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# Toxicity of Metals to an Aquatic Worm, *Nais elinguis* (Oligochaeta, Naididae)

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## ABSTRACT

Impact of metals to the environment is increasing problem worldwide. This study was conducted to determine the acute toxicity of 8 metals to worms *Nais elinguis* in laboratory. Adult freshwater worms *N. elinguis* (Oligochaeta, Naididae) were exposed for a four-day period in laboratory conditions to a range of copper (Cu), cadmium (Cd), zinc (Zn), lead (Pb), nickel (Ni), iron (Fe), aluminum (Al) and manganese (Mn) concentrations. Mortality was assessed and median lethal times ($LT_{50}$) and concentrations ($LC_{50}$) were calculated. $LT_{50}$ and $LC_{50}$ increased with the decrease in mean exposure concentrations and times, respectively for all metals. Ninety-six hour $LC_{50}$ for Cu, Cd, Zn, Pb, Ni, Fe, Al and Mn were 7, 27, 912, 580, 645, 123, 3874 and 364 $\mu g\ L^{-1}$, respectively. Cu was the most toxic to *N. elinguis* followed by Cd, Fe, Mn, Pb, Ni, Zn and Al (Cu>Cd>Fe>Mn>Pb>Ni>Zn>Al). Comparison of $LC_{50}$ values for metals for this species with those for other freshwater worms reveals that *N. elinguis* is equally or more sensitive to metals. This study indicates that *N. elinguis* is a potential organism in toxicity testing and as a bioindicator of metals pollution.

**Key words:** Acute, mortality, sensitivity, toxicity test, heavy metal effect, *Nais elinguis*

## INTRODUCTION

Toxicity testing has been widely used as a tool to derive water quality standards for chemicals and to identify suitable organisms as a bioindicator. Laboratory toxicity tests also are an essential tool for evaluating the potential impact of chemicals on ecological systems and for a comprehensive assessment of contaminated environments. Acute toxicity tests are important steps in establishing suitable water quality criteria and standards (Rathore and Khangarot, 2003). In evaluating the safety of chemical substances and for regulatory purposes, it is necessary to have precise data on the chemical and its effects on organisms. Acute toxicity studies can provide important, valuable and fast information and indicate the trends of toxicity of a chemical and the effects (Calow, 1993; Rand *et al.*, 1995; Watts and Pascoe, 2000). Metals such as Cu, Cd, Zn and Pb are released from natural sources as well as human activity. They are widely used in industry and are common water pollutants. Impact of these metals to the environment is increasing problem worldwide. Malaysia, as a developing country, is no exception and faces metals pollution caused especially by anthropogenic activities such as manufacturing, agriculture, sewage, mining and motor vehicle emissions (Shazili *et al.*, 2006; DOE, 2009; Zulkifli *et al.*, 2010; Yap and Pang, 2011). Metals research in Malaysia, especially using organisms as bioindicator, is still scarce. Therefore, it is important to conduct studies with local organisms that can be used to gain data on metal toxicity, to determine the organism's sensitivity and to derive a permissible limit for Malaysian's water that can protect aquatic communities.

Oligochaetes are most familiar as terrestrial earthworms but there are several families which primarily inhabit marine and/or freshwater environment. Three major families of freshwater Oligochaeta are Naididae, Tubificidae and Enchytraeidae. The freshwater worms occur in a wide range of habitats, from springs and groundwater to river and estuaries and from small temporary pool to the profundal depth of large lakes. Aquatic oligochaetes are typically small and thin, usually less than 1 mm to a few centimeters long. Most aquatic oligochaetes inhabit sediment and are known to affect processes such as sediment-water nutrient exchange, pollutant dynamics and sediment aeration and structure. *Nais elinguis* is from Naididae family, a free-swimming worm and reproduces asexually by dividing into an anterior and a posterior naidid. Naidids generally smaller than tubificids and can be found in a broad range of freshwater habitats, from fast flowing streams to swamps but are not common in deeper parts of lakes. The species is abundant in mats of filamentous algae and cyanobacteria and on macrophytes with dissected leaves (Learner *et al.*, 1978; Vopel and Arlt, 1995). They are also a cosmopolitan species that is abundant in organically enriched sites. *Nais elinguis* were also reported to be the dominated worm in the activated sludge tank (Ratsak, 2001) and sewage filter beds (Learner, 1979). There were also reported to be a few species which occur not only in freshwater but also in brackish waters. There are over 100 species naidids worldwide, arrange into 22 genera (Brinkhurst and Gelder, 2001; Pinder and Ohtaka, 2004).

