nauplii/hydranth/30 min).

The 24-hours *D. magna* feeding test was based on the methodology developed by McWilliam & Baird (2002). The standard control and dilution medium was the same used for the stock cultures, except that no vitamin supplement was provided. For each tested water and control five replicates were set up, each consisting of 175-ml glass vessels filled with 120 ml of test solution plus food (3.5×10^5 cells/ml of *P. subcapitata*) and five organisms. A blank treatment, consisting of control medium with food but without organisms, was also run to control for algal growth during the test period. The test was incubated at 19-21 °C in darkness. After a 24-hours exposure period, the test endpoint was estimated as feeding rate (cells/daphnid/24 hours), calculated from initial and final cell densities; algal counting was performed as described for the 72-hours *P. subcapitata* growth test.

The 6-days *H. incongruens* growth test was conducted according to the Ostracodtoxkit F standard operating procedure (http://www.microbiotests.be/toxkits/OstracodtoxkitFstpl.pdf, last viewed October 2014). The standard control consisted of reference sediment included in the kit plus the same medium used for hatching the cysts, which was also used for samples dilution. The same reference sediment was also used to test all liquid samples whereas in the test with the river sediment samples the respective sediment was used and overlying water was control medium. The tests were conducted in 6-wells plates with each replicate well consisting of 1 ml of sediment plus 4 ml of solution already inoculated with food (3.75×10^6 cells/ml of *Scenedesmus* sp.) and 10 organisms; six replicates were established for the control and three for the tested samples. After the 6-days exposure period, survival and final length (in µm) were recorded. The specific growth rate was estimated from initial and final lengths (r; /day).

The *C. riparius* 48-hours postexposure feeding test was conducted according to procedures described in Soares *et al*. (2005). The same medium as the stock cultures was used for diluting samples and the control. Only the four replicates of the control had sediment (the same used to maintain the culture in the laboratory), whereas the three replicates of each remaining treatment consisted of water only exposures; in the test with the river sediment samples the respective sediment was used and overlying water was control medium. Each replicate consisted of 175-ml glass vials filled with 50 g of sediment, only in the case of the treatments specified above, plus 120 ml of water under continuous aeration. Five larvae were added per replicate. At the end of the 48-hours exposure, larvae were retrieved from the test vials, immediately individually transferred to a 30 ml glass vial filled with 5 ml of control medium and 100 defrosted *nauplii* (< than 24-hours old) of *A. franciscana*. Then they were allowed to feed at 19-21 °C in darkness for 1 hour, time after which each larva was retrieved and the remaining *nauplii* were counted. Feeding rates were calculated as the difference between the initial and the final number of *nauplii* (*nauplii*/larvae/h).

2.6 DATA ANALYSIS

For all except the *V. fischeri* tests, effective concentrations inducing 20 and 50% inhibition in organism responses (EC20 and EC50) and respective 95% CL were obtained by fitting organism responses to a logistic model using the least squares method (OECD, 1998). For the *V. fischeri* tests, median effective concentrations (EC50) and respective 95% CL were calculated using Microtox Omni Software 1.18 (Azur Environmental), whereas EC20 values with respective 95% CL were calculated using the software PriProbit 1.63 (http://ars.usda.gov/Services/docs.htm?docid=11284), applying the probit transformation to luminescence and the logarithmic transformation to concentration..

When EC20 or EC50 values could not be estimated due to the low percentage inhibition at the 100% concentration and for the tests consisting simply of the standard control and 100% sample one-way analysis of variance (ANOVA) or nested ANOVA was used, to test for the existence of significant differences between the organism responses in the standard controls and tested sample. Prior to all analysis of variance, the assumptions of normality (Shapiro–Wilk's test) and homoscedasticity (Bartlett's test) were checked (Zar, 2010).

Chapter 3

Results

All laboratory tests fulfilled the validity criteria for control performance (survival and sublethal endpoint) required in the respective guidelines/standard operational procedures or established for the present study based on known baseline values for each species. Table 6 presents the summary of the pH, conductivity and dissolved oxygen levels measured in the standard control of all toxicity tests performed with *P*. *subcapitata*, *H*. *attenuata*, *D*. *magna,* and *C. riparius*. For the *V. fisheri*, S. *polyrhiza*, *B. calyciflorus*, and *H. incongruens* tests no physico-chemical measurements were performed because test volumes were too small.

Table 6. Range (minimum – maximum) of pH, conductivity (µS/cm) and dissolved oxygen (DO in mg/l) levels measured in the standard control during the toxicity tests with *Pseudokirchneriella subcapitata* (72-hours growth), *Hydra attenuata* (48-hours postexposure feeding), *Daphnia magna* (24-hours feeding), and *Chrinomus riparius* (48-hours postexposure feeding) on the matrices leachate, groundwater, water puddle, soil extracts, and river water and sediment.

Toxicity test	pH	Conductivity	DO
P. subcapitata	6.50 - 7.29	183 - 314	nm
H. attenuata	7.09 - 7.87	258 - 390	nm
D. magna	7.47 - 8.28	482 - 655	8.26 - 9.35
C. riparius	7.79 - 9.10	547 - 631	8.43 - 9.25

nm: not measured

3.1 ECOTOXICOLOGICAL CHARACTERIZATION OF MATRICES WITHIN THE LANDFILL MONITORING PROGRAM

The results described herein are those regarding the matrices included in the monitoring program under the landfill management, i.e., leachates (L1 and L2) and groundwater (Pz2 to Pz5). From all toxicity tests performed, mortality above the validity criterium of either 10 or 20%, the latter only for the ostracod toxicity test, was occasionally registered (Table 7). Mortality was observed for all test organisms in both L1 and L2 and only for *B. calyciflorus* and

H. attenuata for groundwater samples. Despite the observed mortality it was possible to estimate EC50 and EC20 values for all matrices, as sufficient alive organisms remained in at least five test concentrations.

Table 7. Mortality (M, in %) for the test concentrations (C, in %) for which the validity criterium of 10 or 20% (only for the ostracod toxicity test) was not fulfilled during the toxicity tests with *Brachionus calyciflorus* (48-hours population growth), *Hydra attenuata* (48-hours postexposure feeding), *Daphnia magna* (24-hours feeding), *Heterocypris incongruens* (6-days growth), and *Chrinomus riparius* (48-hours postexposure feeding), to evaluate the toxicity of the matrices leachate (L1 and L2) and groundwater (Pz2 to Pz5). Range of concentrations is contiguous from highest to lowest even if at the highest or at intermediate concentrations mortality is within the validity criterium.

