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Effect of Nickel on Bacterial Population in Soil

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Abstract: Soil is considered as the naturally existing physical covering of the earth's surface and are home for innumerable microbes. These microbes help in keeping soil health in its proper manner. Any small variation in natural constituent of soil will alter these microbes, which affects the soils. Anthropogenic activities increased the accumulation of heavy metals in the soil. Nickel is one of the metal which present naturally in the serpentine soils. Micro flora and fauna of serpentine soils are tolerant to the presence of nickel. Nickel is a micronutrient for microbes and plant. High nickel concentration reduces the pH and decreases the bacterial count. Present study reveals that presence of both gram positive and gram negative bacteria in soils and at higher concentrations the number of both gram negative and positive cocci is reduced. Hence data demonstrate the relationship between Nickel and soil bacteria at various concentrations.
Keywords: Heavy metal, Nickel, Serpentine soils, pH, Soil microbes

I. INTRODUCTION

The soil is the cradle of untold forms of plants, animals and microbial life. According to the Soil Science Society of America, soil is defined as, "…the unconsolidated mineral or organic material on the immediate surface of the earth that serves as a natural medium for the growth of land plants..."Soils are considered as the naturally existing physical covering of the earth's surface, it represents the interface of three material states: solids (geological and dead biological materials), liquids (water), and gases (air in soil pores).They are the foundation of all terrestrial ecosystems and are home to a vast diversity of bacteria, archaea, fungi, insects, annelids, and other invertebrates as well as plants and algae. These soil dwellers perform most of the critical roles in the ecosystem. Bacteria are remarkable for an extraordinary variety of metabolic mechanisms. These metabolic activities are affected by the accumulation of chemical compounds like heavy metals etc. Nickel is one of the transition metal which will accumulate in the soil and causes various alterations in the natural metabolism of microflora and fauna of soil. Anthropogenic activities are the main source for nickel contamination. Hence the current study aims on the effect of nickel on soil properties and soil bacteria.

II. MATERIALS AND METHODS

Soil collection – Soil samples were collected from Peechi Wildlife sanctuary. Samples(015) from different locations were collected from a systematic sampling(50mX50m) design. A plot(10X10m) was laid at sample site. The plot was divided into equal blocks, 10 soil cores were obtained from the site and all samples from a plot is pooled together to get a composite sample of the plot. The samples was air dried for 24 hours in shade. The soil samples was then sieved through 2mm sieve and required quantity of soil sample stored at 4^0C till to be analysed.

A. Soil analysis

Soil pH : the level of soil pH of both control sample and test samples is analysed by pH meter.

Heavy metal analysis: The initial level of heavy metals present in the soil sample is analysed through Atomic Absorption Spectrometry by using standard solutions.

Preparation of soil samples by adding different concentrations of Nickel solutions: Six sets of soil samples were taken. One is kept as control sample. Each set with 250g of soil and mixed with 10ml of nickel solution of various concentrations such as, 0.05ppm, 1ppm, 2ppm, 5ppm and 10ppm.

Culture of bacteria- bacterial count is taken by serial dilution method, pour plate method and Gram staining technique at three different intervals with a time period of seven days. Various colonies with different morphological characters were counted and taken for gram's staining technique. One separate colonies were isolated and done the biochemical tests for the identification of bacteria.

III. RESULTS AND OBSERVATIONS

The initial concentration of nickel present in the soil sample is measured with the help of Atomic Absorption Spectrometry and the initial concentration is 2.18ppm.The CFU of soil bacteria is calculated at three different intervals. The intervals were taken as 7^{th} day, 14^{th} day and 21^{st} day after the addition of Nickel to the soil samples. The results are given below.

