DEPARTAMENTO DE CIÊNCIAS DA VIDA

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Toxicity of surface versus subsurface sediments of a protected coastal lagoon under recent remediation (Paramos, Portugal)

Ana Jacinta Oliveira Gonçalves

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor Rui Ribeiro (Universidade de Coimbra) e da Doutora Matilde Moreira-Santos (Universidade de Coimbra)

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Abstract

Wetlands, especially coastal lagoons, are important ecosystems providing services being therefore one of the most developed regions supporting large urban and industrial areas. As a consequence of this development, contamination of these ecosystems is unavoidable. This is the case of the Paramos lagoon, which has a long contamination history and has lately suffered some remediation measures.

The present study aimed to assess the efficacy of the remediation measures already implemented in the Paramos lagoon, by comparing the toxicity of surface versus subsurface sediments to a battery of test organisms, as depth profiles in sediments provide information about the temporal contaminant inputs. For this purpose, a battery of standard toxicity tests was made, with organisms bearing a key role in important ecosystem functions. The following species were selected: the bacteria *Vibrio fischeri* Lehmann & Neumann (decomposer), the unicellular green algae *Pseudokirchneriella subcapitata* Korshikov (primary producer), the crustacean ostracod *Heterocypris incongruens* Ramdohr (epibenthic omnivorous) and the midge *Chironomus riparius* Meigen (benthic insect larvae; deposit feeder). To further explore the efficacy of remediation measures, the toxicity of sediments was assessed by conducting a standard toxicity test with the microalgae *Pseudokirchneriella subcapitata* and a toxicity test with the sediment rooted aquatic dicotyledon macrophyte *Myriophyllum aquaticum* Bernard Verdcourt (under standardization). The obtained results regarding the battery tests demonstrated that further intervention should be taken, since there is no clear remediation of

the site. Results for the primary producers suggest that further testing would be necessary to reduce uncertainties associated to sediment contaminants.

Key words: sediments, toxicity, battery of bioassays, primary producers, remediation

Resumo

As zonas húmidas, em especial as lagoas costeiras são ecossistemas muito importantes por providenciarem serviços do ecossistema, tendo-se tornado por isso regiões muito desenvolvidas abrangendo grandes áreas urbanas e industriais. Como consequência dessa exploração, a contaminação desses ecossistemas ao longo do tempo foi inevitável. A lagoa de Paramos encontra-se nesta situação, tendo já um longo histórico de contaminação, no entanto, mais recentemente foram tomadas algumas medidas de remediação.

Este trabalho teve como objectivo avaliar a eficácia das medidas de remediação implementadas na lagoa de Paramos, por comparação da toxicidade de sedimentos superficias com sedimentos mais profundos, uma vez que estes fornecem informação sobre a contaminação histórica, usando para esse efeito uma bateria de ensaios padronizados. Para isso foi realizada uma bateria de ensaios padrão com organismos representantes de importantes funções do ecossistema. Foram seleccionadas as seguintes espécies: a bactéria *Vibrio fisheri* Lehmann & Neumann (decompositor), a alga verde unicelular *Pseudokirchneriella subcapitata* Koršhikov (produtor primário), o crustáceo *Heterocypris incongruens* Ramdohr (omnívoro epibentónico) e o invertebrado *Chironomus riparius* Meigen (insecto bentónico; detritívoro). Para melhor explorar a eficácia das medidades de remediação, foi avaliada a toxicidade dos sedimentos através da realização do ensaio padrão com a microalga *Pseudokirchneriella subcapitata* e do ensaio de toxicidade com a macrófita *Myriophyllum aquaticum* Bernard Verdcourt. Os resultados obtidos em relação à bateria de ensaios indicam que são necessárias mais

intervenções, uma vez que não há clara remediação do local de estudo. O resultado dos ensaios com os produtores primários revelou ser necessário a realização de mais ensaios de modo a reduzir as incertezas associadas a contaminantes no sedimento.

Palavras-chave: sedimentos, toxicidade, bateria de ensaios, produtores primários, remediação

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Chapter 1 Introduction

1 - Introduction

Wetlands are transitional areas between aquatic and terrestrial environments, estimated to cover about 4 to 6% of the world's land (Mitsch & Gossenlink, 2000). They are among the richest ecosystems concerning biodiversity and primary productivity and are responsible for many processes such as production of biomass, water replacement, retention of nutrients and sediment and control of floods (Sá & Loureiro, 1995; Mitsch & Gossenlink, 2000). As a result, wetlands help to maintain water quality and provide various ecosystem services, being considered very valuable capital assets (Green *et al.*, 1994; Mitsch & Gossenlink, 2000). However, nowadays wetlands are vulnerable ecosystems facing various threats, such as urbanization, industrialization, agriculture, and pollution (Postel & Carpenter, 1997).

As a category of wetlands, coastal lagoons are very dynamic ecosystems, characterized by their relative isolation, shallowness and strong physical and ecological gradients, resulting in high productivity (Mitsch & Gosselink, 2000; Guerra *et al.,* 2009). Coastal areas are among the most developed regions supporting large urban and industrial areas, as well as the overuse of fisheries, tourism and aquaculture, all leading to the deterioration of these ecosystems, and, thus, compromising the productivity of a natural capital (Postel & Carpenter, 1997; Gönenç & Wolflin, 2005).

Contaminants, introduced into the surface water by anthropogenic inputs, accumulate in sediments, which generally act as a repository and source for many toxicants long after the pollution of surface waters (Ingersoll, 1995; De Hass *et al.*, 2002); aquatic organisms, especially benthic organisms, are thus

exposed to contaminants by both water and sediments (Giesy & Hoke, 1989). Consequently, because sediments maintain a record of past pollution, providing information on the temporal evolution of contamination (Degetto *et al.,* 1997; Bellucci *et al*., 2002), studies on the assessment of sediment toxicity fit the purpose of highlighting anthropogenic impacts of pollution (Feiler e*t al.,* 2004).

1.1 – The ecotoxicological line of evidence

To cope with environmental degradation, different approaches, i.e., lines of evidence, can be employed to assess the impacts of contaminants on aquatic ecosystems, being the most commonly used the chemical, the ecological and the ecotoxicological line of evidence (Jensen & Mesman, 2006; Crane *et al.*, 2007). Each of these approaches has strengths and limitations but the uncertainties resulting from each line of evidence can be reduced by integrating the information provided by each (Jensen & Mesman, 2006; Chapman, 2007; Crane *et al.*, 2007). In short, the chemical line of evidence quantifies contaminant levels to compare them with levels at reference sites or with screening values, being its major weakness the fact that such levels provide no information on contaminants bioavailability (Crane *et al.*, 2007). Through the ecological line of evidence, comparisons are made between exposed and non-exposed communities, but such evaluation is highly dependent on the species biology, life history and potential classification as endangered or threatened species (Crane *et al.*, 2007).

The ecotoxicological line of evidence, performs toxicity tests that are designed to measure the effects of contaminants on organisms, either in the

laboratory under controlled conditions, where simple tests provide strong cause-effect relationships, or under more realistic field conditions, where test complexity is high and results are more difficult to interpret (Cairns, 1983; Giesy & Hoke, 1989; Cooney, 1995; Rand *et al.*, 1995; Crane *et al.*, 2007). Although such tests are often performed under conditions with a low realism, both in terms of environmental variables and test species, they are still exceedingly useful for estimating probable damage from anthropogenic stress and provide information on concentrations and durations of exposure to chemicals that can be expressed in changes in behavior, biochemistry, physiology, reproduction, and survival of individuals (Cairns, 1983; Giesy & Hoke, 1989; Cooney, 1995; Maltby, 1999; Crane *et al.*, 2007). Moreover, by performing toxicity tests with a battery of test organisms selected according to their representativeness in the food chain, function at the ecosystem level and sensitivity to the potential contaminants, more comprehensive estimates of contaminant effects can be performed (Giesy & Hoke, 1989; Narracci *et al.*, 2009; Rosa *et al.*, 2010).

