

Characterization the Composition of Industrial Effluents and Isolate Potential Microorganisms for the Possible Application

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Abstract: This study was conducted to determine the physico-chemical parameters of industrial effluents and soil samples polluted by industrial effluents and isolated potential Bacteria and fungi for the possible application. Parameters; pH, Temperature, TSS, TDS, COD, NO_3^- , TP, TN, and SO_4^{2-} , Cl^- , S^{2-} , total alkalinity, Copper and Chromium were analyzed and results were compared with EPA, 2003, WHO, 2001 and USEPA 2001 permissible limits. This study revealed that some parameters pH (7.3-8.5), Temperature (21.6-32°C), TA (107-485mg/l), SO_4^{2-} (0.02-0.05 mg/l), NO_3^- (0.79-2.95 mg/l), TN (40.1-49mg/l), were below the permissible limit and some parameters TDS 3310 -5650mg/l, COD (2140 -341.5mg/l), TP (3.35-24.75 mg/l), SS (371-797 mg/l), S^{2-} (1.00-41.5mg/l), Cl^- (162.5-4982.4 mg/l) were above permissible limits. Cu (0.21, ND, 1.11, and 0.27 (mg/l)) and Cr (0.12, ND, ND, and 0.14 (mg/l)) site A, B, C, and D respectively. Cr, Cu, TN and TP were analyzed from soil samples. The values ranged Cr (157.3 - 109 mg/Kg) and Cu (649.95 - 59.3 mg/Kg) TP (7032.5-1566 mg/Kg and TN (3.64-0.58 mg/Kg). Bacteria's and fungus Isolated from contaminated soil and industrial effluents. Isolated Bacteria and fungi were checked for growth on minimal salt medium amended with (10, 20, 40, 60, 80, 90 and 100 ppm) different concentrations of Cr and Cu salt by flask culture technique. The Bacteria isolated from the soil and industrial effluents including. *Pseudomonas aeruginosa*, *Staphylococcus. au*, *E. coli* and Fungi isolates including *Aspergillus*, and *Penicillium Sp.*, were found to be high tolerant of heavy metals (Cr and Cu) concentration up to 100 ppm. This indicated that potential of these Bacteria and fungi as bio-sorption of heavy metals.

Keywords: Pollution, Industrial Effluent, Heavy Metals, Physico-Chemical Parameters, Bacteria and Fungus

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I. Introduction

Different Industries are major sources of pollution in all environments. Based on the type of industries, various kinds of pollutants can be discharged directly or indirectly into the environment [1]. In Ethiopia industrial wastewater has become one of the most serious problems because of the increase in discharge of untreated effluent to the environment. Many industries fail to afford investments in pollution remediation equipment and technologies, because their profit margin is very low [2]. High level of pollutants which are discharged into rivers pose severe problems for plant, animal and human health due to their toxicity and persistence in the environment [3]. The most common environmental pollutants are BOD, COD, TD, SS sulphid, sulphate chloride nitrate and heavy metals [4]. The presence of heavy metals and essential elements at elevated concentration causes toxic effects if exposed to human population [5]. The accumulation of heavy metals in agricultural soils is increasing concern due to food safety issues and potential health risks as well as its detrimental effects on soil ecosystems [6]. Heavy metals are classified as metallic elements that have relatively high atomic weight and are poisonous at low concentrations. Specifically, heavy metals are those having density greater than 5gcm^{-3} . These include Cd, Cr, Pb, Cu, [7]. Some metals essential metals for health of living organisms (such as cobalt, copper, and manganese), but when their concentrations surpass tolerable limits, they become toxic.

Microorganisms play an important role in removal of metal ions from the environment. In the past years many studies have been carried out for the isolation of metal resistant bacteria and fungi strains. Based on this, microorganisms adopt variety of mechanisms to remove heavy metals from wastewater. They remove metal ions via complexation by exopolysaccharides, adsorption to cell surfaces, binding with bacteria and fungi cell envelopes, biosynthesis of metallothioneins, intracellular accumulation, precipitation, and transformation to volatile compounds and formation of other proteins that trap the heavy metals [8]. The objective of the study to characterize the Composition of Industrial Effluents and Isolate Potential Microorganisms for the Possible Application

II. Materials and Methods

2.1. Description of the Study Area

The study conducted at Modjo Ethio-Japan textile Share Company found in Modjo town, Oromia region 75 km south of Addis Ababa. Modjo town is located at 8° 35' North and 39° 10' East with an altitude of 1,825 m above sea level and Kombolcha which is located on the north central part of Ethiopia placed immediately south east of Dessie in the Amhara region 375 km from Addis Ababa at 11°06' north latitude and 39°45' east longitude. River Borkena crosses the town emerging from the east and running to the west direction. In its way all through the town, it receives effluents indirectly through its tributaries rivers named Worka and Leyole. Most of the factories are found closely together in the middle of the town near by the tributary rivers of Borkena.

2.2. Sample Collection

Wastewater samples were collected from each sampling sites using polyethylene bottles having 2-liters capacity using standard methods for the examination of water and waste water. Waste water samples were preserved with 10% nitric acid at pH less than 2 and digested with concentrated nitric acid and hydrochloric acid 3:1 ratio for analysis of Chromium and Copper were analyzed as shown Tables 1 and 2.

