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Fluid Motion Effect on Metal Toxicity in *Bufo arenarum* Embryos

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Toxicity tests conducted under laboratory standard conditions frequently fail to consider key elements in the environment of test organisms like Fluid Motion (FM) which seems to be an important physical factor affecting nutrient uptake (Reynolds, 1992; Karp-Boss et al., 1996), ventilatory functions (Phillipson, 1954), energy and metabolism (Osborn, 1996) of biological organisms. For example, in the case of rotifers it has been demonstrated that FM could increase the toxicity of pentachlorophenol by almost one order of magnitude respective to static conditions (Preston et al., 2001).

Agricultural and industrial activities cause heavy metal pollution of the aquatic environment and metals are among the chemicals with most adverse effects on biota. In fact, beside oxidative stress, carcinogenic, mutagenic and teratogenic effects, they are related to reproductive impairment (Rivera et al., 1990), ionoregulatory failure (Vijverberg et al., 1994), binding to chromatin DNA (De Boni et al., 1974) and inhibition of enzymatic activities (O´Dell, 1990). These effects seem to be produced by means of establishing strong bindings with sulfhydril groups of proteins and bases of nucleic acids, and a high competition with other divalent cations with physiological functions (Peraza et al., 1998).

Zinc and copper are essential trace elements to all living systems, crucial for many biochemical cell activities. However, they could also be potentially very toxic, as for instance Cu^{2+} which exerts adverse effects even at slightly higher than physiological concentrations (Herkovits and Helguero, 1998). Nickel is the most common transition metal and although it is not considered a broadscale contaminant, certain ecological changes such as a decreased in the number and diversity of species has been observed near Ni-emiting sources (Papachristou et al., 1993). Its toxicity profile (TOP) *in Bufo arenarum* embryos shows a conspicuous increase in toxicity within the initial 96 hr of exposure (Herkovits et al., 2000).

Zinc has a low effect on *Bufo arenarum* embryos, at least during the last developmental stages, compared with results obtained in other aquatic organisms like daphnias and fishes (Suter, 1996). Moreover a beneficial effect of zinc (as $ZnSO_4$) against malformations and/or delayed development and mortality exerted

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by cadmium (Herkovits and Pérez-Coll, 1990), and lethality produced by copper (Herkovits and Helguero, 1998), lead (Herkovits et al., 1996a), aluminum (Herkovits et al., 1997) and mercury has been informed in amphibians. In the case of nickel-zinc interaction, a synergistic or antagonistic effect could be observed depending on its concentration in the maintaining media (Herkovits et al., 2000).

Amphibian embryos, due to their high sensitivity to environmental pollutants (Hall and Henry, 1992), are useful indicators of freshwater contamination (Herkovits et al., 1996b) and therefore they are considered appropriate for ecotoxicological studies and for assessing water quality for wildlife protection purposes (Boyer and Grue, 1995). On the other hand, it has been established that there is a good predictive correlation between embryo-larval tests for measuring in stream toxicity and estimating chronic effects on aquatic biota (US EPA, 1991). The main purpose of this study is to report the effect of FM on the toxicity of copper, nickel and zinc *in Bufo arenarum* embryos. The results are considered in terms of the eventual relevance of FM in toxicity studies conducted in laboratory vs field conditions.

MATERIALS AND METHODS

Ovulation of *Bufo arenarum* females was induced by injecting a suspension of homologous hypophysis. The oocytes were fertilized in vitro with a sperm suspension in AMPHITOX solution (AS) (Herkovits et al., 2001). The embryos obtained were maintained in HS at 20 ± 2 °C until developmental stage 25 (complete operculum) (Del Conte and Sirilin, 1951). The concentrations of Ni, Cu and Zn selected for the FM experiments were based on preliminary acute toxicity data of these metals, reported in this study as LC10, LC50 and LC90 for 24 hr of exposure. The experimental groups were performed by means of triplicate batches of 10 embryos placed in 10 cm glass petri dishes containing 40 ml of HS, which exposed to a range of metal concentrations as follows: $NiCl_2$: 20-25 mg Ni^{2+}/L; $CuCl_2$: 0.05-0.15 mg Cu^{2+}/L and $ZnCl_2$: 20-40 mg Zn^{2+}/L under static and FM conditions. Nickel, copper and zinc experimental solutions were prepared from a.a.s. standard solutions: 1 g Cu^{2+}/L (Riedel-de Haën), 1g Ni^{2+}/L (Riedel-de Haën) and 1 g Zn^{2+}/L (Merck) in AS. FM was produced by means of a shaker (Lab-Line, Junior Orbit Shaker) with a speed of 34 cycle/min on a single axis at a constant frequency. Control embryos, with and without motion, were maintained in HS solution. The results were evaluated after 24 hr of exposure as mortality of control and experimental embryos. No food was provided during the toxicity test. For statistical analysis PROBIT and ANOVA (Bonferoni test) at 0.05 level was employed (SPSS, 1998)

