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**RESEARCH ARTICLE****Bioremediation of Heavy Metals from Crude Oil Polluted Soil Using *Serratia marcescens* and its Toxicology Assessment on Aquaculture****Giwa, Oluwamodupe Emmanuel<sup>1</sup> ✉, Arotupin, Daniel Juwon<sup>2</sup> and Akinyosoye, Felix Akinsola<sup>3</sup>**<sup>1,2,3</sup>Department of Microbiology, Federal University of Technology Akure, P.M.B 704 Akure, 340001, Nigeria**Corresponding Author:** Giwa, Oluwamodupe Emmanuel, **E-mail:** [giwa\\_muyiwa@yahoo.com](mailto:giwa_muyiwa@yahoo.com)

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**ABSTRACT**

*Serratia marcescens* has been studied to show a higher degradation potential for organo-phosphorus pesticides. This property has made it useful as a potential bioremediation agent. The bacterial cell was bio-stimulated in luria beterni broth at a selected experimental temperature ranging from 25 °C – 40 °C. The highest cell biomass was recorded at 35 °C to range from 7.56 - 8.10mg/ml for single strength and double strength yeast extract in luria beterni, respectively. Percentage bio-sorption efficiency of both the cell culture and extracted membrane protein augmented cell culture of *Serratia marcescens* showed 100% removal in heavy metals such as lead, potassium and manganese, respectively. The cell culture percentage sorption also showed 99.2%, 95.9% and 82% removal of copper, nickel and zinc, respectively, while the protein extract augmented cell culture showed improved corresponding heavy metal removal with 100% removal of copper and nickel and 86.5% removal of zinc. The bacterial cell and corresponding protein extracts tend to create oxygen stress in an aquaculture model, reducing the dissolved oxygen level to 3 mg/l. Lethal dose 50 of the bacterial extracts were high a 14.14%; hence this showed high toxicity or pathogenicity of the bacteria. This showed that the bacterial cell and corresponding proteins extract showed promising in the removal of heavy metals in an aquaculture environment; however, its toxicity effect on the test fishes makes it a potential risk to aquatic life.

**KEYWORDS**Heavy metals, bio-sorption, *Serratia marcescens***ARTICLE DOI:** 10.32996/bjes.2022.2.1.5

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**1. Introduction**

The biotechnological process that uses bio-cells and corresponding metabolic by-products to treat and remove the environmental pollutant has been defined as bioremediation (Baghel and Pandey, 2013). This process ranges from oxidation of organic contaminants, biotransformation of organic chemicals into less toxic constituents, reduction of electrophilic halo- and nitro- groups by removing electrons from the pollutant to an acceptor in order to generate energy (Rockne and Reddy, 2003). Genetical engineering of some microbes as a bioreactor to competent cells can pick up foreign gene of interest and secrete the corresponding related protein of interest associated with some specific action, one of which is bioremediation has been explored in the recent decade. *Serratia marcescens* has been studied to secrete laccase proteins, a multi-copper oxidase enzyme that is able to initiate and catalyze the oxidation of one electron of a wide range of phenolic compounds, at a wide temperature and pH range (Kaira *et al.*, 2015). This ability of *Serratia marcescens* to produce active enzymes at a wide temperature and pH range makes it a potential prospect and focal point of interest in the industrial uses for these enzymes, especially in the harnessing of the oxidative potential of this protein in bioremediation purposes. This enzyme has been generally regarded as safe (GRAS) and eco-friendly since it uses molecular oxygen to initiate the process and yields water as the sole by-product (Karla *et al.*, 2020). *Serratia marcescens* has been studied to show a higher degradation potential ranging from (5-13%) for organo-phosphorus pesticides removal hence, suitable for bioremediation of soils contaminated with organo-phosphorus pesticides (Cycoń *et al.*, 2013). *Serratia marcescens* has also been studied and reported to secrete a set of proteins (prodigiosin and pyocyanin/ pyoverdin). As a matter of fact, various mutants have been reported with pigments protein colouration bound to the bacterial cell envelope (Elkenawy *et al.*, 2017).

Microbial proteins may show some involvement in the attraction of heavy metals to the microbial cells via a mechanism of polarity charging the cell wall of the microbes, which has been studied to be anionic therefore providing the binding sites for heavy metal that is cationic in nature (Michalak *et al.* 2013), hence, the attraction of heavy metals, uptake and further sequestration and adsorption from the environments (Deip *et al.*, 2018). Intracellular and extracellular metal sequestration via the aid of microbial proteins has also been reported by Malik (2004) and Mishra and Malik (2013). Little information is available in the exploration of bioremediation potentials of *Serratia marcescens* on heavy metals and its possible toxicity interference with the ecosystem. Therefore, the study focused on the use of *Serratia marcescens* to remediate heavy metals from crude oil-polluted soil and its toxicological effect on aquaculture.

