A comparison of freshwater mussels and passive samplers as indicators of heavy metal pollution in aquatic systems

Utilisation de moules d'eau douce et d'échantillonneurs passifs comme indicateurs de la pollution des milieux aquatiques par les métaux lourds

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RÉSUMÉ

L'utilité de l'échantillonnage passif en tant qu'outil de détermination de l'état écologique des bassins de retenue en eau a été étudiée en tant qu'alternative à l'analyse des organismes vivants. L'accumulation de métaux lourds au cours du temps dans les moules et les échantillonneurs passifs exposés à des eaux pluviales artificielles a été étudiée dans des conditions contrôlées afin de déterminer si l'un ou l'autre système était en mesure de fournir une source de données fiable sur la pollution aquatique. Les résultats de laboratoire indiquent que les moules sont utiles dans ce contexte. Cependant, des échantillonneurs passifs nécessitent un développement supplémentaire pour être utiles car il n'existe pas de corrélation forte entre les concentrations en métaux lourds observées pour les moules et celles des échantillonneurs passifs.

ABSTRACT

The utility of passive sampling as a tool for determining the ecological state of wet retention ponds was investigated as an alternative to the analysis of living organisms. The accumulation of heavy metals over time in mussels and passive samplers exposed to artificial stormwater was examined under controlled conditions in order to determine whether either system was capable of functioning as a reliable source of data on aquatic pollution. The laboratory results indicated that mussels are useful in this context. However, passive samplers will require further development to be useful since there was no strong correlation between the heavy metal concentrations observed in the mussels and those in the passive samplers.

KEYWORDS

Accumulation, Heavy metals, Mussels, Passive samplers, Wet retention ponds

1   INTRODUCTION

Stormwater runoff from urban areas is often conveyed directly into receiving bodies of water without undergoing any treatment to remove harmful substances [Casey *et al*., 2006; Karlsson *et al*. 2010]. Because stormwater runoff often contains significant quantities of pollutants such as heavy metals, nutrients, pesticides, polycyclic aromatic hydrocarbons and volatile organic compounds [Zgheib *et al*., 2011; Birch *et al*., 2011], it is increasingly being recognized as a pollution problem [Vollertsen *et al*., 2006]. Stormwater discharges can contribute to the eutrophication of surface waters and may be toxic to local flora and fauna.

In accordance with this growing recognition, a range of stormwater treatment technologies are being developed in order to control pollution from this source and to maintain a high quality of water throughout the water cycle. These technologies can be incorporated into urbanized areas as they grow or be built into existing urban areas [Karouna-Renier and Sparling, 2001; Marsalek *et al*., 2006].

One widely used method for removing pollutants from surface runoff is based around the conctruction of permanent wet retention ponds, which have proven to be reliable and efficient at removing diverse pollutants from water, including heavy metals and suspended solids [Harper, 1985; Färm, 2002; Hossain *et al*., 2005]. In addition to their roles in water treatment, these ponds are often designed to contribute to the aesthetic and recreational value of the urban environment; they usually take the form of small semi-natural lakes [Hvitved-Jacobsen *et al*., 2010]. Wet ponds eventually turn into habitats for aquatic and terrestrial flora and fauna, because animals and plants do not distinguish between artificial and natural waters. Ponds therefore often support diverse ecosystems that resemble those associated with natural shallow lakes in many respects [Anderson *et al*., 2004; Casey *et al*., 2006].

While this is desirable for several reasons, it also presents cause for concern because flora and fauna within retention ponds may be exposed to urban pollutants [VanLoon *et al*., 2000; Casey *et al*., 2006]. The establishment of wet retention ponds therefore presents a risk of creating small ecological hot spots in which, highly stressed organisms can spread toxic compounds through the food chain. This may ultimately lead to the transfer of contaminants away from the retention pond area [Marsalek *et al*., 2002]. Because wet retention ponds are becoming increasingly common and the existing ponds are ageing, it is important to evaluate the severity of this risk to aquatic fauna and wildlife [Anderson *et al*., 2004]. The most common methods for monitoring heavy metal exposure in organisms that live in wet retention ponds and related environments involve using passive samplers and/or studying living organisms that serve as biomarkers.

A living organism is considered to be a potential biomarker that can be used to monitor environmental pollution if it absorbs contaminants in direct proportion to their abundance in the environment of interest [Ravera *et al*., 2003].

