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The use of *Mytilus* spp. mussels as bioindicators of heavy metal pollution in the coastal environment. A review

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Abstract

The oceans and seas with high population density are submitted to numerous anthropogenic pressures: among them, the chemical pollution by heavy metals. These pollutants, coming from our continental activities, are transported through rivers or by air and accumulate in seas and oceans where they mainly affect coastal areas. During the 70^{ies}, scientist suggested to use organisms, in particular mussels of the genus *Mytilus*, as bioindicator, in order to evaluate the status of chemical contamination of coastal ecosystems. Mussels like the genus *Mytilus* are widely considered as one of the most suitable sentinels and biological indicators of pollution since they possess a multitude of useful characteristics for this purpose: sessile filter feeders that accumulate contaminants in their tissues; exhibit low ability to metabolize organic contaminants; have stable local populations in many places; and have a wide array of sensitive biomarker tools available. The heavy metal concentrations in the *Mytilus* spp. reflect the coastal water concentrations in which they live. Since they living organisms, metal uptake and bioaccumulation may also affected by a number of biotic and abiotic factors such as: body size, location within the intertidal zone, the time of collection, the temperature, the salinity, and the pH. These influences must be identified and measured, so that they may be taken into account during sampling, validation of results, or in the interpretation of monitoring data. We concluded that *Mytilus* spp. was a sensitive bioindicator for monitoring of the past and the present coastal pollution by heavy metals. Therefore provides a global view of the health status of the coastal environment.

1. Introduction

The Industrial, agricultural and urban development is accompanied inevitably by problems of water environment pollution. Indeed, due to human activity, several thousand chemicals substances reach the ocean *via* the atmosphere or continental waters. Some of these, such as pesticides and artificial radioactive elements, foreign to the marine environment; others already exist naturally but their concentrations are altered by human intervention. The impact of these substances on the environment is complex, and can directly or indirectly affect different populations and ecosystems [1-3]. Of course, the role of nutrients salts excess in eutrophication has become widely documented; but other chemical compounds as metals (heavy or traces) on the aquatic life is still poorly known. This situation led to diversify the approaches allowing to understand the state of pollution of aquatic environments.

During the past few decades various biomonitoring strategies have been developed to monitor and evaluate the adverse impact of these compounds on marine and estuarine ecosystems. The Monitoring programs use a great number of bioindicators known as “sentinel organisms”, to detect temporal and spatial variation of chemical pollutants and to contribute to the knowledge of trends in marine contamination [4, 5]. Different organisms, both animal and vegetal species, have been proposed as biomonitors [6-9]. The Mussel Watch [6] are the oldest biomonitoring program in progress worldwide. It has been developed successfully in many countries [10, 11]. Bivalves, and especially mussels, have been widely used as indicators of pollution because of their ability to concentrate many environmental contaminants (**Table 1**). A fundamental assumption has been made that these organisms can provide an accurate time-integrated value for assessing levels of contaminants that are present in coastal environment [11]. Banni et al. [12] and Viarengo et al. [13] founded that genus *Mytilus* is an important candidate in environmental monitoring programs, due to their wide geographical distribution, sedentary lifestyle, filter feeding behavior, and tolerance for a large range of environmental conditions [14, 15], this specie has been widely used as a sentinel organism for marine pollution biomonitoring [16 -18].

This paper reviews the possible use of the mussels genus *Mytilus* as bioindicator organism for biomonitoring of heavy metals in coastal environment. In order to optimize the use of the mussel as an indicator species, the origin of metals and their absorption and metabolism by the genus *Mytilus* will be presented, as well as abiotic and biotic factors affecting their bioavailability will be reviewed.

2. Origin and physicochemical characteristics of heavy metals in aquatic ecosystems

Metals are considered as important toxic pollutants and there is extensive literature concerning their accumulation in ecosystems. Heavy Metals are natural components of the earth's crust. They are naturally occurring in the continental (soil, water), and marine compartment (water and sediments), and in the atmosphere and the biosphere. Other phenomena, such as volcanic activity, biomass fires and thermal sources, contribute to the metal releases in the environment [19, 20]. To these natural inputs, numerous anthropogenic activities, such as mining, combustion of fuels, industrial and urban sewage and agricultural practices, also emitted metals [9]. On a global scale there is now abundant evidence that anthropogenic activities have polluted the environment with heavy metals from the poles to the tropics and from the mountains to the depths of the oceans Different industrial activities such as tannery, textiles industry, pharmaceutical products, painting industry, electroplating, steel industry and domestic waste are responsible for the heavy metals accumulation in the environment [21].

