Bulletin of Environmental Contamination and Toxicology (2021) 107:378–384
https://doi.org/10.1007/s00128-021-03196-6

Comparative Assessment of Cadmium and Copper Toxicity to *Physa acuta* (Draparnaud, 1805)

Emiliano Bálsamo Crespo[1,3] · Gustavo Bulus Rossini[1,2]

Received: 20 December 2020 / Accepted: 13 March 2021 / Published online: 28 March 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

Cadmium and copper toxicity was investigated using bioassays with neonates of a freshwater gastropod *Physa acuta*. Mortality, lethal time, and effects on shell length were studied during 28-day chronic exposure experiments. Relative toxicity was assessed from acute and chronic LC values. Copper showed significantly more toxicity at lower concentrations than cadmium ($p < 0.001$), causing mortality at the same response levels. Conversely, cadmium affected shell length at lower concentrations than copper, although no significant differences ($p > 0.05$) were found in affected shell length between copper and cadmium at the end of the assays. Lethal time (LT_{50}) was significantly affected by metal concentration ($p < 0.001$), with a reduction of 8.28% and 5.90% in time per 0.001 mg/L increase of copper and cadmium, respectively. *Physa acuta* neonates showed medium to high sensitivity to cadmium and copper compared to other freshwater gastropod species, showing it is a suitable test organism, particularly for chronic ecotoxicological assessment.

Keywords Freshwater gastropods · Heavy metals · *Physa acuta* · Acute exposure · Chronic exposure · Relative toxicity

Trace elements such as cadmium (Cd) and copper (Cu) occur in the environment either naturally (Bubach et al. 2015) or due to emissions from anthropogenic activities (Vareda et al. 2019). Since Cu has a key role in catalysis and electron transference as a cofactor of enzymes (Messerschmidt 2010), it is readily transported into cells, where it can cause cellular damage via oxidative stress or displacement of cofactors in proteins (Solioz 2018). Conversely, Cd is not an essential element, but it interferes with biochemical pathways by mimicking other essential elements (Bridges and Zalups 2005). These elements can accumulate in living organisms, which in turn act as vectors of contamination through bioaccumulation processes (Primost et al. 2017).

Gastropods have been widely used in biomonitoring studies (Primost et al. 2017) due to their ecological diversity and role in connecting terrestrial and aquatic ecosystems through food chains (Dodd et al. 2016). *Physa acuta* (Physidae, Draparnaud 1805) is a pulmonate freshwater gastropod found in lentic and lotic ecosystems (Saadi et al. 2020), commonly feeding on detritus and periphyton (Lowe and Hunter 1988). Native to North America, *P. acuta* has been introduced to other continents, and has expanded its distribution as the most cosmopolitan freshwater gastropod in the world (Dillon Jr. et al. 2002). *P. acuta* is an outcrossing protandrous simultaneous hermaphrodite. Eggs are laid in gelatinous coiled/curved masses, and individuals hatch as fully developed juveniles (Nuez 2010).

Despite having been used in many ecotoxicological experiments, most publications with chronic sensitivity assessments derived from mortality or growth of the shell of *P. acuta* were reported in the form of NOEC and LOEC endpoints (Arthur and Leonard 1970; Cheung and Lam 1998; Gao et al. 2017). Furthermore, when LC/EC values were estimated, these usually represented the sensitivity of adult forms (Cheung and Lam 1998; Spehar et al. 1978; Woodard 2005).

Earlier life stages of organisms are generally more sensitive to contamination (Hoang and Klaine 2007), and NOEC/LOEC statistics are progressively considered undesirable statistical endpoints (Warne and van Dam 2008).

✉ Emiliano Bálsamo Crespo
emilianobal@hotmail.com

1 Centro de Investigaciones del Medioambiente (CIM), CONICET, UNLP, Boulevard 120 N 1489, 1900 La Plata Buenos Aires, Argentina

2 Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC PBA), Buenos Aires, Argentina

3 Faculty of Science and Engineering, Southern Cross University, East Lismore, NSW 2480, Australia

Considering this, our objective was to perform a comparative assessment between the toxicity of Cd and Cu to neonates of *P. acuta* based on regression-derived endpoints. We conducted 28-day chronic bioassays to evaluate the effect of Cd and Cu exposure on the mortality and shell length of *P. acuta* neonates. Furthermore, we conducted a relative sensitivity analysis which allowed us to observe how the sensitivity of *P. acuta* to Cd and Cu compares with other freshwater snail species sensitivity data.

