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Tolerance of *Xanthomonas campestris* for heavy metals typically associated with brownfield land

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Abstract

Heavy metals as environmental contaminants often exhibit high latency and persistence in soil systems. The capacity of *Xanthomonas campestris* to tolerate 1mM – 15mM concentrations of lead, cadmium and chromium(VI) which are typically associated with soil pollution due to industrial activities was assessed in this study. Bacterial abundance and enzymatic activity were seen to decrease with increasing heavy metal concentration and contact time. Cadmium was the strongest inhibitor of growth while chromium(VI) was identified as the most toxic to *X. campestris* with regards to enzyme activity. The viable bacterial counts of *Xanthomonas campestris* after exposure to the heavy metals dropped from 0.97 – 1.03 Log CFU/mL on day 0 to mean values ranging from 5.92 – 0.00 Log CFU/mL on day 7 of the study at the different concentrations applied representing reductions of 26.49% – 100%, 33.76% – 100% and 33.38% – 100% for Pb, Cd and Cr(VI) respectively. The changes in growth differed significantly ($p \leq 0.05$) between groups from one heavy metal to the other and within groups from one heavy metal concentration to the other. Pb, Cd and Cr(VI) all inhibited oxidase, catalase and protease activity at higher concentrations. Only Cd exerted an inhibitory effect against α-amylase activity but only at 10 mM and 15 mM concentrations after 5 – 7 days exposure. Pb and Cr(VI) only exhibited inhibition against cellulase activity by the end of the study at the highest concentration of 15 mM. The study established that acute exposure to the heavy metals, lead, cadmium and chromium (VI) had potential to inhibit growth and certain enzymatic activities in *X. campestris*.

1. Introduction

Industrial activities are a major threat to microbial communities in soil and water systems. A robust microbial community contributes to overall ecosystem health. The presence of contaminants in terrestrial ecosystems impacts negatively on the diversity, abundance and function of the resident microbial community. Depreciation of soil quality by heavy metals is a challenge with strong adverse effects on both the environment and the diversity, abundance and metabolic activities of soil microorganisms. *Xanthomonas campestris* is a facultatively aerobic, Gram negative rod-shaped bacterium and the most popular member of the *Xanthomonas* genus. While *X. campestris* is well known to be pathogenic to, cruciferous plants, it has been recognised for its role in the biodegradation of hydrocarbon pollutants in soil and the synthesis of an exopolysaccharide, xantham gum, that has proved beneficial in pharmaceutical, food, petroleum and agricultural industries **(Liu *et al.*, 2022; Osadebe and Okoye, 2023; Makut *et al.*, 2024)**.

The contamination of soil by heavy metals is relatively common, particularly in industrialised regions. Anthropogenic activities like energy production, manufacturing, transportation and urbanisation all lead to the presence of different heavy metals in soil. The most common heavy metals resulting from industrial activities have been noted as lead, copper, cadmium, nickel, zinc, chromium and arsenic **(Kinuthia *et al.*, 2020; Abdullahi *et al.*, 2021)**. Heavy metals are typically persistent because they cannot be converted or degraded to simpler forms. The occurrence of heavy metals in the environment, therefore, tends to be largely irreversible **(Briffa *et al.*, 2020)**. The term "brownfield land" comprises terrain that has been previously utilised for commercial or industrial purposes and now presents a risk of actual or potential pollution. Environmental contamination from brownfield land may be complicated where there have been multiple land use changes. An important part of brownfield re-development, soil quality management and terrestrial ecosystem restoration is an appreciation of the possible impact of contaminants such as heavy metals on individual soil microorganisms and microbial community structure **(Liu *et al.*, 2020; Haque *et al.*, 2023)**.

