International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 02, 2020
ISSN: 1475-7192

Bioaccumulation of cadmium and quantitative characterization of proteins ofSaccharomyces cerevisiae

Sarla Kumari*[1], Chandra Mohan[2], Vinod Kumar[3]

Abstract

Bioaccumulation using microbes is an efficient strategy for heavy metal removal due to its low cost, high efficiency and ecofriendly nature. Recent inventions have been made to understand metal-microbe interaction and their application for metal accumulation. Yeast being a typical eukaryote, has many essential features similar to higher eukaryotes and can be used to investigate various aspects of their cell biology.Saccharomyces cerevisiae can be used as model system because of easy cultivation using normal media.It is therefore, planned to investigate the bioaccumulation of cadmium by S.cerevisiae. Molecules that participate in binding of metal ions have been identified and found in several species of yeast and other fungi.Proteins are able to transport a charged heavy metal ion across biological membrane. Metallothioneins like proteins are important mediatorwhich help in metal uptake and hence accumulation, especially for cadmium.This paper attempts to present the correlation between cadmium concentrations in the environment and accumulation byS.cerevisiae along with the involvement of metal binding proteins.

Keywords: *Bioaccumulation, cadmium, Saccharomyces cerevisiae, proteins.*

I. Introduction

Heavy metals are present in the environment as a consequence of their use in various industries[1,2]andget accumulated throughout the food chain which leads to serious ecological and health problems[3-5].Common sources of cadmium are fertilizers, pesticides, nuclear fission plant, Cd-Ni batteries and electroplating processes in industries[6].It is generally accepted that Cd^{2+} is not essential for growth of plants and micro-organisms, unlike other heavy metal ions such as Zn^{2+}, Cu^{2+}, Mo^{2+} and Mn^{2+}. In the environment, cadmium metal is reported as harmful to living organisms especially animals and microorganisms, its permissible level is 0.06µg/ml[6].

[1] *Department of Chemistry, S P C Government College, Ajmer 305009 Rajasthan, India*
[2] *SMAS, K R Mangalam University, Gurugram, Sohna, 122103, India*
[3] *SMAS, K R Mangalam University, Gurugram, Sohna, 122103, India*

Microorganisms can decontaminate metals by their valence conversion, volatilization or extracellular chemical precipitation[7-10].Microbes have various functional groups on their cell surface those bind with metal cation[11]. It is well known that microorganisms accumulate metals by different processessuch as membrane transport and biosorption[12]. The biosorption of cadmium from synthetic aqueous solutions using yeast has been investigated[13]. *Saccharmyces cerevisiae* is an ideal organism for metal ion removal and to investigate the metal-microbe interaction[14-20]. It has been reported that *S.cerevisiae* accumulate Cd^{2+} cation as well as Cu^{2+}, Pb^{2+}, Zn^{2+} and Co^{2+} ions[21]. Membrane bound proteins play important role in the active transport of nutrients[22-25]. Proteins act as carriers and permeases having binding sites to bind and transport essential nutrients inside the yeast cells[26-37].

The metal transport system of microbes is influenced by the presence of heavy metal ions of the same charge and ionic radius[38] because these metals compete with essential metals and occupy metal binding sites in plasma membrane. These may interact with physiological ions such as $Cd^{2+}$with Zn^{2+} or Ca^{2+}, Ni^{2+} and Co^{2+} with Fe^{2+}, thereby inhibiting the function of the respective cation. Therefore, biochemical properties of microbes can be altered by the presence of heavy metals.The present study was carried out to characterize Cd^{2+}binding proteins in *S.cerevisiae*. For this purpose, extracted proteins in the presence of different concentrations of Cd^{2+}were separated according to their molecular mass by sodium dodecyl sulfate polyacrylamide gel electrophoresis(SDS-PAGE).

II. Experimental

Yeast strain preservation

Saccharomyces cerevisiae (strain 3131) culture waspreserved on standard YEPD solid sterile medium. It is used for the growth and propagation of yeast culture. It primarily contains 1% yeast extract, 2% peptone, 2% dextrose and 2% agar-agar.

