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(Research Article)

Evaluation of *Streptomyces* species from historically sewage sludge contaminated soils for tolerance to copper(II) toxicity

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Abstract

Pollution of the environment by copper(II) exerts numerous adverse impacts on soils, microorganisms, plants, animals and humans. Assessing these impacts on microorganisms is essential as they are potential agents of bioremediation to purify the ecosystem. The aim of this study was to investigate the tolerance of *Streptomyces* species obtained from historically contaminated soils to copper (II) toxicity. Soil samples were collected from farms around sewage sludge dump sites (tests) and where there were no dumps (control). Standard microbiological methods were used to isolate, characterize and identify the three best copper tolerant species. Also, the impact on *Streptomyces* growth characteristics such as radial growth (R2), index of growth (GI) and percentage inhibition of radial growth (PIRG) by copper (II) was examined. Results obtained indicated that; the minimum inhibitory concentration of copper (II) for some of the *Streptomyces* isolates was 600 mg/l. However, three isolates; SW2B, SW7A and SW7B had MIC of 3000 mg/l and were selected for further investigations. Increasing metal concentration from 100 mg/l to 2000 mg/l reduced R2 from 40.2 to 4.0 and GI from 0.77 to 0.08, corresponding to 69.48 % increase in PIRG for SW2B. For isolate SW7A, R2 and GI decreased from 33.7 to 3.8 and from 0.67 to 0.08 respectively as copper (II) concentration increased from 100 mg/l to 2000 mg/l, giving 59.33 % increase in PIRG. The highest increase in PIRG (88.04 %) was obtained for SW7B, corresponding to a decrease of R2 and GI from 39.2 to 5.2 and 0.77 to 0.10 respectively. Molecular method was used to identify the isolates as *Streptomyces chartreusis* (SW2B), *Streptomyces aureoverticillatus* (SW7A) and *Streptomyces subrutilus* (SW7B).

Keywords: Adaptation; Copper toxicity; Metal tolerance; Growth inhibition; Streptomyces isolates

1. Introduction

Heavy metal pollution poses significant threat to health as some heavy metals possess well documented toxic, mutagenic and carcinogenic properties [1-3]. The European Environmental Agency [4] gave the annual estimate of heavy metals release from all sources worldwide to be around (in metric tons) 22,000 of cadmium (Cd); 939,000 of copper (Cu); 783,000 of lead (Pb); 1,350,000 of zinc (Zn); 7,000,000 and 19,300,000 metric tons ferro-chromium (FeCr) and chromites (Cr_2FeO_4) respectively. According to Oves *et al.* [5], heavy metal pollutions are mainly results of

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anthropogenic activities such as chemical wastes from industrial activities and agricultural effluents (such as fertilizer, herbicides and pesticide), combustion of fossil fuels and mining activities.

