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Ecotoxicological Assessment of Chromium (III) and Mercury (II) Ions on *Pseudomonas aeruginosa* Isolated from Kerosene Polluted Ilorin alfisol

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Abstract

The effect of the heavy metal solution of different concentrations on *P. aeruginosa* was studied. Different concentrations of the heavy metal solution (5ppm, 20ppm and 50ppm) were spiked to a fixed volume of *P. aeruginosa* (broth). The changes on the growth of bacteria were monitored for 5days by measuring the optical density of the mixture at 600nm with spectrophotometer. The study revealed that high concentration of some trace heavy metals like Cr^{3+} and Hg^{2+} inhibit the growth of this bacterium. This is shown in the daily growth of the *P. aeruginosa* which kept reducing with an increase in the concentration of the heavy metals. The reduction was attributed to effect of high concentrations of the heavy metals that was taken up by the *P. aeruginosa* which is responsible for the observed inhibition of its growth. The potential harmful effect of these heavy metals can be seen from changes in turbidity of *P. aeruginosa* as the concentration of the heavy metals increases. These effects may also indirectly affect the aquatic animals leading to serious ecotoxicological hazard. Also, owing to the presence of heavy metals and some other pollutants the slight decrease in the growth of *P. aeruginosa* has been attributed to high concentration of heavy metals as a source of pollutants. Therefore, waste that contain either mercury or chromium as one of the major pollutants if discharged into water bodies without treatment may lead to devastating effects on the ecosystem.

Key words: *P aeruginosa,* Heavy metals, Cr^{3+}, Hg^{2+}, Pollutants, Ecosystem, Spectrophotometer.

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Introduction

The effect of heavy metals on aquatic organisms is currently attracting wide attention, particularly in studies related to industrial and anthropogenic pollution. The enrichment of coastal waters with trace metals through sewage and other anthropogenic sources has become a severe problem. This situation has resulted in numerous studies of the effects of heavy metals on benthic marine algae, especially in coastal areas (Strömgren, 1980; Markham *et al*., 1980; Filho *et al.,* 1996). Accumulation of heavy metals in marine environments has been extensively studied using marine macroalgae due to their ability to concentrate and tolerate high metal levels. Marine macroalgae have been shown to be good bioindicators of heavy metal contamination in seawater (Bryan, 1983; Soderlund *et al.,* 1988).

There are several methods used for the removal of heavy metals from waste waters and effluents. They are reverse osmosis, electrodialysis, ultrafiltration, ion exchange, chemical precipitation and phytoremediation. Microbial biomass can bind heavy metals either actively or passively or by a combination of both processes. The passive phenomenon of 'biosorption' has several advantages over the active phenomenon of 'bioaccumulation'. It is a passive non-metabolically-mediated process of metal binding by biosorbent. Bacteria, yeasts, fungi and algae have been used as biosorbents of heavy metals (Gupta and Mohapatra, 2003). Bacteria make excellent biosorbents because of their high surface to volume ratios and a high content of potentially active chemo sorption sites in their cell walls. Bacterial cell walls are negatively charged under acidic conditions and the cell wall functional groups display a high affinity for metal ions in the solution (Akram-Husain *et al*., 2013). The surfaces of bacteria cells are functional groups that act as sorption sites for special components, including heavy metals in the marine environment. An understanding of the surface modification and adsorptive properties of the bacteria cells are necessary to predict how bacteria cells contribute to environmental alterations. Because of their high sorption capacities and low production costs, the application of bacterial biomasses has also attracted the attention of specialists in the field of water treatment (Chubar *et al*., 2008).

Common environmental toxicants include polychlorinated biphenyls (PCBs), pesticides, mold and other mycotoxins, phthalates, volatile organic compounds (VOCs), dioxins, asbestos, heavy metals, chloroform and chlorine (Mercola, 2007). Most of these experimental studies under controlled conditions have been conducted on species from temperate regions (Markham *et al.,* 1980; Anderson and Hunt, 1988; Pellegrini *et al*., 1993) and data on tropical species is limited.

Materials and Methods

Isolation and identification of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa used in this work was isolated from a kerosene contaminated alfisol loam planted with *Zea mays* in Ilorin. Pure culture of bacterial isolate was identified

on the basis of their colonial morphology, cellular morphology and biochemical characteristics according to the protocol of Fawole and Oso, (2007); Barrow and Feltham, (2004). This traditional method of identification was complemented by using Microbact™ ID 24E system for the identification of Enterobacteriaceae and common miscellaneous Gram negative bacilli (MGNB). The Microbact™ ID 24E kit was used according to manufacturer's specifications (Oxoid Ltd., Basingstoke, Hants, UK).

