Heavy Metals in Agricultural Soil and Food Security

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Abstract： Gradually, environmental issues are becoming severe, among which heavy metal pollution in soil is one of the most serious problems. Heavy metal pollution seriously affects the environment, food security and public health because of its toxicity and biological magnification. It is necessary to study the current situation of heavy metal pollution in soil and in an attempt to solve this issue. Instead of physical, chemical and traditional biological methods, synthetic biology provides a new potential solution. With the aid of various microbial and genetic resources, people can develop engineered strains, which can absorb one or multiple heavy metal pollutants, to control heavy metal pollution in soil and support environmental restoration. In this review-format paper, the case study was added; the author of this paper uses a Chinese small town ---- Wuji as an example.

Keywords：Heavy metals, heavy metal pollution, synthetic biology , pollution control.

1 Introduction of heavy metal pollution

1.1 Heavy metal pollution

Heavy metals refer to metallic elements whose densities are higher than 4.0. In the field of environmental pollution, heavy metals mainly refer to elements with significant bio-toxicity, including mercury (Hg), cadmium (Cd), lead (Pb), chromium (Cr) and so on, as well as some semimetals, such as arsenic (As). Heavy metal pollution is that the air, water body, soil and biosphere polluted by heavy metals because of human activity [1].

1.2 Sources of heavy metal pollution

Heavy metals are the natural components of the earth. However, some are released by human activity. Consequently, heavy metals turn into lasting pollutants, since they cannot be degraded or destroyed [5].

1.2.1 Natural resources

High concentrations of heavy metals can be natural, which is caused by weathering of bedrock. For instance, soil at Mendip district, UK, is rich in Pb, Zn and Cd.

1.2.2 Anthropogenic sources

In general, metals are let out during mining and processing. Anthropogenic sources include various industrial areas, such as abandoned and engaged stopes, foundries, and smelteries. By-products of incineration, usage of organic fertilizer and pesticides, and traffic also release metals in the atmosphere. Based on previous studies, anthropogenic sources of metal pollution can be divided into five categories: Mining and metal smelting, industry sources, atmogenic deposit, agriculture sources and waste treatment. [6]

All above considered, heavy metals ultimately flow into solid or liquid wastes (Fig. 1).

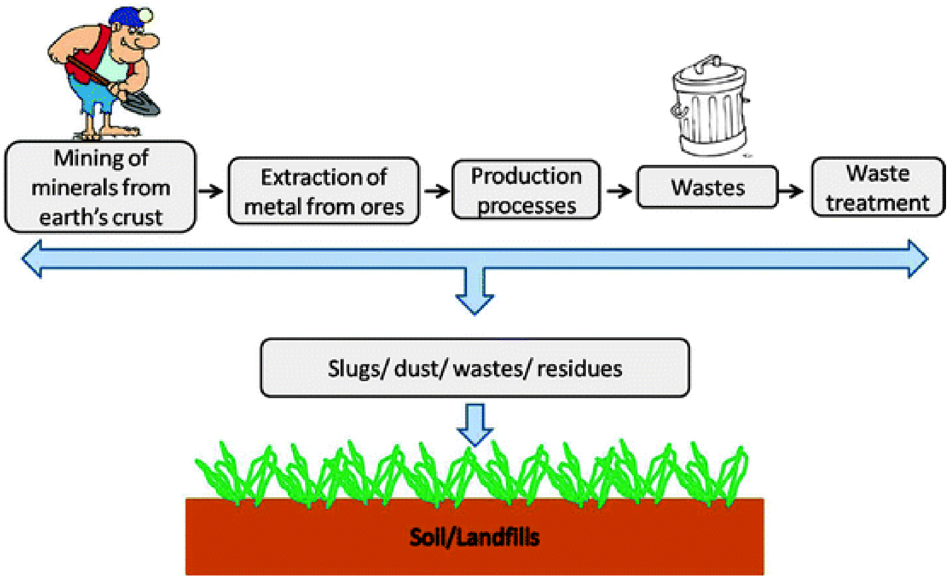

Fig.1 Schematic diagram showing how heavy metals flow [6]

1.3 Hazardousness of heavy metal pollution

Heavy metals can cause direct pollution by getting into the air, water bodies and soil, as well as indirect pollution by migration among the biosphere. As heavy metals cannot be degraded and can be accumulated in organisms through food chains, they threaten the environment, food security and human health. With the quickened industrialization in China, the situation of heavy metal pollution is gradually becoming severer. In recent years, heavy metal pollution in soil has become an increasingly serious issue. Relevant news is raising more and more public concern [2] .