1.2 – The Paramos lagoon

Along the Portuguese northwest coast there are several coastal lagoons, being the Paramos lagoon the one located further north (40º58'N; 08º38'W) (Figure 1). As a coastal lagoon, the Paramos lagoon has a high biodiversity, both in terms of fauna and flora, and, thus, it is: (i) included in the National Ecological Reserve, (ii) classified as a CORINE biotope, (iii) integrated into the second phase of the NATURA 2000 network (site code PTCON0018), and (iv) classified as an Important Bird Area.

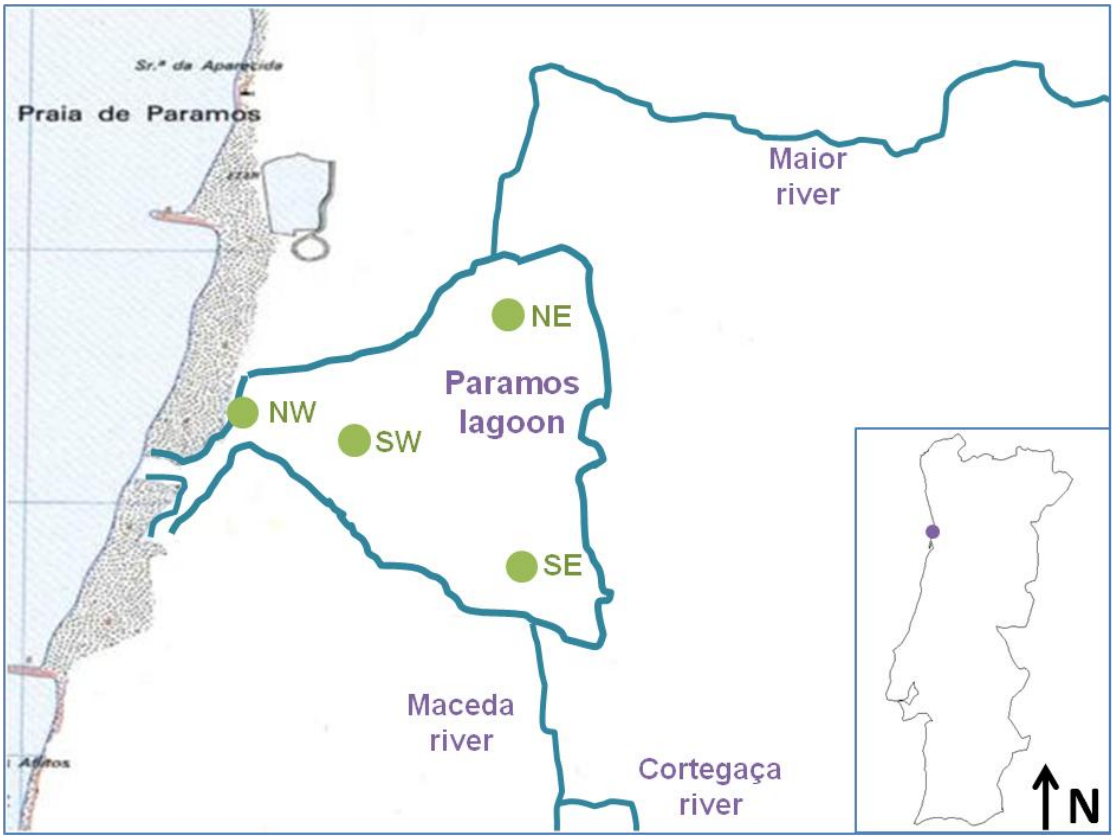

Figure 1 – Schematic representation of the Paramos lagoon (Northwest Portugal) and its two main tributaries (rivers Maior and Maceda), with location of the four selected study sites (NW, NE, SW, and SE).

Despite its ecological significance, the environmental quality of this ecosystem has long been under threat and even deteriorating (SIMRIA, 2002). Sources of contamination are mostly from untreated domestic sewages (in an over-populated region) and effluents from agricultural and industrial activities, the latter including cork, leader, wood, textile, paper, painting, and metallurgical industries, which are directly discharged into the lagoon tributaries (Dias, 2000; SIMRIA, 2002). More specifically, previous studies revealed the presence of contaminants in sediments (e.g., metals: Zn > Cu > Pb = Cr, polychlorinated biphenyls, nitrogen, and phosphorus), water (e.g., metals: similar pattern to that in sediments, pesticides) and groundwater (e.g., chloride, mineral oils) (SIMRIA,

2002; Fernandes *et al.*, 2007b; Fernandes *et al.*, 2008a,b). Additionally, studies carried out with the leaping mullet *Liza saliens* (Risso) – the dominant fish in the Paramos lagoon – revealed the occurrence of hepatic histological alterations in liver histology, changes in plasma blood biochemistry and gill permeability (Fernandes *et al.*, 2007a; Fernandes *et al.*, 2008b) and of bioaccumulation of metals in liver (copper and zinc) and muscles (zinc) (Fernandes *et al.*, 2008a).

Some remediation measures have already been implemented, like the management of the connection channel between the lagoon and the sea, that allows water exchange with the sea and the release of contaminants, and the upgrading of some sections of the tributaries and its major affluents (SIMRIA, 2002). Also as a remediation measure, the treatment centre of the city of Espinho domestic wastes, working since 1998 and covering a total population of 19,800 inhabitants, is considered a major achievement (SIMRIA, 2002). Although the sewage network of the Espinho waste treatment centre does not cover the full area of the Paramos lagoon, the fact that it is already operational for more than ten years suggests that some remediation of water and sediments might have taken place. Nevertheless, despite the ecological significance of the Paramos lagoon and the many contaminant inputs, knowledge on the effects of contaminants on the biological communities and on the efficacy of remediation measures is scarce or even inexistent.

1.3 – Study objectives

The main objective of the present study was to assess the efficacy of the remediation measures already implemented in the Paramos lagoon, by comparing the toxicity of surface (S) versus subsurface (D) sediments to a battery of test organisms, as depth profiles in sediments provide information about the temporal contaminant inputs (Belluci *et al.*, 2002). To attain this goal, two specific objectives were delineated. The first, to perform a battery of standard toxicity tests with organisms bearing a key role in important ecosystem functions. The following species were selected: the bacteria *Vibrio fisheri* Lehmann & Neumann (decomposer), the unicellular green algae *Pseudokirchneriella subcapitata* Korshikov (primary producer), the crustacean ostracod *Heterocypris incongruens* Ramdohr (epibenthic omnivorous), and the midge *Chironomus riparius* Meigen (benthic insect larvae; deposit feeder). The second, to further explore the efficacy of the remediation measures toward primary producers. For this, the toxicity of sediments was assessed by conducting not only the standard toxicity test with the microalgae *P. subcapitata* but also a toxicity test with the sediment rooted aquatic dicotyledon macrophyte *Myriophyllum aquaticum* Bernard Verdcourt, given that the latter test, though still under standardization, allows to evaluate toxicity via the sediment or pore water (Arts *et al.*, 2008).

All toxicity tests selected to perform the present study were considered and designed to focus on the following key features: (1) to assess sediment toxicity as sediments act as a sink and source of contamination, (2) to evaluate sensitive biological responses to maximize their likelihood of being responsive

to the remediation measures, (3) to use species with a key role in different ecosystem functions as a wide range of contaminants was expected, (4) to collect sediment samples representative of a worst-case scenario (dry season, i.e., when the dilution of contaminants in effluents is minimal), as this will cover the entire temporal scale, and finally (5) to collect samples covering relevant areas of concern, i.e., major routes of contaminants discharge.

