King Saud Univ., Vol. 13, *Agric. Sci.* (1), pp. 75-90, Riyadh (1421/2001)

Effect of Heavy Metals on the Biological Activity of Certain Soil and Groundwater Microorganisms

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(Received 7/11/1419; accepted for publication 12/8/1420)

Abstract. *Pseudomonas* P_{13}, *Bacillus* B_{25}, *Streptomyces* S_2 and *Mucor* F_3 (isolated from calcareous soil and groundwater) were grown at different levels of heavy metals (Fe, Mn, Zn , and Cu) in a batch culture. Growth kinetics and biological activity of these organisms were studied. The results obtained revealed that all tested levels of heavy metals (5 - 30 $mg \cdot l^{-1}$) supported growth of *Pseudomonas* P_{13} and *Bacillus* B_{25} on the 1st day of incubation, thereafter the growth rate decreased gradually to less than the control. Low levels of heavy metals (5 and 10 $mg \cdot l^{-1}$) enhanced *Streptomyces* S_2 and *Mucor* F_3 growth. Consumed carbon and carbon utilization efficiency were higher in the control than treatment. Tested organisms also showed higher amount of growth per unit of consumed carbon, nitrogen and phosphorus in heavy metals-free medium than treated one. *Mucor* F_3 was the most resistant microorganism to high concentrations of heavy metals where the growth was reduced from 26.1% to 35.7% at 20 and 30 $mg \cdot l^{-1}$ heavy metal, respectively. *Pseudomonas* P_{13} and *Streptomyces* S_2 were more sensitive to high levels of heavy metals.

Keywords: Microorganisms, soil, ground water, heavy metals toxicity, growth, CNP-consumed.

Introduction

In the last five decades of this century the world had faced a number of formidable environmental problems such as enrichment of soil and water with heavy metals, recalcitrant pesticides, disposal of animal and industrial wastes, acid rain and depletion of the ozone layer, etc. Duxbury and Bicknell [1] and Angle *et al.* [2] reported that industrial activities and disposal of waste products have resulted in the contamination of many terrestrial environments with heavy metals. The extent to which these environments are polluted and whether the metals adversely affect biological systems are difficult to determine. Many heavy metals are essential for microbial growth and metabolism at low concentration, e.g. Cu, Zn, Mn, and Fe, whereas others have no known essential biological function, e.g. Au, Ag, Cd, Pb, Hg and Al [3]. Bacteria

require diverse metals such as Na, K, Mg, Ca, Fe, Co, Mo, Mn, Ni, and Zn as inorganic nutrients. The excessive quantities of these or other metals may be inhibitory or even lethal to organism [4]. Aiking *et al* [5] reported that heavy metals, which might be inhibitory to the cell, could be detoxified by precipitation as insoluble salts by microbially produced sulfide or phosphate. Copper is an essential trace bioelement but is a toxic metal for most micoorganisms at high concentration [6]. Singleton et al [7] studied the ability of *Mucor flavus* to adsorb zinc dust under various conditions. They reported that particulate adsorption was independent of the C, N, or P content of the growth medium used to produce the mycellium. Evdokimov and Mozgoya [8] isolated microscopic fungi such as *Paecilomyces farinosus , Penicillium* sp. and *Rhodotorula glutinis* that one resistant to high concentrations of heavy metals in soil (Podzols) polluted with industrial waste from non-ferrous metallurgical work.

The microbial cell membrane is the obvious first site of action for any toxic agent and extensive membrane damage can be caused by heavy metals resulting in loss of cellular solute permeabilization to the external materials [9,10]. Gadd *et al* [11] stated that copper caused extensive bursting of protoplasts of copper-sensitive yeast strains but not from resistant ones. Rayner and Sadler [12, pp. 39-47] mentioned that small amount of Zn (60 μM) in the medium decreased the lag phase, increased the growth rate and yield of cells. They also reported that the increase in Cd led to an increase in lag phase, prolongation of the exponential phase and decreased yield of *E.coli* K_{12} C_{600}.

The present study was undertaken to evaluate the effect of different concentrations of some heavy metals (Cu, Zn, Mn and Fe) on certain microorganisms isolated from both calcareous soil and groundwater. The biological activity of these organisms such as microbial growth, growth parameters, and consumption of carbon nitrogen and phosphorus and their utilization efficiency were also studied.

Materials and Methods

Microorganisms

The microbial strains used throughout this investigation were obtained from the soil microbiology laboratory, Soil Science Department, College of Agriculture, King Saud University, Riyadh Saudi Arabia. These microorganisms were *Pseudomonas cepacia* P_{13}, *Mucor lamprosporus* F_3 (isolated from calcareous soil pH 7.7 obtained from Al-Safi farm, El-Kharj, Riyadh region) and *Bacillus licheniforms* B_{25} and *Streptomyces sp.* S_2 (isolated from groundwater of El-Kharj well , 69 °C, pH 7.8 and E.C. 4.4 dS m^{-1}). P_{13} and B_{25} were maintained on nutrient agar while S_2 was maintained on starch agar [13, pp.1-430] and F_3 on Czapek's agar at 5°C.

