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Handy, R. D.; Clark, N. J.; Hutt, L. P.; Bescós, R.

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# The microbiomes of wildlife and chemical pollution: Status, knowledge gaps and challenges

R. D. Handy<sup>1</sup>, N. J. Clark<sup>1,2</sup>, L. P. Hutt<sup>3</sup> and R. Bescós<sup>2</sup>

## Abstract

The effect of chemical pollution on the microbiomes of wildlife has been given little attention. A new concept is emerging where microbiomes are vital to host animal or plant health, and for ecosystems. Data are mainly on mammals, birds, and fish. Changing environmental conditions (e.g., salinity, pH, season) and exposure to chemicals alter the composition of gill, gut and skin microbiomes. Gut microbiomes are also modulated by diet, and exposure to chemicals including metals, nano-materials, fungicides or microplastics. However, a change in the microbiome does not necessarily infer adverse effects on the host, with some evidence of co-adaptation. Environmental risk assessment for biocides and new nanomaterials should be revisited in context with microbiome-host interactions to better protect wildlife and ecosystems.

## Addresses

<sup>1</sup> School of Biological and Marine Sciences, University of Plymouth, England

<sup>2</sup> School of Health Professions, University of Plymouth, England

<sup>3</sup> School of Biomedical Sciences, University of Plymouth, England

Corresponding author: Handy, R. D. ([rhandy@plymouth.ac.uk](mailto:rhandy@plymouth.ac.uk))

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Ecotoxicology, Animal health, Nanomaterials, Biocides, Gut flora, Nutrition, Plants.

## Introduction

The precise definition of a ‘microbiome’ is still evolving [1], but from an ecological perspective this is a consortium of microbes that can include bacteria, archaea, fungi, algae, and/or protists that interact together within the body space of a host organism (i.e., plant or animal) or another environment (e.g., biofilms in soils or sediments). The relationship with the host may be

commensal or symbiotic, or in the case of declining wildlife health, pathogenic. Opinion on the importance of microbiomes in wildlife ecology has shifted in recent years, and the microbiome is now considered integral to organism health, ecosystem functions and for the protection of biodiversity [2]. Of course, chemical pollution is well known to adversely affect organism health and ecosystems, and there are numerous reports of the effects of individual chemicals on single species of microbes. For example, the effects of engineered nanomaterials (ENMs) on *Escherichia coli* from soils [3]. However, the effects of chemical pollution on the complex microbiomes of host wildlife and ecosystems is less studied, and how these alter host organism or ecosystem health as well as the biogeochemical processes in the environment are unclear.

It is vital to understand the importance of microbiomes in the fate, behaviour and transformation of chemicals in ecosystem. For example, it has been known for decades that biofilms on aquatic sediments can convert inorganic mercury ( $\text{Hg}^{2+}$ ) to the more bioavailable methylmercury ( $\text{CH}_3\text{-Hg}$ ) form, with the methylation rate being affected by environmental factors such as anoxia, pH, and salinity [4]. However, it also became apparent that the gut lumen of fishes were a crucial route of mercury methylation in aquatic systems [5], but only now are the effects of altering the gut microbiome composition on  $\text{CH}_3\text{-Hg}$  accumulation in fish being understood [6]. Clearly, microbiomes in/on sediments, soils or water may be involved in processes that make chemicals bioaccessible, and it is the microbiomes on epithelia (e.g., mucus layers on organisms, gills and gut especially) that can enable bioavailability. Toxicity to organisms has traditionally been explained by target organ pathology and the processes of adsorption, distribution, metabolism and excretion (ADME). However, the effects of microbiomes go beyond the environment-biological interface. In human toxicology at least, it is now becoming clear that the gut microbiome can influence the internal organs and physiological systems such as the haemopoietic and nervous systems [7]. Given this prospect of ‘cross-talk’ between gut microbiomes and multiple body systems, one must consider that all steps in ADME, the toxic disposition, and health of wildlife will also be affected. Furthermore, some chemicals are designed as ‘antibacterials’ that are directly applied to

ecosystems, such as the fungicides used in agriculture, or antibiotics in animal feeds and aquaculture. Nanomaterials are especially gaining traction as an alternative to traditional antibiotics [8]. These chemicals are already in the environment, but the risks they present to the microbiomes of wildlife are largely unknown.

