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Integrative assessment of sediment quality in acidification scenarios associated with carbon capture and storage operations

Ángel DelValls¹, Lorena da Silva Souza², Alessandra Aloise de Seabra¹ , Camilo Dias Seabra Pereira^{1,3}, Estefanía Bonnail^{4*}, Inmaculada Riba^{2,3}

¹Department of Ecotoxicology. Santa Cecília University (UNISANTA), Santos, São Paulo, Brazil. (delvalls@unisanta.br).

²UNESCO/UNITWIN WiCop. Faculty of Marine and Environmental Sciences, University of Cádiz, Cádiz, Spain.

³Department of Marine Sciences. Federal University of São Paulo (UNIFESP), Santos, São Paulo, Brazil.

⁴Centro de Investigaciones Costeras Universidad de Atacama (CIC-UDA). Universidad de Atacama, Copiapó. Chile.

*corresponding author:
Estefanía Bonnail
Telephone number +56 52225 5496
Fax number +56 52225 5496
estefania.bonnail@uda.cl

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Abstract

Efforts to stem global change include the application of new technological developments that aim to reduce atmospheric carbon dioxide (CO₂) through the capture and storage (CCS) of this greenhouse gas in stable geological structures. In this paper, we assess the potential risks related to the application of CCS technology and the acidification of aquatic ecosystems through CO₂ enrichment. We use the multiple lines of evidence approach (LOEs) to characterize the effects of the acidification with contamination in aquatic sediments. We highlight and discuss the effects of acidification on the LOE: including contamination and mobility of contaminants in sediments, toxicity, macro-benthic community structure, in situ effects and bioaccumulation/biomagnification processes. We further assess the results of acidification on the toxicity of organic contaminants such as antibiotics or illicit drugs like free base form of cocaine (crack). The main goal of using the LOEs approach is to distinguish between adverse effects that are associated with contaminants and those related to acidification by enrichment of CO₂ as a result of CCS technology. Previous assessments have not designed or conducted to incorporate an integrative point of view, nor have they employed a weight-of-evidence approach (WOE) in risk characterization and management of CCS operations and other situations related to acidification by enrichment of CO₂ in the aquatic ecosystem. Based on the findings of this review, the WOE is capable to identify the effects of the acidification on the different LOEs used for sediment quality: contaminant mobility, the adverse effects in organism under laboratory, and field conditions, and the bioaccumulation/biomagnification of contaminants. The main strength in using WOE is the ability of this method to discriminate between LOE responses associated with contamination by different organic and inorganic

substances from those related to CO₂ acidification itself. The WOE will significantly improve the risk assessment in areas affected by potential leakages of CO₂ during CCS operations.

Key-words: bioavailability; emerging pollutants; metal(loid)s; risk assessment; sediment assessment; weight-of-evidence. risk management

1. Introduction

1.1. Aquatic ecosystem acidification and mitigative efforts

The main goal of Carbon Capture and Storage (CCS) technology is to mitigate the increase of CO₂ concentration in the atmosphere, balancing thermodynamic and kinetic chemical processes of organic matter combustion.

The well-established CCS technology has been incorporated into a new initiative that has been introduced over the past few years and is currently applied world-wide. It implies the process of carbon sequestration (IPCC 2012; Global CCS Institute 2016) and its potential to decrease the amount of CO₂ in the atmosphere. Reguera et al. (2009) provided an overview on the initiatives taken by different international conventions, such as London Convention and OSPAR, to regulate the use of this technology. These conventions and protocols have been amended to allow for the storage of CO₂ in the sub-seabed geological formations and to elaborate upon comprehensive guidelines in Risk Assessment

and Management of these storage areas. These regulations will ensure that the CCS activity does not lead to significant adverse consequences either for marine environment, human health or other legitimate use of the sea (London Convention 2006, 2007; OSPAR Guidelines 2007).

The Global Carbon Capture and Storage Institute identified 17 large-scale CCS facilities operating globally, with four more becoming operational in 2018 (Global CCS Institute 2017). Most of these projects are conducted in countries that were signatories to various international conventions. Twelve of the 17-large scale facilities in operation are located in the United States and Canada and two of those began operational within the past twelve months (Petra Nova and Illinois Industrial). In EMEA (Europe, Middle East and Africa), four large scale facilities are currently operating successfully (two in Norway and two in the Middle East), with two more in the early development stages in the United Kingdom. In South America, there is only one CCS large scale system operating. It is located in the Santos Basin (Brazil) where the offshore facilities have injected over 4 million tons of CO₂ (Global CCS Institute 2017). A significant number of these projects may impact natural ecosystems. However, political uncertainties have generated a slow rate in the regulatory development, including countries with sophisticated legal regimes for CCS, such as Australia, Canada, Denmark, the United Kingdom and the U.S.A., all of which have been stalled since 2015. In addition, the Global CCS Institute highlighted the need for establishing (region-relevant) public/private business models that better manage risk allocation between the capture, transport and storage elements of the CCS chain, thus reducing overall risks (Global CCS Institute 2017). In this sense, most of the projects are under the regulations proposed by the different international conventions and are running

different risk characterization and risk management approaches to minimize and monitor them (Global CCS Institute 2016).

1.2. Potential adverse effects associated with CCS in aquatic ecosystems

There are different natural and anthropogenic processes that can increase the acidity in aquatic ecosystems (marine and freshwater). For instance, the atmospheric deposition of CO_2 , submarine volcanic activity, sulfates, and nitrate runoffs from watersheds, organic diagenesis, etc. These processes will produce an acute (short-term) impact on the aquatic ecosystems since the release of CO_2 in it (sediment/water) will decrease the pH (acidification). These changes in pH are directly related to the partial pressure of CO_2 and the chemical buffering capacity of the seawater.

However, an accidental leakage of CO_2 gas from the geological structures used in CCS sites or during its storage operation, may cause sudden acidification in the surrounding environments, which will not only affect water supplies but also onshore and offshore ecosystems (DelValls and Riba 2007). The effects of CO_2 leakage will depend on the amount and/or rate of leakage, the transport and dispersion processes, and the chemical buffering capacity of the sedimentary or water system. For instance, it has been recently demonstrated in a controlled CO_2 gas release under 'in situ' conditions (QICS experiment - Quantifying and Monitoring Potential Ecosystem Impacts of Geological Carbon Storage) by Blackford et al. (2014) that, after an event of leakage followed by the cessation of gas supply, it required approximately 3 weeks for the pore water to return to normal pH values in marine waters. In the experiment, the total dissolved inorganic carbon (DIC) within the sediment, mainly in interstitial and pore waters above the release zone, showed that up to

63% of the carbon dioxide released during the experiment could remain in the dissolved phase within the sediment pore water (Taylor et al. 2015). Thus, impacts associated with an acidification scenario must be addressed. Furthermore, the environment acidification might enhance the adverse biological effects of the existing contaminants and/or make them more bioavailable (Riba et al. 2003; Riba et al. 2004a). In the QICS experiment (Blackford et al. 2014) CO₂ was released into a site with minimal contamination and neither the effects of the contamination combined with acidification, nor the potential adverse effects associated with changes in their bioavailability, were evaluated.

