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Toxicity and bioaccumulation of fluoride ion on *Branchiura sowerbyi,* Beddard, (Oligochaeta, Tubificidae)

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Abstract

Fluoride concentrations are increasing significantly in many aquatic ecosystems as a consequence of human activities (agrochemicals, pharmaceuticals, refrigerants, pesticides, surfactant compounds). Several investigations have revealed that sensitivity to fluorides and safe concentrations vary greatly within classes, families and genera. Aquatic oligochaetes have often been used for pollution assessment and accumulation testing, but no information has been given about tolerance to fluoride ion. Among endobenthic tubificids *Branchiura sowerbyi* is easily identifiable (evident posterior gills, large size) and particularly useful for tissue requirements in chemical analysis. The purpose of this study was to examine the tolerance of this tubificid to fluoride ion and its bioaccumulation capacity by performing short (LC50 96h) and long-term (18 day) experiments at different temperatures (17°C and 22 °C). LC_{50} values (91.3 and 61.7 mg/L for 17°C and 22°C respectively), especially in the presence of sediment (267.6 and 80.1 mg/L for 17°C and 22°C respectively) showed that *B. sowerbyi* is more resistant to fluoride than other freshwater invertebrates. Fluoride became more toxic with increased temperature, demonstrating that seasonal temperature changes could influence the sensitivity of this freshwater tubificid. Bioaccumulation was lower when the organisms were exposed to sodium fluoride in the absence of sediment, indicating that this animal also accumulates fluoride by ingesting sediment.

Key words: *Branchiura sowerbyi*, Fluoride ion toxicity, Bioaccumulation, Temperature influence

Introduction

Natural water fluoride concentrations are mostly dependent on the occurrence of fluoride-bearing minerals in groundwater (Barbier et al. 2010). Fluorides also enter many water bodies via the waste products of anthropogenic activities, such as heavy industry and agriculture. As a result, the fluoride level in many aquatic ecosystems is increasing (Chlubek 2003). The effects of fluoride exposure have been revealed by several studies on many animal and plant phyla: Camargo's review (2003) focused on fluoride toxicity in aquatic organisms, revealing that safe concentrations are significantly different within classes, families, genera and species. The risks for vertebrates are known; most significant are the effects on bone cells that can lead to the development of skeletal fluorosis. However, it is now recognized that fluoride also affects soft tissues cells, such as renal, gonadal, and neurological cells (National Research Council 2006). The main toxic effect of fluoride in cells, involves its interaction with enzymes; it acts as an enzyme inhibitor and interrupts metabolic processes, such as glycolysis and protein synthesis (Kessabi 1984; Casellato et al. 2012). Aquatic invertebrates living in soft waters are more affected by fluoride pollution than those living in seawater, because the bioavailability of fluoride ions (and consequently their toxic action) is reduced with increasing water hardness (Camargo 2003). Various investigators have focused on fluoride toxicity in soft water organisms, but no data have been reported for aquatic oligochaetes, although they have a long history of use in pollution monitoring and in relevant toxicity and bioaccumulation tests (Chapman 2001). In particular, oligochaetes are widely used to assess the toxicity and bioaccumulation of sediment-associated contaminants (Ingersoll et al. 1995). Endobenthic tubificids are particularly appropriate because of their intimate contact with the substrate for their entire life cycle (Reynoldson 1987) and *Branchiura sowerbyi* can be easily

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collected and identified due to their large size and prominent posterior gills (Ducrot et al. 2007). They are widely distributed and have already been used for both toxicity and bioaccumulation tests (Chapman et al. 1982; Casellato & Negrisolo 1989 Casellato et al. 1992; Marchese & Brinkhurst 1996; Bhunia et al. 2000) as they have a low natural mortality rate in the laboratory compared to other tubificids (Bonacina et al. 1994). These worms feed themselves down in the sediment, a behaviour that involves the intake of large amounts of substrate (Wang & Matisoff 1997). Since there are specific tissue requirements for chemical analyses, *B. sowerbyi* is more appropriate for bioaccumulation tests compared to other smaller oligochaete species (Chapman et al. 1980). *B.sowerbyi* dominates other bottom macroinvertebrates in terms of abundance and biomass (Ducrot 2007) and is a significant part of the diet of some fish and crustaceans. The main goal of this research is to provide missing information about the toxic effects of fluoride on *B. sowerbyi* and to investigate the bioaccumulation capacity of this species.

