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## **Orchomenella pinguis (amphipoda) - A Possible Species for Heavy Metal Biomonitoring of Marine Sediments**

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## *Orchomenella pinguis* (amphipoda)—a possible species for heavy metal biomonitoring of marine sediments

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## PEER REVIEW

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**Comments**

This manuscript describes a study of biouptake of metals from manipulated water–only and sediment exposures in an arctic amphipod species (*O. pinguis*). The aim was to investigate the use of this species for biomonitoring of metal contamination in marine sediments in the Arctic. This is a very important task and the performed investigation takes us one step closer to finding a useful monitoring tool for metals in the warming marine Arctic.

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## ABSTRACT

**Objective:** To evaluate the potential local benthic biomonitor organism, *Orchomenella pinguis* (*O. pinguis*), for mining contamination by addressing accumulation and toxicity of mining related metals in this arctic marine amphipod.

**Methods:** A toxicity study exposed *O. pinguis* to four commonly occurring heavy metals (Cd, Cu, Zn and Pb) associated to the mining industry in Greenland using: 1) a 5–day water–only bioassay; 2) a water–only bioassay evaluating the response between metal accumulation in *O. pinguis* and metal concentrations in water during a 5–day period; and finally 3) a sediment bioassay evaluating the response between metal accumulation in *O. pinguis* and metal concentrations in sediment as a function of time during a 20–day period using different mixtures of mining–contaminated sediments.

**Results:** LC<sub>50</sub> values for the four metals were 2.8, 5.4, 10.4 and 21.4 µmol/L, for Cu, Cd, Pb and Zn, respectively, with corresponding modelled metal concentrations of 3.4, 1.0, 11.1 and 6.1 µmol/g dry weight. During the sediment exposure experiments, a similar concentration of Zn did not induce lethal effects at the same level.

**Conclusions:** This study demonstrates that the appliance of metal organism concentrations as an estimate of effects is not a sufficient biomonitor of environmental effects. The organism may sequester metals into cellular compartments thus rendering the metals inert for toxic effects. More studies are needed to investigate effects of metal bioavailability. Additional biomarkers such as effects on functional responses e.g. feeding and burial behavior or effects on reproductive success are suggested in order enhance to the ecological significance.

## KEYWORDS

Invertebrate, Heavy metals, Water–only bioassay, Sediment bioassay, Accumulation, Greenland

### 1. Introduction

Mining for heavy metals possess a well–known environmental concern due to the potential release, spread and uptake of ore related heavy metals and other associated elements in biota. After the mining has started, identification

of the mining related metals and their contribution to the surrounding environment is essential to assess the impact and potentially to reduce environmental effects. Evaluation of the contribution from mining has traditionally been based on concentrations of heavy metals in various biomonitor species<sup>[1–4]</sup>, whereas less effort has been made to further

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identify potential effects<sup>[5,6]</sup>.

In Greenland, dispersal of heavy metals into the marine areas has been an important source of pollution from the mining industry, potentially affecting organisms living both within sediments and in the water–column<sup>[7,8]</sup>. Traditionally, brown algae, mussels and sculpins have been used as biomonitor species in environmental monitoring programs to evaluate dispersal and biological uptake of mining related heavy metals in marine areas<sup>[7]</sup>. Heavy metal uptake in algae and mussels is considered to mainly reflect conditions in the pelagic zone while the uptake in sculpins reflects near–seafloor conditions. However, currently, there is a lack of an appropriate biomonitor species that can be applied to reflect conditions within the sediment near mines in Greenland. Moreover, there is a lack of studies relating heavy metal concentrations in arctic monitor species to possible biological effects.

Biomonitor species can be defined as any biological species or group of species whose function, population or status can be used to determine the state of the ecosystem of which they are a part<sup>[9]</sup>. Amphipods, in general, are considered suitable biomonitor species, as they are relatively sedentary, and are highly abundant in most ecosystems<sup>[10]</sup>. A potential species for marine sediments in the Arctic is the small (5–10 mm length) benthic amphipod, *Orchomenella pinguis* (*O. pinguis*)<sup>[11]</sup>. This sediment–dwelling species is a major component of the shallow waters bottom fauna and densities up to 9000 individuals/m<sup>2</sup> have been found in the coastal area in West Greenland<sup>[4]</sup>. Further, *O. pinguis* as many other amphipod species plays a central role in the benthic ecosystem as an ecosystem engineer and highly affects key processes such as decomposition of organic matter<sup>[12]</sup>. *O. pinguis* has previously shown response to mixed anthropogenic contamination (PAH, metals, etc.) in terms of reduced reproductive success<sup>[13]</sup> and resilience towards other stressors<sup>[4]</sup>. However, little is known on the response of *O. pinguis* towards contamination by metals only.

