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Removal of lead and inhibition of algal growth using prodigiosin produced by *serratia marcescens*

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Abstract

This study examined the removal of lead and inhibition of Anabaena sphaerica and Oscillatoria agardhii growth using prodigiosin produced by Serratia marcescens. Inhibition of the algal growth was studied by the addition of different concentration of prodigiosin 50µl, 100µl, 150µl in 90ml of algal culture. Control was without the pigment prodigiosin. Inhibition rates were determined at the interval of 72 hours of incubation using spectrophotometer. A.sphaerica record highest level of inhibition in all concentrations. It was observed to record highest levels of inhibition at 100µg/L concentration of prodigiosin which was 76.7%, while O.agardhii was 66.3% at the same concentration. At concentration of 50µg/L A.sphaerica record of 66.3% inhibition with O.agardhii recording 64.3% while at concentration of 150µg/L it was 67.2% and 66.5% respectively. Statically analysis shows no significant differences between the rate of inhibition by the two algal species studied but there was significant difference between concentration of prodigiosin and the rate of inhibition. Removal of lead polluted soil sample was studied by the addition of different concentration of prodigiosin 50µl, 100µl, 150µl to 5g of lead polluted soil in 90ml of distilled water. The lead removal rate was determined at the interval of 4 weeks of incubation for six months using atomic absorption spectroscopy (AAS). The result shows that 100µl of prodigiosin enhance high amount of lead removal from soil. The amount of lead immobilized by the pigment at 20 weeks was 52.5% and become stable after 24 weeks. This was followed by 50µL and 150µL of prodigiosin in which there was immobilization of 41.2% and 35.3% respectively at 20 weeks of treatment.The results suggest that the red pigment inhibited cyanobacteria growth and can be used as a potential for the removal of soil polluted with lead.

Keywords: *Serratia marcescens,* prodigiosin, *Anabaena sphaerica*, *Oscillatoria agardhii*, Lead removal, Algal growth inhibition.

Introduction

Heavy metals, especially lead, mercury and cadmium, are important environmental contaminant that presents a greater danger to the ecosystem. Exposure to lead results to health effects on almost every organ in human systems, especially the nervous system, the kidneys, and the cardiovascular, immune and reproductive system[1]. This pollutant finds their way into underground aquifers and drinking water sources, thus posing threat to both human and animal health. The worst heavy metal pollution incidence in Nigeria occurred in Zamfara State in 2010 which claimed the lives of over 500 children following small scale mining operations[2]. Illegal miners took home rocks containing gold ore, unaware of the fact that the ore also contained extremely high levels of the toxic heavy metal, lead. This resulted in the death of hundreds of villagers, mainly children.

Microbial metabolite prodigiosin has been used to reclaim soil, but information on their bioremediation potential is scanty. However, mechanisms of bioremediation of lead by prodigiosin produced by *Serratia marcenscens* have not been adequately studied[3]. Prodigiosin is a tripyrrole first characterized from *Serratia marcescens,* which forms beautiful pillar box red colonies. Its name is derived from "prodigious" - something marvellous. The prodigiosin tripyrrole was reported to be localized in extracellular and cell-associated vesicles and in intracellular granules[4].

Some species of toxic phytoplanktons grow rapidly in seawater leading to a phenomenon called the harmful algal bloom (HAB), which has made a disturbance in the ocean ecosystem by massacring fish, shellfish, and other marine life to result in a massive economic loss in fishery. Harmful algal blooms cause massive economic loss and environmental disturbances[1]. Algal bloom also impacts negatively on fishes, navigation, water sports and angling and can impair the quality of drinking water source. Aquatic organisms such as fishes are affected because they are deprived of oxygen. Mechanical damage to other organisms, such as disruption of epithelial gill tissues in fishes resulting in asphyxiation. The presence of algal blooms in water bodies can prevent the use of the water for livestock, irrigation

and can clog water filter at water treatment works. Algal bloom has been a threat to aquatic life with a slow but considerable damage. Fishes prawns and aerobic bacteria are the major flora damaged[3,2]. Beside, aquatic ecosystem as small as a pond and the seashore are adversely affected[5].