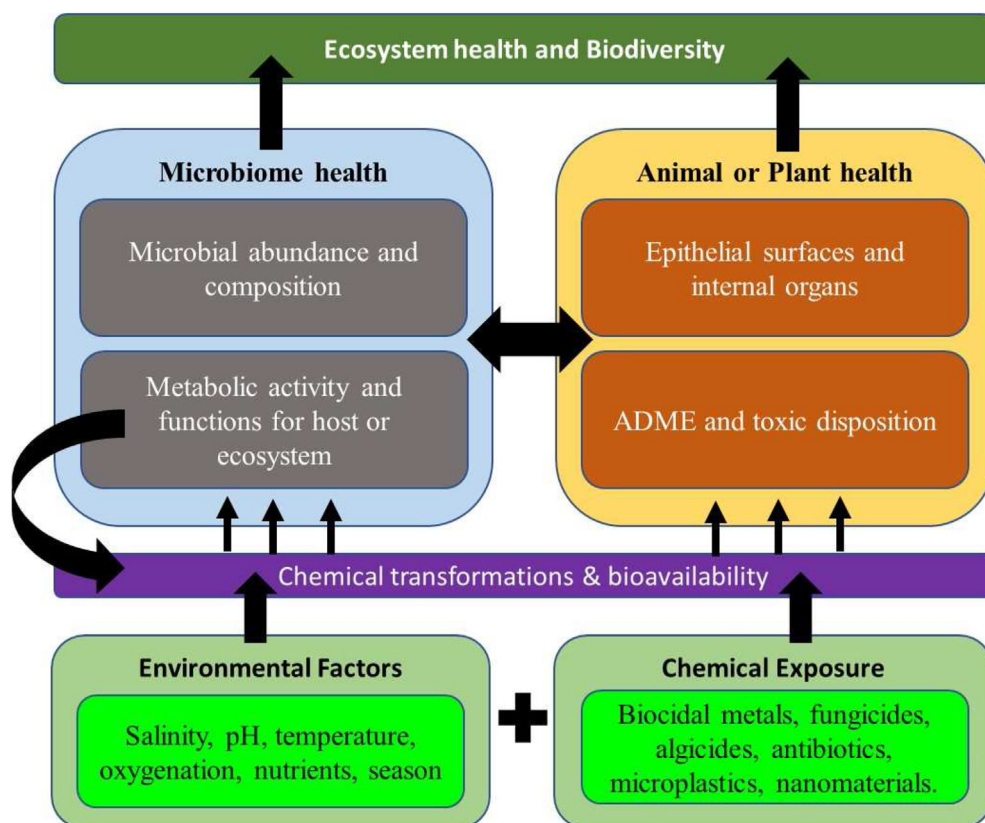
This opinion aims to critically evaluate the effects of chemicals and other environmental factors on the microbiomes of wildlife, with a particular focus on the gut microbiome and new biocides. The specific objectives include reflecting on the normal composition of microbiomes as an integral part of the host wildlife health and consider the environmental factors known to alter both microbiomes and the toxicity of chemicals. Using nanomaterials as an example, the risks and uncertainties posed by new biocides to microbiomes will be explored.

