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Heavy Metal Exposures on Freshwater Snail *Pomacea insularum*: Understanding Its Biomonitoring Potentials

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Abstract: The present investigation focused on the toxicity test of cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn), utilizing two groups of juvenile and adult apple snail *Pomacea insularum* (Gastropod, Thiaridae) with mortality as the endpoint. For the adult snails, the median lethal concentrations (LC_{50}) values based on 48 and 72 h decreased in the following order: Cu < Ni < Pb < Cd < Zn. For the juvenile snails, the LC_{50} values based on 48 and 72 h decreased in the following order: Cu < Cd < Ni < Pb < Zn. The mussel was more susceptible to Cu than the other four metal exposures, although the juveniles were more sensitive than the adults because the former had lower LC_{50} values than the latter. This study provided essential baseline information for the five metal toxicities using *P. insularum* as a test organism, allowing comparisons of the acute sensitivity in this species to the five metals. In conclusion, the present study demonstrated that *P. insularum* was a sensitive biomonitor and model organism to assess heavy metal risk factors for severe heavy metal toxicities. A comparison of the LC_{50} values of these metals for this species with those for other freshwater gastropods revealed that *P. insularum* was equally sensitive to metals. Therefore, *P. insularum* can be recommended as a good biomonitor for the five metals in freshwater ecosystems.

Keywords: metal exposures; biomonitoring; freshwater snails; stresses

1. Introduction

Metals are generally thought to be pollutants, although it is crucial to note that they are naturally occurring compounds. Nevertheless, anthropogenic activities have resulted in higher quantities of heavy metals in environmental matrices and living resources, which surpass natural background values [1,2].

Metals are nonbiodegradable, unlike organic insecticides, they cannot be decomposed into less hazardous components. To effectively manage metal pollution, the concentration dependence of toxicity must be understood. Dose-response relationships serve as the foundation for assessing the dangers and risks associated with environmental contaminants. Toxicity testing is an indispensable method for analyzing the effect and fate of toxicants in aquatic environments, and it has been widely used to select acceptable organisms as bioindicators/biomonitors, and to develop water quality standards for chemicals. There are numerous methods for measuring toxicity, but mortality is the most common endpoint [3,4]. Consequently, it is essential to perform studies with local organisms that may be utilized to obtain data on metal toxicity, to assess the sensitivity of the organisms and to derive an acceptable level for Malaysian water that can protect the local aquatic populations.

Mollusks have been considered attractive bioindicator and biomonitoring subjects for quite some time. They are abundant in numerous terrestrial and marine environments, and are readily collected. They display a high buildup of contaminants, particularly heavy metals [5]. The snail *Pomacea* (family: Ampullaridae) was chosen for testing because of its vast distribution and abundance in aquatic environments, such as rivers, paddy fields, lakes and ponds. Melo et al. [6] suggested that the selection of a test species for toxicity testing is essential for a precise evaluation of environmental impact. The organism must: (a) belong to an important ecological group in terms of taxonomy, trophic level or niche; (b) be widely available in its environment, easily cultivated in the laboratory; (c) have a consistent and measurable response to the toxicant; and (d) be genetically stable. Finally, investigators must be familiar with the organism's physiology, genetics, taxonomy, behavior, etc. *Pomacea insularum* appears to fulfil all of these conditions, with the exception of a database. In the scientific literature, little is known about the hazardous effects of metals on this snail. However, a large body of literature has been published on the toxicity testing of heavy metals employing freshwater snails; Abdel Gawad [7] on *Theodoxus niloticus*, Ab-del-Moati and Farag [8] on *Lanistes bolteni*, and Shuhaimi-Othman et al. [9] on *Melanoides tuberculosus*.

Cadmium (Cd) is a non-essential element which is toxic to organisms, even in trace amounts [10], and it could pose renal toxicity issues in humans in elevated accumulation [11]. Cd accumulates to high levels in various aquatic creatures due to its high solubility in water [10,12]. Copper (Cu), on the other hand, is a vital metal for many animals, including humans, but slightly above the threshold would result in extreme toxicity for aquatic organisms [13,14]. Nickel (Ni)'s biological functions in animals and humans are not well studied, but elevated quantities may have carcinogenic consequences [15–18]. For numerous species of microorganisms and plants, Ni is essential for growth and development [18–20]. Elevated lead (Pb) has been linked to a variety of malignancies, cardiovascular illness, central nervous system disorders, liver and kidney damage, hearing impairment in children and newborns [21–23] and inhibition of enzyme activities [24,25]. Iron (Fe) is a component of hemoglobin in red blood cells, while zinc (Zn) is an important nutrient for plant growth, serving as a cofactor for over 300 proteins [26].

Considering the abovementioned impacts of the five potentially toxic metals (Cd, Cu, Ni, Pb and Zn) to humans, animals, plants and the environment, the goal of this study was to examine the toxicity of exposures to these metals by the use of juvenile and adult *P. insularum* as test organisms.

2. Materials and Methods

2.1. Sample Preparations and Laboratory Experiments

The snails, *P. insularum*, were collected from Universiti Putra Malaysia's Lake (N 02°59′58.84″, E 101°42′39.42″), on the 11 March 2007. The lake is considered unpolluted with low Cu concentration in the surface sediments (27.7 mg/kg dry weight) and with good surface water quality parameters; temperature (32.6 °C), conductivity (77.9 µS/cm), total dissolved solids (0.05 mg/L), dissolved oxygen (7.56 mg/L), pH (6.61) and turbidity (0.00 NTU) (unpublished data).

For acclimation, a maximum of 100 juveniles and 100 adult snails were kept per aquarium (30 cm length × 19 cm with × 18 cm height containing 6000 mL). During the acclimation period, the surface water quality parameters of the dechlorinated tap water in the plastic aquarium tanks ranged from 28.0 to 31.0 °C for temperature, 1.00–5.00 µS/cm for conductivity, 0.01–0.03 mg/L for total dissolved solids, 6.50–7.50 mg/L for dissolved oxygen, 6.70–7.10 for pH and 0.00 NTU for turbidity (unpublished data). Each aquarium was also regularly aerated to give a moderate airflow, in which the snails were acclimatized to laboratory settings for three days. To eliminate any potential bias in the experiments, the snails were divided into two groups, namely juvenile (shell lengths: 0.50 to 0.70 cm) and adult (shell lengths: 1.50 to 2.20 cm) snails for the experimental toxicity study.

Prior to static toxicity testing, range-finding-tests following the standard methods [27] were conducted to determine the critical range, which is defined as the interval between the lowest concentration of metals that kills very few or none of the experimental snails and the highest concentration that kills the majority or all of the experimental snails during the experimental period. The purpose of these tests was to determine the median lethal doses (LC_{50}) of each metal in the snails exposed for 24, 48, 72 and 96 h (h).