1.3.1 Affects to agricultural products brought by heavy metals

Heavy metals damage plants by inactivating proteins or forcing plants to produce hydrogen peroxide or some other substances that can influence the enzymes and metabolism of plants. For example, Cd binds to amino acids or proteins that have hydrosulphonyls, inactivating them and leading to death of the plants. Heavy metals reduce the ability of plants to absorb and transfer N, P, K and other macroelements, and mineral elements, such as Ca, Mg and Fe, causing iron deficiency and other diseases.

The growth of immature wheat leaves and roots are significantly inhibited after Cd treatment [4] . In the leaves, the increase amount of Cd can make the amount of Fe, Mg, Ca and K decrease . High concentrations of Cd damages chlorophyll, promotes the decomposition of ascorbic acid and suppresses the activity of nitrate reductase. High concentrations of Ni in soil significantly reduces the absorption of wheat to N. Similarly, Cd affects P absorption. During studying the distribution of crops, scientists found that Pb severely hindered the physiological activities of crops. In addition, Cr and Hg mainly pollute wheat and corn [3-4] . Cr, Zn and Cu mainly pollute rice and vegetables [4] .

Increasingly, serious heavy metal pollution not only threatens the quality and safety of crops, but also affects ecosystem construction.

1.3.2 Affects to human health brought by heavy metals

Though some heavy metals are necessary microelements, the body cannot bear them over the limit, let alone toxic elements. All heavy metals in the air, water and soil may be absorbed by the human body through the respiratory tract, digestive tract or skin. Once the heavy metals accumulate to a certain concentration, they will directly do harm towards growth and development, physiological and biochemical function, or even result in death (Fig. 2).

In human bodies, heavy metals can react with many components, such as proteins, riboses, vitamins and hormones, which changes the character of these substances and causes damages. Heavy metals can reduce or destroy the activity of enzymes via changing the conformation of active sites or replacing the metal coenzymes. Excess Pb and Hg can affect embryonic development, causing abnormal pregnancy. Long-term exposure in Pb pollution in the environment leads to Pb accumulation in humans tissues, especially in bones, teeth, kindeys and the brain. During the period of brain development within a child, their blood brain barriers are not developed sufficiently to fight off the heavy metals. As a result, Pb can easily enter a child’s brain and interfere with the central nerve system, causing dysfunction and severely hindering a child’s development. What’s more, Pb, Mn, Cu, Hg and Cd in the environment are very harmful, a very low dose is enough to make the body dysfunction, induce diseases and even lead to death. Mn pollution causes pneumonia and other diseases. Cu overplus degenerate hemoglobin, which influences metabolism and causing disease of cardiovascular system. Cd damages renal function and declines renal tubule's re-absorption function, which makes metabolism disordered. Excess Cd in bones may cause bone softening, deformity, fracture, atrophy. At last, Cd can also cause cancer (Fig. 2). Due to these damages and negative influences, the control of heavy metal pollution which guarantees food security, water security and, moreover, human health seems urgent.

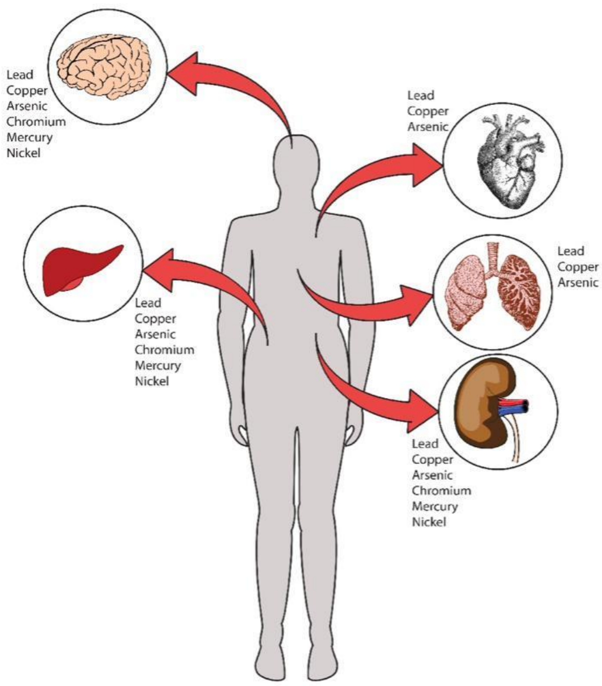

Fig.2 Affects to human health of heavy metals

2 Status of heavy metal pollution in China

Overall, enrichment of heavy metals in soil is the most serious at the southwest region of China. Guangdong, Guangxi and Liaoning are next to it. Compared with background level, the most and second serious pollutant are Cd and Pb, which are out of limits around the country (Fig. 3) [21].