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A. Section A

Table 1
Effect Of Different Concentration Of Nickel On Bacterial Population At Different Intervals

Sample sets with nickel	Ist interval(7th day)		IInd interval (14th day)		IIIrd interval (21st day)	
	CFU at 105	CFU at 106	CFU at 105	CFU at 106	CFU at 105	CFU at 106
Control sample	19.5	15.5	21	3	30	26
0.05ppm	6.5	11.5	13.5	4	14	8
1ppm	16	2.5	12.5	9	12.5	8.5
2ppm	24	6.5	14	4	12.5	10.5
5ppm	12	7	29.5	11.5	14	13
10ppm	50.5	24	38.5	19.5	15	14.5

After the bacterial count, several colonies of different morphological characters are selected for the Gram's Staining method and biochemical tests. The results are given below:

B. Section B

Table 2.1
Biochemical tests and Gram staining at First interval

SOIL SAMPLES CONCENTRATION	RESULT
• Control Sample 1st colony 2nd colony 3rd colony	Micrococcus varians Bacillus sp. Enterobacteriacea
• 0.05ppm 1st colony 2nd colony	Micrococcus varians Bacillus sp.
• 1ppm 1st colony 2nd colony 3rd colony 4th colony	Rod Bacillus sp Staphylococcus aureus Staphylococcus aureus
• 2ppm 1st colony 2nd colony 3rd colony	Micrococcus varians Micrococcus varians Rod
• 5ppm 1st colony 2nd colony 3rd colony	Staphylococcus aureus Rod Bacillus
• 10ppm 1st colony 2nd colony 3rd colony	Bacillus Bacillus Klebsiella

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Table 2.2At Second interval

SOIL SAMPLES CONCENTRATION	RESULT
• Control Sample 1st colony 2nd colony 3rd colony	Rod Bacillus Micrococcus varians
• 0.05ppm 1st colony 2nd colony 3rd colony	Rod Micrococcus varians Rod
• 1ppm 1st colony 2nd colony 3rd colony 4th colony 5th colony	Rod Staphylococcus aureus Staphlococcusaureus Bacillus Rod shaped
• 2ppm 1st colony 2nd colony 3rd colony	Rod Staphylococcus aureus Bacillus
• 5ppm 1st colony 2nd colony 3rd colony 4th colony	Bacillus Rod Rod Rod
• 10ppm 1st colony 2nd colony	Bacillus Bacillus

Table 2.3At Third interval

SOIL SAMPLES CONCENTRATION	RESULT
• Control Sample 1st colony 2nd colony 3rd colony	Bacillus Micrococcus varians Bacillus
• 0.05ppm 1st colony 2nd colony 3rd colony	Bacillus Bacillus Enterobactericeae
• 1ppm 1st colony 2nd colony	Bacillus Rod shaped
• 2ppm 1st colony 2nd colony 3rd colony	Rod shaped Rod shaped Bacillus Rod shaped

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4th colony	
• 5ppm 1st colony 2nd colony	Bacillus Rod shaped
• 10ppm 1st colony 2nd colony 3rd colony 4th colony	Rod shaped Rod shaped Bacillus Rod shaped

The pH of the soil samples were measured through pH meter. The values are given below.

C. Section C

Table 3pH of the soil samples

Concentration of soil samples	pH
Control	6.52
0.05ppm	6.44
1ppm	6.40
2ppm	6.31
5ppm	6.26
10ppm	6.27

