International Research Journal of Environmental Sciences ISSN 2319–1414
Vol. **6(5),** 1-5, May (**2017**) *Int. Res. J. Environmental Sci.*

Biosorption of chromium and zinc by *micrococcus varians* and *staphylococcus aureus* isolated from soil

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Available online at: www.isca.in, www.isca.me
Received 18th May 2016, revised 18th March 2017, accepted 30th April 2017

Abstract

Chromium and Zinc biosorption by Staphylococcus aureus and Micrococcus varians were carried out using 1ml aliquot of 24 hours old bacterial suspensions in 50ml nutrient broth containing distintive concentration of chromium and Zinc (0.5, 1.0, 1.5 ppm) and the pH was adjusted to 7.0 incubated at 37^{0}C with continuous shaking. The study shows that the highest biosorption rate of metal concentration was recorded by Micrococcus varians with 95.1% for Chromium and 89.0% for Zinc and the highest rate of biosorption by Staphylococcus aureus was 84.0% for Chromium and 64.0% for Zinc both on the seventh day. These findings indicates that under favourable condition Staphylococcus aureus and Micrococcus varians can expel heavy metals (Chromium and Zinc) from the earth, with Micrococcus varians having the highest potential.

Keywords: Environment, Heavy Metals, Biosorption, Contaminants.

Introduction

Heavy metal pollution alludes to the intemperate affidavit of lethal substantial elements like Zn, Pb, Mn, Mg, Hg etc present in the soil brought on by anthropogenic exercises. These elements (heavy metals) present on the earth crust incorporate around noteworthy metals of natural danger, for example, arsenic, cadmium, mercury, lead, chromium, and so on. They likewise includes other heavy metals of certain natural toxicity, for example, stannum (Sn), vanadium (V), zinc (Zn), nickel (Ni), copper (Cu), etc. As of late, for improvement of the worldwide budget, all content plus type of soil heavy metals created by anthropoid exercises has continuously expanded, bringing about the decay of the environment[1-6]. Heavy metals are very dangerous to the earth and living beings. Before, before, researchers fail to observed pollution of the soil as critical as water and air contamination, since soil contamination remained frequently harder to be measured and remediate than water and air contamination. In any case, as of late the pollution of soil in nations that are developed turns out to be intense. In this way more consideration is paid to it and turned into a hotly debated issue of environmental protection around the world.

Many heavy metals are vital to people, higher plants and animals, for instance, Mn, Ni, Cu, Zn, Cr, Co among others, aside from Pb, Hg, Pb and Cd. All of these elements have a tendency of availability in more fixations at top soil, that are an impression for expansion of the component by means of climatic deposition, use of humus, phosphate manures and the consolidation of vegetations utilized as aggregators of elements (Table-1)[7]. All in all, the plants promptly retain little concentration of Cu, Cr, Zn and Cd broke up in soil arrangement in chelated or ionic structure, or as complexes[8]. Assimilation and translocation of chromium are low, in spite of the fact that this differs as per the plants observed. Chromium is immobilized when negatively charged, mostly not on the root surface and roots. Above the ground level (shoot), the chromium level are thusly low (about 0.02 to 1 mg·kg−1) and increases just somewhat when manifestations of noxiousness appear[9]. The biomagnifications of this element in biological communities is a noteworthy risk to life[10,11]. To agree to permissible limits, different systems are utilized for the evacuation of heavy metals. The recuperation of these elements utilizing traditional methods is not conomical and not eco-friendly[12]. Customary physico-chemical techniques, for example reverse osmosis, dissipation, electrochemical treatment, ion exchange, sorption and precipitation[13,7].

Table-1a: Heavy metal toxicity effects on plants.

Heavy metal	Plant	Toxic effect on plant	Ref.
Cr	Wheat (*Triticum*sp.)	Reduced shoot and root growth	I4-15
	Tomato (*Lycopersicon esculentum*)	Decrease in plant nutrient acquisition	16-17
	Onion (*Allium cepa*)	Inhibition of development process	18

Table-1b: Heavy metal toxicity effects on plants.

Heavy metal	Plant	Toxic effect on plant	Ref.
Zn	*Cyamopsis tetragonoloba* (Cluster bean)	Reduction in carotenoid, amino acid contented, chlorophyll, sugar, and starch, decrease in germination percentage; biomass and reduced plantheight	19
	Pisumsativum (Pea)	Reduction in photosystem II activity; decrease in chlorophyll content; modification in organisation of chloroplast, decrease plant development	20
	Lolium perenne (Rye grass)	Growth reduction; reduced efficiency of photosynthetic energy conversion.	21

Heavy metals overabundance occurrence in the soil start from numerous bases, that includes sewage watering system, atmospheric deposition, mining exercises, the utilization of pesticides and fertilizers, inappropriate stacking of the industrial solid waste[22]. Transport, particularly the car transport, causes genuine substantial metal pollution (Cd, Cu, Pb, Zn, Cr, and so forth.) of the air and soils[23].