In an effort to develop short-term solutions for controlling HABs, several approaches are being explored, including chemical methods[6,7], physical manipulation (clays, flocculants[8,9] and currently, biological agents [10,11]. For example, treatments of algicidal copper sulfate[12] clay flocculation[11], or UV irradiation[13] have been investigated and applied for the removal of harmful algae. Although effective in controlling blooms, chemical approaches are regarded to be potentially dangerous since chemical agents can cause serious secondary pollution[14], and can indiscriminately kill other organisms in the aquatic ecosystem, altering marine food webs and eventually impact natural fish communities[15]. The high cost of physical manipulation may be impractical[16]. Therefore, biological agents, including bacteria[16], protozoa[15], viruses[17] and macrophytes are now being considered as potential inhibitory agents in controlling the outbreak of algal blooms. This has become a research hotspot in recent years based on the advantages of its efficiency, species-specificity and environment-friendliness[18,19].

The abundance of certain marine bacteria increased during the decline of several algal blooms[20]. Interestingly, some of them has potential to secrete metabolic compounds[21] that can specifically kill harmful phytoplanktons, inferring that the extracellular algicidal compounds might be used as a biological control agent in natural seawaters.

Several algicidal marine bacteria have been reported to produce extracellular algicidal compounds: *Pseudomonas* sp., *Flavobacterium* sp., *Alteromonas* sp.[22], *Pseudoalteromonas*sp.[23], *Bacillus sp*.[15] and *H. chejuensis*[6]. For example algicidal small molecule produced from the marine bacterium *Bacillus* sp. SY-1 was identified as bacillamide and characterized to kill *Cochlodinium polykrikoides* (LC50 of 3.2 µg/mL after 6 h)[15], γ-*Proteobacteria* is one of the most prevalent prokaryotic groups in the marine environments[24], showing a broad spectrum of algicidal activity against bloom-forming red-tide phytoplanktons. *Cochlodinium polykrikoides* (the harmful dinoflagellate resulting to considerable mortality of aquatic organisms and economic loss in Korean coastal waters[7]were treated with crude red pigment solution containing prodigiosin, the algal cells bursted very quickly[6]. Phlorotannins from the brown algae *Ecklonia kuromel*[25] and rhamnolipid biosurfactant produced by a strain of *Pseudomonas aeruginosa*[21] were able to kill the red-tide algae *Heterosigma akashiwo* and *Karenia mikimotoi*.

Therefore, the present study was aimed at utilizing prodigiosin from *Serratia marcescens* for the removal of lead polluted soil and inhibition of algal growth at various concentrations in water as biological mechanism of water purification.

Materials and methods

Organisms: *Serratia marcescens, Anabaena sphaerica* and *Oscillatoria agardhii* were obtained from Department of Microbiology, Federal University of Technology Minna Nigeria.

Algal Growth inhibition: Inhibition of algal growth was studied by the addition of different concentration of prodigiosin 50µl, 100µl, 150µl in 90ml of algal culture in conical flask. Control was without the pigment prodigiosin. Inhibition rates were determined at the interval of 72 hours of incubation using spectrophotometer.

Lead removal using prodigiosin: Removal of lead polluted soil sample was studied by the addition of different concentration of prodigiosin 50µl, 100µl, 150µl to 5g of lead polluted soil in 90ml of distilled water. Lead polluted soil was used for the experiment and the initial concentration was determined before treatment. The removal rate was determined at the interval of 4 weeks of incubation for six months using Atomic absorption spectroscopy (AAS).

Statistical Analysis: All the experiments were conducted in triplicates to ensure the accuracy of the results. Statistical package SPSS for social sciences was used. ANOVA of variance was used to test for significant differences between prodigiosin production in different media, optimization of bioprocess variables for pigment production, anti-algal activity and lead removal.