The heavy metals explored in this study – lead, cadmium and chromium – are well-known for their latency and persistence in soil systems. They display high bioaccumulation and biomagnification capabilities. Contamination with chromium is considered a serious environmental challenge. This heavy metal is used in metal alloy and paint pigments as well as during manufacture of cement, paper and rubber. Fertilisers, pesticides, mining activities, petroleum refining and production of plastics are the most common sources of chromium contamination the environment. Chromium is known to lengthen the lag phase, inhibit growth and hamper the uptake of oxygen in microorganisms. Similarly, Cadmium has been known to damage nucleic acids and proteins at cellular level and inhibit cell division and transcription. It also has adverse effects on carbon

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and nitrogen mineralisation in microbial cells. Cadmium is typically introduced into the environment via dyeing, textile, steel fabrication, electroplating, leather works and paint manufacturing industries. The occurrence of high concentrations of lead in the environment mainly arises from combustion of coal, electroplating, mining, and the manufacture of batteries and paints. Exposure to this heavy metal inhibits enzymatic activity, denatures cellular proteins and nucleic acids and interrupts the transcription process in microorganisms **(Igiri *et al*., 2018; Abdullahi *et al*., 2021; Balali-Mood *et al*., 2021; Khan *et al*., 2022; Xu *et al*., 2024)**.

This study is a preliminary assessment of the capacity of *Xanthomonas campestris* to tolerate the heavy metals, lead, chromium (VI) and cadmium, typically associated with soil pollution as a result of industrial activities.

2. Material and methods

2.1 Isolation and characterisation of the test isolate

The isolates used were obtained from hydrocarbon-contaminated agricultural soil in Bomu, Gokana Local Government Area of Rivers state, Nigeria. The top 20 cm of soil from the area was collected and transferred to the lab in polyethylene bags. Soil samples were homogenised then sieved, using a 2 mm sieve, to remove debris, stone and large particles. about 1 g of the sieved soil was serially diluted and selected dilutions plated out individually on sterile nutrient agar (NA) plates in triplicates. The plates were incubated at 30 ºC ± 2 ºC for 48 h. The streaking method was used to obtain pure isolates from discrete colonies. The pure isolates of *Xanthomonas campsetris* were stored on agar slants until required for further studies **(Cheesbrough, 2006)**. The isolate was identified via 16S rRNA sequencing as described by **Osadebe and Okoye (2023)**.

2.2 Heavy metal toxicity assay for *Xanthomonas campestris*

The heavy metals tested were cadmium, lead and chromium (VI). The nitrate salts of lead and cadmium and potassium dichromate were employed in the study. Testing was carried out at four concentrations of the heavy metals – 1 mM, 5 mM, 10 mM and 15 mM. The media containing the heavy metal salts were sterilised in a closed system using a 0.22 µm membrane filter. The isolate was first cultivated in Lurie Bertani broth for 48 h after which about 1 mL of the broth culture was introduced into nutrient broth. A further 1 mL of the 24 h broth culture of the isolate was then transferred into 100 mL fresh nutrient broth in 250 mL Erlenmeyer flasks amended with different concentrations of the heavy metals. Incubation was at 30 ºC ± 2 ºC for 7 days. The abundance and enzymatic activity of the isolate was monitored at 24 h intervals by plating out on nutrient agar plates to obtain visible colonies before testing. All set-ups were done with replicates. The controls consisted of inoculated media without the addition of any heavy metals.

2.3 Growth response of *Xanthomonas campestris* to the heavy metals tested

Enumeration of the isolate was done via standard plate count technique on nutrient agar. Precisely 0.1 mL aliquots were extracted from the tainted broth cultures at 24 h intervals and inoculated onto fresh YEMA plates via the spread plate method. Following 48 h incubation at 30 ºC ± 2 ºC, the number of discrete visible colony forming units (CFU) was determined using an automated colony counter. Plates having CFU counts in excess of 300 were excluded.

2.4 Enzyme Activity Assays

2.4.1 *Starch hydrolysis assay (α-amylase production)*

The isolate was streaked in parallel lines on starch agar and incubated at 37 ºC for 48 h after which, the plates were flooded with 1 % iodine solution and examined for the appearance of a clear zone around the isolate. The clear zone is indicative of starch hydrolysis due to α-amylase activity.

2.4.2 *Protease production*

The test isolate was screened for extracellular protease production by streaking in parallel lines on 1 % skimmed milk agar plates with incubation at 37 ºC. Protease production was confirmed by clearing of opaque milk proteins around the colony after 24 h **(Purwaningsih *et al*., 2021)**.

2.4.3 *Cellulase production*

The test isolates was streaked onto 2 % cellulose agar and incubated for 48 h at 37 ºC ± 2 ºC. The plates were then flooded with Congo red dye and observed for the appearance of a clear zone around the colony indicating cellulase activity **(Gupta *et al*., 2012)**.