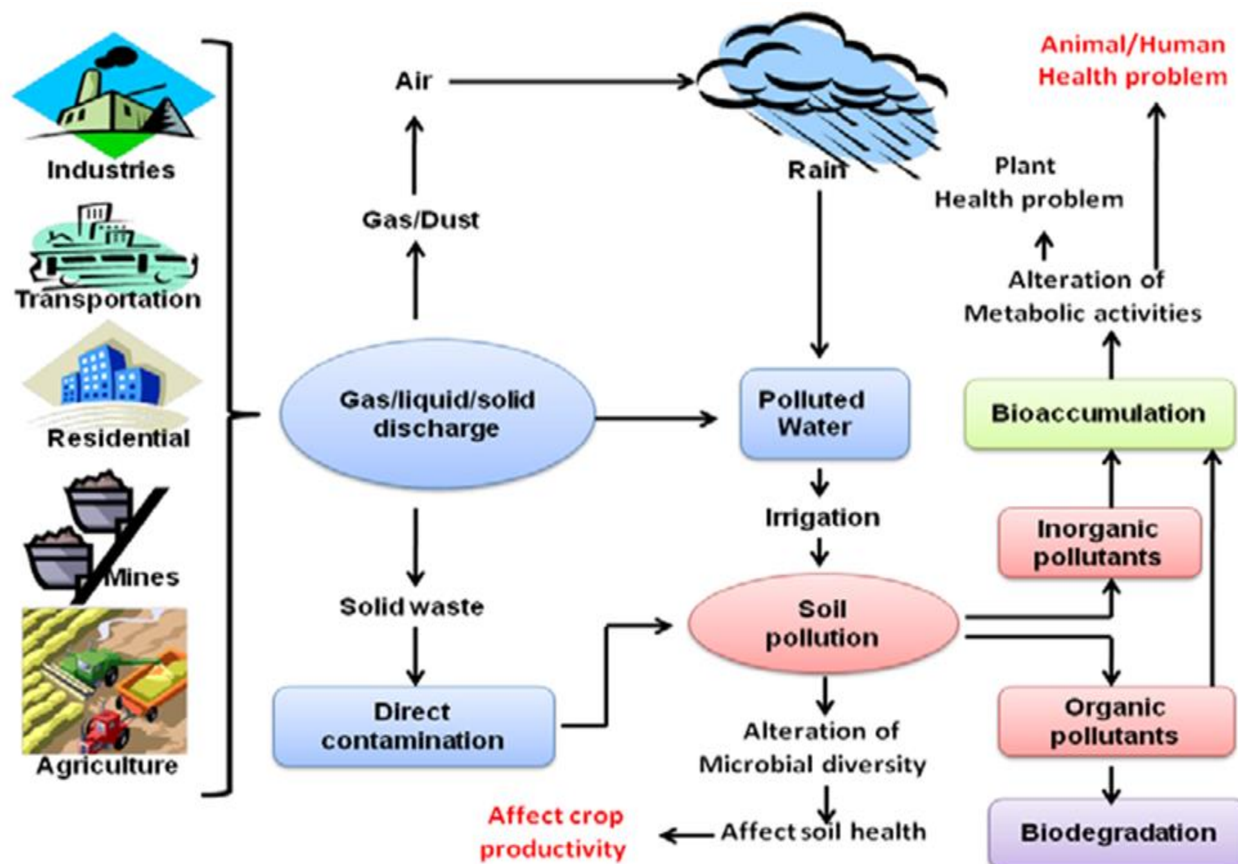

Figure 1 Sources and toxicities of heavy metals [5]

Metals discharged into the environment are not biodegraded rather; they undergo series of chemical or microbial transformations, creating large environmental and public health impacts.

At minimal concentration, say about 5-20 μg/g, copper, for instance, is involved in key physiological processes such as photosynthesis, respiration also an essential co-factor for key enzymes involved in various metabolic pathways such as ATP synthesis, elimination of superoxide radicals. Although Cu(II) is essential for various physiological functions of microorganisms, plants and animals at sub-minimal concentrations, when present beyond its threshold level (i.e. above 20 μg/g), Cu(II) can exert adverse effects on living organisms, soil and water quality [6]. Copper may replace essential metals in living cells and exert toxic effects such as growth inhibition, lower biomass production and disruption of membrane and secondary protein structures and inhibition of enzyme activities [7,8]. The most important distinguishing factors of heavy metals from other toxic pollutants are their non-biodegradability and propensity in living materials. The non-biodegradability and persistence of Cu(II) in the ecosystem poses serious threat to environmental and public health with more severity on microbial life and activities. This severity of Cu(II) toxicity on microorganisms is due to their abundance and high surface area to volume ratio which affords them a large contact area for interaction with the heavy metal in their immediate environment; microbial species that cannot withstand this toxicity die off, impacting negatively on specie biodiversity and ecological balance [9]. Also, Cu(II)can be taken up by plants in polluted soils and persist in the food chain, if such plants are consumed by man, overtime, there will be elevated copper concentration in the body with the health challenges that comes with it [10]. Over time, exposure to heavy metal pollution results in selective pressure leading to the emergence of resistant strains. These resistant strains possess bioremediative capacities, which can be exploited for the control and removal of heavy metal pollution in the environment. *Streptomyces* are of major interest because of their documented capabilities to thrive in metal polluted environment by producing a wide range of exo-polymeric substances and metal ion chelators known as siderophores, which confer protection against adverse effects of heavy metals [11]. This primary aim of this study is to investigate the tolerance of *Streptomyces* species from heavy metal polluted sites to Cu(II) toxicity.