2.3. Physicochemical Parameters

Physicochemical parameters were measured for influents and effluent according to Standard Methods for the Examination of Water and Wastewater [9]. These parameters include; pH, Temperature, Electric conductivity (EC), sulphied, total dissolved solids (TDS), total suspended solids (TSS), total nitrogen, total phosphors, nitrite, COD, alkalinity, and sulfates

2.4. Soil Sample Collection

Soil samples were collected 0-20 cm in depth using an auger sampler from four sampling sites and one control sample from the upper stream of contaminated sampling sites. The samples were air dried to constant weight for a week and large debris and silts were filtered, then the samples were ground and sieved with 2 mm sieve. 0.5g of soil sample were transferred in to a 100ml flask and digested by aqua-regia method (3:1 ratio of HNO_3 67% and HCl 37%) at 80°C until transparent solution obtained. The digested samples were filtered and diluted 100ml distilled water used for Cr and Cu analysis with Atomic Absorption Spectrophotometer.

2.5. Media Preparation for Bacteria and Fungus Isolation

Media was prepared for isolation of bacteria by using nutrient agar medium and Potato dextrose agar. 1000 ml of distilled water in beakers were taken and 28 g of Nutrient agar and Potato dextrose agar powder were dissolved in it followed by sterilization in an autoclave at 121 °C for 15 min and allowed to cool. After that Medias were poured in Petri plates and allowed to solidify and placed in an incubator at 37°C for 24 h in order to check its sterility.

2.6. Preparation of Stock Solutions and Standards

Chromium and copper stock solution (1000 mg/l) were prepared by dissolving 0.38 g of CrO_3 and 0.56 g of copper sulfite in a solution of 20 ml water and 4 ml of concentrated nitric acid and diluted to 200 ml using distilled water. Through serial dilution, standard working solutions of chromium and copper were made. The calibration graph was made using solutions with concentrations of 1, 2, 3, 4 and 5 mg/l.

2.7. Isolation of Fungus

Serial dilution technique, used for isolation of fungal colonies from soil and effluent samples. In each sample 1g soil and 1ml of effluent were taken and placed separately in test tubes containing 10 mL and 9 ml of sterile distilled water, shaking 1 min to homogenize and prepared stock respectively. From these stocks, various dilutions were prepared from 10^{-1} to 10^{-7} using sterile distilled water. One milliliters of each diluted sample was poured into petri-plates containing Potato Dextrose agar medium. Triplicates were maintained for each dilution. Streptomycin was added to the molten medium after autoclave and the plates were incubated at 30°C for 3-5 days to identify the fungi. Distinct fungal colonies with different morphological form were sub-cultured to purity and were preserved on potato dextrose agar slants under refrigeration conditions.

The isolated fungus were characterized and identified on the basis of macroscopic (colonial morphology, color, texture, shape and appearance of colony) and microscopic characteristics (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia and presence of sterile mycelium).

2.8. Isolation of Bacteria

Serial dilution technique was used to isolate bacteria from soil and effluent samples. In each sample 1g soil and 1ml of effluent were taken and placed separately in test tubes containing 10 ml and 9ml of sterile distilled water, shaking 1 min to homogenize and prepared stock respectively. The dilutes were regimented for a short period. Sterile dilution blanks were marked sequentially starting from stock and 10^{-1} to 10^{-4} . One ml from the stock transferred to the 10^{-1} dilution blank using a fresh sterile pipette. One ml from the 10^{-1} dilution was transferred to the 10^{-2} tube for each succeeding step then from the 10^{-2} to the 10^{-3} , then from the 10^{-3} to the 10^{-4} . From each dilution tube 0.1ml of dilution fluid was transferred into Nutrient Agar culture media and incubated at 37°C for 24 hours. Nutrient Agar (NA) culture media contained 0.5% peptone, 0.3% yeast extract, 0.5% NaCl, 0.25% glucose, 1.5% agar, distilled water and pH was adjusted to 7 at room temperature. After successful growth of microorganisms, the pure cultures of bacteria were sub-cultured in NA slants; incubated at 37°C to achieve vigorous growth and then preserved in 20% glycerol vials at -80°C.

Isolated Bacteria were characterized and identified on the basis of their morphological (cultural, physiological) macroscopically and biochemical tests. The tests include Gram staining, Motility, Glucose test, Oxidase test, Lactose test.

2.9. Evaluation of Metal Tolerance Bacteria and Fungus

Isolated Bacteria and fungus were tested for their tolerance to chromium and copper by nutrition broth and Potato dextrose broth dilution method [10]. For this tests, seven different concentrations viz. 10, 20, 40, 60, 80, 90 and 100ppm of heavy metal salts (copper and chromium) containing nutrient broth medium and Potato dextrose broth medium were prepared and dispensed into the test tubes (10ml/tubes) and sterilized in an autoclave. These modified broths were inoculated with equal amounts of individual selected organism isolated from the industrial effluent and soil by preparing suspension. For comparing the growth response at different concentration, one set of nutrient broth and Potato dextrose broth (without the heavy metal salts) containing test tubes were inoculated with the selected organisms and used as control. Then incubated at 37°C for 2-4 days and observed periodical.

III. Result and Discussion

3.1. Physico-chemical Parameters

Industrial influents, effluents and soil samples collected from four sampling sites at Kombolcha Textile (Site, A), Brewery (Site, B), Abattoir (Site, C), and Modjo textile industries (Site, D), were presented in Tables 1, 2 and 3.