Observation of growth characteristics

For studying growth characteristics of yeast synthetic growth medium was prepared in double distilled water by dissolving 0.5% glucose, 0.025% $MgSO_4$, 0.025% $CaCl_2$, 0.3% KH_2PO_4, 0.3% $(NH_4)_2SO_4$, and 0.001% biotin and autoclaved.A loop full of yeast cells was scraped from frozen YEPD slant and suspended in 50ml of sterile liquid synthetic growth medium (SGM) at 30ºC, the flask placed on a horizontal shaker for shaking upto 15h. Simultaneously, optical density of growing culture was measured per hour at 570nmwith the help ofUV-Visible spectrophotometer at 30 ºC upto 15h. A plot, time v/s optical density was drawn (Fig. 1) for the determination of the mid log phase at which the metabolic activities and accumulation of nutrients by the cell become maximum. It was obtained after 7h of growth of the yeast cells.

Estimation of dry mass of the yeast

Yeast cells were grown upto 7h at 30 ºC in SGMcontaining 5µg/ml,10µg/ml,20µg/ml, 50µg/ml and 100µg/ml of Cd^{2+} respectively. At mid log phase of growth, SGM were centrifuged. The harvested cells were

washed using citrate buffer (pH 4.8).Then washed usingdistilled water and dried at room temperature. Dry mass of the cells were measured and results were compared with control (yeast grown in the absence of Cd^{2+})(Table 1).

Analysis ofaccumulated cadmium

Yeast cells grown in SGM containing (5,10,20,50,100) µg/ml of Cd^{2+}upto 7h at 30 ºC then were collected by centrifugation and washed using citrate buffer (pH 4.8). The harvested cells were dried, weighed and digested with 1% HNO_3 solution[39,40]. Accumulated cadmium by yeast cells was measured by Varian 300 atomic absorption spectrophotometer and results were compared with control (Fig. 2).

Estimation of total protein contents in yeast cells

Yeast cells were grown in SGM at all working concentrations of Cd^{2+}as well as without Cd^{2+} upto 7h, collected by centrifugation, dried and treated with 10% trichloroacetic acid 5ml and ethanol-ether (1:1v/v) mixture 5ml, followed with addition of tris glycine buffer(0.2 M, pH 8.6)10ml then boiled for 3min. The cells were again centrifuged and supernatant were used for the estimation of total protein contents by Lowry's method[41] using Follin's reagent respectively. The results were compared with control (Fig. 3).

Estimation of molecular mass ofindividualprotein

The extracted proteins from ***S.cerevisiae*** in the presence of (5,10,20,50,100)µg/ml of Cd^{2+}were treated with stacking buffer (0.5M tris-HCl) and employed to SDS-PAGE. The gel has molecular sieving properties and distribution of pore size of the same order of magnitude as the size of proteins normally found in nature[42], therefore, proteins get separated on the basis of their molecular mass. SDS-PAGE was performed using disk electrophoresis apparatus. Electrophoresis of all the samples was performed under same chemical environment, which results in the discrete protein bands. Standard molecular mass marker protein (14.3 kDa to 97.4 kDa) was also employed for electrophoresis simultaneously. The R_f (relative mobility) values for standards (Table 2) as well as for each discrete protein band were calculated (Table 3). For standards a plot R_f v/s log $M_r$was drawn. The R_f values were used to measure molecular massof SDS-denatured polypeptides by interpolation[43].

III. Results and discussion

Effect of cadmium on dry mass

Cadmium enters the cell by some manganese uptake system in *S.cerevisiae*[44].Moreover, Cd^{2+} and Ca^{2+} show similarity in their chemistry due to similar atomic sizes, so cadmium may get accumulated in place of calcium due to mimicry effect.The effect of Cd^{2+} concentration on accumulation and growth of this strain was studied. Maximum dry mass of *S.cerevisiae* was found in absence of Cd^{2+} while the growth was inhibited by all concentrations of Cd^{2+} tested, which suggests that cadmium is not a preferred metal inside the cell.Its concentration influences various biomolecules. Cd^{2+} start to show biochemical effects on yeast when the concentration increases more than 20µg/ml. When 100µg/ml Cd^{2+}concentration was supplied maximum accumulation of cadmium(Fig. 2)

and lowest growthof yeast was found (Table 1).These results clearly indicate that metal removal capability in *Saccharomyces* increased with decrease in their biomass due to high surface area to volume ratio.

