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Research article

Arsenic acute toxicity assessment on select freshwater organism species in Malaysia

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Abstract: Recently, arsenic has contaminated the aquatic ecosystem, raising government and public environmental concern. In this study, four freshwater organisms were used to evaluate arsenic toxicity levels. Two types of fish, namely *Poecilia reticulata* (guppy) (Poeciliidae) and *Rasbora Sumatrana* (Cyprinidae), an aquatic worm species *Tubifex tubifex* (Oligochaeta) and diptera midge larvae *Chironomus javanus* (Chironomidae) were exposed for a 4-day (96 h) period in laboratory conditions to a range of arsenic (As) concentrations. Mortality was assessed and median lethal times (LT_{50}) and concentrations (LC_{50}) were calculated. The objective of this study is to determine the acute toxicity of arsenic concentration on *Poecilia reticula, Rasbora sumatrana, Tubifex tubifex* and *Chironomus javanus*. Results showed that LT_{50} and LC_{50} increase with the decrease in mean exposure concentrations and times. Results indicated that *Tubifex tubifex* was most sensitive to arsenic toxicity compared to other organisms used in this study in this order; *Tubifex tubifex* > *Chironomus javanus* > *Rasbora sumatrana* > *Poecilia reticulata*.

Keywords: acute; arsenic; LC_{50}; LT_{50}; toxicity

1. Introduction

Over the past few decades, the pollution of natural aquatic resources due to heavy metals released from anthropogenic activities of industrial, domestic and other activities has become a matter of environmental concern [1]. Arsenic is known as one of the toxic elements on the Earth with many applications such as in pesticides and other consumer-related products used in the past 100 years [2]. Arsenate (AsV), arsenite (AsIII), arsenic (As0), and arsine (As-III) are the four main

arsenic states present in aquatic bodies [3]. Inorganic arsenic species are more toxic than organoarsenic species and the toxicity of arsenic to organisms depends on its concentration and speciation [4-5]. Arsenic trioxide makes peoples more vulnerable to exposure as it has been used in pharmaceuticals, pesticides, veterinary products and decolorizing agents. Human activities such as mining, waste disposal and the indiscriminate use of pesticides and herbicides have increased environmental arsenic contamination [6], while smelting operations and the burning of fossil fuels are the major reasons of anthropogenic atmospheric inputs of arsenic on Earth. However, it is still difficult to summarize exactly which human activity contributes to the cycle in the environment as a whole [7].

Wang [8] mentioned that the exposure of metals to aquatic organisms occur through two basic routes; by direct absorption through water, and/or by feeding. For predators such as some species of fish, amphibians and invertebrates, and animals that feed on debris, feeding is the primary route of exposure and accumulation of metals. Fish is one of the important components in the aquatic environment and may be involved in the mobilization of arsenic when feeding occurs throughout the food chain [9]. At any rate, higher trophic level organisms that feed on lower trophic level organisms will accumulate higher amounts of arsenic in the food chain [10]. Aquatic organisms accumulate, retain, and transform arsenic inside their bodies when exposed to it through their diet and other routes [11].

Arsenic contamination in the natural environment is mostly caused by natural processes with a ratio of 60:40 compared to anthropogenic causes [12]. Sources such as man-made, geothermal inputs and atmospheric deposition cause the arsenic concentration to be higher in freshwater than in marine water [13]. Freshwater organisms have a tendency of greater exposure to higher arsenic levels compared to marine organisms, leading to greater bioaccumulation in freshwater food webs [14]. In some areas of the world, high levels of arsenic are naturally present in drinking water, raising environmental concern. In Asia, the impact of arsenic toxicity is particularly alarming in certain areas, for example in the Bengal Basin of Bangladesh and West Bengal, India [15]. Arsenic is also found widely in the United States and Canada as well as in Latin America countries such as Argentina and Nicaragua [16-19] where the sources of arsenic are geo-genic as well as anthropogenic. Alongside the growing concern of arsenic toxicity all over the world, Malaysia as a developing country is also not excluded. Recently, arsenic contamination has become our greatest concern caused by the seeping of bauxite and its residue to rivers and coastal areas near to unsustainable bauxite mining sites in Gebeng, Pahang. Reports showed high levels of arsenic in fish caught in Sungai Pengorak, Pahang, ranging from 70.8 to 104.5 g/kg, which is more than a staggering 70 000 times the permissible limit for arsenic in fish and fishery products (1mg/kg) under the Malaysia Food Regulation 1985 [20].

