Mercury in the submerged macrophytes *Potamogeton crispus* and *Chara virgate* from the brackish, industrial affected Gunneklevfjorden, southern Norway

Av Marianne Olsen, Cecilie Sørensen and Espen Lydersen

Marianne Olsen is scientist at The Norwegian Institute for Water Research (NIVA) and PhD fellow at the University College of Southeast Norway (HSN) (marianne.olsen@niva.no). *Cecilie Sørensen* finished her M.Sci. degree in 2016 at HSN, and this article is based on her M.Sci. thesis. *Espen Lydersen* is professor in limnology at the Department of Natural Sciences and Environmental Health at HSN (espen.lydersen@hit.no).

University College of Southeast Norway, PB 235, 3603 Kongsberg, Norway
The Norwegian Institute for Water Research, Gaustadalleen 21, N-0349 Oslo, Norway

Sammendrag

Kvikksølv i krustjønnaks *Potamogeton crispus* og kransalgen *Chara virgate* i Gunneklevfjorden, en industripåvirket brakkvannsfjord i Norge. Total kvikksølv (Tot-Hg) og metylkvikksølv (Met-Hg) ble analysert månedlig fra mai til september (2015) i krustjønnaks (*Potamogeton crispus)* og i kransalgen *Chara virgate*, for å undersøke opptak og akkumulering av Hg i planter i den forurensede brakkvannsfjorden Gunneklevfjorden. Prosentandel Met-Hg av Tot-Hg (% Met-Hg) var høyere i plantene (1,4-17,3%) enn i sediment (0,01-0,41%) og vann (0,3-1,3%), og dette støtter hypotesen om at Met-Hg akkumuleres i plantene. Tot-Hg og Met-Hg var høyere i *C. virgate* enn i *P. crispus*, noe som støtter hypotesen om ulike mekanismer for opptak av Hg i samsvar med at næring primært tas opp fra sedimentet via røttene i frøplanter mens alger opptar næring via vann gjennom overflaten. Relativ andel Met-Hg (% Met-Hg) var høyere i *P.crispus* enn i *C.virgate*, noe som indikerer ulike kontrollmekanismer for netto akkumulering av Hg.

Summary

Total mercury (Tot-Hg) and methylmercury (Met-Hg) in the vascular plant *Potamogeton crispus* and the green algae *Chara virgate* were analyzed monthly from May to September (2015), to study uptake and accumulation of Hg in macrophytes in the contaminated brackish fjord Gunneklevfjorden, Southern Norway. High values of Met-Hg relative to Tot-Hg (% Met-Hg) in the macrophytes (1.4- 17.3 %), compared to sediment (0.01-0.41 %) and water (0.3-1.3 %), supports the hypothesis that Met-Hg is accumulated within the plants. Tot-Hg and Met-Hg were higher in *C. virgate* than in *P. crispus*, which supports the hypothesis of different mechanisms for Hg uptake, reflecting the main nutrient uptake paths in angiosperms from sediments through roots and in algae from water through

surfaces. The relative proportion of Met-Hg (% Met-Hg) were higher in *P.crispus* compared to *C.virgate*, indicating different mechanisms controlling net accumulation of Hg.

Introduction

Macrophytes comprise a vast diversity of aquatic organisms including large algae, bryophytes and vascular plants, and represent the predominant group of organisms within the euphotic zone in terms of biomass and primary production, in a wide range of littoral ecosystems (Cosio et al., 2014: Noges et al., 2010). They form important and numerous microhabitats for aquatic organisms, offering shelter, substrate and food. Further, submerged macrophytes are thought to create a sediment microenvironment favorable for bacterial activity (Canario and Vale, 2012, Aldridge and Ganf, 2003, Regier et al., 2012). Consequently, at contaminated sites, macrophytes are suggested to improve sediments conditions for microbial methylation of inorganic mercury (Hg) into the organic form methyl-Hg (Met-Hg) (deSouza et al., 1999, Canario et al., 2007, Castro et al., 2009). Hence, submerged meadows are potential important exposure sites for uptake and transfer of Hg into aquatic food webs (Regier et al., 2013, Gothberg and Greger, 2006, Greger and Dabrowska, 2010). Macrophytes may themselves act as vectors for Hg into aquatic food chains through grazing herbivores (Cremona et al., 2009, Bravo et al., 2014, Lafabrie et al., 2011), depending on the distribution of Hg within the plant and the grazing pattern of the present herbivores.

Although elevated bacterial methylation rates have been found in the rhizosphere as well as in roots of submerged macrophytes (Guimarães et al., 2000, Guimarães et al., 1998, Mauro et al., 1999, Windham-Myers et al., 2014, Canário et al., 2010, Windham-Myers, 2014, Regier et al., 2012), the role of roots is not fully understood. However, higher concentrations of Hg in roots compared to leaves and stems (Maury et al., 1988, Regier et al., 2013), indicates uptake from sediment followed by translocation within the plant (Cosio et al., 2014) resulting in heterogeneous accumulation in different plant organs. Further, uptake and accumulation of Hg in shoots directly from the water column have been reported in both laboratory and in the field (Regier et al., 2012, Regier et al., 2013, Anjum et al., 2012, Gothberg and Greger, 2006, Figueira et al., 2012). However, most often no information is available to determine whether inorganic Hg are predominately accumulated in plants from the water column, from sediments or from pore water (Cosio et al., 2014). Several uptake mechanisms are supposed to be in action, such as passive or facilitated diffusion (Regier et al., 2013, Gutknecht, 1981, Schaefer et al., 2011) and carrier-mediated mechanisms (Regier et al., 2012, Hassett and Kosman, 1995, Page et al., 2009), depending on the species of macrophytes. Further, temporal variations in Hg accumulation have been found (Anjum et al., 2012).

