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## 污染胁迫下的蚯蚓蛋白质组学研究进展

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**摘要:**随着蛋白质组学的发展和每年有大量环境污染物进入土壤环境中, 污染胁迫模式动物的相关生物标志物受到日益关注。蚯蚓, 作为土壤中最大的无脊椎动物, 是研究和评价土壤生态污染良好的模式动物。研究蚯蚓的蛋白质组学, 对于寻找环境生态污染相关生物标志物和阐明生态毒理学机制有着十分重要的现实意义。目前已知的污染胁迫下蚯蚓蛋白质组学研究, 提供了几个特定污染物胁迫蚯蚓的蛋白表达谱。这些蛋白涉及许多生物学过程, 例如信号传导、糖酵解、能量代谢、分子伴侣和转录调节, 提示了相关污染物可能的生态毒理学机制, 有望成为潜在的生物标志物, 用于有毒污染物的监测, 但其特异性需要进一步试验的验证。本文就蚯蚓受污染胁迫的蛋白质组表达谱及潜在生物标志物进行简要综述。

**关键词:** 蚯蚓; 蛋白质组学; 双向电泳

## Advances in proteomic studies on earthworms subjected to pollution stress

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**Abstract:** With the advancement in proteomics research and yearly introduction of numerous environmental contaminants into the soil, development of biomarkers to detect contaminant-responsive proteins in model organisms is receiving increasing attention. Earthworms are the largest group of invertebrates in soil; they are a good model system for evaluating ecological soil pollution. Therefore, detecting contaminant-responsive biomarkers and elucidating the ecotoxicological mechanism in earthworms by using proteomics is of important practical significance. The protein expression profiles of earthworms subjected to several specific pollutants have been investigated. These proteins are associated with many biological processes such as signal transduction, glycolysis, energy metabolism, and chaperone and transcriptional regulation and can be used to determine the possible ecotoxicological mechanisms of relevant contaminants and as potential biomarkers for monitoring toxic contaminants. However, their specificity needs to be determined. In this study, we provided a brief overview of the protein expression profiles and identified potential biomarkers in earthworms subjected to pollution stress.

**Key Words:** earthworm; proteomics; two-dimensional electrophoresis

土壤污染是一个环境污染的世界性问题, 我国在这方面也日益突出。2014 年调查结果显示, 部分地区土壤污染较重, 耕地土壤环境质量堪忧, 工矿业废弃地土壤环境问题突出, 全国土壤总的点位超标率为 16.1%, 其中重度污染点位比例为 1.1%。另外, 种植活动中农药和养殖业中抗生素的使用, 也使得土壤生态环境

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受到前所未有的巨大压力。人们更多地关注如何快速、灵敏地监测土壤环境的受污染程度。

在土壤系统中,蚯蚓是最大的无脊椎动物,对分解活动、养分矿化和初级生产有着巨大的影响<sup>[1]</sup>,对土壤中的污染物也有不同程度的生理反应,被经济合作与发展组织(OECD)和国际标准化组织(ISO)公认为用于研究化学物对土壤无脊椎动物毒理效应的模式动物<sup>[2-3]</sup>,已经广泛应用于土壤生态毒理学研究<sup>[4-5]</sup>。目前,有关蚯蚓受土壤污染胁迫的生物标志物主要包括酶活性、溶酶体中性红染色保持时间、金属硫蛋白、热休克蛋白、组织和超微结构变化、大分子加合物、DNA 损伤等,但是研究者越来越意识到,在特定生态系统内进行毒性效应的调查研究极大地得益于多重生物标志物的应用<sup>[6]</sup>。

近几年研究表明,以双向电泳为基础的蛋白质组学在阐明环境污染胁迫蚯蚓产生的毒理学效应和机制方面有着巨大的潜力<sup>[7]</sup>。本文就通过蛋白质组学发现的蚯蚓用于土壤污染潜在生物标志物进行概述。

