Environmental problems in Nanosilver-Enabled Bandages

Analyzed by Bárbara Fernandes, Margarida Barroso, Pedro Fontes

Studies related to the use of nanosilver-enabled bandages shows a big environmental problem in several areas because of the synthesis, production and incineration.

For hundreds of years, silver has been widely used in a range of technologies for its physical and chemical properties. Production and applications of nanosilver have also existed for more than a century, first for ingestion as a medicinal tonic, and later for direct application as a biocidal agent. More recently, nanosilver has been incorporated within a broad range of consumer products.

It was recently published in the article Environmental Life Cycle Assessment of Nanosilver-Enabled Bandages. Jan 2015(1) a study where it is possible to realize the effects of nanosilver-enable bandages.

Over 400 t of silver nanoparticles (AgNPs) are produced globally each year, with applications in cosmetics, textiles, and electronics (1). Almost 30% of the global production of AgNPs is incorporated into medical supplies and devices due to their bactericidal properties, yet there has been limited research on the fate of AgNPs used in health care settings, or on the significance of the life cycle impacts of these nanoparticles in the context of medical devices.

An application of AgNPs in the medical field is nanosilver-enabled wound dressing that is applied directly to severe burns and open wounds. These bandages are produced by embedding silver particles within the fabric of the bandage. The antimicrobial properties of AgNPs allows the bandages to be effective against common bacteria, thus promoting healing of the wounds and preventing infection. AgNPs work against bacteria by releasing silver ions that adhere to the cell membrane, penetrate it, and generate reactive oxygen species that cause oxidative stress and potential DNA damage.

The growing number of nanosilver applications in consumer products worldwide has led to increasing concerns regarding the potential for direct human exposure and releases to the environment. AgNPs can be released from products during the cleaning and disposal, but also from
product manufacturing and use stages, or at any point during a product's life cycle. Several product categories use different concentrations of nanomaterials, and this affects their impacts relative to the larger product.

During the analysis of process contributions to AgNP synthesis based on the production of 1 kg of AgNP was reveal that the top contributing processes to the global warming potential are all combustion related used for power generation. The largest contributor to ecotoxicity is related not to nanosilver releases but to environmental releases from bulk silver processing, emissions that occur far upstream from AgNP synthesis \(^{(1)}\).

Also in the bandage production the data show that the impact of nanosilver is huge, even though AgNPs are just 6% of the bandage mass \(^{(1)}\).

The last part of this product life cycle is the end of life, in other words the incineration. Incineration products of paper and plastic components of the bandage are the largest contributors for most categories of environmental impact, with the exception of ecotoxicity, where the dissociated silver has the highest contribution \(^{(1)}\).

Impacts associated with AgNP synthesis dominate the cradle-to-grave impacts of the bandage, and emissions from AgNP and bandage production are several times more impactful than emissions from bandage incineration, including direct releases of AgNPs \(^{(1)}\).

It has been previously shown that only 10% of AgNPs are released from bandages during use, but additional empirical data of AgNPs leaching from bandages and subsequent fate will support the modeling of additional routes of AgNP disposal and potential release \(^{(1)}\).

A general difficulty of applying LCA to nanomaterials and nanoenabled products is that the use confers superior properties that are not attainable by the current technologies, to which they are to be compared. But other benefits of using AgNPs would be more difficult to capture using current LCA methods, such as reduced bacterial resistance to antibiotics \(^{(1)}\).

Finally, as with many emerging technologies, the data used in the majority of LCA studies for engineered nanomaterials were gathered from bench-scale investigations \(^{(1)}\).

Life cycle impacts will likely decrease as nanomaterial production volumes grow and nanotechnologies become more mature, but life cycle modeling can continue to identify opportunities for process improvements and provide context for understanding relative risks and benefits of nanotechnologies \(^{(1)}\).

Further researches demonstrate that has been some changes in the way of thinking about the advantages/disadvantages brought by these new types of products.

In a 2007 study it was demonstrated that silver-containing dressings are likely to provide a barrier to and treatment for infection however, their bactericidal and bacteriostatic properties are inferior to topical antimicrobial agents \(^{(2)}\).

The available data on the impact of silver nanoparticles on mammalian cells was reviewed in 2010. As pointed out the inflammatory, oxidative, genotoxic, and cytotoxic consequences are associated with silver particulate exposure. However, in this year the data available was not enough to conclude what the toxic effects of silver nanoparticles \(^{(3)}\).

In 2011 was considered the complete life cycle of t-shirts and was conclude that the use phase is the most important in terms of climate footprint \(^{(4)}\).

Also in 2011 the results indicate that current knowledge of AgNP emissions was very uncertain. The main conclusion made in Arvidsson et al. (2011)\(^{(5)}\) was that textiles may become a large source of AgNP emissions in the future and the use of antimicrobial nanosilver for wound dressings may be appropriate, given the efficiency of silver against a wide range of bacteria and because it is not particularly toxic to humans, but they don’t know what is the toxicity to the environment \(^{(5)}\).

On other hand, preliminary evidences in Holder et al. (2013)\(^{(6)}\), suggest that depending on the composition, nanomaterials may undergo physical and chemical transformations within the incinerator and these transformations may also drastically affect the impacts in the environment \(^{(6)}\).

In a 2014 study\(^{(7)}\), the authors propose a new way to assess these advantages/disadvantages in the production of these new products that take into consideration the upstream impacts (material demand, energy requirements and amount of waste generated) and the downstream benefits (benefits to public health, safety use and improved performance)\(^{(5)}\).

In summary is possible to see that the use of nanomaterials is increasing and this can have irreversible damage on the environment.
Pollutants bioavailability and toxicological risk from microplastics to marine mussels

From Avio CG et al., Environmental Pollution, 198:211-222, 2015

Analyzed by Ana Rita Martins, Mariana Gerónimo, Maxime Ferreira

In the last 5 decades, world’s plastic production has increased from half a million, back to 1960, to 300 million tons/year nowadays. \(^1\) Simultaneously, the pollution caused by this material has also increased and it’s estimated that 10% of the annual production ends up in the oceans. \(^1\), \(^2\) Currently the European demand for plastics is 46.3 Mtonne and the most demanding activity sector for this material is the packaging industry, followed by products such as consumer and household appliances due to their relative inexpensiveness and long life span. \(^1\)

Polymer degradation takes much longer as a result of haline environments and the cool-
ing effect of the sea, which allows Plastic Marine Debris (PMD) to persist in the environment and to be swallowed by wildlife. PMD concentration increase in the oceans is linked to human consumption behavior, industrial activities and poor waste management which calls for the attention of a serious environmental problem. [3]

Among the risks that PMD have on marine fauna are the ones of direct ingestion, entanglement, physical damages, exposure to chemicals and they can also act as floating substrates contributing to long-range transport of alien species. Nonetheless, the bigger problems are microplastics. [4]

Microplastics are plastic materials whose diameter is below 5 mm and they can come from two different sources. Primary plastics are manufactured ex-novo and are a direct result of human material and product use while secondary plastics derive from macroscopic debris after some chemical, physical and/or biological fragmentation. Likewise, these smaller plastic fragments can also be ingested by marine organisms and transferred through the trophic chain. [3], [4], [5]

Microplastics are a particular threat not only due to their size but also for their capacity to act as vectors of chemical pollutants and adsorb persistent organic pollutants (POPs). For example, different particles polymers, like polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS), were shown to have a high adsorption capacity for polycyclic aromatic hydrocarbons (PAHs), hexachlorocyclohexanes, among others. [2] Despite their importance in adsorption and transport of hydrophobic pollutants, it’s still unclear whether they also represent a potential source of chemical exposure within marine food webs.

