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Abstract:	The World Health Organization reported that " an estimated 12.6 million people died as a result of living or working in an unhealthy environment in 2012, nearly 1 in 4 of total global deaths ". Air, water and soil pollution, were significant risk factors, and there is an urgent need for effective remediation strategies. But tackling this problem is not easy; there are many different types of pollutants, often widely dispersed, difficult to locate and identify, and in many cases cost-effective clean-up techniques are lacking. Biology offers enormous potential as a tool to develop microbial, and plant-based solutions to remediate and restore our environment. Advances in synthetic biology are unlocking this potential enabling the design of tailor-made organisms for bioremediation.
Author Comments:	Authors are joint corresponding authors

Dear Joanna Aldred,

We have made the requested corrections in the chemical structures in the manuscript Tables, specifically:

- Carboxylic acid on ibuprofen structure is missing a 'H'. Corrected.

- Some methyl groups are labelled 'CH3' (as in ibuprofen) whereas others are not (as in Gemfibrozil), please ensure this is consistent across the table. Corrected.

- Please make sure that all bond angles in the structures are correct with no clashes. We have checked the bind angles.

We hope this meets with your approval.

Yours sincerely

Neil Bruce and Liz Rylott

How Synthetic Biology Can Help Bioremediation

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Abstract

The World Health Organization reported that "*an estimated 12.6 million people died as a result of living or working in an unhealthy environment in 2012, nearly 1 in 4 of total global deaths*". Air, water and soil pollution, were significant risk factors, and there is an urgent need for effective remediation strategies. But tackling this problem is not easy; there are many different types of pollutants, often widely dispersed, difficult to locate and identify, and in many cases cost-effective clean-up techniques are lacking. Biology offers enormous potential as a tool to develop microbial, and plant-based solutions to remediate and restore our environment. Advances in synthetic biology are unlocking this potential enabling the design of tailor-made organisms for bioremediation.

In this review, we showcase examples of xenobiotic clean-up to illustrate current achievements and discuss the limitations to advancing this promising technology to make real-world improvements in the remediation of global pollution.

1. Introduction

"*What I cannot create I do not understand.*" Nobel Physicist, Richard Feynman's quote from over 30 years ago, now encapsulates the burgeoning world of synthetic biology (synbio). The National Human Genome Research Institute defines synbio as "*a field of science that involves redesigning organisms for useful purposes by engineering them to have new abilities*". Synbio achieves its aims by using molecular biology tools, along with cell and systems biology knowledge to model, design and synthesize a series of components (gene promoters, transcription factors, enzymes *etc.*) that together form metabolic pathways with outputs that can be tested, re-modelled and fine-tuned. At the simpler end of the scale, this translates to engineering proteins with the ability to convert substrates to desirable products; at the more complex end, the synthesis of complete, artificial genomes. In the world of bioremediation, synbio could be used to design biosensors, enzymes with unique activities towards persistent organic xenobiotics, organisms that are resistant to challenging environmental conditions, robust biopolymers, artificial storage organelles for toxic metals and much more.

2. Synthetic biology resources

Numerous molecular biology techniques together provide a practical toolkit for synbio, and key among these are massively improved DNA sequencing and synthesis. The wealth of genetic data now available has enabled us to investigate how natural biological systems work. In February 2020, the National Center for Biotechnology Information database contained almost four hundred billion nucelotide bases (www.ncbi.nlm.nih.gov/genbank/statistics/). Advances in Golden Gate cloning and synthetic promoter systems, in tandem with the ability to relatively cheaply synthesize DNA sequence have enabled a modular approach to assembling genes for multiple enzymes and metabolite transporters [1]. Genome-scale engineering is now at the stage where ~4 mega base bacterial genomes are routinely re-engineered [2], with research to redesign and synthesize all sixteen chromosomes encoding the 11.4 Mb genome of the eukaryote *Saccharomyces cerevisiae* near completion (Synthetic Yeast 2.0; http://syntheticyeast.org/). The Genome Project-Write (https://engineeringbiologycenter.org/) is now engineering gigabase genomes of higher-order

eukaryotes [3]. Systems biology and protein design, which use computational and mathematical techniques to model complex biological systems, are also key resources for synbio. In combination with gene editing, which allows small, ideally single-base, changes to an organism's DNA [4] [5], these disciplines allow the fine-tuning of biological systems. Into the future, re-programming the genetic code to include unnatural amino acid will open up the ability to use biological systems to synthesize a near-endless number of different proteins [6].