**2. Materials and Methods**

**2.1 Isolation, Enumeration and Identification of *Serratia marcescens***

Microorganisms were isolated and enumerated from both polluted and unpolluted soil samples using the standard procedure (Benson, 2001; Adamu *et al.*, 2015). The crude oil contaminated soil sample was diluted serially by transferring 1 ml of slurry soil sample into 9 ml sterile distilled water and were mixed homogeneously to dilution fold of 10<sup>-1</sup>. This was further diluted to ten-fold dilutions by serially inoculating 1 ml of consecutive dilutions into test tubes containing 9 ml of sterile distilled water up to the ratio of 10<sup>-9</sup> dilution. The culture medium nutrient agar oxid (NA) and mineral salt media (MSM) of Ijah *et al.* (2008) (1.2 g KH<sub>2</sub>PO<sub>4</sub>, 1.8 g K<sub>2</sub>HPO<sub>4</sub>, 4.0 g NH<sub>4</sub>Cl, 0.2 g MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.1 g NaCl, 0.01 g FeSO<sub>4</sub>.7H<sub>2</sub>O and 20 g agar per litre at pH 7.4; supplemented with 0.1% v/v Bonny Light crude oil) used for the bacteria isolation.

**2.2 Bioremediation potential of *Serratia marcescens***

*Serratia marcescens* heavy metal reduction ability was evaluated using the cells biomass, which was immediately mixed with a slurry of soil polluted with crude oil, while heavy metal evaluation before the mixing and after the mixing was enumerated via atomic absorption spectrophotometer for the period of 3 weeks following procedures described by Tessier *et al.*, (1985) and Omoni *et al.* (2015).

**2.3 Effect of *Serratia marcescens* and corresponding crude protein from on pH, dissolved oxygen (DO) and Lethal dose (LD<sub>50</sub>) on aquaculture**

An aquaria model containing graduated crude protein extract concentration was set up as 0%, 10%, 20%, 30%, 40% and 50% and cultivated with 10 three days old Juvenile catfish (*Clarias gariepinus*), which was inoculated with the *Serratia marcescens* crude protein extract. The dissolved oxygen (DO) and pH were observed and recorded by means of a water quality meter (YSI 85) and pH meter (HANNA, HI 8314) for 24 hours. Lethal dose 50 of each crude protein extract was evaluated using the Lorke principle. The highest dose without mortality (a) and lowest dose with mortality (b) was used to calculate the lethal dose 50 as follows LD<sub>50</sub> = (a x b)<sup>1/2</sup>

**2.4 Screening of interference of extracted crude protein on aquatic flora**

A portion of 100 ml of water from the aquaria model was inoculated into luria beterni broth for 24 hours for cell growth. A 100 ml of selected microbial cell and corresponding protein extract in luria beterni broth were added. Turbidity was measured every 30 minutes by comparing the turbidity of each sample with a standard blank using a spectrophotometer (Jenway, Model 6305) (Rajnovic *et al.*, 2019).

