EFFECT OF EFFLUENT ON BACTERIA COMPARING WITH TAP WATER

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Effluent is discharged from the industrial activities. They consist of water and varieties of hazardous substances (Odigure, 1995). Effluents contain appreciable amount of metallic cations like zinc, copper, iron, manganese, lead, nickel and cadmium (Singh and chandel, 1993). Small amounts of these elements are common in our environment and are actually necessary for good health, but large amount of any of them may cause acute or chronic toxicity. Contamination of wastewater with high rates of heavy metals caused a significant decrease in the number of bacteria in biological system. It is obvious that heavy metals are very danger contaminant in wastewater and disorder of biological wastewater treatment is as a result of this pollution (Sa'idi, 2010). (Pereira *et al.*, 2005) reviewed that heavy metals adversely influenced growth of microorganisms and have dangerous effect on biological treatment, because they have toxic effect on microorganisms, eg. bacteria. Most metal ions have to enter the bacterial cell in order to have physiological or toxic effect. Some microbial species subject heavy metal ions to enzymatic reduction, the reduced from of heavy metal are ions quite insoluble and they precipitate out of solution (Nies, 1999). Microbes can remove heavy metals from contaminated solutions either by intracellular accumulation, sorption or complex formation on cell surface and extra cellular accumulation or precipitation (Kujan *et al.*, 2005). The ability of microbial strains to grow in the presence of heavy metals would be helpful in the wastewater treatment where microorganisms are directly involved in the decomposition of organic matter in biological processes for wastewater treatment, because often the inhibitory effect of heavy metals is a common phenomenon that occurs in treatment of the biological wastewater and sewage (Filali *et al.*, 2000).

Bacterial species are tested for heavy removal and it is found that *Serratia marcescens* might play a role in the biological cycle of metals by mineralizing organic iron and dissolving gold and copper (Eberl *et al.*, 1999). The *R. leguminosarum* bv. *viciae* isolate, STDF-Egypt19, can be useful as an inexpensive and efficient bioremediation technology to remove and recover heavy metal ions from an aqueous solution (Abd-Alla *et al.*, 2012).

Azotobacter vinelandii was better than either *Derxia gummosa* or *Rhizobium trifolii* for sorption of UO_2^{2+}, *A. vinelandii* showed the highest sorption capacity in the early stationary phase. The binding of UO_2^{2+}, Cu^{2+}, Ca^{2+} and Zn^{2+} was affected by the pH of the solution. With HCl as eluent, virtually all the sorbed UO_2^{2+} was released. The presence of Cu^{2+}, Cd^{2+}, Ca^{2+}, and Zn^{2+} inhibited the UO_2^{2+} biosorption whereas Mg^{2+} and K^+ had no effect (Cotoras *et al.*, 1992).

The number of microorganisms depends on the total content and concentrations of particular forms of heavy metals. On the other hand, it is conditioned by several other factors, quantity and quality of organic matter, especially carbohydrate rich organic matter, pH, total exchange capacity, nutrient availability, moisture, temperature and oxygen availability. Heavy metals shift (Sa'idi, 2010).

Structure of microbial populations, impoverish their diversity, and affect species composition reproduction and activity of indigenous microorganisms. Contamination of wastewater with high rates of heavy metals caused a significant decrease in the numbers of bacteria in biological system. The studies for large scale applications demonstrated that passive uptake are more applicable than the active processes because living systems (active uptake) often require the addition of nutrients and hence increase the values biological oxygen demand (BOD) or chemical oxygen microbial population is difficult due to metal toxicity and other unsuitable. Passive uptakes are sensitive to ambient conditions such as pH, ionic strength and the presence of organic and inorganic ligands (Malik, 2004).

Total viable counts of both heterotrophic and *Azotobacter* spp. have been decreased with increased Cd^{2+} concentrations (Durga *et al.*, 2012).

The bacteria *Bacillus sp., Serratia sp. Pseudomonas sp.* and *Staphylococcus sp.* were isolated from industrial effluents (Prassad and Manjuath, 2012).

