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Herbicides and their impact on antimicrobial resistance in the context of food and food safety

Agrochemicals and pesticides as indirect actuators of antimicrobial resistance.

There is a positive correlation between herbicide use and antibiotic resistance.

Introduction

Agriculture is important for a country's economy. With increasing industrialisation and growing populations, land resources for cultivation are decreasing (***Figure 1***). Large amounts of chemicals are used worldwide to control pests and weeds and to increase crop yield. Indiscriminate use of herbicides and pesticides influences microbial processes. Soil microorganisms play an important role in biodegradation of chemicals. Constant exposure to these toxic compounds leads to the genetic modification of these microbes so that they develop specialised mechanisms to degrade the toxic substances.

The genes that code the enzymes that metabolise toxic compounds are present on plasmids (Pérez-Valera et al. 2019). Antimicrobial resistance (AMR) is a phenomenon in which microorganisms resist the growth inhibitory action of antimicrobials. Microorganisms acquire multiple mechanisms to evolve against antimicrobial action. AMR is viewed mostly in clinical settings because it critically affects patient medical treatment. The environmental dimension leading to the genesis and spread of AMR is an important factor. Livestock, water, and soil are interconnected with the food that we consume. The majority of agrochemicals are

easily dissolved in water or absorbed by soil and traces of these agrochemicals that remain on plants are sometimes consumed by animals or humans.

The extensive use of agrochemicals at different levels in these environments leads to the accumulation of residues in the food chain. The vegetables, fruits, and farm products that we consume may have traces of chemicals that were used in their production process.

The exposure of gut microbiota to these chemicals can trigger their genetic modification and lead to AMR. In this review article, we focus mainly on the impact of herbicides on AMR.

Herbicides

Chemicals are used in agriculture to protect crops from the deleterious effects of pests, insects, rats, and weeds. They are usually grouped into pesticides, insecticides, rodenticides, and herbicides. Herbicides are the most widely used chemicals in agriculture. They protect crops from weeds, which are generally other plants that affect crops (Holt 2013). The use of these compounds also exerts indirect damage on microorganisms present in the soil, like bacteria and fungi, potentially changing the ecological properties of microbial communities by targeting evolutionarily-conserved pathways (Liao et al. 2021). The impact of herbicides on AMR development is alarming. Repeated herbicide exposure during weed control may select for increased herbicide tolerance in microorganisms and may vary among and within the species (Raino et al. 2020, Tothova et al. 2010). Herbicide tolerance may be achieved via genetic changes in the herbicide-targeted gene (Wicke et al. 2019) or non-target genes linked with generalised stress tolerance (Comont et al. 2020). There is a positive correlation between herbicide use and antibiotic resistance. Long-term herbicide exposure could indirectly drive antibiotic resistance evolution via cross-resist-

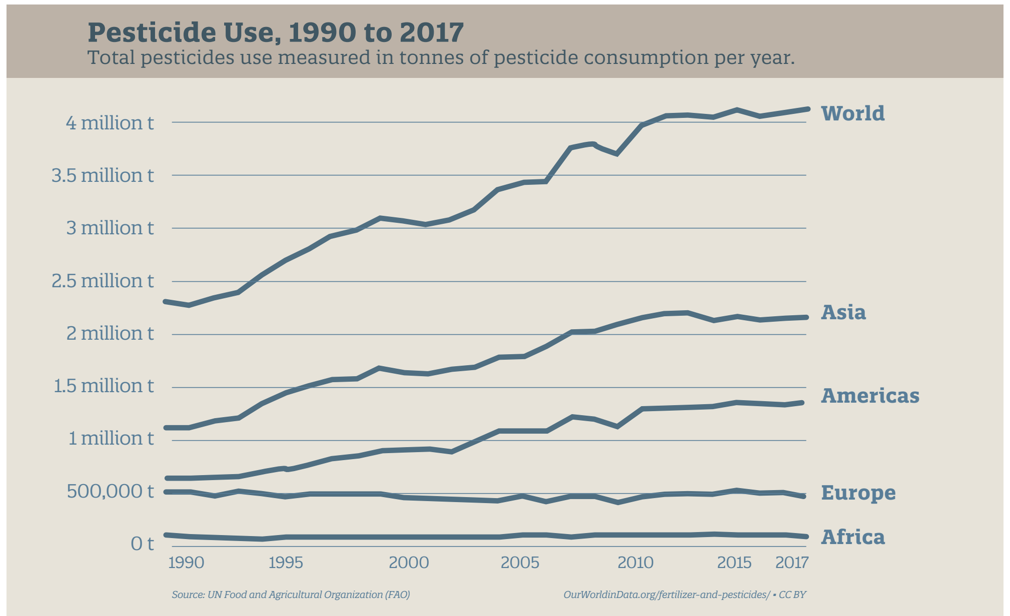

Figure 1:
Worldwide pesticide consumption by region from 1990 to 2017.