Following the range-finding-tests, five nominal concentrations of Cu, Cd, Zn, Pb and Ni were chosen (Table S1). Metal solutions were prepared by diluting the metal stock solutions with dechlorinated tap water to respective nominal concentrations of the five metals. Dechlorinated tap water was used as the negative control. The control samples comprised snails which had not been exposed to any metal solutions and were treated with the identical experimental conditions as the experimental samples. The tests were carried out under static conditions, without renewal of the solution until the end of the experiment. The control and metal-treated groups each consisted of two replicates of 10 healthy snails in a plastic aquarium tank of 21 cm length × 13 cm with × 11.5 cm height, containing 1000 mL of the respective nominal concentrations of the five metals. During the experimental exposure period, the surface water quality parameters in the plastic aquarium tanks were 28.0–30.2 °C for temperature, 1.00–3.00 µS/cm for conductivity, 0.01–0.05 mg/L for total dissolved solids, 6.50–7.50 mg/L for dissolved oxygen, 6.50–7.05 for pH and 0.00 NTU for turbidity (unpublished data).

The standard stock solutions (100 mg/L) of Cd, Cu, Ni, Pb and Zn, were prepared from analytical grade metallic salts of $CdCl_2 \cdot 2.5H_2O$, $CuSO_4 \cdot 5H_2O$, $NiSO_4 \cdot 6H_2O$, $Pb(NO_3)_2$ and $ZnSO_4 \cdot 7H_2O$, respectively (Merck, Darmstadt, Germany), by diluting with deionized water in 1 L volumetric flasks. Acute Cu, Cd, Zn, Pb and Ni toxicity experiments were performed for a four-day (96-h) period using juvenile and adult snails, obtained from stocking tanks. The snail death rates were determined at 24-, 48-, 72- and 96-h periods. The deceased snails were removed from the experimental tanks at each period. The snails that did not recover after being placed in a tank with pure freshwater were considered dead.

No stress was observed for the snails in the solution, indicated by 100% survival for the snails in the control water until the end of the experiment. A total of 10 animals per treatment/concentration were used in the experiment, and a total of 300 healthy juveniles and 300 healthy adult snails were used in the present experimental toxicity study. All the procedures of toxicity testing were modified from Adam [4], Melo et al. [6], Abdel Gawad [7], Abdel-Moati and Farag [8] and Shuhaimi-Othman et al. [9]. Water samples for metal analysis were taken before and after the experimental study. The collected water samples were immediately acidified to 1% with ARISTAR nitric acid (65%) (BDH Inc., VWR International Ltd., Lutterworth, UK) before metal analysis by flame atomic absorption spectrophotometer (FAAS-Perkin Elmer model AAnalyst800, Waltham, MA, USA). The detection limits of the FAAS for Cd, Cu, Ni, Pb and Zn were 0.009, 0.010, 0.010, 0.009 and 0.007 mg/L, respectively.

2.2. Statistical Analysis

The mean values and standard deviations were calculated using Microsoft Excel 2003. The data and median lethal concentration (LC_{50}) values for the toxicity test were examined

utilizing Probit Analysis Biostat 2007 Professional Package 3.7 (Informer Technologies, Inc., Los Angeles, CA, USA).

3. Results

3.1. Toxicity Tests

Using mortality as an endpoint, studies on the toxicity and tolerance of heavy metals in *P. insularum* were conducted by short-term toxicity experiments. Table 1 provides a comparison of the LC_{50} values of five metals between juveniles and adults of *P. insularum*. For the adult snails, the 48-h LC_{50} concentrations (mg/L) of Cd, Cu, Ni, Pb and Zn were 24.73, 3.10, 10.73, 17.24 and 57.99, respectively, while the 72-h LC_{50} concentrations (mg/L) were 11.7, 1.84, 6.88, 11.45 and 26.97, respectively. For the juvenile snails, the 48-h LC_{50} concentrations (mg/L) of Cd, Cu, Ni, Pb and Zn were 3.67, 0.94, 4.77, 10.44 and 30.16, respectively, while the 72-h LC_{50} concentrations (mg/L) were 2.15, 0.50, 3.01, 8.35 and 11.36, respectively.

Table 1. Comparisons of the LC_{50} values (mg/L) of Cd, Cu, Ni, Pb and Zn in both juveniles and adults *Pomacea insularum*, in 48 h and 72 h of exposure, with their standard errors (SE), upper confidential limits (UCL) and lower confidential limits (LCL) of the obtained LC_{50} values.

	Snails	**Juvenile**	**Juvenile**	**Adult**	**Adult**
	Periods	**48 h**	**72 h**	**48 h**	**72 h**
Cd	LC_{50}	3.67	2.15	24.73	11.71
	SE	0.52	0.40	3.64	1.78
	LCL	2.63	1.34	17.38	8.07
	UCL	4.71	2.96	32.09	15.35
Cu	LC_{50}	0.94	0.50	3.10	1.84
	SE	0.21	0.28	0.56	0.58
	LCL	0.53	−0.10	1.98	0.68
	UCL	1.36	1.09	4.22	3.00
Ni	LC_{50}	4.77	3.01	10.73	6.88
	SE	1.16	1.32	1.37	1.42
	LCL	2.42	0.32	7.95	3.90
	UCL	7.12	5.70	13.50	9.85
Zn	LC_{50}	30.16	11.36	57.99	26.97
	SE	4.81	3.50	8.22	5.32
	LCL	20.43	4.19	41.18	15.84
	UCL	39.90	18.52	74.80	38.10
Pb	LC_{50}	10.99	8.35	17.24	11.45
	SE	1.29	1.14	1.99	1.31
	LCL	8.39	6.04	13.22	8.80
	UCL	13.59	10.66	21.25	14.09

After 48-h exposure, the juvenile snail groups were most sensitive to Cu, followed by Cd > Ni > Pb > Zn, whereas the adult snail groups were most sensitive to Cu, followed by Ni > Pb > Cd > Zn. In addition, after 72-h exposure, the juvenile snails were most sensitive to Cu > Cd > Ni > Pb > Zn, while the adult snails were most sensitive to Cu, then Ni > Pb > Cd > Zn. According to Table 1, the juvenile snails were more sensitive and less tolerant to all metals (Cu, Ni, Pb, Zn and Cd) than adult snails.

The LC_{50} values based on 48 and 72 h declined in the following order for adult snails: Cu > Ni > Pb > Cd > Zn. The LC_{50} values based on 48 and 72 h declined in the following order for juvenile snails: Cu > Cd > Ni > Pb > Zn.