Materials and Methods

Individuals of *P. acuta* used in this study came from a well-established population in laboratory aquaria at the Faculty of Exact Science, National University of La Plata. Individuals were originally collected close to La Balandra (34° 56′ 6.27″S, 57° 44′31.35″W), Buenos Aires province in Argentina in 2012. Egg masses were collected from the aquaria and separated under controlled conditions: dechlorinated and oxygenated tap water (conductivity 1.0 mS/cm; hardness 180 mg/L $CaCO_3$; pH range 7.8 ± 0.2; temperature 20 ± 2°C), and photoperiod 16:8 light:darkness. Only 72-h-old neonates (shell length: 0.09 ±0.01 mm) were used to conduct 28-day chronic experiments. Seven concentrations (C1–C7) and a negative control (Ctrl) were tested and run in triplicate with 10 snails per 300 mL polyethylene container (i.e. 30 snails per concentration). Dilutions were renewed every 48 h. Negative controls contained the same water that was used to make treatment dilutions. Snails were fed with 1.5 mL of blended lettuce leaves on each renewal day.

Stock solutions were prepared on each renewal day with double distilled water, using $3CdSO_4{\cdot}8H_2O$ (Merck) and $CuCl_2{\cdot}2H_2O$ (Cicarelli), both ACS analytical grading. Nominal concentrations for Cd were: 0.005, 0.009, 0.022, 0.035, 0.048, 0.061, 0.075 mg/L, and for Cu were: 0.005, 0.007, 0.011, 0.019, 0.026, 0.030, 0.034 mg/L. Sets of samples were randomly collected at different days of renewal for later analysis. Since not every assay replicate and concentration was sampled at each medium renewal event, data of measured and nominal concentrations from each set of samples was used to calculate estimated concentrations by linear regression methods for each assay. Metal concentrations were measured using a Varian Spectr AA 330 atomic absorption spectrophotometer with air-acetylene flame (APHA 1998 Method 3111 B). Quality assurance and control comprised freshly prepared dilutions at the moment of renewal, and the calibration of equipment using certified reference solutions from Accustandard Inc. (Cd:AA08N-1, Cu: AA15N-5). The equipment detection limit for both metals was 0.005 mg/L. All labware was previously cleaned in a 10% HNO_3 bath, and stock solutions as well as assay samples were all refrigerated and acidified with HNO_3 (Analar) analytical grade for storage. Lethal effects were assessed by recording the number of dead snails every 48 hours, and shell length was measured every 7 days to assess chronic sublethal effects. Snails were considered dead if they did not move and no heart beat was observed after stimulation. Shell length was measured from the tip of the protoconch to the anteriormost end of the aperture with a 1/100 mm micrometer ocular. Endpoints were assessed with a stereoscopic microscope.

Statistical analysis was performed in R Core Team (2013). Lethal concentrations (LC) and effect concentrations (EC) for shell length were derived by fitting log-logistic models with the package *drc*. Control group mortality of < 10% was considered acceptable. Relative toxicity between Cd and Cu was estimated utilising the function *EDcomp()*, which models the ratio EC_{Cd}/EC_{Cu} as a function of the response level. EC and LC model parameters were compared with the function *compParm()* (Ritz et al. 2019). We retrieved acute LC values from Bulus Rossini (2013).

Additionally, we estimated LT_{50} values by fitting a two-parameter multinomial log-logistic model (LL.2, type event in *drc*), assuming all individuals would die at some infinite time interval. The trend of LT_{50} as a function of metal concentration in chronic exposure was analysed implementing the *metaphor* package via weighted linear regression of the pooled mortalities and their respective squared standard errors with the function *rma()* (Ritz et al. 2019). Model selection was performed based on Lack-of-fit test and Akaike Information Criterion (AIC) against Weibull models. Normality of residuals was assessed via Shapiro test or by visual assessment with QQ-plots or residual plots and homoscedasticity with Levene's test. When needed, power transformations, or the use of robust standard errors estimates were applied to address model misspecifications.

Relative sensitivity (RS) of *P. acuta* compared to other freshwater gastropods was calculated as RS = $\mathrm{Log}(LC_{50Pa}/LC_{50i})$, where LC_{50Pa} is the geometric mean of all available data for this species and its synonyms (including results from this study), and LC_{50i} represents the lethal concentration estimates for each *i* species. Data was collated from the USEPA ECOTOX database, and only compatible endpoints were used for comparisons. LC_{50} values were normalised to water hardness (100 mg $CaCO_3$/L) using the pooled regression slopes for Cd (USEPA 2016) and Cu (USEPA 2007). We are aware that Cu can be predicted more accurately via the Biotic Ligand Model (BLM), but given the lack of information on other required parameters, we considered the use of pooled slopes a convenient approach. All graphics were built with the *ggplot2* and *cowplot* packages in R.

