



Ranked effects of heavy metals on marine bivalves in laboratory mesocosms: A meta-analysis

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ABSTRACT

Bivalves are commonly used as biomonitors for heavy metal pollution in marine environments because they accumulate heavy metal ions quickly, are sessile, abundant, and widely dispersed, and adult mortality from contamination is rare. However, the breadth of experiments used to measure the effect of heavy metal contamination can obscure general trends. It is unclear which heavy metals cause the most severe effects, how severity varies with exposure concentration and duration, and whether effects vary with level of biological organization. I conducted a meta-analysis of 48 mesocosm studies on the effects of heavy metal ions – silver, cadmium, copper, mercury, lead, and zinc – on marine bivalves. The ordering of effect sizes was $Pb > Hg > Cu > Zn > Cd > Ag$. The significance and direction of concentration and duration as moderators depended on the metal and the biological level. Future studies should consider non-linear effects over time and concentration, and measure both bioaccumulation and effect of the metals being studied.

1. Introduction

The use of bivalves to monitor aquatic pollution, particularly for heavy metal contamination in marine environments, has been common since the 1970s (Goldberg, 1975). Many studies have established that heavy metals have significant effects on bivalves in terms of, for example, genetic diversity (Breitwieser et al., 2016), tissue and cell necrosis (Sheir and Handy, 2010), immune system health (Ivanina et al., 2016), reproductive health (Liu et al., 2014), and filtration rate (Sobrinho-Figueroa and Cáceres-Martinez, 2014). Nonetheless, information regarding the conservation status of marine bivalves is scarce. A search of “bivalve” in the IUCN Red List of Threatened Species, the most exhaustive global database of species' conservation status, returns a list of only ten species all of which are freshwater dwelling.

Because an organism's response to contaminants must be known in order for it to serve as a functional biomonitor, numerous studies have examined the effects of heavy metal contamination on bivalves. The response of bivalve species to heavy metal contamination is complex. Each species responds differently to different metals, even when other biotic and abiotic conditions are equal (Vijayavel et al., 2007). While many metals are toxic in large doses, some heavy metals such as Fe and Cu are micronutrients, and can therefore have a positive effect at low concentration (Yeung et al., 2016). The rate of accumulation of metals in tissues depends on biotic and abiotic factors, such as water salinity

(Gamain et al., 2016) and temperature (Boukadida et al., 2016), which vary seasonally and geographically (Phillips, 1980). These factors can influence the relative toxicity of the metal, in addition to the rate of accumulation. Interaction effects have also been observed: the toxicity of a metal can be increased or decreased by the presence of other heavy metals or contaminants (Fathallah et al., 2013). Finally, metal toxicity often exhibits a non-linear dose dependency (Amachree et al., 2013).

Understanding the effects of heavy metal contamination on marine bivalves is further complicated by the fact that the effects are rarely fatal in adult bivalves, so experiments do not have unambiguous endpoints. While mortality is a common measure for larvae or embryos (for example, Gamain et al., 2016; Fathallah et al., 2013), the majority of experiments performed on adult or juvenile bivalves measure morbidity instead. Low mortality rates from heavy metal contamination are useful in biomonitoring studies because they ensure the organism can be used over a long period of time or in highly contaminated environments. However, the diversity of measures of morbidity – such as lowered filtration rate or increased oxidative stress markers – makes quantitative comparison among studies challenging.

Meta-analysis is a promising means of extracting information on overarching trends from biomonitoring studies, which have a wide range of study sizes, methods, and metrics for measuring biological effects. Meta-analysis is a statistical synthesis that combines and weights published results within a defined group of studies in order to establish a weighted average effect – the “effect size”. It has already

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Table 1 Summary of toxicity, uses, chronology, human impacts, and meta-analytic effect sizes of heavy metals in marine bivalves. Geography-specific information, such as product bans, is for USA (Wang et al., 2009; Banfalvi, 2011).