Freshwater mussels are widely used as biomarkers for studying aquatic pollution because; they are sedentary and long-lived filter feeders that live on the interface between sediment and water [Anderson *et al*., 2004; Naimo, 1995]. They can accumulate contaminants at levels that are significantly greater than their abundance in the surrounding water, without metabolizing them to any appreciable extent [Adjei-Boateng *et al*., 2010]. The abundance of a given contaminant within an individual mussel reflects the average level of that contaminant in its environment over its lifespan [Naimo, 1995; Mersch and Johansson, 1993] as well as the properties of the surrounding water and sediments. Mussels are therefore considered to be useful proxies for monitoring the exposure of aquatic ecosystems to toxic substances such as heavy metals [Anderson *et al*., 2004]. The concentrations of metals within mussel tissue samples is directly dependent on the size of the bioavailable heavy metal pool within the environment the mussel was grown in [Graney *et al*., 1983], and the use of mussels has been recommended in preference to passive samplers for the investigation of food-chain effects [Boehm *et al*., 2005]. The usefulness of mussels for monitoring pollutant loads has been demonstrated in a number of recent studies including those conducted by Perić *et al*. (2012) and Kibria *et al*. (2012), who used caged mussels to assess the bioavailable heavy metal content of different water samples.

The disadvantages of using living organisms as biomarkers are that the procedures involved can be cumbersome, time-consuming and expensive, and the results obtained can be rather variable [Vrana *et al*., 2005; Greenwood *et al*., 2009; Rundberget *et al*., 2009]. Some of these drawbacks can be avoided by instead using passive samplers, in which the pollutant of interest is trapped on an inert chemical matrix of some sort. Passive samplers can provide information on the environmental concentrations, fates and behavior of targeted pollutants [Greenwood *et al*., 2009] over deployment

periods ranging from hours to weeks [Persson *et al*., 2001]. They have a number of important advantages relative to living organisms for this purpose: they usually require less time to set up and analyze, are more affordable to use, and are simpler and more convenient to deploy and analyze. Because passive samplers can provide time-integrated estimates of pollutant bioavailability in aquatic systems [Persson *et al*., 2001; Greenwood *et al*., 2009; Rundberget *et al*., 2009], they should be useful devices for comparing pollutant concentrations in different wet retention ponds. Blom *et al*., (2002) have argued that the low cost and convenience of passive sampling will facilitate significantly more extensive spatial and temporal monitoring of metals in the aquatic environment than has previously been possible.

According to Thomas (2009) and Greenwood *et al*., (2009), passive samplers are more sensitive than monitoring based on living organisms because the former only adsorb dissolved chemical species regardless of their concentration. Since there is a linear relationship between the concentration of the target analyte in the water and that adsorbed by the sampler, passive sampling should provide a measure of the bioavailable/labile heavy metal concentration in wet retention ponds.

In the work presented herein, we compared the performance of passive samplers and biomarkers for the integrative sampling of heavy metals in wet retention ponds.

The accumulation of heavy metals over time in mussels and passive samplers exposed to stormwater was examined under controlled conditions in order to determine whether either method was reliable as an indicator of aquatic pollution.

If there is a strong correlation between the results obtained using mussels and passive samplers, one could reasonably conclude that chemical passive samplers are a viable alternative to biomarkers despite the numerous known and unknown factors that affect the accumulation of different chemical species in living organisms.

2   METHOD

2.1   General Experimental Details

Four tanks with dimensions of 29 cm by 117 cm containing approximately 41 liters of water (the tanks were filled with water to a depth of 12 cm) were set up in the lab. The water in each tank was oxygenated and recirculated within the tank by continuous pumping from one end of the tank to the other (flow: 0.04 l/s) using a Totton Pumps 230V 1PH 50/60 HZ 0.4 A pump (see figure 1).