3.2. Changes in Observed Behavior and Morphology

During the experiment, the behavioral and morphological alterations in snails exposed to varying quantities of Cu, Zn, Cd, Pb and Ni were observed. When the snails were

exposed to lower concentrations of these metals, they could move up and down, stretch their bodies from their shells, and attach themselves to the wall of the plastic container. The majority of the snails survived the experimental period, which corresponded well with the findings of Khangarot and Ray [28]. At moderate concentrations of these metals, the snails secreted mucus with a decreased movement rate. They were inert, incapable of attaching their feet or closing their operculum, and unable to retract their bodies into their shells. The bodies of the snails were mostly exposed in plastic containers. They produced a great deal of mucus, and their feet stretched from their shells but could not retract. Generally, they sunk to the bottom of the plastic containers and became immobile.

The crawling or movement of snails during the experimental periods in an attempt to escape the experimental aquaria was a major drawback of this static toxicity test. As a result, high metabolic rates may have resulted in a large uptake of toxicants at that time, or the snails' activity in a starving condition may have decreased their resistance to toxicants. The size and age of the animals utilized in the toxicity test were additional variables that affected the LC_{50} values. It is well known that as an animal ages, its toxicity and tolerance to metals diminish. Cd and Cu concentrations in the soft tissues of freshwater clams have been found to similarly decrease with increasing age [29]. Young snails may be more sensitive to some metals due to their increased accumulation rates. Wier and Walter [30] revealed that juvenile *Physa gyrina* snails were three times as sensitive to Cd as their mature counterparts. Therefore, larger animals have greater resistance than smaller ones. The present study, using snails of two different sizes, confirmed the predicted result. On the other hand, these snails have an operculum to protect them when the water around them becomes toxic.

In the present experiment, *P. insularum* developed a white slime when they were exposed to high concentrations of Cd (19.98 mg/L) and Cu (4.08 mg/L). Based on the physiological reactions, Ravera [31] discovered that *Biomphalaria glabrata* was more resistant to heavy metals after 24 h of exposure. According to their observations, snails sank to the bottom with their operculum closed, expelled bubbles and slowed down their metabolic processes. When exposed to high levels of Cd and Cu, the snails could not adhere to the tank walls because the metals impede cell dynamics and injure the snails' tissues and cells [32]. The metals eventually infiltrate the cells, resulting in cell necrosis and snail death [33]. Mule and Lomte [34] showed that exposing the freshwater snail *Thiara tuberculosa* to $CuSO_4$ and $HgCl_2$ decreased their oxygen consumption, and the heavy metal absorption from the medium dropped.

4. Discussion

4.1. Cu Is the Most Toxic Metal

The results showed that the mortality of the snails increased as they were exposed to increasing concentrations of metals or for longer durations. Cu was the most toxic metal. The snails were most susceptible to Cu with the lowest LC_{50} values compared to other metals. Comparing the LC_{50} values of Cd and Cu for *P. insularum* with those of other snail species (>10 species), including mussels, clams, sea anemones, cockles and shrimp, revealed that *P. insularum* was more sensitive to Cu than Cd, Ni, Pb and Zn. This is well indicated in the different species of bivalves and gastropods from the literature (Tables 2–6).

Even though juvenile snails were more sensitive to the five metals, Cu was shown to be the most toxic metal for both juvenile and adult snails, when compared to Ni, Pb, Cd and Zn. This is consistently correlative with research on the toxicity of heavy metals to freshwater organisms. For instance, the rank order of toxicity of some heavy metals to *Daphnia magna* was Cu > Zn > Cd > Pb > Ni (48 h) [35]; for rainbow trout (*Salmo gairdneri*) it was Cu > Zn > Cd > Pb > Ni (96 h) [35]; for amphibian tad-poles (*Bufo melanostictus*) it was Cu > Cd > Zn > Ni (96 h) [36]; and for *Lymnaea luteola* it was Cu > Cd > Ni > Zn (72 h) [28].

The findings of the present study showed that the LC_{50} values in the five metals significantly decreased ($p < 0.05$) from 48-h to 72-h periods in both juvenile and adult snails. This indicated that the longer period of toxicity testing resulted in the snails being more

sensitive to the five metal toxicities. Taylor et al. [37] reported that the LC_{50} values of Cu in *Gammarus pulex* decreased from 0.047 to 0.037 after 48-h and 96-h periods, respectively. Similarly, they also found that the LC_{50} values of Cu in *Chironomus riparius* decreased from 1.20 to 0.70 after 48- and 96-h periods, respectively. Using *P. canaliculata* as the test organism, Dummee et al. [38] demonstrated that the LC_{50} values of Cu exposure periods of 4, 48, 72 and 96 h were 0.330, 0.223, 0.177 and 0.146 mg/L, respectively. This indicates a decreasing order of LC_{50} values with increasing Cu exposure period. All of these data demonstrated that the longer the duration of exposure, the more sensitive the invertebrates to pollutants.

Brix et al. [39] showed that the 96-h LC_{50} value of Cu in *Lymnaea stagnalis* was 31 g/L, indicating a moderate acute sensitivity to Cu. However, the projected EC_{20} value (the median effective concentration of a substance to 20% of test organisms) for Cu after a 30-day chronic exposure of juvenile *L. stagnalis* to Cu was 1.8 mg/L, making it the most sensitive organism to Cu investigated to date. In a different experiment with adult freshwater snails, *Melanoides tuberculata*, Shuhaimi-Othman et al. [9] observed an increase in the median lethal times (LT_{50}) and concentrations (LC_{50}) of eight metals after four days of laboratory exposure. Cu was the most hazardous metal to *M. tuberculosis*, followed by Cd, Zn, Pb, Ni, Fe, Mn and Al.

Several investigations suggested that *Pomacea* snails were efficient bioindicators for Cu and Cd. *Pomacea canaliculata* has the capacity to acquire Cu from a variety of metals (20, 30, 45, 67.5 and 101.3 mg/L), but demonstrated behavioral control at Cu concentrations of 67.5 and 101.3 mg/L, as determined by Pena and Pocsidio [40]. This provided evidence for using the golden apple snail (whole tissue analysis) as a sublethal Cu biomonitor (0–45 mg/L). Additionally, Manzla et al. [41] reported acute toxicity of Cu and Cd on the hepatopancreas cells of *Helix pomatia* (toxicity of Cu > Cd). Hoang and Rand [42] showed that $CuCO_3$ was toxic to apple snails (*Pomacea paludosa*) due to the fact that Cu concentrations were higher in living snails than in dead snails. Their results indicated that apple snails could excrete deposited Cu [38]. They demonstrated that *Pomacea* was a suitable bioindicator and biomarker for Cu pollution biomonitoring in aquatic environments. Habib et al. [43] showed that *B. alexandrina* was a suitable organism for assessing Cd toxicity in freshwater environments based on short-term 96-h (LC_{50}) and long-term exposure to Cd.