Chapter 2 Materials and Methods

2 – Material and Methods

2.1 – Study site

The Paramos lagoon is located on the Northwest coast of Portugal (40º58'N; 08º38'W), with a total area of 396 ha, 1,500 m of length (N-S) and 700 m of width (W-E), a 2.5 m maximum depth, and a catchment area of 78 Km^2 (SIMRIA, 2002). The dynamics of this lagoon is dependent mainly on its communication with the sea, which is established through a non-permanent channel, and on the two major tributaries, one in the north (river Maior, also known as river Paramos) and another in the south (river Maceda), via which the system receives most (untreated) effluents (Figure 1) (SIMRIA, 2002). Groundwater and rain also contribute to the dynamics of the lagoon (SIMRIA, 2002). Four sampling sites were selected within the wet area of the lagoon so that they would not only cover the whole lagoon area but also the regions close to the two tributaries (likely to be the most contaminated) and to the non-permanent channel (likely to be the least contaminated). At the north side of the lagoon, sites NW and NE were in the region of the non-permanent channel and river Maior, respectively, whereas at the south side sites SW and SE were located in the regions of the centre of the lagoon and river Maceda, respectively (Figure 1).

2.2 – Experimental design

To assess the ecotoxicological efficacy of the remediation measures so far implemented in the Paramos lagoon, surface (S) and subsurface (i.e., up to 15 cm deep; D) sediment samples were collected once during the dry season (to mimic a worst-case scenario of contamination) at each of the four study sites. All toxicity tests were performed on both S and D sediment samples – only the 100% dilution. Whereas all toxicity tests performed to fulfil specific objective 1 were carried out within two weeks of sediment collection, the toxicity tests with the two primary producers to fulfil specific objective 2 were carried out 12 months after sediment collection. It is a fact that after one year of storage the bioavailability of the contaminants in the sediments might have been altered. Yet, it should be pointed out that the main purpose of the second part of the present study was to compare the responses of two primary producers (microalgae and macrophyte) and not to identify which specific contaminants would have a toxic effect on the primary producers at the Paramos lagoon.

2.3 – Collection of water and sediment samples

At each of the four study sites, surface (5-cm depth) water samples were collected into acid-washed 1.5-L polyethylene–terephthalate bottles (three per site) and transported to the laboratory in thermally insulated boxes (below 15°C in darkness). Upon arrival to the laboratory, part of the water was stored at 4°C in darkness to be used in the toxicity tests (within 2 weeks and 12 months for

specific objectives 1 and 2, respectively) and the remaining was immediately filtered (0.20 µm) and deep frozen for chemical analysis (see below).

Composite sediment samples were collected at each of the four study sites into acid-washed high density polyethylene bottles, immediately transported to the laboratory in thermally insulated boxes (below 15°C in darkness) and stored at 4°C in darkness to be used in the toxicity tests (within 2 weeks and 12 months for specific objectives 1 and 2, respectively) and for sediment physical determinations (see below). At each site, 25 to 30 sediment cores with a depth of 15 cm were retrieved. From each core, the first 5 cm were taken as surface sediment and the subsequent 10 cm as subsurface sediment. Approximately 2 and 3 L of surface and subsurface sediment were collected at each site, respectively.

2.4 – Sediment and water physico-chemical characteristics

At each site, pH (Wissenschaftlich Technische Werkstätten 537 pH meter, WTW, Weilheim, Germany), conductivity (WTW Cond315i/SET conductivity meter), salinity (HANNA Instruments Seawater Refractometer HI 968222, Woonsocket, RI, USA), and dissolved oxygen (WTW OXI 92 oxygen meter) were measured prior to water and sediment collection. The water chemical parameters measured in the laboratory were hardness, ammonia, nitrate, nitrite and reactive phosphorous determinations. All the latter parameters were determined by ion chromatography (DX120 Ion Chromatograph integrated system, Dionex, Sunnyvale, CA, USA)), except

reactive phosphorus, which was determined by the ascorbic acid method (APHA/AWWA/WPCF, 1995),

Laboratory physico-chemical characterization of surface and subsurface sediments included the determination of humidity, organic matter content and particle size distribution. Humidity was determined as the mean percentage loss of the initial wet weight of three sediment aliquots of each sample after drying at 60°C for five days. Organic matter content was determined as the mean percentage loss of the dry weight of the later sediment aliquots after igniting them in a muffle furnace (Nabatherm L3, Lilienthal, Germany) at 550°C for 8 hours (Buchanan & Kaine, 1971). As for the sediment particle size distribution it was determined on approximately 100 g of dried sediment using a standard sieving technique through a sequence of six sieves (from 2000, to 63 µm; Retsch, Haan, Germany) on a sieve mechanical shaker (agitation provided for 15 minutes at 1 mm vibration; Retsch AS 200) (Buchanan & Kaine, 1971). Each sediment fraction was weighted and expressed as a percentage of the total final weight.

2.5 – Toxicity tests

The luminescence test with the marine bacteria *Vibrio fischeri* was conducted according to the basic solid-phase test (Azur Environmental, Carlsbad, CA, USA). The light emission of the test organisms was measured using the microtox toxicity analyzer model 500 (Strategic Diagnosis, Newark, DE, USA) after a maximum exposure period of 30 minutes. According to the test protocol, the maximum sediment concentration that can be tested is

197,400 mg/L of the test diluent. For each sediment sample, the latter concentration was tested in duplicate, whereas the standard microtox control was tested in triplicate.

The 72-hours growth test with the microalgae *P. subcapitata* was done following, as close as possible, the OECD (OECD, 1996) and EC (EC, 1992) guidelines and methodologies described in Moreira-Santos *et al.* (2004) to conduct toxicity tests with microalgae cells immobilized in calcium alginate beads. The latter approach allows performing toxicity tests on the sediment-overlying water since it avoids the loss of algae into the sediment and permits the recovery of all exposed cells at the end of the test (Moreira-Santos *et al.*, 2004). Stock cultures of *P. subcapitata* were maintained in 100-ml nonaxenic batch cultures with Woods Hole MBL growth medium (Stein 1973), at 19 to 21°C under continuous cool-white fluorescent illumination (100 $\mu E/m^2/s$). Beads with *P. subcapitata* cells were prepared as described in detail by Moreira-Santos *et al.* (2004). In short, a volume of an algal cell suspension (obtained from an exponentially growing algal culture) was gently mixed by gentle stirring with a 1.3% (w/v) solution (prepared with distilled water) of sodium alginate (Sigma Chemical, A-7128, Steinheim, Germany) to obtain an alginate-cell suspension with a nominal cell concentration of 10^5 cells/ml of alginate. Beads were formed by dropping the latter suspension through a syringe equipped with a needle into a 2% (w/v) $CaCl_2$ solution, in which they were kept stirring for 45 minutes for gel hardening to take place. They were then washed with distilled water, stored in roughly 20-times diluted MBL medium, in the dark at 4°C, and used within 15 days of preparation. Beads used in the toxicity test performed to fulfil specific objective 1 had a mean diameter of 2.7 mm (n = 50) with a

coefficient of variation (CV) of 7%, whereas correspondent values of beads prepared to conduct the toxicity test of specific objective 2 were 3.1 mm (*n* = 50) and a CV of 8%.

For each sediment three replicates were set up each consisting of 150 ml glass vials filled with 50 g (dry weight) of sediment plus 50 ml of local water previously vacuum-filtered (0.45 µm) to remove indigenous microalgae. A control treatment, also with 3 replicates, consisted simply of 50 ml of MBL medium diluted 2.5 times to be in accordance with the required N/P ratios (OECD, 1996). Vials, except control ones, were prepared 12 to 18 hours prior to the beginning of the test and left with continuous aeration, to allow stabilization between sediment and water. After the letter period, aeration was stopped and 15 to 20 beads were added per replicate. To prevent the possible dissolution of the beads due to the presence of chelating agents in the sediment, beads were placed at the top of a 250 µm mesh screen (also in the control replicates to eliminate possible differences in light intensity). Toxicity tests were conducted under the same temperature and light conditions used for stock culturing. At the end of the 72-hours exposure, the mean specific growth rate per day was estimated. To estimate initial and final cell densities, beads from each replicate were dissolved in 3 ml of a 3% (w/v) solution of trisodium citrate (Sigma, 71404, Steinheim, Germany) with the help of a vortex mixer Unimag Zx (UniEquip, München, Germany). Cell counts were made on well-mixed aliquots of each replicate under a microscope at 400× magnification, using a Neubauer chamber (American Optical, Bufalo, NY).