While the majority of studies on the role of aquatic macrophytes in Met-Hg production have been carried out in agricultural wetlands, freshwater lakes, rivers and saltmarshes in the southern temperate or tropical zone (Bravo et al., 2014), knowledge is scarce for temperate coastal areas and brackish waters. Hence, there is need for more studies of uptake and assimilation of Hg into various species of macrophytes in different climatic zones, as well as the vertical distribution of Hg within the plants, to determine the role of specific macrophytes as vectors for Hg into aquatic food chains. The aim of this study was to investigate the internal vertical distribution of Tot-Hg and Met-Hg concentrations in the vascular plant curly-leaf pondweed (*Potamogeton crispus*) and the green algae *Chara virgate* in relation to rhizosphere sediment and water concentrations of Hg on a temporal scale, within the brackish fjord Gunneklevfjorden in Norway. We hypothesis that Hg accumulates in the macrophytes, whereas the mechanisms for uptake and the accumulation rate are different in the two species studied; primarily from sediments by roots in *P.crispus* and from water by diffusion in *C.virgate*, evident through differences in Hg concentration and in vertical distribution of Hg between the two species.

Material and methods

Study site

Our study site, Gunneklevfjorden, is a 0.7 km^2, brackish fjord with maximum depth 11 meters, located in the temperate zone of Norway (Figure 1). Between 1947 and 1987, approximately 60 tons of Hg were discharged into the fjord from a chlor-alkali plant, and approximately 20-30 tons of Hg are expected to be stored within the sediments (Skei, 1978). Despite heavy sediment contamination, the fjord hosts a submerged meadow of macrophytes, reaching down to approximately 2.7 meters depth. The meadow is classified to be of national value due to its size (> 10 daa) and the presence of threatened species such as horned pondweed, *Zannichella palustris* (Mjelde, 2015). *P. crispus*, *C. virgate* and the Canadian waterweed *Elodea canadensis* dominates the meadow in separate areas. The *P. crispus* is a rhizomatous perennial herb, which primarily assimilate nutrients through roots and shoots. The roots have been found to penetrate 0-15 cm into the sediments with an average total root length of more than 200 cm (Wang, 2013). In contrast, the algae *C. virgate* grows anchored to the substrate by means of branching underground rhizoids, and resemble land plants with stem-like and leaf-like structures, though primarily assimilate nutrients from the water through the surface.

Several investigations, including our study, were undertaken during 2013-2015 to develop a sediment remediation action plan, intended to reduce the ecosystem risk from Hg and other contaminants. Sediment characteristics is prepared for presentation in Olsen et al. (in prep) and is not included in this paper. In short, TOC, grain size, redoks and sulfide showed large vertical and horizontal variations in the fjord.

