Integration of fast screening methods in the management of seafood production systems

Environmental and processing contaminants

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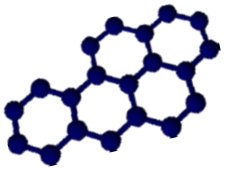
Target contaminants

Environmental contaminants are organic compounds created during industrial processes or synthetized to use in several devices and objects.

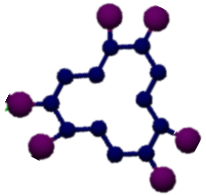
Groups of contaminants commonly used and found in the environment:

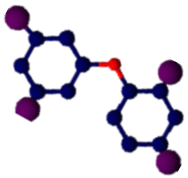
Polycyclic aromatic compounds (PAHs)



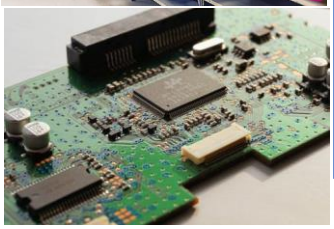
Generated during the combustion of organic material.

Brominated flame retardants (BFRs)

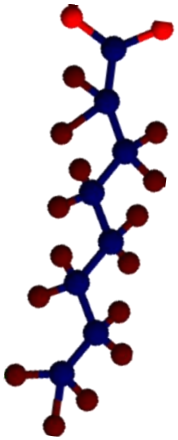




Used as flame retardants in different objects: electronical devices, textiles, plastics…



Polycyclic aromatic compounds (PFCs)



Used in coatings to produce water resistant objects and also as part of fire-fighting floams.

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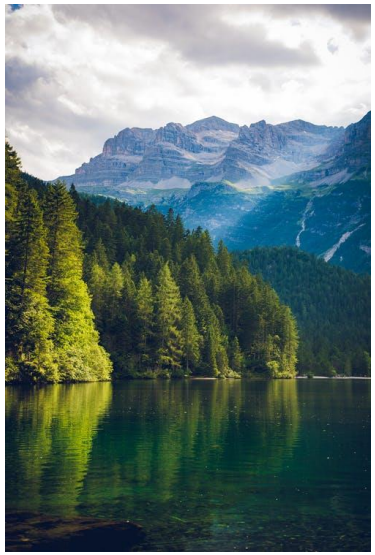
Contaminants in fish

From industrial and other human daily activities contaminants are released to the environment. During the trophic chain they can be accumulated, consequently, critical concentrations have been found in plants and animals which are for human consumption.

HUMAN ACTIVITY

Contaminants



Processing

Contaminants

Through atmospheric deposition, rivers and direct waste, seas accumulate huge amount of contaminants. As a result, they are bioaccumulated, endangering seafood safety. For this reason, authorities have established control methods and legal or recommended concentration limits in fish and seafood products.

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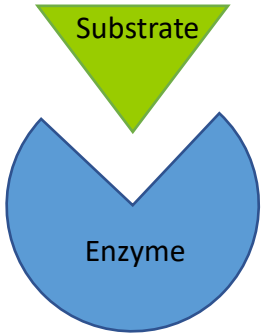
Why an enzymatic biosensor

Biosensors:

- Time saving
- Cost effectiveness
- Unskilled labour
- Portable

Enzymatic biosensor:

- Selectivity
- Multi-target
- Sensitivity
- Rapid



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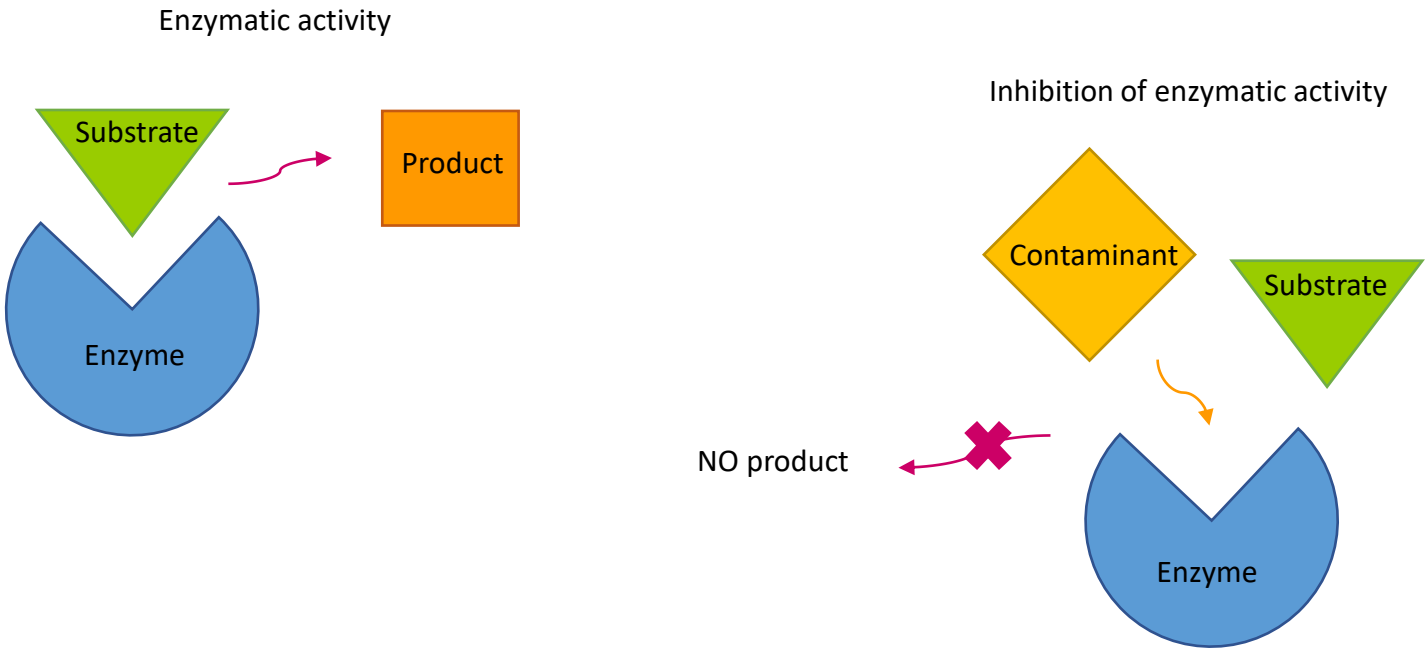
Why an enzymatic biosensor

Enzymatic activity can be affected by contaminants. The most common effect is the inhibition of it, obstructing the production of the product. So, the measurement of alteration in product concentration can alert about the presence of contaminants.



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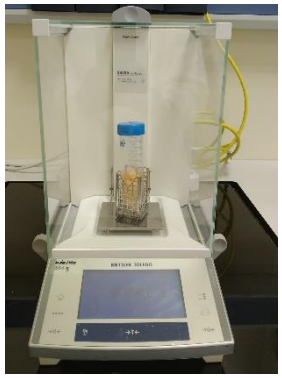
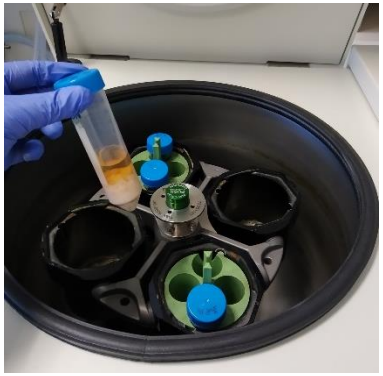
Sample preparation

1 Weight homogenized fish fillets



2 Extraction organic solvent+ QuEChERS/basic medium + 2 min vortex

3 Centrifugate and take the supernatant



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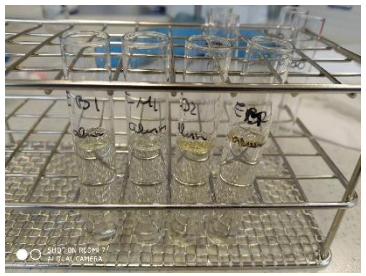
Sample preparation

4 Cleaning steps: freeze-out (PAHs and BFRs) + C18 cartridge (all) + SAX (PFCs) or aluminium oxide with PSA (PAHs and BFRs).

5 Evaporate the organic solvent when it is necessary



6 Reconstitute in working buffer solution.

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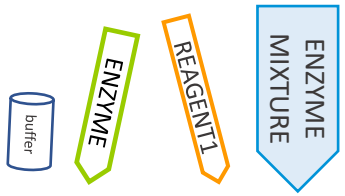
Assay protocol

