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# Effects of temperature and oxygen concentration in sediment toxicity testing ☆

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**Abstract**

Joint effects of temperature and oxygen concentrations for the results of sediment toxicity tests were studied at 10 and 20 °C with 40% and 80% dissolved oxygen (DO) saturation. Growth, feeding rate, and reproduction of *Lumbriculus variegatus* (Oligochaete) and growth, emergence, and survival of *Chironomus riparius* (Diptera) were tested in a polluted and in a reference sediment. Both the feeding of *L. variegatus* and the emergence of *C. riparius* were significantly retarded at low temperature. Additionally, differences in the sex ratio of the emerged adults of *C. riparius* were observed. The oxygen concentration alone did not have any significant effect on the endpoints, but significant combined effects of polluted sediment and low DO were observed on the biomass of *L. variegatus*. The standard sediment toxicity tests might offer only limited data for risk assessment of contaminated sediments at sites where the actual conditions largely differ from the laboratory conditions.
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*Keywords:* *Chironomus*; *Lumbriculus*; Joint effects; Emergence; Feeding rate; Sex ratio; Temperature; $O_2$

## 1. Introduction

Sediment toxicity tests are common approaches to increase weight of evidence in determining whether contamination in any area represented a risk to individual organisms or to the whole ecosystem. Usually, the tests are performed under standardised laboratory conditions to minimise external variation and to insure comparable test results. However, most often the standard test conditions greatly deviate from the natural conditions in aquatic environment. In the standardised sediment toxicity tests with benthic invertebrates, the temperature recommendations range from 20 to 23 °C, and aeration is recommended if oxygen level in the sediment overlying water drops below 2.5 mg/l (ASTM, 2004) or below 60% saturation (OECD, 2004). In the cold temperate region, water temperature seldom reaches 20 °C even in summer (Lowell and Culp, 1999; Holopainen et al., 2002; Hyvärinen and Korhonen, 2003) and the sediment surface can become hypoxic or even anoxic during the stratification periods (Hamburger et al., 1994; Volpers and Neumann, 2005). For the risk assessment of polluted sediments, it would be valuable to know the combined effects of natural stress factors like temperature and oxygen concentration on the responses of organisms to polluted sediments.

Temperature affects poikilothermic animals probably more than any other environmental factor because the animal's inner temperature follows that of the environment. Temperature can affect toxicokinetics (Heinonen et al., 2002; Honkanen and Kukkonen, 2006), the metabolism (Hamburger et al., 1994), and the physiological state (Heugens et al., 2003) of the test animal. Similarly, low dissolved oxygen (DO) conditions in water have been found to change the metabolic activity of organisms (Holopainen and Penttinen, 1993; Hamburger et al., 1994, 1998; Penttinen and Holopainen, 1995; Choi et al., 2000), and affect their growth rate (Canasta and Dodson, 1995) and behaviour (Heinis and Crommentuijn, 1992; Lowell and Culp, 1999; Irving et al., 2004). A few studies

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have examined chemical toxicities at environmentally realistic temperature and oxygen concentrations (Becker, 1987; Lowell and Culp, 1999; Volpers and Neumann, 2005), but studies on the joint effects of low oxygen and low temperature are rare (Hamburger et al., 1998), and no laboratory studies could be found on the multiple effects of these factors on sediment toxicity.

In general, both the low temperature and low oxygen concentration can decrease animal's activity and metabolic rate (Heinis and Crommentuijn, 1992; Heinonen et al., 2002; Landrum et al., 2003). In contaminated environment, a lower metabolic rate may be advantageous since the accumulation of harmful chemicals may be restricted too (Heinonen et al., 2000). However, with lower metabolism also the growth and development can be retarded and for species with short life cycles, like midges, the time spent in the harmful environment increases. In addition, chemicals can reduce the tolerances of organisms to other environmental factors (Canasta and Dodson, 1995; Heugens et al., 2001). Several authors have reported a need for environmentally more realistic toxicity testing and for studies on combined effects of different stress factors, in order to improve the ecological realism in risk assessment of contaminated waters and sediments (Lowell and Culp, 1999; Van der Geest et al., 2002; Coors et al., 2004).

For this study, we selected two benthic invertebrates frequently used as test organisms in whole-sediment bioassays: the midge *Chironomus riparius* (Diptera) and the oligochaete worm *Lumbriculus variegatus* (Oligochaete) (Phipps et al., 1993; Leppänen and Kukkonen, 1998a; ASTM, 2004; OECD, 2004; Ducrot et al., 2005). *C. riparius* is widely distributed in the Northern Hemisphere, most commonly at temperate regions (Hirvenoja and Michailova, 1991; Rossaro, 1991), and is found in eutrophic shallow streams and lakes. This species prefers organically rich sediments and sewage waters (Armitage et al., 1995; De Haas et al., 2005) and is tolerant of toxicants (Stuijfzand et al., 2000; De Haas et al., 2006). *L. variegatus* has adapted to oligotrophic and mesotrophic shallow waters in temperate regions in Eurasia and North America (Laakso, 1967; Dermott and Munawar, 1992). It is a whole-sediment feeder and reproduces asexually by fragmentation. Both species have adapted to environmental conditions in cold temperate regions such as low DO levels and variable temperatures (Bairlein, 1989; Phipps et al., 1993; Penttinen and Holopainen, 1995; Hamburger et al., 1998; Choi et al., 2000).