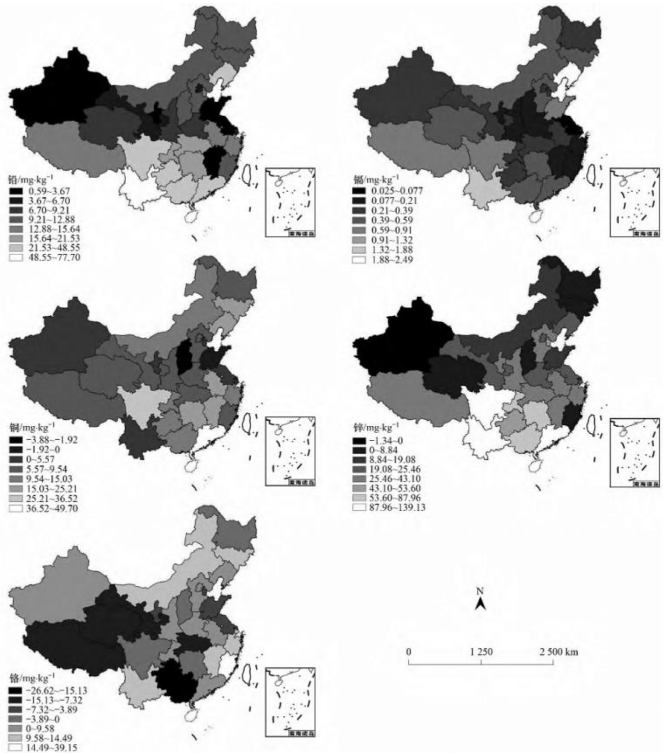

Fig. 3 Status of heavy metal pollution in China [21]

According to the statistical analysis of the concentration of Cr, Cu, Pb, Zn, Ni, Cd, Hg and As in agricultural soil in 8 cities from China, researchers found that the values were higher than background in most cities (Table 1). Via researching agricultural soil in 24 provinces and cities of China, researchers determined 320 heavily polluted areas, and over 80% of the crops with excessive levels of pollutants caused by heavy metal pollution. Most suburban farmlands in China were polluted by heavy metal in varying degrees. For instance, soil in Nanjing was polluted by Pb, Hg and Cd, especially Hg [7]. As for the upper and middle Huangpu River, the mass fractions of Cd, Hg, As, Cr and Pb in agricultural soil were 60%, 68%, 19%, 67% and 45% over the background in 2010 [8]. In Beijing, the soil of suburban farmland held more Hg, Cd and Pb than soil of outer suburbs [9]. In Shenzhen, 37% soil samples contain excess Hg, 6% ones were above middle level pollution [19]. In addition, Hg, Cd, Pb, Cr, As, Cu, Zn and Ni pollution was also found in Guizhou, Fujian, Hebei, Guangxi, Jiangxi, Hainan, Chongqing, Hongkong and some other regions in varying degrees [20].

Table 1 Concentrations of heavy metals in agricultural soils in the cities from China (Unit: mg/kg) [2]

城市	w(Cr)	w(Cu)	w(Pb)	w(Zn)	w(Ni)	w(Cd)	w(Hg)	w(As)	参考文献
北京	75.74	28.05	18.48	81.10	–	0.18	–	–	[10]
广州	64.65	24.0	58.0	162.6	–	0.28	0.73	10.90	[11]
成都	59.50	42.52	77.27	227.00	–	0.36	0.31	11.27	[12]
郑州	60.67	–	17.11	–	–	0.12	0.08	6.69	[13]
扬州	77.20	33.90	35.70	98.10	38.50	0.30	0.20	10.2	[14]
无锡	58.60	40.40	46.70	112.90	–	0.14	0.16	14.3	[15]
徐州	–	35.28	56.20	149.68	–	2.57	–	–	[16]
兰州	–	41.63	37.44	69.58	–	–	–	17.33	[17]
国家背景值	61.00	22.60	26.00	74.20	26.90	0.097	0.065	11.20	[18]