Autotroph serves as a critical component of aquatic systems and food items for higher trophic level organisms. When arsenic enters the aquatic environment, the lower trophic level organisms will be picked up first by higher trophic organisms such as *T. tubifex*, *C. javanus*, *P. reticulata* and *R. sumatrana,* and then transferred to human beings in the food chain via dietary intake. Aquatic organisms accumulate, retain and transform arsenic inside their bodies due by exposure through their diet and other routes such as water, particles and sediment [21]. Maher et al. [22] mentioned that arsenic biomagnification, a process whereby chemical concentrations results in an increase of arsenic levels in aquatic organisms of each successive trophic level due to increasing dietary exposures, other researchers claimed that arsenic concentration in organisms decreases by an order of magnitude

for each trophic step up the food chain as the arsenic is methylated and excreted [23]. Despite the attention on arsenic uptake and accumulation in aquatic ecosystems, many uncertainties still exist on the potential toxicity of arsenic in the environment and its impact on consumers, especially to human beings. Understanding the mechanisms of arsenic accumulation, transformation and magnification in the food chain is critically important in assessing the risks from arsenic contamination, especially from food. Thus, the matter should be investigated further in another research.

The goal of this study is to determine the level of acute arsenic toxicity to *P. reticulata*, *R. sumatrana, T. tubifex* and *C. javanus,* thus showing the negative effects of heavy metal on aquatic life through toxicity tests [24]. The results could be used to understand and make better decisions when dealing with heavy metal pollution [25]. However, managing heavy metal contamination requires the understanding of concentration dependence and toxicity of the compound itself. Lack of metal research data on the use of freshwater organisms as bioindicators may lead to the under-protection or over-protection of aquatic ecosystems, especially in Malaysia. Therefore, toxicity testing should be performed using local freshwater macro-invertebrates as bioindicators in the field and to determine the sensitivity of organisms and derive a permissible limit for Malaysian waters, thus protecting the local aquatic communities for a better future.

2. Methodology

In this study, *P. reticulata* was sampled from a small pond at the National University of Malaysia, *T. tubifex* and *C. javanus* were sampled from a small canal outside Institut Kemahiran Belia Negara (IKBN) Dusun Tua, Hulu Langat while *R. sumatrana* was purchased from aquarium shops in Bangi, Selangor. On their arrival at the laboratory, the organisms were acclimatized to laboratory conditions (28–30 °C with 12 h light and 12 h darkness) in 50L stocking tanks using dechlorinated tap water filtered through several layers of sand and activated carbon; T.C. Sediment Filter® and aerated through an air stone for a minimum of one week time. The organisms were fed with commercial fish food pellets (Super-Gold Tropical Fish Food) daily. Any unwanted particles were removed from the tank after feeding. A standard arsenic stock solution (1000 mg/L) was prepared from analytical grade metallic salt of sodium arsenite ($NaAsO_2$) anhydrous ≥ 95.0% (Sigma Aldrich). The stock solution was prepared using deionized water in a 1 L volumetric flask. Acute toxicity bioassays of arsenic were performed using adult *P. reticulata* (average length 2.8–3.8 cm; average weight 0.22–0.37 g), *R. sumatrana* (average length 5.0–6.0 cm; average weight 3.5–5.0 g), *T. tubifex* (average length 1.0–1.8 cm; average weight 0.0043 g) and *C. javanus* (average length 0.7–1.2 cm; average weight 0.0120 g) obtained from the stocking tanks. Five arsenic concentrations were chosen using the 24 hours finding test range method. The concentrations chosen were 10, 18, 32, 56 and 100 mg/L for *P. reticulata*, *R.sumatrana* and *C. javanus*, while for *T. tubifex,* the concentrations chosen were 1, 10, 32, 56 and 100 mg/L. The test concentrations used in the bioassays are shown in Table 1. Metal solutions were prepared by diluting the stock solution using dechlorinated tap water. Toxicity tests were carried out in the duration of 4 days (96 hours) with the renewal of solution at every 2 days to maintain the arsenic concentration. A tank with only dechlorinated tap water was used for the control experiments.

