# Comparison of sampling methods of blue mussels for environmental monitoring at Greenland mine sites

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# Data sheet


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# 1 Introduction

Environmental monitoring of Greenland mining projects is essential to identify and quantify the environmental impact of the projects and at the same time provides the basis for mitigation initiatives.

## Mussels as bioindicators

Blue mussels are widely used as bioindicators for metal pollution levels due to their filter-feeding behavior and ability to bioaccumulate metals from sediments, water, and food particles, which makes them effective monitoring species. The methodology used at Greenland mine sites typically includes the collection of blue mussels at monitoring stations along a gradient from the potential pollution source. Upon sampling, 20 mussels from each station divided into size groups, 4-5 cm, 5-6 cm, etc., are cut open and left to drain for ~5 minutes before the soft parts are dissected into a pooled sample for each station (Bach et al. 2023). The samples are then transported to the laboratory, freeze-dried, and subjected to analyses for element concentrations in the samples.

### Method optimization

The standard approach used at Greenland mine sites until recently has been the use of non-depurated mussels for metal analysis immediately after collection. Literature (Chapman, 2016; Robinson et al., 1993) has revealed that this practice can lead to inaccuracy with regards to bioaccumulation. This is because the digestive system of the mussels may contain significant quantities of elements associated with food and sediment particles from recent filtering activities. Likewise, sediment particles with possible bound metals can occur in the mantle of the mussel (Robinson et al., 1993). Both occurrences can lead to an overestimation of the pollution level not reflecting the contaminant body burden in the blue mussel tissue. Therefore, it is recommended by several international monitoring programs (European Commission, 2014; Larsen, 2013; OSPAR Commission, 2007, 2013, 2018; UNEP/MAP, 2021; UNESCO/WHO/UNEP, 1996) to subject mussels to depuration prior to analysis to ensure more reliable and representative results of metal bioaccumulation. The methodology of depuration is in most of the guidelines highlighted to be of special relevance for mussels collected at point sources in water with high turbidity or on silt/clay bottoms. To optimize the method and follow international recommendations, a guideline has previously been developed including an internationally accepted standardized method for the depuration of mussels (Bach et al., 2023). Depuration of the mussels before metal analysis, in which mussels purge ingested but unabsorbed particles, is considered to provide a more accurate bioaccumulation measure but may also introduce additional sources of error due to the increased handling of the mussels. The standard method involves depuration in aerated seawater for 24 hours (Larsen, 2013).

This note reports an experiment comparing depurated to non-depurated mussels sampled along a transect from the former Maarmorilik mine. The results are discussed in relation to recommendations for future environmental monitoring using blue mussels at mine sites in Greenland.

# 2 Method

This note compares concentrations of lead (Pb) and zinc (Zn) in depurated and non-depurated mussels collected at stations in a gradient from a pollution source i.e. the major waste rock dump and former mining area in Maarmorilik (Figure 1).

