International Journal of Environmental Monitoring and Analysis
2022; 10(5): 120-126
http://www.sciencepublishinggroup.com/j/ijema
doi: 10.11648/j.ijema.20221005.12
ISSN: 2328-7659 (Print); ISSN: 2328-7667 (Online)

Accumulation of Heavy Metal ion and Ex-situ Remediation of Polluted Soil Using *Escherichia intermedium* and *Shigella sonnei* Isolated from Polluted Sites

Olusola Adelowotan[1, *], Emmanuel Ayodele Oluyemi[1], Abiodun Odunlami Adegunwa[2]

[1]Department of Chemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria

[2]Department of Pure and Applied Chemistry, Osun State University, Osogbo, Nigeria

Email address:

melroseshols@yahoo.com (Olusola Adelowotan)

[*]Corresponding author

To cite this article:

Olusola Adelowotan, Emmanuel Ayodele Oluyemi, Abiodun Odunlami Adegunwa. Accumulation of Heavy Metal ion and Ex-situ Remediation of Polluted Soil Using *Escherichia intermedium* and *Shigella sonnei* Isolated from Polluted Sites. *International Journal of Environmental Monitoring and Analysis*. Vol. 10, No. 5, 2022, pp. 120-126. doi: 10.11648/j.ijema.20221005.12

Received: July 27, 2022; **Accepted**: August 19, 2022; **Published**: September 21, 2022

Abstract: Heavy metals pollution of the soil is of environmental concern and various methods have been applied to reduce its concentration. This study examines the accumulation of metal ion from aqueous solution and remediation potential of indigenous *Escherichia intermedium* and *Shigella sonnei* isolated from polluted site. Standardized 2 mL of inoculum was added to 30 ppm of metal (Cu, Cr, Cd, Pb, and Ni) solution and was incubated at 37°C, the solution was centrifuged and cells were filtered off. The supernatant was analyzed with Atomic Absorption Spectrometer (AAS) for the determination of residual metal concentration. Total metal concentration in the polluted soil was extracted using aqua regia ($HCl:HNO_3$, 3:1) solution. Polluted soil solution was inoculated with 2 mL of standardized inoculum and was incubated at 37°C, the solution was subjected to gradient configuration and cells were filtered off. The resulting soil solution was subjected to acid digestion and analyzed with AAS for the determination of residual heavy metal concentration. Results from the bioaccumulation experiment show the absorbed concentration of metals from the aqueous solution by *Escherichia intermedium* as follows: 19.034 ± 0.26 mg/l (Cd), 5.799 ± 0.26 mg/l (Cr), 18.025 ± 0.17 mg/l (Cu), 9.930 ± 0.22 mg/l (Pb) and 11.179 ± 0.14 (Ni) as well as *Shigella sonnei* with absorbed concentrations of 17.133 ± 0.17 mg/l (Cd), 8.937 ± 0.22mg/l (Cr), 17.686 ± 0.17 mg/l (Cu), 14.347 ± 0.02 mg/l (Pb), 10.776 ± 0.20mg/l (Ni). The range of percentage reduction of metal concentration in the ex-situ remediation of the four polluted soil sampled by *Escherichia intermedium* is as follows: 3.86-34.34% Cu, 18.06-50.39% Cr, 1.72-88.17% Cd, 2.89- 8.53% Pb, 19.31-44.03% Ni and *Shigella sonnei* with 7.66-53.36% Cu, 17.39-50.11% Cr, 12.38%-ND Cd, 6.74-20.41% Pb and 44.17-53.13% Ni reduction. This study establishes that these isolates own good capability of accumulating and reducing metal ions from both aqueous solution and soil solution.

Keywords: Accumulation, Bacteria, Heavy Metals, Pollution, Remediation

1. Introduction

Unlike organic contaminants which by biological means can be possibly degraded, producing carbon-dioxide and water as the final products, heavy metal species discharged to the environment cannot be degraded either by biological or chemical means [2]. Generally, the term 'heavy metal' are used to enclose metals found in soils and sediments that are connected with contamination, toxicity and have high atomic weights [5], due to their increase in concentrations in soils and sediments through anthropogenic inputs. These may be considered dangerous to both plants and animals. They are non-degradable but are incessantly recycled from one soil phase to the other, through natural processes such as erosion, weathering, soil pH, Organic matter etc.

