

## Abstract

Environmental pollution by heavy and toxic metals because of tanning, metallurgic processes, and other chemical industries is a worldwide problem distressing both human health and the environment. Heavy metals are present in the soil, but higher concentration of these elements are damaging to plants, animals, and humans. Prolonged exposure of such heavy metals can have harmful health effects on human life. Bioremediation of these heavy metals like Cu, Cd, Co, Ni, Hg, and Zn can be done by either plants, microorganisms or by the combination of two. Bioremediation is a resourceful and bright technology which is appropriate for the recovery and decrease of heavy metals in water and polluted lands. Microorganism plays an important part in bioremediation of heavy metals. The aim of this study was to examine the synergistic outcome of bacterial biofilms on the bioremediation of Zn, Co, and Cu from contaminated soils. Total 2 biofilm forming strains (QN2 & SN4) were isolated. The isolated strains were characterized morphologically and biochemically. Based on morphological and biochemical characterization these strains were identified upto genus level *Leptospira*. Ribotyping confirmed that *Leptospira* QN2 belongs to *Leptospira* sp. and *Leptospira* (SN4) belongs to *Leptospira interrogans*. *Leptospira* sp. strains showed mature biofilm formation on 5th day of incubation. The mature biofilm of selected strains (*Leptospira* sp. (SN2) & *L. interrogans* (QN4)) showed different potential for metal degradation. Using biofilm of *Leptospira* sp., *L. interrogans* and a mixture of *Leptospira* sp. and *L. interrogans* degraded Zn up to 1.46, 0.51 and 0.42 mgmL<sup>-1</sup>, respectively. Likewise, *Leptospira* sp., *L. interrogans* and a mixture of *Leptospira* sp. and *L. interrogans* degraded Co up to 0.37, 0.35 and 0.47 mgmL<sup>-1</sup>, respectively, within 5 days of incubation period. *Leptospira* sp., *L. interrogans* and a mixture of *Leptospira* sp. and *L. interrogans* degraded Cu up to 0.43, 0.78 and 0.48 mgmL<sup>-1</sup>, respectively period. Among the strains, a mixture of *Leptospira* sp. and *L. interrogans* had highest potential (0.48 ± 0.12 mgmL<sup>-1</sup>) of degradation and showed highest potential of metal degradation.

Owing to the fact, that bacterial isolates showed the significant heavy metal degradation potential, FTIR analysis was used to inspect the quantitative and qualitative variations in different functional groups of treated metal samples compared to control. Five major peaks *i.e.*, 1645 cm<sup>-1</sup>, 1456 cm<sup>-1</sup>, 1635 cm<sup>-1</sup>, 1558 cm<sup>-1</sup> and 3344 cm<sup>-1</sup> were observed in untreated metal (Zn, Cu and Co) samples. These peaks appeared almost flat in metal samples treated with bacterial biofilms. Bond stretching at peak 1645 shifted to 1643cm<sup>-1</sup> and from 3344 to 3317cm<sup>-1</sup> was also observed. The band observed in the region from 2000–2400 cm<sup>-1</sup> was linked with OH, CH, and CHO stretching vibrations in biofilm treated samples. Two strong bands at 1640 and 1540 cm<sup>-1</sup> were attributed respectively to the amide I (—CO—) and amide II (—NH—) in proteins. The significant shift in these peaks indicated the binding of metals with amides I and II in cell surfaces of bacterial strains. This disappearance of the peaks in treated sample could be attributed to the destruction of C-H, O-H, C-O, CO-O-CO and C-Cl groups by the bacteria. Spectroscopy and FTIR analyses confirmed the decolorization and biodegradation of heavy metals by the bacterial biofilm. This study suggested that the tanneries effluent of Sharaqpur regions can be a source of heavy metal degrading microbial flora. In this study, the isolated bacteria (*Leptospira interrogans* and *Leptospira* sp.) showed excellent metals (Zn, Cu and Co)