Aquatic oligochaetes have been used in pollution assessment for a long time (Chapman, 2001). Many of the freshwater pollution assessment and toxicity testing has been reported with oligochaetes especially from the family of Tubificidae and Lumbriculidae such as *Tubifex tubifex* (Khangarot, 1991; Fargasova, 1994; Mosleh *et al.*, 2007) and *Lumbriculus variegatus* (Phipps *et al.*, 1995; Chapman *et al.*, 1999; Sardo and Soares, 2011). However, only few studies have been reported for *Nais elinguis* and most of the studies were on ecology and diversity (Learner, 1979; Bowker *et al.*, 1985; Sundic *et al.*, 2011) and sludge process (Ratsak, 2001; Wei *et al.*, 2009); and very little on toxicity testing. Therefore, the study was undertaken to determine the acute toxicity of Cu, Cd, Zn, Pb, Ni, Fe, Al and Mn to adult worms of *N. elinguis* in laboratory for four days of exposure.

## MATERIALS AND METHODS

**Samples collection:** Worms of *N. elinguis* were collected from fishpond filtration system in Bangi, Selangor, Malaysia. The filter was consisting of several layer of filter mate, made from polyester wool and the water is continuously circulate using water pump from the fishpond to the filter and back to the pond. *N. elinguis* were reported to select algal filaments in preference to monofilaments nylon threads and prefers some algae to others or to glass beads (Bowker *et al.*, 1985). Identification of species was based on method described by Brinkhurst and Gelder (2001) and Pennak (1978). Prior to toxicity testing, the worms were acclimatized for one week under laboratory conditions (28-30°C with 12 h light:12 h darkness) in 50 L stocking tanks using dechlorinated tap water (filtered by several layers of sand and activated carbon; T.C. Sediment Filter® (TK Multitrade, Seri Kembangan, Malaysia) aerated through an air stone. During acclimation the worms were fed with finely ground commercial fish food Tetramin® (Tetrawerke, Germany).

**Toxicity test:** The standard stock solution (100 mg $L^{-1}$) of Cu, Cd, Zn, Pb, Ni, Fe, Al and Mn were prepared from analytical grade metallic salts of $CuSO_4.5H_2O$, $CdCl_2.2½H_2O$, $ZnSO_4.7H_2O$, $Pb(NO_3)_2$, $NiSO_4.6H_2O$, $FeCl_3$, $Al_2(SO_4)_3.18H_2O$ and $MnSO_4.H_2O$ (Merck, Darmstadt, Germany), respectively. The stock solutions were prepared with deionized water in 1 L volumetric flasks. Acute Cu, Cd, Zn, Pb, Ni, Fe, Al and Mn toxicity experiments were performed using adult worms (approximately 1.0 cm body length and wet weight of 0.1-0.3 mg) obtained from stocking tanks. Following a range finding test, five Cu (5.6, 10, 32, 56 and 75 μg $L^{-1}$), Cd (10, 56, 100, 320 and 560 μg $L^{-1}$), Zn (320, 560, 750, 1800 and 3200 μg $L^{-1}$), Pb (56, 100, 320, 560 and 1000 μg $L^{-1}$), Ni (100, 320, 560, 750 and 1000 μg $L^{-1}$), Fe (100, 320, 560, 870 and 1000 μg $L^{-1}$), Al (320, 560, 750, 1800 and 3200 μg $L^{-1}$) and Mn (320, 560, 870, 1000 and 3200 μg $L^{-1}$) concentrations were chosen based on logarithm scale. Metal solutions were prepared by dilution of a stock solution with dechlorinated tap water. A control with dechlorinated tap water only was also used. The tests were carried out for four days (96 h) under static conditions with renewal of the solution every two days. Control and metal-treated groups each consisted of five replicates of four randomly allocated worms in glass petri dishes (diameter 5 cm) containing 10 mL of the appropriate solution. No stress was observed for the worms in the solution, indicated by 100% survival for the worms in the control water until the end of the study. Twenty worms per treatment/concentration were used in the experiment and a total of 820 worms were employed in the investigation (APHA, 1992; Cooney, 1995). Samples of water for metal analysis taken before and immediately after each solution renewal were acidified to 1% with ARISTAR® nitric acid (65%) (BDH Inc, VWR International Ltd., England) before metal analysis by flame or furnace Atomic Absorption Spectrophotometer (Perkin Elmer (Massachusetts, USA) model AAnalyst800) depending on the concentrations.