The outcomes of the present study indicated that both the smaller and bigger snail populations displayed the same decreasing order of metal toxicity: Cu > Cd. The order of toxicity of these metal ions correlates well with the metal toxicity levels of other freshwater organisms. For amphibian tadpoles [36], *Daphnia magna* [35] and pulmonate snails [44], Cu was more poisonous than Cd.

In understanding the toxicity of Cu, Hoang and Rand [42] indicated that the carbonate content of snails may explain the potential toxicity of Cu carbonate to snails. This is because snails need carbonate for shell growth; their carbonate need is greater than that of fish. Cu carbonate may enter snails as Cu, and dissociate after entering the snails by biological and chemical processes. Carbonate would be accessible for shell formation, while Cu would accumulate in soft tissue. Hoang et al. [45] also showed that the majority of deposited Cu in juvenile apple snails (*Pomacea paludosa*) was concentrated in soft tissue (about 60% in the viscera and 40% in the foot), and the shell contained less than 4% of the total accumulated Cu. Nevertheless, a comparison of the absorption rate in aquatic organisms revealed that, generally, the uptake rate constant is Zn > Cd > Cu [46]. This gap is likely related to the four-day metal exposure duration in this investigation. Other factors that may influence the bioaccumulation of heavy metals in aquatic organisms include feeding habits [47], growth rate and age of the organism [5], and the bioavailability of the metals, which is highly dependent on water hardness, pH and acid-volatile sulfide [48]. Hoang and Rand [42] demonstrated that apple snails (*Pomacea paludosa*) accumulated more Cu from soil-water treatments than water-only treatments, implying that apple snails accumulate Cu from environmental media (sediment or water). The rate of increase in the weight of a snail's tissue and shell is typically greater than the rate of accumulation of metals in its body. Lau et al. [5] and Hoang et al. [45] showed that juvenile apple snails

collected Cu during the exposure period and excreted Cu during the depuration phase. Metals accumulated in animals can be stored without excretion, leading to high body concentrations (accumulators), or the metal levels in the body can be maintained at a low constant concentration (regulators) by balancing the uptake with controlled excretion rates [49].

4.2. Juvenile Snails Are More Sensitive to Metal Toxicity

Smaller snails (0.50 to 0.70 cm) were shown to be more sensitive and less tolerant to all metals (Cd, Cu, Ni, Pb and Zn) than larger snails (1.50–2.20 cm). Previous research has demonstrated that younger organisms are more susceptible to toxicity [50,51]. In a study on mussels, Yap et al. [51] demonstrated that the species was most susceptible to Cu, followed by Cd; however, the small size group was more sensitive than the large size group, as the small group had lower LC_{50} values. In addition, it should be emphasized that other environmental variables, such as water quality, might influence the toxicity of a metal [52], and, therefore, can contribute to discrepancies in reported results. Although a standard test on a single species may provide information on the environmental risks of a toxicant, one should not establish safe environmental levels for toxicants based on a small number of test species. As the tolerance of *Pomacea* to metals was influenced by chemical type and test duration, it is imperative that the toxicity test encompasses a wider range of species and exposure times in future studies.

If other aspects of the snail's life cycle had been researched, more information about its sensitivity to heavy metals would have been available. Wier and Walter [30] found that immature *Physa gyrina* snails were three times more vulnerable to heavy metals than their mature counterparts. Cheung and Lam [53] showed that the juvenile stage of *Physa acuta* freshwater snails was the most Cd-tolerant when compared to the embryo. Earlier life stages, such as embryos and larvae, were the most vulnerable to heavy metals, according to multiple investigations [54,55]. These results corroborated the present study's conclusion that snails of a lower size range (0.50–0.70 cm) were more vulnerable to all metals than snails of a larger size range (1.50–2.20 cm) (Cu, Ni, Pb, Zn and Cd). The juvenile stage was found to be more vulnerable to heavy metals than the later stages.

4.3. Comparisons of LC_{50} Values with Those of Other Species of Molluscs

It is difficult to compare the LC_{50} values of metals in this species with those in other gastropod species due to the varying ability of closely related taxa or species belonging to the same genus that inhabit the same environments to accumulate metals in water with varying hardness. Using adult *Theodoxus niloticus* snails, Abdel Gawad [7] reported the 96-h LC_{50} values for Zn, Fe, and Pb to be 12.199, 8.6 and 18 mg/L, respectively. These values grew as the duration of exposure decreased. Fe was the most hazardous element to the snail, followed by Zn and Pb.

The findings of this investigation on the sensitivity of *P. insularum* to the toxicity of heavy metals supported the notion that the susceptibility of an animal to heavy metal toxicity differed among species [55–58]. This is demonstrated by comparing the LC_{50} values of *P. insularum* to those of other species. For example, Arthur and Leonard [59] reported that the 96-h LC_{50} of Cu in *Physa integra* was 0.039 mg/L, which is lower than the 96-h LC_{50} in *P. insularum* in the present study, which was 0.21 mg/L of Cu. The variation may be due to the various test animal species, techniques, and environmental conditions. Throp and Lake [60] reported that the 96-h LC_{50} values of Cd in the freshwater shrimp *Paratya tasmaniensis* were 0.06 mg/L. However, in the present investigation, the 96-h LC_{50} values of Cd in *P. insularum* were 2.55 mg/L. In addition, Lam [61] reported that the 96-h LC_{50} values of the tropical freshwater snail *Radix plicatulus* were 2.55 mg/L of Cd, which was comparable to the 72-h LC_{50} values of Cd in juvenile *P. insularum* (2.15 mg/L) in the present study. Consequently, based on the preceding examples, it can be concluded that the susceptibility of different species [62] and several factors such as experiment procedures, the physical and chemical characteristics of the experimental conditions such as temperature,

DO, pH and water hardness [63], as well as the physiological, size, and age of the animals used, can influence the LC_{50} values in the toxicity study.

Multiple researchers have investigated the impact of environmental characteristics such as temperature, pH, and dissolved oxygen on the toxicity of heavy metals and published their findings in the scientific literature. In general, increasing respiration at higher temperatures directly increased toxicity. Moreover, high temperatures indirectly increase toxicity by reducing oxygen levels in water [64]. Temperature increases had a direct effect on the ramshorn snail, *Helisoma campanulatum*, and the pond snail, *Viviparus benghalensis*, according to Gupta et al. [65]. Eisler [58] also showed that at 20 °C, the mummichog was more vulnerable to Cd than at 5 °C. In contrast, it is well established that increased water hardness reduces the acute toxicity of metals [66]. However, as the temperature utilized in the present exposure investigation was constant, this abiotic parameter had no effect on the snails' toxicity and tolerance to heavy metals.