The overall results of the paper we are analyzing were expected to increase our knowledge on potential toxicological risk of microplastics in the marine environment, so, in this experiment PE and PS particles were size sorted in two groups: One was used to characterize pyrene adsorbing capacity and the other one was used in the exposure of mussels Mytilus galloprovincialis to virgin and contaminated polymers.

In the first case, the adsorption of the PAH pyrene to PE and PS was assessed by suspension of microplastics with a low, medium and high concentration of pyrene and this mixing solutions were maintained in continuously rotating for 6 days. They used these 3 types of pyrene concentrations in order to represent what happens in sea. Low and medium concentrations represent the realistic case and high concentrations represent a possible case of accident such as heavy oil spill or highly contaminated sewage. After the adsorption process was concluded, they distributed all of these solutions to 150 mussels Mytilus galloprovincialis for 7 days. Levels of pyrene adsorbed on polymers were measured after three and six days of treatment. Results clearly confirmed that these two polymers adsorb environmental pollutants. [2] These data support the potential of microplastics in trapping and transporting marine pollutants, as already suggested by studies about adsorption of several hydrophobic chemicals from various typologies of plastic polymers.

After 7 days of exposure, mussels’ haemolymph, digestive glands and gills were rapidly removed from 30 specimen for each treatment. The results obtained with these exposed mussels provided the first clear evidence that pyrene adsorbed on contaminated plastics was transferred to organisms and concentrated in tissues. The bioaccumulation of pyrene was particularly marked in digestive glands where its concentrations appeared up to 3 folds higher than those present on contaminated polymers. It was also verified the presence of particles in gills and haemolymph, although in a lower extent. [2]

They studied immunological response of controls and exposed mussels and they verified that significant effects were observed on haemocytes with a strong shift of the haemocytic cell population, a limited variation of phagocytosis and a significant reduction of lysosomal membrane stability. [2]

Researchers also did comet assay, an “uncomplicated and sensitive technique for the detection of DNA damage at the level of the individual eukaryotic cell” and the results of this method indicated a significant enhancement of DNA strand breaks in haemocytes of mussels treated with virgin microplastics, while nuclear anomalies were higher in all the treatments with either virgin or contaminated polymers, the frequency of micronuclei significantly increased only in specimens exposed to pyrene-treated PS and acetylcholinesterase did not vary in haemolymph and decreased in gills of mussels exposed to both virgin and contaminated microplastics. [2]

The measure The Weight of Evidence value resulted in an overall WOE risk classified as Slight or Moderate for virgin PE and PS and Major or Severe for contaminated polymers. [2]

Mussel’s transcriptional responses were also analyzed using DNA microarrays and it was
revealed a total of 2,143 differentially expressed genes in response to PS. 936 of these genes were up regulated. In response to pyrene-contaminated PS, 1,320 genes were differentially expressed and 496 of these genes were up regulated. Among these, 280 transcripts were significantly affected after both exposures and 135 of these genes were up regulated. Some programs were used programs in order to understand in which pathways those genes were involved. Some of the most interesting enriched KEGG pathways of the up-regulated genes were related to lysosome, coated membrane and endosome which suggested an increased uptake of microplastics via endocytosis and their endolysosomal degradation. It also related genes to NOD-like receptor signaling pathway which is involved with innate immune defenses, such as regulation of inflammatory and apoptotic responses. 

In summary, in *Mytilus galloprovincialis*, plastic particles were accumulated in digestive tissues and translocated to haemolymph. Besides, the uptake of both virgin and contaminated microplastics caused notable histological changes in digestive cells with strong inflammatory responses, formation of granulocytomas and lysosomal destabilization which increased with exposure time.

3. J.P.G.L. Frias, V. Otero, P. Sobral, Evidence of microplastics in samples of zooplankton from Portuguese coastal waters. Marine Environmental Research 95, 89-95 (2014)

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**Environmental health hazards of e-cigarettes and their components: Oxidants and copper in e-cigarette aerosols**

From Lerner CA et al., Environmental Pollution, 198:100-107, 2015

An electronic cigarette is a battery powered vaporized system that simulates de act cigarette smoking delivering nicotine and consequently producing a similar feel.

An e-cigarette is composed by a battery, inhalation pressure sensor, micro-controller and cartomizer which contains an atomizer and a cartridge. When being use, an aerosol is produced, the E-liquid solution contained in the cartridge is atomized by a heating element and the vapor is inhaled. E-liquids are composed by a mixture of propylene glycol, glycerin nicotine and flavorings. 

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An electronic cigarette and their components; Graphical representation of the results obtained by the experimental studies carried out by Lerner, C.A. et al.
Advantages and disadvantages

E-cigarettes may be helpful to quit smoking, hence secondhand smoke isn’t possible, since they are free of tar and other ingredients present in traditional cigarettes. On the other hand, e-cigarettes are not fully tested, and low in regulation, the producers sell unreliable amounts of nicotine. The flavors may appeal to children, can be a gateway to smoking and creating a new addiction. Lastly, poison hazard by leaked nicotine is possible.[2]

Human Health Effects

E-Cigarettes are becoming more and more used by every generation and gender, without any regard for either the short or long term repercussions of its usage.

Information is scarce, studies are being taken ever so frequently, and their conclusions still stand at an impasse; are E-Cigarettes safer or more dangerous than regular cigarettes?

So far, we are aware of the influence of nicotine for the human body, both from its consumption and the withdrawal process; on the other hand, e-Cigarettes are creating a new problem that up until this day was of minimal concern. The quantities of chemicals that are present inside the disposable cartridge are not regulated and lack any control from the producer, leading to a galloping increase in nicotine overdose cases over the last few years, reaching values in the scales of hundreds per month, whereas before wouldn’t even reach the dozen.[3]

Environmental Effects

Scientific Information on the environmental impacts of e-cigarettes manufacturing, use and disposal is very limited. There aren’t yet any studies that formally evaluated the environmental impacts due to the manufacture process or disposal of components. This is why it is crucial that the environmental impacts of e-cigarettes be subjected to further investigation.

The impact that e-cigarettes have on public health includes consequences for the environment such as air quality effects, energy and materials used, as well as issues related with the environmental responsible disposal and land use decisions.[4]

Relatively to the environmental considerations due to e-cigarette manufacture, one of the main concerns is the source of nicotine: the liquid present inside the e-cigarettes (‘e-liquid’ or ‘e-juice’) is formulated to contain a mixture of chemicals, including nicotine. This component can be chemically extracted from tobacco plants or tobacco dust. However, it is not certain if the nicotine used in the e-liquid is in fact chemically synthesized or if it comes from tobacco extracts[4,5]. The chemical synthesis of nicotine uses organic solvents, including formaldehyde, formic acid and dichloromethane, and the intermediate product is purified by high vacuum distillation, which suggests that the waste is emitted to the atmosphere. Regarding the use of resources and energy due to e-cigarette component manufacturing: a typically e-cigarette contains a battery, which contains heavy metals, an atomizer and a cartridge, which is wrapped in a plastic bag. The manufacture process of these components requires the use of energy and specific materials[4,5].

