EXPERIMENTAL RESULTS

Results

(I): Bioremediation of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions using different fungi

(I-1): isolation and identification of fungi:

Fungal isolates were collected from local soil. The isolation technique was described before (material and method) at page (48). These fungal isolates were identified according to Gilman (1971); Watanabe (2002).

The isolated fungi belonging to twelve species namely *Aspergillus niger*, *Aspergillus terreus*, *Cunninghamella echinulata*, *Curvularia lunata*, *Fusarium oxysporum*, *Mucor indicus*, *Nigrospora sphaerica*, *Paecilomyces lilacinus*, *Penicillium chrysogenum*, *Scopulariopsis brevicaulis*, *Stachybotrys chartarum*, and *Trichoderma* viride.

(I-2): Screening for the sensitivity of fungal isolates against different concentrations of heavy metal ions:

It was necessary to study the effect of some heavy metals on the viability and growth of the isolated fungi. twelve fungal species that were isolated by the dilution plate technique (material and method; page 48) and were identified as *Aspergillus niger, Aspergillus terreus, Cunninghamella echinulata, Curvularia lunata, Fusarium oxysporum, Mucor indicus, Nigrospora sphaerica, Paecilomyces lilacinus, Penicillium chrysogenum, Scopulariopsis brevicaulis, Stachybotrys chartarum,* and *Trichoderma* viride as well as the strain *S. cerevisiae* (that was obtained from Holw Elsham Company, 6th of October city, Egypt).

The isolated fungal were grown for seven days at 27 C on the glucose peptone broth media (see page 50) amended with different concentration (50, 100, 150 and 200 mg/l) of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions. After incubation period, the cultures were filtered, and the fungal mats were dried in the oven at 60 C and their weights were determined.

Table 4 includes the average values of dry weights of the isolated fungi grown in glucose-peptone media amended and not amended with different concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions. According to these results, the fungal isolates can be classified, with respect to their viability and growth into four groups:

- **Group A:**

Cultures which are completely inhibited (no growth) in the presence of four metal ions at any concentrations. This group includes four species namely; *C. echinulata*, *C. lunata*, *N. sphaerica* and *P. lilacinus*

- **Group B:**

Cultures which can survive feeble with one metal but completely inhibited with others. This group includes *S. chartarum* which survive feeble with Cd^{2+} and Cu^{2+} (50 mg/l) only; and *S. brevicaulis* which is completely inhibited with Pb^{2+} and As^{5+}.

- **Group C:**

Cultures reveal viability and growth depending on the metal concentration; where the lowest metal concentration is accompanied with the highest fungal growth, and vice versa. This group includes *F. oxysporum*, *M. indicus* and *T. viride.*

- **Group D:**

Cultures show the highest viability and growth (high resistance to metal ions). This group includes four species *A. niger*, *A. terreus*, *P. chrysogenum* and *S. cerevisiae*. *A. terreus* was completely inhibited with Cd^{2+} ion at 150 and 200 mg/l while *P. chrysogenum* was completely inhibited with Cd^{2+} ion at 200 mg/l only.

The data obviously reveal a regular relation between the concentration of metal ions and the amount of fungal growth; where the highest metal concentration is accompanied with the lowest fungal growth, Also, the data

in table 4 clearly establish the *A. niger, A. terreus, P. chrysogenum,* and *S. cerevisiae* showed high tolerant against the highest concentration of four metal ions except Cd^{2+} ion which completely inhibit the growth of *A. terreus* and *P. chrysogenum*.

Based on these results in table 4, *A. niger, A. terreus, F. oxysporum, M. indicus, T. viride, P. chrysogenum, S. cerevisiae, S. brevicaulis,* were tested for further studies in the present investigation due to their highest viability and growth in the presence of different concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions.

Table 4: Average values of fungal dry weights (g/100ml) after 7 days growth of the isolated fungi on glucose-peptone broth media amended and not amended with (50-200 mg/l) of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions

Metal ions / Isolated fungi	Control	Cadmium				Copper				Lead				Arsenate			
		50	100	150	200	50	100	150	200	50	100	150	200	50	100	150	200
Aspergillus niger	7.913	6.341	5.847	3.021	2.519	7.441	5.141	4.077	3.200	5.170	4.631	3.722	3.014	5.563	4.254	3.320	2.436
Aspergillus terreus	7.662	6.731	5.561	---	---	7.521	7.116	6.371	5.075	5.761	4.714	3.841	3.000	5.821	3.325	2.721	2.140
Cunninghamella echinulata	1.005	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Curvularia lunata	1.764	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Fusarium oxysporum	4.301	3.876	3.588	---	---	4.007	3.714	3.210	2.894	3.874	3.545	2.853	2.081	2.875	2.012	---	---
Mucor indicus	3.161	2.308	1.699	---	---	2.721	1.613	1.128	---	2.763	1.888	1.317	0.943	2.874	2.481	---	---
Nigrospora sphaerica	2.029	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Paecilomyces lilacinus	1.772	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Penicillium chrysogenum	4.864	4.164	3.871	2.253	---	3.542	2.855	2.481	2.219	4.121	3.677	3.065	2.431	3.980	3.552	2.652	1.882
Saccharomyces cerevisiae	2.531	2.000	1.421	1.020	0.683	2.158	1.771	1.412	1.084	2.448	2.211	1.764	1.49	2.017	1.821	1.210	0.762
Scopulaupsis brevicaulis	2.004	1.662	1.007	---	---	1.392	1.000	0.832	0.611	---	---	---	---	---	---	---	---
Stachybotrys chartarum	2.115	1.084	---	---	---	1.394	---	---	---	---	---	---	---	---	---	---	---
Trichoderma viride	4.541	4.047	3.226	---	---	3.542	2.714	2.000	1.621	3.723	3.109	2.572	1.988	3.707	3.241	---	---

(II) Determination of minimum inhibitory concentration (MIC) of Cadmium, Copper, Lead and Arsenic against tested fungi:

The minimum inhibitory concentration (MIC) values are important to confirm the degree of resistance of fungi to each metal ions, this experiment was carried out to determine the MIC of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions against the eight fungal species (*A. niger, A. terreus, F. oxysporum, M. indicus, P. chrysogenum, S. cerevisiae, S. brevicaulis and T. viride*) which tested from the total isolates due to their highest resistance to heavy metals.

The eight fungal isolates were grown for seven days at 27 C on the glucose peptone media supplemented with different concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions separately. Triplicate flasks were used for each treatment (see page 53). The MIC was determined here as the lowest concentration of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} salts that will inhibit the viable growth of the tested fungi.

Table 5 includes the average values of the MIC of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions against the tested fungi. The data reveal that the MIC of Cu^{2+} to *A. terreus, A. niger* and *P. chrysogenum.* (600, 550 and 500 mg/l) was higher than that of other tested fungi, while *F. oxysporum.* and *S. cerevisiae* had similar value (450 mg/l). *T. viride, S. brevicaulis* and *M. indicus* had the lowest Cu^{2+} MIC values (350, 300 and 200 mg/l).

A. terreus, S. brevicaulis, F. oxysporum, M. indicus and *T. viride* had the same Cd^{2+} MIC values of 150 mg/l, while *S. cerevisiae, A. niger* and *P.*

chrysogenum could resist more Cd^{2+} concentrations with MIC values 300, 250 and 200 mg/l respectively.

Concerning to As^{5+} resistance, *A. terreus*, *A. niger*, *S. cerevisiae* and *P. chrysogenum* were the most resistance fungi with MIC values 350, 300, 250 and 250 mg/l while *S. brevicaulis* was the most sensitive fungi with MIC values 50 mg/l. *M. indicus*, *F. oxysporum* and *T. viride* showed suitable resistance with the same MIC value of 150 mg/l.

The MIC values of Pb^{2+} revealed that, *A. niger* was the most resistant fungus, where it could resist Pb^{2+} till concentration 650 mg/l. *A. terreus* and *S. cerevisiae* were in the second rank with the same MIC values of 500 mg/l while *T. viride*. was placed in the third rank with 400 mg/l as MIC. *M. indicus*, *F. oxysporum* and *P. chrysogenum* had the same MIC value of 350 mg/l in the fourth rank, finally *S. brevicaulis* was placed in the bottom with 50 mg/l MIC.

Table 5: Average values of the Minimum inhibition concentration (mg/l) of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} against tested fungi

MIC / Fungi	Copper	Cadmium	Arsenate	Lead
A. niger	550	250	300	650
A. terreus	600	150	350	500
F. oxysporum	450	150	150	350
M. indicus	200	150	150	350
P. chrysogenum	500	200	250	350
S. cerevisiae	450	300	250	500
S. brevicaulis	300	150	50	50
T. viride	350	150	150	400

(III) Bioaccumulation of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions by the tested fungi:

The term bioaccumulation refers to heavy metals passing into the cell membranes through the cell metabolic cycle. This mode of metal uptake is dependent on the biological metabolic cycle and is known as "active uptake". This experiment was designed to follow up the ability of the tested fungi to accumulate the selected heavy metals in their growing mats.

The tested fungi were grown separately in conical flasks (250 ml) each containing 100 ml of sterilized glucose-peptone broth amended and not amended with 100 mg/l of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+}. The flasks were then incubated at 27 C for 2, 4, 7 and 14 days. After which, the filtrate of each treatment was analyzed for the final metal concentration (see page 54) and the percentage of metal ions removed by the fungi were calculated from the following equation:

$$\% \text{ removal} = (C_i - C_f / C_i) * 100 \quad \text{where}$$

C_i: initial metal concentration (100 mg/l),

C_f: final or residual metal concentration.

Triplicate set of flasks were carried out for each treatment and the average values were calculated. The data were collected in the tables (6, 7, 8 and 9) and represented graphically in the figures (2, 3, 4 and 5).

It is obvious from the data presented in the tables that all tested fungi able to grow and tolerate 100 mg/l Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions in growth media except *S. brevicaulis* that was inhibited by 100 mg/l Pb^{2+} and As^{5+}. This tolerance was varied among tested fungi and this reflected on their bioaccumulation capacity, where the high tolerance accompanied by high bioaccumulation capacity.

Table 6 and figure 2 showed the bioaccumulation of Cd^{2+} ions by eight fungi after 2, 4, 7 and 14 days of growth. The data reveal that the maximum Cd^{2+} removal was 70.74% by *A. niger* while the minimum Cd^{2+} removal was 12% by *M. indicus* after 14 days incubation. The bioaccumulation capacity of the remaining fungi can be arranged in the following descending order *S. cerevisiae* > *A. terreus* >*P. chrysogenum*> *F. oxysporum*> *T. viride* > *S. brevicaulis* with % removal values 68.29, 56.32, 50.45, 36.72, 34.99 and 17.02 % respectively.

Incubation period played an essential role for cadmium removal and bioaccumulation where the bioaccumulation capacity increased with time till saturation that done for tested fungi during 4-7 days. For example, *A. niger* removed 55.75% Cd^{2+} during first two days incubation but the value increased to 70.74% after 14 days. *A. niger, M. indicus,* and *S. cerevisiae* reached saturation during first 4 days while *A. terreus, S. brevicaulis, T. viride, F. oxysporum* and *P. chrysogenum* reached saturation during 7 days incubation.

Table 6: Average values of Cadmium ions Bioaccumulation (% of removal) by the tested fungi after two, four, seven and fourteen days

fungi / days	*A. niger*		*A. terreus*		*F. oxysporum*		*M. indicus*		*P. chrysogenum*		*S. cerevisiae*		*S. brevicaulis*		*T. viride*	
	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal
2	44.25	55.75	68.39	31.61	73.45	26.56	93.7	6.3	62.26	37.74	53.01	46.99	91.46	8.54	86.14	13.86
4	33.54	66.46	51.07	48.93	68.21	31.79	88.61	11.39	54.23	45.77	34.8	65.2	88.61	11.39	78.54	21.46
7	30.48	69.52	47.77	52.23	63.99	36.01	87.8	12.2	50.67	49.33	32.46	67.54	83.42	16.58	65.21	34.79
14	29.26	70.74	43.68	56.32	63.28	36.72	88	12	49.55	50.45	31.71	68.29	82.98	17.02	65.01	34.99

C_f: final metal concentration (mg/l)
C_i: initial metal concentration (100 mg/l)

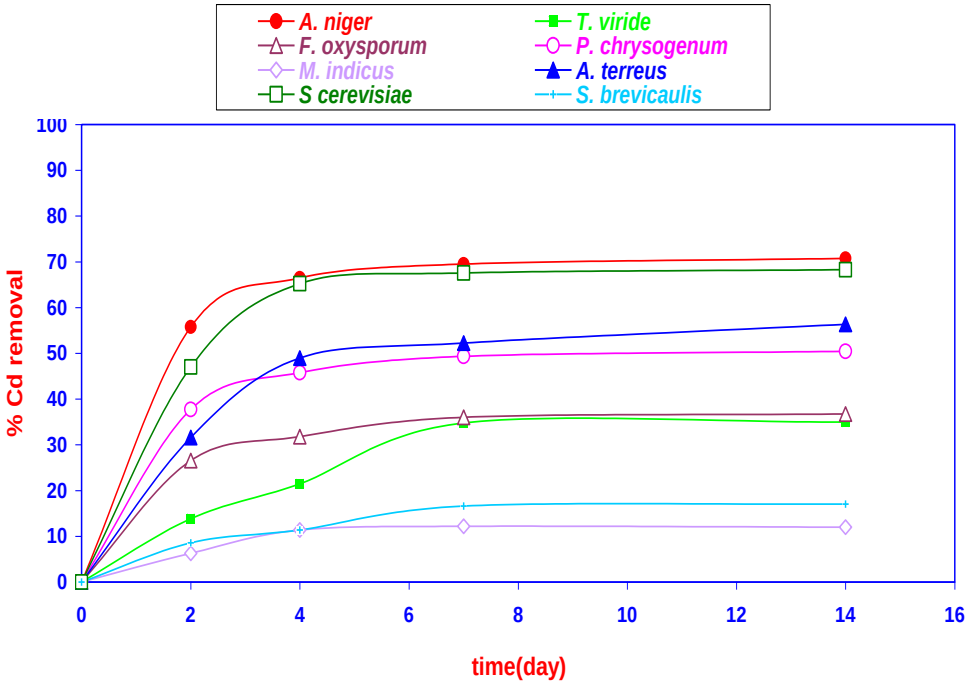


Fig 2: Bioaccumulation of Cd^{2+} ions (% of removal) by the tested fungi after 2, 4, 7 and 14 days of incubation.

Table 7 and figure 3 include the bioaccumulation of Cu^{2+} ions by eight fungal isolates after 2, 4, 7 and 14 days of growth. *A. niger*, *T. viride* and *A. terreus* occupied the first place in the high Cu^{2+} ions removal that were 84.3, 78.85 and 76.34 % respectively while *M. indicus* showed the lowest value that was 14.65% after 14 days incubation. The descending arrangement of the intermediate position for Cu^{2+} ions removal was *P. chrysogenum*> *S. cerevisiae* > *F. oxysporum* > *S. brevicaulis* with % removal values 68.65, 62.87, 52.01 and 42.33% respectively.

S. brevicaulis, *M. indicus*, *T. viride*, and *P. chrysogenum* reached saturation during first 4 days while, *A. niger*, *F. oxysporum*, *A. terreus*, and *S. cerevisiae* reached saturation during 7 days incubation.

Concerning to bioaccumulation of Pb^{2+} ions by the tested fungi, table 8 and figure 4 reveal that yeast *S. cerevisiae* overcome all tested fungi in removing Pb^{2+} ions while *S. brevicaulis* failed to even survive under 100 mg/l Pb^{2+} stress. The fungal bioaccumulation capacity of Pb^{2+} ions can be arranged in the following descending order: *S. cerevisiae* > *A. niger* > *A. terreus* > *F. oxysporum*> *P. chrysogenum*> *T. viride* > *M. indicus* with % removal values 73.98, 61.97, 56.82, 42.24, 31.66, 22.33 and 11.14% respectively.

Here, saturation done during first 4 days for all tested fungi except *T. viride*, and *S. cerevisiae* that involved more time to reach saturation.

Table 7: Average values of Copper ions Bioaccumulation (% of removal) by the tested fungi after two, four, seven and fourteen days

fungi / days	*A. niger*		*A. terreus*		*F. oxysporum*		*M. indicus*		*P. chrysogenum*		*S. cerevisiae*		*S. brevicaulis*		*T. viride*	
	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal
2	53.07	46.93	45.64	54.36	56.73	43.27	89.33	10.67	56.62	43.38	64.02	35.98	76.46	23.54	60.34	39.66
4	24.13	75.87	29.52	70.48	51.39	48.61	87.45	12.55	33.16	66.84	58.02	41.98	58.98	41.02	23.03	76.97
7	16.28	83.72	25.7	74.3	50.35	49.65	87.03	12.97	31.98	68.02	39.13	60.87	57.62	42.38	20.94	79.06
14	15.7	84.3	23.66	76.34	47.99	52.01	85.35	14.65	31.35	68.65	37.13	62.87	57.67	42.33	20.15	78.85

C_f: final metal concentration (mg/l)
C_i: initial metal concentration (100 mg/l)

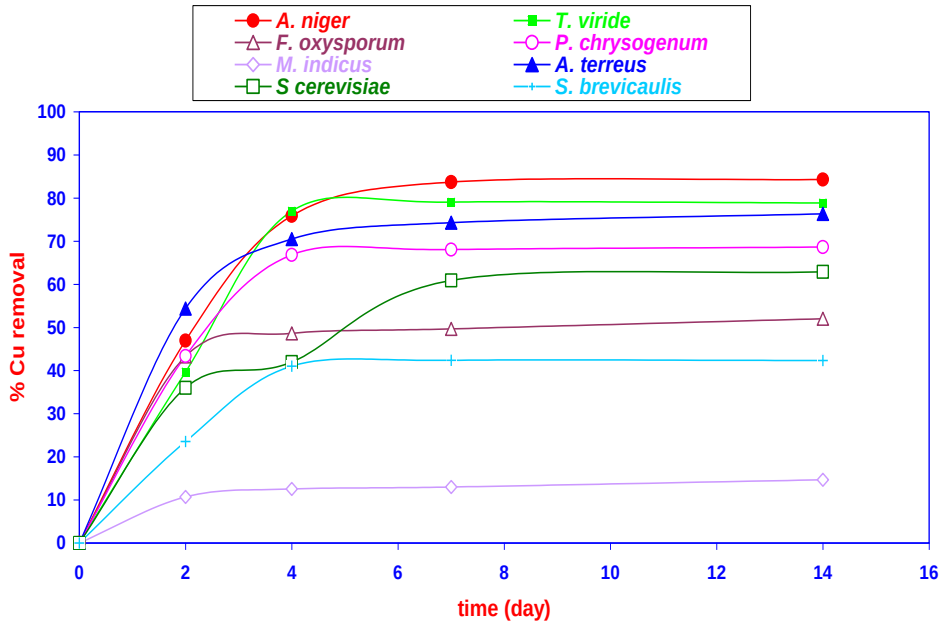


Fig 3: Bioaccumulation of Cu^{2+} ions (% of removal) by the tested fungi after 2, 4, 7 and 14 days of incubation.