The water placed in the tanks originated from a groundwater-fed lake with no inlet or outlet near Aarhus, Denmark (a former gravel pit). Because water evaporated from the tanks on a continuous basis, they were regularly topped up to maintain the desired volume. Unfortunately, severe frosts in December 2010 and January 2011 made it impossible to get water from the gravel pit in those months and so demineralized water was used instead on those occasions. To ensure good living conditions for the mussels, each tank was cleaned and had its the total water content replaced twice during the experimental run-time.
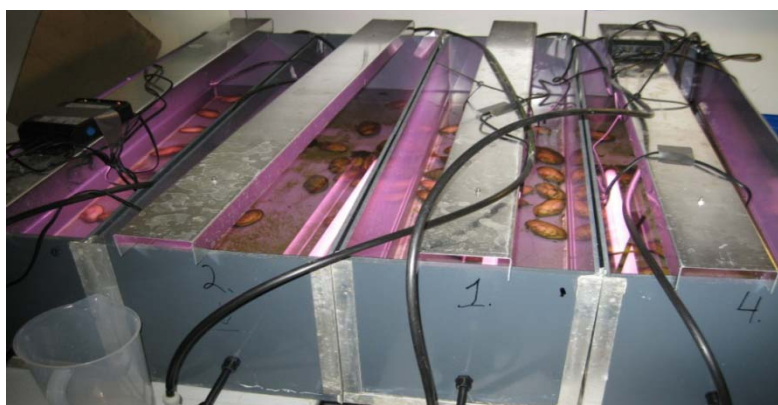

Figure 1: The experimental set up.

To establish an algal bloom that would provide food for the mussels, phosphate ((1 mg P / l) JT Baker CAS: 028-24-7 10, Deventer, The Netherlands) and ammonium ((10 mg N / l), Merck Art 1217, Darmstadt, Germany) were added to the water and lights (Exo Terra nature. PT-2241-2243-2245) were also installed above the tanks (see Figure 1). According to Naimo (1995) analyses of heavy

metal levels in malnourished mussels can be misleading because hunger affects the speed and amount of water transported through the gills. This can increase the accumulation of heavy metals beyond that which would be expected under "normal" conditions.

2.2   Mussels and passive samplers

40 freshwater mussels from the genus *Anodonta* were placed in each tank. The mussels used for the experiment were harvested on the 14th of November 2010 from the same lake as the water. In addition, seven passive samplers containing the strongly acidic ion exchange resin Dowex HCR-W2 (Sigma-Aldrich CAS: 69011-20-7, Steinheim, Germany; Figure 2) were also placed in each tank. For convenience, this material is henceforth referred to simply as "resin". The resin acts as a strong cation exchanger that has comparable affinities for all cationic metal ions. Heavy metals that adsorb on the resin do not desorb under the sampling conditions. Because the resin only adsorbs dissolved metal ions, analyses of the passive samplers should provide an indication of the average bioavailable heavy metal concentration in the water during the measurement period.
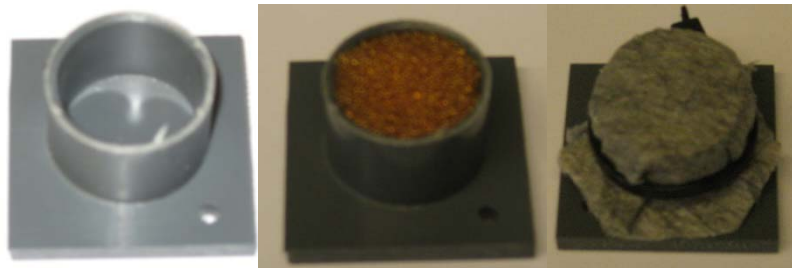

Figure 2: Passive samplers. The leftmost image shows an empty sampler, the central image shows a sampler filled with the ion exchange resin, and the rightmost image shoes a resin-filled sampler that has been sealed with a geotextile.

2.3   Heavy metals

Zn, Cu, Pb, Ni and Cr were added to the tank water to simulate the conditions in a stormwater pond. These metals were chosen because they are most abundant heavy metals in storm water ponds [Hvitved-Jacobsen *et al.*, 2010]. The metals were added as solutions of the following salts: nickel nitrate (pa, Sigma-Aldrich, CAS: 13478-00-7, Steinheim, Germany), chromium potassium sulfate (pa, Merck CAS: 1.01036.0250, Darmstadt, Germany), lead chloride (pa, Merck Schuchardt nature. 807383, Munich, Germany), copper sulfate (pa, Merck CAS: 1.02790.0250, Renningen, Germany) and zinc chloride (pa, Merck CAS: 1.08816.1000, Darmstadt, Germany).