Table 1:

Accumulation of Cd^{2+} by yeast *Saccharomyces cerevisiae* in µg/ng of total proteins

S.N.	Total Cd^{2+} Concentration Supplemented (µg/ml)	Dry Mass (mg)	Total Protein (ng/mg of dry mass)	Absorbance on A.A.S.	Accumulated Cd^{2+}(µg/mg of dry mass)	Accumulated Cd^{2+}(µg/ng of proteins)
1	Control	101.2	2880.43	0.0001	000.0000	0.000
2	005	074.0	1977.03	0.0084	000.0506	00.02563×10^{-3}
3	010	056.0	1926.79	0.0159	000.1294	00.06719×10^{-3}
4	020	081.0	2232.10	0.0205	000.1142	00.05116×10^{-3}
5	050	012.4	7040.32	0.3572	000.8871	00.12600×10^{-3}
6	100	006.4	4531.25	1.1458	191.7968	42.32759×10^{-3}

Table 2:

R_f values of standard proteins from 14.3 kDa to 97.4 kDa

S.N.	Molecular mass of protein (kDa)	Log M_r	R_f
1	97.4	1.98	0.050
2	66.0	1.81	0.083
3	43.0	1.63	0.150
4	29.0	1.46	0.300
5	20.1	1.30	0.666
6	14.3	1.15	0.916

kDa = kilodalton(unit of molecular mass)

Table 3:

R_f values and corresponding molecular masses of proteins extracted from *S.cerevisiae*.

Cd^{2+}concentrations											
Control		**5 (µg/ml)**		**10 (µg/ml)**		**20 (µg/ml)**		**50 (µg/ml)**		**100 (µg/ml)**	
R_f	**Mass**	**R_f**	**Mass**	**R_f**	**Mass**	**R_f**	**Mass**	**R_f**	**Mass**	**R_f**	**Mass**
0.037	110.4	0.087	63.10	0.517	23.28	0.603	21.09	0.060	84.53	0.943	13.65
0.561	22.08	0.522	23.01	0.737	18.07	0.948	13.30	0.600	21.23	–	–
0.727	18.66	0.818	16.37	–	–	–	–	0.800	16.71	–	–
0.909	14.32	–	–	–	–	–	–	–	–	–	–

Molecular masses in kDa

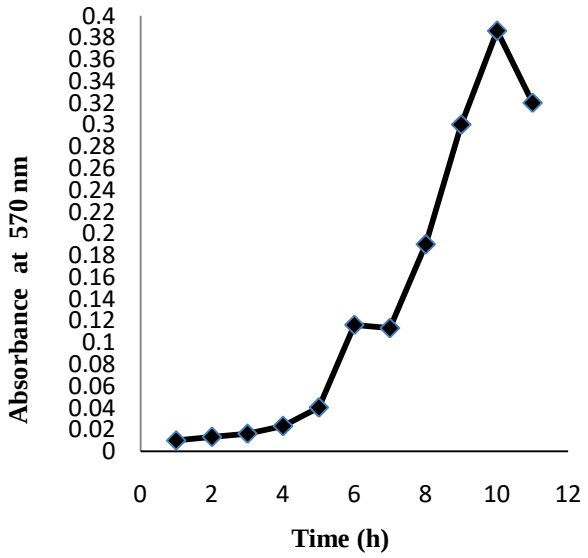

Figure 1: Growth curve of *Saccharomyces cerevisiae* at 25 ^{0}C.