2. Methodology

2.1. Samples Collection and Treatment

Soil sampling was carried out within Ile-Ife metropolis in the South-western part of Nigeria as shown in the coordinates below at a depth of 15cm using soil auger. Samples were mixed thoroughly inside labeled polythene bag and were taken to the laboratory; soil samples for microbial analysis were stored in refrigerator at 4°C and analyzed within 24 hours. The soil sample were dried in open air for two weeks after which were then crushed and sieved through 2.00 mm mesh to obtain powdery forms of the representative samples.

A: Mechanic workshop opposite O. A. U gate (Road 1), Ife (Lat: 07° 29. 776′, Lon: 004° 31. 431′).

B: Ife Iron Smelting Company, Ife (Lat: 07° 29. 723′, Lon: 004° 28. 634′).

C: Mechanic workshop and scraps dumpsite, Modakeke-Ife (Lat: 07° 32. 038′, Lon: 004° 31. 472′).

D: O. A. U Dumpsite, Ife (Lat: 07° 28. 488′, Lon: 004° 32. 399′).

2.2. Sterilization of Materials Used

Materials used in study this were washed with detergent, rinsed with distilled water and soaked in 10% (v/v) HNO_3 for 24 hours to avoid metal contamination. Slides and spatula were sterilized using 95% ethanol. The inoculating wire loop and needles were sterilized by flaming until red hot in Bunsen flame before and after use.

2.3. Isolation and Culture of Isolate

One gram (1 g) of soil sample was suspended in 10 mL of sterile distilled water in test tubes and mixed thoroughly. Ten folds serial dilutions of soil solution were made in a set of test tubes, each containing 9 mL of sterile distilled water. Pour plate method was used for this experiment, one millilitre (1 mL) from each dilution was pipetted into sterile petri dish after which 20 mL of molten nutrient agar (NA) was added. The plates was swirled gently and allowed to set. The cultured plates were incubated at 35°C for 36 to 48 hours. The plate having colonies between 30 and 300 was selected for counting. The average viable count was multiplied by the dilution factor and expressed as number of colony forming unit per gram of sample (cfu/g).

$$\text{CFU/g} = \frac{Colonies}{Volume\ plated} \times Dilution\ factor$$

2.4. Biochemical Characterization and Identification of Bacterial Isolate

The identification of isolates were based on various conventional biochemical tests and compared to Bergey's Manual of Systematic Bacteriology [3]. These are: Triple sugar ion test, Sulphide-indole-motility test, Catalase test, Citrate utilization test, Methyl red test, Voges-proskauer test, Nitrate reduction, Oxidation fermentation test, Oxidase test, Sugar fermentation test.

2.5. Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent that restrains the visible growth of microorganisms. To estimate the heavy metals resistance levels of each of the isolates, procedures according to Sujitha and Jayanthi [7] was adopted with some modifications. Heavy metal solution of the following compounds: $CuSO_4{\cdot}5H_2O$, CrO_3, $3CdSO_4{\cdot}8H_2O$, $Pb(NO_3)_2$ and $NiCl_2{\cdot}6H_2O$ were added separately to nutrient agar media and by gradually increasing their concentration by 20 ppm until each strain failed to grow on the agar media. A sterile wire loop was used to collect a loopful of 24 hours old of each pure isolate, streaked on the surface of the heavy metal incorporated nutrient agar starting with 20 ppm for each of the metal solution in different plates and were incubated at 37°C for 24 hours. After the incubation period, the plates were observed for growth, if any, each isolate was streaked on fresh nutrient agar with increasing concentration of each metal solution by 20 ppm, until each isolate fail to grow. Thus, the last concentration of heavy metal incorporated nutrient agar that restricted visible growth of each isolate was taken as the minimum inhibitory concentration values for each of the isolates. The experiment was done in duplicate.