Most of the CCS projects currently in operation include the regulations established in the International Conventions, such as performing different risk characterizations and management options. Six main steps are followed to improve risk management: (a) problem recognition; (b) selection and site characterization; (c) exposure assessment; (d) effects assessment; (e) risk characterization and its management (including monitoring and mitigation) (Reguera et al. 2009). Several impacts on biogeochemistry levels are characterized during project execution and gas release in the field, such as in the QCIS experiment (Blackford et al. 2014), which requires multiple and multivariate approaches, followed by the correct monitoring of these processes and their impacts (Blackford et al. 2015). However, in recent studies, the ecotoxicological impacts that were determined focused exclusively on acidification "per se", whereas the interaction with contaminants was neither considered nor fully addressed. Moreover, a weight-of-evidence approach was not used to address the environmental and human risks.

The effects of CO₂ leakage in freshwater environments have been receiving little attention in recent years; nevertheless, these ecosystems can be affected by leaks from different sources including: (a) depleted oil and gas fields; (b) Underground storages (since

technical solutions include onshore storage facilities such as aquifers); (c) deep coal seams; and (d) unmineable coal seams. These processes may represent a leakage of CO₂ and the propagation of environmental and human risks, which must also be considered in the risk characterization. A recent study by Lion et al. (2014) addresses some geochemical characteristics related to the CO₂ acidification in freshwater environments. These authors review the effects of CO₂ acidification on geochemical characteristics of groundwater. Mainly, the work focuses on physicochemical effects of acidification and includes the assessment of contaminant mobility and bacteria responses. They recommend conducting studies to address the biogeochemical risks before installing any potential activity that could produce CO₂ enrichment in these environments. In these studies, the authors did not report whether these environments related to adverse biological effects at trophic levels higher than bacteria.

In this review, we discuss the progress made over the last few years regarding the evaluation of adverse effects associated with different scenarios of acidification in aquatic ecosystems caused mainly by potential leaks during CCS operations. In addition, some of these reported results will be examined to better understand the impacts of acidification associated with CO₂ enrichment in aquatic environments. This review will apply the different lines of evidence approach (LOE) in the framework of the international convention guidelines that previously recommended the use of a Weight-of-Evidence (WOE) approach for sediment quality assessments in environments affected by acidification with CO₂. The main aim of this review paper is to highlight the feasibility and strengths of using WOE to environmental risk characterization in aquatic sediments affected by acidification related to enrichment of CO₂.

2. Approach

2.1. Weight-of-evidence (WOE) approach

The WOE implies the use of multiple lines of evidence (LOEs) to determine the sediment quality in different aquatic ecosystems affected by acidification by CO₂ enrichment under an integrative approach. The usual lines of evidence used in the WOE approach includes: (a) Aquatic Contamination: chemical concentration (including the speciation of CO₂) in sediments, interstitial and overlaying waters; (b) Toxicity: laboratory experiments to evaluate the effects of the acidification and contaminants in the aquatic ecosystem (including both lethal and sub-lethal responses, like histopathological, DNA damage, and biomarkers responses); (c) Ecological integrity: in situ exposure experiments to determine the effects of the acidification and contaminants in the aquatic ecosystem (including macrobenthic community structure, residential infaunal analysis of biomarkers and similar responses, etc.); (d) Bioaccumulation/Biomagnification: to establish the effects of the contaminants mobility in organisms and related it with human health by consumption of contaminated species. The WOE can integrate the mentioned lines of evidence using different approaches previously reported by Riba et al. (2004a), which includes multivariate analysis, chart diagrams, pollution index and tabular decision matrix based on a professional best judgment (Riba et al. 2004a; DelValls and Riba 2007). A schematic representation of the WOE is included in the Fig.1.

Regarding the use of WOE in environments affected by potential CO₂ acidification such as CCS operation areas, the application of this method will be paramount importance;

especially, if it is suspected that either the transposition of CO₂ above the CCS formation can get extended to the seafloor (or groundwater) or in cases where the storage site is near sensitive or endangered habitats and species. The WOE will allow monitoring the sediments and overlaid water (in both marine and freshwater ecosystems) to detect and measure possible leakage of CO₂ (and incidental associated substances) into the aquatic environment. The sediment chemistry is used for this purpose as a LOE inside the integrative approach (WOE). There are other techniques and tools often used to detect the leakages of CO₂ such as: visual approach using cameras and remote operated vehicles (ROVs); chemical sensors of CO₂ and/or associated parameters (pH, alkalinity, DIC, etc.); biological monitoring through micro and/or macro organisms characterization and using ROVs; geological and physical monitoring, among others. However, the main objective of the WOE is not only to detect, but also to address under an integrative point of view the risk assessment related to the potential leakage. In this sense, some of the LOEs included in the WOE could be used at the first steps to identify, detect and measure the possible leakage of CO₂ (sediment chemistry, resident infauna, etc.).

The WOE approach will allow measuring the effects of small and diffuse leakage in both short or long term and the degree of contamination originated by this leakage (DelValls and Riba 2007). In addition to acidification impacts on the aquatic ecosystem, the effects of the incidental associated substances and the existing substances mobilized by the injection and storage of CO₂ streams (e.g., brine) can also be assessed with this methodology.

3. Findings

In this section we include and assessment of the different approaches and results recently used to establish the acidification impact on aquatic ecosystems using different lines of evidence. These LOEs were individually used and no WOE was applied in any of the revised studies. We also discuss if these LOEs are used or not in running projects of CCS.

3.1. Sediment Chemistry. Contamination

This line of evidence must address the acidification influence on water/sediment/benthic parameters to establish a potential bioavailability of contaminants, including emergent contaminants. Different environmental samples collected from potential CO₂ storage sites and surrounding areas will be exposed to CO₂-induced acidification. It will be necessary to study the variations of fluxes associated with different contaminants as a function of the acidification by enrichment of CO₂. It will be combined with the changes of pH values and inorganic carbon concentration in the samples. This kind of simulation will help to predict the behavior and potential effects of the contaminants when they change their reactivity, mobility etc., relating to sediment acidification. Also, this kind of approach will be helpful for the site selection during CCS operations.

There are different studies that have addressed the environmental impact of acidification by CO₂ leakages on the aquatic sediments taking into account the modification of the chemical equilibria, moreover those that include the link between the acidification and the contamination in sediments, mainly by metals. Some of them are commented on below.