Metal	Mechanism of toxicity	Industrial uses	Time period of use	Impact on humans	Effect size
Ag	Interferes with sodium-potassium ATPase; low continuous exposure may harm reproduction	Byproduct of Au, Pb, Zn, Cu refining; used in photography and photovoltaic panels	Photography use declining since 1999.	Silver salts toxic to humans; other forms non-toxic.	1.61
Cd	Catalyzes formation of ROS, depletes antioxidants.	Batteries, industrial paints, electroplating, plastics	Still in use but heavily monitored.	Highly toxic in low doses.	1.67
Cu	Impairs enzymes, can cause oxidative stress & Zn deficiency.	Anti-fouling agent on boat hulls (replaced tributyltin)	Banned in 2011 in Washington State for recreational boats.	MCL in water is 1.3 mg/l (EPA)	2.12
Hg	Inhibits selenoenzymes (ex. thioredoxin reductase), increasing oxidative damage	Felting of fur for hats (obsolete), deep earth mining waste product, manufacture of chlorine/caustic soda, electrodes, cosmetics, thermometers, lighting.	Industrial production chlorine/caustic soda phased out 1985. USA: non-prescription Hg fever thermometers banned 2003.	Very toxic: can result in death. Bioaccumulation in fish can be mechanism of poisoning in humans.	2.35
Pb	Reactive radicals damage DNA, cell membrane; interference w/ enzymes; inflammatory protein production	Obsolete: household items, plumbing, leaded paint (inc. marine paint), anti-noc	Anti-knock: 1921–1986 Leaded paint: banned 2000	Highly toxic and highly regulated. Though banned, products that contain lead (ex. paint) are still in use.	4.34
Zn	Suppression Cu and Fe absorption; Zn salts corrode tissue	Batteries, steel/iron coating, metal alloys, roofing, print processes	N/A	Low toxicity in humans	1.82

Table 2

Summary of the random-effects results. p-Values: ***0.001; **0.01; *0.05. none = not significant. The categories in bold have only two studies. “N/A” signifies a single study in the category: effect sizes are not shown for these because the results are not meaningful. While Hg and Pb had the largest effect size, they also had the largest standard error. Cd and Cu, which had the largest number of data points, had some of the smallest standard errors. Interestingly, Ag also had a very small standard error despite not having as many data points (Fig. 2).

Metal	Effect size (SE)	Category	Effect size (SE)
Ag	0.207 (0.036)***	Cellular	0.141 (0.034)***
		Physiological	0.344 (0.13)*
Cd	0.222 (0.026)***	Population	N/A
		Cellular	0.203 (0.019)***
Cu	0.326 (0.040)***	Physiological	0.749 (0.090)***
		Population	0.059 (0.014)***
Hg	0.372 (0.12)**	Cellular	0.124 (0.025)***
		Physiological	0.130 (0.018)***
Pb	0.638 (0.12)***	Population	0.480 (0.074)***
		Cellular	0.0904 (0.043)*
Zn	0.259 (0.067)***	Physiological	0.191 (0.078)*
		Population	0.537 (0.24)*
Ag	0.207 (0.036)***	Cellular	0.242 (0.031)***
		Physiological	1.35 (0.27)***
Cd	0.222 (0.026)***	Population	1.71 (0.47)**
		Cellular	0.019 (0.0076)*
Cu	0.326 (0.040)***	Physiological	0.517 (0.14)***
		Population	0.320 (0.10)**

proven useful in other highly empirical disciplines, such as education (Hedges et al., 1994), ecology (Osenberg et al., 1999), and medicine (Sutton et al., 2000). However, the only formal meta-analysis of heavy metal effects on marine organisms is O'Brien and Keough's (2014) study, which focused on the effects of a single metal (copper) on marine invertebrates.

Here, I use meta-analysis of bivalve heavy metal contamination literature to differentiate the effects of six different heavy metals: silver (Ag), cadmium (Cd), copper (Cu), mercury (Hg), lead (Pb), and zinc (Zn). These metals were chosen based on the number of papers available and their known effects on living organisms (Table 1). Because bivalve responses are increased morbidity (diminished functionality) rather than increased mortality (decreased survival), and because measures of morbidity are highly diverse, this meta-analysis uses dimensionless effect-size measurements. It cannot quantify the severity of an effect in an absolute sense, but can rank effects among contaminants. (See Table 2.)

My hypotheses were that lead and mercury would have the largest effect sizes, as their severely deleterious effect on biological tissue has been quantified in many species, including humans (Papanikolaou et al., 2005; Zahir et al., 2005) and several species of plants (Verma and Dubey, 2003; Patra and Sharma, 2000). I also expected that non-essential metals (i.e. ones that are not required in trace amounts for essential biochemical and physiological processes in metabolism, reproduction, or growth) would have more deleterious effects, because they are more likely to cause damage even at low concentrations: of the metals listed above, Pb, Cd, Ag and Hg are in this category (Tchounwou et al., 2012). I expected effect size to decline from cellular to physiological to population, because damage at lower orders of biological organization is only likely to “scale up” and impede functionality at higher orders within an individual above some minimal level of damage. For example, low levels of lysosomal damage, a cellular level effect, may not cause measurable damage at physiological or population levels unless the damage reaches a certain level of severity. While biomagnification up through trophic levels can and does occur for some heavy metals – for instance Hg – this upward transfer is much weaker when the highest level of organization is a population rather than, for example, a community or entire ecosystem (van der Velden et al., 2013). Lastly, I expected duration of exposure to be a stronger

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