Perfluoroalkyl substances (PFASs) are man-made organic chemicals formed by a carbon backbone where all the hydrogen atoms are replaced with fluorine atoms \[1\]. Due to their excellent surfactant capabilities, stability and amphiphilic properties, PFASs have been widely used in industrial applications and consumer products such as firefighting foams, lubricants, polishes and water and oil repellents \[2\]. Due to their strong carbon-fluorine chemical bonds, these substances have high chemical and biological stability and have been shown to be highly persistent and bioaccumulative in the environment, especially those with carbon chain lengths greater than eight perfluorocarbons \[1\]. As these compounds are not degraded, they end up in soil and water bodies, possibly being ingested by fish, which leads to their accumulation throughout the food chains. Since fluoride compounds have been found in human serum and plasma, the impact of perfluoroalkyl substances on human health have raised serious concerns \[2\]. This led to widespread investigations concerning PFASs, that revealed these compounds are ubiquitous in the environment. As a result, in 2006, the major producers of PFASs joined the US EPA 2010/15 PFOA Stewardship Program to work towards the elimination of long-chain PFASs (i.e., \(\geq C_8\)) and their potential precursors by 2015. Since then, new alternatives have been introduced into the market. Some of the new alternatives to the long-chain PFASs are short chain homologues, such as perfluorobutanesulfonic acid (PFBS) \[3,4\]. Although these new fluorinated substitutes have been on the market for more than one decade, their environmental behavior have not been well studied or documented and information concerning their biodegradability, toxicological effects and bioaccumulation potential are still lacking \[4\].
A study from 2014 compared the byproducts generated in rat liver microsomes by the metabolism of two different types of Scotchgard fabric protector, one with long carbon chains and other with short carbon chains, both produced by the 3M company \[5\]. The major metabolite of the fabric protector with short carbon chains was perfluoro-1-butanesulfonamide (FBSA) \[5\]. As a metabolite of new fluorinated surfactant replacements, the environmental behavior of FBSA and its potential impacts are currently unknown \[2\]. Therefore, the aim of this study was to assess the presence of FBSA in fish samples from different geographical regions to find out whether FBSA is a bioaccumulative contaminant in aquatic biota and to better understand the sources and fate of this new alternative PFAS in the environment. In order to contextualize the results obtained, the long-chain PFASs perfluorooctanesulfonate (PFOS) and its precursor perfluorooctane sulfonamide (FOSA), were also monitored. The identification of these compounds in homogenate samples of fish was performed by liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QToF-MS) and the quantification was accomplished by ultra-high performance liquid chromatography-triple quadrupole mass spectrometry (UHPLC-QQQ-MS/MS).

In the samples collected from different water bodies across Canada and the Great Lakes region, FBSA was detected in 97% of the cases with concentrations ranging from 0.04 to 5.35 ng/g. Additionally, in all lake trout samples, FBSA concentrations were greater than the ultimate PFOS precursor (FOSA) a C8 compound, revealing the bioaccumulative properties of the new alternatives. These results were consistent with those obtained for the flounder from the Western Scheldt, where the FBSA concentration of 80.12 ng/g was also greater than FOSA at 17.52 ng/g. Another important fact is that, in lake trout from remote areas (i.e., Great Bear Lake, Kusawa Lake and Lake Athabasca), the concentrations of FBSA were much lower than those in fish from sampling locations in the more urbanized and industrialized locations. FBSA mean concentration in trout perch samples from Lake Erie was 0.38 ng/g, much lower than those measured in lake trout from the same region. A plausible explanation for this fact is that trout and perch consume primarily insect larvae and some smaller fish and are regarded as forage for lake Trout \[2\]. This lower concentration in forage than predator is consistent with bioaccumulation and biomagnification potential of PFASs in aquatic food webs \[2\]. Thus it is possible to conclude that FBSA is a bioaccumulative contaminant in fish in Canada and possibly in the Netherlands, as results for this last region were not representative enough.

Summarizing, results show that the already known bioaccumulative compound PFOS as well as the new alternative FBSA were present in significant concentrations in fish tissues. Therefore, considering the high volume production, current extensive usage and subsequent disposal of these PFASs alternatives, FBSA-based copolymers can be released into the environment and result in biotic food web accumulation \[2\]. Although it was confirmed the bioaccumulation potential of FBSA in fish, it is presently not clear whether FBSA-based copolymer alternative compounds are first degraded to FBSA in the environment followed by FBSA accumulation in fish or whether the FBSA-based copolymer is first accumulated in fish and then metabolized to FBSA \[2\]. Taking this into consideration and also the fact that little is known about alternative PFASs, it is crucial to give more importance to the study of the sources and fate of these new environmental contaminants, as well as to proceed to a more detailed and extensive analysis of their environmental and biological impact.
Table 1. Arithmetic mean of FBSA, FOSA, PFOS, and Sum (Σ) PFASs (22 compounds) in fish samples from water bodies across Canada and the Great Lakes Region (Canada and USA) and The Netherlands.

<table>
<thead>
<tr>
<th>Location</th>
<th>Species (no)</th>
<th>FBSA ag/g</th>
<th>FOSA ag/g</th>
<th>PFOS ag/g</th>
<th>Σ PFAS ag/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kasumiga Lake</td>
<td>Trout (3)</td>
<td>0.04</td>
<td>-</td>
<td>4.72</td>
<td>4.88</td>
</tr>
<tr>
<td>Great Bear Lake</td>
<td>Trout (3)</td>
<td>0.29</td>
<td>-</td>
<td>2.43</td>
<td>9.60</td>
</tr>
<tr>
<td>Lake Athabasca</td>
<td>Trout (3)</td>
<td>0.14</td>
<td>-</td>
<td>4.79</td>
<td>6.20</td>
</tr>
<tr>
<td>Lake Huron</td>
<td>Trout (3)</td>
<td>2.29</td>
<td>1.49</td>
<td>64.11</td>
<td>117.37</td>
</tr>
<tr>
<td>Lake Huron</td>
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<td>0.21</td>
<td>41.87</td>
<td>64.27</td>
</tr>
<tr>
<td>Lake Ontario</td>
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<td>3.78</td>
<td>0.74</td>
<td>50.08</td>
<td>60.66</td>
</tr>
<tr>
<td>Lake Ontario</td>
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<td>2.89</td>
<td>0.32</td>
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<td>60.90</td>
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<td>0.57</td>
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<td>0.16</td>
<td>111.64</td>
<td>136.54</td>
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<td>The Western Scheldt</td>
<td>Trout (3)</td>
<td>0.58</td>
<td>0.83</td>
<td>10.22</td>
<td>16.65</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>Flounder (1)</td>
<td>80.12</td>
<td>17.52</td>
<td>102.41</td>
<td>321.93</td>
</tr>
</tbody>
</table>

References

Acute and chronic sensitivity, avoidance behavior and sensitive life stages of bullfrog tadpoles exposed to the biopesticide abamectin


Analyzed by: Jean-Baptiste Bove, Joana Vines, Julia Preishuber-Pflügl, Vanessa Freitas

Fig 1 - How does Abamectin affects amphibians (left); how do they react in its presence (up); schematic of apparatus for avoidance testing (right); possible solution to eliminate this toxin (down).
ENVIRONMENTAL PROBLEM

Facing the actual concerns with the destruction of the aquatic ecosystems, considering that the regulation for production of pollutants only contemplates fish harm, it is necessary to understand if amphibians are being put at risk with this consideration.

*Lithobates catesbeianus*, bullfrog, was used as model to perform some chronic and acute toxicity tests. Its ample occurrence and easy culture and maintenance in the lab; its economic and food chain importance; and even the fact that it is usually highly sensitive to a wide range of chemicals at environmental significant concentrations, were some of the facts that led to its use. Since the experiments were performed in two different stages of tadpole's development, at 21G and 25G, the fact that this specific amphibian has a relatively long larval stage was also an important factor to take in consideration in its selection as testing organism.

The pollutant used was Abamectin which is a natural product of *Streptomyces avermitilis*. It is used as an insecticide in mass agricultural productions and as an anthelmintic in vaccines for veterinarian and medicinal purposes, when parasites need to be controlled. This drug is not soluble in water but bounds to soil particles and its half-life can increase from 4 days to 4 weeks in this case [1].

ACUTE LETHAL TOXICITY

For testing acute effects of Abamectin to bullfrog tadpoles, an acute lethal toxicity test was performed. Gosner stage 25G and 21G tadpoles were used; 25G is the most commonly used stage in ecotoxicological tests. 21G tadpoles were used to determine if the life stage influences the sensitivity of tadpoles to chemical substances. The tadpoles were exposed to different concentrations of Abamectin for 96 hours.

