

Study on Detection Technology of Antibiotic Content in Soil

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Abstract. Since the discovery of antibiotics, has been widely used in the treatment of epidemic diseases, affected by this, a large number of antibiotics into the environment of the media, affecting environmental safety. Therefore, it is very important to detect the content of antibiotics in soil accurately and scientifically. At present, microbial assay, immunological analysis and physical and chemical assay are widely used in the determination of antibiotics in soil. The source and ecological risk of antibiotics in soil are studied, the concrete application of the technology is discussed systematically.

Keywords: Soil, antibiotics, content determination, detection technology.

The problem of antibiotic residue in soil has been paid more and more attention, and the detection technology of antibiotic content has been paid more and more attention. Currently, there are no limits on the emission and quality of antibiotics in environmental media, nor are they included in the Environmental monitoring and environmental management system, and there is a lack of technical specifications for the comprehensive utilization of livestock manure. Therefore, the contamination of soil environment by fecal antibiotics will become more and more serious.

1. Sources of antibiotics in soil and ecological risk

The use of veterinary antibiotics and medical antibiotics, as well as the sewage of antibiotic producing enterprises, are the three sources of antibiotics in the environment, among which, the use of veterinary antibiotics has exceeded 52% of the total, become the main source of antibiotics in environmental media. In recent years, the proportion of veterinary antibiotics production has been increasing year by year. In 2013, the production of 36 common antibiotics was 92,700 tons, of which 84.3% of veterinary antibiotics were produced, global meat production will rise by 30 per cent, with concomitant increases in antibiotic production and consumption. Because of the low absorption rate of veterinary antibiotics, 30% ~ 90% of them enter into the soil in the form of the excrement and urine of livestock and poultry, which results in the high residual level of antibiotics in the soil around the farm. Manure runoff is one of the main causes of soil antibiotic pollution in China, six times

greater than in the UK and much of Northern Europe. The residues in soil with pig manure could be as high as 1000 μ g/kg, and the antibiotic content in soil without manure was 2 orders of magnitude lower than that in soil with manure.

Drugs and personal care products (PPCPs), including human and veterinary drugs for treating diseases, personal care products such as bathing and skin care, and daily chemicals such as aromatherapy and disinfectants, are emerging pollutants with potential hazards to the ecological environment and human health. Their environmental presence, risk assessment and toxicology have attracted extensive attention in the environmental science field. The sources of PPCPs in the environment are very extensive. There are a lot of PPCPs in waste water and solid waste from pharmaceutical factory, hospital, livestock and poultry farm, and human daily life. As a class of PPCPs, antibiotics are widely used in animal husbandry, aquaculture and other fields. Most of the antibiotics enter the soil with the excrement, which is easy to cause soil antibiotic pollution. Because of its antibacterial property, the antibiotics entering the soil environment can only be removed by biodegradation, hydrolysis, photolysis or absorption by the roots of plants. However, the remaining trace antibiotics can cause certain toxicity and inhibition to microorganisms, animals and plants and soil ecology.

The ecotoxicity of antibiotics in environmental media was mostly quantified by half effect concentration (EC₅₀). The EC₅₀ of Ciprofloxacin (CIP) and Ofloxacin (ofloxacin) ranged from 1.00×10^{-3} to 2.00×10^{-3} mg/l, the EC₅₀ of OTC and TC were 1.04 and 3.31 mg/L, respectively. The EC₅₀ of sulfamethoxazine, Trimethoprim and Florfenicol were 19.52, 16 mg/L and 1.80×10^4 mg/L, respectively. Antibiotic residues in soil can enter human body through food chain as well as affect soil ecological environment. The daily exposure of Chinese residents to antibiotics through aquatic products was $2.0 \times 10^{-7} \sim 2.7 \times 10^{-3}$ μ g/kg, while the absorption rate of FF in drinking water was low, about 64% was excreted through urine, and the daily exposure of children was $1.90 \times 10^{-4} \sim 2.20 \times 10^{-4}$ μ g/kg. Some antibiotics (FQs, TCS) can accumulate slowly in human body at low dose, cause organ damage and abnormal function, or cause allergic reaction, hormone-like action, teratogenic, carcinogenic and mutagenic action, and increase drug resistance of pathogenic bacteria.