In the aquatic environment, the metals are present in dissolved form, complexed with dissolved organic matter or adsorbed on the particles [22]. Metals presents in ecosystems can be heavy or trace metals. The heavy metals refer to all metal with high atomic mass such as lead, chromium, cadmium, arsenic and mercury [23]. Trace metals exist in the environment at low concentrations; some of them are often biogenic oligoelements with hormetic properties, while other oligoelements, less desirable for living organisms, are often toxic such as cobalt. Metals have a chemical polymorphism depending both on their specific form and the compound, mineral or organic, in which they are incorporated. The physicochemical form of a metal (speciation) determines its mobility and consequently its bioavailability. In general, the free or ionic form, the most bioavailable, is the most toxic [24].

3. Speciation, bioavailability and toxicity of heavy metals

In the aquatic environment, the heavy metals may be present in dissolved or particulate form. They found a mixture of cations, anions and inorganic molecules (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, ...) and dissolved organic matter (urea, amino acids, ...). To this mixture, they added colloids and suspended particles derived from soils and sediments as well as particles of biological origin (phytoplankton and zooplankton in particular). The metals may be present as free ions hydrated, complexed with organic and/or inorganic ligands, adsorbed on the colloids or on the suspension materials [25, 26]. The different speciations influence the behavior, the bioavailability and the metals toxicity against aquatic organisms including bivalve molluscs.

The mobility of metals generally results in the passage of the colloidal and particulate form to the dissolved form. The physico-chemical parameters (pH, oxygen, salinity, hardness and dissolved organic matter) influence mostly this type of exchange in water [27, 28].

Thereby, the mobility of metals generally increases in the medium as the pH decreases. The organic matter also plays a very important role with respect to metals mobility and bioavailability. Organic matter creates a complexing reaction, and decreased the metallic bioavailability [29].

In order to link the contamination level of the different aquatic environment compartments at risk represented by this pollution with respect to organisms that live different compartments, it is essential to know the bioavailable fraction of each contaminant [30]. The metal contaminants are available to mussels through food intake, respiration, and transport across biological membranes [30, 31]. Therefore, the bioavailability of metals depends not only on distribution and their speciation, between different compartments, but also of the physiology and ecology of the organism itself [32, 33].

The metals may be harmful to the health of entire population, to aquatic organisms due to their presence in water above the threshold of tolerance [34]. The toxic effects of metals as well as their behavior in the aquatic environment (mobility, bioavailability) will depend largely on speciation, which corresponds to the distribution of this element in its different species, forms or phases (soluble and/or insoluble) [35], of considered organism (species, sex, age and development stage) and the concentration in a specific organ [36, 37].

The presence of metals in the waters and the sediments can result in aquatic organisms by the appearance of abnormal forms (necrosis, ulceration, atrophy), by the alteration of cell membranes, by the metabolism, the photosynthesis and the DNA repair disturbances [38, 39]. The arsenic, cobalt, cadmium, chromium and nickel also present mutagenic or carcinogenic properties [40, 41].

4. Metal metabolism in the genus *Mytilus*

4.1. Bioconcentration

Bioconcentration is the process by which a chemical concentration in an aquatic organism exceeds that in water as a result of exposure to a waterborne chemical [42]. Vinogradov [43] showed that the bioconcentration could be applied generally to all marine organisms including bivalve molluscs genus *Mytilus* and to most divalent metals. It has been studied in metabolic terms. The first elements studied in this way were those for which a physiological role was apparent, essentially Ca and Si [44]. In addition to these two elements (Ca and Si), Bowen [45] shown that the Cu is associated with active heamocyanine in some mollusks; Mo, Mn, Mg and Zn have an enzyme activation function, and Fe, Cr and Ni are essential to some biological functions.

Simkiss *et al.* [46] suggested an approach to the phenomenon of bioconcentration of divalent cation. The majority of ions are only capable of penetrating into the cytoplasm with the aid of the membrane transporters, because the inorganic ions are hydrophilic and the plasmatic membranes are hydrophobie. Once inside the cell, the ion must be picked up by another ligand in order to avoid its diffusion back outside. These ligands constitute a system of "kinetic trapping" whose efficiency depends on the strength of the binding. Increase in the specificity of the binding is favoured by a series of steady states; the trend towards more stable complexation acts as the basis for metal metabolism within the circulating liquids and the cell. The most stable binding, the final "kinetic sink", may be specific for a metal in the case of a physiological process or more general in the case of a detoxification system [47].

4.2. The absorption

The absorption of metals by bivalves, involves transfer of metals to the circulatory system following: uptake by the apical membrane; movement through the cell as well as interaction with intracellular ligands and efflux across the basal membrane [48].