Experimental setup

The experiments were conducted indoors. All glassware used during the experiment were soaked overnight in 1N HNO_3, then soaked in de-ionized water. *P. aeruginosa* was inoculated into separate salt solution media which had already been supplemented with chromium (III) chloride, mercury (II) chloride and control sample containing no heavy metals. The concentrations used were 0ppm, 5ppm, 20ppm and 50ppm respectively. The culture flasks were incubated at 37°C.The growth rate was measured by optical density. The culture turbidity was measured using a spectrophotometer upon calibration of the optical density in a 1cm cell at 600nm wavelength. These analyses were repeated thrice daily. Growth rate experiments were performed in batch culture.

Results and Discussion

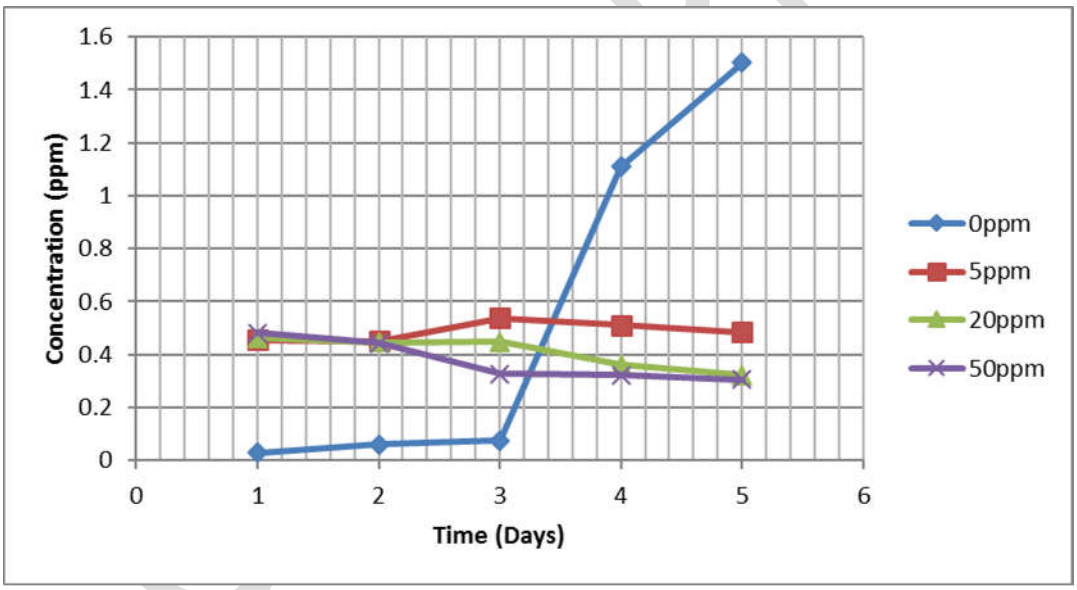

Figure 3.1: Growth pattern of *Pseudomonas aeruginosa* at different concentrations of chromium (III) solution.