Dewar et al. (2013) proposed a model to explore the dissolution of CO₂ in the form of bubbles/droplets, associated with a leakage. It is well-known that the increase of CO₂ determines changes in most of the chemical equilibria as a result of the acidification changes as outlined by Lichtschlag et al. (2015). These authors participated in a controlled release of CO₂ in a field experiment in a marine environmental ecosystem by means of monitoring over one year the physicochemical changes in sediment and water. They found different changes related to increases in the concentrations of inorganic dissolved species of carbonic acid. These high concentrations were measured during the first 5 weeks of the experiment reaching a magnitude of concentration higher than the natural. Also, the researchers found changes in alkalinity and other carbonic acid parameters together with an increase in the dissolution of carbonate and silicate minerals. Three weeks after the CO₂ enrichment was stopped most of the chemical components returned to normal values. These authors did not monitor the influence of these changes on potential contaminant mobility, like metals or organic compounds, probably because these compounds were not present in large concentrations in the study area (Dewar et al. 2013; Lichtschlag et al. 2015).

There are cases where studies addressed the influence of CO₂ on physicochemical changes under laboratory conditions and its effects on contaminant mobility. Payán et al. (2012), for instance, reported the influence of CO₂ leakage on metals using standardized leaching tests. They used different types of waters and acidification conditions (deionized water, natural seawater and acidified seawater at pH 5.0, 6.0, and 7.0) obtained by CO₂ bubbling. They reported that, at the different solutions tested, all the metals increase mobility and bioavailability when the proton concentration increased (decrease in pH values). There are other authors that have identified similar behaviors using different approaches, for instance some authors have modeled equilibrium conditions using

geochemical software (De Orte et al. 2014b; Martín-Torre et al. 2015a), periods before equilibrium (Zheng et al. 2016; Cahill and Jakobsen, 2015) and the kinetic behavior of contaminants (Martin-Torre et al. 2015b, 2016).

De Orte et al. (2014a,b,c, 2018) have reported significant changes in the mobility and chemical speciation of different metals when reached by acidification processes associated with CO₂ enrichment. In general, all the metals analyzed (Fe, Al, Cr, Co, Ni, Cu, Zn, Pb) in different sediments, with different concentrations, showed an increase in mobility when increases concentration of protons. They also reported that the acidification by CO₂ enrichment produces an increase in concentration of some metals and metalloids in different stations relating it to potential environmental risk due to the mobility of metal(loid)s and the increase of their bioavailability. In some cases, like As, concentrations did not increase with acidification but its chemical speciation has changed. These changes were associated with increases of chemical species that are more toxic for metalloids, like As(III).

In summary, all these studies concluded that generally the increase in metal bioavailability was related to the increase of proton concentration by enrichment of CO₂. This effect was significantly intensive when the metals were bioavailable in the interstitial waters (De Schamphelaere et al. 2002; Riba et al. 2010; de Orte et al. 2014b). Similar results were reported by other authors related to different metals in interstitial and sea water (Millero et al. 2009; Wang et al. 2015). There are other studies showing that re-suspension conditions might mobilize some contaminants, like Pb, more than slight acidification levels (Martin-Torre et al. 2017). This can be important during the CCS technology activity, especially in the first steps of the site preparation

The mobility and chemical speciation of different contaminants is one of the LOEs included in the monitoring programs of practically 100% of the running projects (Global CCS Institute 2016). Nevertheless, there is a lack of reported data in the bibliography that address the influence of aquatic ecosystems enriched by CO₂ associated with change in bioavailability of organic contaminants. Sircar (2014) showed that antibiotics change net charge in different pH values. Based on that, the neutral form is considered most bioavailable and therefore most toxic than its metabolites. They postulate that in seawater with high concentration of CO₂ (acidic conditions) polar organic contaminants will be differently charged than in current seawater pH conditions.

This LOE related to the sediment chemistry and contamination studies has the potential to identify, detect and measure the potential leakages of CO₂ associated with CCS operation. However, it shows a significant weakness related to assess the associated biological adverse effect. Even identifying the mobility and speciation of contaminants, this LOE is not capable to determine the biological adverse effect associated with the contamination and the changes in their bioavailability. Thus, additional LOEs to establish this effect are necessary to be integrated under a WOE approach.

3.2. Toxicity

The toxicity of the environmental samples around the CO₂ injection and storage sites is usually carried out by means of bioassays under 'in situ' and laboratory conditions. These bioassays are needed to quantify the exposure pathways of the contaminants and their negative effects by measuring different adverse biological effects (mortality, reproduction, biomarkers of effect such as histopathology or DNA damage, biomarkers of exposure, etc.).

High CO₂ levels in the environment may cause asphyxiation (impair respiration in organisms) (Seibel 2016), acidosis (lowering of pH in animal body fluids) (Esbaugh 2017) and hypercapnia (increased concentrations of CO₂ in body fluids) (Gutowska et al. 2010; Esbaugh 2017) among other adverse biological effects. In the last years, it has been conducted in an effort to increase the knowledge about effects associated with CO₂ acidification and also in its combination with contaminated environmental samples. The main results obtained are summarized below.

Basallote et al. (2012) exposed different species of marine organisms to different scenarios of acidification by enrichment of CO₂. Polychaetes, fish larvae, mollusk and amphipods were exposed to water acidified by CO₂ using gradients of pH values from 5.5 to 8.0. Results show that all the organisms tested, except polychaete, were highly sensitive to increases of proton concentration from CO₂ enrichment. Thus, pH values of 7.0 or lower were associated with significant increases of toxicity in these species. Sokołowski et al. (2017) reproduced the high pressure and determined biological responses of the infaunal bivalve *Limecola balthica* to CO₂-induced seawater acidification through long-term exposition. These authors concluded that even the most acidic conditions (pH= 6.3) did not prove to be fatal. Recently, Bautista-Chamizo et al. (2016) and Passarelli et al. (2017b) have compared the effects of acidification by exposing benthic and pelagic organisms to different scenarios by means of acid mixtures and CO₂. Their results show that the effects of the acidification produced by CO₂ enrichment have significant differences compared to acidification using mixtures of acid. For instance, Bautista-Chamizo et al. (2016) showed that marine microalgae *Pleurochrysis roscoffensis* were healthily growing in a pH range of 8.0-6.5, with the optimal pH value of 6.5 for the species growth when the acidification was produced by CO₂ enrichment. Nevertheless, when HCl was used to reproduce the

acidification condition, the toxicity of the protons on these microalgae was measured at values lower than pH 7.0. Both treatments at pH values lower than 6.5 produced significant toxicity on the population of microalgae, causing their collapse or death. The membrane permeability for protons favors the gaseous CO₂ transfer inside the cell producing different adverse effects in marine organisms. At the same time, this enrichment of CO₂ will benefit microalgae growth (photosynthesis).