## 1 蛋白质组学的定义

蛋白质组学,以细胞、组织或器官内全基因组表达的所有蛋白的集合为研究对象<sup>[8-9]</sup>,包括蛋白质的基本特征和结构、蛋白质表达、翻译后修饰和蛋白质间的相互作用等<sup>[10]</sup>,能够将单个蛋白或蛋白质组与疾病或中毒相联系。与对照相比,在胁迫条件下产生的蛋白图谱复杂程度并不妨碍蛋白表达具体变化的检测和潜在作用方式的阐明<sup>[11]</sup>,之后这些蛋白可以作为某种疾病或毒物暴露的生物标志物。目前用于蛋白质组学研究的技术体系,包括以双向电泳和/或色谱为主的蛋白质分离技术和以质谱分析为主的蛋白质鉴定技术<sup>[12]</sup>。有关环境领域应用蛋白质组学的文献数量日益增多,如今已涉及的范围从微生物、植物到无脊椎动物(蠕虫、昆虫和蛤)、脊椎动物(淡水鱼类和深海鱼类)<sup>[13]</sup>。

## 2 不同污染物胁迫下蚯蚓的蛋白质组学研究

Wang 等人,在镉(Cadmium, Cd)污染胁迫蚯蚓的研究中发现 143 个蛋白差异点,其中至少在一个时间点上 28 个蛋白点上调和 28 个蛋白点下调,成功鉴定出 51 种蛋白<sup>[14]</sup>;在大肠杆菌(*Escherichia coli* O157:H7)污染胁迫蚯蚓的研究中发现 124 个蛋白差异点,其中至少在一个时间点上 11 个蛋白点上调和 41 个蛋白点下调,成功鉴定出 42 种蛋白<sup>[15]</sup>。Wu 等人在菲(Phenanthrene, Phe)污染胁迫蚯蚓的研究中发现 81 个蛋白差异点,其中有 36 个蛋白点上调和 45 个蛋白点下调,成功鉴定出 30 种蛋白<sup>[7]</sup>。Ji 等人在四溴联苯醚(2, 2', 4, 4'-tetrabromodiphenyl ether, BDE 47)污染胁迫蚯蚓的研究中发现 28 个蛋白差异点,其中有 10 个蛋白点上调和 18 个蛋白点下调,成功鉴定出 24 种蛋白<sup>[16]</sup>。吴石金等人在邻苯二甲酸二甲酯(Dimethylphthalate, DMP)污染胁迫蚯蚓的研究中发现 140 个蛋白差异点,成功鉴定 5 种蛋白<sup>[17]</sup>。这些鉴定出的蛋白主要分为五类(见表 1):代谢功能、应激功能、防御功能、转录功能、翻译功能,预测类别和假定类别因为大多功能分类不清,故不在本文讨论范围之内。

表 1 不同污染物胁迫差异蛋白的质谱鉴定结果

Table 1 Identification of proteins differentially expressed in response to various pollutants

| 蛋白名称 Protein name               | 物种 Species                                 | 污染物 Pollutants     |                    |                       |                     |                                        |
|---------------------------------|--------------------------------------------|--------------------|--------------------|-----------------------|---------------------|----------------------------------------|
|                                 |                                            | Cd <sup>[14]</sup> | Phe <sup>[7]</sup> | BDE47 <sup>[16]</sup> | DMP <sup>[17]</sup> | <i>E. coli</i> O157:H7 <sup>[15]</sup> |
| Metabolism                      |                                            |                    |                    |                       |                     |                                        |
| 29-kDa galactose-binding lectin | <i>Lumbricus terrestris</i>                |                    |                    |                       |                     | ↑                                      |
| Actin A3                        | <i>Bombyx mori</i>                         |                    |                    | ↓ ↑                   |                     |                                        |
| Adenylate kinase                | <i>Schistosoma japonicum</i>               | ↑                  |                    |                       |                     |                                        |
| ATP synthase β subunit          | <i>Drosophila melanogaster</i>             | ↓                  | ↓                  | ↑                     | ↑                   |                                        |
| Cathepsin B                     | <i>Branchiostoma belcheri tsingtaunese</i> | ↑                  |                    |                       |                     |                                        |
| CG31075                         | <i>Drosophila melanogaster</i>             |                    | ↑                  |                       |                     | ↓                                      |