The results showed an increased mortality of the tadpoles at the three highest test concentrations (>144 µg a.i. /L Vertimec® 18EC). Also low activity and a loss of equilibrium posture could be seen in all the tested animals what can seriously affect their survival in real life settings. The 21G tadpoles showed a significantly higher sensibility to Abamectin, compared to older tadpoles.

AVOIDANCE

Since avoidance is considered as a sensitive and immediate response of amphibians to chemical stress, this parameter was also tested. The test was conducted by using the methodology developed by Araújo et al. (2014) as shown in figure 1. Initially, a calibration of the avoidance system was performed with NaCl for monitoring the tadpole distribution. For the final test, a dilution gradient of Abamectin was established. After 12 hours the distribution was recorded and the level of avoidance determined by calculating the difference between the expected and observed number of organisms in the compartments.

The results could show that Abamectin (>24 µg a.i. /L Vertimec® 18EC) induces an avoidance behaviour of tadpoles.

CHRONIC TOXICITY

The objective of these tests were to observe the presence (or absence) of any prior to death abnormal behavior due to prolonged exposure to Abamectin. Gosner stage 25G tadpoles were used for the same reason highlighted in avoidance test paragraph, the period of exposure was 24 days.

The results revealed a higher mortality as well as a loss of equilibrium posture in most of the surviving organisms. These organism regained normal equilibrium the last days, which indicates the presence of an adaptation mechanism.

SPECIES SENSITIVITY DISTRIBUTIONS

These distributions represent a comparison between lethal concentration (LC) values of the tadpoles and those of other species. The objective is to determine if pre-existing toxicity data for fish is protective for the amphibian species.

The results show that LC values of 25G tadpoles are well above those of other species. This confirms that fish toxicity data is adequate and supports the sole use of this
data for the acute environmental risk assessments of aquatic vertebrates, including \textit{Lithobates catesbeianus} and other amphibians.

**Possible Solutions**
Since conventional water treatment doesn’t remove pesticides, such as Abamectin, efficiently, there’s a need to develop alternative procedures. It’s not even possible to use biological techniques in order to degrade the pesticides, since the effluents are toxic for the microorganisms involved in those procedures. The use of Advanced Oxidation Processes (AOPs) has achieved good results in the removal of toxic organic compounds in aqueous solutions, so the future regarding the treatment of effluents contaminated with biocides like Abamectin might involve this alternative\cite{3}. In AOPs, hydroxyl radicals are formed and these ionized molecules can mineralize organic compounds to \(\text{H}_2\text{O}, \text{CO}_2\) and mineral acids. There are several types of AOPs, but the Photo-Fenton Process has attracted a lot of attention due to its high efficiency in the production of hydroxyl radicals, during \(\text{H}_2\text{O}_2\) decomposition. This reaction is catalyzed by \(\text{Fe}^{2+}\) in acid solution, under UV irradiation. This process has a lot of advantages such as the use of low cost reagents and solar irradiation as the UV source. By the end of the process, it’s possible to precipitate the iron ions, increasing the pH.

In order to increase the efficiency of the Photo Fenton process, it’s extremely important to control some parameters such as pH and reagents concentration. The optimal pH range for the procedure is 2.5-3, and the use of an excess amount of \(\text{H}_2\text{O}_2\) can reduce the efficiency, since the hydrogen peroxide can generate another radical with lower reducing potential than the hydroxyl.

The application of AOPs in the field it’s not yet established, but certainly the future regarding the removal of harmful pesticides for the environment and the solution for this problem can be achieved by developing and optimizing these procedures\cite{3}.

**References**
\begin{itemize}
\end{itemize}

**Bioaccumulation and biomagnification of classical flame retardants, related halogenated natural compounds and alternative flame retardants in three delphinids from southern european waters**

From Barón E. \textit{et al.}, Environmental Pollution 203 (2015) 107-15

\textbf{Analyzed by: Inês Dias and Karolina Posłowska}

![Fig. 1 - Overview of the procedure adapted of Báron et al.](image)
Flame Retardants (FRs) are a denomination of chemical compounds that, since 1970 \[1\], are being applied to wide range of industrial materials, from textiles to electronics, to prevent fires. Halogenates organic chemicals increase the flame ignition resistance \[2\] and brominated and chlorinated chemicals are the least expensive way to meet flammability requirements \[3\]. FRs are considered anthropogenic contaminants, with many being classified as POP (Persistent Organic Pollutant), PBT (Persistent Bioaccumulative and Toxic) or both. PBT are lipophilic and can cause, among others, immune and reproductive dysfunction (endocrine is a main focus of disruption). Odontocetes, notably, accumulate high concentrations of these kind of contaminants. This was used by Barón et al. to study the bioaccumulation and biomagnification of several FRs and related halogenated natural products (HNPs) – common dolphins, bottlenose dolphins and pilot whales were sampled in the Gulf of Cádiz and the Strait of Gibraltar to compare, in both locations, contaminant levels and stable isotopes (δ\(^{15}\)N) – some tissues of wild animals retain "isotope record" that reflects the average diet in the period when these tissues were formed. Isotopic analysis is therefore taken as an indicator of trophic level (increasing from prey to predator) and the correlation between the concentrations of the contaminant in the sample (lipid-normalized) with the δ\(^{15}\)N is used to evaluate the biomagnification capacity of the contaminant.

FRs are divided in two groups: classical FRs – where the Halogenated Flame Retardants, a popular choice, is included – and emerging FRs (EFRs), which were developed as an alternative in face of the unintended consequences. The EFRs can be themselves separated in Brominated EFRs and Halogenated Norbornenes (HNs), that includes the chlorinated FRs.

Polybromodiphenyl ethers (PBDEs) stand in as the classical FRs, the alternative FRs are represented by α-Hexabromocyclododecane (HBCD), Dechlorane 602 (Dec 602), Dechlorane 603 (Dec 603) and Dechlorane Plus (DP) syn- and anti-isomers. The HNPs detected (2′-MeO-BDE-68 and 6-MeO-BDE-47) are tetra-brominated MeO-PBDEs usually found in marine mammals.

The results of the δ\(^{15}\)N analysis show that bottlenose dolphins had the highest values in both areas, followed by pilot whales and common dolphins. Since the values were statistically different between species of the same area, their trophic position was considered useful to carry out biomagnification studies, with bottlenose dolphins at the top, followed by pilot wales, and common dolphins.

Moving to the examination of contaminants levels, bottlenose dolphins are the most contaminated specie, the second being pilot whales and then common dolphins. There is variation in contaminants levels not only among species, but within the same species too – it was expected seeing no information was available on the age, sex or body condition of the individuals. Comparisons, thus, need to be made with caution.

Due to the great stability and persistence of the classical FRs, it’s reasonable to assume that their levels in the environment, especially in top predators like dolphins, will not decrease immediately after their substitution for alternative FRs that, on other hand, will probably increase in the near future.

Comparing the sum of compounds ratios, PBDEs had a higher concentration than HBCDs and HNs, in that order. Regarding biomagnification, PBDEs has a significant positive correlation with trophic level in both common and bottlenose dolphins in the same way MeO-PBDEs. Dec 602 and Dec 603 seem to have higher bioaccumulation and biomagnification capacities than DP.

A similar situation was observed upon testing the levels of halogenated natural products (MHC-1, TriBHD, TetraBHD,MeO-PBDEs, Q1, and related PMBPs) and halogenated flame retardants (PBDEs, HBB, Dec 602, Dec 603, and DP) in blubber and brain from five Alboran Sea delphinids (Spain) \[5\]. Both HNPs and HFRs were detected in the brain, implying that they are able to surpass the blood-brain barrier and reach the brain, which represents a new finding for some compounds such as Q1 and PMBPs. Moreover, some compounds (TetraBHD, BDE-153, or HBB) presented higher levels in brain than in blubber \[5\]. This is evidence of high concentrations of HNPs in the marine environment, especially in top predators. It shows the im-
portance of further monitoring these natural compounds and evaluating their potential toxicity, when most studies focus on anthropogenic compounds only. While no bioaccumulation was found for HNPs, HFRs increased significantly with body size for both common and striped dolphins.

In conclusion, it is possible to see that the bottlenose dolphins had the highest levels for all types of anthropogenic and naturally-produced contaminants while common dolphins and long-finned pilot whales had similar lower values. The results show that the levels of the classical FRs (PBDEs and HBCD) are higher than levels of alternative FRs (HNs) and that occur correlational evidence of the biomagnification capacity of several compounds (PBDEs, MeO-BDEs and some HNs).

Dolphins are highly exposed to organic contaminants since they accumulate them during many years, toxicological studies of the effects of these compounds in cetaceans should be conducted. In addition, monitoring of flame retardants and halogenated natural product concentrations in cetacean should be implemented. Both aspects are essential to assess their risk for dolphins (combination of potential danger and real exposure).