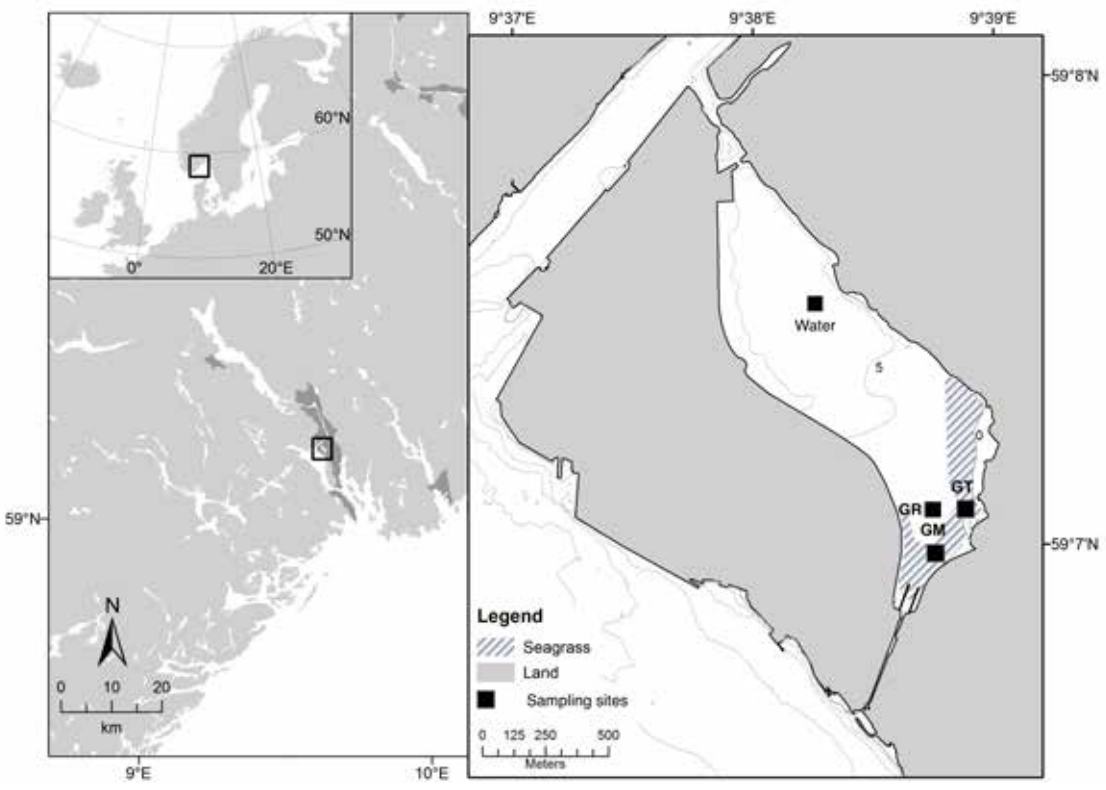

Figure 1. Study area and sampling sites for macrophytes, sediment and water within the Gunneklevfjorden in Norway in 2015. GM: sampling of Potamogeton crispus. GT: sampling of Chara virgate. GR: sampling of sediment outside the meadow. Water = sampling of water.

Within the meadow, sediment water content and TOC concentrations were lower and related to larger grain sizes than outside the meadow, probably following less influence from industrial discharges. Further, low pH and low Eh values within the meadow were found to correspond with high concentrations of H_2S in 2-5 cm sediment depth, supporting the hypothesis of enhanced microbial activity in the root influenced zone (rhizosphere). No similar peak in H_2S were found outside the meadow, even though concentrations were higher compared to within the meadow.

Sampling, analysis and calculations

Specimens of *P. crispus* and *C. virgate* were sampled monthly from May to September in 2015 together with sediments, by use of an Ekman grab. Duplicate grab samples were collected at each of two sites within the meadow; GM and GT, dominated by *P. crispus* and *C. virgate*, respectively, and at a third site outside the meadow; GR (Figure 1). Each month, 1-3 specimens of each plant species were sampled. In addition, two water samples were collected monthly at one meter and five meter depth for analysis of Tot-Hg and Met-Hg, by use of a Ramberg water sampler. Water samples were filtered prior to chemical analysis. Due to insufficient material, the dataset lack analysis of Hg in *P. crispus* in August.

The upper five cm of sediment in the grabs were carefully subsampled with a spoon as close to the plants as possible without rupturing the roots, before plants were removed from the remaining sediment and rinsed in local water. Plants were then washed in 1mM EDTA and 1mM cysteine, following the procedure by Regier et al. (2013). Each individual of *P. crispus* was separated into root, stem and leaf, whereas *C. virgate* was separated into three equal parts; base, center and top. Plant and sediment samples were kept frozen until freeze-dried prior to chemical analysis. A total of 66 plant samples and 32 sediment samples were analyzed for Tot-Hg in triplicates on a Lumex RA-915M instrument with a PYRO 915-unit at The University College of Southeast Norway, table 1.

Tot-Hg and Met-Hg in 10 water samples, as well as Met-Hg in 27 plant samples and 21 sediment samples, were analyzed at The Norwegian Institute for Water Research (NIVA), following methods described in detail by Bloom et al. (1997). No Met-Hg analysis was carried out for top sections of *C.virgate* due to insufficient material. The proportion of Tot-Hg that was found in the organic form Met-Hg was calculated as percentage Met-Hg (% Met-Hg). The bio concentration factor (BCF), i.e. concentration in plant versus concentration in ambient environment, was estimated according to the following equations, assuming uptake from sediments through roots in *P.crispus* and from water through surfaces in *C. virgate*:

$$BCFtot_{P.crispus} = \frac{Tot\text{-}Hg_{root}}{Tot\text{-}Hg_{sediment}} \quad \text{and} \quad BCFtot_{C.virgate} = \frac{Tot\text{-}Hg_{plant}}{Tot\text{-}Hg_{water}} \tag{1}$$

$$BCFmet_{P.crispus} = \frac{Met\text{-}Hg_{root}}{Met\text{-}Hg_{sediment}} \quad \text{and} \quad BCFmet_{C.virgate} = \frac{Met\text{-}Hg_{plant}}{Met\text{-}Hg_{water}} \tag{2}$$

Statistical analysis

All statistical analysis were done using the statistical language and software environment R, version 3.1.0 (R_Core_Team, 2014). One-, two- or three-way ANOVA, as well as non-parametric Kruskal-Wallis test, were used to test for differences in concentrations of Tot-Hg, Met-Hg, and % Met-Hg between species, plant parts and months. TukeyHSD post hoc test and post hoc test for Kruskal-Wallis test were carried out to identify differing plant parts and months. Note that sediment and water statistics were restricted by single monthly samples. Met-Hg concentrations below detection limits in water samples (DL=0.02 ng L^{-1}) were set to half the DL (0.01 ng L^{-1}).

