Microbiological Quality of River Sediments and Primary Prevention

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1. Introduction

The preservation of aquatic ecosystems is fundamental because water quality, plant and animal biodiversity, industrial activities and human health rely on it. During recent centuries, the condition of aquatic ecosystems has become worse due to the increasing their use for irrigation and drinking, excessive land use and deforestation, hydro morphology alteration, riparian zone reduction and not least, climate change.

A strong interdependence between the health of the ecosystem and human health can be demonstrated. Microbiological risks for human health can occur through direct or indirect (fish, molluscs, recreational activities, algal bloom, vegetables, fruits) consumption of contaminated water (Tauxe, 1997; UNEP 1997, 1998; Noji, 1997; Ahem *et al.*, 2005). It is a priority to know the quality of the water and manage the data, in this sense it is necessary to identify the main variables that negatively impact human health.

Indicator organisms are commonly used to evaluate the *microbiological quality* of *aquatic ecosystems (*Berg 1978; Grabow 1996; EU 2006; Tyagi *et al.* 2006). Standard-based water quality assessment is an essential component of monitoring programs and also works to protect human health. As a rule, microbiological indicator detection (i.e. Enterococci and *Escherichia coli*) take place in the water column as necessitated by national and international laws.

In this context, it is appropriate underline the important role of sediment on aquatic ecosystems. This matrix is an extremely heterogeneous habitat characterised by a high microbial biodiversity due to the wide range of functional roles that they perform. Its origin is in the weathering and erosion of minerals, organic material, and soils in upstream areas usually after rainfall or the melting of snow. Transported downstream, it settles along the river bed and banks as sedimentation. Sediments consist of particulate matter that can be transported by fluid flow and which eventually is deposited as a layer of solid particles on the bed or bottom of water bodies. The suspended particle matter (SPM) which settle by sedimentation, include sediments whose diameter falls between 0.1 and 100 µm. Sediment heterogeneity (e.g. grain-size) in fresh and salt water, creates favorable conditions for biodiversity and is a source of life for a healthy river.

The particulate matter that plays a specific role in the aquatic ecosystems is the remaining material on a filter with a nominal porosity between 0.4 and 0.5 µm. The material with smaller dimensions is considered as colloidal and/or dissolved. When there have been found particles with smaller dimensions than this limit, it is not certain if these are the products of aggregates derived from a process of fragmentation during sampling.

The structure and the functions of the microbial communities are strictly linked to the intrinsic peculiarities of the sediment. They are mainly responsible for the recovery of the sediment after the introduction of anthropic perturbations. Microbial processes are highly important for the regeneration of nutrients and nutrient cycles for the whole body of water. To date, estimates suggest that there are up to 10^4 bacterial species per g sediment, of which at least half (and perhaps as many as 95%) are yet unculturable. There is, therefore, the need to assess the contaminated sediment quality by means of standardised and reliable tools developed by advanced research investigation.

Their complex biochemical diversity enables them to exist in many different habitats everywhere on earth where they are essential for the geochemical cycle and the elimination of many pollutants.

By employing bio-indicators, consisting of organisms or communities of organisms which react to environmental effects by modification of their vital functions, it is possible to draw conclusions on the state of their environment. Due to the complexity of services and resources which are supplied by qualitative and quantitative differentiated ecosystems, it is very difficult to find general indicators that characterize the health of an ecosystem. A rich biodiversity, for example indicates a healthy system, but in some cases it can also be a symptom of disturbance when high amounts of nutrients in an aquatic ecosystem cause enhancement of growth. An indicator has to be relevant and useful.

Sulphate-reducing bacteria are a large group of anaerobic organisms that have an important role in many biogeochemical processes as the sulphur cycle and mineralization of organic matter in anoxic marine and freshwater environments and soil (Sitte *et al.* 2010). The sulphite reducing clostridium group, including *Clostridium perfringens*, has been shown to play an important role to assess faecal pollution in sediment ecosystems (Mancini *et al.* 2010, Marcheggiani *et al.* 2008, Marcheggiani *et al.*, 2004). Their presence can be influenced not only by organic matter but also by inorganic contaminants such as heavy metals (*Mancni et al.*, 2011; Mancini *et al.* 2008). *C. perfringens*, gram positive anaerobic spore-forming bacteria of the genus *Clostridum*, that does not carry out a dissimilatory reduction of sulphate, can be used as an alternative indicator for faecal contamination in aquatic ecosystems due to its adaptation to different habitats such as soils, sediments, and sewages . Furthermore, *C. perfringens* presence can be correlated to those of parasitic protozoan and enteric viruses in the water column as *Cryptosporidium sp., Aeromonas sp and Giardia*. In addition, the spores produced by *C. perfringens* are extremely resistant to disinfection and the WHO (1996) suggests that their presence in filtered supplies may be an indication of the need for treatment. Although the World Health Organization recommends *C. perfringens* as a useful indicator of fecal pollution in water quality surveys (WHO, 1978), this microorganism has been adopted in Europe exclusively as an additional source of water quality information (Cabelli , 1978; Olivieri, 1982; Rhodes *et al.*, 1999). The sequence of the 16S rRNA gene has been widely used as a phylogenetic marker to study genetic relationships between different strains of bacteria (phylogeny). The analysis of this gene can therefore be considered a standard method for the identification of bacteria at the family, genus and species levels (Woese , 1987; Weisburg *et al.*, 1991; Jeng *et al.*, 2001; Lehner *et al.*, 2004; Raju *et al.*, 2006; Johansson *et al.*, 2006), and has in fact been included in the latest edition of *Bergey's Manual of Systematic Bacteriology* (Bergey's Manual, 2005).

Therefore, the study of sedimentary microbial communities allows the sedimental damage/contamination state or the verification of the potential risk for human health and the need for primary prevention measures to be identified.

2. Interdependence of ecosystem health and human health

A strong correlation between ecosystem health and human health can be demonstrated and many new approaches to monitoring and environmental conduction are possible (Lackey RT, 2001).
There are four principle categories of ecosystem functions upon which human health is dependant (De Groot, 1992). The first category contains regulatory functions of ecological processes which deliver water, air, and clean soil through energetic and bio-chemical regulation processes, such as the recycling of organic material. The second category is the supply of space and appropriate substrates for human activities such as cultivation, recreation and living spaces. The third category is the production of numerous resources from which food and basic materials derive. The last category of functions has only an immaterial dimension: ecosystems have a large contribution in maintaining mental equilibrium by delivering opportunities for reflection, spiritual enrichment and aesthetic experience. By employing bioindicators, consisting of organisms or communities of organisms which react to environmental effects by modifying their vital functions, it is possible to draw conclusions on the state of their environment. Due to the complexity of services and resources which are supplied by qualitative and quantitative differentiated ecosystems, it is very difficult to find general indicators that characterize the health of an ecosystem. A rich biodiversity, for example indicates a healthy system, but in some cases it also can be a symptom of disturbance when high amounts of nutrients in an aquatic ecosystem cause the enhancement of growth. An indicator has to be relevant and useful.
These properties can be measured by its capacity to measure tendencies with cause of preoccupation, not only by the part of the scientists but also by the public opinion and policymakers. An ecological indicator has to have flexible and measurable characteristics.
Because of the dynamics of an ecosystem and the continuous increase of scientific information, an indicator has to be sufficiently extendable to incorporate new ecosystem components. The measurability of an indicator is determined by its disposability and cost, by the capacity to supply alert signals in respect to alteration, by the distribution in a wide geographic area, by the capacity to supply information about a wide set of stresses (Cecchi & Mancini, 2010). It is obvious that it is very difficult to find indicators or groups of indicators that satisfy all the characteristics mentioned above. The current methods of monitoring are studies about: populations, epidemiology, periodic sampling, toxicity tests and chemical analyses. All these techniques have to be used to supply exhaustive evaluations, but overall are not adequate to evaluate the integrity of the ecosystem in a conclusive way. More research is necessary to supply a truly conceptual picture for the definition of objectives for policy makers.

2.1 Indicators organisms

The acquirement of a reliable evaluation of the environmental quality of water bodies is generally the result of the synergic application of different analytical methods mainly including chemio-physical and biological parameters. The degree of contamination is commonly identified by the presence of a microbial community, which can easily proliferate in presence of particular compounds. For example, fecal bacterial indicators such as *Escherichia coli* and Enterococci are the most common indicators to detect water contamination by sewage pollution (Berg 1978; Grabow 1996; EU 2006; Tyagi *et al.* 2006). The figure showed the main microbiolgical indicators take into account by Italian regulation to assess water quality of main water typologies. (Tab 1).