The target heavy metal concentrations in the four tanks are shown in Table 1. The concentrations were intended to increase gradually on going from tank 1 to tank 4, at a ratio of 1:3:6:10. The concentrations in the two extreme cases were based on the typical minimum and maximum heavy metal concentrations in stormwater. Tank 1 was loaded with the lowest concentrations while tank 4 was loaded to such an extent that heavy metal toxicity was expected to cause significant mussel death during the experimental period.

Unfortunately, it was not possible to maintain constant concentrations of the metals. Therefore, the metal concentrations in the tanks were monitored approximately every ten days and additional metal salts were added as required to restore the target concentrations. At all times during the experiment, the heavy metal concentrations in the tanks remained within the ranges shown in table 2.

Table 1. Initial heavy metal concentrations.

Metal	Tank 1 [µg/l]	Tank 2 [µg/l]	Tank 3 [µg/l]	Tank 4 [µg/l]
Zn	100	300	600	1000
Cu	20	60	120	200
Pb	5	15	30	50
Ni	10	30	60	100
Cr	10	30	60	100

Table 2. Heavy metal concentration ranges during the experiment.

Metal	Tank 1 [µg/l]	Tank 2 [µg/l]	Tank 3 [µg/l]	Tank 4 [µg/l]
Zn	14-113	28-341	59-589	100-823
Cu	5-43	12-87	16-146	30-146
Pb	0-27	0-11	1-43	2-31
Ni	3-24	7-76	12-95	40-311
Cr	3-23	4-31	0-46	0-91

2.4   Sampling procedure

The experiment was conducted over a period of five months. Every third week, five mussels and one passive sampler were collected from each tank. After collection, the passive samplers and mussels were placed in a freezer at -21 °C and stored until required for analysis.

2.5   Chemical analysis

The mussels and the resin from the passive samplers were analyzed for Pb, Cr, Zn, Cu and Ni. In addition, water samples was also analyzed for P. Prior to analysis, the mussels were homogenized using a blender (Braun Vario 300 W type 418) and the resin was homogenized using a glass spatula. The samples were freeze-dried prior to extraction in a microwave oven (Microwave Reaction System, Anton Paar, Multiwave 3000 SOLV). The mussels were extracted according to EPA method 3051 and water samples were extracted according to EPA method 3015. The resin samples were extracted using a specifically-designed protocol in which 500 mg of dry resin was mixed with 6 ml of HNO_3 (67-69 %) prior to extraction. The heavy metal content of each sample was determined using ICP-OES (ICAP 6300 Duo View, Thermo Scientific). After extraction, 1 ml of a 200 µg/l yttrium standard solution (PlasmaCAL, SCP Science, Canada) was added to compensate for variation in the ICP-OES signal intensity. The ICP-OES instrument was calibrated using multi-element standards created by combining a series of certified single-element standards (PlasmaCAL, SCP Science, Canada). As a quality control procedure for the method as a whole, a sample of a certified reference material (EnviroMAT BE Sewage Sludge -1, SCP Science, Canada) was included in each run in the microwave oven. In cases where the measured elemental concentrations were below the method´s minimum detection limits (MDL), the concentration was recorded as being <MDL. In such cases, it was assumed thet the actual concentration was equal to half the MDL when performing calculations.

2.6   Statistical analyses

Potential correlation between the measured heavy metal concentrations in the mussel samples and those for the passive samplers were evaluated by calculating Pearson's correlation coefficient at $p \leq 0.05$.

A two-way ANOVA was performed to investigate the influence of the heavy metal concentration in the water and the experimental runtime on the uptake and accumulation of heavy metals in the mussels and passive samplers. Both of these factors were found to significantly influence metal accumulation/uptake at $p \leq 0.05$. All statistical calculations were performed using Minitab[®] 16, © 2010 Minitab Inc.