Materials and methods

A rich population of *B. sowerbyi,* living in a lily tank at the Botanic Garden, Padova University, has been monitored for a long time, in order to study its life cycle and the regeneration of the sexual apparatus (Casellato 1984; Casellato et al.1987). The tank is filled with thermal water with temperatures ranging between 17°C and 22°C throughout the year. Original water and sediment (both with a concentration less than 0.1 mg F^-/L) were collected together with animals in order to set up an optimal laboratory culture and test conditions. The worms were gradually acclimatized to the test temperatures in glass aquaria (1L) under controlled oxygen conditions. All the experiments were performed at 17°C and 22°C in order to determine if temperature has an influence on fluoride toxicity and bioaccumulation rate. The aquaria were covered to prevent fluoride evaporation. Daily observations of mortality and differences in the animals' behavior and morphology were made. Worms were considered dead when there was no response to physical stimulation; dead individuals were removed and kept in 4% formaldehyde for accumulation analysis. At the end of the experiments, surviving animals were also put in formaldehyde and analyzed. Fluoride accumulation in *B. sowerbyi* soft tissues (dried at 80 °C for 24 h) and in the sediment was evaluated using the alkali fusion method, according to Malde et al. (2001) and McQuacker & Gurney (1977), respectively. Analyses were conducted at pH 5.5 after adding a total ionic strength adjustment buffer (TISAB III) and using an ion selective electrode ISE F800 (0.02 mg F^-/L to saturation, accuracy 0.1%, WTW).

Statistical analysis

An analysis of variance (ANOVA) was carried out to determine differences in bioaccumulation values (Software R ver. 2.15.0). Data relationships were assessed using simple linear regression. The daily mortality percentage was used to estimate LC_{50} values at the 95% confidence level, using multifactor probit analysis (MPA; SAS software ver.9.1.3) (Lee 1991). Concentrations between the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) values, were noted and used to set up long term experiments.

Short-term experiments

Acute toxicity bioassays were performed at 17±0.5°C and 22±0.5°C, for 96 h. For each temperature 15 individuals (three replicates of five worms) were exposed to nominal sodium fluoride concentrations of 80, 160, 320 and 640 mg F^-/L, vs. control (tank water with no added fluoride), either with 125g of original sediment or without sediment.

Long-term experiment

Long term tests were carried out both at 17±0.5°C and 22±0.5°C, for a period of 18 days. All animals were exposed to nominal fluoride concentrations of 80, 120 and 160 mg F^-/L (between NOEC and LOEC) in the presence of sediment. Not only were mortality and behaviour checked in these experiments, but any decrease in water fluoride concentration was also noted. Each week, 10 ml of water were taken from each aquarium and combined with 10 ml of TISAB III for ion-selective electrode analysis. At the end of the experiment, fluoride content analysis was measured for both *B. sowerbyi* (as total body) and the sediment.

Results

Short-term experiments

B. sowerbyi demonstrated great tolerance to fluoride ion toxicity, especially at 17°C. The mortality of *B. sowerbyi* was reduced in the presence of sediment: LC_{50} at 17°C was 91.3 mg F^-/L for animals in water without sediment, and 267.6 mg F^-/L when sediment was added. The same effect of presence of sediment was observed at 22°C (Table 1). The NOEC and LOEC for this species were 80 and 160 mg F^-/L respectively, when sediment was present. Animals treated at 22°C accumulated greater amounts of fluoride in their bodies than did those treated at 17°C. Sediment enhanced bioaccumulation values: at 22°C, animals exposed to 640 mg F^-/L had 1400.9 µg F^-/g when kept in sediment (Fig.1A), while those not kept in sediment had only 790 µg F^-/g (Fig.1B). The accumulation rate increased with increasing fluoride concentration ($p<0.001$, ANOVA). The animals treated with sediment accumulated double the amount of fluoride.

