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ORIGINAL ARTICLE

Bioremediation potential and lead removal capacity of heavy metal-tolerant yeasts isolated from Dayet Oum Ghellaz Lake water (northwest of Algeria)

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Abstract

Seven metal-resistant yeast strains were isolated and selected from Dayet Oum Ghellaz Lake water (northwest of Algeria) known as a highly polluted area by lead and cadmium. The yeast strains were screened on the basis of their resistance to seven heavy metals Hg, Cr, Cd, Pb, Cu, Zn, and Fe and characterized by molecular and phylogenetic analysis. The sequencing of the D1/D2 domain of the 26S rRNA genes revealed the affiliation of the seven yeast isolates to *Rhodotorula mucilaginosa*, *Clavispora lusitaniae*, and *Wickerhamomyces anomalus* species. All yeast strains were halotolerant as they were able to grow in 10–15% NaCl. The yeast isolates were highly resistant to the studied heavy metals and exhibited different tolerance according to the metal type. The highest minimum inhibitory concentrations (MIC) were observed in *R. mucilaginosa* RO7 and *W. anomalus* WO2 strains which were then selected for lead removal assays. The present study is the first to investigate the lead elimination by *W. anomalus*. The lead uptake was significantly affected by biomass concentration in a reverse relationship, with purification percentages estimated at 98.15 ± 0.9% and 97.046 ± 0.47% and removal efficiency of 12.68 ± 0.91 and 15.55 ± 0.72 mg/g for *W. anomalus* WO2 and *R. mucilaginosa* RO7, respectively. The investigated metal-tolerant yeast strains proved to be promising candidates for bioremediation processes of heavy metals. This work amends the metal-resistant yeast bank with new strains having interesting abilities to resist to relatively high concentrations of toxic heavy metals and which can be used in the near future as low-cost biosorbents.

Keywords Lead removal · Heavy metal · Autochthonous yeast · Dayet Oum Ghellaz Lake water

Introduction

Heavy metals released into the environment can be resulted from natural sources or human industrial and agricultural activities, including discharges of pesticides and phosphate fertilizers (El-Shahawi et al. 2010; Lepp 2012). As a result, metals can widely diffuse in all environmental compartments (soil, water, air, and their interface) and lead consequently to serious environmental pollution especially in the case of water, as all the worldwide populations rely on groundwater for drinking and irrigation. Most of heavy metals become toxic, even at low concentrations, and hence, when present in environment, they enter food chain, thereby causing harmful effect to human and animal health. Moreover, the toxicity of heavy metals, including lead, is strongly dependent on the amount available to organisms, the absorbed dose, the time, and the route of exposure (Mani and Kumar 2014). Heavy metals are difficult to remediate through physical and chemical methods due to many disadvantages, including high costs, high energy, and generation of high quantity of chemical wastes (Ayangbenro and Babalola 2017). So far, the use of some yeast indices, like yeast biomasses, is proved as an efficient alternative tool in remediation of heavy metal-contaminated environments (Sun et al. 2020). Noteworthy,

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a long-term exposure of microorganisms to high metal concentrations develops their resistance capacity, and thus, when present in contaminated soils and wastewaters, they become a biological source for metal removal (Parameswari et al. 2010; Rigoletto et al. 2020). Further, microorganisms have developed strategies for continued existence in heavy metal-contaminated environments by means of various biological mechanisms, such as bioaccumulation, biomineralization, biosorption, and biotransformation (Melgar et al. 2007; Ayangbenro and Bobalola 2017; Tarekegn et al. 2020), contributing effectively in heavy metal removal and recovery of polluted environments. The bioremediation of water has become a major concern in the world and highlighted the strong need for the development of efficient microbiological and microbial ecological approaches and encouraged selection of alternative remedies. The omnipresence of yeasts in several natural ecosystems and their exposure to a variety of extreme conditions induced stress responses which accentuated their defense mechanisms. Considering the ecological benevolence of microorganisms for bioremediation of heavy metals, the present study was conducted in order to identify and characterize metal-resistant yeast strains isolated from polluted lake in the northwest of Algeria. The yeast strains exhibiting desirable heavy metal degradative qualities will be a promising microbiological tool for in situ bioremediation operations of natural contaminated water and industrial effluent.

Materials and methods

Sample collection and water physicochemical analysis

In the present study, the water samples analyzed were collected from Dayet Oum Ghellaz, a lake located in Oran city (northwest of Algeria, 35°36′08.0″N 0°25′22.8″W, study area of 300 ha, a maximum altitude of 125 m), at 12 km from Hassi Amer industrial zone II. The lake is characterized by temperature ranging between 9 and 32 °C and an annual mean precipitation of 350 mm. Samples were taken from five randomly chosen places on the surface of the lake at 0.5–1 m of depth and during four seasons (autumn, winter, spring, and summer). The water samples were aseptically collected in sterilized flasks and transported on ice (4 °C) to the laboratory for the analyses of the following physicochemical parameters: pH, electric conductivity, phosphate, biochemical oxygen demand in 5 days (BOD_5), chemical oxygen demand (COD), the ratio COD/BOD_5, and some heavy metals, like chromium (Cr), cadmium (Cd), copper (Cu), zinc (Zn), iron (Fe), and lead (Pb). To assess the quality of lake water content of heavy metals, the metal index (MI) was employed (Tamasi and Cini 2004). This index is calculated by the ratio of heavy metal content in the water (ppm) based on the corresponding values (in Bendjama et al. 2014), where MI > 1 indicates that the water is polluted.

Yeast isolation

The cultivable yeasts were isolated from the sampled water of Dayet Oum Ghellaz Lake using yeast extract peptone dextrose agar plates (YPD medium) containing 0.5% w/v yeast extract, 1% w/v peptone, 2% w/v glucose, and 2% w/v agar. One hundred microliters of water samples were aseptically plated on YPD agar and incubated for at least 3 days at 28 °C. The colonies presenting the typical yeast morphotypes were then picked up and purified by repeated streaking on fresh YPD agar plates. After the purity control, the isolated yeasts were stored at − 20 °C in YPD broth supplemented with glycerol (30% v/v final concentration) (Villegas et al. 2004).