Results and Discussion

On average, estimated concentrations (C1–C7) departed 26% and 19% from nominal values for Cd and Cu, respectively, and mortality in both experiments was dose dependant (Table 1).

Chronic LC values were derived from a four-parameter model with a free lower limit due to the incidence of one death in one control replicate of each metal assay (i.e. lower limit ≠ 0, mortality < 10%). EC values were derived by fitting a four-parameter log-logistic model with shell length data at day 28. The lack of fit test was not significant ($p > 0.05$) in all fitted models, and AIC was the lowest against Weibull models. Shapiro tests for residuals of EC models were not significant for Cd ($p = 0.7379$) or Cu ($p = 0.1839$). LC and EC values at 28 days and their respective 95% confidence limits are shown in Table 2. Bulus Rossini (2013) 4-day LC_{10} for Cd was 0.1720 (CI 0.1314/0.2125 mg/L), LC_{50} was 0.3849 (CI 0.3325/0.4373 mg/L), and LC_{90} was 0.8614 (CI 0.5875/1.1354 mg/L). Cu 4-day LC_{10} was 0.0922 (CI 0.0756/0.1088 mg/L), LC_{50} was 0.1624 (CI 0.1444/0.1804 mg/L), and LC_{90} was 0.2860 (CI 0.2358/0.3362 mg/L).

Data compilation from the ECOTOX database for Cu included 109 entries of 36 freshwater gastropod species, and 21 entries for species which are now considered synonyms with *P. acuta* (e.g. *P. heterostropha*) (Lydeard et al. 2016). Cd data comprised 52 entries for 16 species of freshwater gastropods, of which 5 entries were for *P. acuta* and synonyms (e.g. *P. integra*) (Lydeard et al. 2016). Most data comprised values for 2-, 3-, and 4-day acute exposure. Chronic values for Cu included 7 entries from four different species of gastropods, whereas Cd only had 3 chronic LC values for which only one entry belonged to a species other than *P. acuta* (Das and Khangarot 2010).

Relative toxicity analysis between Cd and Cu for acute and chronic endpoints show that metals are not equally toxic to *P. acuta* at most response levels (i.e. Cd/Cu ratio ≠ 1) (Table 3), and the toxicity ratio is not constant, increasing with the response level in different ways for acute and chronic effects (Fig. 1a, b).

Acute exposure data revealed that Cu had a more pronounced toxic effect than Cd. The Cd/Cu ratio was > 1 for all response levels with an increasing trend (Fig. 1a). The relative sensitivity analysis for acute exposure shows that most available data correspond to 2-day and 4-day exposure times. *P. acuta* sensitivity to Cu falls in the higher end of sensitivity compared to other freshwater gastropods, with the majority of the LC_{50} values falling behind that of *P. acuta* (Fig. 2b). Cd data showed a difference in spread between 2-day and 4-day LC_{50} values. Most freshwater gastropod species showed a similar level of sensitivity for a 2-day Cd exposure, with *P. acuta* falling in the middle range of sensitivity compared to other species. Conversely, 4-day exposure values displayed a wider spread, and *P. acuta* sensitivity sits in the middle-high end of the range compared to other freshwater snail species (Fig. 2a).

With respect to chronic lethal effects, we can observe an increasing mortality with an incipient higher toxicity from Cd, which eventually equates to that of Cu at the 25% response level. For higher response levels, Cu exerted a more toxic effect than Cd (Fig. 1b). This larger toxic effect was reflected in the significant difference detected for the slope

Table 1 Estimated concentrations of heavy metals in mg/L and their respective mortality (%) per group. Estimated values were derived by linear regression from measured concentrations and nominal values

Group	C1	C2	C3	C4	C5	C6	C7
Cd*	0.0037	0.0065	0.0162	0.0259	0.0356	0.0453	0.0550
Mortality	3%	7%	7%	14%	41%	72%	87%
Cu**	0.0049	0.0082	0.0131	0.0231	0.0330	0.0380	0.0429
Mortality	7%	3%	7%	10%	37%	100%	97%

*$R^2 = 0.9997$, SE = 0.0003 **$R^2 = 0.9764$, SE = 0.0025

Table 2 LC and EC values for different response levels at 28-day chronic exposure to Cd and Cu with their respective confidence limits