Toxicity test	**L1**		**L2**		**Pz2**		**Pz3**		**Pz4**		**Pz5**	
	C	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**
B. calyciflorus	100 14.6	15.0 7.50	30.0 20.0 13.3 8.89	35.4 3.13 3.13 25.0	15.0	12.5	100 57.1 32.7	100 25.0 25.0	100	9.38	100 57.1 32.7	50.0 6.30 18.8
H. attenuata	2.10 1.62 1.24	100 58.3 16.7	10.0	75.0	10.0	75.0	100	0	100	0	100	58.3
D. magna	11.5	46.7	70.0	13.3	100	0	100	0	100	0	100	0
H. incongruens	30.0 24.0 19.2 15.4 12.3	100 100 36.7 23.3 10.0	100 66.7	80.0 10.0	100	0	100	0	100	0	100	0
C. riparius	32.5 25.0 19.2 14.8	100 60.0 13.3 33.3	100 40.0 30.8 23.7 18.2	93.3 6.67 0.0 0.0 13.3	100	0	100	0	100	0	100	0

Results of the EC50 and EC20 values estimated for all test organisms for the matrices leachate and groundwater are presented in Table 8. Values of EC50 for L1 could be estimated for all test organisms, ranging from 1 (*H. attenuata*) to 65% (*V. fischeri*), whereas EC50 values for L2 ranged from 6 (*H. attenuata*) to 61% (*C. riparius*), but estimates higher than the 100% concentration were obtained for *V. fischeri*, *P. subcapitata* and *H. incongruens*; with the 100% concentration causing a significant inhibition of 2, 44 ans 33% in the bacteria luminescence, microalgae growth and ostracod growth, respectively. The ratio between EC50 values for L2 and EC50 values for L1 ranged from a factor of 2 (bacteria, macrophyte and insect larvae) to 6

(cnidarian), whereas the correspondent ratio for EC20 values ranged from a factor of 3 (microalgae, macrophyte and insect larvae) to 6 (cnidarian)/7 (cladoceran).

Table 8. Effective leachate (L1 and L2) and groundwater (Pz2, Pz3, Pz4, and Pz5) concentrations inducing 50 (EC50) and 20% (EC20) inhibition in organism responses, for the toxicity tests performed with *Vibrio fischeri* (5-minutes luminescence), *Pseudokirchneriella subcapitata* (72-hours growth), *Spirodela polyrhiza* (72-hours growth), *Brachionus calyciflorus* (48-hours population growth), *Hydra attenuata* (48-hours postexposure feeding), *Daphnia magna* (24-hours feeding), *Heterocypris incongruens* (6-days growth), and *Chrinomus riparius* (48-hours postexposure feeding). Values within curve brackets are 95% confidence limits and those within square brackets are organisms response inhibition caused by the 100% concentration of the sample relatively to the control with * indicating a significant inhibition.

Toxicity test	Parameter	L1	L2	Pz2	Pz3	Pz4	Pz5
V. fischeri	EC50	65.0 (53.0 - 80.0)	> 81.9 [2]	> 81.9 [11]	33.0 (30.0 - 36.0)	> 81.9 [19]	49.0 (48.0 - 50.0)
	EC20	19.8 (-)	> 81.9 [2]	> 81.9 [11]	10.6 (-)	> 81.9 [19]	11.8 (-)
P. subcapitata	EC50	37.6 (32.8 - 42.4)	> 100 [44]*	>100 [30]*	>100 [39]*	> 100 [6]	> 100 [17]*
	EC20	21.3 (16.8 - 25.9)	56.4 (49.9 - 62.8)	57.7 (46.6 - 68.9)	61.4 (55.5 - 67.3)	> 100 [6]	>100 [17]*
S. polyrhiza	EC50	8.00 (7.75 - 8.25)	18.7 (12.4 - 25.0)	43.8 (36.9 - 50.7)	>100 [25]	> 100 [0]	> 100 [35]*
	EC20	5.44 (5.19 - 5.69)	13.9 (6.69 - 21.1)	26.7 (19.3 - 34.1)	75.8 (55.3 - 96.4)	> 100 [0]	> 50 [0]
B. calyciflorus	EC50	5.72 (2.93 - 8.50)	27.1 (21.8 - 32.4)	1.65 (1.53 - 1.76)	18.2 (9.62 - 26.7)	90.2 (77.0 - 103)	12.5 (8.84 - 16.1)
	EC20	2.63 (0.147 - 5.11)	17.0 (10.7 - 23.3)	0.830 (0.710 - 0.940)	7.41 (1.25 - 13.6)	50.5 (36.8 - 64.2)	4.35 (2.02 - 6.67)
H. attenuata	EC50	1.09 (0.931 - 1.24)	6.37 (5.17 - 7.56)	7.64 (6.19 - 9.09)	>100 [7]	>100 [9]	73.9 (38.2 - 110)
	EC20	0.843 (0.631 - 1.05)	4.13 (2.77 - 5.49)	5.82 (3.86 - 7.78)	> 100 [7]	> 100 [9]	37.5 (4.68 - 70.4)
D. magna	EC50	8.28 (6.93 - 9.62)	43.3 (36.3 - 50.4)	71.0 (39.0 - 103)	87.3 (81.6 - 93.0)	>100 [21]*	> 100 [27]*
	EC20	4.17 (2.84 - 5.50)	29.1 (20.9 – 37.3)	12.5 (2.4 -22.6)	61.9 (54.0 - 69.8)	>100 [21]*	88.9 (56.0 - 122)
H. incongruens	EC50	20.0 (18.7 - 21.3)	> 100 [36]*	> 100 [12]*	> 100 [0]	> 100 [0]	> 100 [0]
	EC20	15.1 (13.0 - 17.2)	79.4 (74.3 - 84.5)	> 100 [12]*	> 100 [0]	> 100 [0]	> 100 [0]
C. riparius	EC50	26.1 (17.8 - 34.5)	61.3 (52.0 - 70.6)	> 100 [24]	> 100 [0]	> 100 [18]	> 100 [0]
	EC20	13.9 (6.55 - 21.2)	36.3 (28.6 - 43.9)	74.0 (13.7 - 134)	> 100 [0]	> 100 [18]	> 100 [0]

Regarding groundwaters, the species for which an EC50 value could be estimated varied with the groundwater: *B. calyciflorus*, *H. attenuata*, *S. polyrhiza*, and *D. magna* for Pz2 (EC50 values of 2, 8, 44, and 71%, respectively), *B. calyciflorus*, *V. fischeri* and *D. magna* for Pz3 (EC50 values of 18, 33 and 87%, respectively), and *B. calyciflorus*, *V. fischeri* and *H. attenuata* for Pz5 (EC50 values of 13, 49 and 74%, respectively). As for Pz4, an EC50 estimate was only possible for *B. calyciflorus*, with a value as high as 90%; a significant organism response inhibition at the 100% concentration was only observed for D. magna (by 21%). Pz2 was the groundwater with lowest EC50 values, close to those obtained with L2, except for the rotifer with EC50 values of 2 and 27%, respectively.