2.6. Accumulation of Heavy Metals Ions by Isolates

Procedure base on Varghese *et al.* [10], with some modifications was employed. 8 mL each of 30 ppm of metal solution of $NiCl_2{\cdot}6H_2O$, $Pb(NO_3)_2$, $CuSO_4{\cdot}5H_2O$, CrO_3, $3CdSO_4{\cdot}8H_2O$ were mixed with equivalent amount of nutrient broth in test tubes and sterilized at 121°C using autoclave. Each test tube was inoculated with 2 mL each of standardized inoculum and was incubated for 14 days; cells were separated from the solution by centrifuging at 4000 r.p.m for 30 minutes and filtered with 0.45 µm millipore filter paper. Cells in each test tube was washed with sterile distilled water and added to their respective supernatant collected, which were made up 15 mL for Atomic Absorption Spectrometer analysis.

Bioaccumulation and percentage removal efficiency for each isolate:

$$C_a = C_i - C_f \text{ (mg/l)}$$

Source: [1]

$$R = \frac{C_i - C_f}{C_i} \times 100$$

Source: [6]

C_a= Concentration of metal ion accumulated by each isolate cell (mg/l).

C_i= Initial metal ion concentration before addition of isolate cell (mg/l).

C_f= Final metal ion concentration after removal of isolate cell (mg/l).

R= Percentage Removal Efficiency.

2.7. Extraction of Heavy Metal Concentration from Polluted Soil

The method proposed by Thalita *et al.* [8] with modifications was employed. One gram from each soil samples was weighed into a 50 mL beaker with the addition of 20 mL of aqua regia (HCl:HNO_3, 3:1, v/v) solution and was left to sit overnight. The solution was gradually heated to a reduced volume; an additional 10 mL of aqua regia was added and heated to evaporate off to a reduced volume. The digested solution was cooled with distilled water, filtered with whatman filter paper and were made up to 30 mL in air tied bottles for heavy metals determination of Cd, Cr, Cu, Pb and Ni concentrations using Atomic Absorption spectrophotometer.

2.8. Remediation of Heavy Metal Soil Concentration

Eight mL of nutrient broth was added to 1g of each soil samples in different test tubes and were sterilized by autoclaving at 121°C. Two milliliter (2 mL) each of the standardized inoculum was added to each test tube containing each sample solution. All test tubes were incubated for 14 days at 37°C. To separate cells from soil particles, the suspended cells were filtered with 0.45 μm millipore filter paper. The supernatant was added back to the soil solution and subjected to gradient centrifugation at 500 r.p.m for 10 minutes to releasing the entrapped isolate cells within the soil aggregates on the basis of sedimentation rate according to Viggo and Lars [9], after which the re-suspended cells were filtered again. The supernatant was added back to the soil solution and was subjected to acid extraction. The heavy metals concentration after remediation was extracted with acid 'aqua regia' (HCl and HNO_3 in the ratio of 3:1) solution and the digests was cooled with sterile distilled water, filtered with whatman filter paper and made up to 30 mL in air tight bottles for determination of Cd, Cr, Cu, Pb and Ni concentration using atomic absorption spectrophotometer.

2.9. Fourier Transform Infrared Analysis of Bacterial Isolate Grown in Nutrient Broth

Two mL of standardized inoculum made from 24 hours culture of each isolate was added to 8 mL sterile nutrient broth solution in different test tubes. These mixtures were left to grown for fourteen days. Each mixture was centrifuged at 4000 r.p.m for 20 minutes, decanted and washed with sterile distilled water. The grown isolates in their respective test tubes were dried in oven at 100°C to obtained dry biomass which were analyzed for their chemical functional groups using Fourier Transform Infrared spectrometer.

2.10. Fourier Transform Infrared Analysis of Bacterial Isolate Grown in Nutrient Broth Containing Mixture of Heavy Metal Solution

Ten milliliters each of 20 ppm of the following metal salt solution: $NiCl_4 \cdot 6H_2O$, $3CdSO_4 \cdot 8H_2O$, $CuSO_4 \cdot 5H_2O$, CrO_3, $Pb(NO_3)_2$ were mixed in a beaker and appropriate amount of nutrient broth was added. Eight milliliters was withdrawn from the solution into different test tubes and were sterilized at 121°C, 2 mL of standardized inoculum made from 24 hours culture was added to the solution in test tube. The setup was left to grow. The mixture was then centrifuged at 4000 r.p.m for 20 minutes, decanted and washed with sterile distilled water. The grown isolates in their respective test tubes were dried in oven at 100°C to obtained dry biomass which were analyzed for their chemical functional groups using Fourier Transform Infrared Spectrometer.