During the toxicity test, the worms were not fed. The experiments were performed at room temperature of 28-30°C with photoperiod 12 h light:12 h darkness, using fluorescent lights (334-376 lux). Water quality parameters (pH, conductivity and dissolved oxygen) were measured every two days using portable meters (model Hydrolab Quanta®, Hach, Loveland, USA) and water hardness samples were fixed with nitric acid (ARISTAR®, 65%) and measured by flame atomic absorption spectrophotometer (Perkin Elmer model AAnalyst 800). Mortality was recorded every 3 to 4 h for the first two days and then at 12 to 24 h intervals throughout the rest of the test period. The criterion for determining death was lack of movement and failure to respond to a gentle probing with a blunt dissecting needle. Any dead animals were removed immediately. To avoid possible contamination, all glassware and equipment used were acid-washed (20% $HNO_3$) (Dongbu Hitek Co. Ltd., Seoul, Korea, 68%) and the accuracy of the analysis was checked against blanks. Procedural blanks and quality control samples made from standard solutions for Cu, Cd, Zn, Pb, Ni, Fe, Al and Mn (Spectrosol, BDH, England) were analyzed in every ten samples in order to check for sample accuracy. Percentage recoveries for metals analyses were between 85-105%.

**Statistical analysis:** Median lethal concentrations ($LC_{50}$) for the worms exposed to metals were calculated using measured metal concentrations. FORTRAN programs based on the methods of Lichfield (1949) and Litchfield and Wilcoxon (1949) were used to compute and compare the $LT_{50}$ and $LC_{50}$. Data were analyzed using time/response (TR) and concentration/response (CR) methods by plotting cumulative percentage mortality against concentration and time on logarithmic-probit paper.

## RESULTS AND DISCUSSION

In all data analyses, the actual, rather than nominal, Cu, Cd, Zn, Pb, Ni, Fe, Al and Mn concentrations were used (Table 1). The mean water quality parameters measured during the test were pH 6.51±0.01, conductivity 244.3±0.6 µS $cm^{-1}$, dissolved oxygen 6.25±0.06 mg $L^{-1}$ and total hardness ($Mg^{2+}$ and $Ca^{2+}$) 17.89±1.74 mg $L^{-1}$ as $CaCO_3$.

One hundred percent of control animals maintained in dechlorinated water survived throughout the experiment. Data for median lethal time ($LT_{50}$) were plotted against metal concentration in water (Fig. 1) and results showed that Cu was the most toxic to *N. elinguis*. Data

Table 1: Median lethal times ($LT_{50}$) for *N. elinguis* exposed to different concentrations for Cu, Cd, Zn, Pb, Ni, Fe, Al and Mn

| Concentration (µg $L^{-1}$) | $LT_{50}$ (h) | 95% confidence limits | Concentration (µg $L^{-1}$) | $LT_{50}$ (h) | 95% confidence limits | Concentration (µg $L^{-1}$) | $LT_{50}$ (h) | 95% confidence limits |
|---|---|---|---|---|---|---|---|---|
| **Copper** | | | **Cadmium** | | | **Zinc** | | |
| 8 | 96 | 73-126 | 12 | 283 | 85-940 | 384 | 141 | na |
| 10 | 72 | 51-101 | 46 | 106 | 47-237 | 580 | 132 | na |
| 31 | 52 | 39-69 | 90 | 53 | 26-108 | 814 | 88 | 73-106 |
| 55 | 32 | 23-44 | 367 | 22 | 11-44 | 1924 | 49 | 40-61 |
| 67 | 29 | 15-57 | 515 | 6 | 4-10 | 3078 | 10 | 7-13 |
| Concentration (µg $L^{-1}$) | $LT_{50}$ (h) | 95% confidence limits | Concentration (µg $L^{-1}$) | $LT_{50}$ (h) | 95% confidence limits | Concentration (µg $L^{-1}$) | $LT_{50}$ (h) | 95% confidence limits |
| **Led** | | | **Nickel** | | | **Iron** | | |
| 52 | 2512 | na | 107 | 224 | na | 137 | 79 | 38-164 |
| 91 | 1585 | na | 296 | 135 | na | 319 | 39 | 21-73 |
| 279 | 1318 | na | 553 | 121 | 86-168 | 553 | 15 | 9-28 |
| 522 | 1074 | 83-1396 | 688 | 95 | 66-135 | 863 | 6 | 4-11 |
| 1020 | 27 | 21-35 | 911 | 27 | 20-36 | 1071 | 4 | 3-7 |
| Concentration (µg $L^{-1}$) | $LT_{50}$ (h) | 95% confidence limits | Concentration (µg $L^{-1}$) | $LT_{50}$ (h) | 95% confidence limits | | | |
| **Aluminium** | | | **Manganese** | | | | | |
| 644 | 708 | na | 337 | 119 | 62-229 | | | |
| 1061 | 631 | na | 582 | 68 | 35-131 | | | |
| 1584 | 562 | na | 883 | 35 | 19-65 | | | |
| 3834 | 471 | 113-1965 | 1195 | 17 | 9-32 | | | |
| 6729 | 28 | 18-42 | 3360 | 8 | 5-14 | | | |

