Exposure of caged mussels to metals in a primary-treated municipal wastewater plume

Christian Gagnon a,*, François Gagné a, Patrice Turcotte a, Isabelle Saulnier a, Christian Blaise a, Michael H. Salazar b, Sandra M. Salazar b

a St. Lawrence Centre, Environment Canada, 105 McGill St., 7th floor, Montreal, Quebec, Canada H2Y 2E7
b Applied Biomonitoring, Kirkland, WA 98034, USA

Received 8 February 2005; received in revised form 25 May 2005; accepted 10 June 2005
Available online 6 September 2005

Abstract

The biological availability of metals in municipal wastewater effluents is strongly influenced by the physical and chemical conditions of both the effluent and the receiving water. Aquatic organisms are exposed to both dissolved and particulate (food ingestion) forms of these metals. In the present study, the distribution of metals in specific tissues was used to distinguish between exposure routes (i.e. dissolved vs. particulate phase) and to examine metal bioavailability in mussels exposed to municipal effluents. Caged Elliptio complanata mussels were deployed at sites located between 1.5 km upstream and 12 km downstream of a major effluent outfall in the St. Lawrence River. Metals in surface water samples were fractionated by filtration techniques to determine their dissolved, truly-dissolved (<10 kDa), total-particulate and acid-reactive-particulate forms. At the end of the exposure period (90 days), pooled mussel soft tissues (digestive gland, gills, gonad, foot and mantle) were analyzed for several metals. The results showed that gills and digestive gland were generally the most important target tissues for metal bioaccumulation, while gill/digestive gland metal ratios suggest that both exposure routes should be considered for mussels exposed to municipal effluents. We also found that Ag and Cd in the dispersion plume nearest the outfall, in contrast to other metals such as Cu and Zn, are more closely associated with colloids and were generally less bioavailable than at the reference site in the St. Lawrence River.

Crown Copyright © 2005 Published by Elsevier Ltd. All rights reserved.

Keywords: Metal speciation; Bioavailability; Colloids; Effluent

1. Introduction

Urban effluents represent a major source of chemical contaminants for the aquatic environment (Chambers et al., 1997). Because effluents from municipal wastewater treatment plants are derived from both domestic and industrial sources, they contain a wide variety of natural and industrial products. Surveys in Canada have shown that metals and organic chemicals are present in municipal effluents (OMOE, 1988; Rutherford et al., 1994; Ministère de l’Environnement du Québec and Environment Canada, 2001) and a number of relatively small industrial plants release metals directly into municipal sewer systems (Ministère de l’Environnement du Québec and Environment Canada, 2001). Contaminants released in urban wastewater discharges may be taken
up by the aquatic biota, leading to toxic effects (Rutherford et al., 1994; Wong et al., 1995; Gagné et al., 2002).

Predicting the environmental impact of the metals contained in municipal effluent discharges requires an understanding of how their physical and chemical characteristics, and those of the receiving waters, affect metal uptake by aquatic organisms and the associated toxicity. Although the toxicity of a municipal effluent is dependent on factors related to the treatment processes it undergoes, the physical and chemical characteristics of the receiving waters can also influence the toxicity of contaminants once they are released to the environment (Lijklema et al., 1993). These same properties also affect the speciation of both dissolved and particulate metals (Lijklema et al., 1993; Gagnon and Saulnier, 2003). The biological availability of metals is also influenced by their speciation in both the dissolved and particulate phases (Luoma, 1983; Campbell et al., 1988; Wang et al., 1997; Gagnon and Fisher, 1997b).

Physico-chemical parameters such as pH, organic carbon, suspended particulate matter (SPM) and temperature have an effect on the fate of metals and their biological uptake by both dietary and waterborne vectors (Luoma, 1983; Roditi et al., 2000). Metal partitioning occurs between the dissolved and particulate phases in effluent discharged to the aquatic environment, with geochemical processes playing a major role (Gagnon and Saulnier, 2003; Karvelas et al., 2003). The distribution of particulate metals is considerably influenced by organic matter as well as by reactive particulate iron (Fe) and manganese (Mn) (Gagnon and Saulnier, 2003). Various metal associations with both particulate and dissolved phases are determined by the concentration and strength of specific ligands. Thus, aquatic organisms are exposed to a variety of chemical forms of a given metal (Campbell et al., 1988; Luoma et al., 1992; Fisher and Reinfelder, 1995; Wang et al., 1997). Metal bioaccumulation, toxicity and exposure routes are probably all influenced by the concentration of each form of the metal. It has been suggested that metal concentrations in the gill and digestive gland can be used to estimate waterborne and dietary exposures, respectively (Fisher et al., 1996). Such differences in metal tissue distribution were documented for mussels exposed to waterborne and dietary metals. For example, Long and Wang (2005) recently reported that metal concentrations in mussel gills were higher than those in the remaining tissues after exposure to waterborne metals and reversed patterns after dietary exposure.

