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Tolerance of *Aspergillus niger* to Selected Concentrations of Metals and Sodium Chloride

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Abstract: *The aim of this study was to investigate the tolerance of Aspergillus niger to concentrations of selected metals (zinc, copper, chromium, iron and nickel) and sodium chloride. To achieve this aim, the effects of metal concentration, temperature, pH and inoculum population on tolerance of the test isolate to the metals were investigated. The results revealed that growth of the isolate was inhibited in the presence of nickel at all the concentrations studied. In the presence of sodium chloride, all the concentrations investigated supported the growth of the isolate. In the presence of zinc, iron and copper, growth of the isolate was supported up to a maximum concentration of 200 mg/l while in the presence of chromium, growth of the isolate was supported up to a concentration of 800 mg/l. Optimum temperature and pH for growth of the isolate in presence of the metals was observed to be 25^{o}C and 4, respectively. The study was able to reveal the optimum conditions for tolerance of the isolate in presence of the test metals.*
Keywords: *Aspergillus niger, Metal tolerance*

I. Introduction

Contamination of receiving water bodies is on the rise, due to industrialization and increase in the influx of people into that environment. Contamination of water bodies could be as a result of disposal of controlled and uncontrolled waste from industries, leaching of agricultural products such as plants and animal lots, fertilizers, herbicides, pesticides and herbicides into receiving water bodies and domestic run off of waste materials (Chiromaet al., 2007).The increase in the use of heavy metals is a global issue throughout the world, as industrialization has led to the production of materials with metal incorporated with it. The presence of metals arsenic, cadmium, and lead in both organic and inorganic fertilizers has been established (MDH, 1999; 2008).

Heavy metal discharge is a major constituent of waste water leading to water pollution. Since heavy metals are not readily degradable in nature, its presence would be dependent on the quantity initially discharged into the environment, thus, leading to negative impacts on lives within that environment. Impact of heavy metals in the environment is divided into health and environmental impacts. Uptake of metals are bioaccumulated in the living cells and as they are not readily degradable, they could lead to metal poisoning, asthma, high blood pressure. The major environmental impact caused by the presence of metal in wastewater is that it affects the aesthetic appeal of the water and as a result there could be colorations in that water body (Martin and Griswold, 2009;Shazia and Sumera, 2014).

Heavy metals are bio-accumulated in humans, as these elements are not easily degradable / metabolized by living organisms (Martin and Griswold, 2009). The effect of heavy metals when present in high concentrations affects the human health, which majorly lead to metal poisoning; metals that could cause poisoning includes mercury, lead, copper and silver. Other effect that could arise from continuous exposure to metals includes renal dysfunction by cadmium; low exposure to chromium could cause skin irritation which could cause ulceration after constant exposure. Bone disorder such as fracture, osteopenia and osteomalacia could be caused by aluminium toxicity. Other effects of exposure to heavy metals include liver damage, parkinson's disease, kidney damage, cancer and anemia (Martin and Griswold, 2009; Sailaja and Rajeswari, 2014).

Due to its adverse effect in the environment and on the health of individuals, diverse methods and techniques have been put in place for the control of heavy metals in wastewater, these methods includes; biological treatment of the wastewater, physiochemical treatment and finally phytoremediation of waste water. In the biological treatment of wastewater, microorganisms are used in the bioremediation process of the waste water. Methods taken to achieve this result includes; bioleaching, bioaggumentation, oxidation/reduction reactions and biosorption (EPA, 2013; Pires, 2010).

The toxic nature of heavy metals serves as a limiting factor in bioremediation process by bacteria and fungi. Although, certain microorganisms are capable of secreting enzymes, organic acid and metabolites and also the presence of large extracellular proteins enables them to survive in environments that are highly toxic, thus boosting the use of the organism specie in bioremediation process (Pires, 2010). The fungi Aspergillus niger is effective in the bioremediation process of heavy metals because of its ability to produce a chelating

agent, gluconin and citric acid during its growth process (Mulligan et al., 2004).The aim of this study was to investigate the tolerance of Aspergillus nigerto concentrations of selected metals and sodium chloride.