na: Values could not be calculated from probit software

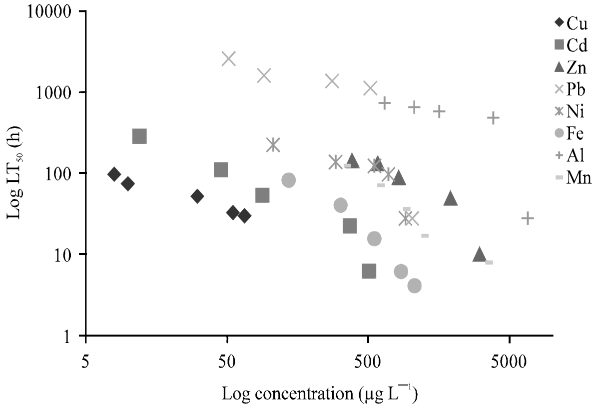

Fig. 1: The relationship between median lethal time ($LT_{50}$) and exposure concentrations for *N. elinguis* exposed to eight different metals

for median lethal concentration (LC$_{50}$) were plotted against time of exposure (Fig. 2) and results also showed that Cu was the most toxic. The median lethal time (LT$_{50}$) and concentrations (LC$_{50}$) increased with a decrease in mean exposure concentrations and times, respectively, for all metals (Table 1, 2). However, the lethal threshold concentration could not be determined since the toxicity curves (Fig. 1, 2) did not become asymptotic to the time axis within the test period. Results showed that trends of toxicity to *N. elinguis* is Cu>Cd>Fe>Mn>Pb>Ni>Zn>Al. Similar results were reported by Khangarot (1991) and Rathore and Khangarot (2002) with tubificid sludge worm *Tubifex tubifex* and the authors concluded that Cu was among the most toxic metals to the worm. With *Tubifex tubifex,* Fargasova (1999) showed the order of toxicity was Cu(II)>Cu(I)>V>Hg>Mn>Ni>Cd>Cr>Mo>Pb>Sn(IV) = Sn(II)>As and Maestre *et al.* (2009) showed the rank of toxicity was Cu>Cd>Cr. The lumbriculidae oligochaeta *Lumbriculus variegatus* was also more sensitive to Cu than to Cd in water-only 10-day acute toxicity tests (Chapman *et al.*, 1999). Contrarily, with *Lumbriculus variegatus.* Bailey and Liu (1980) found the order of toxicity was

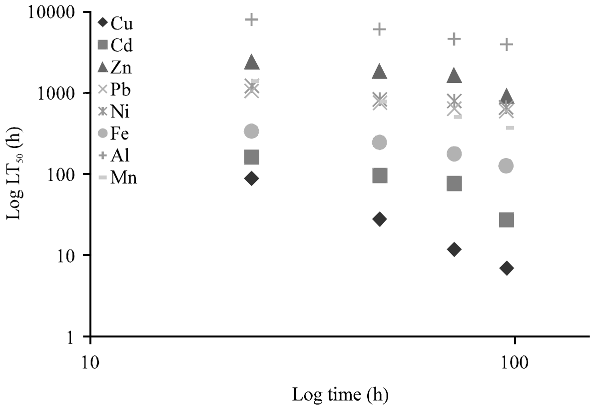

Fig. 2: The relationship between median lethal concentration (LC$_{50}$) and exposure times for *N. elinguis* exposed to eight different metals