Culture conditions

The microorganisms were grown in 250-ml conical flasks containing 100ml basal medium at 25°C on an orbital shaker (120-rpm) for 7 days. Medium containing KNO_3

(2.0 $g \cdot l^{-1}$), K_2HPO_4 (1.0 $g.^{-1}$), $MgSO_4$ (0.5 $g \cdot l^{-1}$) and yeast extract (0.5 $g \cdot l^{-1}$) was used as a basal medium for propagation of tested organisms. This medium was supported with glucose (10.0 $g \cdot l^{-1}$) for bacterial strains (P_{13}, B_{25}), starch (20.0 $g \cdot l^{-1}$) for *Streptomyces* S_2 and sucrose (30.0 $g \cdot l^{-1}$) for *Mucor* F_3. The pH of media was adjusted to 7.0 (by using NaOH). Millipore (0.20 µm) filtered heavy metals solution of $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $MnSO_4.H_2O$ and $CuSO_4$ was added to sterile basal medium to produce separate concentration ranges of 5, 10, 20 and 30 $mg \cdot l^{-1}$ of each metal as Fe, Zn, Mn and Cu. Standard inoculum of each organism was prepared by inoculation of a 100ml conical flask containing 50ml medium (nutrient glucose broth for bacterial strains or *Streptomyces* and malt extract broth for fungal strain) with the tested organism. Bacterial cultures (P_{13}, B_{25}) were incubated at 25°C for 2 days while *Streptomyces* S_2 and *Mucor* F_3 were incubated at 25°C for 5 days [14]. 10-ml portions of culture were used as a standard inoculum.

Samples were withdrawn from growing cultures to determine microbial growth. Bacterial cultures (10ml) were taken aseptically and centrifuged at 4000 rpm for 30 minutes. The sediment (biomass) was washed twice with distilled water and dried at 70 °C for three successive days. Correlation between turbidity of bacterial culture (using Nephelometer) and dry weight of biomass was made. In the case of *Streptomyces* and fungal strains, the whole culture of each flask was filtered and washed twice with distilled water. The biomass was also dried as mentioned before. The supernatant and filtrate of the cultures were used to determine consumed carbon, nitrogen and phosphorus.

Microbial growth as a function of time was plotted on semi-log paper (growth curve). Growth kinetics (specific growth rate, doubling time, saturation constant and yield factor) were calculated according to Rossi [15, pp.179-331] using the following equations:-

$$\{ X = X_o e^{u(t_1 - t_o)} \}$$

where: X = Microbial growth at t_1 time, X_o = Microbial growth at t_o time
µ = Specific growth rate.

$$\{ \mu = \ln 2 \, (t_d)^{-1} \}$$

where: t_d = doubling time .

$$\{ \text{Biomass factor \% (F)} = XS^{-1} \}$$

where: X = Dry biomass , S = Consumed carbon

$$\{ 1/\mu = 1/S.\, Ks + 1/\mu_m \}$$

where: µ = specific growth rate , S = Concentration of heavy metals,
μ_m = Maximum specific growth rate and Ks = Saturation constant .

A plot of 1/µ against 1/S, i.e. a lineweaver- Burk plot, will give a straight line with an intercept on the abscissa at 1/Ks and an intercept on ordinate at 1/µm.

Total carbon, nitrogen and phosphorus of the cultures were determined according to Mebius [16], Olsen and Dean [17, pp.416-431] and Black, *et al.* [18], respectively. C, N, P and their respective ratios were calculated.

Statistical analysis

LSD test at the 5% probability level, regression analyses and consequently correlation coefficient were performed according to SAS [19].

Results

Microbial growth

The data presented in Fig. (1) show the effect of heavy metals on the growth of *Pseudomonas* P_{13}, *Bacillus* B_{25}, *Streptomyces* S_2 and *Mucor* F_3. In the concentration range 5 - 30 $mg \cdot l^{-1}$, it may be observed that the tested organisms are highly varied in their ability to grow at different concentrations of metals. Growth of *Pseudomonas* P_{13} and *Bacillus* B_{25} was supported by all tested levels of heavy metals during the first 24 hours of incubation as compared to control, where exponential growth was recorded. In general, the growth of both bacterial strains was almost stationary at the fourth day of incubation in media containing different levels of heavy metals. After 4 days of incubation, all levels of heavy metals showed a slight increase in microbial biomass but still lower than control. Conversely, the lowest levels of metals (5 and 10 $mg \cdot l^{-1}$) only had a stimulation effect on *Streptomyces* S_2 and *Mucor* F_3 respectively during the first three days of incubation. On the 3rd and 6th day of incubation fungal and *Streptomycetes* strains showed an abrupt decrease in biomass respectively as compared to the control. On the 7th day of incubation, both bacterial strains (P_{13} and B_{25}) and *Streptomyces* S_2 gave the highest biomass in heavy metal-free medium namely 152.44, 93.93 and 94.20 mg/100 ml culture, respectively, while the corresponding figure for *Mucor* was 237.7 mg/100ml at 5 $mg \cdot l^{-1}$.