RESULTS AND DISCUSSION

Figure 1 shows data on Cu^{2+}, Zn^{2+} and Ni^{2+} toxicity on *Bufo arenarum* embryos after 24 hr of exposure. As expected, Cu is almost two orders of magnitude more toxic than Zn and Ni but it is noteworthy that for this last metal the toxicity increases very sharply within the following 72 hr of exposure (Herkovits et al., 2000). In the case of Zn, the $ZnCl_2$ salt employed, exerted a significantly higher

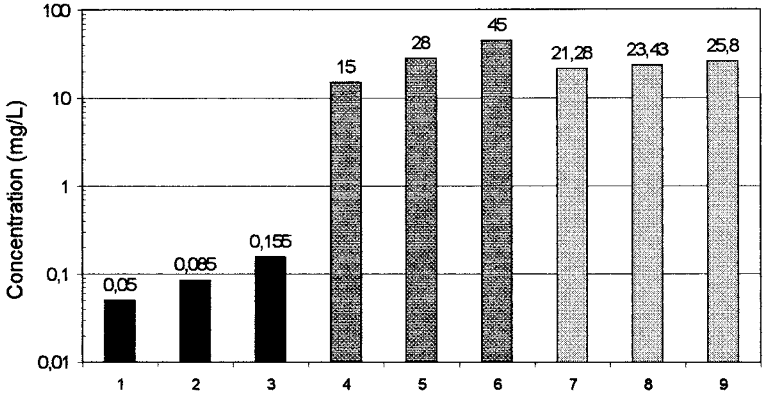

Figure 1. Lethality exerted by Cu, Zn and Ni on *Bufo arenarum* embryos at 24hr of exposure.
Cu: 1) LC 10, 2) LC 50, 3) LC 90; Zn: 4) LC 10, 5) LC 50, 6) LC 90; Ni: 7) LC 10, 8) LC 50, 9) LC 90

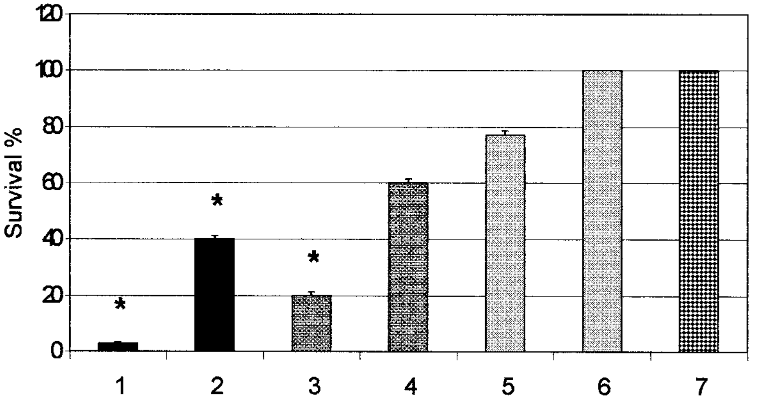

Figure 2. Fluid motion effect on Cu, Zn and Ni toxicity in *Bufo arenarum* embryos at 24 hr of exposure.
Cu: 1) 0.1 FM, 2) 0.1 SC; Zn: 3) 30 FM, 4) 30 SC; Ni: 5) 20 FM, 6) 20 SC (mg/L) 7) Control. FM: Fluid Motion SC: Static Condition * Significant difference at the 0.05 level.

toxicity on *Bufo arenarum* embryos than Zn provided as $ZnSO_4$ (Herkovits et al., 1999).

The most significant results of fluid motion on metal toxicity in *Bufo arenarum* embryos are summarized in Figure 2. As a general pattern, FM conditions did exert an additional adverse effect on the toxicity of the heavy metals employed during this study. The increase in lethality was 50%, 40% and 20% for Zn, Cu and Ni respectively. These results contrast very sharply with the conspicuous increase in toxicity, over one order of magnitude, obtained by means of FM in rotifers employing pentachlorophenol as toxic substance (Preston et al., 2001). For this organisms it has been proposed three hypotheses to explain the significant increase of the toxicity by means of FM: i) an increase in metabolic rates, altering the energetic and metabolic efficiency; ii) an inhibition of rotifers feeding behavior (Miquelis et al., 1998) and iii) an increased uptake of toxicants (Preston et al., 2001). In the case of amphibians embryos, an alteration in energetic and metabolic efficiency and/or an increase in the uptake of the toxic substances, seems to be the more likely mechanisms for the enhanced toxicity obtained by means of FM. In fact, it is expected that under this experimental condition more molecules of the metals could contact the epithelial cells of the embryo thus facilitating the uptake processes.

The very significant difference in the toxicity increases by means of FM between rotifers (Preston et al., 2001) and present data obtained with amphibian embryos, seems to support the assumption that FM could have a more significant implication for low Reynolds number aquatic organisms from phytoplankton to zooplankton species. Therefore, FM could be an important factor specially for toxicity exerted by chemical substances on these aquatic species, a fact which seems to be meaningful to take into account for Ecological Risk Assessment.

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