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Innovative use of foraminifera in ecotoxicology: A marine chronic bioassay for testing potential toxicity of drilling muds

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ABSTRACT

Benthic foraminifera are more and more used as bioindicators of anthropogenic impact in marine environments. In order to increase their potential in bio-monitoring studies, we have developed a chronic bioassay method. We incubated foraminifera for 30 days in natural seawater with different concentrations of cadmium, Fuel Oil no. 2 and two types of drilling muds. Foraminiferal activity in the different experimental setups was evaluated using observations of pseudopodal activity after the 30 days incubation period, and a quantification of newly built chambers. All experiments were conducted in a solution of calcein in natural seawater, so that at the end of the experiment, foraminiferal tests with newly added calcareous chambers could be recognised with an epifluorescence microscope. The first results show that foraminifera have a strong physiological response to a 30-day incubation with high concentrations of all tested pollutants. This response clearly varies in function of the concentrations of the added pollutants. It appears that NABM (non aqueous based mud) has a higher toxicity than WBM (water based mud).

1. Introduction

Benthic foraminifera are marine unicellular organisms protected by a calcareous, agglutinated or organic external shell. Foraminifera have been used as bioindicators of anthropogenic impact in marine environments since the 1960s (e.g., Watkins, 1961; Seiglie, 1968, 1971; Setty, 1976; Rao and Rao, 1979; Schafer, 1982; Setty and Nigam, 1984; Bhalla and Nigam, 1986; Nagy and Alve, 1987; Schafer et al., 1991; Alve, 1995; Coccioni, 2000; Bergin et al., 2006). It has been conclusively shown that the study of their assemblages (standing stocks, diversity and species composition) is a reliable tool to assess the environmental impact of industrial activities, and the recolonization of the affected areas after cessation of the polluting activities (Mojtahid et al., 2006; Denoyelle et al., 2010). In order to validate the evidence based on numerous field studies, culture studies, in which foraminifera are incubated with various concentrations of pollutants are needed. A limited number of culture experimental studies have previously been conducted using mixed pollutants, like oil (e.g., Ernst et al., 2006), Tri-n-butyltin (TBT) (Gustafson et al., 2000), copper (Alve and Olsgardt, 1999; Le Cadre and Debenay, 2005; Munsel et al., 2010), mercury (Saraswat et al., 2004; Nigam et al., 2006).

In the present paper, we will improve the bioindication potential of foraminifera by developing and testing an experimental foraminiferal chronic bioassay method, based on a prolonged study of different physiological parameters. Our approach is comparable to bio-assay methods which have previously been developed for several groups of marine macrofauna, such as bivalves (e.g., Ayling, 1974; Eisler et al., 1978; Cranford et al., 1999), crustaceans (e.g., Neff et al., 1978; Carr et al., 1982; Bookhout et al., 1984; van Weerelt et al., 1984) or polychaetes (e.g., Reish, 1977; Tietjen, 1980). In order to develop this foraminifer-based method, a series of laboratory experiments has been performed, in which foraminifera were exposed for 30 days to various concentrations of cadmium, Fuel Oil no. 2 and two different types of oil drilling muds.

Cadmium is known to be a heavy metal with a high toxicity for marine organisms (e.g., Ketchum, 1975; Calabrese et al., 1977; Watling, 1978; Vlasova and Khristoforova, 1982; Ramirez et al., 1989; Ramachandran et al., 1997). Consequently, cadmium has been used as a routine negative control in ecotoxicological bioassays (e.g., Hamilton et al., 1977 and Quiniou et al., 2005). In a similar way, we have used cadmium as a negative control, to assess the sensitivity of *Ammonia tepida* with respect to a highly toxic substance. Oil drilling muds are complex mixtures, containing several chemical substances. The main fluid component of a drilling mud can be water (water based mud, WBM) or an oil-like fluid (non aque-

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ous based mud, NABM) (Neff, 1987, 2005; Darley and Gray, 1988). These drilling muds are necessary to lubricate the drilling bit and to maintain well pressure. The drilling mud also transports fragments of sedimentary rocks, so-called cuttings, upward to the platform. Once on the platform, the cuttings and adhering drilling mud are treated with special devices (shale shakers, centrifugation, etc.) in order to separate the cuttings from the surrounding drilling mud, which is recovered and as much as possible used again. However, the remaining cuttings still contain considerable amounts of adherent mud. Depending on the specific regulations of countries, there may or may not be restrictions on the discharge of these cuttings into the marine environment (Dalmazzone et al., 2004).

Fuel Oil no. 2 has been chosen because it forms a significant fraction of the mixture composing several types of drilling mud. It is added to the drilling fluid to reduce torque and drag and to improve lubricity. Highly aromatic diesel fuels such as Fuel Oil no. 2 are among the most toxic petroleum products (National Research Council, 1983; Neff, 1987).

