Impact of zinc and nickel on oxygen consumption of benthic microbial communities assessed with microsensors

H. Viret, O. Pringault,*, R. Duran

Abstract

In this study, the effect of zinc and nickel on oxygen consumption in sediments was determined using oxygen microsensors. Sediments from the southwest lagoon of New Caledonia, in the vicinity of the city of Nouméa, were incubated nearby in situ conditions and exposed to Zn and Ni concentrations of 20 and 60 mg l⁻¹. The depth distribution of oxygen consumption was estimated from the steady-state oxygen microprofiles, and the effects of metal were compared on the distributions before and after spiking. In most cases, metal had a strong effect on oxygen consumption at the surface. After 6 h exposure, oxygen consumption was only 10–40% of the initial value. However, the strong decrease in oxygen consumption observed at the sediment surface was counterbalanced by an increase of oxygen consumption deeper in the sediment. This is probably due to (i) a downward migration of aerobic microbial microorganisms living at the surface in order to escape the toxic effect of metal or/and (ii) a switch of the facultative aerobes from the low efficiency fermentation mode to the high-energy aerobic respiration mode.

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1. Introduction

Microorganisms are the main actors in the biogeochemical transformations of organic matter. Bacterial aerobic respiration is the most efficient mechanism for organic matter degradation. In a general scheme of organic matter degradation, the aerobic metabolism is considered as a first step producing metabolites that can then be further degraded by anaerobic microorganisms. Aerobic respiration is therefore an essential stage of organic matter degradation, which controls the other anaerobic pathways of organic matter mineralization. In benthic environments with high amounts of organic matter such as coastal sediments, oxygen can also be used for the oxidation of the reduced compounds such as ammonium or sulfide produced by the anaerobic pathways of organic matter mineralization. As a consequence, in coastal benthic environments, oxygen distribution is restricted to the first top millimeters of sediment (Revsbech and Jørgensen, 1986).

Coastal sediments in anthropogenic areas often receive heavy metals inputs. The microorganisms that inhabit these zones develop different mechanisms of tolerance to survive and grow in such inhospitable environments (Gadd, 1990). Several mechanisms of tolerance may be involved such as, keeping or exporting the metal outside of the cell, or transforming the metal (by oxidation, reduction or methylation) into an innocuous form by production of metal binding
compounds (Gadd, 1990; Ledin, 2000; Valls and De Lorenzo, 2002). Different techniques have been used to study the impact of metals on microbial communities. For example, Vanahala and Ahtiainen (1994) found that ATP content is a good approach to estimate microbial biomass and therefore can serve as an indicator of metal pollution. The degree of inhibition of incorporation of leucine or thymidine can be used as an estimate of the bacterial community tolerance (Diaz-Ravina and Baath, 1996, 2001). Kunito et al. (1999) showed that the dilution plate-count technique could be used to examine the fluctuation of the total and metal-resistant bacteria in soils. Other studies have been based on the measurement of alkaline phosphatase and β-glucosidase activity variations following metallic contamination (Yim and Tam, 1999; Dell’Anno et al., 2003). The major drawback of these different techniques is that they are performed under different conditions that those observed in situ. In benthic systems, metabolic processes such as community respiration or primary production can be estimated using in situ chamber incubations. Hill and Gardner (1987) and Hill et al. (1997) have used such approach to study the effect of metals (Zn, Mn and Fe) on stream sediments (Rocky Mountains). Such techniques privilege a horizontal mapping of the system; however, the depth distribution of the processes can not be determined and therefore the consequences of metal spiking on this depth distribution cannot be inferred.

In this study, we investigated of the impact of metal on oxygen consumption in benthic coastal sediments using oxygen microprobes. These tools allow the high-resolution determination of the spatio-temporal distribution of oxygen in sediments under non-invasive conditions and, therefore, close to those observed in situ. In addition, metabolic processes, which govern oxygen distribution, can also be inferred from the steady-state microprofiles with the same degree of resolution. Similar approaches have been recently used to estimate the impact of organic pollutants in stream sediments (Laursen and Carlton, 1999) and in microbial mats (Grötzschel et al., 2002). The aim of our study was to estimate the impact of nickel and zinc on oxygen consumption in sediments collected in the southwest lagoon of New Caledonia.