2 Detection technology of antibiotic content in soil

In the current study, antibiotics are usually analyzed by ultrasonic extraction, solid-phase extraction and liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS), which are optimized based on EPA 1694. Microbial diversity mainly refers to the study of soil microbial species and relative abundance, that is, microbial community structure. Soil is rich in microorganisms, the main microorganisms include bacteria, fungi, archaea, eukaryotes and so on. For the detection of different types of microorganisms, microbial diversity and related testing is divided into the following testing services:

2.1 Amplification subsequencing detection

Amplification sequencing is mainly used to detect a large class of soil microorganisms, such as bacteria or eukaryotes (16S/18S/ITS, etc.), the species and relative abundance of a certain kind of microorganism, i. e. the community structure of microorganism, were obtained. With the popularization and deepening of the microbial diversity project, many scientific research projects now put forward higher requirements, hoping to detect the absolute number of microorganisms. At present, fluorescence quantitative PCR can be used for the quantitative detection of total bacteria or a certain class of microorganisms in soil. The combination of second-generation high-throughput sequencing and real-time

quantitative PCR can be used to understand the species composition of microorganisms in samples as well as to obtain absolute quantification of specific species.

2.2 Metagenomic detection

Metagenomic detection is the use of the second generation of high-throughput sequencing technology to detect all soil microorganisms, including bacteria, fungi, archaea, eukaryotes and so on, the species, relative abundance and function of all microorganisms were obtained.

2.3 Functional gene sequencing

The second-generation high-throughput sequencing technique was used to detect the species and relative abundance of soil microorganisms with certain functions, such as nitrogen fixation. Soil is a major player in the Roger Fulford, and many of the microbes in the soil function as drivers of chemical cycles, participating in specific carbon, nitrogen, phosphorus, and sulfur cycles. A specific function of microorganisms is determined by a specific functional gene. By detecting a specific functional gene in soil, we can find the species and relative abundance of microorganisms with a specific function in soil.

2.4 Quantitative detection of microorganisms

It is primarily used to measure the absolute quantity of a certain type or specific microorganism. By using fluorescence quantitative PCR technique, not only a large class of microorganisms can be obtained, but also the absolute quantity per unit volume or weight of a particular microorganism can be obtained. This is a good supplement to the second generation high-flux technology, which can only be obtained in species and relative abundance. The combination of the second generation high-throughput sequencing and real-time quantitative PCR can be used to understand the species composition of microorganisms in the samples as well as to obtain absolute quantification of specific species.

2.5 Quantification of antibiotic resistance genes

The common antibiotic resistance genes are tetracycline resistance genes, sulfonamides resistance genes, erythromycin resistance genes, streptomycin resistance genes, etc., the absolute quantification of antibiotic resistance genes can be achieved by fluorescence quantitative PCR or high-throughput qPCR.

3 Conclusion and outlook

With the discovery of ARGs in all kinds of environments, ARGs can migrate and transform in all kinds of environmental media, and it is possible that the resistance gene can be transferred into the food chain, which makes the ARGs pollution global. The existing inspection regulations and most laboratories use the typical internal standard method to analyze several or more kinds of targets. There are structural and property differences between the internal standard and the target, the difference of loss, chromatographic retention behavior and response intensity in the pretreatment process leads to higher detection limit and poorer stability of the analytical method, which can not be used for the accurate quantification of low concentration antibiotics.

Blank test, each batch of samples to do at least 1 laboratory blank, the blank value of the measured elements should not exceed the lower limit of the method. If more than the reason must be found, re-analysis until qualified samples can be analyzed. Calibration curves shall be drawn for each batch of samples and the correlation coefficient of calibration curves shall be greater than or equal to 0.995. Calibration checks shall be performed for each analysis of 50 samples using a calibration curve intermediate concentration standard solution. The relative deviation between the determination result and the concentration of the last calibration curve should be less than or equal to 10% . Otherwise, redraw the curve. Accuracy Control, each batch of samples should contain certified reference material, and the accuracy should be controlled by adding standard to the samples within the given uncertainty, the recovery rate should be in the range of 80%-120% .