3. Results and Discussion

Table 1. *Biochemical characteristics and identification of isolates.*

Code	CS	GR	CT	TSI	SIM	CTU	MR	VP	GLU	MAL	MAN	SUC	LAC	OF/NL	NR	isolate
A	SR	-	++	YG.YG.NC	- - -	+	+	-	YG	YG	YG	YG	YG	F	+	*Escherichia intermedium*
B	SR	-	+	Y.Y.NC	- - -	-	+	+	Y	Y	Y	Y	Y	F	+	*Shigella sonnei*

CS: Cell shape, GR: Gram reaction, CT: Catalase, TSI: Triple sugar ion reaction; SIM: Sulphide indole motility, CTU: Citrate utilization, MR: Methyl-red, VP: Voges proskaur, GLU: Glucose, MAL: Maltose, MAN: Mannitol, SUC: Sucrose, LAC: Lactose, OF: Oxidation-fermentation; NR: Nitrate reduction, YG: Acid and Gas production, NC: No change, Y: Acid production only, F: Fermentative, SR: short rod.

3.1. Minimum Inhibitory Concentration (MIC) of Isolates to Heavy Metal Solution

Metal resistivity of each isolate to each metal solution is shown in Table 2. These results were attained due to no noticeable growth of each isolate on the nutrient agars incorporated separately with increasing concentration of each metal solution.

Shigella sonnei has higher resistivity to increased heavy metal concentration than *Escherichia intermedium* except for copper concentration. The resistance ability of the two isolates declined as the concentrations of each of the metal solutions were increased which resulted to restraint in growth and finally death. Each isolate resistivity response could be attributed to different sources of isolation and degree of pollution which each one has been exposed to before isolation. *Escherichia intermedium* was isolated from Ife iron smelting company soil while *Shigella sonnei* was isolated from mechanic and metallic scraps dumpsite, Modakeke-Ife. Other factor could be ability of each isolate to alter its morphology and biochemical compositions in response to its surrounding.

Table 2. *Minimum Inhibitory Concentration (MIC) of Isolates.*

		Metal salts solution (ppm)				
Code	**Name of Organisms**	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**
A	*Escherichia intermedium*	220	440	700	600	600
B	*Shigella sonnei*	260	720	160	940	780

3.2. Accumulation of Heavy Metals Ions by Escherichia intermedium and Shigella sonnei

Table 3 shows the concentration of heavy metals ions accumulated from their respective metal solution by *Escherichia intermedium* and *Shigella sonnei*.

Shigella sonnei was more effective in the accumulation of chromium, lead and nickel ions with percentages removal efficiency of 30.34, 47.48 and 50.75% respectively while *Escherichia intermedium* was more effective in the accumulation of cadmium and copper ions with percentages removal of 57.66 and 66.23%.

The absorption and adsorption of metal ion by each isolate from their respective metal solution could be due to their increased surface area and cell regeneration during growth with the aid of nutrient broth serving as carbon source. The ionic bonding between the cationic species and the negatively charged chemical functional groups of biomolecules such as nucleic acids, proteins, carbohydrates or lipids present on cell wall and within the cytoplasm.

Table 3. *Accumulated concentration of heavy metal ions from their respective solution by each Isolate.*

	Cd (mg/l)	**Cr (mg/l)**	**Cu (mg/l)**	**Pb (mg/l)**	**Ni (mg/l)**
Escherichia intermedium					
Final conc. (C_f)	10.680±0.05	23.659±0.04	8.679±0.01	20.301±0.04	17.351±0.01
Absorbed conc.	19.034±0.26	5.799±0.26	18.025±0.17	9.930±0.22	11.179±0.14
Shigella sonnei					
Final conc. (C_f)	12.581±0.01	20.521±0.02	9.018±0.01	15.884±0.03	17.754±0.03
Absorbed conc.	17.133±0.17	8.937±0.22	17.686±0.17	14.347±0.20	10.776±0.20
Initial conc. (C_i)	29.714±0.02	29.458±0.03	26.704±0.02	30.231±0.01	28.530±0.01