Table 9 summarizes the results of all toxicity estimates by ranking the EC50 and EC20 values in accordance with five classes of toxicity commonly used in ecotoxicology. It can be observed that based on EC50 estimates only for the species *B. calyciflorus, H. attenuata, S. polyrhiza,* and *D. magna* matrices were ranked as very toxic; L1 for all four species, L2 for *H. attenuata* and Pz2 for *B. calyciflorus* and *H. attenuata*. Moreover, L1 was never ranked as toxic or not toxic, whereas L2 and Pz2 were ranked as toxic or not toxic with the bacteria, microalgae, ostracod toxicity tests, and Pz2 also with the insect larvae test. All remaining groundwaters (Pz3, Pz4 and Pz5) were generally in the categories toxic or not toxic, with a few exceptions appearing in the moderately toxic category; Pz3 and Pz5 with the bacteria and rotifer, Pz3 also with the cladoceran and Pz5 also with the cnidarian, and finally Pz4 with the rotifer. The species identifying samples as very toxic were only *S. polyrhiza*, *B. calyciflorus*, *H. attenuata*, and *D. magna*. However, when EC20 estimates are considered only the rotifer and cnidarian identified samples as extremelly toxic (L1 and Pz2). Overall, results of EC20 values followed a pattern similar to that described for values of EC50.

Table 9. Classification of the matrices leachate (L1 and L2) and groundwater (Pz2, Pz3, Pz4, and Pz5) according to the results obtained in the tests performed with *Vibrio fischeri* (5-minutes luminescence), *Pseudokirchneriella subcapitata* (72-hours growth), *Spirodela polyrhiza* (72-hours growth), *Brachionus calyciflorus* (48-hours population growth), *Hydra attenuata* (48-

hours postexposure feeding), *Daphnia magna* (24-hours feeding), *Heterocypris incongruens* (6-days growth), and *Chrinomus riparius* (48-hours postexposure feeding), estimated by ranking the EC50 and EC20 values in accordance with classes of toxicity commonly used in ecotoxicology: extremely toxic (ET), very toxic (VT), moderately toxic (MT), toxic (T; 100%, when organism response inhibition at the 100% concentration is significant), and not toxic (NT; > 100%, when organism response inhibition at the 100% concentration is not significant). The matrices are ordered from lowest to highest EC50 or EC20 values.

	Toxicity test	ET (< 1%)	VT (1 – 9%)	MT (10 – 99%)	T (100%)	NT (> 100%)
EC50	***V. fischeri***			Pz3 < Pz5 < L1		Pz4 < Pz2 < L2
	P. subcapitata			L1	L2 < Pz3 < Pz2 <Pz5	Pz4
	S. polyrhiza		L1	L2 < Pz2	Pz5	Pz3 < Pz4
	B. calyciflorus		Pz2 < L1	Pz5 < Pz3 < L2 < Pz4		
	H. attenuata		L1 < L2 < Pz2	Pz5		Pz4 < Pz3
	D. magna		L1	L2 < Pz2 < Pz3	Pz5 < Pz4	
	H. incongruens			L1	L2 < Pz2	Pz4 < Pz3 < Pz5
	C. riparius			L1 < L2		Pz2 < Pz4 < Pz3/Pz5
EC20	***V. fischeri***			Pz3 < Pz5 < L1		Pz4 < Pz2 < L2
	P. subcapitata			L1 < L2 < Pz2 < Pz3	Pz5	Pz4
	S. polyrhiza		L1	L2 < Pz2 < Pz3 < Pz5		Pz4
	B. calyciflorus	Pz2	L1 < Pz5 < Pz3	L2 < Pz4		
	H. attenuata	L1	L2 < Pz2	Pz5		Pz4<Pz3
	D. magna		L1	Pz2 < L2 < Pz3 < Pz5	Pz4	
	H. incongruens			L1 < L2	Pz2	Pz3/Pz4/Pz5
	C. riparius			L1 < L2 < Pz2		Pz3/Pz5

3.2 ECOTOXICOLOGICAL CHARACTERIZATION OF MATRICES WITHIN AND OUTSIDE THE LANDFILL AREA

This section describes the results of the toxicity tests performed with the matrices not included in the landfill monitoring program, but potentially contaminated by the landfill leachate: water puddle, soil eluates, river waters and sediments. The sample collected from a puddle (Pd) within the landfill area did not significantly influence the responses of either *V. fischeri*, *P. subcapitata*, *S. polyrhiza*, or *H. attenuata* (one-way ANOVA: $F_{1,2\text{-}22} < 1.10$, $P > 0.31$), but had a significant effect on the responses of *B. calyciflorus*, *D. magna*, *H. Incongruens*, and *C. riparius*

(one-way [nested] ANOVA) (Fig. 2). Yet, in all the latter organisms the Pd water significantly increased the measured responses relatively to the control, by 17, 5, 8, and 30%, respectively.