2. Material and methods

2.1. Sample collection and pretreatment

Soil samples surrounding the University of Nigeria Nsukka's sewage treatment plant contaminated with sewage sludge were collected in polythene bags. Samples were randomly taken from seventeen (17) different sampling points at depth ranging from 0-10cm and taken to the Microbiology lab, University of Nigeria Nsukka immediately where they were air dried for four days and sieved with 2mm mesh sieve before carrying out microbiological analysis.

2.2. Microbiological analysis of soil samples

2.2.1. First stage enrichment of soil for Copper (Cu^{2+}) tolerant Streptomyces

Soil sample (20 g) was aseptically transferred into sterile 250 ml Erlenmeyer flasks followed by the addition of 0.8 g sterile starch and 0.04 g NH_4Cl. 0.1 g Copper ($CuSO_4$) was added to artificially pollute the medium and select for copper tolerant *Streptomyces*. The mixture was rehydrated to 60 % water holding capacity of the soil and incubated at 30 ^oC until microbial growth became visible.

2.2.2. Second stage enrichment for Copper (Cu^{2+}) tolerant Streptomyces

At this stage, 1 g of the soils laden with copper tolerant *Streptomyces*was aseptically transferred into 100 ml Erlenmeyer flasks containing 20 ml of Mineral Salts Tryptone Starch (MSTS) broth. The formulation of this enrichment medium (MSTS) contained per liter of distilled water; 10 g starch, 0.2 g tryptone, 1 g KH_2PO_4, 0.2 g $MgSO_4$ and 0.5 g of Cu^{2+} to enrich for copper tolerant strains. The inoculated medium was incubated at 30 ^oC until microbial growth became visible. This phase of enrichment was repeated using two loopful of the preceding liquid culture as inoculum. Copper tolerant isolates were then streaked on MSTS (solidified with 15 g/l of agar) and incubated at 30 ^oC for 1-2 days.

2.3. Purification of isolates

The isolates were sub-cultured on MSTS copper supplemented agar medium and Potato Dextrose Agar (PDA) to obtain pure cultures. The pure cultures were maintained at 4 ^oC in metal supplemented MSTS agar slants.

2.4. Screening of heavy metal-tolerant *Streptomyces*

The purified isolates were screened to determine their tolerance limits to Cu^{2+} by aseptically inoculating them on Cu^{2+} (500 mg/l) supplemented PDA plates. The inoculated plates were incubated at 30 ºC for seven days and observed for growth of the strains. The index of tolerance was determined by measuring the radius of colony extension (mm) against the control [12].

2.5. Cu^{2+} toxicity testing

The isolates were subjected to Cu^{2+} toxicity test on MSTS supplemented with different concentration of copper (II); 100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l, 500 mg/l, 600 mg/l, 700 mg/l, 800 mg/l, 900 mg/l, 1000 mg/l, 2000 mg/l and 3000 mg/l using agar dilution method [12]. The impact of Cu^{2+} toxicity on *Streptomyces* growth characteristics was evaluated by determining the index of growth (GI) and percentage inhibition of radial growth (PIRG).

GI was calculated as;

$$GI = \frac{R2}{R1}$$

PIRG was determined using the formula;

$$PIRG = \frac{R1 - R2}{R1} \times 100$$

Where; R1 (in mm)= Radial growth of isolates in control plates; R2 (in mm)= Radial growth of isolates in test plates [12].

2.6. Molecular Characterization and Identification of Working Isolates

The best performing isolates were taxonomically characterized by PCR amplification of their 16S rDNA using ITS4 and ITS5 primers followed by nucleotide sequencing.

2.7. Genomic DNA extraction

The method of Lee and Hallam [13] was used. The selected *Streptomyces* strains were grown at 30 °C for 7 days in potato dextrose broth. A small quantity of the hyphal growth was picked, dried and crushed in pestle motor. It was crushed again following addition of 1ml extraction buffer. The crushed semisolid was then transferred into microfuge tube, 10 % of 150 µl sodium dodecyl sulphate (SDS) was added to it and the tube was incubated at 65 °C for 30 minutes and centrifuged at 10,000 rpm for 10 min to collect the supernatant. Equal amount of ice cold solution of phenol:chloroform:isoamyl alcohol (25:24:1) was added to supernatant and centrifuged again. The resulting aqueous layer at the top was transferred into fresh microfuge tube and centrifuged with phenol:chloroform:isoamyl alcohol. This process was repeated twice after which equal volume of 100% ice cold ethanol was added to the top aqueous layer in fresh microfuge tube. Overnight incubation of the tube (at 20°C) followed by centrifugation at 12,000 rpm at 4°C for 10 min was done to precipitate the DNA. The supernatant was discarded and pellet washed in 70 % ethanol, centrifuged again at 12,000 rpm for 10 min and suspended in 100 µl of TE buffer for electrophoresis.