3.3. Remediation and Percentage Reduction of Heavy Metals Concentration in Polluted Soil Samples

Table 4 shows that *Shigella sonnei* was more effective in decreasing Cu, Ni and Cd concentration in sample from mechanic site opposite OAU school gate. This was by 53.36, 45.38 % reduction while Cd concentration was reduced to a non-detective level. Table 5 indicates that *Shigella sonnei* was more effective in decreasing Cd, Pb and Ni concentrations in sample from Ife Iron Smelting Industry with 44.25, 12.49and 44.17% reduction. According to results from table 6, *Shigella sonnei* was more effective in reducing Cr, Cd, Pb and Ni concentration in sample from mechanic and Scraps Dumpsite at our lady, Modakeke-ife with the following percentage reduction 50.11, 26.49, 11.22 and 45.99% respectively. In table 7, *Shigella sonnei* was more effective in reduction of Cu, Cd, Pb and Ni with the following percentage reduction 7.66, 12.38, 20.41 and 53.13% respectively. The result shows the dominance effective of *Shigella sonnei* over *Escherichia intermedium* in reducing metals concentrations in polluted soil. The availability of nutrient broth as a source of nutrient within the polluted soil sample solution enable cell regeneration which aid in adsorption and absorption of metal ions by each isolate. This involves interactions between the ions and the functional groups within the cell and on the cell wall, thus forming metal precipitates or complexes.

Table 4. *Remediation of heavy metals polluted soil sample from mechanic site opp. O.A.U School Gate (Road 1).*

	Cu(µg/g)	**Cr(µg/g)**	**Cd(µg/g)**	**Pb(µg/g)**	**Ni(µg/g)**
Escherichia intermedium					
Final concentration	83.09±0.05	110.13±0.11	0.11±0.02	401.01±0.09	20.43±0.07
Percentage reduction	23.99	24.89	88.17	8.53	19.31
Shigella sonnei					
Final concentration	50.99±0.04	120.33±0.08	ND	408.87±0.10	13.83±0.05
Percentage reduction	53.36	17.93		6.74	45.38
Initial Concentration	109.32±0.13	146.62±0.06	0.93±0.06	438.41±0.08	25.32±0.02

Table 5. *Remediation of heavy metals polluted soil sample from Ife Iron smelting industry, Ile-Ife.*

	Cu(µg/g)	**Cr(µg/g)**	**Cd(µg/g)**	**Pb(µg/g)**	**Ni(µg/g)**
Escherichia intermedium					
Final concentration	371.43±0.09	117.17±0.03	6.62±0.01	715.23±0.09	98.37±0.01
Percentage reduction	34.34	50.39	23.11	7.15	38.69
Shigella sonnei					
Final concntration	400.02±0.07	139.58±0.02	4.80±0.01	670.26±0.03	89.59±0.01
Percentage reduction	29.29	40.90	44.25	12.99	44.17
Initial concentration	565.71±5.81	236.18±0.11	8.61±0.02	770.34±0.03	160.46±0.02

Table 6. *Remediation of heavy metals polluted soil sample from mechanic and scraps dumpsite at our lady, Modakeke-Ife.*

	Cu(µg/g)	Cr(µg/g)	Cd(µg/g)	Pb(µg/g)	Ni(µg/g)
Escherichia intermedium					
Final concentration	7388.63±0.05	71.43±0.09	18.11±0.02	4192.11±0.02	102.44±0.03
Percentage reduction	17.55	18.06	18.28	2.89	44.03
Shigella sonnei					
Final concentration	7623.03±0.08	43.49±0.03	16.29±0.02	3832.77±0.03	98.85±0.01
Percentage reduction	14.94	50.11	26.49	11.22	45.99
Initial concentration	8961.47±0.15	87.17±0.08	22.16±0.08	4316.94±0.11	183.03±0.13

Table 7. *Remediation of heavy metals polluted soil sample from O.A.U campus dumpsite, Ile-Ife.*