TABLE 1: LC50 values at 96 hours for *B. sowerbyi* at different temperature; 95% confidence limits are in parentheses.

Temperature°C	17 ± 0.5	22 ± 0.5	17 ± 0.5	22 ± 0.5
Sediment	yes	yes	no	no
LC_{50} (mg F^-/L) 96 h	267.63 (257.75–277.51)	80.07 (62.10–111.55)	91.28 (84.5–98.05)	61.68 (47.83–90.11)

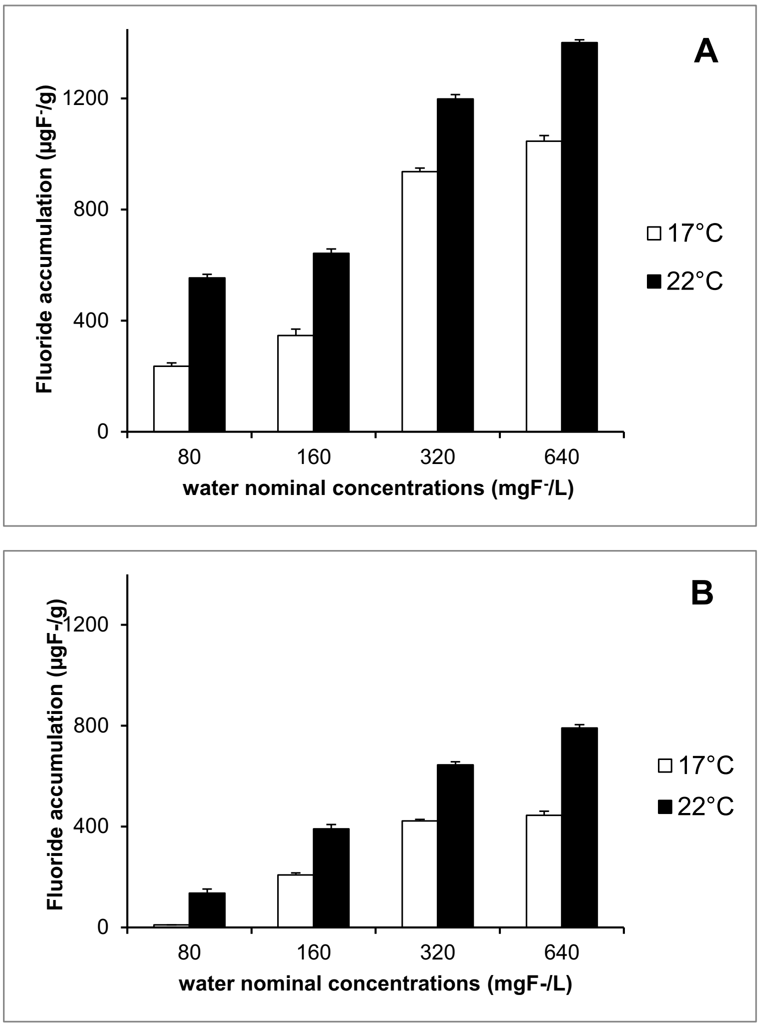

FIGURE 1: Fluoride accumulation in *B. sowerbyi* during short term experiments (96h) (A) With sediment (B) Without sediment.

Long-term experiment

The influence of temperature on fluoride toxicity in *B. sowerbyi* was evident in the long term mortality tests: 6.67% mortality was found for all concentrations tested at 17°C, whereas mortality was higher when the test population was exposed at 22°C and increased ($r^2 = 0.97$) with increasing fluoride concentration (Fig. 2). At 22°C mortality increased by 1.2% for every 10 mg F^-/L added ($r = 0.97$) and more than half the population died when exposed to the highest concentration (160 mg F^-/L) (Fig. 2).