Results

Concentrations in macrophytes

Concentrations of Tot-Hg and Met-Hg in *C. virgate* ranged from 169 to 1321 ng g^{-1} dw and from 3.6 to 52 ng g^{-1} dw, respectively, and were significantly higher than in *P. crispus*, which ranged from 50 to 726 ng g^{-1} dw and from 1.0 to 19 ng g^{-1} dw ($p<0.001$), respectively (Table 1). In contrast, % Met-Hg in *C. virgate* ranged from 1.4 to 11 % Met-Hg and were significantly lower than *P. crispus*, which ranged from 1.6 to 17 % Met-Hg ($p<0.05$).

Tot-Hg in *C. virgate* did not differ significantly between the plant sections, figure 2, in contrast to Met-Hg and % Met-Hg, which were found to be higher in base than in the center ($p<0.05$ and $p<0.1$, respectively). In *P. crispus*, both Tot-Hg and Met-Hg were found to be higher in root than in stem ($p<0.1$), while no significant difference were found for % Met-Hg between plant parts, figure 2.

Both Tot-Hg and Met-Hg differed between months in *C. virgate*, as well as in *P. crispus*, figure 3. In *C. virgate,* Tot-Hg was found to be significantly higher in June than in the other months ($p<0.001$), while neither Met-Hg nor % Met-Hg showed significant difference between months. In *P. crispus*, Tot-Hg was found to be significantly higher in May than in the other months ($p<0.01$), while Met-Hg was higher in July than in June ($p<0.05$). The increase of % Met-Hg observed in *P. crispus* after June were significant ($p<0.05$).

Concentrations in sediment and water

Tot-Hg and Met-Hg concentrations in sediment ranged from 0.2 to 59 µg g^{-1} dw and from 0.1 to 21 ng g^{-1}dw, respectively, while % Met-Hg ranged from 0.01 to 0.41 %, table 1. Tot-Hg and Met-Hg in sediments were significantly higher outside the meadow than within ($p<0.001$). In contrast, % Met-Hg were higher within the meadow than outside ($p=0.09$), figure 4. Neither Tot-Hg nor Met-Hg in sediments differed significantly between the months, while % Met-Hg exhibited a significant peak within the meadow in July ($p<0.001$).

	Tot-Hg	Met-Hg	% Met-Hg	BCFtot	BCFmet
C. virgate	169-1321 (ng g^{-1} dw) (n=39)	3.6-52 (ng g^{-1} dw) (n=13)	1.4-11 (n=13)	$6*10^4$-$4*10^5$ (n=27)	$2*10^5$-$2*10^6$ (n=10)
P. crispus	50-726 (ng g^{-1} dw) (n=27)	1.0-19 (ng g^{-1} dw) (n=24)	1.6-17 (n=24)	0.01-1.7 (n=24)	3-51 (n=11)
Sediment within meadow	0.2-49 (µg g^{-1} dw) (n=27)	0.1-14 (ng g^{-1} dw) (n=16)	0.01-0.41 (n=16)		
Sediment outside meadow	26-59 (µg g^{-1} dw) (n=5)	3.1-21 (ng g^{-1} dw) (n=5)	0.01-0.04 (n=5)		
Water 1 m	2.3-5.0 (ng L^{-1}) (n=5)	0.02-0.04 (ng L^{-1}) (n=5)	0.6-1.3 (n=5)		
Water 5 m	2.9-8.4 (ng L^{-1}) (n=5)	0.01-0.04 (ng L^{-1}) (n=5)	0.3-0.7 (n=5)		

Table 1. Range of concentrations of Tot-Hg, Met-Hg and % Met-Hg in macrophytes (Chara virgate and Potamogeton crispus), sediment and water sampled in Gunneklevfjorden in 2015. BCFtot and BCFmet is given as root-to-sediment ratio for Potamogeton crispus and as plant-to-water ratio for Chara virgate.

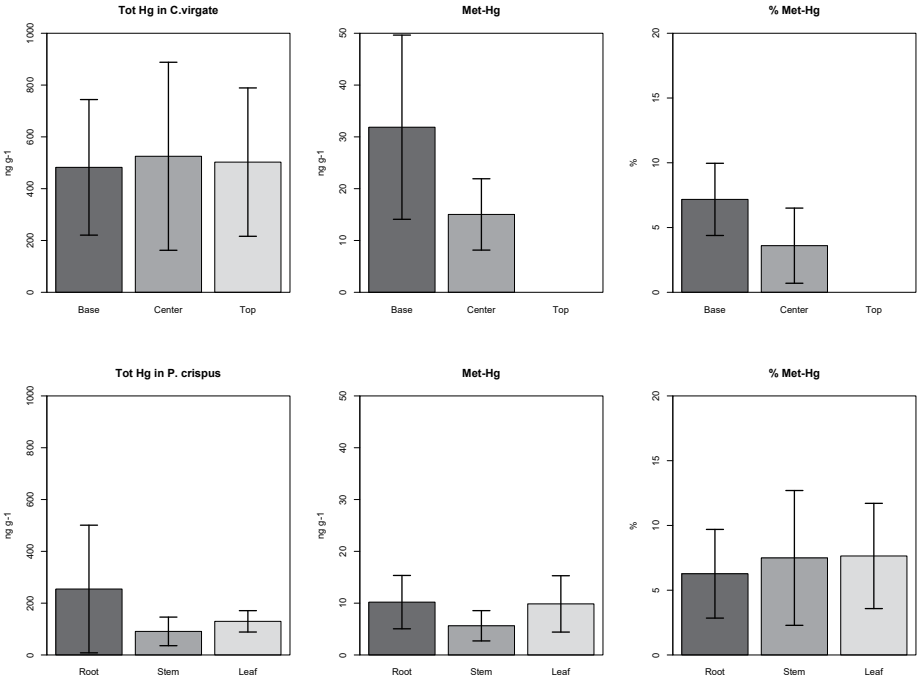

Figure 2. Mean ± sd for concentrations of Tot-Hg, Met-Hg and % Met-Hg in plant parts of Chara virgate (top) and Potamogeton crispus (bottom) sampled in Gunneklevfjorden during May-September 2015. Notes difference in scale for Tot-Hg and Met-Hg.