续表

| 蛋白名称 Protein name                                             | 物种 Species                                                    | 污染物 Pollutants     |                    |                       |                     |                                        |
|---------------------------------------------------------------|---------------------------------------------------------------|--------------------|--------------------|-----------------------|---------------------|----------------------------------------|
|                                                               |                                                               | Cd <sup>[14]</sup> | Phe <sup>[7]</sup> | BDE47 <sup>[16]</sup> | DMP <sup>[17]</sup> | <i>E. coli</i> O157:H7 <sup>[15]</sup> |
| Dihydrolipoamide dehydrogenase                                | <i>Gallus gallus</i>                                          |                    |                    |                       |                     | ↑                                      |
| Elongation factor 1 alpha                                     | <i>Calathus rotundatus</i>                                    |                    |                    | ↓                     |                     |                                        |
| Ewam                                                          | <i>actin-modulator of the earthworm, Lumbricus terrestris</i> |                    |                    |                       |                     | ↓                                      |
| Fibrinolytic enzyme                                           | <i>Eisenia fetida</i>                                         |                    |                    | ↓                     |                     |                                        |
| Fibrinolytic protease 0                                       | <i>Eisenia fetida</i>                                         | ↓                  | ↑                  |                       |                     | ↓                                      |
| Fibrinolytic protease 1                                       | <i>Eisenia fetida</i>                                         |                    |                    | ↓                     |                     |                                        |
| Fructose-bisphosphate aldolase                                | <i>Oligochaeta sp. MR-2009</i>                                |                    |                    | ↓ ↑                   |                     |                                        |
| GDH                                                           | <i>Tigriopus californicus</i>                                 | ↑                  |                    |                       |                     |                                        |
| GDI ( RabGDP dissociation Inhibitor ) family member ( gdi-1 ) | <i>Caenorhabditis elegans</i>                                 |                    |                    |                       |                     | ↓                                      |
| GH12605                                                       | <i>Drosophila grimshawi</i>                                   | ↑ ↓                | ↓                  |                       |                     | ↓                                      |
| GL27266                                                       | <i>Drosophila persimilis</i>                                  | ↑                  |                    |                       |                     |                                        |
| Glyceraldehyde-3-phosphate dehydrogenase                      | <i>Caenorhabditis elegans</i>                                 |                    | ↓                  | ↑                     |                     | ↓ ↑                                    |
| Human CSB                                                     | <i>Cockayne Syndrome B, Caenorhabditis elegans</i>            | ↓                  |                    |                       |                     |                                        |
| Inositol-1,4,5-trisphosphate-3-kinase                         | <i>Gallus gallus</i>                                          | ↑                  | ↓                  |                       |                     |                                        |
| Lombricine kinase                                             | <i>Eisenia fetida</i>                                         | ↑                  | ↑                  | ↓                     |                     | ↓                                      |
| Mitochondrial H+ ATPase α subunit                             | <i>Pinctada fucata</i>                                        | ↓                  | ↓                  |                       |                     |                                        |
| Myosin essential light chain                                  | <i>Eisenia fetida</i>                                         | ↓                  |                    |                       |                     |                                        |
| NRXN1 protein                                                 | <i>Homo sapiens</i>                                           | ↓                  |                    |                       |                     |                                        |
| Phosphoglucomutase 2                                          | <i>M. musculus</i>                                            | ↑                  |                    |                       |                     | ↓                                      |
| Protein disulfide-isomerase 2                                 | <i>Haemaphysalis longicornis</i>                              |                    |                    | ↓                     |                     |                                        |
| Pyruvate kinase, muscle, b                                    | <i>Danio rerio</i>                                            | ↑                  | ↑                  |                       |                     |                                        |
| Succinate dehydrogenase complex, subunit A, flavoprotein      | <i>Bos taurus</i>                                             | ↑                  | ↓                  |                       |                     | ↓                                      |
| Triosephosphate isomerase (TIM)                               | <i>Latimeria chalumnae</i>                                    | ↑                  | ↑                  |                       |                     | ↓                                      |
| Troponin I                                                    | <i>Schistosoma japonicum</i>                                  |                    |                    | ↓                     |                     |                                        |
| Tubulin, α8                                                   | <i>M. musculus</i>                                            | ↓                  |                    |                       |                     |                                        |
| Tyrosine-protein kinase receptor                              | <i>Xenopus (Silurana) tropicalis</i>                          | ↑                  |                    |                       |                     |                                        |
| Myosin regulatory light chain                                 | <i>Lumbricus terrestris</i>                                   |                    | ↑                  | ↑                     |                     | ↓                                      |
| Nucleoside diphosphate kinase A                               | <i>Amphimedon queenslandica</i>                               |                    |                    | ↑                     |                     |                                        |
| SCBP3 protein                                                 | <i>Lumbricus terrestris</i>                                   |                    |                    | ↑                     |                     | ↓                                      |
| Alpha tubulin protein, partial                                | <i>Enchytraeus japonensis</i>                                 |                    |                    |                       | ↑                   |                                        |
| Alpha-1 tubulin                                               | <i>Hirudo medicinalis</i>                                     |                    |                    | ↓                     |                     |                                        |
| Calmodulin-like                                               | <i>Sus scrofa</i>                                             |                    |                    | ↓ ↑                   |                     |                                        |
| Intermediate filament protein                                 | <i>Lumbricus terrestris</i>                                   |                    |                    | ↓                     |                     |                                        |
| Multifunctional chaperone                                     | <i>Glossina morsitans morsitans</i>                           |                    |                    | ↑                     |                     |                                        |
| Smoothelin-like protein 1                                     | <i>Crassostrea gigas</i>                                      |                    |                    | ↓                     |                     |                                        |
| Stress-related                                                |                                                               |                    |                    |                       |                     |                                        |
| Aldehyde dehydrogenase family member (alh-1)                  | <i>Caenorhabditis elegans</i>                                 |                    |                    |                       |                     | ↓                                      |
| Aldehyde dehydrogenase isoform A                              | <i>Lysiphlebus testaceipes</i>                                | ↑                  | ↑                  |                       |                     | ↑                                      |
| Caspase-8                                                     | <i>Danio rerio</i>                                            | ↑                  |                    |                       |                     |                                        |
| Chaperonine protein HSP60                                     | <i>Onchocerca volvulus</i>                                    | ↑                  | ↓                  |                       |                     |                                        |
| Cytochrome oxidase subunit 1                                  | <i>Lespesia sp. posticaDHJ01</i>                              | ↓                  | ↓                  |                       |                     |                                        |
| Extracellular globin-4                                        | <i>Lumbricus terrestris</i>                                   | ↓                  | ↓ ↑                | ↓                     |                     | ↓ ↑                                    |