2.4.4 *Oxidase production*

The oxidase test was conducted using tetramethyl-p-phenylenediamine dihydrochloride (TMPD) as an electron acceptor. With a sterile wooden pick, colonies of an 18 h old culture of the test isolate, *X. campestris*, was placed on sterile Whatmann's filter paper no. 1 and a drop of TMPD reagent added. An immediate colour change to bluish-purple was considered positive for oxidase production.

2.4.5 *Catalase production*

Catalase activity was ascertained by dropping 10 % hydrogen peroxide onto a small portion of the bacterial colony introduced onto a clean glass slide. The evolution of oxygen gas as indicated by effervescence was deemed a positive result for catalase production.

3. Results and discussion

The viable bacterial counts of *Xanthomonas campestris* after exposure to the heavy metals dropped from 0.97 – 1.03 Log CFU/mL on day 0 to mean values ranging from 5.92 – 0.00 Log CFU/mL on day 7 of the study at the different concentrations applied. These values represented reductions in abundance of 26.49 %, 22.34 %, 74.99 % and 100 .00 % for 1 mM, 5 mM, 10 mM and 15 mM concentrations of lead (Pb) respectively by day 7 of the study. With cadmium (Cd), a 33.76 % reduction in growth of *X. campestris* was obtained at 1 mM while a 100 % decline in abundance, indicating no growth, was seen at higher concentrations of 5 mM, 10 mM and 15 mM. The abundance of the test isolate declined by 33.38 % and 71.61 % at chromium (VI) concentrations of 1 mM and 5 mM respectively. The isolate, however, did not tolerate the higher concentrations of 10 mM and 15 mM well as evidenced by the 100 % decline in viable bacterial counts. This showed that short term exposure to lead, cadmium and chromium (VI) at high concentrations totally inhibited the growth of *X. campestris*. These findings are depicted in Figure 1.

