IMPACT OF HEAVY METALS IN SOIL AND THEIR EFFECTS ON SOIL MICROBIAL BIODIVERSITY

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This paper discusses soil metal interactions with microbial diversity and the potential implications for soil productivity and animal and human health. Sources and fate of metal ions in the soil environment, biological interactions and transitions, as well as adverse impacts on soil biodiversity, toxicity. The present works deals the analysis of heavy metals by electrochemical sensors and its effect on microbial communities. For this purpose a no. of bacteria were isolated and the heavy metals of Cu++, Cd++ and Zn++ tolerance in bacteria were studied. It was found that bacteria were highly sensitive to Cu++ compared to Cd++ and Zn++ metals. These finding suggested that the potential of bacteria in developing a electrochemical sensor to analyze Cu++ in soil sample.

Heavy metals such as cadmium, copper, lead, chromium and mercury, are important environmental pollutants particularly in area with high anthropogenic pressure. Heavy metals present in soil cause serious problems to all organisms. Heavy metals are often defined as a group of metals where atomic density is greater than 5 g/cm³. Metals play a vital role in the metabolic processes of the biota. Some of the heavy metals are essential and required by the organisms as micronutrients (Cobalt, chromium, nickel, iron, manganese and zinc etc.) and are known as trace element. On the other hand some other heavy metals have no biological role and determination to the organisms even at very low concentration (Cd, Hg, Pb etc.). However at high level both of the essential and non-essential metals become toxic to any organism. These heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and diversity.

Toxicity of these heavy metals occurs through the displacement of essential metals from their native binding sites or through ligand interaction. The more localized metal contamination found in urban environments. In Heavy metals a few as Cu (II), Zn (II), Cd (II) & Pb (II) arise from number of industrial, mining and agricultural activities. Copper phyto toxicity in soil is difficult to access because copper accumulates and damages roots. Soil chemical properties strongly influence Cu Specification. Usually soil contains 2-100 PPM, overage concentration of Cu is 09 PPM Copper toxicity is uncommon but can occur when soil are contamination with high copper materials. Due to the Selective pressure from the metal in the growth environment, microorganisms have evolved various mechanisms to resist the heavy metal Stress. This has become a threat to public health and microbial community. Cadmium, Copper and Zinc are among those heavy metals that are being released to environment.

In this perspective many approaches have been used to asses the risk posed by the contamination metals in soil, water bodies etc. For this purpose in present work Electro chemical sensor were developed for the assessment of heavy metal such as copper in soil and their important microbes (Bacteria). Copper Electro chemical sensor found very wide application in environmental sample Electro chemical sensor is cost effective than other. By the use of these Electro chemical Sensor, concentration of copper present in soil were determined. After that effect of these heavy metal a (Copper, Cadmium, lead etc.) toxicity is determined on the soil bacteria. In present work the tolerance of soil bacteria to heavy metal has been proposed as an indicator of the potential toxicity of heavy metals to other forms of biota. The objective of this work is to develop tool to asses the heavy metal level in the soil, and their impact an bacteria.

MATERIALS AND METHODS

(A) Development of Electrochemical Sensor: Several different electrochemical sensors have already been developed to asses the heavy metals like. Copper, cadmium and Zinc. In the present work membrane based copper, cadmium and Zinc Electrochemical sensor were prepared. For this purpose copper Sulphide, Cadmium-Thiobarbuturate and Zinc Urate were used as a Electro active material for the preperation of Electro active membrane. Electrochemical Sensor consists of a membrane in which electro active materials are impregnated. These membranes based Electrochemical Sensor selective electrode were employed successfully for preperation of heavy metal Electrochemical sensor. These electrode have good reversion response.
against the copper, cadmium and Zinc ion in concentration change. Copper, cadmium and Zinc Electrochemical sensor pressed diameter of 8 nm Showed in nernstion response from 10-5 to 10-1 M of Copper and Cadmium ion concentration. Further more aiming to its application for analysis of contamination soil electrochemical sensor is remarkable produced of this approach. Before applying these electrochemical sensor it is essential to study the characteristic of electrochemical sensor. The main characteristic of these electrochemical sensor are given in Table-1.

(B) Isolation of the bacteria: Bacterial cultures were isolated from soil sample using standard dilution plate technique A 10 Fold dilutions of fresh soil (1 g) were made in phosphate Buffered saline and 0.1 ml from each of these dilution were spread a triplicate Trypticase soy agar plates. Plates were incubated at 25°C for 2-3 days colonies with different morphological appearance were selected from these culture plates and purified by further subculturing in the same media. All the cultures were stored at -80°C in the Trypticase soya broth with 20% glycerol. Two isolates were selected based on the grams reaction and further characterized using morphological (shape, motility, presence of endospores) biochemical (catalase activity, oxidase activity, acid production from glucose oxidation fermentation reaction characteristics properties).

