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Assessing Microbial Indicators for Heavy Metal Contamination using Automated Image Analysis

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1. Summary

The aim of this project was to develop and evaluate an image analysis protocol for the analysis of specific populations of bacteria in contaminated environments. The protocol was based on molecular detection procedures, i.e. *in situ* hybridization and subsequent epifluorescence microscopy. Processing of captured microscopy images allowed us to specifically enumerate hybridized bacteria, measure their cell sizes, subsequently calculate their biovolumes and estimate their biomass. This procedure was used to analyze bacterial populations in sediment samples from Kearny Marsh, some of which were artificially contaminated with nickel (Ni) and incubated under sulfate-reducing conditions. Compared to non-amended samples, Ni-amended samples generally displayed lower cell numbers, but cell size distributions in a larger range. These result indicated the development of different bacterial populations, and thus demonstrated the usefulness of the developed image analysis protocol.

A second evaluation of the image analysis protocol was attempted in a concomitant greenhouse study using Ni-amended sediment cores with *Spartina patens* obtained from Harrier Marsh. However, within the project time frame only baseline data on heavy metals in sediments and plants could be gathered. Ni concentrations in the original sediments from Harrier marsh averaged at about 20 ppm with decreasing concentrations with depth. Ni-amendment ($NiSO_4$ at 50 mg kg^{-1} core material) resulted in an increased uptake of Ni by *S. patens* with up to 250 ppm compared to 32 ppm for non-amended sediments. Uptake into roots of *S. patens* was accompanied by only little translocation into above-ground material. Similar results were obtained when plants were analyzed for heavy metals present in sediments (i.e. Cu, Cd, Cr, Pb, and Zn). These data provide perfect baseline information for studies on the interactions between different organisms and heavy metals that could be exploited for studies on key organisms with potential bioindicator function. The technologies developed in this project will be useful in achieving this goal in future research.

2. Introduction

New Jersey is one of the most densely populated areas of the U.S. with many industrial sites that border waterfronts of rivers, lakes, estuarine environments, canals, or the ocean. Due to the concentration of industries in these regions, many urban waterfront environments have been extensively contaminated. Industrial activities have generated a variety of waste streams that consist of highly heterogeneous mixtures of organic and/or inorganic material. Estuarine soils and sediments have intercepted these waste streams and are the repositories of decades of accumulated contamination with metals, petroleum hydrocarbons, polyaromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). In New Jersey, which currently tops the list of states with EPA "Superfund Sites", numerous sites are located in densely populated urban settings with soils containing elevated concentrations of heavy metals ("Brownfields"). Contamination is not restricted to urban settings, landfills, industrial sites, or abandoned industrial areas, but extends to adjacent natural habitats. This is the case in many areas in the Northeast, such as the urban salt marshes of the Hackensack Meadowlands, which is part of the larger