续表

| 蛋白名称 Protein name                               | 物种 Species                        | 污染物 Pollutants     |                    |                       |                     |                                        |
|-------------------------------------------------|-----------------------------------|--------------------|--------------------|-----------------------|---------------------|----------------------------------------|
|                                                 |                                   | Cd <sup>[14]</sup> | Phe <sup>[7]</sup> | BDE47 <sup>[16]</sup> | DMP <sup>[17]</sup> | <i>E. coli</i> O157:H7 <sup>[15]</sup> |
| Extracellular hemoglobin linker L2 precursor    | <i>Lumbricus terrestris</i>       |                    |                    | ↓                     |                     |                                        |
| Gelsolin-like proteins                          | <i>Lumbricus terrestris</i>       | ↑ ↓                | ↓                  |                       |                     | ↑                                      |
| Heat shock protein 70                           | <i>Eisenia fetida</i>             |                    |                    | ↓ ↑                   |                     |                                        |
| Heat shock protein 90                           | <i>Opisthophthalmus carinatus</i> |                    |                    |                       |                     | ↓                                      |
| Hemoglobin chain d1                             | <i>Lumbricus terrestris</i>       |                    |                    | ↓                     |                     |                                        |
| Manganese superoxide dismutase (MnSOD)          | <i>Bos taurus</i>                 | ↑                  | ↑                  |                       |                     | ↓                                      |
| Catalase                                        | <i>Eisenia fetida</i>             |                    | ↓                  |                       |                     | ↑                                      |
| Indoleamine 2,3-dioxygenase 2-like              | <i>Anolis carolinensis</i>        |                    |                    | ↓                     |                     |                                        |
| Defense-related                                 |                                   |                    |                    |                       |                     |                                        |
| Antimicrobial peptide lumbricin1                | <i>Lumbricus rubellus</i>         |                    |                    |                       |                     | ↓                                      |
| Coelomic cytolytic factor (CCF)-1               | <i>Eisenia fetida</i>             | ↑                  | ↑                  |                       |                     | ↓                                      |
| Lysenin                                         | <i>Eisenia fetida</i>             | ↓                  | ↑                  |                       |                     | ↓                                      |
| Lysenin-related protein 2                       | <i>Eisenia fetida</i>             | ↓                  | ↑                  |                       |                     | ↓                                      |
| Transcription                                   |                                   |                    |                    |                       |                     |                                        |
| Activating transcription factor                 | <i>Bombyx mori</i>                |                    |                    |                       |                     | ↓                                      |
| Orthodenticle                                   | <i>Achaearanea tepidariorum</i>   | ↑                  | ↑                  |                       |                     | ↓                                      |
| prohibitin 2 isoform 2                          | <i>Homo sapiens</i>               |                    |                    |                       |                     | ↑                                      |
| Translation                                     |                                   |                    |                    |                       |                     |                                        |
| 40S ribosomal protein SA                        | <i>Urechis caupo</i>              | ↑                  | ↓                  |                       |                     |                                        |
| Guanine nucleotide-binding protein subunit beta | <i>Loligo forbesi</i>             |                    | ↓                  |                       |                     | ↓                                      |