3   RESULTS AND DISCUSSION

The accumulation of Cr, Cu, Ni, Pb and Zn in mussels and passive samplers was investigated over a five month period. For the mussels, the day zero elemental concentrations are the means for 12 different mussels. For the passive samplers it was assumed that the initial concentration of each metal was zero. Unfortunately, this assumption proved to be invalid for Cu and Pb: after the experiment had been completed, it was discovered that the product specification for the resin states that it may contain up to 10 mg/kg of Cu and Pb. It would therefore have been preferable to have measured the concentrations of Cu and Pb in the resin samples before the start of the experimental run in order to have more accurate day zero concentrations. The measured concentrations of each element over the course of the experiment are shown in Figure 3. In the graphs for the mussels, each bar represents a pooled sample consisting of tissue from five different individuals; in the graphs for the passive

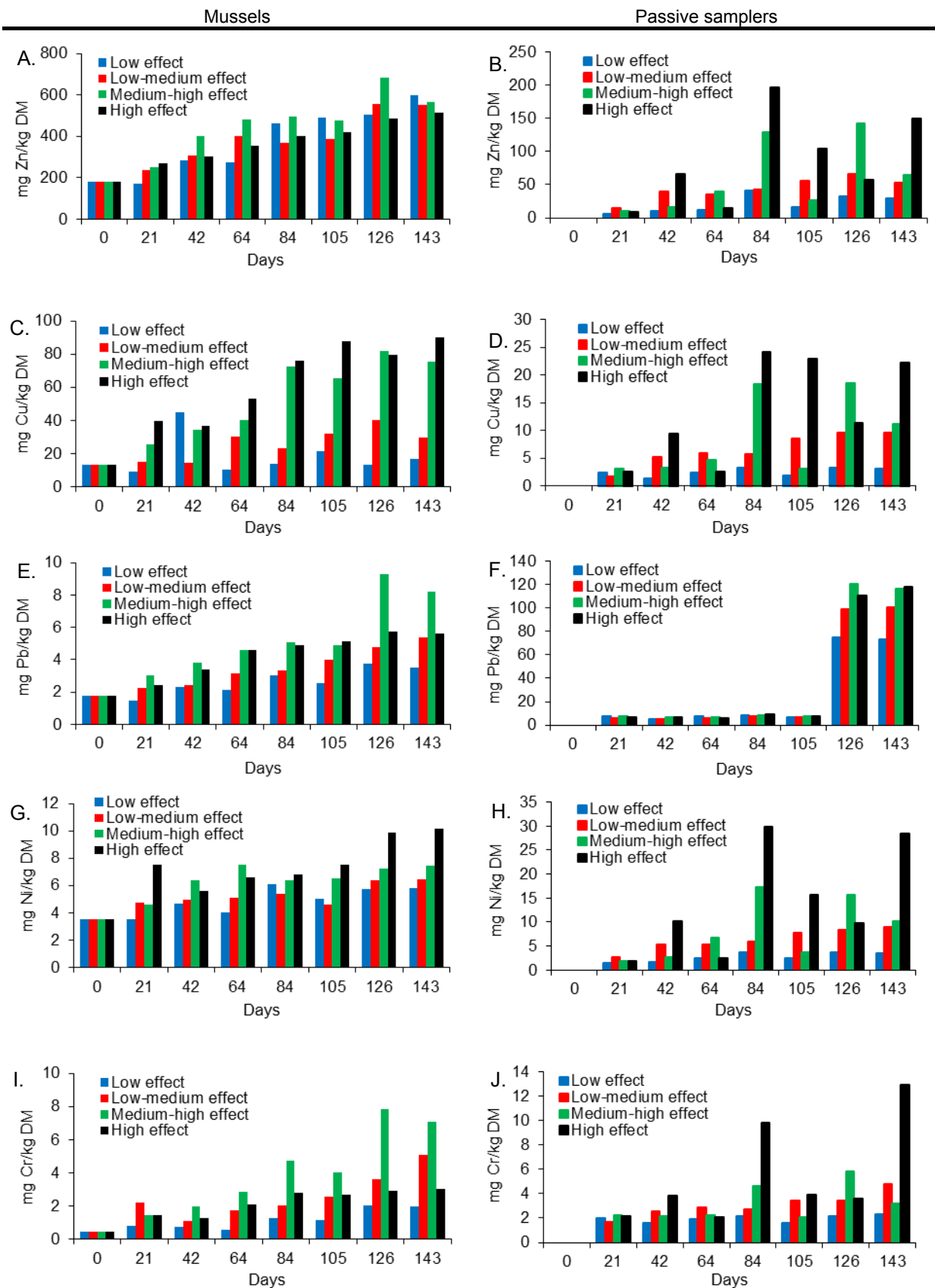samplers, each bar represents data for a single sampler.

