EMERGING THREAT OF ANTIBIOTIC POLLUTION IN SOIL: CAUSES AND CONSEQUENCE

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Soil is a natural medium for plant growth; a system where our food grows, thus it requires to be clean and balanced to produce safe and healthy food. In recent years the inappropriate use of antibiotics has created a selective pressure that drives the emergence and spread of multidrug-resistant microbes in soil environment. Antibiotics are the chemicals meant to kill or reduce the growth of microorganisms. Though these are used mainly to treat human and animal infections, they are also used extensively in agriculture to increase crop productivity and in animal husbandry as growth promoters in animal feed at controlled concentrations. In agriculture, streptomycin is the most commonly used antibiotic to treat bacterial plant diseases (Mann et al., 2021). Since the soil, plants and animal system are highly interlinked, the spread of antibiotics and antibiotic-resistance in environment will not be limited to any one ecosystem. In animal husbandry, use of antibiotics as growth promoter is loosely defined as administration of antibiotics to healthy animals at concentrations below 200 ppm in feed for more than 14 days which amounts to 2ppm, lower than the therapeutic dose of 20 ppm in animals (NAAS policy paper 43, 2010). The low concentration usually non-lethal dose for any bacteria through animal feeds over long periods results in development of resistance against that antibiotic among bacteria. The continued feeding of antibiotics in feed also introduces low levels of antibiotics in soil and water through animal excreta due to poor absorption of many antibiotics. Around 30-80% of antibiotics fed to animals as growth promoters may be excreted as wastes.

ANTIBIOTICS IN SOIL

The concentration of the antibiotics in manure slurry varies from traces to as high as 216 mg L^{-1} of manure slurry. These antibiotics generally remain stable during manure storage and end up in agricultural fields on manure applications. Leaching and runoff of antibiotics from manure-fertilized lands is also threatening the quality of drinking water. Major source of antibiotics entry in soil includes- through application of manure/dung, urine, slurry containing antibiotics, Pesticide application for control of phytopathogens, contaminated irrigation water, application of sewage sludge, dumping in soil (for disposal of unutilized or discarded lot) and native microbes (Actinobacteria and fungi). Commonly used antibiotic in agriculture for control of plant pathogens are given in table 1.

Table1. Antimicrobial used in crop production for control of phytopathogens

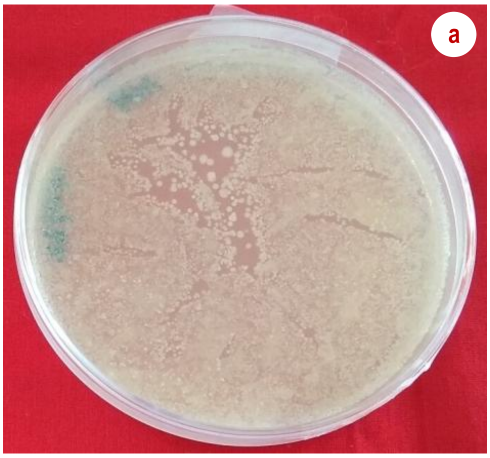
Antobiotics/Antimicrobials	Effective/Recommended Against
Streptocycline (Streptomycin Sulphate + Tetracylin Hydrocloride)	Fire blight in apple; Citrus Canker in citrus; Black leg and soft rot, bacterial brown wilt or ring or the bangle disease of potato; Wild fire in tobacco; Bacterial leaf spot in tomato; Bacterial leaf blight in paddy; Blister blight in tea
Azoxystrobin	Downy mildew& Powdery mildew in grape, Fruit rot & Powdery mildew in Chili, Anthracnose & Powdery mildew in mango, Early & Late blight in tomato, Downey mildew & Powdery mildew in cucumber, Late Blight in potato, Blight & Powdery mildew in cumin
Kasugamycin	Blast in rice
Validamycin	Sheath Blight in rice
Aureofungin	Gummosis in citrus
Pyraclostrobin	Early blight in tomato; Frog eye leaf spot (*Cercospora*) & Alternaria leaf spot in soybean; Alternaria Leaf blight in cotton; Tikka disease in ground nut
Kresoxim-methyl	Blast & Sheath Blight in Paddy; Powdery mildew& Downey mildew in grapes