Exposure in days	Endpoint	Cd			Cu		
		Estimate	Lower	Upper	Estimate	Lower	Upper
28	LC10	0.0231	0.0221	0.0241	0.0281	0.0265	0.0297
	LC50	0.0382	0.0375	0.0389	0.0330	0.0326	0.0334
	LC90	0.0576	0.0559	0.0593	0.0372	0.0349	0.0396
28	EC 10	0.0063	0.0053	0.0073	0.0186	0.0181	0.0191
	EC 50	0.0125	0.0118	0.0132	0.0201	0.0200	0.0202
	EC 90	0.0248	0.0228	0.0269	0.0217	0.0211	0.0223

parameter between curves ($p < 0.001$) for which Cu showed a steeper one ($Slope_{Cd}/Slope_{Cu} = 0.29$). Furthermore, LT_{50} estimates were significantly affected by Cd and Cu exposure ($p < 0.001$), with a decrease in lethal time of 5.90% and 8.28% per increment of 0.001 mg/L of Cd and Cu, respectively. Untransformed fitted models can be observed in Fig. 1c, and LT_{50} estimates are shown in Table 4. Woodard (2005) reported LT_{50} values of 13.07 days for *P. acuta* exposed to their lowest tested concentration (0.4 mg/L Cd), however, those assays included larger snail size classes (shell length > 5.6 mm) and softer water conditions. Conversely, Williams et al. (1985) estimated a LT50 of 19.58 days for 0.4 mg/L of Cd in moderately hard water. With regard to LT_{50} values for Cu, Shuhaimi-Othman et al. (2012) found a similar pattern for Cu toxicity (LT_{50} 6.81 days for 0.081 mg/L of Cu), which decreased the lethal time to a greater degree than Cd (LT_{50}:11.81 days for 0.61 mg/L Cd) in experiments with *Melanoides tuberculata*. Lastly, relative chronic sensitivity analysis showed that *P. acuta* sensitivity to Cd is in the higher range compared to other freshwater gastropods (Fig. 2a), whereas its sensitivity to Cu sits in the middle range of the available data for freshwater snails. Nonetheless, most values fell behind *P. acuta* LC_{50} (Fig. 2b). The lowest 28-day LC_{50} value for *P. acuta* adults exposed to Cd was reported by Spehar et al. (1978), which after normalisation resulted in a lower dose (0.0104 mg/L Cd) compared to our results. With respect to Cu, Besser et al. (2016) reported 28-day LC_{50} values for *P. gyrina* juveniles (0.79-0.85 mm), with values of 0.0420 and 0.0340 mg/L Cu under similar water hardness conditions to our tests. However, Das and

Table 3 Comparisons between Cd and Cu LC and EC values for different response levels with p values, and their respective confidence limits

LC comparisons	4-day exposure				
	Estimate	Std. error	*p* value	Lower	Upper
Cd/Cu: 10/10	1.8646	0.2924	0.0031	1.2914	2.4378
Cd/Cu: 50/50	2.3703	0.2630	0.0000	1.8549	2.8856
Cd/Cu: 90/90	3.0130	0.5848	0.0006	1.8668	4.1591
LC comparisons	28-day expoure				
	Estimate	Std. error	*p* value	Lower	Upper
Cd/Cu: 10/10	0.8237	0.0297	0.0000	0.7655	0.8818
Cd/Cu: 50/50	1.1577	0.0130	0.0000	1.1323	1.1830
Cd/Cu: 90/90	1.5454	0.0553	0.0000	1.4370	1.6538
EC comparisons	28-day exposure				
	Estimate	Std. error	*p* value	Lower	Upper
Cd/Cu:10/10	0.3373	0.0190	0.0000	0.2933	0.3812
Cd/Cu:50/50	0.6214	0.0127	0.0000	0.5921	0.6507
Cd/Cu:90/90	1.1448	0.0357	0.0037	1.0624	1.2272