The 15-day growth test with the rooted macrophyte *M. aquaticum* was performed according to a “Standardized method for investigating test substance

impact on rooted aquatic macrophytes", a protocol that is still under development (Maltby *et al.*, 2010), and to ISO guidelines (ISO, 2009) to determine toxic effects of sediments on the growth of *M. aquaticum*. Stock cultures of the terrestrial form of *M. aquaticum* were maintained in glass aquaria filled with artificial sediment (Maltby *et al.*, 2010) at 19 to 21°C under a 16-hours:8-hours light (cool-white fluorescent illumination at 140 $\mu E/m^2/s$):dark photoperiod.

For each sediment, three replicates were set up, each consisting of small pots (10-cm diameter x 9-cm height) filled with 500 ml of sediment and placed inside glass vials (11-cm diameter x 24-cm height) filled with 2 L of Smart and Barko medium (Maltby *et al.*, 2010). Each pot was previously planted with three shoots apices cut from the culture at a minimum 6-cm height so that the lower two nodes were planted beneath the sediment surface. The sediment surface of each replicate was covered with a thin layer of sand (< 5 mm in particle size) to assist in keeping the sediment in place when adding the 2 L of medium. Test vessels had a minimum of a 12-cm water-column height above sediment surface to allow plants to growth submerged during the entire test period. For the control treatment, 11 replicates were set up with artificial sediment (Maltby *et al.*, 2010). From these, five replicates were removed after a 3-days pre-test culturing phase, during which root formation takes place, to estimate plant biomass (wet weight) at the start of the test. The toxicity test (pre-test and test itself) was incubated under the same conditions as for stock culturing, though the illumination source consisted of a neutral white light and temperature fluctuated from 18.5 to 21.5°C. During the exposure period water levels were daily adjusted with distilled water. At day 11, 1000 and 500 ml of medium were

renewed in the control and treatment replicates, respectively, to prevent microalgae growth during testing. At days 0, 4, 11, and 14 total shoot length (main and lateral shoots) of each plant was measured with a ruler. At the end of the 15-days exposure period, plants were harvested and total shoot length and whole plant biomass (wet weight) were determined. Plant growth was determined as the mean specific growth rate per day.

The 6-day growth test with the ostracod *H. incongruens* was conducted according to the Ostracodtoxkit F standard operating procedure (Creasel, 2001); the purchased kit contains all the necessary materials to perform tests with this organism. The medium used to hatch the organisms and as overlying water for all sediments was reconstituted moderately hard water (ASTM, 2002). For hatching, cysts were incubated in the latter medium at 25°C under continuous illumination (approximately 50 µE/m^2/s) for 52 hours. After the first 48 hours of incubation pre-feeding was carried out with spirulina-powder. For each tested sediment, five replicates were set up, each consisting of 1 mL of sediment plus 2 mL of algal food suspension, and 10 recently hatched ostracods. A standard control treatment was also set up with reference sand. The test was conducted at 25°C in darkness. After the 6 days exposure period, percentages of mortality were determined and growth was estimated as the total body length (in µm).

The *C. riparius* 10-day growth test and 48-hours postexposure feeding test were conducted according to the OECD (OECD, 2004) and EC (EC, 1997) guidelines, and procedures described in detailed in Soares *et al.* (2005), respectively. Whereas first-instar larvae were used in the growth test, the postexposure feeding test used third-instar larvae. Larvae for testing were

obtained form laboratory cultures consisting of crystallizing dishes containing 185 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) every other day (0.1 g/dish, with 30 and 15 larvae/dish up to day seven and from there onwards, respectively), and maintained at 19 to 21 ºC, under a 14-hours:10-hours light (50 µE/m^2/s):dark photoperiod with 90-minutes dawn and dusk periods (for further details see Rosa *et al.*, 2010).

For each sediment and also for the standard control using the same sediment and medium as the cultures, four and three replicates were set up for the growth and postexposure feeding tests, respectively. Each replicate consisted of 175-mll glass vials filled with 50 g (dry weight) of sediment plus 100 ml of local water under continuous aeration. Vials were prepared 12 hours prior to the beginning of each test, to allow stabilization between sediment and water. At the start of the tests, three and five larvae were added per replicate, for the growth and postexposure feeding test, respectively, and 30 minutes latter aeration was restarted. Food was provided only during the growth test at a daily rate of 1 mg of TetraMin per larva up to the second day and 1.5 mg Tetramin per larva from day 2 onwards. Both tests were conducted under the same environmental conditions as those used for culturing. Water levels were daily adjusted with distilled water. At the end of the 10-days exposure period, percentages of mortality were determined and growth was estimated as the body dry weight (in mg). At the end of the 48-hours exposure feeding, larvae were retrieved from the sediment, immediately individually transferred to a 50 ml glass vial filled with 30 ml of ASTM hard water and 100 defrosted nauplii (<

than 24-hours old) of *Artemia franciscana* Kellog, allowed to feed at 20°C in darkness for 1 hour, time after which larvae were retrieved and the remaining nauplii were counted. Feeding rates (number of nauplii/larva/hour) were calculated as the difference between the initial and the final number of nauplii.

Levels of pH, conductivity and dissolved oxygen were measured in overlying water during the following toxicity tests: macrophyte growth at days 0, 4, 7, 11 and 14, microalgae growth and both *C. riparius* tests at test initiation and end. Values measured in the controls were within the limits established in the guidelines, whereas those measured in the treatments were within levels known not to be appropriate for the test organisms.

2.6 – Statistical analyses

For all toxicity tests performed, the effect of the two main factors, sediment depth (two levels: surface and subsurface) and sediment site (four levels: NW, NE, SW, and SE), and their interaction on the organism responses was evaluated. The violations of normality and homoscedasticity were checked using the Shapiro–Wilk's and Bartlett's tests, respectively. For the *V. fischeri* test, a 2-way analysis of variance (ANOVA) was applied on the adjusted absolute luminescence (mean of the two subreplicates) calculated by multiplying the percentage of luminescence of each treatment subsample after a 30 minutes exposure (i.e., 100 minus the % of effect inhibition as given by the Microtox Omni Software 1.18; Azur Environmental) with the overall mean of all control luminescence readings , given that the microtox has a different control

luminescence for each subsample. For both the microalgae tests, growth differences were evaluated through a 2-way ANOVA. For the macrophyte test and for both *C. riparius* tests, organism responses were compared through a 2-way nested ANOVA. Although a nested ANOVA design was used in the ostracod test, a two-way ANOVA was applied to the growth data as a mean to fulfil the assumption of homoscedasticy. When significant effects were detected, the latter analysis were followed by planned comparisons to test for the effects of one factor within the other (if the interaction effect was significant) or for the effects of one factor irrespectively of the other (if the interaction effect was not significant), and by the Tukey HSD test when necessary. All statistical analysis were conducted on Statistic 7.0 software.

Chapter 3 Results

3 - Results

3.1 – Sediment and water physico-chemical characteristics

The percentage of organic matter was much higher in surface than in subsurface sediments at all study sites, except at NW where similar percentages were found (Table I). The particle size distribution was reasonably similar among all sites, both for surface and subsurface sediments (Table I). Whereas for sites NW, SW and SE more than 50% of both the surface and subsurface sediment was composed of medium sand and more than 75% of medium and coarse sand together, sediment from site NE had the highest percentage of very coarse sand (> than 30%) and gravel (> than 9%), especially the subsurface sediment.

Water physico-chemical characteristics in what regards levels of pH, salinity and hardness were very similar among all four study sites, whereas conductivity ranged from 334 to 1460 µS/cm (Table II). Dissolved oxygen levels were much lower at site NE (< than 2 mg/L) than at all other sites (> 5 mg/L). Regarding nutrient levels the highest values were found at site SE and rather similar levels were found among the other three study sites.

