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The Impact of Heavy Metal Ions on Arginase Produced from *Pseudomonas aeruginosa* Isolated from Sewage

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ABSTRACT

Background: Researchers remain captivated by the impact of heavy metal ions, commonly found in sewage, on arginase synthesis. Objective: The study examines the presence and characteristics of Pseudomonas aeruginosa in 24 sewage samples, identifying 18 isolates (75%). It assesses DNA concentration and purity, utilizes PCR-based methods for subtype analysis and investigates the inhibitory effects of heavy metals on purified enzymes.

Methods: Between November 2022 and April 2023, 52 samples were collected from various sewage sites in Baghdad, Iraq. These samples were cultured in brain heart infusion broth and on nutrient agar plates. The isolates were then subjected to laboratory procedures, including biochemical tests, analysis with the Vitek2 system and PCR to identify Pseudomonas aeruginosa. Biochemical testing specifically focused on *Pseudomonas aeruginosa*. DNA extraction was performed on *P.aeruginosa* isolates from sewage, which had already been identified through morphological and biochemical tests as *P. aeruginosa*. This was accomplished by screening for the presence of the 16SrRNA gene using PCR.

Result: Metal ion effects on L-arginase were tested at various concentrations (100 mM, 50 mM, 25 mM, 12.5 mM). Metal ions were obtained via the cutting method and added 15 minutes before the substrate. Enzyme activity was measured and the activity ratio (enzyme activity with and without metal ions) calculated. Activity was assessed in the absence of 100% metal ions. Arginase isolated and purified from *Pseudomonas aeruginosa* from sewage, heavy metals (mercury, cadmium, cobalt) has effect on arginase, mercuric chloride and cadmium chloride inhibit and decrease enzyme activity, while Cobalt chloride increases enzyme activity.

Key words: Arginase, Cd, Co, Hg, Sewage.

INTRODUCTION

Waterway is surface water acquired as of the artificial dispersal of a stream; Distributions could be believed of as streams with a slight structure. The quality of irrigation water obtained from wastewater is better than wastewater or groundwater wastewater, manufacturing). Discarded water is any of the alternative wastewater industries such as leather, leather, textile, packaging and pesticide industries. Furthermore to nourishment, wastewater might comprise one or additional heavy metals that essential or non-essential, for example. copper (Cu), Zinc (Zn), iron (Fe), cadmium (Cd), manganese (Mn), nickel (Ni) and chromium (Cr) with excess amounts of lead (Pb) (toxic) that can be harmless to plants soils and/or humans Ali and Gill (2022).

Toxicity limits for numerous micronutrients or minerals are extended at much minor amounts Denton-Thompson and Sayer (2022). Heavy metals bind to organic and inorganic particles in wastewater Wu *et al.* (2023) and settle with the soil solution after soil irrigation with wasted water. Heavy metals are equipped or deposited in tissues of plant through mechanisms controlled by plant roots: (1) excretion of metallic chelating composites (named phytosiderophores) into the rhizosphere by plant roots and (2) biochemical degradation of plant roots by soil.

Heavy metals are divided addicted to two chief forms: irreplaceable and superfluous. They comprise iron, zinc, manganese, chromium and copper. The high levels of essential heavy metals make them very toxic when exposed to the body. The second type includes secondary species such as lead, cadmium, arsenic and mercury, which even at little concentrations are extremely toxic Simionov *et al.,* (2019). Cadmium and mercury are among the greatest toxic heavy metals and carriage a severe danger to earth ecologies. These heavy metals are associated with soil enzyme action Yang *et al.* (2023), microbial profusion and microbial activity Chen *et al.* (2015) and the structure of the microbial community (He *et al.,* 2023; Ashok *et al.,* 2024; Haryuni *et al*., 2024).