Each control and arsenic-treated group consists of five 2 to 4 randomly allocated replicates in a beaker and petri dish containing the appropriate amount of arsenic solutions. All controls resulted in low mortalities at lower than 10%, indicating the acceptability of experiments until the end of

the study. A total of 10–20 organisms per treatment were used in the experiment and a total of 420 organisms were employed in the investigation. Samples of water were taken before and immediately after the renewal of test solutions for metal analysis. The samples were acidified to 2% with nitric acid (65%) before metallic analysis was conducted using inductive couple plasma mass spectrometry (Model ELAN 9000 Perkin Elmer ICP-MS, USA) with seven concentrations of standard solutions used in the calibration process at 10, 30, 50, 100, 250, 500 and 1000 μg/L. To avoid any possible contamination, all glassware and equipment were acid washed (20% HNO_3) and the accuracy of analysis was checked once for every 10 samples. Procedural blanks and quality control samples made from standard solutions were analyzed in order to check sample accuracy.

During the toxicity test, the organisms were not fed. In acute toxicity testing, test organisms should not be fed while in the test chambers. The experiments were performed at room temperature (27–30 °C) with a 12 h photoperiod and 12 h darkness using fluorescent lights (334–376 lux). For every 2 days, the water quality parameters (pH, conductivity and dissolved oxygen) were measured according to standard procedure [22] using portable meters (model Hydrolab Quanta®), and water hardness samples for the determination of magnesium and calcium concentration (0.45 mm filtered) were fixed with HNO_3 and measured using inductive couple plasma mass spectrometry (Model ELAN 9000 Perkin Elmer ICP-MS, USA). Mortality was recorded every 3 hours for the first and second days, and then every 4 hours for the third and fourth days. Organisms that were unable to respond to gentle physical stimulation were defined as dead. Any dead organisms were removed immediately to avoid contamination.

Median lethal concentrations (LC_{50}) for the fish exposed to arsenic were calculated using measured metal concentrations. FORTRAN programs based on the methods of Litchfield [27] and Litchfield and Wilcoxon [28] were used to compute and compare the LC_{50} values. Data were analyzed using concentration-response (CR) methods by plotting the cumulative percentage mortality concentration on a logarithmic-probit graph.

3. Results and Discussion

In all data analyses, the actual rather than nominal arsenic was used as shown in Table 1. The mean water quality parameters measured during the test were 27.8 ± 0.2 °C for water temperature, 7.1 ± 0.1 for pH, 470 ± 0.8 μS cm^{-1} for conductivity, 7.0 ± 0.2 mg/L for dissolved oxygen, and 29.8 ± 1.4 mg/L as $CaCO_3$ for total hardness (Mg^{2+} and Ca^{2+}). Ninety percent of the control organisms maintained in dechlorinated tap water survived throughout the experiment. Both median lethal times (LT_{50}) (Table 1) and median lethal concentrations (LC_{50}) (Table 2) increased with decreases in mean exposure of concentrations and times. The study showed the LC_{50} values were 17.65, 9.39, 16.07, 2.49 and 3.05 mg/L for *P. reticulata*, *R. sumatrana*, *T. tubifex,* and *C. javanus,* respectively (Table 2). As the concentration was lowered, the survival time increased dependently. Results showed that arsenic contaminants are most toxic to *T. tubifex,* while *P. reticulata* was the least sensitive to arsenic compared to the other freshwater organisms in this study, in this particular order; *P. reticulata* < *R. sumatrana* < *C. javanus* < *T. tubifex*. Under metal exposure, organism mortality mostly occurred during the first 48 h, as inferred from the resultant LC_{50} values that drastically decreased between 24 and 48 h exposure, while the differences between 72 and 96 h LC_{50} values were smaller as the organisms can better tolerate the arsenic concentrations.

