Sohag University **Botany and Microbiology Department** **Faculty of Science**

" BIOREMEDIATION OF WATER FROM HEAVY METAL POLLUTANTS USING YEAST ISOLATES "

A Thesis

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BY

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

8-SUMMARY

Heavy metals pollution become one of the most serious environmental problems. The presence of heavy metals in the environment especially water causes serious health and ecological problems, so that removal of the heavy metals by the microorganisms has given a significant care recently by research community.

The aim of the present investigation is the screening of heavy metal pollutants and mycobiota contamination (yeast and filamentous fungi) in water sources (surface and groundwater) in study area and investigate the ability of yeast isolates to bioremediation of water sources from heavy metal pollutants.

Total of 180 samples were collected from 30 sites (8 surface and 22 groundwater wells) in study area (Sohag governorate, Egypt) during winter and summer season 2018. Six samples for each site, three samples during winter and 3 during summer season.

So the present investigation have been designed and comprised five parts, study of physical properties of water samples tested, determination of heavy metal pollutants (cadmium, lead, chromium, copper and nickel) in surface and groundwater samples tested, isolation and identification of yeast and filamentous fungi, determination the highly active yeast species in heavy metal biosorption as live, dead and pretreatment biomass and study the optimal condition for the highest active yeast isolate used in heavy metal biosorption. The results were summarized as following.

1- The samples were tested for pH which ranged between (7.1-8.7), temperature (15.1-31.1^{o}C), TDS (141-765) and EC (271-1532) in collection sites to avoid sampling errors.

2- All tested samples (surface and groundwater) have been proven to be free from chromium (Cr) pollutant, while copper (Cu) and nickel (Ni) were recorded in most sites but within permissible limit (2.0 and 0.07 mg/dl respectively) according to WHO, (2011).

3- Cadmium was contaminated 87.5% of surface water samples with maximum (0.051 mg/l) and 72.7% of groundwater samples with maximum (0.035 mg/l) during winter as well as during summer season 87.5% of surface water samples were contaminated with cadmium pollutant with maximum (0.071 mg/l) and 90.9% of groundwater samples with maximum (0.048 mg/l) as indicator of cadmium pollution increasing in summer season.

4- Most surface water sites (87.5%) and 36.4% of tested groundwater samples were contaminated with lead (Pb) pollutant over permissible limit (0.01 mg/l) according to WHO, (2011) during winter with maximum 0.071 and 0.089 mg/l, respectively and this contamination increased during summer season to include all sites of surface water (100%) with maximum 0.108 mg/l and 68.2% of groundwater samples with maximum 0.112 mg/l.

5- Cadmium has positive correlation with Pb and negative with Cu, Ni and Cr in both of surface and groundwater. This is good indication that the pollution with these metals is a result of human activities.

6- All water samples were assayed for yeast (YPDA medium) and filamentous fungi (PDA medium) isolation at 28±2^{o}C, where yeast species recorded from different surface water samples were 311 colonies/ml (winter) and 501 (summer), while from groundwater samples were 214 (winter) and 445 (summer) with total count of 812 in surface and 659 in groundwater samples tested.

7- ***Candida*** was the most common yeast genus (80.9% of general total yeast count), represented by 3 species namely, ***C. albicans, C. glabrata*** **and** ***C. krusei. C. glabrata*** was the highly common species that accounted in 46% of general total yeast count), followed by ***C. krusei*** (29.7%), ***Saccharomyces cerevisiae*** (9%), ***Pichia kudriavzeui*** (6.2%), ***C. albicans*** (5.2%) and finally ***Rhodotorula mucilaginosa*** represented by 3.9% of total count.

8- As well as, 21 fungal species and 3 species varieties belonging to 11 genera were isolated, from surface water samples (9 genera, 14 species and 2 species varieties) and groundwater samples (9, 16 and 3). As general, the total count of filamentous fungi recorded in tested surface water samples were 367 (winter) and 741 colonies/ml (summer), while in groundwater samples were 989 (winter) and 1817 colonies/ml (summer).

9- ***Aspergillus*** was the most dominant genus isolated with the richest total count 90.1% of total fungal count. ***A. flavus*** showed the highest count of *Aspergillus* genus represented in 43.2% of general total filamentous fungi count**.** ***A. niger*** came in the second rank (24.8%). With mentioning that, the total count of filamentous fungi was higher in ground than in surface water and in summer than in winter. It was

recorded 1817 and 741 (summer) and 989 and 367 colonies/ml (winter) in ground and surface water, respectively.