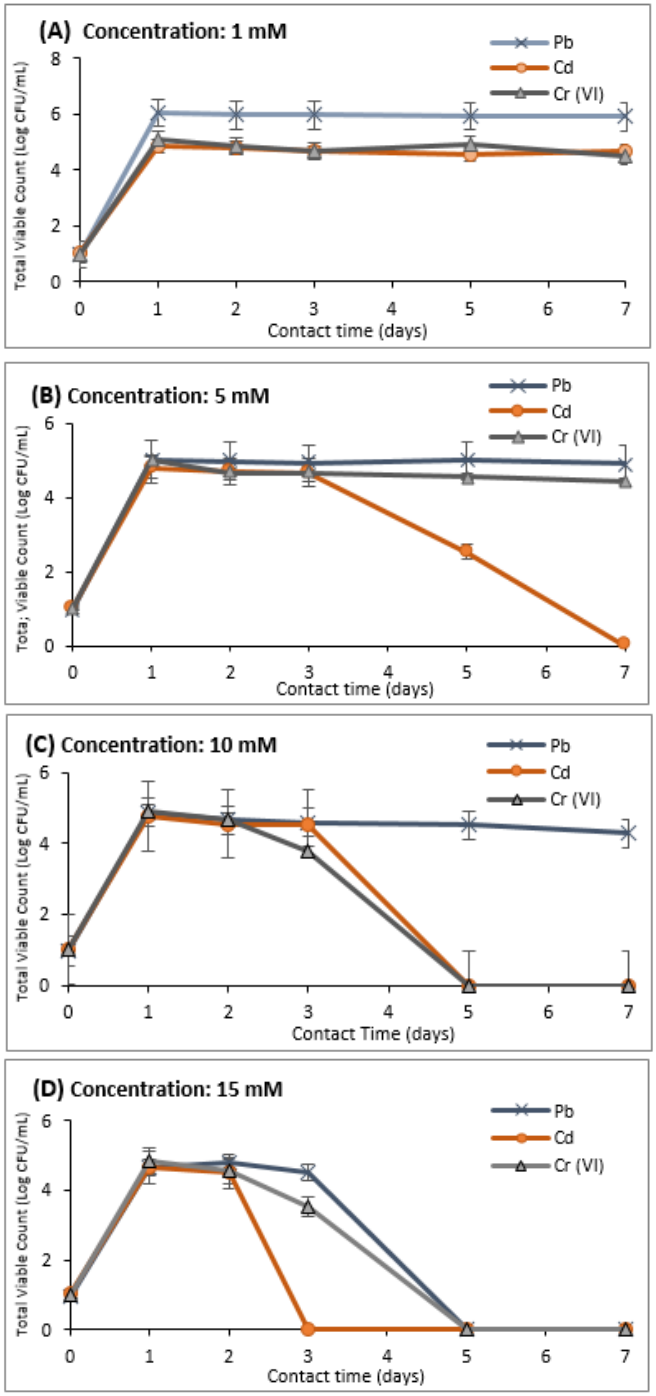

Figure 1 Growth profile of *Xanthomonas campestris* following exposure to (A) 1 mM, (B) 5 mM, (C) 10 mM and (D) 15 mM concentrations of the different heavy metals tested

Cadmium exerted the most toxic effect on the isolate based on the results from *in vitro* growth studies while lead proved to be the least toxic heavy metal in this case. There were statistically significant differences in viable counts of *X. campestris* at 95% confidence interval between the different heavy metals tested. For each heavy metal, viable counts were also seen to differ significantly ($p \leq 0.05$) overall from one application concentration to the other though there were no significant differences within groups at 1 mM and 5 mM concentrations (Pb and Cr only).

Similar to the growth profile, progressive inhibition of enzymatic activity in *X. campestris* was obtained with increasing contact time and increasing heavy metal concentration as shown in Tables 1 – 5. All three heavy metals, Pb, Cd and Cr(VI), inhibited oxidase, catalase and protease activity. Cr(VI) had the strongest inhibitory effect against enzymatic activity overall. Only Cd exerted an inhibitory effect against α-amylase activity but only at 10 mM and 15 mM concentrations after 5 – 7 days exposure. Pb and Cr(VI) only exhibited inhibition against cellulase activity by the end of the study (day 7) at the highest concentration of 15 mM. The inhibitory effect of Pb against the oxidase enzyme was seen at 10 mM and 15 mM after 3 days of exposure and at 5 mM and 1 mM after 5 – 7 days exposure while Cd exerted inhibitory effects at 1 mM to 15 mM after an exposure period of 3 days. Cr(VI) proved to be the most toxic against oxidase activity in *X. campestris* as inhibition of the enzyme was seen after 24 h for 15 mM concentration levels and after 3 days for 1 mM – 10 mM.

Oxidase activity was hampered at all application concentrations of Cr (VI). The activity of the catalase enzyme in the test isolate was only limited at the highest exposure concentration of 15 mM for Cr(VI) and Cd. Pb inhibited catalase activity in *X. campestris* at 10 mM and 15 mM after about 3 days of acute exposure. A similar trend was found with the inhibition of protease activity; while Cr (VI) limited the enzyme's activity at 10 mM and 15 mM after 5 – 7 days' exposure, the inhibitory impact of lead was evident after 3 days' exposure. Cd did not inhibit protease activity.

Changes in microbial growth and enzymatic activity under the influence of heavy metals, as obtained in the current study, have been recognised as a suitable measure of the response of an isolate or microbial community to an environmental stressor **(Strotmann *et al.*, 2024)**. Heavy metals are renowned for their propensity to induce environmental stress responses in microorganisms when present in their environment **(Li *et al.*, 2022)**. The findings of the study demonstrated that the ecotoxic impact of the heavy metals considered on *X. campestris* was strongly dependent on concentration and duration of exposure. Cadmium was the strongest inhibitor of growth while chromium(VI) was identified as the most toxic to *X. campestris* with regards to enzyme activity in the present study. Lead was the least toxic across board. This is consistent with the reports of **Li *et al.* (2020)** and **Angon *et al.* (2024)** regarding the inhibition of overall microbial biomass by heavy metals in soil. The results from the present study also corroborate the toxic effects of heavy metals on individual microorganisms with increasing concentration and exposure period as reported by other parallel studies **(Wang *et al.*, 2018; Bandara *et al.*, 2022; Ngwewa *et al.*, 2022)**.