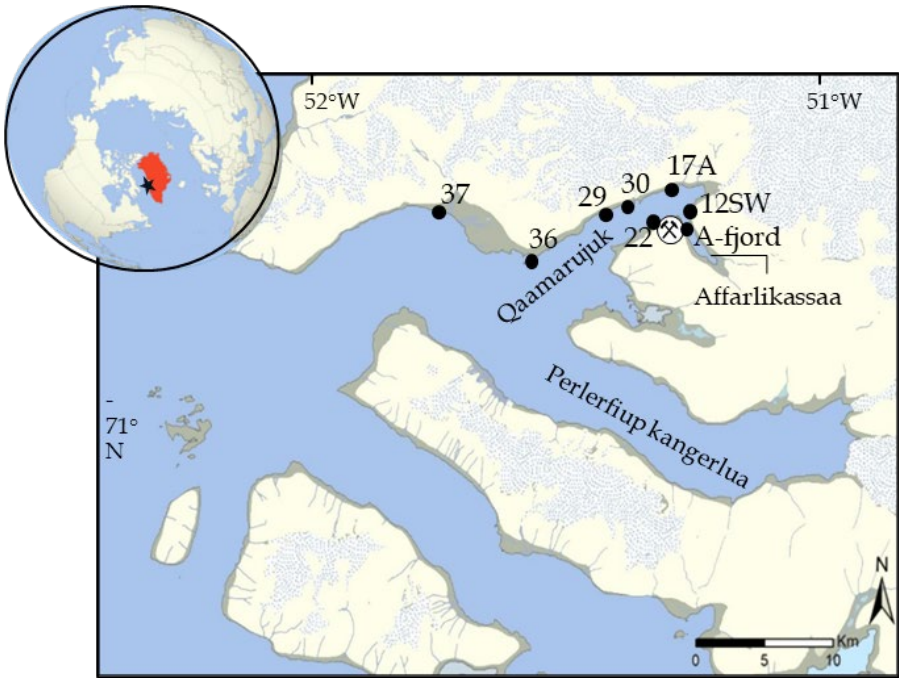


**Figure 1**. Sampling stations visited during the environmental monitoring for pollution in the area of the former Maarmorilik mine.

The study included two parts: 1) analyses of mussel tissue concentrations in individual mussels to provide information on the variability of accumulation of metals between individuals at the same station, in depurated versus non-depurated mussels, and 2) analyses of pooled tissue from 20 individuals from the same station in depurated versus non depurated mussels using the methodology similar to the methodology used in regular monitoring activities at mine sites in Greenland.

For the study of individual mussel concentrations, mussels (4.5-5.5 cm shell length) were collected by hand from two polluted marine stations (st. 12SW and st. A-fjord) in West Greenland (Johansen et al., 2010) (Figure 1). At station 12SW, mussels were collected from beach sediment or smaller rocks, while station A-fjord represents an area where mussels were collected from a boulder/rock coastline. At each site, 10 individual non-depurated mussels were sampled, while another 10 mussels were allowed to depurate individually for 24 hours in 0.5 liters of aerated seawater as illustrated in Figure 2.

The individual mussels were dissected, and the soft parts were sampled, freeze-dried, and analyzed for element content by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Søndergaard et al., 2020). During the extraction procedure, it was recorded whether any sediment residue could be visually observed after chemical digestion. Student's t-test of two unpaired samples was used to test for statistically significant differences between depurated and non-depurated mussels expressed by p-values.

For the determination of concentrations in pooled tissues of 20 mussels, two batches consisting of 20 mussels (4.5 -5.5 cm) each were collected from eight different stations located in the vicinity of the mine, as shown in Figure 1. The stations included both collection at beach sand/small rocks, but also coastline areas of boulders and rocks. One batch was left for depuration for 24 hours in a bucket with 10 liters of aerated seawater before dissection while the non-depurated was dissected directly. Both batches were freeze-dried prior to chemical analysis by ICP-MS.

**Figure 2.** A blue mussel left for depuration for 24 h in clean seawater. Note the depurated particles at the bottom of the beaker. Photo by L Bach.

# 3 Results and discussion

## Individual mussel concentrations

Figure 3 and Table 1 illustrate the average and standard deviation for the Pb and Zn concentrations in the individual mussels (n=10 per group) collected from st. 12SW and A-fjord, comparing the non-depurated and depurated mussels.