**3. Results**

**Table 1: Effect of temperature on *Serratia marcescens* biomass (mg/ml)**

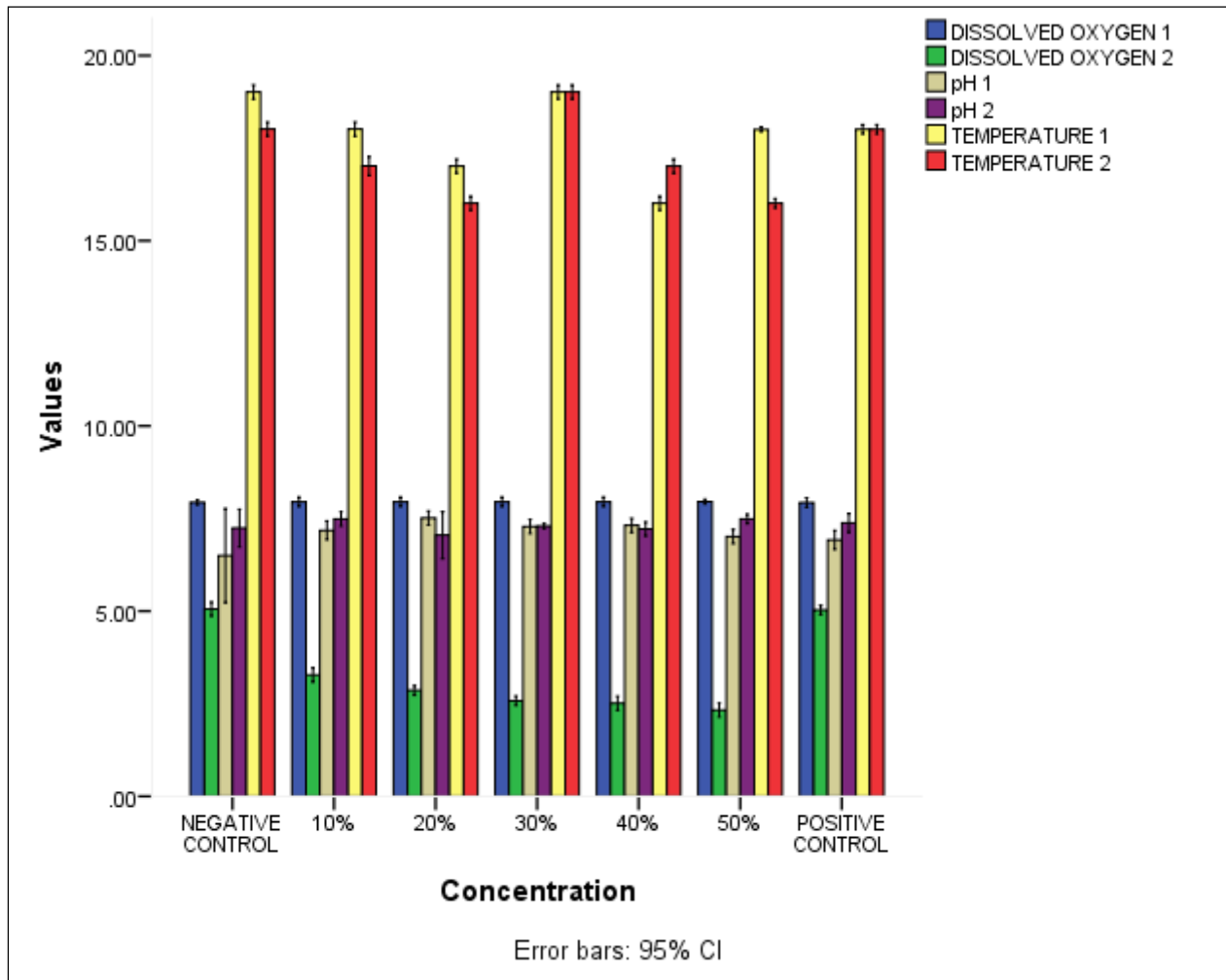
Bacteria isolates	°C	LB	LB+
<i>S. marcescens</i>	25	6.99±0.02	8.32±0.00
	30	7.23±0.00	7.84±0.00
	35	7.56±0.02	8.10±0.00
	40	7.27±0.00	7.63±0.00

**Key:** LB= Single strength yeast extract luria bertani broth, LB+ = Double strength yeast extract luria bertani broth, °C= Temperature

**Table 2: Percentage sorption efficiency of both *Serratia marcescens* cells and their corresponding extracts on heavy metals from soil polluted with crude oil**

Heavy metal	% sorption by cell culture	% sorption by cell culture + EMP	Metal ion concentration in Control sample ppm
Lead	100	100	11
Copper	99.2	100	25
Cadmium	0	0	0
Nickel	95.9	100	34
Zinc	82	86.5	22.2
Potassium	100	100	4.24
Manganese	100	100	1.19
Cobalt	0	0	0

Key: EMP = Extracted membrane protein



**Figure 1: Effect of *Serratia marcescens* crude protein extract concentrations on Physico-chemical parameters of experimental aquaria model**

Key: Negative control = aquifer without fish, Positive control = aquifer with fish

**Table 3: Average weight gain/loss (gram) of juvenile fish culture in an aquarium inoculated with isolates and their corresponding crude proteins over a period of 28 days**

SN/Period (Days)	1	7	21	28
<b>SMP</b>	80.81±1.09	0.00±0	0.00±0	0.00±0
<b>CSM</b>	88.71±0.27	83.92±0.94	66.65±1.00	0.00±0

**Key: SMP= *Serratia marcescens* Protein, CSM= Cell of *Serratia marcescens***

**Table 4: Lethal dose 50 of *Serratia marcescens* isolates cell and crude protein extract**

Protein extract	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%	LD <sub>50</sub>
<b>SMP</b>	0	0	2	3	4	4	4	4	4	4	14.14