Statistical analysis (Table 1) revealed that a positive correlation between incubation time and microbial biomass as affected by heavy metals concentrations was evident. The highest values of correlation coefficient were noticed at 5 $mg \cdot l^{-1}$ for *Pseudomonas* P_{13}, *Streptomyces* S_2 and at 10 $mg \cdot l^{-1}$ for *Bacillus* B_{25} and *Mucor* F_3 ranging from 0.91 to 0.98. Conversely, *Pseudomons* P_{13} and *Streptomyces* S_2 showed the lowest correlation at 30 $mg \cdot l^{-1}$, being 0.74 and 0.54, respectively. All the values of (r) were significant at 0.01 level for all tested organisms except for the case of *Pseudomonas* P_{13}, *Streptomyces* S_2 (30 $mg \cdot l^{-1}$), *Mucor* F_3 (20 $mg.l^{1}$) and at 0.05 for *Streptomyces* S_2 (30 $mg \cdot l^{-1}$). In general, the increase of heavy metals concentration led to a decrease in the microbial biomass and this effect was more pronounced at 30 $mg \cdot l^{-1}$.

With respect to the growth parameters, it was found that the highest specific growth rate (Fig. 2) was observed during the first 24 hours at the concentration of 30 $mg \cdot l^{-1}$ heavy metals for *Bacillus* B_{25} and *Pseudomonas* P_{13}, being 4.1 and 3.9 day^{-1} respectively, while doubling time was 4.1 and 4.3 hours, respectively.

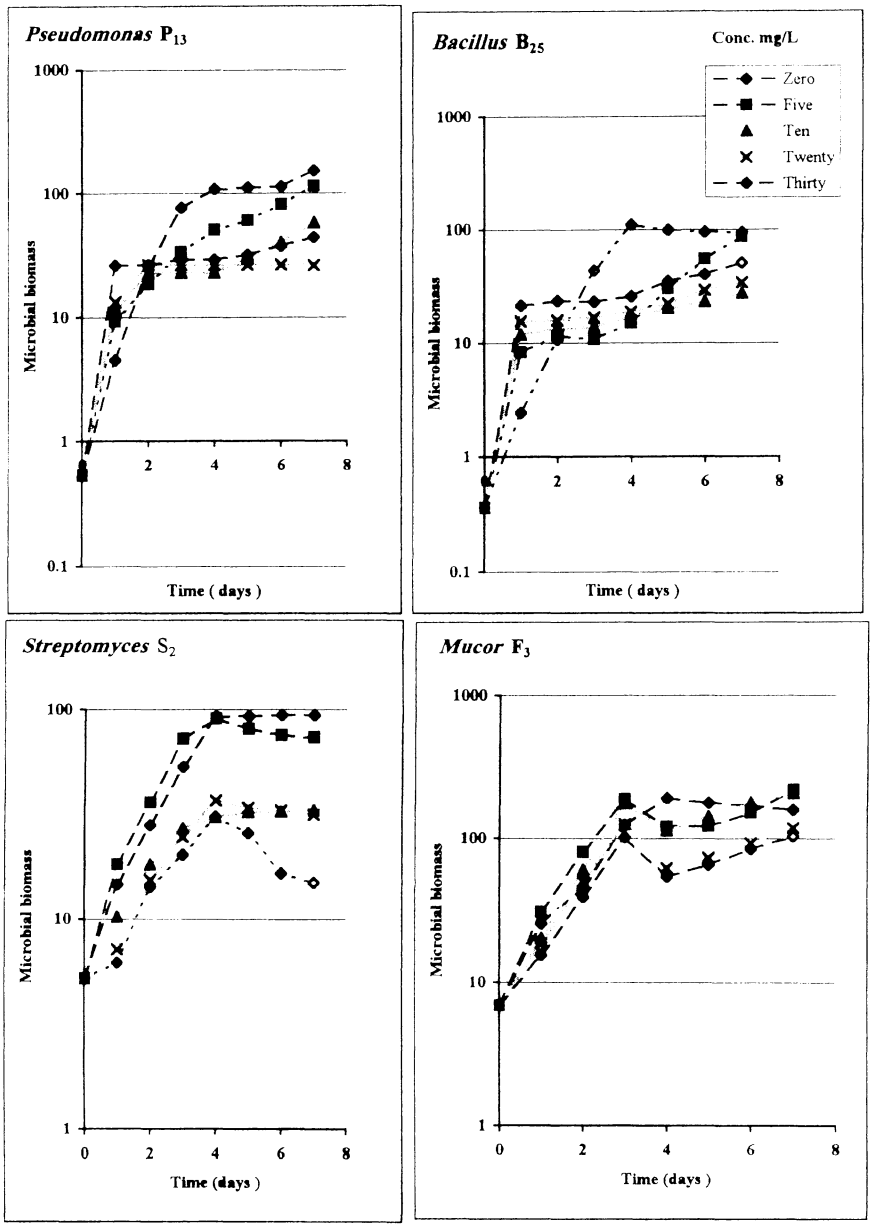


Fig. 1. Biomass (mg/100 ml) of different microorganisms grown at different concentrations of heavy metals (mg/l) .