II. Materials And Methods

The test isolate used for the study was *Aspergillus niger*. The *Aspergillus niger* was part of the laboratory stock in the Department of Biological Sciences, Landmark University, Omu-Aran, Kwara State. Before use, the isolate was first cultured in sabouraud dextrose agar plate at 25 °C±2 °C for 72h to ensure its purity, before transferring into sabouraud dextrose broth and incubated at similar condition. After incubation of the isolate and sufficient growth appeared, the broth culture was then centrifuged at 500 rpm for 30min before suspending in sterile normal saline (0.85%NaCl w/v).

2.1 Preparation of stock solution

The stock solutions of the metals used in the experiments were copper, zinc, chromium, nickel and sodium chloride which were obtained from copper sulphate, zinc chloride anhydrous, chromium (iii)dichloride, nickel sulphate and sodium chloride respectively. In the preparation of 10000 parts per million (ppm) of each of the metals used in the investigation, the mass of each metal was calculated as follows:
The mass of the metals was calculated using
$(Dc \times Vc \times \delta w^{+} \times 10^{-6} / n_A \times F_w)$

Where D_c =10000 ppm of the metals, V_C = volume of water (500 ml), δ_w^{+} = molecular weight of each metals, n_A= atomic number of the metals, F_w = mass number of the metal
The calculated weight was measured and dissolved in 500mL distilled water, sterilized in an autoclave for 15 min at 121 °C. The prepared stock solution was then stored in an amber bottle till when needed.

2.2 Determination of effect of metal concentration on fungi

Exactly 6.4 g sabouraud dextrose agareach was weighed into 11 conical flasks and with 100mL distilled water. Before sterilizing the agar in the autoclave, concentrations of 100ppm, 200ppm, 300ppm, 400ppm, 500ppm, 600ppm, 700ppm, 800ppm, 900ppm and 1000ppm of the metals used in the investigation were incorporated into each conical flask. The agar thatwas not incorporated with heavy metals served as the control in the investigation.

After sterilization, the sterile media was allowed to cool for about 30 min, after which 100µl of the test isolate was inoculated on the agar using pour plate technique. This was then incubated for 72 h at 25 °C. This procedure was repeated in the investigation of each metal.

2.3 Determination of effect of temperature on viable counts of the isolate

Sabourauddextrose agar was prepared in 100 mL quantities in five different conical flasks at respective metal concentrations of 150 mg/L metal concentration and then sterilized in an autoclave for 15 min at 121 °C.
The sterilized agar was allowed to cool, after which it was inoculated with a known inoculum size (100 µL) of the test isolate in a broth culture. The viable counts were carried out using the standard pour plating technique.

The inoculated agar was poured in duplicates into petri dishes and incubated for 72 h at temperatures of 25°C, 30°C, 35°C, 40°C and 45°C respectively. This procedure was repeated in the investigation of other metal stock solutions.

2.4 Determination of effect of pH on viable growth of the isolate

Sabouraud dextrose agar of 1.5 strength was prepared in four conical flasks.To the prepared agar was added the respective metals at concentrations of 150 mg/L. Before sterilization, the pH of the contents in each conical flask was adjusted using either 1 M NaOH or 1 M HCl to pH 4, 6, 8 and 10, after which it was sterilized.The sterilized media was allowed to cool, inoculated with the test isolate and viable counts estimated as described in previous sections.

2.5 Determination of effect of initial inoculum size

In the determination of the effect of the initial inoculums size, using the manufacturer's directives, 6.4 g sabouraud dextrose agar was weighed into 5 conical flasks and each dissolved in 100 mLin distilled water.