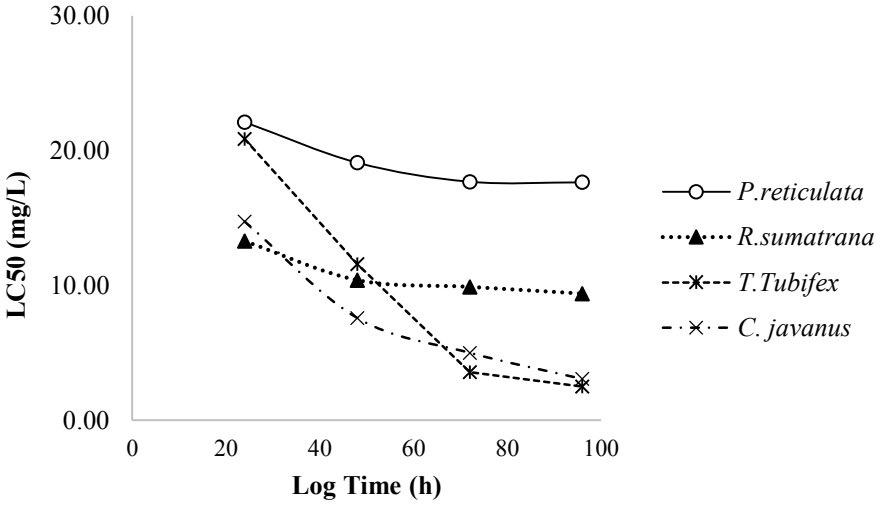

Figure 1. The relationship between median lethal concentration (LC_{50}) and exposure time (h) *for P. reticulata, R. sumatrana, T. tubifex* and *C. javanus* exposed to different concentrations of arsenic.

The results of acute toxicity tests using four aquatic species (Table 2) showed that *T. tubifex* is the most sensitive species, while *P. reticulata* is the most resistant species to arsenic contaminants compared with to the other species in the order of *T. tubifex* > *C. javanus* > *R. sumatrana* > *P. reticulata*. Metal toxicity varies between organisms as the rate of uptake of metals affect the toxicity of the metal itself. It occurs when the accumulation of metal happening in unwanted sites of an organism exceeds the rate of excretion and detoxification. As a result, it will disrupt the function of important molecules in the body [29].

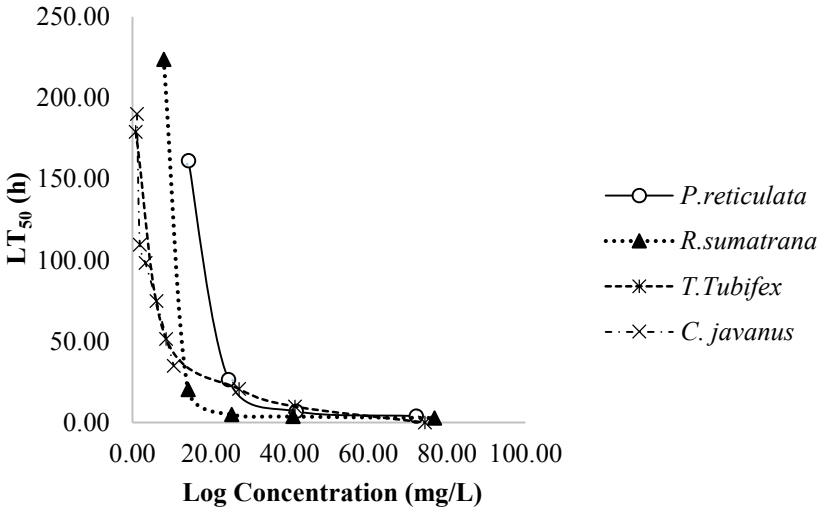

Figure 2. The relationship between median lethal time (LT_{50}) and different concentrations (mg/L) for *P. reticulata, R. sumatrana*, *T. tubifex* and *C. javanus* exposed to different concentrations of arsenic.