For the development of specifically bioremediation technologies, key synbio resources include the use of extremophilic microorganisms. These organisms provide a wealth of enzymes adapted to work in extreme environments under which other proteins would denature. For example, halophilic bacteria with abilities to degrade polycyclic aromatic hydrocarbons, and petroleum from highly saline wastewaters [7,8]; thermo-tolerant microalgae *Galdieria sulphyraria*, in combination with heterotrophic bacteria to remediate ammonium and phosphates from waste water systems providing a biofuel output, without the energy-intensive need to cool the photobioreactor system [9]. Functional metagenomics approaches have enabled the discovery, and characterization, of novel enzymes such as dioxygenases [10*] and cytochrome P450 systems [11]; key players in xenobiotic-degradation.

3. Environmental pollutants

While small areas of contamination can be removed using existing methodologies for example excavation to land-fill, or *ex-situ* remediation, a specific problem with many environmental pollutants is that they are dispersed, often heterogeneously across relatively large areas. Remediating this pollution using current technologies would be too costly, generate huge amounts of waste and be environmentally damaging. Bioremediation can be a cost-effective alternative that can work at large-scale, and as a component of existing ecosystems contribute to the restoration of the environment.

3.1 Inorganic pollutants

The main inorganic pollutants are 'heavy metals' a group that includes Pb, Cd, Cu, Hg, Sn, and Zn (Table 1). Some of these elements are essential micronutrients, but all are toxic at higher levels, with As, Cd, Hg, Pb and Se most readily bioaccumulating in tissues and living organisms. Anthropomorphic inorganic pollution stems predominantly from the petrochemical and agrochemical industries, coal combustion and the mining industry. In addition to heavy metal pollution, global release of N and P from fertilizer, sewage and runoff from animal farms, cause eutrophication of waterways and lakes and thus significant harm to water quality and aquatic life.

3.2 Organic pollutants

Persistent organic pollutants (POPs; Table 2) include some polycyclic aromatic hydrocarbons (PAHs), along with halogenated aromatics, such as polychlorinated biphenyls (PCBs), explosives (2,4,6-trinitrotoluene, TNT; hexahydro-1,3,5-trinitro-1,3,5-triazine, RDX; and pentaerythritol tetranitrate, PETN), dioxins (polychlorinated dibenzo-p-dioxins and –furans), dichlorodiphenyltrichloroethane (DDT) and its metabolites; and more recently, per- and polyfluorinated alkyl substances (PFAS).

POPs commonly have very low water solubility and high hydrophobicity, measured by high octanol/water partition coefficients (log K_{ow}), which generally increase with additional aromaticity. These features reduce bioavailability and uptake, thus hindering natural attenuation by biological systems such as microbes and plants. These compounds are therefore retained in air, water, soils, and sediments for long periods of time; however, once in the food chain the high log K_{ow} values enhance their bioaccumulation in lipid-rich regions of the host organism, with subsequent biomagnification along the food chain. Together, these toxic compounds are some of the most persistent in the environment, with many of them additionally classified as possibly carcinogenic to humans (the International Agency for Research on Cancer ((IARC)) and probable human carcinogens (United States Environmental Protection Agency ((US EPA)). The Stockholm Convention, a global treaty for protecting humans and the environment against toxic contaminants, has listed more than twenty POPs (Stockholm Convention, 2018), with pressure from stakeholders to increase the list to include heterocyclic aromatic compounds and alkyl-derivatives [12]. Even though production and use of many

of these pollutants has significantly decreased since the adoption of the Stockholm Convention, extensive environmental contamination still persists.

