Studies on growth parameters and Protein Content of Yeast Saccharomyces cerevisiae on increasing concentration of Lead (Pb)

Archana Jain

Associate Prof.; Vivekananda Institute of Technology –East Jaipur (RJ)

***Abstract*— The paper presents an effect of Lead (Heavy metal) on growth characteristics and protein content of yeast Saccharomyces cerevisiae. The review of literature on effect of heavy metals on living organisms gives the information about their toxicity and reduction in growth of organism. Lead is a common heavy metal and mankind has known its utility for centuries. It shows toxic effects when accumulated by organism. Saccharomyces cerevisiae is selected for experiment as it is easy to grow in normal laboratory conditions. The study may predict the use of S.cerevisiae in controlling metal ion concentration in the environment.**

Keywords*— *Cell Membrane, nutrients, growth parameters, metallothioneins.

I. INTRODUCTION

All living organisms depend upon external environment for essential nutrients. Air, water and soil are the constituents of our atmosphere which remains in contact with organisms and constitutes eternal environment [1].Essential nutrients like Oxygen, Nitrogen, phosphorous, Potassium, Carbon, Sodium Plants and animals accumulate essential nutrients from environment [2]. Carriers and permeases are the protein molecules which are found in the plasma membrane of living organisms are helpful in the accumulation. Along with essential nutrients other substances present in the environment are non-essential and have toxic effects, when accumulated by organisms [3]. Higher concentrations of these are not tolerable for organisms and they do not survive[4] Industrialization and human activities are the major sources of non-essential elements.The metals having a density more than five are known as heavy metals. This class of heavy metals includes about 38 elements. Toxic levels of heavy metals in environment are found due to the various reasons like soil near roads contains a high level of Lead concentrations, heavy metals are components of fungicides, pesticides and disinfectants, There is also evidence of increasing general environmental pollution from widespread use of compounds of Lead-and Mercury[5].

Influx of heavy metals into the living cells may occur by different ways. It may be diffusion controlled or carrier mediated process. The protein molecules are responsible for the transportation of nutrients as these protein molecules have binding sites, which bind to the metabolites, transporting them across the semi-permeable plasma membrane[6]. Heavy metal binding proteins have been identified and characterized by workers in lower plants[7] Actual mechanism of heavy metal uptake is still not known. Proteins are involved in the action of most heavy metal ions at normal and toxic concentrations. Generally, a metal ion concentration of 1 µg/ml is satisfactory for specific binding. Proteins in intra nuclear inclusion bodies bind Lead, when present at high concentration and much of Cadmium and Mercury absorbed is sequestered by soluble kidney protein, metallothionein. Protein molecules generally act as a multidentate ligand and bind with metal ion at different binding sites. When the essential metals are replaced by toxic metal ions the activity of the protein is affected. Some microorganisms appear to be able to withstand metal concentration. Resistance power of fungi towards heavy metals has been studied but examples are relatively rare. They show that growth was retarted at high concentrations of heavy metals.

The work done on the tolerance of heavy metals in fungi shows that these are able to grow in extreme conditions. Several workers have isolated micro-organisms tolerant to high metal concentration from contaminated soils. Seal (1970) showed that fungus, Aspergillus, from Copper mine soil has a greater Copper tolerance than related fungus from normal soil [8]. Wide variety of bacteria and algae has also been studied for heavy metal tolerance. The most common species of bacteria that are able to resist extreme conditions (high concentrations of Cu and Fe and extreme pH) are Thiobacillus ferroxidans. Chlorella vulgaris, green algae, is tolerant to Ba, Mn, Pb and Cu.Higher concentration of heavy metals, therefore, may not prevent the growth of micro-organisms. Some micro-organisms appear to be able to withstand metal concentration.

II. LEAD

Lead naturally occurs in the form of mineral deposits, mainly as galena (Lead sulphide). It has low melting point and is malleable and relatively inert. These properties of Lead make it an ideal material for many applications. Man has used lead since prehistoric times. It was known even in second century B.C. that working with Lead is a health hazard[9] Later during the technological revolution of the 18th and 19th centuries the wide spread use of Lead in manufacturing processes resulted in industrial Lead poisoning and serious cause of ill health and deaths.

