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Heavy Metals and Antibiotics Resistance of Halophilic Bacteria Isolated from Different Areas in Red Sea, Egypt.

Khalid A. Ali AbdelRahim[1,*], El-Sayed Mohamed Soltan [1,2], Magdy A. Abu-Garbia[1], and Fathia El-Zien[1]

1- Department of Botany, Faculty of Science, Sohag University, 82524 Sohag, Egypt
2- Department of Botany, College of Science, Al-Baha University, Saudi Arabia
E. Mail: khalidfp7@gmail.com

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ABSTRACT

Hundred and thirty eight (138) bacteria were isolated from 3 different areas on Red Sea governorate, Egypt. Among these 138 moderately halophilic isolates, 2.17%, 3.6%, 6.5%, 9.4%, and 7.2% of the isolates were resistant up to 5mM Cd^{2+} and Zn^{2+}, 20 mM for Cu^{2+}, Pb^{2+}, and Co^{2+}, respectively. The majority of halophilic bacterial isolates showed resistance to more than 10 mM to these metals except Cd^{2+}. All strains were resistant to lead, whereas 50% only of the strains were resistant to cadmium. The resistance to (Cd^{2+} + Cu^{2+}); (Zn^{2+} + Cu^{2+}) showed the highest values between bacteria (6.5%), whereas 4.34% of the isolated strains were resistant to (Cd^{2+}, Zn^{2+}) and (Zn^{2+} + Pb^{2+}), but 2.17% of the isolates were resistant to 3 metals (Cd^{2+}, Zn^{2+}, Cu^{2+}); (Zn^{2+}, Cu^{2+}, Co^{2}); (Cu^{2+}, Co^{2+}, Pb^{2+}) and resistant to 4 metals (Zn^{2+}, Cu^{2+}, Co^{2+}, Pb^{2+}); (Cd^{2+}, Zn^{2+} Cu^{2+}, Co^{2}); (Cd^{2},Zn^{2+}, Cu^{2+}, Pb^{2+}); (Zn^{2+}, Pb^{2+} ,Cu^{2+}, Co^{2}). No bacterial isolates were resistant to the 5 metals combined. The isolated halophilic bacteria exhibited sharp peaks of resistance to drugs such as: Velosef 95%, Cceftriaxone, 91.66%, Ampicillin 83.33 %, Nitrofurantion 81.66%, Tarivid 80%, Cephalothin 73.33%, Cephalexin 66.66%, Cefadroxil 83.33%, and Flucloxacillin 85% whereas few isolates (6.6%) were resistant to Vancomycin.

INTRODUCTION

Microorganisms which vary greatly in their salt tolerance can be classified into different categories according to the basis of their optimal growth rates. According to the levels of salinity that can grow in its presence, bacteria can be classified to: non halophiles, which grow best in media containing less than 1% NaCl; slight halophiles (mesophiles) which grow in media containing 1-3% NaCl (include the marine bacteria); moderate halophiles (halotolerant) which grow in media with 3-15% NaCl con.; borderline extreme halophiles that grow best in media containing 9-23% NaCl and extreme halophiles that grow in media up to 32% NaCl (Baxter and Gibbons, 1956; Marquez *et al*., 1987).

Halophilic microorganisms can be classified into two main groups, the first group is extremely halophilic archeae which constitute a very heterogeneous halophilic microorganism and can grow best in the media containing up to 32 % NaCl.

The second group is the moderately halophilic bacteria which can grow in the media containing up to 3% NaCl (Galinski and Tindall 1992).

Many studies were conducted on halophilic bacteria over the last years. Halotolerant and moderately halophilic eubacteria can adapt to wider ranges of salt concentrations (Vreeland *et al*., 1983).

In most cases, a minimum concentration of Na is essential for growth. This may be due in part to the requirement for Na gradients to drive transport processes in the cell membrane. Certain species may also possess a primary respiration–driven outward sodium pump. Addition of high concentrations of compounds such as glucose or glycerol lowered the NaCl requirement to 0.3 M, but no further lowering of the sodium concentration required was achieved (Adams., *et al* 1987).

Studying the interactions between heavy metals and microorganisms has been specially focused on bacterial transformation and conversion of metallic ions by reduction in different polluted environments (Chang and Waltho., 1993), the selection of metal-resistant microorganisms from polluted environments (Hiroki, 1994), and the use of resistant microorganisms as indicators of potential toxicity to other forms of life as well as on mechanisms, determinants, and genetic transfer of microbial metal-resistance (De Rore *et al*., 1987).