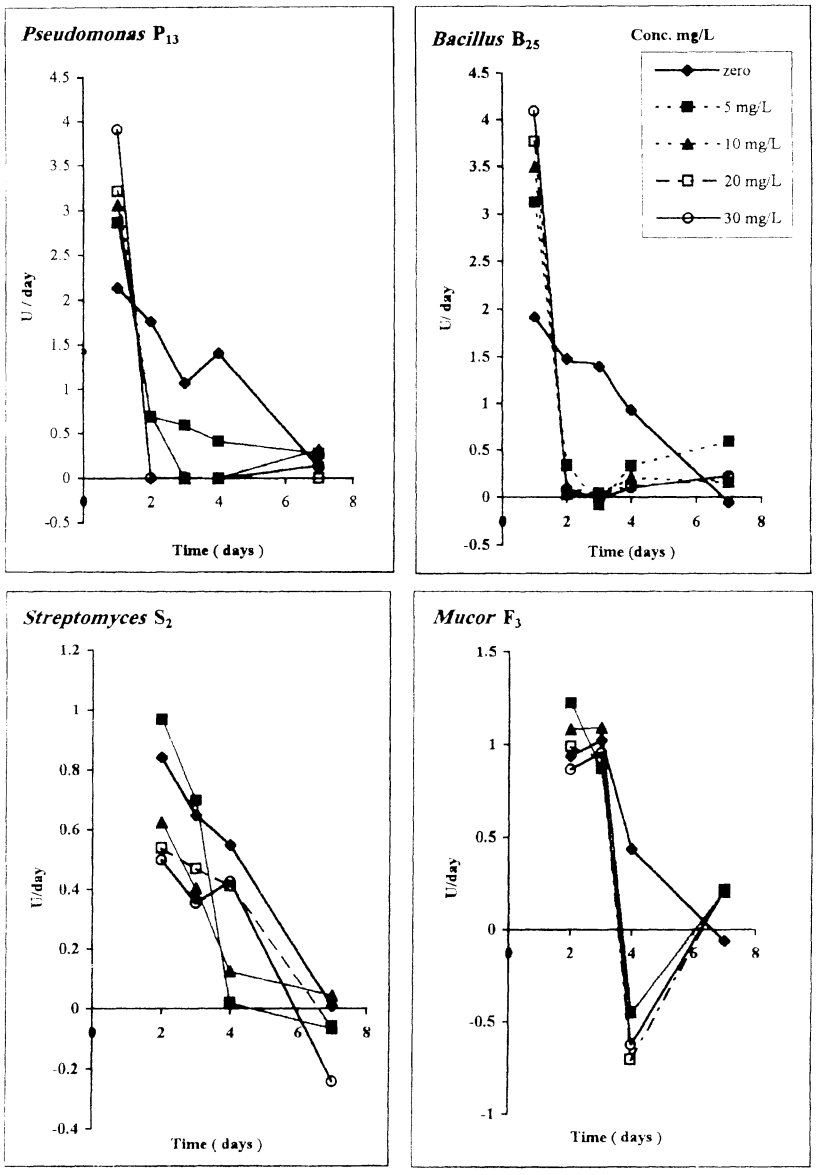


Fig. 2. Specific growth rate (U) of different microorganisms at different incubation periods against heavy metal concentrations (mg/l).

Table 1. Regression equations and correlation coefficient for the relationship between time of incubation and microbial biomass as affected by different concentration of heavy metals

Heavy metals conc. $mg \cdot l^{-1}$	*Pseudomonas* P_{13}		*Bacillus* B_{25}		*Streptomyces* S_2		*Mucor* F_3	
	Regression equation	r	Regression equation	r	Regression equation	r	Regression equation	r
0	y = 22.482x - 4.77	0.9681	y = 17.263x - 3.55	0.8874	y = 10.912x + 18.29	0.8363	y = 27.268x + 18.4	0.8768
5	y = 15.398x - 7.86	0.9802	y = 10.719x - 10.28	0.8893	y = 14.968x + 7.0	0.9407	y = 26.021x + 25.1	0.8528
10	y = 6.814x +2.361	0.9486	y = 3.186x - 4.68	0.9548	y = 4.225x + 8.91	0.9419	y = 28.787x + 13.7	0.9087
20	y = 4.543x +12.47	0.8677	y = 3.8477x - 5.61	0.9370	y = 4.554x + 7.54	0.8812	y = 13.837x + 20.2	0.7821*
30	y = 2.957x +11.33	0.7442*	y = 5.757x - 7.40	0.9405	y = 1.957x + 9.82	0.5446**	y = 12.507x + 14.9	0.8316

* Non significant at 0.01

** Non signoficant at 0.05

The corresponding figures for *Streptomyces* and *Mucor* species were noticed at 5 $mg \cdot l^{-1}$ heavy metals being 0.97 and 1.2 day^{-1} (17.2 and 13.9 hours for doubling time), respectively. After 1-2 days of incubation, the specific growth rate gradually or abruptly decreased and this decrease was more pronounced at high levels of heavy metals. It could be added that the correlation between the reciprocal of the specific growth rate ($1/\mu$) and the reciprocal of heavy metals concentration ($1/S$) was clear in the case of *Pseudomonas* and *Bacillus* as compared to the other two organisms (Fig. 3). Bacterial strains gave 1.7 and 1.8-$mg \cdot l^{-1}$ saturation constants (Ks) and 3.7, 4.2-day^{-1} maximum specific growth rate (μ_m), respectively. It means that *Pseudomonas* had a strong affinity to utilize heavy metals than other tested microorganisms.