Table I. Organic matter content (in %) and particle size distribution (in %) for surface (S) and subsurface (D) sediments collected at each of the four study sites (NW, NE, SW, SE) in the Paramos lagoon (Northwest Portugal).

	Site							
	NW		NE		SW		SE	
	S	D	S	D	S	D	S	D
Organic matter	0.252 (± 0.010)	0.529 (± 0.36)	12.3 (± 1.6)	1.16 (± 0.18)	7.74 (± 3.4)	0.670 (± 0.12)	8.11 (± 0.88)	0.549 (± 0.030)
Particle size (µm)								
>2000	0.14	0.00	9.22	23.27	0.00	0.12	11.32	7.11
1000 – 2000	0.08	0.19	33.45	34.00	0.13	0.42	12.65	11.51
500 – 1000	8.19	16.74	39.71	27.83	13.24	15.40	27.34	27.31
250 – 500	84.05	74.38	16.47	12.92	73.24	75.10	46.07	50.21
125 – 250	7.12	7.39	0.70	1.28	8.30	5.45	2.05	3.53
63 – 125	0.21	0.79	0.24	0.29	1.91	1.14	0.23	0.15
< 63	0.20	0.50	0.21	0.42	3.18	2.37	0.34	0.17

Table II. Water physico-chemical parameters measured at each of the four study sites (NW, NE, SW, SE) in the Paramos lagoon (Northwest Portugal). Values of pH, salinity, conductivity (Cond.), and dissolved oxygen (DO) were measured in the field and those of hardness, nitrite, nitrate, ammonium, and phosphate were measured in samples collected at the study site and kept frozen until analysis (within 1 day).

	Site			
Parameter	NW	NE	SW	SE
pH	7.40	7.20	7.49	7.24
Salinity	0.5	0.1	0.5	0.0
Cond. (µS/cm)	1460	686	1431	334
DO (mg/L)	5.2	1.8	7.2	8.7
Hardness (mg $CaCO_3$/L)	142	145	182	104
NO^{2-} (mg/L)	< 0.1	< 0.1	< 0.1	0.435
NO^{3-} (mg/L)	0.0815	0.0741	2.001	7.32
NH_4^+ (mg/L)	< 0.05	< 0.05	< 0.05	1.296
PO_4^{3-} (mg/L)	0.0374	0.0426	< 0.03	0.6291

3.2 – Remediation efficacy with a test battery

All toxicity tests fulfilled the validity criteria for control performance established in the adopted guidelines/standard operating procedures. In the 30-minutes luminescence test with *V. fischeri*, results of a 2-way ANOVA revealed a significant effect of both main factors (site: $F_{1,3} = 139$, $P < 0.001$; depth: $F_{1,1} = 18$, $P < 0.001$) and of the interaction effect ($F_{1,3} = 9.6$, $P < 0.001$). As shown in Figure 2, the bacteria luminescence was significantly lower in surface than in subsurface sediments at site SW (planned comparisons: $F_{1,16} = 43$, $P < 0.001$), whereas for the other three sites no differences were observed between both sediment depths (planned comparisons: $F_{1,16} < 1.6$, $P > 0.2$). Differences in luminescence among sites were found both for surface and subsurface sediments (planned comparisons: $F_{3,16} > 43$, $P < 0.001$). For both sediment

depths luminescence was significantly lower at site SW than at all other sites (Tukey: $P < 0.001$).

Results of a 2-way ANOVA on the 72-hours growth rate of the microalgae showed that growth was significantly affected by site ($F_{1,3} = 34$, $P < 0.001$) and by the interaction effect ($F_{1,3} = 18$, $P < 0.001$) but not by the sediment depth ($F_{1,1} = 18$, $P = 0.14$). Due to the interaction effect, differences among sites were only revealed for subsurface sediments (planned comparisons: $F_{3,16} > 49$, $P < 0.001$), with growth at NW and SW being significantly lower than at NE (Tukey: $P < 0.001$), and growth at SE being significantly higher than at all other sites (Tukey: $P < 0.05$) (Figure 2). Also, differences between surface and subsurface sediments were found at all sites, with growth at surface significantly higher than at subsurface at sites NW and SW (planned comparisons: $F_{1,16} > 4.6$, $P < 0.05$) and the opposite at sites NE and SE (planned comparisons: $F_{1,16} > 5.2$, $P < 0.05$).

When *H. incongruens* was exposed to the tested sediments, 74% mortality was registered for the surface sediment at site SW (well above the criterion of 20% allowed for the standard control), whereas a 100% survival was registered for all other sediments. A 2-way ANOVA revealed that the 6-days growth was influenced by site ($F_{1,3} = 53$, $P < 0.001$) and by the interaction ($F_{1,3} = 41$, $P < 0.001$), but not by the sediment depth ($F_{1,1} = 3.7$, $P = 0.054$). Differences among sites were observed both for surface and subsurface sediments (planned comparisons: $F_{3,327} > 29$, $P < 0.001$). Growth was higher at site NW for S sediments (Tukey: $P < 0.001$) and at sites NW, SW and SE for subsurface sediments (Tukey: $P < 0.001$), and was higher for surface than subsurface sediments at sites NW and NE (planned comparisons: $F_{1,327} > 16$, P

< 0.001) and lower for surface than for subsurface at site SW (planned comparisons: $F_{1,327} = 82$, $P < 0.001$) (Figure 2).

For the 10-days growth test with *C. riparius*, larval mortality was observed only for subsurface sediments at sites SW (17%) and SE (8%), but both values were below the criterion of 30% allowed for the standard control. A 2-way nested ANOVA showed growth to be affected by site ($F_{1,3} = 17$, $P < 0.001$) and by the interaction ($F_{1,3} = 4.7$, $P < 0.01$), and not by sediment depth ($F_{1,1} = 3.1$, $P = 0.090$). For surface sediments growth was significantly higher at site NW than at all other sites (Tukey: $P < 0.001$), whereas for subsurface sediments growth was significantly higher at NW and SW than at NE (Tukey: $P < 0.05$) (Figure 2). Differences between surface and subsurface sediments were only found at sites SW and SE, with growth at surface being lower than at subsurface (planned comparisons: $F_{1,55} > 5.4$, $P < 0.05$) (Figure 2).

In the 48-hours *C. riparius* postexposure feeding test, a 2-way nested ANOVA revealed a significant effect of site ($F_{1,3} = 53$, $P < 0.001$) and of the interaction ($F_{1,3} = 4.1$, $P < 0.05$), but not of the sediment depth ($F_{1,1} = 0.16$, $P = 0.70$). For surface sediments postexposure feeding was lower at site NE that at all other sites (Tukey: $P < 0.001$), whereas for subsurface sediments postexposure feeding was lower at sites NE and SW than at the other two sites (Tukey: $P < 0.001$) (Figure 2). A difference between surface and subsurface sediment was found only at site NW (planned comparisons: $F_{1,88} = 13$, $P < 0.001$) (Figure 2).