Microbial metal resistance mechanisms include precipitation of metals as phosphates, carbonates, and sulfides; metal volatilization by methyl or ethyl group addition; physical exclusion by electronegative components in membranes and extracellular polymeric substances (EPS); energy dependent metal efflux systems; and intracellular sequestration with low molecular weight, Cysteine-rich proteins (Hughes and Poole, 1989; Gadd, 1990; Silver, 1998).

In general, the microbial metal resistance happened through different strategies to deal with toxic metal concentration in the environments (Roane and Kellogg, 1996; Bruines *et al*., 2000; Nies, 2003). These strategies are either to prevent entry of the metal into the cell or to actively pump the metal out of the cell. Such resistance can be divided into two classes: metal dependent and metal-independent (Roane and Pepper, 2000). As mentioned before, the mechanism of resistance to metals takes several forms, these include accumulation in the form of particular protein–metal association (Ow, 1993; Rohit and Sheela,1994), blockage at the level of the cell wall and the systems of membrane transportation (Tomioka *et al*., 1994; Wehreim and Wettern , 1994), efflux of metal ions outside of the cell, complication of the metal ions inside the cell, reduction of the heavy metal ions to a less toxic state (Nies, 1999) or *in situ* immobilization by extracellular precipitation (Roane, 1999) .

In many cases, resistance to heavy metal ions is determined by plasmids (Silver and Mitra, 1988), which can be used for the creation of novel microbial strains with a high detoxifying activity against heavy metals. As a result of metal resistance ability, microbes play a major role in the biogeochemical cycling of toxic heavy metals also in cleaning up or remediation metal contaminated environments (Jing *et al* ., 2007).

Despite, alternatively low concentration some heavy metals are essential for microorganisms (e.g., Co^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}) since they provide vital co-factors for metallo-proteins and enzymes (Chua 1999; Doelman *et al*., 1994), heavy metals have an inhibitory action on microorganisms by blocking essential functional groups, replacing essential metal ions, or modifying the active conformations of biological molecules (Rajapaksha *et al*., 2004; Wood and Wang, 1985; Doelman *et al*., 1994; Li *et al*., 1994).

In naturally polluted environments, the response of microbial communities to heavy metals depends on the concentration and availability of metals and is dependent on the actions of complex processes, controlled by multiple factors such the type of metal, the nature of medium, and microbial species (De Rore *et al*., 1987; Goblenz *et al*., 1994; Hachemi *et al*., 1994; Tomioka *et al*., 1994). Heavy metal MICs (minimal inhibitory concentrations for bacterial strain present in various natural habitats such as soil, water, sediments, and sewage amended soil have been studied (Chang and Broadbent, 1982; Duxbury and Bicknell, 1983; Abbas and Edwards, 1989; Nieto *et al*., 1989; Hiroki, 1994).

In addition to heavy metals, microorganisms may be resistant to antibiotics too. These resistant strains have been isolated frequently from different environments and clinical samples (Henriette *et al*., 1991; Sundin and Blender 1993). This leads to the suggestion that the combined expression of antibiotic resistance and metal tolerance is caused by selection resulting from metals present in the particular environment (Sevil *et al*., 2009 Calomiris *et al*., 1984).

MATERIALS AND METHODS

Sample Collection and Preparation

Water samples were collected from different sites of the Red Sea, Hurgada, Safaga, and Al-Quseir. For bacteriological analysis, water samples were collected aseptically and transported to the laboratory, where they were analyzed within 8 hrs of collection. To determine total cultural bacteria, a serial dilution method was used to reduce the number of organisms on halophilic agar plates medium. Individual bacterial colonies on nutrient agar plate which varied in shape and color were picked up and purified by repeated streaking. Water samples were acidified with concentrated HNO_3 and stored at 4ºC for heavy metal analysis.

Medium

Halophile medium agar (HA) was used in g/l: NaCl, 100 yeast, 5 tryptone 5; or peptone, 5 or peptone perteose, 5 $MgSO_4.7H_2O$, 20 $CaCl_2$, 0.2 KCl, 5 agar, 25.

Characteristics of Water Samples

Cation concentration and Cl ion content were performed according to the methods described by Abou –Kandil, 2000.