Figure 3. Accumulation of Zn, Cu, Pb, Ni and Cr over time in mussels (left hand side) and resin (right hand side). Diagrams A) and B) show data for Zn, C) and D) for Cu, e) and f) for Pb, G) and H) for Ni, I) and J) for Cr. The scales on the y-axes differ in each case.

It is readily apparent that the heavy metal contents of both mussels and the resins increased over time; the maximum concentrations of the five heavy metals were observed after 126 to 143 days (figure 3). The two-way ANOVA confirmed that the experimental run-time had a statistically significant effect on the measured heavy metal contents in each case. However, this effect was more significant for mussels than for the resin (table 3). For mussels, the heavy metal concentrations increased proportionally over time whereas there was no such proportional relationship between concentration and time in the resin data. Instead, the heavy metal concentrations in the resin samples only began to increase significantly after approximately 12 weeks (with the exception of Pb, which only began to rise after 128 days).

In almost all cases, the mussels with the lowest heavy metal concentrations were those from tank 1. The trend for the other three tanks was somewhat ambiguous since in many cases, the heavy metal concentrations in mussels from tank 3 were higher than those for their counterparts in tank 4 despite the higher metal concentrations in the water of tank 4. Moreover, even though the mussels in tank 4 were exposed to tenfold higher heavy metal concentrations than those in tank 1, the heavy metal contents of the mussels and samplers from tank 4 were less than ten times greater than those in tank 1. Nevertheless, the two-way ANOVA confirmed that heavy metal concentration of the water had a statistically significant effect on the accumulated/adsorbed concentrations of heavy metals, and that this effect was more significant for the mussels than the passive samplers (table 3). The Only case in which the concentration of an element in the water did not significantly affect the measured values was that of Pb accumulation in the passive samplers.

Table 3. Two-way ANOVA results for the effects of time and water heavy metal concentration on the heavy metal contents measured in mussels and passive samplers.

Metal	Type	P_{Time}	$P_{Conc.\ in\ water}$	R^2(adj) %
Zn	Mussels	0.000	0.015	88.2
	Passive samplers	0.005	0.027	49.4
Cu	Mussels	0.009	0.000	63.7
	Passive samplers	0.012	0.008	49.1
Pb	Mussels	0.000	0.000	78.5
	Passive samplers	0.000	0.093	95.3
Ni	Mussels	0.000	0.000	72.7
	Passive samplers	0.012	0.011	48.1
Cr	Mussels	0.000	0.000	69.3
	Passive samplers	0.015	0.037	43.2

In general, both investigated factors (i.e. time and the concentration of the metal in the water) influenced heavy metal accumulation in mussels to a higher degree than in passive samplers; the only exception was Pb (table 3). It was also apparent that the mussels and the resin had different affinities for the five heavy metals. For Pb, Ni and Cr the measured concentrations in the resin were greater than those for the mussels, whereas the opposite was true for Zn and Cu.

As shown in table 4, there was no strong correlation between the metal uptake by the resin and the amount of metal that accumulated in the mussels. The strongest correlation (0.83) occurred for Cu while the weakest (0.46) occurred for Cr.

Table 4. Pearson correlation coefficients for the relationship between the heavy metal concentrations observed in mussels and passive samplers.

Metal	P	Pearson's R
Zn	0.001	0.56
Cu	0.000	0.83
Pb	0.000	0.73
Ni	0.000	0.65
Cr	0.008	0.46

The rates at which metals accumulate in mussels probably depend on the metal concentrations to which the mussels are exposed. However, freshwater mussels can close their shells, thereby avoiding exposure to high concentrations of pollutants [Naimo, 1995]. This may explain why the mussels in tank 4 had lower heavy metal contents than those in tank 3, even though the water in tank 4 had a higher

concentration of each metal. This suggests that mussels are capable of regulating their uptake of toxic metal ions. In keeping with this conclusion, Mersch and Johansson (1993) have previously reported that mussels exercise control over their intracellular metal concentrations. These authors also noted that the metal concentrations in mussel samples remain low even when the mussels are exposed to intermittent high heavy metal loads because the kinetics of their accumulation are slow. This may explain why the mussels from the four different tanks had relatively similar heavy metal contents.