We related the metal forms at different distances within the dispersion plume with those found in the tissues of mussels caged in the plume. The objectives of the study were to: (1) measure the distribution of various metals in mussel tissues; (2) estimate waterborne versus dietary exposure by comparing uptake in the gill versus the digestive gland; (3) compare the relative uptake from water and food by using gill/digestive gland ratios for various sites above and below the outfall; and (4) compare metal accumulation in tissue with metals in operationally dissolved (<0.45 µm), truly-dissolved (<10 kDa), particulate and reactive-particulate phases. Metal accumulation was assessed in mussels placed in the dispersion plume of an effluent whose dilution by receiving water can influence the bioavailability and distribution of metals among target tissues. In parallel, changes in the physico-chemical form of metals were studied in the effluent plume by measuring the dissolved, truly-dissolved, total-particulate, and reactive-particulate fractions of metals. Moreover, the biomarkers of vitellogenin and metallothionein were used to confirm mussel exposure to the effluent and its associated estrogenicity and metal exposure, respectively.

2. Methods

2.1. Water sampling

The effluent dispersion plume was previously characterized by the determination of total and fecal coliforms or chemical contaminants as markers for municipal effluents (Gagné et al., 2001a; Gagnon and Saulnier, 2003) (Fig. 1). Surface water and suspended matter were sampled at several sites ranging from 0.5 to 15 km downstream of a major municipal outfall in the St. Lawrence River (Fig. 1). Samples were also collected at a reference station 1.5 km upstream of the effluent outfall in order to document the initial river water conditions. Surface water samples were collected using a Teflon pump following clean sampling procedures for trace metals, as described in Cossa et al. (1996).

2.2. Mussel exposure experiment

Elliptio complanata mussels were collected during the first week of May 2001 at Lake de L’Achigan in the Laurentians, Quebec, Canada, where mussels have been collected for previous studies. The animals were placed in experimental cages according to standard protocols (ASTM, 2001). Briefly, mussels were sorted by size (weight and shell length) and placed in cylindrical nets, which were attached to a PVC frame (1 m² surface area). The frames were attached to 20 kg blocks and marked with a submerged buoy. Two cages were deployed at each of four sites: 1.5 km upstream (reference station) and 5, 8, and 12 km downstream of the municipal effluent outfall (Fig. 1). The cages could have not been deployed any closer to the outfall due to the high current and suspended matter loads in this stretch of the river. Caged mussels (30 per cage) were submerged in the St. Lawrence River for 90 days, from the beginning of June until the beginning of September 2001.
all mussels at the site 5 km downstream of the outfall died, probably by being smothered by high concentrations of SPM or by being partially covered with bottom sediment. Nevertheless, the three remaining sites provided a metal contamination gradient and, especially, high upstream–downstream differences in water metal concentrations. At the end of the exposure period, the caged mussels were collected and brought to the laboratory within 5 h for shell length, shell weight, and soft tissue weight measurements. In prior biomarker experiments, mussels were kept at 15°C for 24 h in dechlorinated and aerated tap water to purge the tissues of recently ingested material. Mussel soft tissues (digestive gland, gills, gonad, foot and mantle) were collected separately for biomarker and metal determinations and stored at −85°C until analysis. Mussel tissues for metal determination were freeze-dried prior to analysis. Metals in soft tissues were determined by ion-coupled plasma mass spectrometry following an acid digestion (National Laboratory for Environmental Testing (NLET), 1994).

2.3. Water and particle analyses

Water samples (200–500 ml) were filtered through 0.45 μm Teflon filters in a class-100 clean laboratory to obtain the dissolved and particulate fractions. The filtered samples were then acidified with grade Q HNO₃ 1% (Cossa et al., 1996; Gagnon and Saulnier, 2003). Samples of particulate matter (>0.45 μm) obtained by filtration were stored at −20°C until analysis. In addition, 0.45 μm filtered samples were also ultrafiltered within 2 h using a cross-flow tangential filtration system to obtain the high-molecular-weight, colloidal (>10 kDa) and truly-dissolved low-molecular-weight (<10 kDa) fractions. The cross-flow ultrafiltration (CFUF) system was composed of a peristaltic pump and a Millipore Prep/ScaleTM-TFF 1 ft² cartridge regenerated cellulose membrane with a cut off of 10 kDa. The pressure on the membrane was maintained between 14 and 16 psi during the operation. The water flow rate was adjusted to about 6 l/min before the beginning of the ultrafiltration procedure. Ultrafiltered water samples were, then, acidified (1% Q-HNO₃) and stored in acid-clean LDPE bottles until the metal analysis. Dissolved trace metals were determined by ion-coupled plasma mass spectrometry (National Laboratory for Environmental Testing (NLET), 1994). Following their digestion with concentrated acids HNO₃–HCl–HF, total particulate metals were determined by graphite furnace atomic absorption spectrometry and ion-coupled plasma emission spectrometry (Dumouchel et al., 1997). To determine their reactivity and document their potential
bioavailability, metals associated with suspended particles (retained on the 0.45 μm filter) were also partly extracted using 1 N HCl (Huerta-Diaz and Morse, 1990; Raiswell et al., 1994). This dilute acid solution has been suggested as one of the best simple extractants for estimating extractable metals and assessing their bioavailability (Luoma and Bryan, 1978). HCl-extractable metal analyses were performed on samples of particulate matter as described by Gagnon and Saulnier (2003). Suspended particulate matter (SPM), total organic carbon (dissolved and particulate) and other ancillary parameters were determined using standard methods described elsewhere (Cossa et al., 1998; Gagnon and Saulnier, 2003).