Water samples were treated as recommended by Grimalt, 1989 by acid digestion using 0.6 ml of concentrated HNO3, 0.25 ml of 75% H_2SO_4 and 100 ml of unfiltered water. Each sample was then evaporated, diluted to 25 ml and analyzed for metal content using atomic absorption spectroscopy, (Perkin Elmer Analyst 300) metal with acetylene-air flame.

Physico-Chemical Analysis of Water Samples

Temperature and pH values were measured. Electric conductivity, E.C. (mmhos/cm as indicator for the salinity) was also measured according to Mostafa *et al*., 2004 and Dunkle, 1944.

Heavy Metal Analysis

Water samples were analyzed for Heavy metal content by atomic absorption spectrophotometer (Perkin Elmer 2380).

Identification of Bacterial Isolates

Bacterial isolates were identified according to: Morphology (Paik 1980); Gram staining; catalase production (Whittenberg, 1964); growth in 7% sodium chloride; growth in 10% sodium chloride; growth in 15% sodium chloride; growth in 20% sodium chloride; acid and gas from sugars; growth at 15 ºC, 40 ºC and 45 ºC; reduction of nitrate to nitrite (Bachmann and Weaver,1951; Cowans and Steel, 1966); Motility (Rhodes 1958); gelatin hydrolysis; production of indole from tryptophan; starch hydrolysis; casein decomposition; oxidase test (Kovacs ,1956); pigment production (King *et al*., 1954); anaerobic agar; Voges–Proskauer reaction (Cruickshank *et al*., 1975); acetylmethylcarbinol production; and growth on sea water.

Effect of Heavy Metals on Bacterial Isolates

Resistance to heavy metals was determined by an agar dilution method according to Washington and Sutter 1980. Plates containing 20 ml of agar and different concentrations of metals were poured on the day of the experiments. The concentrations for all metals tested were as follows (in mM): 0.1, 0.5,1, 2.5, 5, 10, 20, 40, 80, and 100 (Trevors *et al*., 1985; Garcia *et al*., 1987b). The minimal inhibitory concentration (MIC) was determined to Cd^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, and Co^{2+} as the lowest concentration of metal ion preventing growth (Smith 1967).

Antibiotic Susceptibility Test

Standard disc-agar diffusion method described by FineGold and Martine 1982, was used for determining the antibiotic susceptibility. Discs containing Nitrourantion (NF300 µg), Ceeadroxil (CFR 30 µg), Cephalexin (CL30 µg), Rocephen (CRO30 µg), Ampicillin (AM10 µg), Cephradine (CD 30 µg), Tarivids (OFX10 µg), Flumox (AF 10 µg), Vancocin (Va 30 µg), and Keflin (KF30 µg) were used in this test according to Bauer *et al*, (1966), and the inhibition zone was determined according to Foti *et al.,* 2009; 1974.

RESULTS

Physical and Chemical Characteristics of Water Samples

Thirty six samples were collected from 3 different sites of the Red Sea water. Table 1 shows the average means of physicochemical characteristics of water samples were measured. For example, the physicochemical characteristics of the water samples showed that; the temperature of the water samples varied between 20-25 ºC, and the pH values of the samples were ranged between 7-7.8, which was considered suitable for growth of halophilic bacteria.

Table 1: Mean of the physico-chemical characteristics of water samples collected from different sites of the Red Sea.

Characteristics	Concentrations	Characteristics	Concentrations
Temp (ºC)	20-25 ºC	Ca^{2+} (µg/l)	$7.0\text{-}7.5\times10^2$
PH (-)	7.62-7.81	Mg^{2+} (µg/l)	$12.25\text{-}12.26 \times10^5$
E.C (mmhos/cm)	30.1-30.8	K^+ (µg/l)	$4\text{-}4.5 \times10^2$
Salinity (µg/l)	$1.44\text{-}1.45\times10^5$	HCO_3--(µg/l)	$7\text{-}8 \times10^5$
Na^+(µg/l)	$9.996\text{-}9.997\times10^6$	SO_4^{--}(µg/l)	$4\text{-}5 \times10^6$
Cl^-(µg/l)	$20.14\text{-}20.15\times10^6$		

Each value is an average mean of collected samples.
E.C.: Electric conductivity

Table 2: Heavy metal ions concentrations in water samples collected from different sites of the Red Sea.