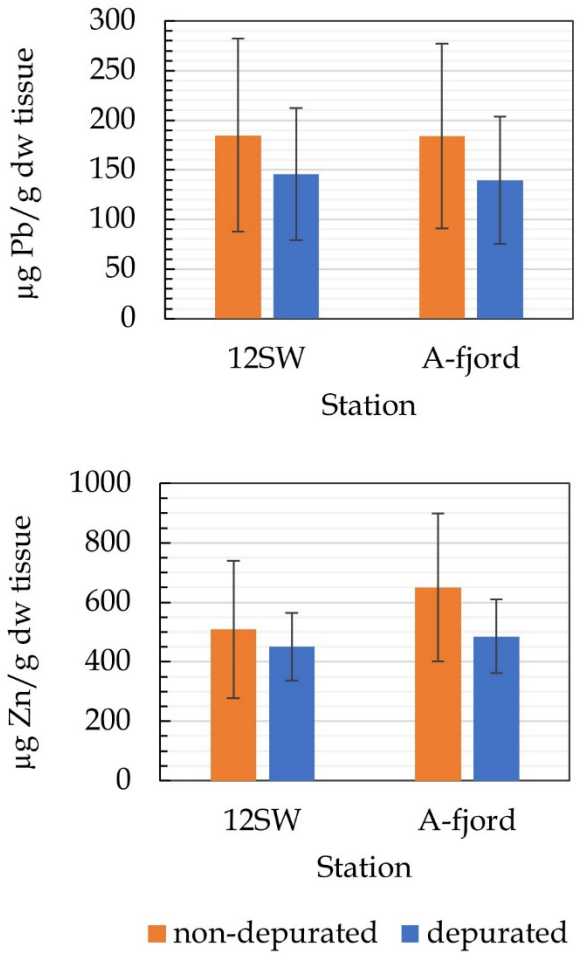


**Figure 3.** Lead (Pb) and zinc (Zn) concentrations from 10 individual non-depurated and depurated (24 hours) mussels collected at two marine sampling stations in Maarmorilik, a former Pb-Zn mine. The bars show the mean +/- one standard deviation.

The results (Figure 3) show that depurated mussels have slightly lower and less variable (lower standard deviation) metal concentrations than non-depurated mussels across the two stations.

Table 1 provides the median, mean, standard deviation, min, and max values of the Pb and Zn concentrations in the analyzed mussel samples, the p-values of the Student's t-test as well as recorded observation of residue particles in the sample upon chemical digestion during extraction. The data demonstrates that all non-depurated mussels retained residue particles after metal analysis, whereas depurated mussels contained minimal to no such residues. This

likely relates to the higher metal concentrations observed in non-depurated mussels (Figure 3), which reflect recently ingested particles or particles captured in the mussel mantle rather than metals bioaccumulated within the mussel tissues. However, the Student's t-test comparing Pb and Zn concentrations between depurated and non-depurated mussels showed a p-value > 0.05, suggesting no statistically significant difference between the mean metal concentrations of the depurated and non-depurated samples of mussels.

**Table 1.** Statistical information on lead (Pb) and zinc (Zn) concentrations in 10 individual non-depurated and depurated mussels from Figure 3.

| | Pb | | Zn | |
|---|---|---|---|---|
| | non-depurated | depurated | non-depurated | depurated |
| **St. 12SW** | | | | |
| median | 185 | 143 | 398 | 444 |
| mean | 185 | 146 | 509 | 451 |
| std | 97.3 | 66.5 | 231 | 113 |
| min | 69.7 | 58.0 | 273 | 297 |
| max | 329 | 240 | 899 | 629 |
| p-values | 0.31 | | 0.49 | |
| **St. A-Fjord** | | | | |
| Median | 175 | 131 | 601 | 487 |
| mean | 184 | 139 | 651 | 486 |
| std | 93.2 | 64.1 | 248 | 125 |
| min | 72.2 | 46.2 | 350 | 336 |
| max | 328 | 276 | 990 | 754 |
| p.values | 0.23 | | 0.08 | |
| Occurence of particles during extraction | 10 of 10 | 10 of 10 | 10 of 10 | 10 of 10 |

## Pooled mussel concentrations

Figure 4 shows the Pb and Zn concentrations in 20 pooled non-depurated and depurated mussels collected from eight stations. For Pb concentrations, depurated mussels showed slightly lower concentrations than non-depurated mussels at all stations except st. 17A. The percentage differences between the two methods ranged between 6-33 %. The lower concentration of Pb in depurated mussels was most pronounced at the more contaminated sites. No statistical analysis was conducted due to the lack of replicative data. These results are in accordance with the higher concentrations found in non-depurated individual mussel concentrations (Figure 3). Regarding zinc, depurated mussels showed lower concentrations than non-depurated at five out of the eight stations, with percentage differences ranging between 2-18 %.