There are recent and numerous studies that address the effects of ocean acidification on marine organisms (i.e., 4th International Symposium on the Ocean in a High-CO₂ World). These studies have significantly advanced to understand the impacts of physicochemical changes in the aquatic system of the carbonic acid. However, most of these studies do not consider the combined effects of acidification with the existing contamination on the biological effects assessment. In other words, they were not able to identify the relationship between the effects of acidification on the physicochemical changes of contaminants and their consequent toxicities to organisms.

Below we review a selection of studies that address the effects of CO₂ on trace metals and their effects using different organisms. The main focus of these studies was to distinguish between the biological adverse effects associated with the acidification itself compared to the increase of bioavailability of metals associated with the acidification by the enrichment of CO₂.

First attempts to address the acidification influence on the toxicity of environmental contaminated samples used mixtures of acid. Riba et al. (2002; 2004a) pointed out the effects of increasing acidification on the mobility, bioavailability and toxicity of metals in contaminated samples in southern Spain. These authors showed that changes in 0.5 units of pH values were associated with significant increase in mobility and bioavailability of

metals as well as their toxicity on mollusks. Basallote et al. (2014) used the benthic amphipod *Ampelisca brevicornis*, to address the effects of the acidification by enrichment of CO₂ in sediments with different levels of metal contamination. These authors reported significant toxicity of metals bound to sediments when the pH values decreased. Thus, for a given sample (contaminated with metals) toxicity was not observed at pH values of 7.5 and 8.0. However, when the same sample was at pH of 7.0 or below, the increase in the mobility of certain metals (Ni, Zn, and Cu, among others) was associated with significant adverse biological effects measured in the exposed amphipods. Recently, Goulding et al. (2017) and Passarelli et al. (2017b) have conducted different research studies that distinguished between the effects of acidification by enrichment of CO₂ ‘per se’ from those biological effects related to contamination of samples (mainly metals). These authors used different amphipod species from different sites in Brazil and Europe. The main results showed to be similar to those reported by Basallote et al. (2015), concerning acidification threshold values. Thus, for the species tested, the values of pH lower than 6.5 produced lethal effects whereas those values between 7.0 - 6.5 showed adverse biological effects but not death. The authors also showed the relationship among adverse effects, acidification, mobility and concentration of metals, demonstrating that the amphipod species used (Tropical, *Hyale youngi* and European *Ampelisca brevicornis*) were sensitive and valid to address the impact of the combined processes (acidification combined with contamination). Other authors that exposed benthic species to different scenarios of acidification by enrichment of CO₂ reported similar results. Rodríguez-Romero et al. (2014) and Basallote et al. (2015) exposed bivalves (*Ruditapes philipinarum*) to different contaminated samples under different scenarios of acidification produced by CO₂ enrichment. The authors reported similar results related to the increase of some metals mobility (Zn and Fe) and the

increase in the toxicity on these organisms. A given sample of sediment demonstrated no toxicity to metal concentration when pH values were higher than 7.0. However, the same sample was toxic to the same organisms when pH values were lower than 7.0. It was concluded by these authors that significant relationships exist between the increase in the mobility of metals from interstitial water, the increase of their bioavailability and the measured biological effect. Although these authors used the same species, the life stage was different, from juveniles to adults. Comparing these results it was concluded that juveniles were more sensitive than adults to the combination of acidification and metal contamination.

Rodríguez-Romero et al. (2014) exposed organisms of polychaeta *Hediste diversicolor* to different contaminated samples at different acidification scenarios by CO₂ enrichment. These authors used the same contaminated samples than those used for the exposure of other organisms in the previous studies (like mollusks). Results showed that individuals of *H. diversicolor* were more tolerant to acidification effects than mollusks, resistant to pH values lower than 6.5 without any significant adverse effects. Besides, these organisms were more resistant to the combination of both effects (acidification and metal contamination) than mollusks. No significant effects were measured in contaminated samples with metals to pH values higher than 6.5.

Recent studies have used mixtures of acid (Riba et al. 2016) and enrichment of CO₂ (Passarelli et al. 2017a; Szalaj et al. 2017) to mimic acidification scenarios. Different species of sea urchin (*Paracentrotus lividus*, *Lytechinus variegatus*), oyster (*Crassostrea gigas*) and mussels (*Mytilus edulis*, *Mytilus galloprovincialis*) were used in these studies to distinguish between the adverse effects related to acidification and those associated with contamination. The contaminated sediments (mainly by metals) used in these studies were

sampled from the same location, allowing a more direct comparison of the responses. The mobility of Zn, Cu, Fe, As and Al was higher, although the last three were not directly related with toxicity suggesting that the acidification conditions could provoke a reaction among non-toxic ligand species. In addition, mobility of metal(loid)s increases their bioavailability and consequently increases the toxicity for the species involved. This was shown by Passarelli et al. (2017a), where toxic effects were not detected in samples between 8.0 and 7.5 using these species, and only reached toxic effects when the pH value was 7.0 or lower. One could conclude from the different data sets that the acidification and consequent metals mobility was the main cause of toxicity, showing sea urchins as the most sensitive species to acidification and mussels as the most tolerant.

In general, studies considering different contaminated samples by metals (and metalloids) and in a different matrix (sediment and water) have been used to expose different organisms to acidification conditions by enrichment of CO₂. Main results show that the increase in concentrations of protons is toxic itself at pH values lower than 7.0. Besides, these studies also demonstrated that environmental samples not toxic at environmental values of pH (7.5 - 8.0) became toxic when pH values decrease to 7.0, or lower, for all the invertebrates used in the experiments under laboratory conditions. In summary, the results of most recent marine/estuarine studies show that acidification by adding CO₂ affects the mobility of metals present in marine sediments. Thus, at a normal marine ecosystem pH, most of the metals are bound to the sediments and were not available to exposed organisms. However, it was also demonstrated that some of the metals became bioavailable and subsequently potentially toxic to the exposed biota when the proton concentration was increase as a result of CO₂ enrichment.

Fig.2 summarizes a gradient of the different organisms sensitivities exposed to combined effects of metal contamination and acidification by CO₂ enrichment. In general, early life stages were more sensitive to acidification effects or to a combination of acidification with metal contamination, than adults of the same species. The figure allows an ordination of the species used in the discussed studies based on the tolerance and sensitivity responses of these taxa to the different CO₂-induced acidification scenarios. It shows that sea urchins and amphipods are the most sensitive species to the conditions tested in the experiments followed by mollusks and finally the polychaete, which was the most resistant species of those reviewed here.