The respective metals were then added to the prepared agar at concentration of 150 mg/L.The sterilized agar was allowed to cool after which known volumes (50 µL, 100 µL, 150 µL and 200 µL) of the broth containing predetermined inoculum sizes were inoculated into the respected flasks. Viable counts were then estimated using the standard pour plating technique as described in previous sections.

All reagents used in the study were of analytical grades. Also, all analyses and determinations were carried out in duplicate and the data expressed as mean ± standard deviation.

III. Results

Table 1 shows the viable counts of the test isolate at the different concentration of the metals used for investigation. As shown in the table, the isolate only showed growth in the presence of zinc and iron at maximum concentration of 200mg/l. In presence of chromium, growth was observed at concentrations of up to 800mg/l while all the concentrations investigated for sodium chloride supported growth. However, none of the concentrations investigated for nickel supported the growth of the isolate. At concentration of 100mg/l, viable counts of the isolate observed for zinc, iron, chromium, copper and sodium chloride were 1.1×10^3spore-forming units/ml, 7.1×10^2spore-forming units/ml, 7.6×10^2spore-forming units/ml, 1.8×10^2spore-forming units/mland 7.0×10^2spore-forming units/ml, respectively (Table 1).

Table 1: Viable counts of the test isolate at different concentrations of the test metals

Concentration	Zn	Fe	Cr	Cu	Nacl	Ni
0 mg/L	1.8×10^3 (±322.44)	1.3×10^3 (±158.39)	1.2×10^3 (±141.42)	1.3×10^3 (±158.40)	9.4×10^2 (±79.20)	1.344×10^3 (±158.39)
100mg/L	1.1×10^3 (±169.71)	7.1×10^2 (±39.60)	7.6×10^2 (±62.23)	1.8×10^2 (±22.63)	7.0×10^2 (±237.59)	0
200mg/L	2.8×10^2 (±56.57)	4.0×10 (±45.25)	3.5×10^2 (±79.20)	0	7.8×10^2 (±22.63)	0
300mg/L	0	0	3.9×10^2 (±130.11)	0	8.1×10^2 (±130.10)	0
400mg/L	0	0	2.6×10^2 (±90.51)	0	8.0×10^2 (±5.66)	0
500mg/L	0	0	2.0×10^2 (±33.94)	0	8.1×10^2 (±96.17)	0
600mg/L	0	0	1.2×10^2 (±39.60)	0	7.4×10^2 (±67.88)	0
700mg/L	0	0	8.8×10	0	1.1×10^3 (±130.11)	0
800mg/L	0	0	5.6×10 (±22.63)	0	9.2×10^2 (±62.23)	0
900mg/L	0	0	0	0	9.7×10^2 (±22.63)	0
1000mg/L	0	0	0	0	8.3×10^2 (±62.23)	0

All counts represent averages of duplicate analysis. Values in parenthesis represent ± standard deviations of measurements. All values are expressed as spore-forming units/ml.

The viable counts of the isolate at the volume of broth cultureused for inoculation of the media containing the respective metals at concentration of 100 mg/L revealed no observable growth in the presence of nickel. In the presence of the other metals and sodium chloride, viable count was observed to increase with increase in volume of broth used for inoculation The highest counts of 1.0×10^2spore-forming units/mL, 3.6×10^2spore-forming units/mL, 2.6×10^2spore-forming units/mL and 4.2×10^2spore-forming units/mL were observed in the presence zinc, iron, chromium and sodium chloride, respectively, when 200 µl of the broth culture of the isolate was used for inoculation(Table 2).

