Evaluation of single and interactive toxicities of lead and iron using filtration rate of Zebra mussels (*Dreissena polymorpha*)

N. Kováts[1], N.-A. Abdel-Hameid[2], K. Kovács[1] & J. Padisák[2]
[1]*Department of Limnology, University of Pannonia, Hungary*
[2]*Department of Zoology, Faculty of Science, Egypt*

Abstract

The filtration rate of zebra mussels (Dreissena polymorpha), as a sensitive biological end point, was used to evaluate the toxicity of lead and iron. Zebra mussels were exposed individually to serial levels of lead and iron (800, 1000, 1200, 1400, 1800 μg L^{-1}). The inhibition of filtration rate was determined after 24 h. The bioassay revealed that the effective concentration causing filtration inhibition 1% (EC1filtration), 5% (EC5filtration), 10% (EC10filtration), 50% (EC50filtration) and 99% (EC99filtartion) of Pb and Fe to D. polymorpha were 427.072, 601.310, 721.648, 1373.448, 4416.951 μg L^{-1} and 409.908, 571.420, 682.141, 1274.149 and 3960.532 μg L^{-1}, respectively. The ECx values recorded for Fe were higher than those recorded for Pb. Therefore, Fe was found to be more toxic to zebra mussels than Pb. The interactive effect of these metals was examined by testing the inhibition of filtration rate of zebra mussels exposed to 1:1 mixture of Pb and Fe. It was established that their interaction was synergistic. The results show that D. polymorpha filtration rate can be a sensitive end-point for assessing ecological risk of Pb and Fe as environmental contaminants.
Keywords: metals, interactive effects, ecotoxicology, zebra mussels, filtration rate.

1 Introduction

Freshwater and marine bivalves are used globally as bioindicators of water quality and as ecotoxicological test organisms [1–3]. Zebra mussels (*Dreissena polymorpha*) are sessile organisms that filter the water columns and live attached

WIT Transactions on Ecology and The Environment, Vol 162, © 2012 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)
doi:10.2495/EID120501

to solid substrates [4]. Although mussels do not filter continuously, it is likely that a single zebra mussel could filter 1 L of water per day [5, 6]. Thus, they have been used effectively to assess the contamination of freshwater ecosystems by inorganic [4, 7, 8] as well as organic pollutants [9–11]. Moreover, zebra mussels have been used in early warning systems [12]. Measuring the mortality as an end point in selected test species allowed the evaluation of environmental disturbances and associated biological effects [13]. For the ecotoxicological assessment of pollutants, there is an ultimate need to use more sensitive biological responses in order to detect the toxic units at low levels. Kraak *et al.* [14] found that the use of filtration rate of *D. polymorpha* to assess the toxicity of metals (Cu, Zn, Cd) is a responsive parameter. They found that, Cu LC50 was more than 25 times higher than the Cu EC50filtration (41µg L^{-1}). Abel [15] reported for the marine mussel *Mytillus edulis* that the 96-h LC50 for Cu and Zn were 2 and 5 times higher than the EC50filtration. Thus, the uses of sublethal end points are far more realistic for ecotoxicological laboratory assessments than traditional mortality alternatives [14]. Zebra mussel is suitable test organism for ecotoxicological laboratory experiments using filtration rate due to little mortality and high filtration rate in the control [14]. Vijayavel *et al.* [16] reported that oxygen consumption; filtration and ATPase systems could be used as valid sublethal biomarkers in aquatic ecotoxicological studies.

Although some metals are necessary for normal physiological processes of aquatic organisms, but contact over threshold induces harmful effects or even death [17]. In the presence of elevated metal levels, the bivalves close their shells for longer time [12], exhibit reduced heart beat [18] and filtration rate [19]. Lead is a toxic metal that is present in the environment in tiny amount. Its largest industrial use worldwide is in the manufacture of batteries and it is also used in paints, glazes, alloys, radiation shielding and tank lining. Lead pollution comes from cars, lead smelters, metal processing plants, incinerators and some old paints (http://www.epa.gov/safewater/contaminants/index.html). Although, Fe is an essential micronutrient, elevated concentrations may limit the animal metabolism [20] and induces oxidative stress [21]. According to EPA, the maximum permissible level of Pb and Fe in surface water is 0.015 and 0.3 mg L^{-1}, respectively (http://www.epa.gov/safewater/contaminants/index.html).

