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The effect of heavy metals on biodegradation of carbofuran by microbial strain enriched from Agricultural areas

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ABSTRACT

The effect of heavy metals on the degradation of carbofuran by immobilized bacterial cells was checked by culturing in the MSM medium at 37^{o}C in the presence of 0-1 mg/L of heavy metals. Bioremediation systems represent a biologically sustainable means to degrading organic pollutants such as carbofuran insecticide, with little energy demand and operational expenses in addition to high efficiency and substrate specificity. Nonetheless, heavy metals present in the agricultural lands may thwart the process efficiency by poisoning carbofuran-degrading microbial isolates. Therefore, we experimentally tested the tolerance of carbofuran-degrading immobilizedbacterial isolated from vegetable plantation area for mercury (Hg) and copper (Cu). The results indicated that the tested metals inhibited carbofuran biodegradation to different extents, depending on concentration. At pH 7.0 and 37°C, complete inhibition of carbofuran biodegradation by Hg occurred at 0.4, 0.5, 0.6 and 0.7 mg/L. Lower concentrations of these metals decreased the rate of carbofuran− biodegradation, with relatively long lag times. Interestingly, the immobilize isolate tolerated higher concentrations, although both the rate and extent of carbofuran− biodegradation were affected.

***Keywords*:** Metal toxicity, metal tolerance, Carbofuran, Biodegradation.

INTRODUCTION

Remediation of sites co-contaminated with organic and metal pollutants is a complex problem, as the two constituents mostly must be treated separately (Sandrin *et al*., 2000). Though some of these metals are essential for biological function, unjustifiable quantities mostly result in the inhibition of essential biological reactions via numerous pathways (Adekomaya, 2020). A number of reports collectively showed that various metals, such as Al, Ni, Cu, Zn, Pb, and Hg at a range of concentrations have adverse effects on the degradation of organic compounds (Huang *et al.*, 2001). Heavy metal contamination is significantly different from many organic chemical pollutants. Many organic chemical pollutants can be achieved through the physical nature of their own physical, chemical or biological effects of self-purification, reduce its toxicity, the lifting of the original harm will be caused by pollutants (Ndungu *et al.,* 2019). Heavy metal affect soil microbial activity, the effect on soil enzyme activity and the composition of soil microbial community.

Carbofuran is one of the harmful substace that affect living organisms. Heavy metals contamination is also a serious threat to the environment, because it has major effect on living organisms in aquatic,

terrestrial and air habitats (Metwally *et al.*, 2021). They are not degradable metabolically but build up in living tissue resulting to health related problems and death of organisms (Weber *et al.*, 2019). Bacteria have developed resistance mechanism to tolerate the toxicity of heavy metal ions; they include efflux of metal ions outside the cell, amassing and complexation of metal ions within the cell and attenuation of heavy metal ions to a less poisonous compounds (Zhang *et al.*, 2019). Therefore, the present study aimed to evaluate the effect of Hg and Cu on previously characterized carbofuran - degrading bacteria isolated from agricultural area.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals utilized for the research were obtained from chemical agents. Heavy metals, Phosphate buffer, nutrient agar and other media were supplied by Merck, Germany. carbofuran was acquired from Sigma Aldrich. For analytical methods Optical Density (at 600nm) was used for measurement of bacterial growth.

Media preparation

Minimal salt medium (MSM) was prepared according to (Onunga *et al.*, 2015) with compositions in g/L): $NaHPO_4$ 5.57g, $KH_2PO_4$2. 44 g, NH_4Cl 2.00 g, $MgCl_2.6H_2O$ 0.20 g, $MnCl_2.4H_2O$ 0.0004, $FeCl_3.6H_2O$ 0.001 and $CaCl_2$ 0.001 g/L. The MSM medium pH was adjusted to 7 then autoclaved at 121 °C for 15 min.