This paper reports the main results of a study focused on accumulation and toxicity of cadmium (Cd), copper (Cu), zinc (Zn) and lead (Pb) in *O. pinguis*. The aim of this work was to investigate the potential of *O. pinguis* to be implemented in monitoring programs as a biomonitor species for sediment contamination in relation to the increasing mining industry in Greenland. This was achieved in three experiments: 1) A toxicity study exposing *O. pinguis* to four commonly occurring heavy metals (Cd, Cu, Zn and Pb) associated to the mining industry in Greenland using water–only bioassays; 2) A water–only bioassay evaluating the response between metal accumulation in *O. pinguis* and metal concentrations in water during a 5–day period; and finally, 3) A sediment bioassay evaluating the response between metal accumulation in *O. pinguis* and metal concentrations in sediment as a function of time

during a 20–day period using different mixtures of mining contaminated sediments.

## 2. Materials and methods

### 2.1. Sampling of sediment and amphipods

Mining–contaminated sediment was collected near the former Black Angel Pb–Zn mine in Maarmorilik on the west coast of Greenland. The mine was operated during 1973–1990 and the activity resulted in significantly elevated concentrations of especially Pb and Zn in seawater, sediments and biota in surrounding fiord system<sup>[7]</sup>. Today, more than 20 years after mining closure, elevated concentrations of metals are still measured in marine biota within a distance of 12 km from the mine<sup>[8]</sup>. Contaminated marine sediment for the sediment bioassay was collected near a major waste rock dump area (71°8′1.32″ N; 51°14′54.60″ W) in August 2011. A detailed description of the Maarmorilik area and the specific sampling site can be found in Sondergaard *et al*<sup>[8]</sup>.

Control sediment for the sediment bioassay and amphipods were sampled near the settlement Sisimiut in West Greenland also in August 2011. Sampling of both sediment and amphipods was conducted at a site outside Sisimiut (66°56′57.99″ N; 53°42′17.27″ W) not considered affected by anthropogenic activity at a depth of 10–15 m (a detailed description is given in Bach *et al*<sup>[11]</sup>). Sampling of *O. pinguis* was done overnight using traps baited with a freshly caught fish (shorthorn sculpin) and the sampling provided more than thousands of individuals. Amphipods were kept in large buckets (10 L) at 7–10 °C in aerated seawater supplied with a handful of sediment to acclimatize for 24 h.

The sampling and laboratory experiments were carried out in August 2011 and August 2013 in Sisimiut, West Greenland.

### 2.2. Experimental setup

#### 2.2.1. Water–only bioassay–toxicity screening (Experiment 1)

The toxicity screening experiment was carried out in order to estimate the effect concentrations of 4 of the predominant metals found in the Maarmorilik sediments: Cd, Cu, Zn and Pb. The experiment was carried out in 280 mL plastic beakers (diameter of 95 mm) containing 100 mL of freshly prepared test substance in aerated seawater. A number of 30 adult amphipods of both sexes were added in discriminately to each beaker. A piece of insect mesh (20 mm×20 mm) was added to each beaker to provide the amphipods with a substratum to cling on to in order to reduce stress and cannibalism. The beakers were kept in cold conditions app. 10 °C and covered by large pieces of cardboard to avoid direct sunlight. To avoid oxygen deficiency and to ensure constant exposure concentrations, 50% of the test substrate

was renewed every 48 h. Exposure media were prepared using aerated seawater (33 ‰, 10 °C) with addition of Cd, Cu, Zn and Pb standards.

The amphipods were exposed in triplicates to 12 concentrations of each metal (Cd, Cu and Pb: 0.625, 0.833, 1.250, 1.670, 2.500, 3.330, 5.000, 6.660, 10.000, 13.300, 20.000 and 26.700 µmol/L and Zn: 3.125, 4.160, 6.250, 8.330, 12.500, 16.700, 25.000, 33.300, 50.000, 66.600, 100.000 and 133.000 µmol/L) and six control replicates exposed to clean seawater. Observations of individual status (number of survivors) were recorded every 24 h for 4 d. Missing animals were considered dead due to the scavenging of the co-fellow amphipods and dead individuals were removed.

LC<sub>50</sub> values, with ±95% confidence limits, were calculated by Probit analysis using the software package XLSTAT Version 2013.4.07 (Addinsoft, USA).