2.8. PCR amplification of 16S rDNA

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each of the 16S ribosomal gene, forward primer (ITS4: TCCTCCGCTTATTGATATGS) and reverse primer (ITS5: GGAAGTAAAAGTCGTAACAAGG) and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermal cycler (Applied BiosystemInc., USA) with a PCR profile consisting of an initial denaturation at 94 °C for 5 min; followed by a 30 cycles consisting of 94 °C for 30 s, 30 seconds annealing of primer at 54 °C and 72 °C for 1.5 minutes and a final termination at 72°C for 10 minutes.

2.9. Agarose gel electrophoresis

Agarose (1% w/v) was added into tris acetate EDTA (TAE) buffer and the mixture was heated in microwave for 2min with shaking at intervals of 30 seconds to release any gas. The agarose solution was allowed to cool, stained with ethidium bromide (5 µl) and dispensed into wells in the electrophoresis plates made using electrophoresis comb. The stained agarose gel was allowed to polymerize for an hour and the comb was carefully removed. The plate was placed in the electrophoresis chamber followed by the addition of TAE buffer until the gel was completely submerged. Bromophenol blue (5 µl) was added to about 4 µl of the DNA and the mixture loaded into the wells after which the chamber was connected to a power supply and the gel was electrophoresed at 92 volts for 3h. The genomic DNA of the isolates was visualized under UV-transilluminator.

2.10. Nucleotide sequencing of amplicons

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis. Sequencing was done at the International Institute of Tropical Agriculture, Bioscience Center, Ibadan, Oyo state

3. Results

3.1. Screening of isolates for growth on Cu^{2+}

As shown in table 1, total of 18 *Streptomyces* species were obtained post-isolation and screened for growth at different Cu^{2+} concentration. Growth was generally observed at relatively low Cu2+ concentration (100 mg/l to 500 mg/l). However, at>500 mg/l of Cu2+, growth inhibition set infor most of the isolates. Three isolates: SW2B, SW7A and SW7B demonstrated tolerance to high Cu2+ concentration, up to 2000 mg/l.

3.2. Cu^{2+} toxicity testing

The three most tolerant isolates; SW2B, SW7A and SW7B obtained from the preliminary screening above were further subjected to toxicity of Cu^{2+} testing. This was done by determining the effect of Cu^{2+} on radial growth and the index of growth (GI). Percentage inhibition of radial growth (PIRG) was also calculated. As shown in table 2, GI decreased from 0.77 to 0.88 while PIRG increased from 22.84 % to 92.32% as Cu^{2+} concentration increased from 100 mg/l to 2000 mg/l

(i.e., GI and PIRG are inversely proportional). Also, the radial growth (in mm) of SW2B was significantly ($p < 0.5$) decreased in the presence of Cu^{2+}.

Table 1 Screening for Cu^{2+} tolerant *Streptomyces*.

		Cu^{2+} concentration (mg/l)											
Isolates	**0 (control)**	**100**	**200**	**300**	**400**	**500**	**600**	**700**	**800**	**900**	**1000**	**2000**	**3000**
SW1A	G	G	G	G	G	G	G	G	G	NG	NG	NG	NG
SW1B	G	G	G	G	G	G	G	G	G	NG	NG	NG	NG
SW1C	G	G	G	G	G	G	G	G	G	NG	NG	NG	NG
SW2A	G	G	G	G	G	G	G	G	G	G	G	NG	NG
SW2B	G	G	G	G	G	G	G	G	G	G	G	G	NG
SW3A	G	G	G	G	G	G	G	G	G	G	NG	NG	NG
SW3B	G	G	G	G	G	G	G	G	G	NG	NG	NG	NG
SW4A	G	G	G	G	G	G	G	G	G	G	G	NG	NG
SW4B	G	G	G	G	G	G	G	G	G	NG	NG	NG	NG
SW5A	G	G	G	G	G	G	NG	NG	NG	NG	NG	NG	NG
SW5B	G	G	G	G	G	G	NG	NG	NG	NG	NG	NG	NG
SW5C	G	G	G	G	G	G	G	G	G	G	NG	NG	NG
SW7A	G	G	G	G	G	G	G	G	G	G	G	G	NG
SW7B	G	G	G	G	G	G	G	G	G	G	G	G	NG
SW8A	G	G	G	G	G	G	G	G	NG	NG	NG	NG	NG
SW8B	G	G	G	G	G	G	G	G	G	G	NG	NG	NG
SW8C	G	G	G	G	G	G	G	G	G	NG	NG	NG	NG
SW8D	G	G	G	G	G	G	G	G	G	G	NG	NG	NG