In principle, the rates of heavy metal adsorption on passive samplers should be directly proportional to the concentrations of the corresponding metal ions in the water being sampled [Vrana *et al*., 2005]. The measured heavy metal concentrations in the passive samplers should therefore have reflected the metal ion concentrations in the different tanks more accurately than they did. On the basis of previous studies, it was expected that the resin would bind 100% of the metals at low and intermediate [100-1000 µg/l] concentrations. The concentrations of the heavy metal ions in the artificial stormwater ranged from 1-300 µg/l, so the measured concentrations in the resin were much lower than one might have expected.

It is possible that the differences in the measured concentrations of the metal ions in the passive samplers occurred because the capacity of the resin was exceeded. According to Greenwood *et al*. (2009), the adsorbed volume is constrained by the sorption capacity of the passive sampler, and Rundberget *et al*. (2009) noted that one cannot generally rule out the possibility that the resin may become saturated during an experiment.

Because the resin used in this work is a strong cation exchanger, it was expected to have identical affinity for Zn, Cu, Pb and Ni cations. Since Cr exists as a complex anion in aqueous solution, it was not expected to be adsorbed at all. This theory was rejected by the results, since the resin adsorbed different concentrations of all five metals, with Zn, Pb and Ni being most strongly adsorbed. Cr was also adsorbed on the resin despite existing as an anion in aqueous solution.

It is possible that these unexpected results were obtained because the resin was stored in small PVC tubes that were sealed with a heavy geotextile. The metals therefore had to diffuse through the geotextile in order to reach the resin. The extents to which the metal ions were capable of passing through the geotextile was not investigated. It is therefore possible that the geotextile reduced the potential for interaction between the dissolved ions and the resin and was thus responsible for the unexpected results. The fact that the heavy metal concentrations in the passive samplers only began to increase after approximately 12 weeks may also have been due to the properties of the geotextile. It is possible that the resistance of the fabric declined over the course of the experiment, thereby permitting larger quantities of metals to pass through. Similar membrane effects on the functioning of passive samplers have been reported by Persson *et al*. (2001).

Another potential reason for the unexpectedly poor performance of the passive samplers is that the geotextile membrane may have been colonized by bacteria or algae, resulting in the formation of a biofilm. This is consistent with the observation that several of the passive samplers became coated with green algae as the experiment progressed. According to Vrana *et al*. (2005), biofilm thickness can vary considerably, even on a single membrane. Both Persson *et al*. (2001) and Rundberget *et al*. (2009) confirm that biofouling can affect the overall resistance to mass transfer by increasing the thickness of the barrier and blocking water-filled pores in the membrane, thereby reducing the scope for contact between dissolved heavy metals and the resin. The sharp increase in uptake that occurred after 12 weeks coincided roughly with the first cleaning of the tanks, during which the mussels and passive samplers were rinsed in clean water. This would have removed algae from the membranes, potentially increasing the scope for the diffusion of metal ions to the resin. The low uptake at the start of the experiment is also consistent with the biofouling theory because there was a run-in period prior to the first sample collection during which algal growth on the membranes may occurred.

Based on the results presented herein, we argue that analyses of living organisms provide more reliable and accurate pictures of the ecological conditions in wet retention ponds than can be achieved using passive samplers.

Further investigations will be required to fully evaluate the performance of passive samplers in biomonitoring. In addition, it will be necessary to obtain more information on the relationship between the properties of the adsorbing media and the performance of passive samplers under different conditions. It may also be necessary to re-evaluate the design of containers for the adsorption media in passive samplers and its functional consequences. The findings presented herein are consistent with those of Wilkie *et al*. (2010), who concluded that metal-chelating resin SIR 300[TM] could not be used to determine metal bioavailability based on a field experiment using metal contaminated

sediments.

4 CONCLUSION

No strong correlation was found between the measured heavy metal concentrations in mussels and those observed in passive samplers. This suggests that passive samplers should not be used as substitutes for living organisms when studying the bioavailability of heavy metals in aqueous environments.

Our results indicate that analyses of mussels provide reliable data on changes in element concentrations over time in the aqueous environment because the metal concentrations in the mussel samples increased as the experiment progressed and as the heavy metal concentrations in the water increased.

Several factors must be considered when deciding whether to use mussels or passive samplers in practical studies. In particular, one must think very carefully when deciding whether or not to use passive samplers to study conditions in wet retention ponds.

Finally, our results show that there are a number of important differences in the accumulation of heavy metals by living organisms and that on passive samplers.

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