### 2.2.2. Water-only bioassay–metal accumulation (Experiment 2)

A metal accumulation experiment was carried out in a water-only bioassay to determine the organism concentration after 5 d. Basically this experiment was carried out similar to the toxicity screening experiment, with the exception that 100–150 adult amphipods of both sexes were added indiscriminately to each beaker. Four experimental concentrations were chosen based on effect concentrations found in the toxicity screening assay (Cd, Cu and Pb: 0.313, 0.625, 1.250 and 2.500 µmol/L and Zn: 1, 2, 4 and 8 µmol/L) and one control exposed to clean seawater. The experiment was performed with one replicate only as one replicate consisted of 100–150 amphipods amounting one analytical sample. At the end of the experiment, at Day 5, the amphipods were sieved out of the test substrate, briefly rinsed with MilliQ-water where after surface water was removed from the animals by drying on good-quality filter paper and kept frozen at –20 °C before transportation to Denmark. In Denmark, samples were freeze dried prior to chemical analyses.

### 2.2.3. Sediment bioassay–metal accumulation (Experiment 3)

A sediment bioassay was conducted to investigate the metal uptake and concentration in *O. pinguis* exposed to different mixtures of metal contaminated and natural sediments as well as the metal accumulation in *O. pinguis* as a function of time.

To obtain sediments with different concentrations of mining contaminated sediment, control sediment from Sisimiut were mixed with Maarmorilik sediments to contain 0%, 25%, 50%, 75% and 100 % of Maarmorilik sediments (Table 1). Prior to mixing, the two sediments were sieved to 1 mm and frozen to kill all meiofauna and microfauna. A total of 150–200 adult amphipods of both sexes were added to 1000 mL beakers (diameter of 110 mm) with 50 mL of sediment and 800 mL of aerated seawater (33‰). To avoid oxygen deficiency, the seawater was constantly aerated. The beakers

were kept in cold conditions, 10 °C, and covered by large pieces of cardboard to avoid direct sunlight.

**Table 1**

Metal concentrations (µmol/g dry weight) in exposure sediments (experiment 3). The sediments were composed of contaminated sediment collected in Maarmorilik (0%–100%) mixed with ‘clean’ sediment.

| Metal             | Concentration |       |        |        |        |
|-------------------|---------------|-------|--------|--------|--------|
|                   | 0%            | 25%   | 50%    | 75%    | 100%   |
| Cd                | 0.002         | 0.018 | 0.045  | 0.060  | 0.099  |
| Cu                | 0.039         | 0.115 | 0.270  | 0.349  | 0.532  |
| Zn                | 1.190         | 6.320 | 15.200 | 19.600 | 32.100 |
| Pb                | 0.020         | 0.830 | 2.190  | 3.200  | 4.710  |
| Ni                | 0.390         | 0.350 | 0.380  | 0.380  | 0.420  |
| Hg                | <d.l.         | <d.l. | <d.l.  | 0.0004 | 0.0009 |
| Fe                | 916           | 853   | 741    | 596    | 424    |
| Al                | 1750          | 1720  | 1560   | 1460   | 1130   |
| Cr                | 2.400         | 2.020 | 1.660  | 1.350  | 0.860  |
| As                | 0.028         | 0.060 | 0.101  | 0.130  | 0.201  |
| % organic content | 0.570         | 2.080 | 4.420  | 6.130  | 9.610  |

<d.l.=below detection limit.

At different time intervals (3, 5, 11 and 20 d), the amphipods were sieved out of the sediment. At the end of the experiment, the amphipods were sieved out of the test substrate, briefly rinsed with MilliQ water and surface water was removed from the animals by drying on good-quality filter paper and kept frozen at –20 °C before transportation to Denmark. In Denmark, samples were freeze dried prior to chemical analyses.

### 2.3. Chemical analyses

The freeze dried amphipod samples were analyzed at the accredited ICP–MS laboratory at Department of Bioscience, Roskilde. A subsample of each homogenized amphipod bulk samples (ca. 300 mg=ca. 80 individuals) and sediment subsamples (ca. 500 mg) were digested using 4 mL Merck Suprapure HNO<sub>3</sub> and 4 mL MilliQ water in Teflon bombs under pressure in a microwave oven (Anton Paar Multiwave 3000). After digestion, solutions were transferred to polyethylene bottles with MilliQ water and analyses of Cd, Cu, Zn and Pb were performed directly on these solutions using an Agilent 7500ce ICP–MS. All metal concentrations are quoted in terms of µmol per g dry weight. Analytical quality assurance was performed by analysing blanks, duplicates and certified reference materials Dorm–3, Dolt–4, Tort–2 and Mess–3. The detection limits (3SD on blank samples) were Cd: 0.00004 µmol/L, Cu: 0.0077 µmol/L, Zn: 0.0028 µmol/L and Pb: 0.0003 µmol/L. In the reference materials, the measured recovery %±SD for Cd, Cu, Zn and Pb were (95±2)%, (96±2)%, (97±2)% and (82±13)%, respectively.