English Version of Table 1 [2]

City	w(Cr)	w(Cu)	w(Pb)	w(Zn)	w(Ni)	w(Cd)	w(Hg)	w(As)	参考文献
Beijing	75.74	28.05	18.48	81.10	–	0.18	–	–	[10]
Guangzhou	64.65	24.0	58.0	162.6	–	0.28	0.73	10.90	[11]
Chengdu	59.50	42.52	77.27	227.00	–	0.36	0.31	11.27	[12]
Zhenzhou	60.67	–	17.11	–	–	0.12	0.08	6.69	[13]
Yangzhou	77.20	33.90	35.70	98.10	38.50	0.30	0.20	10.2	[14]
Wuxi	58.60	40.40	46.70	112.90	–	0.14	0.16	14.3	[15]
Xuzhou	–	35.28	56.20	149.68	–	2.57	–	–	[16]
Lanzhou	–	41.63	37.44	69.58	–	–	–	17.33	[17]
National background value	61.00	22.60	26.00	74.20	26.90	0.097	0.065	11.20	[18]

3 Common recovery techniques of heavy metal pollution in soil

Nowadays, around the world, the main techniques to resolve heavy metal pollution issue include physical, chemical, biological, and integrated recovery techniques. The author selected some common techniques now used by people and listed them in this review. Although these techniques are commonly used, most of them have weaknesses and side-effects and still require perfection.

3.1 Physical recovery technique

The industrial measure: changing the soil or aliening the earth or burying deeply seems to be the very basic measures. It doesn’t require advanced tech but cannot stop the heavy metal pollution radically. Burying polluted soil may cause secondary. Also, this measure requires huge amount of work and costs a lot. To avoid secondary pollution, the polluted soil should be centralized processing. In conclusion, this measure is only suitable for recovery of small polluted areas [22].

The thermal desorption is to heat the polluted soil, letting volatile elements come out from the soil. However, this measure is only suitable for dealing with volatile elements. What’s more, the generated gas, which contains heavy metals and causes secondary pollution is hard to be collected to process.

The common characteristic of physical recovery technique is that the theory is simple but requires cost and work quantities and may cause secondary pollution

3.2 Chemical recovery technique

Soil washing remediation is a process to elute pollutant in soil by getting chemicals into soil to combine with the heavy metal ions, forming the complexes or sediments.When the heavy metals exists as sediments, they cannot be absorbed by plants and people can extract them easily.However, the eluent is expensive when using in a large scale, and the elution effluent may cause secondary pollution to the soil and groundwater [24].

Now, there lacks related technique and complete equipment to scale application, which needs to be solved urgently.

Electrokinetic remediation is to clean the soil by the formed electric field gradient through applying direct voltage at the two sides of polluted soil [23]. In the electric field, heavy metal was brought to the electrodes via electromigration and electrophoresis. The heavy metals exist in soil as positive ions, which will migrate in the electric field and gather around the electric rods that is negatively charged. And when the ions are gathered around the rod, it will be easy for people to extract the ions in this small area. At present, people have carried out some exploratory work in the fields of device design, mechanism study and modeling. However, since the the electrodes can not only gather the heavy metal ions but can also gather other ions and charged particles like H^+, this measure can cause change of chemical components property of the soil.

The common characteristic of chemical techniques is that doesn't require huge work quantities and easy to be put in practise, but still might bring harms and side-effect to soil.

3.3 Bioremediation technique

Bioremediation is to clean the environment and recover ecological functions by utilizing biological absorption, biotransformation, scavenging or degradation to deal with environmental pollutant. It mainly contains phytoremediation, microbial remediation and zoic remediation. The measure is low cost, simple, secondary pollution free, effective and easy to widely spread [27]. As a result, the mechanism and application scenarios receive much attention.