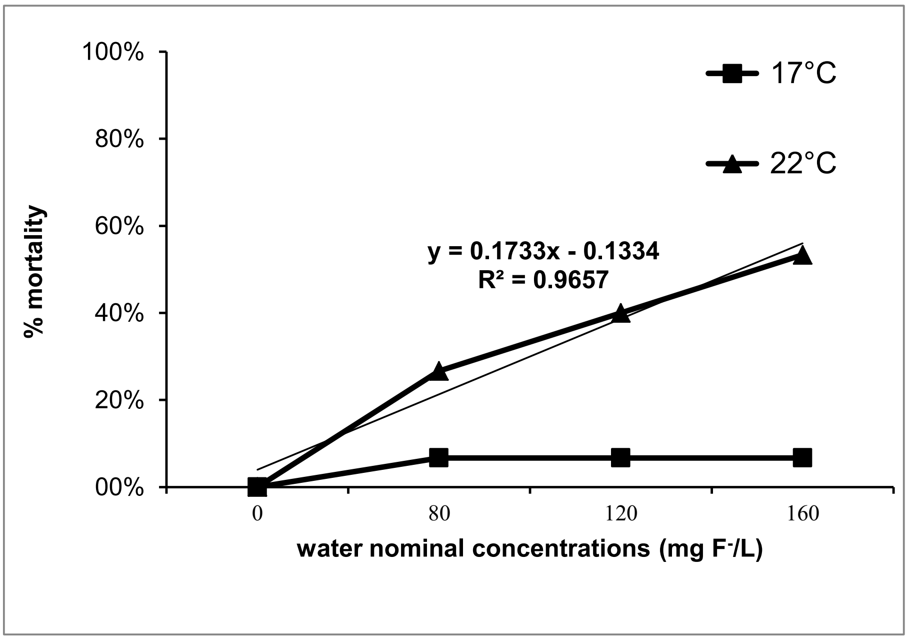

FIGURE 2: Mortality of *B. sowerbyi* during the long term experiments (18 days).

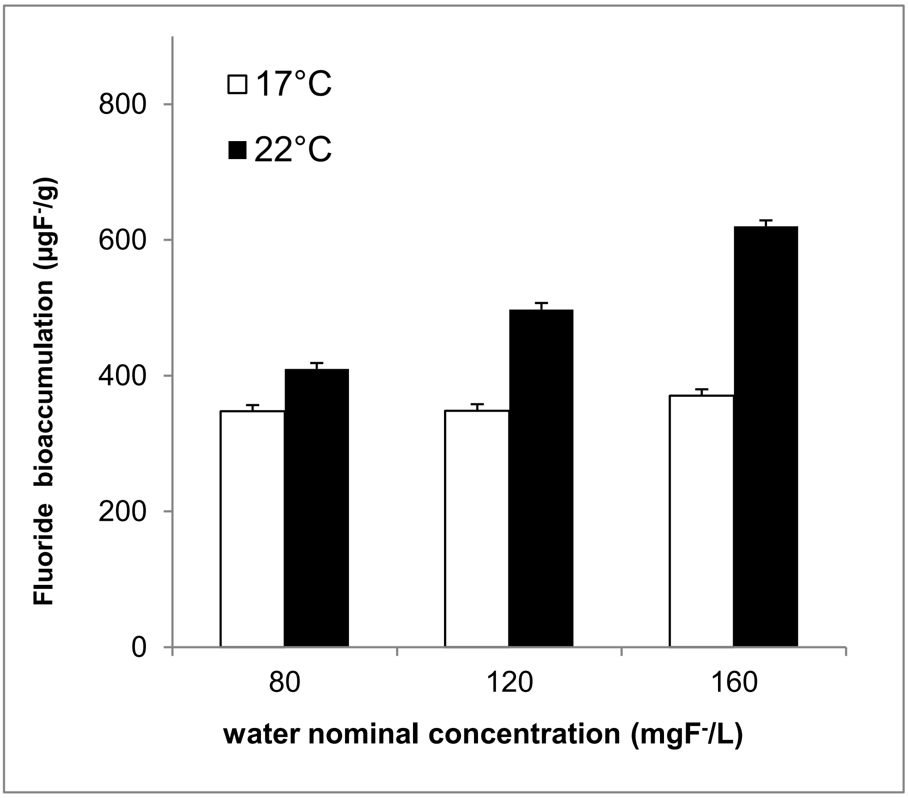

FIGURE 3: Fluoride accumulation in *B. sowerbyi* during the long term experiments (18 days).