Regarding the use of e-cigarettes, the main concern is focused on the secondhand aerosol that is released. The e-cigarette use is a potential source of chemicals and aerosol exposure in the indoor environment. This cigarettes contribute to secondhand aerosols to the environment only by emitting particles (ultrafine particles with low levels of toxins that are toxic for living organism) as they are exhaled by the users.[4,6,7]

No studies have formally evaluated the environmental impacts of the disposal of e-cigarette components, including batteries. However, e-cigarettes are not a pharmaceutical product, and therefore, the disposal of e-cigarettes is not regulated under any program that governs the disposal of solids and hazards substances with proper treatment. This means that the used and unused components containing residual nicotine (like the cartridges) and the batteries, which contain chemical products, can be disposed of without treatment to remove the chemicals, polluting, this way, the environment.[4]

The investigation of the safety of e-cigarettes or Electronic Nicotine Delivery Systems (ENDS) is currently increasing. The study carried out by Lerner and colaborators[8] is focused on investigating, using semi-quantitative measurements, the oxidants/reactive oxygen species (ROS) released from e-cigarette components (cartomizers and batteries), and assess whether these materials contribute in fact to tobacco waste and environmental pollution. The authors also extended their studies to detect the presence of oxidants/ROS associated with e-cigarette aerosols and measured aerosols particle size distribution and copper levels to assess the toxicity of ENDS/e-cigarette aerosols. [8]
The relative levels of ROS produced from e-cigarette vapor was determined using a semi-quantitative measurement of oxidants, through an indicative solution (DCFH solution), which converts the DCFH molecule into a fluorescent molecule (DCF) indicating the presence of free radicals, such as ROS (H₂O₂) or potentially other reactive oxidants. The results obtained are graphically presented in the illustration above (graphs A to D). Based on the data obtained, it is possible to conclude that e-cigarette components (cartomizers and batteries) comprise high amounts of oxidative species, and that the aerosols contain high levels of copper (6.1 times higher that reported for conventional cigarette smoke).[8]

There is a pressing need for stronger regulation of e-cigarettes and their components, including regulation of their storage, leachates, recycling and disposal.


Antibiotics and their effects in the aquatic environment: a growing problem?
From Magdaleno A et al., Ecotoxicology and Environmental Safety, 113:72-78, 2015
Analyzed by Denise Francisco, Omar Mayorga, Vitória Curto

Overview of how do the antibiotics end up in the aquatic environment. Adapted from Boxall et al (2012)
Antibiotics are biologically active molecules that act as antimicrobials and they have been developing throughout the years because of their many advantages. However, they have disadvantages too, and one that has been recently found is the fact that antibiotics might have adverse ecological effects due to toxicity towards non-target microorganisms in the aquatic environments. The consumption of antibiotics has increased, either to treat or to prevent microbial infections, and is now known that they reach the aquatic environments because a significant percentage is non-metabolized by our biotransformation metabolism (Phase 1 & Phase 2) and is non-biodegradable in waste water treatment plants.

Besides the dimension of the problem, and aside the fact that antibiotics are now considered as a highly persistent pollutant due to their continuous introduction in to the aquatic environments and, therefore, an emerging environmental issue, there are places where no data has been reported on the subject. One of those places is Argentina, where the consumption of antibiotics was of 640 tons only in 2007.

In order to prove that antibiotics are affecting aquatic environments, Magdaleno et al. (2015) (paper entitled “Effects of six antibiotics and their binary mixtures on growth of *Pseudokirchneriella subcapitata*”) performed toxicity bioassays (endpoint: microalga growth). The six selected antibiotics were ampicillin (AMP), amoxicillin (AMX), cephalothin (CEP), ciprofloxacin (CPF), gentamicin (GEN) and vancomycin (VAN), because they are the most used ones in hospitals from Buenos Aires, Argentina, where the study took place. The toxicity bioassays were made for both individual and binary mixtures of antibiotics and inhibition of growth was expressed as a percentage of the control. The EC$_{50}$ (median effective concentration) for each one was determined by adjusting the growth inhibition values as a function of the antibiotic concentrations. Values for toxicity were computed based on the predictive equations of the two most widely used definitions of additivity: Concentration Addition (CA) and Independent Action (IA) models. CA is based on the assumption that mixture components have the same sites and similar modes of action and IA is based on the assumption that mixture components act independently and have different sites of action. These predictions for an effective concentration of 50% for each mixture were compared with the experimental results, in order to understand if there were mixed effects in the binary mixtures and, if they existed, determine if a synergistic or antagonistic relationship took place.

In the individual antibiotics toxicity tests, it was found that EC$_{50}$ values for CPF and GEN were low, which means that low concentrations of both antibiotics proved to be highly toxic (Magdaleno et al. 2015). This toxicity on photosynthetic non-target organisms may be caused by the fact that chloroplasts have a prokaryotic nature, making possible to predict that they may be susceptible to at least some of the studied antibiotics (e.g. CPF and GEN). VAN and CEP were moderately toxic, and AMX and AMP proved to be non-toxic for *P. subcapitata* in the range of concentrations tested. This lack of toxicity may be originated because both antibiotics have a very specific mode of action towards bacterial cell walls, therefore, do not affect eukaryotic microorganisms. This study contributed by providing data for the antibiotics CEP, GEN and VAN in microalgae, which did not existed previously.

The results for the binary mixtures showed that for low concentrations all the binary mixtures showed synergism (Magdaleno et al. 2015). This occurred either by mechanisms of facilitating actions (the secondary actions of one drug enhance the activity of another drug in the mixture) or complementary actions (drugs act on the same target at different sites or on different targets of the same pathway). The synergism that is verified between CPF and CEP and between CPF and VAN is explained because CPF acts by inhibiting the DNA enzyme gyrase, thus affecting DNA replication, and CEP and VAN act by inhibiting the synthesis of the cell wall in bacteria. In both cases, the antibiotics have different sites of action and they may act synergistically in green algae also due to non-specific mechanisms. These non-specific mechanisms may be, for example, interactions that
can occur with plasma membrane (or chloroplast membrane) with alteration of its permeability and inhibition of membrane enzymes. As expected, a weaker synergism is observed between CEP and VAN, because they have similar sites of action in bacteria (inhibition of cell wall peptidoglycan) that are not present in the microalga, and the moderate toxicity may be possibly due to non-specific effects of these two antibiotics. A strong synergism is verified between GEN and all the other antibiotics, since GEN has a different mode of action. GEN binds to the 30S subunit of the bacterial ribosome, and can affect protein synthesis in the chloroplast, since the chloroplast also contains subunit ribosomes of type 30S, similar to the ones that occur in bacteria. Both models failed in giving accurate predictions for toxicity of the mixtures.

Risk Quotients (RQ) were also calculated to estimate the actual potential for ecological risk caused by antibiotics and their mixtures. RQ is calculated as the ratio between the predicted environmental concentrations (PEC) and the predicted no effect concentrations (PNEC). PEC values were achieved from the antibiotics data obtained in one of the most important hospitals in Buenos Aires, Argentina, whereas PNEC values were obtained by dividing the EC50 values by 1000, an assessment factor. RQ values below 1 represent no risk, and above 1 represent risk. According to the calculated RQ values, the authors of the work suggest that the individual and binary mixtures of antibiotics represent an ecotoxicological risk for aquatic environments, except GEN (Magdaleno et al. 2015).

Many studies were already made in this field. In 2005, Cluevers et al., proved that the impact of antibiotics can extend to the population level. In tests with activated sludge microorganisms, the outcome also depends on the bacterial susceptibilities, since the whole mixed culture possesses many individual ways of reacting to the antibiotics.

We believe that in the future the experimental approach might consider toxicity bioassay for mixtures with more than two of the antibiotic, since it would provide more realistic conclusions. This has already been done by González-Pleiter et al. (2013), where mixtures up to five antibiotics were tested.

As major implications and conclusions of this study, it can be said that it was proven that antibiotics have mixed effects and environmental risk exists, being hospital effluents a major challenge in terms of risk management. It was also proven that the primary trophic levels can be affected and that these effects can extend to other species and ultimately, affect the whole aquatic environment. Overall, more studies and more data are needed on the matter, and better models should be developed in order to be able to take better conclusions from them. Again, in González-Pleiter et al. (2013), a different method called Combination Index (CI) was used, which tries to quantify synergism or antagonism. The authors concluded that CI method proved to give a more accurate prediction than the classical models. We hope that new knowledge will encourage the scientific community to get regulatory measures taken in order to protect aquatic environments.


