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Environmental Contamination and Toxicology

Behavioral Bioassay with a Local Tadpole (*Pleuroderma cinereum*) from River Rocha, Bolivia, in River Water Spiked with Chromium^{6+}

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The River Rocha has its catchment in the Andean Cordillera surrounding Cochabamba (Bolivia), and by the time it has passed the town it is heavily polluted by more than 100 small industries, *in situ* car washing and untreated domestic waste water (Goitia and Maldonado 1992; Maldonado et al. 1998) and its river banks are used for solid waste disposal (Romero et al. 1998). As indicated by Romero et al. (1998), low water quality downstream of the town seriously restricts the use of the river water for irrigation, cleaning and other purposes. Recent studies by Bervoets et al. (1998) and Solis et al. (1998) concluded that chromium in the downstream reaches of the river originated from Cochabamba, which has tanneries, that typically generate waste water with chromium (Maldonado et al. 1998). The question arose as to which organism should be used in bioassays with river water for a first evaluation of its potential toxicity. No culture of standard organisms existed and unpolluted river sites with potential test organisms (1) were practically non-existent in the vicinity of the town, (2) when far from town, were either inaccessible, or structurally and biologically not representative and provided too low numbers of test organisms. Therefore, we tested a locally abundant tadpole (*Pleuroderma cinereum*) of an intermediary polluted river site, upstream of town. The tadpoles were exposed to distinct concentrations of Cr^{6+} for 48 hours spiked in own river water. Their behavior was measured at different time intervals with the "Multispecies Freshwater Biomonitor" (MFB) (Gerhardt et al. 1994; 1998; Gerhardt 1999; 2000), an automated instrument which quantitatively records different types of movements of aquatic organisms, of different species and size.

MATERIALS AND METHODS

A site on the River Rocha was chozen at "Maylanco" a few km upstream of the town of Cochabamba (alt. 2500 m) to provide locally abundant animals for the laboratory experiment. In order to rapidly assess water and sediment quality of that site, a surber sample was taken in different microhabitats for collecting macroinvertebrates for the calculation of the Belgian Biotic Index (De Pauw and Vannevel 1991) and fourty 4th instar larvae of *Chironomus spp.* sp. were screened for cephalic deformities as indicators of heavy metals and/or organic

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toxicants (Janssens de Bisthoven et al. 1998). The site was very species-poor, harbouring Chironomidae, Corixidae, small fish and tadpoles, and resulting in a BBI=3 (bad water quality). About 7 % of the *Chironomus spp.* larvae were deformed, classifying the sediments as "weak to moderately polluted" (Janssens de Bisthoven et al. 1998; Janssens de Bisthoven 1999). The water temperature was 17 °C, the pH= 7.27, oxygen=6.07 mg/l, 86.4%, conductivity=151 µS/cm, S=5.62 mg/l, Mn=0.066 mg/l, Fe=0.036 mg/l, Cr<0.1 µg/l and sediment Cr=27 µg/g. Downstream, towards the town centre, the water quality further deteriorated with decreasing oxygen, increasing conductivity and increasing Cr-content in the sediments (*unpubl. data*).

The locally abundant tadpoles of *Pleuroderma cinereum* were considered for the behavioral experiment. Tadpoles (N = 120) were exposed for 48 hours in triplicate treatments of 2 litre plastic containers with 10 individuals each. The treatments were a control with river water, and river water spiked with 1, 5 and 10 ppm of $K_2Cr_2O_7$ ($K^+ + Cr_2O_7^{-2}$). Analysis of the water (AAS, *Hamel, UMSS, pers. comm.*) confirmed the nominal concentrations. The temperature was 22 ± 1 °C and the pH varied between 7.06 and 7.75.

The Multispecies Freshwater Biomonitor (MFB) is an online instrument which records behavioral activity, meaning any detectable movement, be it tail movements of stationary tadpole, swimming movements, or head and mouth movements. The animals' movements in the chambers are recorded by quadropole impedance conversion, and sent to a laptop with Windows NT4.0 and the software package "MFB 2.1" (Gerhardt et al. 1994; 1998). Since a small easily transportable MFB with 8 channels was available, we measured 8 animals at a time.