P. aeruginosa is a communal rod -shaped bacterium, gram -negative, encapsulated, aerobic-facultative anaerobic. *P. aeruginosa* are associated with several pathogenic features for instance adhesion, biofilm formation, surface hemagglutinin synthesis, elastase production, pyocyanin synthesis and production, motility, amen lipids, colonization hyphae, type III secretion,

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lipopolysaccharide, alkaline proteases and flagella, Extracellular protein systems and toxins (eg, exoenzyme S and exotoxin A) Mordhorst *et al.* (2023). The previous study showed that arginases as of *P. aeruginosa* purified was a homo-dimer and displayed important action and catalytic constancy close to the circulatory system pH and human body heat. The functions this enzyme as a biochemical typical for mineral hydrolases and is considered a primitive enzyme existe general communal ancestor Christianson (2005). Arginine is intricate in many biological purposes comprising cell signaling, cell proliferation, muscle contraction, neurotransmission immunity, vasodilation and production of growth factors and other amino acids (Abd *et al.,* 2023).

Remarkably, however not amazing, in coelomocytes in vitro cured with Mn^{2+} ions, augmented arginase action was detected, while alike concentrations of Ni^{2+} and Cd^{2+} reduced its action. It takes to be stated that arginase is a metallo-enzyme in which Mn performances as a cofactor in addition to an activator and that essential Mn^{2+} as an important component to exhibition greatest enzyme action; nevertheless, the sum necessary for optimum action of the enzyme diverges rest on arginase isoforms and position Dzik (2014). The relationship between L-arginase and heavy metals has been explored in several studies, highlighting the enzyme's involvement in metal detoxification and tolerance mechanisms (Porru *et al.,* 2024; Ansari *et al*., 2024; Vinogradov *et al*., 2024).

A precise and whole considerate of the apparatus of the association between the arginase enzyme and heavy metals has not stayed deliberate entirely accurately because it might diverge dependent on the kind of minerals in addition to the kind of microorganisms or alive organisms, but however there are numerous straining that have been showed to shelter light on such association in the elimination of toxins from metals (Witkowska *et al.,* 2021). In Conservation ecosystems, there is communication among heavy metal pollutants and the microorganisms that exist in there. Minor amounts of these metals for instance Nickel (Ni), Magnesium (Mg), Chromium (Cr^{3+}), Copper (Cu), Cobalt (Co), Calcium (Ca), Sodium (Na) Manganese (Mn) and Zinc (Zn) are important for metabolism and redox functions but can be toxic at greater concentrations.

The research examines the occurrence and attributes of pseudomonas aeruginosa within a sample of 24 sewage samples, identifying 18 isolates (75%). It specifically investigates DNA concentration, purity and utilizes PCR-based techniques for subtyping analysis and investigates the inhibitory effect of the heavy metals on purified enzymes.

Materials and Methods

Between November 2022 and April 2023 (52) samples were obtained from different sewages in Baghdad, Iraq, all samples were determined by culturing in brain heart infusion broth and nutrient agar plats, followed by isolates in lab, biochemical test, vitek2 system and PCR for *P. aeruginosa*.

Biochemical test was carried out as depicted by Murray *et al.,* (2019), VITEK2 (Biomerieux) for *P. aeruginosa*. DNA was extracted from *P. aeruginosa* isolates from sewage, that is already been identified by morphological and biochemical tests as *P. aeruginosa* by screening the presence of *16SrRNA* gene by using PCR.

Isolation of bacteria from sewage sample

In the laboratory and under aseptic conditions, the collected sample isolated from sewage, were identified by culturing directly on Brain Heart infusion after diluted (1 ml from sewage to 9 ml in DW), then make diluted by take 1 ml from stoke tube to 9 ml and take from tube dilutedto another and all the isolates were incubated for 24 hours at 37°C. Sub-culture on selective media (pseudomonas cetrimide agar) plate by darting and incubation at 37°C for 24 hours to obtain pure well isolated colonies Tormanen (2001).

Purification of *P. aeruginosa* L-arginase enzyme

Purification and Optimization of *P. aeruginosa* L-arginase enzyme was including Precipitation in ammonium sulfite, Ion exchange and Gel filtration. Molecular weight of arginase by SDS gel electrophoresis in *p. aeruginosa* isolated, Table (1).