2.4. Biomarker analyses

Exposure of bivalves to environmental estrogens in municipal effluent was confirmed by the vitellogenin biomarker (Kägi, 1991). Vitellin-like proteins (Vn) were determined in mussel gonads using the organic alkali-labile phosphate (ALP) procedure (Blaise et al., 1999; Gagné et al., 2001b; Blaise et al., 2002). For the ALP procedure, the gonad homogenate was prepared in ice-cold 125 mM NaCl containing 10 mM Hepes-NaOH, pH 7.5, 1 mM dithiothreitol and 1 mM EDTA using a Teflon pestle apparatus. The homogenate was centrifuged at 15000 g for 20 min to obtain the soluble protein fraction. Then, acetone was added to give 35% v/v and centrifuged at 10000 g for 5 min to precipitate high molecular weight proteins. The pellet was resuspended in 200 μl of NaOH 1 M and allowed to stand at 60 °C for 30 min. The release of inorganic phosphate was determined by phosphomolybdenum assay. The results were expressed as μg phosphate per mg gonad protein.

Induction of metallothionein-like proteins related to the detoxification of divalent metals (Kägi, 1991; Baudrimont et al., 1997) were analyzed in digestive gland, gonad and gills by the spectrophotometric thiol assay (Viarengo et al., 1997). Briefly, the tissues were homogenized in the homogenization buffer and the supernatant separated at 15000 g. Metallothioneins were selectively isolated from high molecular weight proteins and low molecular weight thiol compounds by ethanol and chloroform extractions. The levels of thiols were determined by the 5,5'-dithiobis(2-nitrobenzoic acid) reagent with readings taken at 412 nm. Standard solutions of reduced glutathione were used for calibration. The data were expressed as μg of thiol equivalents per mg proteins.

2.5. Quality assurance and quality control

All filtrations and analyses of water samples for trace metals were performed in a class-100 clean room (Cossa et al., 1996). Quality assurance activities included regular processing of reagent blanks and uses of reference materials. The precision and accuracy of metal analyses in the dissolved and ultra-filtrated phase were evaluated using internal reference material. The precision for all metal, expressed as coefficient of variation was 12% or better. The accuracy for all metal, expressed as % of recovery, was comprised between 82% and 100%.

The reference sediment material MESS-3 (National Research Council of Canada) was used for the evaluation of the precision and accuracy of total particulate analyses. The coefficient of variation for Cr, Cu, Zn, Ni, Cd and Pb was 10% or better and the recovery for these metals was between 85% and 106%. For Ag, its concentration in MESS-3 was to low, thus internal reference material was used and the coefficient of variation and the accuracy were 5% and 109%, respectively. For reactive metals (HCl 1 N extraction), the MESS-3 was not used because is not certified for this chemical extraction. For the evaluation of the analysis precision, an internal reference material was used and the precision, expressed as coefficient of variation, was 7% or better.

The reference material DORM-1 (NRCC) was used for the quality control of mussel tissue analysis. The coefficient of variation for Cr, Ni, Cu, Zn, Ag and Cd was 5% or better and 18% for the Pb. The recovery for Cr, Ni, Cu, Zn, Cd and Pb were between 90% and 105%. The reference material DORM-1 was not certified for Ag.

Internal reference materials were used for the evaluation of the variability and recovery of the SPM, DOC and POC. For these three parameters the coefficient of variation was 5% or better and the recovery was between 85% and 95%.

2.6. Data analysis

Differences between various metrics at the cage sites were assessed using Student’s t test when the variance proved to be homogeneous. In the case of variance heterogeneity, a non-parametric Mann–Whitney U test was performed. Significance was set at p < 0.05. A correlation analysis of the metal fractions in receiving waters and in mussels was performed using a stratified version of Kendall’s tau test (τ).