Table 2: Median lethal concentrations (LC$_{50}$) for *N. elinguis* at different exposure times for Cu, Cd, Zn, Pb, Ni, Fe, Al and Mn

| | Cu | | Cd | | Zn | | Pb | |
|---|---|---|---|---|---|---|---|---|
| Time (h) | LC$_{50}$ ($\mu$g L$^{-1}$) | 95% confidence limits | LC$_{50}$ ($\mu$g L$^{-1}$) | 95% confidence limits | LC$_{50}$ ($\mu$g L$^{-1}$) | 95% confidence limits | LC$_{50}$ ($\mu$g L$^{-1}$) | 95% confidence limits |
| 24 | 87 | 67-284 | 158 | 97-278 | 2433 | 2187-2707 | 1047 | na |
| 48 | 28 | 19-42 | 94 | 56-153 | 1827 | 1492-2147 | 730 | 631-844 |
| 72 | 12 | 7-17 | 74 | 42-122 | 1359 | 1131-1650 | 636 | na |
| 96 | 7 | 3-10 | 27 | 14-43 | 912 | 789-1127 | 580 | na |
| | Ni | | Fe | | Al | | Mn | |
| Time (h) | LC$_{50}$ ($\mu$g L$^{-1}$) | 95% confidence limits | LC$_{50}$ ($\mu$g L$^{-1}$) | 95% confidence limits | LC$_{50}$ ($\mu$g L$^{-1}$) | 95% confidence limits | LC$_{50}$ ($\mu$g L$^{-1}$) | 95% confidence limits |
| 24 | 1156 | 947-2025 | 337 | 246-429 | 7920 | 6350-42271 | 1350 | 992-2117 |
| 48 | 793 | 742-851 | 239 | 162-312 | 6048 | 5240-7239 | 745 | 586-936 |
| 72 | 748 | 704-801 | 176 | 106-237 | 4456 | na | 503 | 370-616 |
| 96 | 645 | 560-692 | 123 | 57-174 | 3874 | na | 364 | 222-461 |

na: Values could not be calculated from probit software

Cd>Cu>Pb>Zn. Luoma and Rainbow (2008) explained there is some uncertainty about the shape of the curves describing the stress response of essential and nonessential metals such as Cu and Cd and essential metals are not less toxic than non-essential metals. It is by no means the case that all non-essential metals are more toxic than all essential metals. Khangarot (1991) explained that most of the heavy metal ions are toxic to living organisms because they combine with some ligands of enzymes which are necessary for life. However, for non-transitional metal cation, enzyme inhibition is not likely to be a primary cause in toxicity. But osmotic or other colligative factors working through physical reactions cause physical damage to the cellular system.

This study showed that Cu and Cd had a strong toxicity to tested worm species; Fe showed an intermediate toxicity but the toxicity of Zn, Pb, Ni and Mn was weak. Al was shown to have the weakest toxicity of all metals tested. Toxicity of Cu to the worm were found to be 500-time more toxic than Al and between 52 to 130-time than Zn, Pb, Ni and Mn. High toxicity of Cu has also been noted in this laboratory for other aquatic organisms such as tadpoles *Duttaphrynus melanostictus* (Shuhaimi-Othman *et al.*, 2012a), freshwater fish *Rasbora sumatrana* and *Poecilia reticulata* (Shuhaimi-Othman *et al.*, 2012b) and freshwater snails *Melanoides tuberculata* (Shuhaimi-Othman *et al.*, 2012c). Copper, as an essential biological element, interferes with many physiological functions. It is a constituent micronutrient of the protein component of several enzymes, mainly of those participating in electron flow, catalyzing redox reaction in mitochondria, cell wall and in the cytoplasm of cells (Murthy and Mohanty, 1995). The key role of Cu to animals is mediated through specific Cu proteins. Cytochrome oxidase, the most important Cu protein, is directly associated with the most dramatic form of Cu deficiency in animals (Buck, 1977). In explaining effect of Cu, Aaseth and Norseth (1986) suggested that Cu toxicity seems entirely or in large part to be due to free Cu ions combining with new Cu proteins and altering their physiological functions. However, for Al the toxicity is more influence by pH of the water where at acidic pH it's become more soluble and, hence, potentially more toxic to aquatic biota. Toxicity of Al on organisms are dependent on pH of water, with greater toxicity due to ion regulatory effects occurring at low pH and respiratory distress due to precipitation of insoluble Al complexes at higher pH (Gensemer and Playle, 1999). Toxicity testing in this study was conducted in near to neutral pH water (6.5) and this resulted in low toxicity of Al to the tested organism. According to Gensemer and Playle (1999), Al is relatively insoluble at pH 6 to 8. In general, aquatic invertebrates are less sensitive to Al than are fish (Ormerod *at el.*, 1987; Wren and Stephenson, 1991).