Although there have been increases in toxicity studies combining acidification and contaminants effects using estuarine and marine species, there are only few attempts to determine effects related to CO₂ acidification related to trace elements in freshwater ecosystems, such as aquifers and gas flux measurements in soil (Jones et al. 2015). There is also limited information about metal mobilization caused by a potential CO₂ leak in the freshwater sediment layer and its consequent effects on resident aquatic organisms. To the best of our knowledge, there are only two studies focusing on freshwater sediments that address the toxic effects in acidification scenarios by enrichment of CO₂ and contamination using environmental samples. The first one used the sediment dweller insect larvae of *Chironomus riparius* (Khosrovyan et al. 2014) and the second example the planktonic cladoceran *Daphnia magna* (Khosrovyan et al. 2017). Results of these two studies show similar effects at CO₂ acidification and its combination with metal contamination increasing the toxicity when the proton concentration and the mobility of metals increase. Different endpoints, from survival to behavior, were used in the tests. *D. magna* showed to be sensitive to a gradual CO₂ enrichment, presenting adverse effects by acidic conditions. It

was also identified that metal mobility, especially from Al ions, were related to an increase in mortality and decrease in reproduction of parental *D. magna*. Related to the midge *C. riparius*, results showed an impairment of reproductive functions of these organisms that may lead to population decline and disruption of their functions in the ecosystem. At pH values lower than 7.0, an increase in metal mobility and bioavailability was measured and was found to be associated with the toxicity identified in the experiment.

In recent years, the use of microorganism communities to address the response of different acidification scenarios has been increased. Furthermore, there are few but significant studies addressing the combination of toxic effects of acidification and contamination in aquatic organisms. Tait et al. (2015) reported changes in the microphytobenthos and bacteria community during an in situ experiment with release of CO₂. These authors have found changes related to the effect of the acidification conditions forced in the experiment related to the relative abundance of bacterial taxa. They also discussed that some of the impacts measured could be related to the presence of metals in the sediments but the concentration related with their effects was not reported in the study. Meanwhile, Liu et al. (2017) studied the effects of acidification by enrichment of CO₂ in bacterial community dynamic, in a seawater mesocosm experiment. In the case of low CO₂ treatments, the consumption of the dissolved inorganic nitrogen, the uptake ratios of N/P and N/Si increased significantly during the bloom. The observed responses suggest highly extensive and complex effects related to higher CO₂ concentrations on phytoplankton communities from coastal eutrophic environments.

Recently, Borrero-Santiago et al. (2016a) pointed out an underestimation within the use of bacterial populations and also communities to the risk assessment of acidification by CO₂ enrichment effects. These authors conducted different approaches addressing the role

of these kinds of organisms as an indicator of acidification effects and that could be used in a combination with contaminants scenarios. Borrero-Santiago et al. (2016b) used populations of *Pseudomonas stanieri* to optimize the protocol of using these organisms in this kind of studies and to address the influence of acidification in them. Further studies from the same group (Borrero-Santiago et al. 2016b; 2017a,b) showed responses of two marine strains to different scenarios of acidification by enrichment of CO₂. Strains of *P. litoralis* were more sensitive to acidification than *Roseobacter* sp., representing the first attempts to show the diversity and resistance of marine bacteria and their capacity to adapt themselves under different acidification scenarios. Also, these authors have tested the stress responses in bacterial community exposed to a combination of acidification and contaminants. They carried out a toxicity assessment using bacterial responses (total number of cells, respiring activity, changes in the bacterial community composition and diversity). The main results showed an impact of the acidification on bacterial communities measured by changes in respiring activity, community composition groups and diversity. These results suggested that an acidification by enrichment of CO₂ will remove contaminants from the sediment. In the same study, it was shown that bacterial communities used were able to adapt to the new acidic conditions by means of changing the diversity and structure. Despite the adaptability of bacterial communities in the contaminated sediments used within the acidification experiments, a negative impact was observed on respiration activity under all pH treatments. It demonstrated that this kind of bacterial responses was a rapid and useful indicator for environmental risk assessments in contaminated sediments exposed to different CO₂ acidification scenarios.

There are few studies that tested the influence of acidification on the toxicity of organic compounds (Schiedek et al. 2007; Freitas et al. 2016; Munari et al. 2016). As

observed by Schiedek et al. (2007), new persistent organic contaminants, such as flame retardants and perfluorinated compounds, together with emerging contaminants (such as pharmaceuticals and personal care products), are being produced and released into aquatic ecosystems with limited understanding on their biological effects in different climate changes scenarios. The combined effects of emerging contaminants and pH alteration are scarcely reported in scientific literature. Freitas et al. (2016) found that toxicity of the anticonvulsant or anti-epileptic drug carbamazepine on *Scrobicularia plana* clams was synergistically increased under ocean acidification conditions (pH 7.1). The specimen's survival was reduced and oxidative stress was enhanced when compared to single exposures. Munari et al. (2016) investigated the combined effects of seawater acidification and the nonsteroidal anti-inflammatory drug diclofenac on survival, growth and oxidative stress-related parameters in the larvae of *Ruditapes philippinarum*. This study revealed that mortality was higher under reduced pH in the presence of this substance, whereas shell morphology and larvae growth were negatively affected by both acidification and diclofenac, highlighting that acidification enhances the sensitivity of clam larvae to environmentally relevant concentrations of diclofenac.

Due to the increase in global consumption of illicit drugs and consequently their occurrence in aquatic ecosystems, this class of bioactive compounds has been considered a potential new environmental concern (Binelli et al. 2012). Recent studies have reported coastal water contamination by drugs such as cocaine and its by-products (Klosterhaus et al. 2013; Pereira et al. 2016). However, few studies have focused on biological effects on marine organisms (Maranho et al. 2017). There is a new initiative that is testing the influence of acidification by enrichment of CO₂ in illicit drug crack/cocaine (Pereira et al. 2016). The system of crack-cocaine is a very interesting model to study the influence of

proton concentration. The molecular difference between both compounds (crack and cocaine) is a proton defining a system similar to other inorganic compounds such as $\text{NH}_3/\text{NH}_4^+$, etc.

Although originally the crack compound is considered more toxic based in its neutral molecule compared to cocaine, the first results obtained by these authors show how the increase in proton concentration also produce an increase in the toxicity of these compounds (Souza et al. 2017). They demonstrated the different bioavailability of crack-cocaine exposed to different organisms when modifying the acidification conditions.