Table 8: Average values of Lead ions Bioaccumulation (% of removal) by the tested fungi after two, four, seven and fourteen days

fungi / days	*A. niger*		*A. terreus*		*F. oxysporum*		*M. indicus*		*P. chrysogenum*		*S. cerevisiae*		*S. brevicaulis*		*T. viride*	
	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal
2	74.89	25.11	83.06	16.94	79.44	20.56	97.13	2.87	88.53	11.47	67.24	32.76	100	0	94.3	5.7
4	42.58	57.42	47.41	52.59	58.59	41.41	90.16	9.84	69.97	30.03	45.02	54.98	100	0	84.55	15.45
7	38.17	61.83	43.23	56.77	57.67	42.33	88.82	11.18	69.27	30.73	32.13	67.87	100	0	79.07	20.93
14	38.03	61.97	43.18	56.82	57.76	42.24	88.86	11.14	68.34	31.66	26.02	73.98	100	0	77.67	22.33

C_f: final metal concentration (mg/l)
C_i: initial metal concentration (100 mg/l)

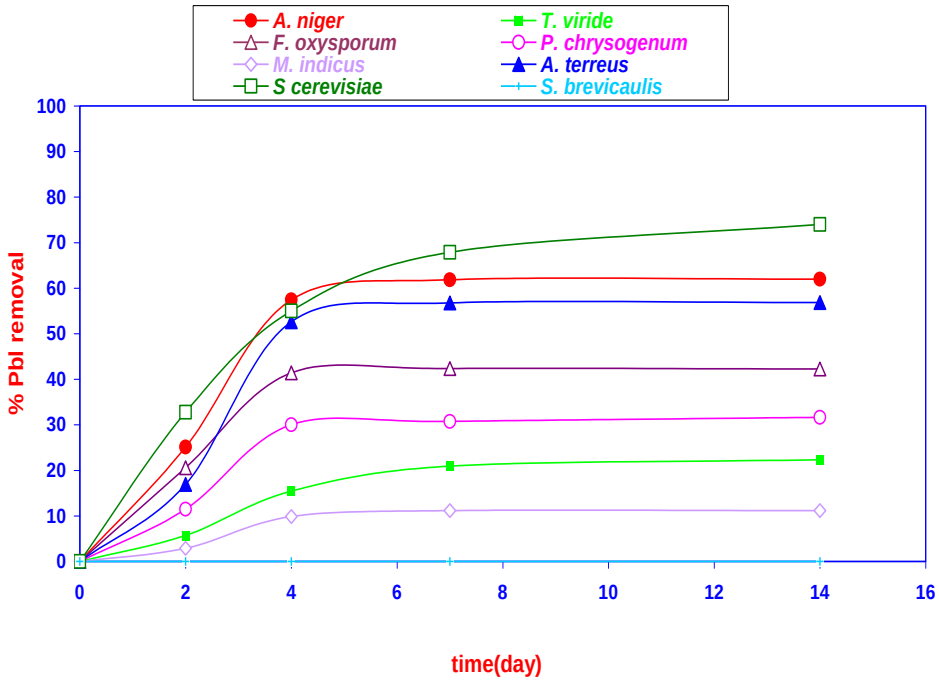


Fig 4: Bioaccumulation of Pb^{2+} ions (% of removal) by the tested fungi after 2, 4, 7 and 14 days of incubation.

From table 9 and figure 5, we can conclude that the tolerance of all tested fungi against 100 mg/l As^{5+} ions in glucose-peptone media reduced dramatically in comparison with other metals, where the removal value of 100 mg/l As^{5+} ions ranged from 61.89 to 7.55%. *P. chrysogenum, S. cerevisiae* and *T. viride* showed high resistance than *M. indicus, A. niger, A. terreus* and *F. oxysporum* while the *S. cerevisiae* failed survive under As^{5+} stress as well as 100 mg/l Pb^{2+} stress. The removal values of the tested fungi were 61.89, 55.05, 45.44, 25.64, 18.17, 11.87 and 7.55% respectively. All fungi reached saturation stat during first 4 days except *A. niger* that required 7 days to reach saturation.

Table 9: Average values of Arsenate ions Bioaccumulation (% of removal) by the tested fungi after two, four, seven and fourteen days

fungi / days	*A. niger*		*A. terreus*		*F. oxysporum*		*M. indicus*		*P. chrysogenum*		*S. cerevisiae*		*S. brevicaulis*		*T. viride*	
	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal	C_f	% removal
2	94	6	83.76	16.24	90.78	9.22	93.67	6.33	55.22	44.78	50.62	49.38	100	0	66.49	33.51
4	91.87	8.13	82.42	17.58	88.64	11.36	93.13	6.87	38.54	61.46	48.21	51.79	100	0	56.86	43.14
7	75.06	24.94	82.9	17.1	88.46	11.54	92.65	7.35	38.01	61.99	46.61	53.39	100	0	54.77	45.23
14	74.36	25.64	81.83	18.17	88.13	11.87	92.45	7.55	38.11	61.89	44.95	55.05	100	0	54.56	45.44

C_f: final metal concentration (mg/l)
C_i: initial metal concentration (100 mg/l)

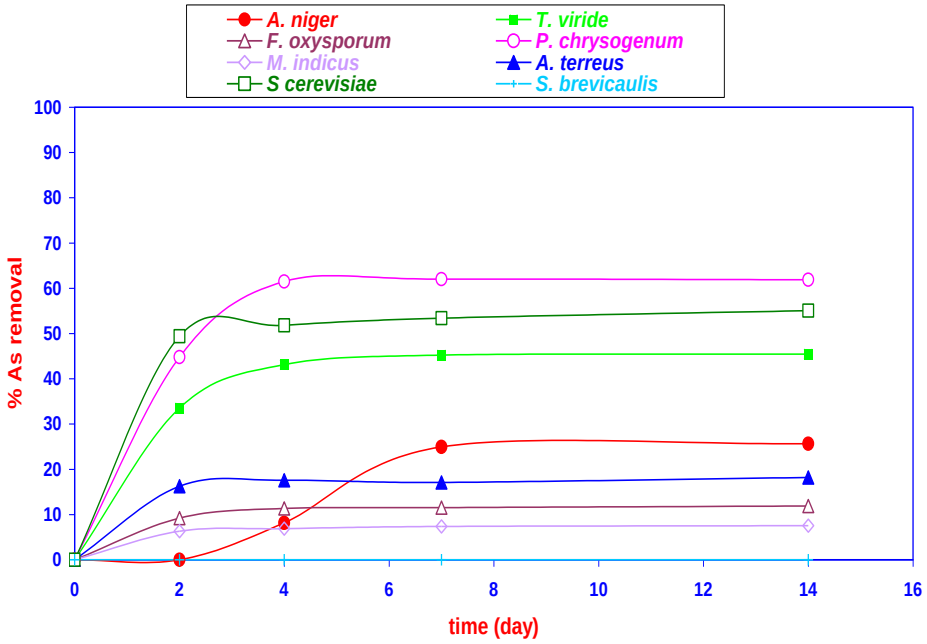


Fig 5: Bioaccumulation of As^{5+} ions (% of removal) by the tested fungi after 2, 4, 7 and 14 days of incubation.

(IV) Biosorption of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions by the dried and killed forms of tested fungi:

The term biosorption, sometimes refer to as physical adsorption, describes the ability of inactive, dead or living biomass to bind to heavy metals or contaminants present in dilute solution (passive sorption solution) there is no need to use growth media but the process was carried out using the aqueous metal solution only. This experiment was designed to follow up the biosorption capacity of the tested fungal biomass (dry and killed) to accumulate the metal ions.

The biomasses were pretreated in two different ways: dried at 50 °C for 12 hours in an incubator (still live) and autoclaved for 15 min at 121 °C (killed). Biosorption experiments were carried out by using 0.5 g biomass (dried or killed) to 50 ml of metal (100mg/l) solution in 100 ml Erlenmeyer conical flasks (pH 5.0). The reaction mixture was agitated at 150 rpm on a rotary shaker after 2, 6, 10, 24, 48 and 96 hours of contact time. The biomasses were separated by filtration and the filtrate was analyzed for residual metal concentration (see page 55 for details), and the metal uptake (q) were calculated form the following equation:

$$q= (C_i-C_f)\ V/W \qquad \text{Where;}$$

q = metal uptake (mg/g).

C_i= initial metal concentration (100 mg/l).

C_f= final metal concentration in the solution.

V= the volume containing solution in the contact with the biomass (l)
W= the amount of added biomass (0.5 g) on dry basis.

Triplicate set of flasks were carried out for each particular treatment and the average values were calculated.

The data were collected in the table (10-17) and represented graphically in the figures (6-13). The data presented in Tables (10, 12, 14 and 16) and figures (6, 8, 10 and 12) show the amounts of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} biosorbed on the dried form of biomasses of *A. niger*, *T. viride*, *F. oxysporum*, *P. chrysogenum*, *M. indicus*, *A terreus*, *S cerevisiae* and *S. brevicaulis* after 2, 6, 10, 24, 48 and 96 hours in their aqueous solution at room temperature on electrical shaker at 150 rpm, while tables (10, 12, 14, and 16) and figures (6, 8, 10 and 12) show the amounts of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} biosorbed on the killed form of biomasses at the same conditions.

Table 10 and figure 6 show the biosorption of Cd^{2+} ions by dried biomasses of *A. niger*, *T. viride*, *F. oxysporum*, *P. chrysogenum*, *M. indicus*, *A. terreus*, *S. cerevisiae* and *S. brevicaulis* after 2, 6, 10, 24, 48 and 96 hours in its aqueous solution at room temperature at 150 rpm. The data reveal that the maximum Cd^{2+} uptake was 6.496 and 6.058 mg/g by *A. terreus* and *A. niger* respectively while the minimum Cd^{2+} uptake values were 0.94 and 1.15 mg/g by *S. brevicaulis* and *M. indicus* respectively after 96 hours. The biosrption capacity of the remaining fungi can be arranged in the following descending order as *S. cerevisiae* >*P. chrysogenum*> *T. viride* > *F. oxysporum* (q values 5.546, 4.566, 3.429, and 3.053 respectively) after 96 hours contact.

Table 10: Average values of Cadmium ions Biosorption (mg/g) after 2, 6,10,24,48 and 96h using dried biomasses of the selected fungi with concentration (0.5g/50ml):

Fungi / Time (h)	*A. niger*		*A. terreus*		*F. oxysporum*		*M. indicus*		*P. chrysogenum*		*S. cerevisiae*		*S. brevicaulis*		*T. viride*	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
2	57.79	4.221	63.92	3.608	75.29	2.471	94.8	0.52	77.7	2.23	70.38	2.962	94.54	0.546	87.14	1.286
6	44.45	5.555	55.25	4.475	74.85	2.515	91.04	0.896	66.54	3.346	62.17	3.783	95.02	0.498	81.41	1.859
10	40.34	5.966	51.14	4.886	71.18	2.882	90.21	0.979	57.44	4.256	51.27	4.873	93.05	0.695	79.71	2.029
24	40.21	5.979	48.26	5.174	69.87	3.013	89.29	1.071	55.65	4.435	47.12	5.288	92.39	0.761	72.88	2.712
48	39.55	6.045	38.59	6.141	69.87	3.013	89.29	1.071	54.9	4.51	44.67	5.533	90.6	0.94	65.79	3.421
96	39.42	6.058	35.04	6.496	69.47	3.053	88.5	1.15	54.34	4.566	44.54	5.546	90.6	0.94	65.71	3.429

C_f: final metal concentration (mg/l)
q: metal uptake or biosorption (mg/g)

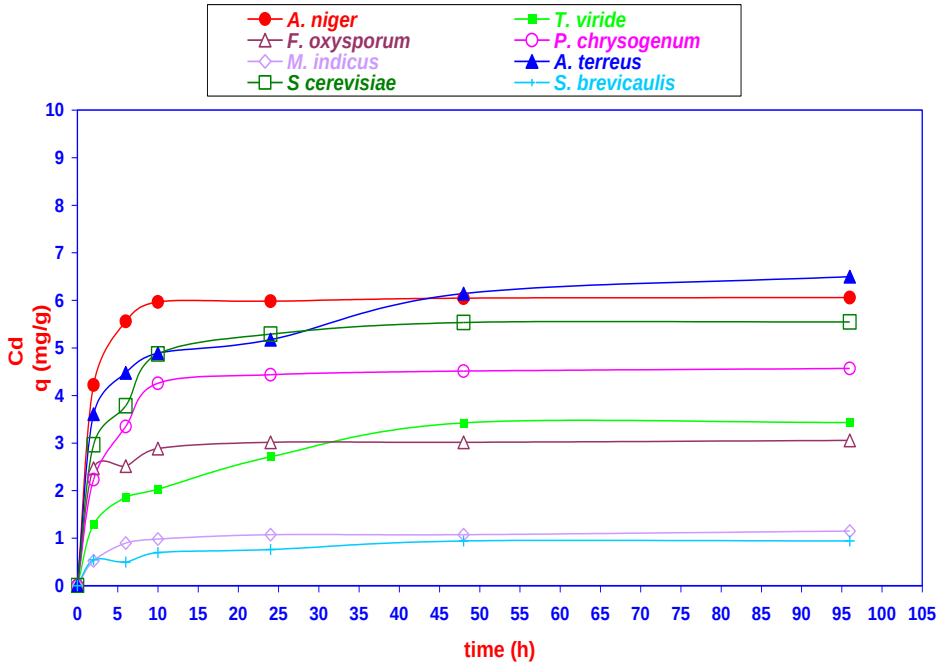


Fig 6: Biosorption of Cd^{2+} ions by dried biomasses of tested fungi after 2, 6, 10, 24, 48 and 96 hours of contact.

The biosorption of Cd^{2+} ions by killed biomasses of tested fungi after 2, 6, 10, 24, 48 and 96 hours in its aqueous solution at room temperature at 150 rpm was shown in table 11 and figure 7. Despite the dried forms of *A. terreus* and *A. niger* occupied the first position in biosorption of Cd^{2+} ions; they lowered to intermediate position (with q values 3.487 and 3.845 mg/g respectively) in biosorption of Cd^{2+} when they were killed. The highest Cd^{2+} biosorption capacities were by *P. chrysogenum* and *S. cerevisiae* with q values 6.408 and 4.352 mg/g respectively while the *M. indicus*, *S. brevicaulis*, *T. viride* and *F. oxysporum* had the lowest q values as follow: 0.936, 1.776, 1.29 and 2.506 mg/g respectively, all after 96 hours contact.

Table 12 and figure 8 demonstrate the biosorption of Cu^{2+} ions by dried biomasses of *A. niger*, *T. viride*, *F. oxysporum*, *P. chrysogenum*, *M. indicus*, *A. terreus*, *S. cerevisiae* and *S. brevicaulis*. The data reveal that all dried biomasses could perform well in biosorption of Cu^{2+} where the lowest q value was 3.075 by *M. indicus*. The descending order of Cu^{2+} biosorption by the remaining biomasses was: *A. niger* > *A. terreus* > *P. chrysogenum*> *S. brevicaulis* > *F. oxysporum*> *S. cerevisiae* > *T. viride* with q values 7.179, 6.602, 6.269, 6.081, 5.767, 5.666 and 5.033 mg/g respectively after 96 hours contact.

The killed form of *P. chrysogenum* and *S. cerevisiae* got the same results in biosorption of Cu^{2+} ions with q values 7.541 and 7.452 mg/g respectively as shown in table 13 and figure 9, while *M. indicus* still in the bottom even with its killed form with q value 1.516 mg/g. The remaining killed biomasses got the intermediate position with q value ranged from 6.684 to 3.32 mg/g after 96 hours contact.

Table 11: Average values of Cadmium ions Biosorption (mg/g) after 2, 6,10,24,48 and 96h using killed biomasses of the selected fungi with concentration (0.5g/50ml):

Time (h) \ Fungi	*A. niger*		*A. terreus*		*F. oxysporum*		*M. indicus*		*P. chrysogenum*		*S. cerevisiae*		*S. brevicaulis*		*T. viride*	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
2	76.6	2.34	80.88	1.912	81.89	1.811	92.7	0.73	86.27	1.373	57.57	4.243	85.13	1.487	89.9	1.01
6	72.1	2.79	79.79	2.021	81.85	1.815	91.91	0.809	72.23	2.777	57.57	4.243	83.16	1.684	89.77	1.023
10	70.91	2.909	73.36	2.664	81.11	1.889	91.47	0.853	70.91	2.909	57.49	4.251	82.73	1.727	89.78	1.022
24	64.35	3.565	71.02	2.898	79.66	2.034	90.6	0.94	47.77	5.223	57.18	4.282	82.94	1.706	89.33	1.067
48	61.73	3.827	67.57	3.243	78.66	2.134	90.38	0.962	38.11	6.189	56.96	4.304	82.33	1.767	87.54	1.246
96	61.55	3.845	65.13	3.487	74.94	2.506	90.64	0.936	35.92	6.408	56.48	4.352	82.24	1.776	87.1	1.29

C_f: final metal concentration (mg/l)
q: metal uptake or biosorption (mg/g)

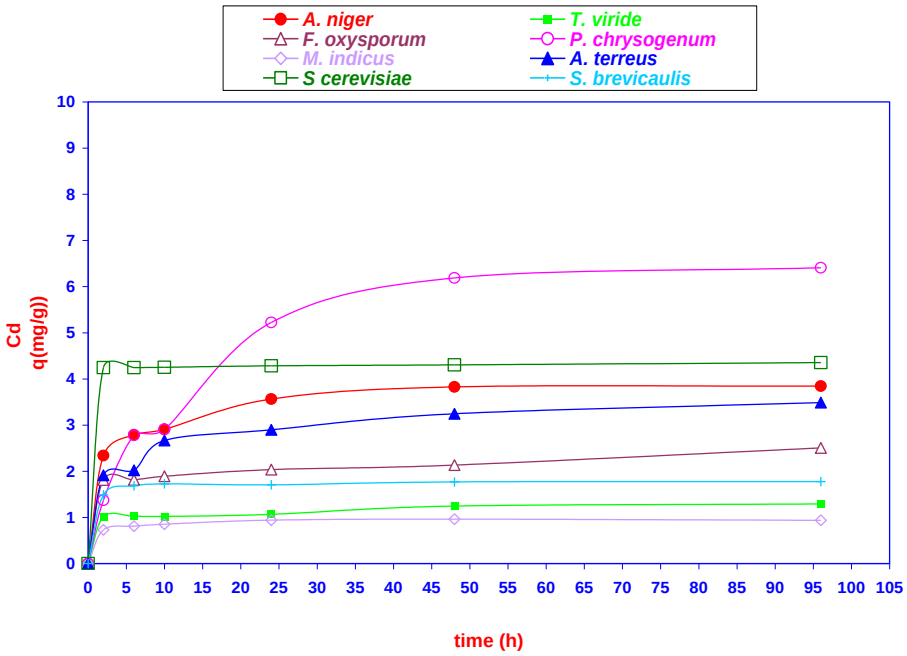


Fig 7: Biosorption of Cd^{2+} ions by killed biomasses of tested fungi after 2, 6, 10, 24, 48 and 96 hours of contact.