Semiquantitative screening for heavy metal resistance and salt tolerance

The agar well diffusion assay was used to test the resistance of the isolated yeast strains (Gadd et al. 1986) against seven selected soluble heavy metals ($CdCl_2$, $ZnSO_4$, $Pb(NO_3)_2$, K_2CrO_4, $CuSO_4$, $HgCl_2$, and $FeCl_3$). Wells of approximately 6-mm diameter were created in YPD agar plates previously inoculated with 100 µl of yeast strain suspensions. Agar wells were then filled with 40 µl aliquots of heavy metal solutions with various concentrations (10, 25, 50, 100, 150, and 200 mM) prepared by dissolving the appropriate quantities in bi-distilled water followed by a sterilization cycle at 120 °C for 20 min (Benmalek and Fardeau 2016). The inhibition zone diameter was determined after incubation of strains at 28 °C during 72 h. Wells filled with sterilized distilled water were used as controls. Thus, yeast strain is considered as tolerant to the corresponding heavy metal when their inhibition zone diameter is equal or lower than 10 mm. Yeast strains resistant to the studied heavy metals were selected and subjected to molecular identification and evaluation of MIC.

Halotolerance of yeasts was measured by analyses of growth capacity under various concentrations of NaCl. Each yeast strain was streaked onto YPD solid medium containing different concentrations of sodium chloride (0%, 3%, 5%, 10%, 15%, 20% (w/v)) and incubated at 28 °C for 72 h.

DNA extraction, PCR amplification, and sequencing

The yeast genomic DNA was extracted and amplified at the Biological Resource Center CIRM-Levures, Micalis Institute, INRA/AgroParisTech, France following the procedures described by Hoffman and Winston (1987) and

Burgaud et al. (2016), respectively. For the DNA isolation, 10 ml of the yeast cultures was grown until saturation. The yeast cells were then collected by centrifugation and resuspended in 0.5 ml of water. The cells were additionally transferred to a new microfuge and pelleted by a 5-s centrifugation. After cell harvesting, the supernatant is decanted and the pellet is resuspended by brief vortexing in the residual liquid. 0.2 ml of 2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris pH 8.0, and 1 mM EDTA are then added followed by the incorporation of 0.2 ml phenol–chloroform-isoamyl alcohol (25:24:1; the addition of isoamyl alcohol is optional). 0.3-g acid-washed glass beads (Thomas Scientific, Philadelphia, PA; 0.45–0.52-mm glass beads, solid, for cell homogenizers) are then needed. The glass beads are prepared by soaking in concentrated nitric acid for 1 h, washing extensively with water, and baking until dry. The solution is vortexed for 3 to 4 min followed by the addition of 0.2 ml TE. The microfuge is spun for 5 min and the aqueous layer is transferred to a new tube and mixed by inverting with 1.0 ml 100% ethanol. Another fast spin for 2 min is necessary before the resuspension of the pellet in 0.4 ml TE plus 30 µg RNase A (Sigma Chemical Co., St. Louis, MO) for 5 min at 37 °C. 10 µl of 4 M ammonium acetate plus 1.0 ml of 100% ethanol are then added and mixed by tube inverting. The pellet is finally spun for 2 min, dried, and resuspended in 50 µl TE. The amplification was performed on the D1/D2 domain of the LSU rRNA gene. The D1/D2 rRNA gene was symmetrically amplified by PCR using the universal fungal primers NL1 (5′-GCATATCAATAAGCGGAGGAA-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) (O'Donnell 1993). The PCR reaction mixture contained 1 U Takara ExTaq, 0.8 mM dNTPs, 0.4 µM forward and reverse primers in the recommended buffer, and 25–50 ng of template DNA in a final volume of 50 µl. Cycling conditions consisted in an initial denaturation step at 94 °C for 4 min, followed by 30 cycles of 30-s denaturation at 94 °C, 40-s annealing at 54 °C, and 90-s elongation at 72 °C, with a final extension step of 7 min at 72 °C. Successful amplification products were verified by electrophoresis using a 1% agarose gel. Sequencing reactions were performed on both strands with the corresponding primers (NL1 and NL4) by Eurofins MWG Operon (Ebersberg, Germany) at the Biological Resource Center CIRM-Levures (France).

Nucleotide sequence accession numbers

The nucleotide sequences of the selected yeast isolates generated in the present study were deposited in the NCBI GenBank database under accession numbers reported in Table 2.

Phylogenetic analysis

A search for homology among the D1/D2 domain from the LSU (26S) rDNA sequences was done using the BLAST algorithm (Altschul et al. 1997) available in public databases such as GenBank at the National Center for Biotechnology Information (NCBI), USA (http://www.ncbi.nlm.nih.gov) in order to determine the closest relatives and then aligned with them. The sequence alignments were performed using the MUSCLE algorithm in MEGA 7.0 software (Kumar et al. 2016) and were adjusted manually. Each DNA sequence was assembled and edited manually if needed. Phylogenetic trees were constructed on the final alignment using the maximum likelihood program implemented in MEGA 7.0 with 1000 bootstrap replications and Kimura two-parameter model with gamma distribution (K2 + G) (Kimura 1980). Confidence values were estimated from bootstrap analyses of 1000 replicates. *Schizosaccharomyces pombe* UCDFST 40–277 (MH595199) was used as outgroup species to root the tree.