2.1 CHEMISTRY OF LEAD

The alchemist believed Lead to be the oldest known metal and associated it with the planet Saturn. The atomic weight of Lead is 207.19; atomic no. 82, melting point 327°C, boiling point 1744°C and sp.gr. 11.35 at 20°C. It has a valence of 2 and 4. Lead is obtained chiefly from galena (PbS) by roasting process. It is a bluish white metal of bright luster, very soft, highly malleable and ductile and a poor conductor of electricity. It is very resistant to corrosion

Uses: Lead is very effective as a sound absorber, and is used as a radiation shield around x-ray equipment and nuclear reactors. It is used to absorb vibrations. White Lead, the basic Lead carbonate, sublimed white Lead (PbSO4) chrome yellow (PbCrO4), red Lead oxide (Pb3O4) and other Lead compounds are extensively used in paints. Lead oxide is used in producing fine crystal glass and flint glass of high index of refraction for achromatic lenses. Its nitrate and acetate are soluble salts and are used in chemical laboratories. Lead salts are sometimes used in medicines, as antiseptic and astringents. Care must be taken while using Lead as it is a cumulative poison [10].

2.2 ATMOSPHERIC LEAD

In the early part of this century smelting and refining were the main sources of atmospheric Lead. Today the combustion of Leaded petrol in automobiles is the largest contributor of Lead in our atmosphere.

Approximately 90% of air borne Lead in the United States, which can be traced, comes from the combustion of Petrol (Data from Nat Inventory Air Pollution Emission and control 1972). Three Lead isotopes 206Pb 207Pb 208Pb from radioactive decay of the Uranium chain elements contributes to total environmental Lead. Forest fires are known to be another source of atmospheric Lead. Refuse incineration is also a significant source of Lead. A negligible natural source of Lead may be from the process of evaporation and volatilization of elements associated with "bubble bursts" from surface of ocean waters.

2.3 SURFACIAL LEAD

Significant quantities of Lead get deposited on surface from waste and sewage. Soils near highways and industrial areas are also important source of Lead. Lead compounds are widely spread on the surface of soil and during the rainy season due to the flow of water, salts of Lead accumulate in water bodies. Precipitation from the atmosphere tends to increase the Lead contents of soil and aquatic systems. Most of the Lead compounds entering aquatic system are not water-soluble and are removed by sedimentation. Plants through their roots also absorb lead; hence there is a direct relationship between natural contents of Lead in plants and soil [11].

Street dust is a potential source of ingested Lead, especially in children. Continuous exposure to Lead, results in its gradual accumulation in the body. No beneficial role of Lead has yet been known and there is no substantial evidence to suggest that it is an essential trace element. On the contrary, at the cellular level Lead is f found to interfere with the respiratory pigment, energy production and membrane function.

2.4 BIOLOGICAL EFFECTS

Biological interest in Lead was initiated as a result of its toxic properties as an industrial hazard to man and plants. The concentration of Lead estimated to produce Lead poisoning is well documented. Lead is toxic because it mimics many aspects of the metabolic behavior of Ca and inhibits many enzyme systems. Attention has, therefore, been confined to the biological consequences of long-term exposure to low concentration of Lead present in the environment and factors that influence the retention of Lead in living organisms.

It was observed that exposed children have lower score on psychometric intelligence, auditory and speech processing and show more non-adaptive behavior. Lead accumulates in bones and tissues and in high concentration causes characteristic symptoms of Lead poisoning resulting in anemia. Anemia in man is caused by disorders in the porphyrin metabolism. It seems that harm affects virtually all steps in the process of harm synthesis. Other problems created by high concentration of Lead are impairment of the function of liver, kidney, spleen, spinal deformities and ultimately death. Both the central and peripheral nervous systems are affected by absorbed Lead. In man one of the chief concern of Lead toxicity is its effect in causing brain damage particularly in the young. Lead pollution can also induce aggressive behavior in animals.