The capacitity of *Bacillus circulans* isolated from industrial effluent to grow and concentrate iron from the different concentrations of iron and manganese containing medium. Maximum accumulation of metal was observed when cells are grown in media containing 4.0 mM iron and manganese. The cell membrane profile of the cells grown in metal free medium showed the presence of twelve different proteins. The cells total protein expression was reduced by the stress of iron and iron and manganese grown in iron, iron and manganese induced the production of same type of proteins, indicating the role of these proteins in bioaccumulation of iron

So can consider *Bacillus circulans* in Biological filter systems for removal of iron and manganese present in industrial effluents and wastewater (Pavani *et al.*, 2011). Many bacteria have developed means of resistance to the toxic metal ions and this is one factor that makes microbial cells special organisms, the bacterial cells acquire a gene or genes which normally code for proteins and enzymes that perform specific functions either to protect the bacterial cell, or block or alter the incoming toxic metal, or both. Heavy metals uptakes are sensitive to ambient conditions such as pH, ionic strength and the presence of organic and inorganic ligands.

This work aims to study the distribution of a number of trace metals (Zn, Cd, Pb, Fe, Mn and Cu) in effluent from Alexandria and Sadat City to determine the effect of different types and concentrations of effluent on the bacterial growth. Investigate the bioremediation potentials of the bacteria in metal in effluent. The effects of different types and concentration of effluent on SOD enzyme and protein profiles in bacteria.

MATERIAL AND METHOD

Sample of effluent

Effluent samples were collected from oxidation ponds of Sadat City and Alexandria City (waste treatment agamy region) Egypt. The collected samples were transferred to a sterile glass container and maintained at 4°C for further studies (Apha, 1998). Metals concentrations were analyzed using Atomic Absorption Spectrophotometer (AAS).

Bacteria, media and growth conditions

Bacteria used were *Rhizobium leguminosarum* 301, *Azotobacter chrocoocum* 184, *Serratia marcescns* 8000 and *Bacillus circulans* 251. Media used were nutrient agar (NA) medium was prepared by beef extract 3 g., Peptone 3 g. Agar 20 g. Distilled water up to 1000 ml. pH adjusted to 7. The medium was sterilized by autoclaving at 120°C for 20 minutes. Plates were incubated at 37°C. Nutrient Broth (NB) medium was as previous without agar.

Effects of different effluent concentration on bacterial growth

The effects of different effluent concentration on bacterial growth were determined by growing the bacteria on concentrations (0, 20, 40, 60, 80 and 100%) of effluent. Plates were spread with the same value (1ml) of cultures of appropriate organism and incubated at 37°C for 24 hours, after that colony forming units were counted. This investigation was carried for three times.

Removal of heavy metal from effluent by bacterial used

The metal were removed from effluent by inoculated 100 µl of bacterial cells in Erlenmeyer flasks containing 200 ml of sterile nutrient broth medium supplemented with effluent instead of distilled water. The flasks were kept under a rotary shaker at 100 rpm for 24 hours at 37°C, cells were separated from the medium by centrifugation (14000 rpm for 15 minutes) followed filtration using 20µ pore filter. The residual metals concentrations were determined by Atomic Absorption Spectrophotometer (AAS).

Polyacrylamide gel electrophoresis of protein

The bacteria were grown in effluent by inoculated 100 µl of bacterial cells in Erlenmeyer flasks containing 200 ml of sterile nutrient broth medium supplemented with effluent instead of distilled water on concentrations (20, 40, 60, 80 and 100%) comparing with tap water from Alexandria (control). The flasks were kept under a rotary shaker at 100 rpm for 24 hours at 37°C; cells were separated from the medium by centrifugation (14000 rpm for 15 minutes). The bacterial protein extraction and electrophoresis were carried out according to Laemmli (1970).

Super Oxide Dismutases (SOD) isozymes activity

Crude extract of *Rhizobium leguminosarum, Azotobacter chrocoocum, Serratia marcescns* and *Bacillus circulans* was used for SOD activity, 200 µl of 0.05 M potassium phosphate at pH 7.8 added for each sample and sonicated 5 times for 20 Seconds at 40 pluses with Sonicator to obtain crude extract followed by centrifugation at 20,000 xg for 10 min to remove particulate matter was separated on 10% nondenaturing polyacrylamid and visualized as achromatic bands by staining with nitro blue tetrazolium choloride according to (Beauchamp and Fridovich, 1971). The bacteria were grown in effluent from Alexandria and Sadat City comparing with control (tap water).