Industrial and municipal wastewaters and urban storm water commonly contain combinations of metals such as copper, lead, and zinc in addition to organic residues, all of which may be directly or indirectly released into recipient aquatic systems [22]. Therefore, studying the ecotoxicological impacts of aquatic ecosystems by mixtures of pollutants is of increasing concern worldwide [23], [24]. The interactive toxic effects of metals in a mixture may be augmented (synergistic) or antagonistic [25]. Thus, the aim of the present study was to assess the lone as well as the interactive toxicity of Pb and Fe using filtration rate of *D. polymorpha*.

2 Materials and methods

2.1 Mussels collection and laboratory care

Zebra mussels (*D. polymorpha*, Pallas) were collected (November 2009) from Lake Balaton at Balatonaliga, (GPS, N 46 59′ 111″−E 18 09′500″), Hungary. Mussels were acclimatized at a laboratory static system in well aerated tanks. The water of the aquaria was well aerated and changed weekly with Csigere stream water (near Devecser village west Veszprèm, GPS; N 47 07′ 081″ – E 17 25′ 244″, Hungary) to provide the mussels with natural food and to remove the possible accumulation of ammonium. The physical and chemical parameters, total phytoplankton biomass of Csigere stream water are presented in Table 1. They were measured using multi probe portable equipments (Multi HQ 40 d, Hach for DO, PH, conductivity and Hach Lange for turbidity).

Table 1: Some physicochemical characteristics and total phytoplankton noumber and biomass of Csigere stream water.

Items	Value
pH	8.1
Dissolved oxygen, mg/l (%)	9.7 (88.1)
Temperature (°C)	10.4
Conductivity (μs/cm)	830
Turbidity (FNU)	7.84
Phytoplankton noumber ($X10^3$/ml)	40.959
Phytoplankton biomass (mg/m^3)	15904.76

2.2 Bioassay and estimating the total phytoplankton biomass

Stock solutions of 10 mg L^{-1} for both $PbCl_2$ and $FeCl_3$ were used to prepare 5 serial concentrations of each metal (800, 1000, 1200, 1400, and 1800 μg L^{-1}). The exposure experiment was done in aerated plastic container (10 liters), containing 2 liters (1 liter Csigere stream water and 1 liter distilled water). The initial total phytoplankton biomass was 7952.38±302.21 mg m^{-3} for the control and test groups (Table 1). There were two replicates per each serial concentration and control group. Five mussels (shell length 10-15 mm) were used for each replicate. The bioassay was terminated after 24 h.

The total phytoplanktons in Csigere stream water as well as those in the test water were fixed by adding by few drops of Lygol solution. The total count of every phytoplankton species of each sample was done after at least 24 h of fixation. Total phytoplankton biomass as mg m^{-3} was computed by Hamilton computer software (algamica) based on the method of Gosselain and Hamilton [26].

2.3 Data analysis

Using this software, automated calculations of densities, biovolumes, surface areas and carbon biomass were available at the termination of each sample count.

The data of total phytoplankton biomass were expressed as means± standard error. The filtration rate was calculated from the percentage of reduction of total algal biomass in each test concentrations as well as in the control group using the following formula: Filtration rate (%) = 100 (Initial biomass-Final biomass/Initial biomass). Inhibition of filtration regarding those of the control was computed using the following formula: Filtartion inhibition (%) = 100 (Filtration rate of control-Filtration rate of test/Filtration rate of control). The EC_x of tested metals and its confidence limit was computed using EPA probit analysis program version 1.5.

The interaction of both Pb and Fe was examined by exposing duplicate group of mussels to equitoxic (EC_{50}) mixture of both metals. The observed inhibition (observed effect) of filtration rate was estimated from the reduction of total algal biomass. The theoretically expected (PE) effect of the binary mixtures on the test organisms was evaluated using the formula proposed by Kungolos *et al*. [27] and Hadjispyrou *et al*. [11]. It was computed using the following formula: $P(E)=P_1+P_2-P_1P_2/100$. Where, P (E) is the theoretical expected effect, P_1 is the inhibition caused by chemical A and P_2 is the inhibition caused by chemical B. This model is based on the hypothesis that the toxicants act concurrently and not successively on the affected organism [24].