These mechanisms were mentioned in the case of Zn in *Mytilus edulis* by George and Pirie [49]. Authors assumed that the eventual metabolism of zinc would be independent of the route of uptake from water, food or particulate material. In fact, a considerable proportion of the zinc was absorbed via the gut, and may have been bound to mucus or absorbed as particulate zinc hydroxide. Zinc accumulates rapidly in the soft tissues from sea water. There is a rapid uptake by the superficial tissues (mantle and gills) although, the greatest proportion is found in the gut. The uptake kinetics of metals is characterized by a direct proportionality between the accumulated doses and the time of exposure, whereas the final concentration in the organism is determined by the intracellular availability of metal-binding ligands and by the turnover of the formed complexes [46].

Fowler and Benayoun [50] studying the kinetics of the Cd^{109} radioactive isotopes in *Mytilus galloprovincialis* show that the rate of absorption of Cd is in direct proportion to its concentration in seawater, and that it is affected neither by temperature nor by the presence of different Zn concentrations. Fowler and Benayoun [50] also show that equilibrium between the animal and its environment is reached with time exposure. Moreover, there does not seem to be any equilibrium between radioactive Cd^{109} and stable Cd already accumulated in the mussel. Schulz-Baldes [51] reported extensive studies of lead uptake by *Mytilus edulis* and suggested a relationship between the concentration of lead in mussel tissues and the average concentration of lead in the surrounding water. The uptake of iron might be expected to be unusual since this metal normally occurs in sea water as a colloidal precipitate of hydrated ferric hydroxide [52] and yet readily accumulates to high concentrations in the common mussel *Mytilus edulis* [53].

4.3. Sequestration and cellular storage

Molluscs accumulate metals principally in two organs, the hepatopancreas and the kidney, although certain elements can be accumulated in other tissues such as gills and mantles. Two of the best-studied intracellular structures involved in metal sequestration and storage are the metallothioneins and the intracellular vesicle-bound granules, classified as granules.

The capacity for metallothionein induction is greatest in tissues that are active in metal uptake, storage and excretion [54]. In *Mytilus edulis*, metallothioneins have been identified in the gills [55]. Binding of metals to metallothionein enhances bioaccumulation in these organs, as well as in the liver and kidney.

Measurement of metallothionein induction has been proposed as a cellular indicator of metal exposure and toxicity in *Mytilus edulis* [55, 56]. This induction confers enhanced metal tolerance to intact individuals of *Mytilus edulis* [57]. The bivalves contain a wide variety of granules, may of which bind metals. The metal content of the granules varies considerably. The granules are generally have a low percentage (< 10% of organic

matter [58]), although granules in the kidney of *Mytilus edulis* are an exception to this pattern, with 46% metal [59]. Metal-rich Ca phosphate granules are generally associated with digestive and excretory tissues [59]. The granules have been identified in the hemocytes of a number of marine bivalves [60]. The granules can be composed of pyrophosphate or orthophosphate, depending on species [61], and are virtually insoluble in saline, making them excellent `sinks' for immobilizing metals in a nontoxic, unavailable form.
The cellular lysosomal system is involved in most instances of granule formation [59]. Lysosomal systems are particularly well developed in digestive and excretory cells such as those of the molluscan digestive gland and kidney. In mussel kidney, the breakdown of metallothionein/metal complexes results incorporation of Cu, Cd, Hg and Zn into lysosomal granules [62].

4.4. Cellular excretion mechanisms
Bivalve molluscs utilize a variety of mechanisms to eliminate metals from their bodies (**Figure. 1**). The overall process is a species-specific, organ and tissue-specific and metal and ligand-specific process. The kidney is the most important organ of excretion in the bivalve molluscs. The metals found there in the lysosomes are excreted by exocytosis of the vacuole contents, by elimination from the renal podocytes or else by diapedesis of whole granules in the urinary tract of *Mytilus edulis* [63].

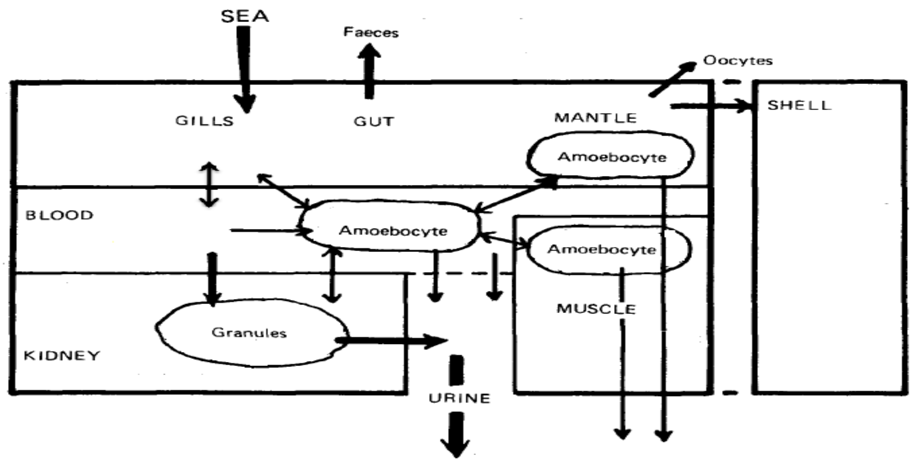

Figure 1. A schematic representation of cellular excretion mechanisms of metals in *Mytilus* [64].