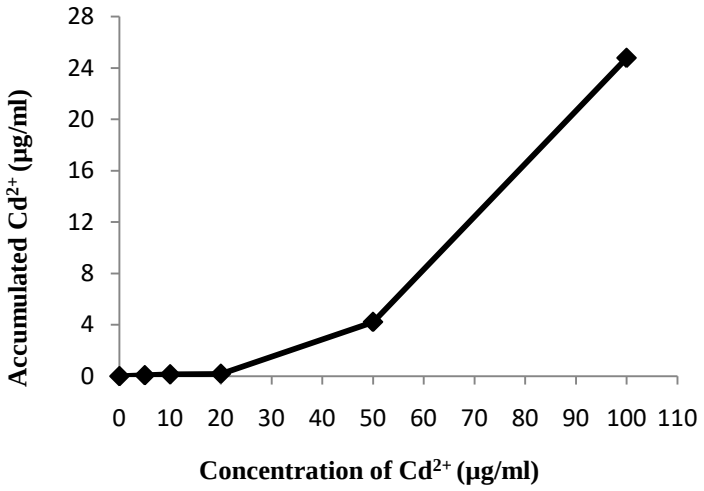

Figure 2: Accumulated Cd^{2+} by *Saccharomyces cerevisiae*.

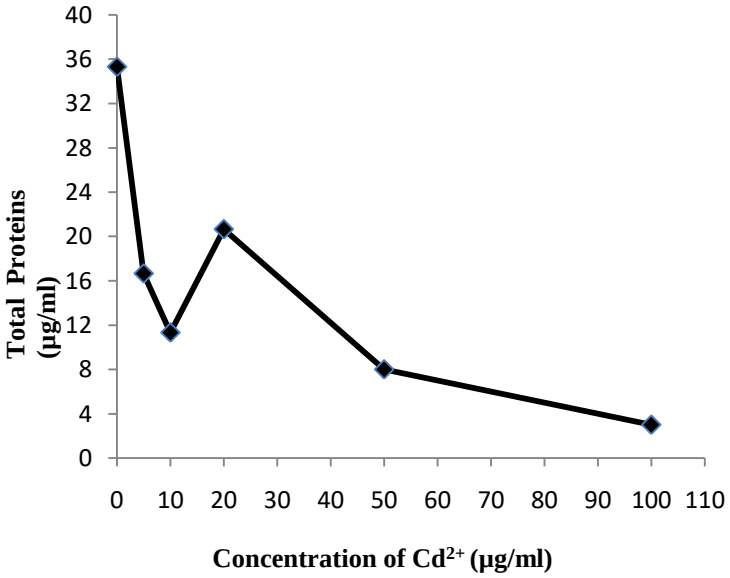

Figure 3: Total proteins determined in *S. cerevisiae* when grown in Cd^{2+} concentrations.

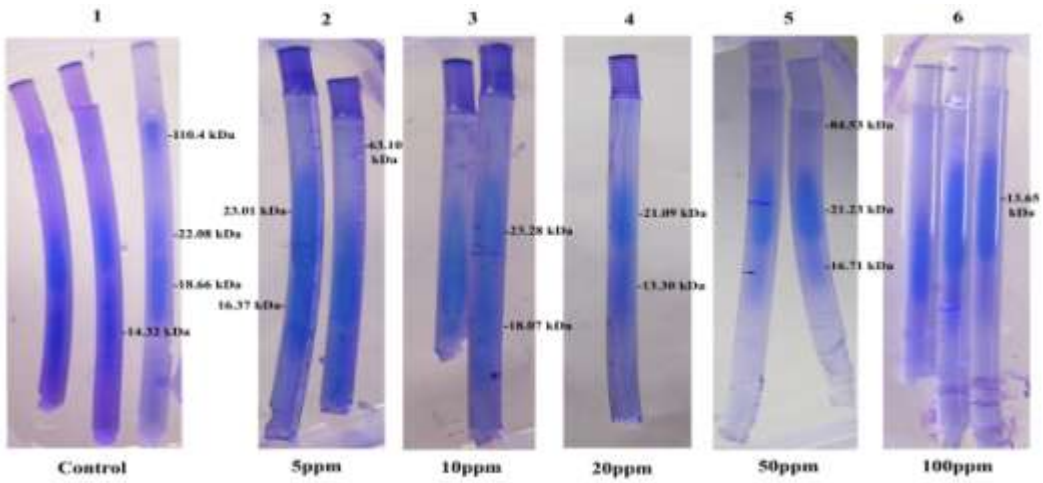

Figure 4: Discrete protein bands obtained by SDS-PAGE in the absence and presence of different concentrations of Cd^{2+}.