RESULTS AND DISCUSSION

Determination of the Effect of metals on bacterial Growth: Toxicity of the Selected metals to the bacteria isolation was determined using seven concentration of each metal. these concentration ranged from 0 to 8.0 mg/L medium. Several well sterile polystyrene micro plates were used in this study as growth vessels sterile MES buffered minimal medium was amended with each heavy metal and inoculated with experimentally growing culture (24 h old, optical density of 0.090 at 600 nm) of bacterial isolated prepared in the same medium. Medium without metal but the bacterial inoculums (bacterial growth control) and medium with metal but without bacteria (abiotic controls) Served as controls. All the experiment were conducted in triplicate. Microplates were then closed with their lids with condensation ring and sealed using additional lab film. The test microplates were incubated at 25°C a orbital shaker at 100 rpm. Bacterial growth was measured in terms of optical density at 600 nm for 4 days at 24 hours intervals using the multi detetion Microplate Reader with equipped with KC4 software.

Identification of the Bacteria: The results of the morphological and biochemical identification experimental are shown in the Table -2 Both of these bacterial isolates are rod shaped, spore formers. They showed almost same response to the biochemical reactions tested during the study. Both of these bacterial genera represent the common soil bacterial and have been reported as soil inhabitants.

Determination of the Effect of metals on Bacterial Growth: The metal response experiments were carried out in a minimal medium which maintains a high free metal concentration in solution. Two gram positive isolator exhibited a growth patterns in the presence of different heavy metals. The growth curve for both of bacteria in the presence of different metals conc. are shown in Figs. 1 & 2. Bacteria exhibited a growth curve similar to the typical bacterial growth curve over the experiment time period (96 hrs). Bacteria B1 increased its growth and reached its maximum growth at 72 hrs. A decrease in growth (measured in terms of optical density) was observed upon increasing metal conc. at any given time interval compared to the control without metal amendment. The lower optical density values revealed that the bacterial growth was affected due to the presence of metals in the growth medium. On the other hand in the case of bacteria B2 the growth increased stealthy over the entire 96 r experimental period. However, the reduction of the growth in the presence of increased concentration of the metal used in the study was evident through out the experiment compared to the control without metal. But its growth was not effected much in the presence of Zn2+. Two bacterial isolates showed different levels of tolerance of the metal under investigation and the dose-response data for these bacteria are shown in Table-3 Estimated EC 50 Value for B1 and B2 Bacteria.

Table 1: Characteristics of electrochemical sensors

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Electroactive material</th>
<th>Lower detection limit</th>
<th>Slop of curve</th>
<th>Response Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper II</td>
<td>Copper Sulphate</td>
<td>5X10^3</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>Cadmium II</td>
<td>Cadmium Thio Burbuturate</td>
<td>1X 10^-3</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Zinc II</td>
<td>Zinc Urate</td>
<td>5X 10^-3</td>
<td>34</td>
<td>20</td>
</tr>
</tbody>
</table>
Table 2: Morphological and biochemical characters of bacterial isolates

<table>
<thead>
<tr>
<th>Code</th>
<th>Shape</th>
<th>Motility</th>
<th>Gram’s Reaction</th>
<th>Endosperm oxides</th>
<th>Collahye</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁</td>
<td>Short rod</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>B₂</td>
<td>Rod</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 3: Dose response data bacteria.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Cd⁺⁺ mg/L</th>
<th>Cu⁺⁺ mg/L</th>
<th>Zn⁺⁺ mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁</td>
<td>1.77 ± 0.16</td>
<td>0.011 ± 0.003</td>
<td>7.02 ± 0.44</td>
</tr>
<tr>
<td>B₂</td>
<td>1.53 ± 0.01</td>
<td>0.82 ± 0.04</td>
<td>2.98 ± 0.2</td>
</tr>
</tbody>
</table>

Fig. 1: Growth curves of Bacteria B1 in the presence of different heavy metals

Fig. 2: Growth curves of Bacteria B2 in the presence of different heavy metals

CONCLUSION

The current work demonstrated that the tolerance of heavy metal varied between bacteria even though they were isolated from the same soil. Both the gram positive bacteria were highly sensitive to Cu²⁺ than the other two metals. There finding revealed the potential of bacterial to assess the (copper) in soil sample. This Leads to developing a electrochemical sensor to detect Cu²⁺ in varies environmental samples.

REFERENCE