Determination of minimum inhibitory concentration (MIC) of heavy metals

The yeast isolates were grown in YPD liquid medium supplemented with various soluble metals, with increasing concentrations of 0.1, 0.25, 0.5, 0.75, and 1 mM for mercury solution and of 10, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, and 300 mM for the other heavy metal solutions. The inoculated flasks were incubated at 28 °C for 72 h. Non-metal supplemented liquid media were also inoculated and incubated to act as control. The yeast growth was measured using spectrophotometry by determining their optical density at 600 nm. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the heavy metal at which complete inhibition of visible growth of the yeast isolate was observed (Rossbach et al. 2000).

Effect of lead on growth and cell morphology of the metal-resistant yeast strains

The selected metal-resistant yeast strains were cultivated in 50 ml of YPD liquid medium adjusted to pH 6.5, non-supplemented (control) or supplemented with increasing concentrations (by a factor of two) of lead (31.25, 62.5, 125 ppm). The cultures were then grown at 28 °C with continuous shaking on a rotary shaker (150 rpm). Cell growth was determined spectrophotometrically by measuring the optical density of the culture broth at 600 nm after 24, 48, and 72 h. Following incubation for 72 h, yeast cells were collected and examined under optical microscope. The measurements of the cell cross-sectional area (the length and the width) were performed based on the morphological evaluations of 50 yeast cells and shape coefficient (ratio between

length and width), which can determine the spherical shape of the cells when it equals 1 (Kieliszek et al. 2016).

Evaluation of lead removal capacity by actively growing yeast cells

After 72 h of yeast culture in shaking conditions (150 rpm) at 28 °C, aliquots of wet cells were obtained through centrifugation at 10,000 g for 15 min. All cell samples were washed twice with distilled de-ionized water before being contacted with the metal-bearing solutions. The effect of biomass concentration on the elimination of various concentrations of lead prepared from $Pb(NO_3)_2$ compound was studied. Briefly, suitable aliquots of live biomass (0.6, 1, and 1.5 g) were added in 250-ml conical flasks containing 100 ml of lead solution with known initial metal concentration (31.25, 62.5, and 125 ppm) and pH 6.5 and incubated for 24 h at room temperature. Control flasks without yeast biomass were also maintained for each concentration of lead solution. The evaluation of the residual lead concentration was carried out after centrifugation at 10,000 g for 5 min and analysis of the supernatant from each sample using atomic absorption spectrometry.

The biosorption efficiency (E (%)) was determined according to the following formula (Xin et al. 2012):

$$E\ (\%) = (Ci - Cf/Ci)\ 100$$

The metal uptake value (q) was calculated using the following equation (Salinas et al. 2000):

$$q\ (\text{mg metal/g of biomass}) = V\ (Ci - Cf)/1000m$$

where V is the volume of metal solution (100 ml), Ci and Cf are the initial and final concentrations of metal, respectively (mg/l), and m is the mass of yeasts (g).

Statistical analysis

The different data obtained in the present study were statistically processed using the SPSS statistical computer software (version 23.0). Mean values were analyzed by two-way analysis of variance (ANOVA) and generalized linear model (GLM). The Tukey test was also performed to test the statistical level of significance ($p < 0.05$).

Results

Physico-chemical characteristics of the lake water

As shown in Table 1, the water sampled from Dayet Oum Ghellaz Lake was characterized by a slightly basic pH and high COD and BOD_5 levels. Accordingly, the ratio COD/BOD_5 was superior than 3, and water substances were hardly biodegradable (Ammar and Ueno, 1999). The level of electrical conductivity was elevated, which is somehow resulted from the high chloride concentrations. The amount of total phosphorus in water was relatively high. On the other hand, the results indicate significant contamination by lead, iron, and cadmium (Table 1).

Table 1 Physicochemical characterization of water from Dayet Oum Ghellaz Lake

Parameters	Values
pH	7.80 ± 0.46
EC (mS cm^{-1})	5.74 ± 0.75
Cl^- (mg l^{-1})	1777.34 ± 642.89
COD (mg l^{-1})	376.27 ± 672.40
BOD_5 (mg l^{-1})	29.70 ± 23.86
COD/DBO_5	16.429 ± 32.66
Total phosphorus (mg l^{-1})	0.6455 ± 0.64
Metal index (MI)	
Cu^{2+}	0.22 ± 0.25
Cd^{2+}	3.42 ± 6.85
Cr^{2+}	0.16 ± 0.19
Pb^{2+}	1.970 ± 2.61
Zn^{2+}	0,15 ± 0.13
Fe^{3+}	1.7 ± 0.72

Isolation and molecular characterization of heavy metal-resistant yeasts

A total of seven heavy metal-resistant yeast strains were isolated from water samples of Dayet Oum Ghellaz Lake named as WO1, WO2, WO3, WO4, WO6, CO5, and RO7 belonging to three species of three yeast genera (Table 2). RO7 was the only yeast species which developed intense red colonies when grown at 28 °C on plated solid media.

The BLAST analysis of the D1/D2 domain of the 26S rDNA gene belonging to the seven studied yeast strains revealed very high homologies between the WO1–WO6, CO5, and RO7 sequences and those of *Wickerhamomyces anomalus*, *Clavispora lusitaniae*, and *Rhodotorula mucilaginosa*, respectively, deposited at GenBank (Table 2). *Wickerhamomyces anomalus* was present as the most abundant (WO1–WO6) species in the lake water. The phylogenetic analysis of the studied sequences strongly supported also the affiliation of the seven yeast strain sequences to three clusters representing each of the pre-cited species with significant bootstrap or posterior probability values. Moreover, two major clades were highlighted corresponding to the ascomycetous yeasts where the two minor clades of *Wickerhamomyces anomalus* and *Clavispora lusitaniae* strains belong and the second major clade was represented by basidiomycetous

Table 2 Nucleotide sequence identity (%) and GenBank accession numbers of the yeast strain sequences investigated in the present study

Strain code	GenBank accession number (NCBI)	Length (bp)	Closest GenBank relative (NCBI)	Accession number of the closest GenBank relative (NCBI)	Identities (% homology)
WO1	MT328415	592	*Wickerhamomyces anomalus*	MT279706	592/592 (100%)
WO2	MT331854	592	*Wickerhamomyces anomalus*	MT279706	592/592 (100%)
WO3	MT331858	592	*Wickerhamomyces anomalus*	MT279706	592/592 (100%)
WO4	MT331856	592	*Wickerhamomyces anomalus*	MT279706	592/592 (100%)
WO6	MT332097	592	*Wickerhamomyces anomalus*	MT279706	592/592 (100%)
CO5	MT328421	532	*Clavispora lusitaniae*	MG871742	532/532 (100%)
RO7	MT328422	586	*Rhodotorula mucilaginosa*	KY109112	586/587 (99.83%)

yeasts and enclosed *Rhodotorula mucilaginosa* strain studied in the present work (Fig. 1, Table 2).

