# CHAPTER 8

# Impact of Pollutants on Coastal and Benthic Marine Communities

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**Abstract:** In recent years, sources, types and levels of contaminants in the marine environment have increased as a consequence of anthropogenic activities worldwide. Chemical substances are usually present in the marine environment at different concentrations. They are accumulated in the tissues of marine organisms exerting damaging effects at different levels of organization, from organisms to communities and ecosystems. The understanding of their effects and distribution has increased substantially since the early reports on the biological effects of marine pollution and associated monitoring problems. This Chapter has been divided into five main sections: (i) molecular, cellular and tissue level biomarkers in assessing effects, reviewing the use of biomarkers in monitoring effects; (ii) biological effects at organism and population level; (iii) bioassay studies at organism level, focusing on ecotoxicology and discussing toxicity estimation and bioassay limitations; (iv) ecological effects at community level, including structural parameters, such as richness, diversity, *etc.*, but also the proportion of opportunistic and sensitive species, discussing the multiple pressure interactions; and (v) measuring pollutant effects in integrative assessments, both in evaluating risk and assessing the ecological status of the ecosystems. The main objective of the Chapter is to bring together the knowledge on the biological effects of pollution, developed in recent years, at different biological levels of organization.

## INTRODUCTION

In recent years, sources, types and levels of contaminants in the marine environment have increased as a consequence of anthropogenic activities worldwide [1, 2] (Table **1**). Chemical substances are present usually in the marine environment in different concentrations; they are not necessarily accumulated in the tissues of marine organisms, but they still can cause toxic effects (e.g. herbicides) even at very low concentrations, exerting damaging effects [3]. The understanding of their effects and distribution has increased substantially since the early reports on the biological effects of marine pollution and associated monitoring problems [4] (Table **1**). However, there is a need to develop methods for the identification, estimation, comparative assessment and management of the potential risks posed by chemical pollutants to aquatic living resources and marine ecosystems [5, 6]. Hence, the relationships between pollutants and direct and indirect effects on the marine environment, across different levels of organization, together with the operational framework for establishing causal-effect relationships are presented in Fig. (**1**).

Assessment of environmental pollution cannot be based solely upon chemical analyses, because this approach does not provide clear indication of the deleterious effects of contaminants [5, 7]. In addition, the increasing number and types of potential pollutants (*i.e.* polybrominated diphenyl ethers, endocrine disruptors, pharmaceuticals, *etc.*) entering the marine environment requires novel strategies for pollution assessment. Consequently, there is general agreement that the most appropriate approach for the assessment of environmental pollution is by integrating a suite of chemical and biological measurements [5, 7-9].

In this respect coastal organisms are of high importance in environmental toxicology as sentinel species, because they are can be used in the assessment of the effects of pollution through biological effect measurements [10]. Bivalve molluscs have been one of the most widely-used indicators to determine the existence and toxicity of chemical substances. Therefore, due to their sessile nature, wide geographical distribution and high bioaccumulation capacity, they have been considered as ideal for the detection of the biological effects of pollutants [11].

In order to analyse the extent of disturbances of a biological system and to quantify the state of health, the integration of several biological effects at different levels of biological organisation has been suggested by several authors [6, 12]. Biological effect measurements incorporate three approaches: (i) biomarker studies at molecular, cellular and tissue level; (ii) bioassay studies at whole organism level; and (iii) ecological surveys at community and population level [13].

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**Table 1:** Effects on marine life, from different types and sources of pollution. Source: adapted from WorldWatch Institute (http://www.gdrc.org/oceans/marine-pollution.html).

| Type | Primary Source / Cause | Effect |
|---|---|---|
| Nutrients | Runoff - approximately 50% sewage, 50% from agriculture, cattle, and other land use. Also airborne nitrogen oxides | Feed micro- and macroalgal blooms in coastal waters. Decomposing algae depletes water of oxygen, and releasing toxins can kill marine life |
| Sediments | Erosion from mining, forestry, farming, and other land-use; coastal dredging | Water turbidity; impede photosynthesis. Clog gills of fish. Smother and bury coastal ecosystems. Toxins |
| Pathogens | Sewage, livestock | Contaminate coastal swimming areas and seafood, spreading cholera, typhoid and other diseases |
| Alien Species | Transported in ballast water, aquaculture, *etc.* | Outcompete native species, reduce biodiversity, introduce diseases, increased incidence of red tides |
| Persistent Toxins (PCBs, metals, DDT *etc.*) | Industrial discharge; wastewater discharge from cities; pesticides from farms, forests, home use, *etc.*; seepage from landfills | Poison or disease in marine life, especially near major cities or industry. Contaminate food-webs and seafood. Fat-soluble toxins that bio-accumulate in predators can cause disease and reproductive failure |
| Oil | 46% from cars, heavy machinery, industry, other land-based sources; 32% from oil tanker operations and other shipping; 13% from accidents at sea; also offshore oil drilling and natural seepage | Low level of pollution can kill larvae and cause disease in marine life. Oil slicks kill marine life, especially in coastal habitats. Tar balls from coagulated oil litter beaches and coastal habitat. Oil pollution is down 60% from 1981 |
| Plastics | Fishing nets; cargo and cruise ships; beach litter; wastes from plastics industry and landfills | Discarded fishing gear continues to catch fish and crustaceans. Other plastic debris entangles marine life or is mistaken for food. Plastics litter beaches and coasts and may persist for 200 to 400 years |
| Radioactive substances | Discarded nuclear submarine and military waste; atmospheric fallout; also industrial wastes | Hot spots of radio activity. Can enter food web and cause disease in marine life. Concentrate in top predators and shellfish, which are eaten by people |
| Thermal | Cooling water from power plants and industrial sites | Kill off corals and other temperature sensitive sedentary species. Displace other marine life |
| Noise | Supertankers, other large vessels, military exercises, and machinery | Can be heard thousands of kilometres away under water. May stress and disrupt marine life. |

This Chapter has been divided into five main sections: (i) molecular, cellular and tissue level biomarkers in assessing effects; (ii) biological effects at organism and population level; (iii) bioassay studies at organism level (as a part of the previous section, focusing on ecotoxicology); (iv) ecological effects at community level; and (v) measuring pollutant effects in integrative assessments. The main objective of the Chapter is to bring together knowledge on the biological effects of pollution developed recently at different biological levels of organization.

## MOLECULAR, CELLULAR AND TISSUE LEVEL BIOMARKERS IN ASSESSING EFFECTS

Recent studies have applied the "biomarker approach" to assess deleterious effects in biological systems [8, 16]. However, there is still some debate relating to their definition and, especially, their use in environmental risk assessment [17, 18]. According to McCarthy and Shugart [19], biomarkers are defined as measurements of body fluids, cells, or tissues, that indicate in biochemical or cellular terms, the presence of contaminants (exposure biomarkers) or the magnitude of the host response (effect biomarkers). Owing to the short time of response, biomarkers are used as early warning signals of biological effects caused by environmental pollutants in order to predict changes at higher levels of biological organisation; *i.e.* populations, communities or ecosystems [5]. In general, responses at lower biological organisation levels are more specific, sensitive, reproducible and easier to determine, but more difficult to relate with ecological changes. Conversely, responses at higher biological organisation levels are indicative directly of ecosystem health; hence, more relevant to environmental management. However, they are more difficult to determine, less specific and only manifest at a late stage, when environmental damages have already occurred [20, 21].

