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Toxicity of Heavy Metals to Bacteria in Sediments

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Several biological parameters may be used to evaluate toxic effects on bacterial populations: growth rate (Macaskie & Dean 1984), biomass measurement (Pritchard & Bourquin 1985), activity variations or specific enzymatic activities (Bitton et al. 1992). Most experiments are *in vitro* tests, with pure bacterial strains, isolated or not from the study site (mainly soil or aquatic environments). Some of the studies are *in situ* toxicity versus bacterial populations, especially in sediments. These studies are in fact complex: bacterial activities are highly variable, according to sediment localization (marine, estuarine or continental) and chemical composition, environmental parameters (oxygen level, pH, organic matter content, temperature, ...). This induces an important heterogeneity during sampling. In the same way, environmental factors have a very marked influence on heavy metal toxicity (Babich & Stozky 1986).

The aims of the present study were (1): to determine changes in respiratory (dehydrogenase) and exoenzymatic (ß-glucosidase) activities of a microbial community in a non-polluted river sediment, in the presence of lead and cadmium and (2): to determine and compare the impact of these two heavy metals on pure bacterial cultures isolated from the same sediment and from a neighboring one with the same physicochemical charactereristics, but contaminated with high levels of heavy metals, particularly lead (114 ppm) and cadmium (10,1 ppm).

MATERIALS AND METHODS

Study sites were two small rivers, at 35 km downstream from Grenoble, (Isère, in eastern France). Sediments were sampled with a CEMAGREF (French Institute of Agricultural and Environmental Engineering Research) hand corer at three sites in a small unpolluted river (la Mayenne) and three in a polluted one (la Morges) running across the town of Rives and an industrial park (paper mill, mechanic industries,

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wastewater treatment plant). A preincubation with heavy metals was carried out at the laboratory for 36 hr in gently shaken flasks at 20°C. Heavy metals were added as $CdCl_2$ and $Pb(OOCCH_3)_2$, with concentrations varying from 0 up to 1470 ppm, respectively, of Cd^{2+} and Pb^{2+}. Sediment pH was 6.9, Eh was -232mV.

Glucosidase activity was determined by fluorimetry (on a Jobin-Yvon fluorimeter, at 365nm for excitation and 455nm for emission), after incubating the sediments in the presence of 4-methylumbelliferyl-ß D-glucoside for 30 min at 20°C. This technique is commonly used for ecological studies on organic matter biodegradation in aquatic environments (Montuelle and Volat 1993). Dehydrogenase activity (electron transport system activity) was carried out as described by Broberg (1985) and measured by spectrophotometry at 490nm on a Kontron Uvikon 860.

Bacterial strains were isolated from sediment previously treated with sodium pyrophosphate (0.1 % for 1hr). Suspension-dilutions of the floating phase allowed us to isolate 33 strains on a soil extract medium (1/2 diluted soil extract, agar 12g/l). Each strain was then cultivated at 20°C on a PGY medium (bactopeptone 2g/l, yeast extract 1g/l, glucose 1g/l, agar 12g/l). The medium contained different Pb or Cd concentrations (0.1; 1; 10; 100mM). Metal-free medium was used as control growth medium. One mM of Pb equals 207 ppm and 1mM of Cd equals 112 ppm.

Growth was monitored over 2 wk and results are expressed as the percentage of strains grown on the metal-growth medium in comparison with control-growth medium. The same protocol was used for strains isolated from the contaminated site (La Morges river). Two strains were selected: a *Coryneform sp.* (N30) and a *Bacillus sp.* (N16) growing on medium containing up to 1mM Pb and up to 0.1 mM Cd, respectively. A Macaskie & Dean's liquid medium was used, modified by a bactopeptone complement (0.5 g/l) that does not complex with Cd and little with Pb and yeast extract (0.25g/l); this medium contained Cd as $CdCl_2$ (from 0 up to 0.15mM) or Pb as $Pb(NO_3)$ from 0 up to 2mM.

Growth medium contained all the essential ions and was selected because it limits metal insolubility. Metabolic phosphate was added in the form of glycerol-2-phosphate which precipitated less metals than free phosphate (Hambuckers-Bertin and Remacle 1990).