The measure of heavy metals that gets into the soil by raining sedimentation and normal deposition are identified with the level expulsion of metal by microorganisms is a mind boggling process that relies on several factors, for example, cell wall structure of microorganisms, chemistry of metal ions, cell physiology and physico-chemical components, for example, contact time, ionic quality, pH, temperature and metal concentration[25]. The point of this study was along these lines, to analyze the capability of Micrococcus varians And Staphylococcus aureus in Zinc and chromium removal.

Materials and methods

Assortment of soil sample: Soil sample was collected from the botanical garden of Biological Science Department, Federal University of Technology, Minna, Nigeria.

Isolation of organisms: The soil sample obtained was used to prepare a soil slurry by adding 1 gram of the soil to 9ml of sterile distilled water and was mixed for 15 minute, 0.1ml of the serially diluted sample were cultured on nutrient agar by method of spread plate and incubated at 37^0C up to 24hours.The colonies were numbered and recorded in cfu/g. Sub-culturing of the colonies were done repeatedly on nutrient agar to obtain pure-culture and then stored in an agar slants in their pure forms for further characterization and identification.

Preparation of metal solutions: The stock solution of Potassium dichromate [$K_2Cr_2O_7$] and zinc sulphate [$ZnSO_4.7H_2O$] were prepared by dissolving 2.8g and 2.2g respectively in half a litre (500ml) distilled water, agitation for fifteen minutes and then allowed to stand for a period of one day in order to obtain a whole dissolution of the salt. The initial Zinc and chromium concentration were determined using the AAS (Atomic Absorption Spectrophotometer). The pH of the solutions were also adjusted to pH 7 using sodium hydroxide (NaOH) and hydrochlroric acid (HCl).

Experimental design: Two millilitres (2ml) broth of a day old bacteria culture (*S.aureus* and *M. varians*) all inoculated into fifty milliliters of nutrient broth having the different concentrations (0.5, 1.0, 1.5ppm) of Cr and Zn separately. The metal pH solution was adjusted to the pH value of 7 before the different isolates were added to the solution. This was done by adding appropriate amount of NaOH and HCl respectively, and the pH reading was taken using pH meter. The conical flasks were incubated at a temperature of 37^{O}C. Each conical flask was withdrawn at specific intervals of 0, 7, 14, 21, 28 days of inoculation, centrifugation was done at 4000rpm for 25minutes.

After centrifuging, the supernatant was digested in correspondence to their varying concentration using nitric acid of 4ml for every metal solution sample. The concentration of metal was determined by AAS. The biosorption percentage was determined by Beer Lambert's law:

$$\text{Percentage (\%)Biosorption} = \frac{\text{Initial metal concentration} - \text{final metal concentration}}{\text{Initial metal concentration}} \, x \, 100$$

Results and discussion

Biosorption of Zinc: Remediation of Zinc through biosorption potential of *Staphylococcus aureus, Micrococcus varians* were studied in different concentrations (0.5, 1.0, 1.5ppm) and also at different time intervals (7, 14, 21 and 28days) (Figure-1). Highest biosorption rate was observed in *M. varians* on day 7 for the 0.5ppm concentration of Zinc at 89%. While the highest biosorption observed in *Staphylococcus aureus* was also on seventh day for 0.5 ppm concentration of Zinc was 64%. At the 28 day, there was insignificant sorption by both organisms. The variation in percentage varied in order of 0.5>1.0>1.5 concentration of Zn, and 7>14>21>28 days. The change in percentage sorption could be as a result of Zinc at a higher concentration and a longer period could cause damage to the cell of the organisms.

Munoz *et al.*[7] reported that population of microbes in metal contaminated environment adjust to dangerous groupings of heavy metals and get to be metal safe. Consequently, they can sorbs the heavy metals[26]. Utilization of microbial assets coupled to other cutting edge procedures is a standout amongst the most encouraging and practical systems for evacuating environmental pollutants[27].