This study showed that $LC_{50}$s for 24 and 96 hours of Cu, Cd, Zn, Pb, Ni, Fe, Al and Mn were 87, 158, 2433,1047, 1156, 337, 7920 and 1350 $\mu g\ L^{-1}$ and, 7, 27, 912, 580, 645, 123, 3874 and 364 $\mu g\ L^{-1}$, respectively (Table 2). A comparison of $LC_{50}$ values with other freshwater worms is shown in Table 3. In the literature, the range of values of 96 h-$LC_{50}$ of the metals reported for other freshwater oligochaetes in aqueous acute toxicity tests is wide. This study showed that for most of the metals tested, *N. elinguis* showed highest sensitivity compared with other worm species such as *Lumbriculus variegatus* (Bailey and Liu, 1980; Schubauer-Berigan *et al.*, 1993), *Limnodrilus hoffmeisteri* (Wurtz and Bridges, 1961) and *Tubifex tubifex* (Rathore and Khangarot, 2002). However, for some metals such as Cu, Ni and Mn, this study showed higher $LC_{50}$ values compared with study reported by Fargasova (1999) with *Tubifex tubifex* (Table 3). These differences probably because of different species used, age, size of the organism, test methods and water quality such as water hardness (McCahon and Pascoe, 1988; Phipps *et al.*, 1995; Rathore and Khangarot, 2003). Therefore, comparisons should be made cautiously. In the present study, water hardness was considered low and the water was categorized as soft water (<75 mg $L^{-1}$ as $CaCO_3$). Rathore and

Table 3: Comparison of $LC_{50}$ values of *N. elinguis* with other freshwater worms

| Metal | Species | Water hardness (mg $L^{-1}$) | Test duration (days) | $LC_{50}$ (µg $L^{-1}$) | Reference |
|---|---|---|---|---|---|
| Copper | *N. elinguis* | 18 | 4 | 7 | This study |
| | *Lumbriculus variegatus* | 280 | 4 | 130 | Schubauer-Berigan *et al.* (1993) |
| | *Tubifex tubifex* | 120 | 4 | 0.16 | Das *et al.* (1993) |
| | *Tubifex tubifex* | - | 4 | 120 | Milani *et al.* (2003) |
| | *Lumbriculus variegatus* | - | 2 | 191 | Meyer *et al.* (2002) |
| | *Tubifex tubifex* | - | 4 | 2 | Fargasova (1999) |
| | *Limnodrilus hoffmeisteri* | 100 | 4 | 400 | Wurtz and Bridges (1961) |
| | *Lumbriculus variegatus* | 30 | 4 | 150 | Bailey and Liu (1980) |
| | *Tubifex tubifex* | 245 | 4 | 158 | Khangarot (1991) |
| Cadmium | *N. elinguis* | 18 | 4 | 27 | This study |
| | *Limnodrilus hoffmeisteri* | - | 4 | 2900 | Williams *et al.* (1985) |
| | *Lumbriculus variegatus* | 30 | 4 | 74 | Bailey and Liu (1980) |
| | *Tubifex tubifex* | - | 4 | 400 | Reynoldson *et al.* (1996) |
| | *Tubifex tubifex* | 119 | 4 | 30 | Bouche *et al.* (2000) |
| | *Tubifex tubifex* | - | 4 | 1032 | Fargasova (1999) |
| | *Limnodrilus hoffmeisteri* | - | 4 | 170 | Chapman *et al.* (1982) |
| | *Tubifex tubifex* | 245 | 4 | 47530 | Khangarot (1991) |
| Zinc | *N. elinguis* | 18 | 4 | 912 | This study |
| | *Lumbriculus variegatus* | - | 10 | 2984 | Phipps *et al.* (1995) |
| | *Lumbriculus variegatus* | 30 | 4 | 6300 | Bailey and Liu (1980) |
| | *Tubifex tubifex* | 224 | 2 | 130000 | Qureshi *et al.* (1980) |
| | *Limnodrilus hoffmeisteri* | 100 | 4 | 10000 | Wurtz and Bridges (1961) |
| | *Tubifex tubifex* | 245 | 4 | 17780 | Khangarot (1991) |
| | *Tubifex tubifex* | 34 | 2 | 2980 | Brkovic-Popovic and Popovic (1977) |
| Lead | *N. elinguis* | 18 | 4 | 580 | This study |
| | *Tubifex tubifex* | - | 4 | 14620 | Fargasova (1999) |
| | *Lumbriculus variegatus* | - | 10 | 740 | Phipps *et al.* (1995) |
| | *Lumbriculus variegatus* | 280 | 4 | 8000 | Schubauer-Berigan *et al.* (1993) |
| | *Lumbriculus variegatus* | 30 | 4 | 1800 | Bailey and Liu (19800 |
| | *Tubifex tubifex* | 245 | 4 | 42 | Khangarot (1991) |
| Nickel | *N. elinguis* | 18 | 4 | 645 | This study |
| | *Lumbriculus variegatus* | - | 10 | 12160 | Phipps *et al.* (1995) |
| | *Lumbriculus variegatus* | 280 | 4 | 75000 | Schubauer-Berigan *et al.* (1993) |
| | *Tubifex tubifex* | - | 4 | 537 | Fargasova (1999) |
| | *Tubifex tubifex* | 245 | 4 | 66750 | Khangarot (1991) |
| Iron | *N. elinguis* | 18 | 4 | 123 | This study |
| | *Tubifex tubifex* | 237 | 4 | 28550* | Rathore and Khangarot (2002) |
| | *Tubifex tubifex* | 245 | 4 | 101840 | Khangarot (1991) |
| Aluminium | *N. elinguis* | 18 | 4 | 3874 | This study |
| | *Tubifex tubifex* | 245 | 4 | 50230 | Khangarot (1991) |
| Manganese | *N. elinguis* | 18 | 4 | 364 | This study |
| | *Tubifex tubifex* | 237 | 4 | 239390* | Rathore and Khangarot (2002) |
| | *Tubifex tubifex* | - | 4 | 295 | Fargasova (1999) |
| | *Tubifex tubifex* | 245 | 4 | 170610 | Khangarot (1991) |