It’s also important to limit bioavailability of negative compounds in marine food webs and actions should be made to reduce the discharges into the marine environment.

References

Effects of toxic leachate from commercial plastics on larval survival and settlement of the barnacle Amphibalanus amphitrite

From Heng-Xiang L et al., Environmental Science and Technology, 50:924-931, 2016

Analyzed by: Ana Rita Lourenço, Carina Galhofa and Jéssica Correia
Plastics are the material of the 21st century. They provide a key enabler of innovation in the most various sectors of the economy, like healthcare, energy generation, construction, electronics, packaging and textile. The plastic industry is thus a billion euro industry that keeps growing. In fact, global production of plastics was an estimated 299 million tons in 2013, a 3.9% increase from 2012 (PlasticsEurope, 2015). Indeed it seems that the future relies on plastics. But this is not without consequences.

Plastic pollution represents a major global problem. Recent estimates predict that more than 5 trillion plastic pieces weighing over 250 000 tons are currently afloat at sea (Eriksen et al., 2014). And so, studying the effects of plastics in marine environments becomes relevant.

The present study suggests that biofouling of plastics (accumulation of living organisms on wetted plastic surfaces) may play a key role in determining the environmental fate and effects of plastic debris. As such, the authors focused on the impact of the seven most common categories of recyclable plastics (polyethylene terephthalate, PET; high-density polyethylene, HDPE; polyvinyl chloride, PVC; low-density polyethylene, LDPE; polypropylene, PP; polystyrene, PS; polycarbonate, PC) on survival and settlement of the barnacle *Amphibalanus amphitrite*. This barnacle is a frequent fouling organism and can grow on a wide range of hard surfaces. Its life cycle is divided in three main stages: the nauplii is released from fertilized eggs and corresponds to the feeding form; the cypris doesn’t feed and is responsible for finding a suitable surface for settlement; and the barnacle is a filter feeder attached to the chosen surface (Fofonoff et al., 2003).

To test the impact of plastics in the survival and settlement of the studied barnacle, the authors used the nauplii and cypris stages respectively. These tests were performed with plastic leachates, which were prepared from the contact of plastic pieces with filtered aged seawater (FASW), allowing the plastic to leach.

The toxicity of the plastic leachates on the nauplii larvae was assessed as the larval mortality under incubation with each type and concentration of plastic; this was quantified by the proportion of immotile larvae after 24 hours. At all concentrations tested, the larval mortality was below 30%, although it was variable between concentrations and materials. Since plastics are composed by a complex chemical mixture that releases specific chemicals when leached, it is only natural that the variation in mortality observed was material-dependent. Moreover, PVC was shown to produce the most toxic biological response in nauplii at the highest concentration (0.5 m² plastic/L solution), being associated with some of the compounds found in its composition that have biocidal properties – vynil chloride monomers, lead, cadmium, dibutyltin dilaurate, phthalates. Another plastic that produced significant results is the LDPE, which was the second most toxic at the highest concentration level, perhaps due to the release of phtalate esters. These chemical compounds are known to be endocrine disrupting, interfering with hormone biosynthesis, metabolism and action; furthermore, phthalates are non-covalently associated with the polymer matrix, making it very easy to be leached into the media.

For the settlement tests, two approaches were used, in both of which settlement inhibition was determined as the percent of larvae that had not attached to the test surface and metamorphosed into young barnacles. The first approach tested the settlement of cyprid larvae in glass vials with leachates from each category of plastics. Here, settlement was significantly inhibited by leachates from all categories of plastics at all tested concentrations, having LDPE, PET, and HDPE consistently exhibited the greatest percent inhibition. Interestingly, no material-specific relationship between leachate settlement inhibition of barnacle cyprids and mortality of barnacle nauplii could be established, suggesting that the underlying mechanisms exhibiting adverse outcomes in this model are life-stage dependent. The second approach tested the settlement of cyprid larvae directly onto the plastics. The results show that cyprid settlement was significantly depressed after 24 hours, and after 96 hours, four plastics exhibited significant inhibition of settlement in the order: HDPE > PC > LDPE > PET.
In 2013, however, Li et al. had shown that specific secondary metabolites (furanon and furan) isolated from soft corals, gorgonians, brown algae, and fungi collected along the coast of China could act as antifouling agents, inhibiting the settlement of barnacles. This article shows that other factors have to be considered when the settlement inhibition of barnacle is studied in the presence of plastic leaching (Li et al., 2013); something that the authors of the present study have not considered.

Additionally, the authors studied some physicochemical properties of the plastics, namely the hydrophobicity and chromatographic features, in order to understand the underlying features of inhibition of settlement. Assessment of the surface energy values of each plastic, or hydrophobicity, showed a positive correlation with the previous results, suggesting that the most hydrophobic surfaces are the most toxic. However, these tests were only performed in short term; therefore, long term studies need to be conducted in order to see if this correlation still occurs.

Settlement tests with these plastic leachates were also done in glass vials and inhibition of settlement also occurred in all tested concentrations which may be related with chemicals that are not acutely toxic but strongly biologically active.

Chromatographic analysis (high-performance liquid chromatography–high-resolution accurate-mass mass spectrometry; HPLC-HR/AM-MS) of the plastic leachates revealed the presence of unique chromatographic peaks between each plastic type; for example, in the case of PVC, 61 of the 158 chromatographic peaks were only observed in that plastic type. This variation may explain the variable rank order in toxicity and settlement inhibition and the resultant toxic effects likely represents the sum of the chemical stressors that are exclusively present in the plastic.

The authors also tried to identify the chemicals given by the chromatographic peaks by using a database with data of more than 3000 compounds relevant to toxicology and environmental sciences. Only one compound was identified, DEET (N,N-diethyl-meta-toluamide), suggesting that the compounds present in the leachates are not commonly monitored toxic chemicals.

Several other works that had been performed in other species, such as *Daphnia Magna* and *Nitocra spinipes* (Bejgarn et al. 2015; Lithner et al. 2012), corroborate the results obtained in this study, stressing the idea of the variability and material-dependence of the toxicity of plastic leachates, as well as the significant role of PVC on the introduction of toxicity in aquatic organisms.

As such, this work compliments other studies about the toxicity of plastics and their associated leachates to aquatic environments, where barnacle nauplii larvae can be constrained with plastic debris in shallow and stagnated pools. The results also showed that plastic debris in near surface environments may resist settlement by barnacle cyprids which has implications for the fate of plastics in the near shore marine environments. The challenges with assessing the potential effects associated with exposure of marine fauna to plastic debris are also highlighted and scarcity of data regarding the chemical composition and toxic effects of these leachates to ecologically model organisms limits comprehensive risk assessment of the environmental impacts of plastic debris in aquatic environments.

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New Insights on the Influence of Organic Co-Contaminants on the Aquatic Toxicology of Carbon Nanomaterials


Analyzed by: André Rodrigues, Carolina Gonçalves, Cátia Pereira

Carbon nanomaterials (CNM) are carbon nanostructures, characterized by their large surface-to-volume ratio, aggregation properties in suspension, and diffusion capacity. In light of these properties, CNM play a big role in nanoscience, due to their applications in electronics, analytical chemistry, as potential drug carriers or even in the formulation of new materials.

Given their high production, both from anthropogenic and natural sources, CNM are being considered as emerging pollutants, presenting a risk for the environment and living organisms. However, the fate, behavior, and potential risks of CNM in the environment is still poorly understood, reason for which the study of their chemical properties, and interaction mechanisms with other contaminants, is of great importance.

The present study targets the mainly used CNM: fullerenes, multiwall carbon nanotubes (MWCNT) and graphene nanoflakes, focusing on their toxicity in aquatic ecosystems. To do so, bioassays of the immobilization of the invertebrate *Daphnia magna* and the bioluminescence inhibition of the marine bacteria *Vibrio fischeri* were performed. In order to infer on the effects of aging in CNM’s toxicity,
the particles were aged under realistic conditions, on an estuary simulated microcosm. The estuary scenario was chosen because of its pollution rates, resuspension phenomena, high evaporation rates and intermediate salinity.

Firstly, it was conducted the characterization of aqueous suspensions by Nanoparticles Tracking Analysis (NTA), which confirmed a disperse size distribution of fullerene aggregates. Three maximum peaks were identified between 63 and 175 nm. With regards to MWCNT, a wide distribution centered at 170 nm was found. Regarding the graphene suspension, the size distribution profile was a symmetrical function centered at 300 nm.