2.1 代谢方面

文献中共鉴定出 41 种蛋白,涉及糖酵解、三羧酸循环、蛋白质合成与分解等生物学过程,其中至少有四种污染物胁迫研究中共同鉴定出的蛋白有,ATP 合成 β 亚基(ATP synthase β subunit)、胍乙基磷酸丝氨酸激酶(Lombricine kinase)、纤溶蛋白酶(Fibrinolytic protease)。ATP 合成酶是一种利用跨膜质子泵催化 ADP 与磷酸反应生成 ATP 的蛋白复合体,在线粒体中存在的主要是 F1-F0 型 ATP 合酶。线粒体是细胞内供能物质氧化和产生 ATP 的场所,其功能紊乱可能是导致修复病人肌肉糖代谢受损的关键因素<sup>[18]</sup>。胍乙基磷酸丝氨酸激酶,在动物体内能量产生和利用的耦合过程中起关键作用<sup>[19]</sup>。两者表达量发生显著变化表明,蚯蚓在受污染胁迫时,能量需求变化较为显著,运动系统可能发生障碍。纤溶酶能够溶解血栓,是一种重要的化疗药物<sup>[20]</sup>,污染胁迫后以下调为主,表明其在防御应答溶解纤维蛋白凝块时起重要作用。

2.2 应激方面

文献中共鉴定出 14 种蛋白,涉及氧化还原、电子转移、氧气运输等生物学过程,其中至少有三种污染物胁迫研究中共同鉴定出的蛋白有,醛脱氢同工酶 A (Aldehyde dehydrogenase isoform A)、胞外球蛋白(Extracellular globin-4)、类凝溶胶蛋白(Gelsolin-like proteins)、锰超氧化物歧化酶(Manganese superoxide dismutase, MnSOD)。老鼠在多种环境胁迫条件下体内可诱导产生醛脱氢酶<sup>[21]</sup>。Willuhn 等人发现,基因 Ebaldh 可以编码一种假定的醛脱氢酶,其表达能被镉诱导增强<sup>[22]</sup>。这证明了线粒体中醛脱氢酶的上调可能是蚯蚓应对污染物胁迫的有效解毒机制。胞外球蛋白与氧运输有关,胁迫下以下调为主。有研究称,血红蛋白的功能可能包括抗氧化防御作用<sup>[23]</sup>。凝溶胶蛋白在血管平滑肌收缩功能和运动能力发生改变时很可能起重要作用,其表达量的增加可能是蚯蚓抵御和消除污染物的机制。活性氧,是有氧代谢或氧化剂暴露后的副产物,当他们损伤核酸、蛋白和膜脂时,具有毒性或致命性。为了对抗这些具有潜在损伤特性的活性氧,需氧生物已经进化出一套由抗氧化酶系统(SODs)组成的酶防御体系<sup>[24]</sup>。锰超氧化物歧化酶就是其中之一,它能