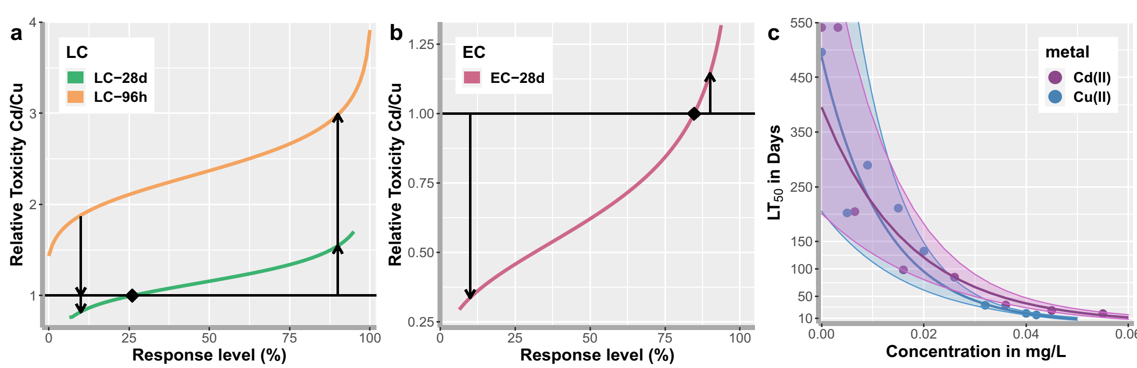

Fig. 1 Relative toxicity between Cd and Cu as a function of response level for **a** acute and chronic LC, and **b** chronic EC. Black dots and guide lines show where metals were equally toxic, and arrows show divergence from that point. Subfigure **c** shows the relationship of LT_{50} as a function of metal concentration

Khangarot (2011) obtained a 4-week LC_{50} value of 0.0159 mg/L Cu for adults of *Lymnaea luteola* in very hard water. The adjusted LC_{50} to 100 mg/L $CaCO_3$ was 0.0078 mg/L Cu; a much higher sensitivity than the one estimated from our experiments and from Besser et al. (2016).

In regard to chronic sublethal effects, Cu also showed a significantly steeper slope parameter than Cd ($p < 0.05$, $Slope_{Cd}/Slope_{Cu} = 0.11$), implying a more pronounced toxic effect. Results from our 28-day exposure tests showed the highest concentrations of Cd and Cu affected shell length to the same extent. Mean shell length at the highest concentrations at day 28 were 0.9230 ±0.0619 mm and 1.0238 ± 0.0478 mm for Cd and Cu, respectively. Therefore, no significant differences were found ($p = 0.2119$) between lower limits for Cu and Cd model parameters. Upper limits (i.e. control treatments mean response) did not show significant differences ($p = 0.2185$), with values of 2.8017 ± 0.0647 mm and 2.9048 ± 0.0415 mm for Cd and Cu experiments, respectively (See Fig. 3a, b). However, we found significant differences between EC values for Cd and Cu (Table 3), suggesting a more toxic effect exerted by Cd, particularly at lower response levels (Fig. 1b). Literature reported 28-day EC_{50} for growth (shell length) of *Lymnaea palustris* and *L. stagnalis* were 0.1420 mg/L and 0.0580 mg/L of Cd and Cu, respectively (Coeurdassier et al. 2003). Furthermore, Dorgelo et al. (1995) estimated an EC_{50} for the growth (shell length) of *Potamopyrgus jenkinsi* under Cd (0.016 mg/L) and Cu (0.013 mg/L) exposure. Unfortunately, there were no details about water hardness values for the experiments, but their EC_{50} estimations seem to suggest a greater sensitivity than those of *P. acuta*.

Inhibitory effects of Cd and Cu on the growth of freshwater snails have been previously reported (Arthur and Leonard 1970; Cheung and Lam 1998; Das and Khangarot 2011; Gao et al. 2017). Inhibition of the shell mineralisation is a known effect of metal ion exposure in molluscs, since they displace enzyme cofactors that participate in the process of biomineralization, and induce acidosis (Lionetto et al. 2016). Although it is beyond the scope of this study, it would useful to explore the ecotoxicology of biomineralisation in order to understand whether different mechanisms of action could explain differences in toxic response in *P. acuta* (e.g. EC Cd/Cu ratios as seen in Fig. 1b). For example, Brix et al. (2011) and Grosell and Brix (2009) found that the sensitivity of