Table 1. Average values of industrial influent and effluent (mg/l).

Parameters	Site A				Site B			
	Influent mg/l	Effluent mg/l	%Efficiency	EPA St.	Influent mg/l	Effluent mg/l	%Efficiency	EPA St.
pH	7.75	7.7	0.645	9-Jun	11	8.5	24.78	9-Jun
TDS	3780	3310	12.4	*1000	11700	8365	28.5	*1000
TOC	22.1	21.6	50	40	32.4	25.3	21.9	40
SS	280	371	32.5	30	829	798	3.74	50
TA	154	107.1	32.5	*400	127	110.9	12.68	*400
COD	823	341.5	58.57	150	2598	2410	7.24	250
SO ₄ ²⁻	0.5	0.02	96	*200	0.05	0.02	60	*200
S ²⁻	8.5	41.3	385.9	2	0.6	1	66.67	*2
NO ₃ ⁻	8.97	1.05	88.3	*50	2.5	2.75	10	*50
TN	40.2	39.3	2.24	40	55.65	43.6	21.8	40
TP	14	11.7	16.44	10	41.6	3.35	91.95	5
Cl-	5982	4983	16.7	*1000	5986	4119	31.19	*1000
Cr	-	0.12		0.1		ND		
Cu	-	0.23		2		ND		

Parameters	Site C				Site D			
	Influent mg/l	Effluent mg/l	%Efficiency	EPA St.	Influent mg/l	Effluent mg/l	%Efficiency	EPA St.
pH	8.06	7.45			7.3	7.3	29.7	9-Jun
TDS	6010	4615	23.2	*1000	7730	5630	32.1	*1000
TOC	25.3	20.5	19	40	32	22.5	30	40
SS	584	578	1.03	80	829	563	17.13	30
TA	448.3	416.3	7.18	*400	467.3	485.6	87.5	*400
COD	2653	1618	39	250	2715	2250	17.02	150
SO ₄ ²⁻	0.8	0.01	98.8	*200	0.4	0.05	83.12	*200
S ²⁻	4	14.8	-2.5	*2	1.6	3.5	-118.8	2
NO ₃ ⁻	4.2	2.95	30.24	*50	4.68	0.79	83	*50

TN	415.7	39.5	90.5	40	30.2	49	50.8	40
T P	26.75	24.35	9	5	9.35	4.6	97.8	10
Cl-	5360	162.5	97	*1000	8152	178.5	27.2	*1000
Cr		ND				0.14		
Cu		1.11				0.27		

([2], [11] and [12])

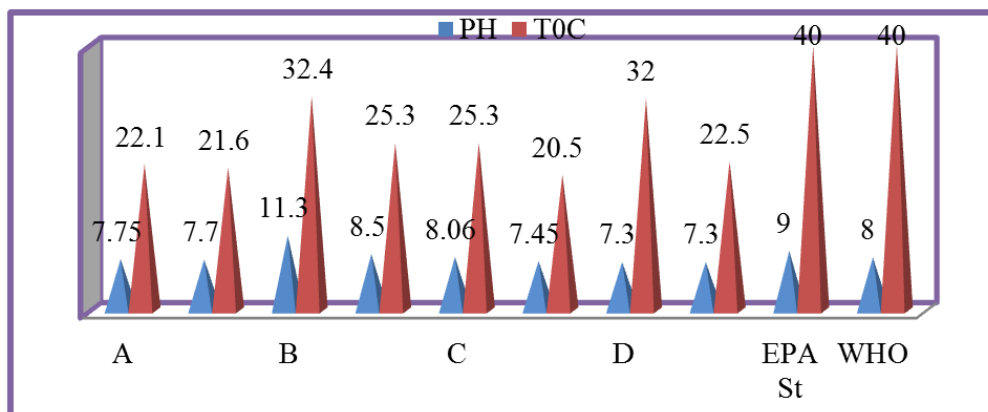


Figure 1. PH and T°C Concentration in different industries

PH: The pH results were shown in Table 1 and figure1 ranged from 7.3-8.5 indicated that the pH of the industries effluents within the permissible limit set by ([2] and [13]) (6-9).

Temperature (T°C): Results in Table 1 and figure 2 showed that the temperature of industrial effluents varied from a minimum of 21.6°C and maximum 32°C. The Temperature of the effluents discharged from the factory ranged within the permissible limit set by ([2]and [13]) (40°C).