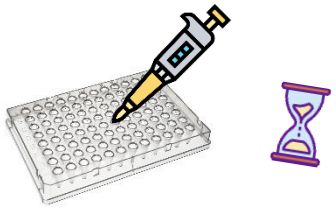


7 Prepare the enzyme mixture.

8 Add into well plate sample solution and enzyme mixture and incubate 10 minutes.

9 Incubate 10 minutes at 37˚C, the same time prepare the substrate mixture.

10 Add the substrate to wells, incubate 60 minutes at 37˚C and read the signal with a reader.



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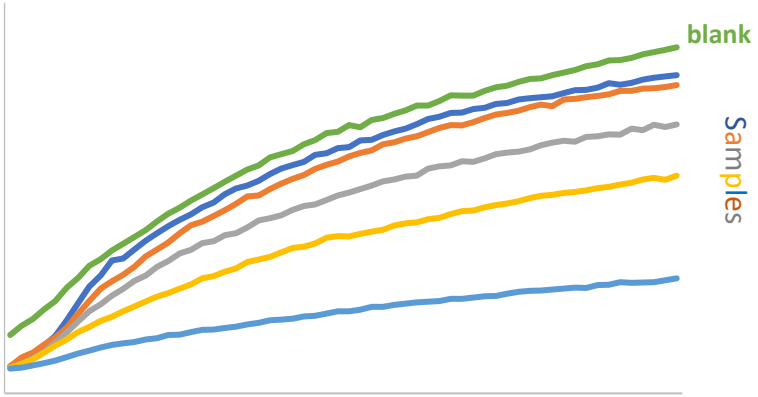
Analysis of results

Measured signal is proportional to product concentration. As we measure a blank (buffer solution) together with samples, the differences between signals can give the percentage of enzymatic inhibition.

$$Inhibition\ (\%) = \left(1 - \frac{S_{sample}}{S_{blank}}\right) x\ 100$$



A Cut-off value has been calculated for each contaminant and correlated with a concentration.

A positive sample (contaminant present) will provide and inhibition higher than the cut-off.

A negative sample (free of contaminants) will provide and inhibition lower than the cut-off.

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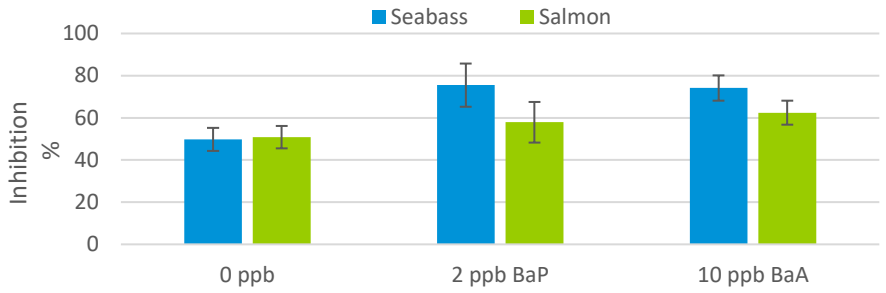
Results

PAHs

LOD: 2 ppb BaP
10 ppb ∑4PAH

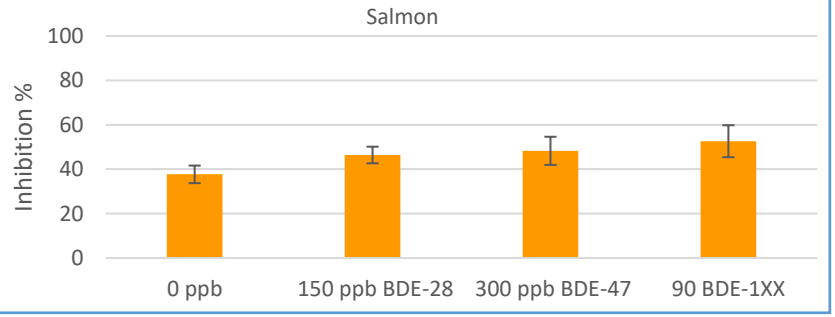
Cut-off value ≈ 50%



BDEs

LOD: 90 ppb BDE-100/BDE-153/BDE-154
150 ppb BDE-28/BDE-99
300 ppb BDE-47

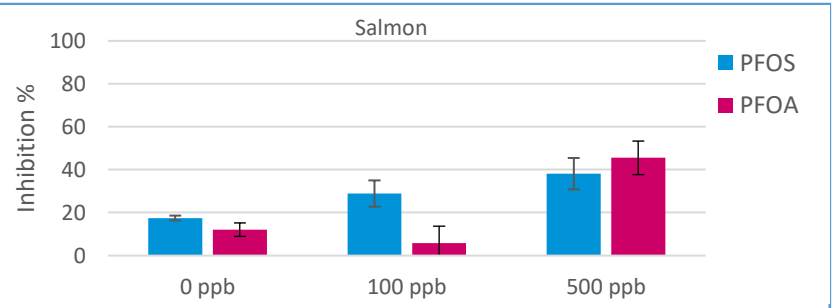
Cut-off value ≈ 40%



PFCs

LOD: 100 ppb PFOS
500 ppb PFOA

Cut-off value ≈ 15%



Conclusions

- Developed enzymatic bioassays allow the detection of hazardous contaminants present in the environment. The methods have been developed in salmon and seabass.
- This fast screening method can be used to avoid waste of time, reagents and trained labour during precise analysis. When a sample is a positive result, subsequent analysis by chromatography should be performed.

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Thank You

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