Endocrine activity of alternatives to BPA found in thermal paper


Analyzed by Maria do Rosário Nazaré, Sofia Salsinha, Martim Cardador

Bisphenol A, or simply known as BPA, is an industrial chemical widely used as a monomer or additive a variety of polymeric materials such as plastics and certain paper products. One such example is thermal paper.

Thermal paper, a smooth type of paper to which a coating of leuco dye and developer is applied (like BPA), is commonly used for tickets, cash receipts and laboratory. Since its use is so widespread, it’s not difficult to imagine how one could be exposed to BPA due to transfer of BPA to the skin. As such, individuals who handle large quantities of thermal paper (such as cashiers) could run the risk of developing symptoms related to prolonged exposure.

Due to the already identified risks associated with long-term exposure to this compound, the study (Goldinger et al, 2015) in what was

Schematic representation of the study performed by Goldinger et al, 2015, concerning the effects of BPA and its alternatives (BPS, BPF, Pergafast and D-8) in steroidogenesis.
based this article attempts to analyze the current situation in Switzerland when it comes to BPA substitutes already in use and their effects on the human endocrine system.

In this work (Goldinger et al., 2015), 124 thermal paper receipts were analyzed and four BPA alternatives were selected: BipshenoS, Bispheno1F, Pergafast and D-8. After this market analysis the thermal paper was extracted and the final solution was used for a screening assay that was done by liquid chromatography high-resolution mass spectrometry and the quantitation of the chemicals by liquid chromatography tandem mass spectrometry. The authors also did a steroidogenesis to identify xenobiotics that target intracellular components that comprise the steroidogenic pathway. They also used Virtual-ToxLab, an in silico tool used to predict the endocrine and metabolic disruption potential of the compounds in study. It calculates the toxic potential and the binding affinity of any molecule to 16 proteins. And finally they did a statistical analysis.

BPA was found most often in a range of 5.6 to 30.4 mg/g. Additionally, only three alternative substances: BPS, Pergafast 201 and D8 were detected in a range from 3.3 to 13.2 mg/g. In fact, in many reported studies BPA was found with a detection frequency between 44 and 100% and with a concentration up to 28 mg/g. In a study performed by Geens et al in 2012, 44 thermal paper samples collected in Belgium were analyzed. BPA was detected in all the samples and 73% of the samples had concentrations between 9 and 21 mg of BPA/g of paper. In another study performed by Ted Mendum et al, in 2010 on the concentration of BPA in thermal paper, 8 of the 10 receipts that were tested had quantifiable concentrations of BPA.

By studying cell line H295R viability, cytotoxicity could be measured and only in the highest concentration (100 µM) this parameter was significantly affected when considering BPA, that shows a 23% drop in cell viability, Pergafast, a 43% decrease and D-8, a 26% drop (Goldinger et al., 2015).

In what concern to the studies in vitro, made in order to understand the effects of these compounds in steroidogenesis, it was observed a significant increase of 17β-estradiol concentration for BPA and BPF. It was also observed that BPF seem to be more potent than BPA, since the increase in 17β-estradiol concentration was approximately 15% higher. In what concerns to BPS, Pergafast 201 and D-8 they did not show any significant effects on 17β-estradiol level (Goldinger et al., 2015).

Additionally in a study performed by Rosenmai et al, 2014 they also investigated the effects of BPF and BPS on steroidogenesis and those results are in agreement with the ones presented in this study. This group investigation aimed to compare the hazards of bisphenol B (BPB), Bisphenol E (BPE), Bisphenol F (BPF), Bisphenol S (BPS) to BPA. Thus, they also studied the other hormones intermediates of the steroidogenesis pathway. They saw that all test compounds cause the same qualitative effects on estrogen receptors and androgen receptors activites, and most of the alternatives exhibited potencies within the same range as BPA.

On the other hand, concerning the effects on free testosterone level, a decrease was seen for BPA and BPS, whereas no significant effects were seen for BPF and D-8 (Goldinger et al., 2015).

To support the findings that were made in the in vitro steroidogenesis assay was performed a toxic analysis in order to study the toxic potential (TP) and the binding activities of BPA, BPF, BPS, Pergafast 201 and D-8 to 16 proteins. The TP values were defined as a range from 0 to 1, where 0 correspond to non-toxic effect and 1 to an extreme effect and could thus be interpreted as toxic alert. The TP values that were calculated for BPS, D-8, BPF and BPA lay between 0.380 and 0.476, showing a moderate risk of binding the receptors. Only Pergafast 201 showed a low risk of binding, with a TP value of 0.269. In the binding activity assay, it was observed that the main target for all compounds except Pergafast 201 was the estrogen receptor β (Goldinger et al., 2015).

This study (Goldinger et al., 2015) is one of the first to find substitutes of BPA other than bisphenol in thermal papers, like D-8 and Pergafast 201. There is only limited data, up to now, available on the endocrine activity of D-8 and Pergafast 201 and in fact, this study (Goldinger et al., 2015) represents the first time that a steroidogenesis assay was made with these two substances. There is no indication that Pergafast does exhibit hormonal activity. In what concerns to D-8, although it is structurally related to BPS, it was found no effect neither on the concentration of 17-β estradiol nor on free testosterone, which suggests that this substance does
not influence steroidogenesis. However, the metabolic activity is very limited in this test system, so it could not be excluded that a metabolic activation of Pergafast and D-8 would lead to estrogenic activity.

Concluding, substitution of BPA by its structural analogs BPF and BPS should be considered with caution, since the endocrine activity exhibit by them is similar to the one showed by BPA. With this study other alternatives were analyzed and although D-8 and Pergafast were presented as possible good alternatives for the replacement of BPA when considering their steroidogenesis effect, the truth is that further studies are required since steroidogenesis could be affected by several mechanisms such as binding to pathway enzymes or modulation of metabolism.


Living on the edge: Populations of two zooplankton species living closer to agricultural fields are more resistant to a common insecticide

From Bendis RJ et al., Environmental Toxicology and Chemistry, 33:2835-2841, 2014

Analyzed by Marília Silva, Rui Martins, Sónia Ruivo

The paper analyzed in this news article is a study of the resistance of freshwater zooplankton to a common insecticide, chlorpyrifos and also the correlation between resistance of popula-
tions and their distance to agricultural fields (Bendis et al. 2014).

Nowadays it’s clear the environment is the target of anthropogenic actions that force organisms to adapt to newly imposed conditions in order to survive. Pollution itself has been implicated in a range of detrimental outcomes and pesticide pollution is one of the biggest concerns in the agricultural industry. The worldwide large scale use of pesticides has led to the evolution of resistance in targeted-pest species being a major motivation to determine how insecticide resistance evolves in pest species to prevent further evolution in the future.

Although resistance in targeted species is a downside of pesticide use because the physiological mechanisms that confer resistance in targeted species are largely evolutionarily conserved, we might expect non-target organisms to be capable of evolving resistance as well. Freshwater zooplankton is an example of these non-targeted species. Previous studies have confirmed that the evolution of increased resistance in freshwater zooplankton is possible and also suggest that the exposure of natural populations of zooplankton to pesticides may impose selection for resistance. However, considering the natural populations as a study target the determination of whether natural populations with different proximities to pesticides are more resistant when located closer to areas of pesticide application needs to be further studied being one of the aims of the studied carried out by Bendis and collaborators (2014).