In environmental studies, particular attention has been paid to the environmental impact of oil drilling fluids, because of their potential toxicity to marine faunas (Neff et al., 1980; Conklin et al., 1983; Holdway, 2004). Acute lethal effects of NABM have been reported for a variety of marine organisms (e.g., Xiao and Piatti, 1995; Terzaghi et al., 1998; Papp and West, 1999) and are consequently a serious concern for oil companies. Bioassay tests usually give an assessment of the toxicity of chemical contaminants based on the response of the test organisms during a 24–96 h exposure period. Long term exposure, which is the common situation at the impacted sites, does not only mean prolonged contact with toxic substances, but may also be accompanied by physical disturbance and/or organic enrichment. Until today, relatively few studies have been conducted on marine organisms to assess the long-term toxicity of drilling muds (e.g., Macauley et al., 1990; Cranford and Gordon, 1991; Cranford et al., 1999; Payne et al., 1995; Raimondi et al., 1997). These earlier experimental studies suggest that long term exposure with drilling muds does have an impact on marine organisms and that this impact is stronger with NABM than with WBM.

We decided to use benthic foraminifera as a new test organism because it appears that the sensitivity for chemical substances varies strongly between organisms of different trophic levels (Marchand and Tissier, 2005; Ramade, 2007). It appears interesting to develop new bioassay with unicellular organisms which are most times at the base of the food web, and may have a different sensibility than metazoans. In fact, it is difficult to set standards for the protection of marine fauna by extrapolating ecotoxicological results from a single, or a very limited number of taxa. The sensitivity of the various investigated taxa may be very different, especially when they have different cellular, metabolic and physiological organisations, and may not be the same for all pollutants. It is evident that in such a context, the use of a single species for an ecotoxicological evaluation (as is often the case) is strongly reductive. Foraminiferal bioassays could yield valuable additional information, because these unicellular heterotrophic organisms are found in all marine areas. Since many species are facultative anaerobes (Risgaard-Petersen et al., 2006; Piña-Ochoa et al., 2010), foraminifera are particularly resistant to hypoxia, that may result from drill mud disposal. In this context, our study has two main purposes:

1. To examine the long term sensitivity of foraminifera to cadmium, NABM, WBM and Fuel Oil no. 2, and
2. To compare the long-term toxicity of NABM and WBM.

2. Materials and methods

Tests are based on a 30 day incubation of foraminifera in sea water at different concentrations of the tested pollutants. Foraminifera are heterotrophic unicellular organisms protected by a shell (usually composed of several chambers, which are added during the ontogeny) through which a pseudopodal network can be extended. In order to investigate chamber addition during the incubation period, we added the fluorescent tracer calcein to the culture medium. This allows us to recognize newly built chambers, which were formed during the exposure period. The parameters measured after 30 days exposure were (1) the pseudopodal activity of each individual, and (2) chamber addition of the incubated foraminifera by observing fluorescent newly built chambers.

2.1. Collecting and storage of the foraminiferal faunas

Sediment samples containing a large amount of the foraminiferal species *A. tepida*, provided with a calcareous test, were collected in August 2009 and May 2010 at the intertidal area in the Bay of Aiguillon, located on the West coast of France. During low tide, the 5 first millimeters of the sediment were sampled in diatom-rich areas (presenting as green spots on the muddy sediment), where foraminifera are abundantly present. The sediment was stored carefully in rectangular plastic bottles (5 cm × 5 cm × 10 cm). The same day, in the laboratory, each sediment sample was washed over sieves of 600 μm and 150 μm. The 600 μm sieve was used to remove most of the polychetes, gastropods and algal waste. The 150 μm sieve residue allowed us to discard all clay and silt particles, and thus, to concentrate adult specimens of *A. tepida.* We added natural seawater (sampled at a water depth of 250 m in the Bay of Biscay) until the bottles were half full. This seawater had been previously microfiltered through a 0.45 MicronSep Cellulosic membrane and stored in plastic containers at the same temperature as the culture.

In order to keep the foraminiferal specimens alive and active, 4 mL of a rehydrated *Chlorella* sp. solution at 1 g/L (lyophilized green algae from AQUAMER S.A.) and 2 mL of a solution containing living diatoms *Phaeodactylum tricornutum* (*CCAP*), both prepared as explained by Barras et al. (2009), were added as food every 3 days. Murray (1963), Wilson-Finelli et al. (1998), Toyofuku et al. (2008), Havach et al. (2001), Heinz et al. (2001), Le Cadre and Debenay (2005) and Barras et al. (2009) have shown in several previous studies that living foraminifera use these types of food. Two thirds of the water was replaced weekly in order to avoid evaporation and salinity variations. Salinity was checked several times, and was always around 36, which is an optimal value for the growth of *A. tepida* (Bradshaw, 1957; Murray, 1991). The temperature and light cycles were not controlled artificially; bottles containing cultures of living foraminifera were stored in the culture room where the temperature was around 22 °C and light followed the natural cycle.

2.2. Selection of living individuals

For our bioassays, it was essential to choose alive and active specimens of *A. tepida*. Green/brown colored individuals surrounded by sticky algal detritus were picked. For shallow water foraminifera, the coloration of the cytoplasm is frequently used as an indication of viability (e.g., Goldstein and Corliss, 1994; Bernhard et al., 2004; Le Cadre and Debenay, 2005), just as the accumulation of organic particles around the aperture (e.g., Goldstein and Corliss, 1994; Heinz et al., 2005). However, this method of observation does not allow to distinguish between dead and living foraminifera with 100% certainty (Bernhard, 2000). Therefore, we decided to verify the vitality of each individual by observing the extension of pseudopods. All foraminifera were placed in groups of 30 individuals