Afterwards, some toxicity measurements were performed. Relatively to the toxicity of CNM suspended in artificial estuary water, fullerene soot presented moderate toxicity to V. fischeri while MWCNT and graphene were not toxic for this organism. On the other hand, D. magna was more sensitive to CNM with toxicity as follows: fullerene soot > MWCNT > graphene. This can be related to the aggregates size distribution in the suspensions. Those with the highest proportion of small aggregates are expected to be the most toxic, although other parameters such as their morphology can also influence their final toxicity. In this case, the influence on the final toxicity of size was predominant over the shape.

Due to CNM’s sorptive capacity, with a possible role in partition, transport and toxicity of other contaminants, binary toxicity assays were also performed, using fullerene and five organic co-contaminants: nonylphenol, an alkylphenol used in the manufacturing of detergents and plastics; triclosan, an antibacterial and antifungal agent; malathion, a pesticide used in agriculture; diuron, and glyphosate were studied. Antagonism effect was the most common effect, as it had been observed in V. fischeri. However, a different behavior was observed for malathion-fullerene soot mixtures. In this case, synergism was observed. This behavior was not observed in V. fischeri experiments and can be related to daphnid filtration: daphnids actively accumulated fullerene aggregates doped with malathion molecules. In those binary mixtures where fullerene soot concentrations were high enough, malathion was efficiently immobilized, whereas in those binary mixtures where fullerene aggregates surface was saturated, loosely attached malathion molecules were released inside the microcrustacean organisms. Therefore, it can be concluded that the affinity of malathion to fullerene soot was moderate due to the hydrophilicity of malathion, which leads to weaker immobilization by the CNM.

Studies focused on CNM toxicity in D. magna, under conditions that did not promote aging, allowed to infer on the influence of this factor in toxicity. In the case of fullerene, the aging of the material lead to an increase in toxicity \[^{[1]}\], while for MWCNT and graphene, the toxicity was decreased \[^{[2,3]}\].

The dynamic between CNM and aquatic contaminants was also highlighted in other papers, demonstrating the influence of fullerene in the toxicity of phentachlorophenol (decreasing it) and phenantherene (increas-
ing it) [4]. The influence of MWCNT also lead to increased toxicity in the case of diuron [5] and phenantherene [6].

In summary, the findings on CNM show how their toxicity can be modulated by environmental conditions over time. Important relationships of antagonism and synergism were also demonstrated between fullerene and organic co-contaminants, highlighting the effects of CNM sorptive properties in the contaminants toxicity. These findings emphasize the importance of studying CNM behavior upon release to the environment, since they can lead to various deleterious effects.

References

Fin whales and microplastics:
The Mediterranean Sea and the Sea of Cortez scenarios

From Fossi M. C. et al., Environmental Pollution, 209:68-78, 2016
Analysed by: Kamila Sumara, Pedro Silva, Vera Esgueira

In the course of the last decades, plastics have assumed a great role in the modern world and revolutionized our daily lives. An excess of 260 million tonnes of plastic are used per year (8 per cent is for world oil production). In the meantime the concern about the environmental consequences resulting from the accumulation of waste plastic has increased. Scientific studies have been recently performed regarding the effects of plastic debris on wildlife and concerns for human health [1]. At global scale 80% of litter present in oceans consists in plastics and is globally distributed across all oceans due its properties and durability [2]. The impact of plastic pollution through ingestion and entanglement of marine fauna is well documented, however microplastics (fragments
smaller than 5mm) and the impact that those have on baleen whales still remains largely unexplored. It’s known that ingestion of microplastics occurs directly during feeding activities and indirectly via consumption of contaminated zooplankton. Fin whales are the second-largest filter feeder inhabiting in two semi-enclosed marine basins. These organisms feed near the surface, engulfing an average of 71 m$^3$ of water per mouthful, being consequently exposed to a high risk of microplastic ingestion. Fin whales are listed as “endangered” worldwide and “vulnerable” in the Mediterranean Sea on the IUCN red list of threatened species.

In this study the interaction between fin whales and microplastics was examined by comparing the populations living in two semi-enclosed basins, the Mediterranean Sea, that has become a dumping ground for the anthropogenic waste, and Sea of Cortez, considered one of the most pristine areas in the world. Both zooplankton and microplastic samples were collected from the Gulf of Asinara and the Sardinian Sea (in Mediterranean Sea) over the course of three expeditions in the summer of 2011, 2012 and 2013. The data was subdivided into three sampling periods: July, August and September. Fin whale skin biopsies were also collected in these three periods in the Ligurian and Sardinian Sea and geographical position of each sampling location was registered. The currents that concentrate plankton may also act to concentrate microplastics debris in the same convergence zones during the summer season which, as previously mentioned, constitutes a potential risk for large filter feeders preying on plankton, as they ingest microplastics along with their prey.

In order to study the whale’s toxicological bioreponse in the different habitats, the authors analysed different biomarkers: cytochrome P450 1A (CYP1A1), cytochrome P450 2B (CYP2B) and lipid peroxidation (LPO), several plastic additives were quantified such as phthalates Di-(2-ethylhexyl) (DEHP) and mono-(2-ethylhexyl) (MEHP). Some organochlorine compounds (OCs) were also monitored such as Hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyl (PCB) congeners. The research also included statistical analysis of biomarkers and contaminants levels. This examination was useful to define clusters on the basis of area and time variables. The results of these studies have proved that microplastics are far less abundant in the Sea of Cortez than in the Mediterranean. Moreover, microplastic density differed between the Ligurian and Sardinian Sea. There was no statistical difference between genders in terms of the parameters, LPO and phthalates in the Mediterranean fin whale population, but the parameters of organochlorine compounds were significantly different between whales from the Ligurian and Sardinian seas.

It is important to know if whales feed in areas containing a high density of microplastics. This study demonstrated that there is a clear overlap between areas with high levels of microplastic pollution and the fin whales summer feeding grounds in the Mediterranean Sea, an indication that fin whales are subjected to a high level of exposure to microplastic ingestion during feeding in the areas. Temporal and regional ecotoxicological differences in fin whales were identified, suggesting that the Mediterranean Sea, particularly the Pelagos Sanctuary, is exposed to the risk of microplastics in comparison to other basins. Mediterranean fin whale population displayed LPO values twice those of the Sea of Cortez. Mediterranean whales might be exposed to a higher toxicological pressure (namely oxidative stress) due to the presence of plasticisers.

Gene analysis revealed that there was a significant divergence between the tested populations. Nevertheless the authors proved neither genetic background nor sampling period affect the fin whales toxicological responses in the Pelagos Sanctuary. The three sampling periods studied differed significantly. Contaminant levels varied with different patterns, the highest values of OCs and biomarkers occurred in September, towards the end of the summer foraging season so it’s possible to infer that exposure to microplastic contamination increases over the span of permanence in the summer feeding grounds.

The laborious and remarkable work deposited in this study was unable to answer an important question: to what extent PBT (Per-
sistent, Bioaccumulative, and Toxic) chemicals are absorbed from direct microplastic ingestion in comparison to the amount ingested indirectly from feeding on contaminated prey? However, the obtained results represent a warning for the vulnerable Mediterranean fin whale population. Although this species may use adjacent areas for feeding purposes, the continuous decline in the fin whale population in the Pelagos Sanctuary raises concerns about the status of this species in the Mediterranean Sea. Additionally there are more fish in the sea than just whales, so this is a transversal problem that comprises more organisms and not only fin whales. To exemplify this fact, similar studies have been performed in other species such as sharks [3]. Moreover, microplastics high density is not a problem exclusive in marine environments, recent studies assessed the impact of this debris on earthworms [4].

References:

Tracking the global distribution of persistent organic pollutants counting for e-waste exports to developing regions


Analysed by: Rafael Bustillo, Diana Hernandez, Sabina Kolšek, Jaka Žagar

BACKGROUND
A lot of chemicals may travel around the world through the different ways far away from the locations where they are produced. These kinds of molecules are persistent organic pollutants (POPs). Water and air are usually main mediums for transportation, but there are also other ways by which POPs could move around of globe. These ways are non-environmental and usually illicit (the main driving force is profit). Typical example is export of e-waste (discarded electrical and electronic equipment). Developed countries accumulate e-waste and then pay to devel-
oping countries near equator to receive it. E-wastes which come in developing countries have economic value (valuables parts are gathered and sold). After that the e-wastes are subject to informal recycling (shredding and burning). Consequently volatile polychlorinated biphenyls (PCBs) are released in an atmosphere. The authors of the article want to caution that informal recycling is not appropriate in poor communities in countries where wealth and population do not correlate well.