(Adapted from Sarkar et al., 2018)

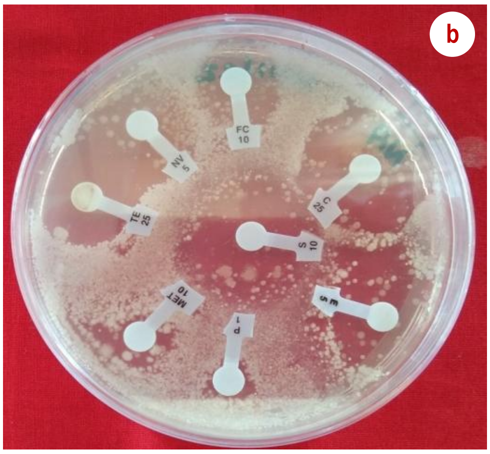
PREVALENCE OF ANTIBIOTIC RESISTANCE IN SOIL BACTERIA

Increased antibiotic concentrations in the soil create a selection pressure and promotes preferential outgrowth of antibiotic-resistant bacteria, which may lead to change in antibiotics sensitivity of entire microbial populations. An even sub lethal concentration of antibiotics in the soil creates conditions for genetic changes in bacterial genomes and transfer of antibiotic resistance genes and mobile genetic elements, such as plasmids and transposons, between and among microbial populations (Cycon et al., 2019).

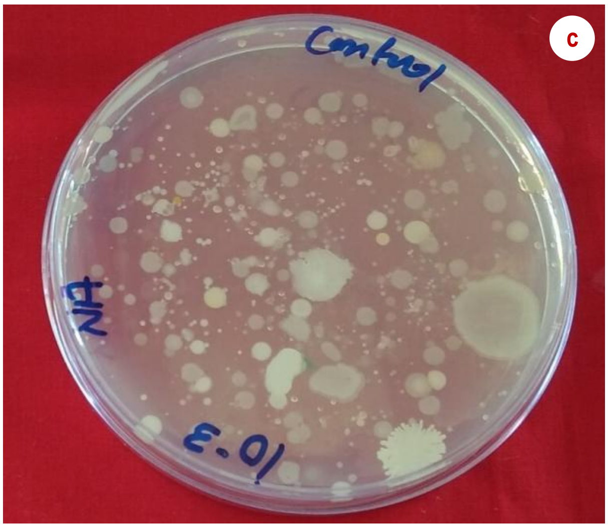
Data on prevalence of antimicrobial resistance and resistome in Indian soil is scanty however reports from other countries indicated manure applied to the soils contain drugs like oxytetracycline, doxycycline and sulphadiazine, sludge mainly contains drugs that are less water-soluble like ofloxacin, ciprofloxacin, norfloxacin and trimethoprim. The 'resistome' or the collection of genes capable of conferring resistance has been found to persist long after the manure or sludge is decomposed (Taneja & Sharma, 2019). In a study in China, 156 new antimicrobial resistant genes and mobile genetic elements were identified in the composted manure and sludge (Su et al., 2015).