Herbicides such as glyphosate, glufosinate, and dicamba have been implicated as a cause of major alterations in bacterial sensitivity to various antibiotics.

ance evolution if the same mutations increase bacterial tolerance to both herbicides and antibiotics (Kurenbach et al. 2018, Comont et al. 2020). The magnitude of the induced response may undermine antibiotic therapy and substantially increase the probability of spontaneous mutation to higher levels of resistance. The combination of the intensive use of herbicides and antibiotics in proximity to farm animals and important insects, such as honeybees, might also compromise their therapeutic effects and drive greater use of antibiotics (Kurenbach et al. 2015).
Addressing the crisis of antibiotic resistance requires broadening our view of environmental contributors to the evolution of resistance. Antimicrobial-resistant gene (ARG) transfer from environmental bacteria to pathogenic bacteria is a key determinant for antibiotic resistance (Malagón-Rojas 2020). The environmental dimension of AMR has been identified in recent years as an important factor in the development and spread of resistance. Resistant bacteria originating in an altered environment and sharing their resistance with clinically relevant pathogens could become a major problem for public health (Ramakrishnan et al. 2019). Available evidence suggests that the multiple interactions between livestock and soil and water in a natural environment polluted by agricultural chemicals may be a key dissemination route for antibiotic-resistant genes (Van Bruggen et al. 2018).

Major herbicides and their indirect effect on bacteria to promote AMR

2,4-D (2,4-dichlorophenoxyacetic acid), dicamba (3,6-dichloro-2-methoxybenzoic acid), and glyphosate (N-(phosphonomethyl)glycine) are the most extensively used herbicides in the world (Duke and Powles 2008, Benbrook 2016). Atrazine, simazine, carbamates, diquat, paraquat, fluazifop, and sethoxydim are other commonly used herbicides (Holt 2013). The continuous application of herbicides to soil results in selection pressure among soil microbiota. Herbicides such as glyphosate, glufosinate, and dicamba have been implicated as a cause of major alterations in bacterial sensitivity to various antibiotics. Glyphosate, dicamba, and 2,4-D induce a change in the susceptibility of the potentially pathogenic bacteria E. coli and S. enterica to multiple antibiotics (Kurenbach et al. 2017). Herbicide toxicity tested at sub-lethal concentrations induces an adaptive multiple-antibiotic resistance phenotype characterised by an increase in the expression of efflux pumps, reduced synthesis of outer membrane porins, or both; these effects vary among species, herbicides, and antibiotics (Kurenbach et al. 2015). Common surfactants used in herbicide preparation such as carboxymethyl cellulose (CMC) and Tween 80 also showed effects on the antibiotic susceptibility of bacteria though to a lesser extent than those of the active ingredients (Kurenbach et al. 2018).

Herbicides promote efflux pump activity in bacteria and horizontal gene transfer

Adaptive resistance is characterised by the induction of resistance to one or more antibiotics in response to the presence of an environmental signal. When the inducing signal is removed, antibiotic resistance generally returns to the original level though in some cases it cannot be restored (Fernández et al. 2011). As an adaptive feature, long-term herbicide exposure may also promote antibiotic resistance indirectly via cross-resistance evolution if the same mutations increase bacterial tolerance to both herbicides and antibiotics (Comont 2020).
The over-expression of efflux pumps has been linked to induced tolerance to antibiotics in a variety of organisms, such as *E. coli*, *Pseudomonas aeruginosa*, *Campylobacter jejuni*, *Streptococcus pneumoniae*, and *Salmonella enterica serovar Typhimurium* (Webber and Piddock 2003). The activation of the AcrAB-TolC efflux pump in *E. coli* and *S. enterica serovar Typhimurium* has been shown to result in reduced susceptibility to fluoroquinolone, β-lactams, tetracycline, and chloramphenicol (Fernández et al. 2011). Herbicides have also been shown to promote antibiotic resistance via horizontal transfer of ARGs within bacterial strains and between species.
Herbicide exposure leads to chemical stress that causes oxidative damage to DNA, accumulation of intracellular reactive oxygen species (ROS) and SOS response, and increased cell membrane permeability in bacteria that further promotes conjugation frequency (Liao et al. 2021).