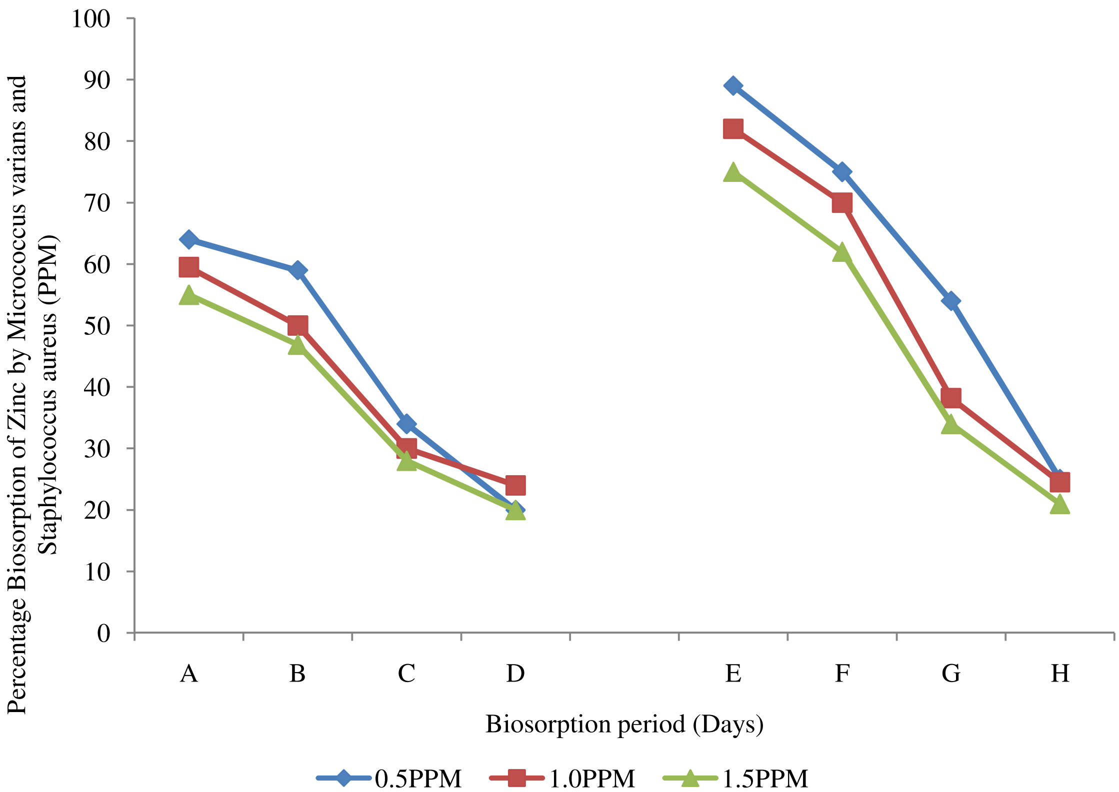

Figure-1: Percentage biosorption of Zinc by *Micrococcus varians* and *Staphylococcus aureus.*
Key: A= Day 7 of Biosorption with *Staphylococcus aureus,* B= Day 14 of Biosorption with *Staphylococcus aureus,* C= Day 21 of Biosorption with *Staphylococcus aureus,* D= Day 28 of Biosorption with *Staphylococcus aureus,* E= Day 7 of Biosorption with *Micrococcus varians,* F= Day 14 of Biosorption with *Micrococcus varians,* G= Day 21 of Biosorption with *Micrococcus varians,* H= Day 28 of Biosorption with *Micrococcus varians.*

Biosorption of Chromium: The highest sorption percentage was recorded in *Micrococcus varians* on the seventh day for the 0.5ppm concentration of Zn was 95.1%. The highest sorption observed in *Staphylococcus aureus* was also on the seventh day for 0.5ppm concentration of Cr was 84%. After day 28, there was insignificant sorption by the two organisms. The change in percentage varied in order of 0.5>1.0>1.5 concentration of Cr, and 7>14>21>28 days. The change in percentage biosorption could be as a result of the fact that Chromium at a higher concentration and a longer period could cause damage to the cell of the organisms (Figure-2). However, Fred et al.[28] found that, fungi *Gomus intraradices* might enhance resilience and retention of sunflower to Cr. Species of Sporophyticus, Aspergillus, Pseudomonas, Phanerochaete and Bacillus have been accounted for as effective chromium reducers[29]. Hamza et al.[30] reported normal procedure utilizing microorganisms as an extremely powerful and ecological benevolent technique for cleaning.