Statistical analysis

All results were the mean of three replicates ± SE. Data analysis were performed using SPSS16.0.

RESULTS AND DISCUSSION

Measurement of Physicochemical

Samples of effluent were taken from oxidation ponds of Sadat City and Alexandria and assessed for various physicochemical parameters like pH, Electrical conductivity (EC), concentrations of salts, osmotic pressure, BOD and COD were listed in Table (1). The pH was 7.78 and 7.58, respectively compared with tap water (control) pH from Sadat City and Alexandria 7.4-7.2, respectively Table (2).

It is denoted that wastewater are suitable for biological treatments and also within the World Health Organization (WHO) (1996) tolerance limits of 6.0-9.0 for the discharged of wastewater. Electrical conductivity is widely used to indicate the total ionized constituents of water. It is directly related to the sum of the cations or anions, E. C was higher than WHO standard value. Concentration of solids mg /L in Alexandria and Sadat City were 1753.6 and 2899.2, respectively. The total suspended solids (TSS) acceptable WHO (1996) limits 100 mg/L (Metcalf and Eddy, 2003). The parameters of BOD and COD were widely used to characterize the organic and chemical matter content of wastewater (Puangrat and Nattapol, 2010). Table (1) demonstrated the concentrations of BOD and COD which obtained from the studied area, Sadat City 65 and 135 mg O_2/L, Alexandria 35 and 94 mg O_2/L, respectively. The average of BOD and COD concentration of domestic wastewater with regard to its pollution strength are in the ranges of 110-350 and

250-800 mg O_2/L, respectively (Metcalf and Eddy, 2003). The COD/BOD ratio was in the range of 2.8-2.07, therefore the organic matters in Sadat City and Alexandria effluent has low biodegradability in comparison with domestic wastewater (ratio1.5) and unacceptable value with WHO (1996) limits BOD 30 and COD 60 mg O_2/L) (WHO, 1996).

Determination of metals concentrations in effluent

The concentrations of metals in the effluent samples was determined by Atomic Absorption Spectrophotometer (AAS). The metals Pb, Cd, Cu, Fe, Mn and Zn. The trend of metals in effluent of Alexandria was Zn > Fe > Pb > Cu > Mn > Cd. The concentration of Pb is higher in Alexandria than Sadat City whereas the highest amount of Zn > Fe > Cu > Pb > Mn and Cd are represent in Sadat City than Alexandria, Table (3). The high concentration (mg/L) of metals in in Alexandria and Sadat City were Zn (1.3 and 1.4), Fe (0.75 and 0.83), Cu (0.67and 0.79), Pb (0.7 and 0.65), respectively.

Effect of different sources and concentrations of effluent on bacterial growth

Figure (1) showed bacterial growth in the presence of different concentrations of effluent from two different sources Alexandria and Sadat City. The results indicated that effluent from Sadat City was more toxic to *Rhizobium leguminosarum* , *Azotobacter chrocoocum and Serratia marcescns* than effluent from Alexandria and no significant difference (P=0.983) in case of *Bacillus circulans*. The bacteria grown at different concentrations of effluent (20, 40, 60, 80 and 100%) compared with (control) tap water (0% effluent) was decreased, respectively. This might due to the effluent of Sadat City is highly contaminated than Alexandria, according to Tables (1 and 2). Many studies showed that the amount of metal ion accumulated per unit of a cell mass is directly proportional to the concentrations of the metal ion in a solution (Omar, 2002). (Pons and Fuste, 1993) reported that the high biosorbents concentrations are known to cause cell agglomeration and consequent reduction in the inter-celllar distance. High concentration of heavy metal ions within the microbial cells are very toxic, microorganisms have been forced to develop metal-ion homeostasis factors or metal resistance determinants (Nies and Silver, 1995).