Effect of accumulated cadmiumon total protein

Yuet al. has been reported the presence of various functional groups on the surface of modified baker's yeast such as carboxyl, hydroxyl and amide groups, those participates in the adsorption of Cd(II) and Pb(II)[45]. In *S.cerevisiae*, cadmium is bound by glutathione (GSH) and the resulting cadmium-bi-glutathionato complex is transported by the YCF-1p transporter, an ABC transporter, into the vaculoe[46,47].Accumulated cadmium may affect total protein of yeast cells.As we can see that, in the presence of 5µg/ml and 10µg/ml of Cd^{2+}, total protein content decreasedas compared to control whereas increasedtotal proteins were observed when 20µg/ml Cd^{2+} was supplied (Fig 3).At concentrations higher than 20µg/ml regular decrease in total proteins was also observed. The present data illustrated that, as the initial metal ion concentration increasesCd^{2+} leads to binding with biologically sensitive molecules like proteins changing their conformations and reactivity. Due to its larger size and more atomic weight it may cause the protein molecules to disintegrate and therefore get denatured as evidenced by the observed minimum total proteins at higher concentrations of Cd^{2+}(Fig 3).Fashola M. et al also reported that cadmium denatures protein, destroy nucleic acid and hinder cell division[48].Inouhe et al in 1996 has been observed from, the amino acid compositions of Cd-binding complexes extracted from various species of yeast, that cadmium binds with Glu, Gly, Cys, Lys amino acid residues[49]. Furthermore, it may replace Zn^{2+} in enzymes thus preventing them from working normally. As we know that the trace element zinc is required for proper functioning of a large number of proteins including various enzymes, its deficiency reduces the protein synthesis. There may be some efflux systems or Cd-resistant moieties developed inside the cells when 20µg/ml of Cd^{2+} was supplied, which lead to the increase in protein content. Cd-MTs are important Cd-binding complexes that function in the sequestration of cadmium.

The aforesaid conclusions trulysupportedby the accurate characterization of Cd^{2+}binding proteins by measuring their molecular mass.Initially yeast cells were grown in SGM without Cd^{2+}concentration andproteins

wereextracted then separated by SDS-PAGE method. Four discrete protein bands were observed, their molecular masses were measured by corresponding R_f values (Table 3). First band depicts a protein of high molecular mass and other three bands showproteins of low molecular mass respectively (Fig. 4).At working concentrations of Cd^{2+} (5, 10, 20, 50,100) µg/ml many individual protein bands were also seen. Cadmium affected the high molecular mass protein (110.4kDa) seen in control, at all working concentrations. In the present study it was found that only low molecular mass proteins (23.01kDa, 16.37kDa, 23.28kDa, 18.07kDa, 21.09kDa, 13.30kDa, 21.23kDa, 16.71kDa and 13.65kDa respectively) exist with increasing concentrations of Cd^{2+}(Fig. 4).The present data illustrated that binding with high molecular mass proteins Cd^{2+}denatured them or may be effluxed by them. Metallothionein synthesizedin response to cadmium as a protective detoxifying mechanism[50]. As a result of such binding, the Cd^{2+} is rendered indiffusible and prevented from binding with enzymes. Two new proteins of high molecular mass (63.10kDa and 84.53kDa) were seen in the presence of 5µg/ml and 50µg/ml Cd^{2+} respectively.Cd^{2+}binding with proteins results into aggregation and production of new molecules with large molecular mass. Cadmium binding complexes have been found in wild type yeasts such as *Saccharomyces exiguus*, *Pichia farinosa*, *Torulaspora debrueckii*, *Schizosaccharomyces octosporus* and in *S.cerevisiae* 301N[50]. In the presence of 100µg/ml Cd^{2+} only one protein of low molecular mass (13.65kDa) was identified, which proves that some biological mechanisms running inside yeast cells were hindered by cadmium ion. It supports the conclusion of highest accumulation of Cd^{2+} in µg/ng of total proteins by the cells among all the concentrations (Table 1).

IV. Conclusion

Finally it may be concluded, when cadmium get accumulated by target protein molecules in the cell, these proteins may be required by the metal or get attached due to competitive accumulation.Only low molecular mass proteins exist with increasing concentrations of Cd^{2+}. Cadmium bound with high molecular mass proteins and denatured them or may be effluxed by them. Newly synthesized protein molecules which arise due to metal stress, have specific function of effluxing unwanted heavy metal ion from the cell. As we have identified those proteins getting bonded with cadmium we can work out to develop the mechanism to reducecell poisoning. It is a step in the direction for keeping environment healthy through the knowledge of biochemical effects of heavy metals.