Ngwewa *et al.* (2022) confirmed that the growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* had an inversely proportional relationship with the concentrations of cadmium and lead alongside copper, zinc and cobalt at concentrations up to 1000 ppm. A study on toxicity of five heavy metals identified cadmium and chromium as the most toxic heavy metals against growth and physiological processes in *Enterobacter cloacae* at exposure levels of 25µg/mL – 200 µg/mL **(Syed *et al.*, 2021)**. They found that cadmium reduced the synthesis of several key macromolecules by 59 % – 80 % at 200 µg/mL concentration. Growth of *E. cloacae* was totally eliminated by chromium and cadmium at concentrations of 150 µg/mL – 200 µg/mL while its phosphate solubilising capacity was inhibited by chromium at 94.6 µg/mL and cadmium at 127.2 µg/mL. Cadmium limited biofilm formation capacity as well. Akin to the current study, lead was identified as one of the least toxic heavy metals.

The study by **Ngwewa *et al.* (2022)**, like the present study, also identified lead as the least toxic heavy metal with regards to microbial growth patterns due to its inability to fully inhibit growth even at higher concentrations compared to other heavy metals. Another comparable study reported that lead, chromium, zinc, copper, nickel and manganese diminished microbial bioactivity, species richness and microbial diversity by mean values of 55.1 % – 87.7 % **(Li *et al.*, 2020)**. The reduction

levels in microbial abundance in response to lead obtained in those studies are somewhat similar to the 26.49 % – 100 % reduction in the counts of *X. campestris* reported in the current study. In contrast, prolonged exposure to cadmium has been linked to considerable rises in the abundance of members of the Bacteroidata and proteobacteria and marked declines in the occurrence of the acidobacteria, firmicutes, chloroflexi, myxococcota and Gemmatimonadota phylum taxonomic ranks **(Bandara *et al*., 2022)**.

Table 1 Effect of varying concentrations of lead, cadmium and chromium (VI) on oxidase activity in *X. campestris*

Heavy Metal	Concentration	CONTACT TIME					
		Day 0	Day 1	Day 2	Day 3	Day 5	Day 7
Lead	1 mM	+	+	+	+	+	–
	5 mM	+	+	+	+	–	–
	10 mM	+	+	+	–	–	–
	15 mM	+	+	+	–	–	n/a
	Control	+	+	+	+	+	+
Cadmium	1 mM	+	+	+	–	–	–
	5 mM	+	+	+	–	–	–
	10 mM	+	+	+	–	–	–
	15 mM	+	+	+	–	–	n/a
	Control	+	+	+	+	+	+
Chromium(VI)	1 mM	+	+	+	–	–	–
	5 mM	+	+	+	–	–	–
	10 mM	+	+	+	–	–	n/a
	15 mM	+	–	–	–	–	n/a
	Control	+	+	+	+	+	+

+ positive oxidase activity; – Negative for oxidase activity; n/a – not applicable as no growth observed

Table 2 Effect of varying concentrations of lead, cadmium and chromium (VI) on catalase activity in *X. campestris*

Heavy Metal	Concentration	CONTACT TIME					
		Day 0	Day 1	Day 2	Day 3	Day 5	Day 7
Lead	1 mM	+	+	+	+	+	+
	5 mM	+	+	+	+	+	+
	10 mM	+	+	+	+	–	–
	15 mM	+	+	+	–	–	n/a
	Control	+	+	+	+	+	+
Cadmium	1 mM	+	+	+	+	+	+
	5 mM	+	+	+	+	+	+
	10 mM	+	+	+	+	+	n/a
	15 mM	+	+	+	–	–	n/a
	Control	+	+	+	+	+	+
Chromium(VI)	1 mM	+	+	+	+	+	+
	5 mM	+	+	+	+	+	+
	10 mM	+	+	+	+	+	n/a
	15 mM	+	+	+	+	–	n/a
	Control	+	+	+	+	+	+

+ Positive result;– Negative result; n/a – not applicable as no growth observed

Table 3 Effect of varying concentrations of lead, cadmium and chromium (VI) on α-amylase activity (starch hydrolysis) in *X. campestris*