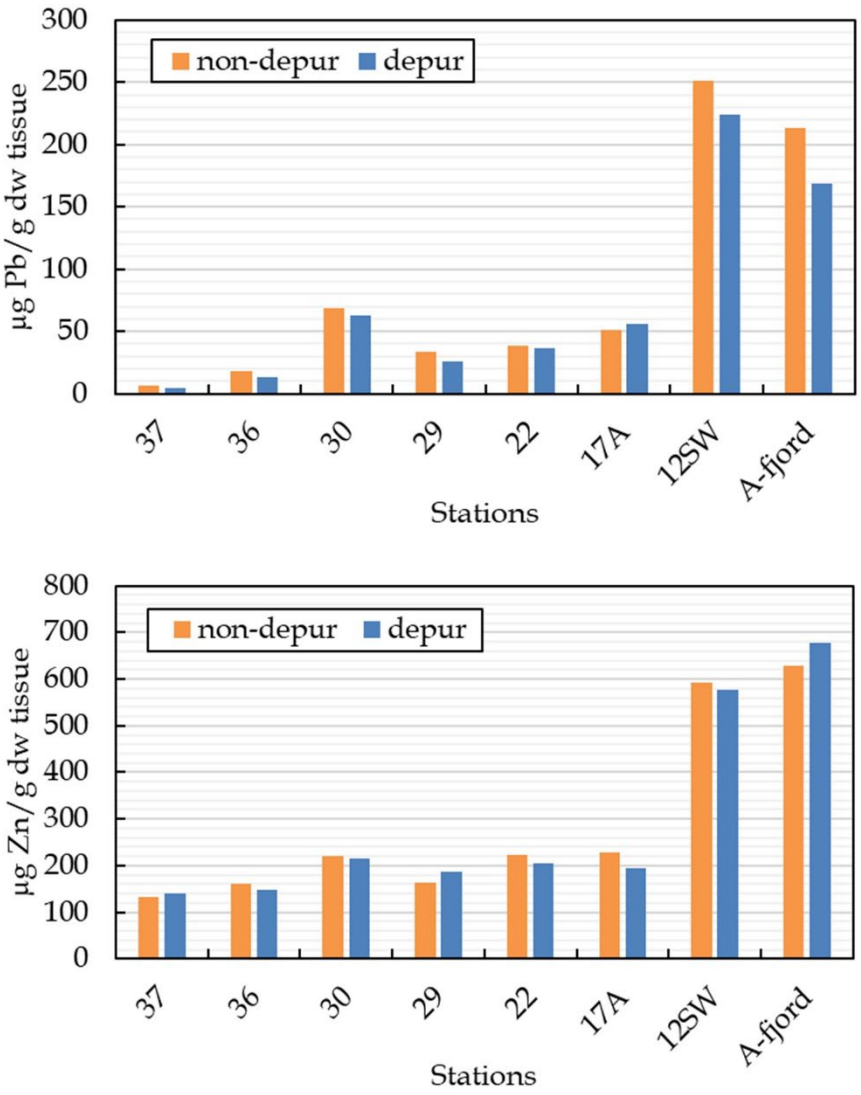


**Figure 4.** Lead (Pb) and Zinc (Zn) concentrations in 20 pooled non-depurated and depurated (24 hourd) mussels collevted from eight different stations in Maarmorilik.