Fig.3 shows the first results by Souza et al. (2017) of this kind of experiment linking this with ocean acidification scenarios. They performed a study on the interaction between the toxic effects of illicit drugs and pH alterations in marine organisms. These authors showed that toxicity of crack-cocaine to the gametes and larvae of *Perna perna* mussel gametes and larvae increased due to acidification. The enhanced toxicity of contaminants is of emerging concern as the effects of illicit drugs on the quality of gametes and their interaction, as well as embryo-larval development under different pH values, sheds light on more relevant adverse effects of bioactive compounds in ocean acidification scenarios.

Sircar (2014) reported results of a combined effect between the increase of proton concentration by enrichment of CO_2 and the presence of antibiotics that were significantly observed in a community of bacteria. In general, the bacteria communities that typically occurred in winter appeared to be more sensitive compared to those spring bloom communities that are expected to be more diverse than that at winter time. The toxic effect of the antibiotic amoxicillin increases when the concentration of protons increases. These results suggest that organic contaminants will be changing their toxicity on the organisms at different CO_2 acidification scenarios.

The results reviewed in this section are related to the use of the LOE toxicity under laboratory conditions. The main strength associated with this LOE is the capability to address the adverse effect related to the CO₂ acidification and to the contamination existing in the samples. However, this LOE by itself suffers from information related to the cause that provokes the effects measured. Also, it is assumed that the battery of tests is adequate to identify all the possible responses to all the contaminants presents in the sample and it should be taken into account that these effects were measured under controlled laboratory conditions.

3.3. Field studies and resident infauna

The difficulty of adverse biological effects evaluation resides on the measurement of potential 'in situ' biological adverse effects associated with CO₂ acidification and in combination with the 'in situ' contamination. There are no easy ways to reproduce CO₂ leakage rates under field conditions and only big approaches allow these kinds of surveys compared to laboratory surveys. It has been proposed to use different approaches to mimic these conditions: (a) In situ releases of CO₂ streams to observe the behavior of the stream (Brewer et al. 2006; Adams et al. 2013; Blackford et al. 2014) and its effects on the marine environment; (b) Use natural analogues to better understand the consequences of the release of significant amounts of CO₂ (Benson 1980); or (c) To conduct the -in situ- experiments at either natural analogs or in environments with specific pH variations (e.g., estuaries, acidic environments as Rio Tinto in Spain, etc.) (DeI Valls and Riba 2007).

Regarding the use of macrobenthic community structure as a methodology to address the -in situ- biological effects, there are few studies conducted under field and mesocosms

conditions. Widdicombe et al. (2015) reported results of changes in benthic macrofauna distribution exposed to an artificial release of CO₂ under field conditions. They collected macrofauna samples within the area of CO₂ release and up to 450 m away from the CO₂ emission source. Their results showed that macrofaunal community structure changed significantly. The authors only related the changes in macrofauna to CO₂ in the area closest to the emission of gas. They also showed that the structure of the macrobenthic community recovery was detected 18 days after the CO₂ gas injection had stopped. It was concluded that short-term CO₂ enrichment events are likely to cause localized impacts on macrofaunal communities and it is expected that a rapid recovery would occur, depending on the characteristics of the communities and habitats impacted.

Almagro-Pastor et al. (2015) used a mesocosm experiment to mimic different scenarios of acidification by enrichment of CO₂ and expose macrobenthic communities from a contaminated area. Results showed that, in general, the increase in proton concentration was associated with the decrease in the number of species, diversity, richness and abundance of macrobenthic community structure. They reported that abundance of each species was affected in different ways by the increase of proton concentration. Their results pointed out the differential vulnerabilities of different species, as previously documented in similar mesocosm experiments (Widdicombe et al. 2009; Hale et al. 2011). They showed that polychaeta are the most tolerant taxa to acidification conditions reproduced in the mesocosms. These authors also reported a decrease in the biomass of the resident infauna related to the increase of proton concentration. Also, Passarelli et al. (2018b) showed similar results comparing two different macrobenthic populations from two different ecosystems located in the south and the north hemispheres.

There are other different approaches that could be used to determine the adverse biological effects under field conditions. It could be conducted by sampling resident infauna in the field affected by CO₂ acidification events and measure different responses such as histopathology at different tissues, DNA damage, or other sub-lethal responses like general biomarker approach, etc. These responses may be associated with the CO₂ acidification and also with the contamination occurring in the area before and after the CO₂ leakage. However, to our best knowledge, there are no available data related to the use of these approaches under field conditions. Only studies related to potential use of these sub-lethal measurements have been used to address field CO₂ acidification, but without linking this to existing (or non-existing) contamination in the area. For instance, Pratt et al. (2015) used molecular ecophysiology of resident mollusks affected by the release of CO₂ under field conditions. They did not find any impacts associated with the acidification of the ecosystem by enrichment of CO₂ in the field experiment using selected gene expression in the bivalves. The use of sub-lethal responses has been very helpful in addressing the toxicity of CO₂ acidification and contamination of environmental samples but under laboratory conditions as commented here in section 3.2.

In any case, the use of these measurements and/or the studies using macrobenthic community structure has identified some weaknesses related to the biological effect measured. In contrast to the toxicity evaluations under laboratory conditions, the adverse biological responses measured under field conditions are affected by natural variability and therefore cannot be unequivocally identified if this LOE is used in isolation.

3.4. Bioaccumulation/Biomagnification

There are only a few studies that have considered the bioaccumulation of metals (or other contaminants) at different scenarios of CO₂-induced acidification (Riba et al. 2010; Rodríguez-Romero et al. 2014; Basallote et al. 2015). In these studies, different species of polychaeta and mollusk were exposed to different values of pH (from 8.0 to 6.0) and to different contaminated samples. The main conclusions obtained were that polychaeta are more tolerant to acidification than mollusks. Mollusks and polychaetes did not show significant bioaccumulation of metals to pH values higher than 7.0, as previous authors have reported at ocean acidification scenarios reproduced under laboratory conditions at pH values higher than 7.0 (i.e., Belivermis et al. 2015). In general, none of these studies showed significant bioaccumulation of metals when exposed to different CO₂ acidification scenarios. In a recent study, Passarelli et al. (2018a) identified pH values between 7.0 and 6.0 that had significant correlations between metal bioaccumulation (Fe, Ni and Zn) and the acidification by enrichment of CO₂. In general, the bioaccumulation factor of the contaminants at these conditions of CO₂ acidification must be addressed. It will be necessary to determine the risk of contaminant transference through the trophic chain. There are not enough studies available assessing this problem and we recommend future studies to mainly focus on those potential contaminants that could be affected by biomagnification processes. The results commented in previous sections of this review addressed the increase in the mobility of metals bound or trapped in environmental samples when the acidification increases. Future studies should attempt to establish correct risk evaluation criteria not only at the ecosystem health level but at the human health level as well.