In this work we focused on PCBs. A lot of historical research examined the influence of PCBs on environment and because of that we have a lot of references. PCBs are species with two benzenes which are bonded and on the other free carbons are bonded chlorines. More chlorines substituents on PCBs mean more harmful and less biodegradable molecule. In the environment PCBs can stay for a very long time. They can easily cycle between air, soil and water. In the environment they can undergo bioaccumulation, bioconcentration and biomagnification. PCBs are very carcinogenic. Exposed organisms risk cancer, neurotoxicity effects, reproductive and development toxicity, immune system suspension.

OBJECTIVE
To develop a global scale of various emission scenarios of industrial-use organic contaminants (IUOCs) and PCBs, based in a study of the consequences of emission, long-range transport and disposal of this chemicals, attributed to a change in source and deposit regions of e-waste in subtropical and tropical areas.

METHOD
To understand the behaviour of IUOCs and PCBs it was necessary to use two possible situations. The first case focused on the study of global emission scenarios developed for representative IUOCs (RIUOCs) in a world with and without exports of e-waste and the emissions occur only via passive volatilization from disposal/recycling sites. In this part the main purposes are:
1. Simulate the distribution of RIUOCs in the environment.
2. Assess possible implications for transport and exposures attributed to e-waste exports in isolation.
3. Restrict the analysis to a set of transparent assumptions with a mechanistic basis.
4. Develop generic scenarios to compare and contrast findings for PCBs and beyond.

The second case explores the implications of e-waste exports for global emissions of selected PCBs, with the help of an emission inventory. Then, this study simultaneously account for historical emissions and long-range transport of selected PCBs as well as emissions during other stages of the chemical life-cycle. In this case, it is assumed that a fraction of the e-waste collected to a formal recycling of electrical equipment containing PCBs in OECD countries is diverted for export to developing regions that are non-OECD countries. In these areas, it is assumed that the e-waste is mainly subject to informal recycling. After dismantling, the e-waste can be disposed in landfills, dumped onto soils, subject to open burning.

Three emission scenarios were assumed:
- The “baseline” scenario (no export) to facilitate a comparison with the other two scenarios
- Default (exports), 5% of the disposed e-waste is subject to open burning
- High (exports) 20% of the disposed e-waste is subject to open burning

Finally, all simulations were carried out using the geographically explicit multimedia contaminant fate model BETR-Global 2.0

NOVELTIES
This study was probably the first attempt to numerically integrate long range transport (LRT) by both environmental and non-environmental ways to research global chemical fate of PCBs.

CORE FINDINGS
- The hypothetical scenario for emissions of PCBs with export was the best approximation for the historical values of reported concentrations of PCBs.
- Emissions of PCBs in historic source regions are predicted to have only a small
The general decline in PCB concentrations over the past few decades is expected to continue in these locations.

- The emission scenarios for PCBs highlights in developing regions: informal recycling and disposal may make emissions worst, if the e-waste becomes subject to open burning. Warmer temperatures and informal recycling in developing regions are probably going to lead to increased global emissions compared to the no-export scenario. Magnitude of these effects are uncertain because of the lack of reliable data.

The authors used an integrated approach that could help organizations (which are trying to prevent harmful effects on environmental and human health) to better understand non-environmental long-range transport (LRTs) of PCBs.

BRIEF OVERVIEW OF OTHER WORKS

There are lot of articles describing accumulation of PCBs in animal species and effects of PCBs on human beings, both living in e-waste dismantling areas in China.

- Scientists examined bioaccumulation of PCB in wild aquatic animals (Chinese mystery snail prawn, fish, water snake) living near e-waste recycling workshops in China. Elevated levels of PCBs was found in the exposed biota (20-2598 ng/g) compared to that in the reference sample and (75-83 ng/g) (1).

- PCBs levels were measured in 40 citizens of an e-waste dismantling area in China and in 15 citizens living in control areas. PCBs levels were much higher in exposed organisms (964 ng/g) than in control group (68 ng/g) (2).

ENVIRONMENTAL RELEVANCE

Authors try to explain the relation between increased concentration of PCBs and e-waste recycling in developing countries. The purpose of that article is to caution the production of e-wastes causes negative impacts because the electronic equipment is not disposed correctly after its lifetime use. E-waste consists of metal, plastic and glass parts, thus it should receive different treatment in the recycling process. Majority of e-waste goes to landfills, because people don’t know the proper way to treat it. Besides that, companies generating electronics are poorly involved with the disposal and treatment of their own products.

E-waste is not only a threat for environment contaminating water and soil, but it also represents a danger for people health and the other living organisms. It contains also As, Hg, Pb, Cd and other elements, which cause serious damage in the short or/and long term.

CONCLUSION

Further efforts to reduce emissions of PCBs from informal e-waste recycling in developing areas will be necessary to minimize impacts on environment and human health and have positive impact not only in a remote areas, but also on a global scale. First little step could be informing people and raising awareness to minimize the consumerism and subsequently decrease the amount of e-waste. Also in order to have more information about the PCBs, more researches should be devoted to that problem.

References:
(3) Regulations.gov, Polychlorinated Biphenyls (PCBs); Reassessment of Use Authorizations: https://www.regulations.gov/#/home
Triclocarban Influences Antibiotic Resistance and Alters Anaerobic Digester Microbial Community Structure


Analyzed by: Miguel Nascimento, Nuno Marques and Nuno Pedro

Nowadays, the use of many compounds to fight bacteria growth plays an important role in our society. These compounds are called antibacterial and they are used in many fields, like cosmetic and household products.

These antibacterial products, like Triclocarban (TCC) prevent products from bacterial growing and spoiling. In particularly these compounds interact with the synthesis of cell membrane, by inhibiting ACP reductase, which is an enzyme that participates in the elongation of fatty acids \(^1\). So, in that way, why study the effects of TCC?

It was described that 100% of the biosolids analyzed from WWTP’s (wastewater treatment plants) contained TCC \(^2\). This study shows the persistence of TCC in many environments, so could TCC increase the bacterial resistance?

In this paper, the authors decided to establish two goals: The first one was to determine if TCC affects the abundance of Antibiotic Resistance Genes and what happens with microbial community structure and function in methane production; The second one was to determine if the rate at which TCC concentrations increase in digesters would impact this Antibiotic Resistance Genes profiles, community structure and methane production.

In order to research the effects of TCC, it was initially performed a study to understand how TCC inhibit the methane production. For that they used eight different conditions of TCC addition in the digesters. These conditions were related with: TCC concentration (130 mg/kg, 430 mg/kg and 830 mg/kg) and addition rate (gradual and immediate).

In the beginning, methane production was evaluated inside the digesters using gradual addition of TCC and only when the concentration was high (850 mg/kg), methane production decayed which suggests that microbial communities were able to adapt to the new conditions from 30 mg/kg (background) to 450 mg/kg (medium) of TCC in gradual digesters. After immediate addition of TCC, the authors could detect that the microbial communities can only adapt to new
conditions below 130 mg/kg (low) of TCC. From these results, we can say that the loading rate of TCC influences the capability of the communities to adapt to TCC.

To study the influence of TCC on the abundance of Antibiotic Resistance Genes (ARGs), the abundance of 4 genes in different TCC concentrations and loading rates was evaluated. In this step the authors choose two antibiotic resistance genes that encode for efflux pumps (mexB and tet(L)), because, it was described that Triclosan increase antibiotic resistance by bacteria, promoting overexpression of multidrug efflux pumps\(^\text{[3]}\), so it is fundamental to use them to study the resistance to TCC. A negative control, ermF, which codifies for an rRNA methylase, was used for the validation of the results since it is not related with any efflux bomb system. The last gene, intI1, is related with horizontal gene transfer\(^\text{[4]}\).

After TCC addition, mexB abundance increased in all the digesters when compared to control digester. TCC selects for mexB, resulting in the increasing of efflux pumps. On the other hand, tet(L) gene showed no relation in its expression with TCC addition. ermF was not enriched in any of the digesters, however, inhibited digesters show lower abundance of this negative control. This result is explained by microbial community shift in structure and function. intI1 showed no difference between the digesters. This shows equal potential in the digesters for bacteria to transfer genetic material through integrons between the species.

In the last part of the article the authors studied the impact of TCC on the microbial community inside the digesters. In this survey, it was used 16S rRNA technique to identify the species present inside the digesters and their abundance in the end of the work. From this 16S rRNA analyses, they concluded that TCC-exposure conditions that inhibited methane production changed microbial community structure. Methanobacteria and Actinobacteria were enriched in the inhibited digesters possibly because of their capability to tolerate acidic conditions (there was a decrease in the pH of these digesters). Since methane production decreases until almost zero and Methanobacteria were enriched, a future study can be done to understand biomolecular changes inside this class of bacteria. Then they evaluated the 30 most abundant genera present in the digesters and they saw that Prevotella was enriched in the inhibited digesters because they tolerate acidic conditions and Proteiniphilum was enriched in uninhibited digesters and this makes sense because these genera encompasses acetate-producing organisms that is essential in the digesters for methane production\(^\text{[5]}\). These results prove that communities shifted away from control as TCC concentration increases and they shift towards bacteria that have capability to resist to this compound.