Two experiments were performed with Simocephalus vetulus and Daphnia pulex respectively where the authors considered the natural population of zooplankton. (Coors et al. 2009) The used pesticide was the organophosphate chlorpyrifos that is a widely used and also commonly found in water bodies being highly toxic for aquatic invertebrates, such as zooplankton. The populations collected from ponds with higher percentage of agricultural land were more resistant to the insecticide than those collected from ponds with lower percentage of agricultural land. This finding evidences the evolution of resistance of the species analyzed to chlorpyrifos. (Bendis et al. 2014) This can be triggered either by a selection with the insecticide in study or by other insecticides that act in a similar physiological way providing a cross-resistance.

Prior studies have shown that low concentrations of insecticide can decimate zooplankton populations. This event triggers an exponential increase of phytoplankton that eventually may lead to a bloom which ultimately causes reduced growth and development of the remaining pond species changing the dynamic of the aquatic ecosystem. The existence of populations with a level variation of resistance to pesticides may have community-wide consequences. The authors claim that this evolution in resistance can be actually beneficial in the point of view of the whole dynamic of the aquatic ecosystems, once it can buffer the detrimental effects that agrochemicals initiate. (Bendis et al. 2014) This is one hypothesis that the authors intend to verify in further studies.

Major core finding of this work was the positive correlation between evolved resistance of zooplankton to chlorpyrifos and proximity of the populations to agricultural fields were this pesticide was used, being on one of the first reports to obtain statistically significant results when considering the natural populations as a study target. The use of natural populations which better reflect the behavior of populations exposed to the pesticide is a way to guarantee the accurateness of the results obtained. This will enable a better prediction of how ecosystems are affected and react to the presence of pesticides leading to a better evaluation of the risk of pesticide use in agriculture.

Other related studies include findings regarding adaptation to pesticides by marine invertebrates when exposed to Cyfluthrin (an insecticide) and Napthalene (a polyaromatic hydrocarbon), both can easily enter aquatic environments either by agricultural application or by run-off. This resistance allows these organisms to adapt to the existence of pesticides in the ecosystem, and there are cases where adaptation to a certain pesticide confers cross-resistance to other pesticides that are not present in the ecosystem (Brauch et al 2009). Other Studies have also been conducted to analyze if the adaptation to the pesticides affects the fitness of the populations. It was found that evolved resistance to Carbaryl confers a greater susceptibility to an endoparasite P. ramose. The populations adapted to this pesticide show greater rate of infection by this endoparasite, which reduces the rate of natural increase of the populations adapted to the pesticide and may be one negative aspect of the evolution
of resistance (Jansen et al. 2011; Coors et al., 2008; Jansen et al., 2011a). Lastly one important capability of marine invertebrates is the capability of undergoing rapid microevolution acting as a “buffer” for the community, consequently enhancing the ability of the community to resist to some small environmental stresses (Doorslaer et al. 2007).

In summary, the findings of this work are important and relevant in the prediction of the adaptation of aquatic ecosystems to anthropogenic stressors. By studying how the natural populations respond to the presence of pesticides, in concentrations that are found in nature, it will be possible to perform a more rigorous risk assessment for the use of these compounds. Future work may include unravel of the mechanisms behind resistance and also verify if different levels of resistance are beneficial to the aquatic ecosystems dynamics.


Shale gas wastewater and formation of disinfection byproducts (DBPs) during disinfection processes
From Parker KM et al., Environmental Science and Technology, 48: 11161-11169, 2014
Analyzed by Jéssica Machado, Jássica Nereu

The development of shale gas (methane) has changed the energy portfolios and economies around the world. Many have considered that natural gas is and will be a sustainable alternative
to a conventional energy sources.[1]

Hydraulic fracturing is one of the techniques used to extract natural gas. In this technique fluids are pumped under high pressure in order to fracture low permeability geologic formations. This way, in this process three types of fluids can be considered: injected water (necessary to the perforation), flow back fluids (fluids that return to the surface when the pressure decreases) and produced waters (waters from the formation).[2][3] Unfortunately, this technique is associated with several risks related with water resources, namely: water withdrawals for hydraulic fracturing; wastewater treatment, discharge and disposal; methane and fluid migration in the subsurface; spills and erosion at the surface.[1]

One major concern is the contamination of shallow drinking water sources due to accidents or failures that culminate with the migration of contaminated waters. Another problem associated to this practice, as referred, is the spills and leaks of waste fluids and chemicals compounds, due especially to accidents, poor management and planning and illicit dumping.

The extraction process requires a huge amount of water from any source, superficial or groundwater, wherein we should emphasize that waste fluids can be drilling muds, flowback waters, produced waters or brine. These waters are frequently contaminated with chloride, bromide, calcium, barium, strontium, radium, and iron, demanding subsequent treatment (via publically owned treatment works – POTWs, commercial wastewater treatment plants – CWTs or on-site treatment approaches) in order to be reused, or discharge to the environment.

An effective disinfection system is automatic, simply maintained, safe and inexpensive that provides an ideal treatment of all the water. The chemicals used in this process should be easily stored and not make the water unpalatable, however the chlorination, chloramination and ozonation techniques don’t fulfill this last statement. The main difference between those three methods lies in the disinfectant utilized. In chlorination various types of chlorine or chlorine-containing substances are used. For chloramination, chloramine is employed, with further addition of chlorine and small amounts of ammonia which react to form chloramine. Lastly, ozonation uses ozone that is produced by an electrical corona discharge or UV irradiation of dry air or oxygen.[4] Related with this aspect is important refer that usually treated wastewaters are discharge directly in surface waters, and also that many of the contaminants, specially halides (chloride, bromide and iodide) are difficult to remove from water.[1]

Halides can react with decontamination chemicals, like: chlorine, chloride dioxide, ozone or chloramines, used in disinfection methods, and the respective products can react with dissolved organic matter (DOM) leading to the production of halogenated disinfection by-products (DBPs), among them tri-halomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs), haloacetalddehydes and N-nitrosodimethylamine (NDMA).[3][5][6] The presence of DBPs in drinking water can be very harmful to the public health, since the majority of these DBPs are mutagenic (can change the sequence of DNA) and/or genotoxic (can cause mutagenesis and DNA damage).[6] For instance, THMs are compounds derived from methane in which one or more hydrogens were substituted by halogens. These compounds can be formed mainly during chlorination and chloramination.

The formation of brominated trihalomethanes (THMs) have been associated with high levels of bromide and they are tendentiously more toxics than their chlorinated analogous; in particular, the ratio chloride/bromide seems to be an important factor for the production of these toxic compounds. As well the ratio Br/I seems to contribute for the formation of iodinated THMs, which are more cyto- and genotoxic than their brominated homologous.[7][8][1]

Considering chloramination, high levels of bromide and iodide in water can originate a human carcinogenic and extremely hepatoxic N-nitrosodimethylamine (NDMA). Its carcinogenic effects are associated to the conversion into potent genotoxic metabolites, causing tumors on esophagus and specially liver.[6]

During ozonation in waters rich in bromide, bromide (carcinogenic) is formed, while iodate (not so problematic) can occur in waters rich in iodide. These brominated by-products, like aldehydes or ketones, are produced by the oxidation of Br to hypobromus acid or hypobromite. The major concerns related with the formation of these compounds is associated with salt waters intrusions, where the ozone has the potential to produce THMs or HANs if the Br
ion is present along with natural organic matter.[6][9]

Finally, the halocetonitriles (HANs), that results from halogenated acetonitriles, produced during chlorination process are extremely reactive, inducing DNA damage in mammalian cells[6]. These nitrogenous by-products that tends to be more cytotoxic and genotoxic than THMs, present the ability to turn gun tissues radioactive by covalent bonding and increasing of the lipid peroxidation.[6][9][10]

Several articles, including the work performed by Parker et al.[7], have reported that the levels halides in surface waters next to wastewater treatment plants are elevated.