Halotolerance of the yeasts

All the yeast strains exhibited high halotolerance as they were able to grow in the presence of 10–15% of NaCl (Table 3), although they did not require salts for their physiological activities. These results suggest that all the studied yeast strains have superior osmotic tolerance.

Heavy metal resistance and minimum inhibitory concentration (MIC)

The results of the present study showed the ability of seven yeast strains belonging to the species of *Wickerhamomyces anomalus*, *Clavispora lusitaniae*, and *Rhodotorula mucilaginosa* to resist and remove several heavy metals which may constitute a potential environmental pollutant.

The diameter of the inhibition zone differed significantly according to the yeast strain, the heavy metal type, and metal concentration (Table 3). In the present study, the MICs of the six studied heavy metal strains were between 10 and 300 mM (Table 4). The cadmium MIC ranged from 10 to 150 mM and the chromium MIC was between 10 and 75 mM. The zinc and iron MIC values reached 300 mM. The minimum inhibitory concentration of copper was between 50 and 250 mM. The seven yeast strains exhibited considerable MIC values of lead (25–200 mM). However, mercury was the most toxic metal for yeast strains with MIC between 0.1 and 0.5 mM (Table 4). Finally, *W. anomalus* WO2 and *R. mucilaginosa* RO7 strains displaying the highest MIC values were selected for lead removal assays.

Effect of lead on cell growth and morphology of the selected metal-resistant yeast

The growth rate of the selected yeast isolates exposed to different increasing concentrations of lead was good until 24 h of incubation and slightly decreased after 24 h and 48 h compared with control (without metal amendment) (Fig. 2). This result demonstrates the ability of the selected yeast strains to grow in the presence of lead.

The microscopic examination demonstrated also a significant increase in yeast cell size (length and width), following 72 h of yeast exposition to lead in the experimental media (Table 5; Fig. 3). In fact, in absence of lead, *W. anomalus* WO2 strain shows a cell size of $4.13 \pm 1.23 \times 3.18 \pm 1.03$ µm and a shape coefficient of 1.31 ± 0.17, giving to cells a slightly oval shape. However, in the presence of lead, the size increases progressively up to $6.34 \pm 1.19 \times 5.68 \pm 1.26$ µm with a shape coefficient of 1.14 ± 0.14. Thus, the increasing of the cell size induced form changes in yeast cells which adopted a spherical shape (Fig. 4a, b). *R. mucilaginosa* RO7 presents normally an average cell size of $4.35 \pm 1.01 \times 4.22 \pm 0.92$ µm with a shape coefficient of 1.03 ± 0.17 corresponding to the spherical form. After lead exposure, the cell size increased up to $6.02 \pm 1.66 \times 6.02 \pm 1.66$ µm, and the shape coefficient reduced slightly to 1 which really did not affect the spherical shape of cells (Fig. 4c, d).

Lead removal by living yeast cells

As shown in Fig. 5, the Pb uptake and the percentage of yeast cell removal efficiency was highly significant affected by the initial lead concentration and the amount of biomass. No significant difference was noticed in the effect of strains (Table 5).

In this study, lead concentration of 31.25 ppm makes the purification activity significant in the presence of 1 g of biomass, with a high percentage of purification estimated at $85 \pm 2\%$ and $95.32 \pm 1.06\%$ for WO2 and RO7 strains, respectively. However, in the presence of 1.5 g of yeast biomass, the purification percentage becomes $80.5 \pm 4.28\%$, and $84.5 \pm 2\%$ for WO2 and RO7 strains, respectively.

The maximum efficiency capacity of yeast strains in lead removal was established at high concentrations up to 125 ppm, with 1 5g of biomass for both strains which provided a remarkable purification activity ($98.15 \pm 0.90\%$