Water typology	**Mcrobiolgical indicators**	**Regulation**
Surface water	*E. coli*	**D.Lgs 152/1999 (Italia, 1999)**
Surface water destined to drinking water productions	*Total Coliforms, Fecal Coliforms, Fecal Streptococci, Salmonellae*	**D.Lgs 152/2006 (Italia, 2006)** (2000/60/CE)
Wastewater (urban and indrustrial sewage in soil and surface water and fognature)	*E. coli*	**D.Lgs 152/2006 (Italia, 2006)** (2000/60/CE)
Waste used in shellfish culture	*Fecal Coliforms*	**152/2006 (Italia, 2006)** (2000/60/CE)
Bathing water (inland, costal and transitional waters)	*Intestinal Enterococci* and *Escherichia coli*	**D.Lgs 116/2008 (Italia, 2008)** **(2006/76/CE)**
Water for human consumption	*E. coli* and *Enterococci* , *C. perfringens* (spore includes) only for water originated from surface waters. *Pseudomonas aeruginosa*, *E. coli* and *Enterococci,* Colony count at 22° and 37° C only in packaged or bottle water	**D.Lgs 31/2001 (Italia, 2001)** **(98/83/CE)**

Table 1. Italian legislation ruling water management

Further studies have focused more attention on sediments due to their capacity to retain pollutants within the layers of the matrix thus giving rise to suitable habitats for the growth of particular species of microbes, mostly anaerobes (Davies *et al.* 1995; Robles *et al.* 2000; Desmarais,Solo-Gabriele and Palmer 2002).

The use of an appropriate set of microbiological indicators gives detailed information of pathogens present in freshwater and also represents the basis for emergency procedures. Micro-organisms connected to emerging diseases that are linked to contaminated water belong to different taxonomic groups of bacteria, viruses' protozoa and helminths (Fecham *et al.* 1983). The advantages of using a set of indicators during monitoring activities, is to obtain a more complete information about the state of the water quality; in this way it is possible to draw up emergency and prevention procedures (Tyagi *et.al*, 2006).

Set microbiological indicators should be included not only with *Escherichia coli*, an intestinal Enterococci whose presence indicates those of others pathogenic bacteria, but also with *Coliphages*, an indicator of enteric virus, and also with *Clostridium perfringens*, an obligate anaerobe which indicates the presence of parasitic protozoan and enteric viruses in the water column as *Cryptosporidium sp.,* Aeromonas sp e *Giardia* (Payment and Franco, 1993; Gleeson and Gray, 1996; U.S. EPA, 2007). In addition, the spores produced by *C. perfringens* are very resistant to disinfection and the WHO (1996) suggests that their presence in filtered water supplies may be an indication of the need for treatment.

2.2 Microbiological Indicators and sanitary significance for human health

Microbiological risks related to the consumption of contaminated water can occur directly or indirectly. Indirect consumption involves the consumption of fish, molluscs, vegetables and fruits contaminated through water used for irrigation, recreational activities and algal blooms (Tauxe, 1997; UNEP 1997, 1998). The risk of diarrhoeic disease outbreaks, due to enteric pathogens, is higher in developed than underdeveloped countries (Noji, 1997; Ahem *et al.*, 2005).

Several studies reported that the consumption of contaminated water and food are a probable source of Salmonellosis, Giardiasi, Hepatitis A and Criptosporidiosis outbreaks. (Mancini et al, 2010; Conio *et al.* 2000; Ballone *et al.*, 2001; Stroffolini *et al.* 1990; Leoni *et al.* 1998; Selvaggi *et al.* 1996). Indicator organisms are commonly used to evaluate the *microbiological quality* of *aquatic ecosystems (*Berg 1978; Grabow 1996; EU 2006; Tyagi *et al.* 2006). Standard-based water quality assessment is an essential component of monitoring programs and also works to protect human health. As a rule, microbiological indicator detection (i.e. Enterococci and *Escherichia coli*) takes place in the water column as necessitated by national and international laws.

2.3 Role of sediment in aquatic ecosystems

Sediments play an important role in the evaluation of the health of aquatic ecosystems (Carere *et al.*, 2008, Heise, 2008, Salomon, 2004, Heise, 2004, De Groot, 1992). Originating in the weathering and erosion of minerals, organic material and soil in upstream areas usually after rainfall on snow melt, sediments are then transported downstream, settling along the river bed and banks by sedimentation. Due to embankments and the loss of flooding areas naturally occurring sedimentation areas are very limited. Sediment heterogeneity, for example grain-size, provide favourable conditions for a high biodiversity and a good health of the river. Microbial processes are very important for the regeneration of nutrients and nutrient cycles for the whole water body (Sitte, 2010).

Sediments consist of particulate matter that can be transported by fluid flow and which eventually are deposited as a layer of solid particles on the bed or bottom of water bodies. Sedimentation is defined as the process of deposition by settling of a suspended material. The sediment will be transported as a suspended load if the flow is greater than the settling velocity. Sediments with a sufficient diameter to settle but still move are known as bed load and the particles are transported by mechanisms like saltation (jumping up into the flow, being transported a short distance then settling again). To characterize and identify pedological structures it is important to use a basic language to facilitate scientific communication. In the year 1926 the International Society of Soil Science developed, based on the research of Novak, the following conventional classification system (Table 2). The physical mechanical constitution of the sediment is a fundamental element. The particle dimensions can provide helpful information about to draining by superficial water, erosions and other forces. In additional, there may be a correlation between sediment grain-size composition and ecotoxicology (Benton, 1995).

Fraction number	Name	Diameter (mm)
1	Clay	<0.002
2	Silt	0.002-0.02
3	Sand (fine and very fine)	0.02-0.2
4	Sand (medium and big)	0.2-2
5	Gravel	2-20
6	Pebbel	>2

Table 2. Classification of the granulometric fractions after the system of Novak

The particulate matter that plays a specific role in the aquatic ecosystems is the remaining material on a filter with a nominal porosity between 0.4 and 0.5 µm. The material with smaller dimensions is considered as colloidal and/ or dissolved; even when there have been found particles with smaller dimensions than this limit (Gordon , 1970; Eisma *et al*., 1970). It has not been proven that these are the products of aggregates derived from a process of fragmentation during sampling.

The upper dimensional limit of the particulate matter is not well defined, even when Visher (Visher, 1969) proposed the value of 100 µm as the limit between particles that move by saltation in laminar flow and these which are transported in suspension. Moss (Moss, 1963) indicates the limit between 70 and 100 µm. Baudo and Bertoni (Baundo & Bertoni, 1984) gave the dimensions of the following chemical and physical species present in the aquatic environment (Table 3).

Species	Molecular Weight Dalton	Diameter (mm)
Ions, inorganic complexes and small organic molecules	200	d≤1
Bigger, dissolved compound (Fatty acid, fulvic acids, polyhydoxid complexes, polysilicates, etc)	200 -10000	1≤d ≥10
Colloids, humic acids, proteins, metal hydroxid complexes and clayey minerals	106	10≤d ≥100
Suspended solid portions organic and inorganic particles		d≥100

Table 3. Dimensional indications of the chemical and physical species in the aquatic environment [Hunt, 1980]