Legend: G =Growth; NG = No Growth.

Table 2 Cu^{2+} toxicity on the growth of SW2B

Conc. of Cu^{2+} (mg/l)	**R1 (mm)**	**R2 (mm)**	**GI**	**PIRG (%)**
100	52.1	40.2	0.77	22.84
200	52.1	33.7	0.65	35.32
300	52.1	31.9	0.61	38.77
400	52.1	26.6	0.51	48.94
500	52.1	21.4	0.41	58.93
600	52.1	19.7	0.38	62.19
700	52.1	16.0	0.31	69.29
800	52.1	11.0	0.21	78.89
900	52.1	8.9	0.17	81.66
1000	52.1	10	0.19	80.81
2000	52.1	4.0	0.08	92.32

Legend: R1 = Radial growth of isolates in control plates; R2 = Radial growth of isolates in test plates
GI = Index of Growth; PIRG = Percentage of Inhibition of Radial growth

Similarly, table 3 depicts the impact of Cu^{2+} on the growth characteristics of isolate, SW7A. Here, as Cu^{2+} concentration increased from 100 mg/l to 2000 mg/l, GI decreased from 0.67 to 0.08 while PIRG increased from 33.13 % to 92.46 %. as Cu^{2+} concentration increased from 100 mg/l to 2000 mg/l (i.e., GI and PIRG are inversely proportional). Also, in the presence of Cu2+, radial growth was decreased significantly ($p < 0.5$).

Table 3 Cu^{2+} toxicity on the growth of SW7A

Conc. of Cu^{2+}(mg/l)	R1 (mm)	R2 (mm)	GI	PIRG (%)
100	50.4	33.7	0.67	33.13
200	50.4	29.5	0.59	41.47
300	50.4	29.1	0.58	42.26
400	50.4	15.5	0.31	69.25
500	50.4	13.0	0.26	74.21
600	50.4	12.5	0.25	75.20
700	50.4	11.2	0.22	77.78
800	50.4	7.6	0.15	84.92
900	50.4	4.0	0.08	92.06
1000	50.4	6.0	0.12	88.10
2000	50.4	3.8	0.08	92.46

Lastly, Cu^{2+} toxicity was investigated on SW7B. Result obtained shows that, higher concentration of Cu^{2+} had higher negative impacts on R2 and GI as shown in table 4.

Table 4 Cu^{2+} toxicity on the growth of SW7B

Conc. of Cu^{2+}(mg/l)	R1 (mm)	R2 (mm)	GI	PIRG (%)
100	51	39.2	0.77	1.76
200	51	36.1	0.71	11.96
300	51	31.5	0.62	17.65
400	51	27.0	0.53	17.25
500	51	21.0	0.41	41.18
600	51	15.5	0.30	49.02
700	51	17.4	0.34	50.78
800	51	13.4	0.26	51.57
900	51	9.5	0.19	61.37
1000	51	11.5	0.23	77.45
2000	51	5.2	0.10	89.80

3.3. Molecular identification of the Cu^{2+} tolerant isolates

Three isolates selected based on high tolerance to Cu2+ salts were characterized and identified as *Streptomyces chartreusis* (SW2B); *Streptomyces aureoverticillatus* (SW7A) and *Streptomyces subrutilus* (SW7B) (Figure 2).

Isolate SW2B has 100 % pairwise Identity to *Streptomyces chartreusis* strain ATCC 14922 with NCBI accession number CPO23689.1 and E-value of 0.