Table 1: The purification steps.

Purification step	Volume (mL)	Enzyme activity (U/mL)	Protein conc. (mg/mL)	Specific activity (U/mg)	Total activity (U)	Purification fold	Yield %
Crude enzyme	250	4.058	11.144	0.364	1014.5	1	100
Precipitation with (60-80) % saturation of $(NH4)_2SO_4$	30	5.019	10.056	0.499	157.57	1.371	15.531
Desalting and concentration by dialysis	20	4.750	9.213	0.516	95	1.418	9.364
Ion exchange chromatography (DEAE-Cellulose)	25	3.343	3.416	0.979	83.575	2.690	8.238
Gel filtrationG100	25	2.861	3.271	0.875	71.525	2.404	7.05

Heavy metals solutions

It was made by dissolving the concentration in Table (2) heavy metals with (10 mL of D.W).

Statistical analysis

The statistical Analysis System (SAS) program [19] to assess the impact of various factors on the study parameters. The significance of differences between means was determined using the Least Significant Difference (LSD) test, which is a form of analysis of variance (ANOVA). Additionally, the Chi-square test was utilized to compare the significance of differences between percentages, with a significance level set at 0.05 and 0.01 probabilities, in this study.

Results and Discussion

Fifty-two samples of isolates were collected from various sources of sewage water. Isolates from the sewage water are capable of producing the L-arginase enzyme in M9A media. The result indicates that the L-arginase enzyme in the sewage isolate had a slightly higher molecular weight (32 kDa) Fig (1).

Effect of heavy metals on purified L -arginase activity

The effect of diverse metal ions on L-arginase activity was resolute by pre-incubating the enzyme with 100 mM, 50 mM, 25 mM, 12.5 mM and Metal ions were obtained by the cutting method 15 minutes before the addition of the substrate. Next, the enzyme activity was resolute and the relation activity (the ratio of enzyme activity in the existence and lack of metal ions) was calculated. The activity evaluated was taken in absence of 100% metal ions [20], Table 3 shows the effect of different metal solution on L-arginase activity.

Effect of mercuric chloride

Mercuric chloride ($HgCl_2$) has been shown to have an inhibitory effect on the activity of the enzyme L-arginase 0.5 (28%). Several studies have demonstrated this (Rybak *et al.,* 2023; Zhou *et al*., 2023; Haroun *et al*., 2024). The inhibitory effect of mercuric chloride on arginase activity is likely due to its ability to interfere with the manganese cofactor required for arginase activity, as all arginases are metalloenzymes that require manganese.

Since 1955, a study carried out by Meister *et al*., published in the Journal of Biological Chemistry in 1955, also investigated the inhibitory effects of various metal ions on arginase activity. They observed that mercuric chloride was a potent inhibitor of arginase activity. However the current study reveals that mercury chloride has an inhibitory effect on the activity of the arginase enzyme. The findings indicate a reduction in arginase enzyme activity of the enzyme purified from *P. aeruginosa* to 28% for the enzyme from sewage, as shown in Table (3), when exposed to a concentration of 100 mg/ml, this finding aligns with previous research by Sheweita *et al.* (2023).

Chen *et al.* (2020) did a study about the effect of mercuric chloride tyrosinase., the study suggest the formation of $HgCl_2$-tyrosinase complex prompts conformational alterations in the enzyme suggesting inhibition effect of $HgCl_2$ that exhibits by binding with the enzyme (tyrosinase) Chen *et al.,* (2020).

Fig 2 shows the decreases in enzyme activity due to increases in the concentration of mercuric chloride.