**Key: SMP= *Serratia marcescens* proteins**

#### 4. Discussion

Table 1 shows all cellular biomass of fermentation samples derived at different temperatures. An increase in temperature was observed to have a linear effect on the biomass measured in mg/ml until a decline was recorded after the temperature of 35°C for both LB and LB+ as 7.56 and 8.10 mg/ml, respectively. This significant increase in biomass weight in all the selected fermentation temperatures ranging from 25 °C, 30 °C, 35 °C and 40 °C with an increase from 6.99 to 8.32 mg/ml, 7.23 to 7.84 mg/ml, 7.56 to 8.1 mg/ml and 7.27 to 7.63 mg/ml respectively at the probability level P<0.05 showed that double-strength yeast extract in luria bertani broth has a significant effect on the biomass growth. Also, the highest biomass weight gain observed was under 25 °C fermentation temperature with double strength yeast extract luria bertani broth (LB+). Some of the proposed mechanisms for heavy metal biosorption, removal and detoxification depend on polarity charging of the microbial cell wall (Michalak *et al.* 2013; Formina and Gadd 2014), which aids in heavy metals uptake, sequestration and adsorption from the environments (Mishra and Malik, 2013; Deip *et al.*, 2018). Hence, the 100% reduction was observed for lead, potassium and manganese in table 2. However, zinc, nickel and copper sorption percentage decreased when cell culture alone was applied but, increased reduction/removal was recorded when the membrane protein extracted was added to the cell culture. This increased sorption potential could be a synergy, probably since the copper may be halting the secretion of the cytochrome protein (Korashy and El-Kadi, 2005). The cell culture with the addition of the extracted microbial protein tends to remove more heavy metal sorption percentages quantity compared with sorption capacity of the cell biomass. The application of these proteins extracellularly may not be stable. This has been reported that prodigiosin and pyocyanin/ pyoverdin, pigments protein produced by *Serratia marcescens*, are non-diffusible and water-insoluble pigment bound to the bacterial cell envelope (Elkenawy *et al.*, 2017). This property implies that these microbes, too, may need adsorption and sequestration of heavy metal mechanisms. The release of its protein extracellularly to act has biosurfactant metabolites and probably interferes with the ecological balance may be near impossible because of the long-term stability. Al-Ghanem (2018), has reported the photodegradation of the protein pigment prodigiosin of *Serratia marcescens*.

The addition of the isolates containing corresponding crude protein extract in figure 1 may impact and increase the microflora competing with the available oxygen. Therefore, the more the broth culture of each isolate is added to each aquaria model, the significant reduction in the observed dissolved oxygen over a period of 24 hours at probability level P≤0.05. Pigmented *Serratia marcescens* has been reported to possess enzymes that protect it from reactive oxygen species (superoxide dismutase, catalase or peroxides) and ozonation; hence, it can survive the presence of oxygen and its derivatives (De-Ondarza, 2017), and however, it is a documented facultative anaerobe. The significant reduction in dissolved oxygen can also be associated with interference with the quality of life of the fishes hence leading to death and reduction of life in the aquaria organisms. The extracted crude protein from *Serratia marcescens* appears more virulent than the cultured cell (Table 3). The cell culture reduced the weight of the fishes from day 1 till day 21, after which every cultivated fish died. However, the crude protein extract was observed to kill all cultivated fishes before day 7. Therefore, the virulence of the crude protein extract appears to be more potent compared with the cultured cell of *Serratia marcescens*. *Serratia marcescens* cell and protein both kill the cultivated fish. The organisms have been reported to be pathogenic on honey bees and fishes (Wamala *et al.*, 2018). The death of fishes in the pond infected with *S. marcescens* may have been due to the pathogenicity of the bacterium. *Serratia marcescens* have been reported to be pathogenic towards *Phyllophaga ravid* (Scralab) insect larvae as well as causing anti-feeding effects, change in colour and mortality (Pineda-Castellanos *et al.*, 2015). Furthermore, the cell-free extract of the *S. marcescens* culture was found to be toxic to the insect larvae causing significant mortality. These reports corroborate the findings of this study. The organism may have produced proteinous toxins, which may have a lethal effect on the fish fingerlings. Earlier, *Serratia marcescens* was reported as the bacterium that causes disease in plants and in a wide range of both invertebrate and vertebrate hosts. It is even reported as an opportunistic human pathogen that can be life-threatening (Kurz *et al.*, 2003; Sutherland *et al.*, 2011). Many *S. marcescens* strains are also resistant to multiple antibiotics, according to Moradigaravand *et al.* (2016). These showed that the bacterium might be pathogenic to catfish by both exotoxin

production and active infection by the bacterial cells. Pineda-Castellanos *et al.* (2015) had reported that *Serratia* isolates produce an extracellular single 50 kDa protease which may be responsible for the mortality induced by the isolates in fish observed in this study. The LD<sub>50</sub> of crude extracted protein from *Serratia marcescens* are very virulent at a concentration of 14.14 % lethal dose required to kill 50% of the cultivated fishes. This value further showed how virulent the isolate is against the test aquatic fishes.

## 5. Conclusion

Though *Serratia marcescens* showed promising in the removal of heavy metals from soil polluted with crude oil in the aquatic region, the recommendation of its usage in the bioremediation of heavy metal is questioned due to its high toxicity effect on the physicochemical parameters and the pathogenicity of the cell and the extracted proteins.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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