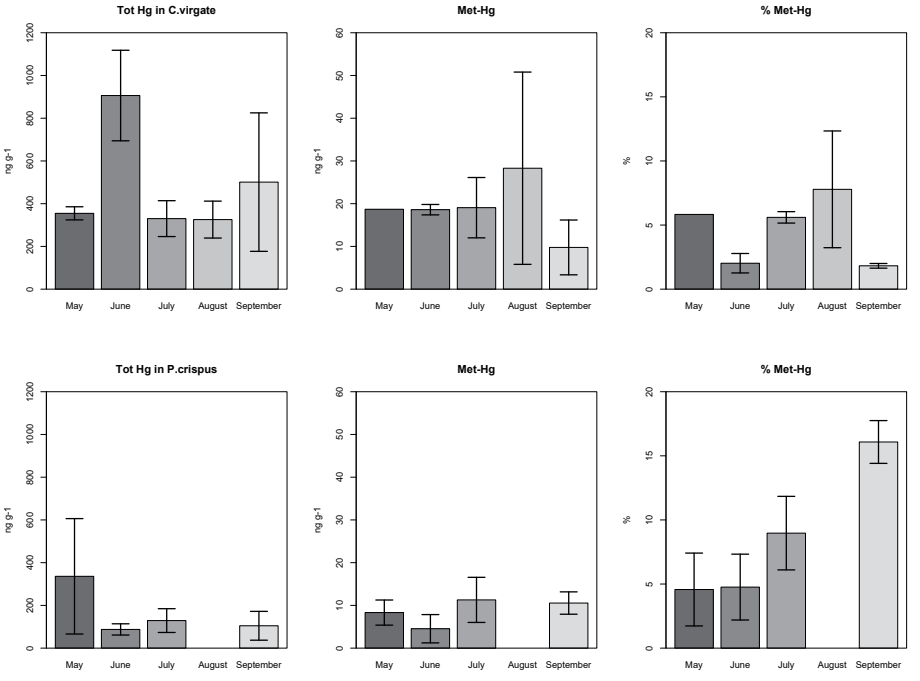

Figure 3. Mean ± sd for concentrations of Tot-Hg (ng g^{-1} dw), Met-Hg (ng g^{-1} dw) and % Met-Hg in Chara virgate (top) and in Potamogeton crispus (bottom) from May to September in Gunneklevfjorden in 2015. Notes difference in scale for Tot-Hg and Met-Hg.

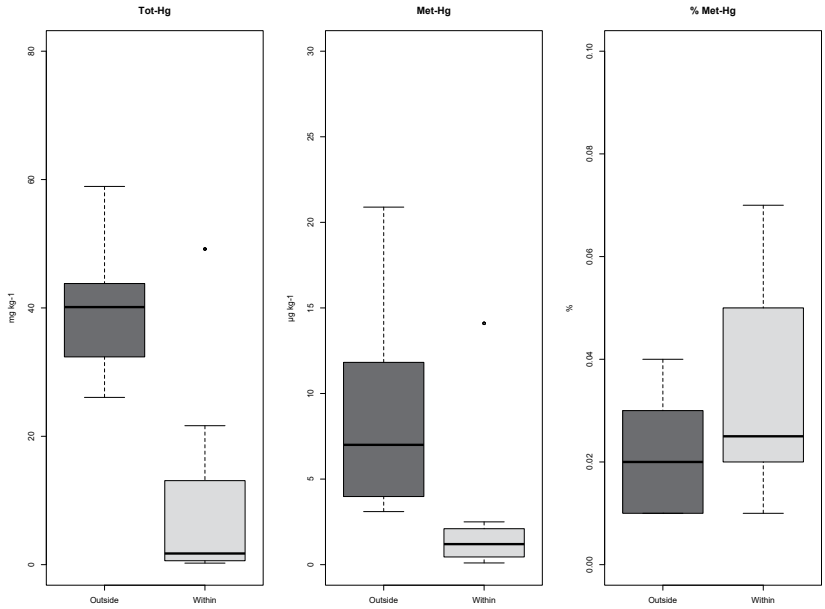

Figure 4. Mean ± sd for concentrations of Tot-Hg (µg g^{-1} dw), Met-Hg (ng g^{-1} dw) and % Met-Hg in sediment samples collected from May to September, outside and within the meadow in Gunneklevfjorden. Notes difference in scale for Tot-Hg and Met-Hg.