Effect of cadmium chloride

Cadmium chloride exposure has been associated with various toxic effects on biological systems, including enzyme inhibition Peana *et al.* (2022). Arginase is one of the enzymes that can be affected by cadmium chloride exposure Witkowska *et al*. (2021), however, arginase is a metalloenzyme that plays a crucial role in the urea cycle, converting arginine to ornithine and urea. Studies have demonstrated that cadmium chloride can inhibit arginase

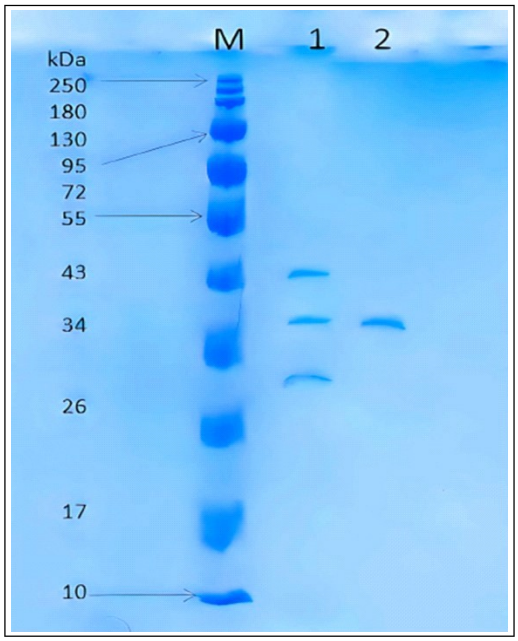


Fig 1: Molecular weight of arginase by SDS gel electrophoresis in *P. aeruginosa* isolated from sewage.

Table 2: Heavy metals with their concentration.

Volume of D.W Water in mL	$HgCl_2$ Wt. in Grams	$CdCl_2.2H_2O$ Wt. in Grams	$CoCl_2$ Wt. in Grams	Concentration (Mm)
10	0.03375	0.023	0.03	12.5
10	0.0675	0.05	0.60	25
10	0.135	0.1	0.12	50
10	0.270	0.2	0.24	100

activity by binding to the enzyme's active site or by disrupting its cofactors Caldwell *et al*. (2018); Witkowska *et al*. (2021); Peana *et al*. (2022).

The current research noted a notable decline in arginase enzyme activity following exposure to cadmium chloride at a concentration of 100 mg/mL, specifically; the arginase enzyme's activity from sewage and the remaining activity was 39.32%, with an initial measurement of 0.7 U/ml (Table 3).

Prior studies have corroborated the inhibitory impact of cadmium chloride on arginase activity. Mishra *et al.* (2020) explored this phenomenon in their research, focusing on cadmium chloride's effects on arginase activity in rat liver. Rats were subjected to different cadmium chloride concentrations, including 100 mg/ml. Their results unveiled a marked reduction in arginase activity, aligning with the findings of the current study.

The exact mechanism by which cadmium chloride inhibits arginase activity remains unclear. However, it is suggested that cadmium ions may compete with essential metal ions (such as zinc or manganese) at the enzyme's active site, thus disrupting its catalytic function. Additionally, cadmium might cause conformational changes in the enzyme structure, resulting in reduced enzymatic activity Rafati *et al*. (2017). Fig 3 illustrates the effect of cadmium chloride on arginase activity.

The effect of cobalt chloride

The outcome of the present study reveals that cobalt chloride significantly enhances the activity of the arginase enzyme, as shown in Table 3. At a concentration of 100, there was a 40.44% increase in activity. These results are consistent with previous research by Chakraborty *et al.* (2017), which showed that cobalt chloride ($CoCl_2$) is frequently used as a hypoxia-mimetic agent, mimicking the cellular effects of a low-oxygen environment.

Furthermore, inhibition of hypoxia-inducible factor (HIF) hydroxylases by citric acid cycle intermediates suggests potential connections between cell metabolism and HIF stabilization. Additionally, cobalt ions are recognized for their influence on various cellular functions, including the induction of HIF-1α, which in turn can elevate arginase activity.