	Cu(ppm)	Cr(ppm)	Cd(ppm)	Pb(ppm)	Ni(ppm)
Escherichia intermedium					
Final concentration	498.69±0.01	26.66±0.03	27.95±0.02	344.79±0.03	34.85±0.03
Percentage reduction	3.86	47.39	1.72	7.85	38.10
Shigella sonnei					
Final concentration	478.99±0.02	34.31±0.05	24.92±0.02	297.81±0.04	26.39±0.01
Percentage reduction	7.66	32.29	12.38	20.41	53.13
Initial concentration	518.70±0.17	50.67±0.11	28.44±0.10	374.18±0.25	56.30±0.04

3.4. Infrared Spectrum of Escherichia intermedium Grown in Nutrient Broth

The spectrum of *Escherichia intermedium* in Figure 1 shows a weak broad band at 3295 cm^{-1} indicating OH stretching of alcohol, while bands at 2944 cm^{-1} and 2832 cm^{-1} signify C-H stretching of alkane. Bands at 1625 cm^{-1} indicates Sp^2 C-H stretching of alkene and 1401 cm^{-1} signifies OH bending of alcohol. Bands at 1021 cm^{-1} signifies C-N stretching of amine and broad band at 622 cm^{-1} represents C-Br/Cl/F of halo-compound [4].

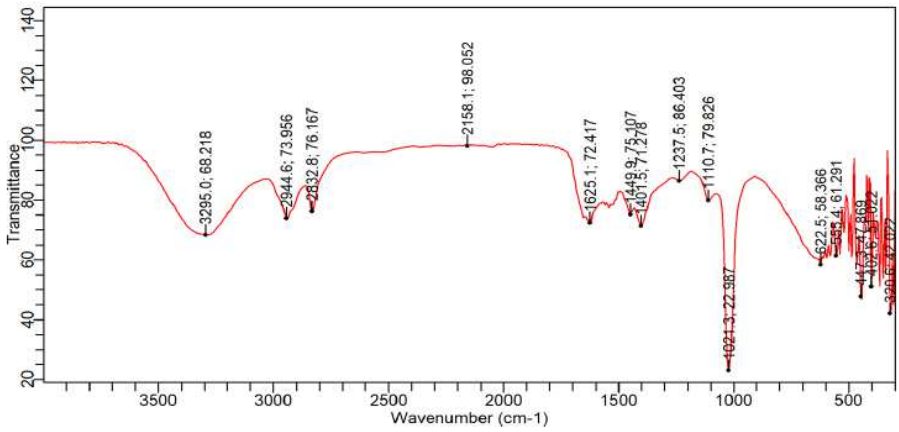

Figure 1. *Infrared spectrum of Escherichia intermedium grown in nutrient broth.*

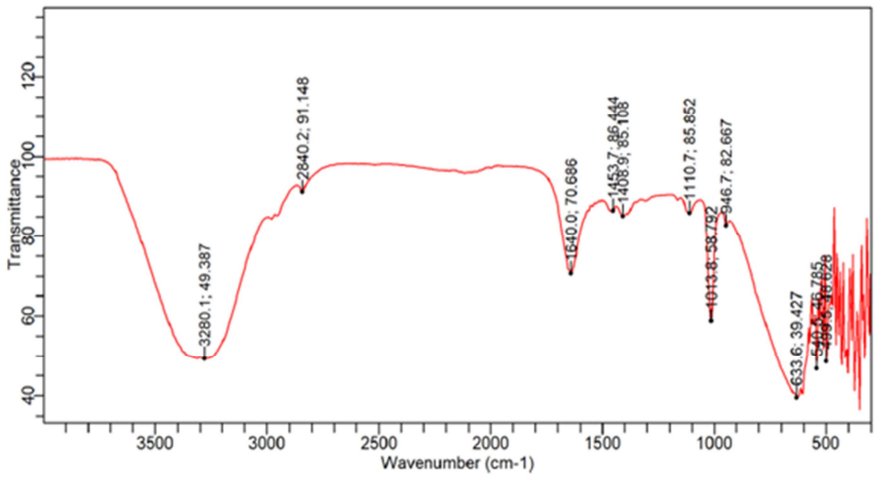

Figure 2. *Infrared spectrum of Escherichia intermedium grown in nutrient broth containing mixture of heavy metals solution.*