Heavy Metal	Concentration	CONTACT TIME					
		Day 0	Day 1	Day 2	Day 3	Day 5	Day 7
Lead	1 mM	+	+	+	+	+	+
	5 mM	+	+	+	+	+	+
	10 mM	+	+	+	+	+	+
	15 mM	+	+	+	+	+	n/a
	Control	+	+	+	+	+	+
Cadmium	1 mM	+	+	+	+	+	+
	5 mM	+	+	+	+	+	+
	10 mM	+	+	+	+	+	–
	15 mM	+	+	+	+	–	n/a
	Control	+	+	+	+	+	+
Chromium(VI)	1 mM	+	+	+	+	+	+
	5 mM	+	+	+	+	+	+
	10 mM	+	+	+	+	+	n/a
	15 mM	+	+	+	+	+	n/a
	Control	+	+	+	+	+	+

+ Positive result;– Negative result; n/a – not applicable as no growth observed

Table 4 Effect of varying concentrations of lead, cadmium and chromium (VI) on protease activity in *X. campestris*

Heavy Metal	Concentration	CONTACT TIME					
		Day 0	Day 1	Day 2	Day 3	Day 5	Day 7
Lead	1 mM	+	+	+	+	+	+
	5 mM	+	+	+	+	+	+
	10 mM	+	+	+	+	–	–
	15 mM	+	+	+	–	–	–
	Control	+	+	+	+	+	+
Cadmium	1 mM	+	+	+	+	+	+
	5 mM	+	+	+	+	+	+
	10 mM	+	+	+	+	+	+
	15 mM	+	+	+	+	+	n/a
	Control	+	+	+	+	+	+
Chromium(VI)	1 mM	+	+	+	+	+	+
	5 mM	+	+	+	+	+	+
	10 mM	+	+	+	+	+	–
	15 mM	+	+	+	+	–	n/a
	Control	+	+	+	+	+	+

+ Positive result;– Negative result; n/a – not applicable as no growth observed

Table 5 Effect of varying concentrations of lead, cadmium and chromium (VI) on cellulase activity in *X. campestris*

Heavy Metal	Concentration	CONTACT TIME					
		Day 0	Day 1	Day 2	Day 3	Day 5	Day 7
Lead	1 mM	+	+	+	+	+	+
	5 mM	+	+	+	+	+	+
	10 mM	+	+	+	+	+	+
	15 mM	+	+	+	+	+	–
	Control	+	+	+	+	+	+
Cadmium	1 mM	+	+	+	+	+	+
	5 mM	+	+	+	+	+	+
	10 mM	+	+	+	+	+	n/a
	15 mM	+	+	+	+	+	n/a
	Control	+	+	+	+	+	+
Chromium(VI)	1 mM	+	+	+	+	+	+
	5 mM	+	+	+	+	+	+
	10 mM	+	+	+	+	+	–
	15 mM	+	+	+	+	+	n/a
	Control	+	+	+	+	+	+

+ Positive result;– Negative result; n/a – not applicable as no growth observed

The actions of catalase, oxidase and α-amylase were the most sensitive to the test heavy metals in the present study. Low heavy metal concentrations of 1 mM and 5 mM had only negligible impact on enzymatic activity in the test isolate. Responses to the presence of the heavy metal were only seen as concentrations increased. Inactivation of the catalase and oxidase enzymes would lead to a build-up of toxic hydrogen peroxide in the cell leading to the accumulation of deleterious free radicals in the case of the catalase enzyme; and for the oxidase enzyme, inactivation would negatively impact mitochondrial metabolism and energy production within the cell it could trigger a build-up of reactive oxygen species inducing oxidative stress in aerobic bacteria **(Korshunov *et al.*, 2016; Sen and Imlay, 2021; Ramzan *et al.*, 2022; Osés *et al.*, 2024)**. The activities of α-amylase, protease and cellulase ensure that the organism is able to utilise a diverse array of compounds as sources of energy which, in turn, strengthens the capacity of the organism to thrive in varying environments under changing nutrient conditions while also driving the cycling of nutrients within the ecosystem. In the absence of these enzymes, the bacterium would be severely limited nutritionally and less likely to survive changing environmental conditions **(Gonzalez and Aranda, 2023; Narayanan *et al.*, 2023; Singh *et al.*, 2024)**. This decrease in enzyme activity has been linked to a decline in enzyme production **(Syed *et al.*, 2021)**.