Table 12: Average values of Copper ions Biosorption (mg/g) after 2, 6,10,24,48 and 96h using dried biomasses of the selected fungi with concentration (0.5g/50ml):

Time (h) \ Fungi	*A. niger*		*A. terreus*		*F. oxysporum*		*M. indicus*		*P. chrysogenum*		*S. cerevisiae*		*S. brevicaulis*		*T. viride*	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
2	46.63	5.337	41.98	5.802	60.16	3.984	86.06	1.394	59.11	4.089	59.53	4.047	77.98	2.202	63.48	3.652
6	31.57	6.843	36.08	6.392	48	5.2	81.06	1.894	44.26	5.574	44.93	5.506	44.26	5.574	53.17	4.683
10	30.8	6.92	34.82	6.518	45.16	5.484	72.43	2.757	37.68	6.232	43.44	5.656	43.73	5.627	51.07	4.893
24	28.28	7.172	34.26	6.574	43.73	5.627	71.06	2.894	37.62	6.238	43.44	5.656	43.73	5.627	50.06	4.994
48	28.14	7.186	34.15	6.585	42.33	5.767	69.35	3.065	37.34	6.266	43.36	5.664	39.29	6.071	49.71	5.029
96	28.21	7.179	33.98	6.602	42.33	5.767	69.25	3.075	37.31	6.269	43.34	5.666	39.19	6.081	49.67	5.033

C_f: final metal concentration (mg/l)
q: metal uptake or biosorption (mg/g)

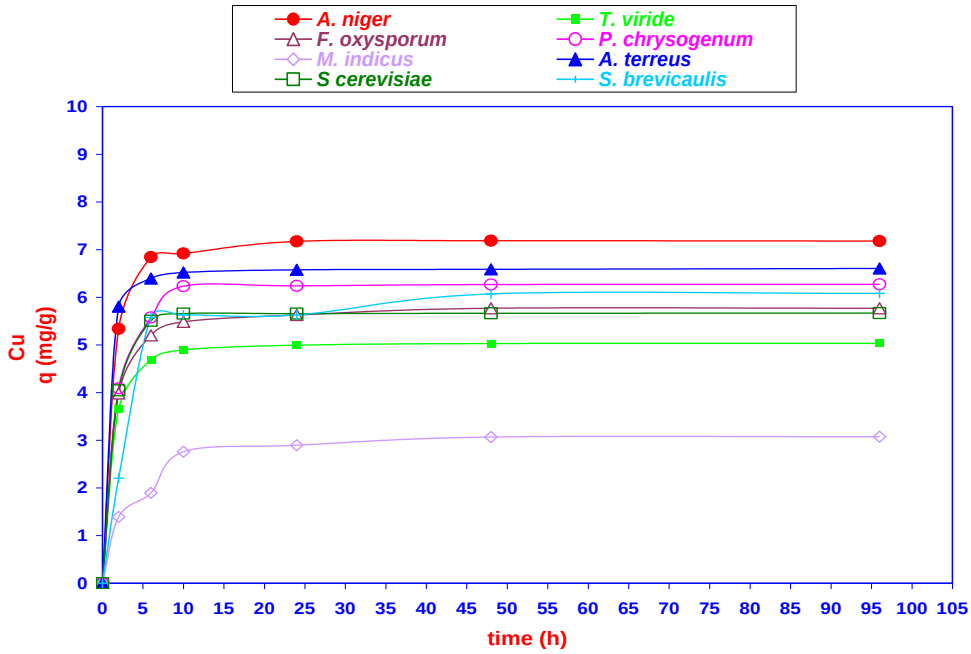


Fig 8: Biosorption of Cu^{2+} ions by dried biomasses of tested fungi after 2, 6, 10, 24, 48 and 96 hours of contact.

Table 13: Average values of Copper ions Biosorption (mg/g) after 2, 6,10,24,48 and 96h using killed biomasses of the selected fungi with concentration (0.5g/50ml):

Time (h) \ Fungi	*A. niger*		*A. terreus*		*F. oxysporum*		*M. indicus*		*P. chrysogenum*		*S. cerevisiae*		*S. brevicaulis*		*T. viride*	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
2	69.67	3.033	53.27	4.673	67.85	3.215	88.16	1.184	60.3	3.97	35.62	6.438	38.02	6.198	92.06	0.794
6	69.6	3.04	49.32	5.068	67.92	3.208	86.02	1.398	54.85	4.515	29.82	7.018	37.82	6.218	75.54	2.446
10	68.76	3.124	47.61	5.239	67.5	3.25	84.98	1.502	53.17	4.683	25.94	7.406	33.98	6.602	63.69	3.631
24	67.5	3.25	44.43	5.557	67.22	3.278	84.91	1.509	27.12	7.288	25.42	7.458	33.44	6.656	60.93	3.907
48	66.14	3.386	44.43	5.557	67.01	3.299	84.84	1.516	26.99	7.301	25.34	7.466	33.16	6.684	53.52	4.648
96	58.24	4.176	44.44	5.556	66.8	3.32	84.84	1.516	24.86	7.514	25.48	7.452	33.16	6.684	53.17	4.683

C_f: final metal concentration (mg/l)
q: metal uptake or biosorption (mg/g)

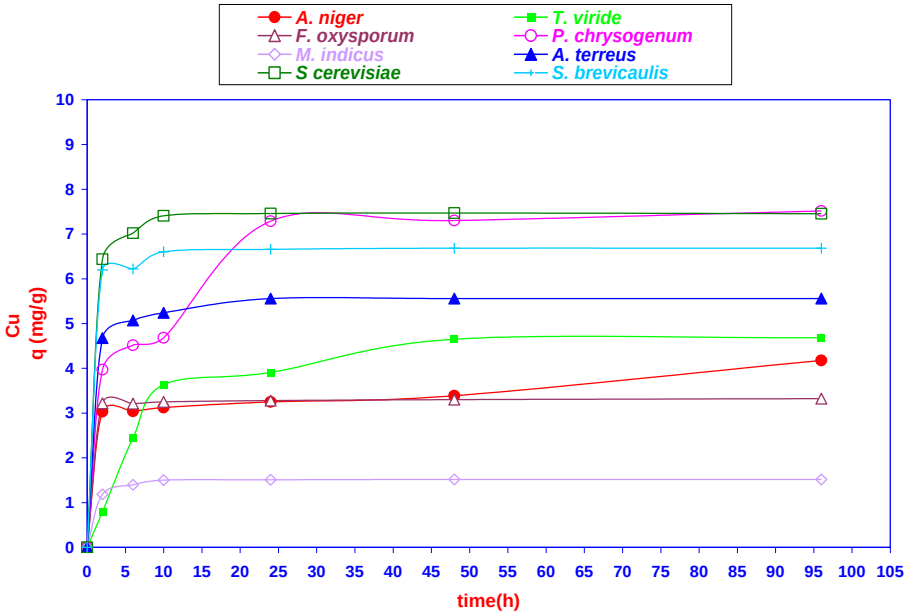


Fig 9: Biosorption of Cu^{2+} ions by killed biomasses of tested fungi after 2, 6, 10, 24, 48 and 96 hours of contact.

The biosorption of Pb^{2+} ions by dried and killed biomasses of tested fungi after 2, 6, 10, 24, 48 and 96 hours in its aqueous solution in tables 14,15 and figures 10,11 respectively. According to the results obtained in two tables, the dried form of *S. cerevisiae* and *P. chrysogenum* had the same and high Pb^{2+} biosorption capacity with q value 7.435 and 7.215 mg/g respectively while the killed form of *S. cerevisiae*, *A. niger* and *A. terreus* had the same and high Pb^{2+} biosorption capacity with q value 6.253, 6.245 and 6.211 mg/g after 96 hours contact. Both dried and killed forms of *T. viride* and *M. indicus* had the lowest Pb^{2+} biosorption capacity with q value 3.826, 2.67 mg/g for dried form, and 4.187, 2.381 mg/g for killed form respectively after 96 hours contact.

According to our data, the equilibrium stat of dried and killed biomasses with Pb^{2+} ions differ according the fungal type, where dried form of *M. indicus* and killed form of *F. oxysporum*, *S. cerevisiae* involved only 2 hours to reach saturation stat with Pb^{2+} ions. In contrast, the dried form of *P. chrysogenum*, *S. cerevisiae* and the killed form of *M. indicus* consumed more than 24 hours to reach equilibrium stat with Pb^{2+} ions.

Table 16 and figure 12 include the biosorption data of As^{5+} ions by dried biomasses of *A. niger*, *T. viride*, *F. oxysporum*, *P. chrysogenum*, *M. indicus*, *A. terreus*, *S. cerevisiae* and *S. brevicaulis*. The data reveal *A. niger* and *P. chrysogenum* showed high biosorption capacity with q value 5.586 and 5.073 mg/g respectively, while the descending order of As^{5+} biosorption by the remaining biomasses was: *A. terreus* > *S. cerevisiae* > *F. oxysporum*> *T. viride* > *S. brevicaulis* > *M. indicus* with q values 4.836, 3.991, 3.056, 2.6, 1.589 and 1.167 mg/g respectively after 96 hours contact.

Table 14: Average values of Lead ions Biosorption (mg/g) after 2, 6,10,24,48 and 96h using dried biomasses of the selected fungi with concentration (0.5g/50ml):

Time (h) \ Fungi	*A. niger*		*A. terreus*		*F. oxysporum*		*M. indicus*		*P. chrysogenum*		*S. cerevisiae*		*S. brevicaulis*		*T. viride*	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
2	47.55	5.245	53.67	4.633	56.94	4.306	80.41	1.959	59.18	4.082	50.54	4.946	77.25	2.275	64.8	3.52
6	45.03	5.497	48.58	5.142	54.09	4.591	78.63	2.137	44.39	5.561	30.37	6.963	64.76	3.524	64.94	3.506
10	35.71	6.429	45.52	5.448	52.07	4.793	74.4	2.56	42.04	5.796	26	7.4	62.2	3.78	64.05	3.595
24	31.76	6.824	44.85	5.515	49.51	5.049	72.59	2.741	42.57	5.743	25.68	7.432	52.35	4.765	63.73	3.627
48	31.82	6.818	44.53	5.547	49.51	5.049	73.51	2.649	35.25	6.475	25.75	7.425	52.03	4.797	62.81	3.719
96	31.91	6.809	44.53	5.547	43.43	5.657	73.3	2.67	27.85	7.215	25.65	7.435	49.65	5.035	61.74	3.826

C_f: final metal concentration (mg/l)
q: metal uptake or biosorption (mg/g)

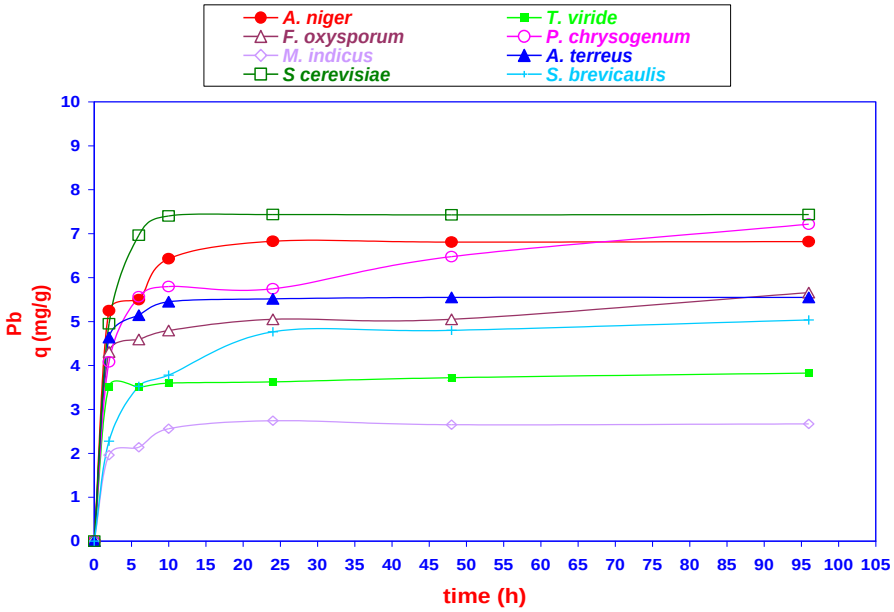


Fig 10: Biosorption of Pb^{2+} ions by dried biomasses of tested fungi after 2, 6, 10, 24, 48 and 96 hours of contact.

Table 15: Average values of Lead ions Biosorption (mg/g) after 2, 6,10,24,48 and 96h using killed biomasses of the selected fungi with concentration (0.5g/50ml):

Time (h) \ Fungi	*A. niger*		*A. terreus*		*F. oxysporum*		*M. indicus*		*P. chrysogenum*		*S. cerevisiae*		*S. brevicaulis*		*T. viride*	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
2	78.92	2.108	49.47	5.053	61.14	3.886	96.77	0.323	68.25	3.175	58.72	4.128	56.94	4.306	72.12	2.788
6	43.43	5.657	45.88	5.412	60.5	3.95	89.3	1.07	64.05	3.595	41.68	5.832	56.62	4.338	60.85	3.915
10	42.68	5.732	38.31	6.169	56.26	4.374	86.46	1.354	53.81	4.619	38.08	6.192	56.62	4.338	59.78	4.022
24	42.68	5.793	38.09	6.191	55.52	4.448	85.42	1.458	51.56	4.844	37.78	6.222	56.41	4.359	59.82	4.018
48	37.91	6.209	37.95	6.205	54.77	4.523	76.5	2.35	51.48	4.852	37.69	6.231	53.79	4.621	58.65	4.135
96	37.55	6.245	37.89	6.211	54.23	4.577	76.19	2.381	51.1	4.89	37.47	6.253	52.93	4.707	58.13	4.187

C_f: final metal concentration (mg/l)
q: metal uptake or biosorption (mg/g)

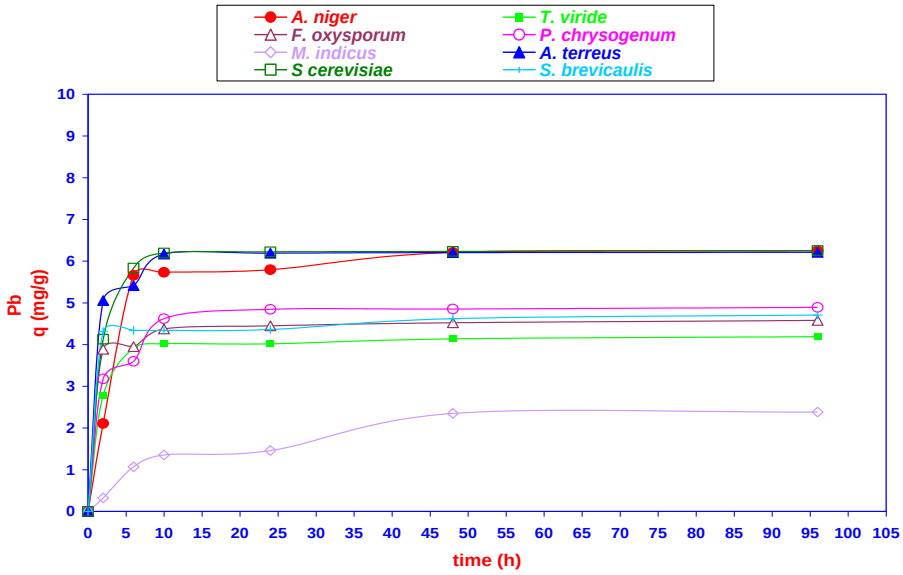


Fig 11: Biosorption of Pb^{2+} ions by killed biomasses of tested fungi after 2, 6, 10, 24, 48 and 96 hours of contact.

Table 16: Average values of Arsenate ions Biosorption (mg/g) after 2, 6,10,24,48 and 96h using dried biomasses of the selected fungi with concentration (0.5g/50ml):

Time (h) \ Fungi	*A. niger*		*A. terreus*		*F. oxysporum*		*M. indicus*		*P. chrysogenum*		*S. cerevisiae*		*S. brevicaulis*		*T. viride*	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
2	57.29	4.271	64.74	3.526	86.53	1.347	95.73	0.427	72.29	2.771	70.29	2.971	89.28	1.072	82.16	1.784
6	53.3	4.67	57.95	4.205	77.56	2.244	91.27	0.873	66.07	3.393	62.7	3.73	86	1.4	76.51	2.349
10	44.9	5.51	51.83	4.817	72.67	2.733	90.04	0.996	53.26	4.674	60.8	3.92	84.53	1.547	75.04	2.496
24	44.67	5.533	51.55	4.845	71.81	2.819	88.9	1.11	49.89	5.011	60.38	3.962	84.44	1.556	74.57	2.543
48	44.29	5.571	51.69	4.831	69.87	3.013	88.57	1.143	49.84	5.016	60.14	3.986	84.11	1.589	73.9	2.61
96	44.14	5.586	51.64	4.836	69.44	3.056	88.33	1.167	49.27	5.073	60.09	3.991	84.11	1.589	74	2.6

C_f: final metal concentration (mg/l)
q: metal uptake or biosorption (mg/g)

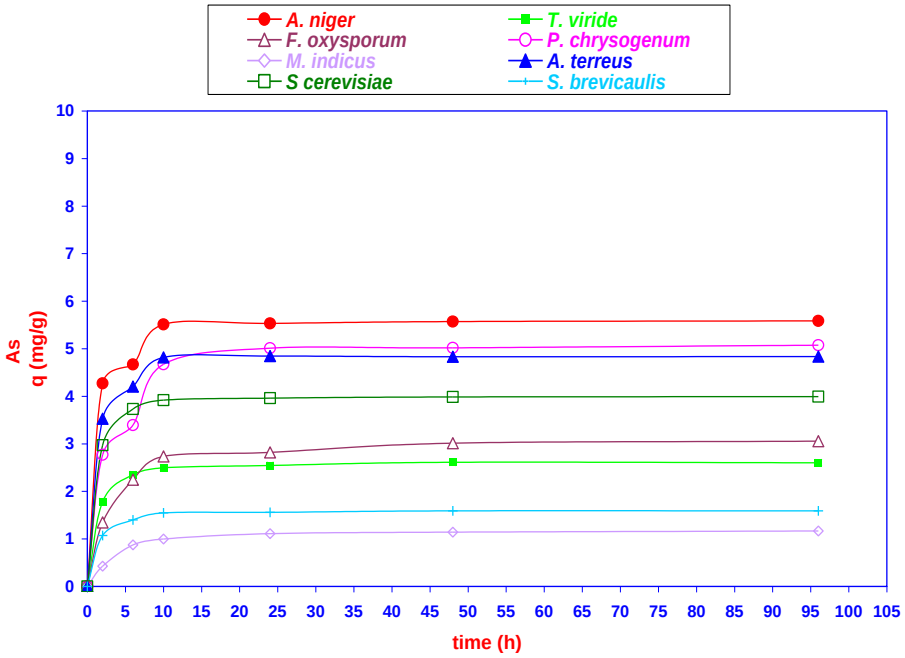


Fig 12: Biosorption of As^{5+} ions by dried biomasses of tested fungi after 2, 6, 10, 24, 48 and 96 hours of contact.