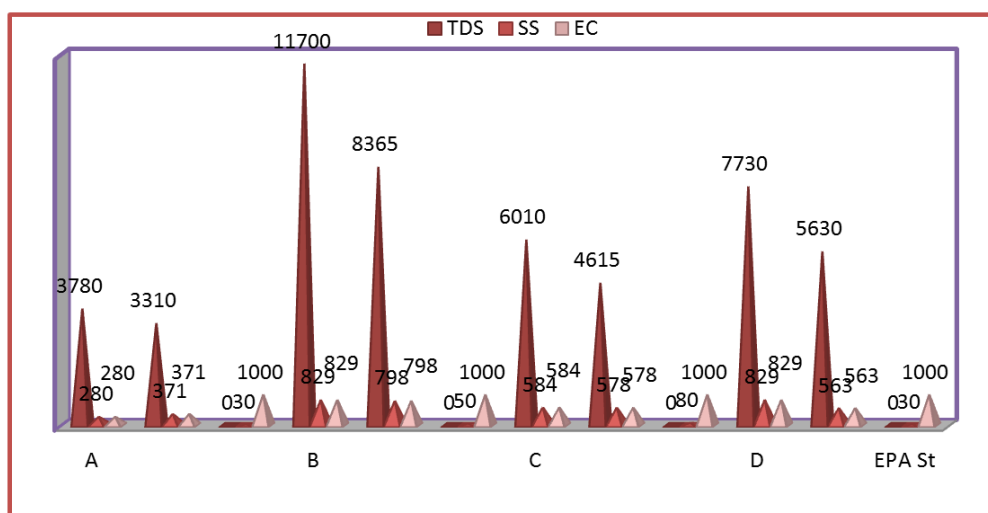


Figure 2. SS, TDS and EC concentration in different industries.

Total suspended Solids (TSS): are solid materials, including organic and inorganic, that are suspended in the water sample. High concentrations of suspended solids can lower water quality by absorbing light, hence causes depletion of oxygen level in the water samples. The values showed in table 1 and figure 2. Total suspended Solid concentration in the industrial waste water currently measured varied between minimum average values of 371mg/L(site A) to a maximum average values of 797.5mg/L (site B). The values at all site above the standard permissible limit. According to reference ([2],[11]and[14]) (100 mg/l)due to the presence of high amount of organic and inorganic matter present in the effluent which is supported by [15].

Total Dissolved Solids (TDS): Total dissolved solids refer to any minerals, metals, cations or anions dissolved in water. The values showed in table 1 and figure 2 total dissolved solid concentration in the industrial effluents varied between a minimum average values of 3310.5mg/L (site A) to a maximum average values of 5650.3mg/L (site B). The values at all site above the standard permissible limit set by ([10] and [11]) (2100mg/l) which may be due to the presence of large amount of organic and inorganic matter present in the effluent which

is supported by [15].

Electrical conductivity (EC): The results of electrical conductivity showed in table 1 and figure.2 minimum average values of 1947 μ S/cm to a maximum average values of 4728 μ S/cm. which was found to be beyond the permissible limits set by [14] (400 μ S/cm). Untreated effluent showed higher level of electrical conductivity which could reflect the presence of dissolved organic and inorganic substances would have increased the conductivity [16].

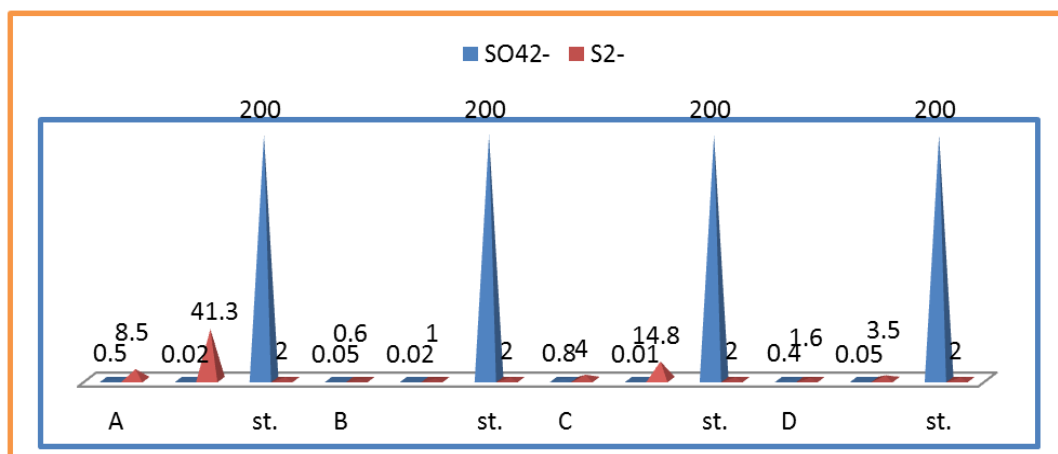


Figure 3. Sulphate and sulphid concentration of different industries.

Sulphate (SO₄²⁻): The results in Table 1 and figure 3. depicted the average value of sulphate ion concentration in the four sites ranged from a minimum of 0.02mg/L (site A) to a maximum of 0.05mg/L (site D). In the entire Sites the values were below the standard limit set b ([2] and [13]). Sulphate is one of the toxic anions and large quantities would have to be ingested in order to health disorder to occur (especially diarrhea type symptoms).

Sulphid S²⁻: The results table 1 and figure 3 showed at different industrial effluents were within the permissible limit set by [2] (2mg/l).