Results and discussion

Comparative study of prodigiosin produced by *S. marcescens* on inhibition of *Anabaena sphaerica* and *Oscillatoria agardhii*at 15th day's incubation from 0.60µg/L: The data documented in Figures-1, 2, 3 shows the comparative study of prodigiosin on growth inhibition of *Anabaena sphaerica* and *Oscillatoria sp*. *Anabaena sphaerica* record highest level of inhibition in all concentrations. It was observed to record highest levels of inhibition at 100µg/L concentration of prodigiosin (Figure-2) which was 76.7%, while *Oscillatoria agardhii* was 66.3% at the same concentration. At concentration of 50µg/L *Anabaena sphaerica* record of 66.3% inhibition with *Oscillatoria agardhii* recording 64.3% (Figure-1) while at concentration of 150µg/L it was 67.2% and 66.5% respectively (Figure-2). Statically analysis shows no significant differences between the rate of inhibition by the two algal species studied but there was significant difference between concentration of prodigiosin and the rate of inhibition.

Rate of lead removal by different concentrations of prodigiosin produced by *Serratia marcescens:* The results of the rate of lead removal by different concentrations of prodigiosin produced by *Serratia marcescens* is presented in Figure-4. The result clearly shows that 100µl of prodigiosin

enhance high amount of lead (Pb) removal from soil. The amount of lead (Pb) immobilized by the pigment at 20 weeks was 52.5% and become stable after 24 weeks. This was followed by 50µL and 150µL of prodigiosin in which there was immobilization of 41.2% and 35.3% respectively at 20 weeks of treatment.

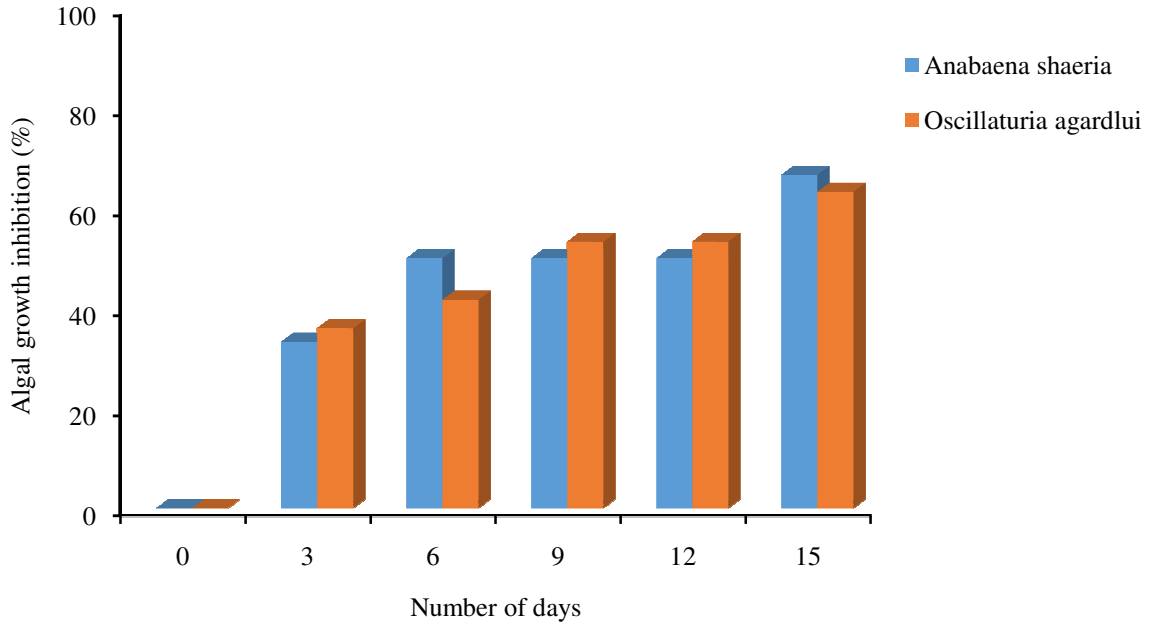

Figure-1: Inhibition of algal growth rate at concentration of 50µL of prodigiosin produced by *Serratia marcescens*.