The Isolate sequence for sample SW2B is as shown;

TAGGCTTGKTSTTWTTWTTTATTTGCTGATCTCYGCTMWMGGTACCWCCKAYCGRGTCACCTGGAAGATTGATTGGGGTCG
CCGGCGGGCGCCGGCCGGGCCTACAGAGCGGGTGACGAAGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCG
GGCCCGCCCCCCGGGAGCCGGGGGGCGAAGCCCAACACACAAGCCGTGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCC
CCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGC
TGCGTTCTTCATCGATGCCGGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTGATTTAGCTAATCTGCTCAGACTGCAATCTT
CAGACAGAGTTCATTGGTGTCTTCGGCGGGCGCGGGCCCGGGGGCGAGTGCCCCCCGGCGGCCGTGAGGCGGGCCCGCCGAAGC
AACAAGGTAGAATAAACACGGGTGGGAGGTTGGACCCAGAGGGCCCTCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGA
AACCTTGTTACGACTTTTACTTCC.

Isolate SW7A has 96.77 % pairwise Identity to *Streptomyces aureoverticillatus* strain HN6 with NCBI accession number CP048641.1.The E-value is 0.

Isolate sequence for sample SW7A;

CMCCTKGAWCCSARGGYCAACCTGGAAAGATTGAGGGGGGTCGCCGGCGGGCGCCGGCCGGGYCTACAAGAGCGGGTGACGAA
GCCCCATACGCTCGAGGACCGGACGCGGTGCCKCCGCTGCCTTTCGGGCCCGCCCCCCGGGAGCCGGGGGGCGGGGGCCCAACAC
ACAAGCCGTGCTTGAGGGCAGCAATGACGCTCGKACAGGMATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACT
CKATGATTCACTGAATTCTGCAATTCRCATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGRAACCAASAGATCCGTT
GTTGAAAGTTTTAACTGATTTAGCTAATCGCTCARACTGCAATCTTCAGACAGCGTTCAATGGTGYCTTCGGCGGGCGCGGGCC
CGGGGGCGGATGCCCCCCGGCGGYYGTGAGGCGGGCCCGCCGAAGCAACAAGGTACGATAAACACGGGYGGGAGGTTGGACCCA
GAGGGCCCTCACTCGGTAATGATCCTTCCGCWGGTTCCCCTACGGAA

Isolate SW7B has 89.74 % pairwise Identity to *Streptomyces subrutilus* strain ATCC 27467 with NCBI accession number CP023701.1The E-value is 0.

3.4. Isolate sequence for sample SW7B

TTCCAATGSATCCGAGGTYACCTGGAAASATTGATTGGGGTCGCCGGYGGGCGCCGGCCGGGYCTACAGAGCGGGTGACGAAGC
CCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGCCCCCCGGGAGCCGGGGGGCGAAGCCCAACACACA
AGCCGTGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGA
TGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCGTTGTT
GAAAGTTTTAACTGATTTAGCTAATCTGCTCAGACTGCAATCTTCAGACAGAGTTCATTGGTGTCTTCGGCGGGCGCGGGCCCG
GGGGCGAGTGCCCCCCGGCGGCCGTGAGGCGGGCCCGCCGAAGCAACAAGGTAGAATAAACACGGGTGGGAGGTTGGACCCAGA
GGGCCCTCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTTCCA.