Excretion of certain metals may also occur through the tegument, with faeces, by byssus production [63, 65], through the shell, or by way of gamete emission during spawning [66]. George *et al.* [63] shows when elimination iron is considered, with *Mytilus edulis* a significant proportion of absorbed iron, is also excreted *via* the faeces as indicated from the faecal analyses and the presence of iron in the rectal lumen, a significant amount is transferred *via* the byssal gland into the byssal threads. George and Pirie [49] also studied the excretion of zinc in *Mytilus edulis*, and founded that the zinc elimination is by defaecation, exocytosis of the kidney granules into the urine and diapedesis of the amoebocytes.
A better knowledge of uptake-excretion kinetics and of the subcellular distribution of metals in field conditions may contribute to the understanding of the time-integration capacity of mussels for different elements, and provide useful information for interpreting heavy metal levels in *Mytilus spp.* mussel population.

5. Factors affecting the variation of metal bioavailability in the genus *Mytilus*
Mussels (*Mytilus spp*.) are recognized as sentinels of heavy element marine and freshwater pollutions since their metal concentrations reflect the water concentrations in which they live [53, 51, 67]. Since they are living organisms, metal uptake and accumulation may also affected by a number of intrinsic and extrinsic factors [68, 69]. These influences must be identified and measured, so that they may be taken into account during sampling, validation of results, or in the interpretation of monitoring data. Factors such as size [70], sex [71, 28] location within the intertidal zone [72, 70], the time of year of collection [73-75], the pH, the coexistence of several metals, the pre-exposure to metals, the temperature, and the surrounding salinity levels, likely to influence metal concentrations in the mussels were reviewed.

5.1. Effect of age and body size
The age of the organism seems to be an important factor affecting the metal accumulation capability of sentinel species like mussels. Youngest stages (embryos) accumulate a lot, whilst the oldest, which are less sensitive, possess the lowest rate. This could be also linked to the higher surface to volume ratio of embryos over mature

organisms. Yap *et al.* [76] suggested that there might be differences in physiology between young and older mussels. Since large and aged mussels tended to pump less water, through their bodies per unit of body weight, the uptake of metals was lower than that in smaller individuals. The surface area to volume ratios decreased with size, and this affected the relative contribution of the adsorbed metal content to the total body burden of heavy metals [77, 78]. Therefore, the decrease in metal concentrations with body size indicated that a significant proportion of the metal content was surface-adsorbed as smaller mussels have a larger surface area to volume ratio [28]. As the concentrations of heavy metal decreased with an increase in the body size (length and thickness of shell); this indicated that the chemical pollutants were more concentrated in young mussels due to their faster growth rate [70]. Boyden [79] reported relationships between organism size, metal concentration and total metal content for several species of bivalves, and founded that Mn, Fe, Ni, Cu, Zn and Pb were more concentrated in smaller *Mytilus edulis* while Cd concentrations were size-independent. Similarly, Jones and Walker [80] found that concentrations of Fe, Mn, Zn and Cd in the whole soft body parts of freshwater mussels decreased with increasing body weight.

Other studies revealed that the body size might change the heavy metal uptake due to the changes in the kinetic steady-states as mussels grew [68]. Schulz-Baldes [51] found a similar effect for the uptake of lead by *Mytilus edulis*: again, uptake was faster in smaller animals than in larger ones. Obviously, body size would affect metal bioaccumulation in the rates of uptake and excretion [81]. Besides, the effects of the body size on different physiological rates such as pumping, filtration and respiration, have been reported in the *Mytilus edulis* mussels [82, 83].

5.2. Effect of reproduction cycle and sex

The possible effects of the reproductive cycle have often been mentioned as an explanation for the variations in metal content in the mussel. The fact that during spawning up to 40% of soft tissue weight is lost shows the importance of gametogenesis in the physiology of the *Mytilus* [47]. The body weight changes seasonally according to the relative rate of somatic or gonadal growth occurring at different periods of the year: highest values in summer and smallest through winter. Body weight decreases sharply to a minimum in spring-summer coincident with spawning, and this is followed by a rapid increase in the summer as animals build up reserves to be used in the following autumn and winter for gonad development. Therefore, the unpredictability of the extent of gonad development also makes it more difficult to use adult animals as indicators in pollution monitoring programs. For this reason, it has been suggested the use of immature specimens to reduce the influence of metabolism on the metal content and thereby give a more precise indication of pollution levels.