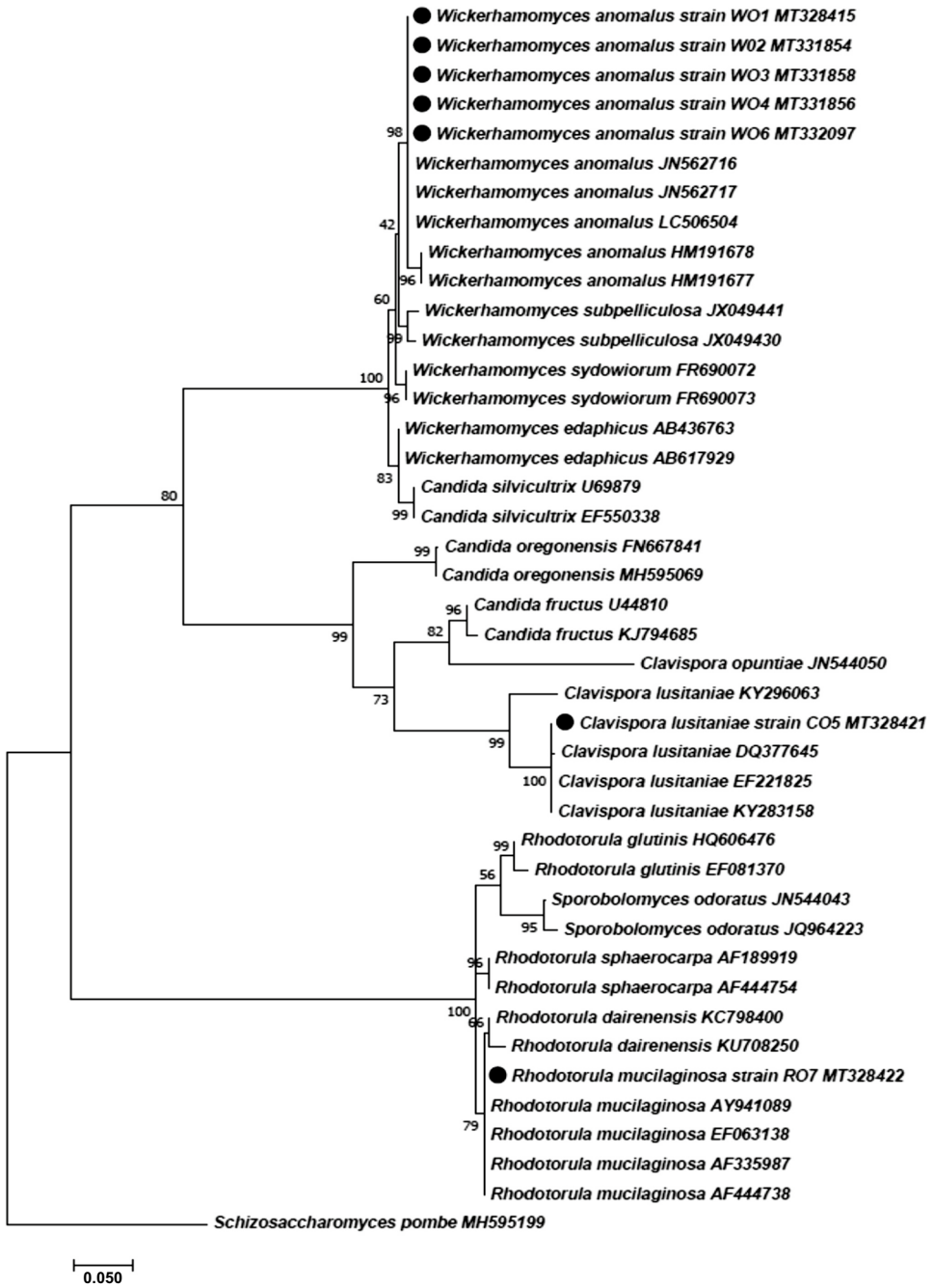

Fig. 1 Phylogenetic tree derived from maximum likelihood analysis of the D1/D2 domain of 26S rDNA, showing the taxonomic position of the seven studied yeast species (indicated by filled circles). *Schizosaccharomyces pombe* UCDFST 40–277 (MH595199) was used as an outgroup. Bootstrap values (%) over 50% based on 1000 replication are given at nodes. The scale bar represents 0.05 substitutions per nucleotide position

for WO2; 97.046 ± 0.47% for RO7). Nevertheless, with 1 g of biomass, *W. anomalus* WO2 and *R. mucilaginosa* RO7 could efficiently remove 87.67% and 82.8% of lead, respectively (Fig. 5a, b).

The removal efficiency of lead by the two strains reached its maximum levels, 12.68 ± 0.91 mg/g and 15.55 ± 0.72 mg/g for WO2 and RO7, respectively, under the lowest concentration of fresh biomass (0.6 g) and for the highest lead concentration (125 ppm) (Fig. 5c, d).

Discussion

Oum Ghellaz Lake water was characterized by significant lead and cadmium contamination. Metal values were higher than maximum concentration foreseen in the environmental water quality guidelines. Furthermore, MI values exceeding 1 are a threshold of warning (Khazaal et al, 2019), indicating the worse quality of lake water. That lake

Table 3 Average inhibition zone diameter of the different studied heavy metals. Results are expressed as means ± SD (n = 3 * 6) and maximum tolerable concentration of NaCl for the seven selected yeast strains

	WO1	WO2	WO3	WO4	WO6	CO5	RO7
Hg (mM)	27.56 ± 4.69	27.56 ± 4.91	28.67 ± 5.52	26.22 ± 7.55	27.67 ± 5.94	28.89 ± 6.57	23.89 ± 6.72
Cr (mM)	16.4 ± 12.82	17.26 ± 12.40	15.93 ± 13.90	19.07 ± 11.84	19.73 ± 13.73	38.73 ± 4.99	26.87 ± 23.37
Cd (mM)	11.13 ± 7.54	7.80 ± 7.35	11.40 ± 7.60	17.93 ± 5.46	12.33 ± 7.16	28.40 ± 6.56	5.60 ± 5.19
PB (mM)	9.07 ± 4.91	1.53 ± 3.18	6.27 ± 6.27	9.20 ± 8.19	7.00 ± 5.96	13.40 ± 8.03	3.73 ± 4.93
Cu (mM)	1.67 ± 3.46	0	3.93 ± 5.01	5 ± 4.26	4.87 ± 6.19	9.33 ± 5.09	3.4 ± 4.36
Zn (mM)	0	0	0	5.6 ± 4.95	0	11.27 ± 7.28	0
Fe (mM)	6.13 ± 3.29	0	0	5.13 ± 4.58	1.93 ± 4.01	6.73 ± 3.56	1.17 ± 3.04
NaCl (%)	15	15	15	10	10	10	10

Table 4 Minimal inhibition concentration of the different studied heavy metals

	WO1	WO2	WO3	WO4	WO6	CO5	RO7
Hg (mM)	0.5	0.25	0.25	0.1	0.1	0.1	0.25
Cr (mM)	75	75	75	25	25	10	50
Cd (mM)	100	100	100	25	25	10	150
PB (mM)	50	200	150	50	50	25	150
Cu (mM)	200	250	150	250	100	50	200
Zn (mM)	300	300	300	100	300	50	300
Fe (mM)	250	300	300	150	250	250	300