Based on the effect of metal concentration on percentage sorption of Zn and Cr by *Staphylococcus aureus* and *Micrococcus varians,* the highest sorption rate 7-28 days was noted for the two metals at the concentration of 0.5 ppm and the lowest rate of sorption at 1.5 ppm. Be that as it may, the lethal result of metals to organisms at high concentrations could have caused this. The highest rates of sorption of Zn and Cr were recorded on day 7 for 0.5 ppm at 89% and 64% for *Micrococcus varians* and *Staphylococcus aureus* respectively for Zn, 95.1% and 84% for *M. varians* and *S. aureus* respectively for Cr. The lowest rates of removal were recorded on day 28 for the two metals. Comparing the same concentration of 0.5 ppm, *Staphylococcus aureus* and *Micrococcus varians* were having a biosorption rate of 21% and 20% respectively for Zn on 28 days and 22% and 15% respectively for Cr on 28 days. The reason for the decline in the rate of biosorption from day 7 to day 28 could be as a result of the saturation of the organism-metal binding sites. Biosorption of Chromium and Zinc by *M. varians* also gave a positive result in recent work by Kabala et al.[31]. Abioye et al.[32] found that *Pseudomonas aeruginosa* and *Bacillus subtilis* were able to effectively carry out biosorption of Chromium at varying optimum conditions (Temperature, pH, contact time), but it was found that the Gram positive *Bacillus subtilis* was more efficient than Gram negative *Pseudomonas aeruginosa*, this may be due to the fact that Gram positive bacteria are rich in techoic acid which serves as a source of carboxyl groups that are the main agents in heavy metal uptake

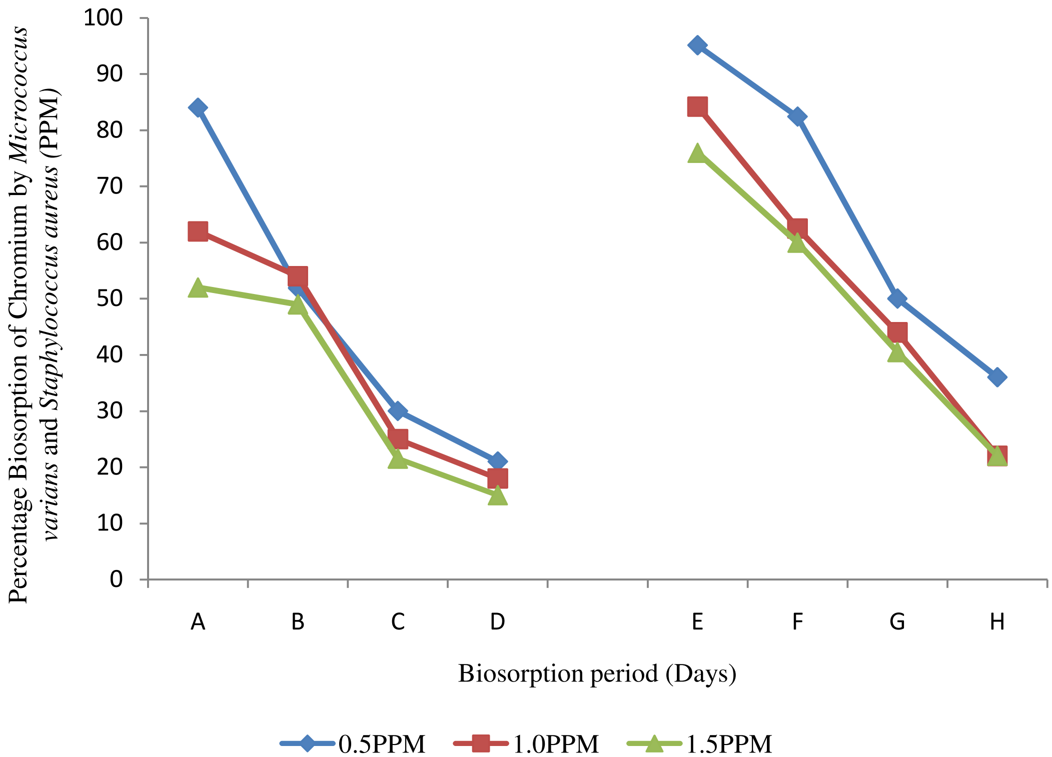

Figure 2: Percentage biosorption of Chromium by *Micrococcus varians* and *Staphylococcus aureus.*
Key: A= Day 7 of Biosorption with *Staphylococcus aureus,* B= Day 14 of Biosorption with *Staphylococcus aureus,* C= Day 21 of Biosorption with *Staphylococcus aureus,* D= Day 28 of Biosorption with *Staphylococcus aureus,* E= Day 7 of Biosorption with *Micrococcus varians,* F= Day 14 of Biosorption with *Micrococcus varians,* G= Day 21 of Biosorption with *Micrococcus varians,* H= Day 28 of Biosorption with *Micrococcus varians.*

Conclusion

The procedure of sorption by organisms have been shown as a helpful option techniques for the removal of lethal metals from contaminated environment over other conventional methods. Conclusively from this research, *M. varians and S. aureus*can possibly evacuate heavy metals like Zinc and Chromium from the environment under a favorable growth conditions. Hence, they can be used as a bio-agent for the biosorption of Zinc (Zn) and Chromium (Cr).

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