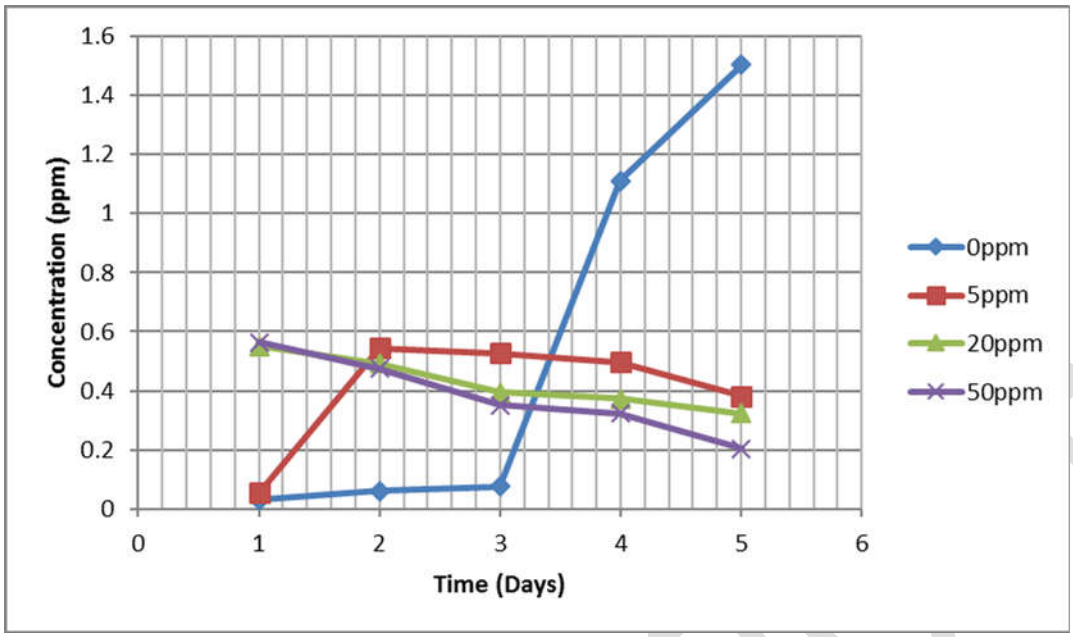

Figure 3.2: Growth pattern of *Pseudomonas aeruginosa* at different concentrations of mercury(II) solution.

Effect of different concentrations of heavy metals, chromium and mercury on *P. aeruginosa* is shown in figure3.1 and 3.2. It was observed that the growth of *P. aeruginosa* was seriously affected by various concentrations of the heavy metals within the 5 days of exposure. The optical density decreased from 0.554 to 0.204. This study shows that the growth of the bacterium reduced with an increase in heavy metals concentration which revealed the toxic effect of the heavy metals on the microorganism. Heavy metals have reported to inactivate the reaction centers and reduced performance index (Li *et al*., 2010).

Higher concentration of heavy metals solution was responsible for the inhibition of the growth of *P. aeruginosa.* Following the metals transport into the cell wall of the organism, metals may react with SH enzyme group of the *P. aeruginosa*, disrupting enzyme active sites and cell division hence reducing growth (Stauber and Florence, 1987).

Abalde *et al*. (1995) reported that biologically, heavy metals can be divided into two categories; essential and non-essential. However, high concentrations of essential heavy metals have also been reported to be toxic. Many of these metals have a direct influence on various physiological and biochemical processes, including reduction in growth, or inhibition of enzyme activities. Some heavy metals, such as chromium, mercury, lead, iron and cadmium have been observed to be essential micronutrients as trace metals though may limit microbial growth if their concentrations are too low, but they can be toxic at high concentrations (Asku *et al.*, 1991).

The slight decrease in the growth of *P. aeruginosa* has been attributed to high concentration of heavy metals solution prepared. This result is in agreement with the findings of DaCosta and Duta (2001) which reported an increase in uptake of metals with higher concentration.

Many microorganisms, such as bacteria, yeast and algae can take up dissolved metals from their surroundings onto their bodies and can be used for removing heavy metal ions successfully (Asku *et al*., 1991). Bacteria occupy the lower trophic level within food web and changes in their diversity and abundance could have an indirect but significant effect on the rest of the freshwater community. This study has shown to be in agreement with findings that pollutants are toxic to some bacteria (Holten-Lutzhoft *et al.*, 1999; and Wollenberger *et al.*, 2000). Since it has been reported that pollution are either from natural or anthropogenic sources, the heavy metals that have been discharge into the ecosystem might affect the good living of *P. aeruginosa* which might be responsible for the effect.

The effect is shown in the daily growth of the *P. aeruginosa* which were reduced with an increase in the concentrations of the heavy metals which may be due to toxicity effects of the heavy metals that was taken up by the *P. aeruginosa* which is responsible for the observed inhibition of daily growth. The potential harmful effect of these various concentrations of heavy metals can be seen from changes in turbidity of the bacterium as the concentration of the heavy metal increases. These effects may also indirectly affect the aquatic animals leading to serious ecotoxicological hazard.

Also, owing to the presence of heavy metals from different pollution sources, the slight decrease in the growth of *P. aeruginosa* has been attributed to high concentration of heavy metals as one of the major world pollutants. Therefore, if the polluted material is discharged into the rivers without treatment, it may have devastating effects on the ecosystem.

Conclusion

It has been revealed that the presence of Cr(III) and Hg (II) ions affect the growth activities of *P. aeruginosa* hence it can be inferred that the ions has effect on the ecosystem. Therefore, it would be necessary for the governmental agencies to enact laws that protect the ecosystem from the future effects of Cr (III) and Hg (II) ions.

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Conflict of interest: Nil

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