Phytoremediation have been gradually developing since early 1980s. It is a kind of methods that are to deal with soil polluted by heavy metals by natural or transgenic plants [28]. Based on the mechanism of action, the technique mainly includes phytostabilization, phytovolatilization and phytoextraction [29]. Through these measures, the heavy metals can be absorbed by plants and either go into the air or store in the plants themselves. This measure seems practical and the cost is low, however, there are some other drawbacks. Phytovolatilization enables heavy metals in soil to be transformed into gas and emit into the atmosphere, which brings pollution to the air. And if the plants that absorb heavy metals enter the food chain, it may influence animals in the food chain. And at last, the author is also worrying about the plants. If the heavy metal pollution in soil is too severe, the plants may not even withstand the pollution.

Microbial remediation is to reduce the pollution level of heavy metals by absorbing them or transforming them to low toxic product [31]. It now seems that microbial remediation is of most development potential and application prospective technology. However, microorganism is so tiny that is difficult to be separated from soil. In addition, the microorganism introduced to restore soil would compete with the aborigines. In all, the research focus in the future are domestication and screening efficient strains, building strain libraries, and optimizing combination technology.

4 Application of synthetic biology in controlling heavy metal pollution

Though microorganism has unimaginable environmental restoration capability, its evolution speed is slower than that of emerging contaminants. As a result, synthetic biology is needed to solve the problem. A possible solution is to directly design and transform existing pollutant-degrading strains, and construct genetically engineered strains that can degrade one or various kinds of pollutant, on the basis of fully understanding of the pathway of microorganism to degrade refractory organics and utilizing the various microbial and gene resources of China. At the same time, some measures should be taken against compound pollution, such as waste water. After building the BioBrick libraries related to organic pollutant metabolism, regulation and stress resistance, people can introduce artificial strains, reasoningly design and assemble biosystem and construct efficient pollutant-degrading strains. In all, such actions would promote the study for microbial degradation and metabolism of emerging contaminants, and support engineering application of environmental remediation [33].

4.1 Synthetic biology

During the last 20 years or so, synthetic biology, systems biology, developmental biology, DNA synthesis and sequencing, gene editing have developed a lot. With the addition of computer technique and bioinformatics, scientists can possibly use computer aided design and artificially synthesize gene and even genome. It becomes possible to cross the bottleneck of industrialization of biological engineering. The interaction among bioindustry, engineering science, systems biology, biotechnology and synthetic biology is closer and closer，which will rapidly shorten the distance between biological technical research and application.

Rather than editing lives by nuclear transplantation or gene modification, scientists want to introduce the idea and of engineering science to biology. Via combining chemistry, computer science, physics and other subjects, scientists could finally design and create new biomolecules, regulatory networks and metabolic pathways. If so, the dream would come true that creating beneficial substances and lives with special functions by human will.

4.2 Biosensor [34-35]

Biosensors are composed of sensing modules and signal transduction modules. Many things can be used to make sensing modules, such as enzymes, enzyme components, living bodies, tissues, cells, antibodies, nucleic acids and organics. The main function of sensing modules is specially recognizing its targets. The main function of signal transduction modules is changing the physical or chemical signal generated at the sensing modules into exportable electrical or visible color signal.

The basic of biosensors is the interaction between DNA and repressor or activator proteins. Repressor proteins is transcription factors that regulate gene expression. They inhibit transcription when bonding to the operons. Only if the target is available, will the repressor-operon complex be broken. The target binds the repressor and changes its conformation. Then the repressor is released and transcription initiates, as the RNA polymerase is no longer blocked. On the contrary, the binding of activators and DNA can promote transcription. When the target binds the activator, the complex binds exact DNA region and enhances the affinity of RNA polymerase to the promoter. As a result, the transcription efficiently started (Fig. 4).

Biosensor can generate a signal whose intensity is proportionable with the concentration of the target. The signal can be further amplified, processed or stored, then recorded by suitable equipment, so that the aim of analysis and detection would be achieved.