Cu is more hazardous than Zn and Hg to the two intertidal snails *Planaxis sulcatus* and *Trochus radiatus*, according to an acute toxicity test performed by Kulkarni et al. [67] using static bioassay procedures. The availability of heavy metals due to different anthropogenic metal inputs could be attributed to their metal toxicities [68].

For all metals, Shuhaimi-Othman et al. [9] found that (LC_{50} increased with decreasing mean exposure concentrations and periods. Cu was discovered to be the most hazardous metal to *M. tuberculosis*, followed by Cd, Zn, Pb and Ni. Other studies demonstrated divergent patterns in the toxicity of certain snails. According to Luoma and Rainbow [40], the rank order of metal toxicity varies among organisms; Khangarot and Ray [28–30] demonstrated that the order of toxicity was Cd > Ni > Zn in *Lymnaea luteola*,; Gupta et al. [69] and Gadkari and Marathe [70] showed that the order of toxicity was Zn > Cd > Pb > Ni in *Viviparus bengalensis*.

According to Shuhaimi-Othman et al. [9], the LC_{50} values of Cu, Cd, Zn, Pb and Ni for 48 and 96 h were 0.39, 11.85, 13.15, 10.99 and 36.46 mg/L, and 0.14, 1.49, 3.90, 6.82 and 8.46 mg/L, respectively. Metals' acute toxicity to *M. tuberculosis* was the subject of only a few studies. Nebeker et al. [71] showed that the 96-h LC_{50} value of Cu in *Fluminicola virens* was 0.08 mg/L, and that of Zn in *Physa gyrina* was 1.27 mg/L, which were lower than those reported by Shuhaimi-Othman et al. [9]. Bali et al. [72] and Mostafa et al. [73] reported 96-h LC_{50} values of Cu in *M. tuberculosis* were 0.2 and 3.6 mg/L, respectively, which were greater than those reported by Shuhaimi-Othman et al. [9].

Abdel Gawad [74] investigated the effect of different doses of Cd on the toxicity of *Corbicula fluminalis*. The 96-h LC_{50} and daily survival rates were evaluated to determine the acute toxicity. Their results indicated that the *C. fluminalis* mortality rate was proportional to the Cd concentration. After 96 h of exposure, the LC_{50} was 0.52 mg/L. After 96 h of exposure, the bioaccumulation value of the pollutant in the soft portions of the clam was greater than the comparable value in the shell.

Shuhaimi-Othman et al. [9] showed that the LC_{50} values in *M. tuberculata* were generally lower or comparable to those of other freshwater gastropods. It was difficult to make direct comparisons between the toxicity values found in this study and those in the literature due to changes in the test waters' properties (mainly water hardness, pH, and temperature). Different species, ages, and sizes of the organisms as well as different test methods (water quality and water hardness) can influence toxicity [50,75–78]. In the present investigation, the water hardness was low (18.7 mg/L $CaCO_3$), and the water was classified as soft (75 mg/L as $CaCO_3$).

The snail *M. tuberculata* was found to be less sensitive to metals compared to other species [9]. Von Der Ohe and Liess [79] demonstrated that 13 Crustacea taxa were among the most sensitive to metal compounds, and they concluded that Crustacea taxa are comparable to one another and to *Daphnia magna* in terms of sensitivity to organics and metals, and that mollusks have an average sensitivity to metals. Mitchell et al. [80] observed that the snail has a tightly sealed operculum, which enables it to tolerate desiccation and, presumably, also boosts its chemical tolerance.

Table 2. Comparison of LC_{50} values (mg/L) of Cd in *Pomacea insularum* with other mollusks reported in the literature.

Molluscs	Species	Water Hardness (mg L^{-1})	Live Stage	Test Duration	LC_{50} (mg/L)	References
Bivalves	*Donax faba*	29.9 ppt	Adult	96-h EC_{50}	0.99	Din and Ong [81]
	Anadara granosa	29.5 ppt	Adult	96-h EC_{50}	0.94	Din and Ong [81]
	Perna viridis	NA	NA	24-h EC_{50}	1.53	Yap et al. [45]
	Modiolus phillippinarum	NA	NA	96-h EC_{50}	0.02	Ramakristinan et al. [82]
Gastropods	*Lymnaea luteola*	195	Adult	48-h EC_{50}	2.10	Khangarot and Ray [28]
	Amnicola sp.	50	Adult	96-h EC_{50}	8.40	Rehwoldt et al. [83]
	Biomphalaria glabrata	100	NA	96-h EC_{50}	0.30	Bellavere and Gorbi [84]
	Viviparus bengalensis	180	NA	96-h EC_{50}	1.20	Gupta et al. (1981a) [69]
	Viviparus bengalensis	NA	NA	NA	2.54	Gadkari and Marathe [70]
	Aplexa hypnorum	45	Adult	96-h EC_{50}	0.09	Holcombe et al. [85]
	Physa fontinalis	NA	NA	96-h EC_{50}	0.08	Williams et al. [86]
	Radix plicatulus	NA	NA	96-h EC_{50}	2.50	Lam [62]
	Lymnaea luteola	195	Adult	72-h EC_{50}	1.60	Khangarot and Ray [28]
	Lymnaea luteola	195	Adult	96-h EC_{50}	1.52	Khangarot and Ray [28]
	Physa acuta	NA	NA	48-h EC_{50}	1.05	Cheung and Lam [48]
	Potamopygus antipodarum	NA	NA	96-h EC_{50}	0.72	Hall and Golding [87]
	Pomacea sp.	NA	NA	24-h EC_{50}	2.25	Piyatiratitivorakul et al. [88]
	Pomacea sp.	NA	NA	48-h EC_{50}	2.07	Piyatiratitivorakul et al. [88]
	Pomacea sp.	NA	NA	72-h EC_{50}	0.68	Piyatiratitivorakul et al. [88]
	Pomacea sp.	NA	NA	96-h EC_{50}	0.47	Piyatiratitivorakul et al. [88]
	Filopaludina martensi martensi	NA	NA	24-h EC_{50}	27.8	Piyatiratitivorakul and Boonchamoi [54]
	Filopaludina martensi martensi	NA	NA	48-h EC_{50}	5.01	Piyatiratitivorakul and Boonchamoi [54]
	Filopaludina martensi martensi	NA	NA	72-h EC_{50}	3.96	Piyatiratitivorakul and Boonchamoi [54]
	Filopaludina martensi martensi	NA	NA	96-h EC_{50}	2.33	Piyatiratitivorakul and Boonchamoi [54]
	Melanoides tuberculata	18.7	Adult	96-h EC_{50}	1.49	Shuhaimi-Othman et al. [9]
	Cerithedia cingulata	NA	NA	96-h EC_{50}	9.19	Ramakristinan et al. [82]
	Biomphalaria alexandrina	NA	NA	96-h EC_{50}	0.22	Habib et al. [43]
	Pomacea canaliculata	NA	NA	48-h EC_{50}	4.26	Huang et al. [89]
	Pomacea canaliculata	NA	NA	72-h EC_{50}	2.24	Huang et al. [89]
	Pomacea canaliculata	NA	NA	96-h EC_{50}	1.98	Huang et al. [89]
	Pomacea insularum (small)	65	Juvenile	48-h EC_{50}	3.67	This study
	Pomacea insularum (small)	65	Juvenile	72-h EC_{50}	2.15	This study
	Pomacea insularum (large)	65	Adult	48-h EC_{50}	24.73	This study
	Pomacea insularum (large)	65	Adult	72-h EC_{50}	11.7	This study

Note: NA = data not available.