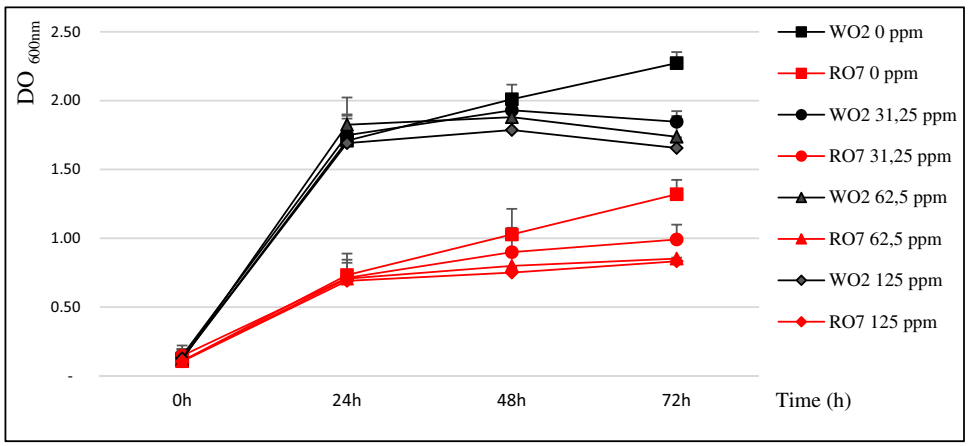

Fig. 2 Growth of the two selected yeasts *W. anomalus* WO2 and *R. mucilaginosa* RO7 in the presence of 0, 31.25, 62.5, and 125 ppm lead concentrations at 28 °C

Table 5 Statistical significance of variation sources in both strains and lead concentration effects on length, width, and shape coefficient and relation with yeast strains, lead concentration, and biomass effects on lead removal and lead uptake

Parameters	Length (µm)	Width (µm)	Shape coefficient	Lead removal efficiency (%)	Lead uptake (mg/g)
Strain (S)	0.03 ns	17.73***	101.80***	1.81 ns	7.63 ns
Pb concentration	19.44***	23.13***	4.33**	30,062.09***	395.18***
Biomass (B)	/	/	/	896.92***	61.96***
S * Pb	0.50 ns	0.97 ns	2.13 ns	0.73 ns	6.13*
S * B	/	/	/	9.68***	9.69***
Pb * B	/	/	/	53.14***	121.68***
S * Pb * B	/	/	/	22.69***	18.70***

Significance levels: *ns* not significant, *p < 0.05, **p < 0.01, ***p < 0.001

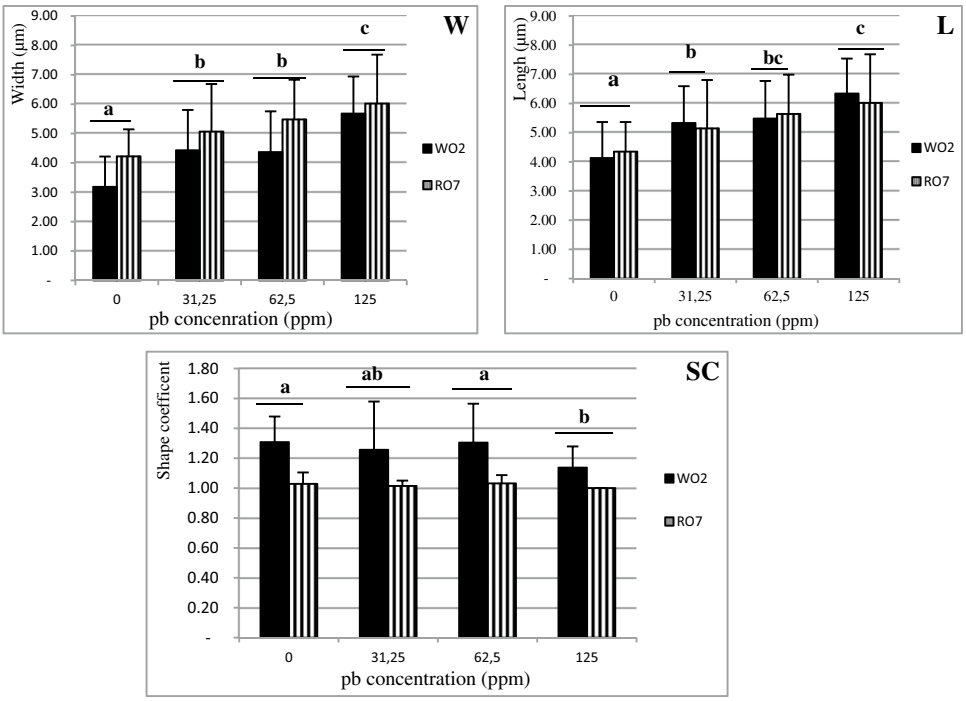

Fig. 3 Lead concentration effect on width (W), length (L), and shape coefficient (SC) of two selected yeast strains. Results are expressed as means ± SD (n = 50). Different letters are significantly different from each other ($P < 0.05$) according to the Tukey test