The SPM (suspended particle matter) which settle by sedimentation, include sediments whose diameter falls between 0.1 up to 100 µm. Significant quantities of particulate matter, biogenic with low density, can be found as a subform of particles with d>100 µm, the so called floccules. Among these chemical compounds, present in the aquatic environment, a dynamic equilibrium with continuous changes and transformations by re-suspension and resettlement exists and dimensions of the particles can change in the long term. The composition of the particulate matter is extremely variable depending on the aquatic environment. The largest amount of sediment with continental origin is transported by the rivers into the oceans. The concentration of the particulate matter is not homogenous in the inner of the water body and is various due to the meteo-climatic characteristics of the

catchment basin, the contribution of single tributaries and of hydraulic conditions. Concentrations of different particles can be measured over the course of one day or as variations on a monthly or seasonal scale at different water levels (high, medium, low).
To estimate the solid transport of a course of a river, the relation between the suspended solid fraction and the transported liquid fraction must be known. Even when this relation is not always linear the concentration of SPM is generally elevated during periods with a higher water level. Not all the SPM is transported to the ocean, in many cases it remains in areas of the river with a low energy level, deposition zones, lakes, or plains in artificial basins.
In their natural state, the particles are found in the form of aggregates called floccules with dimensions of 1-2 mm but occasionally in some cases even more than 10 mm. The flocculation of the SPM has a great influence on the transport of the particles and associated substances. The formation of floccules depends on an elevated number of factors such as the salinity of the water body, particle concentration (which augments the potentional contacts between the particles), turbulence, Brown movements (Hunt, 1980, McCave, 1988), polymerisation and adsorption to granules of dissolved organic substances (in particular carbon hydrates) (Sieburth J , 1965; Wassermann *et al*., 1986), bacterial mucus and exudates or phytoplankton that reacts like adhesive, and aggregation of air or gas bubbles.
The transport of SPM in rivers is generally connected with the morphological characteristics of the basin and can be classified according to the length, the expansion and the erosionibility of the catchment basin. Furthermore, the concentration of the suspended solid fraction depends on hydraulic characteristics (e.g. the water content of the river and the roughness of the lower bed) as well as the meteorological conditions and the climate characteristics of drained air, and finally on the antrophic activity in the course of the river as well as the basin.
The SPM consists of an organic and inorganic fraction. **The organic fraction** consists of living organisms (phytoplankton, nanoplankton and bacteria), detrital material (products from the degradation of cells from plants or algae, fragments from diatoms) essences, secretions and faecal pellets (expression for the faecal products predominantly from invertebrates). The organic substances form a thin layer on the mineral particles consisting of clay (with a high specific surface), thus it can be concluded that the clay fraction has a high absorbance potential. As a consequence, the molecules or ions can be easily adsorbed into the SPM, and then transported and accumulated in the aquatic environment. The organic substances, which consist of a few chemical elements like oxygen, hydrogen, carbon, nitrogen, phosphor and sulphur are a source of food for a lot of heterotrophic aquatic organisms. Nevertheless, the biological oxidation of too much particulated organic substance by anaerobic microorganisms leaves insufficiently dissolved oxygen for the survival of the flora and fauna and causes a higher mortality due to eutrophication processes (Madej, 2005). The **suspended inorganic fraction** is derived by the modification and erosion of the continental crust, snow smelt and the atmosphere. Through volcanic activity the emission of inorganic substances can become locally and/or temporaneously important. The contribution of cosmic dust is nominal, but the content of cosmic particles in the oceanic sediments can provide important clues for the reconstruction of cosmic events (Grieve, 1987).
In conclusion, based on features of the sediment can be divided into two parts: an active part represented by the surface layer (approximately the first centimetre, silk , sand clay) an piece and quiet part represented by the deepest layer .

Several studies have highlighted the value of sediments as indicators of pollution (Mancini *et al.*, 2010 ; Marcheggiani *et. al.*, 2008, Mancini *et al.*, 2008). Indeed, sediments – as potential reservoirs for bacteria and viruses in aquatic environments – are able to provide information on past instances of pollution which are no longer detectable in water samples, as well as on the presence of pathogens, which may in some cases pose a future threat to human health. River sediment is ideal habitat of *C. perfringens* and of others species because in this matrix the main factors that enhance their capability for survival coexist.
In conclusion, indicator sets give useful information about microbiological risks for human health and can improve emergency and prevention plans

2.4 Sediment and microbiological indicators

Microorganisms are the main source of fertility and of degradation of organic matter and pollutants in sediments. Their complex biochemical diversity enables them to exist in various habitats throughout the planet where they are essential for the geochemical cycle of many elements and the elimination of many pollutants. Many of these reactions are performed by specialized organisms that cannot be easily substituted. Furthermore, microorganisms are indispensable for many symbiotic and pathogenic relationships with higher life-forms. For example the rhizosphere microflora gives plants additional competitive abilities and some microbial species serve as a food source for many soil animals. The disappearance of these communities could cause extinction of many species (van Beelen *et al.*, 1997). Due to their ubiquitous presence, microorganisms are very important as environmental indicators of contamination and provide an excellent subject for the establishment of quality guidelines (Mancini *et al.*, 2008). The sulphide content of soil is a leading influence on the bioavailability of metals, because trace metals are able to react with FeS (ferrous sulphide) (major component of acid-volatile sulphides) and form metal sulphides according to Me^{2+} FeS(s) ↔ MeS (s) + Fe^{2+}.

2.5 Sulphate-reducing bacteria

Sulphate-reducing bacteria are a large group of anaerobic organisms that have an important role in many biogeochemical processes such as the sulphur cycle and mineralization of organic matter in anoxic marine and freshwater environments and soil (Sitte *et al.* 2010). The sulphite reducing clostridium group, including *Clostridium perfringens*, is important in the assessment of faecal pollution in soil ecosystems (Mancini *et al.* 2010, Marcheggiani *et al.* 2008, Marcheggiani *et al.*, 2004). Their presence can be influenced not only by organic matter but also by inorganic contaminants like heavy metals (Mancini *et al.* 2008). *C. perfringens*, gram positive anaerobic spore-forming bacteria of the genus Clostridum, that does not carry out a dissimilatory reduction of sulphate, can be used as an alternative indicator for faecal contamination in aquatic ecosystems due to its adaptation of different habitats such as soils, sediments and sewages. Furthermore, *C. perfringens* presence can be correlated to those of parasitic protozoan and enteric viruses in the water column as *Cryptosporidium sp.*, *Aeromonas sp* and *Giardia*.
The role of *C. perfringens* as an environmental fecal indicator has been recently acknowledged by several studies. Despite this, the ruling Italian National legislation (D.Lgs. 152/99) names *C. perfringens* exclusively as a 'supplemental' indicator in recreational waters. In contrast to running water, sediments are also able to provide evidence of former fecal contamination by the analysis of different layers at various depths. Especially in situations of discontinuous disposal events, water quality assessment can be misleading while

sediments are characterized by a higher stability favoring sludge accumulation. During the last decade molecular methods have been developed to study the diversity of indigenous microbial communities independent of the classical techniques such as cultivation and microscopic identification (Amann, Ludwig, and Schleifer 1995; Schäfer and Muyzer).

2.6 The microorganisms Clostridia

The genus Clostridium is considered a biological indicator because its presence in the sediment can be natural or caused by anthropogenic discharges.
The genus Clostridium was described for the first time in 1880 by Prazmowski who separated the genus from the genus Bacillus to include the Gram positive and obligatory anaerobic rods with central or subterminal heat resistant endospores. At that time there was less opportunity to separate the genus into smaller genera for a better. Bergey *et al.* made a slightly expanded definition of that of Prazmowski in 1923 (Cato *et al.*,1986; Cato *et al.*, 1989). The genus Clostridium is characterised by anaerobic or microaerophilic spore-forming rods that do not form spores in the presence of air, are usually Gram-positive and do not carry out a dissimilatory sulphate reduction. *C. perfringens* produces a variety of toxins that play an important role in the pathogenesis of infections. The strains are classified into five groups (types A-E) on the basis of their production of lethal toxins (Sterne & Warrack,1984). At the end of the 18th Century the first diseases of man and animal associated with *C. perfringens* were documented (Rood *et al.*, 1984). The five types of *C. perfringens* cannot be differentiated reliably on the basis of cellular or colonial morphology, biochemical reactions, or gas-liquid chromatographic analyses of fatty and organic acid metabolic end products (Cato *et al.*,1986).
Pathogenicity: Some species produce exotoxins and are pathogenic to humans, however, the infectious dose is 10^8-10^9 (Bitton, 1984; Pahren, 1987).
C. botulinum and *C. difficult*, responsible respectively for botulism and pseudomembranous colitis in humans and animals, other clostridia are associated mostly with wound infections, including the most important are: *C. tetani*, head of tetanus; *C. perfringens*, *C. novyi*, *C. septicum*, *C. histolyticum*, *C. brothels*, often associated with gas gangrene. *C. botulinum* and *C. perfringens* cause serious infectious phenomena associated to consumption of contaminated food. Other species of clostridia, *C. bifermentans* and *C. sporogenes*, are only rarely associated with infections and they are not consider pathogenic.
Bacilli are widespread in the environment can be divided into : Clostridia invasive: produce toxins less powerful (hystolitic enzymes), among these, *C. perfringens*, *C. novy, C. chauvoei*, *C. septicum* and *C. haemolyticum*. Clostridia non-invasive: the pathogenic action is due to the production of potent exotoxins and their dissemination in the body among these, *C. botulinum.*
Clostridium perfringens, a Gram-positive anaerobic spore-forming bacterium of the genus Clostridium, has been suggested (Bisson &C abelli,1980; Leeming *et al.*,1998) and successfully used as an alternative indicator of fecal contamination in aquatic environments due to its wide distribution in nature and to its adaptation to a variety of habitats such as soils, sediments and sewages. Moreover, this organism has been found in air, dust, water, and even food (Niilo, 1980, Van Metre *et al.*, 2000). Spores produced by this organism are very resistant to disinfection and their presence in filter units indicate treatment inefficiencies (WHO, 1996).