Eight animals were collected randomly from the 30 animals present in the triplicate treatments at the beginning (only the control), at 24 h and at 48 h of exposure. The animals were placed in 8 individual MFB-measurement chambers (cylindrical, diameter = 2 cm , length = 3 cm, lids capped with nets of 1 mm mesh size), submerged in the respective Cr-spiked conditions. After an acclimation time of at least 10 minutes, the individual behavioral activities of the 8 animals per concentration and time of exposure were recorded during 240 s. After the recording the tadpoles were set back in their respective tanks, meaning that the recordings at 0, 24 and 48 h of exposure concern different randomly chosen animals. The activity signals obtained were automatically converted by a discrete Fast Fourier Transformation (FFT) into classes of frequencies of movement (from 0.5 to 8.5 Hz with increments of 0.5 Hz) (Gerhardt et al. 1998; Gerhardt 1999; 2000), e.g. in graph B3 of Fig. 1 the tadpoles moved with a frequency of 1 Hz during 35 % of the recording time of 240 s. Complex swimming movements may be composed of several frequencies (e.g. 0.5, 1.0 and 1.5 Hz), while a regular beating of the tail will rather be monofrequent (including only one frequency, e.g. 3 Hz).

Table 1. Percentage of MFB-recording time during which Cr^{6+}-exposed tadpoles were active (mean ±Std. Dev., n = 6-8).

Water	0 h	24 h	48 h
LOCOMOTION			
Control	24±1.1	29.2±9.8	29.2±8.9
1 ppm Cr^{6+}	-	32.0±13.0	24.0±12.0
5 ppm Cr^{6+}	-	22.6±14.1	26.0±7.2
10 ppm Cr^{6+}	-	21.3±14.8	24.6±9.5
VENTILATION			
Control	6.8±3	4.2±1.5	4.2±1.5
1 ppm Cr^{6+}	-	3.3±5.2	3.6+3.4
5 ppm Cr^{6+}	-	5.1±7.2	18.4±15.7*
10 ppm Cr^{6+}	-	10.4±9.7	11.4±8.6*

* High ventilation was significant in half of the animals (see text).

The FFT of an individual represents a summary of its behavior for 240 s. The slow movements were summarised by the MFB-software in band 1, which represents the summation of all frequencies <2.5 Hz. These movements include all swimming behaviors. The fast movements were summarised in band 2, representing the summation of all frequencies > 2.5 Hz. These movements are fast ventilating movements of the tail or mouth movements and often have a regular and monofrequent pattern. Swimming and ventilation are not mutually exclusive. After testing violations of ANOVA assumptions, the frequency data were arcsine $x^{1/2}$ – transformed and analysed with one-way MANOVA (factor: concentration, dependent variables band 1 and band 2) and post-hoc Tukey test differences were judged significant at $p<0.05$.

RESULTS AND DISCUSSION

Mortality after 24 hours was 0 % except in 10 ppm Cr (3.3 % ± 4.7 SD). After 48 h, the mortality was 10 % ± 14 SD in unspiked river water, 10 % ± 8.2 in 10 ppm Cr and 0 % in the other conditions. No dose-dependency in mortality was found (Chi-square test, p>0.05) and we can conclude that the tadpoles were exposed to sublethal ranges of Cr.

Some typical behavioral signals of *P. cinereum* are illustrated in Fig. 1. The locomotory activity of the tadpoles remained similar to the control in all treatments at all recording times (Table 1). Ventilation during the first 24 hours of exposure remained similar to the control in all the chromium treatments as well (Table 1).

After 48 h of exposure, also the ventilation of tadpoles exposed to 1, 5 and 10 ppm Cr did not differ from the control.