Emerging pollutants (Eps; Table 3) are those not yet commonly monitored but have the potential to enter and negatively affect the environment and human health. These compounds include pharmaceutical and personal care products (PPCPs), many of which are biologically active [13]. PPCPs enter urban wastewater streams but are not removed by conventional treatment technologies and can recycle back into the food chain via their land application as fertilizers [14]. Furthermore, antimicrobial agents in PPCP waste have the potential to promote bacterial resistance in the environment [15].

Additional emerging pollutants include plasticizers and nanoparticles (NPs). Plasticizers are additives used to increase flexibility or plasticity, such as bisphenol A (BPA) and phthalates, and are particularly recognized as endocrine disruptors [16]. Manufactured NPs are present in many commercial products, including agricultural herbicides and pesticides and while the true effects of NPs in the environment is not yet well understood, there is evidence they are taken up by and have deleterious effects on crop plants [17].

4 Bioremediation using synbio

In purist terms, synbio techniques are currently used to modify, or artificially create, prokaryotic systems; the application of synbio to more complex, multicellular organisms is still in its infancy. Ambitious projects such as the C4 Rice project [18], (c4rice.com/the-project-2), and engineering nitrogen fixing cereals [19,20] are underway, with astonishing possibilities. But, in the area of bioremediation, the application of synbio techniques is still focused on the development of microbial-based systems. Plants contribute a major role in bioremediation, yet technologies are at the level of expressing one, or a few transgenes with true synbio techniques still to be established. In this section, we outline examples of current achievements in the application of synbio to bioremediation.

4.1 Biosensors

A lack of information on the presence of pollutants in soils, particularly in developing countries, [21], compounded by a lack of adequate controls and bad practice, has led to significant pollutant dumping sites in some Asian countries [22].

Bacteria, such as *Geobacter sulfurreducens* and *Shewanella oneidensis* have the ability to grow as highly conductive biofilms, composed mainly of hair-like structures called pili. These bacteria form the basis of microbial fuel cells (MFCs), which can produce electrical current from the degradation of organic pollutants [23]. The output voltage from MFCs has been used to demonstrate biosensors for *p*-nitrophenol in industrial wastewater [24], atrazine [25], formaldehyde [26] and continuous biomonitoring of copper from mine effluent [27]. New innovative designs are incorporating MFC-based biosensors onto paper to produce a low-cost, portable and easy-to-use format, that can biodegrade after use [26*].

4.2 Artificial organelles

A significant challenge to remediating inorganic pollutants is the inherent toxicity associated with accumulating these pollutants within sensitive cellular environments. Artificial organelles could enable the concentration of inorganic pollutants away from these areas [28]. Einfalt et al. demonstrated that reduction-triggered nanocompartments could be synthesized *in vivo* in HelA cells [29**]. And towards utilization for bioremediation, encapsulation of a polyphosphate kinase in *Escherichia coli* led to the increased uptake, and compartmentalization of phosphate [30*]; a potential application for phosphate removal. A recent study has successfully targeted proteins to the luminal side of an artificial bacterial microcompartment [31**]. These advances pave the way for the incorporation of proteins that can bind specific metals within artificial organelles, enabling the hyperaccumulation of specific metals.

4.3 Bioremediation of mercury

Mercury is ranked third in the priority list of hazardous substances by the Agency for Toxic Substances and Disease Registry (www.atsdr.cdc.gov) with hotspots of pollution coming from mining

and metal manufacturing. Microbial activities in the environment readily convert Hg to methylmercury, which bioaccumulates. Both forms are effective neurotoxins [32].