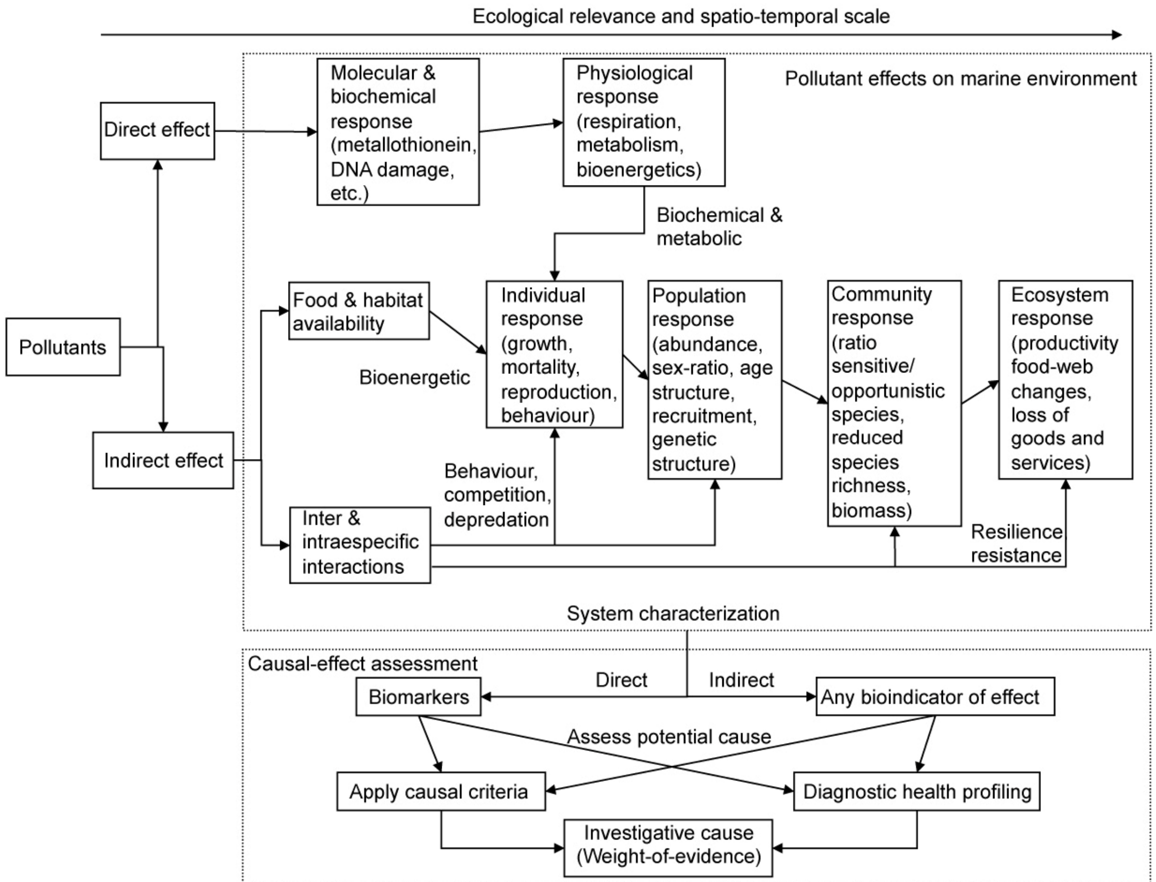


**Figure 1:** Relationships between pollutants and their direct and indirect effects on the marine environment along a gradient of ecological relevance and spatiotemporal scale, together with the operational framework for establishing causal-effect relationships using multiple lines of evidence (adapted and modified from Clements [14] and Adams [15]).

The biomarker approach should be applied as a 'multi-marker' approach, since the use of a single biomarker in environmental studies does not reflect the integrated response of the organism [5, 6]. Furthermore, for each biomarker, specificity, temporal relevance, inter- and intra- individual variability, baseline values and the existence of confounding biotic and abiotic factors should be defined before its application [8].

Despite the above mentioned shortcomings, biomarkers could be a very valuable tool to determine cause-effect relationships, in the assessment of environmental pollution in the operational and investigative monitorings defined in the European Water Framework Directive (WFD) [22]. Thus, against the background of the development of the European Marine Strategy and of relevant Directives, the biomarker approach has been recommended and introduced in monitoring programmes for the surveillance of the marine environment, as an approach that complements the use of diagnostic chemical analysis with the assessment of biological effects [7, 23].

Over the last 20 years, an increasing number of ecotoxicological papers or research dealing with pollutants have focused on the study of causality between pressure of xenobiotics and responses at the molecular, cellular and tissue level. The possible effects of various contaminants on the health status of several coastal organisms are reviewed herein.

Metal toxicity occurs when the rate of metal uptake into the body exceeds the combined rate of excretion and detoxification of available metal [24]. The first measurable effect of metals is on the inducibility of metallothionein (MT) genes and MT protein synthesis [25, 26]. In mussels, two families of MT isoforms have been characterised recently [27]; it has been suggested that these isoforms may accomplish different functions in presence of different metals and stressors. Whereas the monomeric MT10 isoforms have been reported to be synthesised constitutively and involved in essential-metal regulation, particularly of Cu and Zn, the dimeric MT20 isoforms are inducible and are involved mainly in Cd and hydroxyl radical detoxification [26, 28].

Organic pollutants are taken up readily into the tissues of aquatic organisms; here, biotransformation via Phase I (functionalisation) and Phase II (conjugation) metabolism can in part, determine the fate and toxicity of the contaminants [29]. Of central importance to Phase I metabolism is the mixed function oxygenase system, whose terminal component cytochrome P450 (CYP) exists as a superfamily of proteins, capable of oxidising a wide variety of substrates in numerous aquatic species [30]. One specific form, CYP1A1, can be estimated in fish liver by the measurement of 7-ethoxyresorufin-O-deetilase (EROD) activity and has been used as a biomarker of exposure to organic aromatic xenobiotics [23, 31]. In contrast with fish, marine invertebrates, especially mussels, have a low ability to metabolise organic pollutants, due to the lack of efficient isoforms of P450, which are only found in terrestrial organisms. Thus, one of the most important sentinel species (mussels) does not possess this important biotransformation system, discouraging the utilisation of EROD induction in pollution biomonitoring programmes, using mussels as sentinels. However, recent studies have suggested that the assessment of peroxisome proliferation and multixenobiotic resistance (MXR) may be more appropriate, as biomarkers of exposure to organic xenobiotic compounds in molluscs [32]. In this respect, both laboratory and field studies have shown that organic xenobiotics (such as phthalate ester plasticizers, Polycyclic Aromatic Hydrocarbons (PAHs) and oil derivatives, Polychlorinated Biphenyls (PCBs), certain pesticides, bleached Kraft pulp and paper mill effluents, alkylphenols and estrogens) all provoke peroxisome proliferation in aquatic organisms [33, 34]. Accordingly, MXR-related gene expression has been detected, either in terms of induction or inhibition, in molluscs exposed to anthropogenic organic pollutants [35, 36].