3 Results and discussion

The data presented in Table (1) showed that Csigere stream water quality was normal and contained high amount of total algal biomass originating from an upstream fish pond. Its algal population was rich with 46 total numbers of taxa and total biomass of 15904.76 mg m^{-3}. In Table 2 gives the filtration rate of *D. polymorpha* expressed as percentage clearance of total algal biomass of the control and those of serial concentrations of Pb or Fe. The bioassay revealed that the filtration rate of *D. polymorpha* was repressed due to the exposure to serial levels of Pb and Fe. Its magnitude varied according to the metal and its levels. In

Table 2: Acute effect (24 h) of lead and iron on filtration rate of zebra mussel (D. polymorpha).

Metals	Levels (µg L^{-1})	Total biomass (mg m^{-3})		Filtration rate (%)	Filtration inhibition (%)
		Initial	Final		
Lead	Control	7952.38±302.21	3702±187	53.45±4.21	-
	800	-	4283±254.35	46.14±4.55	13.68±1.05
	1000	-	4868±172.37	38.75±3.87	27.5±2.85
	1200	-	5400±195.56	32.09±2.66	39.96±2.39
	1400	-	5812±146.22	26.91±2.46	49.65±4.61
	1800	-	6726±198	15.42±1.87	71.15±3.98
Iron	Control	7952.38±302.21	3702±187	53.45±4.21	-
	800	-	4471.58±206.23	43.77±3.46	18.11±1.25
	1000	-	5100±197.35	35.87±3.11	32.89±2.47
	1200	-	5398.46±205.45	32.11±2.68	39.92±2.68
	1400	-	6137.24±205.22	22.82±1.79	57.31±3.94
	1800	-	7026.47±179.25	11.64±1.09	78.22±4.79

case of Pb exposure, the lowest filtration inhibition (13.68±1.05%) was recorded for the lowest Pb level (800 μg L^{-1}) and the highest filtration inhibition (71.15±3.98%) was recorded for the highest one (1800 μg L^{-1}). Iron exposure induced a higher filtration inhibition than Pb. The lowest Fe level induced 18.11±1.25% filtration inhibition and the highest one induced 78.22±4.79% filtration inhibition (Table 2).

Data are Mean ±SE. Filtration rate (%) = 100 (Initial biomass-Final biomass/Initial biomass). Filtration inhibition (%) =100 (Filtration rate of control-Filtration rate of test/Filtration rate of control).

Table 3: Comparison of different ECx of Pb and Fe based on filtration rate of *D. polymorpha.*

EC_x	Pb (μg L^{-1})	Fe (μg L^{-1})
EC_1	427.072(312.925-521.647)	409.908(302.194-500.149)
EC_5	601.310(484.577-692.258)	571.420 (460.313-659.162)
EC_{10}	721.648(610.877-806.230)	682.141 (575.394-764.626)
EC_{50}	1373.448 (1293.381-1475.834)	1274.149(1203.732-1355.619)
EC_{99}	4416.951(3474.785-6423.668)	3960.532(3187.951-5526.334)

95% confidence limit indicated between parentheses.

The data in Table 3 present the computed $EC_{1filtration}$, $EC_{5filtration}$, $EC_{10filtration}$, $EC_{50filtration}$ and $EC_{99filtration}$ of Pb and Fe to *D. polymorpha* which were 427.072, 601.310, 721.648, 1373.448, 4416.951 μg L^{-1} and 409.908, 571.420, 682.141, 1274.149 and 3960.532 μg L^{-1}, respectively. Although the ECx recorded higher values for Pb than those for Fe, their 95% confidence limit overlap indicating nonsignificant differences, meanings that the toxicity of Fe was non-significantly higher than those of Pb. It was found that *D. polymorpha* exposed to equitoxic mixture of both Pb and Fe exhibited a significantly higher filtration inhibition than the theoretically expected one. Thus, the interactive effect of both tested metals was synergistic (Table 4).

