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Comparative analysis of trace element accumulation in seagrasses *Posidonia oceanica* and *Cymodocea nodosa*: Biomonitoring applications and legislative issues

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ABSTRACT

This study aimed to compare the bioaccumulation patterns and translocation of trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn) from the environment in the seagrasses *Posidonia oceanica* and *Cymodocea nodosa*. Results showed that *P. oceanica* has a higher capacity of accumulation. *P. oceanica* and *C. nodosa* accumulate mainly in roots and leaves, the main organs acting as potential bioindicators. No significant correlation was found between water and both seagrasses. In turn, *P. oceanica* and *C. nodosa* were correlated, to a different extent, with As, Cd, Cu, Ni and Zn in sediments. This study showed also that current European regulations do not provide an exhaustive set of legal concentration limits of trace elements in marine water and sediments. Seagrasses *P. oceanica* and *C. nodosa* can act as effective bioindicators of trace elements only if quality limits are set for the most toxic elements present in marine ecosystems.

1. Introduction

Pollution in marine ecosystems has become a worldwide issue due to the dramatic levels reached in the last few decades (Millennium Ecosystem Assessment MEA, 2005; Halpern et al., 2008; Bonanno and Orlando-Bonaca, 2017; Sánchez-Quiles et al., 2017). Trace elements are a category of ubiquitous pollutants that are increasingly spreading across marine environments as a main result of human activities (Small and Nicholls, 2003; Roberts et al., 2008; Boudouresque et al., 2009). Trace elements are generally considered as serious pollutants because of their toxicity, their persistence in the environment, and their capacity to accumulate in organisms (Greger, 2004; Rainbow, 2007; Tchounwou et al., 2012; Bonanno et al., 2013). Although their inhibiting potential depends on various factors such as levels of concentration, ability to form complexes and capacity of oxidation (Szyczewski et al., 2009), trace elements, especially heavy metals, are generally considered as inhibitors of life processes (Nagajyoti et al., 2010; Charlesworth et al., 2011). The ecological risk of trace elements is also difficult to assess because of their complex behavior and numerous interrelations, especially in aquatic ecosystems (Bargagli, 1998; Mitsch and Gosselink, 2007; Bonanno, 2011, 2012; Bonanno and Vymazal, 2017). Moreover, unlike most organic pollutants, trace elements are usually not removed from aquatic ecosystems by natural processes (Greger, 1999; Kabata-Pendias and Pendias, 2001).

In coastal waters, marine organisms (e.g. algae, mussels) have been regularly used as bioindicators of trace element pollution (Campanella et al., 2001; Conti and Cecchetti, 2003; Richir and Gobert, 2014). In particular, seagrasses are generally considered as potential indicator species because of their longevity and capacity to integrate biological, physical and chemical parameters (Pergent-Martini and Pergent, 2000; Orth et al., 2006; Orlando-Bonaca et al., 2015). Seagrasses have also a high capacity of metal accumulation since they interact directly with both the water column (through the leaves) and the sediment pore water (through the roots), as both leaves and roots are sites of ionic uptake (Romero et al., 2006). Seagrasses contribute significantly to the primary production of marine environments in the littoral zone since they have a fundamental trophic role in aquatic ecosystems and an important function in the recycling of nutrients. Consequently, they can extract large amounts of metals from the environment (Kaldy, 2006). Several authors showed also that seagrasses may be suitable for trace element biomonitoring (Prange and Dennison, 2000; Ferrat et al., 2003; Lewis and Devereux, 2009).

Posidonia oceanica (L.) Delile is a widely distributed marine plant, endemic to the Mediterranean, which forms dense communities (meadows) with bathymetric range of 0–40 m depth (IUCN, 2015). *P. oceanica* meadows plays a central role in the ecology of the Mediterranean

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not only as one of the most important contributors to coastal primary production but also as a source of spawning areas, nurseries, and permanent habitats for numerous plant and animal species (Hemminga and Duarte, 2000). *Cymodocea nodosa* (Ucria) Asch. is another coastal seagrass of tropical origin, nowadays restricted to the Mediterranean Sea and some locations in the North Atlantic, from southern Portugal and Spain to Senegal (Green and Short, 2003; OSPAR Commission, 2010). Similarly to *P. oceanica*, it forms mono-specific meadows, and can be found up to a depth of 40 m (Mazzella et al., 1993). *C. nodosa* is considered as a pioneer species able to colonize bare areas of the sea bottom, with its rhizomes growing several meters per years (Borum and Greve, 2004). *P. oceanica* and *C. nodosa* have several characteristics that make them suitable bioindicators of the environment since they are abundant, widely distributed, long-lived, sensitive to natural and anthropogenic stresses, easy to identify and sample (Reizopoulou and Nicolaidou, 2004). Consequently, *P. oceanica* and *C. nodosa* have been previously investigated as potential bioindicators of trace element pollution (Marín-Guirao et al., 2005; Lafabrie et al., 2008; Malea and Kevrekidis, 2013; Richir et al., 2015).