### 2.4. Statistics

The toxicity of the metals (experiment 1) was compared and for a given pair of LC<sub>50</sub> values, non-overlapping confidence

intervals provided evidence, at a 5% significance level ( $P < 0.05$ ), that the  $LC_{50}$  values were significantly different. For the metal accumulation experiments (experiment 2 and 3) logistic regression analyses were performed on amphipod concentrations as a function of exposure concentration applying the least square method on linear regression.

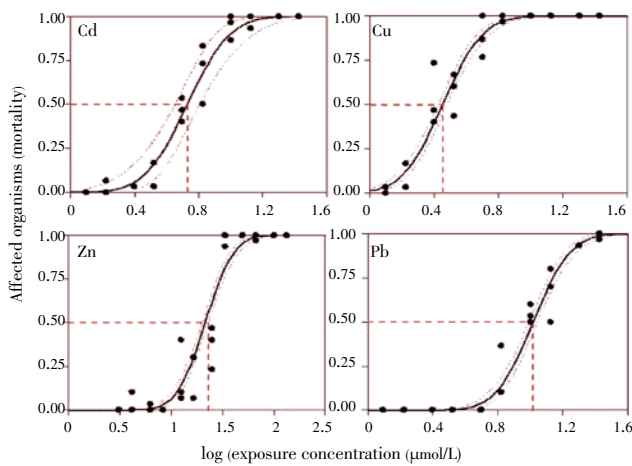
### 3. Results

#### 3.1. Water-only bioassay

##### 3.1.1. Toxicity screening (Experiment 1)

To assess the toxicity of the metals, organisms were exposed to 12 different concentrations of each of the four metals (Cd, Cu, Zn and Pb) for 96 h with daily observations. For all metals there was an increase in metal toxicity with increasing exposure time.

After 96-h exposure, the  $LC_{50}$  values were estimated (Figure 1) based on linear interpolation between the data points using logistic regression (for all metals the probability of  $\chi^2$  test  $< 0.0001$ ). Copper showed to be most toxic element with a  $LC_{50}$  value of 2.83 (95% CI: 2.66–3.00)  $\mu\text{mol/L}$  followed by Cd, Pb and then Zn with  $LC_{50}$  values of 5.35 (95% CI: 5.02–5.71)  $\mu\text{mol/L}$ , 10.38 (95% CI: 9.79–11.01)  $\mu\text{mol/L}$  and 21.36 (95% CI: 20.00–22.83)  $\mu\text{mol/L}$ , respectively ( $\text{Cu} > \text{Cd} > \text{Pb} > \text{Zn}$ ). The controls (seawater exposure) recorded a lethality of  $< 1\%$ .

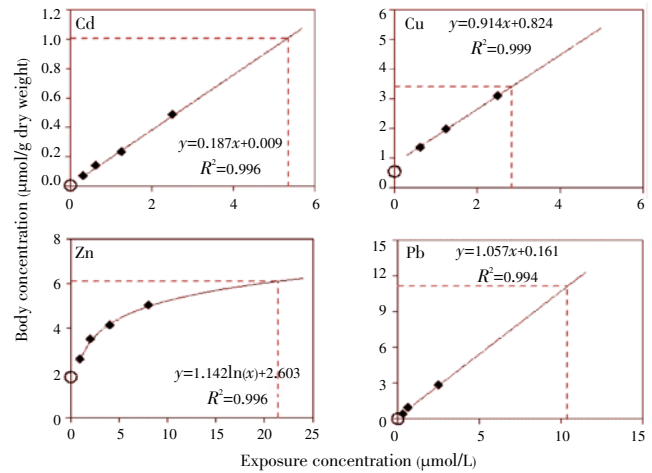


**Figure 1.** Affected organisms (mortality) of the amphipod species *O. pinguis* when exposed to a range of different concentrations of Cd, Cu, Zn and Pb in water-borne setup systems for 5 d (experiment 1). The graphs show the logistic regression of affected organism by log (exposure concentration ( $\mu\text{mol/L}$ )) and display the data points, the model and the 95% confidence range (blue) around the model. The punctuated red lines represent the values of  $LC_{50}$ s.

##### 3.1.2. Metal accumulation in *O. pinguis* (Experiment 2)

To assess the metal accumulation in the amphipods in relation to exposure concentrations in water, amphipods were exposed to sub-lethal concentrations of Cd, Cu, Zn and Pb for 5 d (Figure 2). The concentration dependent accumulation followed linear regressions ( $R^2 = 0.99$ ) for Cd,

Cu and Pb, but not for Zn, which best fitted line followed a logarithmic curve ( $R^2 = 0.99$ ). The regressions and their significance are given in Table 2 (least square method;  $P < 0.05$ ). Steady state of uptake could thus not be confirmed for any of the metals within the given timeframe of the study.