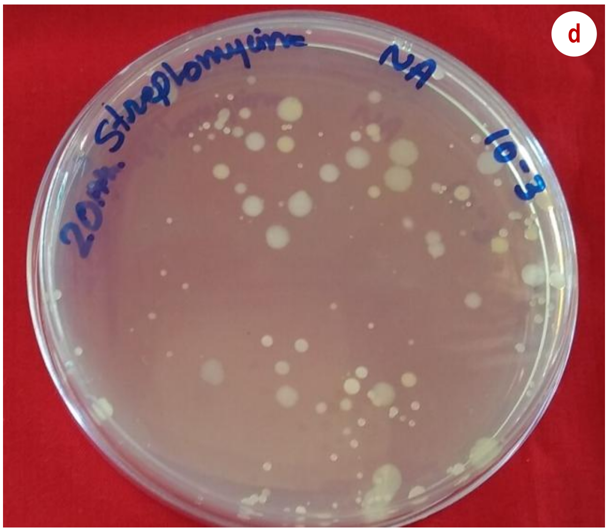
Soil microbes from soil suspension



Antibiotic resistant microbes present in soil



Microbial population in soil in the absence of streptomycin



Streptomycin tolerant soil microbes

Figure 1. Prevalence of antibiotic resistance in native soil microbes

FATE/PERSISTENCE OF ANTIBIOTICS IN SOIL

In soil, antibiotics may subject to transformation or degradation due to various abiotic and/or biotic processes, including (Cycon et al., 2019). Photolysis, hydrolysis, biodegradation and binding on to soil particles through adsorption process influence their persistence in soil. Antibiotics like ciprofloxacin, ofloxacin, and virginiamycin degrade very slowly and may persist in soil in its original form up to 30-80 days while bambermycin, tylosin, and erythromycin completely degrade in a period of one month at temperatures ranging from 20-30°C. Soil properties that influence persistence of antibiotics in soil includes

pH: Determines ionization of antibiotics in soil and also influence dissociation of antibiotics. Antibiotics which are stable under wide range of pH may have greater persistence in soil

Adsorption Reaction: Binding to mineral and organic colloid in soil. Charge in the soil and in antibiotic molecule will determine the strength of adsorption. Soil pH, clay content and organic matter in soil determined adsorption

Soil Texture: Sand, silt and clay proportion in soil also influence its degradation and residence time in soil. Half-life of ceftiofur was more than 49 days in sand and only 22 days in clay loam.

Clay Minerals: In soils dominated by montmorillonite or illite or kaolinite, clay mineral reacts with strongly basic and amphoteric antibiotics to form complexes. However, acidic and neutral antibiotics are adsorbed only in soil that dominantly contains montmorillonite type of clay mineral. Amount of antibiotics adsorbed by clays varies from 9 mg g^{-1} for kaolinite clays and strongly basic antibiotics to more than 300 mg g^{-1} in case of montmorillonite clays and amphoteric antibiotics.

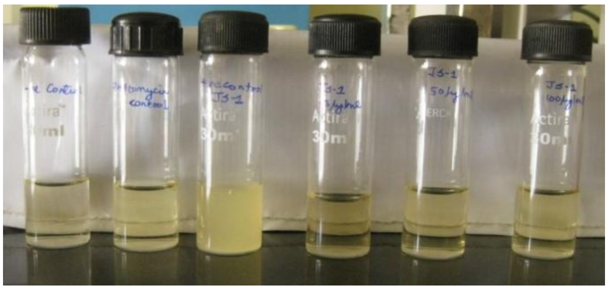
Soil Temperature: As the temperature decreases from the normal range of 25-30^0C, persistence of antibiotics increases. At 30^0 C, 44% of chlortetracycline and 23% of bacitracin remained in the soil after 30days of their application. However, when temperature decreased to 20^0C, 88% of chlortetracycline, 33% of bacitracin, remained in soil. A rapid degradation and quick elimination from the soil can be expected in *kharif* season compared to *rabi* season due to relatively higher temperature in *kharif* season.

SOIL ANTIBIOTIC POLLUTION: POSSIBLE EFFECT ON SOIL MICROBES

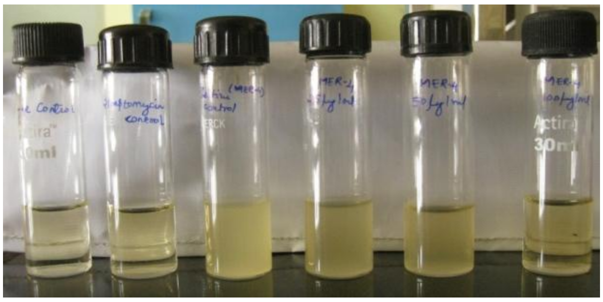
- Alter the composition of indigenous soil microbial communities that are of fundamental importance for ecosystem function
- Change function in nutrient cycling, especially of nitrifying bacteria.
- Inhibit decomposition of organic matter.