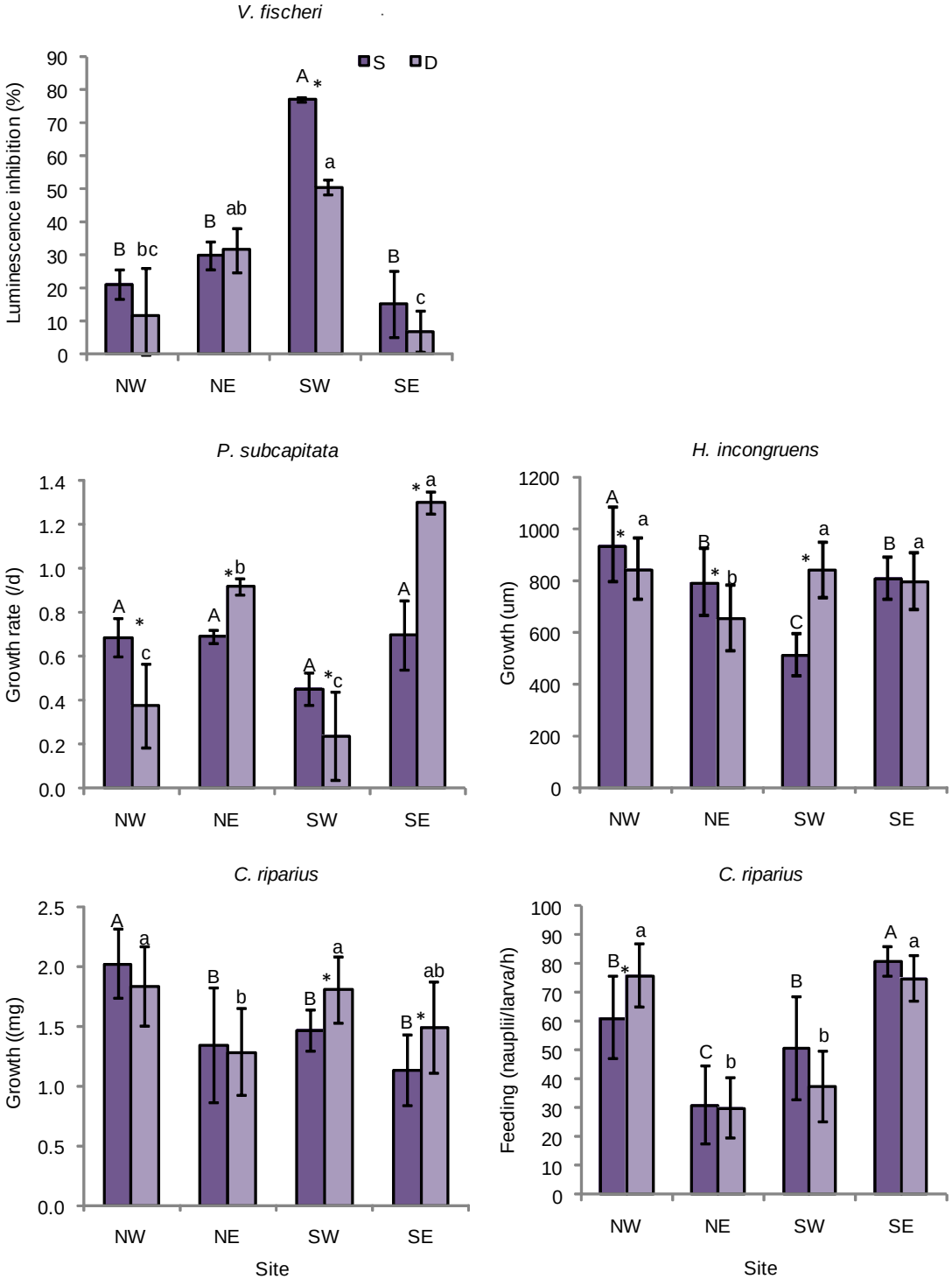

Figure 2. Sublethal effects of surface (S) and subsurface (D) sediments collected at each of the four study sites (NW, NE, SW, SE) in the Paramos lagoon (Northwest Portugal), on *Vibrio fischeri* (30-minutes luminescence), *Pseudokirchneriella subcapitata* (72-hours growth), *Heterocypris incongruens* (6-days growth), and *Chironomus riparius* (10-days growth and 48-

hours postexposure feeding). Error bars indicate ± 1 standard deviation; common letters above error bars indicate means not significantly different between sites – capital letters for differences within S sediments and small letters for differences within D sediments – and asterisks denote significant differences between S and D sediments within each site (by planned comparisons and Tukey tests when necessary).

3.3 – Further investigation on primary producers

The microalgae test fulfilled the validity criteria established in the adopted guidelines. A 2-way ANOVA revealed that only site had an effect on the growth of *P. subcapitata* ($F_{1,3} = 11$, $P < 0.001$), with growth at site NW being lower than at all other sites (Tukey: $P < 0.05$) (Figure 3).

The macrophyte growth test fulfilled the validity criterion established in the under-development protocol of Maltby et al. (2010) regarding biomass increase, but not that established in the ISO (2009) guidelines regarding the specific growth rate; according to the latter protocol the test is to be started with shoots of a much smaller length which are expected to have a different growth rate. A 2-way nested ANOVA showed that growth was significantly affected only by site ($F_{1,3} = 21$, $P < 0.001$). Growth at site NW was lower than at sites NE and SW (Tukey: $P < 0.05$) and at site NE was higher than at all other sites (Tukey: $P < 0.001$) (Figure 3).

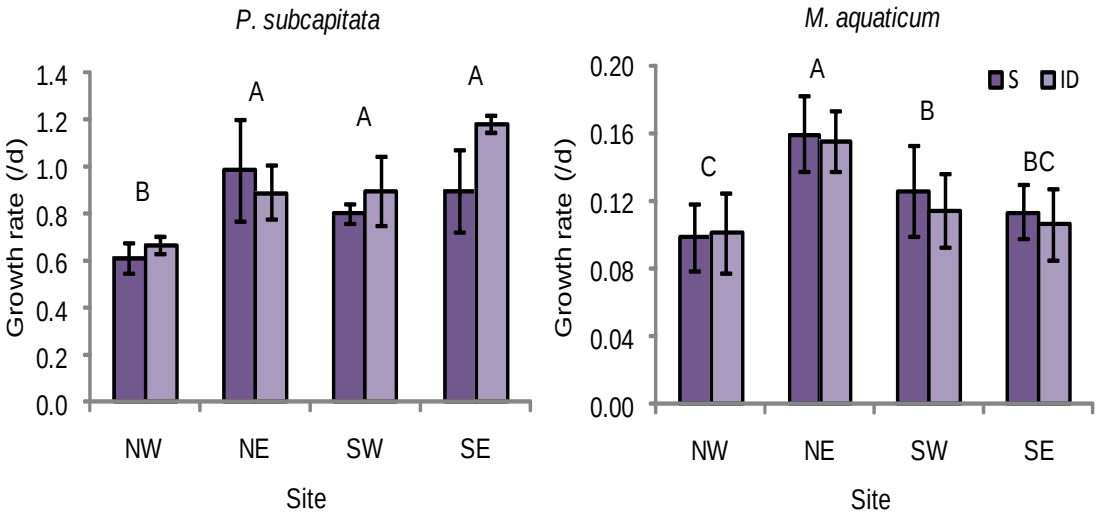

Figure 3. Sublethal effects of surface (S) and subsurface (D) sediments collected at each of the four study sites (NW, NE, SW, SE) in the Paramos lagoon (Northwest Portugal), on *Pseudokirchneriella subcapitata* (72-hours growth) and *Myriophyllum aquaticum* (15-days growth). Error bars indicate ± 1 standard deviation; common letters above error bars indicate means not significantly different between sites – capital letters for differences within sites (NW, NE, SW, SE).

Chapter 4 Discussion

4 – Discussion

4.1 – Remediation efficacy with a test battery

To evaluate the efficacy of the remediation measures that have taken place up to date in the Paramos lagoon (Northwest Portugal), a battery of toxicity tests on surface and subsurface sediments from four selected study sites was carried out with organisms representative of different: (i) throphic levels in the food web, (ii) taxonomic groups and (iii) functions at the ecosystem level. Previous studies indicated that sediments from this lagoon were contaminated mainly with metals, such as zinc, copper, lead and chromium, but also with polychlorinated, biphenyls, phosphorous, nitrogen (SIMRIA, 2002; Fernandes *et al.*, 2007a; Fernandes *et al.,* 2008a,b).