On the contrary, there are other studies shows that cobalt chloride inhibits the activity of arginase Kumanto *et al*. (2017). The researchers demonstrated that cobalt chloride inhibited arginase activity in a dose-dependent manner. They also proposed a mechanism for the inhibition involving the binding of cobalt to the active site of arginase. However, George *et al.* (2017) results indicate that the arginase enzyme from *H. pylori* has some unique properties compared to other arginases. Specifically, the *H. pylori* arginase has an acidic pH optimum of 6.1 and exhibits optimal catalytic activity with cobalt as a metal cofactor, rather than the more common manganese cofactor, the

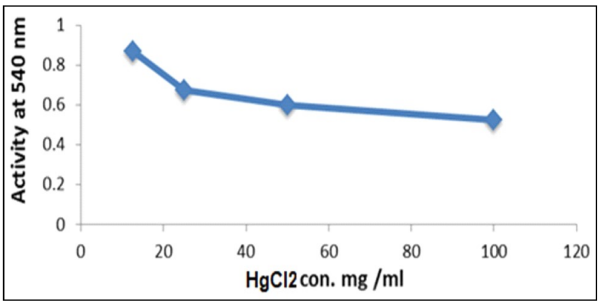


Fig 2: Effect of $HgCl_2$ on activity of L-arginase enzyme.

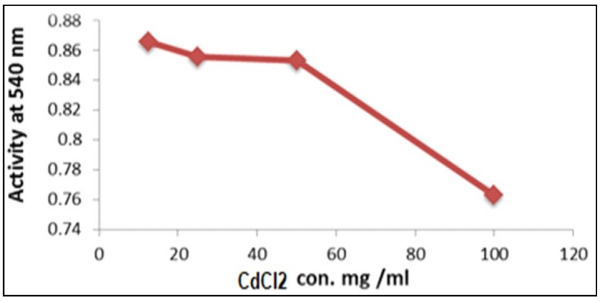


Fig 3: Effect of cadmium chloride on inhibition of Arginase.

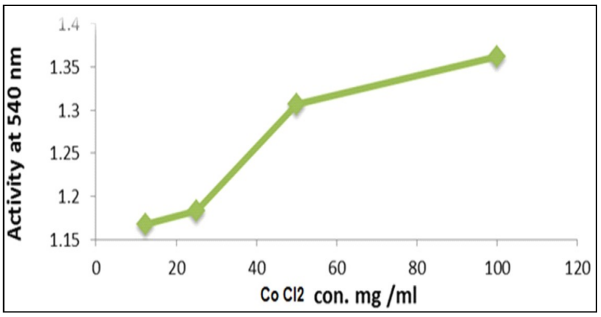


Fig 4: Effect of Cobalt chloride as a heavy metal on activity of arginase enzyme.

Table 3: Effect heavy metals ($HgCl_2$, $CdCl_2$ and $CoCl_2$) of L-arginase enzyme production by measuring activity (U /mL).

Crude enzyme	Inhibition Activity			
Con. heavy metals	12.5%	25%	50%	100%
$HgCl_2$	1.02 (57.30%)	0.9 (50.56%)	0.7 (39.32%)	0.5 (28%)
$CdCl_2$	1.2 (67.4%)	1.1 (61.79%)	0.9 (50%)	0.7 (39.32%)
$CoCl_2$	1.8 (1.1%)	1.9 (6.74%)	2.11 (18.53%)	2.5 (40.44%)
LSD value	18.75 *	17.56 *	25.63 *	31.05 *

*($P \leq 0.05$)

study shows that cobalt can enhance the activity of the *H. pylori* arginase enzyme when present in the growth medium which is match with the current findings. Fig 4 shows the effect of cobalt chloride on arginase.

CONCLUSION

- Arginase was successfully isolated and purified from *Pseudomonas aeruginosa* Isolated from Sewage.
- Effect of heavy metals (mercury, cadmium and cobalt) on arginase was investigated.
- Mercuric chloride and cadmium chloride shows inhibition and decreases in enzyme activity.
- Cobalt chloride shows increases in the enzyme activity.

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Conflict of interest

Authors declare no conflict of interest.

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