Based on the sex, Watling and Watling [84] separated soft tissues of black mussel, and they founded that Mn, Cu and Zn were more concentrated in females but that Pb and Bi were more concentrated in males and no significant differences were found in the concentrations of Cd, Fe, Co, Ag, Ni and Cr. However, in a subsequent study, Orren *et al.* [85] analysed soft tissues of the same species on two occasions and found that Mn, Fe, Cu and Zn were more concentrated in females only before a major spawning event; no significant differences were found after spawning had occurred.

5.3. Effect of location within the intertidal zone

Among the many natural factors affecting sessile intertidal organisms, shore height is probably the most important and obvious factor with greatest potential to cause high individual variability in the contents of heavy metals in individuals within a mussel bed. However, the influence of tidal exposure has not received the attention it deserves and in *Mytilus edulis* only few studies involving Cd, Cu, Pb and Zn could be found [72, 86]. Besides, De Wolf [87] observed that the concentrations of Hg in *Mytilus edulis* and *Mytilus galloprovincialis* were in general, for the same site, higher for individuals in the intertidal zone than for continually immersed animals. Bourget and Cossa [88], no founded any clear difference for Hg concentration on the location of the mussels *Mytilus edulis* in the intertidal zone. Nielsen [89] found that concentrations of zinc, cadmium, lead and iron in the cultured mussel *Perna canaliculus* varied with sampling depth at one location. This was suggested to be due possibly to differences in available food or in soluble:particulate metal ratios in the water column with depth, or to the presence of hydrogen sulphide from sediment-living bacteria affecting metal solubilities in the water column. Concentrations of the same metals in mussels from a second location, of greater water circulation, showed no vertical gradients of this kind. Coleman [90] noticed that mussels subjected to periods of emersion accumulated less Cd than those which were continuously immersed, with differences of accumulation which did not depend simply upon immersion times. Different concentrations of metals in mussels may be generated by the effects of stress from immersion, differences in growth rates or bioavailability of the metal according to the mussel location in the intertidal area [71].

5.4. Effect of season and periode collection

The influence of season on the concentrations of trace metals found in bivalves has been partly elucidated. In the *Mytilus edulis,* a numerous of studies have appeared which increase the body of knowledge about the structure of seasonal variations of metal concentrations [85, 33, 91]. Pentreath [53] reported seasonal differences in total concentrations and in tissue distributions of non-radioactive zinc, iron and manganese in *Mytilus edulis*. Bryan [92] found that the concentrations of zinc, lead, copper, cobalt, iron, manganese and nickel in tissues of the mussels were greatest in autumn and winter, and it was suggested that metal concentrations were inversely related to phytoplankton productivity.

Some authors observed in different species of filter-feeding shellfish that the peaks of metal accumulation might be reached in winter and the lowest levels in summer [93, 94]. Rouane-Hacene *et al.* [33] founded the enrichment in metallic elements (Zn, Cu, Cd) in mussel tissues collected in winter may result from the inputs of urban and industrial activities. But authors founded, the opposite tendency for Pb. The strong Pb accumulation in summer could be related to an increase of urban population during this period, leading to higher urban wastewater discharges and atmospheric Pb levels, due to a more intense traffic. In the latter case, the contamination could be both direct by the atmospheric deposition and indirect by the leaching of roads by rainwaters [95]. Fowler and Oregioni [96] in studies of the variation in the concentrations of ten metals in *Mytilus galloprovincialis*, suggested that the seasonal maximum seen in samples collected in March (spring) was due to the reproductive state of the animals and to the high winter run-off increasing the amount of available metals. Phillips [72] reached very similar conclusions concerning the seasonal variation of concentrations of zinc, cadmium, lead and copper in *Mytilus edulis.* The seasonal fluctuations of trace metal concentrations were reciprocal to the seasonal changes in tissue weights of individual animals; thus the total metal content of each individual changed little throughout the year. Weight changes were in turn related to the sexual cycle, with a minimum in late winter or early spring, coincident with the seasonal maximum of trace metal concentrations. Giarratano and Amin [97] founded that heavy metals (Cu, Zn, Fe, Cd) in Mussels showed a seasonal trend of bioaccumulation being higher in winter. This is probably due to the slower growth at cooler temperatures, when the availability of food is lower. Reserves are used for metabolism, leading to adecreasing tissue mass and, therefore, increasing the metal content ratio. Rouane-Hacene *et al*. [33] suggested that the variation in Cd concentrations in *Mytilus galloprovincialis* was due to the changes in weight of the animal during the reproductive cycle. The seasonal changes in the various metal concentration in mussel tissues could result from a combination of factors directly correlated to the weight of animals (cycle of reproduction, metabolism, development and age, food availability, temperature), and with others, more independent, such as salinity, modification of biogeochemical cycle and bioavailability of metals related to the site [98, 99]. The physicochemical characteristics of water, which vary according to seasons, modulate the bioavailability of pollutants and therefore influence both their bioaccumulation and the biological responses of organisms to these pollutants.