Growth studies

In this study 1 ml of study sample was collected by the use of a sterilized micropipette and placed in a 1.5 ml sterilized centrifuge tube. The sample was centrifuged by Sigma 2-3 Sartorius centrifuge at 10,000 x g for 10 minutes. About 100 µl of sample was drawn using sterilized micropipette and conveyed into a 15 ml centrifuge tube containing 9.9 ml of deionized water to dilute the sample. The sample was stirred by the use of a vortex machine. 500 µl of diluted sample was transferred to a sterilized 1.5 ml centrifuge tube. The tube was tightened and mixed gently. It was allowed to stay at room temperature for 2-3 minutes. The mixture was incubated at 25 °C for 5 minutes. Absorbance was determined (at 600 nm) using Shimadzu U.V. Mini 1240, spectrophotometer against reagent blank.

Heavy metals effect on biodegration of carbofuran

The effect of heavy metals Hg and Cu was tested on the biodegradation of carbofuran by *Enterobacter* sp. Strain BRC05. Thirty-six hours old bacterial culture with an Optical Density of 0.7-0.8 was used as inoculum for free cells and immobilized cells. The bacteria (free or immobilized) were inoculated into the MSM medium supplemented with 0-1 mg/L of heavy metals. For each heavy metal used the experiments were carried out in triplicate. Medium without the addition of heavy metals was used as control (Sow *et al.*, 2013). The medium was incubated using a shaker incubator (150 rpm) at 37^oC. Carbofuran concentration was taken every six hours until the reduction of Carbofuran was observed. Bacterial growth measured by taking the OD600 for free cells. The heavy metals that affected the carbofuran degradation were observed, and the experiments were repeated for immobilized cells and the incubation period was extended until the maximum degradation of carbofuran has been realized. All experiments were carried out in triplicates.

RESULTS AND DISCUSSIONS

Heavy metals Hg and Cu were tested against carbofuran degrading strain isolated bacteria to investigate their effect on their ability to degrade carbofuran. The toxicity of heavy metals ranges in an array of highest toxic to the lowest toxic element. Mercury (Hg^{2+}) is considered to be highly toxic while Cu is considered to be lowest in toxicity. In the present study, the effect of mercury on carbofuran degradation was determined using 0.1 to 1 mg/L concentrations. The outcomes (Fig. 1) revealed that concentrations of mercury from 0 to 1 mg/L have affected the degradation performance of the compound carbofuran. Increasing the concentration of mercury to 0.5 mg/L causes a reduction in degradation of carbofuran even by the immobilized cells which can be classified as detrimental. An analysis of variance showed no significant difference ($P \geq 0.05$) in the degradation of carbofuran by free cells between 0.4, 0.5, 0.6 and 0.7mg/L. Also, no significant difference ($P \geq 0.05$) was observed for immobilized cells between 0.1, 0.2, 03 and 0.4. While, there was a significant difference between other concentrations of mercury ($P \leq 0.05$). It has been reported that metals appear to affect organic compound biodegradation by affecting both the physiology and ecology of organic compound degrading microorganisms (Sokhn *et al.*, 2001). Radjendirane *et al.* (1991) showed that mercury can hinder hydroxylases activity like aryl 2,4-dichlorophenol hydroxylase and 3-hydroxybenzoate-6-hydroxylase. Ibrahim *et al*. (2015) found that concentrations of mercury from 0.1 to 1 mg/L affected the degradation of caffeine by *Leifsonia* sp. isolated from soil.