Carbon consumption

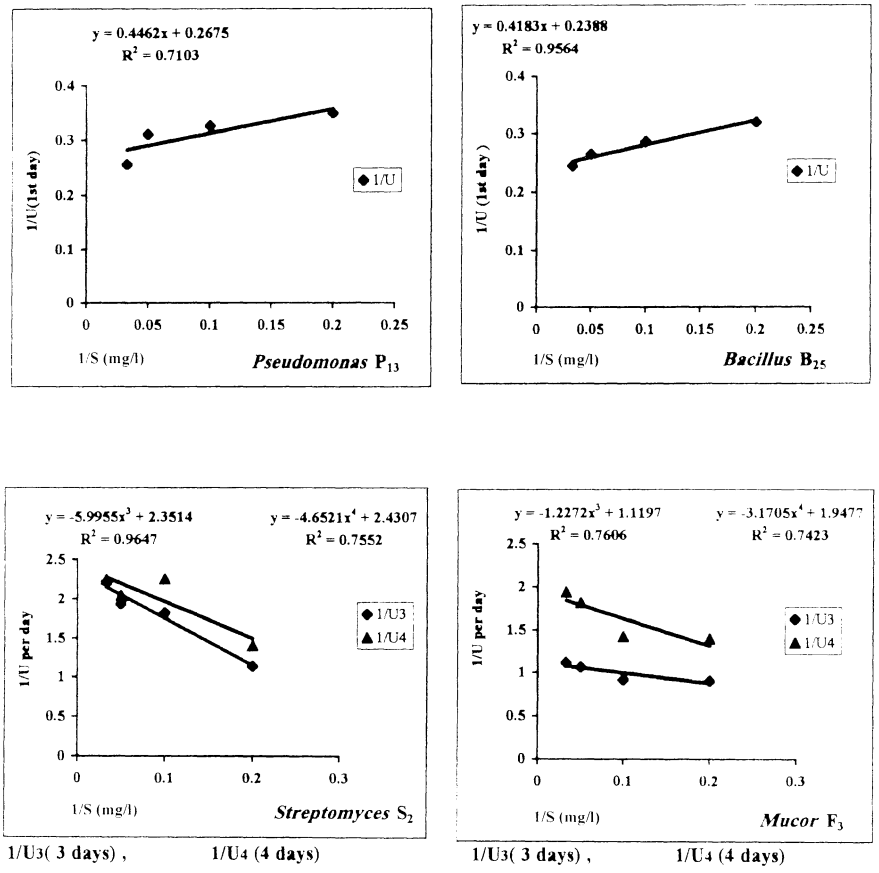
Results in (Table 2) showed that consumed-carbon by different microorganisms increased gradually during the first four days of incubation either in heavy metals-free medium (control) or in treatment containing 20-$mg \cdot l^{-1}$ heavy metals mixtures. At a late stage of growth, consumed carbon was higher in control than in media containing 20-$mg \cdot l^{-1}$ heavy metals. Carbon utilization efficiency (CUE) also exhibited the same trend where the highest value of this parameter was recorded in the case of *Pseudomonas* P_{13} grown in control, being 81%, followed by *Bacillus* B25 (73%), *Streptomyces* S_2 (31.8%) and *Mucor* F_3 (29.9%). CUE in media containing 20 $mg \cdot l^{-1}$ was lower than control where the corresponding figures were 64, 55.5, 26.9 and 23.1%. In contrast, CUE values were approximately equal in both control and treatment for each organism during the first 1-2 days of incubation.

With respect to the amount of biomass produced per unit of consumed carbon (Biomass factor %), it was found that this factor was higher in control than media containing heavy metals (Table 3) being 51.2, 49.3, 37.6 and 32.6 % for *Mucor, Paeudomonas*, *Bacillus* and *Streptomyces* species growing in control (4^{th} day of incubation), respectively. The corresponding figures for media containing heavy metals were 21.3, 13.0, 8.5, and 13.1 %, respectively.

Nitrogen consumption

The amount of consumed nitrogen and nitrogen utilization efficiency (NUE) is shown in (Table 4). The results revealed that the efficiency of tested microorganisms to utilize nitrogen source from the media (NO_3^-) was not greatly affected by heavy metals. The control and treated media did not show statistically significant difference. Nitrogen utilization efficiency ranged from 22.0 to 70.8 %. In general, it may be stated that about 22.0 to 38.4% of the nitrogen source were consumed in treated media during the first 1-2 days of incubation. Thereafter, the consumed nitrogen slightly increased by time tending to be more constant, at the end of incubation period (the concentrations ranged from 12.7 to 19.6 mg 100 ml^{-1}).