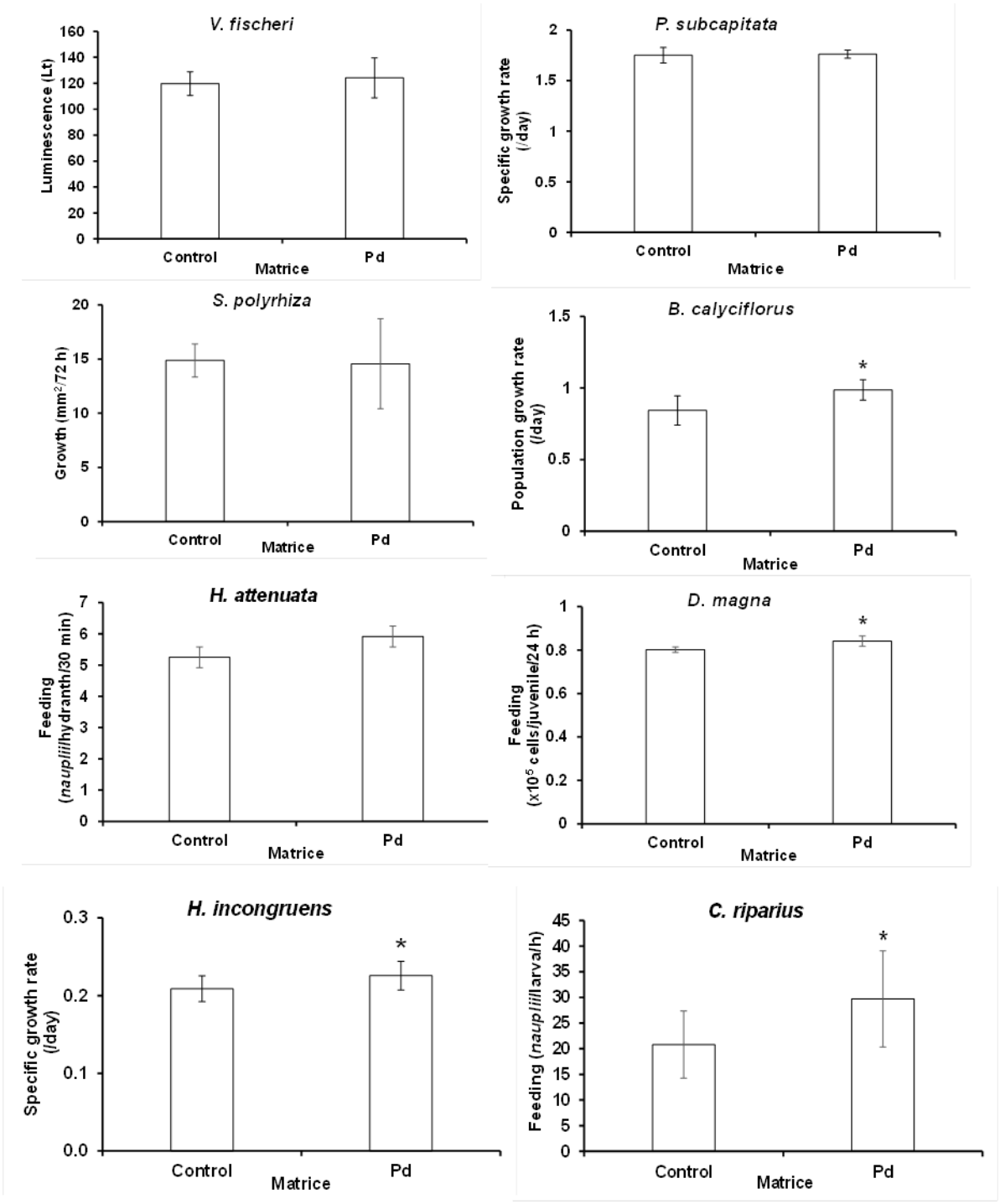

Fig. 2. Sublethal effects of the matrice puddle (Pd) on *Vibrio fischeri* (5-minutes luminescence), *Pseudokirchneriella subcapitata* (72-hours growth), *Spirodela polyrhiza* (72-hours growth), *Brachionus calyciflorus* (48-hours population growth), *Hydra attenuata* (48-hours postexposure

feeding), *Daphnia magna* (24-hours feeding), *Heterocypris incongruens* (6-days growth), and *Chironomus riparius.* Error bars indicate ± 1 standard deviation; * indicates means significantly different from respective control (by one-way [nested] ANOVA).

Regarding the soil eluates (Fig. 3), significant differences among the different matrices were observed with *P. subcapitata*, *B. calyciflorus* and *D. magna* (one-way ANOVA: $F_{7,17\text{-}56} > 4.60$, $P < 0.001$). In detail, significant differences across eluates were only found between S83 and S63 in the microalgae test, though of merely 10%, between S38/S70 and S63 by around 20% in the rotifer test, and between S28 and all other eluates except S73 in the cladoceran test, by 12 (S28 versus S63) to 38% (S28 versus S38). On the other hand, no significant differences were detected among eluates in the *H. attenuata* and *H incongruens* tests (one-way [nested] ANOVA: $F_{7,87\text{-}243} < 4.56$, $P > 0.20$); whereas with hydrants the percentage of feeding rate differences between eluates ranged from 12 (S58 versus S38/S63) to 29% (S58 versus S73), with ostracods percentages of growth rate differences attained a maximum of 10% (S58 versus S73).