Bioassays using biological responses other than mortality are more precise and generally the values of EC50 are lower the values of LC50 [28]. Kraak *et al.* [4] found that zebra mussels collected from Eijsden on the river Meuse (Belgian and Dutch border, km 620) containing elevated levels of Cu, Zn, Cd and Pb exhibited also low filtration rate, the results compared with reference contamination free water (Lake Markermeer, The Netherlands). Kraak *et al.* [14] estimated the 48 h $EC_{50filtration}$ of Cu (41 μg L^{-1}), Cd (388 μg L^{-1}) and Zn (1350 μg L^{-1}). Loayza-Muro and Elliás-Lettas [3] assessed the inhibition of filtration rate of Anodontites trapesialis (Unionidae) and found that Cd exerted the most toxic effects (EC50filtration 64 μg L^{-1}) followed by Cu (605 μg L^{-1}) and Zn (4064 μg L^{-1}).

Table 4: Acute effect (24 h) of equitoxic binary mixtures of lead and iron on % inhibition of filtration rate and comparison between theoretically expected and observed interactive effects of zebra mussel (D. polymorpha).

Groups	Total biomass (mg/m^3)		Filtration rate (%)	Observed Filtration inhibition, % (O)	Theoretically Expected effect (E)
	Initial	Final			
Control	7952.38 ±302.21	3702 ±187	53.45 ±4.21	-	-
Pb+Fe	-	7364.42 ±275.44	7.39 ±0.79	86.17 ±3.26↑	75

Data are Mean ±SE. Filtration rate (%) = 100 (Initial biomass-Final biomass/Initial biomass). Filtration inhibition (%) =100 (Filtration rate of control-Filtration rate of test/Filtration rate of control). ↑ is significantly higher than E, more than additive.

The results herein reported that the exposure of *D. polymorpha* to Pb and iron causes inhibition of the filtration rate. This phenomenon was previously reported for other heavy metals and for different species [3, 4, 16, 28]. The present study recorded 24 h $EC_{50filtration}$ for Pb (1373.448 µg L^{-1}) and Fe (1274.149 µg L^{-1}). This means that Pb was more toxic than Fe to *D. polymorpha*. The recorded 24 h $EC_{50filtration}$ of Pb was closer to Zn 48 h $EC_{50filtration}$ (1350 µg L^{-1}) recorded previously for zebra mussel by Kraak *et al*. [14]. The 24 h EC50 value is usually approximately around the half of 48 h EC50. Thus, it could be concluded that the toxicity of Zn is greater than Pb and Fe. Neal *et al*. [29] reported higher level of Pb (6.3 µg L^{-1}) in river Swale (one of Humber rivers, UK) than the background (0.2 µg L^{-1}) but they are less than those reported for major industrial rivers of Europe (57 µg L^{-1}) (e.g. the Rhine) and less than the average of environmental quality standard, EQS, (8-18 µg L^{-1}). They also found 113 µg Fe L^{-1} in river Swale water which is higher than those of Rhine water (35 µg L^{-1}) but also below than EQS level (1000 µg L^{-1}). Moss *et al*. [30] reported ecological relevant levels of Pb 30 µg L^{-1} and Fe 20-1195 µg L^{-1} in crawfish (*Procambrus clarkia*) pond. By comparing these results with $EC_{50filtration}$ recorded in the present study, it was found that the Pb level was far low from the $EC_{50filtration}$, whereas the upper limit of Fe was less below those of Fe $EC_{50filtration}$.

The present results found that the mixture of Pb and Fe exerted synergistic toxic effect to *D. polymorpha*. This result was in agreement with those reported earlier by Cooper *et al*. [31] for mixture of copper, lead and zinc using immobilization test of Cladoceran species, *Daphnia carinata* and *Ceriodaphnia dubia.* Interaction of metals may be influenced by the species being tested, combination of metals or water quality [31]. Sephar and Fiandt [32] declared that the same combinations of metals (arsenic, cadmium, chromium, cooper, mercury and lead) showed different interactive effects depending on both species exposed and the end point tested. The same authors found that when daphnids are used as the test organisms the effects are almost strictly additive or nearly additive for acute and chronic exposures, respectively.