It is important to note that biomarkers can be affected by mixtures of different chemicals present in the field, giving rise to additive, synergistic and/or antagonistic effects. Several studies have demonstrated decreases in the activities of several enzyme markers of organic compounds in molluscs from polluted sites, probably due to complex interactions occurring in the marine environment between mixtures of pollutants [16, 37]. Similarly, acetylcholinesterase activities, inhibited by some pesticides (in particular carbamates and organophosphorus compounds), can be inhibited also by metals [38].

At a cellular level, tolerance to metals is based upon sequestration by a range of cellular ligands, such as MTs, followed by compartmentalisation within lysosomes [25]. Thus, metals bound to metal-binding proteins may enter lysosomes, and follow the catabolic pathway as would any other cellular protein. However, excessive concentrations of metals can cause alterations of structure, permeability and integrity of the lysosomal membrane when storage capacity of the lysosomes is overloaded [39, 40]. Impairment of lysosomal functions and, thereby, of food assimilation, can result in severe alterations in the nutritional status of the cells and whole organisms, and could be indicative of disturbed health. Previous studies have reported lysosomal enlargement, increased intralysosomal accumulation of metals and enhanced production of lipofuscins in metal exposed mussels, as a detoxification mechanism to minimise toxic effects of excess metals [28, 41, 42]. Additionally, a decrease in lysosomal membrane stability has been documented upon exposure to a progressively higher Cu concentration in laboratory experiments [43, 44] and in a transplant study in a copper mine [45].

Nevertheless, lysosomal responses are not metal-specific and, thus, are considered as a biomarker of general stress [46]. For instance, destabilised lysosomal membranes have been found also in marine organisms exposed to organic compounds, or collected from sites with mixture of contaminants [16, 47, 48]. Likewise, enhanced lipofuscin deposition has been observed in the digestive cells of mussels exposed to PAHs under both laboratory and field conditions [47, 49]. Intralysosomal neutral lipid accumulation in aquatic organisms (*i.e. Mytilus edulis*) has been reported upon exposure to organic chemicals in laboratory experiments [50, 51] and in sites polluted with oil derivatives [41, 52].

There is evidence [48] that reactive oxygen species (ROS) are formed in the presence of a wide range of contaminants, such as metals (e.g. Cu, Fe), PCBs and some pesticides, provoking alterations in proteins, DNA and membrane structures/functions. ROS are detoxified by antioxidant enzymes and scavenger molecules. In marine mussels caged for 4 weeks, in an industrialised harbour of north-west Italy, a biphasic trend for single antioxidants (catalase, glutathione *S*-transferases, glutathione reductase, total glutathione) and the total oxyradical scavenging capacity was shown. There was no variation or increase during the first 2 weeks of exposure to the polluted site, followed by a progressive decrease up to a severe depletion of ROS in the final part of the experiment [48]. The decreased capacity to neutralise specific ROS has been shown to correlate with the occurrence of alterations at various sub-cellular targets, including lysosomal membranes and DNA [53, 54]. Oxidative DNA damage, revealed as high levels of the mutagen 8-oxo-7,8-dihydro-2'-deoxyguanosine and lipid peroxidation, measured in terms of high malondialdehyde levels, has been found in mussels from polluted areas [55, 56].

Genotoxic compounds, such as persistent organic pollutants (POPs), can alter the integrity of DNA structure, either directly or through their metabolites [57], causing mutagenesis [58]. Biomarkers of genotoxicity include DNA damage, which is based upon potentially pre-mutagenic lesions (such as DNA adducts, base modifications, DNA-DNA and DNA-proteins cross-linking and DNA strand breaks) and chromosomal damage [59]. The presence of micronuclei is an indicator of chromosome breakage or chromosome loss [60]; this technique has been used extensively in invertebrates [61, 62]. Caged mussels exposed to seawater polluted by aromatic hydrocarbons, displayed a continuous increase of micronuclei frequency in gill cells, reaching a plateau after a month of caging [63]. The incidence of micronuclei has also been linked to the induction of leukaemia cells in the clam *Mya arenaria*, suggesting that the micronucleus test is a very good indicator of the potentially life-threatening consequences of genotoxic exposure [64].

The effects of environmental pollution have been identified also at tissue level and, therefore, histopathological changes in target tissues have been proved to be sensitive markers of health status in aquatic organisms [65, 66]. In bivalve molluscs, highly significant correlations between tissue pathologies and contaminants (Pb, Hg and PCBs) have been observed in mussels from the east coast of the USA [67]. In the digestive gland of mussels exposure to Cu, Cd and the water-accommodated fraction of different oils induced alterations in cell type ratios of digestive tubules, with basophilic cells increasing in number, in relation to digestive cells [28, 68]. This morphological change of digestive tubules has been observed, accompanied by atrophy of the digestive epithelium in molluscs [45, 69], apparently involving augmented autophagic processes [70]. Following the *Prestige* oil spill [69] that occurred in November 2002 in the Atlantic, north-west coast of Spain, digestive gland atrophy was demonstrated in mussels, indicating disturbed health, due partly to disturbances in digestion and metabolism [71]. Another severe tissue alteration, the incidence of granulocytomas, has been observed in molluscs from highly polluted areas [72].

There is a growing concern that chemicals in the environment, either natural or synthetic, can interact with the endocrine system, causing reproductive disturbances in aquatic organisms that may affect recruitment and lead eventually to deleterious population effects [73, 74]. Endocrine–disrupting chemicals such as phytoestrogens, alkylphenols, synthetic estrogenic hormones and bisphenol A mimic estrogenic hormones and thus cause estrogenic or feminising effects, whereas other chemicals such as tri-butyltin (TBT), used in antifouling paints for ships, and synthetic androgenic hormones cause androgenic effects [73, 75]. In invertebrates, the endocrine regulation of reproduction and development is not as clear as in vertebrates [76]. Vitellogenin (VTG)-like proteins, yolk proteins produced only in sexually mature females, have been observed in male molluscs exposed to different xenoestrogens such as alkylphenols [34, 77, 78]. In contrast, reduced VTG-like protein levels have been described in female molluscs inhabiting PAH contaminated sites or, after exposure to North Sea oil, indicating a possible anti-estrogenic effect of PAHs [34, 79]. Moreover, exposure of females to certain metals, such as Cu, provoked an increase in VTG-like protein levels and accelerated spawning, maybe as a result of possible acute toxic effects, or as an effect on hormone regulation of gamete development [45, 80]. These controversial results in females highlight the need for more basic research to understand biomarker responses, before their implementation in monitoring studies. On the other hand, it should be noted that the development of more advanced techniques for biomarker quantification could also be helpful in the interpretation of the multi-marker approach.

Overall, the utility of stress indices, based upon molecular, cellular and tissue responses in sentinel species, could provide a comprehensive indication of the impact of chemical pollutants in coastal marine environments. However, the need to increase the knowledge on biomarker baseline values and confounding factors is also highlighted.

## BIOLOGICAL EFFECTS AT ORGANISM AND POPULATION LEVEL

The impacts of metallic and organic pollutants on marine biota cover a plethora of direct and indirect effects on organisms and populations. These effects have been reported in a wide variety of biota such as bacteria, fungi, plants and animals.

