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## ***Asterias rubens* (Echinodermata, Asteroidea) in polluted Sørfjord (SW Norway): molecular biomarkers and bioavailability of heavy metals**

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The present work aimed

(1) To validate the use of *A. rubens* as a bioindicator of heavy metal contamination using experimental exposures and sampling in the field.

(2) To assess the impact of heavy metals in asteroid populations from a 'hot spot' in the field using a multilevel approach:

- Molecular level: metallothionein (MT) concentrations were quantified and alkaline phosphatase (AP) activity was measured (MT is a low molecular mass protein involved in mechanisms of heavy metal detoxification and AP is an enzyme whose co-factor is Zn).
- Organismic level: The skeleton development in the body wall was measured
- Population level: The population structure was studied over a gradient of heavy metal contamination.

One of the most important points when calibrating a bioindicator of heavy metal contamination is to ascertain that it is not a regulator of internal metal concentrations and that there is a simple relationship between internal concentrations and concentrations in the environment. If the selected species is able to regulate internal metal concentrations, it should not be used as a bioindicator because it will not indicate variations in the environment.

Regarding uptake of heavy metals in *A. rubens*, it has been shown that there is a simple relationship between Cd bioconcentration factors and Cd concentrations in sea water. Similarly, there is a simple relationship between Pb accumulation ratio and Pb concentrations in sea water.

As far as elimination of heavy metals is concerned, it has been shown that Cd elimination from pyloric caeca is rapid; on the contrary, it is not significant from the skeleton. Concerning elimination of Pb,

similar kinetics were calculated: a rapid decrease of Pb concentrations in the pyloric caeca during the decontamination period while no significant loss was observed in the skeleton.

Therefore, uptake models led us to ascertain the value of *A. rubens* as a bioindicator. The pyloric caeca were proposed as a short-term bioindicator of Cd and Pb contamination because uptake and elimination of these metals is rapid. In contrast, the skeleton was proposed as a long-term integrator of Cd and Pb contamination because of the high retention time in this compartment.

As part of the international effort to assess the quality status of the North Sea, we used *A. rubens* as a bioindicator to describe heavy metal bioavailability in Sør fjord (SW Norway), one of the most contaminated sites in the North Sea catchment area. Huge quantities of heavy metals have been discharged into the fjord for more than 65 years from a zinc smelter located at its head. Inputs were drastically reduced in 1986 but release of heavy metals from sediments continue to act as a secondary source, stimulated by benthic activity. Therefore, severity of contamination has been little affected by the drop in inputs. In an effort to decrease the secondary source, a membrane was placed in 1992 to cap the hot spot sediments.

In order to assess the effect of such remedial actions, asteroids were sampled in 1995 at 4 sites in the fjord: S1 (100 meters South of the polluting smelter), S2 (in front of another smelter, 5 km North), S3 (17 km North) and S4 (outside of the fjord, 40 km North). Concentrations of Cd and Pb were measured in pyloric caeca (the short-term bioindicator) and skeleton (the long-term integrator) by flame absorption spectrometry.

In the pyloric caeca, there was a significant decreasing trend from 3-6  $\mu\text{g Cd, Pb g}^{-1}$  DW in populations close to the smelters to  $< 1 \mu\text{g Cd, Pb g}^{-1}$  DW at S3 and S4. In skeleton, the general decrease from the head to the mouth of the fjord was steeper. At S1, concentrations were very high: 12  $\mu\text{g Cd g}^{-1}$  DW and 155  $\mu\text{g Pb g}^{-1}$  DW. At S2, intermediate concentrations were measured: 5  $\mu\text{g Cd g}^{-1}$  DW and 47  $\mu\text{g Pb g}^{-1}$  DW. At S4, lower concentrations were measured: 3  $\mu\text{g Cd g}^{-1}$  DW and 16  $\mu\text{g Pb g}^{-1}$  DW. Concentrations of Pb in the skeleton reflect closely the decreasing gradient of contamination in the fjord. The pyloric caeca, a short-term bioindicator, indicate that the head of the fjord is still heavily contaminated.

In attempt to assess temporal trends, asteroids representing the whole size range of the population in the 'hot spot' in Sør fjord were sampled and Cd and Pb concentrations were measured in the pyloric caeca and the skeleton. In the pyloric caeca, concentrations did not vary significantly with size. In the skeleton, concentrations of Cd did not vary either. In contrast, there was a clear and significant increase of Pb concentrations with size. Pb concentrations in the skeleton of the largest individuals (12-14 cm; 132-155  $\mu\text{g Pb g}^{-1}$  DW) were up to 8 times higher than those in the smallest individuals. Asteroids larger than 10 cm in size were likely to be 4-7 years-old which means they were present before the remedial action of 1992 and may have already accumulated high concentrations of Pb. Small asteroids ( $< 4$  cm) developed after the remedial action, presumably in a cleaner environment. The absence of a

significant allometric relationship for Cd indicates this metal has different flux rates in relation to growth compared to Pb.

In order to assess the ecotoxicological relevance of the high metal concentrations measured in asteroids of the Sjørfjord, the impact of metal contamination on the biology of *A. rubens* was studied. The presence of *A. rubens* in the hot spot, and absence of most other macrobenthic species of the fjord, indicates this asteroid population is relatively resistant to heavy metals. However, several parameters were studied to detect sublethal effects.

Metallothionein (MT) concentrations were quantified by differential pulse polarography. MT concentrations were significantly higher in 2 populations of the fjord (S2 and S3; 5.0 - 5.6 mg MT g<sup>-1</sup> dw) than in control asteroids (S4: 2.8 - 3.0 mg MT g<sup>-1</sup> dw) but surprisingly, no significant increase was measured in asteroids sampled close to the first smelter (S1). Thus, although there was significantly higher concentrations of MT in some populations of the fjord, no close relationship with Cd, Cu or Zn bioavailability could be detected.

On the contrary, AP was significantly inhibited (45% of activity) in populations close to the smelters while activity was not significantly different from controls in asteroids from S3 and S4 (the clean sites of the fjord).

Impact on other levels of biological organization was investigated. The development of the skeleton in the body wall (w/w) was affected in the hot spot (30% versus 50%) which fits well data reported in the literature which indicates that skeletogenesis in echinoderms is highly sensitive to heavy metal exposure.

Finally, at the population level, large asteroids in the hot spot were rare and reduced in size compared with the largest size classes at the other stations, despite the abundance of the mussel *M. edulis* on which they feed.

## Conclusions

The present study validates the use of *A. rubens* as a bioindicator of heavy metal contamination in the field, the pyloric caeca and the skeleton being used as short-term and long-term bioindicators, respectively. According to our results in Sjørfjord, it is concluded that heavy metal bioavailability to asteroids in the hot spot has decreased recently and that capping the contaminated sediment is a relatively efficient method of decreasing heavy metal bioavailability. However, the latter is still higher than in the other parts of the fjord. Although *A. rubens* is particularly resistant to heavy metals in comparison to other macrobenthic organisms, the high metal loads in the most contaminated sediments of Sjørfjord probably results in toxic effects in *A. rubens*, as observed with AP activity which was affected in populations close to the smelters. On the other hand, based on current evidence, MT quantification in pyloric caeca is not an appropriate biomarker of heavy metal impact in asteroids as there was no simple relationship between MT concentrations and Cd bioavailability. Higher levels of biological organization were most probably affected by the pollution.