Previously, studies have used MerR transcriptional regulator to develop mercury biosensors. When Hg^{2+} ions bind to MerR, MerR is derepressed and the *mer* operon genes are expressed. Replacing *mer* genes with reporter genes such as GFP or luciferase produced mercury inducible biosensors; important tools, but not directly useful for bioremediation [32]. Subsequent studies have engineered bacteria able to sequester Hg^{2+} [32], but this is limited by the intracellular toxicity of the Hg^{2+}, and requires the continual production of cellular biomass to absorb the metal. An exciting study by Tay et al. [33], combined MerR and an operon encoding a mercury-absorbing, extracellular protein nanofiber, or curli, into *E. coli*. These curli fibers form a biofilm that is only produced when mercury contamination is present, and provide a large surface area for Hg^{2+} absorption, to negate the toxicity of intracellularly accrued Hg^{2+} ions. Furthermore, this circuit is responsive at environmentally relevant concentrations. The nanofiber specifically binds Hg^{2+}, and recovery and recycling not hindered by the presence of other metals. This work paves the way for the development of on-demand living biofilm materials that can operate autonomously as heavy-metal absorbents. There are, however, still hurdles to the advancement of this technology. For example, *E. coli* exhibits sensitivity towards mercury toxicity, and mercury-resistant microbial species are required.

4.4 Biodegradation of polyethylene terephalate (PET)

Originally designed to resist degradation, it is this very feature of plastics that is now a huge environmental problem. The build-up of plastics in our environment, particularly oceans, is causing devastating damage to animals. Accumulation of microplastics in the environment and food chain, is also of increasing public concern.

PET, a plastic used intensively in textile production, and as packaging for food and liquids, comprises about 10% of the synthetic plastic polymers produced globally. A number of enzymes with activity towards PET, albeit low, have been characterized. To date, the most promising species mined for PET-degrading enzymes is *Ideonella sakaiensis* 201-F6, isolated from sediment at a PET bottle

recycling site, and able to use PET as its main energy and carbon source. The two enzymes isolated from *I. sakaiensis* were a PET hydrolase, which converts PET to mono(2-hydroxyethyl) terephthalic acid (MHET); and MHET hydrolase, a structurally-unique enzyme, which converts MHET to terephthalic acid (TPA) and ethylene glycol (EG) [34]. Subsequently MHET was engineered with improved activity and additional polyethylene-2,5-furandicarboxylate (PEF)-degrading ability [35**]. Towards the use of this system in aquatic environments, extracellular MHET activity has been successfully conferred to the marine microalga *Phaseodactylum tricornutum* [36*]. Enzymes have also been found with activity towards the ester-based polyurethane (PUR), but as yet, enzymes with activity towards the remaining major plastic polymers (polystyrene, polyamide, polyvinyl chloride, polypropylene, ether-based polyurethane and polyethylene), which comprised over 250 million tonnes in 2016, have not been discovered [37].

4.5 Biodegradation of aliphatic chlorinated compounds

Bacteria, including *Xanthobacter autotrophicus*, have been isolated that can break down a broad range of halogenated aliphatic compounds [38]. Using 1,2-dichloroethane (1,2-DCA), which is listed as a priority pollutant and "*probable human carcinogen*" by the U.S. Environmental Protection Agency (EPA), as an exemplar, dehalogenase genes *dhlA* and *dhlB* from *X. autotrophicus* were incorporated into tobacco (*Nicotiana tabacum*) and together with endogenous alcohol and aldehyde dehydrogenase, used to create a synthetic route for the degradation of 1,2-DCA [39]. More recently, a complete, artificial pathway for the metabolism of 1,2,3-trichloropropane, a toxic pollutant and listed by the EPA as "*likely to be carcinogenic to humans*", has been engineered into *E. coli* [40]. The authors used computational models to identify bottlenecks in the five-gene pathway, and employed forward engineering to optimize 1,2,3-TCP degradation [41]. Many microbes have activity towards aliphatic chlorinated compounds, including microbial communities in the rhizosphere of plants. It has been demonstrated that metabolites released by plant roots into this zone can enhance the biodegradation of 1,2-dichloroethylene (DCE) [42*]. Combining genetically modified -plant and -rhizosphere-dwelling bacteria seem to be the next logical step.

