A SIMPLE METHOD FOR SAMPLING INTERSTITIAL WATER OF RIVER SEDIMENT FOR EVALUATION OF ACUTE AND CHRONIC TOXICITY WITH *DAPHNIA MAGNA*.

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ABSTRACT

A method for extraction of interstitial water from river sediment is proposed. The method simplifies the common procedures of sampling and processing for toxicity tests on natural river pore water. Acute and chronic toxicity tests from interstitial water of a polluted section of the Butrón river were conducted to evaluate the method. Toxicity of the sediment pore water is demonstrated by survival and reproduction data from chronic bioassays, in first and second generations.

INTRODUCTION

Assessment of river quality includes three subsequent levels of evaluation: measurement of concentration of toxic chemical elements, data on field biological communities and toxicological bioassays (Chapman, 1990). This last aspect is being incorporated to the already classical studies on freshwater communities (Reynoldson & Zarull, in 1989) in order to complete the information about the effects of pollutants on the ecosystem. Giesy *et al.* (1988a) state the limitations of traditional field surveys in pollution control programs concerning the answers to questions such as "what concentration" causes "what effects". In Spain, so far only the first two aspects have been developed, but the management of freshwater ecosystems requires the knowledge of toxicological information and it is a field which needs to be explored in the near future.

Bioassays on aquatic animals have been developed since the last century (Anderson, 1980). Nowadays, there are a number of tests based on different species that can be classified into a scale ranging from short-term / easy / inexpensive to long-term / complex / expensive. The first ones include very standardised bioassays, like Microtox or *Daphnia magna* acute-toxicity tests. But on many occasions, these bioassays are not useful for evaluating the toxicity of natural waters, although very affected by pollution, because of the effect of dilution or precipitation of many toxic chemicals into the sediment. Therefore, we have noticed a growth in popularity of long-term tests that measure sublethal or chronic effects induced by exposure to natural sediments. In the freshwater ecosystem, sediments play a potential role as a reservoir of toxic substances (Reynoldson *et al.*, 1991) therefore they may cause alterations to biota not only in the present but also over long time periods. Consequently, many research programs have concentrated on developing methods for measuring the toxicity of natural sediments (Nebeker *et al.*, 1984; Long & Chapman, 1985; Chapman *et al.*, 1985; Giesy *et al.*, 1988a; Giesy & Hoke, 1990; Hoke *et al.*, 1990; Reynoldson *et al.*, 1991; Sasson-Brickson & Burton, 1991; Schubauer-Berigan & Ankley, 1991). Depending on the pH and chemical nature of the water, toxicants may be found dissolved, forming complexes or precipitated. Therefore the assessment strategy might include measurements of indicator species exposed to interstitial (or pore) water as well as to the solid sediment. Methods and test-species are different in each case, and protocols are not as standardised and easy as we would like.

Bioassays with pore water allow us to use the most standard test-species, *Daphnia magna* Straus, a planktonic organism, which is very easy to cultivate in the laboratory, allowing reproducible experiments. Toxicity tests with pore water have been conducted by a number of authors (Schubauer-Berigan & Ankley, 1991; Giesy *et al.*, 1988a, 1988b, 1990; Giesy & Hoke, 1989).

We discuss a method for the extraction of interstitial water, that is simple and avoids the processing of a huge quantity of sediment, and very sophisticated or time consuming procedures, which are not possible in small laboratories.

MATERIAL AND METHODS

1. **LOCALITY.** Interstitial water from sediment was sampled from a polluted locality in the Butrón river, in Munguía (Bizkaia, Spain) (Fig. 1). Coordinates in projection U.T.M. (Hayford Ellipsoid): 30TWP 123014. The locality is not far from Bilbao and it has an industrial area that discharges effluents straight into the river from 13 metallurgy and 3 chemical factories.

FIG.1.- Sampling site on the Butrón river.

2. **CULTURE OF *DAPHNIA MAGNA*.** This species has been widely recommended for bioassays because it is easy to culture in the laboratory (Ten Berge, 1978), has parthenogenetic reproduction, fast response (24 or 48 h in acute tests and 4 to 28 d in chronic tests) and the fact that it is considered a standard organism by many international guidelines for toxicity control of pollutants (BOE, 1989; ISO, 1982; OECD, 1983; Ward & Parrish, 1983; Horning & Weber, 1985; NPR, 1980; SCA, 1983).