The data of this study reiterate that the use of filtration rate of *D. polymorha* could be useful biomonitoring test for evaluating the toxicity of Pb and Fe. Also, the toxicity of Fe was higher than Pb and their interaction was strictly additive.

Acknowledgements

The authors are appreciative of the immense efforts by Mrs. Zámbóné Doma Zsuzsa, the technician at Department of Limnology, University of Pannonia for counting and estimating the total algal biomass.

References

[1] Boening, D. W., An evaluation of bivalves as biomonitors of heavy metals pollution in marine waters. *Environmental Monitoring and Assessment*, 55, 459-470, 1999.

[2] Kádár, E., Salánki, J., Powel, J., White, K.N., McCorhan, C.R., Effect of sublethal concentration of aluminium on the filtration rate activity of the freshwater mussel Anodonta cygnea L. at neutral pH. *Acta Biologica Hungarica* **53**, (4), 485-493, 2002.

[3] Loayza-Muro, R., Elliás-Letts, R., Response of the museel Anodonties trapesialis (Unionidae) to environmental stressors: Effect of pH, temperature and metals on filtration rate. *Environmental Pollution*, **149**, 209-215, 2007.

[4] Kraak, M.H.S., Kupiers, F., Schoon, H., de Groot, C. J., Admiraal W., The filtration rate of the zebra mussel Dreissena polymorpha used for water quality assessment in Dutch rivers. *Hydrobiologia*, **294**, 13-16, 1994.

[5] Griffiths, R. W., Schloesser, D. W., Leach, J. H., Kovalak, W. P., Distribution and Dispersal of the Zebra Mussel in the Great Lakes Region. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 1381-1388, 1991.

[6] Balogh, C., The settling, quatitative trend, filtration rate of zebra mussel (Dreissena polymorpha) and the effect of the mussel on the matter-energy flux and on the bacterial community. Ph.D. Thesis, University of Pannonia, Environmental Sciences Ph.D. School, Veszprèm, pp., 11, 2008.

[7] Johns, C., Timmerman, B.E., Total cadmium, copper, and zinc in two dreissenid mussels, Dreissena polymorpha and Dreissena bugenesis, at the outflow of lake Ontario. *Journal of Great lakes Research*, **24**, 55-64, 1998.

[8] Camusso, M., Balestrini, R., Binelli, Use of zebra mussel (Dreissena polymorpha) to assess trace metal contamination in the largest Italian subalpine lakes. *Chemosphere*, **44**, 263-270, 2001.

[9] Kraak, M.H.S., Ainscough, C., Fernández, A., van Vlaardingen, P.L.A., de Voogt P., Admiral, W.A., Shor-tem and chronic exposure of the zebra mussel (Dreissena polymorpha) to acridine. *Aquatic Toxicology*, **37**, 9-20, 1997.

[10] Hendriks, A. J., Pieters, H., Boer, J., Accumulation of metals, polycyclic halogenated aromatic hydrocarbons and biocides in zebra mussel and eel from the Rhine and Menuse rivers. *Environmental Toxicology and Chemistry*, **17**, 1885-1898, 1998.

[11] Hadjispyrou, S., Kungolos, A., Anangostopoulos, A., Toxicity, bioaccumulation and effects of organotin, cadmium and chromium on Artemia franciscana. *Ecotoxicology and Environmental Safety*, **49**, 179-186, 2001.

[12] Krammer, K.J.M., Jenner, H.A., de Zwart, D., The valve movement response of mussels: a tool in biological monitoring. *Hydrobiologia*, **199/189**, 433-443, 1989.

[13] Soucek, D.J., Schmidt, T.S., Cherry, D.S., In situ studies with Asian clams (Corbicula fluminea) detected acid mine drainage and nutrients input in low order streams. *Canadian Journal of Fisheries and Aquatic Science*, **58**, 602-608, 2001.

[14] Kraak, M.H.S., Toussaint, M., Lavy, D., Davids C., Short-term effects of metals on the filtration rate of the zebra mussel Dreissena polymorpha. *Environmental pollution*, **84**, 139-143, 1994b.

[15] Abel, P.D., Effect of some pollutants on filtration rate of Mytillus. *Marine Pollution Bulletin*, **7**(12), 228-231, 1976.