Among the inorganic metallic pollutants, Cd, Cu, Cr, Pb, Hg, Ni and Zn are some of the best studied in terms of speciation, toxicity, bioavailability or bioaccumulation in marine ecosystems. Other metals with toxic effects in marine communities are Al, Sb, As, Se or Ag. Unlike other contaminants, all of these metals occur naturally in the environment; some of them (e.g. Cu and Zn) have essential functions in several biota at low concentrations. The degree of increase in levels of these metals in the environment, with respect to the background levels, and the degree to which they have toxic effects on biota, depends upon a wide number of factors such as: (i) their geochemical behaviour; the physiology and condition of the target biota; (ii) chemical speciation and; (iii) the presence of other toxicants, or environmental conditions [81]. In order to assess the lethal responses of marine biota to the metallic pollution, several approaches have been carried out since the 20$^{th}$ Century. Laboratory bioassays have permitted comparisons of toxicity between different pollutants and among different species. Moreover, these bioassays have shown that the first developmental stages of several species of invertebrates are highly sensitive to several toxicants [82, 83]. Research has also been carried out in order to evaluate sublethal responses in marine biota to inorganic metals; this has included effects on growth, sexual maturity, diseases, luminescence, metabolism, *etc.*

The research undertaken on the biological effects of the organometallic pollutants has shown that some of these compounds are substantially more toxic than the inorganic metal to marine organisms. As an example, methylmercury is substantially more toxic to several biota than inorganic mercury, because it is more efficiently transported across the gut [84] and because of its ability to diffuse through lypophilic media and cross cell membranes. Another highly researched pollutant is TBT, which has toxic effects in a wide variety of biota, whereas inorganic tin is less toxic. TBT effects include lethal toxicity and effects on growth, reproduction, physiology, and behaviour [85]. Several of the negative effects are due to interferences with the endocrine function, and manifested as the imposex phenomenon. In dioecious gastropods, imposex presents as the superimposition of male organs in females [86]. In some species, such as the dogwhelk *Nucella lapillus,* this masculinisation has provoked the local extinction of populations in several polluted areas, during the 1970s and 1980s [87]. Because TBT has negative effects at a very low concentration (e.g. ~ 1 ng Sn/L [87]), it has been considered as one of the most toxic xenobiotics ever produced and introduced deliberately into the environment [88]. At those sites where measures were taken to reduce the input of TBT into marine ecosystems, the previously affected species have shown a recovery [89].

PAHs are relatively ubiquitous organic molecules, within marine ecosystems. There exists more than 100 different PAHs, with both natural (e.g., forest fires) and anthropogenic (e.g., burning processes or oil spills) inputs into the ecosystems. The fact that PAHs occur generally as complex mixtures in the environment makes the evaluation of toxicity more difficult [90, 91]. This difficulty is increased by the fact that some have shown higher toxicity as a consequence of photoactivation [92]. Although there is a need for research to clarify the biological effects of PAHs in marine ecosystems [90], toxic (carcinogenic) effects have been found in phytoplankton, zooplankton, invertebrates and vertebrates because they form DNA adducts. Most of these toxic effects are related to a reaction with macromolecules (like nucleic acids or proteins), or an interaction with lipids in cell membranes or other cellular constituents [93, 94]. These processes can cause diseases (including tumours) and negative effects on immunosystems, growth, reproduction and behaviour.

Chlorinated pollutants (*i.e.* PCBs, organochlorine pesticides, herbicides and fungicides) have effects on the physiology and behaviour of marine biota. Almost all of these compounds are not known to occur naturally in the environment and some are very persistent. Negative effects were found on growth, reproduction, luminescence, metabolism, *etc.* Some of these effects are due to endocrine disruption. Although some of these effects in marine organisms are relatively well known, others are still poorly understood [95].

Pollution effects on organisms can imply consequences at the population level. These can occur in different degrees, from changes in the population dynamics or genetic diversity, to the local extinction (as the above-mentioned case of some gastropod extinctions due to TBT pollution). The linkage between pollution and population can be more complex to assess than is the case for individual organisms. Nevertheless, the recent development of genetic techniques presents some assessments of this issue [96]. Populations might respond with increased genetic variation (e.g. resulting from new mutations), or decreased genetic variation (e.g. resulting from population bottlenecks) [97]. The loss of genetic diversity can imply a reduced adaptive potential of populations to changes in environmental characteristics or to the presence of new pollutants [96, 98, 99]. Because of the complexity of the processes involved, the loss of genetic diversity is not predictable based solely upon knowledge of the mechanism of toxicity of the chemical contaminants and the life cycle of the biota [97].

Several studies of the effects of contaminants in populations have been carried out with meiobenthic species. This approach is related to the fact that several meiobenthic species have short generation times; this permits a study for the duration of a full life cycle. Studies carried out near offshore platforms, combined with laboratory experiments, found genetic diversity in meiobenthic copepods (*Nitocra lacustris, Cletodes sp., Enhydrosoma pericoense, Normanella* sp.*, Robertsonia* sp.*, and Tachidiella* sp.) correlated inversely with the degree of sediment contaminants (hydrocarbons); however, other causes could be attributed [100, 101]. Laboratory assays have found that the exposure to polybrominated diphenyl ether (BDE-47) and copper can reduce genetic diversity and alter genotype composition without affecting population abundance of some meiobenthic species [102, 103].

Genetic studies have also been carried out on macrobenthic taxa. Research undertaken on mussel, barnacle, prawn and isopod species suggests that pollution may reduce genetic diversity, but other causes cannot be discarded. Moreover, long-term exposure to metal pollution does not necessarily result in decreased genetic diversity [104, 105].

The exposure of the populations to pollutants can select rapid genetic changes or small-scale evolutionary processes associated with a genetically-inherited increase in tolerance to the pollutants (a process called microevolution [106]). This microevolution can buffer, partially, the effects of pollution in populations. As an example, an estuarine oligochaete species (*Limnodrilus hoffmeisteri*) was found to be more resistant to cadmium and nickel pollution following two generations of selection in laboratory assays [107].

## BIOASSAY STUDIES AT ORGANISM LEVEL

An important component of pollutants arriving in the marine environment is retained in the bottom sediments, where they can reach concentrations several orders of magnitude higher than in the overlying waters [108]. Hence, in coastal systems, bottom sediments need to be characterized in environmental studies. Historically, such characterization has been limited to physicochemical analysis [109]. However, the chemical analyses by themselves do not provide evidence of biological effects on organisms; therefore, they do not assist in confirming the effect that they induce on ecosystems [110, 111]. Thus, toxicity tests on marine systems, among other biological methods of ecotoxicological evaluation, are necessary as a complement to physicochemical analyses to assess the potential effects of pollutants on organisms and biological communities [109].

Ecotoxicology, as the science that studies all of the adverse biochemically-mediated effects of all chemicals on all living organisms, including all their interactions within organisms and among species in the environment [112], is applied to the evaluation of the effects caused by pollution on the marine environment by means of bioassays.

### Bioassays

Bioassays are used to evaluate the environmental quality through the measures of toxicity in natural samples, and to predict the ecological risk of contamination. These tests show numerous advantages: (i) the test organisms only respond to the bioavailable fraction of a pollutant; (ii) also, as opposed to chemical analyses that detect only previously well-known compounds, bioassays can help identify new toxic elements whose noxious effects had not been described previously [113]. In addition, (iii) they offer quantitative information on sediment toxicity, which provides a basis for discriminating between impacted and unimpacted sites. The results from these tests are also relevant ecologically because they commonly use resident species. As such, the tests undertaken provide a way to compare the sensitivities of different organisms.