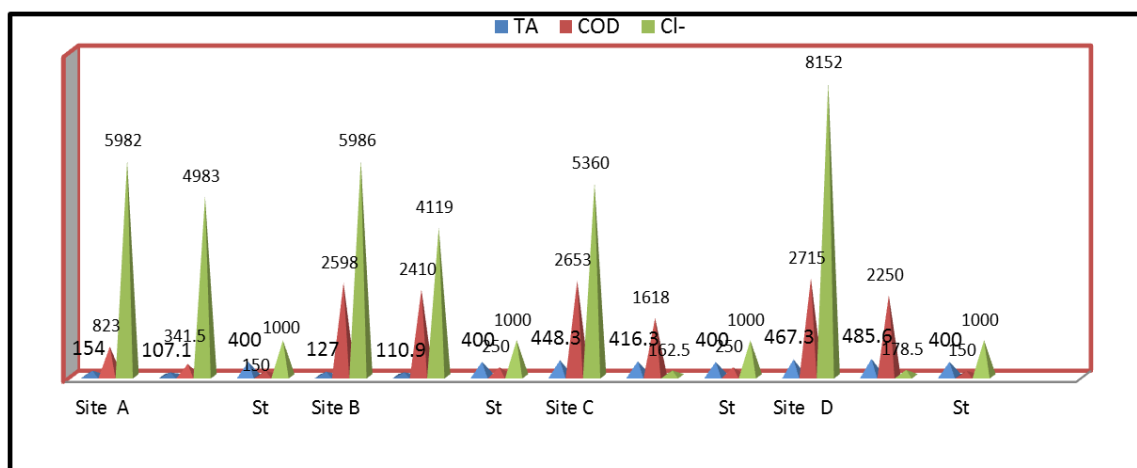


Figure 4. Chloride, alkalinity and COD Concentration in different industries.

Chloride: Chloride levels showed in table 1 and figure 4 ranged from minimum 1780.5 mg/l and maximum 4982.4 mg/l with this all values were higher than the limits set by ([2], [14] and [12])(1000mg/l), 600mg/l) and (250 mg/l) respectively. The high chloride levels recorded could have resulted from the use of calcium hypochlorite Ca(OCl)₂ or sodium hypochlorite (NaClO) as major primary disinfectants in wastewater treatment operations. Also, various concentrations of chlorine solution are employed for sanitation/disinfection and bleaching purposes in industries like textile, breweries, and meat processing companies.

Alkalinity: Alkalinity of water is its acid neutralizing capacity. It is the sum of all the bases. The alkalinities of industrial effluents are due to the salt of carbonates, bicarbonates, and borates silicates and phosphates along with hydroxyl ions in the Free State. However, the major portion of the alkalinity is due to

hydroxides, carbonates and bicarbonates. The results of present study revealed that alkalinity level from each industrial waste given in Table 1 and figure 4.

Chemical oxygen demand (COD): is used as a measure of oxygen requirement of a sample that is susceptible to oxidation by strong chemical oxidant. The COD is a test which is used to measure pollution of domestic and industrial waste. The result showed in Table 1 and figure 4 ranged from a minimum of 2410 mg/l to a maximum of 341.5 mg/l which has exceeded the permissible limit supported by ([2] and [14]) (250 mg/l). COD test is the best method for organic matter estimation and rapid test for the determination of total oxygen demand by organic matter present in the sample.

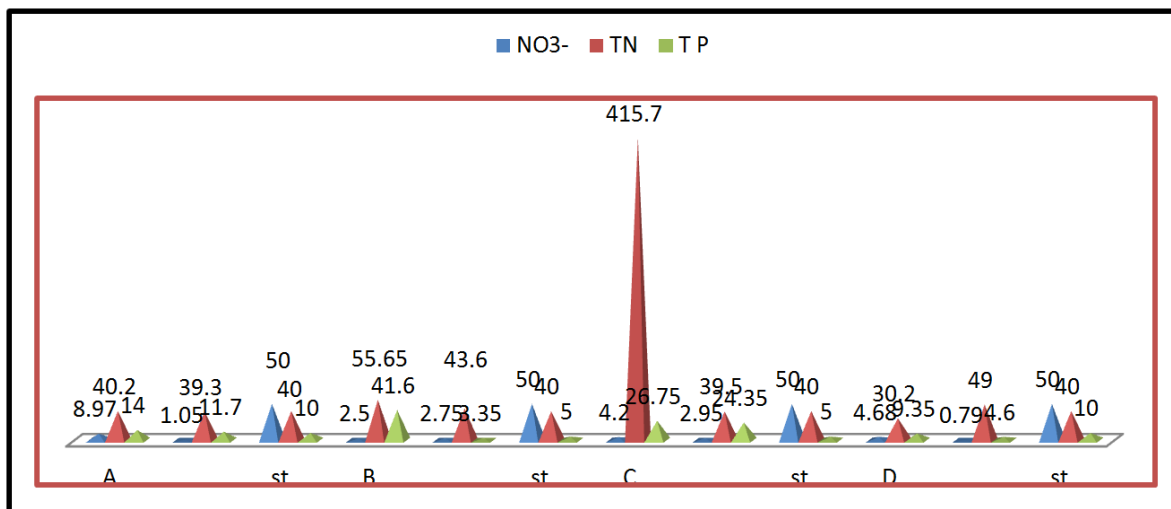


Figure 5. Nitrate, TN and TP concentration in different industries.

Nitrate NO_3^- : The results showed in Table 1 and figure 5 ranged from a minimum of 0.79mg/L (site D) to a maximum of 2.95mg/l (site C). In all sample sites were below permissible limit set by ([13] and [2]) (10 mg/L) and (20mg/L) respectively. Nitrate represents the most oxidized form of nitrogen and the product of oxidation of nitrogenous matters and its concentration may depend on the nitrification and de-nitrification activities of microorganisms.

Total Nitrogen: Total nitrogen levels depicted in table 1 and figure 5 obtained minimum 40.1 mg/l and maximum 49 mg/l within all site values were lower than the limits set by [2] (80mg/l).