Fluoride bioaccumulation showed a statistically significant relationship ($p<0.001$) with increasing test concentration. The highest values were reached by test populations at 22°C (Fig.3); the exposure of *B. sowerbyi* to 160 mg F^-/L resulted in accumulation at 22°C 40.25% higher than at 17°C. Sediment accumulation was directly proportional to test concentration ($p<0.01$), with peak values of 722 and 815 µg F^-/g, for experiments at 17°C and 22°C respectively (Fig.4). Fluoride ion was rapidly absorbed by animals and sediment, as shown by declining fluoride concentrations in water: the highest percentage decrease (26%) was reached in 6 d at 22°C from the initial nominal concentrations (Fig.5).

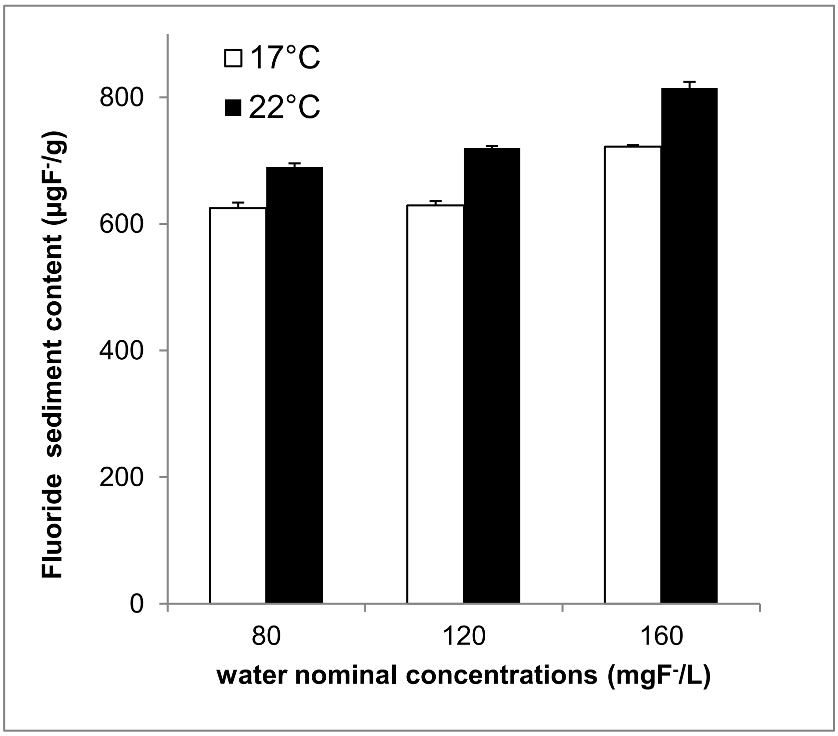

FIGURE 4: Fluoride content in sediment after the long term experiment (18 days).