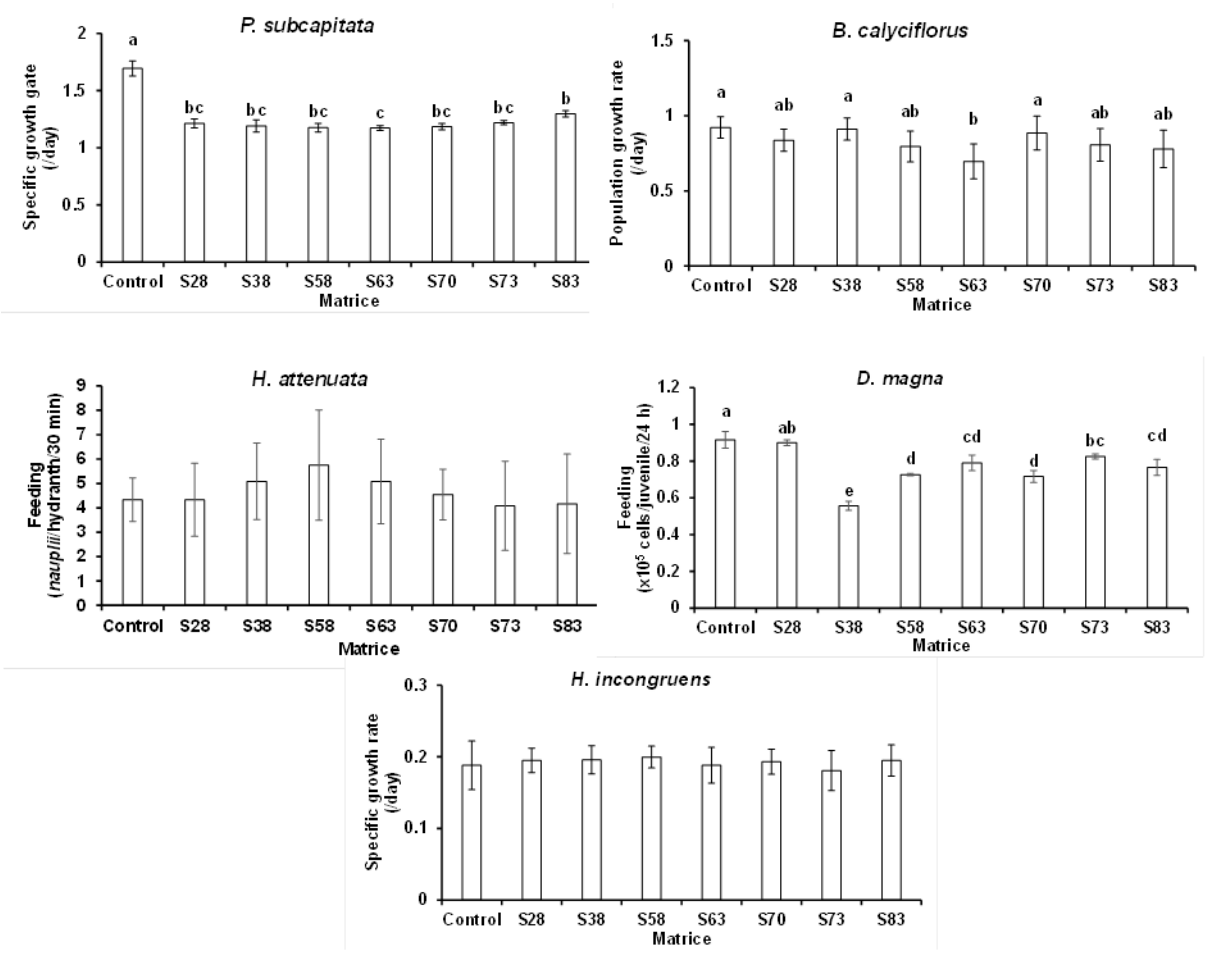

Fig. 3. Sublethal effects of the matrices soil eluates (S28, S38, S58, S63, S70, S73, and S83) on *Pseudokirchneriella subcapitata* (72-hours growth), *Brachionus calyciflorus* (48-hours population growth), *Hydra attenuata* (48-hours postexposure feeding), *Daphnia magna* (24-hours feeding), and *Heterocypris incongruens* (6-days growth). Error bars indicate ± 1 standard deviation; common letters above error bars indicate means not significantly different from each other within each toxicity test (by Tukey HDS test).

The toxicity tests performed to the river waters showed significant effects on the responses of *P. subcapitata*, *B. calyciflorus*, *H. attenuata*, *D. magna*, and *H. incongruens* (one-way [nested] ANOVA: $F_{5,13\text{-}183} > 3.17$, $P < 0.038$). Tukey HSD tests revealed significant differences between Pw4 and Pw3 (by 12%) in microalgae growth, between Pw5 and Pw1/Pw2 (by 20%) in the rotifers population growth, between Pw2 and Pw4 (25%) and Pw2 and Pw1 (32%) in the feeding of hydranths, and between Pw3/Pw4 and P1 (by 14%) in cladoceran feeding; in the ostracod test maximum percentage growth difference between waters was 6%. No significant differences were observed among river waters in the *V. fischeri*, *S. polyrhiza* and *C. riparius*

tests (one-way [nested] ANOVA: $F_{5,6\text{-}68} < 2.78$, $P > 0.064$), with percentages of difference between waters ranging from 12 (Pw3 versus Pw4/Pw5) to 16% (Pw3 versus Pw1/Pw2) in the bacteria test, from 9 (Pw2 versus Pw5) to 16% (Pw2 versus Pw1) in the macrophyte test, and from 20 (Pw3 versus Pw4/Pw5) to 30% (Pw3 versus Pw1/Pw2) in the insect larvae test.