Total Phosphate (TP): The Results of TP showed in table 1 and figure 5 in the studied industries effluents ranged from the minimum of 3.35mg/L (site B) to the maximum of 24.35mg/L (site C). In the entire site, the concentration of phosphate were above the maximum limit set by [14] (3mg/L).

The breakdown of phosphorus complexes in detergent wastewater (and other household products, as well as human and industrial wastes that contain phosphate) creates freely available phosphates; these can contribute to an oversupply of phosphate in waterways and cause imbalance of the aquatic ecosystem. This result in excessive algal growth and increasing the number of decomposer organism that requires oxygen, which can deplete the amount of oxygen dissolved in the water. Excessively large number of decomposers may reduce the oxygen levels to the extent that other aquatic organisms die from lack of oxygen.

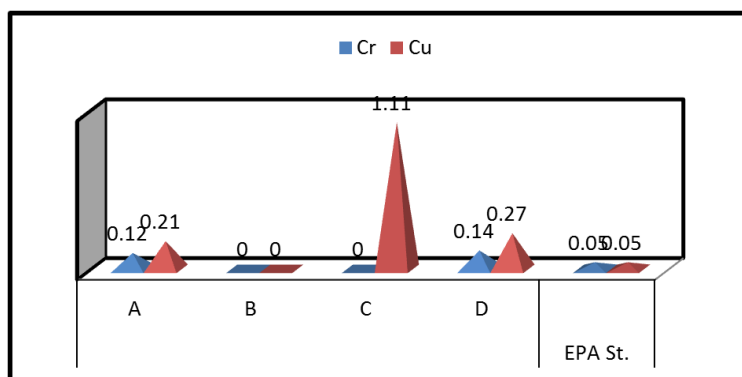


Figure 6. Cr and Cu concentration in different industries effluents.

Chromium (Cr): The results depicted in Table 1 and figure 6. The concentrations of Cr in different industrial effluents were ranged from a minimum of 0.12mg/L (site A) to a maximum of 1.14mg/L (site D). The results of all sampling sites were below the recommended limit set by [2] (0.5mg/l) and above the recommended limit set by [11] (0.1mg/L) guidelines. Chromium causes cancer, dermatological disorders and anemia.

Copper (Cu): The results in Table 1 and figure 6. Showed that concentrations of Cu in different industrial effluents were ranged from a minimum of 0.23mg/L (site A) to a maximum of 1.11mg/L (site D). The results of all sampling sites were below the recommended limit set by [2] (2mg/l) except site B above the recommended limit set by [11] (0.1mg/L) guidelines. Cu is more of essential trace element, but copper in the dissolved form is potentially very toxic to aquatic animals and plants, especially to young life-stages such as fish larvae Soil samples analysis

Table 2. Heavy metals, TP and TN concentration in (mg/Kg) the soil polluted by industrial effluents.

parameter	S ₁	S ₂	S ₃	S ₄	Control	EPAST.2003
Cr	157.25	76.1	17.5	109.3	53.3	100
Cu	649.95	59.3	174.5	132	20.2	100
TP	7032.5	15370	1740	1566	1370.5	-
TN	1.76	3.64	1.83	0.58	0.21	-

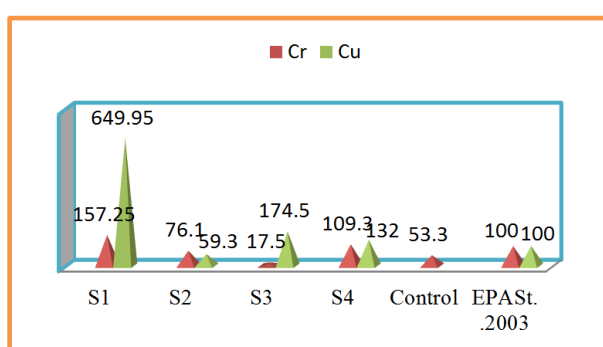


Figure 7. Cr and Cu concentration in soil polluted by industries effluents.

Table 2 and figure 7. The concentration of chromium and copper in the soil polluted by industrial effluents were above the permissible limit set by [11] (100 mg/L) except Cu at brewery industry and Cr at brewery and abattoir industries. Table 1 and figure 8. The concentration of TP and TN in the soil polluted by industrial effluents varies from the minimum of 1566mg/kg (site S₃) to the maximum of 15370 mg/kg (site S₂) and minimum 0.58 mg/kg to maximum 3.64 mg/kg respectively.

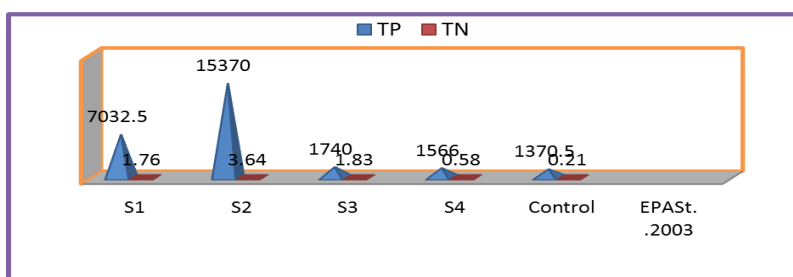


Figure 8. TN and TP concentration in soil polluted by industrial effluents.