Table 3. Comparison of LC_{50} values (mg/L) of Cu in *Pomacea insularum* with other mollusks reported in the literature.

Molluscs	Species	Water Hardness (mg/L)	Live Stage	Test Duration	LC_{50} (mg/L)	References
Bivalves	Clam *Donax faba*	NA	NA	96-h EC_{50}	0.93	Sommanee [90]
	Donax faba	29.9 ppt	Adult	96-h EC_{50}	0.20	Din and Ong [81]
	Anadara granosa	29.5 ppt	Adult	96-h EC_{50}	0.23	Din and Ong [81]
	Perna viridis	NA	NA	24-h EC_{50}	0.25	Yap et al. [45]
	Anadara granosa	NA	NA	48-h EC_{50}	0.29	Yap et al. [91]
	Modiolus phillippinarum	NA	NA	96-h EC_{50}	0.22	Ramakristinan et al. [82]
Gastropods	*Biomphalaria glabrata*	100	NA	96-h EC_{50}	0.04	Bellavere and Gorbi [84]
	Viviparus bengalensis (at 27.3 C)	180	NA	48-h EC_{50}	0.27	Gupta et al. [66]
	Viviparus bengalensis (at 27.3 C)	NA	NA	72-h EC_{50}	0.12	Gupta et al. [66]
	Lymnaea luteola	NA	NA	96-h EC_{50}	0.172	Mathur et al. [92]
	Physastra gibbosa	NA	NA	96-h EC_{50}	0.041	Skidmore and Firth [93]
	Melanoides tuberculata	NA	Juvenile	24-h EC_{50}	0.20	Bali et al. [72]
	Potamopyrgus jenkinsi	NA	Adult	96-h EC_{50}	0.08	Watton and Hawkes [94]
	Lithoglyphus virens	21	Adult	96-h EC_{50}	0.08	Nebeker et al. [71]
	Juga plicifera	21	Adult	96-h EC_{50}	0.015	Nebeker et al. [71]
	Lymnaea luteola	195	Adult	48-h EC_{50}	0.025	Khangarot and Ray [28]
	Lymnaea luteola	195	Adult	72-h EC_{50}	0.027	Khangarot and Ray [28]
	Lymnaea luteola	195	Adult	96-h EC_{50}	0.027	Khangarot and Ray [28]
	Biomphalaria glabrata	44	Adult	48-h EC_{50}	0.18	De Oliveira-Filho et al. [95]
	Melanoides tuberculata	NA	NA	48-h EC_{50}	3.60	Mostafa et al. [73]
	Pomacea sp.	NA	NA	24-h EC_{50}	4.84	Piyatiratitivorakul et al. [88]
	Pomacea sp.	NA	NA	48-h EC_{50}	1.85	Piyatiratitivorakul et al. [88]
	Pomacea sp.	NA	NA	72-h EC_{50}	0.92	Piyatiratitivorakul et al. [88]
	Pomacea sp.	NA	NA	96-h EC_{50}	0.12	Piyatiratitivorakul et al. [88]
	Pomacea paludosa	68	60 d	96-h EC_{50}	0.14	Rogevich et al. [96]
	Melanoides tuberculata	18.7	Adult	96-h EC_{50}	0.14	Shuhaimi-Othman et al. [9]
	Cerithedia cingulata	NA	NA	96-h EC_{50}	0.52	Ramakristinan et al. [82]
	Pomacea canaliculata	NA	NA	24-h EC_{50}	0.33	Dummee et al. [32]
	Pomacea canaliculata	NA	NA	48-h EC_{50}	0.22	Dummee et al. [32]
	Pomacea canaliculata	NA	NA	72-h EC_{50}	0.18	Dummee et al. [32]
	Pomacea canaliculata	NA	NA	96-h EC_{50}	0.15	Dummee et al. [32]
	Pomacea insularum (small)	65	Juvenile	48-h EC_{50}	0.94	This study
	Pomacea insularum (small)	65	Juvenile	72-h EC_{50}	0.50	This study
	Pomacea insularum (large)	65	Adult	48-h EC_{50}	3.10	This study
	Pomacea insularum (large)	65	Adult	72-h EC_{50}	1.84	This study

Note: NA = data not available.

Table 4. Comparison of LC_{50} values (mg/L) of Ni in *Pomacea insularum* with other mollusks reported in the literature.

Molluscs	Species	Water Hardness (mg/L)	Live Stage	Test Duration	LC_{50} (mg/L)	References
Bivalves	*Utterbackia imbecillis*	60	Juveniles	96-h EC_{50}	0.19	Keller and Lam [97]
	Utterbackia imbecillis	80	Juveniles	96-h EC_{50}	0.252	Keller and Lam [97]
	Hamiota perovalis	43	Juveniles	96-h EC_{50}	0.313	Gibson et al. [98]
	Villosa nebulosa	43	Juveniles	96-h EC_{50}	0.51	Gibson et al. [98]
Gastropods	*Amnicola* sp.	50	Embryo	96-h EC_{50}	11.4	Rehwodlt et al. [83]
	Amnicola sp.	50	Adult	96-h EC_{50}	14.3	Rehwodlt et al. [83]
	Viviparus bengalensis	180	NA	96-h EC_{50}	9.92	Gupta et al. [69]
	L. acuminata	375	NA	96-h EC_{50}	2.78	Khangarot et al. [99]
	Lymnaea stagnalis	100	Juveniles	96-h EC_{50}	0.9	Nebeker et al. [71]
	Physa gyrina	26	NR	96-h EC_{50}	0.239	Nebeker et al. [71]
	L. luteola	195	Adult	48-h EC_{50}	1.7	Khangarot and Ray [28]
	L. luteola	195	Adult	72-h EC_{50}	1.7	Khangarot and Ray [28]
	L. luteola	195	Adult	96-h EC_{50}	1.43	Khangarot and Ray [28]
	Melanoides tuberculata	18.7	Adult	96-h EC_{50}	8.46	Shuhaimi-Othman et al. [9]
	Leptoxis ampla	43	Juveniles	96-h EC_{50}	0.033	Gibson et al. [98]
	Somatogyrus sp.	43	Adult	96-h EC_{50}	0.301	Gibson et al. [98]
	Pomacea insularum (small)	65	Juvenile	48-h EC_{50}	4.77	This study
	Pomacea insularum (small)	65	Juvenile	72-h EC_{50}	3.01	This study
	Pomacea insularum (large)	65	Adult	48-h EC_{50}	10.73	This study
	Pomacea insularum (large)	65	Adult	72-h EC_{50}	6.88	This study

Note: NA = data not available.