2.7 Sulphite reducing bacteria in river sediment of Italy

The health of river ecosystems can be assessed using indices and indicators, the study of which is oriented to detect health prevention actions. In this contest the aim of these works

are to identify species of sulphite-reducing clostridia in river sediments. This can be obtained through a combined approach involving standard microbiological and molecular tools. In these paragraphs we have examined the available information deriving from studies specifically dedicated to assessing the genetic variability of the anaerobic microbial community in river sediments of Italy, and its relation to different fecal pollution sources (Study 1 and 2).
The *Clostridium perfringens* presence in sediments can be influenced not only by organic matter but also by inorganic contaminants such as heavy metals. Below is shown a study, performed in vitro to evaluate the potential effect of lead on the vitality of *C. pergringens* population (Study 3). At the end, a study was performed to evaluate the direct or indirect effect, of methyl mercury on sulphite reducing bacteria of lagoon sediments (Study 4).

2.7.1 Study 1

A study was performed to investigate the anaerobic community in river sediment samples of the lower Tiber catchment area, in central Italy, through a combined approach involving granulometric analysis of sediment samples, followed by microbiological and molecular (16S rRNA) analyses of bacterial strains isolated from these samples (Marcheggiani *et al.*, 2008). The study area includes the lower course of the Tiber river basin and the sampling sites have been selected to represent the upstream-downsteam gradient of pressure. The Analysis of 16S rRNA fragments was molecular tool used to investigated sulfate reducing bacteria community of sediment rivers. Eighty three PCR products were aligned and matched against NCBI database sequences. The resulting phylogenetic tree, based on the neighbor-joining method, is showed in Fig. 1. The genetic analysis assigned the bacteria to one of three clusters: *C. perfringens* (I), *C. bifermentans* (II) and *B. cereus* (III), grouped into two genuses (Clostridium and Bacillus). Cluster I included the taxa *C. perfringens, C. barati*, *C. thiosulfatireducens* and *C. butyricum*, and cluster II included *C. bifermentans*, *C. glycolicum* and *C. ghoni*. The cluster III was B. cereus. *C. perfringens* was found to be the most prevalent in all but one of the sampling sites. As expected, more biodiversity was observed downstream than upstream, both along the stem of the Tiber and along its tributaries.
Authors conclusion were the important role of *Clostridium perfringens* as a microbial indicator of fecal contamination in river sediments. The presence of this bacterium in all sediment sampling sites, as well as in both seasons (along the main stem of the Tiber, where samples were collected seasonally lend support to its suitability as an alternative indicator of fecal pollution in water quality surveys.
While further studies are still needed to explore possible relationships between the presence of specific microorganisms in sediments and the effects of such pollution on human health, one may safely assume that sulphite reducing bacteria, being useful sources of information, are destined to play a key role in the management of freshwater environments. Moreover, information derived from the analysis of sulphite-reducing bacteria in river sediments may prove valuable in water reclamation plans. In this context, the information may be used for purposes such as the evaluation of health risks in a given area, or quality control, to ensure that the health of the water ecosystem in question has in fact been restored or improved.
Further quantitative studies will be useful in order to state the suitability of this group of bacteria as indicators. Despite the usefulness of qualitative molecular tools, however, these cannot currently replace classical methods of routine water quality assessment, the latter being quantitatively more informative and easier to execute. An alternative, faster molecular methodology, which may be examined in future studies, is the direct extraction of microbial

genome from the matrix and its amplification using selective primers specifically designed for each taxonomic unit.

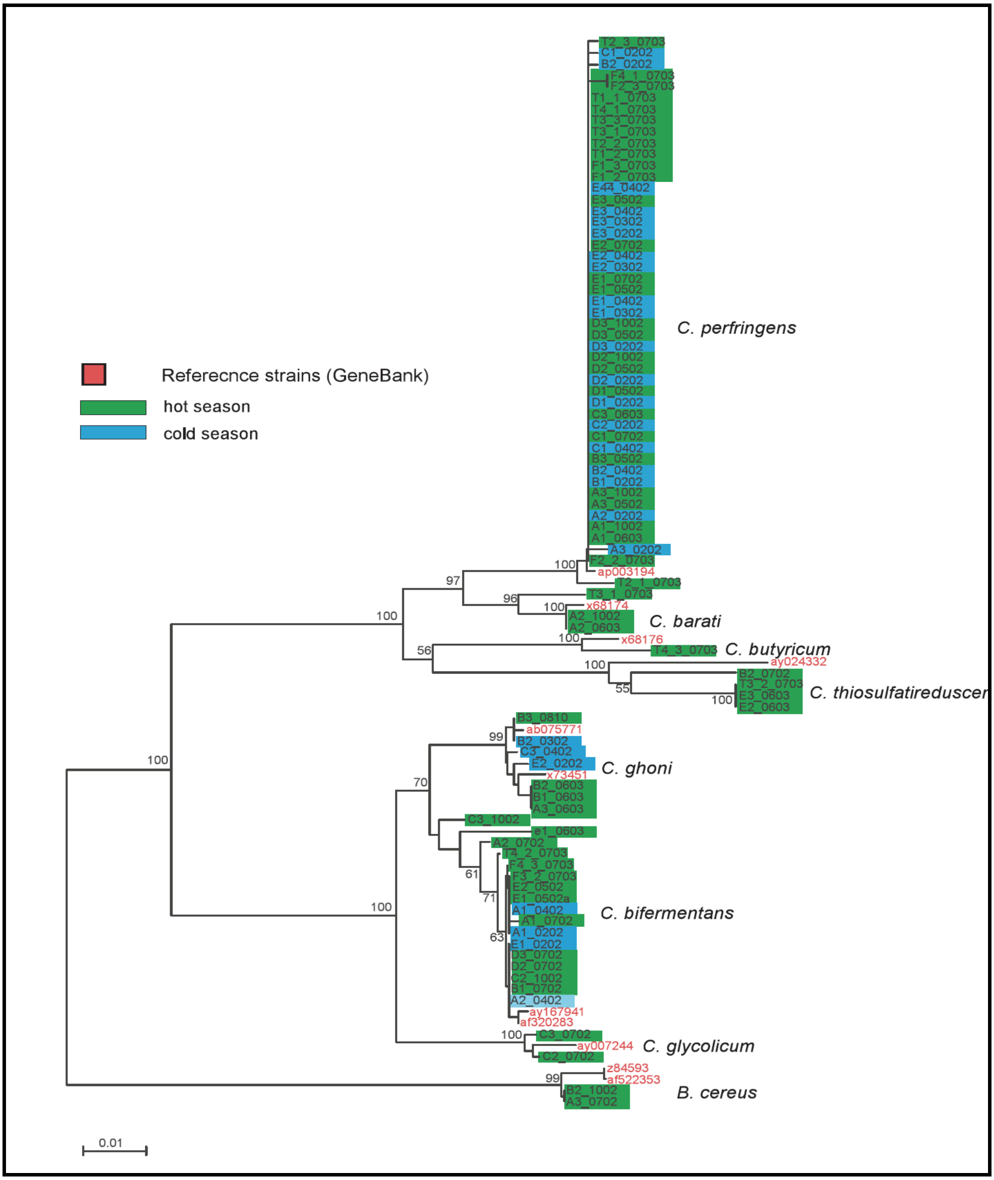

Fig. 1. Phylogenetic tree constructed with the Neighbor-Joining method-distance Kimura 2-, for a 702 bp fragment of the 16S rRNA coding region. Numbers above branches show bootstrap values expressed as percentages of 100 replications. and distribution of the genetic profiles observed in different seasons

2.7.2 Study 2

A study conducted on the river systems Foglia, Tevere, Astura, and Sitzerri located in three Italian regions, was performed to assess the genetic variability of the anaerobic microbial community in freshwater sediments as a preliminary step for future possible assessment of the relation of its members to different fecal pollution sources (Mancini *et al.*, 2010). Also, in this study was compared the concentration of Clostridia in sediments and *E. coli* in water column.