3. Results

3.1. Chemical and physical water characteristics

Concentrations of various parameters such as suspended particulate matter (SPM) and dissolved and particulate organic carbon (DOC, POC) were measured to characterize the receiving waters and determine their influence on the fate and effects of metals released in urban effluent (Table 1). The results demonstrate the
relative enrichment of urban effluent compared to unaffected receiving waters, with SPM, DOC and POC concentrations being, respectively, 13%, 7% and 36% higher than those at the reference site. After mixing with receiving waters, SPM and POC concentrations at the site 12 km from the outfall dropped to levels similar to those measured upstream (reference station). These variations reflect dilution in the dispersion plume. Around 75% of the effluent was reported to be diluted 5 km downstream, with 90% dilution occurring in the first 10 km downstream of the outfall (Gagnon and Saulnier, 2003).

3.2. Trace-metal distributions in receiving waters

Dissolved-metal concentrations decreased rapidly along the effluent dispersion plume and were typically higher downstream than at the upstream site (Fig. 2). At 8 km downstream (i.e. the site nearest the outfall), enrichment factors reached values of 10, 1.3, 20, 1.1, 1.2, 0.5, respectively, for dissolved Ag, Cd, Cr, Cu, Ni and Zn.

In contrast, particulate concentrations of certain metals (i.e. Cr, Ni) tended to increase within the first 5 km downstream, while others (e.g. Ag, Cu and Zn) were similar to levels for dissolved metals (Fig. 3). Particulate concentrations tended to stabilize further downstream.

Municipal effluents release a substantial amount of colloids, which can decrease the relative proportion of metals in the truly-dissolved phase. Distributions of the truly-dissolved metal fraction (i.e. <10 kDa), expressed as a proportion (%) of the total dissolved phase (<0.45 µm), are shown in Fig. 4. Truly-dissolved proportions of Ag and Cd were considerably lower at the site 8 km downstream than at the reference site. Cadmium and Ag were the two metals with the lowest truly-dissolved fraction at the site 8 km, with proportions as low as 30% and 58%, respectively. The other metals presented different trends: the truly-dissolved fraction, for example, was rather stable or decreased along the dispersion plume. Much higher proportions of colloidal metals were found at sites closer to the outfall. For example, 65% of dissolved Cd, 95% of Ag and, to a lesser extent, half of the dissolved Cu and Zn, were measured as colloids at a site 0.5 km downstream of the outfall (unpublished data). These high colloidal metal fractions observed would be the result of their association with colloidal material released from the municipal

---

Table 1

<table>
<thead>
<tr>
<th>Distance from outfall</th>
<th>SPM c (mg/l)</th>
<th>POC d (mg C/l)</th>
<th>C in SPM c (%)</th>
<th>DOC e (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upstream</td>
<td>3.0 (0.2)</td>
<td>0.25 (0.01)</td>
<td>8.3 (0.6)</td>
<td>2.3 (0.1)</td>
</tr>
<tr>
<td>8 km downstream</td>
<td>3.4 (0.2)</td>
<td>0.34 (0.02)</td>
<td>10.0 (0.7)</td>
<td>2.5 (0.1)</td>
</tr>
<tr>
<td>12 km downstream</td>
<td>2.9 (0.2)</td>
<td>0.25 (0.01)</td>
<td>8.6 (0.6)</td>
<td>2.4 (0.1)</td>
</tr>
</tbody>
</table>

a Values in parentheses are standard deviations.
b Water samples were collected on August 14, 2001.
c Suspended particulate matter.
d Particulate organic carbon.
e Dissolved organic carbon.

---

Fig. 2. Distribution of dissolved trace metals in the dispersion plume.

Fig. 3. Distribution of particulate trace metals in the dispersion plume.

---

wastewater effluent. Despite the influence of the effluent outfall on metal speciation, the colloidal metal proportions observed are comparable to values found in the literature. For example, Wen et al. (1997, 1999) reported high proportions of metal in colloidal forms: Cd: 45%, Cu: 55%, Ni: 36%, Pb: 64%, Ag: 89% and Zn: 91%. The relatively low proportions of the truly-dissolved fraction observed for some metals in the plume highlight the influence of colloids in the distribution of dissolved metals and their low potential bioavailability.

Municipal effluent is a major source of particulate metals that are potentially labile or weakly associated with suspended particles. The distributions of reactive/labile particulate metals (partial extraction with 1 N HCl), expressed as a proportion (%) of total particulate metal concentration, are shown in Fig. 5. Partial chemical extractions on suspended particles indicate that released particulate metals are generally more reactive in the effluent dispersion plume than at the reference site. Reactive metal fractions in the dispersion plume exceeded 50% for all metals except Pb. The reactivity of particulate Pb was very low (<20%) at all sites. The highest reactivity value for all metals, except Ag, was observed at the site 8 km downstream of the outfall. Generally, the reactive fraction was higher at this site than at the reference site, and decreased along the dispersion plume. A contrasting trend was observed for Ag and Pb, probably explained by their strong affinity for POC.