The monthly concentrations of Tot-Hg and Met-Hg in water ranged from 2.3 to 8.4 ng L^{-1} and from 0.01 to 0.04 ng L^{-1}, respectively, and % Met-Hg ranged from 0.3 to 1.3 % (Table 1). The highest values of Tot-Hg were found at five meters, while % Met-Hg showed highest values at one meters depth. Tot-Hg decreased from May to September at both one meter (54 % reduction) and five meters depth (65 % reduction), while no such trends were observed for Met-Hg or % Met-Hg.

Discussion

The concentrations of Tot-Hg, Met-Hg and % Met-Hg were found to differ between the vascular plant *P. crispus* and the green algae *C. virgate* (Table 1), with the highest % Met-Hg found in *P.crispus*. This supports the hypothesis of species-specific mechanisms for uptake and accumulation of Hg, also suggested by others (Regier et al., 2013, Gutknecht, 1981, Schaefer et al., 2011). In *P. crispus,* the highest Tot-Hg concentrations were in the roots followed by leaves and stems. This supports the hypothesis that sediment-to-root is the primary uptake path for Hg in vascular macrophytes, causing heterogeneous distribution, which agrees well with previous studies. However, BCF_{tot} for *P.crispus* were low (0.01-1.72), indicating that relatively small amounts of the Hg in sediments is taken up by macrophytes, as has been suggested by Cosio et al (2014). The low concentrations of both Tot-Hg and Met-Hg in the stem of *P. crispus*, demonstrates that the stem functions primarily for transport of Hg rather than storage. This is in accordance with other studies indicating translocation from root to other plant organs (Regier et al., 2012, Regier et al., 2013, Boudou et al., 1991). (Maury et al., 1988, Regier et al., 2013).

The high Tot-Hg in *P.crispus* in May supports more available Hg and/or active uptake at the onset of the growth season. From May to September, the % Met-Hg in *P. crispus* increased, which might be explained by bioaccumulation during growth season, as suggested by Gothberg and Greger (2006), or production of Met-Hg within the plants, as hypothesized and suggested in several studies (Guimarães et al., 2000, Guimarães et al., 1998, Mauro et al., 1999, Windham-Myers et al., 2014, Canário et al., 2010, Windham-Myers, 2014, Regier et al., 2012,

Gothberg and Greger, 2006). However, % Met-Hg was similar in all plant parts of *P.crispus*, indicating that no major net methylation occurs in any particular plant organ after translocation from the roots. Hence, we suggest that the primary cause of increase in % Met-Hg during growth season is bioaccumulation, but cannot exclude production of Met-Hg within *P.crispus*.

In *C. virgate*, the peak of Tot-Hg in June coincides with the peak in water at five meter depth, indicating that water is the source to Hg, as hypothesized. The high BCF_{tot} (10^4) and BCF_{met} (10^5) of *C. virgate* indicates that a relatively large amount of Hg in the water is taken up by the plants, reflecting that the Met-Hg bioaccumulation step from water to primary producers represent the largest single increase for Met-Hg concentrations in aquatic ecosystems, as described by Mason et al. (1996). Hence, the BCF-values are in accordance with ratios previously reported from water to phytoplankton and to other seston (Baeyens et al., 2003, Watras and Bloom, 1992, Mason et al., 1996). Surprisingly, Met-Hg was significantly higher in the base of the plant than in the center, indicating that uptake and accumulation are not homogenous within *C. virgate*, or that the exposure of Met-Hg is relatively higher in the sediment boundary layer. This should be further investigated. In *C. virgate*, no significant increase in % Met-Hg during growth season was seen, as would be expected due to bioaccumulation, and % Met-Hg was significantly lower than in *P. crispus*. The absence of increase and the low % Met-Hg in *C.virgate* may be explained by mechanisms controlling the net production and accumulation of Met-Hg within the plant, such as demethylation which has been suggested to occur within plants by Simon and Boudou (2001). Still, the % Met-Hg found for both species of macrophytes were high (1.4-17.3%) compared to sediment (0.01- 0.41 %) and water (0.3-1.3 %), which supports accumulation and/or methylation with the plants. Higher % Met-Hg in the sediments within the meadow compared to outside, supports that macrophytes enhance microbial methylation in sediments, as reported in several previous studies (Regier et al., 2012, Canario and Vale, 2012, Aldridge and Ganf, 2003, deSouza et al., 1999, Canario et al., 2007, Castro et al., 2009) and elaborated in Olsen et al. (in prep). The high sediment concentrations of Hg found outside the meadow compared to within, are probably explained by the distance to the previous point of discharge from the chlor-alkali plant.

Conclusion

Our results improves insight to the complexity of the fate of Hg in aquatic environments and the role of different species of macrophytes. The differences in concentrations of Hg and in % Met-Hg found between P. crispus and C. virgate, supports species-specific mechanisms for uptake and accumulation of Hg. We suggest that Hg uptake in P. crispus primarily is from sediment to roots, whereas C. virgate primarily takes up Hg from water. Our results indicate accumulation in roots and leaves of P. crispus during the growth season, while in C.virgate there might be mechanisms in action controlling the net accumulation rate of Met-Hg. In C. virgate, Met-Hg seems to be higher in the base of the plant and the reason for this should be further investigated. Our results of accumulation of Met-Hg in plants and higher % Met-Hg in sediments within the meadow compared to outside, reflects that macrophytes play important roles to Hg fate in aquatic systems. Depending of the species present, macrophytes may act as vectors for Hg into aquatic food webs, demonstrating the role of site-specific environmental parameters on the fate of Hg in the environment.