### Composition of microbiomes and wildlife health

A central idea emerging from ecology is that the composition of the microbiomes affects the health of the host, and therefore the ecosystem (Figure 1). First

consider animals as hosts, where the microbiome is on the external barriers (e.g., respiratory epithelium, gut, or skin) and in close contact with the external chemistry. So far, these microbiomes have been studied mostly using human or mammalian models, with much less on wildlife. Or have been performed on organisms where the genomes are known (e.g., zebrafish; the nematode worm, *Caenorhabditis elegans*) to enable some differentiation of the host and microbiome genomics [9]. Nonetheless, data on the composition of microbiomes in some wildlife are emerging, including the gills of fish and shellfish (e.g., oysters [10], Atlantic salmon [11]) and some organisms that are used in regulatory ecotoxicity testing (e.g., marine mussels [12], earthworms [13]). It is too early in the research to make generalisations about the 'normal' composition of those microbiomes in wild animals, partly because the animals contain many microbes that are not yet annotated in omics databases (i.e., unidentified clones), and/or the number of studies on a single species of animal are not enough to agree the normal range. Without the latter for reference, it is not possible therefore to define what is pathological or

Figure 1



**Microbiomes, ecosystem health, and the environmental toxicology of chemicals.** Environmental factors and chemical exposure will influence the composition and metabolic functions of the microbiome(s), that in turn modulates chemical transformations and the bioavailability of substances to the host animal or plant. The microbiome is also critical to organ functions and health of the host. A cascade of healthy/diverse microbes enables wildlife health and therefore promotes biodiversity and health at the ecosystem level.

abnormal for wildlife health. However, some points are worth considering. The microbiome should reflect the normal environmental chemistry of the place where it is found, so one would expect salt tolerant microbes on the gills of marine animals, as observed in oysters [10], or facultative/anaerobic microbes in the reducing conditions of the gut lumen, and so on.

For animals and humans, the gut microbiome has arguably been the most studied, and with a composition that changes from the oral cavity to the anus to reflect the different anatomical regions and luminal chemistries in the gut (e.g., redox status, pH, digesta flow rates and composition of secretions from the host). This microbiome has co-evolved with the animal hosts and is essential to systemic health through the regulation of the digestion of food [14], the immune system [15], and inhibiting the colonization of the gut with pathogens [16]. The gut microbiome might even drive the phenotypic evolution of the host, such as ruminants that have evolved specialised organs for digesting plant material [17]. Analysis of faecal samples from wildlife show the microbiome varies with taxonomic group of the host, lifespan of the host animal and perhaps even social status [18]. In the human gut microbiome there is heterogeneity in composition between populations and individuals [19], and there is some heterogeneity also in studies of wildlife from different locations [10], suggesting influences of both the external environment and the genetics of the host population. Some of the apparent heterogeneity in the literature may also arise from different experimental methods, with early studies using labour intensive culture methods that tended to work better (bias) for some microbes, in contrast to modern global or 16 S ribosomal RNA amplicon sequencing that are less problematic. In humans the small intestine is dominated by mostly facultative anaerobes, while the colon is dominated by mainly obligate anaerobes [20]. A similar situation is noted in the composition of gut microbiomes from zoo animals (primates, elephants, big cats, deer, meerkats, etc) where the microbiomes reflect the type of gut morphology (e.g., fore- or hind-gut fermenters) and feeding habit (e.g., carnivores have similar microbiomes) [21]. Similar observations on feeding habit, gut type and trophic position in food webs have been noted for the diversity of microbiomes in wild animals [18,22]. Notably, laboratory animals such as mice have poor microbial diversity in the gut compared to those of wild animals [23] and even bringing primates into captivity also reduced the diversity of their gut microbiome [24]. Thus, it is important to collect data from animals and plants in the wild, not in captivity.

In birds, the composition of gut microbiome depends on the diet of the animal, its phylogeny, and environmental factors such as climate and season [25]. Similarly in fish, at least one report indicated diet, phylogeny, and

location in the ecosystem as important factors [26]. Data on the gut microbiomes of reptiles [27] and amphibians are very sparse, but at least one study on the latter noted effects of microplastic exposure [28]. The small size of invertebrates species makes it very challenging to investigate their microbiomes, but a study on Enchytraeid worms in soil found that both the gut and soil microbiomes were altered by fungicide (difenoconazole) exposure [29]. However, consensus is yet to be agreed on precisely what environmental conditions and biotic factors (age, size, sex, etc.) influence the gut microbiomes, and other microbiomes of wild animals.