4.6 Phytoremediation of explosive compounds

Explosive compounds 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are used extensively by the military and are significant environmental pollutants [43]. In the U.S. alone, 10 million hectares of military land is contaminated with munitions components, of which TNT and RDX are major components [44]. Numerous studies have characterized the biochemistry behind microbial detoxification pathways for TNT and RDX [43], and bacteria able to degrade RDX have been used in bioaugmentation studies in RDX-contaminated aquifers [45,46]. The genes responsible, *xplA* and *xplB* have been engineered into rhizosphere-colonizing bacteria [47], and *Arabidopsis thaliana* [48,49]. More recently, this technology has been advanced to produce RDX-degrading plant species suitable for remediation in-the-field. Both *xplA* and *xplB*, along with a bacterial nitroreductase that detoxifies the co-contaminant and phytotoxic TNT, have been engineered into switchgrass (*Panicum virgatum*), wheatgrass (*Pascopyrum smithii*) and creeping bentgrass (*Agrostis stolonifera*) [50*,51]. This advance brings the technology a step closer to using engineered, native species for *in situ* remediation of organic pollutants.

5. Future directions

The environment has only been relatively recently exposed to many organic pollutants, with anthropomorphically-derived chemicals only in widespread use from the 1900s. However, the existence of enzyme systems with degradative abilities towards these xenobiotic compounds demonstrates the remarkable speed at which microorganisms have evolved to exploit these substrates.

The slower regeneration times in eukaryotic, plant and algae organisms, compared to prokaryotes, means that they have simply not had sufficient time to evolve biochemical activities towards many organic xenobiotics. However, plant-based remediation systems offer many advantages, detailed in [43]. To maximize *in situ* bioremediation capacity, Figure 1 outlines where studies could focus on using synthetic biology to confer xenobiotic detoxification abilities to plants, in combination with their use as hosts for genetically-modified endophytic and rhizospheric microbial populations.

Many organic pollutants have chemical structures that are extremely challenging for biochemical-based mineralization. For example, PCBs often comprise of up to 130 different individual compounds; with biochemical degradation routes characterized for only a few [52]. Developing synbio techniques and mining expanding nucleotide databases will, in time, enable the design of enzyme-based systems to mineralize these compounds.

In contrast to organic pollutants, which have the potential to be mineralized, the bioremediation of inorganic pollutants presents a different challenge. There is a significant volume of literature demonstrating *in situ* removal of inorganic pollutants from soil, water and air into biological systems, but cost-effective systems to recover toxic metals and metalloids from this biomass is currently lacking. For elements of higher market value such as Ni, the biochemical mechanisms used by plant hyperaccumulator species need to be further understood. But, given the sheer quantity of relatively low-value metal and metalloid contamination, *in situ* approaches should perhaps also focus on trapping these pollutants into biological chelators such as metallothioneins, and absorbents such as nanofibers, which over time can become locked into soils and sediments and monitored with biosensors. Towards these goals, synbio approaches will enable the design of synthetic bio-based compounds and self-assembling artificial storage organelles to trap inorganic pollutants, or enable their cost-effective recovery.

The use of synbio technologies for bioremediation is still in its infancy, but already offers exciting possibilities towards the use of engineered organisms to provide a cleaner, safer environment.

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Tables

Table 1. Major inorganic pollutants
ATSDR 2019 Substance Priority List rankings (www.atsdr.cdc.gov/spl/index.html#2019spl).

Name	Chemical symbol	ATSDR 2019 Rank
Arsenic	As	1
Lead	Pb	2
Mercury	Hg	3
Cadmium	Cd	7
Chromium (hexavalent)	Cr^{6+}	17
Phosphorus (white)	P_4	19
Cyanide	CN^-	35
Beryllium	Be	43
Colbalt	Co	52
Nickel	Ni	58
Chromium(VI) trioxide	CrO_3	66
Zinc	Zn	75
Chromium	Cr	78
Thiocyanate	SCN^-	92
Asbestos	Silicate minerals	94
Radium-226	Ra	95
Uranium	U	97

Table 2. Persistent organic pollutants
All structures and chemical names are from PubChem, National library of Medicine (www.pubchem.ncbi.nlm.nih.gov). Numbers in brackets are the ATSDR 2019 Substance Priority List rankings (www.atsdr.cdc.gov/spl/index.html#2019spl).