Results showed that the frequency of recorded haplotypes appeared to be almost heterogeneous for all the sampling sites. *Clostridium perfringens* was found to be the only ubiquitous species with frequencies of occurrence between 22% (Astura and Sitzerri Rivers) and 100% (Tiber). A relative higher biodiversity was recorded in summer samples. *C. perfringens* was the exclusive taxonomic unit identified in winter samples; up to four more species *(C. bifermentans, C. thiosulfatireducens, C. ghoni*, and *Bacillus cereus*) were recorded on late summer samples. Even the Sardinian samples showed a close correlation with seasonal changes but also with the typology of outfall. Samples collected in a brook running through a disused blende and galena minefield expressed a prevalence of *C. sporogenes*, while other water bodies were generally dominated *by C. perfingens*. Samples from the Foglia River were characterized only by *C. perfringens* in winter samples while those collected in summer expressed lower percentages of *C. bifermentans*. Genetic profiles detected on the only seasonal sample from the Astura River could be ascribed to the species *C. perfringens* and to a strain closely related *to C. thiosulfatireducens* identified on the NCBI database as 'swine manure gramþbacterium'. Two other strains, *Providencia stuartii* and *Proteus mirabilis*, optional anerobes, were identified but not included as results of a possible mismanagement in samples heat pretreatment.

The genetic relationships between the detected species are described by a dendrogram (Figure 2) that identifies six main clusters of which five are ascribed to the genus Clostridium and one to the genus Bacillus. The comparison with prototype sequences performed with BLAST, lead to the identification of the following taxonomic units: *C. perfringens*, *C. sporogenes, C. thiosulfatireducens, C. ghoni, C. bifermentans,* and *B. cereus*. The cluster referring to *C. perfringens* was found to be the most homogeneous in respect to the others. With the exception of C. *sporogenes*, none of the other systematic units showed complete homology with the respective prototypes.

The comparison between the concentration of Clostridia in sediments and *E. coli* in water for each site did not provide any significant correlations. According to the increment of pollution, relative concentrations of Clostridia and *E. coli* were characterized by an increasing gradient proceeding downstream on the water bodies.

In conclusion the lack of correlation between the occurrence of *E. coli* in water and *Clostridium sp* in sediments is to be attributed to the higher storage ability of sediments enhanced by accumulation and/or by the typology and seasonal regimes of the water bodies; the stability of sedimental layers is indeed inversely correlated with the irregularity of the water flow. Frequent changes are more common in small streams which make the settlement of suitable habitats for microbial growth more difficult. This may explain the higher number of profiles recorded in the Tiber River in respect to other streams. Second, the microbial composition of sediments can be temporarily influenced by particular climatic events such as floods or drought.

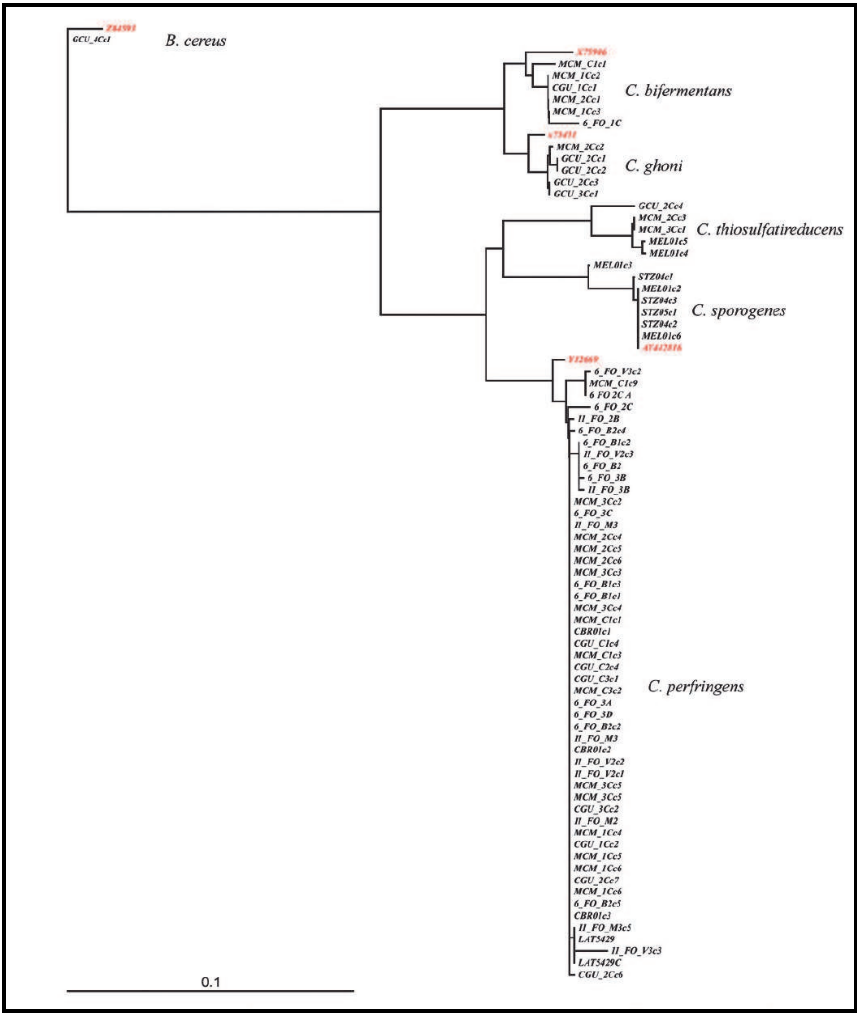

Fig. 2. Phylogenetic relationships observed between strains of sulphite-reducing isolated from sediment .genetic distance have been computed by CLUSTALX using Kimura evolutionary model (from Mancini *et al.*, 2011)

Finally, the disposal of industrial or urban waste waters could play an important role in the development of suitable habitats for the selective growth of particular species of microbes. The results of this study seem to confirm this hypothesis: *C. perfringens* and *C. bifermentans* resulted in being prevalent in urban and countryside areas where domestic and agricultural sewages are the most common impact source. The higher presence of *C. sporogenes* in the Sardinian brook running through a disused minefield suggests a role of mine wastes in providing suitable habitats for the selective growth of this species.
The evaluation of the relationships between the influence of particular impact sources and the selective growth of microbes should be investigated further in order to assess their

function as indicators of pollution. Within this context the knowledge of the genetic profiles by molecular tools of the environmental microbial community is a precondition for a correct approach to assess possible linkages with pollution sources. However, further studies should be performed in this field in order to employ the anaerobes as indicators of environmental pollution.

2.7.3 Study 3

This study aimed at detecting the effect of lead on the vitality of *C. perfringens* population in a determined, experimentally controlled sediment matrix using a luminescent reaction (Mancini *et al.*, 2011).

Lead is a priority substance of the Water Framework Directive (European Parliament, 2000) and for this reason the emissions, releases and losses should be reduced within a certain deadline (European Parliament, 2008; 2000). Native lead occurs in nature very rarely and currently lead can be found bounded to zinc, silver and copper (Visher, 1969).Due to these activities, lead is so common in the environment that it can reach man via air, food, water, dust or soil. Deposition from atmosphere is major contributor to lead inputs to water and land. After deposition in water, depending on salt content of the water and the presence of organic complexing agents, lead splits between water and sediment. In order to contribute to the knowledge of these mechanisms, this study aimed at detecting the effect of lead on the vitality of *Clostridium perfringens* population in a determined, experimentally controlled sediment matrix using a luminescent reaction. Artificially polluted sediment was contaminated with a pure culture of *C. perfringens*. Bacterial vitality was measured for period of 40 hours by luminescence method using the BacTiterGlo™-, both in the blank sediment and in the sediments added with 50 ppm, 250 ppm and 760 ppm lead concentrations.

The results of the performed ecotoxicity test using the BacTiterGlo™-assay showed a significant decrease in bacterial vitality at 760 ppm concentration. This effect occurs within the first 16 hours and therefore the initial experimental time adopted in the protocol can be reduced to this range of time. The results showed that lead concentrations that influence *C. perfringens* vitality are similar to those found in hazardous waste sites.

Next steps, the effect of lead should be observed in this range of concentration and within this period of time with more replicates and shorter time distances between the measurements. In addition, the ecotoxicological effect observed in this way, should be tested also in different types of sediments and with different environmental strains to determine sensitivities through combination of sediment properties and properties of microorganisms.