Metal ions	Concentration
Pb^{2+} (µg /l))	$7\text{-}7.2 \times10^2$
Co^{2+} (µg/l)	$3\text{-}3.3 \times10^2$
Zn^{2+} (µg/l)	20-25
Cd^{2+} (µg/l)	75-76
Cu^{2+} (µg/l)	$7.9\text{-}8.0 \times10^2$

The chemical analysis of water samples showed that the chloride ions of the water samples varied between 20.14×10^6-20.15×10^6 µg/ml, the sodium concentrations of the samples ranged between 9.996×10^6-9.997×10^6 µg/ml, whereas it had low amount of calcium and potassium (7.0×10^2-7.5×10^2 µg/ml, 4×10^2-4.5×10^2 µg/ml), respectively. The sulphate concentration fluctuated between 4×10^6-5×10^6 µg/ml, while magnesium levels ranged between 12.25×10^6-12.26×10^6 µg/ml.

Heavy Metal Analysis

Table 2 shows the average of 5 heavy metal ions (Pb^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, and Cu^{2+}) concentrations in sea water.

Table 3: Total count of halophilic bacteria isolated from different sites of the Red Sea.

Sites	CFU×10^3/ml water samples			
	winter	Autumn	Summer	Spring
Hurghada	3	2.9	2.9	3
Safage	1.6	1.6	1.8	1.7
Al-Quseir	1.3	1.5	1.3	1.3

Bacteria

Hundred and thirty eight (138) bacterial isolates were obtained after incubation of water samples on HA medium (Table 3). The mean value of total count of halophilic bacterial isolates from the three sites were ranged in summer between1-3.8 (CFU/10^3ml), in autumn was between1.2-4.7 (CFU/10^3ml), in winter was between1.1-5 (CFU/10^3ml), whereas in spring was between 1.1-3.8 (CFU/10^3ml).

Table 4: Incidence of metal resistance (138 isolates) halophilic bacterial isolates isolated from the Red Sea.

Metals	No. of resistant isolates (%of resistant isolates)								
Metals ions (mMol)	**0.05**	**0.1**	**0.5**	**1**	**2.5**	**5**	**10**	**20**	**40**
Cd^{2+}	69(50)	55(39.8)	37(26.8)	35(25)	25(18)	3(2.17)	0	0	0
Zn^{2+}	119(86.2)	58(42)	30(21.7)	21(15.2)	16(11.5)	5(3.6)	0	0	0
Cu^{2+}	106(76.8)	83(60)	69(50)	58(42)	51(36.9)	46(33.33)	23(16.6)	9(6.5)	0
Pb^{2+},	138(100)	138(100)	133(96.6)	92(66.66)	78(56.5)	71(51.44)	22(15.9)	13(9.4)	0
Co^{2+}	124(89.8)	104(75.36)	92(66.66)	69(50)	58(42)	33(23.9)	19(13.76)	10(7.2)	0

Screening of Halophilic Bacterial Isolates for Heavy Metal Resistance

The 138 bacterial isolates were screened for their metal resistance using media containing different concentrations of the five heavy metal, namely, Cd^{2+}, Zn^{2+}, Cu^{2+} ,Co^{2+}, and Pb^{2+} ranging from 0.05 to 100mM (Table 4). Among the 138 moderately halophilic isolates isolated from the Red Sea, 2.17%, 3.6%, 6.5%, 9.4%, and 7.2% of the isolates were as resistant up to 5mM Cd^{2+} and Zn^{2+}, 20 mM for Cu^{2+}, Pb^{2+}, and Co^{2+}, respectively. The resistance determination (Table 4) indicated that a majority of halophilic isolates showed resistance to more than 10mM to these metals except Cd^{2+}, other resistance values up to 20 mM were recorded. As shown in Table 5 and Figure 1, all strains were resistant to lead, whereas 50% only of the strains were resistant to cadmium.

Table 5: Percentages of the halophilic bacterial isolates resistant to used five heavy metal.

Metal ion	No of resistant isolates	% of resistant isolates
Cadmium	69	50
Zinc	119	86.2
Copper	106	76.8
Lead	138	100
Cobalt	124	90

Table 6: Multiple metal resistance pattern of the (138) halophilic bacterial isolates recovered from different sites of the Red Sea.