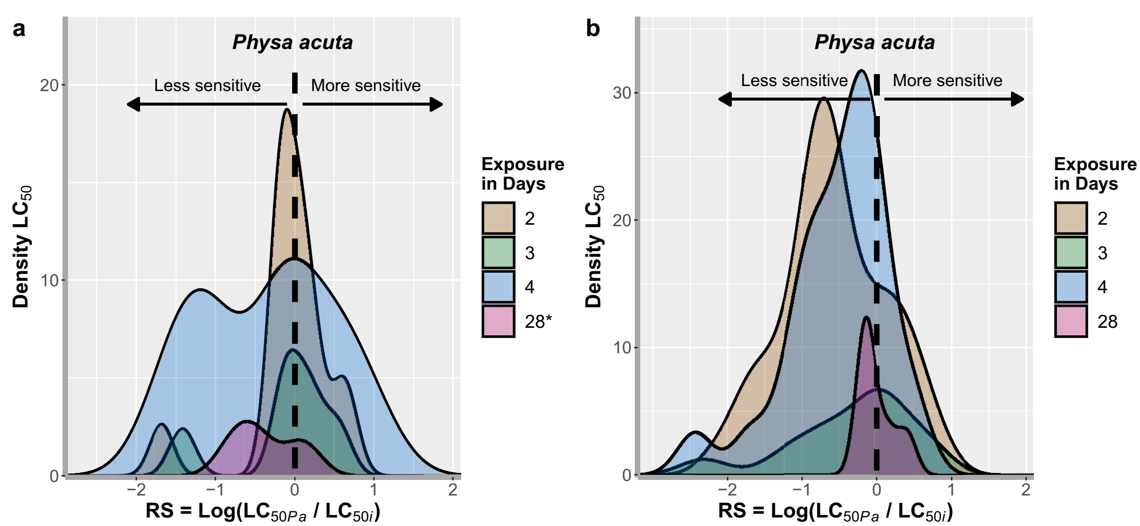

Fig. 2 Relative sensitivity (RS) calculations for LC_{50} values from ECOTOX database for **a** Cd and **b** Cu. *P. acuta* chronic LC values were included for Cd comparisons (28*). Positive values represent a greater sensitivity, and negative values correspond to a lesser sensitivity than *P. acuta* (dashed line)

Table 4 LT_{50} estimations, and their respective standard errors in days for Cd and Cu tested concentration groups

LT_{50} Estimates*								
Metal	Ctrl	C1	C2	C3	C4	C5	C6	C7
Cd	541.15 (316.49)	541.15 (316.49)	204.57 (93.55)	208.63 (303.87)	134.62 (160.27)	33.79 (11.01)	23.91 (3.53)	18.18 (2.73)
Cu	496.01 (312.93)	237.10 (284.54)	289.44 (259.99)	210.86 (257.38)	132.35 (138.05)	33.42 (9.57)	18.61 (2.18)	15.73 (2.13)

*LT_{50} units in days, and Standard Errors in brackets

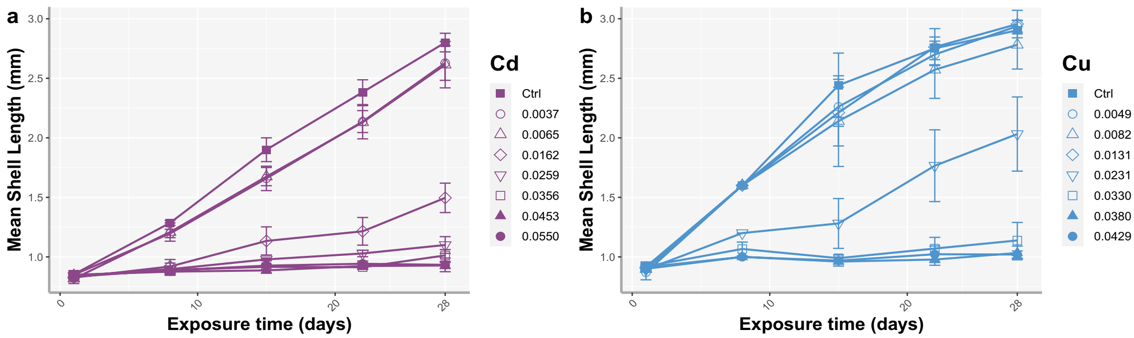

Fig. 3 *P. acuta* mean shell length for 28-day exposure of each concentration group for **a** Cd and **b** Cu tests

L. stagnalis to Cu and lead was the result of calcium being outcompeted at the apical membrane level where channels regulate intake. Although Cd toxicity is hindered by calcium in solution, it has been found to interfere with carbon anhydrase (CA) function and the homeostasis of calcium in mussels (Machado and Lopes-Lima 2011). Furthermore, Cu studies show different degrees of inhibition of CAs and specificity for other binding sites too (Lionetto et al. 2016), and histopathological effects such as desquamation and necrosis of the mantle epithelium (Otludil and Ayaz 2020). In saying this, growth inhibition could also be explained by a decrease in feeding rates (Das and Khangarot 2010). Further study is required to understand the influence of water chemistry and speciation on toxicodynamic processes (USEPA 2007, 2016).

The extensive distribution of *P. acuta*, its suitability to laboratory culture, and its sensitivity range, demonstrate the usefulness of this freshwater gastropod for chronic biomonitoring projects. The results from this study show that *P. acuta* presents a good range of sensitivity for the assessment of lethal and sublethal effects under the exposure to metal ions such as Cd and Cu. Neonates of *P. acuta* showed a higher acute sensitivity for Cu than Cd, and compared to other freshwater snails. Conversely, chronic sensitivity to Cd was higher than Cu for sublethal effects, whereas for lethal effects, it sits within the middle range compared to other freshwater gastropods. On a final note, this study also contributes to the generation of regression based sensitivity data, particularly for chronic exposure assessments which are mostly reported as NOEC/LOEC values in freshwater gastropods studies.