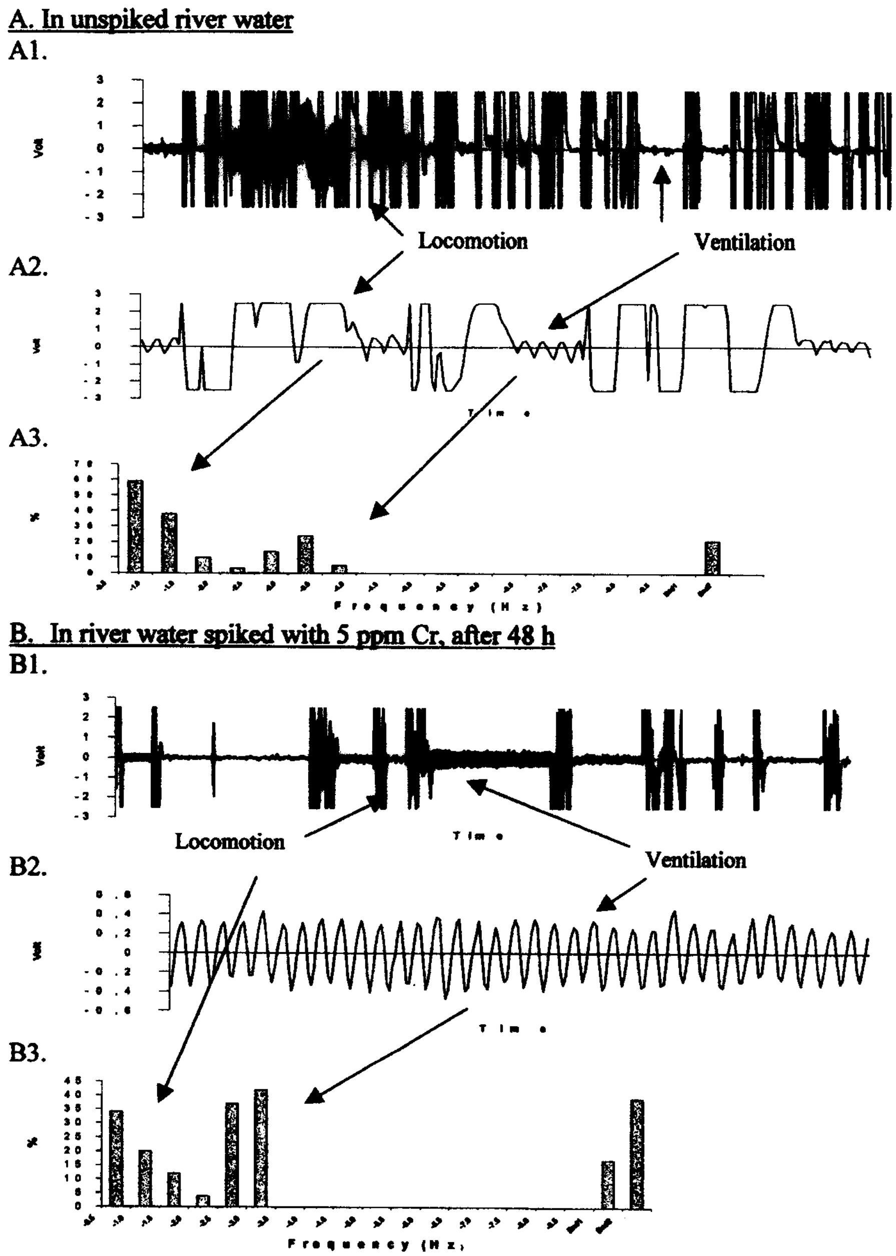

Figure 1. Behavioral signals of *Pleurodema cinereum* tadpoles generated by the Multispecies Freshwater Biomonitor. A, unstressed tadpole from unspiked river water, mostly locomotion with few ventilation; B, stressed tadpole from 5 ppm Cr after 48 h exposure with ventilation. A1., original signals during 240 s, A2. original signals during 12 s (zoomed from A1), A3, Fast Fourier Transformation diagram (FFT) of the data in A1. The same appplies for B1., B2. and B3.