够在细胞溶质内合成并修饰后进入线粒体基质<sup>[25]</sup>。

### 2.3 防御方面

文献中共鉴定出 4 种蛋白,主要作用是抵抗细菌,其中至少有三种污染物胁迫研究中共同鉴定出的蛋白有,体腔细胞溶解因子(Coelomic cytolytic factor, CCF),胞溶素(Lysenin),胞溶素相关蛋白 2(Lysenin-related protein 2, LRP-2)。Engelmann 等人已证明蚯蚓的自然免疫依赖于体腔细胞合成和分泌的体液抗菌分子(CCF, Lysenin, and Lumbricin I 等)<sup>[26]</sup>。在环节动物体内,体腔细胞溶解因子是一种类似于哺乳动物肿瘤坏死因子的防御分子,其在免疫应答调节中起重要作用<sup>[27-28]</sup>,细菌刺激可引起其生物合成量上调<sup>[27-29]</sup>。胞溶素是一种存在于赤子爱胜蚓体液中的造孔毒素,由包括胞溶素相关 1(LRP-1, lysenin 2)和胞溶素相关蛋白 2(LRP-2, lysenin 3)在内的蛋白家族构成<sup>[30]</sup>。Yamaji 等人研究发现,能够特异性的与鞘磷脂结合并引起红细胞溶解<sup>[31-33]</sup>,已被证明可以引起大鼠血管平滑肌的收缩<sup>[34]</sup>。另外,胞溶素相关蛋白 2 可以清除线粒体内具有潜在毒性的超氧自由基<sup>[35]</sup>。

### 2.4 转录和翻译

转录方面,文献中共鉴定出 3 种蛋白,其中至少有三种污染物胁迫研究中共同鉴定出的蛋白相关基因有 Orthodenticle (Otd)。Otd 是无脊椎动物体内调节激活转录相关的成形成基因<sup>[36-38]</sup>,也是果蝇嗅觉突触神经元和局部中间神经元发育所必需的<sup>[39]</sup>。翻译方面,文献中共鉴定出 2 种蛋白,其中至少有两种污染物胁迫研究中共同鉴定出的蛋白有 40s 核糖体蛋白 SA 型(40S ribosomal protein SA)和鸟嘌呤核苷酸结合蛋白  $\beta$  亚基(Guanine nucleotide-binding protein subunit beta)。前者属于核糖体家族,主要作用是把 RNA 和蛋白质组装成核糖体亚小单位,而后者主要参与信号传导活动<sup>[40-41]</sup>。

## 3 存在问题和展望

蛋白质组学以组织、器官或生物体所包含的全部蛋白质为出发点,考察外源化学毒物对生物体所产生的毒性效应及其导致的生物体内代谢通路的改变,可以更加全面发现生物体内蛋白水平细微的变化及其变化之间的联系,为研究者分析污染物的毒性机制和生物体防御机制提供了基础。目前已知的污染胁迫下蚯蚓蛋白质组学研究,提供了几个特定污染物胁迫蚯蚓的蛋白表达谱。这些蛋白涉及许多生物学过程,例如信号传导、糖酵解、能量代谢、分子伴侣和转录调节,提示了相关污染物可能的生态毒理学机制,有望成为潜在的生物标志物,用于污染物毒性监测,但其特异性需要进一步试验的验证。

双向电泳技术分离蛋白的范围较窄,实验操作的主观性强,难以保证实验的重复性。而目前分离范围更加广泛、通量更高的二维液相串联质谱技术与包括 iTRAQ (Isobaric Tags For Relative And Absolute Quantitation) 技术在内的同位素标记方法的结合,极大地提高了蛋白质的检测量,进而找到更多的潜在生物标志物。蛋白质组学研究不仅依赖高通量的蛋白分离技术和质谱鉴定手段,还需要蛋白质组和基因组数据库的支持。蚯蚓基因组没有进行完整的测序,许多鉴定出的蛋白质功能也只能依靠与其基因表达序列高度相似和同源性较高的基因转录翻译的蛋白功能进行注释。因此,质谱所鉴定出的蛋白质序列或经过测序所得 DNA 序列的功能一般是不能在相关数据库中准确地匹配。

总之,蛋白质组学在环境污染生态领域,尤其是污染物胁迫蚯蚓的研究中,有着极为广阔的应用前景,如能对蚯蚓的全基因组测序,将对后续蛋白质组学研究及土壤生态污染诊断和污染修复也会有很大的帮助。

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