*$EC_{50}$ value

Khangarot (2003) conducted a study on effect of different water hardness on toxicity of heavy metal to *Tubifex tubifex* and shows that 96 h $EC_{50}$ values of Cd, Co, Cr, Cu, Fe, Pb, Mn, Ni and Zn were higher in hard (300 mg $L^{-1}$) and very hard (170 mg $L^{-1}$) water compared with soft (45 mg $L^{-1}$) and very soft (12 mg $L^{-1}$) water. The authors also demonstrated that the rank order toxicity of the metals (Fe, Pb and Cd) varied in different water hardness. Increase in the concentrations of these cations ($Ca^{2+}$ and $Mg^{2+}$) will increased the competition to bind at the receptor sites and this further decreases the amount of metal that is bound at the receptor sites (Meyer *et al*., 1999). In another experiment, Rathore and Khangarot (2002) conducted a study on effect of different temperature on toxicity of heavy metal to *Tubifex tubifex* and conclude that acute toxicity of Cd, Cr, Co, Cu, Pb, Hg, Ni and Zn increases with temperature increase. In addition, test methods will also affect toxicity result especially with benthic organisms such as worm. Some of the reported studies conducted testing with sediment or spiked sediments and this normally will reduce sensitivity of the worm to metals compare to water-only toxicity test. Chapman *et al*. (1982) reported that the presence of sediments resulted in increased tolerances on toxicity of pollutants to 12 aquatic oligochaeta species, demonstrating the role of sediments as important modifiers of toxic effects on oligochaetes. Other factors such as collection site of the worm from polluted or non-polluted areas and pre-exposure also affect the results of toxicity tests (Reynoldson *et al*., 1996; Reinecke *et al*., 1999).

## CONCLUSIONS

This study showed that *N. elinguis* was equally or more sensitive to metals compared to other freshwater worms. Toxicity of metal to freshwater worms was influenced by many abiotic and biotic factors. Copper was the most toxic to *N. elinguis* followed by Cd, Fe, Mn, Pb, Ni, Zn and Al. This study indicates that *N. elinguis* is a potential organism in toxicity testing and as a bioindicator of metals pollution.

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