V. Acknowledgement

The corresponding author is thankful to University Grants Commission New Delhi for financial assistance as research scholarship. We would like to express our deep gratitude to the director of Geological Survey of India, Jaipur for sample analysis by atomic absorption spectrophotometer.

References

1. Martins B.L., Cruz C.C.V., Luna A.S. et al., *Biochem. Eng. J.*, **2006**, 27(3), 310-14.

2. Turgay T. et al., Biosorption of Cadmium Ions from Aqueous Solution onto Non-living Lichen *Ramalina fraxinea* Biomass, Clean: Soil, Air, Water, **2009**, 37, 249-255.
3. Zouboulis A.I., Loukidou M.X., Matis K.A., Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils, *Process Biochem.*, **2004**, 39, 909–916.
4. Iyer A., Mody K., Jha B.K., Biosorption of heavy metals by a marine bacterium, *Marine Poll. Bull*., **2005**, 50, 340–343.
5. Kim S.U., Cheong Y.H., Seo D.C., Hur J.S., Heo J.S., Cho J.S., Characterisation of heavy metal tolerance and biosorption capacity of bacterium strain CPB4 (*Bacillus* spp.),*Water Sci. Technol.*, **2007**, 55,105–111.
6. Alluri H.K., Ronda S.R. et al., *African J. of Biotechnol*., **2007**, 6(25), 2924-931.
7. Ramasamy K., Kamaludeen S., and Parwin B., "Bioremediation of Metals Microbial Processes and Techniques," In S. N. Singh & R. D. Tripathi (ed.) *Environmental Bioremediation Technologies,* Springer Publication, NY**.,2006**,173-187.
8. Bruins R.M., Kapil S., and Oehme W.F., "Microbial resistance to metals in the environment," *Ecotoxicological and Environmental Safety*, **2000**, 45(2), 198-207.
9. Sharma P.K., Balkwill D. L., Frenkel A. and Vairavmurthy M.A., Ä new klebsiella plantticola strain (Cd-1) grows anaerobically at high cadmium concentrations and precipitates cadmium sulfide,"*Applied and Environmental Microbiology,* **2000**, 66 (7), 3083-3087.
10. Wang C.L., Ozuna S.C., Clark D.S., Keasling J.D., 'A deep-sea hydrothermal vent isolate, *Pseudomonas aeruginosa* CW961, requires thiosulfate for Cd^{2+} tolerance and precipitation,' *Biotechnology Lett*, **2002**, 24 (8), 637- 641.
11. Gavrilescu M.,"Removal of heavy metals from the environment by biosorption," *Engneerng Life Science,* **2004**, 4, 219-232.
12. Brady D. and Duncan J.R., *Enzyme Microb.Technol*., **1994a**,16, 633-8.
13. Goksungur Y., Uren S. and Guvenc U., *Bioresour. Technol.*, **2005**, 96,103-9.
14. Kapoor A. and Viraraghavan T., *Bioresour. Technol*., **1995**, 53, 195-206.
15. Peregol P. and Howell S.B., *Toxicol. Appl. Pharmac*., **1997**, 147, 312-8.
16. Brady D. and Duncan J.R., *Appl. Microbiol. Biotechnol*., **1994**, 41, 149-154.
17. Wang J. and Chen C., *Biotechnology Advances*, **2006**, 24, 427-51.
18. Blackwell K.J., Singleton I. and Tobin J.M., *Appl. Microbiol. Biotechnol*., **1995**, 43, 579-84.
19. Avery S.V. and Tobin J.M., *Appl. Environ. Microbiol*., **1992**,58, 3883-9.
20. Soares E.V., Duarte A.P.R.S. et al., *Appl. Microbiol. Biotechnol*., **2002**, 58, 836-41.
21. Huang C.P., Huang C.P. and Mbrehart A.L., *Water Res*., **1990**, 24, 433-439.
22. Vanaja M., Charyulu NVN and Rao KVN, *Proc. Ind. Natl. Sci. Acad*., **1989**,55,489-94.
23. Steward F.C. and Barber J.T., *Amer. J .Bot*.**1965**, 52(2), 155-164.
24. Steward F.C. and Barber J.T., *Ann. N. Y. Acad. Sci*.,**1964**, 121(2), 525-531.
25. Bharti N. and Singh R.P., *Phytochem.*, **1993**, 33, 531-534.
26. Lodish H.F. and Rothman J.E., *Sci. American*, **1979,** 240, 38-53.