Nowadays, biosensors, optical sensors and electrochemical sensors based on CDs have been widely used in the detection of antibiotics in environment and food matrix. The small size effect and amphiphilic characteristics of CDs make it easy to be combined with chromatography for the detection of antibiotics, this may be due to the complex substrate for detection of antibiotics, the limited number of CDs available for antibiotic pretreatment, and the fact that the mechanism of CDS as chromatographic separation material is not completely clear. The substrate for the detection of antibiotics based on CDs materials is relatively single, and the analysis and detection of antibiotics in lake water, soil and other complex environmental samples are relatively few, because the size and function of CDs are easily affected by the temperature, time and the level of the experimenter, the synthesis of CDs with small size and special function is still in the laboratory stage. Some antibiotics have the characteristics of poor stability. When using the internal standard method to determine them, the storage time of the samples is short, and the requirements for analysis and determination are severe, try To finish pretreatment within 3 days and instrument analysis within 45 days.

SRGS and INTL1 were enriched by single and combined contamination of antibiotics and heavy metals, and the higher the concentration of antibiotics, the higher the relative abundance, the results showed that the resistance genes had common resistance mechanism to antibiotics and heavy metals. Tetracycline resistance Gene Tet A. Tet G. Tet X did not increase significantly, indicating that low concentrations of antibiotics and heavy metal contamination had no significant effect on TRGs induction. A representative soil sample was selected for high-throughput sequencing to study the microbial community structure under different initial addition amount. Actinomycetes and Proteus were the dominant bacteria in all the samples, 50.6%-70.4% . The relative abundance of Proteobacteria was 26.1%-41.3% , which was significantly higher than that of the control. The relative abundance of chloro flexi increased with the increase of the concentration of antibiotics, and the proportion of chloro flexi in the combined pollution of antibiotics and heavy metals was obviously lower than that in the single pollution, the results showed that the combined pollution of antibiotics and heavy metals could inhibit chloro flexi.

Antibiotics is a global pollution problem, which is directly related to the health and development of human beings. The pollution of soil antibiotics is closely related to the production and life of human beings. There are many ways for antibiotics to enter the soil. To control the pollution problem, we should further Quantitative analysis the forms of antibiotics in the soil and study the physical and chemical properties of the soil in the treated area, to find out the influence of soil environment on the transformation law of the forms of antibiotics in soil, so as to take effective measures to intervene and control the rate of the migration and transformation of antibiotics in soil, both economic and environmental benefits should be taken into account. We can also take measures to control the source and actively explore new repair techniques.

4 Conclusion

In summary, antibiotics have high toxicity and high ecological risk, soil as an environmental carrier of antibiotic enrichment, after the change of environmental conditions, antibiotics in soil can be released into water environment through surface runoff, soil runoff, underground runoff and so on to accelerate the diffusion and increase human exposure. Therefore, it is very important to do a good job in the detection of antibiotics in soil.

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References

1. Nan Qiong; Tang Jingchun; Hu Yucheng; et al. Research progress of antibiotic detection methods in different environmental media [J]. *Chemical Research and Application*, 2017,(11):1609-1621.
2. Jin Lu; Chen Pinpin; Wang Xiao; et al. Research progress on pretreatment and detection methods of antibiotics in environmental samples [J]. *Journal of Tianjin Agricultural University*, 2017, (3): 92-96.
3. Zeng Qiaoyun; Ding Dan; Tan Xiao. Research progress on the pollution status and sources of tetracycline antibiotics in agricultural soils in China [J]. *Journal of Ecology and Environment*, 2018, (9): 1774-1782.
4. Ruan Linlin; Lin Hui; Ma Junwei; et al. Degradation and microbial response of single and compound antibiotics in soil [J]. *China Environmental Science*, 2018, (3): 1081-1089.
5. Qi Heming; Yan Hua; Zhang Lei; et al. Research progress of antibiotic detection technology in crops [J]. *Journal of Food Safety and Quality Inspection*, 2019, (15): 5098-5103.