- Develop resistance in organisms in the soil environment.
- Antibiotics like streptomycin-laden manure decrease bacterial count in soil up to 50-75% over several months depending upon the nature of bacterial population.
- Broad spectrum antibiotics like tetracyclines, aminoglycosides, and sulfonamides in manure and soil are expected to inhibit the nitrification process.
- Narrow spectrum antibiotics such as sefdiazine, oxolinic acid, and tylosin, on the other hand, stimulate the nitrification process.
- Build up of tylosin in soil can cause shifting of bacterial communities from Gram positive to a Gram negative.

HOW IT MAY AFFECTS SOIL PROCESSES?



Azotobacter strain JS1



Burkholderia sp. strain MER4

Figure 2. Sensitivity of *Azotobacter* and *Burkholderia* to increasing concentration of Streptomycin

- A decrease in the nitrogen fixation, nitrification ability of soil may result from antibiotics pollution in soil even at lower concentration.
- Strong inhibition of Fe (III) reduction was also observed in soil contaminated with sulfamethoxazole and oxytetracycline at concentrations >10 mg/kg soil (Molaei et al., 2017).
- The presence of Enrofloxacin in irrigation water reduces the fixation of atmospheric carbon in stems and leaves by up to 6.14% in alfalfa. It reduces the number of nodules by 91% when the plant is exposed to a concentration of 200 μg L^{-1} of ENR. At the same time, root elongation has an inverse relationship to the presence of this antibiotic, reducing its length by up to 32% compared to the average (Vilca, 2022).
- Inhibition of Dehydrogenase and Urease was observed in soil amended with tetracycline @ 1 μg/kg soil (Pinna et al., 2012)
- Reduction in Dehydrogenase and arylsulphatase activities with increasing concentration of oxytetracycline at 1 to 200 mg/kg over 7 weeks (Chen et al., 2013).

SOLUTION FOR ANTIBIOTICS PROBLEM IN SOIL

Multipronged approach is required to tackle the problem of antibiotic pollution in soil. Since the soil receives antibiotic mostly from other sources, management at source level will have greater impact in combating the problem. Some of the points to be considered include

- Use safe growth promoters in animal/bird feeds and proper disposal of animal carcass
- Alternative therapeutic measures including biocontrol and antagonism, phage therapy (Polyvalent and cocktail of phages) etc.
- Use of effective botanical extracts for control of microbial pathogens in plants.
- Proper decontamination and treatment of hospital and pharmaceutical effluents
- Agriculturally beneficial microbes should also be checked for antibiotic resistance before release as biofertilizers to prevent the transfer of trait in other organisms and soils

- Lab organisms and genetic materials should not escape lab in viable form
- Awareness and training to pesticide dealers involved in sale of such chemicals (course for pesticide dealers and agri-clinic professionals)

CONCLUSION

In soil, the antibiotic pollution mainly occurs due to application of manure, contaminated irrigation water and use of pesticides. Emergence of resistance against many of the commonly used antibiotics such as penicillin, methicillin and streptomycin are frequently encountered among soil microbes. Fluoroquinolones antibiotics have relatively higher persistence in soil. Antibiotics in soil may alter the microbial composition and abundance and thus will affect the soil productivity and ultimately food security. Since soil is the habitat for microbes and important source of novel genes, it is urgently required to prevent any unnecessarily imposed selection pressure to drive the evolution in wrong direction. Research is required to quantify the loss in ecosystem services due to presence of antibiotic in soil, contribution of different sources in loading antibiotics in soil, estimating prevalence of genes (copy number, types) under different land use system, effect on composition of microbial community due to antibiotic pollutions in soil etc. Degradation as well as the byproducts formed need to be assessed for ecotoxicity and movement in food chain at different trophic levels to precisely assess the impact of antibiotic pollution and spread of antimicrobial resistance in different habitats.

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