More deeply, the study performed by Parker et al. 2014 focused on the impact of diluted HFW, from shale gas extraction, on DBPs formation in the course of disinfection water processes, like regulated THMs and nonregulated HANs as well as Iodo-THMs during chlorination, NDMA and Iodo-THMs during chloramination and bromate during ozonation. This article exposes the environmental problems associated with high levels of bromide and iodide present in waters from hydraulic fracturing of shale gas, which may promote the formation of DBPs during chlorination, chloramination and ozonation. Hydraulic fracturing is an established technology that has been used in the oil and gas industries for many decades and shale gas has diversified domestic energy supplies and reduced US dependence on imports of liquefied natural gas. Since this gas is in such expansion it’s important to review the impacts that this extraction process causes to the environment and consequently to human health. That’s why the study by Parker et al 2014 is important to improve the safety and environmental performance of shale gas extraction.

Upgrading the monitoring (before and after fracturing), planning, good practices of management and legislation revisions enforce operational best practices and are the first step to minimize technical complications.[1] The production of methane and harmful DBPs should be monitored, as well as potential leakages of methane and other gases to the atmosphere. The formation of DBPs can be prevented with the prior removal of organic matter and/or discriminatory removal of bromide from water treatments, as well, by control of some conditions of this procedure (pH, temperature).[2][11]

In conclusion, the most important is ensuring well integrity remains the highest priority to prevent contamination.

Global energy is mainly produced by combustion of coal, with its numbers surpassing all non-fossil fuel sources combined. Its activity leads to greater carbon emissions and to large quantities of waste water. A typical 1000MW power station produces 500,000L of metal-contaminated water per year\textsuperscript{[1]}. Increasing water security and power requirements in water scarcity parts of the world will lead to the direct competition of water resources for human consumption and Industrial use in the next decade\textsuperscript{[1][2][3]}.

Coal-Fired power stations produce Ash-Water (AW) by washing and disposing the residual ashes produced by combustion of coal. This AW is rich in toxic elements, cannot be discharged and is stored in “Ash Dams” (AD), posing a threat to watersheds and representing an inefficient use of scarce water resources\textsuperscript{[4]}. AW poses a persistent threat after power-stations are decommissioned, and few treatment options exist\textsuperscript{[5]}. Some doubts arise concerning the rentability of macroalgae-based bioremediation. This strategy could develop more market acceptance if biomass produced was used as feedstock for Biochar production. Biochar is used to ameliorate soils, improving nutrient retention and reducing greenhouse gas emissions\textsuperscript{[8]}. Furthermore, “syn-gas” – a by-product of biochar production – can be used as bioenergy, returning high energy yields from the process\textsuperscript{[9]}

Cultivation of macroalgae in conjunction with biochar production has the potential to deliver bioenergy with biological carbon capture and storage\textsuperscript{[10]}. In this study\textsuperscript{[11]} a world-first validation of large-scale macroalgal cultivation and bioremediation is conducted at an Australian coal-fired power station demonstrating a sustainable means of producing biomass for value-added applications.
Tarong power station is in activity since 1984; this station has a capacity of 700MW and has accumulated 46,000ML of AW contaminated with metal elements, such as Aluminum, Arsenic, Cadmium, Chromium, Copper, Nickel, Selenium and Zinc which, according to the Australian and New Zealand Environment and Conservation Council (ANZECC), are in excess of the water quality guidelines.

An endogenous macroalgae - *Oedogonium* - was isolated from the AD, cultured in controlled conditions, and reintroduced in smaller AW ponds. Its growth was measured in 3-4 day cycles, achieving growth rates equivalent to a 50% increase in dry weight per cycle - placing biomass productivity at 5.6 g DW m\(^{-2}\)d\(^{-1}\) (20.4 metric tons ha\(^{-1}\)yr\(^{-1}\)). Carbon capture rates were, on average, 1.9 g C M\(^{2}\)d\(^{-1}\) (6.9 t C ha\(^{-1}\)yr\(^{-1}\)) which are very close to those observed in species used in terrestrial carbon capture, such as *Miscanthus giganteus*, with similar growth rates, and carbon capture rates of 5.2-7.5 t C ha\(^{-1}\)yr\(^{-1}\). This would correspond to less than 0.01% of annual carbon emissions from the power plant\(^{[11]}\).

Concentrations of metallic elements were measured with each harvest and a direct correlation between biomass production and metal bioremediation was found; three groups of metallic elements were identified, according to bioremediation rates: Al and Zn had the highest rates (3.1 and 1.5 mg m\(^{-2}\)d\(^{-1}\), respectively); As, Cu and Ni had intermediate rates (0.22-0.43 mg m\(^{-2}\)d\(^{-1}\)); Cd, Cr and Se had the lowest rates (below 0.1 mg m\(^{-2}\)d\(^{-1}\)). All had final concentrations below the initial concentration in AW, but above the regulatory limits for discharge.\(^{[11]}\)

All bioremediation rates exceeded the estimated rates of new metal inputs each year, meaning that bioremediation could nullify the new inputs of metals from the power station; were the AD be dedicated to bioremediation, this power plant could, theoretically, achieve zero discharge of all elements within a decade (except for Se) and bioremediation of Al, Cu, Cr and Zn within 2-5 years.\(^{[11]}\)

This study demonstrates, for the first time at this scale, that the integrated cultivation of macroalgae is an effective waste water bioremediation technology which can deal with multiple contaminants simultaneously, making it interesting for application in new or existing power plants or in the deactivation of decommissioned stations.\(^{[11]}\)

The main uncertainty is how the sequestration rates of metals would change as the initial concentrations in AW change.\(^{[11]}\)

For biochar production, dried biomass was submited to pyrolysis at diferent highest heating temperatures (HHT), following MS analysis of the different elements that compose this bioproduct. Pyrolysis is the key feature for biochar production devoid of CO\(_2\) since thermochemical conversion occurs under an oxygen depletion environment, allowing carbon sequestration\(^{[12]}\).

Results showed that HHT is reversly proportional to yield and O:C ratio. Intermediate temperatures (450-750°C) revealed lower element concentrations in biochar leachates (55-64 mg L\(^{-1}\)), compared to biomass leachates (4123 mg L\(^{-1}\)) or biochar produced at 300 and 900°C (324 and 413 mg L\(^{-1}\) respectively). Leaching effect from biochar is an important criteria regarding metal immobilization, since contaminants present in biochar may have detrimental effects on soil properties and functions\(^{[13]}\).

A balance between C recalcitrance, metal immobilization and yield must be achieved to optimize biochar production. Overall, regarding this parameters, the best suited biochar was that produced at 750°C.

This study reveals that *Oedogonium*
is promising for biochar production and HHT is a critical factor considering different parameters. Biochar can be suited for soil improvement, as a carbon sink and a way of mitigating CO₂ emissions.

Other studies reveal that biochar can be a good adsorvent, regarding other parameters such as pH, cation exchange capacity, heterogeneous macroporous structure and surface group functionality. This parameters are also crucial for nutrient retention, especially when applied to degraded and more acidic soils, increasing fertility.

However, little experimental evidence is available on the short and long-term occurrence and bioavailability of metal contaminants in biochar and biochar-enriched soil. Biochar pH and cation exchange capacity can also compromise more delicate soil proprieties, and ecosystems of those soils. Also, since biochar is a much more stable carbon source comparing to any organic matter or coal, administration procedures must be optimized when applied to soil amelioration.