The killed form of also *A. niger* and *A. terreus* got the same and high results in biosorption of As^{5+} ions with q values 4.679 and 4.271 mg/g respectively as shown in table 17 and figure 13 while *M. indicus* still in the bottom even with its killed form and even with different metal ions with q value 0.873 mg/g. The remaining killed biomasses got the intermediate position with q value ranged from 3.588 to 1.381 mg/g after 96 hours contact.

The biosorption of As^{5+} ions by both killed and dried forms of biomasses required nearly 6 hours in order to reach the saturation stat.

In our biosorption study, dried and killed biomasses were used. It is obvious from the data presented in tables 10 to 17 and figures 6-13 that *A. niger* and *A. terreus* were the best microorganisms in removal of all metals except Pb^{2+} that was removed better by dried form of *S. cerevisiae*; also Cd^{2+} and Cu^{2+} were removed better by killed form of *P. chrysogenum.* The highest metal uptake values by dried biomasses were 6.49, 7.17, 7.43 and 5.58 mg/g while by killed biomasses were 6.4, 7.51, 6.2 and 4.67 mg/g at pH 5 for Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+}, respectively during the 96 h (saturation done during the first 6 h).

The factor of time was not essential with the same degree as in the bioaccumulation process for most tested fungi because this process is non-metabolism dependant, and faster than bioaccumulation process. Saturation of fungi with metals varied according to the individual fungus and the type of metal ions. For most fungi, saturation not requires long time but it takes place during the first 4-6 hours.

It is clear from the data presented in tables 10 to 17 that all tested fungi able to adsorb an amount of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions. This amount (q) was varied among tested fungi according to the type of metal ions, the main composition of cell wall.

Table 17: Average values of Arsenate ions Biosorption (mg/g) after 2, 6,10,24,48 and 96h using killed biomasses of the selected fungi with concentration (0.5g/50ml):

Time (h) \ Fungi	*A. niger*		*A. terreus*		*F. oxysporum*		*M. indicus*		*P. chrysogenum*		*S. cerevisiae*		*S. brevicaulis*		*T. viride*	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
2	77.56	2.244	69.63	3.037	81.69	1.831	93.08	0.692	71.39	2.861	83.11	83.11	95.26	0.474	85.48	1.452
6	57.86	4.214	63.46	3.654	75.04	2.496	92.55	0.745	69.39	3.061	76.94	76.94	88.85	1.115	84.11	1.589
10	57.1	4.29	61.32	3.868	74.99	2.501	92.6	0.74	65.07	3.493	70.63	70.63	87.95	1.205	83.68	1.632
24	53.68	4.632	60.8	3.92	73.14	2.686	91.65	0.835	65.03	3.497	70.29	70.29	86.43	1.357	83.63	1.637
48	53.45	4.655	57.53	4.247	73	2.7	91.46	0.854	64.41	3.559	69.87	69.87	85.96	1.404	83.63	1.637
96	53.21	4.679	57.29	4.271	72.81	2.719	91.27	0.873	64.12	3.588	69.44	69.44	85.19	1.481	83.58	1.642

C_f: final metal concentration (mg/l)
q: metal uptake or biosorption (mg/g)

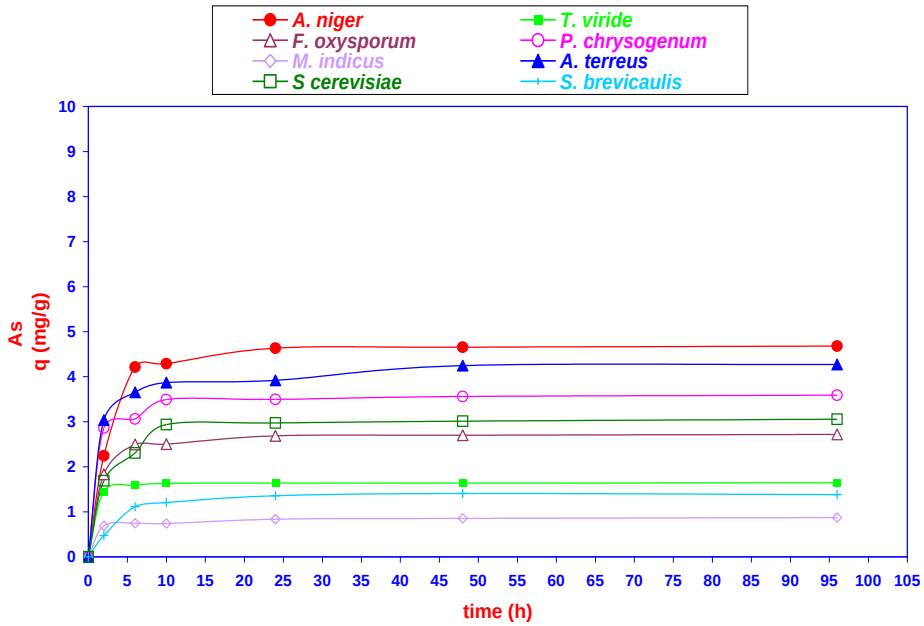


Fig 13: Biosorption of As^{5+} ions by killed biomasses of tested fungi after 2, 6, 10, 24, 48 and 96 hours of contact.

Studying various parameters that affect on Bioremediation of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions by *Saccharomyces cerevisiae*

(I-1): Effect of Different nutrient media on bioaccumulation of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions by *Saccharomyces cerevisiae*:

Five different liquid nutrient media (Glucose-peptone, Malt 4% dextrose peptone yeast broth, Malt broth-Blakeslee, YM broth and Glucose Yeast peptone broth, see material and methods page 51 and 52) were used to study their effect on the bioaccumulation of 100 mg/l of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions by *S. cerevisiae* after 4 days of growth at 27 C and pH 5. Triplicate set were used for each treatment.

Table 18 and fig 14; a &b include the dry weights and metal removal capacity of *S. cerevisiae* that was grown in different five liquid nutrient media as control and in the presence of 100 mg/l of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions. The data revealed that the nutrient media number 1 and 2 (Glucose-peptone, Malt 4% dextrose peptone yeast) showed high *S. cerevisiae* growth rate (high dry weight) and also high metal removal capacity, while the remaining three liquid media (Malt broth-Blakeslee, YM broth and Glucose Yeast peptone broth) showed lower growth rate and also lower metal removal capacity.

The dry weights of *S. cerevisiae* in the Glucose-peptone, Malt 4% dextrose peptone yeast broth, Malt broth-Blakeslee, YM broth and Glucose Yeast peptone broth media under control condition were 2.432, 2.651, 1.765,

1.118 and 1.481 g/100 ml medium respectively. These dry weights decreased in different values after the treatment of 100 mg/l of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions. The bioaccumulation process was strongly related to the yeast growth, where high *S. cerevisiae* growth rate, accompanied with high metal removal capacity.

Table 18: Average values of the effect of Different culture media on the dry weight (g/100ml) and % metal removal by growing *S cerevisiae*

Treatments / media	Control	Cadmium			Copper			Lead			Arsenate		
	Dry weight (g/100ml)	C_f	% removal	Dry wt.	C_f	% removal	Dry wt.	C_f	% removal	Dry wt.	C_f	% removal	Dry wt.
Nutrient medium 1	2.432	34.8	65.2	1.188	58.02	41.98	1.321	45.02	54.98	1.688	48.21	51.79	1.732
Nutrient medium 2	2.651	38.7	61.3	1.331	57.8	42.2	1.234	49.4	50.6	1.154	43.9	56.1	1.472
Nutrient medium 3	1.765	49.4	50.6	0.922	63.2	36.8	1.102	75.6	24.4	0.621	53.2	46.8	1.231
Nutrient medium 4	1.118	67.1	32.9	0.516	86.3	13.7	0.411	76.9	23.1	0.551	59.3	40.7	0.731
Nutrient medium 5	1.481	66.7	33.3	0.681	82.8	17.2	0.491	78.6	21.4	0.622	59.7	40.3	0.533

The growth was measured by determine the dry weight (g), incubation period was 4 days in static culture at 27 C°, pH=5, amount of inoculated cells =square disc (0.5cm^2) of four days biomass old in 100ml liquid medium, initial metal concentration was 100mg/l for all ion species.

Nutrient medium 1: GP broth
Nutrient medium 2: MDP broth
Nutrient medium 3: MB broth
Nutrient medium 4: YM broth
Nutrient medium 5: GYP broth

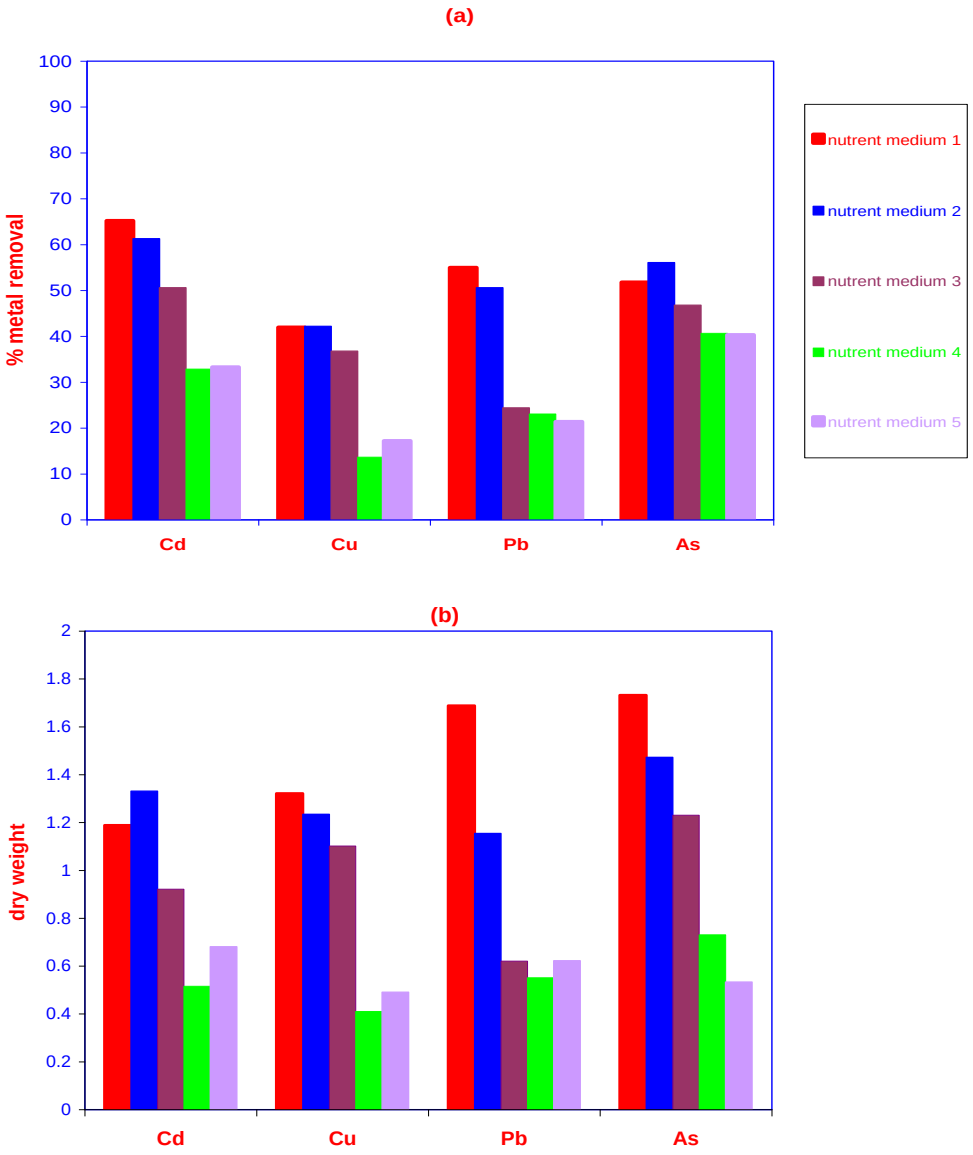


Fig (14): effect of different nutrient media on (a) metal bioaccumulation capacity of *S. cerevisiae* and (b) its growth (resembled by dry weight g/100ml medium).

(I-2): Effect of incubation period on bioaccumulation of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions by *Saccharomyces cerevisiae*:

The effect of increasing incubation period (2, 4, 7 and 14 days) on the bioaccumulation of 100 mg/l of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions by *S. cerevisiae* grown in glucose-peptone medium at pH 5 was studied.

The data concerning the dry weights and metal removal capacity of *S. cerevisiae* that was grown in glucose peptone media as control and under the effect of 100 mg/l of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions was included in table 19 and fig 15; a &b. It showed that by increasing time, the growth increase till the stationary phase at which the growth stopped. The bioaccumulation capacity of *S. cerevisiae* also is affected by increasing time, where it increases with time till saturation state.

The dry weights of *S. cerevisiae* in the Glucose-peptone after 2, 4, 7 and 14 days were 1.332, 2.432, 2.531 and 2.566 g/100 ml medium as control. These dry weights decreased in different values after the treatment of 100 mg/l of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions. The data obviously revealed a regular relation between the amount of *S. cerevisiae* growth and the bioaccumulation process, the highest the biomass, the highest is the metal bioaccumulation.

Table 19: Average values of the effect of incubation period on the dry weight (g/100ml) and % metal removal by growing *S cerevisiae*

Treatments / Incubation period (d)	Control	Cadmium			Copper			Lead			Arsenate		
	Dry weight	C_f	% removal	Dry wt.	C_f	% removal	Dry wt.	C_f	% removal	Dry wt.	C_f	% removal	Dry wt.
2	1.332	53.01	46.99	1.002	64.02	35.98	0.771	67.24	32.76	1.216	50.62	49.38	1.121
4	2.432	34.8	65.2	1.188	58.02	41.98	1.321	45.02	54.98	1.688	48.21	51.79	1.732
7	2.531	32.46	67.54	1.421	39.13	60.87	1.771	32.13	67.87	2.211	46.61	53.39	1.821
14	2.566	31.71	68.29	1.521	37.13	62.87	1.819	26.02	73.98	2.217	44.95	55.05	1.901

Medium used was glucose-peptone, incubation period was 2,4,7 and 14 days in static culture at 27 C°, pH=5, amount of inoculated cells =square disc (0.5cm^2) of four days biomass old in 100ml liquid medium, initial metal concentration was 100mg/l for all ion species.

C_f final metal concentration (mg/l)

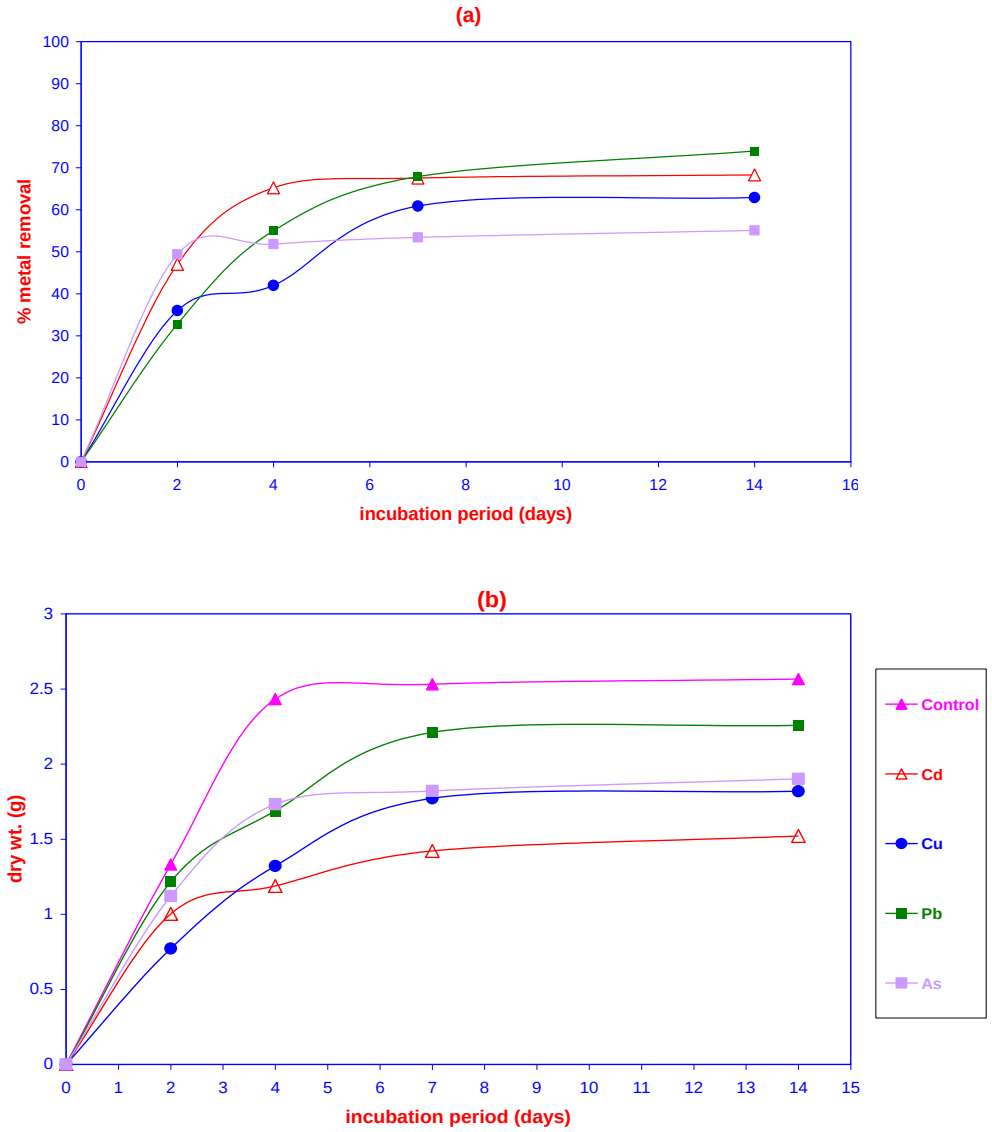


Fig (15): Effect of different incubation periods on (a) metal bioaccumulation capacity of *S. cerevisiae* and (b) its growth (resembled by dry weight/100ml medium).

(I-3): Effect of initial concentration on bioaccumulation of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions by *Saccharomyces cerevisiae*:

The effect of increasing Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions concentrations (50 -500) on their bioaccumulation by *S. cerevisiae* grown in glucose-peptone medium at pH 5 after 4 days incubation was studied. The data revealed that by increasing metal ions concentration the growth and also its bioaccumulation capacity decrease.

Table 20 and fig 16; a &b show the dry weights and metal removal capacity of *S. cerevisiae* that was grown in glucose peptone media as control and under the effect of different Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions concentrations ranged between (50 -100mg/l). *S. cerevisiae* could not grow under 300 mg/l Cd^{2+}, 450 mg/l Cu^{2+} and 250 mg/l As^{5+} but it resisted all Pb^{2+} concentrations. The increasing metals concentrations were negatively reflected on its bioaccumulation capacity, where the lowest bioaccumulation values were obtained under the sub-lethal metal concentrations and vice versa.