3.3. Biomarkers

The exposure of mussels to the effluent was confirmed by the estrogenic effects observed in the dispersion plume. Mussels exposed to the municipal wastewater plume had higher levels of vitellin-like proteins (Vn) (Fig. 6). The mean value of Vn in mussel gonads was 0.05 μg alkali-labile phosphate (ALP)/mg protein at the reference site, while mean values of 0.13 and 0.12 were measured at the sites 8 and 12 km downstream of the outfall, respectively.

Levels of metallothionein-like proteins (MT) analysed in the digestive gland, gonad and gills also helped confirm effluent exposure (Fig. 7). The highest MT
values were observed in gonads, followed by digestive glands and then gills. Differences in MT levels for each tissue were significant (ANOVA, \( p < 0.05 \)) among sites. For a given tissue, the highest MT value was observed at 8 km downstream, with maximum values of 0.09, 0.07 and 0.04 \( \mu \text{mol thiols/mg proteins} \), respectively, in gonads, gills and digestive glands. MT values measured in the tissues of mussels at the site 12 km downstream of the outfall were comparable or even lower than those at the reference site. These results indicate the presence of MT-inducing agents in the vicinity of the effluent outfall.

### 3.4. Metal bioaccumulation in mussels

Mussels that were exposed to the effluent dispersion plume accumulated all the metals measured in their tissues (Fig. 8). Accumulated metal concentrations were above or similar to beginning-of-test (\( T = 0 \)) concentrations (Table 2). Based on published metal efflux rates for mussel (1–5% daily: Fisher et al., 1996; Wang et al., 1997), the duration of our exposure experiment would have allowed caged mussels to depurate from initial contamination (i.e. at \( T = 0 \)). The highest concentrations of accumulated metals in exposed mussels were generally observed gill and digestive gland. Copper and Zn were the most abundant metals in the effluent dispersion plume and the most concentrated in gills, with concentrations exceeding 15 and 350 mg/kg dw, respectively. However, metal levels were not higher in the gills of mussels at the downstream sites, nor were metal accumulations in gills significantly different (\( p > 0.05 \)) between upstream and downstream sites. The other metals (Ag, Cd, Cr, Ni, Pb) were also found in mussel gills, but at much lower concentrations (<7 mg/kg dw). The levels were significantly lower (\( p < 0.05 \)) in mussel gills at the 8 km site than at the reference site (Fig. 8). Metal concentrations were significantly higher at the site 12 km downstream and similar to those at the reference site.

Based on the observed concentrations of bioaccumulated metals, the digestive gland also appears to be an important target tissue. Concentrations of Cu and Zn in the digestive gland reached 20 and 150 mg/kg dw, respectively, while concentrations of the other metals were similar to levels in gills. Concentrations of Ag, Cr, Ni and Pb in digestive glands were significantly different (\( p < 0.05 \)) among sites. Concentrations of these metals were significantly lower (\( p < 0.05 \)) in the digestive gland of mussels placed nearest the outfall (8 km site) than further downstream (12 km) or at the upstream reference site.

Gill/digestive gland metal ratios were determined to establish the relative importance of these two main target tissues and to help estimate waterborne and dietary metal exposures. Although ratios were often higher than 1 at the reference station, the ratios were close to 1 for most metals (except Ni and Zn), suggesting comparable exposures from water and food ingestion at the 8 km site (Table 3).

Concentrations of metals were generally lower in gonads and in the two non-target tissues (i.e. foot and mantle) than in gills and digestive glands (Fig. 8). These data support the hypothesis that the gill and the digestive gland, at a lesser extend, are the primary target tissues for metal accumulation. Concentrations of Cu were below 15 mg/kg in the non-target tissues, while Zn concentrations were less than 150 mg/kg. Accumulation of
the other metals in non-target tissues and gonads was generally observed at levels slightly lower than or similar to levels in gills and digestive glands. Data on gonads and non-target tissues were given little consideration in this study on metal exposure because metal concentrations in these tissues are the result of re-distribution among all tissues. On the other hand, more attention was paid to the metal accumulation in gills and digestive glands, as they are expected to have more direct contact with metals, respectively, in the dissolved phase and particulate phase through particle ingestion.

4. Discussion

The exposure of mussels to the municipal wastewater plume was confirmed through their estrogenic response, i.e. higher levels of Vn (Fig. 6). The synthesis of Vn, which is estrogen-dependent, can be modulated by environmental chemicals known to interact with the estrogen receptor (Palmer and Selcer, 1996; Gagné et al., 2001b).