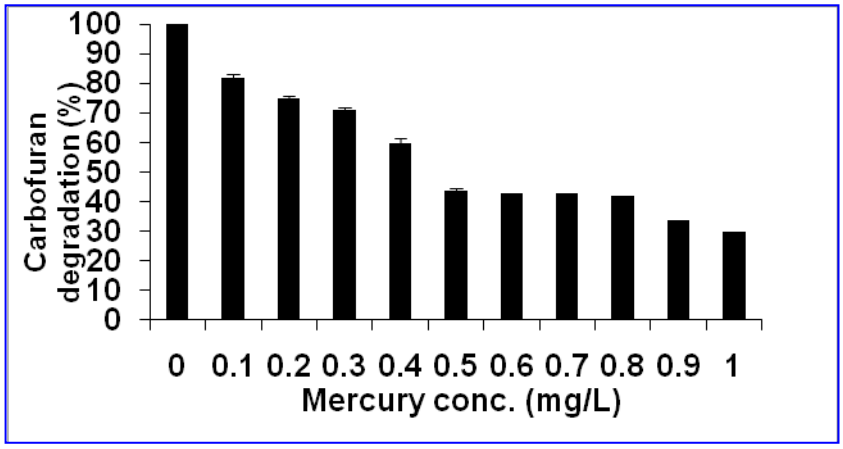

Fig. 1. Effect of Mercury concentrations on Carbofuran degrading Activities by Immobilised Cells of *Enterobacter* sp.

Mercury (Hg) belongs to one of the noxious non-radioactive heavy metals which is widely dispersed in nature (Zhou *et al.*, 2015). It is a persistent and bio-accumulative compound. Many polluted sites are co-contaminated with organic and metal pollutants. Data from both aerobic and anaerobic systems showed that biodegradation of the organic compound may be reduced by metal toxicity (Ibrahim *et al.*, 2015; Kamashwaran and Crawford, 2003; Liu *et al.* 2014; Radjendirane *et al.* 1991). Growth and degradation of dibenzofuran by *Sphingomonas wittichii* RW1 were strongly inhibited by mercury at 1 mg/L (Hong *et al.* 2007).

The effect of copper (Cu) with concentration ranging from (0.1 to 1 mg/L) on carbofuran degradation was evaluated. It was obvious from Figure (2) that Cu concentration from 0.1 to 0.4 mg/L has a negligible effect on carbofuran degradation by the cells ($P \geq 0.05$). The lower the metal concentration the higher the degradation of carbofuran. Conversely, the degradation of carbofuran was affected tremendously when the concentrations of Copper was increased from 0.4 mg/L ($P \leq 0.05$). It has been reported that metals upset pesticide degradation through catalyzing the hydrolysis of target pesticides and at the same time influencing the microbial activity in soil (De *et al.*, 2003).

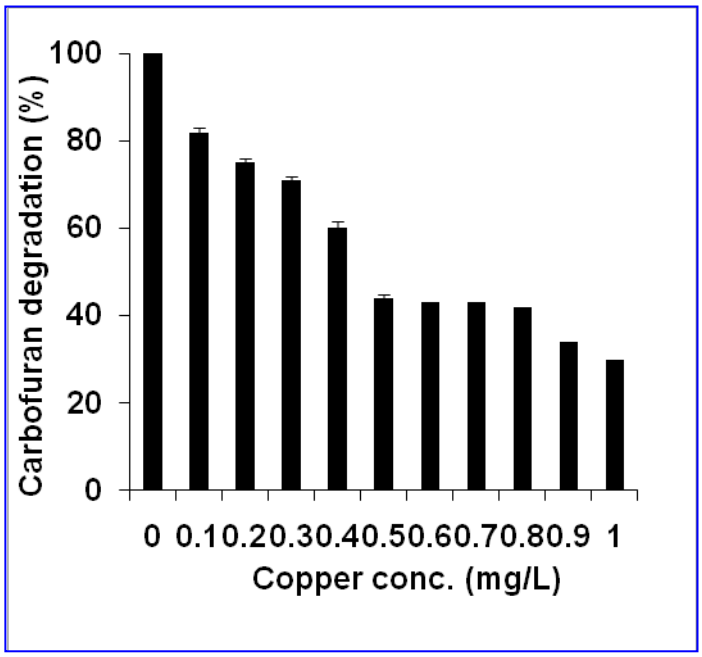

Fig. 1 Effect of Copper concentrations on carbofuran degrading activities by immobilised Cells of *Enterobacter* sp.