No. of metals	Resistance pattern	No. of resistant isolates	% of resistant isolates
2	**Cd^{2+}, Zn^{2+}**	6	4.34
	Cd^{2+}, Cu^{2+}	9	6.5
	Cd^{2+} , Pb^{2+}	3	2.17
	Zn^{2+} , Cu^{2+}	9	6.5
	Zn^{2+}, Pb^{2+}	6	4.34
3	**Cd^{2+}, Zn^{2+} , Cu^{2+}**	3	2.17
	Zn^{2+} , Cu^{2+}, Co^{2}	3	2.17
	Cu^{2+}, Co^{2+}, Pb^{2+}	3	2.17
4	**Zn^{2+} , Cu^{2+}, Co^{2+}, Pb^{2+}**	3	2.17
	Cd^{2+}, Zn^{2+} Cu^{2+}, Co^{2}	3	2.17
	Cd^{2},Zn^{2+} , Cu^{2+}, Pb^{2+}	3	2.17
	Zn^{2+}, Pb^{2+} ,Cu^{2+}, Co^{2}	3	2.17

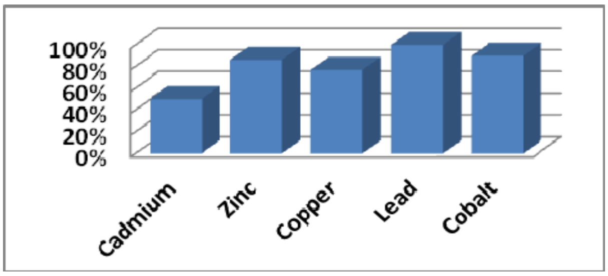

Fig. 1: Percentages of the halophilic bacterial isolates resistant to heavy metal.

The results of multiple metal resistances of these halophilic bacteria isolated from different sites of the Red Sea were listed in Table 6 and drawn in Figure 2. The resistance to (Cd^{2+} + Cu^{2+}); (Zn^{2+} + Cu^{2+}) showed the highest values between bacteria (6.5%), whereas 4.34% of the isolated strains were resistant to (Cd^{2+}, Zn^{2+}) and (Zn^{2+} + Pb^{2+}), but 2.17% of the isolates were resistant to 3 metals (Cd^{2+}, Zn^{2+} , Cu^{2+}); (Zn^{2+} , Cu^{2+}, Co^{2}); and (Cu^{2+}, Co^{2+}, Pb^{2+}) and resistant to 4 metals (Zn^{2+} , Cu^{2+}, Co^{2+}, Pb^{2+}); (Cd^{2+}, Zn^{2+} Cu^{2+}, Co^{2}); (Cd^{2},Zn^{2+} , Cu^{2+}, Pb^{2+}); and (Zn^{2+}, Pb^{2+} ,Cu^{2+}, Co^{2}). No bacterial isolate were resistant to the 5 metals combined.

Table 7: Percentages of isolates resistant to antibiotics.

Antibiotic	Conc. (ug)	NO of resistant strains	% of resistance
Velosef (Cephradine) (CD)	30	131	95
Ceftriaxone (CRO)	30	127	91.66
Ampicillin(AM)	10	115	83.33
Nitrofurantion (NF)	300	113	81.66
Tarivids (OFX)	10	110	80
Cephalothin (KF)	30	101	73.33
Cefalexin (CL)	30	92	66.6
Cefadroxil (CFR)	30	115	83.33
Flucloxacillin (AF)	10	117	85
Vancomycin(VA)	30	9	6.6

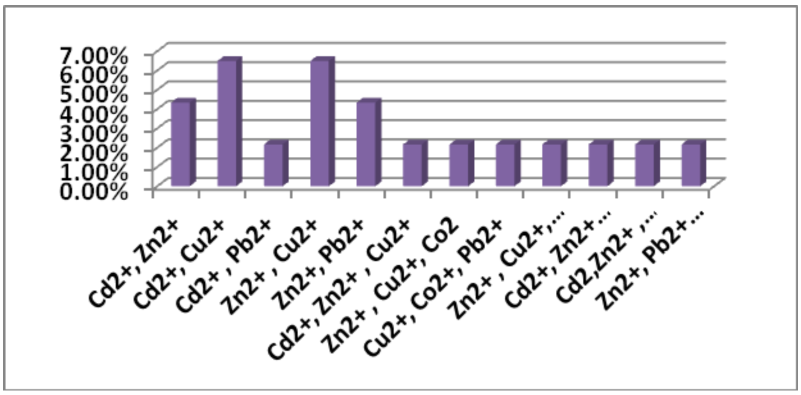

Fig. 2: Multiple metal resistance pattern of the isolated halophilic bacteria.