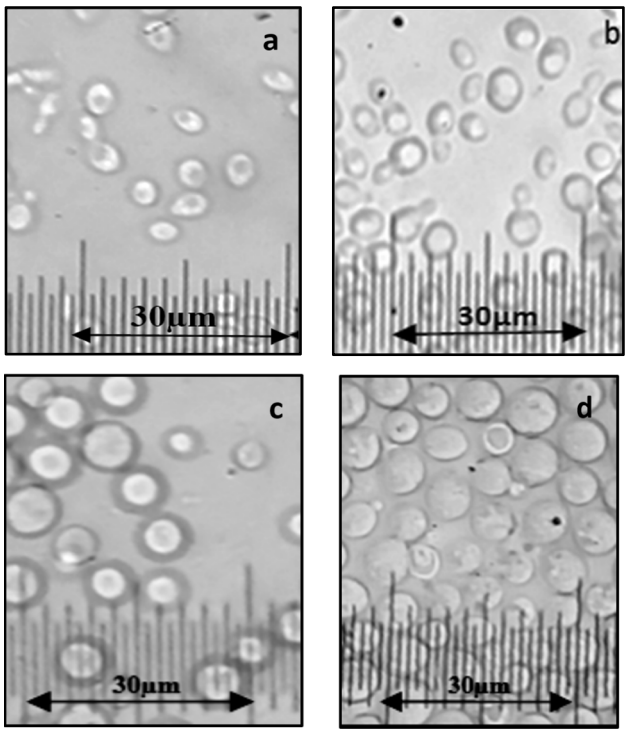

Fig. 4 Cell morphology of *Wickerhamomyces anomalus* WO2 (**a** without lead; **b** with 125 ppm of lead) and *Rhodotorula mucilaginosa* RO7 (**c** without lead; **d** with 125 ppm of lead) cultivated for 72 h in YPD liquid medium

was also characterized by high COD and BOD5. The ratio COD/BOD5 was superior to 3, and water substances were hardly biodegradable (Ammar and Ueno 1999). Based on the Interim National River Water Quality Standard (INRWQS) classification, the lake water is very polluted (in Akinbile et al. 2013). Extensive industrialization has brought about huge changes in the distribution of elements at the surface of the Earth. Metal-resistant microorganisms become dominant in habitats contaminated with relevant heavy metals (Rehman and Anjum 2011).

A total of seven heavy metal-resistant yeast strains were isolated from water samples of Dayet Oum Ghellaz Lake. *Wickerhamomyces anomalus* formerly known as, *Hansenula anomala* and *Pichia anomala*, this ascomycetous species is commonly reported from river water and lake (Coelho et al. 2010; Naito et al. 2019). In addition, *W. anomalus* is widely distributed in various ecological niches, suggesting thus a ubiquitous character of the yeast species. *W. anomalus* can survive in unfavorable conditions and grow in stressful environments, owing to its physiological versatility evidenced by the large number of metabolized carbon and nitrogen sources. Furthermore, this species presents interesting abilities in producing killer toxins and growing in a low active water level and osmotic stresses due to the presence of lactic acid, contributing in its overall competitiveness (Polonelli et al. 1983; Kurtzman and fell 1998; Heide-Marie et al. 2011; Restuccia et al. 2020). *Clavispora lusitaniae* is another ascomycetous yeast isolated from the studied lake water. This species is known as a cosmopolitan, saprobial, fermentative