## Application of depuration methodology

The trends of the present study focused on the importance of accounting for gut sediment contents when assessing metal body burdens in blue mussels. Depuration resulted in generally lower concentrations of Pb and Zn, reduced variability, although there was insufficient statistical evidence for the differences, besides an observed purge of residue particles. Ignoring the contributions of gut sediment contents in non-depurated mussels could instead result in overestimation and deviations from accurate assessments of metal bioaccumulation.

However, the Student's t-test indicated no statistically significant difference in metal concentrations between depurated and non-depurated mussels ($p > 0.05$), suggesting that while depuration reduces variability and metal concentrations, this effect was not statistically conclusive in this dataset. Also, the percentual difference between non-depurated versus depurated mussels was relatively small (6-33 % for Pb, and 2-18 % for Zn). This implies that the observed differences between the groups could be random variations rather than a consistent, meaningful effect of depuration, or more likely natural variability of feeding behavior, i.e., time of and feeding items between the individual mussels. Further studies with larger sample numbers may confirm the impact of depuration on metal burden assessments with statistically significant results.

Additionally, it is still unclear whether particle-associated elements in the gut were effectively purged or if some residual particles remained captured in the mussel gut or mantle. Even though the findings showed that residue particles were mostly found in non-depurated mussels, it is worth looking into whether extending the depuration time could further reduce the standard deviation and provide a more accurate assessment of the bioaccumulation. Identifying the optimal depuration duration is essential for refining the method, since overly extended depuration may also lead to the loss of tissue-bound contaminants. Also, a study on optimizing the method with a short rinse under running water after opening the mussel to ensure all visible silt and sediment particles are removed before elemental analysis could be relevant.

Applying the depuration method should, however, depend on the specific study's objective and the species involved. While depuration is relevant when assessing body burden, ecotoxicological risks, and risk for human consumption, it may be less relevant when assessing contaminant concentrations in prey species, as most predators would ingest both the organism and its gut content (Rust et al., 2004; Weston & Maruya, 2002).

# 4 Conclusion and recommendations

The findings of the present study demonstrated that depurated mussels generally showed lower metal concentrations and reduced variability compared to non-depurated mussels. These findings likely reflect the ability of the depuration methodology to reflect the bioaccumulated metals rather than recently ingested particle-bound metals present in the digestive system.

The statistical analysis (Student's t-test) did not provide sufficient evidence to support a significant impact of depuration, and the observed differences in metal concentrations of Pb and Zn were minimal within this case study. The potential influence of depuration may, however, vary depending on the collection site and could thereby show a more pronounced impact for mussels collected at other locations.

Depuration is recommended in international guidelines (European Commission, 2014; Larsen, 2013; OSPAR Commission, 2007, 2013, 2018; UNEP/MAP, 2021; UNESCO/WHO/UNEP, 1996) and in particular for mussels collected near point sources with high turbidity or on silt/clay bottoms, where there is an increased exposure to sediment particles. However, a complete transition to depuration practices for environmental monitoring may disrupt the long-term data series achieved for Greenlandic mines, which have historically relied on non-depurated mussels. To maintain comparability with historical baseline data, it is therefore essential to collect both depurated and non-depurated samples during a transition period. This approach could be implemented for a duration of approximately three to five years, allowing for comparisons between past and current datasets. After this transition, sampling programs may shift exclusively to depurated mussels. Importantly, whether or not samples have been depurated before storage and analysis should be explicitly reported to ensure transparency and data consistency.

In conclusion, in alignment with international guidelines, and to avoid any uncertainties related to the specific conditions of the site of sampling, it is generally recommended to apply the methodology of depuration of the mussels for baseline studies and environmental monitoring.

For ongoing monitoring with long-term data series and where monitoring has been conducted using non-depurated mussels, it is recommended to continue the monitoring without depuration. Addition of a set of depurated mussels to reveal a possible difference between depurated and non-depurated mussels may be an advantage in some cases.

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