The presence of manganese with iron together was found to increase the growth of *Bacillus circulans* as compared to the growth in the media containing iron alone. Noticed that the after reaching a certain limit, the growth of the microorganism was decreased. This indicates that after a certain saturation level the heavy metals becomes toxic to the organism and thus growth was decreased. The amount of zinc accumulation in *Pseudomonas putida* increased in the medium but was stabilized at 2.0 mM. The possibility cannot be ruled out that the bacteria adapt it to survive high concentrations of metals by decreasing metal transport into cells (Pavani *et al*., 2011).

Removal of metal from effluent by bacteria

The percentage reduction of heavy metal concentrations in the effluent sample by growing the bacteria in sterile nutrient broth media supplemented with effluent Fig. (2), *Rhizobium leguminosarum* indicated the highest removal in Cu (44% and 39%) and the lowest removal in Zn (15% and 14%). When applied *Azotobacter chrocoocum*, the highest removal was detected percentage of metals in Cu (42% and 50%) and the lowest removal in Zn (23% and 12%). When *Serratia marcescns* inoculated showed the highest heavy metal removal in Cu (50% and 50%) and the lowest removal in Mn (20% and 16%). Cultivation of *Bacillus circulans* indicate the highest removal of heavy metals were (Fe 36% and Cu 46%) whereas and the lowest removal Pb were (22% and 12%) from Alexandria and Sadat City, respectively. Rani *et al.* (2010) reported that *Bacillus sp.*, *Pseudomonas sp.* and *Micrococcus sp.* were identified as efficient strains that were resistant to Cu, Cd and Pb and also showed maximal growth of biomass in the presence of Cu, Cd and Pb at level of 3, 2 and 4%, respectively. Salehizadeh and Shojaosadati (2003) reported that *exopolysaccharide produced* by *Bacillus firmus* able to remove Cu, Pb and Zn from aqueous solutions, and *Serratia sp*, able to absorb Cd (Valdman *et al.,* 2005). (Hongwei *et al*., 2011) reported that alginates are salts of alginic acid, which is a linear heteropolysacchride, may be found in various soil bacteria species such as *Azotobacter chrocoocum* and *Azotobacter vinelandii* in wastewater treatment, alginates could play an important role in removal heavy metal ions. High intercellular carbohydrates and large cell inclusions increase the resistance of *Rhizobium leguminosarum* to Cd, Cu, Ni, and Zn, whilst production of thiols has also been shown to counter heavy metal induced oxidation. Thiols bind to metal ions, forming a complex and preventing any cell damage by inactivating the ions redox potential and have been shown to be against Cd, Au, Hg, and Pb toxicity.

Effect of effluent in protein profiles

The SDS- PAGE of protein patterns of *Rhizobium leguminosarum, Azotobacter chrocoocum, Serratia marcescns* and *Bacillus circulans* are shown in Fig. (3). In comparison to the control a number of protein bands were obtained different when bacteria grown in effluent. (Saxena *et al*., 1996) reported that any stress on bacteria results in adaptive responses that lead to changes in the regular metabolic process in the cell, which are then reflected in the alteration of the protein profiles. It is generally assumed that the increases in gene expression for protein profiles reflect a positive attempt of cell to adjust to the new environmental conditions, whereas the decreases of gene expression, in special if they occurred in a high number of proteins, is indicative of disruption of cellular metabolism, a higher in gene expression of protein was usually related to tolerance mechanisms in *Rhizobium*. *Rhizobium* is a sensitive species that can help to predict the impact of heavy metals on agricultural soil submitted to contamination (Pereira *et al.*, 2005).

Super Oxide Dismutases (SOD) isozyme activity

Figure (4) SOD gel electrophoresis activity the blue dye color was absent at sites of O_2 scavangening i.e at site of SOD isozymes activity. In comparison to the tap water (control) a number of bands controlled by genes were obtained that represents enhanced enzyme activity and variation in different type of effluent. Organisms have developed several protective to scavenge reactive oxygen species (ROS). The effects of heavy metals generating antioxidative defense systems (i.e. superoxide dismutase, ascorbat peroxydase, glutathione reductase and catalase) (Okamoto *et al.*, 2001). Microorganisms produce detoxifying enzymes such as SODs and catalases enzymes to protect themselves from highly toxic redicals (Daz *et al*., 2006).