The culture is maintained at a constant temperature ($20 \pm 1^\circ\text{C}$), with a photoperiod of 16 h light and 8 h dark and fed on the green alga *Chlorella vulgaris*. Algae were supplied three times a week; 10 mL/L were added to each baker from a suspension of 10^8 cells/mL. The density of *Daphnia* in the culture is 40 *Daphnia* in 3 L (75 mL per individual).

Culture and dilution water were made up following ISO standard guidelines, adding 5mg/L of yeast (Peters, 1987) and 1 $\mu\text{g/L}$ of Se. This concentration of Selenium is equivalent to that present in natural waters (Ingersoll *et al.*, 1990) and it is supplied in the form of SeO_3Na_2 (Elendt & Bias, 1990). Selenium has proved to be efficient in preventing culture failures occurring in monoaxenic cultures after a number of generations (Peters, 1987). Selenium also prevents the appearance of some abnormalities in the secondary antennae (Elendt & Bias, 1990), as we have also observed associated with a behaviour revealing increasing weakness. Once a week, half of the volume of water is renewed. The culture is periodically tested with Potassium Dichromate and the response has always been within the appropriate range (ISO, 1982).

Cultures are characterised as follows (mean $\pm$ SD): time to the first brood: 9.57 $\pm$ 1.30 d; number of broods in 21 d per female: 4.0 $\pm$ 0.0; number of neonates per brood (in 21 d): 14.25 $\pm$ 4.76; interbrood period: 3.44 d $\pm$ 1.01d; average cumulative number of neonates in 3 broods per female: 44.4 $\pm$ 9.69; total number of neonates per female in the life cycle: 103.2 $\pm$ 14.133. The fecundity per individual is adequate, following the validity criteria of a minimum of 20 neonates in the 3 first broods (OECD, 1983) or 70 neonates per *Daphnia*, as proposed the validity criteria of the EC ring-test (Cabidenc, 1986, in Baird *et al.* (1989).

3. SAMPLING PROCEDURE. Interstitial water was sampled in the river bank, at approximately 25 cm from the water line, using the Karaman procedure (Chapuis, 1950). The method consists of a well of 20 -30 cm depth, where the pore water flows and it can be sampled easily with a bottle. In order to avoid biased information, 4 samples were collected from 4 wells along the bank, separated from each other by approximately 4 m. A total of 24 L were transported in ice to the laboratory and maintained at 4°C until they were processed (within 7 days) (Giesy *et al.*, 1988a). Water was filtered by a Millipore OM 100 vacuum pressure system (142 mm ϕ , cellulose nitrate membrane filter 0.45 μ m).

A sample of the benthic invertebrate community was taken for a calculation of a biotic index in the studied area.

4. PHYSICAL AND CHEMICAL ANALYSIS OF THE WATER. Additional sampling were made in a different date to describe the characteristics of the studied water and also to study the differences between a traditional core sampling method and the Karaman well procedure, both contrasted with the superficial river water. Three separated samples from wells were made at 25 cm of the watershed; several cores were taken to get one sufficient sample of supernatant; and one sample was taken from superficial running water. Measures of Temperature and pH were made in the field; alkalinity and conductivity were measured in the laboratory; concentrations of five common metals (Zn, Fe, Ni, Cu, Cd) were measured in water fixed with nitric acid in the field at pH < 4, by flame atomic absorption spectrometry (Perkin Elmer, 2380) for zinc and iron, and AAS using graphite furnace HGA 500 technique for nickel, copper and cadmium.

5. BIOASSAYS. Acute toxicity was measured by the test of bioluminescence inhibition of *Photobacterium phosphoreum* (EC₅₀-15 min.) with a Microtox Model 500 Toxicity Analyzer (Microbics) and by the test of immobilisation of *Daphnia magna* (EC₅₀-24h and 48 h) (ISO, 1982; BOE, 1989). The chronic bioassays with *Daphnia* were conducted until the third brood was concluded, with four replicates exposed to a series of dilutions in beakers of 400 mL with 10 individuals of *Daphnia* within each (OECD, 1983). The dilution series were calculated as proposed by Petrocelli (1985) [C₆: 1.00, C₅: 0.750, C₄: 0.562, C₃: 0.422, C₂: 0.316, C₁: 0.237, C₀(control): 0.00]. Giesy & Hoke (1990) recommend performing dilutions of the interstitial water to determine dose-response relationships. Water was weekly renewed and *Daphnia* were fed three times a week to maintain the same conditions as in the culture.

Daily observations on survival, reproduction, behaviour and health were made; neonates were removed and counted. Dead individuals were also removed and the volume of water and food were always adjusted so as to maintain density and nourishment per individual as constantly as possible.