Table 5. Comparison of LC_{50} values (mg/L) of Pb in *Pomacea insularum* with other mollusks reported in the literature.

Molluscs	Species	Water Hardness (mg/L)	Live Stage	Test Duration	LC_{50} (mg/L)	References
Bivalve	*Mussel Modiolus phillippinarum*	NA	NA	96-h EC_{50}	2.88	Ramakristinan et al. [82]
Gastropods	*A. hypnorum*	60.9	NA	96-h EC_{50}	1.34	Call et al. [100]
	Viviparus bengalensis	165	NA	96-h EC_{50}	2.54	Gadkari and Marathe [70]
	L. emarginata	150	NA	96-h EC_{50}	14	Cairns Jr et al. [101]
	E. livescens	150	NA	96-h EC_{50}	71	Cairns Jr et al. [101]
	Filopaludina sp.	NA	Adult	24-h EC_{50}	319	Jantataeme et al. [102]
	Filopaludina sp.	NA	Adult	48-h EC_{50}	271	Jantataeme et al. [102]
	Filopaludina sp.	NA	Adult	72-h EC_{50}	235	Jantataeme et al. [102]
	Filopaludina sp.	NA	Adult	96-h EC_{50}	192	Jantataeme et al. [102]
	Melanoides tuberculata	18.7	Adult	96-h EC_{50}	6.82	Shuhaimi-Othman et al. [9]
	Snail Cerithedia cingulata	NA	NA	96-h EC_{50}	15.5	Ramakristinan et al. [82]
	Freshwater snail Theodoxus niloticus	NA	Adult	96-h EC_{50}	18	Abdel Gawad et al. [7]
	Archachatina papyracea	Land snails	Adults	28-days EC_{50}	1121	Owojori et al. [103]
	Pomacea insularum (small)	65	Juvenile	48-h EC_{50}	10.44	This study

Table 5. *Cont.*

Molluscs	Species	Water Hardness (mg/L)	Live Stage	Test Duration	LC_{50} (mg/L)	References
	Pomacea insularum (small)	65	Juvenile	72-h EC_{50}	8.35	This study
	Pomacea insularum (large)	65	Adult	48-h EC_{50}	17.24	This study
	Pomacea insularum (large)	65	Adult	72-h EC_{50}	11.45	This study

Note: NA = data not available.

Table 6. Comparison of LC_{50} values (mg/L) of Zn in *Pomacea insularum* with other mollusks reported in the literature.

Molluscs	Species	Water Hardness (mg/L)	Live Stage	Test Duration	LC_{50} (mg/L)	References
Bivalves	*Corbicula fluminea*	64	NA	96-h EC_{50}	6.04	Cherry et al. [104]
	Actinonaias pectorosa	170	Glochidia	96-h EC_{50}	0.31	Cherry et al. [104]
	Medionidus conradicus	170	Glochidia	96-h EC_{50}	0.57	Cherry et al. [104]
	Phychobranchus fasciolaris	170	Juveniles	96-h EC_{50}	0.21	Cherry et al. [104]
	Utterbackia imbecillis	60	Juveniles	96-h EC_{50}	0.27	Keller and Lam [97]
	Utterbackia imbecillis	80	Juveniles	96-h EC_{50}	0.44	Keller and Lam [97]
	Utterbackia imbecillis	60	Juveniles	96-h EC_{50}	0.36	Keller and Lam [97]
	Utterbackia imbecillis	80	Juveniles	96-h EC_{50}	0.59	Keller and Lam [97]
	Villosa nebulosa	170	Glochidia	96-h EC_{50}	0.66	Cherry et al. [104]
	Actinonaias pectorosa	40	Juveniles	96-h EC_{50}	0.36–0.37	McCann [105]
	Actinonaias pectorosa	160	Juveniles	96-h EC_{50}	1.06–1.19	McCann [105]
	Villosa iris	50	Juveniles	96-h EC_{50}	0.34	McCann [105]
	Villosa iris	160	Juveniles	96-h EC_{50}	1.12	McCann [105]
	Villosa umbrans	43	Juveniles	96-h EC_{50}	1.30	Gibson et al. [98]
	Villosa nebulosa	43	Juveniles	96-h EC_{50}	0.44	Gibson et al. [98]
	Donax faba	29.9 ppt	Adult	96-h EC_{50}	3.61	Din and Ong [81]
	Anadara granosa	29.5 ppt	Adult	96-h EC_{50}	7.76	Din and Ong [81]
	Modiolus phillippinarum	NA	NA	96-h EC_{50}	2.34	Ramakristinan et al. [82]
Gastropods	*Helisoma campanulatum*	20	Adult	96-h EC_{50}	0.87–1.27	Wurtz [106]
	Helisoma campanulatum	100	Adult	96-h EC_{50}	1.27–3.03	Wurtz [106]
	P. heterostropha	20	Adult	96-h EC_{50}	1.11	Wurtz [106]
	P. heterostropha	100	Adult	96-h EC_{50}	3.16	Wurtz [106]
	Physa heterostropha	20	Juveniles	96-h EC_{50}	0.30–1.39	Wurtz [106]
	Physa heterostropha	100	Juveniles	96-h EC_{50}	0.43–1.39	Wurtz [106]
	Amnicola sp.	50	Adult	96-h EC_{50}	14.0	Rehwodlt et al. [83]
	Amnicola sp.	50	Embryo	96-h EC_{50}	20.2	Rehwodlt et al. [83]
	Viviparus bengalensis	180	NA	96-h EC_{50}	0.64	Gupta et al. [69]
	Lymnaea luteola	NA	NA	96-h EC_{50}	6.13	Mathur et al. [92]
	L. acuminata	375	NA	96-h EC_{50}	10.5	Khangarot et al. [99]
	Physa gyrina	36	Adult	96-h EC_{50}	1.27	Nebeker et al. [71]
	Lymnaea luteola	195	Adult	96-h EC_{50}	11.0	Khangarot and Ray [28]
	Lymnaea luteola	195	Adult	48-h EC_{50}	3.80	Khangarot and Ray [28]
	Lymnaea luteola	195	Adult	72-h EC_{50}	3.80	Khangarot and Ray [28]
	Lymnaea luteola	195	Adult	96-h EC_{50}	1.68	Khangarot and Ray [28]
	Lanistes bolteni	NA	NA	NA	58.0	Abdel-Moati and Farag [8]