CONCLUSIONS

The results of this study revealed that effluent caused a significant decrease in the numbers of bacteria used in this study. All the bacteria used in this study can remove metals and the most effective in the removal Cu. Different sources of effluent were effective in the protein banding pattern. The variation of the SOD isozymes activity from the investigated bacteria were increased under effluent stress.

SUMMARY

Water is important for all the living things but nowadays, it has been polluted with inorganic contaminants which are discharged from industries. This study was carried out to evaluate the potential of *Rhizobium leguminosarum, Azotobacter chrocoocum, Serratia marcescns* and *Bacillus circulans* to survive in effluent obtained from Alexandria and Sadat City and the ability of these bacteria to remove metals from effluent. Samples of effluent were assessed for various physicochemical parameters like pH, Electrical conductivity (EC), concentration of salts, osmotic pressure, biological oxygen demand (BOD) (35 and 65), chemical oxygen demand (COD) (94 and 135) in Alexandria and Sadat City, respectively. The pH was 7.58 and 7.78, respectively compared with tap water (control) pH from Sadat City and Alexandria 7.4-7.2, respectively. It was found that different the concentrations of media containing effluent obtained from Alexandria and Sadat City having negative effect on bacterial growth and the more effective of Sadat City effluent, except *Bacillus circulans* had the same trend. The examined bacteria were capable of removing metals from different sources of effluent (Alexandria and Sadat City), Cu^{++} ions was highest removed by *Rhizobium leguminosarum* (44% and 39%), *Azotobacter chrocoocum* (42% and 50%), *Serratia marcescns (*50% and 50%) and *Bacillus circulans* (36% and 46%, respectively). The SDS- PAGE profiles of the extracted protein showed change in gene expression for protein profiles com-

paring with control. The incubation these bacteria in effluent increase superoxide dismutase (SOD) isozyme activity.

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Table (1): Characteristics of effluent used for bacterial growth.

Parameter	Effluent source	
	Alexandria	Sadat City
pH	7.58	7.78
Electrical conductivity (ms)	2.74	4.53
Concentration of salts in effluent mg /L	1753.6	2899.2
Osmotic pressure%	98.64%	163.08%
BOD mg O_2/L	35.00	65.00
COD mg O_2/L	94.00	135.00
COD/BOD ratio	2.68	2.07

Table (2): Characteristics of tap water from Alexandria comparing and Sadat City.

Parameter	Tap water source	
	Alexandria	Sadat City
pH	7.20	7.40
Electrical conductivity(ms)	1.45	2.04
Total dissolved solids mg /L	928.00	1305.6
Osmotic pressure %	52.20%	73.44%

Table (3): Metal contents in the effluent samples.

Sample	Pb mg/L	Cd mg/L	Cu mg/L	Fe mg/L	Mn mg/L	Zn mg/L
Alexandria	0.70±0.028	0.50±0.017	0.70±0.017	0.75±0.040	0.53±0.026	1.3±0.014
Sadat City	0.65±0.020	0.54±0.008	0.79±0.020	0.83±0.013	0.59±0.017	1.4±0.031