The toxicity tests under laboratory conditions are carried out with the purpose of establishing any relationship between exposure of pollutants and the effects caused on individual organisms. By means of these tests, dose-response relationships are established, determining the relationship between the concentration or dose of the toxin and the noxious effect on an organism. Following the proposal of the use of toxicity tests as an appropriate tool for the valuation of marine pollution [83], they have become a fundamental part of the evaluation of environmental risk; they provide a direct measure of toxic adverse effects, complementing the traditional physicochemical measures [114].

At present, the list of bioassays to assess toxic effects of exposure to marine bottom sediments, as a measure of risk to populations is very extensive [115-118], given the multiple combinations that exist between the elements and the conditions to be selected. These bioassays, carried out in highly defined, controlled and reproducible conditions, can be applied to total sediment, suspended sediment, elutriate, pore water and/or sediment extract. The response variables to be measured include long-term toxicity, acute toxicity, bioaccumulation, endocrine effects, effects on reproduction, carcinogenesis and mutagenesis. Different marine organisms belonging to different trophic levels (bacteria, algae, molluscs, echinoderms, annelids, fishes, *etc.*) and in different development phases can be used in bioassays. This approach offers a wide range of biological possibilities for investigation [119, 120].

The organisms used in these tests are selected on the basis of: (i) their sensitivity; (ii) their ecological, commercial or recreational relevance; (iii) their high availability and abundance; (iv) their ease of culture or maintenance in laboratory; (v) and the simplicity of the analysis of results [121-123]. Finally, the responses obtained in test species can be qualitative or quantitative, but should be unequivocal, easily observable, describable, measurable, biologically significant and reproducible [124].

In a typical bioassay, the response of an aquatic organism to a toxic substance is related to the toxic concentration in water/sediment, together with the time of exposure. A commonly used technique to measure the dose-response relationship requires exposure time to be held constant over a series of different concentrations in order to record the proportion of individuals that present a specific biological response; e.g. mortality-survival, fertilization-non fertilization, or mobility-non mobility. The final objective is to obtain a toxicity curve of a substance or compound for an organism that defines the relationship between concentration (dose) and response (see Chapter 1).

**Toxicity Estimation**

To estimate the toxicity of a substance or sample, the EC50 is used extensively since it is a statistically-reliable measure. The EC50 is defined as the Effective Concentration that produces a specific effect on 50% of a population based upon experimental laboratory tests. It is used as a standard measure of toxicity and it permits a comparison of the toxicity of different compounds on an organism, or the toxicity of the same compound on different organisms. However, on the basis that the final objective of toxicological studies is to protect ecosystems, the EC50 alone is not sufficient. Therefore, it is necessary to obtain a second parameter that defines the toxicity threshold; *i.e.* the concentration above which they begin to show adverse effects. In this sense the NOEC (the highest experimental concentration in which the response does not present statistically-significant differences, with regard to the control) and LOEC (the lowest experimental concentration in which the observed response is significantly different from that of the control) are defined. These last reference parameters present a higher potential for utilisation from the point of view of their ecological application. Nevertheless, some debate exists presently on the suitability of NOEC and LOEC as estimates of the toxicity threshold because of their strong dependence on the experimental design, and an alternative frequently used is the concentration causing a lower level of effect, such as the EC10 [125, 126]. Moreover, the reason for not using LOEC and NOEC as endpoints in many instances (e.g. regulatory, water quality guidelines) is the statistical unreliability of the calculated values for such endpoints [125, 126].

**Bioassays Limitations**

The laboratory bioassays have some limitations since they do not necessarily reproduce the range of potentially relevant environmental factors present in nature [127, 128]: (i) the species used are not necessarily part of the communities that inhabit the studied sediments and, as such, they may not be representative of the species found in the area of interest [129]; (ii) the laboratory conditions are controlled, whilst the factors (i.e. abiotic:. climate, temperature, hydrodynamics, quality of water and/or sediment; and biotic: development stage, reproductive state, health, presence of other individuals and/or species, etc.) are changing in the environment [125, 130]; (iii) biomagnification through trophic webs, effects of nutrients, habitat alteration, inter- and intraspecific predation or competition relationships are not taken into account [131]; and (iv) laboratory bioassays do not predict the indirect effects that often characterize the responses of the ecosystems to stress [132]. Therefore, although these bioassays have improved our understanding of the effects of pollutants, their results are difficult to extrapolate to the environment, because they lack ecological "realism" [133], since too many components exist in an ecosystem that make it impossible to predict accurately the effect that a toxic substance can exert. For example, the effect of a toxicant varies not only between species within an ecosystem, but also in the same species in different ecosystems. On the other hand, in natural environments, a substance may not produce adverse effects on a particular species, but does so in its predators or its food source, which influences finally the survival of the organism. Thus, in the absence of a thorough understanding of natural systems and their processes there is always a high degree of uncertainty [134]. Hence, it is necessary to obtain the highest amount of data on ecosystem dynamics, abiotic compartments and xenobiotics' toxic effect on the species. The analysis of these data permits the prediction, with a higher reliability, of the ecological risk associated with an episode of contamination (Environmental Risk Evaluation). Similarly, all this knowledge needs to be applied together for the restoration of ecological health in contaminated systems [135, 136]. This is the reason why ecotoxicological information is important when establishing environmental monitoring programmes [137].

Because of the necessity of obtaining more complete and useful information from an ecological perspective, testing on the basis of a single species has not been considered sufficient in recent years. When evaluating potentially contaminated samples from the environment, the use of a test battery, including species belonging to different habitats, development stages, multiple trophic and evolutionary levels, and (even) different times of exposure, is recommended as a more appropriate strategy [138, 139].

For the purpose of chemical registration, regulatory authorities require bioassays with individual chemicals.. These bioassays need to be conducted under standard conditions and there is no interest in reproducing the broad variability of natural ecosystems. On the other hand, bioassays with environmental samples are normally conducted in order to detect the presence of chemicals a priori unknown. In that case, the bioassay is a tool in the 'tool-box'. Once the hot-spots are identified, subsequent more expensive techniques may be applied to study the ecological effects.

## EFFECTS ON COMMUNITIES AND ECOSYSTEMS

There is general agreement amongst marine investigators that measuring a suite of indicators across levels of biological organization is often necessary to assess ecological integrity [14]. As the effects of pollutants on the marine environment may be identified at all levels of biological organization, these indicators should include biochemical, population, community, and ecosystem responses (Fig **1**). In previous sections, the most common approaches in assessing those effects at low organizational levels have been presented. In turn, warnings of pollutant effects at community and ecosystem level are scarcer. However, as ecological integrity requires the protection of a good structure and functional processes at those high levels [140, 141], demonstrating biochemical and physiological responses to pollutants may not be sufficient.

Following Clements [14], the key to predicting the effects of contaminants on communities and ecosystems, is to understand the underlying mechanisms. Thus, establishing a cause and effect relationship between stressors and responses at higher levels of organization is problematic. The structure and functioning of these systems may be altered for many reasons other than contaminant exposure. Hence, Clements [14] suggests that one of the major goals of ecotoxicology is to develop an improved mechanistic understanding of ecologically-significant responses, to contaminants.