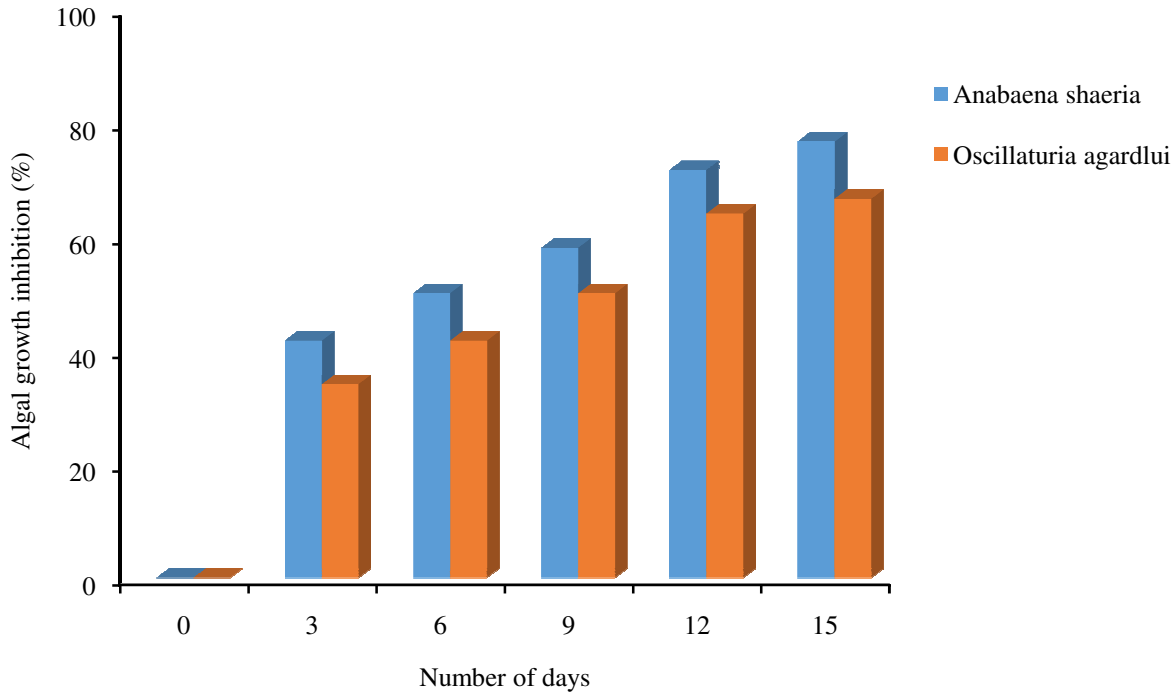

Figure-2: Inhibition of algal growth rate at concentration of 100µL of prodigiosin produced by *Serratia marcescens*.