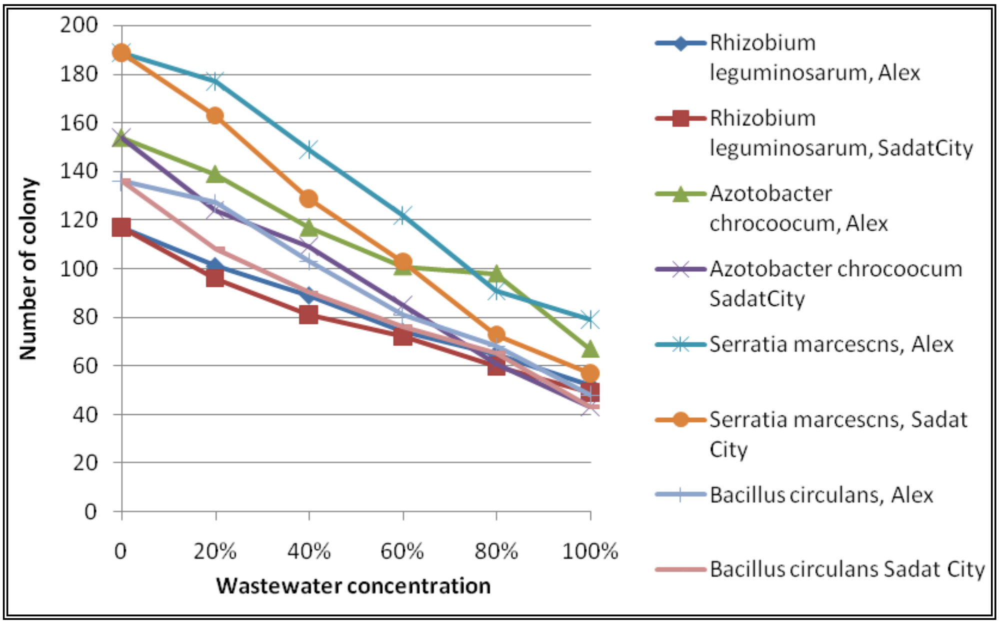

Fig. (1): Effect of wastewater concentration on bacterial growth.

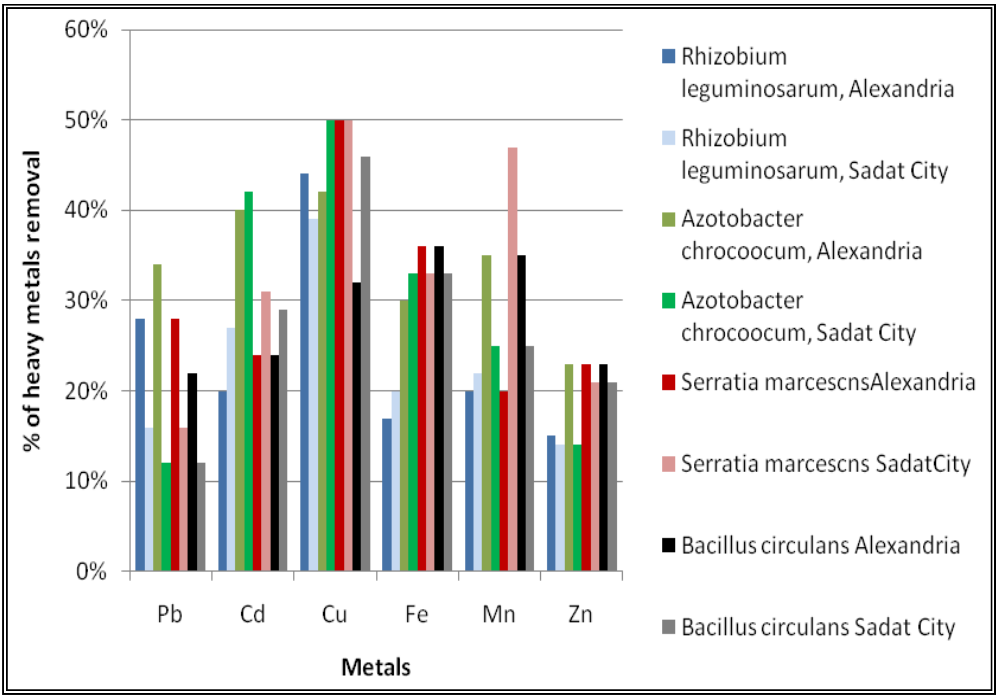

Fig. (2): Percentage removal of metals by bacteria.