Major soil microorganisms relevant to human health and disease

Soil microbiomes are extremely complex and diverse; each gram of soil contains more than 10 000 varieties of microorganisms. It is estimated that 25% of the life on Earth is in soil.
Soil microbiome studies have revealed a wealth of information about soil biodiversity, soil-microbe interactions, and ecosystems. Soil microorganisms play multiple roles and the majority of these microorganisms do not pose a threat to human health. They participate in soil formation, nutrient cycling, soil fertility maintenance, and a multitude of complex interactions between organisms within the soil and with the soil itself. However, some soil microbes are opportunistic pathogens that take advantage of susceptible individuals, such as those who are immuno-compromised, and some are obligate pathogens that must infect humans in

Euedaphic Pathogenic Organisms (EPO)		Soil-Transmitted Pathogens (STP)	
Name of organism	Disease caused	Name of organism	Disease caused
Actinomyces sp.	Actinomycetoma	*Coxiel/a burnetii*	Q Fever
Bacillus anthracis	Anthrax	*Borrelia sp.*	Lyme disease
Clostridium botulinum	Botulism	*Ascaris lumbricoides*	Ascariasis
Campylobacter jejuni	Campylobacteriosis	*Salmonella enterica*	Salmonellosis
Leptospira interrogans	Leptospirosis	*Pseudomonas aeruginosa*	Multiple Infections
Listeria monocytogenes	Listeriosis	*Escherichia coli*	Multiple Infections
Clostridium tetani	Tetanus	*Entamoeba histolytica*	Amoebiasis
Francisella tularensis	Tularemia	*Trichuris trichiura*	Trichuriasis
Clostridium perferingens	Gas Gangrene	*Echinococcus multicularis*	Echinococcosis
Yersinia enterocolitica	Yersiniosis	*Strongyloides Stercoralis*	Strongyloidiasis
Aspergillus sp.	Aspergillosis	*Trichinella spiralis*	Trichinellosis
Blastomyces dermatitidis	Blastomycosis	*Cryptosporidium parvum*	Cryptosporidiosis
Coccidiodes immitis	Coccidioidomycosis	*Balantidium coli*	Balantidiasis
Histoplasma capsulatum	Histoplasmosis	*Cyclospora cayetanensis*	Cyclosporiasis
Sporothrix sclzenckii	Sporotrichosis	*Isospora belli*	Isosporiasis
Rhizopus sp.	Mucormycosis	*Giardia lambila*	Giardiasis
Nocardia sp.	Mycetoma	*Toxoplasma gondii*	Toxoplasmosis
Strongyloides Stercoralis	Strongyloidiasis	*Shigella dyseneriae*	Shigellosis

Table 1:
Major euedaphic pathogenic organisms (EPO) and soil transmitted pathogens (STP) and the diseases they cause.

order to complete their life-cycles, such as parasites. These organisms may be capable of surviving in soil for extended periods of time before infecting humans who come into contact with contaminated soil. Factors that may affect the incidence of soil-borne diseases are interrelated or associated with land management practices, the purpose of land use, climate change, the use of antibiotics/antiseptics in livestock, environmental disinfectant use, and methods of water purification.
Organisms in the soil that cause human infections are divided into two major groups: euedaphic pathogenic organisms (EPO) and soil-transmitted pathogens (STP). EPOs are potential pathogens among true soil organisms and STPs are not true soil organisms but obligate human pathogens that can survive in soil for extended periods of time until they enter a human body to complete their life cycle (see ***Table 1***).