2.7.4 Study 4

A study performed on evaluation of the quality of cotaminate sediment using sulphite reducing bacteria was performed in Orbetello lagoon –Italy (Marcheggiani *et al.*,2011)

Orbetello lagoon is a site of main ecological and biodiversity interest for its peculiar characteristics of brackish wetland and is located along the southern coast of Tuscany; the lagoon has also a relevant interest due to the activities of intensive and extensive aquaculture. Orbetello lagoon over last thirty years has showed an increase of the eutrophication phenomena (Giusti *et al.*, 2005). Additionally, an establishment of anaerobic conditions has formed in some parts of the lagoon (Giusti & Marsili Libelli, 2009). Ecotoxicological and chemical studies performed in this lagoon have highlighted the presence of methylmercury in the sediments and biota (Beccaloni *et al.*, 2011).

In order to improve the knowledge about the state of the sediment contamination of the Orbetello lagoon a study to investigate the composition of sulphite reducing bacteria from sediment samples will be performed. Sampling sites have been selected into critical point of the lagoon to detect the main pressures into the area; in the same sites ecotoxicological parameters, mercury and methylmercury will be also analysed (Beccaloni *et al.*, 2011). Each sediment sample will be submitted to granulometric (Shepard, 1954) and microbiological analysis.
Results showed an increase of bacteria concentration in the sites characterized by low concentration of methylmercury was observed particularly in the south-east sites and the north east sites. These sites are characterised by a silty clay sediment composition. Furthermore, the lagoon has a large amount of organic matter probably due to intensive fish farming and agriculture activities as well as the discharge of treated/untreated urban watstewater, which has significantly increased as a consequence of the tourist trade (Lenzi, 1992, Lenzi 1998). However, a low concentration of bacteria in sites with high metylmercury concentration was also observed (Beccaloni *et al.*, 2011). A probable reason for this phenomena could be the effect of metylmercury on the sulphite-reducing community. Other investigations performed in vitro for the evaluation the effect of heavy metals on these bacterial communities showed a decrease of their growth (Mancini *et al.*, 2011; Sitte *et al.*, 2010).
Preliminary results showed an increase in the rate of supply of organic matter probable due to the intensive fish farming and agriculture activities. The results of this study will be integrated with the ecotoxicological and chemical studies and will support also the knowledge related to the transformation of mercury in methylmercury in the sediments. The multicriteria approach, including a microbiological indicator, applied to a specific site of relevant ecological interest could represent a useful tool for the preservation of environmental resources, remediation actions and assessment of the risks for human beings. The authors concluded that The multi-criteria approach including a microbiological indicator which is applied to a specific site of relevant ecological interest could be a useful tool for the preservation of environmental resources, remediation actions and assessment of the risks to human beings. Further studies should be performed in order to consider the relationships between pollution and species occurrence in sediments. It is possible to assess the key role of sulphite reducing bacteria as useful information holders for the correct management of aquatic ecosystems. Moreover, this feature plays a special role in reclaiming plans such as the evaluation of the sanitary risks of a given area or to verify the improvement of the health of a water ecosystem.

3. Conclusion

The risk assessment of microbiological water sources is not an easy task Microbial risk assessment differs significantly from chemical risk assessment. Because microorganisms can proliferate in the host; they have different die-off rates in the environment; there are risks of secondary transmission; hosts may or may not acquire partial or complete immunity, and animal responses may be very different from human responses to the same microbe.
For these reason, often the mathematical models available are not an useful tool to predict microbiological risk. It is not easy to identify water-borne infectious diseases, it is possible only when at least 1% of the population of a community became ill within a few months

(epidemic). Furthermore, another important aspect is that microbiology risk depends on exposure to pathogens and not by accumulation.

The microbial risk analysis should be based on information on the infectious dose of the organism, the exposure response, the spread of the organism in water, the likelihood of infection and the secondary spread in the environment.

Indicator organisms are commonly used to evaluate the *microbiological quality* of *aquatic ecosystems (*Berg 1978; Grabow 1996; EU 2006; Tyagi *et al.* 2006). Standard-based water quality assessment is an essential component of monitoring programs and also works to protect human health. As a rule, microbiological indicator detection (i.e. Enterococci and *Escherichia coli*) take place in the water column as necessitated by national and international laws. Therefore, the study of sedimentary microbial communities permits both the sedimental damage/contamination state and the verification of the potential risk for human health to be evaluated and the need for primary prevention measures to be identified.

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5. References

AAVV: Bergey's Manual of Systematic Bacteriology. 2nd edition (2005). Edited by: Garrity G. Springer, London, UK;

Amann, R.I., Ludwig, W. & Schleifer K.H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews* March 59: 143–69.

Ahem, M.; Kovats; R.S.; Wilkinson, P.; Few, R. & Matthies, F. (2005);. Global health impacts of floods: epidemiologic evidence. *Epidemiol Rev* 27:36-46.

Ballone, E. ; Fazii, P.; Riario Sforza, G.; Scassa, E. ; Di Nicola, M.; Ippolito, N. ; Di Mascio, C. & Schioppa, F. (2001). Indagine Sulla diffusione della giardiosi nell’area pescarese. *Ann Ig* ;13:111-120.

Baudo, R. & Bertoni R. (1984). Analisi del materiale particellato: significato e limiti. *Acqua-Aria*, 6: 722-727.

Beccaloni, M. R. ; Cicero, A.M.; Mancini, L.; Marcheggiani, S.; Miniero, R.; Scenati, R. ; Vendetti, C.; Ziemacki, G. & M. Carere.(2011) Approaches for the Derivation of Sediment Quality Criteria for the protection of Human Health in Italian water bodies. Proceedings *Sixth International Conference on Remediation of Contaminated Sediments* (New Orleans, Louisiana; February 7–10, 2011)-in press

Benton, M.J. ; Malott M.L.; Knight S.S.; Cooper C.M. & Benson, W.H. (1995). Influence of sediment composition on apparent toxicity in a solid-phase test using bioluminscent bacteria. *Environ. Toxic. and Chem.*, 14 (3): 411-414.

Berg, G. (!978). The indicator system. In Indicators of Viruses in Water and Food Edited by: Berg G. *Ann Arbor*, MI: *Ann Arbor Science*.1-13.

Bisson, J.W. & Cabelli, V.J. (1980). *Clostridium perfringens* as a water pollution 22. indicator. J *Water Pollut Control Fed* 1980, 52:241-248.

Bitton, G. ; Henis, Y. & Lahav, N. (1972). Effect of Several Clay Minerals and Humic Acid on the Survival of aerogenes Exposed to Ultraviolet Irradiation. *Appl Microbiol* 23:870-874

Cabelli, V.J. (1978). Obligate anaerobic bacterial indicators. In *Indicators of Viruses in Water and Food* Edited by: Berg G. Ann Arbor, MI: *Ann Arbor Science*; 1978:171-200.

Carere M. , Marcheggiani S., Miniero R., Pilozzi A. e Mancini L. Ed, (2008). Risk assessment elements for the management of contaminated sediments Ann Ist Super Sanità 44. pg 268

Cato, E.P. & George, W.L. (1986). Finegold. Genus clostridium. In: *Bergey´s Manual of Systematic Bacteriology.* Vol. 2. Streath PHA, Mair NS, Sharpe ME, Holt JG (Ed.). Baltimore: *Williams and Wilkins*; pp. 1141-200.

Cato, E.P. & Stackebrandt, E. (1989). Taxonomy and Phylogeny. In Clostridia- Biotechnology handbooks, III series Edited by: Minton NP, Clarke DJ. New York and London: *Plenum Press*; 1989:1-26.

Cecci & Mancini(2010). Salute degli ecosistemi come priorità della gestione ambientale. Roma: Istituto Superiore di Sanita (*Rapporti ISTISAN 06/10*).

Conio, O.; Palombo, F.; Borelli, E.; Pignata, C.; Gilli, G. & Carraio, E. (2000). Contaminazione da *Giardia* spp. e *Cryptosporidium* spp. nelle acque destinate al consumo umano in Italia. *Ig Mod*;114:77-100.

Davies, C.M.; Julian, A. ; Long, H. ; Donald, M. & Ashbolt, N.J. (1995). Survival of fecal microorganisms in marine and freshwater sediments. *Applied Environmental Microbiology* 61: 1888–96.

De Groot, R.S. (1992). Functions of Nature: evaluation of nature in environmental planning, management and decision-making. Groningen: *Wolters Noordhoff BV*.

Desmarais, T.J.; Solo-Gabriele, H.M. & Palmer. C.J. (2002). Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Applied Enviromnmental Microbiology* 68: 1165–72.