5.5. Effect of the pH of water

The availability of metals for uptake by organisms is also influenced by the pH of the water. The form of metal available for uptake is often depending on the metal and the specificity of the pH. The toxicity of heavy metals may be reduced when they are adsorbed to suspended organic matter, thus reducing their ionic fraction in the water column. Both pH and redox potential affect the toxicity of heavy metals by limiting their availability [100, 101]. At low pH, metals generally exist as free cations; at alkaline pH, however, they tend to precipitate as insoluble hydroxides, oxides, carbonates, or phosphates [102].

5.6. Effect of the co-occurrence of metals.

Mussels are exposed to a mixture of metals in natural waters. Many studies have focused on a single metal to investigate metal-specific uptake and tissue distribution [103]. Such studies are critical to understanding contaminant dynamics, but cannot be used to assess effects of multiple-metal exposures. The concomitant presence of various pollutants may have synergistic or antagonistic effects on bioaccumulation rates and/or toxicity [104]. Competition between chemically similar ions for binding sites can significantly affect ion bioaccumulation by mussels. Hemelraad *et al*. [105] demonstrated that Zn in freshwater mussels was antagonistic to Cd, inhibiting Cd uptake in gills and accelerating the redistribution of Cd from the gills to internal organs. They hypothesized that Zn competes with Cd for binding sites in the gills. Whereas, Jackim *et al*. [106] show that the absorption of Cd by *Mytilus edulis* is lowered in the presence of increasing dose of Zn. Phillips [107] remarks that the effect noticed by Jackim *et al*. [106] only occurs when levels of Zn are extremely high (up to several hundreds of $\mu g\ l^{-1}$). The results of Fowler and Benayoun [50] do not show evidence of any influence of Zn on the uptake of Cd by *Mytilus galloprovincialis*. In other work, Romeril [108] reported that both iron and cobalt depress the rate of Zn uptake by the mussel *Ostrea edulis.* The effects were different for soft parts and for the shell. Ion competition at uptake or absorption was suggested as the possible interfering mode of action. Studies found in mussel oysters, that Zn with Cu and Hg, potentiate the lethal effect of each other. Cadmium-Cu co-exposure produces a decreased accumulation of Cd in freshwater molluscs. In the case of zebra mussel, however, Cd plus Cu short-term exposures result in greater than additive bioaccumulations, whereas chronic exposure to Cd plus Cu results in the opposite response, indicating a loss of potential for bioaccumulation during prolonged exposure. Phillips [72] has shown that the net uptake of copper by *Mytilus edulis* is affected by the presence and change in concentration of zinc, cadmium and lead. Boukadida *et al*. [104] demonstrated that copper and silver interact additively on the embryonic development of the Mediterranen sea *Mytilus galloprovincialis*. Effects of metals in mixture are much more complicated than single metal and many hypotheses can explain these divergent results. The mode of action of copper and silver has been already documented [109, 111]. Copper was reported to affect membrane permeability by increasing the membrane potential and by blocking ion channels. At low concentrations, silver ions act as a blocker of K^+ channel and at larger concentrations, they increase membrane permeability [111]. This may explain the interactive effects observed between Cu and Ag ions acting in the same way by affecting membrane permeability and ion channels. Moreover, toxicity of Cu-Ag mixtures may depend of metal concentrations and combinations [109, 110]. According to Lucas and Horton [112] silver has greater impact when it is combined with low concentration of copper; otherwise copper has predominant deleterious effects. The effects of the coexistence of several metal pollutants on the uptake of any single metal by an organism are extremely difficult to study because of the large number of metal combinations and biological responses possible.

5.7. Effect of the pre-exposure to metals

Enhanced tolerance to the potentially toxic metals Cu, Cd, Hg and Zn is known to occur in a number of organisms which have had a prior history of short-term exposure to these metals. It appears that some prior conditions must be satisfied during the pre-exposure for the enhanced tolerance to be elicited: pre-exposure should be sufficiently high to initiate cellular compensatory responses, and pre-exposure should below toxic levels. Kahle and Zauke [113] had reported that some aquatic organisms having the ability to concentrate contaminants in their tissue and organ systems to more than a million times, compared to their concentration in their habitat. Mussels can accumulate certain metals to high concentrations without adverse effects, for example, cadmium, can concentrate in the kidney for detoxification (such as by chelation) without adversely affecting that organ [114]. In addition, the rate of metal accumulation within an organism likely varies as a function of the exposure concentration; the importance of exposure concentration on test results cannot be overemphasized. Brooks and Rumsby [115] found that the concentration factors for Cd in oysters *Ostrea sinuata* decreased steadily with increase in exposure concentration and the concentrations of Cd in the tissues decreased in the order gill > heart > visceral mass > mantle> white muscle > striated muscle. Eisler *et al*. [116] and Shuster *et al.* [117] founded that *Crassostrea virginica* exposed to Cd rapidly accumulated high levels of the metal in soft tissues. Maximum concentration of Cd accumulated in the animals was independent of exposure concentration. George and Coombs [118] reported an initial lag period in uptake of Cd in mussel *Mytilus edulis,* but a subsequent linear relationship with time and exposure concentration. Similar results were obtained by Jackim *et al*. [106] for *Mytilus edulis*. At low inorganic arsenic (As) exposure the *Mytilus edulis* are capable of biotransforming the inorganic As to organoarsenic species, presumably as a detoxification process, but at higher exposure to inorganic As the biotransformation threshold is exceeded and the animals deposit and accumulate the inorganic As in their tissues.