III. MATERIALS

Yeast strain 'Saccharomyces cerevisiae' was obtained from NCIM, Pune. It is easy to culture in moderate laboratory conditions and serves as an important tool to study the vital metabolic activities going on inside the cell. Saccharomycetes are the ascomycetous yeasts. (The term yeast refers to simple morphological forms of Ascomycetes.) Ascosporogenous yeasts are known particularly for their ability to ferment carbohydrates, hence the name Saccharomycetes. These are well distributed over the surface of Earth and are particularly abundant in sugar-rich substrates such as nectar of flowers and surfaces of fruits, vegetative parts of plants etc. In contrast to most other ascomycetes, Saccharomycetes are unicellular organisms and do not forms a mycelium. Cells occur in variety of shapes such as globose, elongated or rectangular. Individually, cells are colourless but on solid media they produce coloured colonies. There are three main functions of yeast plasma membrane. Firstly, it forms an expandable cover and protective barrier for the protoplast, secondly, it controls the entry and exit of solutes to and from the cells, in other words, allows communication of cells with the external environment, and thirdly, it works as an organelle on which enzyme reactions leading to synthesis of many important molecules take place. The structure of plasma membrane is best explained by fluid mosaic model.

According to this model, phospholipid and sterols form a lipid bilayer in which the non-polar tail parts of lipids face each other at the core of the bilayer and polar head parts face outwards forming the basic matrix in which two types of proteins are embedded at irregular intervals. First type of proteins are loosely attached extrinsic proteins which are relatively easy to remove from the membrane. While the

second type of proteins are the proteins which cannot be easily removed. These proteins are asymmetrically oriented in the lipid bilayer. The individual lipid and protein subunits in a membrane form a fluid mosaic and these are free to move laterally in the plane of the membrane. Most of these proteins are carriers and permeases, responsible for the influx and efflux of nutrients.

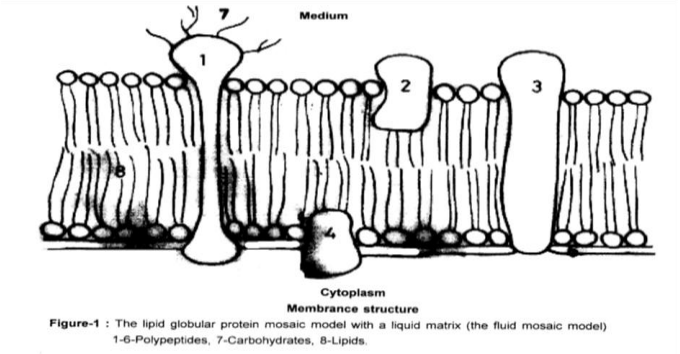


Figure-1 : The lipid globular protein mosaic model with a liquid matrix (the fluid mosaic model) 1-6-Polypeptides, 7-Carbohydrates, 8-Lipids.

Previous Work done on Yeast S.cerevisiae

The literature reveals that many workers have used the yeast cells as the model system in the past study the structure and functional relationships.

Lipids modulate the activities of several microbial membrane proteins, including those related to cellular permeability. In membrane transport amino acid uptake in S.cerevisiae has been studied by Keenan et al (1982). Ion transport in S.cerevisiae has been studied by Kleinhans et al, (1979). Sugar transport has been studied by Franzusoff and Arillo (1983). Thiamine transport has been studied by Iwashima and Nose (1975). Dye uptake has been studied by Bard et al, (1978). Polyene antibiotic senstivity has been studied by Gale et al, (1975). In membrane bound enzymes the AT pase activity in S.cerevisiae was studied by Bottema et al (1983). L-Kynurenine hydroxylase activity was studied by Janki et al, (1975), Cytochrome oxidase activity has been studied by Thompson and Parks (1974).

All glass apparatus used was of Corning/ Borosil make. They were washed first with dil. Chromic acid and then successively with distilled water.

Agar, Peptone and Yeast extract were obtained from Difco, U.S.A. Bovine serum albumin and Coomassie Brilliant Blue - G were obtained from Sigma Chemical Company, U.S.A. All the chemicals used were of Anal R, CDH grade.

The yeast cells were grown in SGM containing different concentrations (like 5 ppm, 10ppm, 50ppm, 100ppm and 200ppm) of Lead. Temperature was noted during the experiment and the flask containing the cells was kept on a magnetic stirrer for 10 hrs.