| Name	Use/Source	Chemical structure	ATSDR 2019 Rank
Polycyclic aromatic hydrocarbons (PAHs) Emitted during combustion of organic material. Found in coal tar, oil and gas, and in vehicle exhaust fumes, cigarette smoke			9
Benzo(a)pyrene	Not commercially produced		8
Benzo(b)fluoranthene	Not commercially produced		10
Polychlorinated biphenyls (PCBs) Historically used as electrical insulators and coolants in mixtures such as Arochlor 1260, which is composed of 12% penta-, 38% hexa-, 41% hepta-, 8% octa-, and 1% nona-chlorobiphenyls			5
Explosives Used globally on military training and conflict, manufacturing and decommissioning sites, also used in mining and quarrying industries			-
2,4,6-trinitrotoluene (TNT)	Used with RDX, as the main components of military explosives		80
Hexahydro-1,3,5-trinitro-1,3,5-triazine(RDX)	Used with TNT, as the main components of military explosives		96
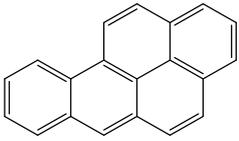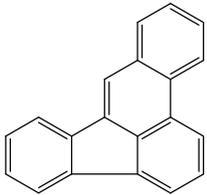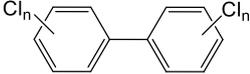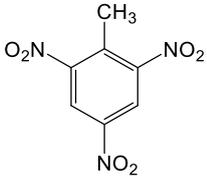
Pentaerythritol tetranitrate (PETN)	Used in plastic explosives such as Semtex, and also as a vasodilator to treat specific heart conditions		-
Dioxins (polychlorinated dibenzo-p-dioxins and –furans) Produced as unwanted by-products of chemical synthesis, and from the combustion of organic materials			-
Polychlorinated dibenzo-p-dioxins (*n* and *m* can range from 0 to 4)			-
2,3,7,8-Tetrachlorodibenzo-P-dioxin	Infamous as a production contaminant in Agent Orange		72
Polychlorinated dibenzofurans (*n* and *m* can range from 0 to 4)			-
Heptachlorodibenzo-*p*-dioxin	Unwanted industrial by-product. No known commercial applications		156
DDT p,p'-Dichlorodiphenyl-trichloroethane Insecticide widely used to control malaria and typhus, and on food crops			13
Per- and polyfluorinated alkyl substances (PFAS) Used as fire extinguisher foams, as a stain- and water-resistant coating on fabrics and carpeting, and in cleaning products and paints			-
Perfluorooctanesulfonic acid (PFOS)	Historically, a key ingredient in the stain resistant product Scotchgard		143
Perfluorooctanoic acid (PFOA)	Previously used in Teflon production		155
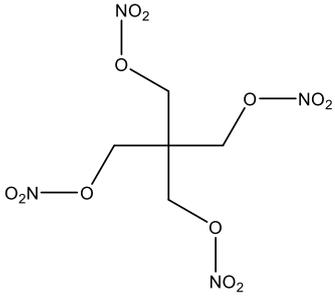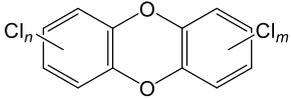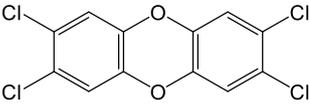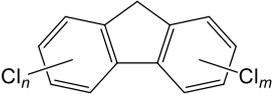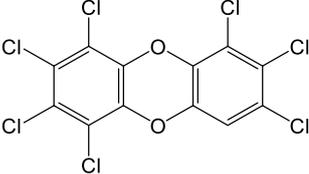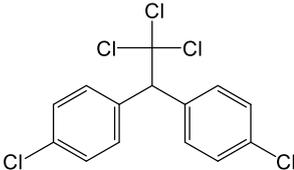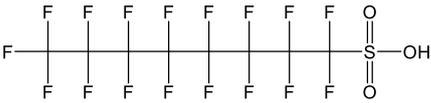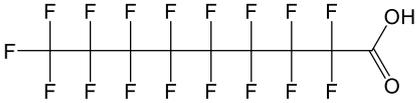
Table 3. Emerging pollutants including pharmaceutical and personal care products (PPCPs)
All structures and chemical names are from PubChem, National library of Medicine
(www.pubchem.ncbi.nlm.nih.gov).