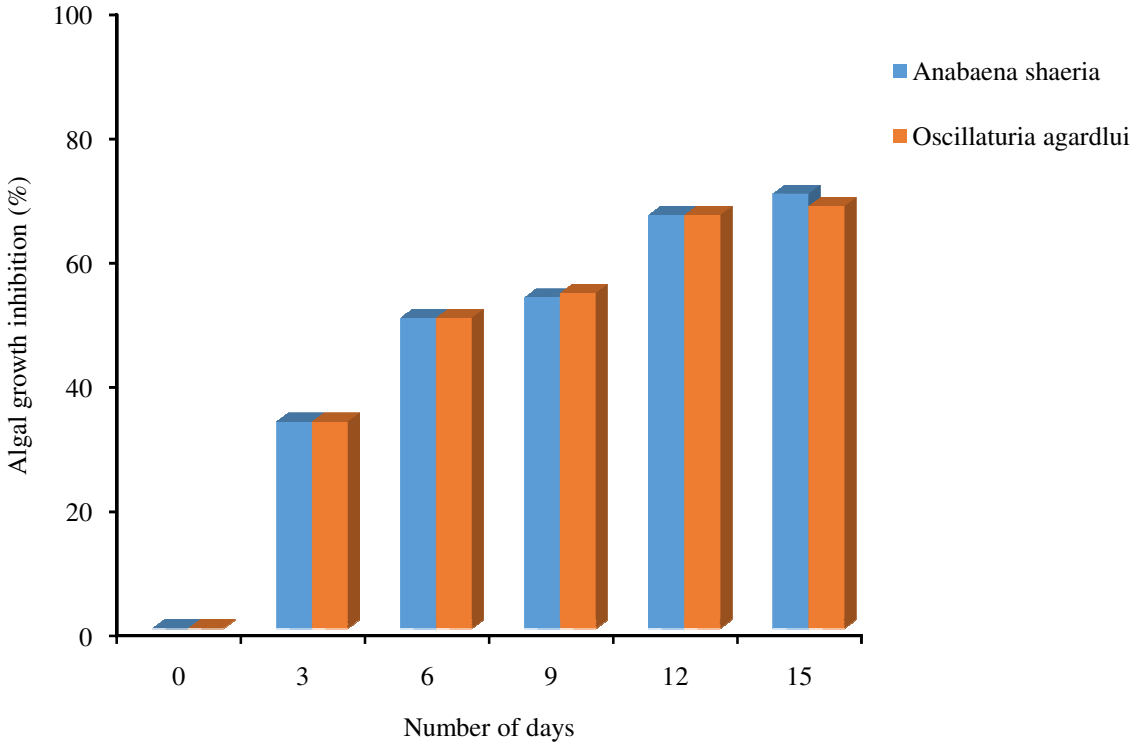

Figure-3: Inhibition of algal growth rate at concentration of 150µL of prodigiosin produced by *Serratia marcescens*.