Also, mixed interactions between pesticides and metals might decrease the rate of pesticide biodegradation. Kools *et al.* (2005) have reported a positive correlation between glyphosate degradation rates and soil metal pollution. The effect of copper on the degradation of phenanthrene by soil micro-organisms shows that copper has a positive influence on the degradation of the compound (Sokhn *et al.*, 2001). Copper is one of the essential nutrients used by bacteria, however, in high doses, copper ions can cause a series of negative events in bacterial cells. It is needed as a trace element, but at high concentration of Cu would become unfavorable to the microbes (Dewey, 2010). The heavy metal affects the biodegradation of organic pollutants in polluted soils (Liu *et al.*, 2007). Ecological effect of Cu and its compounds is prevalent due to their utilization by many industries (Wall and Stratton, 1994). Soil microorganisms play a significant role in the degradation of the target compound and other poisonous chemical mixtures found in soil (Khalid *et al.*, 2016). The presence of heavy metals co-contaminated with pesticides may decrease the activity of microbes in the soil as metals affect the general microbiology of

soil (Kools *et al.*, 2005). Copper was also reported to have affected the degradation of Atrazine (Dewey, 2010). Biodegradation of organic pollutant co-existed Cu(II) ions using *Stenotrophomonas maltophilia* a gram-negative bacteria was studied and the degradation of BaP was inhibited when the concentration of Cu(II) ions was low (1 mg/L) (Chen *et al.*. 2013).

Conclusion

This study shows the ability of carbofuran-degrading bacterial strain to tolerate the toxicity of heavy metals in the degradation of carbofuran. Mercury from 0 to 1 mg/L have affected the degradation performance of the compound carbofuran, while Cu tends to be less effective. Although the enormous difference among reported inhibitory concentrations of metals, it remains clear that metals have the potential to inhibit organic biodegradation in both aerobic and anaerobic systems.

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The effect of heavy metals on biodegradation of carbofuran by microbial strain enriched from Agricultural areas

تأثير المعادن الثقيلة على التحلل البيولوجي للكاربوفيوران بواسطة السلالة الميكروبية المخصبة من المناطق الزراعية

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المستخلص

تم فحص تأثير المعادن الثقيلة على تحلل الكاربوفيوران بواسطة خلايا بكتيرية غير متحركة عن طريق الزراعة في وسط MSM عند 37 درجة مئوية في وجود 0-1 ملغم / لتر من المعادن الثقيلة. تمثل أنظمة المعالجة الحيوية وسيلة مستدامة بيولوجيًا لتدهور الملوثات العضوية مثل مبيدات الحشرات الكربوفيوران ، مع طلب ضئيل على الطاقة ونفقات تشغيلية بالإضافة إلى الكفاءة العالية وخصوصية الركيزة. ومع ذلك ، قد تؤدي المعادن الثقيلة الموجودة في الأراضي الزراعية إلى إعاقة كفاءة العملية عن طريق تسميم العزلات الميكروبية المهينة بالكاربوفيوران. لذلك تم اختبار تجريبي لتحمل البكتريا المعزولة عن طريق الكاربوفيوران المعزولة من منطقة زراعة الخضروات للزئبق (Hg) والنحاس (Cu). أتضح من النتائج ان المعادن الثقيلة التى تم اختبارها قد أدت الى إعاقة التحلل الأحيائي للكاربوفيوران بدرجات متفاوتة حسب التركيز. عند درجة حموضة 7.0 وحرارة 37 درجة مئوية ، حيث حدث تثبيط كامل للتحلل البيولوجي للكاربوفيوران بواسطة الزئبق 0.4 و 0.5 و 0.6 و 0.7 ملغم/ لتر على التوالي. أدت التركيزات المنخفضة لهذه المعادن إلى انخفاض معدل التحلل البيولوجي للكاربوفيوران ، مع فترات تأخير طويلة نسبيًا. ومن المثير للاهتمام ، أن العزلة التي تم تجميدها تحملت تركيزات أعلى ، على الرغم من تأثر كل من معدل ومدى التحلل البيولوجي للكاربوفيوران.

الكلمات المفتاحية : سمية المعادن، تحمل المعادن ، الكاربوف.