3.5. Infrared Spectrum of Escherichia intermedium Grown in Nutrient Broth Containing Mixture of Heavy Metal Solution

The spectrum of *Escherichia intermedium* in Figure 2 shows shifting of bands and wavelengths which are as a result of metal bonding activities within the solution. Decreased wavelength of broad absorption bands at 3280 cm^{-1} signifies OH stretching of alcohol as a result of deprotonation. Bands at 2840 cm^{-1} and 1640 cm^{-1} indicate C-H stretching of alkane and C=C stretching of alkene respectively as for the formation of metal alkyl complex. Peak at 1408 cm^{-1} signifies O-H bending of alcohol while 1110 cm^{-1} and 633 cm^{-1} signify C-N stretching of amine which could also be as a result of amine-metal complex formation and C-Br/Cl/F of halo-compound [4].

3.6. Infrared Spectrum of Shigella sonnei Grown in Nutrient Broth

The spectrum of *Shigella sonnei* isolate shown in Figure 3 displays broad band at 3369 cm^{-1} indicating hydroxyl (-0H) stretching of alcohol, while bands at 2959 cm^{-1} and 2922 cm^{-1} indicate sp^3 C-H stretching of alkane. Band at 1636 cm^{-1} indicated C=C stretching of alkene, while 1405 cm^{-1} bands indicates OH bending of alcohol. Weak absorption bands at 1308 cm^{-1} and 1088 cm^{-1} indicates S=O stretching of sulfone and C-N stretching of amine [4].

3.7. Infrared Spectrum of Shigella sonnei Grown in Nutrient Broth Containing Mixture of Heavy Metal Solution

The spectrum of *Shigella sonnei* isolate in Figure 4 reveal shifts in bands and wavelengths of chemical groups present due to metal bonding activities between the isolate and metal ions in the solution. A shift of broad band at 3261 cm^{-1} indicates OH stretching of alcohol which is as a result of deprotonation process. A new band at 2109 cm^{-1} indicating Sp CH stretch of alkyne. Band at 1636 cm^{-1} indicates Sp^2 CH of alkene while 1464 cm^{-1} indicates C-H bending of alkane indicating metal alkyl complex formation. Absorption band at 1386 cm^{-1} indicated OH bending of phenol and a band at 622 cm^{-1} indicates C-Br/Cl/F of halo-compound [4].