Acknowledgements

This study received financial support from "Statsminister Gunnar Knudsen og hustru Sofie født Cappelens familielegat" (grant to MO). We thank Mari Olsen for help during field sampling and Hans Fredrik Veiteberg Braaten for analysis of water and for Met-Hg analysis in sediment and biota. We also thank Morten Schaanning and Frithjof Moy for valuable comments in the development of the manuscript.

References

ALDRIDGE, K. T. & GANF, G. G. 2003. Modification of sediment redox potential by three contrasting macrophytes: Implications for phosphorus adsorption/desorption. *Marine and Freshwater Research,* 54**,** 87-94.

ANJUM, N. A., AHMAD, I., VALEGA, M., PACHECHO, M., FIGUERIA, E., DUARTE, A. C. & PEREIRA, E. 2012. Salt marsh macrophyte *Phragmites australis* strategies assessment for its dominance in mercury-contaminated coastal lagoon (Ria de Aveiro, Portugal). *Environ Sci Pollut Res,* 19**,** 2879-2888.

BAEYENS, W., LEERMAKERS, M., PAPINA, T., SAPRYKIN, A., BRION, N., NOYEN, J., DE GIETER, M. & GOEYENS, L. 2003. Bioconcentration and biomagnification of mercury and methylmercury in North Sea and Scheldt Estuary fish. *Archives of Environmental Contamination and Toxicology,* 45.

BLOOM, N. S., COLMAN, J. A. & BARBER, L. 1997. Artifact formation of methyl mercury during aqueous distillation and alternative techniques for the extraction of methyl mercury from environmental samples. *Fresenius Journal of Analytical Chemistry,* 358**,** 371-377.

BOUDOU, A., DELNOMDEDIEU, M., GEORGES-CAULD, D., RIBEYRE, F. & SAOUTER, E. 1991. Fundamental roles of biological barriers in mercury accumulation and transfer in freshwater ecosystems (analysis at oranism, organ, cell and molecular-levels). *Water Air and Soil Pollution,* 56**,** 807-822.

BRAVO, A. G., COSIO, C., AMOUROUX, D., ZOPFI, J., CHEVALLEY, P.-A., SPANGENBERG, J. E., UNGUREANU, V.-G. & DOMINIK, J. 2014. Extremely elevated methyl mercury levels in water, sediment and organisms in a Romanian reservoir affected by release of mercury from a chlor-alkali plant. *Water Research,* 49**,** 391-405.

CANARIO, J., CAETANO, M., VALE, C. & CESARIO, R. 2007. Evidence for elevatred production of methylmercury in salt marshes. *Environmental Science & Technology,* 41**,** 7376-7382.

CANARIO, J. & VALE, C. Mercury in wetlands: A contribution to the definition of a global mercury policy. SETAC North America 3rd Annual Meeting, November 11-15, 2012, 2012 Long Beach, CA, USA. 55.

CANÁRIO, J., VALE, C., POISSANT, L., NOGUEIRA, M., PILOTE, M. & BRANCO, V. 2010. Mercury in sediments and vegetation in a moderately contaminated salt marsh (Tagus Estuary, Portugal). *Journal of Environmental Sciences,* 22**,** 1151-1157.

CASTRO, R., PEREIRA, S., LIMA, A., CORTICEIRO, S., VALEGA, M., PEREIRA, M., DUARTE, A. & FIGUERIA, E. 2009. Accumulation, distribution and cellular partitioning of mercury in several halophytes of a contaminated salt marsh. *Chemosphere,* 76**,** 1348-1355.

COSIO, C., FLUCK, R., REGIER, N. & SLAVEYKOVA, V., I. 2014. Effects of macrophytes on the fate of mercury in aquatic systems. *Environmental Toxicology & Chemistry,* 33**,** 1225-1238.

CREMONA, F., HAMLIN, S., PLANAS, D. & LUCOTTE, M. 2009. Sources of organic matter and methylmercury in littoral invertebrates: A stable isotope approach. *Biogeochemistry,* 94**,** 81-94.

DESOUZA, M. P., HUANG, C. P. A., CHEE, N. & TERRY, N. 1999. Rhizosphere bacteria enhance the accumulation of selenium and mercury in wetland plants. *Planta,* 209**,** 259-263.

FIGUEIRA, E., FREITAS, R., PEREIRA, E. & DUARTE, A. 2012. Mercury uptake and allocation in *Juncus maritimus*: Implications for phytoremediation and restoration of a mercury contaminated salt marsh. *J Environ Monit,* 14**,** 2181-2188.

GOTHBERG, A. & GREGER, M. 2006. Formation of methyl mercury in an aquatic macrophyte. *Chemosphere,* 65**,** 2096-2015.

GREGER, M. & DABROWSKA, B. 2010. Influence of nutrient level on methylmercury content in water spinach. *Environmental Toxicology & Chemistry,* 29**,** 1735-1739.