As shown in (Table 5) cadmium was the most toxic metal since 50 % of the isolates were inhibited by only 0.05 mM. Zinc was very toxic after cadmium, since 86.2% of the isolates were resistant by the same concentration while copper, lead, and cobalt were the less toxic metals than cadmium and zinc, since 76.8%, 100%, and 90% of the isolates were resistant by the same concentration respectively.

Antibiotics Susceptibility Test

In our study, the isolated halophilic bacterial strains exhibited sharp peaks of resistance to drugs such as: Velosef 95%, Cceftriaxone 91.66%, Ampicillin 83.33 %, Nitrofurantion 81.66%, Tarivid 80%, Cephalothin 73.33%, Cephalexin 66.66%, Cefadroxil 83.33%, and Flucloxacillin 85%, whereas few isolates (6.6%) were resistant to Vancomycin as shown in Table 7 and Figure 3.

Table 8: Incidence of multiple antibiotic resistances.

NO. of antibiotics to which isolates were resistant	No. of isolates	solates (%)
10	9	6.6
9	50	33.66
8	55	40
7	57	41.45
6	60	43.63
5	68	49.27
4	73	52.89
3	87	63
2	122	88.40
1	131	95

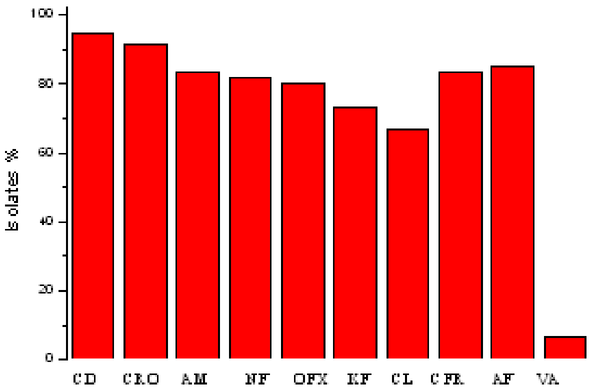

Fig. 3: Percentages of isolates resistant to antibiotics.

As shown in Table 8 and Figure 4, the greater the number of antibiotics that bacteria were exposed to it, the less the numbers and percentage of resistant isolates. Table 9 shows the antibiotic resistance pattern of the isolates.

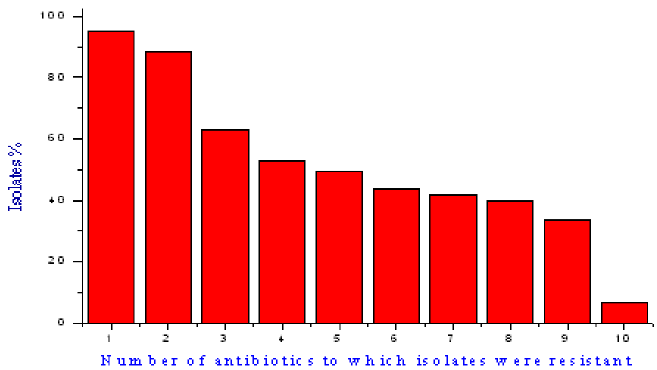

Fig. 4: Incidence of multiple antibiotic resistances.

Table 9: Antibiotic resistance pattern of 138 isolates recovered from different sites of the Red Sea.

NO of antibiotics	Resistance pattern	NO of resistant isolates
2	AF, KF CRO,AM SFR,CL OFX.NF NF,VA CD.AF	27(19.5) 30(21.3) 27(23.1) 30(21.3) 5(3.6) 3 (2.1)
3	AF.KF.CRO AM.CFR.CL OFX.NF.VA	20(14.4) 15(10.8) 5(3.6)
4	AF.KF.CRO.AM CFR,CL,OFX,NF VA,CD,AF,KF	17(12.3) 13(9.4) 4(2.8)
5	AF,KF,CRO,AM,CFR CL,OFX,NF,VA,CD	13(9.4) 3(2.17)

DISCUSSION

The chemical characteristics of the Salter deposit from Alexandria, and Port-Saeed, since the pH values are 8.5-9 and 7.6, respectively. These two sites had temperature range between 22ºC-27ºC. (Rodriguez-Valera *et al* .1981; Rodriguez-Valera *et al*.1985).