**Figure 2.** Mean metal concentration in the amphipod species *O. pinguis* when exposed to different concentrations of Cd, Cu, Zn and Pb in water-borne setup systems for 5 d (experiment 2). The open circle (○) represents the initial body concentration in the amphipods. The punctuated lines (red) represent the concentration at the  $LC_{50}$  values by extrapolation of the dose response matrices.

**Table 2**

Statistic regression for metal concentration in the amphipod, *O. pinguis* as a function of exposure concentration.

| Amphipod                        | Metal | $x^*$ or $\ln(x)^{**}$ | 95% CI       | $R^2$ | P      |
|---------------------------------|-------|------------------------|--------------|-------|--------|
| Amphipod conc water exposure    | Cd    | 0.187*                 | 0.152–0.221  | 0.996 | 0.002* |
|                                 | Cu    | 0.913*                 | 0.836–0.991  | 0.999 | 0.000* |
|                                 | Zn    | 1.142**                | 0.911–1.370  | 0.996 | 0.002* |
| Amphipod conc sediment exposure | Pb    | 1.057*                 | 0.036–2.080  | 0.994 | 0.048* |
|                                 | Cd    | 0.013*                 | –0.025–0.051 | 0.274 | 0.365  |
|                                 | Cu    | 0.580*                 | –0.225–1.380 | 0.637 | 0.106  |
|                                 | Zn    | 1.910**                | 0.783–3.040  | 0.907 | 0.012* |
|                                 | Pb    | 0.317**                | 0.189–0.445  | 0.954 | 0.004* |

\*indicates significant correlation at  $P < 0.05$ .

For all metals, metal concentration of the amphipods was modelled at the  $LC_{50}$  value (Figure 1 and Table 3) by extrapolation of the dose response matrices.

**Table 3**

Resulting  $LC_{50}$  and 95% CI of toxicity tests based on water-only bioassays of *O. pinguis* (experiment 1) and corresponding amphipod concentration derived on models from sub-lethal concentration exposures (experiment 2).

| Metal | Model                                   | $LC_{50}$ | 95% CI      |
|-------|---|-----------|-------------|
| Cd    | Exposure conc. ( $\mu\text{mol/L}$ )    | 5.35      | 5.02–5.71   |
|       | Amphipod conc. ( $\mu\text{mol/g dw}$ ) | 1.01      | 0.95–1.07   |
| Cu    | Exposure conc. ( $\mu\text{mol/L}$ )    | 2.83      | 2.66–3.00   |
|       | Amphipod conc. ( $\mu\text{mol/g dw}$ ) | 3.41      | 3.25–3.56   |
| Zn    | Exposure conc. ( $\mu\text{mol/L}$ )    | 21.40     | 20.00–22.80 |
|       | Amphipod conc. ( $\mu\text{mol/g dw}$ ) | 6.10      | 6.02–6.18   |
| Pb    | Exposure conc. ( $\mu\text{mol/L}$ )    | 10.40     | 9.79–11.00  |
|       | Amphipod conc. ( $\mu\text{mol/g dw}$ ) | 11.10     | 10.50–11.80 |



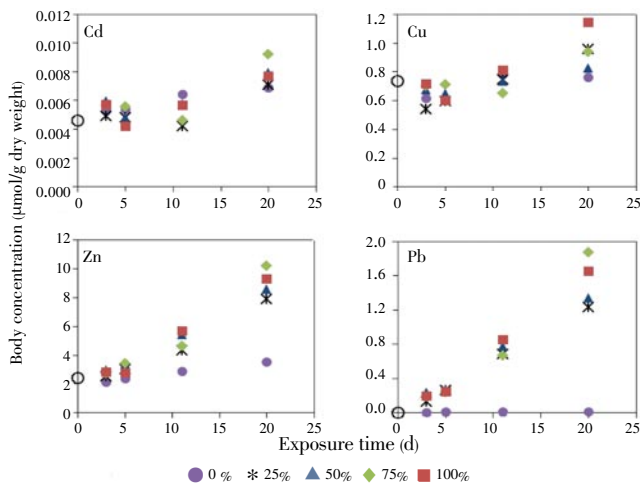
### 3.2. Sediment bioassay

#### 3.2.1. Metal concentrations in sediment mixtures (Experiment 3)

The metal concentrations were determined in the 5 different sediment mixtures by ICP-MS (Table 1). In the Maarmorilik sediment, particularly Zn and Pb were found in high concentrations (32.1 and 4.7  $\mu\text{mol/g}$  dry weight, respectively), both exceeding the categorized 'Severe Effect Level' at 4.1 and 0.5  $\mu\text{mol/g}$  dry weight as defined in Fletcher *et al.*[14]. Notably, in the Sisimiut sediment, high concentrations of Cr and Fe (2.4 and 917  $\mu\text{mol/g}$  dry weight, respectively) were found both at the level of 'Severe Effect Level' (2.1 and 716  $\mu\text{mol/g}$  dry weight)[14]. The occurrence of both Cr and Fe is however expected to be a result of natural occurrences and the site is not considered affected by anthropogenic contamination.