The aim of this study is to investigate the single and combined effects of temperature and oxygen concentration on sediment toxicity. The survival, biomass, adult emergence, and sex ratio of *C. riparius* and the biomass, feeding (egestion rate), and reproduction of *L. variegatus* were studied in two different sediments. The environmental stress was evaluated by measuring the interactions of temperature, low oxygen concentration, and polluted sediment on the endpoints measured. In addition, differences between the results of standard sediment toxicity tests and those run in environmentally realistic conditions are further discussed. The effects of these factors to *in situ* testing in temperate waters in general are considered.

## 2. Materials and methods

### 2.1. Sediments

Sediments included a highly polluted sediment from a sawmill pool (SP) contaminated with several PAHs ($\sum$299 μg/g dry weight, DW), metals (Cr 300 μg/g DW, Cu 486 μg/g, As 393 μg/g, Cd 12 μg/g, Hg 0.6 μg/g, Pb 69 μg/g DW), and dioxins ($\sum$PCDD/Fs 226 ng/g DW) (Consulting Engineers Paavo Ristola Ltd., 1996; Lyytikäinen et al., 2001) and an unpolluted sediment from the oligotrophic Lake Höytiäinen (LH) (Ristola et al., 1999), both located in Eastern Finland, near the town of Joensuu. SP sediment samples were collected from 3 m depth with an Ekman grab (5–10 cm sample depth) and LH sediment with a pump from a depth of 20 m. Additional water on top of the sediments was removed and the sediments were stored in the dark at $4\pm1$ °C until the test start. Dry weight (105 °C/24 h), organic carbon and nitrogen content (Carlo Erba Elemental Analyser 1105) and the loss of ignition (550 °C/12 h) were analysed. Particle size distribution was determined using sieves with following mesh sizes: 400, 250, 125, 63, and 20 μm.

### 2.2. Bioassays

Both test species originated from laboratory cultures kept at $20\pm2$ °C in artificial fresh water (61.625 g $MgSO_4\cdot7H_2O$, 147 g $CaCl_2\cdot2H_2O$, 2.875 g KCl, 32.375 g $NaHCO_3$) and at the light:dark period of 16:8 h in the Laboratory of Aquatic Ecotoxicology, University of Joensuu. The experimental design was constructed to follow a $2\times2$ factorial set-up of two temperatures (high and low temperature, HT and LT) and two oxygen concentrations (high and low DO concentration, HO and LO) resulting in four different treatment combinations (HT–HO: 20 °C, >80% $O_2$ saturation; HT–LO: 20 °C, <40% $O_2$ saturation; LT–HO: 10 °C, >80% $O_2$ saturation; LT–LO: 10 °C, <40% $O_2$ saturation). At the treatment HT—HO, the conditions correspond to a standard laboratory test defined by ASTM (2004), in which aeration is recommended if oxygen level in the sediment overlying water drops below 2.5 mg/l. Conditions in the treatment LT–LO corresponds to the average conditions in natural waters at the sediment surface in cold climate regions during spring and autumn. Overlying water in the HO treatments was aerated throughout the experiment. The low oxygen concentration in the LO treatments was created with a controlled flow of $N_2$ into the water throughout the experiment. The $N_2$ flow and aeration were started when the animals had burrowed into the sediment. The intended low oxygen saturation was reached within the first hours from the test start. The test beakers for low temperature were placed in a climate chamber ($10\pm1$ °C). Water was added after evaporation every second day in the 10-day growth test and emergence test with *C. riparius* and in the 10-day test with *L. variegatus*. In the 28-day test with *L. variegatus*, water was renewed almost completely on the days when the faecal pellets were collected (every second or third day). For the low oxygen treatment the added water was first treated with $N_2$ flow until the intended oxygen concentration was reached in the water. Water temperature and oxygen concentration were measured daily ($N=4$) during the test, and pH in the beginning and end of the tests ($N=4$) (WTW Multiline P4-meter, Germany).

### 2.3. Chironomus riparius

Two tests (modified from ASTM standards E1706-00) with *C. riparius* were performed: a 10-day test (weight and survival) ($N=10$) and an emergence test ($N=20$). For both tests, three egg sacs were incubated in aerated artificial freshwater provided with paper slip as substrate at 20 °C. The hatched larvae were fed with a fish food suspension (TetraMin®) and

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