27. Walker N.A., *Enc. of Plant Physiol*., New-Series, Springer, Verlog, Berlin Heidelberg, New York, **1976,** 2, 3-11.
28. Singer S.J. and Nichalson G.L., *Science,***1972,** 175,720-731.
29. Sood I.S., Sindhu R.S. and Sharma K.K., *Poll. Res*., **1994,** 13(4), 331-343.
30. Sood I.S., Sindhu R.S. et al., *Perpective in Pteridology, present and future,* **1992,** 14,361-64.
31. Bhardwaj S., Ph.D. Thesis submitted to M.D.S. University, Ajmer (Raj.), **1992.**
32. Blundell T.L. and Jenkins I.A., *Chemical Soci.Rev.*, **1977,** 6(2)**,** 139-71.
33. Kleeman S.G., Keung W.M. and Riordan J.F., *J. Inorg. Biochem. USA*, **1986,** 26(2), 93-106.
34. Markovac J. and Goldstein G.W., *C. Nature*, **1988**, 334, 71-73.
35. Kumar V, Sharma AK, Rajput SK, Pal M, Dhiman N. Evaluation of phytochemical, toxicological and pharmacological profile of Eulaliopsisbinata leaf extracts. Toxicol Res. 2018; 7: 454-464
36. Kumar V., Pal M., and Dhiman, N., Determination of sun protection factor in different extract of Eulaliopsisbinata, Plant Archives, 2019; 19(2), 185-187
37. Kumar V, Mohan C and Kumari S, "Synthesis, characterization, and antibacterial activity of the schiff bases derived from thiosemicarbazide, 2-acetyl thiophene and thiophene-2 aldehyde" International Research Journal of Pharmacy, 2018, 9(7), pp 153-158
38. Brierley C.L., *J. Geomicrobiol*., **1990**, 8, 201-223.
39. Baker A.J.M. and Brooks R.R., *J. Ecol. and Phytochem.; Biorecovery;* **1989**, 1, 81.
40. Verma R.S., Rao T.V.G. and Prasad R., *Biochem. Biophys. Act;* **1984**, 778, 289.
41. Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J., *J. Biol. Chem.;* **1951**, 193, 265.
42. Dewhurst F., *J. of Chem. Edu.*, **1969**, 46(12), 864-865.
43. Weber K. and Osborn M., *J. Biol. chem*., **1969,** 244, 4406.
44. Liu X.F., Supek F., Nelson N. et al., *J. Biol. Chem.*, **1997**, 272, 11763-11769.
45. Yu J. X., Tong M., Sun X. M., Li B. H., Cystine-modified biomass for Cd(II) and Pb(II) biosorption, *J. Hazard. Mater.* **2007**, 143, 277-284.
46. Li Z.S., Lu Y.P., Zhen R.G. et al., *Proc. Natl. Acad. Sci.USA*, **1997**, 94, 42-47.
47. Li Z.S., Szczypka M., Lu Y.P., Zhen R.G. et al., *J. Biol. Chem.*, **1996**, 271, 6509-517.
48. Fashola M., Ngole-Jeme V., and Babalola O.,"Heavy metal pollution from gold mines: Environmental effects and bacterial strategies for resistance," *International Journal of Environmental Research and Public Health,* 13, 1047, **2016**.
49. Inouhe M., Sumiyoshi M. et al., *Plant Cell Physiol*., **1996,** 37(3), 341-46.
50. Higham P.D., Sadler J.P., and Scawen D.M., "Cadmium-binding proteins in *Pseudomonas putida*: pseudothioneins," *Environmental Health Perspective*, 65(3), 5-11, **1986**.