It is well known that toxicity tests/bioassays provide relevant information on the biological damage caused by contamination, i.e., on the environmental quality of sediments (Chapman, 2002; Ghirardini *et al.,* 2005). Assessing sediment toxicity by using standard bioassays with a battery of test species is essential because: (i) species sensitivity varies among toxicants, no single species is more sensitive to all contaminants (Burton, 1991), (ii) provides insight into the potential effects of contaminants on the population dynamics (Maltby, 1999), (iii) gives a direct measure of functional responses (Giesy & Hoke, 1989), and (iv) integrates additive, synergistic and antagonistic effects of all contaminants (Pandard *et al,.* 2006). Thus, using a battery of bioassays is an important tool to reduce uncertainties, to provide robustness in toxicity

assessments, and, depending on the type of assays selected, it has also the potential to be a rapid screening tool (Giesy & Hoke, 1989; Bailey & Young, 1997; Narracci *et al.,* 2009).

In what regards bioassay endpoints, growth and reproduction are among the most classically and commonly used sublethal organism measurements, mostly because they are generally sensitive responses whose consequences at the individual level are expected to be transferred to population, community and finally to ecosystem structure and functions in a time-delayed process (Giesy & Hoke, 1989; Maltby, 1999). However, effects on reproduction and growth imply time-delayed extrapolations from individuals to population and ecosystems (Krell *et al.*, 2011). Yet, postexposure feeding has shown to be an important endpoint to be evaluated since exposure to stressors can have direct effect on the feeding rate which in turn induces changes in growth and reproduction of the population and thus has eventually effects on ecosystem functioning (Matlby, 1994; Maltby, 1999). Feeding answer has been proved to be a sensitive and fast endpoint to be measured, since it takes only few days (Alonso *et al.*, 2009; Maltby, 1994; McWilliam & Baird, 2002). Furthermore using feeding as an endpoint has advantages because a depression in feeding may have direct and indirect effects on the ecosystems by preventing the functioning of the ecosystem before its effects at individual level may have consequences at higher levels of biological organization (Krell *et al.*, 2011). At the screening level, and because bacteria have an important role as decomposers, the luminescence bioassay with the marine bacteria *V. fischeri* has been demonstrated to be a sensitive, easy and rapid test, and as a result has been

widely incorporated into batteries of bioassays to asses both water-column and sediment toxicity (Giesy & Hoke, 1989; Burton, 1991; Pandard *et al.,* 2006).

The bacteria *V. fischeri* has been used in toxicity tests due to its functions in the ecosystem, as the degradation process and the nutrient cycling (Giesy & Hoke, 1989). The luminescence test with *V. fischeri* was highly sensitive to sediment samples for surface and subsurface sediment at site SW that is luminescence was inhibited by 96% and 59% respectively. This was the only site showing differences between the two types of sediments for site SW. In fact surface sediments reveal to be more contaminated than deeper ones. Burton (1991) stated that contaminants are associated to fine sediment particles, due to the relatively large surface area and transport. This fine sediment particles promotes also bacterial adhesion, that settle out at the bottom, thus affecting light emission (Davorean *et al.,* 2005, Parvez *et al.,* 2006). Fine sediment fractions tend to predominate in deposition areas, in fact that is what happened to this sampling site, with an average of 3% fine particles for site SW contrasting to < 0.5% for the other sampling sites. Probably the reason for these results is related to the sediment particle size and to the presence of chemicals such as, polycyclic aromatic hydrocarbons (PAHs) and metals, which have confirmed to be toxic to *V. fischeri* (Salizzato *et al.,* 1998; Wolksa *et al.,* 2007). Bacteria are known to be sensitive to organic compounds rather than chlorinated organic compounds and can uptake contaminants from the sediments and water in a short period of time (Giesy & Hoke, 1989; Liss & Ahlf, 1997).

The microalgae *P. subcapitata* as a primary producer and as a food source for invertebrates and fish has demonstrated to be an important species within aquatic trophic chains, being therefore an important species to be applied in a toxicity test (Pérez et al., 2010). *P. subcapitata* proved to be a sensitive test species to effluents and contaminated sediments and effects on primary producers may have important effects for the whole aquatic ecosystem (Burton, 1991). The microalgae growth test revealed to be sensitive to each type of sediment, surface and subsurface and at each site. The growth rate was inhibited by 71% in subsurface sediments from site SW, being the lowest growth rate observed. The highest growth rate was observed for subsurface sediments from site SE with an inhibition of 17%. The methodology of using immobilized algae has proved to be efficient in toxicity assessments (Hameed & Ebrahim, 2007). This methodology prevent sedimentation of the algae, facilitates the handling and therefore the recovery of the cells after the assay (Faafeng et al., 1994; Moreira-Santos *et al.,* 2004). However immobilization of algae in beads can prevent the diffusion of nutrients, carbon dioxide and light penetration (Van Donk *et al.,* 1993; Faafeng *et al.,* 1994; Moreira-Santos *et al*., 2004). Contrary to the Microtox, the growth inhibition test with *P. subcapitata* showed clearly differences between both types of sediments, surface and subsurface, for all sampling site. Also this was the assay which effects were more noticed, maybe because of turbidity which can affect the growth of microalgae by reducing light diffusion into water (Burton, 1991; Moreira-Santos *et al*., 2004). In addition microalgae are very sensitive to pesticides in general including herbicides such as atrazine (Pérez *et al.,* 2010), which are known to be present in the sediments from the lagoon due the agricultural practices

(SIMRIA, 2002). Interactions between pesticides can have an overall higher effect due to synergism between them (Pérez *et al.,* 2010). Water sample from site SE had the highest content of available N and P, this could have enhanced growth of algae that was observed for site D-SE.

The ostracod *H. incongruens* is a freshwater cosmopolitan species, is an omnivorous species and is considered to be an indicator of organic pollution (Ganning, 1971; Külköylüoglu, 2004). The growth test with *H. incongruens* was very sensitive to SW surface sediment with a growth inhibition of 33%. For surface sediments from site NW the highest growth was observed (21%). This assay has proved to be simple to do, sensitive and precise (Belgis *et al.,* 2003). Gills are the major site for metal uptake in crustaceans (Maltby, 1999), and fine sediment particles can decrease respiration rate by affecting respiratory structures (Lemly, 1982). Ostracods are also affected by metals, especially zinc and PAHs (Wang *et al.,* 2009), which was confirmed to exist in the sediments (Fernandes *et al.*, 2007b; Fernandes *et al.,* 2008a). These could be the main causes of stress that ostracod individuals faced to. Nevertheless, results from sampling sites were not very different from each others with the exception for surface sediments from site SW. This result is probably due to high mortality (74 %) rate observed for surface sediments.

The midge *C. riparius* is an important aquatic key species in decomposition process and is in constant contact with sediment being therefore a good organism for assessment of sediment toxicity (Giesy & Hoke, 1989; Pérez *et al.*, 2010). The growth test with *C. riparius* revealed that the midge had a lower growth rate in surface sediments for site SE (95%), as the higher growth rate was detected for surface sediments for site NW (214%). Chironomid larvae

are in constant contact with sediments being therefore a good organism for assessment of sediment toxicity (Giesy & Hoke, 1989). The *C. riparius* growth test revealed some differences between treatments. *C. riparius* is known for being an opportunistic species and for being resistant to contaminants (Burton et al., 1991; De Hass *et al.,* 2002). During the test individuals were feed at a minimum level to compensate for the physicochemical characteristics of sediments and to avoid them to starve (Akerblom & Goedkoop, 2003; Ristola *et al.,* 1999). However, some authors suggest that this species respond more to sediment nutritional levels than to associated contaminants, indeed, chironomids can incorporate a significant nutritive value from detrital matter associated to sediments (Ankley *et al.,* 1994, De Hass *et al.,* 2002). Furthermore, feeding can mask the effects of contaminants on larval development (De Haas *et al.,* 2002), by reducing (Stuijtzand *et al*., 2000), or increasing (Akerblom & Goedkoop, 2003) their bioavailability meaning that results may not be directly related to effects of contaminants. In fact, this relation of food levels with growth of *C. riparius* occurs in eutrophic environments, where an overcompensation of toxic effects by food was observed (Stuijtzand *et al*., 2000). This could be the reason for the obtained results since the study site is highly eutrophic.

