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Title	Using recombinant E. coli displaying surface heavy metal binding proteins for removal of Pb2+ from contaminated water [an abstract of dissertation and a summary of dissertation review]
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Citation	北海道大学. 博士(環境科学) 甲第13311号
Issue Date	2018-09-25
Doc URL	http://hdl.handle.net/2115/71950
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Type	theses (doctoral - abstract and summary of review)
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File Information	Shehata_Mohamed_abstract.pdf (論文内容の要旨)

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学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Using recombinant *E. coli* displaying surface heavy metal binding proteins for removal of Pb^{2+} from contaminated water

（汚染水からの鉛除去のための表在性重金属結合タンパク質提示組換え大腸菌の利用）

Water pollution remains a serious problem with economic and public health concerns worldwide. Lead (Pb^{2+}) is one of the dangerous metals related to chronic diseases and is responsible for many deaths around the world. Exposure to Pb^{2+} is cumulative over time. High concentrations of Pb^{2+} in the body can cause death or permanent damage to the central nervous system, the brain, and kidney. This damage commonly results in behavior and learning problems (such as hyperactivity), memory and concentration problems, high blood pressure, hearing problems, headaches, retardation of growth, reproductive problems in men and women, digestive problems, muscle and joint pain. Despite the advances in technologies for removal of heavy metals e.g. Pb^{2+} from water, all current techniques have shown some limitations that obstructed their application. Taking advantages of usage of surface-displaying proteins by *E. coli* for improving the removal efficiencies of Pb^{2+}; Recombinant *E. coli* were engineered to display metallothionein (SmtB) and lead binding protein (PbrR) onto outer membrane. DNA fragments encoding these proteins were fused to DNA fragment encoding β- domain of antigen 43 (Ag43) for translocation of both heavy metals binding protein

The resultant recombinant *E. coli* exhibited a capability to adsorb Pb^{2+} successfully from water samples containing 100 mg/L of Pb^{2+}, and concentrations of Pb^{2+} reached to undetectable level after 18 hours. Heat-inactivated *E. coli* displaying PbrR and SmtB on outer membrane showed comparable removal

efficiencies to live *E. coli* cells. The present study revealed that *E. coli* cells have the characteristics to remove Pb^{2+} from acidic solution, which is a difficult issue because of the high solubility of Pb^{2+} compounds in acidic condition. Unlike the most common methods for removal of Pb^{2+} from wastewater (chemical precipitation in alkaline solution), this method could be considered for removal of Pb^{2+} at acidic solution. It is believed that removal of heavy metals by bacteria depending on their specific binding activity to metal ions has a major concern toward releasing of recombinant organisms into the environment. However, it is noteworthy to mention that heat-treatment (at 60°C for 60 min) is enough to remove viability of recombinant *E. coli* strains without losing its activity to bind Pb^{2+}, which is an important finding for the possibility of application of this system. These observations suggest that our method can be used as a promising, specific and efficient approach for removal of Pb^{2+} from contaminated water.