Mussels at the downstream sites had elevated levels of MT in tissues (Fig. 7). Indeed, there was significant ($p < 0.05$) induction of MT in mussel tissues, with the highest MT value being observed at the site 8 km downstream of the outfall. However, those MT values measured could not be explained by metal uptake in tissues at this downstream site. This raises the issue of the specificity of the MT biomarker towards heavy metals in complex mixtures. Although MT is generally recognized as a biological marker for metal exposure and metal detoxification in less complex contaminated aquatic environments (Roesijadi, 1992; Couillard et al., 1995), the biomarker does not appear to be specific enough for assessing metal exposure in this municipal effluent which releases various domestic, clinical and industrial contaminants. Indeed, this protein was shown to be induced in aquatic species by other stressors than metals such as inflammation from infection (Regala and Rice, 2004) and oxidative stress (Viarengo et al., 1997; Wright et al., 2000). A previous study at the same sites, showed an increased phagocytic activity which is associated to infection/inflammation conditions after 60 days (Blaise et al., 2002). Lipid peroxidation in mussels exposed for 1 year to the municipal effluent plume was increased in gills and gonad tissues (Gagné et al., 2004). MT induction in aquatic environments contaminated by a large municipal effluent seems highly dependent on the duration of exposure to urban pollutants and its specificity towards metals is difficult to appraise in such chemically complex environments. To best of our knowledge, this is the first study showing that MT induction in aquatic environments contaminated by a large municipal effluent could be associated to other factors than metals. Hence the value of measuring metal speciation and bioavailability to tailor out the causative agents underlying MT induction are of values in environments contaminated by complex urban pollutants.

4.1. Metal bioaccumulation vs. dissolved phase

Based on the relative tissue levels of metals, the gills appear to be the major target tissue (Fig. 8). These findings support the dissolved phase as an important exposure route for mussels (Carpene and George, 1981). The relatively high Cu and Zn accumulation in gills is related to the relative abundance of these metals in the receiving waters (Figs. 2 and 3). Metal accumulation, however, was generally lower ($p > 0.05$) for Ag, Cd, Cr, Ni and Pb in mussel gills at the site nearest the
outfall than at the reference site or further downstream (Fig. 8). The low bioaccumulation observed at the 8 km site could not be explained by the total dissolved metal concentrations in the water. In fact, mussel gills were not exposed to lower dissolved metal concentrations. Rather, they were exposed to concentrations similar to or higher than those at the upstream site (Fig. 2). As mussel gill can be seen as the target tissue for metal bioaccumulation through the dissolved phase (Long and Wang, 2005), dissolved metal concentrations in the receiving water were compared to levels in this mussel tissue.

A statistical analysis (Kendall’s tau test) was performed on data for all the metals combined (Table 4). The analysis showed very few meaningful relationships between metal exposure and mussel responses and that metal concentrations in mussel gills were significantly \( p < 0.05 \) inversely correlated to the levels in the dissolved phase of the receiving water. This suggests that low- and high-molecular-weight dissolved matter contribute differently to metal availability in gills. Thus, the dissolved metal concentration in receiving waters is not directly proportional with metal accumulation in mussel gills. Fig. 9 provides examples (Ag and Cd) of how total dissolved concentrations of metals cannot predict the metal bioaccumulation in mussel gills. Other field studies on the effects of environmental conditions on metal uptake by mussels showed poor relationship between total metal exposure and metal tissues concentrations (Gundacker, 1999).

### 4.2. Metal bioaccumulation vs. particulate phase

The digestive gland can be viewed as the target tissue through particle ingestion. Therefore, metal concentrations in this tissue were compared against those in particles. No significant correlation \( (p > 0.05) \) was found between metal concentrations in the digestive gland and concentrations in suspended particles (Table 4). Thus, total concentrations of particulate metals alone cannot predict metal bioaccumulation in the digestive gland. However, the metals in the remainder of tissues (after gills, gonad and digestive gland removal) is explained by the particulate phase of metals in the plume \( (\tau = 0.52; \ p = 0.01) \). As the plume is characterized by suspended particles rich in organic matter (Table 1), the bioavailable fraction of particulate metals may change along the dispersion plume. This is shown in Table 3, where the gill/digestive gland metal ratio steadily dropped for most metals along the dispersion gradient.

### 4.3. Metal bioaccumulation vs. truly dissolved phase

The high concentration of dissolved organic carbon may be related to colloidal enrichment close to the outfall. The effluent contains large amounts of organic matter, and relatively high concentrations of particulate