In postexposure feeding test, *C. riparius* feeding rate was mainly affected at sites NE and SW. Surface sediments at site SE promoted a high feeding rate of larvae on artemia (187%), while for subsurface sediments from site NE had the lowest feeding rate (16%). Chironomid where exposed to water and sediment samples for 48 hours without being feed. Thus the physicochemical characteristics of the sediment were the main stress cause to these organisms.

Ankley *et al.* (1994) demonstrated that sediment characteristics can have effects on the response of chironomids (Ankley *et al.,* 1994). In fact he suggests that better growth rate of chironomids is observed in slightly coarse sediments particles (particle size: 250 – 500 µm). Sampling site NE is mostly composed by coarse sediments (Table 1), this could be a stress factor which effect was observed in the feeding rate. Other important result is the one for site SW, which effects could be also related to particle size. For this sampling site, a higher percentage of very small particles were present. Often, contaminants are associated to small particles (Burton, 1991) being an important exposure route and acting therefore as another stressor factor. Moreover, metals can be linked to small particles, which is also an important stress factor. Fine sediment composition can prevent the performance of chironimid (Ankley *et al.,* 1994). Even for a short time duration it could be that larvae feed on the organic matter present in sediments which in turn are highly associated to metals (Fernandes *et al.*, 2007b; Fernandes *et al.,* 2008a).

4.2 – Overall toxicity

A positive correlation between organic matter and metals was found, being an important factor contributing to the decline of water and sediments quality of this lagoon (Fernandes *et al.,* 2007b; Fernandes *et al.*, 2008a). The present data clearly show that, depending on the endpoint measured (growth, feeding and luminescence), that differences in toxicity were observed. Some considerations can be made regarding the different toxic responses of the various species. Even though, a battery of bioassays may not provide a perfect

correlation between assays due to the relative sensitivity of the different test species to the variety of contaminants, it has been proved to reduce uncertainties (Giesy & Hoke, 1989; Tuikka *et al.,* 2011). Uncertainties related to contamination of sediments were reduced, even if no clear trend between the toxicity bioassays was observed.

In overall, responses from the battery assays in surface sediments were higher than or equal to responses in subsurface sediments at all sites except at SW. The only exceptions were once at site NW (S < D by 19% in the *C. riparius* postexposure feeding test), once at site NE (S < D by 25% in the *P. subcapitata* growth test) and twice at site SE (S < D by 24% and 46% in the *C. riparius* growth test and the *P. subcapitata* growth test respectively). Only at site SW, responses at surface sediments were lower than responses in subsurface sediments for all tests, with exception to the *C. riparius* postexposure test (S > D by 26%). Site NW together with site SE were among the sites which presented higher organism responses. These results were expected since site NW is located next to the sea which promotes some renewal of the water and sediments, and site SE located in the Maceda river where remediation measures first took place. Contrary, sites NE and SW were sites with lower organism responses and with higher uncertainties in what regards their toxicity. These sites were expected to have lower responses since site NE is located in the Maior river which has been very contaminated for many years and site SW, which is located in the centre of the lagoon where probably contaminants accumulate. For the microalgae test no specific responses were detected, revealing some uncertainties. In order to establish if these uncertainties are due

to the sensitivity of the selected species or due to the presence of other contaminants not detected by the other assays, a macrophyte growth test with *M. aquaticum* and microalgae growth test with *P. subcapitata* was performed to distinguish differences among primary producers.

In a case study with such a contamination history, confounding factors are usual due to the high complexity of compounds in sediments and water Pandard *et al.*, 2006). However, this study along with others (Bailey & Young, 1997, Rosa *et al.*, 2010), demonstrated that a battery of assays is an important approach for impacted environments where industrial and domestic contamination is observed providing information with ecological realism.

4.3 – Primary producers

Primary producers are very important in ecosystems because they convert solar energy and carbon dioxin into organic matter, produce of oxygen, sequestrate carbon dioxin and therefore sustain higher levels. Submersed macrophytes can be regarded as key species since changes in the marophyte community can have consequences for the aquatic ecosystem (Arts *et al.,* 2008). Macrophytes maintain important ecosystem structures and functions, such as cycling and retention of nutrients, stabilization of sediments, provision of food, habitat resources for aquatic fauna and promote biodiversity (Maltby *et al.*, 2009). The *M. aquaticum* growth test has been recently developed to better understand the exposures routes through sediments to aquatic plants, this because the only aquatic plant used in risk assessment, *Lemna minor,* is not exposed by roots (Arts *et al.,* 2010).

Results for the growth test with *P. subcapitata* clearly revealed a growth inhibition for site NW (46%). No differences between the other treatments, NE, SW, SE, were observed, however site SE showed a growth inhibition of 13%. No big differences were detected for this assay since that no differences between surface and subsurface sediments were observed and between treatments only one site showed differences. Site NW was the only site being significantly different with a lower growth rate. Sites NW and SW had the lowest organism response for the microalgae test, as happened for the first microalgae test. Contaminants that caused toxicity to this species in the previous assay were probably degraded.

For the *M. aquaticum* growth test site NE clearly represent the highest growth rate (59%) whereas for site NW the lowest growth rate (1%) was observed. *Myiophyllum* spp. are suitable test organisms to assess the phytotoxicity of herbicides or contaminated sediments (Knauer *et al.,* 2008). In fact, results revealed some differences between treatments however no differences between surface and subsurface sediments were observed. The reason why site NW had the lowest growth rate could be related to organic matter content in the sediments of this sampling site, which was the lowest. Contrary, site NE had a high organic matter content that could enhance plants growth. This differences observed among treatments could be related to persistent herbicides and other contaminants in the sediments since this study site has a long contamination history. Actually herbicides are designed to inhibit dicotyledonous, which is the case of *M. aquaticum* (Feiler *et al.,* 2004).

Results obtained for the primary producers are in conformity with each other, regarding the absence of toxicity between surface and subsurface

sediments. The microalgae test revealed big differences that could be due to contaminants degradation, between the first microalgae test and the second one, revealing some uncertainties. Nevertheless the macrophyte growth test could detect more differences between treatments than the microalgae growth test, however contrary to what happened in the battery test, site NW was no more the one with a higher growth rate, as site NE was the site with higher response. Uncertainties regarding primary producers' responses at all sites, dictate that it is necessary to carry further testing. Yet, the possibility that herbicides with a sediment distribution different than the other contaminants are responsible for the results found in the microalgae test cannot be ruled out. For instance, some herbicides like atrazine are highly persistent could justify this results.

Chapter 5 Conclusion

5 – Conclusion

The quality of water and sediments within this lagoon has been gradually degraded over the last few years, by industrial and domestic discharges (Fernandes *et al.*, 2007b; Fernandes *et al.*, 2008a). This degradation was confirmed by results obtained from the toxicity assays. Along with other studies (Bailey & Young, 1997, Rosa *et al.*, 2010), this study demonstrated that a battery of assays is an important approach for impacted environments where industrial and domestic contamination is observed providing information with ecological realism.

Sites NW and SE were the ones that demonstrated higher organism responses. Contrary sites NE and SW were sites with lower organism responses and with higher uncertainties in what regards their toxicity. Concerning surface versus subsurface sediments, site SW was the only with responses for surface sediments lower than responses in subsurface sediments for all test, with exception to the *C. riparius* postexposure feeding test.

The macrophyte growth test proved to be, as in other studies, a valuable complement assay to add to a battery of bioassays (Feiler *et al.*, 2004). Concerning primary producers, both revealed for site NW the lowest response, as for site NE the higher response was observed. Different uptake routes were the reason for the differences between the microalgae and macrophyte test, still contaminants that promoted that behavior remained unknown. Uncertainties regarding primary producers' responses at all sites dictate that it is necessary to carry further testing.

This kind of assays proved to be useful for rapidly establish the state of the lagoon indicating that further interventions should take place in order to remediate this ecosystem.

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