[16] Vijayavel, K., Gopalakrishnan, S., Balasubramanian, M. P., Sublethal effect of silver and chromium in the green mussel Perna viridis with reference to alterations in oxygen uptake, filtration rate and membrane bound ATPase system as biomarkers. *Chemosphere*, **69**, 979-986, 2007.

[17] Bossuyt, B.T.A., Janssen, C.R., Acclimation of Daphnia magna to environmentally realistic copper concentrations. *Comparative Biochemistry and Physiology Part* C, **136**, 253–264, 2003.

[18] Grace, A. L., Gainey Jr., The effects of copper on heart rate and filtration rate of Mytillus edulis. *Marine Pollution Bulletin*, **18**(2), 87-91., 1987.

[19] Redpath, K.J., Davenport, J., The effect of copper, zinc and cadmium on the pumbing rate of Mytilus edulis L. *Aquatic Toxicology*, **13**, 217-226., 1988.

[20] Halliwell B., Gutteridge J.M.C., Free radicals in biology and medicine. Academic Press, London, 1985.

[21] Koster, J.T., Slee, RG, van Berkel, C., On the lipid peroxidation of rat liver hepatocytes, the formation of fluorescent chromolipids and high molecular weight protein. *Biochemica et Biophysica Acta*, **710**, 230-235., 1982.

[22] Gobeil, C.; Rondeau, B., Beduin, L., Contribution of municipal effluents to metal fluxes in the St. Lawrence River. *Environmental Science and Technology*, **39**, 456-464., 2005.

[23] Shaw, J. R., Dempsey, T.D., Chen, C. Y., Hamilton, J.W., Folt, C. L., Comparative toxicity of cadmium, zinc and mixtures of cadmium and zinc to daphnids. *Environmental Toxicology and Chemistry*, **25**, 182-189, 2006.

[24] Kungolos, A., Emmanouil, C., Tsiridis, V. N., Tsiropoulos, N., Evaluation of toxic and interactive toxic effects of three agrochemicals and copper

using a battery of microbiotests. *Science of the Total Environment*, **407**(16), 4610-4615., 2009.

[25] Enserink, E.L., Maas-Diepeveen, J.L., van Leeuwen, C.J., Combined effects of metals; an ecotoxicological evaluation. *Water Research*, **25**, 679-687, 1991.

[26] Gosselain, V., Hamilton, P.B., Algamica: revisions to a key-based computerized counting program for free-living, attached, and benthic algae. *Hydrobiologia*, **438**, 139-142, 2000.

[27] Kungolos, A., Samaras, P., Kipopoulou, A.M., Zoumboulis, A. Sakellaropoulo, G.P., Interactive toxic effects of agrochemicalson aquatic organisms. *Water Science and Technology*, **40** (1), 357-364., 1999.

[28] Arambašic, M.B., Bjelić, S., Subakov, G., Acute toxicity of heavy metals (copper, lead, Zinc), phenol and sodium on Allium cepa L., Lepidium sativum L. and Daphnia magna St.: Comparative investigations and the practical applications. *Water Research*, **29**, 497-503., 1995.

[29] Neal, C., Smith, C.J., Jeffery, H.A., Jarvie, H.P., Robson, A. J., Trace element concentrations in the major rivers entering the Humber estuary, NE England. *Journal of Hydrology*, **182**, 37-64., 1996.

[30] Moss, J. C., Hardaway, C. J., Richert, J. C., Sneddon, J., Determination of cadmium copper, iron, nickel, lead and zinc in crawfish (Procambrus clarkii) by inductively coupled plasma optical emission spectrometry: A study over the 2009 season in Southwest Louisiana. *Michrochemical Journal*, **95** (1), 5-10., 2010.

[31] Cooper, N. L., Bidwell, J. R., Kumar, A., Toxicity of copper, lead and zinc mixtures to Ceridaphnia dubia and Daphnia magna. *Ecotoxicology and Environmental Safety*, **72**, 1523-1528., 2009.

[32] Sephar, R.L., Fiandt, J. T., Acute and chronic effects of water quality criteria based mixtures on three aquatic species. *Environmental Toxicology and Chemistry*, **5**, 917-931., 1986.