#### 3.2.2. Metal accumulation in *O. pinguis* (Experiment 3)

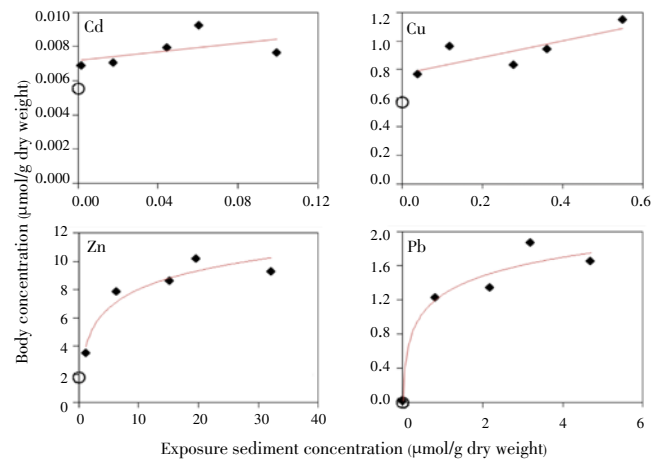
The amphipods accumulated the metals to different degrees dependent on time (Figure 3) and concentrations (Figure 4). The time dependent uptake was evident for all metals but most pronounced for the uptake of Zn and Pb.



**Figure 3.** Time dependent mean metal concentration in the amphipod species *O. pinguis* when exposed to different mixtures of metal contaminated Maarmorilik sediments for 20 d (experiment 3) with focus on Cd, Cu, Zn and Pb. Symbols represent proportions of Maarmorilik sediment according to Table 2 and open circles (○) the initial concentration in the amphipods.

Similarly, was the concentration dependent uptake also pronounced for Zn and Pb and no good correlation between exposure concentration and accumulation could be established for Cd and Cu (Table 2). At the end of the exposure period (20 d), the accumulation of both Cd and Cu (0.007–0.009  $\mu\text{mol Cd/g}$  dry weight and 0.83–1.14  $\mu\text{mol Cu/g}$  dry weight) differed only slightly from the initial amphipod concentration (0.006  $\mu\text{mol Cd/g}$  dry weight and 0.57  $\mu\text{mol Cu/g}$  dry weight) (Figure 4). In contrast, the accumulation of both Zn and Pb was higher and correlations between exposure concentration and amphipod concentration for both metals were significant and followed logarithmic uptake curves (least square method;  $P < 0.05$ –Table 2). After 20 d of exposure, the accumulation of Zn was approximately 4 times (7.9–10.2  $\mu\text{mol/g}$  dry weight) the initial body concentration (1.8  $\mu\text{mol/g}$  dry weight) and the accumulation of Pb was even greater with an initial

concentration of 0.001  $\mu\text{mol/g}$  dry weight to 1.2–1.9  $\mu\text{mol/g}$  dry weight after 20 d.



**Figure 4.** Final mean metal concentration in the amphipod species *O. pinguis* after exposure to different mixtures of contaminated Maarmorilik sediments for 20 d (experiment 3) with focus on Cd, Cu, Zn and Pb.

Open circles (○) represent the initial concentration in the amphipods.

Amphipod metal accumulation derived of the sediment exposure metrics was compared to the body-burden associated toxicity at the water-only bioassays. The amphipod concentrations of Cd, Cu and Pb (max body-burden at 0.009, 1.14 and 1.87  $\mu\text{mol/g}$  dry weight, respectively) in the sediment exposed amphipods were all lower than expected toxicity levels. In contrast, the concentration of Zn in sediment exposed amphipods reached levels (10.2  $\mu\text{mol/g}$  dry weight) where considerable toxicity for the single metal would be expected. Even though we did not quantify the mortality precisely in the sediment bioassay, we estimate that the mortality was less than 10%.

## 4. Discussion

### 4.1. Metal accumulation in *O. pinguis*

The studies presented in this paper are intended as an approach in biomonitoring to expand the use of biomonitoring organisms in Greenland and include a local sediment living organism. Further, it is a first study linking toxicokinetics to the toxicodynamics with respect to metal exposure to a Greenlandic amphipod.

Sediment burrowing amphipod species, including *O. pinguis*, are exposed to metals from surrounding aquatic medium and from ingestion of sediment particles as a food source. Though *O. pinguis* is a sediment dwelling scavenger that primarily feeds on detritus and carcasses, sand grains have also been detected in their gut[15,16]. The exposure from surrounding aquatic medium includes both when feeding on surface or swimming freely in the pelagic water and when they are burrowing and are exposed to the sediment interstitial water. The bioavailability of metals from latter may differ largely from the overlying water exposure due to physicochemical factors as redox potential, oxygen concentration, salinity, *etc.* The two scenarios are represented in the present study that describes

the differences between metal exposure concentrations in sediment and water, toxicity and accumulation.