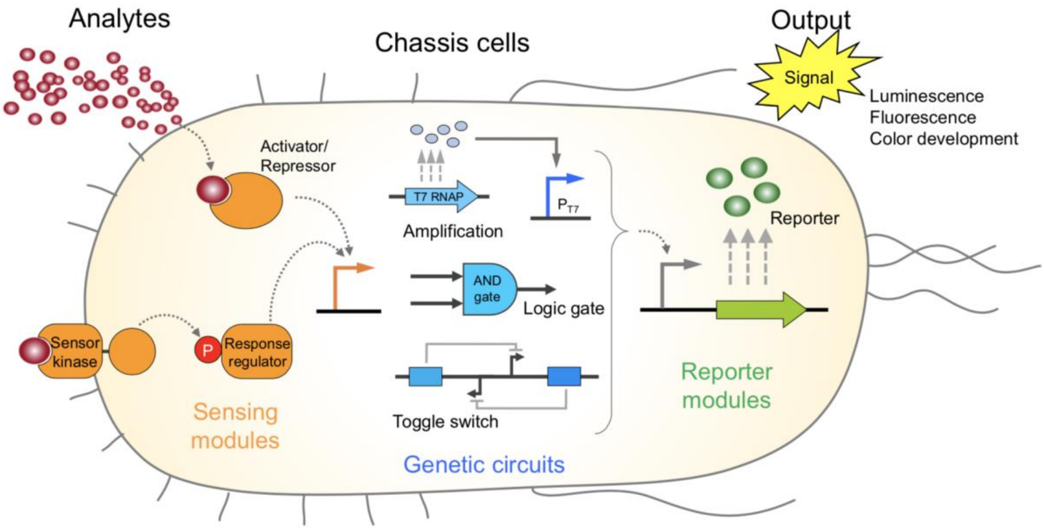

Fig. 4 Biosensor [34-35]

Till now, scientists have discover several proteins that can sense specific heavy metals, and construct kinds of genetic circuits, such as As, Cu, Hg, Pb biosensors [43-46]. PbrR is a Pb-sensing regulatory protein found with the Pb (II) resistance determinant, pbr, was found in Ralstonia metallidurans in 2001 [47]. PbrR binds at the operon upstream the *pbrABCD* gene cluster and inhibits the genes' expression. When Pb^{2+} binds PbrR, the dimer releases the DNA and the Pb resistance related genes express. As a result, scientists develop a Pb biosensor with PbrR and pbr-promoter-operator region (*pbr P/O*) [45]. PbrT is also useful because it contribute to Pb uptake. Another means to replace PbrT is surface display of PbrR [48]. The biosensors for other elements more or less vary. ArsR forms homodimer first and then responds to As^{3+} [46]. CueR dimer binds Cu^{+} first, and then binds DNA and activates gene expression, rather than inhibition [49].

Most of the original biosensors are not sensitive enough. On one hand, scientists triy to change the reporter modules. For example, the pH reporter system was used to replace traditional fluorescence or LacZ /Xgal reporter system [43]. On the other hand, scientists also debug the whole circuit. By integrated modulation, which mainly contained adjusting promoter strength, introducing amplifiers and controlling plasmid copy number, the detection limit and output of As biosensor were improved up to 5,000 and 750 fold [46].

4.2 Bio-enrichment device [36-40]

The principles of bio-enrichment device include: (1) enriching metal by biosensor, (2) using artificial biological material, such as biofilm [42] and spytag-spycatcher [36-41]. When the target touch the material, it is absorbed and forms complex with the material.