IV. Methods and Observations

To study the growth characteristics of Saccharomyces cerevisiae, the cells were transferred from the YEPD (which is the solid medium in the form of slants containing Glucose, Agar agar, Yeast extract and peptone) to SGM (which is a suitable liquid media) in sterile condition of laminar flow. Growth of cells was monitored by taking the Optical Density every hour and drawing the growth curve. In growth curve time period between the points A and B is called the lag phase, in which the cells start the growth and the metabolic activities are very slow. The stage from point B to C is the mid log stage and point Z is mid log phase which is 10 hours at 27°C. Mid log phase is the time period in hours at which the rate of multiplication is the maximum and indicates maximum metabolic activity as well as maximum accumulation of nutrients by cells. Stage between point C to D is the saturation phase in which the metabolic activity shows the constant rate. All the experiments were done at mid log phase i.e./after the growth of 10 hrs. in SGM.

The essential conditions for the normal growth of yeast cells

1. Suitable media which contains Carbohydrates, Nitrogen source, Agar-agar (for the solidification), if required, source of vitamin, metals etc.
2. Temperature between 20°C to 30°C.
3. pH of the media between 3.5 to 4.5 i.e. acidic condition. pH above and below the above range may retard the growth of cells.
4. Completely sterilized media to avoid the interference of other microorganisms.
5. Continuous stirring of the liquid media is necessary, for the continuous supply of oxygen to the yeast cells during the growth. If the media is kept stable, the cells die.

Observations for the growth characteristics:

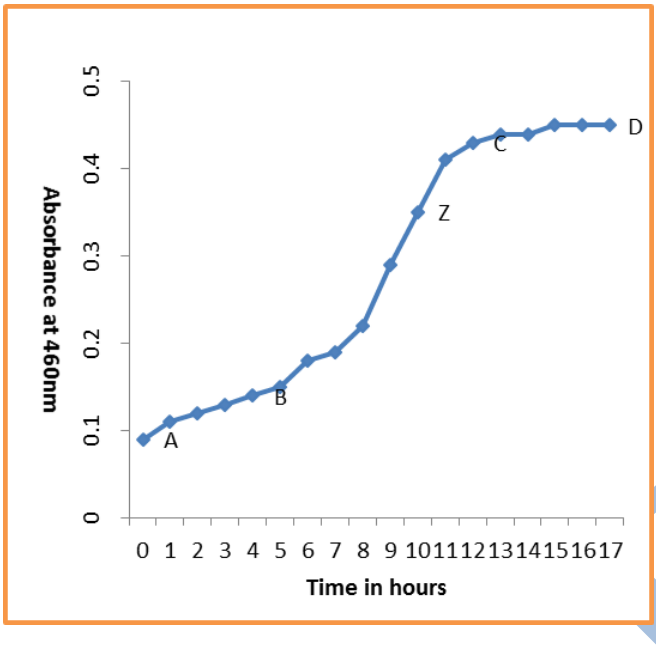
From the growth curve the time at which cells were growing at the maximum rate was estimated. This is called midlog phase. All the experiments were performed at mid log phase. Growth was monitored by observing Absorbance at 460 nm at different time interval.

Growth parameters of Saccharomyces cerevisiae

Table-1

S. No.	Time in hours	Absorbance at 460 nm
1	0	0.09
2	1	0.11
3	2	0.12
4	3	0.13
5	4	0.14
6	5	0.15
7	6	0.18
8	7	0.19
9	8	0.22
10	9	0.29
11	10	0.35
12	11	0.41
13	12	0.43
14	13	0.44

15	14	0.44
16	15	0.45
17	16	0.45
18	17	0.45



Graph-Showing Growth parameter

RECORD OF THE WEIGHT OF S.CEREVISIAE IN THE PRESENCE OF Pb (NO3)2

The growth of S.cerevisiae cells was also monitored by determining the dry weight of the cells in the presence and absence of lead concentrations. The results were compared and it was observed that the dry weight of cells reduced remarkably at heavy metal concentration of 200µg/ml

Table-2

S.No.	Lead Concen-tration (µg/ml)	wt. of emtpy weighing tube (A) gm.	wt.of weighing tube + dry yeast(B)gm.	wt. of yeast cells(B-A) gm.
1	Control	3.7205	4.0216	0.3011
2	5	3.6194	3.8206	0.2012
3	10	3.7453	3.9368	0.1915
4	50	3.4505	3.5448	0.0943
5	100	2.8754	2.9596	0.0842
6	200	3.7105	3.7705	0.06

Protein contents in yeast saccharomyces cerevisiae

To study the total proteins of yeast Saccharomyces cerevisiae, extraction of proteins were done. For this purpose the yeast cells were grown separately in SGM in the absence as well as in the presence of Pb in different concentrations for 10 hours. The culture was centrifuged, and treated with 5 ml of 10% TCA and again centrifuged for 3min. The mixture was centrifuged now with ethanol ether mixture, (1:1 v/v) to remove the excess of TCA. Cells were then boiled in glycine buffer on water bath for 3 min and centrifuged. Supernatant liquid was used as the source material for protein estimation and separation.