3.2. Characterized Isolated Microorganism

Nineteen Bacterial isolated were identified using morphological (cultural, physiological) macroscopically and biochemical tests. Isolated Bacteria's were identified showed some repeats and finally, five bacterial strains were identified as *Streptococcus Sp*, *Staphylococcus Sp*, *Pseudomonas aeruginosa*, *E. coli* and *Salmonella Sp*. In addition, nineteen isolated fungal were identified based on colony morphology and microscopic examination. Some repeats were also detected. Seven fungal strains were identified as two strains of *Aspergillus Niger*, *Aspergillus flavor*, *Aspergillus awamori*, *Aspergillus tamari*, *Cladosporium sphaerospermum* and *Penicillium Sp*.

3.3. Evaluation of Metal Tolerance Bacteria and Fungus

Bacteria isolated were evaluated for growth on minimal salt medium amended with different concentrations of Chromium and Copper salts by flask culture technique. The test was carried out in nutrient

broth(for bacteria) and Potato Dextrose broth (for fungi) supplemented with chromium and copper salt solution in different concentrations range from 10, 20, 40, 60, 80, 90 and 100 ppm showed in Table 5 and 6.

Table 3. Evaluation of heavy metal ($K_2Cr_2O_7$) tolerance Bacteria.

S.N	Control	10	20	40	60	80	90	100
Streptococcus Sp	++++	++++	+++	++	+	+	-	-
Staphylococcus Sp	++++	++++	+++	+	+	-	-	-
Pseudomonas aeruginose	++++	++++	+++	+	+	+	+	-
E. coli	++++	++++	+++	++	+	-	-	-
Salmonella Sp.	++++	++++	+++	++	+	-	-	-

+++ Massively visible ++moderately visible + poor visible - not visible

Table4. Evaluation of heavy metal ($CaCO_4$) tolerance Bacteria.

S.N	Control	10	20	40	60	80	90	100
Streptococcus Sp	++++	+++	++	+	+	-	-	-
Staphylococcus Sp	++++	++++	+++	++	+	+	+	-
Pseudomonas aeruginose	++++	+++	++	+	+	-	-	-
E. coli	++++	++++	+++	++	+	+	+	-
Salmonella Sp.	++++	+++	++	++	+	+	-	-

+++ Massively visible ++moderately visible + poor visible - not visible

Table 3 and 4. Showed different bacteria species were tolerate different heavy metal salt concentration. The bacteria isolated, Streptococcus Sp. was able to tolerate Cr ($K_2Cr_2O_7$) and Cu ($CuSO_4$) up to 80 and 60 ppm respectively. Staphylococcus Sp. was able to tolerate Cr ($K_2Cr_2O_7$) and Cu ($CuSO_4$) up to 60 and 90 ppm. Pseudomonas aeruginosa was able to tolerate Cr ($K_2Cr_2O_7$) and Cu ($CuSO_4$) up to 90 and 60 ppm. E. coli was able to tolerate Cr ($K_2Cr_2O_7$) and Cu ($CuSO_4$) up to 60 and 90 ppm respectively while the isolate salmonella Sp. was able to tolerate Cr ($K_2Cr_2O_7$) and Cu ($CuSO_4$) up to 60 and 80 ppm respectively. Most of the bacterial isolates grew well in the presence of low concentration of the heavy metals, while some bacteria could grow at higher concentrations. A bacterial tolerance to chromate has been found in Pseudomonas strains and copper sulphate has been found in Staphylococcus and E. coli.

Table 5. Evaluation of heavy metal ($K_2Cr_2O_7$) tolerance fungus.

S.N	Control	10	20	40	50	60	70	80	90	100
Aspergillus tamaraii	++	++	++	+	+	-	-	-	-	-
Aspergillus flavus	++	++	++	++	+	+	-	-	-	-
Aspergillus niger	+++	+++	+++	+++	+++	++	++	+	+	-
Aspergillus awamori	++	++	++	+	+	-	-	-	-	-
Aspergillus niger	++	++	++	++	++	++	++	+	-	-
Cladosporium sphaerospermum	++	++	++	++	+	+	-	-	-	-
Penicillium Sp.	+++	+++	++	++	+	+	+	-	-	-

Table 6. Evaluation of heavy metal ($CaCO_4$) tolerance fungus.

S.N	Control	10	20	40	50	60	70	80	90	100
Aspergillus tamaraii	+++	++	++	+	+	+	+	+	-	-
Aspergillus flavus	++	++	++	++	+	+	+	+	-	-
Aspergillus niger	++	++	++	++	++	++	+	+	+	-
Aspergillus awamori	++	++	++	++	+	+	-	-	-	-
Aspergillus niger	+++	+++	+++	++	++	++	++	+	+	-
Cladosporium sphaerospermum	++	++	++	++	+	++	+	+	+	-
Penicillium Sp.	+++	+++	+++	++	++	++	++	+	+	+

+++ Massively visible ++ moderately visible + poor visible - not visible

Tables 5 and 6. Showed different bacteria species were tolerated by different heavy metal salt concentrations. The isolated fungus, *Aspergillus Niger* (C_5 and C_7) were able tolerate Cr ($K_2Cr_2O_7$) up to 90 and 80 ppm and Cu ($CuSO_4$) up to 90 ppm respectively. *Penicillium Sp.* was able to tolerate Cr ($K_2Cr_2O_7$) up to 70 ppm and Cu ($CuSO_4$) up to 100 ppm, *Aspergillus flavus* was able to tolerate Cr ($K_2Cr_2O_7$) up to 60 ppm and Cu ($CuSO_4$) up to 80 ppm, *Aspergillus tamaraii* was able to tolerate Cr ($K_2Cr_2O_7$) and Cu ($CuSO_4$) up to 50 ppm and 80 ppm respectively. The isolate *Aspergillus awamori* was found to tolerate Cr ($K_2Cr_2O_7$) up to 50 ppm and Cu ($CuSO_4$) up to 70 ppm, *Cladosporium sphaerospermum* was able to tolerate Cr ($K_2Cr_2O_7$) up to 60 ppm and Cu ($CuSO_4$) up to 90 ppm.