Acknowledgements The authors thank the Faculty of Exact Science of the National University of La Plata (FCEx-UNLP) for providing the space to conduct the experiments involved in this article. Furthermore, we thank Prof Amanda Reichelt-Brushett from Southern Cross University (SCU) in Australia, for kindly assisting with the English editing, and to the reviewers whose valuable observations helped us improve our manuscript.

References

Arthur JW, Leonard EN (1970) Effects of Copper on *Gammarus pseudolimnaeus*, *Physa integra*, and *Campeloma decisum* in Soft Water. J Fish Res Board Can 27(7):1277–1283. https://doi.org/10.1139/f70-149

Besser JM, Dorman RA, Hardesty DL, Ingersoll CG (2016) Survival and growth of freshwater pulmonate and nonpulmonate snails in 28-day exposures to copper, ammonia, and pentachlorophenol. Arch Environ Contam Toxicol 70(2):321–331

Bridges CC, Zalups RK (2005) Molecular and ionic mimicry and the transport of toxic metals. Toxicol Appl Pharmacol 204(3):274–308. https://doi.org/10.1016/j.taap.2004.09.007

Brix KV, Esbaugh AJ, Grosell M (2011) The toxicity and physiological effects of copper on the freshwater pulmonate snail, *Lymnaea stagnalis*. Comp Biochem Physiol Part C 154(3):261–267. https://doi.org/10.1016/j.cbpc.2011.06.004

Bubach D, Macchi P, Catn S (2015) Influence of volcanic activity and anthropic impact in the trace element contents of fishes from the North Patagonia in a global context. Environ Monit Assess. https://doi.org/10.1007/s10661-015-4910-y

Bulus Rossini G (2013) Evaluacin de la sensibilidad de diferentes especies acuáticas, presentes en la cuenca matanza riachuelo, expuestas a diversos contaminantes determinados en la misma, 31 p. Informe vii de convenio elevado a la autoridad de cuenca matanza riachuelo, ACUMAR, Buenos Aires, Argentina

Cheung C, Lam P (1998) Effect of cadmium on the embryos and juveniles of a tropical freshwater snail, *Physa acuta* (Draparnaud). Water Sci Technol 38(7):263–270. https://doi.org/10.1016/S0273-1223(98)00626-X

Coeurdassier M, Vaufleury AD, Badot PM (2003) Bioconcentration of cadmium and toxic effects on life-history traits of pond snails (*Lymnaea palustris* and *Lymnaea stagnalis*) in laboratory bioassays. Arch Environ Contam Toxicol 45(1):102–9. https://doi.org/10.1007/s00244-002-0152-4

Das S, Khangarot B (2010) Bioaccumulation and toxic effects of cadmium on feeding and growth of an indian pond snail *Lymnaea luteola* l. Under laboratory conditions. J Hazard Mater 182(1–3):763–770

Das S, Khangarot B (2011) Bioaccumulation of copper and toxic effects on feeding, growth, fecundity and development of pond snail *Lymnaea luteola* l. J Hazard Mater 185(1):295–305

Dillon RT Jr, Wethington AR, Rhett JM, Smith TP (2002) Populations of the European freshwater pulmonate *Physa acuta* are not reproductively isolated from American *Physa heterostropha* or *Physa integra*. Invertebr Biol 121(3):226–234. https://doi.org/10.1111/j.1744-7410.2002.tb00062.x

Dodd SR, Haynie RS, Williams SM, Wilde SB (2016) Alternate food-chain transfer of the toxin linked to avian vacuolar myelinopathy and implications for the endangered Florida snail Kite (Rostrhamus Sociabilis). J Wildl Dis 52(2):335–344. https://doi.org/10.7589/2015-03-061

Dorgelo J, Meester H, van Velzen C (1995) Effects of diet and heavy metals on growth rate and fertility in the deposit-feeding snail *Potamopyrgus jenkinsi* (smith) (gastropoda: Hydrobiidae). Hydrobiologia 316(3):199–210

Gao L, Doan H, Nidumolu B, Kumar A, Gonzago D (2017) Effects of copper on the survival, hatching, and reproduction of a pulmonate snail (Physa acuta). Chemosphere 185:1208–1216. https://doi.org/10.1016/j.chemosphere.2017.07.101