Anaerobic biodegradation of nonylphenol in river sediment under nitrate- or sulfate-reducing conditions


Analyzed by Bernardia Teixeira, Pedro D. Teixeira, Prashneel Kumar, Nicholus J. Mukhwana

Nonylphenols are a family of closely related organic compounds called alkylphenols with linear or branched carbon chains. They are produced industrially, naturally and by the environmental degradation of alkylphenol ethoxylates. Nonylphenols (NPs) and sub-products are commonly used in manufacturing antioxidants, lubricating oil additives, detergents among others and are released to the environment through the waste water system from industries. They accumulate in environmental compartments with high organic content, such as sewage sludge and river sediments due to their low solubility and high hydrophobicity. They are semi-volatile capable of water/air exchange due to their vapour pressure ($2.07\times10^{-2}$ Pa) and Henry's law constant ($8.39\times10^{-1}$ Pa m$^3$/mol), and can be transported to aquatic and terrestrial ecosystems by wet deposition. They have a log $K$ value of 4.48 and low solubility in water. Their high partition to organic matter, cause them to be adsorbed to sludge solids instead of being biodegraded in standard Wastewater Treatment Plants (WWTP’s). These characteristics prolonged their persistence in sediments, with an estimated half-life of more than 60 years.

Nonylphenols are hormone disruptors and mimic the natural hormones like estrogen which makes them harmful to aquatic ecosystems. Studies conducted by Wang et al and Chang et al show that some bacterial species present in the fresh water sediments can biodegrade NP thus creating interest in identifying the microorganisms using molecular tools. Since the sediment environment is anaerobic, the researchers of the paper under analysis in this news article focused in identifying the best reducing conditions that favors NPs biodegradation.

Nonylphenol Biodegradation

In the study of Wang et al., the anaerobic degradation of nonylphenol was assessed under nitrate and sulphate reducing conditions, analyzing the difference in the biodegradation rate and microbial community complexity. Samples of sediment microcosms obtained from Wenyu River (Beijing city, China) were treated with sodium nitrate and sodium sulphate independently and the respective controls were composed of sterilized sediments. They were incubated during 90 and 110 days for nitrate and sulphate reducing conditions, respectively, and the residual NP was measured by High Performance Liquid Chromatography at specific time intervals. It was found that under both conditions linear NP was degraded, although at a higher rate in nitrate-reducing conditions (Figure 1 – [I]).
Information on NP anaerobic biodegradation in sediment ecosystem is still very limited. Two independent studies suggest that NP in sediment could be more easily biodegraded under sulfate than nitrate-reducing conditions \cite{10, 11}. Other authors claim that NP could only be slowly biodegraded under nitrate-reducing condition \cite{12}, contradicting the results shown in this article: NP biodegradation (100 mg kg$^{-1}$) in river sediment microcosms occurred under both nitrate and sulfate-reducing conditions, at higher rate for nitrate conditions. The contradicting results can be explain by the different test conditions and the inoculum used. It cannot be assumed that nitrate and sulphate are the only nutrients that will affect the biodegradation of NP; there are several nutrients available in varying quantities in the environment which could affect the results.

### Bacterial community diversity and composition

The Illumina MiSeq method was used to identify the bacterial taxa present in the studied conditions. It was found that for nitrate-reducing conditions in the sediment, the bacterial diversity increased while for sulfate ones a slight decreased occurred. In nitrate-reducing conditions, 11 bacterial phyla were identified, of which the largest comprise Proteobacteria, Bacteroides and Chloroflexi. For samples under sulfate-reducing conditions, 12 bacterial phyla were spotted, of which Proteobacteria and Firmicutes were the most predominant groups (Figure I – [II]). Given the abundance of Proteobacteria phylum, further analy-
sis for classes within this phylum was assessed, as shown in Figure 1 – (III).

Some articles have pointed-out the phylogenetic composition of aerobic NPs-degrading sediment microbial community indicating Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Bacteroidetes as the most abundant classes in river sediments (13, 14). The present study showed a more complex bacterial community structure, especially for nitrate reducing condition. Further analysis suggested a variation in the genera and quantity of microorganisms between the two reducing conditions.

However, the Illumina sequencing method requires previous sequencing information for correct microorganism identification, and this may not identify novel microorganisms that might be playing a major role in NP biodegradation.

In this study, only the linear NP was used, which is not representative for the branched NPs that have different biodegradation profile. Branched NPs are not as easily biodegraded as linear ones. A few bacterial species like Sphingomonas spp isolated are able to degrade the branched chain NPs (15). Furthermore, the incubation temperature used (28°C) is above the natural conditions, which could cause bacterial community analyzed to be much different from the natural one.

These results indicate that NP is much more persistent than previously reported, with 90 and 110 days tests in nitrate and sulphate conditions, respectively. Furthermore, this is the first metagenomics approach specifically designed to define the most important microbial taxa involved in NP bioremediation by natural river sediment community. Several important phyla were identified as the most abundant, with special presence of Proteobacteria, which include species with known capacity for biodegradation (16).

The presented study sets the base information for the improvement of NP biodegradation rate in the environment, which could lead to higher understanding in NP impact and natural bioremediation. Furthermore, new bioremediation strategies could be design through biostimulation of the identified taxa or by bioaugmentation with important microorganisms.

More research is still required to understand all variable effects (temperature, water quality, microbial community structure) to improve the complete biodegradation of this harmful environmental compounds produced by human activities.

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5. K.H. Langford, J.N. Lester, Fate and behaviour of endocrine disrupters in wastewater treatment processes


The lifestyle of our modern’s societies may be generating – to both the environment and wildlife – unpredictable effects and consequences, to which currently, we may not be aware and prepared. With technology and knowledge evolving every day in such a mutable speed, we risk to do not let enough time pass to evaluate its outcomes. By inserting foreign compounds into the environment, even in small quantities, we may be damaging wildlife itself and its life cycles – meaning that the repercussions may reoccur again in human beings.

One of the most direct ways of introducing foreign agents into nature is through the effluents of wastewater treatment plants (WWTP). Pharmaceuticals and personal care products (PPCPs) are contaminants that have been found in wastewater and surface waters around the world. A major source of these compounds is the incomplete metabolism in humans and subsequent excretion in human waste. [1]

According to the study performed by Westerhoff et al. (2005)[3], endocrine-disrupting compounds (EDCs) and PPCPs are nowadays not satisfactorily removed by common drinking water treatment processes, raising some concerns.

One pharmaceutical found in particularly high abundance in recent WWTP effluent and surface water studies, is metformin, one of the world’s most widely prescribed antidiabetic drugs, and also a
treatment for cancers as polycystic ovary syndrome. Metformin is thought to act primarily by inhibiting the complex I of the electron transport chain, increasing the cellular AMP: ATP ratio, and activating the regulatory kinase (K) AMPK. Acting as a cellular energy sensor, AMPK phosphorylates enzymes and transcription factors, inhibiting gluconeogenesis and activating glycolysis.

However, the effect directly related to endocrine disruption is the improvement in glucose uptake. By acting on metabolic pathways to promote catabolism and glucose uptake, metformin sensitizes cells to insulin, affecting pathways regulated by insulin signaling – that will subsequently influence steroidogenic pathways.

The endocrine system is a set of glands and hormones that help in development, growth, reproduction and behavior. Some compounds, known as EDCs, may interfere with the glands, the hormones, or their site of action, altering the circulating amounts.

It has been shown before that metformin affects the expression of steroidogenic enzymes such as cytochrome p450 (CYP) 11A and 3b-hydroxysteroid dehydrogenase (3b-HSD), as well as the cholesterol transporter steroid acute regulatory protein (and in mammals, the CYP17 activity). Although metformin is one of the most prevalent pharmaceuticals in WWTP effluent, its impact on aquatic life has only been explored for its metabolic effects, in aquaculture or drug screening, and the generally considered endocrine-disrupting effects have not been studied at all in aquatic organisms. Interactions between insulin signaling and steroidogenesis may suggest potential disruption. It has been suggested that the appearance of intersex fish in watersheds may be the result of WWTP effluent with its cocktail of (potential and known) disruptors.