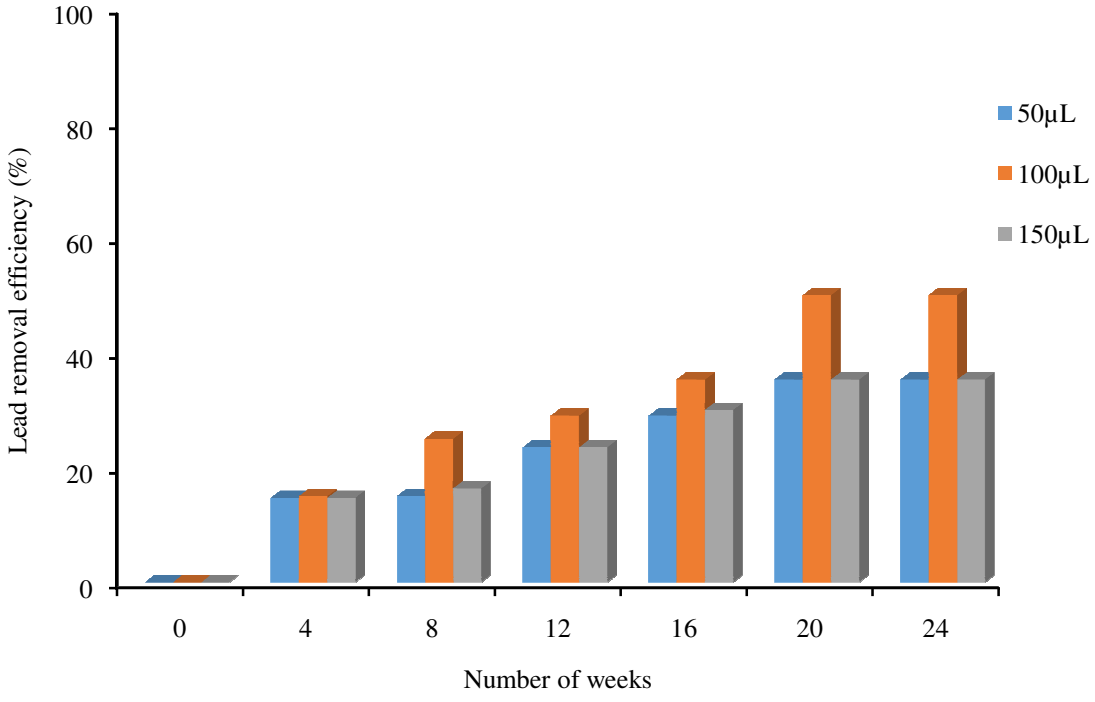

Figure-4: Removal of lead from soil by prodigiosin produced by *Serratia marcescens*: Fermentation conditions: 50µL, 100µL and 150µL of prodigiosin, six months of incubation.

Discussion: The result presented in Figures-1, 2 and 3 showed that prodigiosin produced by *Serratia marcescens* inhibited algal growth at different concentrations in a short period of time and recorded appreciable level of inhibition seventy-five percent (76.7%). The present study agrees with observation made by Jin *et al.*[26], who use microbial pigment produced by *Serratia marcescens* SL08 to inhibit the growth of harmful algal bloom in marine water and obtained seventy percent (70%) inhibition, but in contrast to report by Van Hullebusch *et al.*[12], who used microbial pigment produced by *Serratia marcescens* BTWJ8 to treat algal growth and recorded twenty percent (20%) inhibition.

In the present study, biological inhibition using prodigiosin from *Serratia marcescens* was used as an environmental friendly strategy. The result documented in Figures 1, 2, 3 indicated that prodigiosin has ability to inhibit *Anabaena sphaerica* and *Oscillatoria agardhii* growth especially at concentration of 100µL (76.7%) inhibition compared to concentrations of 50µL and 150µL (63- 66%) inhibition respectively. The results obtained in this study is in accordance with the reports made by Jeong *et al.*[6] and Jin *et al.*[26] who use crude red pigment containing prodigiosin, to treat *Cochlodinium polykrikoides* which resulted in rapid bursting of algal cells, which resulted in 78% inhition. This result also agrees with the report made by Furstner A.[27], Takamatsu *et al.*[28] and Dembitsky *et al.*[29] that natural pigment has cytotoxic effects. In the present study, there was a significant difference in the pattern of inhibition of *Anabaena sphaerica* and *Oscillatoria agardhii* by the pigment. The inhibition decreased in pigment coated substrates when compared with controls.

The result obtained using prodigiosin against cyanobacteria growth inhibition showed the higher anti-cyanobacteria potentials of the red pigment on *Anabaena sphaerica* than *Oscillatoria agardhii* and are in accordance with the observation of Mekhael H. and Yousif H.[30] who study the inhibitory effect of prodigiosin against both Gram positive and Gram negative bacteria and found higher inhibitory effect of prodigiosins against Gram positive than Gram negative bacteria. The antalgal activity of prodigiosin could be as a result of their ability to pass through the outer membrane and their capacity for inhibiting target DNA modulating enzymes. The present result is also in agreement with the report of Berlanaga *et al.*[31], that DNA gyrase and topoisomerase IV, has potential to inhibit the cell growth.

The present study also agrees with the reports of Jeong *et al.*[6] who used prodigiosin produce from *Serratia marcescens* to inhibit the growth of cyanobacteria bloom in water. In addition, the present study also look at the removal of lead from polluted soil by different concentration of prodigiosin (50µL, 100µL, 150µL). This was due the fact that prodigiosin binds to heavy metal to both detoxify and remove the lead. The result obtained in this study shows that prodigiosin at concentration of 100µL gives higher removal of lead (52.3%) from soil as compared to other concentrations. The results suggest that the red pigment inhibited cyanobacteria growth and can be used as detoxifer of soil polluted with lead at different concentrations.

Conclusion

The result obtained using prodigiosin produced by *Serratia marcescens* in the present study against algal growth inhibition showed the broad anti cyanobacteria potential of the prodigiosin and also immobilization of lead polluted soil as form of environmental pollution control measure that is cost effective with little or no side effects.

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