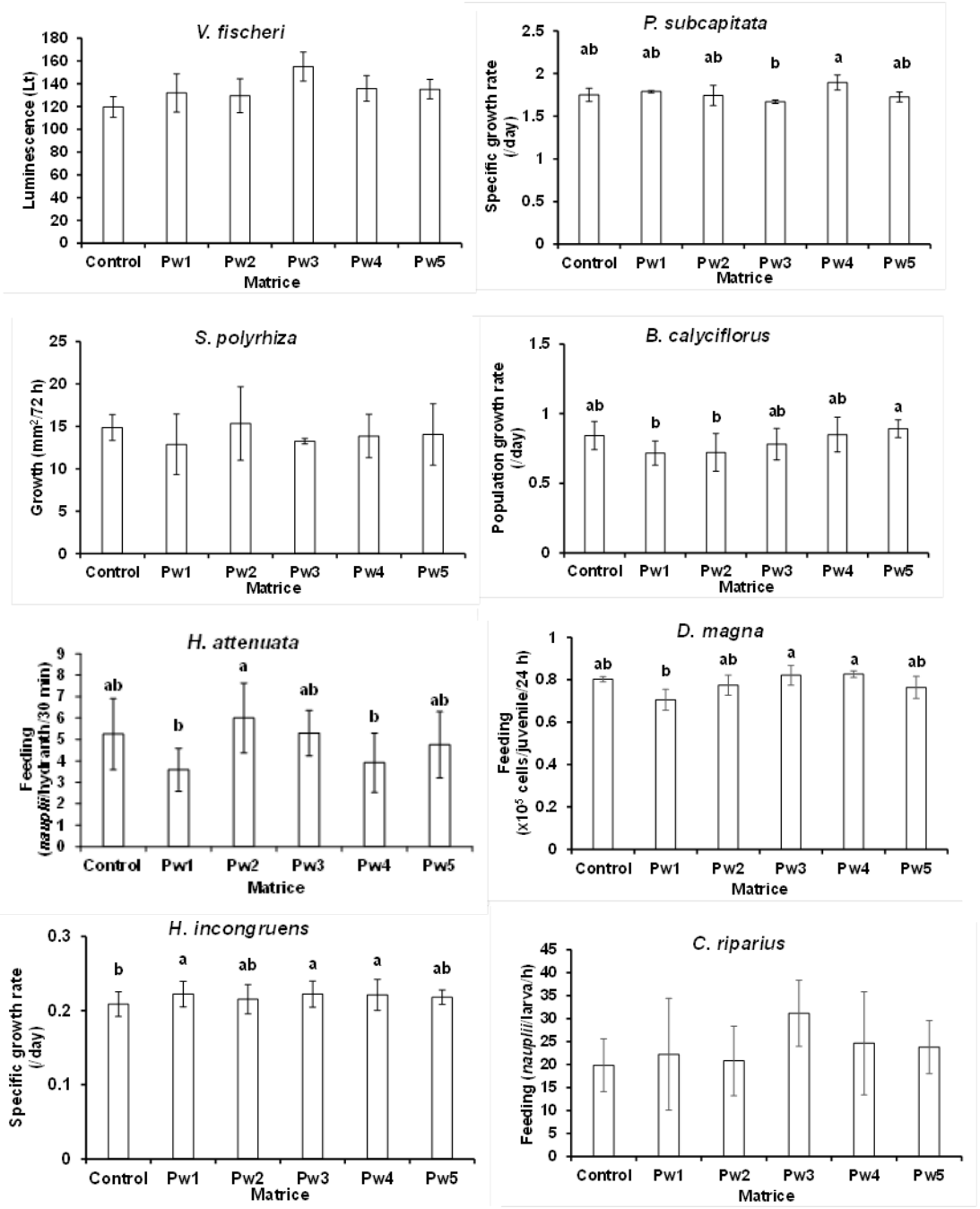

Fig. 4. Sublethal effects of the river waters (Pw1, Pw2, Pw3, Pw4, and Pw5) on *Vibrio fischeri* (5-minutes luminescence), *Pseudokirchneriella subcapitata* (72-hours growth), *Spirodela polyrhiza* (72-hours growth), *Brachionus calyciflorus* (48-hours population growth), *Hydra attenuata* (48-hours postexposure feeding), *Daphnia magna* (24-hours feeding), *Heterocypris*

incongruens (6-days growth), and *Chironomus riparius.* Error bars indicate ± 1 standard deviation; common letters above error bars indicate means not significantly different from each other within each toxicity test (by Tukey HDS test).

Regarding the river sediments (Fig. 5), significant effects were observed in the responses of *V. fischeri*, *H. incongruens* and *C. riparius* (one way [nested] ANOVA: $F_{5,6\text{-}183} > 14.2$, $P < 0.001$). The highest effects were observed with the bacteria, with luminescence at Ps1 significantly lower than at all other sediments, by 69 (at Ps5) to 77% (at Ps2); luminescence at Ps2 was also significantly higher than at all other three sediments, by 11 (Ps4) to 26% (Ps5). Growth of *H. incongruens* was significantly higher at P2 than at Ps4 (by 9%), Ps3 (by 10%) and Ps1 (by 13%). As for *C. riparius*, its feeding was significantly lower at Ps1 than at Ps3 (by 30%) and Ps4 (by 39%).

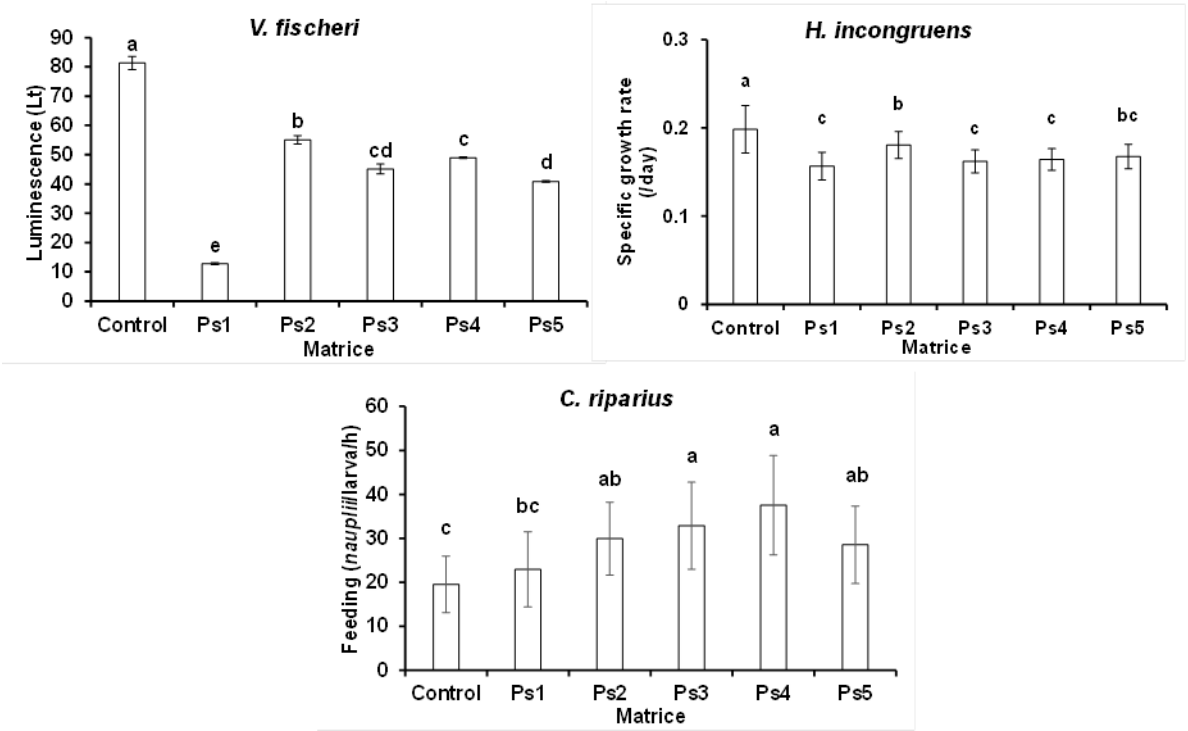

Fig 5. Sublethal effects of the river sediments (Ps1, Ps2, Ps3, Ps4, and Ps5) on *Vibrio fischeri* (5-minutes luminescence), *Heterocypris incongruens* (6-days growth) and *Chironomus riparius* (48-hours postexposure feeding). Error bars indicate ± 1 standard deviation; common letters above error bars indicate means not significantly different from each other within each toxicity test (by Tukey HDS test).