5.8. Effects of the temperature

The role of temperature on metal uptake and accumulation in marine mussels has not been well studied [119]. This is despite the fact that mussels are important biomonitoring organisms. Furthermore, there seem to be no general consensus among the few available studies on the subject. Denton and Burdon-Jones [120] reported in bivalve mollusks, Cd concentrations in body soft tissues increased with increasing temperature. Cd bioaccumulation by molluscs increases with temperature because of its effect on the metabolic activity of the animals. However, *Mytilus galloprovincialis* has shown that temperature does not seem to have an influence on the accumulation of Cd but does induce a slight increase in Hg uptake [50]. In contrast Fischer [121] has shown that a rise in temperature accelerates the building of Cd in soft tissues while their growth is reduced. It was noticed that the relation between Cd body burden and shell weight is independent of temperature between 7ºC and 25°C. Boukadida *et al*. [104] founded that temperature increased the toxicity of both metals (copper and silver) as proved with a 50% effective concentration at 20°C at 3.86 mg/L and 16.28 mg/L for Ag and Cu respectively. These results highlight a possible impairment of *Mytilus galloprovincialis* reproduction in the

Mediterranean Sea in relation to increase of both pollutants and water temperature due to global warming. Boening [122] reported that Mercury toxicity increases with temperature and decreases with water hardness. Some recent studies have also reported uptake that is dependent and increases with increase in temperature [123, 124]. Baines *et al*. [125] also found a positive relationship between assimilation efficiency of dietary metals with temperature. These studies are consistent with results involving many different organisms, which tend to show a positive correlation between temperature and metal uptake, accumulation or toxicity [126, 119]. Sokolova and Lannig [127] hypothesised that the increase sensitivity of organisms to metals at elevated temperatures could result from the increase of water solubility and hence bioavailability of metals and by higher contaminant uptake due to metabolism increase. However, Attig *et al*. [14] provided evidence about the reduction of the ventilation rate in *Mytilus galloprovincialis* exposed to higher temperatures, thus affecting uptake rate of contaminants.

5.9. Effects of the salinity

Salinity is an important environmental variable, especially in estuarine and coastal regions. Metals become more toxic as salt content of the water decreases. Salinity also modifies metal speciation, different metal species inducing different toxic effects [128, 129]. Phillips [72] has shown that Cd is better absorbed at lower than at high salinities by *Mytilus edulis*. Jackim *et al*. [106] reported that decrease in salinity from 30 to 20 ‰ increased Cd uptake by *Mytilus edulis*, by 24 to 400% at 10 and 20ºC. Those observations are confirmed by George *et al*. [130] who's observed that mussels accumulate more Cd in dilute seawater than in 100% seawater. They concluded that this increase in uptake of cadmium is due to the effect of osmolarity of the surrounding medium. Cd (bound to chloride ion) becomes more toxic at low salinity because in this situation it is transformed into free ion. In the case of Pb and Zn, for example, great concentrations of metal are needed to cause toxic effect because they are mainly present in the environment as hydroxides.

Conclusions

Many different species of bivalve mussels have been used in attempts to monitor the concentrations of heavy metals in the the coastal environment of many countries. The genus *Mytilus* is capable of acting as an efficient time-integrated indicator of heavy metal over a wide variety of environmental conditions.

It is well known that metal concentration levels in *Mytilus spp*. are not only the result of their bioavailability in the environment. Biotic and abiotic factors are acting. However, the extent or magnitude of the effect of some of these factors on metal concentrations in *Mytilus spp.* requires further clarification.

Although additional variables may remain to be investigated, the *Mytilus spp.* is recommended as an alternative to studies metal concentrations existence in marine and estuarine water, allowing rapid, reliable and inexpensive control of water quality. *Mytilus spp.* provides a global view of the health status of the coastal environment.

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Table 1. The use of bivalves as bioindicators of heavy metals contamination in environmental waters.