Hanan (2002) reported that, the Red Sea coast marshes were formed by the evaporation of sea-water on the Red sea-side and encouraged by high temperatures and low rainfall, the temperature during sampling was 28ºC-30ºC. Schweinfurth and Lewin 1998, have collected two samples from soda lakes in Wadi–Naturon, Egypt, which are located in a desert depression west of the Nile Delta. The temperature of this sampling area was in the range of 30ºC-32ºC, and pH values ranged from 9.6 to 10. In the present work, the electric conductivity fluctuated between 30.1-30.8 mmhos/cm, salinity ranged between $1.44\text{-}1.45x10^5$ which reflected high salinity of collected samples.

Metal concentrations ranged at the time of sampling from 0.075µg l^{-1} for Co^{2+} to 181µg l^{-1} for Pb^{2+}. These values represent up to a 100-fold increase above those reported internationally and are even higher than data from polluted marine environments (Spivak 1981; Abosamra *et al*.1989). These high metal concentrations may attribute to sewage disposal and to the ship-maintenance activity (El-Sayed *et al*. 1981; Claisse and Alzieu 1993).

The marked variations in the microbial numbers of the different water samples could be interpreted on the basis of the differences in the physical and chemical characteristics of water samples (e.g., salinity temperature and others). These factors interact and produce a complex interrelated effect on the microbial counts and activities. In this respect, El-Abyad *et al*., (1979) suggested that the salinity is not only the factor that affects the microbial numbers, but the environmental conditions during incubation have a major influence on the appearance of specific bacterial types.

Pollution of the environment by metals has increased dramatically in recent years, largely as a result of industrial activity; although agricultural products and sewage disposal also contribute (Gadd, 1990; Gadd White, 1993). The ability of microorganisms to grow in the presence of high metal concentrations may result from specific mechanisms of resistance (Sabry *et al*., 1997; Nies, 1999). These resistance mechanisms take several forms, such as extracellular precipitation and exclusion, binding to cell surface and intracellular sequestration (Blackwell *et al*., 1995). Duckworth *et al*., (1996) reported that Soda Lake in Wadi-Natrun contains dense populations of aerobic organotrophic and alkaliphilic bacteria and recorded numbers of $10^7\text{-}10^8$ bacteria ml-1 in dilute soda lakes.

Ramarnoorthy and Kushner (1975) showed that the availability of lead in the growth medium is generally very low, since this ion binds to the components of the media. That may explain the resistance of all isolates to applied lead ions. Also, the high resistance to lead could be attributed to the high lead content (181) µg l-1 in sea water. These findings are in accordance with data from similar work (Nieto *et al*., 1989; Riley and Taylor 1989). It has been reported that lead can be accumulated in the cell wall and membrane (Tornabene and Edwards 1972). Nevertheless, very little research has been conducted on the genetic basis of lead resistance in bacteria.

The high zinc susceptibility (21%) of the isolates detected in this study is probably due to the increased toxicity to zinc in media containing NaCl, due to the formation of a soluble zinc- chloro complex which increases the availability of the cation to the bacterial cell (Hughes and Poole, 1989).

The majority of halophilic bacterial isolates were strongly multi-resistant to metal ions, as the resistance often occurred for a range of metals rather than for specific metal alone (Dressler *et al*., 1991: Trojanovska *et al*., 1997). Sabry *et al*., (1997) suggest that all of the 81 isolates they studied were penta-metal resistant with 11 different resistances combinations. The resistant of haophilic bacteria to heavy metals may attributed to presence of phosphates and carbonates naturally occurring in sea water and can protect bacteria against metal toxicity (Hughes and Poole 1989), in addition, halophilic isolates are good extracellular polysaccharide producers which may further protect the cell from the toxic effect of heavy metals (Geesey and Jange, 1990).

Plaut *et al*., (2013) reported that, under similar experimental conditions, three strains (3.7%) were resistant to 11 tested antibiotics and hepta-resistance (9.88%) occurred within eight strains. In addition, seven isolates (8.64%) could tolerate nine different antibiotics.

As pointed by Hsu *et al*., (1992), the differences in percentage of bacterial resistance to various antibiotics may reflect the history of antibiotic application and hence there is a possibility of using bacterial drug resistance as an indicator of antibiotics application. The adaptive responses of the bacterial community to several stress agents observed in the present investigation seemed to be the result of sewage disposal as previously stated by Baldini and Cabezali, 1991.