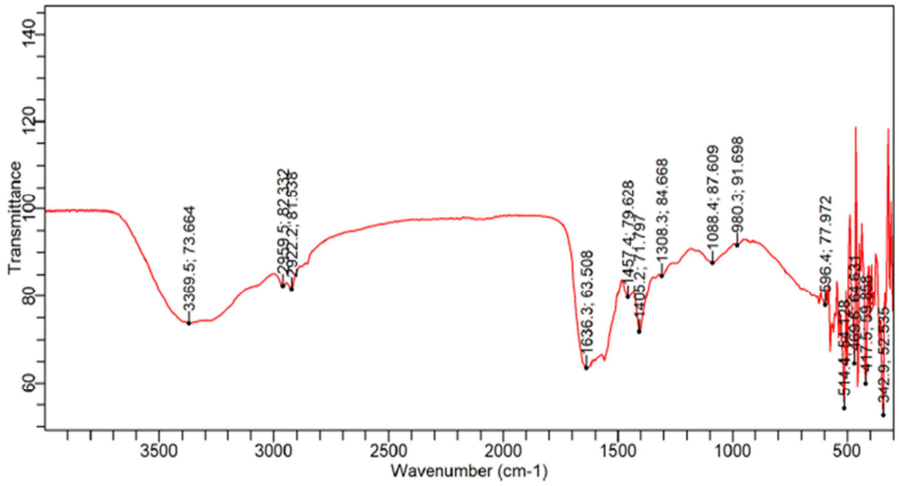

Figure 3. *Infrared spectrum of Shigella sonnei grown in nutrient broth.*

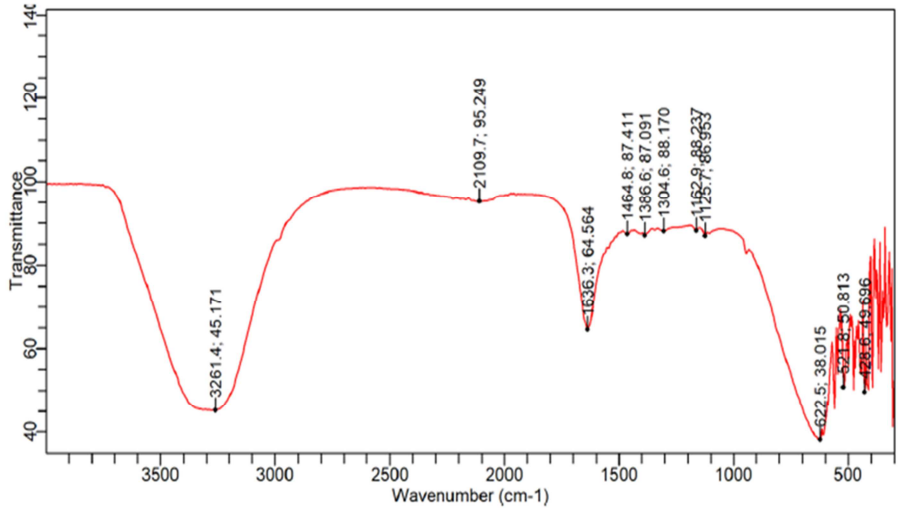

Figure 4. *Infrared spectrum of Shigella sonnei grown in nutrient broth containing mixture of heavy metal solution.*

4. Conclusion

This study concluded that the two isolates from polluted sites showed high resistance to selected heavy metal concentrations and were effective in accumulation of metal ions. It also revealed their capability in the reduction of heavy metals concentrations in polluted soil and more information were given on chemical functional groups responsible for their absorption and adsorption mechanisms. This shows the scientific significant of soil microorganisms in recycling toxic pollutants to less harmful compounds or gases and their usefulness in reclaiming polluted environmental media such as soil and water.

References

[1] Achmad A., Apriyanti F. D, Purwaningsih P, Kalqutny S. H, Agustina D, Surtiningsih T, Shovitri M, Zulaika E. (2017). Lead (Pb) Bioaccumulation; Genera Bacillus Isolate S1 andd SS19 as a case study. Cite as: *AIP Conference Proceedings 1854, 020003.*

[2] Bosecker, K. (2001). Microbial leaching in environmental clean-up programmes. *Hydrometallurg, Vol.* 59: 245-248.

[3] Holt J. G., Kreig N. R., Sneath P. H. A., Staley J. T. and Williams S. T., (1994). Bergeys's manual of determination bacteriology. 9th ed., Lippincott Williams and wilkins, Baltimore, U.S.A., ISBN-10: 0683006037.

[4] IR Spetrum Table and Chart (2020). Marck KGaA, Darmstadt. Germany www.sigmaaldrich.com

[5] Ranasinghe P., Weerasinghe S., and Kaumal M. N., (2016). Determination of heavy metals in tilapia using various digestion methods. *International Journal of Scientific Research and Innovative Technology*. 3: 38-48.

[6] Rui G., Yiming W., Yefei Z., Jin T. and Wei D. (2017). Cobalt (II) bioaccumulation and distribution in Rhodopseudomonas palustris. *Biotechnology & Biotechnological Equipment*, 31 (3): 527-534.

[7] Sujitha D and Jayanthi M, (2014). Efficiency of immobilized microbial combination for the bioremediation of tannery effluents in Vellore District, Tamil Nadu, India. *International Journal of Advanced Research in Biological Sciences* 1 (8): 113–120.

[8] Thalita F. A., Carlos A. S., Daniela A. A and Waldete J. O. C. (2014). Influence of digestion methods on the recovery of Iron, Zinc, Nickel, Chromium, Cadmium and Lead Contents in 11 organic residues. *Revista Brasileira de Ciencia do Solo (RBCS).* 38: 166-176.

[9] Viggo Lindahl and Lars R. Bakken (1995). Evaluation of methods for extraction of bacteria from soil. *FEMS Microbiology Ecology* 16: 135-142.

[10] Varghese R., Krishna M. P., Babu A. V. and Mohamed Hatha A. A (2012). Bioaccumulation of cadmium by *Pseudomonas* sp. isolated from metal polluted industrial region. *Environmental Research*, *Engineering and Management*, 61 (3), 58-64.