Table 20: Average values of the effect of initial metal concentration on the dry weight (g/100ml) and % metal removal by growing *S cerevisiae*

Init. Conc. / Metals		Cadmium	Copper	Lead	Arsenic
500mg/l	Dry Wt.	-	-	0.215	-
	% removal			2.44	
	C_f	-	-	487.8	-
450mg/l	Dry Wt.	-	-	0.328	-
	% removal			5.71	
	C_f	-	-	424.3	-
400mg/l	Dry Wt.	-	0.282	0.416	-
	% removal		5.86	16.9	-
	C_f		376.5	332.4	-
350mg/l	Dry Wt.	-	0.331	0.712	-
	% removal		12.42	18.45	-
	C_f	-	306.5	285.4	-
300mg/l	Dry Wt.	-	0.521	0.91	-
	% removal	-	15.83	35.8	
	C_f	-	252.5	192.6	-
250mg/l	Dry Wt.	0.441	0.591	1.121	-
	% removal	7.84	15.48	40.72	
	C_f	230.4	211.3	148.2	-
200mg/l	Dry Wt.	0.766	0.812	1.332	1.032
	% removal	31.2	18.8	43.75	35.75
	C_f	137.6	162.4	112.5	129.5
150mg/l	Dry Wt.	1.121	1.144	1.621	1.243
	% removal	41.45	31.13	46.05	37.08
	C_f	87.82	103.3	80.92	94.37
100mg/l	Dry Wt.	1.188	1.321	1.688	1.732
	% removal	65.2	41.98	54.98	51.79
	C_f	34.8	58.02	45.02	48.21
50mg/l	Dry Wt.	1.472	1.649	2.261	1.795
	% removal	57.58	30.16	60.56	54.86
	C_f	21.21	34.92	19.72	22.57

Medium used was glucose-peptone, incubation period was 4 days in static culture at 27 C°, pH=5, amount of inoculated cells =square disc (0.5cm²) of four days biomass old in 100ml liquid medium, initial metal concentration was 50-500mg/l for all ion species.

$$\% \text{ removal} = (C_i - C_f/C_i) * 100$$

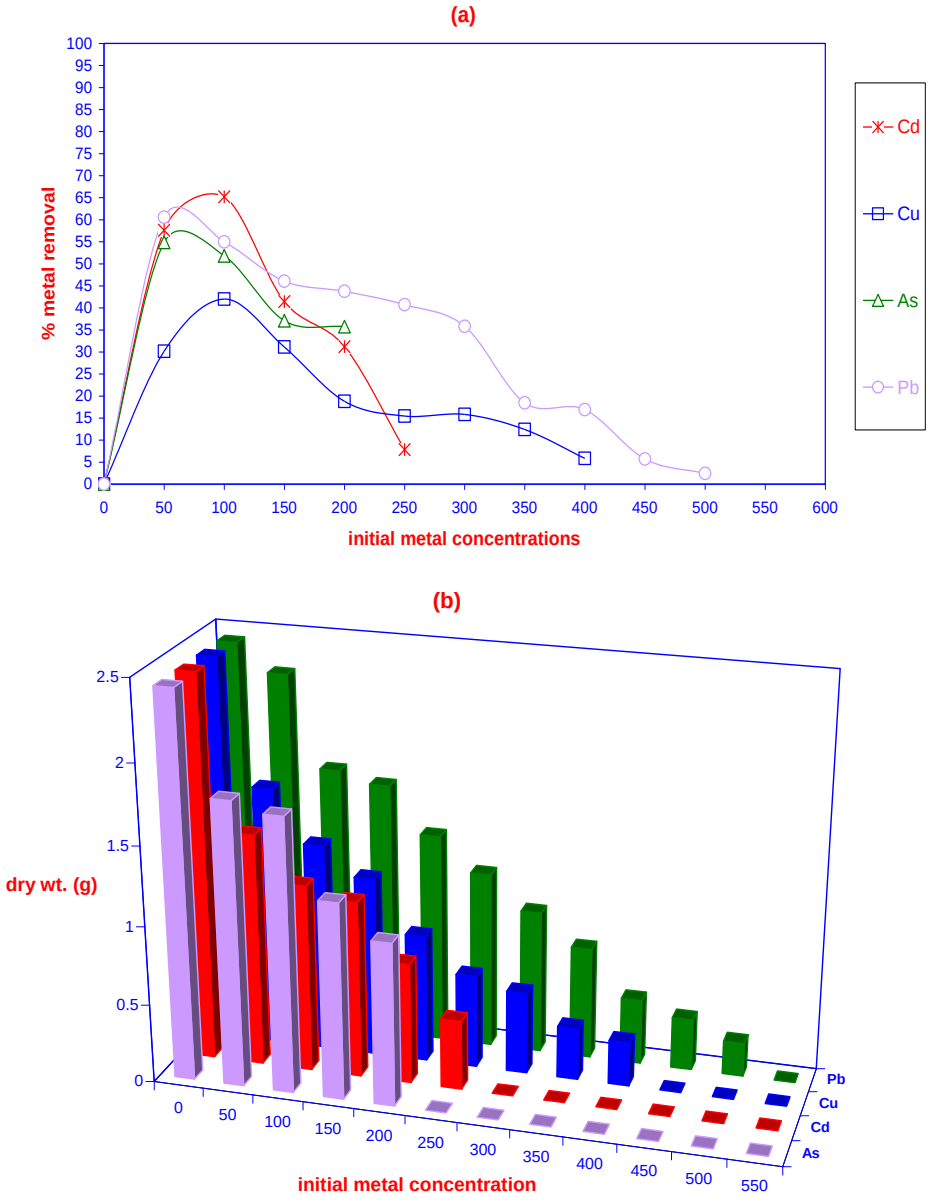


Fig (16): Effect of different metals concentrations on (a) metal bioaccumulation capacity of *S. cerevisiae* and (b) its growth (resembled by dry weight/100ml medium).

(II) Effect of different physical and chemical pretreatments on metals biosorption by *Saccharomyces cerevisiae* cells:

In order to investigate the effect of different pretreatments on metal uptake by *S. cerevisiae* cells, the cells were treated with heat, ethanol, sodium hydroxide, formaldehyde, hydrogen peroxide and acetic acid solutions (see method and materials page 60 and 61). Table 21 showed the q values of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} obtained by these cells and untreated cells. The data revealed that, physical and chemical pretreatments of yeast cells showed different metal uptake capacities.

The data presented in table 21 and fig. 17; a, b, c and d, generally indicated that all physical and chemical pretreatments of *S. cerevisiae* participated in Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} removal, but in different values. The highest metal uptake values (q) which were 6.78, 7.54 and 6.12 mg/g for Cd^{2+}, Pb^{2+} and As^{5+} respectively, were obtained by ethanol treated yeast cells, but the highest Cu^{2+} ions uptake was obtained by sodium hydroxide treated yeast cells (8,00 mg/g). On the other hand the lowest As^{5+} and Cu^{2+} biosorption was obtained by fresh biomasses with q values 3.7 and 2.5 mg/g, while the lowest Cd^{2+} and Pb^{2+} biosorption was obtained by formaldehyde and acetic acid treated cells with q values of 2.45 and 2.03 mg/g respectively.

The physical treatment (dried and killed forms) of *S. cerevisiae* cells showed suitable Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} biosorption capacity. The highest biosorption capacity were obtained by dried biomass with q values 7.4, 4.87,

and 3.92 mg/g for Pb^{2+} , Cd^{2+} and As^{5+} respectively, while autoclaved biomass was the best for Cu^{2+} ions biosorption (q value was 7.4 mg/g).

The Cd^{2+} uptake values by sodium hydroxide, acetic acid, formaldehyde, hydrogen peroxide and ethanol treated *S. cerevisiae* cells were 5.6, 2.45, 4.04, 4.4, and 6.78 mg/g respectively.

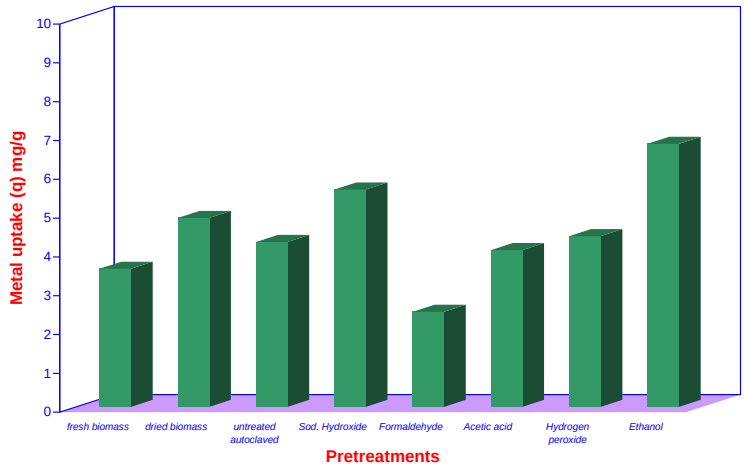
The q values of Cu^{2+}, Pb^{2+} and As^{5+} biosorption by previous treated *S. cerevisiae* cells were 8, 3.8, 5.53, 4.32, 5.18 f mg/g or Cu^{2+}, 5.15, 2.65, 2.03, 4.41, 7.54 mg/g for Pb^{2+} and 5.3, 5.54, 4.35, 5.89, 6.12 mg/g for As^{5+} after 10 hours contact.

Table 21: Average values of the effect of different physical and chemical pretreatments on metals Biosorption (q) by *S cerevisiae* cells

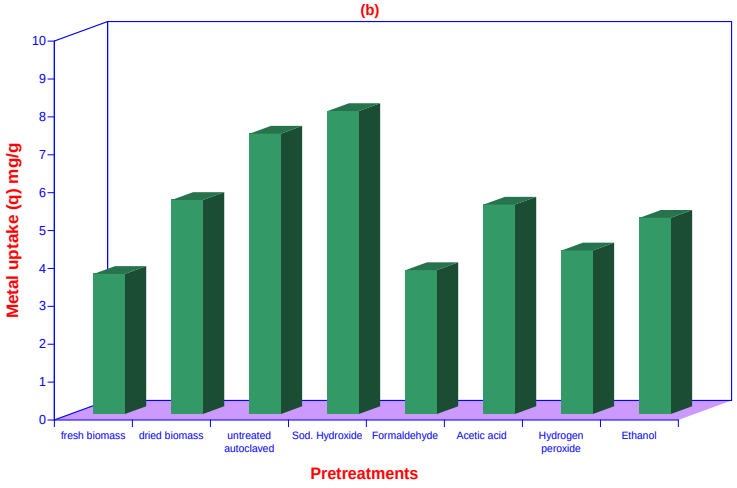
Metal ions / Type of pretreatments	Cd^{2+}		Cu^{2+}		Pb^{2+}		As^{5+}	
	C_f	q	C_f	Q	C_f	q	C_f	q
Fresh biomass	64.39	3.56	62.95	3.7	70.76	2.92	74.99	2.5
Dried biomass	51.27	4.87	43.44	5.65	26	7.4	60.8	3.92
Autoclaved biomass	57.49	4.25	25.94	7.4	38.08	6.19	70.63	2.93
Sod. Hydroxide treated cells	43.96	5.6	19.99	8	48.5	5.15	46.93	5.3
Formaldehyde treated cells	75.45	2.45	62	3.8	73.46	2.65	44.59	5.54
Acetic acid treated cells	59.53	4.04	44.63	5.53	79.63	2.03	56.47	4.35
Hydrogen peroxide treated cells	55.99	4.4	56.76	4.32	55.9	4.41	41.03	5.89
Ethanol treated cells	32.15	6.78	48.19	5.18	24.52	7.54	38.74	6.12

C_f: final metal concentration (mg/l)
q: metal uptake (mg/g)

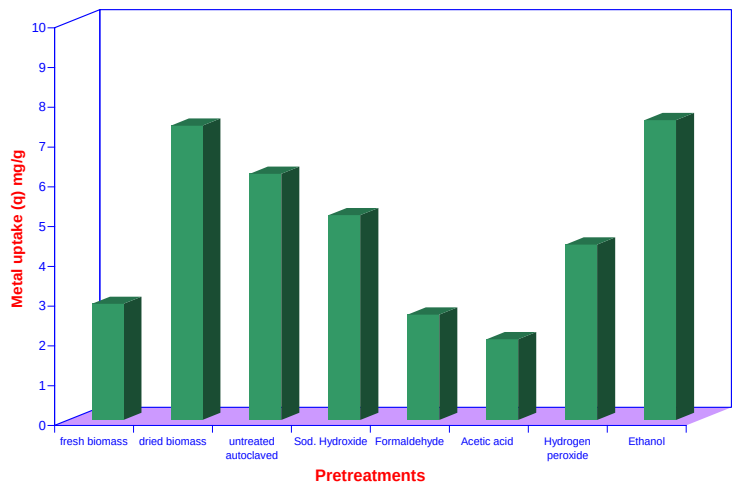
(a)


Metal uptake (q) mg/g
0
1
2
3
4
5
6
7
8
9
10
fresh biomass
dried biomass
untreated autoclaved
Sod. Hydroxide
Formaldehyde
Acetic acid
Hydrogen peroxide
Ethanol
Pretreatments

(b)


Metal uptake (q) mg/g
0
1
2
3
4
5
6
7
8
9
10
fresh biomass
dried biomass
untreated autoclaved
Sod. Hydroxide
Formaldehyde
Acetic acid
Hydrogen peroxide
Ethanol
Pretreatments

(c)


Metal uptake (q) mg/g
0
1
2
3
4
5
6
7
8
9
10
fresh biomass
dried biomass
untreated autoclaved
Sod. Hydroxide
Formaldehyde
Acetic acid
Hydrogen peroxide
Ethanol
Pretreatments

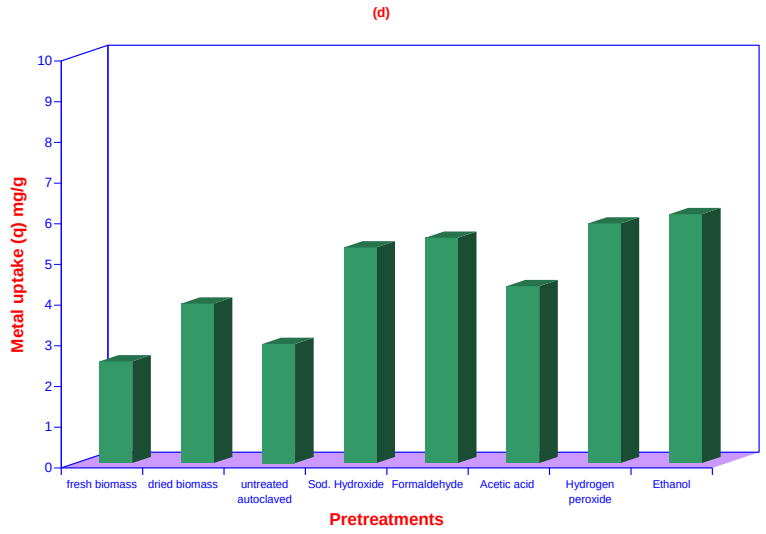


Fig (17): Effect of different physical and chemical pretreatments on the Cd^{2+} (a), Cu^{2+} (b), Pb^{2+} (c), and As^{5+} (d) biosorption by *S. cerevisiae* cells.

(III) Effect of different pH values on Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} biosorption by *Saccharomyces cerevisiae* cells:

The aim of the present experiment was to test the effect of pH values on the Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} biosorption capacity of *S. cerevisiae*. The pH ranges of 2 – 6.8 for all metal ions were used (pH values higher than 7 could not be used due to the rapid precipitation of $Cu^{2+}$and Pb^{2+} ions). Biosorption experiments were carried out by adding dry biomass 0.5 gm of yeast cells to the pH metal solutions which were adjusted to the desired pH value with either 2M NaOH or 2M H_2SO_4 solution. NaOH pretreated cells (0.5 gm) was contacted with Cu^{2+} ions, ethanol treated yeast cells was contacted with Cd^{2+}, Pb^{2+} and As^{5+} ions in separate solutions (50 ml) at a metal ion concentration 100 mg/l. The reaction mixture was agitated at 150 rpm for 1, 2, 4, 6, 8, and 10 hours. Triplicate set were used for each treatment.

Table 22, 23, 24 and 25, and fig 18, 19, 20 and 21 showed the effect of different pH values (2, 4, 5, 6, 6.5, and 6.8) on biosorption of 100 mg/l Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions by treated *S. cerevisiae* after 1, 2, 4, 6, 8, and 10 hours of contact time on electrical shaker at room temperature.

At an initial pH 4 or lower, little biosorption occurred. Especially at pH 2, almost no biosorption was observed for all metals. A sharp increase in biosorption capacity took place in the pH 5. Above pH 5, biosorption of Pb^{2+}, As^{5+} and Cu^{2+} was found to be relatively reduced; biosorption of Cd^{2+} still increased but to a lesser extent. Thus, different metals have different pH optima. The factor of time was important for saturation of *S. cerevisiae*

biomass with metal ions but not for growth because the process was growth independent.

Table 22 reveals that the effect of different pH values on Cd^{2+} biosorption by ethanol treated *S. cerevisiae* cells. Increasing pH value lead to increase Cd^{2+} biosorption till pH 6, after that (i.e. pH 6.5 and 6.8) the biosorption process decreased. The highest Cd^{2+} biosorption was at pH 6.

Tables 23, 24, and 25 show the effect of different pH values on Cu^{2+} biosorption by sodium hydroxyl treated cells and Pb^{2+}, As^{5+} biosorption by ethanol treated cells at 1-10 hours contact time. The highest biosorption of the three metal ions was at pH 5, after that the biosorption rate decreased, while the lowest biosorption occurred at lower pH (2 and 4).

Table 22: Average values of the effect of pH on the biosorption of Cadmium ions by Ethanol treated *S. cerevisiae*

Incubation periods	pH											
	2		4		5		6		6.5		6.8	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
1	82.41	1.75	66.09	3.39	36	6.4	22.79	7.72	33.85	6.61	31.1	6.89
2	79.65	2.03	65.92	3.4	35.03	6.49	22.83	7.71	31.53	6.84	28.6	7.14
4	78.95	2.1	64.08	3.59	32.95	6.7	22.3	7.77	31.1	6.89	28.6	7.14
6	78.91	2.1	63.47	3.65	32.66	6.73	22.09	7.79	30.13	6.98	27.99	7.2
8	78.04	2.19	63.51	3.64	32.32	6.76	21.52	7.84	29.39	7.06	27.64	7.23
10	77.9	2.2	63.16	3.68	32.15	6.78	21.3	7.87	29.13	7.08	27.29	7.27

C_f: final metal concentration (mg/l)
q: metal uptake (mg/g)
Initial metal concentration 100 mg/l

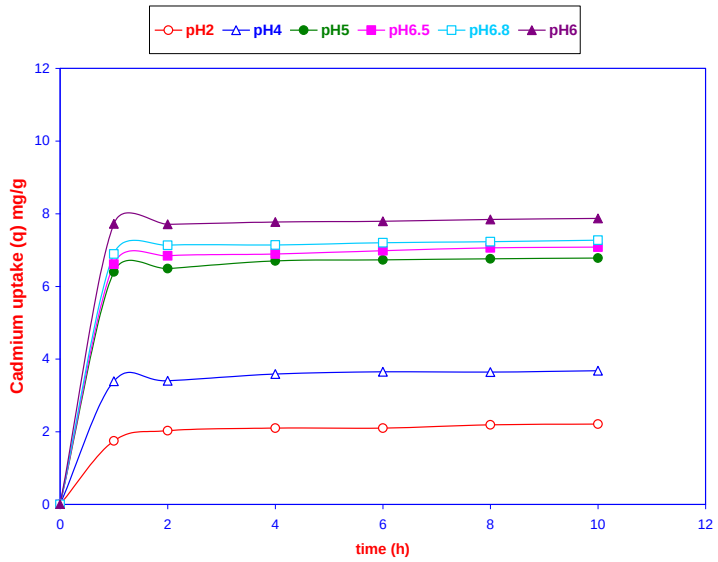


Fig (18): Effect of different pH values on the biosorption of Cd^{2+} by ethanol treated *S. cerevisiae* cells.