Table 6. *Cont.*

Molluscs	Species	Water Hardness (mg/L)	Live Stage	Test Duration	LC_{50} (mg/L)	References
	Melanoides tuberculata	18.7	Adult	96-h EC_{50}	3.90	Shuhaimi-Othman et al. [9]
	Cerithedia cingulata	NA	NA	96-h EC_{50}	8.99	Ramakristinan et al. [82]
	Leptoxis ampla	43	Adult	96-h EC_{50}	0.07	Gibson et al. [98]
	Somatogyrus sp.	43	Adult	96-h EC_{50}	0.33	Gibson et al. [98]
	Theodoxus niloticus	NA	Adult	96-h EC_{50}	12.2	Abdel Gawad et al. [7]
	Pomacea insularum (small)	65	Juvenile	48-h EC_{50}	30.16	This study
	Pomacea insularum (small)	65	Juvenile	72-h EC_{50}	11.36	This study
	Pomacea insularum (large)	65	Adult	48-h EC_{50}	57.99	This study
	Pomacea insularum (large)	65	Adult	72-h EC_{50}	26.97	This study

Note: NA = data not available.

4.4. Implications from Biomonitoring Perspective

The use of small prosobranch snails, such as *P. insularum*, as one of the biological indicators in toxicity testing, offers several benefits. First, because these snails are prevalent in still (ponds) and running (streams) waters, they can be utilized as ecologically significant target species in both lotic and lentic environments. Secondly, they are affordable, easily harvested and manageable. In addition, they are sensitive indicators of dangerous amounts of heavy metals such as Cu, Pb, Cd, Ni and Zn identified in this study, comparable to that reported by Ravera [52] and Lam [62]. They are possibly more susceptible to metals than larger snails, *Brotia hainanensis*, because they possess the same trait [107]. For a realistic approach to pollution consequences, additional research on the acute and chronic toxicity of various environmental contaminants under various environmental and biological circumstances is necessary. It is also necessary to assess the combined toxicity of substances. The mechanisms of contaminants at the cellular and molecular levels in these animals must also be comprehended.

Under controlled laboratory conditions, Pyatt et al. [108] evaluated the effects of Pb (5 or 10 mg/L) on the survival of the freshwater snail *Lymnaea stagnalis* (L.) collected from lead-contaminated or uncontaminated environments. Significantly more animals from the polluted environment survived subsequent acute (up to 24 days) Pb exposure than animals from the unpolluted environment. Acute exposure to Pb (72 h) hindered various behavioral activities, including movement, eating, tentacle elongation and emerging from the shell. Pb bioaccumulated in snail tissues, specifically the buccal mass and the stomach. The freshwater snail is an excellent system for researching the bioaccumulation and development of environmental Pb tolerance.

Nebeker et al. [71] observed that three snail species from western Oregon were exposed to metals: *Juga plicifera* and *Lithoglyphus virens*, which occupy temperate coastal streams, and *Physa gyrina*, which inhabits ponds in the Willamette Valley. *J. plicifera* was subjected to Cu and Ni in laboratory flow-through testing, while *L. virens* was exposed to Cu, and *P. gyrina* was exposed to Ni and Zn. *J. plicifera* had a 96-h LC_{50} Cu value of 0.015 mg/L, and a no observable effect level (NOEL) of 0.006 mg/L (at which mortality was not substantially different from that in control groups) (30-d survival). The 96-h LC_{50} and NOEL for Ni in *J. plicifera* were 0.23 mg/L and 0.124 mg/L, respectively. The 96-h LC_{50} and NOEL for Cu in *L. virens* were 0.008 mg/L and less than 0.008 mg/L, respectively. The 96-h LC_{50} for Ni in *P. gyrina* was 0.239 mg/L, the 96-h LC_{50} for Zn was 1.274 mg/L and the NOEL for Zn was 0.570 mg/L.

Piyatiratitivorakul et al. [88] investigated the acute toxicity of Cd and Cu to *Pomacea* sp collected from Thailand. The findings revealed the possibility of using the freshwater

snail *Pomacea* sp. as a biomonitor for heavy metal levels in freshwater resources. Huang et al. [89] revealed the acute toxicity of Cd, in which the metal bioaccumulation in tissue was measured in *P. canaliculata* and its native competitor *Sinotaia quadrata* under experimental settings. The LC_{50} concentrations (mg/L) for the invasive species were 4.26, 2.08 and 1.98 after being exposed for 48, 72 and 96 h, respectively, which were approximately three times greater than those of the native species. The viscera gathered the highest concentration of Cd, followed by the foot and shell in both species. The metal concentrations in the aforementioned three tissues of *P. canaliculata* were significantly greater than those of *S. quadrata*, regardless of Cd dose and exposure time. They concluded that a high Cd tolerance, may partially explain *P. canaliculata*'s capacity to displace *S. quadrata* from Cd-contaminated habitats. Cd primarily accumulated in the hepatopancreas and kidneys of invading species, thus altering the activity of antioxidant enzymes and helping the animals to deal with the toxicity.

5. Conclusions

This investigation revealed that *P. insularum* exhibited the same metal sensitivity as other freshwater gastropods. Cu was the most harmful to *P. insularum*, followed by Cd, Zn, Pb and Ni. The acute toxicity tests revealed that *P. insularum* is more susceptible to Cu than Cd, Ni, Pb and Zn, which is consistent with the LC_{50} values reported in the literature for most invertebrate species. This study indicated that *P. insularum* may also be used as a biomonitor for acute and subacute Cd, Cu, Ni, Pb and Zn exposures. Since *P. insularum* is widely spread in urban and suburban regions, it is incredibly valuable for ecotoxicological research. This study demonstrated that *P. insularum* may be a biomonitor of potentially toxic metal contamination. Using *P. insularum* as a test organism, this study provided essential baseline data for PTM toxicity. Changes in the snail population due to PTM exposures may potentially influence the predation behavior of predators, which is an interesting area for future studies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13021042/s1, Table S1: Nominal and measured concentrations (mg/L) of Cd, Cu, Ni, Pb and Zn in the toxicity test for the *Pomacea insularum* of two different sized groups (Shell lengths, small 0.50–0.70 cm; large: shell 1.50–2.20 cm); Table S2: Mortality of individuals (*Pomacea insularum*) for the small sized group (Shell length: 0.50–0.70 cm) collected after four different periods of exposure to a series of different concentrations of Pb, Ni, Cd, Zn and Cu; Table S3: Mortality of individuals (*Pomacea insularum*) for the large-sized group (Shell length: 1.50–2.20 cm) collected after four different periods of exposure to a series of different concentrations of Cd, Cu, Ni, Pb and Cu, Zn.

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