Neonates from the first brood were used for a second generation test of the pore water, in such a way that those born in a particular concentration were again exposed to the same concentration in the second generation test. Both tests (first and second generations) were conducted until the third brood was complete (18 d for the first generation and 19 d for the second).

RESULTS

Table I shows the values of conductivity, alkalinity, pH, temperature, and the concentration of the some important metals in the pore water extracted by the Karaman method and by a core, and in the superficial river water of the studied section of the Butrón river. Temperature and pH readings are relatively homogeneous in the three compared waters. Alkalinity and conductivity are in general high but they are higher in those waters extracted by the Karaman procedure, being the waters sampled by a core intermediate between channel and well waters. Heavy metal content was likewise relatively homogeneous from sample to sample. Concentrations of Zn, Fe and Cu of superficial water are higher than the proposed guidelines for protection of freshwater aquatic life (Canadian Water Quality Guidelines, 1987).

Interstitial water used for the toxicity test had a high degree of turbidity. The sediment was made up of sand, clay and mud of blackish colour. Both gave off a penetrating, disagreeable odour.

The community of macroinvertebrates in the section of the river contained a large number of tubificid worms (*Limnodrilus* spp.) forming a continuous carpet on the bottom of the river, some specimens of *Physa acuta*, *Chironomus* spp., the introduced crayfish *Procambarus clarki* and empty shells of unionids, probably transported from upstream unpolluted sites. That configures a community of a very low biotic index [I.B. (Verneaux & Tuffery, 1967)=4]. This value allows us to interpret the site to be subject to an important pollution stress.

TABLE I.-The values of conductivity ($\mu\text{S}/\text{cm}$), alkalinity ($\text{mg}\cdot\text{L}^{-1}\text{CO}_3\text{Ca}$), pH, temperature ($^{\circ}\text{C}$), and heavy metal concentrations in superficial and interstitial water of Butrón river

	$\mu\text{S}/\text{cm}$ Cd (μg)	$\text{mg}\cdot\text{L}^{-1}\text{CO}_3\text{Ca}$	pH	$^{\circ}\text{C}$	Zn(μg)	Fe(mg)	Ni (μg)	Cu (μg)	
Karaman (range)	546-948	522.6-757.8	6.8-6.9	16.5-17.5	100-200	12.5-17.0	20-40	<15-53	0.7-1.5
Karaman (mean)	709	609.73	6.83	17	160	13.86	30	-	1.2
Core	442	402.4	7.2	18	400	17.4	40	<15	1.3
Superficial water	362	186.05	7.5	17.5	100	16.0	10	19	1.0

Acute-Toxicity tests.

Acute tests with Microtox(15 minutes) and *Daphnia magna* (48 h) did not reveal apparent toxicity, therefore chronic tests were conducted further to evaluate the toxicity of the sediment. An alternative method could have been to concentrate the contaminants until they were in the range that produced acute responses (Guzzella, 1991).

Long-term toxicity tests with *Daphnia magna*.

Survival. In the first generation, the concentration C_6 , equivalent to the natural pore-water, caused mortality to 47.5 % of the individuals within 18 d (Fig.2). ANOVA of the transformed data (arcsine $\sqrt{[\% \text{ accumulated survival}]}$) shows that different concentrations of the pore water cause harmful effects in populations of *Daphnia* ($F=17.26$, $p<0.05$). Scheffe's F test showed significant differences for the control C_0 and the highest concentration C_6 ($F= 3.82$, $p<0.05$). Dunnet's t test did not show significant differences between different concentrations and the control.

In the second generation, there was a marked falling off in survival (Fig. 3). But the response was much more irregular than in the first generation. ANOVA of the transformed data showed that the pore water caused a significant decrease in survival of *Daphnia magna* ($F= 6.01$, $p<0.001$). Scheffe's F test showed significant differences in survival of control and both C_3 ($F= 4.25$, $p<0.05$) and C_6 ($F= 3.82$, $p<0.05$). Dunnet's t test did not show significant differences between different concentrations and the control.

Reproduction. In the first generation, the number of neonates per *Daphnia* in the first brood were markedly higher in batches with interstitial water than in the control. In contrast, the second and third broods were both sharply lower in number than the first (Fig. 4). Kruskal-Wallis non parametric test for the total number of neonates per *Daphnia* showed significant differences in the total number of neonates for the six concentrations and the control ($H\text{-adj.} > X^2_{0.05[6]} = 12.592$). However, the number of neonates in the control were lower than expected (a mean of 17.8 neonates/*Daphnia* /3 generations).