However, studies comparing the performance of *P. oceanica* and *C. nodosa* as bioindicators of trace element pollution are generally scarce in literature. Previous studies pointed out the significant correlation between seagrasses and abiotic components such as bottom sediments (e.g., Bonanno and Di Martino, 2016, 2017; Bonanno et al., 2017), but neither the magnitude of this correlation was determined nor the causal relationship was inferred between element concentrations in seagrasses and concentrations in water and sediments. This study aimed thus to identify which trace elements in water and sediments are correlated with trace elements in seagrasses. In particular, this study analyzed the concentrations of the elements As, Cd, Cr, Cu, Hg, Ni, Pb, Zn in the roots, rhizomes and leaves of *P. oceanica* and *C. nodosa*, and in samples of water and sediments. Similarly, trace element mobility was investigated in the various plant organs. Finally, European Community regulations concerning marine pollution resulting from trace elements are discussed in the light of the findings from the present study.

2. Materials and methods

2.1. Study area

This study was carried out in three costal sites of Sicily (Italy), which include one protected area and two urban sites. (Fig. 1; Table 1). The protected site (Vendicari) is an important marine reserve that was chosen as a control site. The urban sites are seaside resorts that are affected by trace element inputs due to untreated municipal wastewaters and pollution spills from marine traffic. The climate of the study areas is characterized by wet and mild winters, and dry summers. The annual mean temperature is 18 °C, and precipitation ranges between 400 and 600 mm annually. *P. oceanica* and *C. nodosa* meadows were abundant in the study areas, and formed wide monospecific populations distributed up to a depth of 10 m, within about 100 m from the shoreline.

2.2. Sampling

This study followed the same sampling protocols reported in Bonanno and Di Martino (2016). Sampling was carried out bimonthly for three years (2014–2016). During sampling, weather conditions were regular, specifically sunny, not windy, without recent rains and with smooth sea. The same sampling protocol was used for *P. oceanica* and *C. nodosa*. Both seagrasses were collected on the same day. Samples were carried out in once-off trips per site, and then transported to laboratory on the same day of collection. In each study site, sampling was conducted within the area occupied by dense meadows of seagrass, which were far from the coast from 1 to 100 m, and with a variable sea depth of 1–10 m. The size of seagrass meadows varied from 5 × 5 m to 25 × 25 m. The samples included water, sediment and individuals of seagrass. Plant individuals consisted of roots, rhizomes and mature leaves. In each sampling site, 20 samples per typology were collected. Each of these 20 samples was obtained by mixing subsamples. Regarding the seagrass, the generic analytical sample was obtained by mixing 10 mature individuals randomly and manually collected within a subplot of average size of 5 × 5 m. In the same subplot, 10 subsamples of sediment and 10 of water were also randomly collected. The generic analytical sample of sediment was obtained by mixing such subsamples into a representative composite sample. This protocol was repeated twenty times for every typology of sample in each study site (N = 20). After sampling, plant individuals were carefully shaken to remove gross attached material, rinsed with distilled water to remove minor sediment particles, and dried with a clean linen cloth. Finally, the 10 plant individuals of each subplot were sealed in one sterilized and airtight plastic bag. Sediment subsamples were collected from the top 5 cm of the upper layer through a Plexiglas corer (internal diameter 10 cm). Regarding water, subsamples were collected at mid-height between sea bottom and water surface. Sediment and water samples were put in 0.5-L polyethylene sterilized bottles. All samples were kept in PVC containers at 4 ± 1 °C until laboratory analysis.

2.3. Chemical analysis

The concentrations of As, Cd, Cr, Cu, Hg, Ni, Pb, Zn were analyzed in water, sediment and organs of *P. oceanica* and *C. nodosa*. In the laboratory, plant samples were preliminary washed through running tap water to remove gross superficial particles, and then rinsed with bi-distilled water to remove possible residual materials. Seagrass organs were dissected into roots, rhizomes and leaves, and then stored at 4 °C until analysis. Plant organs and sediment were dried to constant weight at room temperature to avoid interferences during the detection of some elements. After drying, plant samples were ground and homogenized in an agate mortar, whereas sediment samples were passed through a 1 mm diameter sieve. After that, these samples were weighed at 0.1 ± 0.05 g, and oven-digested at 90 °C overnight (microwave oven Mars 6, CEM Corporation) in an acid solution (H_2O_2/HNO_3, 2:3 ratio; Carlo Erba). Water samples were acidified with 63% HNO_3 to pH ≤ 2, before being filtered through a filter paper 2.0 μm (Whatman® GF/A glass microfiber filters). After digestion, plant and sediment samples were diluted with ultrapure Milli-Q water to a final volume of 25 mL and analyzed via ICP-MS (Cd, Cr, Cu, Ni, Pb, Zn), and FAAS (As and Hg) (respectively through PerkinElmer Elan® 6000 PerkinElmer® AAnalyst™ 400 AA Spectrometer). The element rhodium (Rh) was used as internal standard. Regarding quality, the instruments were periodically checked against the low level standards (once every five samples) and recalibrated either when signs of drift were noted or after every 10 samples. The standard reference material *Ulva lactuca* (B.C.R. reference material No. 279/504) was analyzed with the same protocol of field collected samples. Student's *t*-test (α = 0.05) was performed to ascertain the good agreement between analyzed values for the reference material and certified values. The percent recovery ranged between 91.4 and 98.4% (Table 2). All analyses were carried out in three replicates. Instrument detection limits were expressed as three times the standard deviation from the mean blank. Mercury concentrations were not reported because below detection limits.

2.4. Statistical processing

Translocation and bioaccumulation factors were calculated to assess element mobility:

- Translocation factors (TF):
  $C_{rhizome}/C_{root}$
  C_{leaf}/C_{root}
  $C_{leaf}/C_{rhizome}$where C_{root}, $C_{rhizome}$ and C_{leaf} are the concentrations