Table 2: Viable counts of the isolate at different initial inoculum population in presence of the test metals

Metal	50 µl	100 µl	150 µl	200 µl
Zinc	1.0×10^1 (±2.83)	2.6×10^1 (±8.49)	6.4×10^1 (±11.31)	1.0×10^2 (±5.66)
Iron	1.3×10^2 (±31.11)	1.8×10^2 (±22.63)	2.2×10^2 (±56.57)	3.6×10^2 (±11.31)
Chromium	1.2×10^2 (±25.46)	1.9×10^2 (±45.25)	2.1×10^2 (±14.14)	2.6×10^2 (±11.31)
Copper	1.2×10^1 (±2.83)	1.7×10^2 (±2.21)	3.9×10^1 (±5.66)	7.8×10^1 (±2.83)
Sodium Chloride	1.5×10^2 (±14.14)	2.4×10^2 (±25.46)	3.2×10^2 (±11.31)	4.2×10^2 (±11.31)
Nickel	0	0	0	0

All values represent averages of duplicate analysis and are spore-forming units/mL. Values in parenthesis represent ± standard deviations. Metal concentration used for each metal was 100 mg/L. 50 µl, 100 µl, 150 µl and 200 µl represent initial broth volume used for inoculation

As shown in Table 3, in the presence of iron, chromium, copper and sodium chloride, the lowest viable counts of 2.1×10^1spore-forming units/ml, 2.2×10^1spore-forming units/ml, $1.5\times10^1$and 2.7×10^1, respectively

were observed at pH 6. In the presence of copper, although viable counts increased with increase in pH, at pH 10, no growth was observed. In the presence of nickel, no growth was observed at the different pH used for investigation (Table 3).

Table 3: Effect of pH on viable counts of the isolate

Metals	pH 4	pH 6	pH 8	pH 10
Zn	2.0×10^2 (±33.94)	2.3×10^1 (±1.41)	1.9×10^1 (±6.36)	5.6×10^1 (±28.28)
Fe	2.8×10^2 (±1.41)	2.1×10^1 (±2.83)	3.4×10 (±8.49)	3.8×10^1 (±4.95)
Cr	5.8×10^2 (±2.83)	2.2×10^1 (±0.71)	2.5×10^1 (±6.36)	5.6×10^1 (±5.66)
Cu	1.3×10^2 (±3.54)	1.5×10^1 (±0.71)	1.9×10^1 (±4.24)	0
NaCL	3.0×10^2 (±7.78)	2.7×10^1 (±4.95)	2.9×10^1 (±5.66)	$3.5x10^1$ (±0.71)
Ni	0	0	0	0

All values represent averages of duplicate analysis and are spore-forming units/mL. Values in parenthesis represent ± standard deviations. Metal concentration used for each metal was 100 mg/L

At the different incubation temperatures investigated, no growth of the isolate was observed at40°C and 45°C. This trend was irrespective of the metal used. In presence of the respective metals, maximum growth was observed at 25^{o}C. This trend was also irrespective of the metal used. As was observed in other experiments, there was no growth of the isolate at the different temperatures in presence of nickel. At 25°C,growth of the isolate was observed to be 6.6×10^2spore-forming units/mL, 4.4×10^2spore-forming units/mL, 9.8×10^2spore-forming units/mL, 5.0×10^2spore-forming units/mL and 4.2×10^2 spore-forming units/ml in the presence of zinc, iron, chromium, copper and sodium chloride, respectively (Table 4).

Table 4: Effect of temperature on viable count of the test isolate

Metal	25^{o}C	30^{o}C	35^{o}C	40^{o}C	45^{o}C
Zn	6.6×10^2 (±8.49)	5.6×10^1 (±11.31)	1.8×10^1 (±8.48)	0	0
Fe	4.4×10^2 (±36.77)	2.2×10^1 (±2.83)	2.0×10^1 (±26.77)	0	0
Cr	9.8×10^2 (±42.42)	7.4×10^1 (±36.77)	1.8×10^1 (±14.14)	0	0
Cu	5.0×10^2 (±2.83)	3.0×10^1 (±2.83)	1.0×10^1 (±8.49)	0	0
NaCl	4.2×10^2 (±2.83)	2.2×10^1 (±2.83)	3.0×10^1 (±25.46)	0	0
Ni	0	0	0	0	0

All count values represent averages of duplicate analysis and are spore-forming units/mL. Values in parenthesis represent ± standard deviations. Metal concentration used for each metal was 100 mg/L.