A review of the effects of pollutants on aquatic ecosystems in different parts of the world can be seen in Islam and Tanaka [142]. These authors describe a decrease in species diversity, changes in community structure, degradation of habitats, decline in abundance and biomass, diminution in yield of marine resources, *etc.* Hence, Wolfe [143] and other authors (see also Fig. **1**) have systematized the bioindicators of pollution for marine monitoring programmes at community and ecosystem levels. These approaches include measurements of abundance, biomass, richness, dominance, similarity, ratio opportunistic:sensitive species, age-size spectra, trophic interactions, energy flow, productivity, and the loss of goods and services. The response to pollutants (metals, organic compounds), of some of these indicators, is examined below.

## Richness, Diversity and Evenness

In a recent review, Johnston and Roberts [144] make a meta-analysis of 216 papers in which the most frequently used measures of diversity and evenness were species richness (number of species per unit area), the Shannon–Wiener index and Pielou evenness (Margalef's richness and Simpson's diversity were used occasionally). The vast majority of the contributions concluded that there were significant negative effects of pollution upon species richness, with occasional increases in species richness and diversity associated with nutrient enrichment. Only 20% of the papers did not detect the effects of contamination upon diversity. When an effect was detected, its response ratio based upon species richness and Shannon–Wiener diversity tended to be greater than reductions in the Pielou evenness. Hence, a 30–50% reduction in species richness and diversity were identified in all habitats exposed to all contaminant types. In turn, Dauvin [145] does not consider species richness a good indicator of disturbance in estuaries, due to marked changes linked to salinity gradients.

There is also good evidence that offshore discharges of oil-based drilling fluids by the oil and gas industry have caused reductions in benthic species diversity or other changes in community structure at distances of <1–3 km from drilling locations [146, 147].

Although there is a large degree of variability when comparing laboratory toxicity values and benthic measures, Long *et al.* [148, 149] have demonstrated that (from almost 1500 samples) in 92% of the samples classified as toxic, at least one measure of benthic diversity or abundance was less than 50% of the average reference value. These findings have been used in the derivation of sediment quality guidelines (SQGs), as commented below.

Pollution of marine habitats has been associated with a reduction in biodiversity, either as a result of reduced species richness, increased dominance of tolerant species (i.e. decreased evenness), or a combination of both factors following the Pearson and Rosenberg paradigm [150]. However, the abovementioned meta-analysis [144] indicates a remarkable similarity in the response ratios across habitat and contaminant types. Hence, pollution was never associated with the complete exclusion of life from a particular location (commonly 50–70% of species were able to tolerate the contaminant load). In some cases, estuarine communities showed very high abundance and biomass values together with very high levels of contaminants [145, 151]. This paradox is explained by the delay between the period with the maximum runoff and the maximum contaminant input (at the end of autumn and during the winter) and the recruitment period for the dominant species (throughout the spring and summer), together with the absence of anoxic conditions.

## Ratio Opportunistic/Sensitive Species

From the previous Section, it is clear that the identity of pollution-tolerant and –intolerant species is of great interest. Pollution-tolerant and opportunistic species have long been recognized as potential bioindicators of impacted systems [130, 152, 153]. Macrobenthic communities respond to pollution by means of different adaptive strategies [130]: (i) r-selected species, with short life-span, fast growth, early sexual maturation and larvae throughout the year; (ii) k-selected species, with relatively long life, slow growth and high biomass; and (iii) T, stress-tolerant species, not affected by alterations.

These strategies have been used in developing different indices that can be used to assess the environmental quality status in estuarine and coastal systems (see reviews in [154, 155]). From the high number of indices based upon sensitive/opportunistic ratio of species, probably the most successful are AZTI Marine Biotic Index (AMBI) [156] and the Benthic Quality Index (BQI) [157], which are on the basis of many other methodologies, most of them used within the WFD [158].

From the extensive publications using these indices (and especially from that of AMBI), it can be observed that increases in metals [159-161], organic compounds and TBTs [146, 162, 163] produce a decrease in benthic community quality detected by this index. Hence, a primary mechanism driving these changes, as a result of exposure to contaminants, is the elimination of sensitive species and the subsequent monopolization of resources by tolerant and opportunistic species [144].

In some cases such as at Restronguet Creek in the Fal estuary system [147], copper and zinc pollution from mining is strongly suspected to have caused the exclusion or restriction of several species of bivalves, including

*Cerastoderma edule, Macoma balthica, Mytilus edulis* and *Scrobicularia plana*, as well as the changes in nematode metal tolerance. However, some species (such as *Nereis diversicolor*) are able to adapt to pollution. Normally, in these extreme situations of metal pollution, infaunal communities are dominated typically by metal-tolerant opportunistic deposit-feeding polychaetes [144]. In fact, sediment metal chemistry and benthic infauna surveys undertaken over 33 years with sampling before, within and after tailings deposition from a metal (Pb, Zn) mine in Greenland [161] have shown dramatic changes of benthic fauna composition. Faunal recolonisation 15 years after closure of the mine was slow. Of the metals, Pb had the greatest impact, with deterioration of benthic communities above a threshold of 200 mg/kg, decreasing diversity and dominance of sensitive species, and increasing tolerant and opportunistic species; *i.e.* long-lasting effects on the biological system.

In turn, from the relatively few data on hard-bottom substrata communities, it has been suggested that macroalgal communities are relatively resilient to pollution [144]. However, some research has shown metal- and nutrient-impacted rocky shores to contain degraded communities of macroalgae. Opportunistic algal species with rapid growth rates, including *Ulva* and *Enteromorpha* dominated, replacing relatively diverse communities of large perennial algae and sessile filter feeders seen in more pristine areas [164-168].

### Trophic and Other Interactions

Pollution may affect diverse components of the ecosystem, through primary species structuring the community. Hence, Roberts *et al.* [169] review the ways in which the contamination of biogenic habitats may affect other compartments (*i.e.* epifauna), describing four pathways: (i) colonisation by mobile fauna; (ii) inhibition of larval settlement; (iii) feeding by herbivores and predators; and (iv) post-ingestive effects on fauna.

The effects of habitat-bound contaminants, on the abundance of epifauna, may be driven by the behavioural responses of dispersing organisms [169]. For instance, recruitment of epifauna is reduced to macroalgae experimentally-spiked with copper as a result of behavioural preferences for uncontaminated algal hosts. Thus, exposure to habitat-bound contaminants is likely to be spatially complex. Hence, small-scale variation in contaminant concentrations interacts with variation between organisms in their ability to disperse among alternate habitats.

In addition, the accumulation of metals by macroalgae and seagrasses represents a potentially important pathway of contaminant exposure to grazing organisms (herbivores and detritivores), which are responsible for much of the transference of metals to higher trophic levels [169]. In fact, the algae *Ulva lactuca* and *Enteromorpha intestinalis*, collected from contaminated sites and used to feed herbivorous gastropods, produced complete mortality of the latter organisms within 1–4 weeks of continuous dietary exposure [170]. Similar effects have been described at other trophic levels [169].