Species	Studied area and date	Heavy metal studied	Nature of study	References
Mya arenaria	Norway,Coast off Spitsbergen and, Poland, Bay of Gdansk	Fe, Co, Ni, Pb, Zn, Cu, Cr, Cd and Mn	Interspecific comparison study	[131]
Astarte borealis	Norway,Coast off Spitsbergen and, Poland, Bay of Gdansk	Fe, Co, Ni, Pb, Zn, Cu, Cr, Cd and Mn	Interspecific comparison study	[131]
Mytilus galloprovincialis	Morocco, El Jadida coastline, (1994 - 1995)	Cd, Cu, Zn, Fe and Mn	*In situ* study, monthly monitoring, studied seasonal pattern	[132]
Mytilus galloprovincialis	France, French Mediterranean Sea, (2000)	Cd, Hg, Zn, Pb, Cu, Ni, Cr, and As	*In situ* study, Interspecific comparisons	[133]
Mytilus galloprovincialis	Morocco, Casablanca and Mohammadia coastal, (1998 – 2002)	Cd, Cr, Cu, Pb and Hg	*In situ* study, Interspecific comparisons	[134]
Chamelea gallina	southern Spanish Atlantic coast, (2003)	Cr, Ni, Cu, Cd, Pb, Zn, As and Hg	*In situ* study, Interspecific comparisons	[135]
Donax trunculus	southern Spanish Atlantic coast, (2003)	Cr, Ni, Cu, Cd, Pb, Zn, As and Hg	*In situ* study, Interspecific comparisons	[135]
Mytilus galloprovincialis	Morocco, El Jadida, (2004)	Cu, Cd, Zn and Hg	Punctual sampling, Interspecific comparisons	[136]
Chamelea gallina	Turkey, Sea of Marmara, (2005-2006)	Hg, Cd, Pb, Co, Ni, As, Sn, Cu, Zn, Fe, Al and Mn	*In situ* study, monthly monitoring, Interspecific comparison	[137]
Donax trunculus	Turkey, Sea of Marmara, (2005-2006)	Hg, Cd, Pb, Co, Ni, As, Sn, Cu, Zn, Fe, Al and Mn	*In situ* study, monthly monitoring, Interspecific comparison	[137]
Mytilus galloprovincialis	Spain, Atlantic and Northern coasts, (2005)	Hg, Cd, Pb, Cu and Zn	*In situ* study, Study of spatial distribution,	[138]
Mytilus edulis chilensis	Argentina, Beagle Channel, (2007-2008)	Fe, Zn, Cu, Cd and Pb	*In situ* study, seasonal monitoring, Interspecific comparison	[97]
Mussels	Turkey, Aegean Sea, (2011)	Hg, Cd, Pb, Cr, Cu, Zn and Mn	*In situ* study	[139]
Mytilus galloprovincialis	Italy, Apulian coast, (2009)	Cd, Cr, Cu, Hg, Pb, Zn and As	Study of spatial distribution, seasonal monitoring	[17]
Mytilus galloprovincialis	South Africa, Cape Town, (1985 – 2008)	Cu, Cd, Pb, Zn, Hg, Fe and Mn	*In situ* study, annual and seasonal sampling	[140]
Mytilus galloprovincialis	Western Algeria, Oran Bay, (2011)	Hg, Cd and Pb	Study spatial distribution	[141]
Mytilus spp. (Mytilus edulis)	North of Norway, Bøk fjord, (2014)	Pb, Zn, Fe, Ni, Cd, Cu, Al, As	*In situ* study, Punctual sampling	[18]
Mytilus galloprovincialis	Algeria, Algerian west coast, (2010)	Zn, Cu, Pb, and Cd	*In situ* study, seasonal monitoring	[33]
Mytilus galloprovincialis	France, Arcachon Bay, (2015)	Cu and Ag	*In situ* study, Temperature effect	[104]
Mytilus spp.	German, Island of Helgoland, and river Elbe in Cuxhaven. (2011-2014)	Ag, Al, As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Se and Zn	*In situ* monthly monitoring,	[142]
Perna perna	Brazil, Southeastern Brazil (Guanabara and Ilha Grande Bay), (2015)	Ni, Cu, Zn, As, Se, Cd and Pb	Intraspecific comparison study, Active biomonitoring	[54]
Mussels	North Atlantic and European regional seas, (2008-2009)	As, Cu, Zn, Cd, Hg and Pb	Multiparametric study	[9]
Perna viridis	Indonesia, Semarang, (2015)	Pb, Cr and Cd	*In vitro* study	[21]
Mytilus galloprovincialis	Morocco, Cala Iris Sea (2017)	Fe, Zn, Cu, Pb and Cd	Study of spatial distribution, monthly monitoring	[143]
Mytilus galloprovincialis	Morocco, Coast of Safi (2014)	Cd	Geographical distribution	[144]

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