Table 23: Average values of the effect of pH on the biosorption of Copper ions by Sodium hydroxide treated *S. cerevisiae*

Incubation periods	pH											
	2		4		5		6		6.5		6.8	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
1	77.52	2.24	64.8	3.52	28.34	7.16	33.72	6.62	42.81	5.71	49.49	5.05
2	75.63	2.43	63.64	3.63	26.59	7.34	32.68	6.73	43.09	5.69	48.51	5.14
4	75.14	2.48	62.77	3.72	24.15	7.58	29.74	7.02	40.68	5.93	48.02	5.19
6	74.69	2.53	60.57	3.94	21.74	7.82	27.54	7.24	37.43	6.25	47.25	5.27
8	73.26	2.67	60.25	3.97	20.41	7.95	26.84	7.31	37.32	6.26	47.36	5.26
10	72.98	2.7	59.97	4	19.99	8	26.24	7.37	36.59	6.34	46.87	5.31

C_f: final metal concentration (mg/l)
q: metal uptake (mg/g)
Initial metal concentration 100 mg/l

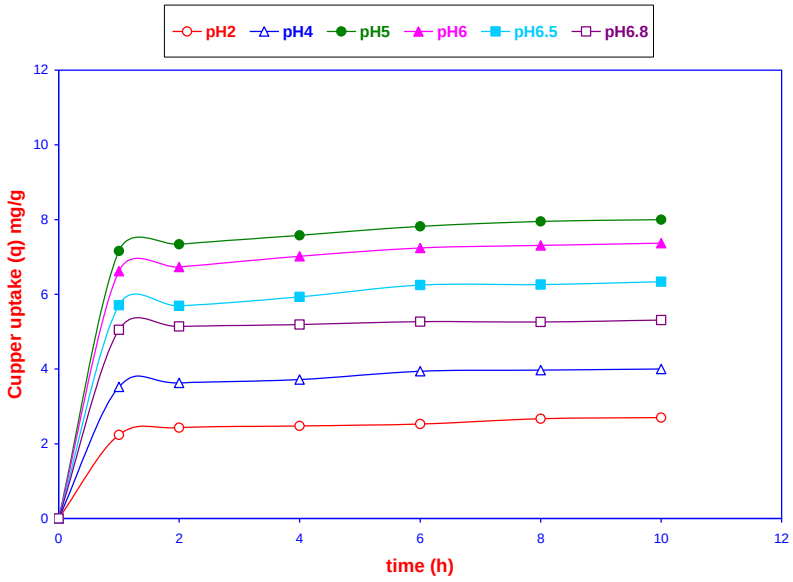


Fig (19): Effect of different pH values on the biosorption of Cu^{2+} by sodium hydroxide treated *S. cerevisiae* cells.

Table 24: Average values of the effect of pH on the biosorption of Lead ions by Ethanol treated *S. cerevisiae*

Incubation periods	pH											
	2		4		5		6		6.5		6.8	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
1	89.79	1.02	38.76	6.12	33.31	6.66	50.67	4.93	46.97	5.3	58.81	4.11
2	88.58	1.14	37.48	6.25	31.25	6.87	37.48	6.25	46.4	5.36	49.03	5.09
4	88.44	1.15	29.19	7.08	28.38	7.16	36.77	6.32	44.48	5.55	50.74	4.92
6	87.69	1.23	27.56	7.24	27.91	7.2	35.84	6.41	37.87	6.21	51.56	4.844
8	87.73	1.22	27.34	7.26	27.17	7.28	35.06	6.49	36.77	6.32	52.66	4.73
10	87.41	1.25	27.16	7.28	24.52	7.54	34.38	6.56	36.16	6.38	53.3	4.67

C_f: final metal concentration (mg/l)
q: metal uptake (mg/g)
Initial metal concentration 100 mg/l

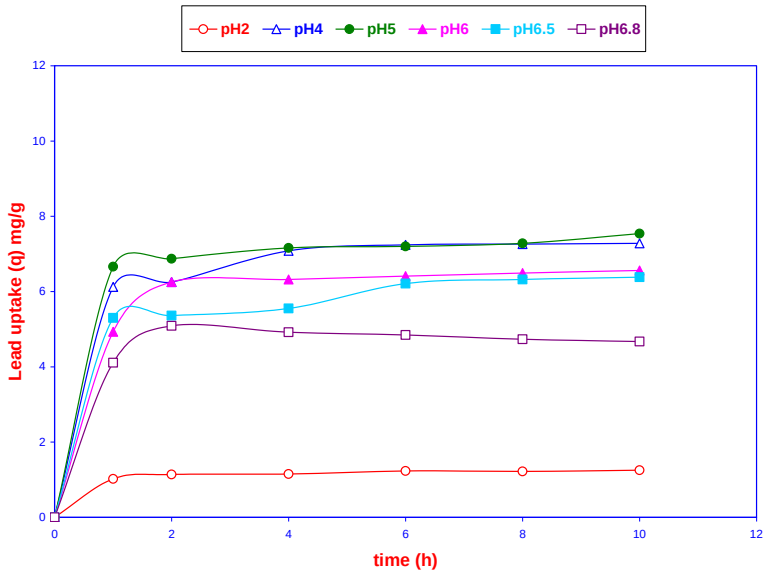


Fig (20): Effect of different pH values on the biosorption of Pb^{2+} by ethanol treated *S. cerevisiae* cells.

Table 25: Average values of the effect of pH on the biosorption of Arsenate ions by Ethanol treated *S. cerevisiae*

Incubation periods	pH											
	2		4		5		6		6.5		6.8	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
1	96.67	3.33	79.92	2	63.31	3.66	47.93	5.2	51.58	4.84	60.98	3.9
2	95.44	0.45	77.21	2.27	53.48	4.65	45.75	5.42	49.07	5.09	60.6	3.94
4	94.63	0.53	73.8	2.62	44.72	5.52	43.75	5.62	46.22	5.37	48.45	5.15
6	92.73	0.72	72.47	2.75	41.32	5.86	42.28	5.77	45.75	5.42	47.79	5.22
8	91.21	0.87	71.23	2.87	39.48	6.05	41.95	5.8	44.8	5.52	46.7	5.33
10	90.65	0.93	70.57	2.94	38.74	6.12	39.72	6.02	43.04	5.69	45.42	5.45

C_f: final metal concentration (mg/l)
q: metal uptake (mg/g)
Initial metal concentration 100 mg/l

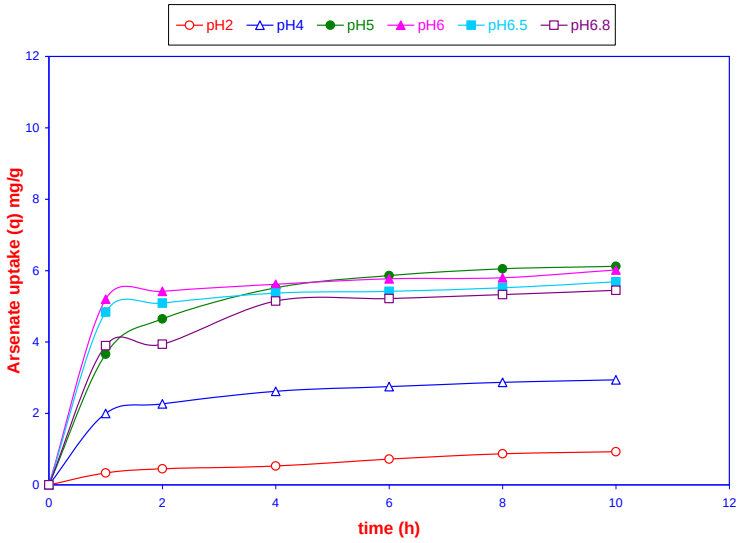


Fig (21): Effect of different pH values on the biosorption of As^{5+} by ethanol treated *S. cerevisiae* cells.

(IV) Effect of different concentration of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} on their biosorption by *Saccharomyces cerevisiae*:

The Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions were prepared at different concentrations in order to study the effect of initial metal concentrations on the biosorption process by *S. cerevisiae*. The range of metal concentration was 25-200 mg/l at pH 6 for cadmium and pH 5 for other metal ions. The biosorbent concentration was 0.5g per 50 ml metal solution (NaOH pretreated cells was contacted with Cu^{2+} ions, ethanol treated yeast cells was contacted with Cd^{2+}, Pb^{2+} and As^{5+} ions in separate solutions). The reaction mixture was agitated at 150 rpm for 1, 2, 4, 6, 8, and 10 hours. Triplicate set were used for each treatment.

Tables 26, 27, 28, 29 and fig 22, 23, 24, and 25 include the effect of various Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} concentrations (25, 50, 75, 100, 150 and 200 mg/l) on their biosorption by *S. cerevisiae* after 1, 2, 4, 6, 8, and 10 hours of contact time on electrical shaker at room temperature.

The data reveal that increasing of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} concentrations in their aqueous solutions containing suitable form of *S. cerevisiae* , was accompanied by increasing their biosorption process. We noted that the biosorption was very fast for all metal ions during the first 2 h but, in the remaining time period, final metal concentrations in the liquid reached an equilibrium concentration value.

Concerning the effect of different Cd^{2+} concentrations on its biosorption by ethanol treated *S. cerevisiae* cells at pH 6; it was appeared from the results which are presented in table 26 and fig 22 that the highest concentration of Cd^{2+} led to its highest biosorption in comparing to its lower concentrations. Saturation occurred during first 2-4 hours.

Table 27 showed the biosorption of 25–200 mg/l Cu^{2+} by sodium hydroxyl treated *S. cerevisiae* cells at pH 5. The data presented indicated that, biosorption process capacity increased with increasing the concentration of target metal ions. The factor of time was not important because saturation was quickly occurred and the process was metabolism independent.

The data presented in Tables 28, 29 and plotted in fig 23, 24 showed the effect of the same previous concentrations of Pb^{2+} and As^{5+} on their biosorption by ethanol treated yeast cells at pH 5. The same previous conclusion was also noted here; that by increasing Pb^{2+} or As^{5+}concentration, their biosorption would increase.

Table 26: Average values of the Effect of initial Cadmium concentration on its biosorption (q) process by *S. cerevisiae* biomass

Incubation periods	Initial concentration mg/l											
	25		50		75		100		150		200	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
1	9.25	1.57	12.54	3.74	15.27	5.97	22.79	7.72	43.54	10.64	97.32	10.26
2	6.84	1.81	9.54	4.04	14.65	6.03	22.83	7.71	39.64	11.03	93.5	10.65
4	4.62	2.03	9.34	4.06	13.84	6.11	22.3	7.77	38.15	11.18	92.54	10.74
6	4.58	2.04	9.26	4.07	13.66	6.13	22.09	7.79	37.88	11.21	92.41	10.75
8	4.29	2.07	9.18	4.08	13.54	6.14	21.52	7.84	37.64	11.23	92.33	10.76
10	4.22	2.07	9.08	4.09	13.37	6.16	21.3	7.87	37.29	11.27	92.17	10.78

C_f: final metal concentration (mg/l)
q: metal uptake (mg/g)

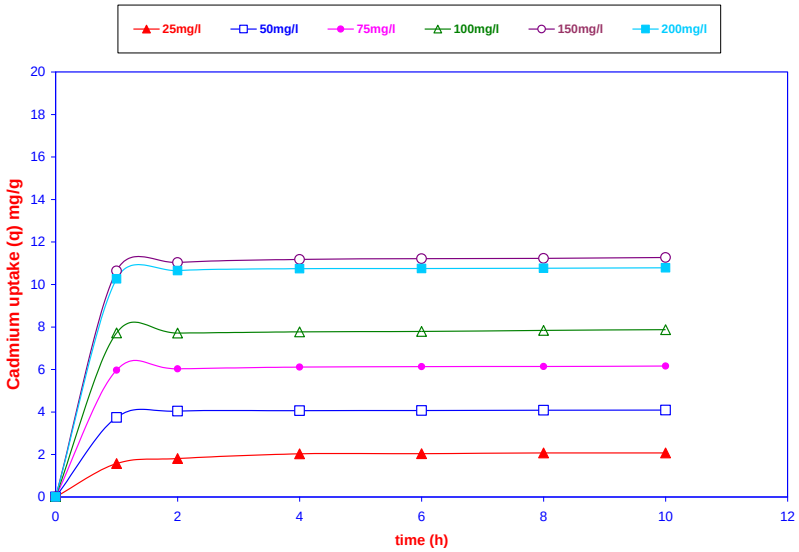


Fig (22): Effect of different initial metal concentrations on the biosorption of Cd^{2+} ions by ethanol treated *S. cerevisiae* cells.

Table 27: Average values of the Effect of initial Copper concentration on its biosorption (q) process by *S. cerevisiae* biomass

Incubation periods	Initial concentration mg/l											
	25		50		75		100		150		200	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
1	7.65	1.73	17.66	3.23	20.22	5.47	28.34	7.16	35.22	11.47	62.64	13.73
2	6.24	1.87	16.92	3.3	18.34	5.66	26.59	7.34	33.65	11.63	61.33	13.86
4	5.84	1.91	16.24	3.37	17.66	5.73	24.15	7.58	29.46	12.05	60.55	13.94
6	5.24	1.97	15.22	3.47	17.51	5.74	21.74	7.82	29.4	12.06	60.21	13.97
8	4.21	2.07	15.13	3.48	17.34	5.76	20.41	7.95	24.22	12.57	59.91	14
10	4.17	2.08	14.86	3.51	17.14	5.78	19.99	8	24.01	12.59	59.87	14.01

C_f: final metal concentration (mg/l)
q: metal uptake (mg/g)

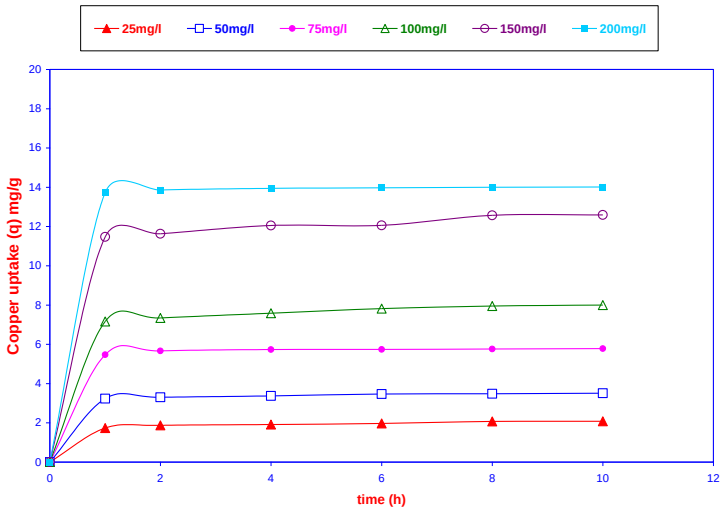


Fig (23): Effect of different initial metal concentrations on the biosorption of Cu^{2+} ions by sodium hydroxyl treated *S. cerevisiae* cells.

Table 28: Average values of the Effect of initial Lead concentration on its biosorption (q) process by *S. cerevisiae* biomass

Incubation periods	Initial concentration mg/l											
	25		50		75		100		150		200	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
1	5.66	1.93	10.05	3.99	12.35	6.26	33.31	6.66	41.61	10.83	79.64	12.03
2	4.65	2.03	9.61	4.03	11.84	6.31	31.25	6.87	39.54	11.04	75.32	12.46
4	3.57	2.14	8.21	4.17	11.65	6.33	28.38	7.16	38.62	11.13	71.24	12.87
6	3.31	2.16	7.51	4.24	11.24	6.37	27.91	7.2	38.22	11.17	69.54	13.04
8	3.26	2.17	7.23	4.27	11.13	6.38	27.17	7.28	37.94	11.2	68.35	13.16
10	3.04	2.19	7.14	4.28	11	6.4	24.52	7.54	37.84	11.21	68.17	13.18

C_f: final metal concentration (mg/l)
q: metal uptake (mg/g)

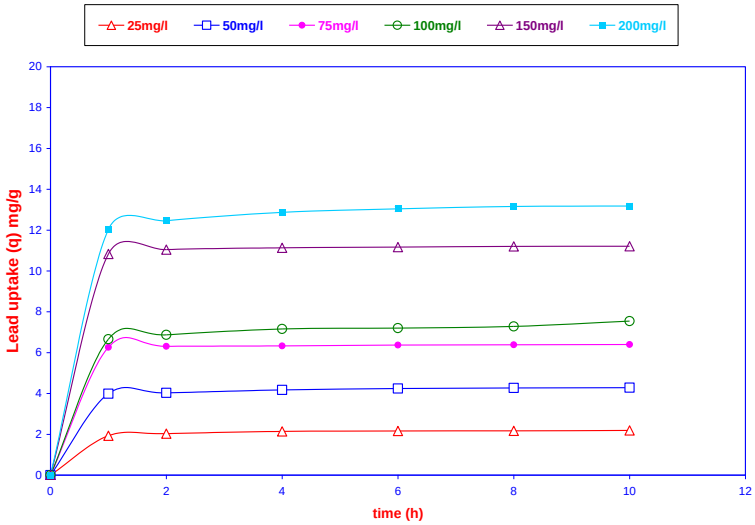


Fig (24): Effect of different initial metal concentrations on the biosorption of Pb^{2+} ions by ethanol treated *S. cerevisiae* cells.