A LOE that addresses the bioaccumulation/biomagnification of contaminants could be applied either under laboratory or field conditions. However, in both cases it shows a

weakness related to the information offered when used alone. The results obtained do not provide information regarding the toxicity of the environmental samples with acidification by CO₂ enrichment. This LOE informs about a process related to the incorporation of contaminants in the organisms and detects a potential cause for possible adverse effects measured in them when used with other LOEs that are capable addressing toxicity (sections 3.2 and 3.3).

3.5. Weight of evidence

The Weight-Of-Evidence (WOE) approach is needed because of the weaknesses identified in the use of the different LOEs used in isolation. The WOE approach will be able to link the information obtained by LOEs reported in the current study. We are unaware of any study that has integrated these kinds of LOEs to distinguish between the adverse effects of acidification and those related to the changes in contaminants. There are a few studies that have used different LOEs to address the impact of acidification by enrichment with CO₂. One of the recent studies (Blackford et al. 2014) reported an integration of different methods carried out to address the impact of a CO₂ release with field conditions. They concluded that the geochemical and biological impacts of the enrichment of CO₂ (<1 ton CO₂ d⁻¹) were limited to meters surrounding the source of the gas. This was a significant advance in the understanding of the impacts of CO₂ enrichment on the marine environment. However, they did not link the changes measured in their experiment to the effects associated with the presence (or not) of potential contaminants in the sediments or water.

The WOE must answer four questions related to determining environmental and human health: What contaminants? b) What level of contaminants? c) What are the biological effects? d) Is there a bioaccumulation/biomagnification of contaminants (Fig.1). Furthermore, under acidification scenarios, it must be able to distinguish between the adverse biological effects associated with acidification 'per se' and those related to changes in the physicochemical characteristics of those contaminants presents in the area of study that are subjected to acidification.

The integration of results can be conducted using different approaches (DelValls 2007). A classical use of statistical analysis by means of performing ANOVA and multivariate analysis (MAA) will allow the predictive identification of nonlinear and mechanistically unpredictable relationships among properties of natural complex systems like sediments (DelValls 2007). The integration of the different set of data will allow quantifying the environmental quality of the studied ecosystems. Also, this integration will allow establishing the mobility of some of the contaminants in the environment, including their mobility through the food chain. The use of WOE by integrating all the results (chemistry, laboratory toxicity, field adverse effects and potential bioaccumulation/biomagnification) using multivariate analysis as a tool should allow researchers to derive sediment quality values (SQGs) as recently reported by Passarelli (2018a). Using the same approach, it will be possible to determine tissue quality values (TQGs) linking the concentration of contaminants measured in the tissue of the organisms and the associated sublethal effects (for instance, using biomarkers of exposure and effects), as previously reported Riba et al. (2005a,b). Also, there are other tools that can link the different data sets obtained for the WOE approach. For instance, the use of different mathematical tools as Self-Organizing Maps (SOMs) or artificial neural networks with unsupervised learning will be able to

691 handle a very high number of cases. These techniques are rare in the integration of the
692 WOE results, probably because trained operators are required to apply this approach in
693 contrast to simply using classical techniques, although SOMs are more visual. In this sense
694 SOMs are more powerful than other techniques used for WOE when large data sets and
695 high quality visualization are required. However, they are not sufficiently developed to
696 establish sediment quality criteria/values or benchmarks that usually are available using
697 classical approaches. Also, classical techniques are developed based on graphical
698 representations, like pie chart diagrams (Riba et al. 2004b), i-Index values (Chapman et al.
699 2002), tabular matrix (Chapman et al. 2002; Riba et al. 2004b) and Best Professional
700 Judgement –BPJ– (Chapman et al. 2002). All of these approaches will be useful in
701 integrating the results and addressing risk assessment with the combination of qualitative-
702 graphical (BPJ, etc.) and quantitative (PCA, etc.) methods, especially when site-specific
703 quality values are need.

704 The use of WOE is recommended in a synoptic way (samples collected at the same time
705 and in the same site, as recommended by Chapman et al. (2002). This tactic will avoid the
706 typical weaknesses associated with the method, such as inconsistencies between the set of
707 data to be integrated when they are separated by different time scales or when they are
708 spatially separated. It will need an additional effort in the design and the execution of the
709 method that will need the application of multiple lines of evidence.

710 The integration of WOE approaches will contribute in the risk characterization and
711 management for the development of a decision-making framework in CO₂ storage sites.
712 This will be based on the characterization of the sediment quality after the leakage and its
713 relationship with the ecosystem health, and the effects of leakage on the marine/freshwater
714 biological community that could be conducted at the same time when applying the

previously described LOEs. Other techniques discussed in this review and can detect leakage but cannot at the same time evaluate the adverse effect. Furthermore, WOE will contribute to establishing the risks associated with the bioaccumulation and/or biomagnifications processes.

Recent amendments to the London Protocol and the OSPAR agreement established Guidelines for Risk Assessment and Management of Storage of CO₂ Streams in Geological Formations (2007) (OSPAR Guidelines 2007). These conventions ask for the “*improvement of impact prediction by gaining knowledge on the effects on species and ecosystems as a result of leakage of CO₂*” to improve risk assessment and management. The use of WOE as part of the guidelines recommended by the International Conventions is shown in Fig.4. It shows the role of WOE during a risk characterization schema approved by the regulatory guidelines for the management of risk in CCS areas. This figure includes a brief scheme of the contents of this risk assessment and management proposed in the international conventions. The different LOEs included in the WOE are related to the cause, effects and exposure assessment within the risk characterization schema proposed. They are needed to characterize the risk and to design an appropriated management plan. Moreover, it is required that the link of LOEs under a WOE will be able to identify potential hazards related to the activity by estimating the probability of these hazards occurring and the severity of effects to exposed species and ecosystems. It determines the need of using the WOE under a correct application of the LOEs and integration of data using the qualitative and quantitative approaches previously discussed. Also, it will be necessary to describe risk estimates in the context of any adverse effects, and to identify and also summarize the uncertainties in the risk assessment. In the EU Directive for CO₂ capture and storage there is a specific article (art.13) concerning the requirements of the

monitoring of the site selection for CCS, including the storage complex and the surrounding environment (EC 2009).

The WOE includes the physicochemical and ecotoxicological characterization of sediments. Also, it includes the bioavailability assessment of contaminants under laboratory and field surveys that will allow for the identification of biomagnification processes. All these lines of evidence (LOEs) will be carried out in a synoptic approach using field-collected sediment and biological samples. This method will allow for the measurement of contamination and its effects related to the acidification associated with small and diffuse CO₂ leakages, including both short and long term episodes (DeValls and Riba 2007; Reguera et al. 2009; Passarelli et al. 2018b).