2. Materials and methods

2.1. Study sites

The southwest lagoon of New Caledonia is subject to terrigenous metallic contamination due to mining activities and natural soil leaching. The lagoon is also subject to increasing anthropogenic metallic contamination due to urban activities, as a result of an increasing population in the coastal zones and a lack of adequate wastewater treatment (Labrosse et al., 2000). Three different sampling sites were chosen in the southwest lagoon of New Caledonia (Fig. 1): station N12 in Sainte-Marie bay, subject to an anthropogenic metallic contamination; station D49 in Dumbéa bay, subject to a terrigenous metallic contamination; and station M33 in the lagoon under oceanic influence. This last station is considered as a reference station (Fig. 1).

2.2. Sediment respiration measurement

Sediments were sampled using a Kajak sediment core sampler (KC, Denmark). Cores were 50 cm long and 5.2 cm in diameter. Upon return in the laboratory, sediment cores were maintained in a “flow cell” (Epping et al., 1999) that contains 500 ml of seawater from the sampling station. The overlying water was maintained at air saturation via an airlift that also creates a small flow over the sediment interface. Oxygen concentration was measured using a Clark type oxygen microelectrode (Revsbech, 1989) manufactured by Unisense (Denmark). The electrode had a 90% response time lower than 1 s and a stirring sensitivity of 1%. The electrode was mounted on a motorized micromanipulator and oxygen vertical profiles were performed with a vertical resolution of 100 μm. The oxygen microprobe was manually positioned at the sediment surface, profiling and data acquisition were then controlled by computer. Linear calibration of the microprobe was determined from the electrode readings in air-saturated water above the sediment and in the anoxic part of the sediment. Oxygen concentration in air-saturated water was calculated from the solubility equation according to Garcia and Gordon (1992). Oxygen flux \( J \) was estimated from the steady-state oxygen profiles according to the first Fick’s law of diffusion:

\[
J = -D_o \times \left( \frac{\delta C(z)}{\delta z} \right)
\]

where \( D_o \) represents the oxygen diffusion coefficient in water (Broeker and Peng, 1974) and \( \frac{\delta C(z)}{\delta z} \) the oxygen gradient in the diffusive boundary layer. Depth distribution of oxygen consumption was estimated using a numerical model described by Berg et al. (1998). This procedure is based on a series of least square fit to the measured steady-state
concentration profiles, assuming an increasing number of consumption zones. Statistical F-testing compares the fits, so that the simplest consumption profiles results, which reproduces the measured concentration within the chosen statistical accuracy (Berg et al., 1998). Porosity ($\phi$) in the top 5 mm was assumed to be equal to 0.8 and sedimentary diffusion coefficient ($D_s$) was estimated from the diffusion coefficient in
water \((D_s)\) according to the relation of Ullman and Aller (1982):

\[
D_s = \frac{D_o}{2} \times D_o.
\]  

(2)

For each experiment, the \(T_o\) (initial measurement) was based on three to four oxygen microprofiles that were measured before metal spiking. Cores were then incubated with the defined metal for a period of 6 h. Oxygen measurements were then performed using the same procedure as described for the initial profiles comparing oxygen fluxes and oxygen depth consumption profiles before and after spiking assessed metal impact.

Metals solutions used for spiking treatment were prepared with salts of metals: \(\text{ZnCl}_2\text{NiCl}_2\text{H}_2\text{O}\). The concentrations used for the experiments were 20 mg l\(^{-1}\) and 60 mg l\(^{-1}\) of zinc and 20 mg l\(^{-1}\) of nickel.

2.3. Dissolved metals analysis

At the end of the treatment, the quantity of the remaining metal in the overlying water was measured. The seawater of the “flow cell” was filtered through 0.45 μm pore membrane and the analyses of dissolved metals were realized using an ICP-OES with three replicates.

3. Results

3.1. Impact of nickel

The impact of Ni was studied in two stations, D49 and M33. For M33 (reference station), the spiking with 20 mg l\(^{-1}\) of Ni led to a significant decrease of the oxygen flux at the water sediment interface (Table 1). Six hours after spiking, the oxygen flux represented only 61% of the initial value. The impact of Ni resulted in a strong decrease in oxygen consumption in the top millimeter (Fig. 2), but significant oxygen consumption was still measurable at the water sediment interface. Metal spiking led to an expansion of the oxic zone (Fig. 2). Under initial conditions, oxygen penetration did not extend to more than 2 mm depth, whereas after spiking oxygen was measured up to 2.7 mm depth. This increase of the oxic zone was accompanied by an expansion of the oxygen consumption layer.