Chapter 4

Discussion

The aim of this study was to ecotoxicologically characterize the risks posed by a closed landfill to adjacent aquatic systems. To attain this objective, a battery of toxicity tests was performed on the potentially leachate-contaminated matrices to which aquatic organisms might be exposed inside and outside the landfill: the leachate itself, groundwater collected from piezometers at the fringe of the landfill, water from a puddle within the landfill, soil extracts from sites within and adjacent to the landfill area, and water and sediment samples from adjacent aquatic systems. The studied landfill was constructed in 2006 and has an area of 19 ha; it was active for 30 years which led to the deposition of 2 500 000 tons of MSW.

In environmental quality assessments, the use of precise reproducible methods, which includes standardized ecotoxicity tests with organisms representing the food chain of an ecosystem, contribute to guarantee a more comprehensive interpretation of the results of the studies. Consequently, in the last decade, the conception of a battery of toxicity tests involving the use of producers, consumers and decomposers as model organisms has been introduced (Słomczyńska and Słomczyński, 2004). In the present study, a battery of species from different taxonomic and functional groups was selected to reduce uncertainties. In what regards toxicity test endpoints in this study, mortality but mainly the sublethal relevant endpoints luminescence, feeding, reproduction and growth selected.

4.1 ECOTOXICOLOGICAL CHARACTERIZATION OF MATRICES WITHIN THE LANDFILL MONITORING PROGRAM

In the present study, the toxicity tests performed on the matrices included within the landfill monitoring program, namely the leachate (L1 and L2) and groundwaters (Pz2 to Pz5), revealed

toxicity for all the matrices, but the degree of toxicity and the species affected depended on the matrice. Overall, the leachate collected in Autumn-Winter (L1) was toxic for all test organisms; based on EC50 values it was very toxic (EC50 ≤ 1-9%) for the cnidarian, rotifer, macrophyte and cladoceran and moderately toxic (EC50 = 10 to 99%) for all remaining species, whereas based on the EC20 values the degree of toxicity was similar except that it was extremely toxic (EC50 < 1%) for the cnidarian. As for the leachate sample collected in Spring-Summer, EC50 values revealed it was very toxic only for the cnidarian, moderately toxic to the macrophyte, rotifer, cladoceran, and insect, and toxic or not toxic (EC50 ≥ 100%) for the remaining species (bacteria, microalgae and ostracod); a rather similar ranking was obtained for the EC20 values. Moreover, ratios between L2 and L1 EC50 values and also between their EC20 values, ranged between a factor of 2-3 to 6-7, with the highest ratios for the species identifying samples as extremely or very toxic.

The toxicity observed in the present study with both leachate samples was an expected result as the landfill was closed merely eight years ago and leachates are known to contain high levels of contaminants and have been shown to pose toxic effects to a wide range of aquatic organisms, including microalgae, daphnids and fish (Binet *et al*., 2003; Oshode *et al*., 2008; Alkassasbeh *et al*., 2009; Pablos *et al*., 2011). Although no physico-chemical analysis of the leachate samples used in the present study are yet available, based on the physico-chemical analysis conducted within the landfill monitoring programme in the last seven years, it can be seen that the leachate is neutral to alkaline, it presents extremely high conductivity values, metal concentrations (mainly As, Cr, Ni, Zn, Fe, and Mn) and ammonium and chloride levels, all factors known to be associated to leachates toxicity (Słomczyńska *et* Słomczyński, 2004; Alkassasbeh *et* al., 2009). In agreement, from the physico-chemical parameters measured in the 100% leachate samples during the performance of the toxicity tests, pH is alkaline and conductivity appears extremely high, being both potentially toxic factors. Indeed, whereas leachates in the initial period of waste deposition (up to 5 years) on landfills have pH ranging

from 3.7 to 6.5 due to the presence of carboxylic acids and bicarbonate ions, in landfills exploited for a long period of time, leachates become alkaline (pH 8.0 - 8.5) (Słomczyńska *et* Słomczyński, 2004).

It has been suggested in a few studies that certain physico-chemical parameters could be used for a preliminary ranking of the toxicity of leachates for aquatic organisms (Alkassasbeh *et al*., 2009; Pablos *et al*., 2011). However, such approach should not be considered a cause-effect relationship but an association between the physico-chemical parameters and the overall content of toxic substances in the leachate, as complex interactions determine contaminants bioavailability (Pablos *et al*., 2011). In the latter study, Pablos and co-authors found a moderate to strong relationship between physico-chemical variables - chlorides (Cl), alkalinity (HCO3), conductivity, ammonia (NH3) and COD - and the toxicity observed towards *D. magna* and *Xenopus* (Pablos *et al*., 2011). Corroborating the results of the present study, Pablos *et al*. (2011) reported toxic leachates to present high levels of chemical oxygen demand, conductivity and nitrogen, among others, with pH values already ranging from 6.50 to 8.96 (since the landfill was already exploited from a long time).

As above mentioned, in the present study the leachate collected in Autumn-Winter (L1) was more toxic than that collected in Spring-Summer (L2), most likely due to the leachate dilution by rain water, since L2 was collected after a long rainy period. Actually, Pablos *et al*. (2011) also reported that one of the factors influencing leachate toxicity was precipitation rate.

Regarding the groundwaters, their toxicity ranged from extremely toxic to not toxic, depending on the toxicity tests. Based on the EC50 values, Pz2, Pz3 and Pz5 were either very (EC50 ≤ 1-9%) or moderately toxic (EC50 = 10 to 99%) to all species except the microalgae, ostracod and insect, for which the three groundwaters were either toxic or not toxic (EC50 ≥ 100%). Nevertheless, Pz2 was the only groundwater being very toxic (to the rotifer and the cnidarian), whereas it presented moderate toxicity to the macrophyte and cladoceran. As for Pz3 and Pz5,

they presented moderate toxicity to the rotifer and bacteria, and Pz3 also to the cladoceran and Pz5 also to the cnidarian. Therefore, overall, Pz2 was the most toxic groundwater followed by Pz3 and Pz5. However, the ranking of species sensitivity across groundwaters was not similar either among them or relatively to the leachate, suggesting that the observed groundwater toxicity might not be leachate-related. The rotifer was the most sensitive species to all groundwaters. Then: (i) for the cnidarian Pz2, Pz3 and Pz5 were very toxic, moderately toxic and not toxic, respectively, (ii) for the macrophyte Pz2, Pz3 and Pz5 were moderately toxic, not toxic and toxic, (iii) for the bacteria Pz2 was not toxic and Pz3 and Pz5 were moderately toxic, and (iv) for the cladoceran Pz2 and Pz3 were moderately toxic and Pz5 not toxic. The Pz4 groundwater was only moderately toxic to the rotifer and for all remaining species was either toxic or not toxic.