IV. Discussion

In this study, nickel was observed to inhibit the growth of the test isolate at the different concentrations and experimental conditions investigated. A similar finding has been reported in a study by Rajendran et al, 2002. In testing the toxicity of nickel chloride to *Aspergillus niger*, bioassay carried out revealed 50% conidial inhibition at 1.7 mM nickel. They indicated that hyphal extension was affected even at a lower concentration (0.4 mM), thus suggesting the more sensitivity of hyphae than conidia to nickel. Further increase in nickel concentration was reported to result in proportionate decreases in hyphal extensions (Rajendran et al. (2002).

At the different temperatures and pH used for investigation, maximum growth was observed at 25^{o}C and at pH 4. Earlier workers have studied the influence of copper ions on growth and accumulation properties of cultures of *Aspergillus niger* growing at low pH (Tsekova and Dentchev, 2003).It was indicated that at 130 and 2650 mg/L of copper (II) ions, growth of the organisms was remarkably inhibited. *Aspergillus niger* is indicated to have the ability of accumulating large amounts of copper (II) ions at low pH, without losing its biological activities (Tsekova and Dentchev, 2003).Also, the optimum temperature for the growth of the test isolate in the investigation was within the range of 20 °C – 30 °C, temperatures above this range would either lead to a slow down of the metabolic growth process of the test isolate or kill the organism (Payne et al., 2001;Tsekova and Dentchev, 2003;Gunasekaran et al., 2002).

It is indicated that metal ion concentration, adsorption time, co-ions and pH have great impact on metal biosorption. In a study on heavy metals biosorption ability of *Saccharomyces cerevisiae*, high metal biosorption was indicated to be obtained at initial metal concentration that ranged between 50 and 100 mg/L at 30 ^{o}C and pH 5.0 (Thippeswamy et al., 2014).

In an investigation of metal tolerance ability of different species of Aspergillus, *Aspergillus flavus* was indicated to be more tolerant of metals while *Aspergillus niger* was reported to be moderately tolerant to metal concentrations. *Aspergillus niger* has been reported to be tolerance against Pb and sensitive to chromium. The variation in metal tolerance by different organisms is indicated to be the result of the presence of one or more strategies of tolerance or resistance mechanisms displayed by different fungi (Iram et al., 2013).The present study revealed growth of the isolate in the presence of chromium up to concentration of 800 mg/L. This observation corroborated the findings of Iramet al., (2013).

In this study, an increase in the concentration of the test isolate and contact time favored the proliferation of the test isolate. Metals such as copper, nickel and iron inhibited the growth of the test isolate at a lower concentration of the metal due to its high level toxicity comparedto chromium which supported the growth of the test isolate up to a concentration of 800 mg/l. This observation could imply that the metals with high toxicity level are not readily degraded and could take a longer time for bioremediation process to be successful (Thippeswamyet al., 2014).

V. Conclusion

This present study that was based on evaluating the tolerance of *Aspergillus niger* to selected concentrations of metals and sodium chloride was able to reveal the following:

- In the presence of nickel, no growth of the test isolate was observed at the different concentrations and experimental conditions investigated.
- All the concentrations of sodium chloride used for the study supported the growth of the isolate.
- In the presence of chromium, the growth of the isolate was supported up to a concentration of 800 mg/l while in the presence of zinc, copper and iron, growth of the isolate was supported up to concentrations of 200 mg/L.
- Optimum temperature and pH for growth in presence of the metal were observed to be 25^{o}C and 4, respectively. Temperature of 40^{o}C and above did not support the growth of the isolate.

The study was able to reveal the tolerance of the test isolate to the metals investigated under the experimental conditions studied.

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