For the station D49 (regularly subjected to Ni inputs via the Dumbéa river; Fig. 1), the impact of Ni on

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Initial (O_2) flux (nmol cm(^{-2}) min(^{-1}))(^a)</th>
<th>(O_2) flux after spiking (nmol cm(^{-2}) min(^{-1}))(^a)</th>
<th>Surface activity after spiking(^b)</th>
<th>% of metal remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>M33 20 mg/l Ni</td>
<td>1.795±0.123</td>
<td>1.098±0.312* (61%)</td>
<td>37%</td>
<td>96%</td>
</tr>
<tr>
<td>D49 20 mg/l Ni</td>
<td>2.175±0.325</td>
<td>1.328±0.180* (61%)</td>
<td>26%</td>
<td>92%</td>
</tr>
<tr>
<td>D49 20 mg/l Zn</td>
<td>2.842±0.171</td>
<td>0.928±0.342* (33%)</td>
<td>10%</td>
<td>n.d.</td>
</tr>
<tr>
<td>N12 20 mg/l Zn</td>
<td>1.134±0.138</td>
<td>0.843±0.261* (74%)</td>
<td>53%</td>
<td>57.5%</td>
</tr>
<tr>
<td>N12 60 mg/l Zn</td>
<td>1.855±0.194</td>
<td>1.190±0.180* (64%)</td>
<td>38%</td>
<td>72.7%</td>
</tr>
</tbody>
</table>

\(^a\) Average±standard deviation \((n=3–4)\), values in brackets represent the percentage of the initial flux. Significant differences \((p<0.05)\) are marked with *; n.s.: not significant.

\(^b\) Percentage of the initial oxygen consumption at the water–sediment interface. n.d.: not determined.
oxygen consumption at the surface was more pronounced as compared with the reference station (M33). After 6 h of spiking with 20 mg l\(^{-1}\) of Ni, the oxygen consumption at the water-sediment interface represented only 26% (Table 1) of the initial value (0.42 nmol O\(_2\) cm\(^{-2}\) s\(^{-1}\)). This strong decrease in oxygen consumption led to a decrease in the oxygen flux. Below the first millimeter, oxygen consumption was unaffected by metal spiking. However, as observed for M33, metal spiking resulted in an expansion of the oxic zone together with the appearance of oxygen consumption below 2 mm and up to 2.9 mm depth. This deep oxygen consumption partly counterbalanced the decrease observed at the surface and, as a consequence, the areal rate (i.e. oxygen flux) represented 61% of the initial value (Table 1).

### 3.2. Impact of zinc

The impact of Zn was studied in two stations D49 and N12 with a concentration of 20 mg l\(^{-1}\). As observed for Ni, Zn had a strong impact on oxygen consumption for the sediments subject to terrigenous influence. After 6 h of Zn exposure, oxygen consumption in the top millimeter in D49 decreased strongly to reach only 10% of the initial value (Fig. 3, Table 1). This strong decrease was concomitant with an expansion of the oxic zone. Before spiking, maximum oxygen penetration was 0.9 mm, whereas after spiking oxygen penetrated down to 2.5 mm below the sediment surface. Conversely, in the station N12 exposed to urban influence, spiking with 20 mg l\(^{-1}\) of Zn did not result in a decrease of the areal oxygen consumption (Table 1). In the top millimeter, oxygen consumption decreased to reach 53% of the initial value; however, this decrease was counterbalanced by an increase of oxygen consumption below 1 mm depth. As a consequence, oxygen flux after 6 h of spiking was not significantly different from the initial flux (Table 1).

Spiking with 60 mg l\(^{-1}\) of Zn had greater effects on oxygen consumption for the sediment of St. Marie Bay than for the spike with 20 mg l\(^{-1}\) of Zn. The oxygen consumption strongly decreased in the top millimeter.
(74% of the initial value), but the effect of Zn was also observed deeper in the sediment, since significant decreases in oxygen consumption were observed down to 1.8 mm depth (Fig. 4). Oxygen penetration expanded, together with, the appearance of an oxygen consumption zone between 1.9 and 2.4 mm depth. As a consequence, areal rate after spiking still represented 64% of the initial oxygen demand (Fig. 4, Table 1).