### Interactions of Pressures within the Ecosystems

The potential for interactions to occur between chemical contaminants and habitat factors (e.g., food and habitat availability) has been identified as being important for understanding the ecological effects of pollutants [171]. Thrush *et al.* [171] demonstrated a way of determining likely interactions and also that multiplicative effects, such as stressors, frequently interact across environmental gradients. This pattern suggests a strong role for regression-based analysis of field gradients in the determination of contaminant effects. This study highlights the potential variation in response to metal contaminants across ecological landscapes; it provides an insight into fitting ecotoxicological responses into ecosystems. The complex community effects, mediated by impacts on foundation or key species and ecosystem engineers, have been assessed by Thrush *et al.* [171], highlighting the need for improved integration of ecological patterns with contaminant-stress responses.

Moreover, Crain *et al.* [172] in an analysis of 171 studies that manipulated two or more stressors in marine and coastal systems, found that cumulative effects in individual studies were additive (26%), synergistic (36%), and antagonistic (38%), which is very close to what would be expected from a random distribution (33-33-33%). The overall interaction effect across all of the studies was synergistic, but interaction type varied in relation to response level (antagonistic for community, synergistic for population), trophic level (antagonistic for autotrophs, synergistic for heterotrophs), and specific stressor pair (seven pairs additive, three pairs each synergistic and antagonistic). Addition of a third stressor changed the interaction effects significantly in two-thirds of all of the cases; it doubled the number of synergistic interactions. Hence, although pollutants can affect communities and ecosystems, their effects can be reinforced when other pressures or stressors (*i.e.* nutrient inputs, habitat loss, hypoxia, *etc.*) are present. Generally, organisms living under conditions close to their environmental tolerance limits appeared to be more vulnerable to additional chemical stress [173].

## MEASURING POLLUTANT EFFECTS IN AN INTEGRATIVE ASSESSMENT

Much discussion has taken place about the lack of a coherent terminology to differentiate the various assessment types and the diverse nature of aquatic environmental integrative tools and methods in assessing ecological integrity [141, 174]. Following Borja *et al.* [141], these approaches can be divided into two categories: (i) those evaluating risk and state of a particular system (*sensu* the Drivers-Pressures-State-Impacts-Response (DPSIR) approach); and (ii) those assessing the ecological integrity status of the whole ecosystem under an ecosystem-based approach.

### Evaluating the Risk and State of a System

The ecotoxicological effect measurements must be used, within the context of ecological risk assessment (ERA), as a tool to assess the likelihood of harm being caused to ecosystems, or their components through exposure to a specific concentration of a chemical.

Among the approaches used to overcome the limitations shown above, some authors propose the use of multispecies tests, or using different compartments of the system (*i.e.* chemical analysis, bioassays, impacts on benthic communities) in the assessment. This approach is developed within the context of an integrative assessment, considering several lines of evidence (LOE); *i.e.* sediment contamination, toxicity and benthic fauna.

Another relatively recent approach is the weight of evidence (WOE) approach, which is the result of combining different measures of environmental quality to establish an overall assessment of environmental health. The philosophy behind WOE is a preponderance/burden of evidence approach, where the conclusions drawn from individual components are considered not as a sum of these components, but relative to one another [175]. WOE determination incorporates judgements concerning the quality, extent, and congruence of the data contained in the different LOE. It includes also observational (e.g. ecology) and investigative or manipulative (e.g., toxicology used to determine cause-and-effect) components. Ideally, any WOE framework will be easily understandable by lay personnel or decision-makers; it will also appropriately differentiate between hazard (the possibility of impact) and risk (the probability of impact) [176].

One of the first sediment quality WOE frameworks was the sediment quality triad (SQT). The triad concept was conceived more than 20 years ago by Long and Chapman [177] to provide a sediment quality evaluation based upon three components: (i) chemistry, to determine chemical contamination; (ii) bioassays to evaluate toxicity; and (iii) benthic community structure to determine the status of resident fauna exposed to the sediment contaminants. These three original components provide the basis for the SQT, or contaminated sediment risk assessment [178]. However, the traditional SQT is based on correlation, not causation; it can provide definitive conclusions regarding the pollution status of contaminated sediments, but cannot provide definitive conclusions in all cases, and cannot derive causation without further studies. Hence, the traditional SQT can be considered as a screening-level ERA, with causation examined at a higher tier [179]. Hence, the SQT needs to include additional LOE to address all aspects of ERA.

Individual LOE involved in contaminated sediments evaluation should include [175]: (i) measures of sediment chemistry to determine the level and extent of pollution and modifying factors (e.g., grain size, total organic carbon) compared to SQGs, and to answer the question “are contaminants present at levels of concern?”; (ii) measures of resident benthic community structure to determine whether community structure has been altered, possibly due to pollution; and (iii) measures of toxicity to determine whether the contaminated sediments are affecting the biota.

Additional LOE can include: (i) measures of biomagnification, usually involving measurements of body burdens in sediment-dwelling invertebrates, and food chain modelling to answer the question “are any contaminant of concern capable of biomagnifying and likely to do so?”; (ii) measures of exposure such as biomarkers or body burdens (bioaccumulation) to determine which sediment contaminants, if any, are bioavailable and to try to determine causation; (iii) toxicity identification evaluations (TIE) to attempt to assign causation; and (iv) determinations of sediment stability to determine whether only surficial sediments should be evaluated or whether deeper sediments, which may be exposed during storm or other events, need to be evaluated by answering the question “is the sediment stable or is it prone to erosion resulting in exposure of deeper, more contaminated sediments and/or contamination down-current?”

In summary, a battery of different LOE selected for specific purposes should be developed, maximizing flexibility in the use of WOE within a wide variety of situations and locations which exist in the environment.

For sediment risk assessment, the recommended WOE is the tabular decision matrix (TDM); this is the most effective and logical basis for presenting WOE in a manner readily understandable. TDM was used under the SQT first proposed by Chapman [125] and improved by other authors [176, 180, 181]. Such a matrix must be based on a strong quantitative, statistical evaluation / summarisation prior to merging into more qualitative matrix tables. Each LOE is established on the basis of a graduation (a scoring system) to rate each measurement endpoint as indicative, moderate, or negligible/low ecological risk. These LOE are summarized in SQGs, toxicity test results and biotic indices. The classification of the toxicity tests to use in the ordinal ranking scheme is based upon comparison with sediment toxicity guidelines and/or standards established previously in national ring, or intercalibration tests using the same species of organisms [182]. The integration of data-reducing techniques is very useful to incorporate into a tabular matrix, as emphasised by Chapman [183]. Some steps in the SQT are assigned more weight than others based upon expert knowledge of the sediment assessment, system behaviour and factors interpretation computed from Best Professional Judgement. As mentioned previously, to develop a tabular matrix, a ranking scheme must be applied for categorisation. An example of this application is shown in Tables **2** and **3** [184].

**Table 2:** Ordinal ranking scheme applied for ‘weight of evidence’ categorisation. PIAE = Potential Impact for Adverse Effects.

| Rank | Pollutant | Classification | | |
|---|---|---|---|---|
| Contamination[1] | Metals and metalloid | average < AL1<br>(low PIAE) | AL11<average<AL2<br>(moderate PIAE) | average > AL2<br>(high PIAE) |
| | PAHs | values< AL1<br>(low PIAE) | AL1< values < AL2<br>(moderate PIAE) | AL2 > 1<br>(high PIAE) |
| | PCBs | values< AL1<br>(low PIAE) | AL1< values < AL2<br>(moderate PIAE) | AL2 > 1<br>(high PIAE) |
| Toxicity[2] | Amphipods mortality | < 25%<br>(not toxic) | 25-50%<br>(moderate toxicity) | > 50%<br>(high toxicity) |
| | Microtox | EC50>1000<br>(not toxic) | 1000>EC50>500<br>(moderate toxicity) | EC<500<br>(high toxicity) |
| Benthos[2] | Biotic index | 0–3.3<br>(no alteration) | 3.3–5<br>(moderate alteration) | 5–7<br>(high alteration) |
| Overall Risk Assessment | | No significant adverse effects (0-1 positive) | Potential significant adverse effects (1-3 positive) | Highly significant adverse effects (more than 3 positives) |

[1] For sediment pollution, two guideline values, obtained from previous studies undertaken in Spanish Ports [185], are used: Action Level 1 (AL1) and Action Level 2 (AL2).