Briefly, TCC selects for mexB gene involved in resistance and this happen at the background concentrations (30 mg/kg) that are the concentrations of TCC usually present in the biosolids\(^\text{[4]}\). So, we can assume that bacteria can become resistant to this compound that we use in our home in the concentrations that already exist in the environment. More than that, we can ask ourselves what danger this represent. In the future, how can we overcome this antibiotic resistance? Is our health threatened? Is this resistance irreversible? It is equally important to see the impact of this research: We humans, affect our own environment, day-by-day, just by washing our teeth and hands.

Despite these interesting facts, there are some weaknesses in this study: The number of genes tested should be higher so that the conclusions and mechanisms could be more supported. The information related with TCC is also scarce. Our perspective from this work is that this compound is yet far from fully understanding, and a lot of work relating TCC with antibiotic resistance and microbial structure is still required.

References
Lipid regulator gemfibrozil, an increasing problem for treated water quality, may have an end


Analyzed by: Beatriz Lopes, Filipa Pessoa e Marisa Faustino

Gemfibrozil is a lipid regulator drug extremely used to control high concentration of triglycerides and cholesterol in the blood stream. In 2009 it was prescribed in North America as much as 500 000 times. Due to its high usage, it’s a common feature of water that arrives to waste water treatment plants (WWTPs). Despite the treatment procedures, there are several areas that registered very low yields in the removal of gemfibrozil from water.

Fish are the main targets of this bad water management as gemfibrozil bioaccumulates in these animals. Several researches indicate the ability of gemfibrozil to disrupt the endocrine activity and in some cases decrease testosterone levels, disturbing sexual behavior of fish (Mimeault, et al. 2005). Another report shows its ability to induce oxidative stress, to change the conformation of proteins and also to negatively affect the immune response in marine mussels (Wiebke Schmidt 2014).

As the core issue is the low treatment efficacy, the authors proposed the analysis of a recently discovered strain of Bacillus (Bacillus sp. GeD10, isolated from activated sludge (Zhou, et al. 2013) since it is capable of using gemfibrozil as a carbon source in rich nutrient medium, degrading it to concentrations lower than 60 ng/L. The full sequence of DNA of the Bacillus strain was sequenced and analyzed. Afterwards proteins produced by this microorganism that were differently abundant in exposure to gemfibrozil were identified. And finally a metabolomics analysis was carried out to identify specific metabolites related to gemfibrozil degradation. Combining the results obtained, the authors predicted a pathway for degradation of gem-
fibrozil. With this approach, the authors aimed to fully characterize the new strain found in order to study its potential as a bac-
bacteria for bioaugmentation.

Bioaugmentation is a phenomenon in which the addition of a population of microorganisms (for instance, one or more species of bacteria, fungi, etc) to an environment or certain medium contaminated with a pollutant compound increases the rate and efficiency of degradation of that compound. *Bacillus* sp. GeD10 was found to degrade gemfibrozil to low concentrations but not fully degrading it. Therefore this bacteria is not a sole solution to the high concentrations of the compound found in wastewater. Nevertheless, some enzymes were identified as possible future biomarkers of the degradative process of this drug, such as a cytochrome P450 hydroxylase, an alcohol dehydrogenase and a catechol-2,3-dioxygenase.

Other treatments such as the use of bioreactors with submerged polysulfone ultrafiltration hollow fiber membrane for the removal of gemfibrozil were tested by other authors, obtaining a complete removal of gemfibrozil (Gutierrez-Macias & Nacheva, 2015). Additional studies reveal that oxidation techniques have proven effective to quantitatively remove potential pollutants in wastewater that cannot be degraded biologically. One example is the electroperoxone process, a method that involves the electrochemical generation of H₂O₂ in-situ from O₂ in a sparged O₂ and O₃ gas mixture (i.e., ozone generator effluent) during ozonation, in which the pharmaceutical is oxidized and degraded. (Weikun Yao 2015) Another study investigated the effectiveness of chlorine dioxide (ClO₂) and peracetic acid (PAA) as oxidants, demonstrating that ClO₂ is more effective than PAA at removing pharmaceuticals like gemfibrozil in wastewater (Hey, et al. 2012).

In summary, the results obtained in the analyzed work allowed the prediction of a degradative pathway for gemfibrozil in *Bacillus* sp. GeD10, but it is evident that further study is required to develop an efficient strategy for the elimination of this drug from treated wastewater. There were several observations that the authors could not explain, thus the study was not very conclusive. However, it provided the initial components for a new way to go about the mentioned problem and additional knowledge on the metabolic pathway and the proteins involved in it may enable genetic engineering of the *Bacillus* strain in order to increase its efficiency in gemfibrozil degradation.

**References**


*Trichoderma longibrachiatum* Evx1 is a fungal biocatalyst suitable for the remediation of soils contaminated with diesel fuel and polycyclic aromatic hydrocarbons

From Andreoli, M et al., Environmental Science and Pollution Research, 23(9):9134-43, 2016.

Analysed by: André Costa, Inês Gonçalves, João Cotovio

In the wake of humanity’s never ending and ever growing demand for energy, great environmental disasters have occurred with grievous consequences for the habitats of countless animal and plant species. Accidents related to fossil fuel usage, in specific, oil spillages, have great and everlasting impacts on the ecosystem. Bioremediation processes’ efficiency varies inversely with time\(^1\), thus, a growing interest in the development of adequate, relatively fast methodologies, dominates the paradigms of social and environmental responsibility.

Following an environmental disaster of this nature, both crude and refined oil compounds release molecules like aliphatic and aromatic hydrocarbons into the soil and water. Most of these constituents are biodegradable under aerobic conditions; however, some of the more complex molecules have near imperceptible biodegradation rates\(^2\). Along with heavy metals, polycyclic aromatic hydrocarbons (PAHs’) are one of the major pollutants resulting from fossil fuels. PAH’s may account for up to 11% of a diesel fuel and even being considered a minor constituent, these organic pollutants are among the most toxic to plant and animal life, with reported mutagenic and carcinogenic effects\(^3\).

The present work aims to identify and characterize a potential bioremediation agent in the *Trichoderma* genus. These hardy, ubiquitous fungi represented in the *Ascomycota* division thrive wherever cellulosic substrates are found. It is well established as an interest genus and its usefulness in biocontrol approaches has been extensively studied, acting as fungal parasites while establishing positive interactions with plant life. It is also widely used in the production of recombinant proteins. As recent studies suggest these organisms also possess an ability to process diesel fuel...
residues and degrade PAHs[4]. The authors set out to isolate, identify and metabolically characterize a *Trichoderma* strain (Evx1) from the soil of a semi-deciduous forest in Southern Italy. Bioremediation agents may prove to be the way to nurture our contaminated soils back to health, as soil health directly correlates to human well-being and quality of life.

The experimental approach was divided in two great objectives: in first place, a PCR-DGGE analysis followed by BlastN was performed so as to accurately identify the organism; secondly, gallic acid oxidation and PAH tolerance tests were performed, with the latter being described as proxy for PAH removal capabilities[5] and the former qualitatively evaluating polyphenol oxidase production. Additionally, dye decolorization tests, both in solid and liquid medium, were performed using different dyes mimicking pollutant organic compounds. After proving Evx1 strain potential for bioremediation the authors proceeded to inoculate diesel fuel spiked soil and determined the C\textsubscript{12-40} fraction over the course of 30 days.

Following phylogenetic analysis *Trichoderma* isolate Evx1 was correlated to the *longibrachiatum* species with 99% similarity. Gallic acid oxidation also tested positive with the medium turning a dark brown, proving the production of polyphenol oxidases and encouraging further testing. Dye decolorization tests showed great promise as of the three used dyes (Congo Red, Methylene Blue and Malachite Green) Evx1 was the first strain of *T.longibrachiatum* that proved able to decolorize Congo Red (60% decolorization) and Methylene Blue (35%). These dyes mimic azo-compounds and polynuclear heterocycles, respectively. Azo-compounds are present in complex wastewater effluents, tend to bioaccumulate and have teratogenic, allergic, carcinogenic and mutagenic effects on humans[6]. These compounds relate not only to soil but also water contamination, posing a concern for all sectors from animal husbandry to crop farming and water consumption.