Herbicides and food contamination

Herbicides are used to protect crops against weeds and they play a significant role in food production. Herbicides are toxic to humans and can have both acute and chronic health effects. The effects of herbicides depend on the quantity and route of exposure.
Herbicide production, distribution, and use require strict regulation and control because herbicides are intrinsically toxic and deliberately spread in the environment. Regular monitoring for pesticide residues in food and in the environment is also required. There are many regulations in place today around the world to ensure food safety and to eliminate the adverse effects of herbicides on food consumers. The United States Environmental Protection Agency (EPA), the European Food Safety Authority (EFSA), and the Food Safety and Standards Authority of India (FSSAI) are some of the apex agencies that regulate the quality of food and ensure food safety. The World Health Organisation (WHO) reviews evidence and develops internationally-accepted maximum residue limits (MRLs) for herbicide residues. None of the herbicides that are authorized for use on food crops in international trade today are genotoxic. Adverse effects from these chemicals occur only above safe levels of exposure. Agricultural workers who apply herbicides are directly exposed and are most at risk. The 2018 European Food safety Authority (EFSA) report on pesticide residues in food products commonly consumed by European citizens found that of 11 679 samples analysed, 58% of the samples were found to be without quantifiable levels of residues (residues < limit of quantification [LOQ]) (European Food Safety Authority 2020). Foods tested included aubergines (eggplant), bananas, broccoli, cultivated fungi, grapefruit, melons, sweet peppers/bell peppers, table grapes, wheat grain, virgin olive oil, bovine fat, and chicken eggs. (ibid.). One or more pesticide residues in concentrations above the LOQ were found in 40.6% of the samples. 1.4% of the samples contained residue concentrations exceeding the MRLs (ibid.). 26 countries submitted 9 573 food samples (including processed products) for glyphosate residue testing (ibid.) Glyphosate was quantified at levels above the LOQ but below the MRL in 1.9% of the samples (179 samples) and exceeded the MRL in 12 samples (0.1%), a decrease from the exceedance rate in 2017 (0.2%) (ibid.). MRL exceedances were identified in samples grown in Argentina (one dry bean sample), Germany (one apple sample), India (one dry lentil sample), Lithuania (four honey and other apicultural product samples), Poland (two buckwheat and other pseudo-cereal samples and one honey and other apicultural product sample), and Ukraine (two millet samples) (ibid). The FSSAI, India's food regulatory authority, reported that in 2017-18 a total of 23 660 samples that included vegetables, fruits, spices, rice, and tea were analysed for pesticide residues (Food Safety and Standards Authority of India 2019). Pesticide residues were detected in 19.1% of the samples, of which 2.2% were found to exceed FSSAI's MRL, including 1.9% of vegetable samples, 1.1% of fruits samples, 12.1% of spice samples, 7.2% of rice samples, 1% of wheat samples, and 1% of pulses samples (ibid.). None of the tea, packaged milk, or fish/meat samples were found to exceed FSSAI's MRL (ibid.). Understanding the interrelationship between water, soil, pesticides, and livestock is necessary for environmental monitoring programs and for raising awareness to overcome the current AMR crisis. The Food and Agriculture Organization of the United Nations (FAO) is home to a vast reservoir of knowledge, with experts that include food safety specialists, agronomists, and veterinarians. The FAO plays a unique

role in supporting producers and other actors in food systems in addressing antimicrobial resistance (AMR) risk. The FAO has engaged in significant, sustained efforts to raise awareness of AMR risks and advocate for improved behaviours and policies among cross-sectoral fields (Food and Agricultural Organization 2020). The FAO has set up an action plan for AMR with main focus areas that include:

1. Promoting good practices in food and agriculture systems with prudent use of antimicrobials,

2. Strengthening governance related to antimicrobial use (AMU) and AMR in food and agriculture, and

3. Developing capacity for surveillance and monitoring of AMR and AMU in food and agriculture.

These guidelines and regulations exist in part to keep food in compliance with maximum residue limits to significantly reduce the exposure of the general population to all sorts of chemicals used in agriculture. People can further limit their exposure to these residues by following these household practices:

1. Ensure safe, purified drinking water.

2. Scrub and rinse firm fruits and vegetables like melons, potatoes, and carrots before consuming.

3. Use drinking water to rinse vegetable surfaces (this will remove about 80% of chemical residues).

4. Use 2% salt water to rinse vegetable surfaces (this will remove nearly 100% of chemical residues).

5. Peel the outer layer of fruits and vegetables like mangoes and citrus.

6. Blanch vegetables or soak them in vinegar.

These practices reduce the presence of chemical residues and also prevent other foodborne hazards, such as consumption of harmful bacteria.
While food safety agencies regulate pesticide and agrochemical use on food, there is still a need for guidelines to monitor the effects of agrochemicals on soil microorganisms. Only a few in vitro studies have been published that investigate the effects of commonly used pesticides and herbicides on soil microorganisms. Genotoxicity studies conducted to receive regulatory approval for herbicides and pesticides should consider studying the effects of those chemicals on the genome plasticity of microorganisms that are relevant to human health and present in agriculture and livestock environments. In spite of all the action plans and measures taken by governments and scientific agencies, every individual should be aware of the concept of AMR and its implications. Every one of us should be vigilant in how our actions relate to our health and our environment. On behalf of AMR Insights, we engage in activities promoting knowledge about the AMR crisis and we initiate steps to save the world from the threat of AMR for the betterment of society.

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The AMR Insights Ambassador Network consists of an integrated global and cross-professional community discussing, devising, and driving actions to combat AMR. The Network aims to inspire, connect, and empower our Ambassadors to take individual and collective actions to curb AMR. Here are the authors' affiliations: [1]Department of Biotechnology, Indian Institute of Technology, Roorkee, India; [2]Barcelona Institute of Global Health, Barcelona, Spain; [3]Infectious Diseases Institute, Makerere University, Kampala, Uganda; [4]Department of Microbiology, University of Ibadan, Nigeria; [5]Department of Veterinary Extension, Banaras Hindu University, India; [6]Department of Microbiology, Lanka Hospital Diagnostics, Sri Lanka; [7]AMR Insights, Amsterdam, The Netherlands.

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