Eisma, D.; Kalf, J. & Veenhuis, M. (1980).The formation of small particles and aggregates in the Rhine Estuary. *Neth J Sea Res*;14:172-8.

EU, European Union: Directive 2006/7/EC 15 February 2006. Concerning the management of bathing water quality and repealing directive 76/160/EEC. Official Journal of the European Union 2006 L64/37: 1–15.

Eurometaux, 2000: Information and data on cadmium, lead and nickel in letter to the European Commission (DG ENV.E.1), 3 October 2000.

European Parliament and the Council. Official Journal of the European Communities, Directive 2000/60/EC of the 23 October 2000 establishing a framework for Community action in the field of water policy. L327. p. 1 ff. Official Journal of the European Communities 18 May 1976 L 129, 0023-0029.

European Parliament and the Council. Official Journal of the European Communities, Directive 2008/105/EC of 24 December 2008. Priority Substances and Certain Other Pollutants Annex II . OJ L348, p.84-97

European Parliament and the Council. Directive 2006/7/EC 15 February 2006. Concerning the management of bathing water quality and repealing directive 76/160/EEC. Official Journal of the European Union 2006 L64/37: 1–15.

European Parliament and the Council . Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption Official Journal of the European Union 1998 L 330, 51- 32.

Feachem, R.G.; Bradley, D.J.; Garelick, H. & Mara D.D. (1983). In: *Sanitation and Disease: Health Aspects of Excreta and wastewater management*. New York, John Wiley and Sons, 1983.

Giusti, E. & Marsili-Libelli S. (2005). Modelling the interactions between nutrients and the submersed vegetation in the Orbetello Lagoon. *Ecological Modelling* 184: 141 - 161.

Giusti, E. & Marsili-Libelli, S. , (2009). Spatio-temporal dissolved oxygen dynamics in the Orbetello lagoon by fuzzy pattern recognition. *Ecological Modelling*, 220: 2415–2426

Gleeson, C. & Gray, N . (1996). In: *The coliform index and water borne disease*. London, E and F N Spon.

Gordon, DC. (1970). A microscope study of organic particles in North Atlantic Ocean. Deep-*Sea Res Ocean Abstract*;17:175-8.

Grabow. W.O.K. (1996). Waterborne diseases: Update on water quality assessment and control. Water SA, 22:193-202.

Heise, S. (2008). Risk management of sediments. In Risk assessment elements for the management of contaminated sediments. *Ann Ist Super Sanità* Vol. 44, No. 3: 224-232

Heise, S.; Apitz, S.E.; Babut, M.; Bergmann, H.; Besten, P.D.; Ellen, G.J.; Joziasse, J.; Katsiri A.; Vera Maaß, Oen A.; Slob, A. & White, S. (2004). Risk management of sediments and communication. Synthesis of the SedNet working group 5 outcomes. *J Soils & Sediments* 4:233-5.

Hunt, J.R. (1980). Prediction of ocean particle size distribution from coagulation and sedimentation mechanisms. *Am Chem Soc Washington* Adv Chem Ser;189:243-57.

Italia, (1999). Decreto legislativo 11 maggio 1999 n. 152. Disposizioni sulla tutela delle acque dall'inquinamento e recepimento della direttiva 91/271/CEE concernente il trattamento delle acque reflue urbane e della direttiva 91/676/CEE relativa alla protezione delle acque dall'inquinamento provocato fai nitrati provenienti da fonti agricole. *Gazzetta Ufficiale- Supplemento Ordinario* n. 124 del 29 maggio 1999.

Italia, (2006). Decreto Legislativo 3 aprile 2006, n. 152. Norme in materia ambientale. *Gazzetta Ufficiale Supplemento Ordinario* n. 88 del 14 aprile 2006.

Italia, (2008). Decreto Legislativo 30 maggio 2008, n. 116. Attuazione della direttiva 2006/7/CE relativa alla gestione della qualità delle acque di balneazione e abrogazione della direttiva 76/160/CEE. *Gazzetta Ufficiale - Supplemento Ordinario* n. 155 del 4 luglio 2008.

Italia, (2001). Decreto Legislativo 2 febbraio 2001, n. 31 .Attuazione della direttiva 98/83/CE relativa alla qualita' delle acque destinate al consumo umano. *Gazzetta Ufficiale Supplemento Ordinario* n. 41 n. 52 del 3 marzo 2001.

Jeng, R.S.; Svircev, A.M.; Myers, A.L.; Beliaeva, L.; Hunter, D.M. & Hubbes, M. (2001).The use of 16S and 16S-23S rDNA to easily detect and differentiate common Gram-negative orchard epiphytes. *J Microbiol Methods*, 44:69-77.

Johansson, A.; Aspan, A.; Bagge, E.; Båverud, V.; Engström Björn, E. & Johansson, K.E.(2006). Genetic diversity of *Clostridium perfringens* type A isolates from animals, food poisoning outbreaks and sludge. *BMC Microbiol*, 6:47.

Lackey, RT (2001). Value policy and ecosystem health. *BioScience*;51:437-44.

Leeming, R.; Nichols, P.D. & Ashbolt, N.J. (1998). Distinguishing sources of fecal pollution in Australian inland coastal waters using sterol biomarkers and microbial fecal indicators. In Research Report No. 204 Melbourne: *Water Services Association of Australia*.

Lehner, A.; Tasara, T. & Stephan, R, (2004). 16S rRNA gene based analysis of Enterobacter sakazakii strains from different sources and development of a PCR assay for identification. *BMC Microbiol*, 4(1):43.

Lenzi, M. & Mattei, N. (1998). Risultati di quattro anni di gestione dell'ecosistema lagunare di Orbetello attraverso la raccolta delle biomasse algali. *Biologi Italiani*, XXVIII, 2, 7-12.

Lenzi M.(1992). Experiences for the management of Orbetello Lagoon : eutrophication and fishing. Science of the Total Environment (suppl. 1992), 1189-1198. *Elsevier Science* Pubbl. B.V. Amsterdam.

Leoni, O.; Cosentino, M.; Michielotto, D.; Oria, C.; Massimo, E.; Lecchini, S. & Frigo, G.M (1998). La segnalazione spontanea delle reazioni avverse ai farmaci: indagine conoscitiva in ambito ospedaliero. *Le Basi Razionali della Terapia,* XXVIII (3-4): 233

Madej, M.A. (2005).The role of organic matter in sediment budgets in forsted terrain. Sediment budjets 2. In: *Proceedings of symposium 81 held during the Seventh IAHS Scienfific Assembly*. Foz do Iguacu, Brazil, April 2005). IAHS Publication 292.

Mancini, L.; Rosemann, S.; Puccinelli, C. ; Ciadamidaro, S. ; Marcheggiani, S. & Aulicino, F. A., (2008). Microbiological indicators and sediment management. *Annali dell'Istituto Superiore di Sanità*; 44(3):268-72.

Mancini L., Rosermann S., Aulicino A.F., Carere M., Miniero R., & Marcheggiani S., (2011). *Clostridium perfringens* Vitality as an Ecotoxicity Test for Measuring the Lead Concentration in Sediment. *Proceedings of Sixth International Conference on Remediation of Contaminated Sediments*- Battelle February 7-10 2011 New Orleans Louisiana.

Mancini, L.; Marcheggiani, S.; Puccinelli, C., Iaconelli, M.; D'Angelo, A.M.; Pierdominici, E.; Formichetti, ; Equestre, M.; Aulicino, F.A.; Floris, B.; Rosselli, P.; Ammazzalorso, P.; Le Foche, M.; Zaottini E. & C. Fabiani. (2010). "A molecular approach for the impact assessment of fecal pollution in river ecosystems." *Toxicological and Environmental Chemistry*, 92(3):581-591.

Marcheggiani S., Scenati R., Vendetti C., Mario C., Musmeci L.,. Cicero M. R, Beccaloni E., Mancini L.. (2011) Evaluation of the Quality of Contaminated Sediment using Sulphite-reducing bacteria - Orbetello lagoon (Italy)". *Sixth International Conference on Remediation of Contaminated Sediments* (New Orleans, Louisiana; February 7–10, 2011) (abstract and poster)

Marcheggiani, S.; Iaconelli, M.; D'Angelo, A. & Mancini, L. (2004). Health of river ecosystems: sulphite reducing clostridia as indicators of the state of sediments. Edited by ISS, 38 p. *Rapporti ISTISAN* 04/37.