Various behavioral abnormalities were observed during the experimental exposure of arsenic to tested fishes, diptera and oligochaete. In the first few minutes of exposure to arsenic concentrations, jumping out from the test solutions, rapid movement and erratic swimming of the fishes were observed. Fishes and mollusk release cloudy mucus into the solutions, especially in higher concentrations of arsenic. While in lower concentrations of arsenic, no significant abnormal reactions or little were observed. The concentration of metal exposure relates closely to such behavioral observations of the aquatic organisms. There are similarities between this present study with Akter et al. [30], which had observed abnormal reactions such as rapid movement of operculum, excess secretion of mucus, erratic swimming, jumping out from the test solutions and etc. during exposure to arsenic. Such behaviors are caused by neurotoxic effects and also by irritation to the perceptive system of the body in reaction to the test solutions [31]. Arsenic enters the food chain directly through dietary and non-dietary channels, and indirectly through epithelia and skin uptake. Gills, skin, and the digestive tract are potential sites for the absorption of water soluble arsenic species for fishes [14]. Fish are ideal organisms to work with in toxic response studies due to the rigid fish models to establish biomarkers of exposure [32].

Experimental results showed that *P. reticulata* is a more resistant species of fish (LC_{50} 96 h 17.65 mg/L) to arsenic contaminants compared to *R. sumatrana* (LC_{50} 96 h 9.39 mg/L). The difference in toxicity tolerance may be due to different fish species. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in animals. It was also showed that *R. sumatrana* is more sensitive to all eight metals compared to *P. reticulata* [33]. This indicates that different organisms have different sensitivity levels to metal toxicity. Since fishes respond to toxicants in a similar way as higher vertebrates, they can be used to screen for chemicals that are potentially harmful to humans [32]. Our 96 h LC_{50}, 17.65 and 9.39 mg/L for *P. reticulata* and *R. sumatrana* respectively was lower to the range of the 96h LC_{50} of arsenic to freshwater tilapia (71.7 mg/L) reported by Hwang and Tsai [34]. The present study's result for the 96 h LC_{50} of arsenic to both species of fishes was also lower compared to the range of the 96 h LC_{50} of arsenic to the rainbow trout *Oncorhynchus mykiss* (23–26.6 mg/L) [35], the bluegill *Lepomis macrochirus* (29–35 mg/L), the stonefly *Pteronarcys californica* (38 mg/L) [36], and the Perch *Anabas testudineus* (18.211 mg/L) [30]. Other studies on 96h LC_{50} of arsenic to *O. latipes* resulted in 14.6 mg/L for *P. reticulata* and *R. sumatrana* [37]. The toxicity levels reported by other studies differ from that reported in this study, owing to the different species, ages, and sizes of organisms used, as well as the varied test methods (water quality and water hardness) [38]. Landrum et al. reported that the rapid metabolism process in smaller fishes compared to larger fishes may result in lower metal accumulation and toxicity [39]. Other than that, high surface area for smaller fishes may affect toxicity as the skin serves as an important arsenic absorbing site [14].

The physical state, solubility and purity of arsenic compound influences toxicity. Arsenic toxicity is correlated with temperature. Lower pH also increases toxicity due to As^{3+} formation [40]. The oligochaete acts differently compared to other organisms in this study, as the exposure of arsenic occurs through skin absorption while the toxic action is due to the formation of a mucus-metal complex which precipitates on the body wall of worms and blocks the exchange of oxygen and carbon dioxide [41]. The present study showed that *T. tubifex* is the most sensitive species to arsenic exposure with the LC_{50} 96 h 2.49 mg/L. During the experiment, the controls remained active throughout the test period. They clustered at the bottom of the dishes with typical movements. In the arsenic treated beaker, *T. tubifex* remained separated at the beginning of the experiment and showed