IV. Conclusion

In this study the physicochemical parameters pH (7.3-8.5), Temperature (21.6-32°C), TA (107-485mg/l), SO_4^{2-} (0.02-0.05 mg/l), NO_3^- (0.79-2.95 mg/l), TN (40.1-49mg/l), were below the permissible limit and TDS 3310 -5650mg/l , COD (2140 -341.5mg/l , TP (3.35-24.75 mg/l), SS (371-797 mg/l), S^{2-} (1.00-41.5mg/l), Cl (162.5-4982.4 mg/l) were above permissible limits.

Among the fungus strains isolated from wastewater and soil, *Aspergillus Niger* species was more tolerance of chromium salt at 90 ppm while *Penicillium Sp.* showed more tolerance of copper salt 100ppm and Among the Bactria strains *Pseudomonas aeruginosa* species was more tolerance of chromium salt at 90 ppm and *Staphylococcus Sp.* and *E. coli* were more tolerance of copper salt at 90 ppm. These Isolates were the most tolerance of metals tested.

References

- [1]. Tilt, B., 2013. Industrial pollution and environmental health in rural China: Risk, uncertainty and individualization. *China Q.*, 214: 283-30.
- [2]. EPA, (2003), Standard for the use or disposal of sewage sludge, pollutants limit, Washington DC: US. Environmental Protection13http://www.epa.gov/epahome/cfr40.htm. june,06, 2000.
- [3]. Zinabu Gebre-Mariam and Zerihun Desta. (2002). The chemical composition of the effluent from Awassa Textile factory and its effects on aquatic biota. *SINET: Ethiop. J. Sci.*,25(2): 263-274.
- [4]. Papatilipaki, A.; Kotti, M. And Stavroulakis, G. (2008). Seasonal variations in dissolved heavy metals in the Keritis River Chania, Greece. *Global Nest Journal*; 3:320-325.
- [5]. Fong.; Du, P. X.; Cao, J. J.; Posmentier, E. S. Multivariate analysis of heavy metal contamination in urban dusts of Xi'an, Central China. *Sci. Total Environ.* 2008, 355,176–186.
- [6]. Qishlaqi, A. And Moore, F.(2007). Statistical analysis of accumulation and sources of heavy metals occurrence in agricultural soils of Khoshk River Banks, Shiraz, Iran. *American –Eurasian Journal of Agriculture and Environment Science*; 2:565-573.
- [7]. Rodier, J. An Analysis of Water, Natural Water, Waste Water, Sea Water: Chemistry, Bacteriology, Biology. Dunod. (1975).
- [8]. Chen L, Xiao X, Luo S, Zeng G, Wei W, Wan Y. Biosorption of cadmium by endophytic fungus *Microsphaeropsis* sp. Isolated from cadmium hyperaccumulator *Solanum nigrum* *Bioresource Technol* (2011b).
- [9]. APHA, (2005). Standard methods for the examination of water and wastewater, 21st Ed. American Public Health Association. Washington, DC.
- [10]. Holt, J. G., Krig, N.R., Sneath, P. H. A., Staley, J. T. and Williams, S.T. (1994). *Bergey's manual Of Determinative bacteriology* (9th edn). Baltimore, Maryland: Williams and Wilki.
- [11]. WHO. 2001. Food additives and contaminants. Joint Codex Alimentarius Commission, FAO/WHO Food standards Programme.
- [12]. World Health Organization (2002) Environment Protection Act. Standards for effluent discharge regulations. General Notice No 44, Geneva, Switzerland.
- [13]. CPCB (1995) Pollution Control: Acts, rules and modifications. Central Pollution Control Board, New Delhi.
- [14]. U.S.EPA (2003). US Environmental Protection Agency, safety drinking water Ac, EPA 816-F-03-016.
- [15]. Aftab, S.Y. and Noorjahan, CM. 2006. Biodegradation of fertilizer effluent. *Asian. J. Kalita B, Bhuyan K C & Sharma D K* (2003), Physico-chemical qualities of effluents discharged from nagaon paper mill in Assam, *J Ecotoxicology Environ Monit.* 1, 77-79.
- [16]. S.R. Mishra, and D.N. Saksena, "Planktonic fauna in relation to physico-chemical characteristics of Gauri Tank at Bhand, M. P. India," *Advanus in limnology* Narendra Publishing house, New Delhi, pp. 57-61, 1993.

Tesfalem Belay Woldeamanuale. "Characterization the Composition of Industrial Effluents and Isolate Potential Microorganisms for the Possible Application." *IOSR Journal of Environmental Science, Toxicology and Food Technology* (IOSR-JESTFT), 15(1), (2021): pp 50-58.