To determine the total protein contents, Follin's reagent was used (also called Follin - Ciocalteau reagent). It is quite complex and contains phosphomolybdic acid and tungstate. The aromatic amino acids-tyrosine and tryptophan-present in the proteins react with these chemicals and produce a dark blue colour. A number of reagents listed below were also prepared for the experiments under study.

2% Sodium Carbonate in 0.1 N NaOH

0.5% Copper Sulphate solution in 1% Sodium potassium tartrate solution (Freshly prepared solution was used.)

C, 50 ml of reagent A was mixed with 1 ml of reagent B, just prior to the use.

Follin-Ciocalteau reagent—The reagent was commercially available, however it had to be diluted with equal volume of water just before use. The reagent can also be prepared in the laboratory into a 2 liter flask by mixing out 100 gm. sodium tungstate, 25 gm. sodium molybdate, 50 ml 85% phosphoric acid and 100 ml. concentrated HC1 with 500 ml. distilled water. The mixture" was refluxed gently for about 10 hours with air condenser. After cooling, 150 gm. of lithium sulphate, 50ml of distilled water and a few drops of Bromine were added and boiling continued for another 10 minutes without the condenser. This helps in removing excess Bromine. After cooling, the volume was made up to 1000 ml and the solution filtered, if necessary. It was taken care that the filtrate did not have any greenish tint. If greenish tint persisted it was boiled with Bromine once more. This was the stock reagent, which was diluted with equal volume of water just before use.

The standard protein Bovine Serum Albumin (BSA) solutions having different concentrations were prepared.

0.5 ml of sample solution was diluted with 0.5 ml of distilled water to make the total volume to 1 ml. to each experimental test tube, 5.5 ml. of the alkaline mix (reagents) was pipette out, mixed well and allowed to stand at room temperature for 15 minutes. 0.5 ml of the reagent was pipette out into each experimental test tube, mixing rapidly after each addition. The test tubes were left for 30 minutes and readings of the solutions formed were taken at 650 nm.

A proper blank solution without protein sample was prepared side by side, by mixing 1 ml distilled water and the reagents as that of the sample. From the absorbance of sample solutions and blank, standard curves between concentration of protein and absorbance were drawn.

Different solutions of known concentrations of BSA were prepared from the stock solution. Then 5.5 ml of reagent C and 0.5ml of Follins reagent were mixed. Distilled water was added to make the final volume to 7.0 ml. Optical Density (O.D.) was determined at 650 nm for different concentrations and standard curve drawn. Similar process was followed for proteins extracted from yeast cells and O.D. observed at 650 nm. From the standard curve total proteins were then estimated. The above method is known as Lawry's method [12].

Table-2
Table &graph for standard curve (protein estimation)

S.no.	Concentration(ml of BSA solution) (with conc. 1 µg/ml)	Dilution with Distilled water in ml	Absorbance at 650nm
1	0	1000	0.07
2	20	980	0.1
3	40	960	0.11
4	60	940	0.13
5	80	920	0.14
6	100	900	0.15
7	120	880	0.19
8	140	860	0.21
9	160	840	0.22
10	180	820	0.25
11	200	800	0.28
12	220	780	0.29
13	240	760	0.3
14	260	740	0.32
15	280	720	0.35
16	300	700	0.36
17	320	680	0.38
18	340	660	0.4
19	360	640	0.42
20	380	620	0.45
21	400	600	0.46