4. Final Remarks

The physicochemical approach is one of the most commonly used lines of evidence in risk assessment related to the combined effects of acidification and contamination. It includes different techniques and is highly valuable in detecting and identifying the leakages of CO₂ in the CCS. However, it is not capable of establishing biological adverse effects and then the risk assessment will be partial if only this LOE is applied. The equilibrium of carbon dioxide and its relationship with mobility and bioavailability (including speciation) of metal(loid)s is the most addressed issue in this kind of studies.

Few studies addressing the effects of acidification in bioavailability of organic contaminant and its toxicity have been reported. In general, these studies concluded that the increase in metal bioavailability was related to the increase of proton concentration by enrichment of CO₂. Additional efforts must be conducted to address the influences of

acidification in the behavior of organic contaminants and their bioavailability, including toxicity.

The use of the toxicity LOE has increased in the last few years, but it is not widely applied in CCS risk assessment. It is capable of identifying adverse biological effect related to acidification and/or contamination, but applied alone it is not able to identify the cause of the biological effects measured. The toxicity results discussed here showed that most of the revised marine/estuarine/freshwater studies demonstrated that the CO₂-induced acidification affects the mobility of metals bound to marine sediments. At a normal marine ecosystem pH value, those metals that were previously bound to sediments and were not available to the exposed organisms became available, and subsequently toxic to the exposed biota when the proton concentration increased as result of CO₂ enrichment. The results reviewed here show a gradient of tolerance at different life stages and taxa, with early life stages being more sensitive to acidification and to the combined effects of contaminants than adults. Regarding taxa, sea urchins and amphipods were more sensitive to these effects than mollusks and polychaetae. These two last taxa are likely better candidates for conducting bioaccumulation/biomagnification studies than sea urchin or amphipods.

Although there is information available related to the metal mobilization in freshwater ecosystems affected by CO₂ acidification, there are only two works in the bibliography that relate this mobilization with the toxicological effects on resident aquatic organisms. Although the two studies discussed here show similar results to those obtained using seawater environments, these kinds of studies should be increased in the future.

Studies conducted using mesocosms and under field conditions, using macrobenthic communities or caged animals, showed impacts on the diversity, richness and biomass of

the affected communities. Recovery of the community is reported in studies conducted under field conditions, at acidification scenarios, without considering the potential contamination of the sites studied. When linking both effects (acidification and contamination) in mesocosm studies, using macrobenthic communities, significant adverse effects in the structure of the community were measured and related to metal mobility. This LOE is capable of determining the biological effects, but is not capable of identifying the cause or to discriminate the effect measured from natural changes, when used alone.

There are only a few studies that characterize the effects of CO₂ acidification on the bioaccumulation/biomagnification of contaminants in sea and fresh water ecosystems. The results discussed here addressed the increase in the mobility of metals, bound or trapped in environmental samples, when the proton concentration increases by CO₂ enrichment. It is to be expected that a potential increase in the bioaccumulation/biomagnification of certain contaminants when the proton concentrations increase. However, it has not been clearly demonstrated and additional efforts should be conducted in future studies to establish a potential risk evaluation, not only at the ecosystem health level, but also affecting human health. Special efforts should be conducted regarding substances capable of biomagnification (organic, emerging contaminants, etc.) at different acidification scenarios.

The integration of the multiple LOEs under a WOE approach is recommended in the different steps included in the guidelines for the risk characterization and management proposed by different international regulations. However, these LOEs applied in isolation have some weaknesses, as previously discussed. There are as yet no studies that have fully applied the WOE to determine the combined effects of acidification and contamination. It is strongly recommended to increase the research and technological efforts to improve the use

of WOE in the risk management and characterization of aquatic ecosystems affected by enrichment episodes of CO₂ (CCS, or other sources).

The combination of acidification and contamination studies must be used with an integrative assessment. The best approach is to use multiples lines of evidence, which will avoid the weaknesses associated with each of them if applied in isolation. The integration of the LOEs results will be linked based on a weight of evidence approach. We must solve this problem, which has competing risks and benefits.

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Data Accessibility

All data is available upon request directly to corresponding author.

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FIGURE CAPTIONS

Figure 1.- Schematic representation of a weight-of-evidence approach used to address the four questions to risk characterization (adapted of DelValls 2007): What contaminants? b) What level of contaminants? c) What are the biological effects? d) Is there a bioaccumulation/biomagnification of contaminants? These four questions must be addressed to risk characterization in scenarios combining CO₂-induced acidification and contamination of environmental samples. The main aim is to distinguish the risk associated with the acidification, with the contamination and with the combination of these two processes.

Figure 2.- Diagram representation of the sensibility of different taxa to the combination effects of acidification and contaminant concentration in environmental samples that have been acidified to mimic different scenarios by enrichment with CO₂.

Figure 3.- Results from larval development success of *Perna perna* mussel. The larvae were exposed to different concentrations of crack-cocaine (Control, 6.25, 12.5 and 25 mg/mL) at different scenarios of acidification defined by pH values (8.5; 8.0; 7.5 and 7.0). Asterisks (*) mean significant difference to the control ($p < 0.05$).

Figure 4.- Schematic description of the framework for risk assessment and management of CO₂ storage in sub-seabed geological formations established in different international regulations such as London Convention and OSPAR (adapted from Reguera et al. 2009, and DelValls 2007). The main role of the WOE approach is to characterize the risk associated with the ecosystem and the human health, as described in the figure.

Schematic representation of a weight-of-evidence approach used to address the four questions in risk characterization (adapted of DelValls 2007): a) What contaminants? b) What level of contaminants? c) What biological effects? d) Does it exist a bioaccumulation/biomagnification of contaminants? These four questions must be addressed to risk characterization in scenarios of CO₂-induced acidification and contamination of environmental samples. The main aim is to distinguish the risk associated with the acidification, with the contamination and with the combination of these two processes.

210x172mm (96 x 96 DPI)

Diagram representation of the sensibility of different taxa to the combination effects of acidification and that associated with contaminant concentration in environmental samples acidified by CO₂ release that mimic different scenarios of enrichment of CO₂.

223x107mm (96 x 96 DPI)

Results from larval development success of *Perna perna* mussel. The larvae were exposed to different concentrations of crack-cocaine (Control, 6.25, 12.5 and 25 mg/mL) at different scenarios of acidification defined by pH values (8.5; 8.0; 7.5 and 7.0). Asterisks (*) mean significant difference to the control ($p < 0.05$).

223x170mm (96 x 96 DPI)

Schematic description of the contents included in the framework for risk assessment and management of CO₂ storage in sub-seabed geological formations established in different international regulations (adapted from Reguera et al. 2009, and DelValls 2007). The main role of the WOE approach is described in the figure and related to the characterization of the ecosystem and human risks.

184x129mm (96 x 96 DPI)