[2] Toxic thresholds for amphipods from DelValls *et al.* [182], whilst benthic reference values have been derived for Basque Country ecosystems based on Borja *et al.* [156].

In WOE assessments, there is no single correct way to relate sets of variables and other approaches are considered, such as multivariate analyses (e.g., Principal Component Analysis) which are used typically in WOE determinations [183, 186].

Some authors [183, 187] have also detailed a tiered scheme for WOE components. The tier testing approaches are recommended for regulatory purposes as it permits keeping risk assessment cost-effective and feasible. It allows an assessor to undertake only as much sampling and analysis as are needed to come to a reasonable decision. Moving through the tiers (steps), one moves from a broad to a more focused scope and from general benchmarks to more detailed, directed tests [188]. Although WOE assessments (such as the SQT) have improved since their initial development, future WOE applications should consider specific LOE in terms of risk assessment, ensuring that both exposure and effect assessment are addressed adequately, as are both causation and ecological relevance. As emphasised by Chapman *et al.* [180], WOE assessment (such as the SQT) should not be used to develop a single numerical index; any simplification of WOE should be site- or region-specific, not generic.

**Table 3:** Example of tabular matrix with the Sediment Quality Triad (Lines of Evidence, for management), using a port from the Basque Country (northern Spain).

| **Stations** | **Chemistry** | | | **Toxicity** | | ***In situ* alteration**[1] | **Overall risk assessment**[2] | **Explanation / contamination of concern** |
|---|---|---|---|---|---|---|---|---|
| | Metals and metalloids | PAHs | PCBs | Amphipods mortality | Microtox | AMBI | | |
| 1 | - | +/- | + | + | + | + | + | Sediments polluted. Biological effects associated with PAHs and PCBs. |
| 2 | - | - | - | - | - | +/- | - | Clean sediments. Not polluted. Moderated *in-situ* alteration not associated with chemicals in sediments and no effect in laboratory. |
| 3 | - | - | - | - | + | + | +/- | Moderate toxicity and alteration. Either biological effects are associated with other contaminants or with confounding factors such as organic matter, ammonia and/or sulfides. |
| 4 | - | - | +/- | +/- | +/- | +/- | +/- | Moderated degradation by organics. These sediments could become polluted. |
| 5 | - | + | - | - | - | + | + | Degradation associated with PAHs. Sediment toxicity tests must be cross-checked. |

Note: use of symbols provides for a convenient and rapid visual assessment of all endpoint results, as well as assessment of the concordance among endpoints, for a given site. Symbols indicate: +, contamination, effect or alteration are observed; +/-, moderate contamination, effect or alteration; -, no contamination, effect or alteration.

PAH = Polycyclic Aromatic Hydrocarbons; PCB = Polychlorinated Biphenyls

[1] Benthic community *in-situ* alteration using the AZTI Marine Biotic Index (AMBI) [156].

[2] For overall risk assessment (see Table **2**): two moderate (+/-) equal to one positive.

However, the components of WOE assessments will change as new, more ecologically-relevant measurement endpoints are discovered and applied. In particular, it is expected that: (i) particular WOE "tools" will be refined and validated; (ii) interpretative guidelines will be more fully developed; and (iii) chronic toxicity tests and community responses will be further incorporated into WOE assessments.

In summary, environmental quality, assuming the persistence of a suitable habitat, can be determined only by the responses, or condition of multiple (never single) measures undertaken as part of integrative assessments.

The uncertainty and high variability, inherent in both ecosystems and methods of measurement, require a burden of evidence approach. The WOE approaches are, and will continue to be, most useful where they are flexible and responsive to study goals, ecological realities, and social concerns [175].

**Assessing the Ecological Integrity of an Ecosystem**

One criticism of the sole use of diversity indices, as measures of ecological impacts is that such measures do not consider alterations to the structure of communities; therefore, they may mask more effects than they elucidate [144]. Following these authors, indices which consider taxonomic relatedness and multivariate analyses of community structure are more sensitive and powerful means of detecting ecological impacts than studies that consider diversity and species richness (or other indices) alone. These criticisms, together with the interactions and synergistic effects described above, have led to the use of multiple indices and integrative methods to assess ecological integrity in marine waters [189]. These methods take into account the concept of environmental or ecological status, which includes the structure, function and processes of marine ecosystems, bringing together natural physical, chemical, physiographic, geographic and climatic factors; subsequently, integrating these conditions within the anthropogenic impacts and human activities in the assessment.

Hence, the environmental status concept defines quality in an integrative way, using several biological parameters (*i.e.* macroalgae, macroinvertebrates, *etc.*) together with physico-chemical and pollution elements, including ecosystem attributes (such as food web dynamics, species diversity, and the distribution of life histories) that are not direct biological properties but functions of the entire ecosystem. They are important because they provide information about the functioning and status of the ecosystem; they have been perceived widely as additional and potentially useful indicators of environmental status [141]. This approach is intended to permit an assessment of the ecological status at the ecosystem level (‘ecosystem-based approach’ or ‘holistic approach’ methodologies), more effectively than can be carried out at a species or chemical level (*i.e.* quality objectives). However, there are few examples of pollutant impacts at the whole marine ecosystem level [142, 190], in an integrative way, because they are masked by other human pressures as commented upon above.

An overview of these kinds of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems world-wide can be seen in Borja *et al.* [189]. Overall, the legislative measures world-wide tend to converge in defining environmental water quality in an integrative way. However, the degree of convergence is variable, based generally upon studies carried out in single systems, which do not permit generalisation.

In practical terms, managers and decision-makers need simple, but scientifically well-established methodologies capable of demonstrating to the general public the evolution of a zone (estuary, coastal area, *etc.*), taking into account pollution and other human pressures or recovery processes [155, 189]; likewise, capable of guiding the implementation of successful management. Within this context, there is a major scientific challenge to develop tools to define adequately the scale and present condition of marine ecosystems in terms of biological performance, as well as to monitor changes through time, and similarly, to identify and address, through management, the causes of observed impairments [189].

Some of these challenges have been addresses in assessing ecological status in large ecosystems in the USA and Europe [166, 191]. However, with the success of these tools come additional challenges. The proliferation of indices to assess the status adds an element of confusion back into what they had been intended to simplify [140]. Some of the confusion arises because of the different processes used for developing, calibrating and validating methods in different regions; this leads to inconsistencies in assessment across regions. Additional confusion results from indices developed for multiple types of biota, providing managers with multiple, often conflicting, answers for a single water body [140]. Whereas the last decade was characterized by an explosion of methods, the next decade should be one of consolidation and agreement [140].

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