IV. DISCUSSION

Soil microbes play an important role in the recycling of plant nutrients, maintenance, detoxification of noxious chemicals, and the control of plant pests and plant growth. Alternations in the activity of microorganisms have often been proposed as a sensitive indicator of anthropogenic effects on soil ecology. (Wang et.al. 2007.) The alternations in the activity of soil microbes is mainly due to the presence of certain chemicals in an abnormal level. One of the important compounds belong to this category is heavy metals. They are highly toxic to the environment at certain concentrations. Their toxicity depends upon on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. Nickel is considered as one of the heavy metals which promotes the plant growth and bacterial growth if it is present in an adequate level. But if the concentration of nickel is increased it will negatively affect the soil pH, microbial count, soil fertility etc Soil pH is an important control on the chemical reactions that take place in soil. Each microorganism has its own optimum pH. The optimum pH range for most of the plants and microbes is between 5.5 to 7 and the maximum growth can be seen at the range of pH 6. (Section C).In the present study it is found that there is a slight decrease in the pH of the soil with increase in the concentration of Nickel in the soil. In the control sample the pH is 6.52. Whereas it is decreased upto 6.26 at the concentration of 10ppm. Each unit decrease in pH results in approximately 2-fold increases in the concentration of metals such as Zn, Ni and Cd. (Ken et.al.1997). pH interacts with heavy metals to diminish their toxicity (Roman and Margaret 1996). Nickel is well known as an essential trace elements for plants and cyanobacteria. In trace level it promote the growth of both plants and bacteria. Furthermore, Ni can be regarded as the catalyst of life. Here in the present study, the bacterial count of the control sample is steadily increasing at three different intervals, because the initial level of Nickel is about 2.18ppm which is trace level and it promotes the growth of the bacteria. The bacterial count increases from 19.5 x 10-5, 24x10-5 and 30x10-5 CFU/ml. Nickel is present in large amounts mainly in Serpentine soils. The plants and microbes in serpentine soils are highly adaptive and resistant for Nickel. The fundamental role of nickel in microbial metabolism may be the function of Ni in the synthesis of urease. Serpentine soils are rich in Nickel and chromium. Certain studies characterized the properties of nickel resistant microflora from Serpentine soils from Andaman. The potent Ni^{2+}-resistant species were identified as Pseudomonas and Bacillus. Growth kinetics of these Ni^{2+}-resistant bacteria showed a prolonged lag phase in Ni^{2+}-containing media, which extended with increasing nickel concentration.(Arundhathi.et.al. 2004.). But here in the present study the soil belongs to alluvial, clay soil. As a result, the Nickel level will be in a trace amount and the plants, bacteria will not be tolerant to the toxic effects of Nickel. The results shows that the bacterial count is decreasing with the increase

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in the Nickel concentration. A drastic decrease in bacterial count can be seen at the soil sample with 10ppm. Here, at the first interval the bacterila count is 50.5x10-5cfu/ml, whereas in the third interval it is 15x10-5cfu/ml. In the soil samples with low level of Nickel concentrations like 0.05ppm, 1ppm and 2ppm the bacterial count is increasing at certain intervals. This shows that Nickel present in trace level will promote the growth of the bacteria to a certain level. Further observation on the results shows that there is a sudden increase in the bacterial count at high concentrations like 5ppm and 10ppm at two different initial intervals. It is due to the sudden stress caused by the heavy metal. It represents the increased energy demand for repair and maintenance.(Fliebach et.al.1994).Odum (1985) pointed out that any detectable stress at the ecosystem level is a real cause for alarm as it may signal a breakdown in homeostasis. The results of the gram staining methods shows that most of the bacteria is Gram positive rod and cocci. In this the cocci shaped ones are present upto the 5ppm at the first interval. But when it reached the third interval the cocci like Staphylococcus aureus and Micrococcus aureus is present only in the control sample.

V. CONCLUSION

From this study, it comes to the conclusion that, when the concentration of Nickel in soil is increased the bacterial count falls. . The soil used for the experiment belongs to alluvial, clay category. So the microbes are not adaptive or resistant to the high concentrations of nickel. At trace amounts of Nickel such as 0.05ppm and 1ppm the bacterial count is not affected or it is normal. A sudden increase in bacterial count is seen at the concentration of 10ppm, it could be due to the repair mechanism in bacteria. Both Gram positive and gram negative bacteria were observed in the samples after staining. It was found that comparatively number of gram positive was more than negative and also gram negative cocci was negligible at certain high concentrations. The bacteria identified are Bacillus, Staphylococcus, Micrococcus and Klebsiellaspecies. When nickel concentration increases the count of Staphylococcus aureus and Micrococcus varians decreases. The present study also reveals that when Nickel concentrations increase on the soil samples the pH also decreases. The initial pH was 6.25 and it decreased to 6.26 at the nickel concentration 10ppm. When the pH lowered to acidic level it will affect the plants and microbes in a soil. More in depth studies are required to identify relationship between Nickel and soil bacteria and what extent it affects the pH of the soil and its fertility. Such studies will help in future to take precautions to preserve the natural quality of the soil.