---

**Table 4**

<table>
<thead>
<tr>
<th>Metal fraction</th>
<th>Gill</th>
<th>Digestive gland</th>
<th>Gonads</th>
<th>Remainder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \tau ) P</td>
<td>( \tau ) P</td>
<td>( \tau ) P</td>
<td>( \tau ) P</td>
</tr>
<tr>
<td>Dissolved</td>
<td>-0.62 0.0041</td>
<td>0.05 0.6959</td>
<td>0.05 0.6950</td>
<td>-0.14 0.4332</td>
</tr>
<tr>
<td>Truly-dissolved</td>
<td>-0.20 0.3544</td>
<td>0.20 0.3544</td>
<td>-0.07 0.6451</td>
<td>-0.07 0.6451</td>
</tr>
<tr>
<td>Particulate</td>
<td>-0.24 0.2377</td>
<td>0.33 0.1130</td>
<td>0.33 0.1138</td>
<td>0.52 0.0153</td>
</tr>
<tr>
<td>Labile particulate</td>
<td>-0.56 0.0159</td>
<td>0.22 0.2890</td>
<td>0.22 0.2890</td>
<td>0.00 0.8332</td>
</tr>
</tbody>
</table>

\( ^{a} \) Stratified version of Kendall's tau test \( (\tau) \).

\( ^{b} \) \( P > \text{Abs}(\tau) \).

---

Fig. 9. Metals in mussel gills vs. concentrations in water total measured concentrations of Ag and Cd, for example, in the dissolved and particulate phases do not explain metal accumulation in mussel gills.
(POC; 0.34 mg C/l) and dissolved (DOC; 2.47 mg C/l) organic carbon were measured at the 8 km site (Table 1). Organic carbon favors the formation of colloidal metals (Wen et al., 1999), which can considerably change the speciation of dissolved metals. The relatively high concentrations of POC and DOC can therefore decrease the fraction of truly-dissolved (<10 kDa) metals. Since total dissolved (<0.45 μm) metal concentrations do not provide adequate information on the availability for metal uptake by gills, these tissue/metal ratios were instead compared to the results of water ultrafiltrations (Fig. 4). The truly-dissolved (i.e. <10 kDa) fraction was low for Ag and Cd at the site 8 km from the outfall and increased along the dispersion plume. The relatively low proportions observed in the plume highlight the importance of colloids to the distribution of certain dissolved metals. Cadmium and Ag were the two metals presenting the lowest truly-dissolved fractions at the 8 km site, and this may explain the results reported for metal bioaccumulation in gills at this site. Because natural colloids can influence metal uptake by mussels differently, distinctive metal bioaccumulation behaviors were observed (Wang and Guo, 2000; Pan and Wang, 2004). No relationship (ρ > 0.05) was observed between metal concentrations in tissue and truly-dissolved phase (Table 4). Both truly-dissolved and colloidal metals are bioavailable to mussels, but with a relative importance that is metal-specific (Pan and Wang, 2004). This corroborates the statement that it is the binding properties of colloids rather than the concentration of metals in this fraction that drives the bioavailability in this system. The speciation of metals within the tissues or cells is also of consequence for toxicity. For example, metals associated to high molecular weight proteins were related to toxicity in trout hepatocytes (Gagné et al., 1990). In another study, the level of the soluble fraction of Cd (cytosolic fraction) was closely related with oxidative-mediated damage in gill tissues (Giguère et al., 2003). The presence of calcium concretions in gill tissues was also shown to act as sink for metals although seems not to be inducible by site quality (Bonneris et al., 2005).

4.4. Metal bioaccumulation vs. labile particulate phase

The partial extraction of metals on suspended particles with 1 N HCl (Fig. 5) provides key information for determining the reactivity and lability of particulate metals and documenting their potential bioavailability (Luoma and Bryan, 1978; Gagnon and Saulnier, 2003). Iron and manganese hydr(oxides), which are generated by the mixing of wastewater (more reduced than upstream waters) with receiving waters and their oxygenation, were reported as important carriers of trace metals and the metals associated with these mineral phases remained reactive (Gagnon and Saulnier, 2003). Metals associated with Fe-rich particles were reported as bioavailable for freshwater and marine mussels (Luoma and Bryan, 1978; Gagnon and Fisher, 1997b). The high ratios of HCl-extractable metal/total metal observed at the 8 km site help explain the reactivity of most metals in the particulate phase (Fig. 5). The ratios of many metals, including Cr and Zn, were higher at the downstream sites than at the reference station, and remained high along the dispersion plume. In contrast, the reactivity of Ag and Cd was low at the 8 km site, with ratios reaching values comparable to those at the reference site, which indicates that the acid reactivity of suspended matter changes spatially in the plume. Interestingly, the bioaccumulation of these metals in the digestive gland showed a similar trend, with minimum values at the site 8 km downstream albeit was not correlated with all the tissues examined in mussels (Table 4). Nevertheless, the reactivity of particulate metals suggests that food ingestion can also be considered a potential exposure pathway for mussels. However, particulate acid reactivity for Ag (lower at 8 km) and Cd (higher at 8 km) does not seem to contribute to the gill/digestive gland metal ratio.