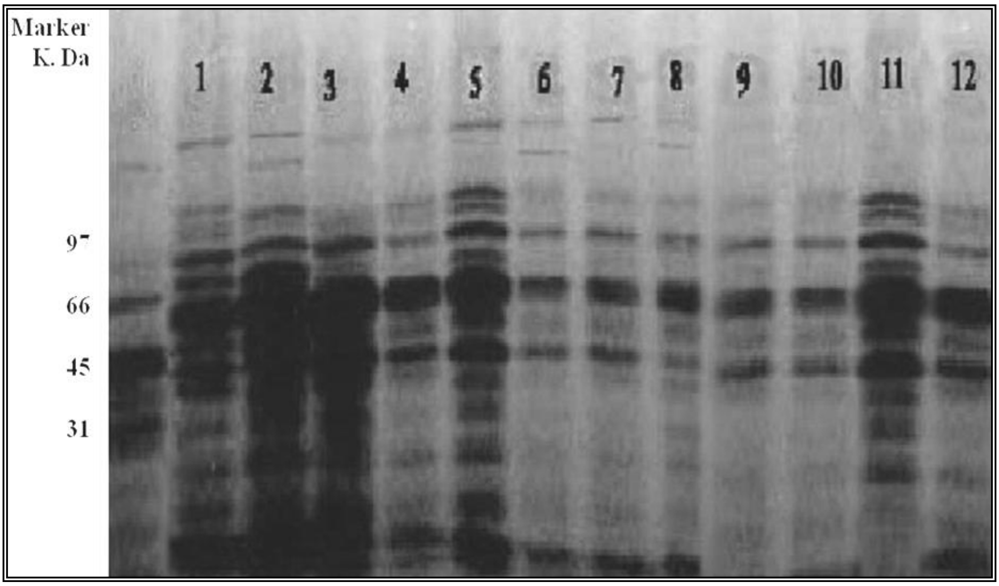

Fig. (3): The SDS- PAGE of protein patterns, lanes1, 2, 3 and 4 bacteria were treated with effluent of Sadat City, 5, 6, 7 and 8 represent that bacteria were treated with effluent of Alexandria and 9, 10, 11 and 12 represent control of *Rhizobium leguminosarum, Azotobacter chrocoocum, Serratia marcescns* and *Bacillus circulans*, respectively.

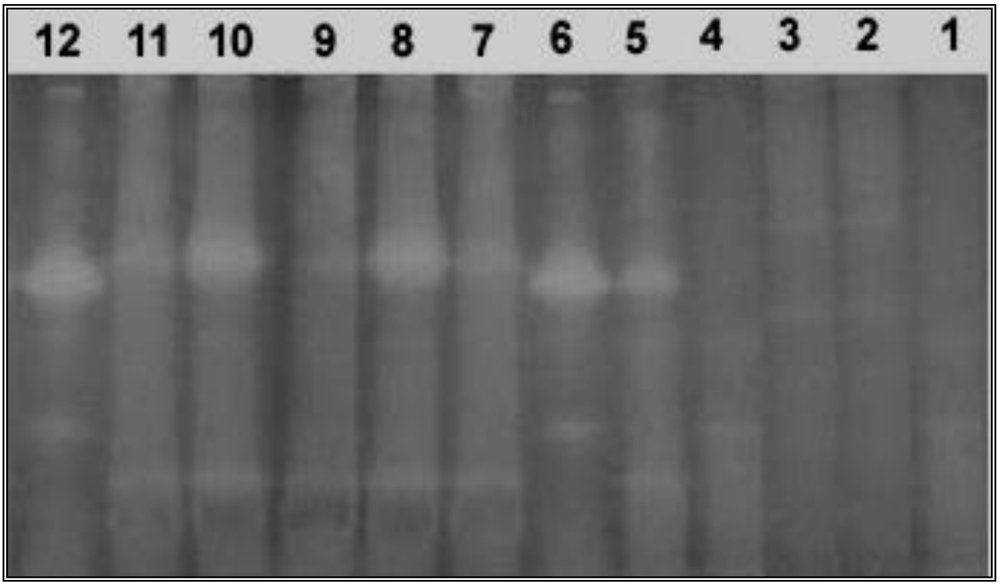

Fig. (4): SOD polyacrylamid gel electrophoresis activity demonstrating, lanes1, 2, 3 and 4 represent control, 5, 6, 7 and 8 represent that bacteria were treated with effluent of Alexandria and 9, 10, 11 and 12 represent that bacteria were treated with effluent of Sadat City of *Rhizobium leguminosarum, Azotobacter chrocoocum, Serratia marcescns* and *Bacillus circulans*, respectively.