Tested microorganisms also showed higher amount of growth per unit of consumed nitrogen in control than treated media (Table 5). The highest value was noticed in the



1/U3(3 days) , 1/U4 (4 days)

1/U3(3 days) , 1/U4 (4 days)

Fig. 3. Lineweaver-Burk plot for the relationship between reciprocal of specific growth rate (1/U) and reciprocal of heavy metal concentration (1/S).

dried biomass on the 4th day of incubation. Conversely, this organism synthesized 4.8 units of biomass in treated media. The corresponding figures for *Pseudomonas* and *Bacillus* species were 7.9 and 8.2 units in control and 12.0 and 1.4 in treated media respectively. *Streptomyces* species gave the lowest figures in this respect.

C-consumed: N-consumed ratio (Table 5) was slightly lower in media containing heavy metals than the control on the 4th day of incubation (it ranged from 15.2: 22.4 to 15.9: 24.1, respectively). *Pesudomonas and Bacillus* species gave the lowest values whereas *Streptomyces* and *Mucor* showed the highest values.

Table 2. Carbon- consumed and carbon utilization efficiency (CUE) exhibited by different microorganisms as affected by heavy metals

Micro-organisms	Time (days)	Carbon - consumed			
		Control media		Treated media *	
		mg 100ml^{-1}	CUE %**	mg 100ml^{-1}	CUE %**
Pseudomonas	1	134	33.5	129	32.3
P_{13}	2	139	34.8	137	34.3
	3	210	52.5	174	43.5
	4	219	54.8	205	51.3
	7	324	81.0	256	64.0
Bacillus	1	86	21.5	95	23.8
B_{25}	2	94	23.5	105	26.3
	3	199	49.8	157	39.3
	4	292	73.0	222	55.5
	7	243	60.8	203	50.8
Streptomyces	2	178	20.0	182	20.5
S_2	3	193	21.7	225	25.3
	4	283	31.8	239	26.9
	7	274	30.8	197	22.2
Mucor	2	257	20.3	227	18.0
F_3	3	340	26.9	267	21.1
	4	378	29.9	292	23.1
	7	311	24.6	249	19.7
LSD(0.05)	Time (T)	45.04		45.04	
	Microrganism(m)	48.47		48.47	
	T . M	101.18		101.18	

* Media containing 20 mg·l^{-1} heavy metals solution (Fe, Mn, Zn, Cu) .
** CUE = (C – consumed / C – initial) 100 .

Table 3. Microbial biomass, consumed carbon and biomass factor for different microorgansms in control and media containing 20 mg·l^{-1} heavy metals on the 4th day of incubation

Microorganisms	Biomass mg100ml^{-1}		Consumed carbon mg100ml^{-1}		*Biomass factor (%)	
	Control	Treated	Control	Treated	Control	Treated
Pseudomonas P_{13}	107.86	26.60	219	205	49.25	12.98
Bacillus B_{25}	109.81	18.83	292	222	37.61	8.48
Streptomyces S_2	92.20	31.23	283	239	32.58	13.07
Mucor F_3	193.6	62.3	378	292	51.22	21.34

* Biomass factor = (Biomass mg100ml^{-1} / Consumed carbon mg100ml^{-1}) 100.

Table 4. Nitrogen- consumed and nitrogen utilization efficiency (NUE) exhibited by different microorganisms as affected by heavy metals

Microorganisms	Time (days)	Nitrogen - consumed			
		Control media		Treated media *	
		mg 100ml^{-1}	NUE % **	mg 100ml^{-1}	NUE % **
Pseudomonas	1	9.72	35.1	8.94	32.3
P_{13}	2	11.65	42.1	10.65	38.4
	3	12.67	45.7	11.67	42.1
	4	13.74	49.6	13.45	48.6
	7	13.75	49.6	13.75	49.6
Bacillus	1	8.34	30.1	7.03	25.4
B_{25}	2	10.68	38.6	9.73	35.1
	3	11.69	42.2	10.46	37.8
	4	13.35	48.2	13.53	48.8
	7	13.21	47.7	13.75	49.6
Streptomyces	2	6.65	24.0	6.11	22.1
S_2	3	8.60	31.0	9.39	33.9
	4	13.65	49.3	12.89	46.5
	7	13.74	49.6	12.65	45.7
Mucor	2	9.68	34.9	8.85	31.8
F_3	3	13.85	50.0	11.25	40.6
	4	15.72	56.8	13.06	47.1
	7	19.57	70.6	14.87	53.7
LSD(0.05)	Time (T)	1.24		1.24	
	Microrganism (m)	1.34		1.34	
	T. M	2.78		2.78	

* Media containing 20 mg·l^{-1} heavy metals solution (Fe, Mn, Zn, Cu) .