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ARABIC SUMMARY

دراسه مقاومه المعادن الثقيلة والمضادات الحيوية في البكتيريا المحبة للملوحة والمعزولة من مناطق مختلفة من البحر الأحمر في مصر

خالد عبدالله علي عبدالرحيم[1&2] - السيد محمد سلطان[1&3] - مجدي عبدالسميع أبو غربيه[1] - فتحيه الحامدي الزين[1]
١- قسم النبات - كليه العلوم- جامعه سوهاج
٢- قسم النبات والميكروبيولوجى- كليه العلوم- جامعه الملك سعود-الرياض- المملكة العربية السعودية
٣-قسم النبات - كليه العلوم- جامعه الباحة – المملكة العربية السعودية

تعتبر مقاومة العناصر الثقيله والمضادات الحيويه من الظواهر المهمه والتي بدأت تتزايد هذه الأيام، وهي تعتبر أيضا مؤشر على إزدياد معدلات التلوث بهذه المركبات نتيجة لصرف مخلفات وعوادم الصناعه في مصادر المياه المختلفه وكذلك نتيجه لسوء الإستخدام من جانب الإنسان للمضادات الحيويه على سبيل المثال.

في هذه الدراسه تم عزل مائة وثمانية وثلاثين (١٣٨) عزله بكتيريه من ٣ مناطق مختلفة في محافظة البحر الأحمر، بجمهورية مصر العربيه (الغردقه – سفاجا – القصير)، وتم إختبار مقاومتها وتحملها لعدد من العناصر الثقيله والمضادات الحيويه. وأظهرت نتائج الدراسه أن ٢.١٧ ٪ ، ٣.٦ ٪ ، ٦.٥ ٪ ، ٩.٤ ٪ و ٧.٢ ٪ من هذه العزلات كانت مقاومة لتركيز ٥ ملي مول من الكادميوم، ٢٠ ملي مول من النحاس والرصاص والكوبلت على الترتيب. وأظهرت غالبية العزلات البكتيرية المحبة للملوحة المقاومة لأكثر من ١٠ ملم إلى هذه المعادن ما عدا الكادميوم. وكانت جميع العزلات مقاومة للرصاص، في حين أن ٥٠ ٪ فقط من السلالات كانت مقاومة للكادميوم.

ولقد وجد أن أعلى نسبه للمقاومه كانت لكل من الكادميوم والنحاس مجتمعين، وكذلك الزنك والنحاس مجتمعين وكانت حوالي ٦.٥% من إجمالي العزلات البكتيريه تقاوم كل من زوجي العناصر المشار اليهم مجتمعين. في حين أن ٤.٣٤٪ من السلالات المعزولة كانت مقاومة للزنك والكادميوم مجتمعين، الزنك والرصاص مجتمعين، في حين أن ٢.١٧ ٪ من العزلات مقاومة لل ٣ من المعادن مجتمعه (الكادميوم، الزنك، النحاس)، (الزنك والنحاس والكوبلت)؛ (النحاس، الكوبلت، الرصاص) ومقاومة ل ٤ من المعادن مجتمعه (الزنك، النحاس، الكوبلت، الرصاص)، (الكادميوم، النحاس، الزنك، الكوبلت)؛ (الكادميوم، الزنك، النحاس، الرصاص)؛ (الزنك، الرصاص، النحاس، الكوبلت). لم يسجل مقاومة أي من العزلات لكل من الخمس عناصر المستخدمه مجتمعه.

أظهرت الدراسه أن نسب عاليه من العزلات البكتيريه المحبه للملوحه كانت مقاومه للمضادات الحيويه المستخدمه. فعلى سبيل المثال، كان ٩٥% من العزلات كانت مقاومه للفيلوسيف (سبفرادين)، ٩١.٦% كانت مقاومه للسي سيفترياكسون، ٨٣.٣% كانت مقاومه للأمبسيلين، ٨١.٦% كانت مقاومه للنيتروفيورانتيون، ٨٠% مقاوم للتاريفيد، ٧٣.٣% مقاوم للسيفالوتين، ٦٦.٦% مقاوم للسيفالكسين، ٨٣.٣% مقاوم للسيفادروكسيل، ٨٥% مقاوم للفلوكوكساسيلين، في حين أن أقل مقاومه كانت للمضاد الحيوي فانكومايسين (٦.٦%).