RESULTS AND DISCUSSION

Expression of IC_{20} (concentration inhibiting 20% of the enzymatic activity) allows for the differentiation of the *in situ* effects of Pb and Cd on the two enzymatic activities. Results from sediment incubations (Fig. 1, a and b) show that Pb was clearly more toxic for glucosidase activity (IC_{20} = 182 ppm) than for dehydrogenase activity (IC_{20} = 1280 ppm).

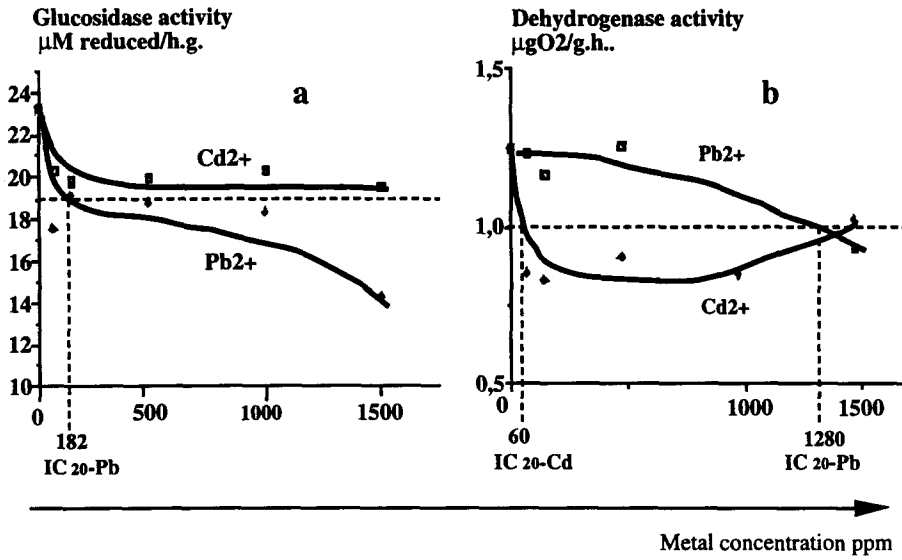

Figure 1. Influence of Pb and Cd on glucosidase activity (a) and dehydrogenase activity (b).

Conversely, Cd did not show IC_{20} for glucosidase activity at the concentrations used. Dehydrogenase activity was very sensitive to Cd (IC_{20} = 60ppm).

The metal concentrations used in these experiments were high compared to those generally found in sediments, but De Vevey et al. (1993) found Pb concentrations up to 4495 ppm in sediment of lake Yojoa (Honduras). The high concentrations tested resulted in just small inhibitory effects on enzymatic activities. This may have been due to a shelter effect of the sediment and its organic matter content. Adsorption, chelation, precipitation are physicochemical processes that neutralize Pb^{2+} and Cd^{2+} ions which consequently lower the bioavailability of the soluble metal. This effect has been demonstrated by Hamdy and Wheeler (1978) and Babich and Stotzky (1986).

As a preincubation was carried out, it was impossible to differentiate the immediate effects of the metal on the enzymatic activities (immediate toxic effect) from the effects on microbial growth (short-term toxic effect). Thus, these results accounted for most of the biological responses of the microbial community, except the genetic response (long-term toxic effect), due to a short incubation time. Furthermore, lead does not induce mutagenic effects in bacteria, unlike cadmium (Babich and Stoztky 1986). The organic matter content of the sediment could explain the small toxic effect: Babich and Stotzky (1986) showed that organic composition of a specific growth medium influences the toxicity of the heavy metal tested.

It is generally observed that cadmium has a higher general toxic effect on microbial organisms than lead. We observed this effect on the electron transport system activity, but not on glucosidase activity. Other experiments demonstrated the toxic effect of cadmium on dehydrogenase, the activity of which is correlated with living microbial organisms (Tynecka et al. 1981). Surowitz et al. (1984) showed that the effects of Cd^{2+} on respiration of sensitive strains seemed to involve metabolic mechanisms prior to the entry of electrons into the electron transport system, rather than affecting the transport system itself. Furthermore, Cd^{2+} and Pb^{2+} are able to alter the outer and the inner cell membranes (Bitton et al. 1988) and disturb the proton flux through the membrane.