Table 29: Average values of the Effect of initial Arsenate concentration on its biosorption (q) process by *S. cerevisiae* biomass

Incubation periods	Initial concentration mg/l											
	25		50		75		100		150		200	
	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q	C_f	q
1	9.31	1.56	19.32	3.06	28.34	4.66	63.31	3.66	61.25	8.87	95.35	10.46
2	8.64	1.63	18.24	3.17	27.12	4.78	53.48	4.65	58.25	9.17	93.21	10.67
4	8.02	1.69	17.62	3.23	26.34	4.86	44.72	5.52	57.64	9.23	92.6	10.74
6	7.66	1.73	17.51	3.24	25.54	4.94	41.32	5.86	57.11	9.28	91.64	10.83
8	7.41	1.75	17.22	3.27	24.66	5.03	39.48	6.05	57.04	9.29	91.31	10.86
10	7.11	1.78	17.13	3.28	24.31	5.06	38.74	6.12	56.87	9.31	91.22	10.87

C_f: final metal concentration (mg/l)
q: metal uptake (mg/g)

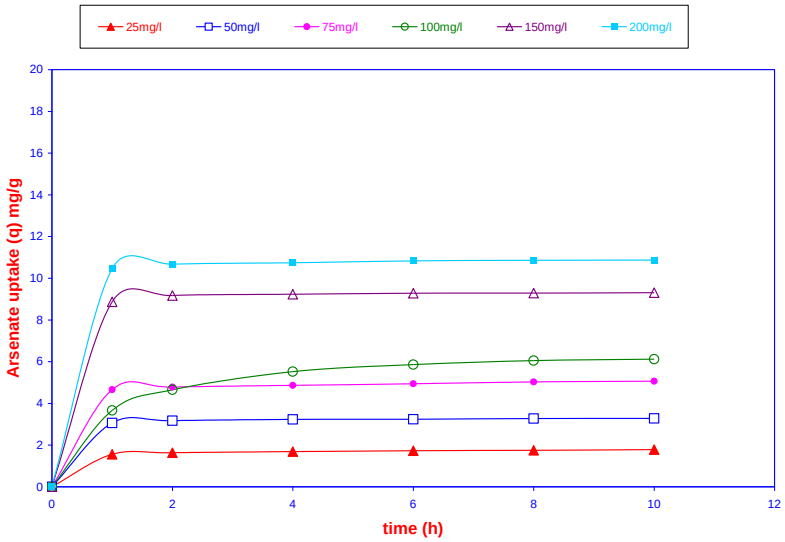


Fig (25): Effect of different initial metal concentrations on the biosorption of As^{5+} ions by ethanol treated *S. cerevisiae* cells.

(V) Regeneration and reuse of pretreated *Saccharomyces cerevisiae* for biosorption of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions

Elution is defined as the process of extracting a substance (adsorbed metal ion) adsorbed to another (*S. cerevisiae* cell wall) by means of a suitable solvent or buffering agent. After biosorption of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions, biomass was eluted using HNO_3 as solvent, and then regenerated by washing with deionized water or NaOH. The aim of this experiment is to test the best regenerated agent (water or sodium hydroxyl), and to exploit the same biomass in other biosorption processes.

Initial metal concentration was 100mg/l, pH was 5 for Pb^{2+}, As^{5+} and Cu^{2+}, 6 for Cd^{2+} biosorption. Biomass was ethanol treated cells for biosorption of Cd^{2+}, Pb^{2+}, and As^{5+} while it was sodium hydroxide for Cu^{2+} biosorption. Contact time was 10h, rpm= 150 at room temperature. Biomass concentration was 0.5g/50ml (dry mass per volume). The elution was carried out using 0.05M HNO_3.

S. cerevisiae biomasses were used for 5 cycles of biosorption– elution–regeneration, where the first cycle was the main experiment without any elution or regeneration but the 2-5 cycles undergo elution and regeneration. Tables (30) and fig (26; a &b) showed that biomass regenerated by washing with only deionized water lost some of its metal removal capability in comparison with that of NaOH-pretreated biomass. However, biomass regenerated with NaOH regained its initial metal removal capacity. The q values in the cycle 2–5 were fluctuated around the q value in first cycle using

NaOH-pretreated biomass, while the q values in biomasses that regenerated with deionized water were always lower than that of first cycle.

For example, in regeneration with deionized water, the q value of Cd^{2+} in first cycle was 7.87 mg/g and then decreased in 2-5 cycles with values 3.75, 3.99, 4.3 and 4.07 mg/g, while in regeneration with sodium hydroxyl the q values remain around the first value (7.96, 8.13, 7.7 and 8.07 mg/g) and may exceed it in same cases. The same was done for all metal ions.

Table 30: Average values of Regeneration by NaOH and deionzed water for recycling of biosorption process

Metal ions	Metal uptake (q) mg/g									
	Regeneration by deionzed water					Regeneration by NaOH solution				
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Cadmium	7.87	3.75	3.99	4.3	4.07	7.87	7.96	8.13	7.7	8.07
Copper	8	5.97	5.78	6.44	4.97	8	8.29	8.45	7.87	7.39
Lead	7.54	6.37	6.67	5.59	5.73	7.54	6.97	7.13	7.06	6.67
Arsenate	6.12	2.44	2.4	2.99	3.32	6.12	3.98	4.67	4.97	5.38

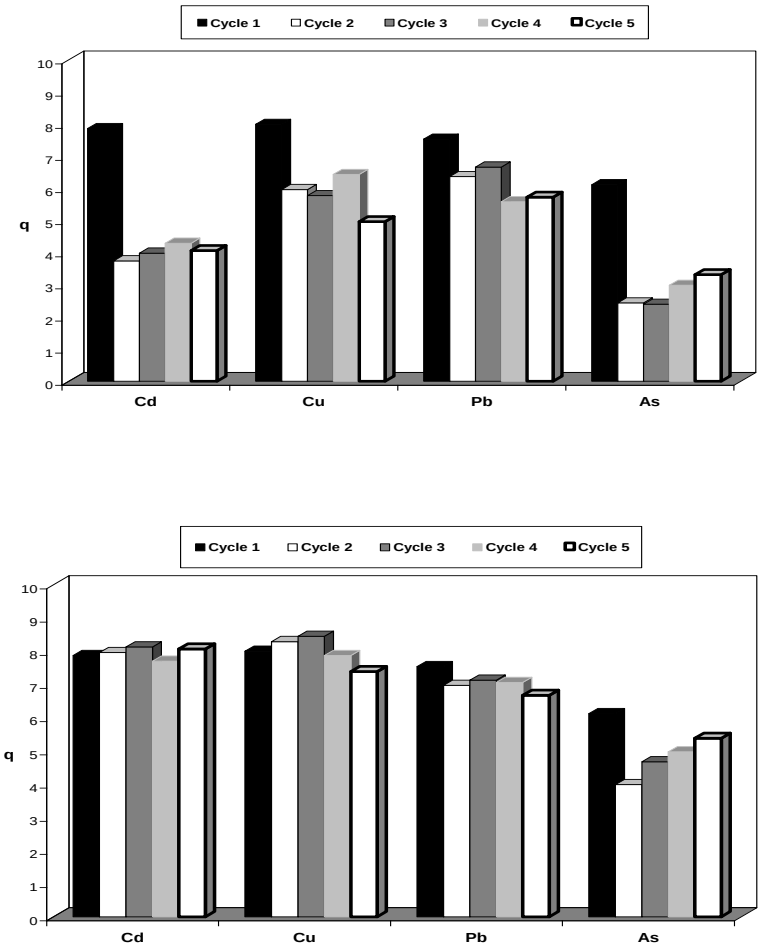


Fig (26): Regeneration by (a) deionzed water and (b) NaOH for recycling of biosorption process

Effect of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions on the growth and metabolism of *Saccharomyces cerevisiae*

(I) Growth curves of *Saccharomyces cerevisiae* under the effect of different Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} concentrations:

The effect of , Cu^{2+}, As^{5+}, Pb^{2+} and Cd^{2+}ions with different concentrations (50 -250 mg/l) on growth phases of *S. cerevisiae* that grown in glucose-peptone broth medium at pH 5 was studied. The growth rate of *S. cerevisiae* cells was determined after 4, 8, 12, 24, 48, 72, 96 and 120 hours through measuring the dry weight .The data were recorded and plotted in tables 31, 32, 33, 34 and figures 27, 28, 29, and 30.

This experiment showed the negative influence of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions on all growth phases of *S. cerevisiae*. Although this negative influence, *S. cerevisiae* resisted all Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions with all concentrations except 250 mg/l As^{5+} ions.

The shape of the absorbance curve during the growth of cultures indicates the inhibition of the yeast cell reproduction (fig 27, 28, 29 and 30). This inhibition differs from metal to another according to the degree of metal toxicity. The growth curve of the control cells was higher than the curves of the treated cells. With increasing concentration of Cd^{2+}, Cu^{2+}, Pb^{2+} or As^{5+} ions the metal uptake gradually decreased and the curves were flatter and flatter. The speed of *S. cerevisiae* growth lowered under the effect of Cd^{2+},

Cu^{2+}, Pb^{2+} or As^{5+} ions with any concentration than the control cells. The concentration of 250 mg/l As^{5+} ions caused total inhibition of yeast growth.

Table (31): Average values of the effect of different concentrations (mg/l) of Cu^{2+} ions on the growth (dry weight g/100ml) of *S. cerevisiae*

Cu^{2+} (mg/l)	Dry weight g/100ml after								
	0.00h	4h	8h	12h	24h	48h	72h	96h	120h
0.00	0.07	0.13	0.38	0.55	1.28	2.26	2.36	2.38	2.41
50	0.07	0.12	0.25	0.41	0.96	1.88	1.91	1.93	1.93
100	0.07	0.09	0.21	0.37	0.79	1.64	1.67	1.68	1.7
150	0.07	0.08	0.18	0.25	0.6	1.33	1.34	1.34	1.36
200	0.07	0.07	0.15	0.2	0.45	0.94	1.02	1.04	1.08
250	0.07	0.07	0.1	0.18	0.3	0.6	0.63	0.67	0.68

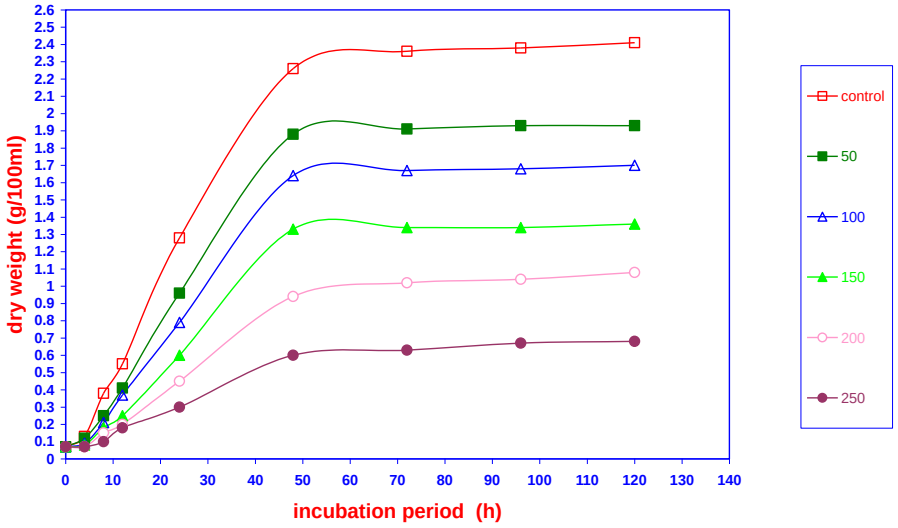


Fig (27): Effect of Growth curves of *S. cerevisiae* under different Cu^{2+}ions concentrations (50, 100, 150, 200 and 250 mg/l) on its growth rate (g/100ml dry weight).

Table (32): Average values of the effect of different concentrations (mg/l) of As^{5+} ions on the growth (dry weight g/100ml) of *S. cerevisiae*

As^{5+} (mg/l)	Dry weight (g/100) after								
	0.00h	4h	8h	12h	24h	48h	72h	96h	120h
Control	0.07	0.13	0.38	0.55	1.28	2.26	2.36	2.38	2.41
50	0.07	0.09	0.19	0.23	0.86	1.42	1.42	1.42	1.44
100	0.07	0.08	0.12	0.19	0.8	1.33	1.34	1.36	1.39
150	0.07	0.07	0.09	0.11	0.56	1	1.06	1.06	1.06
200	0.07	0.07	0.07	0.09	0.35	0.84	0.87	0.88	0.88
250	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07

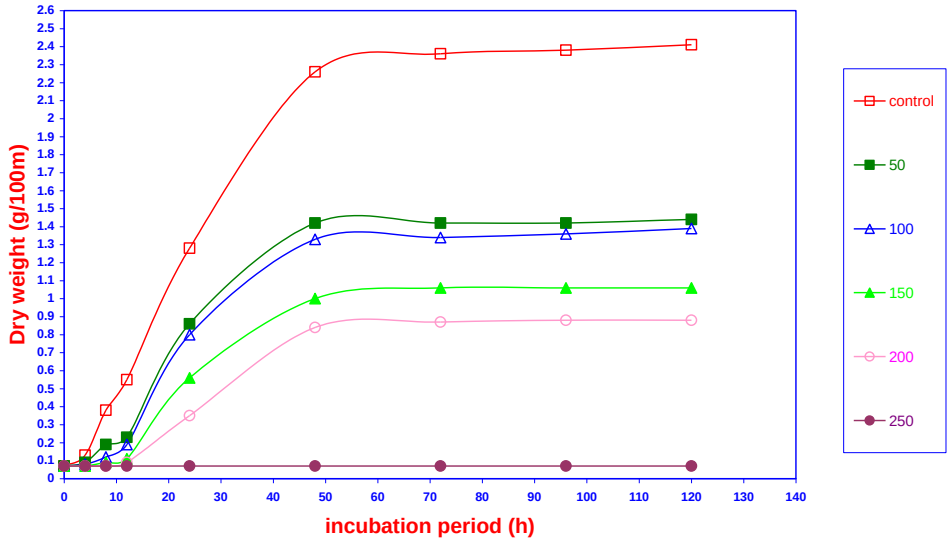


Fig (28): Effect of Growth curves of *S. cerevisiae* under different As^{5+}ions concentrations (50, 100, 150, 200 and 250 mg/l) on its growth rate (g/100ml dry weight).

Table (33): Average values of the effect of different concentrations (mg/l) of Pb^{2+} ions on the growth (dry weight g/100ml) of *S. cerevisiae*

Pb^{2+} (mg/l)	Dry weight (g/100ml) after								
	0.00h	4h	8h	12h	24h	48h	72h	96h	120h
Control	0.07	0.13	0.38	0.55	1.28	2.26	2.36	2.38	2.41
50	0.07	0.13	0.34	0.51	1.18	2.23	2.31	2.33	2.35
100	0.07	0.1	0.24	0.32	1.04	2	2.02	2.03	2.03
150	0.07	0.09	0.17	0.23	0.7	1.41	1.45	1.48	1.48
200	0.07	0.09	0.12	0.18	0.6	1.1	1.11	1.11	1.13
250	0.07	0.07	0.09	0.14	0.43	0.88	0.89	0.9	0.92

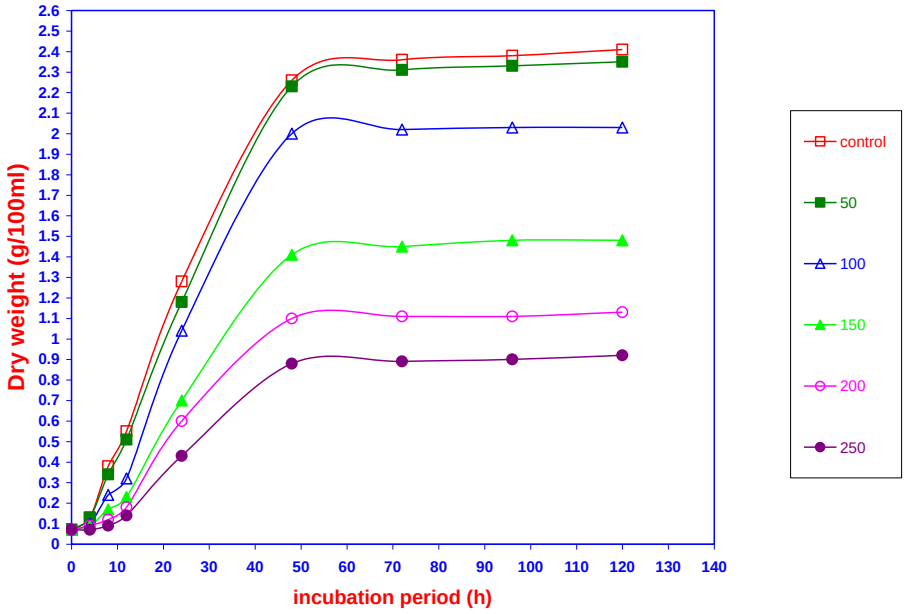


Fig (29): Effect of Growth curves of *S. cerevisiae* under different Pb^{2+}ions concentrations (50, 100, 150, 200 and 250 mg/l) on its growth rate (g/100ml dry weight).

Table 34: Average values of the effect of different concentrations (mg/l) of Cd^{2+} ions on the growth (dry weight g/100ml) of *S. cerevisiae*

Cd^{2+} (mg/l)	Dry weight (g/100ml) after								
	0.00h	4h	8h	12h	24h	48h	72h	96h	120h
Control	0.07	0.13	0.38	0.55	1.28	2.26	2.36	2.38	2.41
50	0.07	0.08	0.25	0.45	0.9	1.33	1.34	1.36	1.37
100	0.07	0.08	0.18	0.37	0.68	1.1	1.16	1.16	1.16
150	0.07	0.07	0.11	0.22	0.56	0.95	0.97	0.97	0.98
200	0.07	0.07	0.07	0.09	0.43	0.9	0.92	0.92	0.94
250	0.07	0.07	0.07	0.07	0.39	0.81	0.83	0.86	0.87

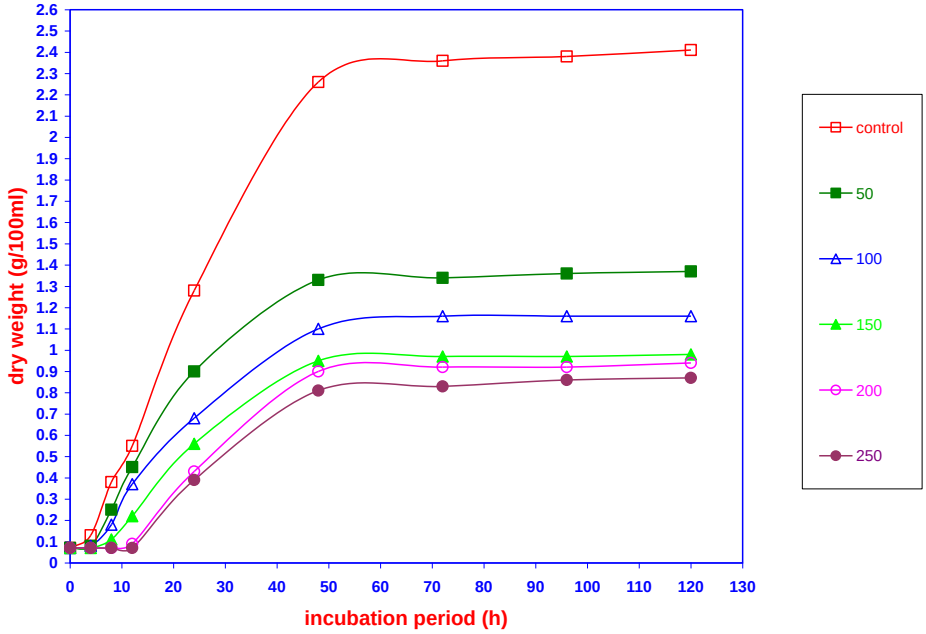


Fig (30): Effect of Growth curves of *S. cerevisiae* under different Cd^{2+}ions concentrations (50, 100, 150, 200 and 250 mg/l) on its growth rate (g/100ml dry weight).