4. Discussion

4.1. Note on the methodology

The impact of metals on microbial activity is often assessed with techniques that do not preserve the in situ conditions observed in the studied microbial habitat. The use of microelectrodes offers the possibility to determine with high vertical resolution microbial metabolism under conditions, which are very close to in situ. Although, it should be noted that the high vertical resolution is somewhat counterbalanced by a very low spatial resolution as compared with other techniques used to estimate benthic oxygen consumption (Viollier et al., 2003). A recent study performed in the southwest lagoon of New Caledonia has shown that, for muddy sediments, benthic oxygen consumption rates assessed with microsensors were similar to the rates estimated with techniques that take into account the spatial heterogeneity of the sediments (Grenz et al., 2003). In our study, the three stations were mainly composed of muddy sediments with very low bioturbation effects, conditions that are favorable to a realistic estimation of benthic oxygen consumption with microsensors.

From an oxygen profile, it is possible to estimate the oxygen consumption using the empirical approach as described by Nielsen et al. (1990). Similarly, Laursen and Carlton (1999) have studied the effects of atrazine on oxygen consumption, nitrification and denitrification in stream sediments. They showed that atrazine exposure could severely affect oxygen consumption and denitrification. In our study, the vertical distribution of oxygen consumption was estimated using a modeling approach (Berg et al., 1998). This technique uses a statistical approach to calculate the depth distribution of oxygen consumption, by solving the second Fick’s law of diffusion. This statistical approach in the oxygen profile interpretation minimizes the bias incurred by the empirical approach suggested by Nielsen et al. (1990).

The concentration of metal used in our study to test the inhibition effects might be inappropriate regarding the range of metal concentration that are observed in the southwest lagoon of New Caledonia. The maximum values for Zn (5–20 μg l⁻¹) or Ni (1–2 μg l⁻¹) are observed in the bay surrounding the city of Nouméa (Benjamin Moreton, unpublished results). We spiked the sediment with metal concentration that is known to be the minimum inhibitory concentration (MIC) calculated for the bacteria Escherichia coli (Nies, 1999). Therefore, we assumed that the concentrations in the milligrams per liter range should be sufficient to induce an inhibition effect. The metal effect was assessed after 6 h of metal exposure in order to minimize the changes in the microbial community structure. This short time was however long enough to allow a diffusion of metal within the top millimeters of the sediment where oxygen consumption occurs. Zn and Ni have a similar diffusion coefficient, 0.703 and 0.665 10⁻⁵ cm⁻² s⁻¹ for Zn and Ni, respectively. According to the Einstein–Smoluchowski equation, the distance covered by diffusion can be calculated as follows:

\[ s = (2D \times t)^{0.5} \]

where \( s \) represents the vertical distance covered by diffusion during the time \( t \) as a function of the diffusion coefficient \( (D) \). After 6 h of metal exposure, \( s \) equals 5.5 mm for Zn and 5.4 for Ni. In the different sediments studied, the thickness of the oxygen consumption zone never exceeded 4 mm. Therefore, the 6 h of metal exposure were long enough to allow a penetration of both metals into the oxygen consumption zone as illustrated by the time course of the metal effect on oxygen consumption (Fig. 5). Areal oxygen consumption immediately decreased after metal spiking and reached a plateau after 4 h following metal exposure.

![Fig. 5. Time course of the metal effect (60 mg l⁻¹ of Zn) on oxygen consumption in the sediment of N12 station during 6 h of incubation.](image-url)
The decrease in oxygen consumption could be due to change in microbial activity consecutive to laboratory incubation. In order to test the effects of the incubation on oxygen consumption, we compared initial rates with values measured after 6 h of laboratory incubation without metal spiking (Fig. 6). After 6 h of incubation, oxygen profiles and vertical distribution of oxygen consumption were comparable to the initial values. Oxygen fluxes measured at the end of the incubation were not significantly different to those measured at the beginning. This demonstrates that the 6 h incubation was not long enough to allow natural changes in microbial activities but long enough to allow a metal effect on oxygen consumption.