rapid twisting movements. Toxic response can be seen when the oligochaete showed reduced tactile movement. Before their death, necrosis and disintegration of the body as described by Khangarot [42], no other noticeable signs were observed. Their hemoglobin completely disappeared and the rear part of the body became white and disintegrated. Direct comparison of toxicity values obtained in this study with those in the literature is difficult because of the differences in the characteristics (primarily water hardness, pH, and temperature) of the test waters. The LC_{50} value reported by Khangarot [42] at 8.87 mg/L is greater than the current report. In the present study, water hardness was considered low (29.8 mg/L $CaCO_3$) and the water was categorized as soft water (< 75 mg/L as $CaCO_3$), while in the other study, the water hardness level (245 mg/L $CaCO_3$) was categorized as hard. This has known to give effect as water hardness decreases as the toxicity of metals increase [43]. The sensitivity of chironomids depend on environmental conditions. The temperature effect on metal toxicity appears to be contradictory. Generally, temperature and toxicity are positively correlated for most chemicals [44]. It is clear that temperature has an important role in the toxicity of metals. Jeyasingham and Ling [45] discovered that LC_{50} 96 h for *C. zealandicus, C. sp.a* and *C. pavidus* were 50.0, 13.0 and 33.1 mg/L respectively compared to the present study where the LC_{50} 96h was much lower (3.05 mg/L). The same relationship was observed with *C. zealandicus* collected during summer which were more resistant than those collected during winter, as the water temperature was lower than during summer. Other than that, genetics differences among laboratory stock, life stage and natural populations are also reflected in different sensitivities to environmental stress.

Experimental results show that *T. tubifex* is the most sensitive compared to the other three species organisms. This indicates that *T. tubifex* is a potential bioindicator of arsenic pollution and is suitable as a toxicity-testing organism in assessing the effect of arsenic to human beings.

Table 1. Median lethal times (LT_{50}) for *P. reticulata*, *R. sumatrana*, *T. tubifex,* and *C. javanus* exposed to different concentrations of arsenic.

Nominal Concentration (mg/L)	Measured Concentration (mg/L)	LT_{50} (h)	95% confidence limits	Nominal Concentration (mg/L)	Measured Concentration (mg/L)	LT_{50} (h)	95% confidence limits
P. reticulata				*R. sumatrana*			
10	8.07	Na	Na	10	7.95	224	26–1927
18	14.26	161	60–437	18	14.15	20	8–50
32	24.44	27	17–41	32	25.21	5	3–8
56	41.70	7	5–9	56	40.86	2	2–6
100	72.26	4	3–5	100	76.91	2	2–5
C. javanus				*T. tubifex*			
1	1.17	190	76–474	1	0.83	179	61–527
1.8	1.90	109	78–155	10	8.55	52	39–69
3.2	3.32	98	67–144	32	27.10	21	14–30
5.6	6.10	75	54–103	50	41.34	10	8–12
10	10.48	35	26–46	100	74.44	Na	Na

Na: Not available

Table 2. Median lethal concentrations (LC_{50}) for *P. reticulata*, *R. sumatrana*, *T. tubifex* and *C. javanus* exposed to different concentrations of arsenic.

Time (h)	LC_{50} (mg/L)	95% confidence limits	Time (h)	LC_{50} (mg/L)	95% confidence limits
P. reticulata			*R. sumatrana*		
24	22.10	20–26	24	13.28	10–27
48	19.11	16–22	48	10.39	7–13
72	17.69	15–20	72	9.88	7–13
96	17.65	14–21	96	9.39	7–12
C. javanus			*T. tubifex*		
24	14.74	11–1133	24	20.87	17–25
48	7.58	6–11	48	11.56	7–16
72	4.99	4–7	72	3.55	2–6
96	3.05	2–4	96	2.49	1–4

4. Conclusion

It is concluded that *T. tubifex* is most sensitive to arsenic exposure compared to other freshwater organisms used in this present study in the order of *P. reticulata* < *R. sumatrana* < *C. javanus* < *T. tubifex*. All of these observations show that different organisms and metals have different patterns in metal accumulation and toxicity, which depend on various factors such as types of species, physiology and environmental conditions. Therefore, the data gained from the laboratory experiments for each species are important in helping us understand the relationship between arsenic concentrations in the environment and toxicity effects to organisms before the organisms can be used as a bioindicator in the field for toxicity testing in Malaysia. Further studies using different species from different taxa is recommended for better understanding to protect the Malaysian freshwater aquatic system

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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