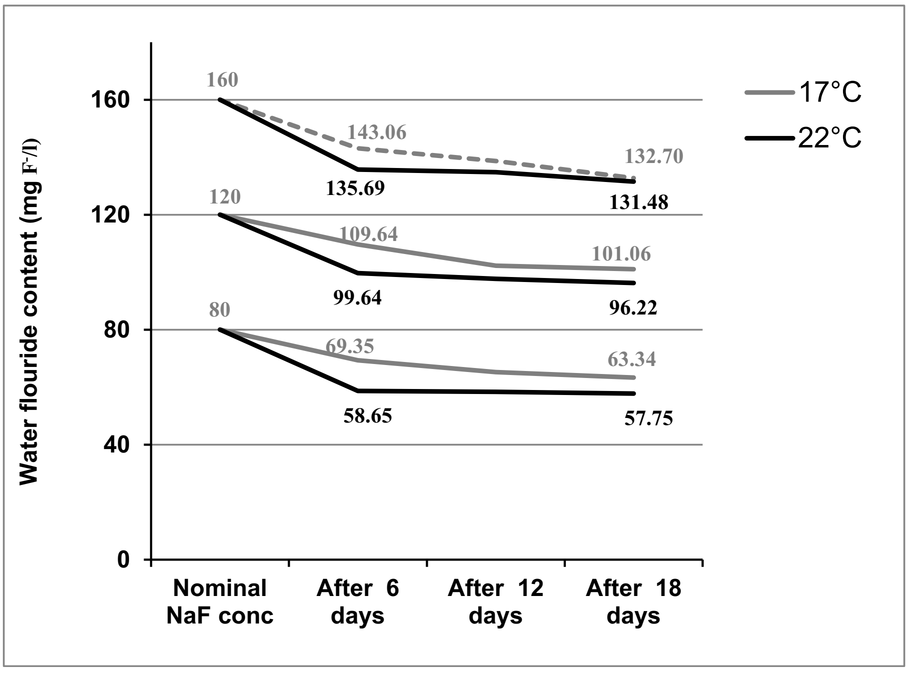

FIGURE 5: Decreasing concentration of fluoride in water during the long term experiment (18 days).

Discussion

This research clearly indicated that *B. sowerbyi* is very resistant to fluoride ion dissolved in water, especially in presence of sediment, much more than other aquatic invertebrates. Gonzalo et al. (2010) reported a much lower LC50 value (5.8 mg/L at 17°C) for the gammarid *Dikerogammarus villosus*. Camargo (2003) summarized many literature results regarding fluoride toxicity in freshwater invertebrates, showing that the mean 96h LC_{50} ranges from 10.5 mg F^{-}/L for some species of crustaceans to 45 mg F^{-}/L for the most resistant species of caddisfly (*Chimarra marginata*). *Branchiura sowerbyi* survival is two times higher compared to various species of *Hydropsyche* (Janice et al. 2003) and showed a 96h LC_{50} value close to the 24 h LC_{50} value of *Daphnia magna*, which is considered one of the less sensitive invertebrate species (Dave 1984). In addition, our results indicate a temperature –dependent effect: fluoride toxicity increases with increasing temperature, probably because the temperature increases the rate of metabolism, resulting in faster fluoride absorption and faster poisoning, as reported by Angelovic et al. (1961).

A similar effect was recently described for the mollusc *Dreissena polymorpha* (Del Piero et al. 2012).

A further consideration with respect to the presence or absence of sediment in the bioassays is that the resistance of *B. sowerbyi* during short term exposure was enhanced by the presence of sediment, probably because this presence prevents an additional stress for the tubificid worm, which is a limicolous species, feeding on sediment. In his "*ad libitum*" feeding test involving *B. sowerbyi*, Ducrot (2007) found that part of the total amount of the food ingested by this worm is the sediment itself. Moreover, Casellato & Negrisolo (1989), Chapman et al. (1982) and Naqui (1973), using various toxicity bioassays, demonstrated that sediment reduces the bioavailability of the contaminants, limiting the toxic effects on organisms exposed to pollutants dissolved in water. But this is true for *B. sowerbyi* only in the short term bioassays. When the duration of the test was extended to 18 days, the prolonged feeding on the contaminated sediment increased fluoride bioaccumulation, particularly at 22°C (Fig.3). Lee et al (2000); Volparil & Mayer (2000); Weston et al (2000) suggested that the contaminated sediments ingested by deposit-feeding animals should be considered the major source of absorbed contaminants.

Thus, fluoride absorption through ingestion could explain the difference in the bioaccumulation values between the performed bioassays with and without sediment, both at 17°C and 22°C (Fig1).

Finally, as far as we know, no study has been previously conducted to examine fluoride bioaccumulation in deposit-feeding freshwater invertebrates at different times and exposures. Our research on *B. sowerbyi*, an important component in terms of abundance and biomass of the diet of some fish and crustaceans, is not exhaustive but can represent a first contribute to evaluate properly capacity of an invertebrate species to take up and retain fluoride ions from the environment.

Acknowledgements

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