However, glucosidase activity is not strictly associated with living bacterial cells. It has been shown that exoenzymatic activities are associated with membrane fragments or could be found free in the water phase (Chrost 1991). Glucosidase activity could persist after cell death and/or lysis. The metal toxicity is then caused by a partial or total denaturation of the protein resulting in enzyme inactivation (Tynecka et al. 1981). For example, Cd caused a decrease in the alkaline phosphatase activity because of the replacement of Zn^{2+} by Cd^{2+} in the enzyme itself (Macaskie and Dean 1984). Some authors use a bacterial test (MetPAD test, Bitton et al. 1992) which measures the galactosidase activity of a pure strain of *Escherichia coli* for testing industrial wastewater toxicity. This test appears to be very sensitive to toxics in a water phase. Conversely, the use of MetPAD on sediment and on a sediment-water extract indicate negative results due to the very small bioavailability of heavy metals (DeVevey 1993). Respiratory activity thus seems to be a more sensitive *in situ* "descriptor" of heavy metals toxicity on a sediment microbial community.

Table 1. Pb and Cd influence on bacterial strains: % of strains growing on PGY medium + metal compared to total number of strains isolated on PGY medium (blank medium).

		Cd				Pb			
	metal concentration (mM)	0.01	0.1	1	10	0.1	1	10	100
resistant strains (%)	polluted river (Morges)	36	21	0	0	85	12	0	0
	unpolluted river (Mayenne)	63	16	0	0	92	18	0	0

In pure culture with solid medium, the proportion of strains resistant to 0.01 mM Cd was higher in contaminated sediment (63%) than in uncontaminated sediment (36%). Thus, the difference was weaker with Pb 0.1mM: 92% vs 85% (table 1).

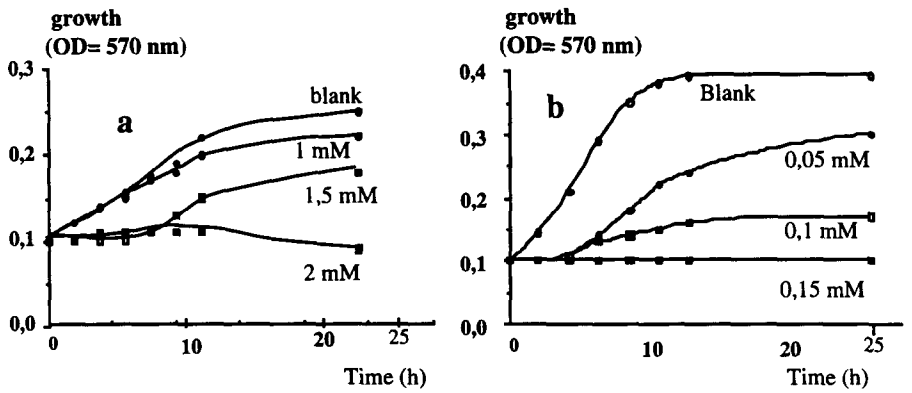

Figure 2. Influence of Pb on the growth of a *Coryneform* strain (N30) (a) and influence of Cd on the growth of a *Bacillus sp.* (N16) (b).

The presence of metals would generate a strain selection or a strain acclimation, as has been shown in other sediments (DeVincente et al. 1990). At high concentrations (0.1mM Cd and 1mM Pb), there were no more differences between the bacterial strains of the two sites: only the more resistant strains grew.

This result is not surprising as it is common to find, in an uncontaminated site, potentially resistant strains to a pollutant without previous contact (Houba and Remacle 1980). A liquid medium (Fig. 2 a and b) allowed more precise study of bacterial growth. The inhibitory effect of Cd on strain N16 was detected at 0.05 mM concentration; it was marked at 0.1 mM (residual growth) and lethal at 0.15 mM (no growth). Pb was lethal at 2 mM concentration on the *Coryneform* strain N30.

Comparisons between *in situ* bacterial populations and pure cultures underline the protective effect of sediments on bacterial populations and their enzymatic activities. In the Morge River, only 16 % of the isolated strains from a sediment containing nearly 0.1mM Cd (10.1 ppm) were able to grow on an agar medium with similar concentrations. The chemical forms of the metal in the sediment or its bioavailability were probably different from those in the agar medium where the soluble toxic fraction was more available. Study of biochemical parameters show that for ecotoxicity studies in sedimentary environments, the dehydrogenase activity, in aerobic conditions, seems to be better adapted than exoenzymatic activities.

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