GUIMARÃES, J. R. D., MEILI, M., HYLANDER, L. D., SILVA, E. D. C. E., ROULET, M., MAURO, J. B. N. & DE LEMOS, R. A. 2000. Mercury net methylation in five tropical flood plain regions of Brazil: high in the root zone of floating macrophyte mats but low in surface sediments and flooded soils. *Science of the Total Environment,* 261**,** 99-107.

GUIMARÃES, J. R. D., MEILI, M., MALM, O. & MARIA DE SOUZA BRITO, E. 1998. Hg methylation in sediments and floating meadows of a tropical lake in the Pantanal floodplain, Brazil. *Science of the Total Environment,* 213**,** 165-175.

GUTKNECHT, J. 1981. Inorganic mercury (Hg^{2+}) transport through lipid bilayer-membranes. *Journal of Membrane Biology,* 61**,** 61-66.

HASSETT, R. & KOSMAN, D. J. 1995. Evidence for Cu(II) reduction as a component of copper uptake by *Saccharomyces cerevisae. Journal of Biology and Chemistry,* 270**,** 128-134.

LAFABRIE, C., MAJOR, K. M., MAJOR, C. S. & CEBRIAN, J. 2011. Arsenic and mercury bioaccumulation

in the aquatic plant, *Vallisneria neotropicalis*. *Chemosphere,* 82.

MASON, R. P., REINFELDER, J. R. & MOREL, F. M. M. 1996. Uptake, Toxicity, and Trophic Transfer of Mercury in a Coastal Diatom. *Environmental Science & Technology,* 30**,** 1835-1845.

MAURO, J. B. N., GUIMARÃES, J. R. D. & MELAMED, R. 1999. Mercury methylation in a tropical macrophyte: influence of abiotic parameters. *Applied Organometallic Chemistry,* 13**,** 631-636.

MAURY, R., BOUDOU, A., RIBEYRE, F. & ENGRAND, P. 1988. Experimental study of mercury transfer between artificially contaminated sediment (CH_3HgCl) and macrophytes (*Elodea densa*). *Aquatic ecotoxicology,* 12**,** 213-228.

MJELDE, M. 2015. Vannvegetasjon i brakkvann, med spesiell vekt på Gunneklevfjorden i Telemark. Delrapport fra aktivitet 2a, Beslutningsgrunnlag og tiltaksplan for forurensede sedimenter i Gunneklevfjorden. NIVA report nr. 6767-2014.

OLSEN, M., SCHAANNING, M. T., BRAATEN, H. F. V., EEK, E., MOY, F. E. & LYDERSEN, E. in prep. Enhanced methyl mercury production within a macrophyte meadow in a temperate brackish Norwegian fjord

PAGE, M. D., KROPAT, J., HAMEL, P. P. & MERCHANT, S. S. 2009. Two *Chlamydomonas* CTR copper transporters with a novel Cys-Met motif are localized to the plasma membrane and function in copper assimilation. *Plant Cell,* 21**,** 928-943.

R_CORE_TEAM 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

REGIER, N., FREY, B., CONVERSE, B., RODEN, E., GROSSE-HONEBRINK, A., BRAVO, A. G. & COSIO, C. 2012. Effect of *Elodea nuttallii* roots on bacterial communities and MMHg proportion in a Hg polluted sediment. *PloS one,* 7**,** e45565.

REGIER, N., LARRAS, F., BRAVO, A. G. & UNGUREANU, V.-G. 2013. Mercury bioaccumulation in the aquatic plant *Elodea nuttallii* in the field and in microcosm: Accumulation in shoots from the water might involve copper transporters. *Chemosphere,* 90**,** 595-602.

SCHAEFER, J. K., ROCKS, S. S., ZHENG, W., LIANG, L. Y., GU, B. H. & MOREL, F. M. M. 2011. Active transport, substrate specificity, and methylation of Hg(II) in anaerobic bacteria. *Proceedings of the National Academy of Science, USA,* 108**,** 8714-8719.

SIMON, O. & BOUDOU, A. 2001. Direct and trophic contamination of the herbivorous carp *Ctenophatyngodon idella* by inorganic mercury and methylmercury. *Ecotoxicology and environmental safety,* 50**,** 48-59.

SKEI, J. 1978. Serious mercury contamiation of sediments in a Norwegian semi-enclosed bay. *Marine Pollution Bulletin,* 9**,** 3.

WANG, L. 2013. Response of Potamogeton crispus root characteristics to sediment heterogeneity. *Acta Ecologica Sinica,* 33**,** 282-286.

WATRAS, C. J. & BLOOM, N. S. 1992. Mercury and methylmercury individual zooplankton-implications for bioaccumulation. *Limnology and Oceanography,* 37**,** 1313-1318.

WINDHAM-MYERS, L. 2014. Methylmercury cycling in wetlands managed for rice agriculture and wildlife: Implications for methylmercury production, transport, and bioaccumulation. *Science of The Total Environment,* 484**,** 219-220.

WINDHAM-MYERS, L., MARVIN-DIPASQUALE, M., A. STRICKER, C., AGEE, J. L., H. KIEU, L. & KAKOUROS, E. 2014. Mercury cycling in agricultural and managed wetlands of California, USA: Experimental evidence of vegetation-driven changes in sediment biogeochemistry and methylmercury production. *Science of The Total Environment,* 484**,** 300-307.