(II) Effect of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions concentrations on DNA of *S. cerevisiae*

(II-1): Effect of different concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions on total DNA of *S. cerevisiae*:

The effect of different concentrations (50- 200 mg/l) of Cd^{2+}, Cu^{2+}, Pb^{2+},and As^{5+} ions on total DNA of *S. cerevisiae* that was grown after four days growth on glucose peptone broth media (in static culture at 27 C°, pH=5), amended and not amended with metal ions was studied.

Table 35 shows the total concentrations of *S. cerevisiae* DNA as control and also under the effect of different Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions concentrations ranged between (50-100mg/l). The data revealed that by increasing the concentration of Cd^{2+}, Cu^{2+}, Pb^{2+} or As^{5+} ions, the total DNA decreased. The degree of the negative effect of metal ions on DNA was varied according to the type of metal ions.

The response to metal toxicity appeared first under arsenate treatment, where at even low As^{5+} concentration (50 mg/l), total yeast DNA decreased from 0.521 to 0.154 µg/µl, unlike under 50 mg/l of Cd^{2+}, Cu^{2+} and Pb^{2+} treatment. At higher Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} concentration (200mg/l), total *S. cerevisiae* DNA concentration decreased dramatically with values 0.049, 0.182, 0.028 and 0.021 µg/µl respectively.

Table 35: Average values of the effect of different concentrations (mg/l) of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions on total DNA (µg/µl) content of *S. cerevisiae*

Metal conc. mg/l	DNA content (µg/µl)			
	Cadmium	Copper	Lead	Arsenate
Control (0)	0.521	0.521	0.521	0.521
50	0.301	0.483	0.322	0.154
100	0.154	0.441	0.147	0.133
150	0.07	0.224	0.056	0.126
200	0.049	0.182	0.028	0.021

Total DNA content (µg/µl) of *S. cerevisiae* after growth for 4days on glucose peptone broth media (in static culture at 27 C°, pH=5), amended and not amended with metal (Cd^{2+}, Cu^{2+}, Pb^{2+},and As^{5+})concentrations varied between 50-200 mg/l.

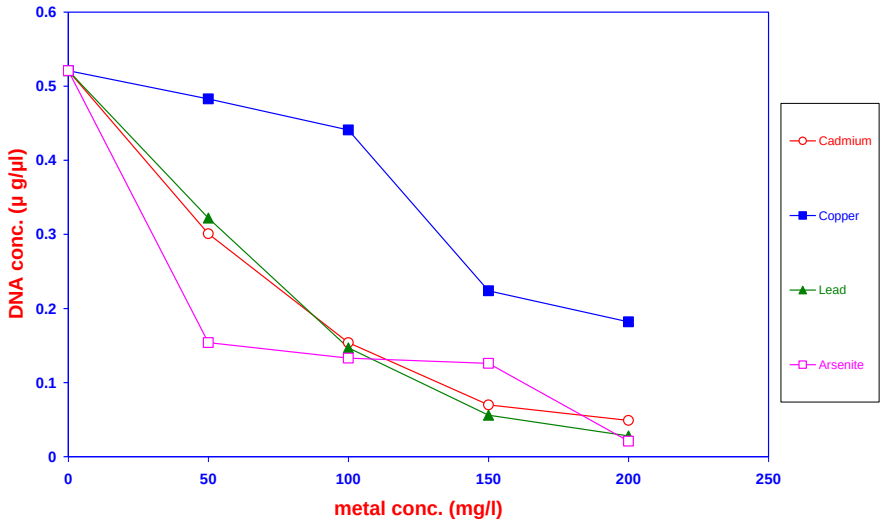


Fig (31): Effect of different Cd^{2+}, As^{5+}, Cu^{2+} and Pb^{2+} concentrations on total DNA content (µg/µl) of *S. cerevisiae*

(II-2): Effect of sub-lethal Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} concentrations on intact DNA of *S. cerevisiae*:

S. cerevisiae was subjected to further molecular study. This experiment aimed to evaluate the molecular level changes in total genome DNA as a result of metal treatment. Thus total DNA's from harvested *S. cerevisiae* cells in the presence of sublethal concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} as well as in the absence of such metals, were detected.

Agarose gel electrophoresis of *S. cerevisiae* total DNA (Table 36 and fig.31) revealed that the intact DNA of the control sample of *S. cerevisiae* was separated into one band without any fragmentations with molecular weight 1089 bp representing 100%.

Three DNA bands were detected in Cd^{2+} treated cells of *S. cerevisiae*, their molecular weight were 556, 396 and 211 bp accounted for 36.4%, 36.9% and 26.8% respectively. With respect to As^{5+} treated cells of *S. cerevisiae* in lane 2 table 36, the data revealed the separation of four DNA bands. These bands had the molecular weights; 1078, 540, 379 and 168 bp representing 26.6%, 26.9%, 26.9% and 19.6% respectively. In lane 3, Cu^{2+} treated cells of *S. cerevisiae* did not showed any fragmentation of DNA and appeared in one band with 1072 bp DNA representing 100%. Finally, in lane 4, DNA was fragmented and four bands were detected after Pb^{2+} treatment. These bands had molecular weights of 1039, 600, 483 and 193 bp constituted 29.2%, 22.4%, 29% and 19.4% respectively.

In conclusion, the intact *S. cerevisiae* DNA undergone fragmentations when was treated with sublethal concentration of Cd^{2+}, Pb^{2+} and As^{5+} but not under Cu^{2+} treatment.

Table 36: Changes in number and molecular weights of electrophoretic DNA bands for *S. cerevisiae* treated and not treated with Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions .

Lanes:	Marker		Control		Lane 1		Lane 2		Lane 3		Lane 4	
Bands	(mol.w.)	(amount)	(mol.w.)	(amount)%	(mol.w.)	(amount)	(mol.w.)	(amount)	(mol.w.)	(amount)	(mol.w.)	(amount)
1	650	9.09	1089	100	556	36.4	1078	26.6	1072	100	1039	29.2
2	600	8.46			396	36.9	540	26.9			600	22.4
3	550	8.24			211	26.8	379	26.9			483	29
4	500	7.43					168	19.6			193	19.4
5	450	8.19										
6	400	17.1										
7	350	6.7										
8	300	5.82										
9	250	5.28										
10	200	14.4										
11	150	7.46										
12	100	0.724										
Sum		98.9		100		100		100		100		100
In lane		100		100		100		100		100		100

***S. cerevisiae* DNA profile analysis by software. Total DNA profile prepared from *S. cerevisiae* cells treated with sub-lethal concentrations of Cd^{2+} (lane 1), As^{5+} (lane 2), Cu^{2+} (lane 3) and Pb^{2+} (lane 4). *Sccharomyces* DNA was extracted by "Biospin fungus genomic DNA extraction kit". The DNA analysis was carried out by agarose gel electrophoresis.**
% percentage of DNA in each band

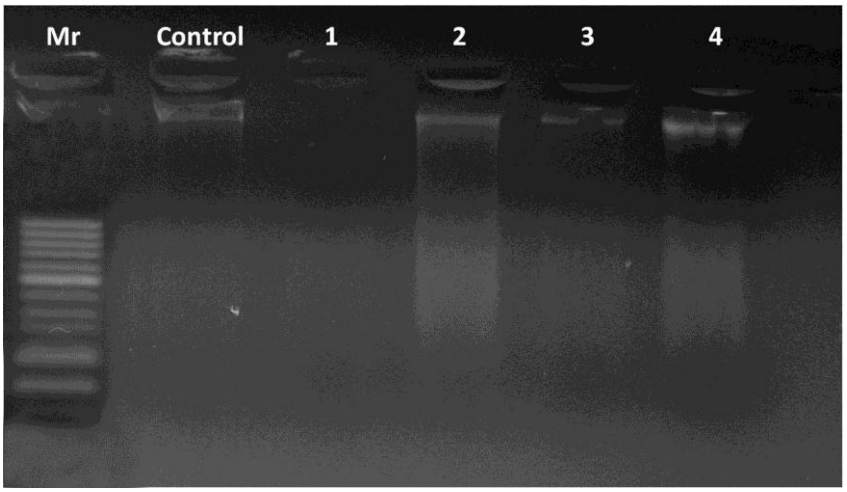


Fig (32): *S. cerevisiae* DNA profile analysis by electrophoresis. Total DNA profile prepared from *S. cerevisiae* cells treated with sub-lethal concentrations of Cd^{2+} (lane 1), As^{5+} (lane 2), Cu^{2+} (lane 3) and Pb^{2+} (lane 4).

(III) Effect of different concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions on total protein of *Saccharomyces cerevisiae*:

The effect of different concentrations (50- 200 mg/l) of Cd^{2+}, Cu^{2+}, Pb^{2+},and As^{5+} ions on total proteins of *S. cerevisiae* that was grown after four days growth on glucose peptone broth media (in static culture at 27 C°, pH=5), amended and not amended with metal ions was studied.

Table 37 and figure 33 showed the total concentrations of *S. cerevisiae* protein as control and also under the effect of different Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions concentrations ranged between (50-100mg/l). The data revealed that by increasing the concentration of Cd^{2+}, Cu^{2+}, Pb^{2+} or As^{5+} ions, the total protein decreased. The degree of the inhibitory effect of metal ions on total protein was varied according to the type of metal ions.

Under control conditions, *S. cerevisiae* total protein was 305.2 mg/g. This value decreased till 123.5 mg/g under 200 mg/l Cd^{2+} treatment, 187.9 mg/g under 200 mg/l Cu^{2+} treatment, 127.1 mg/g under 200 mg/l Pb^{2+} treatment, and 97.2 mg/g under 200 mg/l As^{5+} treatment. These values reflected that the degree of metal toxicity towards total protein increased in order; As^{5+} >Cd^{2+} >Pb^{2+} >Cu^{2+}.

Table 37: Average values of the effect of different concentrations (mg/l) of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions on the total proteins content (mg/g) of *S. cerevisiae*

Metal conc. (mg/l)	Total protein content (mg/g)			
	Cadmium	Copper	Lead	Arsenate
Control (0)	305.2	305.2	305.2	305.2
50	270.4	291.5	285	222.8
100	220.7	266.8	247.2	138.5
150	177.9	218.4	187.8	114.8
200	123.5	187.9	127.1	97.2

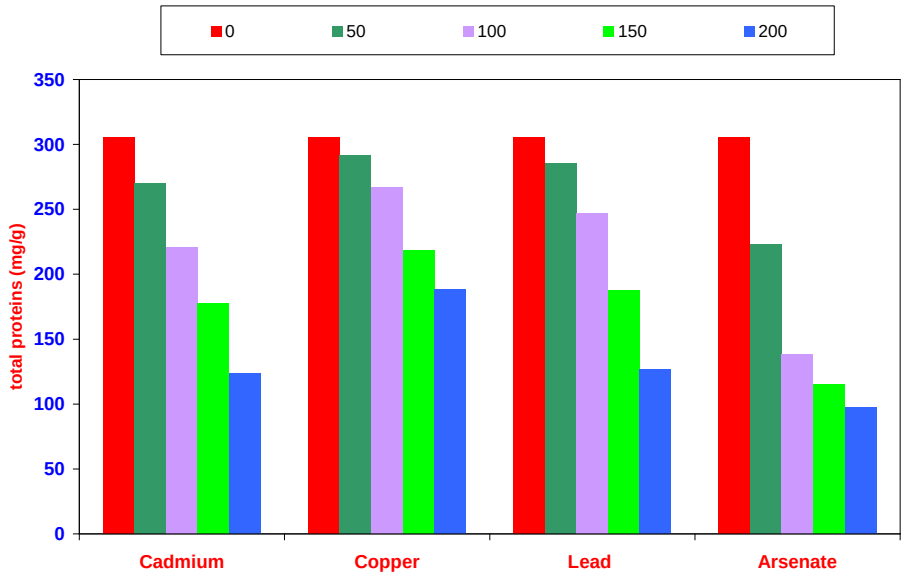


Fig (33): Effect of different Cd^{2+}, Cu^{2+}, Pb^{2+}, and As^{5+} ions concentrations (50, 100, 150 and 200 mg/l) on the total protein content (mg/g) of *S. cerevisiae*

(IV) Effect of different concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions on total carbohydrates of *Saccharomyces cerevisiae*:

Effect of different concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions on total carbohydrates of *S. cerevisiae* was placed secondary after total proteins. Different concentrations (50- 200 mg/l) of Cd^{2+}, Cu^{2+}, Pb^{2+},and As^{5+} ions were used to study their effect on total carbohydrates of *S. cerevisiae* that was grown after four days growth on glucose peptone broth media (in static culture at 27 C°, pH=5), amended and not amended with metal ions was studied.

Table 38 and figure 34 showed the total concentrations of *S. cerevisiae* carbohydrates as control and also under the effect of different Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions concentrations ranged between (50-100mg/l). The data also revealed that by increasing the concentration of Cd^{2+}, Cu^{2+}, Pb^{2+} or As^{5+} ions, the total carbohydrates decreased. The degree of the negative effect of metal ions on total carbohydrates was varied according to the type of metal species.

Under control conditions, *S. cerevisiae* total carbohydrates were 115.6 mg/g. This value decreased till 50.2 under 200 mg/l Cd^{2+} treatment, 73.8 under 200 mg/l Cu^{2+} treatment, 52.7 under 200 mg/l Pb^{2+} treatment, and 30.6 under 200 mg/l As^{5+} treatment. These values reflected that the degree of metal toxicity towards total carbohydrates increased in the previous same order that was; $As^{5+} > Cd^{2+} > Pb^{2+} > Cu^{2+}$.

Table 38: Average values of the effect of different concentrations (mg/l) of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions on the total carbohydrates content (mg/g) of *S. cerevisiae*

Metal conc. mg/l	Total carbohydrates contents (mg/g)			
	Cadmium	Copper	Lead	Arsenate
Control (0)	115.6	115.6	115.6	115.6
50	91	108.5	100.6	89.4
100	77.4	95.1	83.9	67
150	66.5	84.7	74.3	59.6
200	50.2	73.8	52.7	30.6

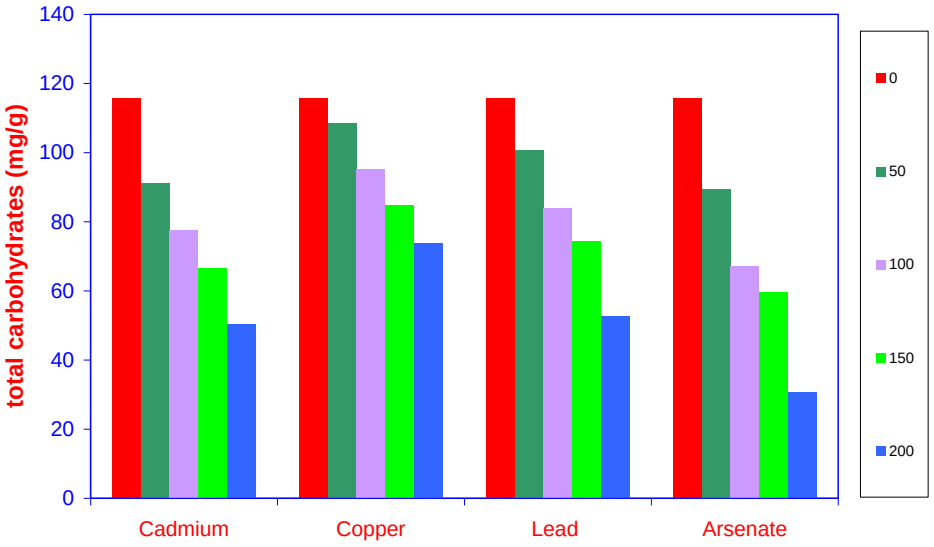


Fig (34): Effect of different Cd^{2+}, Cu^{2+}, Pb^{2+}, and As^{5+} ions concentrations (50, 100, 150 and 200 mg/l) on the total carbohydrates content (mg/g) of *S. cerevisiae*

(V) Effect of different concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions on total Lipids of *Saccharomyces cerevisiae*:

Finally, the effect of different concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions ranged between 50 – 200 mg/l on total lipid of *S. cerevisiae* was studied. *S. cerevisiae* was grown after four days growth on glucose peptone broth media (in static culture at 27 C˚, pH=5), amended and not amended with metal ions was studied.

Table 39 and figure 35 showed the total concentrations of *S. cerevisiae* lipids as control and also under the effect of different Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions concentrations ranged between (50-100mg/l). The data also revealed that by increasing the concentration of Cd^{2+}, Cu^{2+}, Pb^{2+} or As^{5+} ions, the total lipid decreased. The degree of the negative effect of metal ions on total lipid was varied according to the type of metal species.

Under control conditions, *S. cerevisiae* total lipid was 52.3 mg/g. This value decreased till 23.2 under 200 mg/l Cd^{2+} treatment, 33.1 under 200 mg/l Cu^{2+} treatment, 23.7 under 200 mg/l Pb^{2+} treatment, and 16 under 200 mg/l As^{5+} treatment. These values reflected that the degree of metal toxicity towards total lipid increased in the previous same order that was; As^{5+} >Cd^{2+} >Pb^{2+} >Cu^{2+}.

Table 39: Average values of the effect of different concentrations (mg/l) of Cd^{2+}, Cu^{2+}, Pb^{2+} and As^{5+} ions on the total lipid content (mg/g) of *S. cerevisiae*

Metal conc. (mg/l)	Total lipid contents (mg/g)			
	Cadmium	Copper	Lead	Arsenate
Control (0)	52.3	52.3	52.3	52.3
50	46.9	50.3	48.8	37.5
100	41.7	44.6	42.5	28.9
150	34	39.6	36.3	20.1
200	23.2	33.1	23.7	16

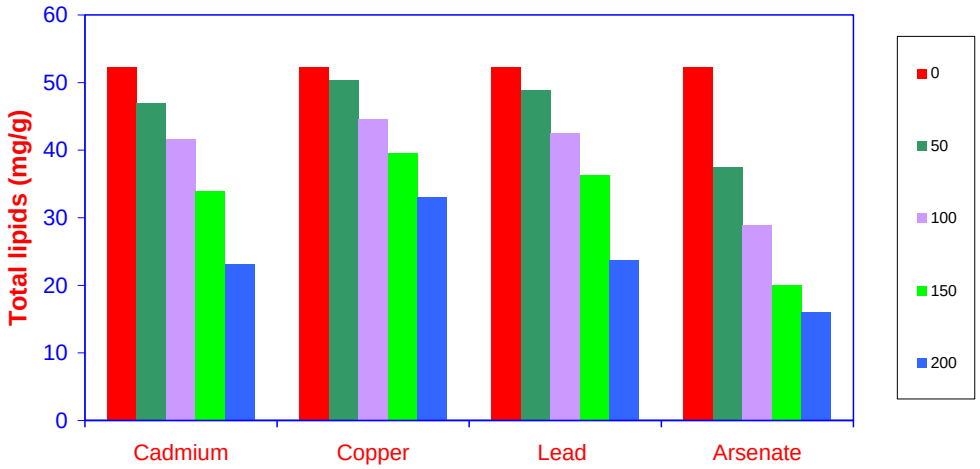


Fig (35): Effect of different Cd^{2+}, Cu^{2+}, Pb^{2+}, and As^{5+} ions concentrations (50, 100, 150 and 200 mg/l) on the total lipids content (mg/g) of *S. cerevisiae*