In plants, most of the research on microbiomes has been in the context of agriculture, and with special interest on the root-soil interface (the rhizosphere) and the microbial communities it contains. For the latter, environmental conditions such as pH and climate, soil quality and the type of plant root tissue influences the composition of the microbiome [30]. However, one very neglected aspect in both plants and animals are the compositions and functions of microbiomes found inside tissues and the internal organs. These structures are not sterile and carry their own populations of microbes. Data is beginning to emerge on the woody tissue of plants [31], and the lymph nodes of pigs [32]. Furthermore, how microbiomes achieve stability in microbial composition and function with changing environmental conditions is unclear, when many of the species present are capable of quorum sensing via the release of signalling molecule that promote growth and survival of conspecifics, as well as communicating with other species of microbes in the consortium [33]. The cell-to-cell communication in the microbiomes and with the wild-life host has especially been neglected.

### **Environmental cause or indirect host effect on microbiome composition?**

Inevitably, chemical pollution will be a stress for the microbiome and the host. There is likely two-way communication between the host and its microbiome(s) and so any changes in microbiome could be either a direct consequence of chemical toxicology or arise from the host adapting to the pollution, or both (Figure 1). For example, exposure to chemicals will affect the growth and development of the early life stages of fishes (zebrafish [34]). This could manifest as delayed hatching, delays in first feeding of the hatched larvae, and/or abnormal growth of the larvae and subsequent juvenile fish. However, the gut microbiome changes with stage of development, likely because the gut anatomy matures and the animal alters its feeding habits as it grows [35]. One experimental approach to unravel these factors could be to use zebrafish larvae that have been maintained in sterile conditions (i.e., assumed minimal or absent microbiome) and can then be colonised with an experimentally bespoke consortium of microbes [36]. Such steps could be done with/without the hazardous

chemical present, and isolates of the microbiome (i.e., not on the fish) examined separately.

Similarly, dietary exposure to chemicals in the environment is a main cause of chronic effects and bioaccumulation in wildlife. The exposure may alter food intake, gut functions including the absorption of nutrients, and therefore alter the overall nutrition and health of the animal. In addition, exposure to chemicals can alter the gut microbiome and its metabolic activity for host nutrition. For example, exposure to microplastics alters lipid metabolism in the gut microbiome of tadpoles [28], and causes inflammation of the gut and an altered microbiome in seabass [37]. Clearly, chemical exposure can affect nutrient availability, but nutrients can also modulate the composition of the microbiome (e.g., in fish, dietary zinc [38], dietary taurine [39]).

Chemicals in the environment will affect the microbiomes in water, sediment and soils, and one assumption is that these microbiomes colonise the host organism in that environmental compartment. If the ecosystem loses microbes, then the prospect is the host organism microbiome will decline as well. Antibiotic pollution is especially a concern for the loss of microbiomes and subsequently for the declining biodiversity of wildlife [2]. However, an apparent environmental stress can modulate the microbiome so that the host is less susceptible to chemical toxicity. For example, adapting zebrafish to lower temperatures promotes Gram-positive bacteria that convey some improved resistance of the host to radiation exposure [40]. Flame retardants such as polybrominated diphenyl ethers (PBDEs) can reduce diversity and abundance of several microbial species in the gut of fish, but are also endocrine disruptors. There is some evidence that changes in the gut microbiome during PBDE exposure in zebrafish work to protect the physiology of the animal, with for example a reciprocal relationship between the hepatosomatic index (liver health) and the abundance of Firmicutes organisms in the gut [41].