4.2. Direct effects of the spiking

In most of the spiking experiments performed in our study, we observed a very strong decrease of the oxygen consumption in the top millimeters of the sediment. The only exception was the spiking treatment performed with 20 mg l\(^{-1}\) of Zn in the Sainte Marie Bay sediment where no effect on oxygen consumption was observed after the spike. The metal concentrations added were in the range of the MIC for \textit{E. coli} (see above). However, it is well known that the environment where microorganisms evolve can significantly enhance their tolerance to metallic compounds. Indeed, a study dealing with metal tolerant bacteria showed that bacterial populations of a polluted soil contained a subgroup of bacteria that could tolerate metals over a greater range of concentrations than bacteria from a non-polluted soil (Duxbury and Bicknell, 1983). In our study, the oceanic station was chosen as a reference station since sediments are not exposed to terrigenous or anthropogenic influence. Spiking with 20 mg l\(^{-1}\) Ni led to a strong decrease in oxygen consumption; similar results were obtained with Zn spiking (data not shown). This would suggest that the M33 station could be considered as a reference station for both metals. The sediments in the Dumbéa bay are exposed to high inputs of Ni via the Dumbéa River; therefore, the microbial community could be considered as being well adapted to high Ni concentrations. The spiking treatment performed in the Dumbéa sediments with 20 mg l\(^{-1}\) Ni seems to contradict this hypothesis. In order to develop a potential tolerance to a metal, microorganisms should regularly be in contact with the defined metal (Duxbury and Bicknell, 1983; Duxbury, 1985; Pennanen et al., 1996). Metal has to be bioavailable to the microorganism in order to have a potential effect (positive or negative) (Hines and Jones, 1982). In the muddy sediments of the Dumbéa bay, we can expect that the regular inputs of Ni from the river are trapped by the silt particles leading to a low bioavailability of Ni for the microorganisms as described by Doelman and Haanstra (1979) and Ranjard et al. (2000). These authors have shown a reduction of metal bioavailability in silty substrates compared to light-textured substrates. Recently, Borgmann and Norwood (2002) studied nickel and zinc bioavailability in sediments as a function of depth. They found that nickel was less bioavailable and therefore less toxic at the surface than deeper in the sediment.

When a decrease of oxygen consumption occurred due to metal spiking, it is interesting to notice that in all cases, addition of metal did not totally inhibit oxygen consumption in the sediments. At the top surface, oxygen consumption was still detectable. It is likely that this remaining oxygen consumption was partly due to abiotic oxygen demand for the oxidation of reduced compound. In sediments, oxygen consumption represents the sum of the microbial respiratory activity and the oxidation of the reduced compound produced by the anaerobic microbial respiration processes such as ammonia or sulfide. Although oxidation of reduced compounds is mainly due to microbial activity,
oxidation can also occur with much less efficiency under abiotic conditions. Therefore, in our study, it is likely that the inhibition observed after metal spiking only affects microbial oxygen demand and, therefore, the remaining oxygen consumption observed at the sediment surface can be partly due to abiotic oxidation of reduced compounds but also to biotic oxygen consumption from microbial communities, which were not totally inhibited by metal spiking.

The effect of Zn in the sediment of the St Marie Bay seems indicate a possible tolerance of the microorganisms to Zn. Spiking with 20 mg l\(^{-1}\) of Zn did not result to a significant difference in oxygen consumption (Table 1). The small decrease in oxygen consumption observed at the top was counterbalanced by the slight increase deeper in the sediment (Fig. 3). A spike with three fold more Zn (60 mg l\(^{-1}\)) had a greater effect on oxygen demand. The oxygen consumption observed in the first two millimeters strongly declined and represented only 50% of the initial value after 6 h of exposure. These results would suggest that microbial community present at the sediment of the St. Marie Bay have mechanisms of tolerance to Zn since the bay is regularly subjected to Zn inputs via water runoff from the surrounding urbanized area. The concentration of dissolved Zn can reach more than 20 \(\mu g\) l\(^{-1}\) in the water column; this represents concentrations of 1000 times higher than those measured in the open ocean (Ben Moreton, unpublished results). These conditions are favorable to the development of mechanisms of tolerance (Duxbury, 1985; Duxbury and Bicknell, 1983). Moreover, despite high inputs of Zn, sediments from St Marie Bay appear to still have some empty binding sites for Zn since after spiking with 20 and 60 mg l\(^{-1}\), the remaining concentration in the water was 11.5 mg l\(^{-1}\) (57.5%) and 43.6 mg l\(^{-1}\) (72.7%) for 20 and 60 mg l\(^{-1}\), respectively (Table 1). This decrease in the metal concentration during spiking has to be taken into account for a precise determination of the real inhibitory Zn concentration. However, from our results, it is impossible to clearly define the threshold concentration, which would result in a complete inhibition of the microbial oxygen consumption.