10- Yeast isolates were tested for heavy metals tolerance and the result showed that, ***Rhodotorula mucilaginosa and Pichia kudriavzevii*** were the strongest isolates for lead tolerance with MIC (3600 and 3500 mg/l, respectively), while ***Rhodotorula mucilaginosa*** and ***Saccharomyces cerevisiae*** were the strongest isolates in cadmium tolerance with MIC (1200 and 1100 mg/l respectively). Therefore, ***Rhodotorula mucilaginosa, Pichia kudriavzevii*** and ***Saccharomyces cerevisiae*** were selected for ongoing experiments.

11- Several media were used for yeast biomassing. Dates-extract based medium ($M_{2)}$ was the best one for yeast biomassing due to its high content of elemental nutrients, provides a rich substrate for yeast biomass production more than potatoes- and beans-extract based media. The biomassing weights (g/l) on M_2 medium were; ***S. cerevisiae*** (10.3), ***P. kudriavzevii*** (8.7) and ***R. mucilaginosa*** (6.9).

12- The uptake capacity of lead (Pb) and cadmium (Cd) increased with increasing initial metal concentration and decreased with increasing biosorbents concentration.

13- The uptake capacity increased with increasing pH until maximum at pH 5.0 for lead (Pb) and 6.0 for cadmium (Cd).

14- Biosorption of heavy metals was a highly pH-dependent process, and pH was imperative during the adsorption process to ensure that biosorption was the only mechanism responsible for metal removal.

15- The ability of yeast biomass to heavy metals removal from water was tested under different conditions of pH, contact time, biomass

weights and initial heavy metals concentration to determine optimum conditions. The results revealed that, the optimum conditions for Cd removal were at; pH 6, contact time 120 min, biomass weight 0.25g/l and initial metal concentration 100mg/l, as well as, for Pb at pH 5.

17- Among all tested strains ***Rhodotorula mucilaginosa*** was the most efficient strain gave the highest ability t***o*** remove Cd and Pb being 44.12% and 97.9%, respectively, whereas ***Saccharomyces cerevisiae*** removed 30.2% and 83.4% of Cd and Pb ions from solution. Also, ***Pichia kudriavzevii*** was the lowest efficient strain in Cd and Pb metal removal which reached to 27.3% and 79.2%, respectively.

18- FTIR spectra indicated the high significant role of hydroxyl group on surface of ***S. cerevisiae*** yeast cell wall, as important sites for metals (cadmium and lead) uptake, in addition to, carboxylic, amido and amino groups. While in ***P. kudriavzevii***, alcohols amines and amide groups showed the highly significant role as important sites for metals (cadmium and lead) uptake, in addition to, hydroxyl, carboxyl and alkanes groups. As well as, the hydroxyl group of alcohols and carboxylic acid on surface of ***R. mucilaginosa*** yeast cell wall, played important role for metals (cadmium and lead) uptake, in addition to, carboxylic, amido and alkanes groups.

19- According to Langmuir isotherm the maximum biosorption capacity q_{max} (mg/g) under optimum conditions for Cd and Pb by ***S. cerevisiae*** was 16.81 and 54.05, while by ***P. kudriavzevii*** was 15.31 and 47.17 and by ***R. mucilaginosa*** was 22.73 and 89.29 respectively**.**

20- The effect of different pretreatment methods on biosorption process was tested and the results revealed that, alkaline pretreatment

method by NaOH (0.5M) was the best improvement method, where the uptake capacities of Cd and Pb (mg/g), respectively by alkaline treated biomass were (18.1 and 30.16), (15.68 and 27.64) and (20.14 and 37.92) by ***S. cerevisiae, P. kudriavzevii*** and ***R. mucilaginosa***, respectively.

21- The order of treatment methods was; alkaline >citric >heating (dead cells) >ethanol >untreated >hot-water and glycerol method.

22- Under the optimum conditions, the maximum uptake capacity **(q_{max})** of cadmium and lead was 27.32 and 97.1 mg/g, respectively by ***R. mucilaginosa*.**

23- Alkaline treatment by NaOH 0.5 M was the best method in improvement Cd and Pb uptake capacity by tested yeast strains.

24- Under the optimum conditions (the best isolate, best conditions and best treatment methods) the maximum uptake capacity **(q_{max})** of Cd and Pb was 27.32 and 97.1 mg/g, respectively.

25- This study confirmed that, ***R. mucilaginosa*** are very efficient in removing Pb ions from lead-bearing solutions with maximum uptake capacity (q_{max}) 97.1 and 27.32 mg/g for Cd under optimum conditions.

26- Research performed in this work can serve as a potential incentive for promoting biosorption as a promising alternative to conventional metal treatment methods.

27- Finally, the alkaline pretreatment of ***R. mucilaginosa*** biomass by NaOH (0.5 M) can be applied as a low-cost and environment friendly method as a biotechnology method for water bioremediation**.**