### Responsible innovation with new biocides including nanomaterials

Environmental risk assessment is an iterative process, and when new science emerges such as the importance of microbiomes to organism and ecosystem health (Figure 1), it would be beneficial to revisit the risk analysis so that ecosystems remain protected. For microbiomes, the concern would be for existing licenced antibiotics, disinfectants, algicides, or fungicides already in the environment. The traditional disinfectants used in agriculture such as products containing bleach, hydrogen peroxide, peroxyacetic acid, or quaternary ammonium compounds, are expected to kill pathogens and may also decrease microbiome diversity in the host. For example, the disinfectants used in poultry housing [42]. However, it is unclear how those effects would

manifest in wildlife exposed to disinfectant residues. Data are emerging on the effects of fungicides [43] and herbicides [44] on the microbiomes of wildlife. There are also concerns that pesticides have unintended antimicrobial activity, and for example, may interfere with the microbiomes of pollinators such as bees [45]. Thus, there is a need to revisit the hazards of existing agrochemicals, but importantly also to re-evaluate approvals of new novel antimicrobials. Engineered nanomaterials (ENMs) are a good example of innovation to provide new antimicrobials with hopefully less risk of the antibiotic resistance seen with traditional drugs [8]. ENMs are being released into the environment, but the metallic forms made of biocidal metals such as zinc and copper are also proposed as feed additives for animal nutrition, or as biocides in agriculture. Some ENMs made of non-essential metals such as silver are very potent antimicrobials that could replace the use of in-feed antibiotics [46]. Such applications of ENMs raise concerns about effects on the gut microbiome and immune health of the host animal. [47], and while regulatory agencies are beginning to recognise the need to control ENMs in the food chain for animal and human health [48], the risks to microbiomes have not yet been considered in regulations.

Nonetheless, the approach should be to consider bioavailability of ENMs in the context of gut anatomy and their colloidal behaviours in the lumen and other physico-chemical properties such as dissolution or the release of dissolved metals [49], as well as how ENMs may interact with microbes [8]. For example, intestinal mucus of trout seems to bind high quantities of silver nanoparticles [50], and the mucus layer is also integral to the microbiome on the gut surface. Metal salt controls are especially important to differentiate a 'nanoparticle' effect (i.e., due to form, shape, size) from a 'substance' effect (i.e., due to dissolved metal) in order to reach the correct conclusions about hazard. Unfortunately, studies on microbiomes and ENMs often lack these metal salt controls. When present in fish feeds, the ENMs can alter the endogenous bacteria found in the intestine, with some evidence suggesting these changes are different compared to the dissolved metal control [9]. A recent systematic review summarised the evidence from 68 studies (*in vivo* and *in vitro*) by ranking the quality of the studies and found there is moderate evidence for gut microbiota dysbiosis caused by Zn, Cu, TiO<sub>2</sub> (anatase form) and Ag-based ENM exposure in invertebrates and vertebrates [51]. The effect of the ENM exposure was dependent on the ENM size, dose, exposure duration and the presence of functional groups [51], making comparisons between studies, even on the same materials and species, difficult. Therefore, standardised protocols are required to systematically assess the effect of ENMs on the gut microbiome, as well as addressing concerns about the normal healthy composition of microbiomes for reference controls (see above).



Despite data showing changes to the gut microbiome with ENMs, evidence of effects on the host organism remains elusive. In fish, dietary ENM exposure concentrations that are lethal for bacteria are sublethal to animal physiology (Ag ENMs, [52]; CuO ENMs [53]). This is likely attributed to differences in bioavailability between organisms, as well as the systemic defences against chemical insult in vertebrate animals (metal chelators, antioxidants, etc.). Poor health can be revealed with a stressor or additional challenge, but even earthworms exposed to CuO ENMs, despite changes in the gut microbiome, showed no change in immune markers when challenged with a pathogen [54].

## Conclusions

Overall, research on the effects of chemical pollution and other environmental stressors on the microbiomes of wildlife is still at an early stage. A change in the microbiome does not necessarily indicate an adverse effect on the host animal or plant. Indeed, it may be a co-adaptation to a new homeostatic setpoint in a polluted environment. For ecology, the expectation is that microbiomes infer aspects of animal or plant health, and that should lead to healthy wildlife populations and therefore ecosystems. However, data is needed to incorporate those ideas into environmental risk assessments so that ecosystems are truly protected.

## Author credit statement

All the authors contributed to the drafting and writing of the manuscript, led by RDH who also prepared the illustrations. All authors approved the final submission.

## Declaration of competing interest

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 article.

## Data availability

No data were used for the research described in the article.

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