Regarding PAH tolerance tests, Evx1 showed high Tolerance Rate Indexes when grown in the presence of phenanthrene (TRI=95.2%), anthracene (TRI=96.8%), pyrene (TRI=89.7%), fluoranthene (TRI=88.1%) and even in a mix of all four PAH’s (TRI=79.4%). As per the literature, this would indicate a capability of the Evx1 strain to remove PAH’s from the soil, as proved by the following experiment in which PAH content was measured over the course of 12 days for control and inoculated flasks. These results revealed a PAH removal percentage ascribable to Evx1 of 69.20-70.78%.

Having established a basis for the bioremediation potential of this strain the authors proceeded to spiked soil tests where the C\textsubscript{12-40} fraction was assessed over the course of 30 days for soil samples spiked with 5g kg\textsuperscript{-1} diesel fuel. Over the course of the experiment the fastest removal of hydrocarbons was verified in the Evx1 inoculated soil samples with a 54.2% C\textsubscript{12-40} reduction.

Persistence of the inoculum must also be characterized and controlled to the furthest extent, as there have been reports of *Trichoderma* spp. persisting up to 270 days[7] as well as of pathogenic interaction with humans[8]. In this sense, knowledge is lacking as it hasn’t kept up with the number of applications and usage frequency of *Trichoderma*.

These results show great promise for bioremediation efforts using fungi as biocatalysts. However, bioremediation protocols must be approached very carefully so as not to disturb local equilibria. In this sense extensive studies are required to characterize interactions and synergistic effects between the inoculum and autochthonous organisms.

**References**


Fluorescence-based biosensor for monitoring of environmental pollutants: From concept to field application


Analyzed by: Catarina Bombaça, Daniel Figueiredo, Miguel Antunes

Halogenated aliphatic compounds are one of the most relevant groups of industrially produced chemicals. The large quantities produced and their widespread use ultimately results in environmental quality damage such as contamination of soils, water, air, and food [1].

These compounds have a xenobiotic character, which means they are not naturally degraded as there is a general absence of metabolic routes utilizing them. As a consequence, the compounds are essentially inert, and their partial degradation may result in toxic intermediates.
that are chemically reactive, leading to their accumulation in living organisms and disturbs in the normal metabolism [2].

Currently, detection of these contaminants in the environment is made using quantitative analytical methods, namely gas chromatography and mass spectrometry. These methods have major disadvantages such as the use of expensive equipment, the sample preparation (time-consuming) and the need of highly trained staff. Biosensors come as an effective quantitative alternative method for contaminant detection. They solve most of the problems encountered when using traditional methods of quantification. Furthermore, they are highly sensitive and can be made portable, small and automatic. However, they do not come without challenges; owing to the use of a biorecognition element, such as an enzyme, the biosensor device is fragile and can have problems associated with stability and reproducibility.

In the analyzed article it is proposed the creation of an advanced optical biosensor that can be used as an on-site monitoring and mapping tool of environmental pollution. The working mechanism relies on the proton release that results from the enzymatic reaction between the biorecognition element and the analyte. Proton concentration is determined based on the fluorescent light (later converted into an electric signal) produced by the fluorescent pH indicator after being excited by a light source from the fluorometer [3].

The biosensor is composed of two channels (reference and test) and two tips that consist of a disposable glass disc with the biorecognition element (biosensor) or BSA (reference) co-immobilized with a pH indicator (5(6)-carboxynaphthofluorescein (CNF)) via cross-linking with glutaraldehyde. Glass sticks work as optical transducers [3].

The well-characterized haloalkane dehalogenase LinB from Sphingobium japonicum UT26 can be used to detect the compounds 1,2-dibromoethane and 3-chloro-2-(chloromethyl)-1-propene with limits of detection (LOD) of 2.4 and 1.4, respectively. The detection of 1,2-dichloroethane makes use of the haloalkane dehalogenase DhA from Xanthobacter autotrophicus GJ10 giving a LOD of 4.6 mg/L, and for the compound 1,2,3-trichloropropane, a mutated haloalkane dehalogenase Dha (Dha31), from Rhodococcus rhodochrous is needed. Dha31 offers a catalytic efficiency 26-fold higher than the wild type and a LOD of 1.4 mg/L. It is further possible to detect γ-hexachlorocyclohexane using the dehydrochlorinase LinA from S. japonicum UT26, with a LOD of 12.1 mg/L. Field-testing quantification assays were successfully performed in the vicinity of various sites in the Czech Republic and Serbia, where a leakage of 1,2-chloroethane and other chlorinated aliphatic compounds was previously reported [3].

An example of other biosensor is the work developed by Voplensk et al., which presents a biosensor based on Saccharomyces cerevisiae cells with modifications in two steps of the purine biosynthesis pathway, immobilized in alginate beads under conditions of low aeration. This biosensor allows the detection, and rough quantification of copper ions in water in the range of 1-100 µM. The detection is readable by the naked eye, and the working mechanism is based on changes in the color of the modified S. cerevisiae strain from white to red, proportionally to the concentration of copper ions and depending on the level of oxygen. Upon exposure to aerial oxygen, the development of the final coloration of the beads takes from 30 to 60 minutes [4].

Another approach for the detection of water contaminants is surface-enhanced Raman spectroscopy (SERS). Chemical modification of a metal surface can be made to promote the capture of particular analytes, making possible the SERS sensing of analytes with very low affinity for SERS-enhancing surface. The work developed by Kubackova et al., involved the functionalization of a metal nanoparticle (NP) surface with alkyl dithiols, which induced the NP linkage to obtain high SERS enhancement from hot spots present in the interparticle junctions, and create a specific environment in the nanogaps making them suitable for the assembly of the analyzed pesticides. This
strategy can be implemented in low-cost optical sensing devices and used for in situ analysis of samples. Furthermore, the SERS detection eliminates the need for sample pretreatment and the LOD of organochlorine pesticides obtained, was in the order of $10^{-8}$ M [5].

The biosensor described in the analyzed article (Bidmanova et al. 2015), offers the possibility of on-site detection of common chlorinated aliphatic compounds as opposed to conventional methods, with a good correlation with the latter. The very short measurement times of one minute, could allow the monitoring of changes in the concentration of those analytes at different sites as well as tracking of the water pollution sites by creating contamination maps through the interlink with the Global Positioning System (GPS). Thus, constituting an early warning system and minimizing ecological and human health risks. Contrary to traditional methods, which have very low LOD (ng/L), the biosensor only achieves LOD of mg/L. This limitation impairs the measurement of these contaminants in drinking water and the monitoring of agricultural run-offs. However, this issue only becomes a major problem if the LOD does not contemplate the maximum concentration set by regulatory entities for the tested contaminant. Environmental conditions and a complex matrix may also pose some complications in the measurements since the biosensor only maintains more than 60% of its response in the pH range of 4 to 10 and temperatures ranging from 15 to 50 °C.

In summary, the developed biosensor is suitable for field measurements of halogenated aliphatic hydrocarbons and presents one of the fastest systems for their detection. Its selectivity is dependent on the substrate specificity of the biorecognition element used, which means it detects classes of substrates. However, even though it falls behind SERS, this selectivity can be increased by engineering the enzyme, narrowing the substrate specificity. Applications can range from the identification of contamination hot spots to the determination of the efficiency of hazardous waste site remediation procedures on site.

References
The rapid development of the industry related to paper production, fertilizers, pesticides and other has conducted to the discharge of large amounts of metal-contaminated residues into the environment, resulting in a serious problem of environmental contamination. Unlike organic contaminants, metals are not biodegradable and tend to accumulate in living organisms, becoming toxic and carcinogenic.

For instance, chromium is a metal with different oxidation states although only hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)] are stable in the environment. The Cr(VI) compounds exist mainly as chromate and dichromate and have high solubility, bioavailability and mobility. These compounds are associated with several diseases such allergic reactions, contact dermatitis and cancer of the lung. The Agency for Toxic Substances & Diseases Registry (ATSDR) from USA included the Cr(VI) in hazardous substance list since 2001. On the other hand, Cr(III) is much less toxic so its presence in environment does not constitute a problem.

Chromium is commonly used in metal finishing and tanning industries and, therefore, soil may be contaminated with chromium through wastewaters and land disposal of sewage sludge [Zheng et al., 2007]. The soil is a very complex and heterogeneous matrix whose biodiversity supports the provision of several ecosystem services (nutrient cycling and soil formation) of most importance for food production and maintaining socioeconomic activities.

The preservation of soils depends on monitoring soil contamination in order to prevent the dispersion of pollution and avoid drastic consequences. Therefore, it is important to map the concentration of toxic compounds present in soils.