| Name	Use	Chemical structure
Pharmaceuticals		
Ibuprofen (2-(4-Isobutylphenyl)propanoic acid)	Nonsteroidal anti-inflammatory treatment	
Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol)	Antimicrobial used in products such as toothpaste, soaps, detergents	
Carbamazepen (5H-Dibenzo[b,f]azepine-5-carboxamide)	Anticonvulsant and mood-stabilizing drug used primarily to treat epilepsy and bipolar disorder	
Gemfibrozil (5-(2,5-Dimethylphenoxy)-2,2-dimethylpentanoic acid)	Used to treat abnormal blood lipid levels	
Naproxen ((2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid)	Primarily used to treat pain or inflammation caused by arthritis	
Estradiol (8R,9S,13S,14S,17S)-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[a]phenanthrene-3,17-diol)	Synthetic estrogen hormone	
Personal care products		
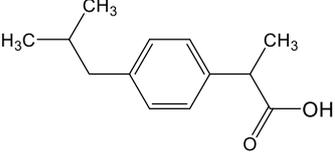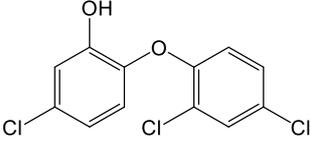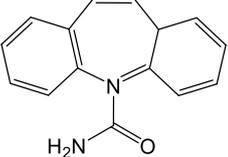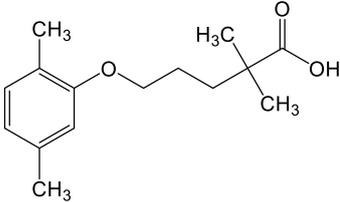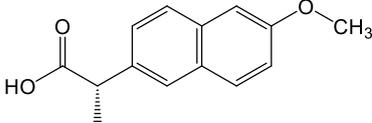
|		
DEET (N, N-Diethyl-meta-toluamide)	Common active ingredient in insect repellents	
Oxybenzone (2-hydroxy-4-methoxyphenyl)-phenylmethanone	Used as a UV filter in sunscreens, and in plastics to reduce UV degradation	
Additional emerging pollutants		
Bisphenol A (4-[2-(4-hydroxyphenyl)propan-2-yl]phenol)	Precursor molecule for polycarbonates and epoxy resins	
Phthalates e.g. Bis(2-ethylhexyl) phthalate	Commonly used as a plasticizer	
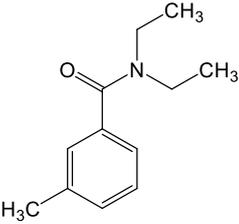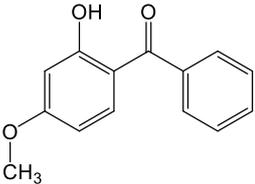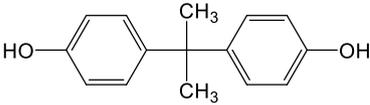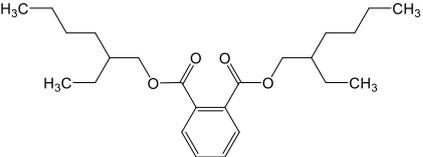
Figure legends

Figure 1. Schematic demonstrating how synbio techniques could be applied to develop and enhance

bioremediation with plants and microbes.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Declarations of interest: none

Figure 1   Click here to access/download;Figure;Figure1.jpg ⬇

Figure 1

Dear Joanna Aldred,

We have made the requested corrections in the chemical structures in the manuscript Tables, specifically:

- Carboxylic acid on ibuprofen structure is missing a 'H'. Corrected.

- Some methyl groups are labelled 'CH3' (as in ibuprofen) whereas others are not (as in Gemfibrozil), please ensure this is consistent across the table. Corrected.

- Please make sure that all bond angles in the structures are correct with no clashes. We have checked the bind angles.

We hope this meets with your approval.

Yours sincerely

Neil Bruce and Liz Rylott