Grosell M, Brix KV (2009) High net calcium uptake explains the hypersensitivity of the freshwater pulmonate snail, *Lymnaea stagnalis*, to chronic lead exposure. Aquat Toxicol 91(4):302–311. https://doi.org/10.1016/j.aquatox.2008.10.012

Hoang TC, Klaine SJ (2007) Influence of organism age on metal toxicity to *Daphnia magna*. Environ Toxicol Chem 26(6):1198–1204. https://doi.org/10.1897/06-515R.1

Lionetto MG, Caricato R, Giordano ME, Schettino T (2016) The complex relationship between metals and carbonic anhydrase: new insights and perspectives. Int J Mol Sci 17(1):127. https://doi.org/10.3390/ijms17010127

Lowe RL, Hunter RD (1988) Effect of grazing by *Physa integra* on periphyton community structure. J N Am Benthol Soc 7(1):29–36. https://doi.org/10.2307/1467828

Lydeard C, Campbell D, Golz M (2016) *Physa acuta* Draparnaud, 1805 should be treated as a native of North America. Not Europe. Malacologia 59(2):347–350. https://doi.org/10.4002/040.059.0213

Machado J, Lopes-Lima M (2011) Calcification mechanism in freshwater mussels: potential targets for cadmium. Toxicol Environm Chem 93(9):1778–1787. https://doi.org/10.1080/02772248.2010.503656

Messerschmidt A (2010) Copper metalloenzymes. Compr Nat Prod II 8:489–545. https://doi.org/10.1016/B978-008045382-8.00180-5

Nuez V (2010) Differences on allocation of available resources in growth, reproduction, and survival in an exotic gastropod of Physidae compared to an endemic one. Iheringia Série Zoologia 100:275–279. https://doi.org/10.1590/S0073-47212010000300014

Otludil B, Ayaz S (2020) Effect of copper sulphate ($CuSO_4$) on freshwater snail, *Physa acuta* draparnaud, 1805: a histopathological evaluation. Bull Environm Contamin Toxicol. https://doi.org/10.1007/s00128-020-02846-5

Primost MA, Gil MN, Bigatti G (2017) High bioaccumulation of cadmium and other metals in Patagonian edible gastropods. Mar Biol Res 13(7):774–781. https://doi.org/10.1080/17451000.2017.1296163

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna http://www.R-project.org/

Ritz C, Jensen S, Gerhard D, Streibig J (2019) Dose-response analysis using R. CRC Press, Boca Raton. https://doi.org/10.1201/b21966

Saadi AJ, Davison A, Wade CM (2020) Molecular phylogeny of freshwater snails and limpets (Panpulmonata: Hygrophila). Zool J Linnean Soc. https://doi.org/10.1093/zoolinnean/zlz177

Shuhaimi-Othman M, Nur-Amalina R, Nadzifah Y (2012) Toxicity of metals to a freshwater snail, melanoides tuberculata. Sci World J

Solioz M (2018) Copper toxicity. Springer, Cham, pp 11–19. https://doi.org/10.1007/978-3-319-94439-5_2

Spehar RL, Anderson RL, Fiandt JT (1978) Toxicity and bioaccumulation of cadmium and lead in aquatic invertebrates. Environ Pollut 15(3):195–208. https://doi.org/10.1016/0013-9327(78)90065-4

USEPA (2007) Aquatic Life Ambient Freshwater Quality Criteria Copper, 2007th edn. EPA-822-R-07-001, United States Environmental Protection Agency (US EPA), Office of Science and Technology

USEPA (2016) Aquatic Life Ambient Freshwater Quality Criteria Cadmium, 2016th edn. EPA-820-R-16-002, United States Environmental Protection Agency (US EPA), Office of Science and Technology

Vareda JP, Valente AJ, Dures L (2019) Assessment of heavy metal pollution from anthropogenic activities and remediation strategies: a review. J Environ Manag 246:101–118. https://doi.org/10.1016/j.jenvman.2019.05.126

Warne M, van Dam R (2008) Noec and loec data should no longer be generated or used. Aust J Ecotoxicol 14:1–5

Williams K, Green DW, Pascoe D (1985) Studies on the acute toxicity of pollutants to freshwater macroinvertebrates. pt. 2. phenol. Arch Hydrobiol;(Germany, Federal Republic of) 103(1)

Woodard V (2005) Feasibility for utilization of a freshwater pulmonate snail, Physa acuta, as a model organism for environmental toxicity testing, with special reference to cadmium ion toxicity. PhD thesis, Graduate School, The University of Texas at Arlington, Arlington,Texas, United States

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.