The aim of the study performed by Niemuth et al. (2014), was then to determine whether a chronic exposure of adult Pimephales promelas, the North American fathead minnow, to the levels of metformin found in WWTP effluent would cause detectable endocrine disruption. Fathead minnows are commonly used to evaluate endocrine disruption in fish.

The experience consisted in twelve tanks, divided, with a male-female pair in each compartment. Six tanks remained as control, while the other six were supplemented with a solution of metformin to achieve the concentration of 40µg/L. A 16:8h light:dark cycle was kept, and food given twice a day. A 100% water change occurred each 4th and 7th day of a week, for 28 days, before euthanization. Weight and length were measured and blood taken out of the caudal artery/vein. The liver and gonad were collected and flash-frozen in liquid nitrogen; part of the gonad was stored in formalin for histopathological evaluation.

RNA was extracted from liver and gonad to evaluate the expression of genes associated with metabolism and reproduction (metabolism-related genes like the ones encoding GK, FBPase, and FASN, had been observed before to be affected by metformin exposure in mammals and other fish studies), other fathead minnow genes known to be affected in other studies due to exposure to chemicals in wastewater (e.g. encoding CYP3A4 and the pregnane X receptor (PXR), both involved in steroid synthesis and metabolism of xenobiotics), and, lastly, the steroid synthesis gene CYP19A.

The mRNA transcripts encoding for the egg yolk precursor protein vitellogenin (VTG) were also examined. These are usually produced in the liver of oviparous females, and the expression of VTG (mRNA and protein), in male, became a metric indicator of endocrine disruption. Reproductive endpoints were also considered: egg production (eggs counted daily), plasma hormone levels – VTG and testosterone in males (only 5 fish per treatment were used due to difficulties in obtaining samples) and the expression of genes in adult fathead minnows – GK, FBPase, FASN, and PXR (only 8 samples per group due to low RNA liver yields).

Of all the tested parameters, one sustained the proposal of endocrine-disruption: VTG mRNA was expressed at a significant higher level in metformin-
treated males comparing to control males.[1]

As expected, females expressed levels higher than males, but with no difference to control. However, no differences were observed in the expression of the endocrine-related parameters (steroid acute regulatory protein or anti-Müllerian hormone, in the liver, and no difference in between treatments was found for CYP17, CYP19A, or 3β-HSD, in male gonad).

There were also no spotted differences in expression found for GK or FBPase, both metabolic genes, or for CYP3A4, CYP11A and CYP17, detoxification genes – either in treatment or sex. There was, however, a difference between sexes for FASN and PXR, metabolic and detoxification genes – both higher in males. [1]

Although plasma VTG protein levels were found to be higher in metformin-treated male fathead minnows, the difference was not significant, and there was no difference found in plasma testosterone levels between control and treated males. There was also no difference in mean of total eggs, spawning frequency or clutch size, and histopathology of male gonad (normal appearance of gonadal cells in testis).[1]

To conclude, the study calls for more research to understand fully how metformin induces VTG expression in male, and why it does not affect the other indicative-parameters. Also to understand the differences in metabolism between gender. Perhaps the study was too brief, or the species were not sensitive enough through the route of administration used. A study with higher concentrations of metformin, longer time of exposure, and different species should be performed for further clear conclusions.


Lead toxicity to the performance, viability and community composition of activated sludge microorganisms

From Yuan L CA et al., Environmental Science and Technology, 49: 824-830, 2015

Analyzed by Antoine Kühner, Carsten Pichler, Josquin Puntos-Dami

Background

Lead (Pb) is a heavy metal. That means it has a density between 3.5 g/cm³ and 7 g/cm³ and fills other criteria. It is the least toxic element of the heavy metals, but is listed second, after mercury (Hg) and before arsenic (As), on the substance priority list (SPL). That list takes into account the frequency, toxicity and potential for human exposure at national priorities list sites.
(hazardous waste sites). It has such a place due to its ubiquitous presence in the environment because Pb was and is still used in so many applications that our environment is downright contaminated with this element.

Through different causes and ways Pb ends up in big waste water treatment plants (WWTPs) where it causes issues for the microorganisms. Although there are short-term studies (<24 h) about the effect of lead to microorganisms, no long-term studies were done so far. So the researchers of this paper made a study about the long-term effect (7 days) of microorganisms in activated sludge. This study is of great importance because activated sludge is the most widely used aerobic treatment option and lead might have a big effect on it.

Results
To do the experiment, the authors took fresh activated sludge from a local WWTP and exposed it to different Pb concentrations (from 0 mg/L to 10 mg/L), knowing that the maximum found in real situation was 15 mg/L. They studied the effects of different exposure times to lead contamination, from 1 day up to 7 days and also the recovery period from day 8 up to day 19. The researchers of this paper were interested in monitoring the following parameters in an activated sludge exposed to different concentrations of lead:

- **COD (chemical oxygen demand):** it measure the amount of organic compounds in water
- **ammonia-nitrogen removal rates**
- **SOUR (specific oxygen uptake rate):** it measures the rate of different metabolic processes that take place in the sludge
- **bacterial viability**
- **community structures**
- **sorption processes**
- **Sensitivities of microorganisms** especially of heterotrophs, ammonia oxidizing organisms and aerobic microorganisms.

Through this experiment, they found out that the lead toxicity was greatly underestimated so far and even spontaneous events (unique contamination) could have long-lasting effects (see fig.1). For example, the ammonia-removal communities never recovered even from a 1 mg/L exposition. So the lead has big impacts for ammonia-oxidizing bacteria (AOB) compared to COD removal rate and SOUR as you can see in Figure 1 - Figure 4.

![Figure 1](image1.png)

**Figure 1.** Rate of NH3-N removal, adapted from Figure 1. in Yuan et al.

![Figure 2](image2.png)

**Figure 2:** COD removal rate, adapted from Figure 1. in Yuan et al.

As you can see in Figures 1-3, every lead concentration after 7 days has its own effect on the microorganism community in the activated sludge. It is also evident from the
Figures that after a recovery period of 11 days the microorganisms had recovered their former level or even increased as you can see in Figure 3, except for ammonia removal communities. It implies that if it is possible to reduce the lead concentration input in the WWTP, most of the communities would be able to recover.

We can see in Figure 4 that the higher the lead concentration in the samples the more decrease the quantity of living microorganisms. That means if it is possible to reduce the lead concentration in our environment the impact on WWTP will be smaller, which is very important for the sewage treatment.

Figure 4: The impact of different Pb concentrations after 7 days to the microorganisms. Green are living bacteria and red are dead bacteria. (LIVE/DEAD coloration process). Adapted from Figure 2. in Yuan et al.1

Figure 5 shows that after the recovery period the microorganisms were able to increase in the samples except of the Nitrospira. This Bacteria group disappeared almost totally.

Figure 5: This graphic shows the community composition of day zero and day 20 of communities exposed to different lead concentrations, obtained through 16S rRNA gene sequencing. Adapted from Figure 3.a) in Yuan et al.1

Another important conclusion of this paper is that they found out that the absorbed lead played a role in the contamination calculations (total lead gave better correlation than only the dissolved one)1. This is in complete contradictions to the previous studies on this subject1-7 that proved absorbed heavy metals to be irrelevant in this case, except for one that assessed the importance of absorbed copper ions8.

Conclusion

This study is very important regarding WWTP’s management, since it states that lead (that is present everywhere) has a huge effect on the communities present in activated sludge. It has important implications regarding the management of the Plants and can change the way we see this heavy metal regarding contamination. Furthermore, the conclusion about absorbed lead having a toxic effect is important, but would require more studies to be proven or refuted.

2 http://www.atsdr.cdc.gov/SPL/
5 Hsieh, K. M.; Murgel, G. A.; Lion, L. W.; Shuler,