However, the 5 and 10 ppm Cr treatments generated bimodal behavioral ventilation data, because the data contained two groups of tadpoles: an active group and a passive one. Among about 50 % of the organisms exposed to 5 ppm Cr (ventilation 30.8 % ± 12.7) and 40 % of organisms exposed to 10 ppm Cr (ventilation 17.3 % ± 6.2) ventilatory activity was significantly longer than in the control by a factor 5 to 6 ($F(9, 43) = 13$, $p < 0.001$). The other more passive animals were not different from the control and ventilated for significantly shorter times than the active animals (5 ppm Cr: 6.0 ± 2.9 and 10 ppm Cr: 3.7 ± 2.5, $F(3, 11) = 9.9$, $p = 0.002$).

In a previous study by Romero et al. (1998), biological oxygen demand was relatively low at Maylanco especially in the dry season between May and October. Sites in and around the town centre are known to be polluted (more than 14 % deformed *Chironomus spp.* larvae, Trappeniers et al. 1997). In the present study, Maylanco had a very impoverished fauna, while the sediments were characterised with chironomid deformities as moderately polluted (Janssens de Bisthoven 1999). The concentrations in the present study (up to 10 mg/l Cr) were representative of sublethal Cr^{6+}- toxicity to freshwater invertebrates and vertebrates: chromium LC_{50} values (96-h when not indicated otherwise) range from less than 1.8 mg/l (48-h) for *Daphnia* (Fargasova 1994), to 1.4 mg/l for *Gammarus fossarum*, 3.9 mg/l for *Heptagenia sulfurea*, 9.5 mg/l for *Physa fontinalis*, 15.3 mg/l for *Asellus aquaticus*, 30.4 mg/l for *Hydropsyche pellucidula*, and between 33 and 69 mg/l for freshwater fish (Canivet et al. 2001). The obtained survivals showed that the tadpoles were relatively resistant to $K_2Cr_2O_7$. This is not surprising, given the fact that this indigenous test organism had adapted to a relatively polluted environment. Stress of chromium on amphibians is reported as part of a pollutant mixture in coal ash, provoking oral deformities in tadpoles (e.g. Rowe et al. 1996). Behavioral measurement of *Rana temporaria* tadpoles with the MFB in unpolluted water has been described in Gerhardt et al. (1994), showing a typical pattern of locomotion (<2 Hz, irregular multifrequent slow movements of tail and body) and ventilation (=>2 Hz, regular monofrequent fast movements of mouth and tail). The swimming behavior of adult frogs has recently been analysed and described with video technique by Nauwelaerts and Aerts (2002). Exposure of larval salamanders to low pH resulted in hypoactivity and was proposed as an activity test (Kutka 1994). Hypoactivity was also observed in goldfish exposed to sublethal concentrations of nickel (Ellgaard et al. 1995). Amphibia tend to be underrepresented in ecotoxicological studies, although their worldwide decline is cause for concern (Semlitsch et al 1995). Semlitsch et al (1995) demonstrated that 5 µg/l triphenyltin chloride (a fungicide) can cause a decrease in swimming activity, and hence in feeding (both are associated) of *Rana esculenta* tadpole.

Variance in the ventilatory response increased after 48 h exposure to 5 and 10 ppm Cr, as about half of the organisms increased ventilatory activity, thus not only magnitude of response but increase of variation in response could be important indicators of the sublethal toxicity of Cr. The fact that the response to

Cr was stronger in 5 ppm than in 10 ppm Cr could be indicative of a non-linear dose-response relationship, but needs further confirmation. Also is not clear why hypoactivity was not observed like in other studies with amphibians and fish (Kutka 1994; Ellgaard 1995). A possible explanation is that the used Cr-concentrations were too low for the pre-adapted local tadpoles. Variance of biological response has earlier been reported as a possible response parameter to stress (Odum et al. 1979; Cairns 1986). The resulting increased means and variances of ventilation could be used in surveillance by online biomonitoring with tadpoles.

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