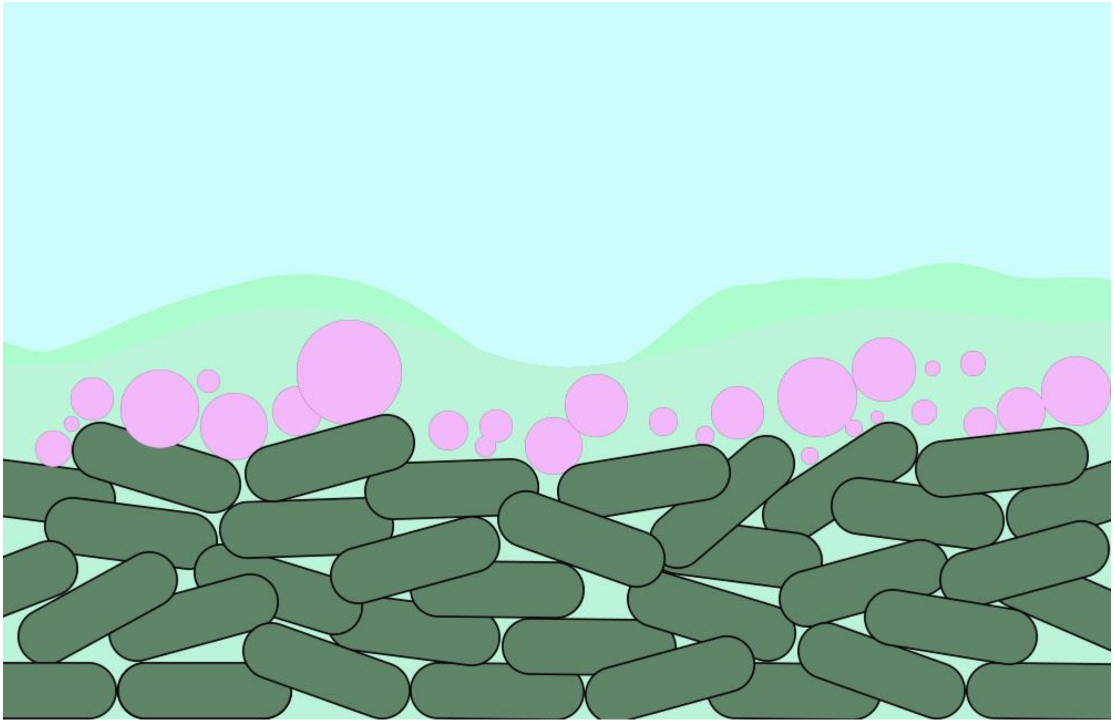

Fig. 5 Bio-enrichment device (*E. coli* biofilm catches heavy metals)

Biofilms are ubiquitous as they can be found both in human and some extreme environments. They can form on the inert surfaces of devices and equipment, which will be hard to clean and cause dysfunction of the device.

However, scientists view it through different lenses to transform this ill impact into goodness. Researchers envision to establish the Solar Hunter system on *E. coli*'s biofilm. Biofilm can substantially increase the resistance of bacteria to adverse conditions like acid or oxidative stress and form a stable and balanced system. These traits make them easily grow with low cost and elevate bacteria's adaptability. So it will be a good practice to be applied to polluted environment.

What's more, biofilm can automatically assemble extracellular growth by static adherence, which facilitates regeneration and recycling in mass production in industry. Startlingly, biofilms can also serve as a synthetic nonconductive biological platform for self-assembling function materials. The amyloid protein CsgA, which is the dominant component in *E. coli*, can be programmed to append small peptide without losing biological activity of peptide and self-assemble function of CsgA. Also, it has been tested that CsgA subunits fused with not too large peptide can be tolerated by curli export machinery and maintain the self-assembly function. In all, scientists highlight the recyclability, stability, scalability, and versatility of biofilm in our system as a design that is truly applicable.

Using synthetic biological technique, people edit the genes of micro-organisms to enable them to produce proteins that can absorb heavy metals and cultivate these micro-organisms to secrete biofilm, which contains these proteins. Biofilms will thus have the function of absorbing heavy metals and can serve as bio-enriching devices. With good environmental adaptability and self assemble ability, biofilm will be promising to be put into practise.

5 Prospect

As the understanding of heavy metal pollution in soil goes deep and the requirement of environmental protection and health rises, people raise higher claim to restore the soil. Single remediation technique is hard to acquire perfect result. As a consequence, it becomes a development tendency to study how to reasonably combine different techniques and make a breakthrough in the field of remediation of soil pollution.

To degrade heavy metals in the environment effectively, it has become a research in the field of environmental microorganism to develop effectively strains with the help of synthetic biology. Synthetic biology aims at modifying natural or creating non-natural parts, and combining parts from different organisms to make metabolic non-natural pathways in cells and generate new cell factories for pollutant degradation. So it differs from traditional environmental biotechnology, whose key point is to screen suitable natural strains. To achieve this aim, people may apply synthetic biology technique, directly transform microbial metabolic pathway. By this means, people will give some strains the ability to degrade certain pollutant, enhance the degradation efficiency, and improve the environmental suitability, which means the strains can work at high-salt, acidic or alkaline, and hypertonic environment. It is sure that such effort is of great benefit to environmental modification. For example, people can modify the genes of rhizobiums, enabling them to absorb heavy metal ions instead of nitrogen and enabling them to attach to plants which don’t enter the food chain, which may be effective in limiting heavy metals entering the crops.

However, there also exist some limits. Biofilms do well in liquid environment, while it is questionable whether the devices work in soil with poor liquidity. Integrated usage of the above methods would be effectively. For example, use washing remediation to clean the soil and then use synthetic biology to clean the washing buffer. In addition, it is worrying that the genetically engineered strains would influence the natural organisms or sent engineered DNA outside. As a result, a suicide system is supposed to be added. So far, scientists have designed several such systems, based on auxotroph, unnatural amino acid, etc.[50] However, scientists should go on studying to further reduce the escape probability. Also, they must consider the possibility for the modified gene segment to have gene mutations, which destruct the expected function. Without the suicide system, humans cannot take risks to put the genetically organisms into application since the risks and consequences cannot be anticipated. People should always use genetic modified products with caution.

