DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category 3: Biological effects

Toolbox component: General stress

Fact Sheet 3.12: Catalase activity (CAT)

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What is it?

Catalase (CAT) is an enzyme that catalyses a reaction where hydrogen peroxide (H_2O_2) is broken down to water and oxygen ($2H_2O_2 \rightarrow 2H_2O + O_2$). CAT is a key enzyme in the antioxidant defence system (ADS).

What does it tell you?

Elevated levels of CAT activity indicate activation of the cellular ADS due to an accelerated production of reactive oxygen species (ROS) that need to be neutralized to prevent oxidative damage to macromolecules. On the other hand, lowered CAT levels may indicate that the ADS is already overloaded and the organism cannot properly cope with the excessive amounts of ROS (the so-called bell-shape response).

Type of Indicator (tick box)

☒ non-specific stress indicator
☐ specific for groups of contaminants incl. CWA or explosives
☐ CWA-specific indicator
☐ specific for substances related to explosives (e.g. TNT)

How to measure it?

***Species*:** CAT can be measured in a large variety of organisms, including fish and mussels.

***Matrix*:** Fish liver, mussel digestive gland and gill tissue homogenates.

***Equipment*:** Spectrophotometer /microplate reader able to measure at 240 nm in intervals; UV microplates; basic laboratory equipment (pipettes, decanters). For reagents, see, e.g., Turja et al., 2014 and Vuori et al. (2015).

***Measurements and units*:** For a detailed description, see, e.g., Claiborne (1985) and Turja et al., (2014) and Vuori et al. (2015). Briefly, the activity of CAT is measured as the change in UV absorption in a mixture containing 4.3 µM H_2O_2 at a final concentration in phosphate buffer. The

activity of CAT (OD/min) is adjusted to the protein concentration of the sample, measured with, e.g., the Bradford method (Bradford, 1976).

***Calculations*:** Change in absorbance (OD/min). Subtract blank measurement values from the sample measurements.

CAT activity is calculated with the formula

$$activity = \frac{-\text{absorbance change} * analysis\ volume}{molar\ attenuation\ factor\ of\ H2O2 * light\ path * sample\ volume * sample\ protein\ concentration}$$

where molar attenuation factor of H_2O_2 is 0.04 $mM^{-1}\,cm^{-1}$.

***Sample size*:** Measurements are made from at least 15-20 individual specimens from each study site.

How to analyze and assess the data?

Compare the CAT activity levels measured from organisms collected from the target area to those from the reference area. An elevated or lowered activity level (bell-shape response) compared to the reference area indicate a negative effect. If the difference in mean activity level is more than one standard deviation (SD) of the mean values measured in the reference area, stress is considered moderate. If the level differs more than two SDs, stress is severe.

References

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Turja, R., Höher, N., Snoeijs, P., Baršienė, J., Butriavičienė, L., Kuznetsova, T., Kholodkevich, S.V., Devier, M.H., Budzinski, H., Lehtonen., K.K. 2014. A multibiomarker approach to the assessment of pollution impacts in two Baltic Sea coastal areas in Sweden using caged mussels (*Mytilus trossulus*). *Science of The Total Environment*. 473-474:398-409.

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