A 45-day study on the effects of heavy metals on soil enzymatic activities reported that catalase activity, alongside those of dehydrogenase and urease, were sensitive to the concentration of cadmium in farmland soil **(Yeboah *et al.*, 2021)**. In tandem with the current study, catalase activity in this study was seen to decline with increasing cadmium concentration and exposure time. The changes were more drastic than obtained in the current study probably due to differences in the applied concentration of the heavy metal. The current study had much lower concentrations than the 0.1 mg/L – 10 mg/L seen here. **Ngwewa *et al.* (2022)** opined that cadmium, lead, copper, zinc and cobalt did not impact on the biochemical properties of *E. coli* and *S. aureus* but found that cadmium, in addition to copper, zinc and cobalt triggered a positive urease result in the characteristically urease negative *P. aeruginosa*. Although opposite to the results from the current study, this speaks to the possible influence of heavy metals on enzymatic activity in bacterial isolates. Unlike the findings in the current study, **Bartkowiak *et al.* (2020)** found that there was significant positive correlation between nitrate reductase (an enzyme in the class of oxidoreductases) activity and the concentrations of bioavailable lead and copper in rhizosphere soil region of selected tree species. They stated that increased heavy metal concentrations resulted in a corresponding increase nitrate reductase activity.

Lead and cadmium have been highlighted for their capacity to interrupt microbial processes including nutrient cycling capabilities, metabolic processes and enzymatic activity **(Abedi *et al.*, 2022; Rehman *et al.*, 2023)**. Various reasons have been

put forward for the observed reduction in abundance and activity including heavy metal-induced changes in physiochemical environmental factors like pH. Studies have highlighted the occurrence of soil acidification due to lead contamination resulting in limited the growth and activity of soil microorganisms (Collin *et al*., 2022). Cadmium, has also been known to induce acidification in soil systems with a similar impact on the soil microbial community and their enzymatic activities **(Ukalska-Jaruga *et al*., 2022)**. Heavy metal levels over specific threshold concentrations are known to exert inhibitory effects on microorganisms by modifying key molecules or by transforming the active conformations of biological molecules **(Syed *et al*., 2021)**. While the exact mechanisms of inhibition are still being studied, research has shown that certain bacteria are able to resist the toxic effects of heavy metals via intracellular and extracellular sequestration by complexation using metal-binding peptides as well as using cellular systems such as efflux pumps common to the P-type ATPase transport systems, blocking membranes, ATPase complexes and biofilm formation (**Mathivanan *et al*., 2021; Li *et al*., 2022; Jeyakumar *et al*., 2022; Roy *et al*., 2023)**.

The results from the present study contrasts somewhat with heavy metal toxicity studies regarding *X. campestris*. The bacterium has been recognised for its resistance to certain heavy metals. One study found that *X. campestris* previously exposed to lower concentrations of cadmium, exhibited resistance to both cadmium and zinc even at higher lethal concentrations **(Banjerdkij *et al*., 2003)**. **Ramnarine *et al*. (2024a)** also confirmed resistance of *X. campestris* and *X. melonis* isolated from infected crucifers to multiple heavy metals including cadmium at concentrations up to 25 mM which is higher than the cadmium concentrations employed in the current study. Another similar study highlighted the resistance of *X. campestris* to copper **(Ramnarine *et al*., 2024b)**. This study found that a fifteen acute exposure to 0.8 mM copper triggered gene-related stress responses in the bacterium that helped to mitigate the effect of the heavy metal.

4. Conclusion

This preliminary study established the potential toxicity of the heavy metals, lead, cadmium and chromium as chromium (VI) to soil isolate, *X. campestris*. The growth of *X. campestris* declined with increasing concentrations and contact time with significant differences ($p \leq 0.05$) from one heavy metal concentration to the other. The findings further revealed that the different heavy metals exerted varying inhibitory actions against enzyme activity in the isolate with the cytochrome c of oxidase being the most susceptible and cellulase being the most resistant. Adequate remediation of brownfield land for elimination of non-biodegradable inorganic pollutants is recommended to ensure the integrity of indigenous microbial communities. Further studies that confirm the results using quantifiable techniques is essential. Studies that explore the exact mechanisms of action of these enzymatic systems in microorganisms would provide a more robust understanding of the results obtained.

Conflict of Interest Statement

The authors declare that, to the best of our knowledge, there are no conflicts of interest associated with this paper.

Abbreviations: NCBI, National Centre for Biotechnology Information; CFU, Colony forming Units

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