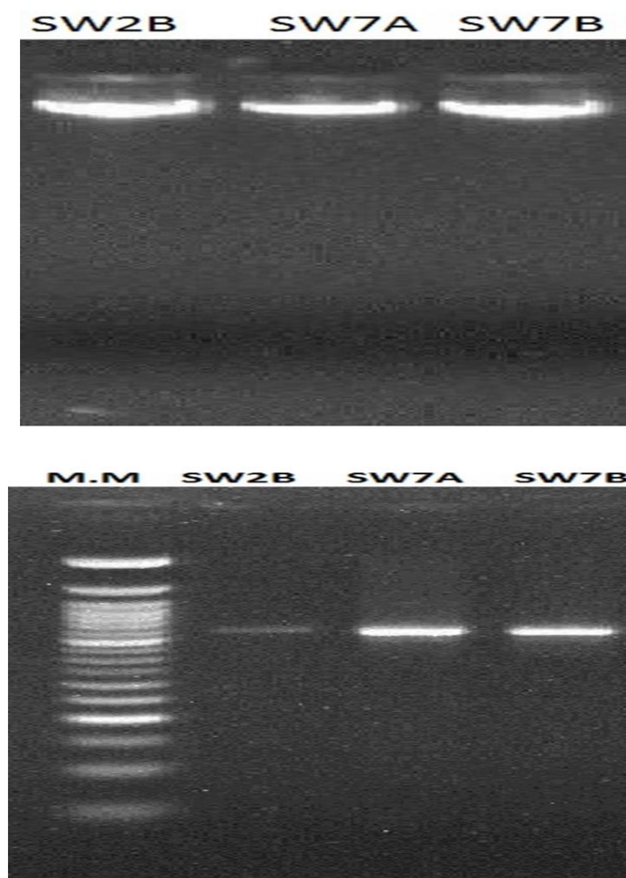

Figure 2 Gene amplification (a) Image showing Genomic DNA from PCR Amplification of gene. (b) Internal transcribed spacer (ITS) gene amplification,

4. Discussion

The present study investigated tolerance of *Streptomyces* isolates from historically sewage sludge-contaminated soils to the divalent metal ion (Cu^{2+}). Eighteen copper tolerant isolates were recovered and the most tolerant were identified molecularly via their 16S rDNA as *Streptomyces chartreusis* (SW2B), *Streptomyces aureoverticillatus* (SW7A) and *Streptomyces subrutilus* (SW7B). The impact of heavy metals on the growth of the *Streptomyces*isolates was firstly assessed on the basis of mycelia diameter. At lower metal ions concentrations, all the isolates were found to exhibit strong radial growth. Nevertheless, at higher metal ion concentrations, considerable reduction in radial growth attributable to increased toxicity occurred. This concurs with the results of other studies on the impact of Cu2+ on microbial growth and metabolism [9,14]. In contrast, the toxicity of Cu^{2+} to *Streptomyces* appeared not to be concentration dependent according to a study by Arisa et al. [15].

The minimum inhibitory concentration (MIC) values of the heavy metal were determined and complete inhibition of *Streptomyces* growth occurred at as high as 3000mg/l. This adaptive behavior of growth in the presence of heavy metals could be due to acclimatization of the strains as a result of prolonged exposure which leads to considerable modification of their physiology [16]. Resultantly, these organisms develop numerous strategies towards the resistance of heavy metals even at high concentration. In some instances, metal resistance has been connected to differences in uptake/ transport of the toxic metal or enzymatic transformation of the toxic metal into less toxic chemical species while in other cases, defense is attributed to the use of low molecular weight chelating agents like siderophores in *Streptomyces* to immobilize soluble metal ions or complexes, thereby reducing their bioavailability and increasing tolerance [17,18].

Based on significant reduction on mycelial growth diameter, the percentage inhibition of radial growth escalated past 50 % at; 400 mg/l, 500 mg/l and 700 mg/l for *S. aureoverticillatus*, *S. chartreusis* and *S. subrutilus* respectively, indicating a dependence of metal resistance on microbial specie or type. This corroborates a study by El Baz et al. [19] which demonstrated the resistance of different *Streptomyces* strains to lead ions (Pb2+) in the following order; *Streptomyces* sp. BN2>*Streptomyces* sp. BN3>*Streptomyces* sp. BN48. According to Iram et al. [20], the tolerance capacity of isolates to heavy metals differs because they exhibit different tolerance strategies or resistance mechanisms, as such, isolates belonging to the same genus could display varied resistance to metals. In addition, the mycelial morphology of the *Streptomyces* isolates changed in the presence of copper. At elevated concentration, radial extension, tip growth and branching at the edge of the mycelia dramatically reduced. Such morphological changes might be a detoxification mechanism to increase tolerance to heavy metals [21].

5. Conclusion

The ability of the *Streptomyces* isolates to grow in the presence of copper(II) shows metal tolerance of the organisms. Also, their ability to withstand copper(II) toxicity is an indication of an innate or acquired adaptive mechanism to such toxicities. Hence, these organisms can be employed as cost effective and ecologically-benign bioremediation options for the removal of heavy metal pollution from contaminated environments

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest in this work.

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