** NUE = (N – consumed / N - initial) 100 .

Table 5. Consumed Nitrogen, biomass per unit of consumed nitrogen and C/N ratio for different microorgansms in control and media containing 20 mg·l^{-1}heavy metals on the 4th day of incubation

Microorganisms	Consumed –N mg100ml^{-1}		Biomass / Consumed -N		C : N Ratio	
	Control	Treated	Control	Treated	Control	Treated
Pseudomonas P_{13}	13.74	13.45	7.85	1.97	15.94	15.24
Bacillus B_{25}	13.35	13.53	8.23	1.39	21.87	16.40
Streptomyces S_2	13.65	12.89	6.75	2.42	20.73	18.54
Mucor F_3	15.72	13.06	12.32	4.77	24.05	22.36

Phosphorus consumption

It is apparent from the data presented in (Table 6) that all tested bacteria and *Streptomyces* species consumed a higher amount of phosphorus in media containing 20 mg·l^{-1} heavy metal mixtures than that observed in control during the first 1-2 days of incubation whereas *Mucor* species showed an opposite trend. With advancement of incubation time phosphorus utilization efficiency (PUE) was increased by both bacterial strains in control as compared to media containing heavy metals. *Mucor* and *Streptomyces* species also exhibited the same trend on the 7th day of incubation. Comparing PUE by the microorganisms under study, it was found that the highest PUE was recorded in the case of *Mucor* (57.4 %) followed by *Bacillus* (38.2 %) whereas the lowest PUE was detected in the case of *Streptomyces* and *Pseudomonas*.

Results also revealed that the amount of biomass per unit of consumed phosphorus exhibited large variations among microorganisms, being 31.5, 27.5, 23.2 and 18.7 units in heavy metal-free media for *Mucor*, *Bacillus*, *Streptomyces* and *Pseudomonas*, respectively, on the 4th day of incubation. The corresponding values for media containing 20 $mg \cdot l^{-1}$ heavy metals were 6.4, 5.0, 10.1 and 7.3.

Table 6. Phosphorus- consumed and phosphorus utilization efficiency (PUE) exhibited by different microorganisms as affected by heavy metals

Micro-organisms	Time (days)	Phosphorus - consumed			
		Control media		Treated media *	
		mg $100ml^{-1}$	PUE %**	mg $100ml^{-1}$	PUE %**
Pseudomonas	1	1.97	11.1	3.48	19.6
P_{13}	2	2.03	11.4	3.14	17.7
	3	4.89	27.5	3.70	20.8
	4	4.78	25.2	4.65	26.2
	7	5.51	31.0	2.40	13.5
Bacillus	1	0.83	4.7	3.03	17.0
B_{25}	2	1.85	10.4	3.20	18.0
	3	6.82	38.4	3.30	18.6
	4	4.00	22.5	3.80	21.4
	7	5.45	30.7	2.39	13.4
Streptomyces	2	0.98	5.5	3.19	17.9
S_2	3	1.77	10.0	4.20	23.6
	4	3.97	22.3	3.67	20.6
	7	4.62	26.0	2.70	15.2
Mucor	2	10.34	58.2	9.45	53.1
F_3	3	7.40	41.6	9.22	51.9
	4	6.14	34.5	9.77	54.9
	7	10.24	57.6	8.72	49.0
LSD(0.05)	Time (T)	1.06		1.06	
	Microrganism(M)	1.07		1.07	
	T . M	2.21		2.21	

* Media containing 20 $mg \cdot l^{-1}$ heavy metals solution (Fe, Mn, Zn, Cu)
** PUE = (P – consumed / P - initial) 100 .

Moreover, it may be observed that C-consumed: P- consumed ratios were lower in media containing heavy metals as compared with control on the 4th day of incubation, ranging from 29.9 to 65.1 and from 45.8 to 73.0, respectively, whereas N:P ratios did not show the same trend for all treatments. C:N:P ratios ranged from 45.8: 2.87 :1 to 73: 3 : 1 for control and from 29.9 : 1.97 :1 to 65 : 2.9 : 1 for treated media, i. e. the microorganisms grown in control media consumed more carbon than treated media.

Discussion

There is now great awareness of the potential dangers of environmental pollution of heavy metal compounds. The discharge of metals bearing wastewaters and industrial wastes cause severe damages to aquatic and soil microorganisms. The high

concentrations of heavy metals are toxic for most microorganisms causing a deleterious effect on many biological processes and recycling of nutrients in soil and consequently soil fertility, such as nitrogen fixation, minerilization of organic matter, ammonification and nitrification [20, p. 88].

In this investigation four microorganisms isolated from calcareous soil and ground water, were tested for their ability to grow at different concentrations of heavy metals (Fe, Mn, Zn, and Cu). These heavy metals were chosen because they are essential trace bioelements as micronutrients for both microorganisms and plants in spite of their toxicity at high concentrations for most living organisms. The results clearly indicated that heavy metals concentrations (5 to 30 $mg \cdot l^{-1}$) had a stimulatory effect on both bacterial strains (*Pseudomonas* P_{13} and *Bacillus* B_{25}) during the first 24 hours of incubation (exponential phase), thereafter a deleterious effect was noticed up to the end of the experiment (7 days). This may be attributed to adsorption of high amount of heavy metals on the cell surface thus decreasing the rate of their uptake by bacterial cells. The elapse of time led to accumulate heavy metals in bacterial cells to become more toxic on the biological system of cells. *Streptomyces* and *Mucor* species were more sensitive to high concentration of heavy metals where they showed a considerable growth only at 5 to 10 $mg \cdot l^{-1}$ as compared to bacterial strains. This finding is in line with that observed by Rayner and Salder [12, pp.39-47]. They found that small amount of Zn (60 uM) in the medium decreased lag phase , increased the growth rate and yield of *Pseudomonas putida*.