Marcheggiani, S.; Iaconelli, M.; D'Angelo, A.M.; Pierdominici, E.; La Rosa, G.; Muscillo, M.; Equestre, M. & Mancini L. (2008). Microbiological and 16S rRNA analysis of sulphite reducing clostridia from river sediments in central Italy. *BMC Microbiology* 8:171.

McCave, IN. (1988). Particulate size spectra, behaviour and origin of nepheloid layers over the Nova Scotian Continaental Rise. *J Geophysical Res* (C12):7647-66.

Minton, N.P. & Clarke, D.J. (1989). Clostridia. Biotechnology handbooks. 2 Series. New York and London: *Plenum Press*;. pp. 2-28. 24.

Moss, A.J. (1963). The physical nature of common sandy and pebbly deposit. Part II. *Am J Sci*;260:97-343.

Niilo, L. (1980). *Clostridium perfringens* in animal disease: A review of current knowledge. *Can Vet J*, 21:141-148.

Noji, E.K. (1997). The Public Health Consequences of Disasters. New York, *Oxford University Press*.

Olivieri, V.P. (1982). Bacterial Indicators of Pollution. In *Bacterial indicators of pollution* Edited by: Minton NP, Clarke DJ. Boca Raton, FL: *CRC Press*;21-41.

Pahren, H. (1987), Microorganisms in municipal solid waste and public health implications. CRC *Critical Reviews in Environmental Control* 17, 187–228.

Payment, P. & Franco, E. (1993). *Clostridium perfringens* and somatic coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoan cysts. *Appl Environ Microbiol*;59:2418-2424.

Raju, D.; Waters, M.; Setlow, P. & Sarker, M.R. (2006). Investigating the role of small, acid-soluble spore proteins (SASPs) in the resistance of *Clostridium perfringens* spores to heat. *BMC Microbiol*, 6:50.

Rhodes, M.W. & Kantor, H. (1999). Sorbitol-fermenting bifidobacteria as indicators of diffuse human faecal pollution in estuarine watersheds. *J Appl Microbiol*, 87:528-535.

Robles, S.;, Rodrìguez J.M.; Granados, I. & Guerrero, M.C. (2000). Sulfite-reducing clostridia in the sediment of high mountain lake (Laguna Grande, Gredos, Spain) as indicators of faecal pollution. Int Microbiol, 3:187-91

Rood, J.I.; Mc Clane, B.A.; Songer, J.G. & Tithall, R.W. (1997). The clostridia: molecular biology and pathogenesis. San Diego, California: *AP press*;. pp. 126-133

Salomons, W. & Brils, J. (2004). Contaminated sediments in European river basins. European Sediment Research Network SedNet. EC Contract No. EVKI-CT-2001-20002, Key Action 1.4.1 Abatement of Water Pollution from Contaminated Land, Landfills and Sediments. Den Helder, TNO. The Netherlands

Schäfer, H. & Muyzer, G. (2001). Denaturing gradient gel electrophoresis in marine microbial ecology. In Methods in microbiology, ed. J.H. Paul, Vol. 30, 425–68. London: *Academic Press*.

Selvaggi T.M.; Rezza, G.; Scagnelli, M.; Rigoli, R.; Massu, M.; De Lalla. F.; Pellizzer, G.; Tramarin, A.; Bettini, C.; Zampieri, L.; Belloni, M.; Dalla Pozza, E.; Marangon, S.; Marchioretto, N.; Togni, G.; Giacobbo, M.; Todessato, A. & Binkin, N. (1996).

Investigation of a Q fever outbreak in Northern Italy. *Eur J Epidemiol*;12:403–408.

Shepard, J. (1965) .Organic aggregation in seawaterby alkaline precipitazion of inorganic nuclei during the formation of ammonia by bacteria. *Jour Gen. Microbial* 41:20.

Sieburth, J. (1965). Organic aggregation in seawater by alcaline precipitation of inorganic nuclei during the formation of ammonia by bacteria. J Gen Microbiol;41:20.

Sitte, J.; Akob, D.M.; Kaufmann, C.; Finster, K.; Banerjee, D.; Burkhardt, E.M.;. Kostka, J.E; Scheinost, A.C.; Büchel, G. & Küsel, K. (2010). Microbial links between sulfate reduction and metal retention in uranium- and heavy metal-contaminated soil. *Appl. Environ. Microbiol.* 76(10):3143-52.

Solo-Gabriele, H.; Wolfert, M.; Desmarais, T. & Palmer, C. (2000). Sources of *E. coli* to a SubTropical Coastal Environment. *App Environ Microbiol*, 66(1):230-237.

Sterne, M. & Warrack G.M. (1984). The types of *Clostridium perfringens. J Pathol Bacteriol* 88:279-83.

Stroffolini, T.; Biagini, W.; Lorenzoni, L.; Palazzesi, G.P.; Divizia, M. & Frongillo, R. (1990). An outbreak of hepatitis a in young adults in central Italy. *Europ J Epidemiol*;6(2):156-159.

Tauxe, R.V. (1997). Emerging Foodborne Diseases: An Evolving Public Health Challenge. *Emerg Infect Dis*;3,4:425-434.

Tyagi VK; Chopra, A.K.; Kazmi, A.A. & Kumar, A. (2006) Alternative microbiol indicators of faecal pollution: Current Perspective. *(IAEH)Iran Ass Environ Health*, 3:205-216.

UNEP - United Nations Environment Programme. *Global Environment Outlook*, 1st ed., New York and Oxford, Oxford University Press, 1997.

UNEP - United Nations Environment Programme. *Human Development Report*. New York and Oxford, Oxford University Press.

US EPA - U.S. Environmental Protection Agency Ashbolt N., Fujioka R., Glymph T., McGee C., Schaub S., Sobsey M., Toranzos G. Chapter 2 "*Pathogens, Pathogens indicators and indicators of fecal contamination*" Pgs 35-56. In: Report of the expert scientific workshop on critical research needs for the development of new or revised recreational water quality criteria. EPA823-R-07-006. Washington, DC: US EPA , 2007.

van Beelen, P. & Doelman P. (1997). Significance and application of microbial toxicity tests in assessing ecotoxicological risks of contaminants in soil and sediment. *Chemosphere*;34:455-99.

Van Metre, P.; Mahler B. & Furlong, E. (2000). Urban sprawl leaves its PAH signature. *Environ Sci Technol*, 34:4064-4070.

Visher, G.S. (1969). Grain size distributions and depositional processes. *Jor. Of Sed. Petrol.*, 39 (3): 1074-1106.

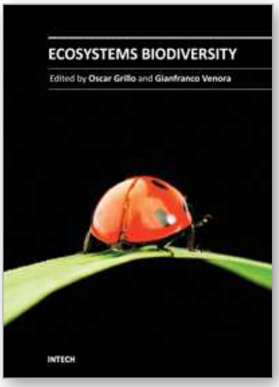
Wassermann, P.; Naas, K.E. & Johannessen, PJ. (1986). Anual supply and loss of particulate organic carbon in Nordasvannet, a eutrophic, land-locked fjord in western Norway. Rapp P *Rèun Cs Int Explo Mer*;186: 423-31.

Weisburg, W.G.; Barns, S.M.; Pelletier, D.A. & Lane, D.J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol*, 17:697-703.

WHO - World Health Organization. *Guidelines for drinking water quality.* Second Edition, vol. 2, Health criteria and other supporting information. Geneva, World Health Organization, 1996

WHO - World Health Organization. *Guidelines for drinking water quality.* Second Edition, vol. 2, Health criteria and other supporting information. Geneva, World Health Organization, 1996.*Water Res* 1989, 23:191-197.34. International Hydrological Decade – World Health Organization (IHD-WHO): Water quality surveys; a guide for the collection and interpretation of water quality data. In *Studies and Reports in Hydrology (UNESCO), no. 23/International Hydrological Decade, 75 – Paris (France)* Geneva: World Health Organization; 1978:57-54.

Woese C.R. (1987). Bacterial evolution. *Microbiol Rev*, 51:221-271.



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Ecosystems can be considered as dynamic and interactive clusters made up of plants, animals and micro-organism communities. Inevitably, mankind is an integral part of each ecosystem and as such enjoys all its provided benefits. Driven by the increasing necessity to preserve the ecosystem productivity, several ecological studies have been conducted in the last few years, highlighting the current state in which our planet is, and focusing on future perspectives. This book contains comprehensive overviews and original studies focused on hazard analysis and evaluation of ecological variables affecting species diversity, richness and distribution, in order to identify the best management strategies to face and solve the conservation problems.