4.5. Environmental factors controlling metal availability

The high Corg concentrations (both POC and DOC) observed near the effluent outfall (Table 1) could be expected to influence the bioaccumulation of most metals. The high binding capacity of organic carbon has been shown to play a major role in metal availability (Santschi et al., 1999). Decreased bioaccumulation of most metals was observed in mussels located downstream of the effluent outfall; surprisingly, neither particulate nor dissolved metal concentrations can explain these results. DOC and POC appear to be one of the key factors that modulate the bioaccumulation of organic-reactive metals (Gagnon and Fisher, 1997a; Guo et al., 2001; Voets et al., 2004; Lorenzo et al., 2005).

Metal speciation within the dissolved phase, which can be considerably modified by DOC, might influence metal uptake by mussel gills. The presence of dissolved organic carbon such as humic acids was reported to clearly damper Cu uptake in mussel gills, but did not have effect on whole-animal uptake (Lorenzo et al., 2005). Those reported results suggest that dissolved organic substances influence differently metal uptake by mussel gills and remaining tissues. We observed that the truly-dissolved proportion of Ag and Cd decreased, while the gill/digestive gland ratio decreased. The fact that metal concentrations in gills decrease while DOC increases may point out DOC as a key factor controlling the speciation of organic-reactive metals such as Ag and Cd. However, other factors (such as MT expression) must also be considered in those complex aquatic systems. We also observed that the reduced gill/digestive gland metal ratio appears to be caused by a decrease
in Ag and Cd levels in gills, but not in the digestive gland. As the proportion of metals in the dissolved phase was also reduced by a concomitant rise in the particulate phase, it can be suggested that the particulate form of the metals contributes to reduced gill/digestive gland metal levels.

Changes in the distribution of metals between the dissolved and particulate phases in the effluent dispersion plume was shown to considerably influence the exposure routes of contaminants for aquatic organisms as evidenced by gill/digestive gland metal ratios. A comparison of metal accumulation levels in gill and digestive gland allowed us to estimate the metal uptake pathways in effluent-exposed mussels. The results of gill/digestive gland metal ratios were mixed (Table 3). Cadmium, Cr, Ag and Zn showed the highest ratios (>1), suggesting a higher metal uptake in gills in effluent receiving waters (i.e. upstream site). Gill/digestive gland metal ratios at the 8 km site, however, were close enough to 1 to suggest that metal uptake by gills and digestive glands were equivalent at the 8 km site nearest the effluent outfall, despite the considerable metal concentrations measured in the total dissolved phase. These results suggest that metal speciation in the effluent dispersion plume is crucial to understand the importance of waterborne and dietary exposure routes and their environmental relevance to metal accumulation by these filter-feeding invertebrates.

5. Conclusions

The distribution of metals in target tissues of mussels was used to distinguish the exposure routes (dissolved and particulate forms) for mussels exposed to municipal effluents. Gills and digestive gland but at a lesser extent for the latter, are target tissues for most metals in both effluent dispersion plumes and receiving waters. Metal accumulation was generally lower (i.e. less bioavailable) in mussels placed nearest the effluent outfall (8 km downstream) than at the upstream reference site, although MT was significantly induced. MT induction is a response to overall exposure to metals, but the biomarker appears not specific enough to metal exposure in complex contaminated sites such as environments impacted by municipal effluents. The bioaccumulation of metals in mussel tissues (except the remainder) was not proportional to total concentrations of either dissolved or particulate metal forms in receiving waters. Physicochemical conditions of receiving waters (e.g. organic carbon content) can control metal uptake in the dissolved phase. Truly-dissolved metal fractions were relatively low near the outfall and may reduce the bioavailability of specific metals in the dissolved phase for mussel gills. The results of selective metal extractions using 1 N HCl indicated that the particulate metals in the dispersion plume are in the labile form (i.e. 1 N HCl-reactive). Metals in the particulate phase should probably be considered potentially bioavailable by food ingestion, and they contribute to metal accumulation by mussels through this exposure route. Although metal accumulation from the dissolved phase appears to be a logical route of exposure in most cases, both dissolved and particulate sources must be considered when evaluating the bioavailability of metals released in municipal effluents. Metal accumulation in the gill and digestive gland suggests that metal exposure and accumulation from both waterborne and dietary sources in municipal effluent receiving waters are significant and should be the subject of further study.

Acknowledgements

We are grateful to M. Bouachrine, L. Gougeon, M. Arseneau and G. Brault for their help in the field and the laboratory. Statistical analyses were performed by P. Gagnon and M. Bouachrine. The manuscript was edited by P. Potvin. This project was funded by the St. Lawrence Centre, Environment Canada (to C.G.) and the International Copper Institute (to M.H.S.).

References


Chambers, P.A., Allard, M., Walker, S.L., Marsalek, J., Lawrence, J., Servos, M., Busnarda, J., Munger, K.S.,


OMOE (Ontario Ministry of the Environment), 1988. Thirty-seven Municipal Water Pollution Control Plants: Pilot