Based on the physico-chemical measurements taken within the landfill monitoring programme in the last seven years, it can be observed that threshold legal limits for certain parameters, mainly metals and chlorides were exceeded often in Pz2, Pz3 and Pz5 and rarely in Pz4. However, a pattern within such exceedances was not disclosed, in agreement with the observed lack of ranking in species sensitivity. Also, during the performance of the toxicity tests Pz3 was the groundwater presenting highest conductivity values and the only with a pH above the legal limit of 9.0.

All in all, leachate exposures resulted in greater ecotoxicity than control exposures, as leachate is still being produced within this closed landfill with an extensive area of 19 ha, with lowest pollution levels after a rainy season, most likely due to leachate dilution by rainwater despite the counter potential effect of increased leachate generation by the augmented weight of the top layer impermeable cover. Also, although groundwater exposures resulted in greater ecotoxicity than control exposures, a direct association to potential deficiencies (e.g. leakages) in the system of capture and drainage of leachate at the base of the landfill cannot be made

due to the suggested lack of correspondence between leachate and groundwater toxicity and also due to the lack of information to characterize the bodies of groundwater within and at the fringe of the landfill.

4.2 ECOTOXICOLOGICAL CHARACTERIZATION OF MATRICES WITHIN AND OUTSIDE THE LANDFILL AREA

To more comprehensively investigate the extent of the potential contamination of adjacent aquatic systems via exposure to the landfill leachate, other matrices located within and outside the landfill area, but not included in the landfill monitoring programme, were also ecotoxicologically characterized, namely: water from a puddle within the landfill, soil extracts from sites within and adjacent to the landfill area, and water and sediment samples from adjacent aquatic systems.

The water puddle did not reveal toxicity towards either of the tested species. On the contrary, for the rotifer, cladoceran, ostracd, and insect, this matrice significantly increased their performance relatively to the standard control. The obtained results are most likely due to the fact that puddle sample was mainly composed by rainwater rather than by a leachate overflow, since it was collected after a few days of heavy rainfall. To better investigate the possible occurrence of soil contamination associated to puddles formed by leachate overflow a more intensive monitoring should be established.

Regarding the soil eluates, significant differences in organism responses across sites were detected for the microalgae, rotifer and cladoceran. However, maximum percentages of inhibition between standard control and sites or across sites were either lower than 10% or of the same order of magnitude of those found for the remaining tests organisms; the exception being the microalgae test as no nutrients were added to the soil eluates and thus nutrient

levels were not standardized across treatments. Therefore, a link between the obtained results and a potential soil contamination due to leachate was not suggested. Nevertheless, the possible influence of a slight acidic pH on the observed results should not be excluded as it was at the sites with a pH between 4.4 and 5.5 (S38, S58 and S63) that the highest percentages of inhibition were observed. A slightly acidic soil pH is within normal values for soil organisms but may be circumvented when preparing eluates by using artificial reconstituted waters rather than distilled water.

The samples of the river water at five sites caused significant effects on organism responses for all species except the bacteria, macrophyte and insect. The site at which highest inhibition of organism responses were observed was clearly Pw1: 20% rotifer population growth (relatively to Pw5), 32% cnidarian feeding (relatively to Pw2), 14% cladoceran feeding (relatively to Pw3/Pw4). Such tendency was observed even for the bacteria, macrophyte and insect, for which highest percentages of inhibition (16 to 30%) were always observed at Pw1 relatively to Pw2/Pw3. The results obtained with the river sediments corroborated those obtained with the river waters, as significant effects in organism responses across sites were observed for the three tested species with highest percentages of inhibition clearly at Ps1, by 69 to 77% for the bacteria, by 9 to 13% for the ostracod, and by 20 to 39% for the insect. However, site P1 is not only the one located furthest from the landfill area but also the only one located upstream the confluence of the rivers that flow closest to the landfill (Fig 1). Moreover, because all sediments were rather similar presenting high percentages of particles with a size range equal to or higher than very coarse to coarse sand (82 to 91%) and low percentages of silt and organic matter ($\leq$ 2%), no differences in contaminants bioavailability is to be expected. Therefore, the toxicity observed at this site is undoubtedly not related with the landfill activities but rather with other anthropogenic activities upstream in this river.

Overall, exposures to a water puddle and soil extracts from sites within and nearly the landfill did not result in greater ecotoxicity than control exposures. Although river and sediment from a single site in an aquatic systems nearby the landfill resulted in greater ecotoxicity than control exposures, this site was located highest upstream the landfill and before the confluence of the rivers flowing closest to the landfill. Therefore, the obtain results did not suggest either the occurrence of leachate overflow or deficiencies in the drainage of surface water to the rain water drainage system of the landfill, or of infiltration of contaminated groundwater.

Chapter 5

Conclusion

The battery of toxicity tests performed to the various matrices within the landfill monitoring program and not included in the landfill monitoring program, but potentially contaminated by the landfill leachate, allowed to ecotoxicologically characterize all matrices, revealing that the level of pollution on groundwaters cannot be ascribed to the leachate and that the aquatic systems adjacent to the landfill are most likely not impacted by the leachate.

Based on the results obtained in the present study, the leachate was revealed to present a high degree of toxicity. Although the leachate sample collected in Spring-Summer was less toxic than the one collected in Autumn-Winter, presumably due to the leachate dilution by rain, both leachates were toxic. Thus, it is recommended that the leachate continues to be monitored by the landfill management and treated in the water treatment plant. Given that the toxicity observed for groundwater collected at some of the piezometers was not suggested to be strongly related with the leachate, further studies to discriminate sources of groundwater contamination and more information on groundwater bodies at the fringe of the landfill are highly recommended that this study continues in order to find the source of contamination, it can be the landfill activities or not. Importantly, the results of the present study of the toxicity tests on the matrices not included in the landfill monitoring programme did not reveal toxicity, at least due to leachate, suggesting that either infiltration of contaminated groundwater, the occurrence of leachate overflow, or deficiencies in the drainage of surface water to the rain water drainage system of the landfill, were not likely to take place. Nevertheless, it should be emphasized that the matrices tested in the present study were collected once in time and thus that some level of motorization on the matrices presenting small degrees of toxicity should be taken into account (e.g. soil eluates).

Chapter 6

References

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