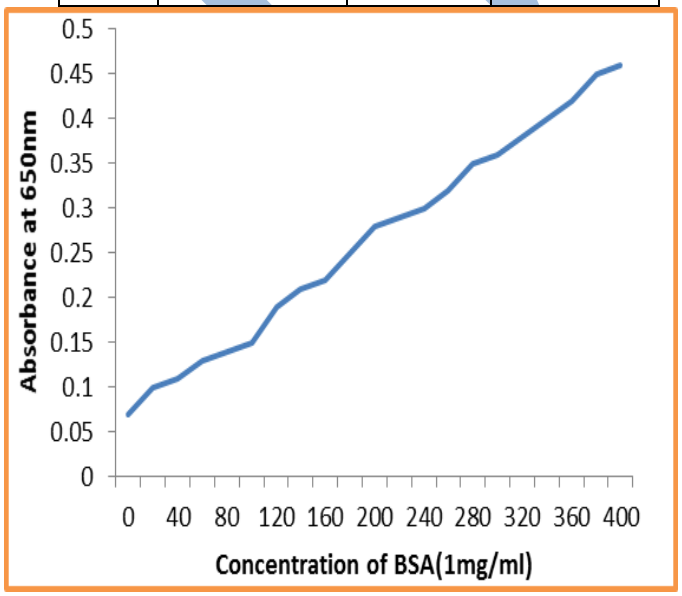


Table-3
Total protein contents in S. Cerevisiae in the presence of different concentrations of lead

Experiment no.1

S.No.	Final Concentration of Lead (µg/ml)	Absorbance at 650nm	Total Proteins(µg/ml)
1	Control	0.44	380
2	5	0.38	320
3	10	0.35	290
4	50	0.32	250
5	100	0.27	200
6	200	0.24	170

Experiment no.2

S. No.	Final Concentration of Lead (µg/ml)	Absorbance at 650 nm	Total Proteins (µg/ml)
1	Control	0.44	380
2	5	0.39	325
3	10	0.35	290
4	50	0.3	240
5	100	0.27	200
6	200	0.23	165

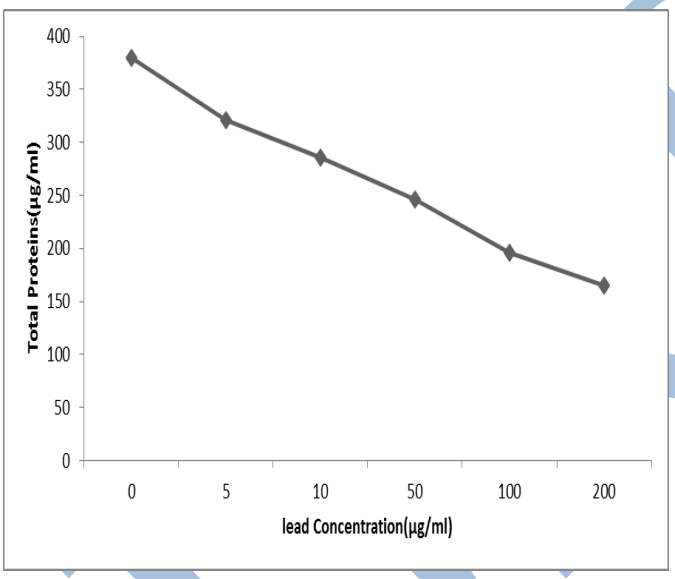
Experiment no.3

S.No.	Final Concentration of Lead (µg/ml)	Absorbance at 650 nm	Total Proteins (µg/ml)
1	Control	0.44	380
2	5	0.38	320
3	10	0.34	280
4	50	0.32	250
5	100	0.26	190
6	200	0.22	160

Average Total Protein Content

S. No.	Final Concentration of Lead (µg/ml)	Absorbance at 650 nm	Total Proteins (µg/ml)
1	0	0.44	380
2	5	0.38	321
3	10	0.34	286
4	50	0.31	246
5	100	0.26	196
6	200	0.23	165

Variation of Total Proteins with different concentration of lead



V. Results and Discussion

It was observed that on increasing the concentration of Lead from 5µg/ml to 200µg/ml, the mass and protein contents in the yeast decreased continuously. The minimum dry weight at 200(µg/ml concentration was found, 0.06gm in the case of Lead. It shows that the lead interference reduces the dry weight of the cells considerably.

The metal toxicity is a reasonable determinant of reduction in growth and that the degree of response of the organism is dependent on the amount of metal which traversed through the cell membrane and was not bound by detoxifying mechanism [13].