In the water-only bioassay, the major pathway of uptake is considered to be directly through permeable surfaces including the gills. The concentrations of Cd, Cu, Zn and Pb in *O. pinguis* increased with increasing exposure concentrations and exposure periods and even though it happened with a decreasing rate, steady state could not be confirmed within the experimental period, neither could it be rejected that steady state was reached. Standards for assessing sediment bioaccumulation recommend testing for 28 d to obtain certainty for achieving steady state<sup>[17]</sup>, but it was not possible to extend the exposure experiment from 20 to 28 d due to logistical issues. The uptake of Zn in the present study differed however from the other metals. Whereas the correlation between concentrations and uptake for Cd, Cu and Pb revealed no indication of an uptake regulation in *O. pinguis* within the given time line of the study, a saturation curve was observed for Zn, indicating at least a partial concentration induced regulation. The general perception is that the accumulation of non-essential metals as Cd and Pb is not regulated by the organism and that the accumulated concentration varies only in line with the bioavailability<sup>[18]</sup>. Available data suggest however that essential metals, as Cu and Zn, are regulated, though in varying degrees<sup>[19–23]</sup>. In agreement with the present study, no indications of regulation of either Cd or Cu were reported for the amphipod, *Paramorea walkeri*, in water exposures<sup>[24]</sup>. In a 10 weeks' water bioassay on the amphipod, *Hyaella azteca*, there was found no regulation of Pb but indications of a partial regulation of Zn and an entire regulation of Cu<sup>[20]</sup>. It was suggested that part of the general discrepancies on Cu regulation by amphipods may be due to the time required for the amphipods to adapt to Cu stress. While indications of Zn regulations were found in this study, no regulation was found for Cu. The exposure period in this study was only 5 d for the water bioassays, and it cannot be excluded that a regulation of Cu may be induced after a longer time of exposure. In general, the metal accumulation obtained in our experiments is largely within the range reported for marine amphipods in literature<sup>[18,25,26]</sup>. Though comparison of metal accumulation from contaminated sediments is more difficult than from water because of differences between partitioning behavior or binding strength of the contaminant to the sediment used, sediments are ecologically important because they mediate chemical exchange among the particulate, dissolved and biological phases and provide a valuable indication of overall environmental contamination.

The higher accumulation found in *O. pinguis* in the water-only bioassay relative to the sediment bioassay taking exposure concentrations into account, is consistent with the findings in a study on accumulation of Cd in the freshwater amphipod, *Hyella curvispina*, in both water-borne and sediment bioassays<sup>[27]</sup>. These findings are however not surprising as dissolved or weakly adsorbed contaminants are more bioavailable to aquatic biota compared to more structurally complex mineral-bound contaminants which may only become bioavailable upon ingestion with food<sup>[28]</sup>. In the

sediment bioassay a positive correlation between exposure and metal accumulation was observed for Zn and Pb, but not for Cd or Cu. The Maarmorilik sediments contained high concentrations of Zn and Pb and the organisms accumulated the metals correspondingly, thus with an uptake rate of both metals decreasing with increasing concentration. Whether this is related to the assumption of regulation of essential metals for the uptake of Zn, it is also possible that the bioavailable fractions of the metals do not relate linearly to sediment concentrations.

#### 4.2. Metal effects to *O. pinguis*

In the sediment bioassay, *O. pinguis* were exposed for 20 d and whereas the accumulation of both Cu and Cd at the end of experiment were not different from initial concentrations, the amphipods accumulated both Zn and Pb considerably relative to the initial body concentrations and it is likely that the exposure resulted in some effects on *O. pinguis*. Relating amphipod concentration to toxic effects is however not straight forward. In the present study, body-burdens of Zn were found exceeding the level where considerable lethality would be expected, but no such effect was observed. This discrepancy indicates that relation between metal concentration in whole organisms and toxicity is complex and that the organism concentration as such can't be unaccompanied as an indicator of toxicity. Metal accumulation resulting in toxic effects is influenced by intrinsic processes such as accumulation rates, transport, detoxification and excretion of the metals in addition to other environmental factors such as co-exposure, exposure time and species sensitivity<sup>[29]</sup>. Amphipods are known to possess detoxification mechanisms to prevent toxicity by sequestering metals and thereby rendering it inert<sup>[29–32]</sup>. Such detoxification processes may involve deposition of the metal into insoluble intracellular granules or induction of metallothioneins, which are low molecular proteins with a high affinity for binding metals, hence decreasing the intracellular availability and thus toxicity<sup>[19]</sup>. Consequently it is not necessary the total organism concentration that correlates with toxicity rather it is the concentration of metabolically available metal that corresponds to the toxicity onset<sup>[29,32]</sup>. More studies are therefore needed to relate the intracellular available fraction, whole organism concentration and the amount sequestered in detoxification organelles as lysosomes and in metallothioneins to toxic effects.