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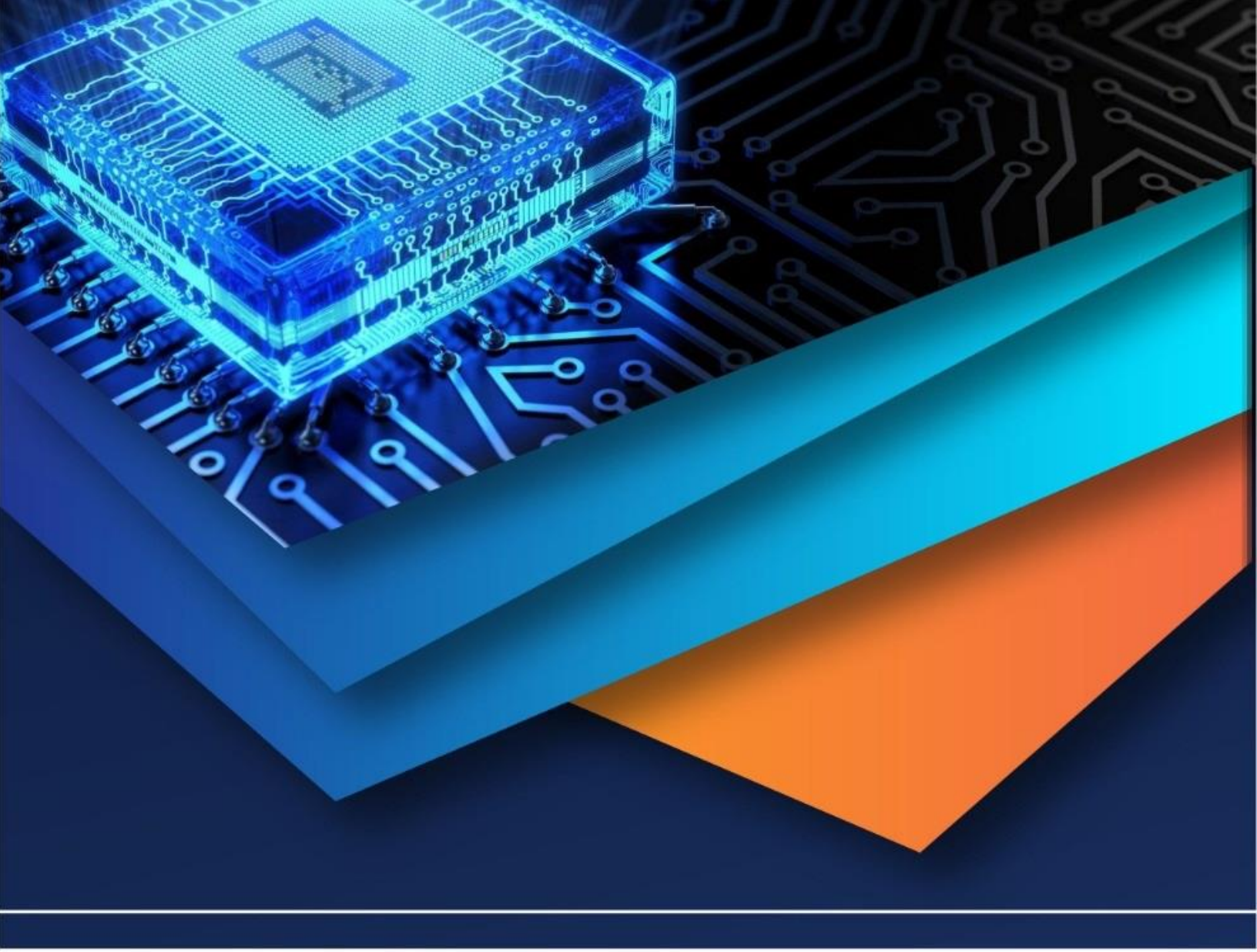
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