4.3. Indirect effects of the spiking

In the different spiking treatments performed in this study, the decrease in the oxygen consumption in the top millimeter was always concomitant with an expansion of the oxic zone. In some cases, the oxygen penetration increased by more than 1 mm (Fig. 2). The increase in the oxygen penetration leads to the development of an oxygen consumption zone, in a region where no oxygen respiration was observed before spiking. The appearance of this “new” oxygen consumption could be due to several reasons. Firstly, as a result of metal spiking at the top surface, oxygen consumption strongly decreased, which in turn led to an increase in the bioavailability of oxygen for microbial communities living deeper in the sediment. In coastal sediments where accumulation of organic matter is important, oxygen could be a limiting factor for aerobic bacteria. The low amount of dissolved oxygen in the water column (0.211 mM at 25 °C and for a salinity of 35) results in strong oxygen gradients within the first millimeters of sediment. In contrast, due to the high amount of sulfate in seawater (30–32 mM), sulfate reducing bacteria are not limited by the sulfate concentration but rather by the quantity and the quality of the organic matter. This low availability of oxygen results in strategic adaptations of bacteria inhabiting coastal sediments. Facultative aerobic bacteria have the capability to switch from oxygen respiration to fermentation and vice versa, as a function of oxygen presence. When oxygen is available, facultative aerobic bacteria preferentially perform oxygen respiration due to its high-energy supply, whereas, as soon as oxygen is depleted, they switch to a fermentative mode of growth. When oxygen is less used in the top millimeters due to inhibition of oxygen respiration, it can penetrate deeper in the sediment where it becomes available for facultative aerobes. This would result in an appearance of oxygen consumption in a layer where no oxygen respiration was observed prior to spiking. This “new” oxygen consumption observed deeper in the sediment could also be due to a migration of aerobic motile microorganisms living in the top millimeters of the sediment that migrate in order to escape the toxic effects of the metal (Alexandre et al., 2004). The metal exposure was chosen to be short in order to minimize the changes in the microbial community structure. The diffusion model predicts that, after 6 h of exposure, metal should be present at up to 5 mm depth (see above). However, the diffusion of metal consecutive to spiking leads to the creation of a metal gradient with high concentration at the top and low concentration deeper in the sediment. Therefore, it is likely that motile microorganisms inhabiting the top surface of sediment would migrate deeper in the sediment where metal concentration is lower in order to escape the potential toxic effects of the metal. Similar behaviors have been observed in microbial mats where migration of cyanobacteria was observed after exposure to UV radiation (Bebout and Garcia-Pichel, 1995).

The appearance of this oxygen consumption deeper in the sediment partly counterbalanced the inhibition of
oxygen consumption observed at the top. As a consequence, spiking with metal has less effect when considering areal oxygen consumption (oxygen flux at the sediment surface), which represents the integration of the oxygen consumption over the oxic zone. For example, Ni spiking in Dumbéa sediment (D49) resulted in a strong decrease of oxygen consumption in the first millimeter, which only represented 26% of the initial value, whereas oxygen flux after spiking still represented 61% of the initial flux (Table 1). By using a technique, which takes into account the spatial heterogeneity of the sediment such as in situ (Clavier and Guarrigue, 1999) or ex situ core incubations (Glud et al., 1994; Grenz et al., 2003), impacts of metal on oxygen consumption could be underestimated. Those techniques estimate the areal oxygen consumption from the change in oxygen concentration observed in the overlying water during the dark incubation. In this study, we have observed that metal spiking has different effects on microbial communities as a function of sediment depth. This emphasizes the fact that oxygen microsensors should be preferentially employed to assess metal effects in benthic environment rather than techniques that based on changes in oxygen concentration in the overlying water.

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