The concentrations of Zn and Pb in the Maarmorilik sediment (100%) were almost a 10 factor higher than the categorized 'Severe Effect Level' concentration as defined by Fletcher *et al.*<sup>[14]</sup>. The arctic amphipod was, however, not severely affected in terms of mortality. The 'Severe Effect Level' concentrations (like other sediment and water quality criteria) are based on data compiled from temperate and tropic species toxicity tests and it has been and still remains a question whether arctic species are more or less sensitive to stress including contaminants than temperate species<sup>[33–36]</sup>. Only few studies have been performed on effects of contaminants to arctic marine invertebrates, and only a small fraction of these on metals. Chapman and Riddle<sup>[37]</sup> reviewed the available data on metal toxicity (Cu, Cd, Cr, Zn and Pb) to

arctic species and found that the marine water quality criteria/guidelines on short term tests based on temperate data would be protective of the reviewed tested arctic species, as would *O. pinguis* from the present study.

#### 4.3. *O. pinguis* as a biomonitor species

As an arctic biomonitor species for sediment contamination the studied arctic species, *O. pinguis*, may have potential due its wide abundance and significant uptake of heavy metals from both water and sediment. Further, due to its occurrence in shallow coastal waters, it is likely to be one of the first species to be affected by contaminants originating from land.

This study highlights, however, that though organism accumulation can be applied as a biomarker of exposure it is more complicated to relate organism concentration to toxic effects as described above as unknown amounts of the quantified metals may be sequestered into for example methallothioneins and lysosomes, thus rendering the metals analytic measureable—but inert for toxic effects. We suggest additional biomarkers of effects such as effects on functional responses *e.g.* feeding, burial behavior and tolerance, or effects on life characteristics *e.g.* juvenile growth, time to first reproduction, reproductive success *etc.*

This study further points out the challenges in linking results obtained by traditional 120 h water-only toxicity tests to more ecological relevant settings for sediment dwelling organisms and we suggest to include more studies on effects of metal bioavailability and especially on effects of dietary metal uptake. Acknowledging that more knowledge on uptake, accumulation and effects of heavy metals on *O. pinguis* is needed, we positively add potential for this species to contribute as a valuable bioindicator species for metal contamination from industries such as mining. A challenge remains in linking contaminant concentrations with toxicological effects on population processes and hence enhance the ecological significance.

A noteworthy outcome of this study is that the general environmental quality guidelines developed on temperate species for the metals under study (Cd, Cu, Zn and Pb) are found to be appropriately protective for at least this particular arctic species, *O. pinguis*.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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#### Comments

##### Background

This research investigates the accumulation of metals in an Arctic amphipod (*O. pinguis*) from water only—and sediment exposures. The purpose was to investigate the possibility of using *O. pinguis* as an indicator species in field monitoring of metal contamination in Arctic marine areas affected by mining activity.

##### Research frontiers

Metal accumulation from marine sediments has been extensively investigated for decades. The most important scientific efforts in terms of developing management and monitoring tools in aquatic environments have been carried out in the 1990 by the US–EPA. Yet, good tools are still lacking in order to accurately predict biological and ecological effects of metals in the marine environment. This is especially true for the Arctic. Having proper tools and methods for monitoring is essential in times of climate change, retraction of ice cover and increased exploitation of natural resources in the Arctic. This research takes one step towards solving this problem.

##### Related reports

Ankley GT, *et al.* (1996), reported that assess the ecological risk of metals in sediments, and also reported technical basis and proposal for deriving sediment quality criteria for metals. Hanna SK, *et al.* (2013) studied the accumulation and toxicity of metal oxide nanoparticles in a soft–sediment estuarine amphipod.

##### Innovations and breakthroughs

A new species is tested for biomonitoring purposes. The study shows that the sensitivity of *O. pinguis* is the same as the level for metal toxicity in temperate species, validating the use of general assessment criteria in the arctic.

##### Applications

Arctic amphipod (*O. pinguis*) is a good choice for monitoring of metal contamination in sediments.

##### Peer review

This manuscript describes a study of bio-uptake of metals from manipulated water-only and sediment exposures in an arctic amphipod species (*O. pinguis*). The aim was to investigate the use of this species for biomonitoring of metal contamination in marine sediments in the Arctic. This is a very important task and the performed investigation takes us one step closer to finding a useful monitoring tool for metals in the warming marine Arctic.

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