Data of reproduction in the second generation showed interference with the production of ephippia, related to accidental damage of lamps causing diminution of light intensity during one weekend. As a result of this accident, sexual reproduction was induced. Control population produced a high number of ephippia compared to populations exposed to pore water which presented a lower production, reaching complete inhibition in the higher concentration C_6 (Fig. 5).

FIG.2

FIG. 2.- The survival of the first generation of *Daphnia magna* exposed to a series of dilutions of interstitial water of the Butrón river. Means and SD shown.

FIG.3

FIG. 3.- The survival of the second generation of *Daphnia magna* exposed to a series of dilutions of interstitial water of the Butrón river. Means and SD shown.

DISCUSSION

The method of Karaman for sampling interstitial water from river sediment is simple and rapid and it has no important requirements in terms of staff or laboratory infrastructure for storing and processing samples. This method was originally described by Chapuis (1950) for sampling freshwater interstitial fauna associated to river system. This technique avoids sampling huge quantities of sediment (e.g. 100 kg/sample; see Giesy *et al.*, 1990) and centrifugation for extraction of pore water. The construction of wells for extracting of water has been used in different kind of research works to study both hyporheic and phreatic waters (Pennak & Ward, 1986; Stanford & Ward, 1988). Mestrov & Lattinger-Penko (1977/78) (in Hynes, 1983) found little evidence of lateral spread of the pollution effects outwards from the banks. However, the spatial extent and strength of hyporheic-channel waters interactions undoubtedly vary from river to river, much depending on the nature of sediment, and it is not possible to give an universal model that allows us to know until which extent hyporheic waters in the river bank are the same as those below the river bed. But we can assume that there is a situation that allows for lateral exchange with the water of the stream as some works describe stream-dwelling fauna as far as 2 km. from a river (Stanford & Ward, 1988).

Clearly, it is necessary more study on the nature of water extracted by this method in the stream bank, but it probably gives a good picture of the situation of the sediment pore-water of the river. The existence of local high or low concentrations of some chemical compounds is solved by sampling several wells along the bank of the selected site. Physical and chemical differences between interstitial water extracted by a core and by Karaman method do not seem to be important. However, a conclusive answer could be given comparing tests performed with pore waters extracted by different methods. Giesy & Hoke (1990) briefly discuss some pore water extraction techniques. Nebeker *et al.* (1984) do not filter because it is said to be a less efficient method than centrifugation, removing undissolved components from water, although no numerical data are provided to justify this assertion. Likewise, Schubauer-Berigan & Ankley (1991) (and P. Chapman, personal communication) consider the filtration of waters to be unsatisfactory because of the loss of toxicity. However, in some protocols water is filtered after centrifugation (Giesy *et al.* 1990).

The lack of a lethal response in the acute tests with Microtox (15 minutes) and *Daphnia* (48 h) is not surprising, since Giesy *et al.* (1988a) have shown that most of the sediments analyzed in the Detroit river could not be classified as hazardous by these same assays.

Chronic toxicity test data must be analyzed with caution due to the number of neonates that appeared in the control, slightly lower than the recommendations of OCDE (1983). However, survival results and the number of neonates are still of interest. Sediment interstitial water causes lethal effects in the population of *Daphnia* in long-term tests and its effect is more marked in the second generation. On the other hand, the addition of interstitial water causes in the first generation an increment in the number of neonates. This effect has been described by Hoke *et al.* (1990) working with elutriates of sediments from Lake Erie (North America). The production of ephippia in the second generation, however, demonstrates that although asexual reproduction could be enhanced by the presence of pollutants, sexual reproduction is sharply reduced and even completely inhibited.

Fig.4

Fig.5

Fig.4- Parthenogenetic production of neonates in the first generation of *Daphnia magna* exposed to a series of dilutions of interstitial water of the Butrón river. Means and SD are shown.

Fig. 5.- Production of ephippia in the second generation of *Daphnia magna* exposed to a series of dilutions of interstitial water of the Butrón river. Means and SD are shown.

CONCLUSIONS

1. Extraction of interstitial water by the Karáman method is simple and inexpensive, but it must still be tested with the centrifugation method to measure its effectiveness and adequacy for bioassays.
2. Contaminants in the sediment interstitial water of Butrón river (Munguía) affect survival of populations of *Daphnia magna* in long-term toxicity tests.
3. Parthenogenetic reproduction is favoured in the first generation by the presence of contaminants in the interstitial water.
4. Production of ephippia is diminished by the presence of contaminants, reaching its complete inhibition.

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