Stanier *et al.* [21, pp. 276-292] mentioned that cells in the stationary phase are small relative to cells in the exponential phase, since cell division continues after increase in mass has stopped, and they are more resistant to adverse physical and chemical agents. Martin [22] and Mac Donald and Martin [23] reported that the essential metals might also exert toxic effects if their concentrations are raised to high levels. Alig *et al* [24] stated that the binding of heavy metals by microorganisms referred to adsorption on the cell surface, transport through the cell membrane and entrapment by cellular components. Bacterial cells have a larger interface for interaction with external metalic ion than other microorganisms. It means that bacterial cells offer the greatest amount of space for interaction with the external milieu [25-27]. Silver and Misra [28] reported that resistance strategies generally involve a decrease in susceptibility to heavy metals by an alteration in structure and /or quantity of the susceptible enzymes, a decrease in heavy metal membrane permeability, active transport (efflux) of heavy metal from the cell or a variety of detoxification mechanisms which are often plasmid mediated. Eradi *et al.* [27] stated that metal sulfide compounds and many metal phosphate compounds represent simple microbial detoxification mechanisms because the resistant insoluble heavy metal precipitates are relatively non toxic. This precipitate is quite often retained in the vinicity of the cell by being bound to the capsule of the walls.

High levels of heavy metals also affected consumption of C, N and P. This may be referred to the effect of heavy metals on the cell wall and plasmalemma and

consequently on permease enzymes decreasing the permeability of these essential nutrients into the cytoplasm. On the other hand, the accumulation of heavy metals inside microbial cells had an inhibitory effect on the utilization of these macronutrients decreasing the biosynthesis rate, which led to a decrease in the growth parameters [25,28].

Conclusion

In conclusion, the results reported in this investigation showed that the high concentration of heavy metals led to a decrease in the microbial biomass as well as their biological activity, specially the consumption of carbon source. *Mucor* F_3 was the most resistant organism to the high concentrations of heavy metals (Fe, Mn, Zn and Cu) where 26.1 % and 35.7 % reduction of growth was observed at 20 and 30 $mg \cdot l^{-1}$, respectively. This was followed by *Bacillus* B_{25} (36.9 % and 45.9 % reduction in growth respectively). Conversely, *Pseudomonas* and *Streptomyces* were more sensitive to the highest levels. The reduction of their growth ranged from 60.6 % to 82.7 % as compared to control.

It is clear from the foregoing discussion that the discharge of metal bearing materials, like waste waters on soil and natural waters can lead to severe damage of the microflora especially those playing an important role in soil fertility. On the other hand, these metals may interact with other components of soil and water producing more toxic materials. Misra *et al.* [29] reported that the discharge of metal bearing waste waters into natural water do not stay in isolation; but interact with other components of water producing multiple species with different levels of toxicity. Smith and Cook [30] stated that ferrous iron, which is the trigger for ethylene production in soil was produced anaerobically. The ethylene then diffuses to inhibit the growth of aerobes.

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تأثير الفلزات الثقيلة علي النشاط الحيوي لبعض ميكروبات التربة والمياه الجوفية

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ص ب : ٢٤٦٠ ، الرياض ١١٤٥١ المملكة العربية السعودية .

(قدم للنشر في ١٤١٩/١١/٧هـ؛ وقبل للنشر في ١٤٢٠/٨/١٢هـ)

ملخص البحث: تم دراسة النشاط الحيوى لأربعة سلالات من الكائنات الحية الدقيقة (*Pseudomonas* P13 و*Bacillus* B25، *Streptomyces* S2؟ *Mucor* F3) المعزولة من التربة الجيرية والمياه الجوفية في منطقة الرياض- المملكة العربية السعودية وذلك أثناء نموها في تركيزات مختلفة من الفلزات الثقيلة (الحديد- المنجنيز - الزنك -- النحاس) . ووجد أن جميع المستويات المختبرة من العناصر الثقيلة (٥ - ٣٠ مجم/ لتر) شجعت نمو *Pseudomonas* P13 و *Bacillus* B25 خلال اليوم الأول من التحضين وبعد ذلك انخفض معدل النمو ليكون أقل من تجربة المقارنة . كما أن التركيزات المنخفضة من الفلزات الثقيلة (٥- ١٠ مجم /لتر) شجعت نمو *Streptomyces* S2 و *Mucor* F3 . وكانت كمية الكربون المستهلك وكفاءة الإنتفاع منه عالية في تجربة المقارنة عنه في المعاملات. كما لوحظ أيضا أن الميكروبات المختبرة قد أظهرت قدرا أكبر من النمو لكل وحدة من كل من الكربون والنيتروجين والفوسفور المستهلك في المزارع الحالية من الفلزات الثقيلة بالمقارنة مع المعاملة. وكان فطر *Mucor* F3 أكثر الميكروبات تحملا للتركيزات المرتفعة من الفلزات الثقيلة حيث أنه حفض النمو بمقدار ٢٦,١ ٪ و ٣٥,٧ ٪ عند تركيز ٢٠ و ٣٠ مجم / لتر علي التوالي. بينما أظهرت كل من *Pseudomonas* P13 و *Streptomyces* S2 حساسية ملحوظة للتركيزات العالية.