To sum up, synthetic biological techniques are potential solutions to heavy metal contamination. Using this technique can effectively lower the cost of controlling heavy metals since the genetically modified microorganisms can reproduce rapidly and be put into industrial production. And this measure seems much more efficient than the traditional ones. It requires more researches and perfections to improve safety and efficiency. Believing that with this technique, humans may make a breakthrough in combating heavy metal pollution and guaranteeing food security.

6 Case study

Wuji ------- a small town in China is located in southern Shijiazhuang, Hebei Province. Wuji was a important agriculture area before the 1980s. But with the quickened industrial development in China, leather industry became prosperous in Wuji after the 1980s. For this reason, Wuji was called The Home of Leather in China. Plenty leather factories were established in this small town. However, the leather factories pull waste water into the river, and soil. Though the government strengthened the supervision, it was surely difficult to absolutely stop some secret sewage discharge done by some illegal manufacturers. The waste water contains heavy metals, Cr mainly, which was used to process leather in the factories. The soil in a large area around Wuji was polluted by heavy metals, which made the agriculture soil deserted and wasted since people can no longer grow crops there. Also the ground water within that region was also contaminated by heavy metals, which have adverse influence on the agricultural areas nearby since the ground water serves as irrigation water. In 2014, China Central Television made this issue go public. The news reports that Cr concentration in soil in Wuji was 30 times higher than the normal value. Since then, under the much more strengthened supervision, the waste water discharge was controlled effectively. However, the soil and water around was polluted by heavy metals seriously already and still requires remediation before putting these soil into use again.

The government have already taken plenty of actions actively. The government strengthened the management including shutting down those factories that are not up to standard and establishing a industrial district in order to more the leather factories there and strengthen supervision. There are more investment on developing remediation technology and building relevant facilities. The government built a sewage treatment plant, which can process the sewage as well as the already contaminated underground water. This plant uses chemical methods mainly, adding chemicals to form sediments complexes, to separate water and heavy metals. This measure was effective and the government have made progress in controlling more heavy metal pollution and cleaning contaminated water.

The heavy metals in soil, however, are much difficult to be separated. Usually, people uses soil changing, or burying the contaminated soil deeply, which cannot stop the pollution radically and causes secondary pollution. In some places, people burn the polluted soil directly, similar to the thermal desorption technique, in order to separate the heavy metals and soil. However, the heat can turn Cr^{3+} into Cr^{6+} which is more toxic. The remediation of contaminated soil has been on. The traditional techniques are not effective and causes secondary pollution. Changing the soil can only be applied to a small area since removing soil in large area requires labor and seems expensive. The author of this paper suggests that Wuji can use synthetic biological technique to clean the soil. A simple genetic modification can enable microorganisms to detect and absorb heavy metals, which doesn’t require so much labor and money and also work more efficiently. Biofilms are easy to be put into industrial production and reusing. And to achieve the highest efficiency and best result, the author suggests that people should develop a systematic soil remediation process, which uses combined techniques instead of only one technique.

Step 1: The government strengthen the supervision and assure that there won’t be new pollutants adding in the soil.

Step 2: Use the synthetic biological technique, install bio-sensors to detect the heavy metal pollution.

Step 3: If detected, install biofilm that contain proteins that can absorb heavy metals. The soil should be divided into sections. Strains and the biofilm that they formed will be cultivated in the sections and ensure the strains can absorb the heavy metal ions within its section. What people need to do is to install biofilm and take it out after the absorption. The biofilm can be reused, so this step can be repeated so as to remove heavy metals effectively. The division of sections can make it easy to detect problems and work targeted. If any problem occur or any further actions should be taken, people can locate the area according to section coordinate, which can improve the efficiency and avoid repeat working.

Step 4: for those soil that the pollution is too severe to cultivate strains or plants, use chemical or physical techniques to complete the remediation.

By using combined techniques, and systematic recovery process, the heavy metals in the soil can be removed efficiently. The food security will be guaranteed and the agriculture soil that are contaminated already can be put into use again.

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