Total protein contents after treatment with lead 165µg/ml at 200µg/ml concentration. The control had 380µml protein contents under the same conditions.

These results confirm the findings of other workers. The protein concentration decreased due to lead exposition induced proteinemia In the case of Lead, it appears that it affects the metabolic activities of S.cerevisiae.. Anemia produced by lead treatment in rats has also been reported [14].

Occurrence of metallothioneins (MTs) in the organisms exposed to toxic heavy metals has been reported long back. Experiments have given information concerning heavy metal protein interactions. Metallothioneins and MT like protein molecules and polypeptides containing high cysteine contents have also been identified in higher plants. Induction of protein synthesis and accumulation of metailothionein in liver and kidney of some fishes has also been reported. All these reports suggest that the presence of heavy metal concentration inside the cells and in the immediate environment of the cells have metabolic effects on the organisms.

Present observations suggest that heavy metals may bind to certain proteins with enhancement, modification or inhibition of the normal biological activity. Protein molecules undergo a regulation in response to changes in the microenvironment. The total binding proteins decrease in proportion to heavy metal concentration. It is also confirmed in the past that proteins are utilized in response to the uptake of heavy metal ions. The other possible reason for the decreased protein synthesis could be the replacement of metal ions in protein synthesizing enzymes. Binding of heavy metals with specific protein molecules might 'therefore' affect its structure and function significantly. These metal bonded protein molecules are either excreted out by the cells or may be stored in some organelles of the cells. The heavy metals as environmental contaminants are toxic virtually to every system in the organisms. These effects lead to the decreased protein contents of the cells, and in turn in decrease of the body weight.

It is quite likely that cells come under great stress to synthesize new molecules to face the environmental stress to remove heavy metals accumulated inside the cells. It may be possible that free metal ions denature the protein portion or may have inhibitory effect on the interaction between the proteins and lipids in cell membrane.

References

[1]. Anarku, Y. (1968). J. Biol. Chem. 243: 3116-3122.
[2]. Baker, A.J.M. and Brooks, R.R.(1989). A review of metallic elements distribution ecology and photochemistry bio recovery. 1: 81-126.
[3]. Baker, A.J.M. and Walker, PL. (1989). Chemical speciation and bio-availability. 1(1): 7-17.
[4]. Antonovitfs, J., Bradshaw, A,O. and Turner, R.g. (1971). Adv. Ecol. Res. I : 1-85.
[5]. Ruhling, A. And Tyler, G. (1969). Bot.Notiser 122: 248-259.
[6]. Hasan, M.Z. and Pande, S.P. (1978). Indian Jour. Environ. Hlth.20: 232-248.9.
[7]. Blundell. T,L. and Jenkins, J.A. (1977). Chemical Society Review. X ' 6 (2): 139-170.
[8]. Seal, K. (1970). M.Sc. Thesis, University of Wales.
[9]. Egan, H. (1972). Lead in Environment ed. P. Hepple, Just of Petroleum, London. 34.
[10]. Hall, S.K. (1983). Journal of Chemical Education. 60 (2): 145.

[11]. Ma., W. (1989). Arch. Environ. Contam. Toxicology 18: 617-622.
[12]. Lowry, O.H., Rosebrough N.J., Farr, A.L. and Randal, RJ. (1951). J. Biol. Chem. (193): 265-275.
[13]. Faulkes, E.C. (1988). Toxicology. 52 (3) : 263
[14]. Garcia - Arribas, O, Perez - Calvo, M., Ozonas, B. Ribas andSangerman. J. M. Escribano (1998). An. R. Acad. Farm. 64(4) : 599-614.
[15]. Raeva, N.N., Sidorov, A.V., Yu. G. Izv. Akad (1999). Nauk, Ser. Biol (6) : 748-755.
[16]. Chen, Z., Ren, L. ShaoQ, Shi, D, Ru, B. (1999). Mr. Pollut. Bull. 39 (1-12) : 155-158.
[17]. Angandi, S. B. and Mathad, Pratima (1998). J. Environ. Biol. 19 (2): 119-124.
[18]. Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, et al. (2003) Global analysis of protein expression in yeast. Nature 425: 737–741.
[19]. Kushnirov VV (2000) Rapid and reliable protein extraction from yeast. Yeast 16: 857–86.