Soils composition and physicochemical properties – such as organic matter content, the concentration of metal ions (iron and manganese) that influence the oxidation-reduction cycle of chromium [Kotas´ and Stasicka, 2000], soil texture and pH dictate the bioavailability of metals in these complex matrix. The chromate concentration in soil decrease over time, independently of the soil.
properties. However, this concentration decreases differently depending on the presence of certain chemical elements (iron and manganese), low pH, granulometry (sand, clay and silt percentage) and mostly organic matter that promotes chromate reduction to Cr(III).

There are various methods for metal detection however classic methodologies involve expensive instrumentation, require additional chemical compounds, which are pollutants and are unable to detect metal bioavailable concentrations. Nevertheless, the measurements of the bioavailable fraction of metals is a parameter of high interest since it determines the toxicity of metals to the organisms.

Assays with microorganisms have been seen as an excellent methodologies since they have short life cycle, can be easily maintained in laboratory cultures at low cost and are highly sensitive and selective to specific analytes. In this way, the development of metal-specific biosensors tools functioning on the basis of reporter systems has been acquiring increasing attention.

The use of biosensors constructed through fusion of the regulatory gene, chrB of the chromate resistance determinand of Ochrobacterium tritici 5bv1 with the reporter gene green fluorescence protein (gfp), has been reported as an efficient and sensible approach to detect chromate in environmental waters spiked with chromate [Branco et al., 2013].

In the paper to be analysed, two biosensors, pCHRGFP1 E. coli and pCHRGFP2 O. tritici, were used to detect and monitor Cr(VI) in different chromate contaminated soils showing the usefulness of the biosensors as an alternative tool for monitoring chromate.

The constructed pCHRGFP1 E. coli showed higher sensitivity for chromate than pCHRGFP2 O. tritici. Other analytical methodologies, such as colorimetric diphenylcarbazide method and ionic chromatography, were also employed in order to compare the efficiency of the different approaches in chromate measurements. Biosensors and ionic chromatography methods demonstrated to be very sensitive and very accurate, whereas the colorimetric method showed a large discrepancy between the quantity measured and the chromate concentration in solution. Since the ionic chromatography is very expensive and time consuming, the application of these biosensors seems to represent an advance in chromate detection and quantification.

The rate of chromate disappearance in soils were also monitored and seemed lower at concentration higher than 40 mg Cr(VI). Kg⁻¹. This can be explained by the fact that high concentrations of metal lead to decreasing microbial communities and consequently, to low ability of microorganisms to reduce Cr(VI).

Collembolan reproduction tests with Folsomia candida, following standard procedures (usually performed for assessing the effects of chemical on the reproduction of collembolans in soil), were also performed as a way to relate the quantification of Cr(VI) with the toxicity toward this invertebrate species.

The reproductive output of collembolans in the reproduction tests showed that the slower the disappearance of chromate in soil, the higher is the effects detected in the reproduction of collembolans. Collembolans activity influences the concentration of chromate in soil due the fact that springtails use soil bacteria as nutritional source, thus, limiting the bacterial capacity in the chromate reduction process. Despites these latest facts and since the collembolans F. candida represent one specific route of exposure to chromate, further tests could not be excluded to verify if the same correlation can be observed with other soil key-species having different routes of exposure.

References
Bioaccumulation of pharmaceuticals and personal care products in the unionid mussel *Lasmigona costata* in a river receiving wastewater effluent

From de Solla, S.R. et al., Chemosphere 146:486-496, 2016

Analysed by Joana Costa

Recently, the concerns about effluent quality have expanded beyond traditional pollutants to include organic contaminants that can alter responses in aquatic organisms at very low concentrations. Among the organic contaminants in wastewater treatment plant (WWTP) effluents are considered pharmaceuticals and personal care products (PPCPs) (Arlos et al. 2014).

PPCPs such as stimulants, nonsteroidal anti-inflammatory agents, antidepressants and other prescription drugs have been detected at concentrations reaching low μg/L in municipal wastewaters. The majority of pharmaceuticals are relatively water soluble and are generally non-bioaccumulative. Resident biota can be chronically exposed to PPCPs due to the continuous release of PPCPs into ecosystems downstream of WWTPs (de Solla et al. 2016).

The continuous introduction of this organic contaminants and their bioactive metabolites into the environment may lead to high, long-term concentrations and promote adverse effects on aquatic and terrestrial organisms. For the last few years researchers have been trying to better understand toxicological issues including low-dose, multi-generational exposure to multiple chemical stressors and how human and ecological risks might be affected by these recent changes in the aquatic environment (Barceló & Petrovic 2007).

Sola et al. (2016) were characterizing the seasonal changes in PPCP concentrations in water using freshwater mussels. The poor
water quality is deleterious to this popula-
populations of mussels. And, this marine
animals are useful for monitoring water
quality in aquatic ecosystems because they
are benthic filter-feeders that are exposed to
contaminants dissolved in water,
sequestered in surficial sediment, or bound
to particulates in the water column (de Solla
et al. 2016).

In the paper analysed the objectives
were to characterize the seasonal changes in
PPCP concentrations in water, to calculate
bioaccumulation factors (BAFs) of PPCPs in
mussels, and to determine the chemical and
physical properties of PPCPs driving the
bioaccumulation on the Grand River
watershed in southern Ontario (de Solla et
al. 2016).

The authors found out that the PPCP
concentrations in the Grand River tended to
be highest in the summer or early fall. Which
revealed that flow-driven transport
processes (advection and dispersion) greatly
influenced the behaviour of the target
contaminants in the aquatic environment
(Arlos et al. 2014). Photolysis and
biodegradation also appeared to contribute
to the attenuation of these compounds in the
Grand River.

The PPCPs detected at the highest
concentrations were dimethylxanthine (this
drug is a psychoactive central nervous system
stimulant), iopamidol (a blood medication),
triclosan (is an antibacterial/antifungal
agent), sertraline (included in the
antidepressants class), and theophylline (a
blood medication and muscle relaxant).
When the PPCPs were summed into four
general classes (antibiotics, antidepressants,
blood medication, and painkillers),
concentrations were all higher downstream
of the WWTP compared to upstream for the
wild mussels, and for all classes except
antibiotics for the caged mussels (de Solla et
al. 2016).

Considering the long life span of
mussels, bioaccumulation of PPCPs in
mussels with both short term exposures for
the caged mussels (4 weeks) was compared
to the life span exposures of the wild mussels
(> 20 years). And given that the majority of
PPCPs are relatively water soluble, they
anticipated that the time to reach
equilibrium between the water/sediment
exposures and body burdens would be
relatively quick, and within the time frame
that the caged mussels were deployed (de
Solla et al. 2016).

In other studies the authors used
biomonitoring to study the changes in water
using fish and mussels. Once, biomonitoring
has many advantages as integrate the
response temporally, accounts for
bioavailability and more directly assesses
exposure under ambient conditions (Jasinska
et al. 2015). The PPCPs were monitoring with
biomarker expression in the freshwater
mussels, so this could evaluate if the
contaminants affected the receiving water.

With monitoring biomarkers, Jasinska
et. al (2015) found out that in marine
mussels (Mytilus edulis) collected from
intertidal regions impacted by municipal
wastewater, biomarker responses included
reduced levels of Vtg-like proteins
(vitellogenin) in females and elevated lipid
content in males. The gene for vitellogenin,
an egg yolk protein precursor, can be
induced by estrogen exposure and for this
reason, it has been used as an indicator to
predict impacts on mussels’ reproductive
success. It was also observed induction of
oxidative stress biomarkers and modulation
of immune function in freshwater mussels
(Lasmigona costata) caged in a river
influenced by wastewater (Jasinska et al.
2015). They conclude that it is possible this
immune response was due to exposure to
bacterial pathogens in the wastewater, and
not to exposure to contaminants of emerging
concern (CECs), like pharmaceutical and
personal care products. In summary, more
work is needed to elucidate whether CECs
found in wastewater can cause oxidative
stress in aquatic organisms before a
definitive link can be made between
exposure to CECs in wastewater and
biomarkers of oxidative stress.

In the other hand, for Harvey & Angela
(2011) the effects are already evident: they
include the feminization of fish by residues of
the contraceptive pill, and the deaths of
millions of vultures on the Indian
subcontinent following ingestion of the anti-
inflammatory drug diclofenac. Antibiotic
overuse has led to the emergence of
resistant pathogenic bacteria in the wider environment, and not just in medical settings (Harvey & Angela 2011).

In conclusion, many pharmaceuticals, if consumed together at therapeutic doses, can cause severe adverse interactions in humans. If aquatic organisms respond to pharmaceutical compounds in the same way as humans, effects on the environment could be greater than predicted (Boxall et al. 2012). Antimicrobial PPCPs may also increase persistence of other PPCPs, thus affecting the overall risk because many PPCPs will be emitted continuously into the environment, and organisms in the environment will be exposed throughout their lifetime.

References