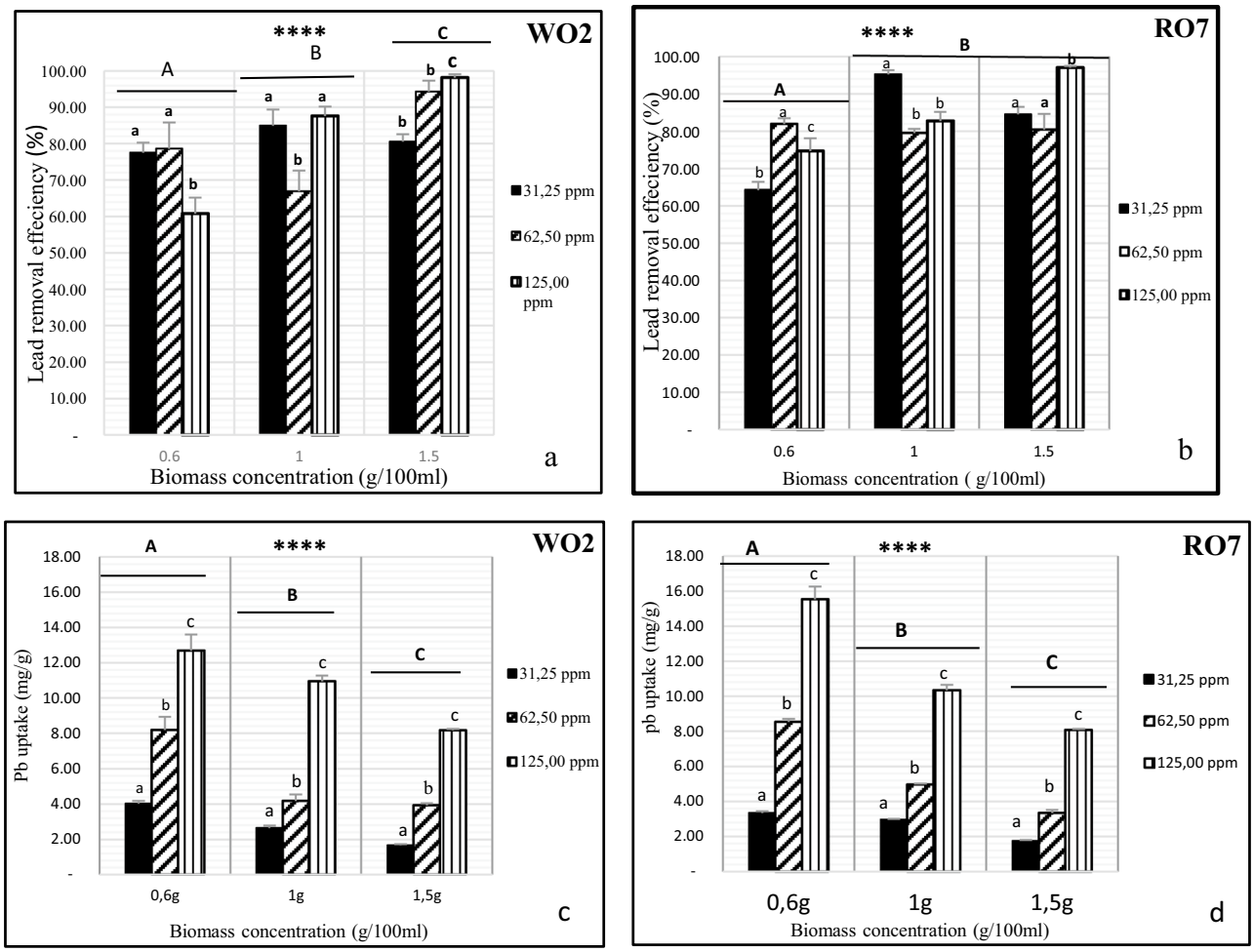

Fig. 5 Effect of biomass concentration of *Wickerhamomyces anomalus* WO2 and *Rhodotorula mucilaginosa* RO7 on lead removal efficiency (**a**, **b**) and lead uptake (**c**, **d**) in different concentrations of lead solution. Results are expressed as means ± SD (n = 3). Asterisks depict interaction between biomass and Pb concentrations at $P < 0.0001$ using generalized linear model. Uppercase letters indicate significant differences between biomass treatments and lowercase letters indicate significant differences among Pb concentrations within each biomass treatment using Tukey's multiple comparison post hoc test ($P < 0.05$)

yeast whose ecological niche is still poorly elucidated. In fact, *C. lusitaniae* can be isolated from soil and water and from different plant substrates and hosts (de Almeida 2005; Coelho et al. 2010; Pérez-Brito et al. 2015). In the present work, we signaled the presence of *Rhodotorula mucilaginosa*, the red yeast species reported as a frequently occurring yeast species in several aquatic environments (Libkind et al. 2003; 2004; D'Elia et al. 2009; Silva-Bedoya et al. 2014). Moreover, this carotenogenic yeast is considered to be ubiquitous due to its worldwide distribution in terrestrial, freshwater, and marine habitats and to its ability to colonize a large variety of substrates (Libkind et al. 2008). The synthesis of carotenoids by these colored yeast species may represent an interesting issue for biotechnological studies on these microorganisms as a potential source of carotenoid pigment production (Tkáčová et al. 2015; Zhao et al. 2019; Garcia-Cortes et al. 2021).

The investigation of halotolerance property showed that all the yeast strains were salt-tolerant. This type of adaptation has received great attention from many researchers interested in studying genes of biotechnological importance, in order to improve the halotolerance of industrially important yeasts. Previous studies have reported that salt stress-tolerant yeast cells have generally a negatively charged molecules in the exopolymeric layer or uronic acids, which are directly proportional to the heavy metal chelation (Cho and Kim 2003).

The diameter of the heavy metal inhibition zone differed significantly according to the yeast strain and the heavy metal type. Similar results were obtained by Balsalobre et al. (2003) who reported that the metal tolerance and the uptake

capacity are dependent on the ionic metal type and the yeast species. Moreover, our findings revealed that the seven studied strains displayed tolerance thresholds for heavy metals higher than those observed in many yeast strains isolated from metal-polluted aquatic or terrestrial environments (Muñoz et al. 2012; Oyetibo et al. 2014). Several studies have shown that the yeasts isolated from heavy metal-contaminated sites exhibit a marked adaptation mechanism of resistance against stress conditions due to the selective effect of metals (Ramirez-Ramirez et al. 2004; Lopez-Archilla et al. 2004; Srivastava and Thakur 2006). The cadmium MIC indicated a high level of yeast tolerance to heavy metals. The studied *Rhodotorula mucilaginosa* strain was found to be more highly resistant than other previously reported yeasts such as *Candida tropicalis* isolated from industrial wastewater with a cadmium MIC of 2.8 mg/l (Rehman and Anjum 2010). However, the *Rhodotorula mucilaginosa* AN5 strain isolated from Antarctic sea ice remains much more resistant to cadmium with a 1000 mM MIC (Kan et al. 2018). The chromium MIC values recorded in the present study are in accordance with those obtained for three yeast strains isolated from tannery sediment of mining site (30, 50, and 60 mM) (Villegas et al. 2008). Nevertheless, these MIC values remain much higher than that found in yeast isolated from industrial wastewater in Pakistan with a maximum of 2 mg/l (Rehman and Anjum 2010). The chromium tolerance observed in the studied *Wickerhamomyces anomalus* strains was also reported earlier in another strain of the same species isolated from a site receiving textile dye effluents and which could resist up to 50 mM of chromium and was able to reduce this metal from the state hexavalent (Cr (VI)) to trivalent state (Cr (III)) (Fernández et al. 2013). Thus, the *Wickerhamomyces anomalus* strains WO3, WO4, and WO6 displaying a considerable MIC (75 mM) could be a promising candidate in the bioremediation of chromium-contaminated waters. The zinc and iron values of MIC reached in the present work 300 mM and were significantly lower than those of copper. Previous studies have also demonstrated that the tolerance of yeast strains to Zn is higher than that for Cu (Vadkertiova and Slavikova 2006; Singh et al. 2013). The tolerance capacity of the studied strains to Zn is lower than that obtained in 53% of the yeast strains isolated by García-Béjar et al. (2020), which reaches a threshold of 100 mg/l. Despite the essentiality of zinc and iron for living organisms including yeasts, these metals can be nonetheless strongly harmful for microorganisms at high concentrations (Askwith et al. 1996; Balsalobre et al. 2003).

It is noteworthy that all the studied yeast strains exhibited higher tolerance to copper than many earlier described yeasts isolated from deep waters (maximum MIC of 1400 mg/l) (Singh et al. 2013) or industrial sewage (maximum MIC of 20 mM) (Irawati et al. 2017). However, some *Cryptococcus* sp. (Abe et al. 2001) and *Rhodotorula mucilaginosa* (Kan et al. 2018) species seem to display similar copper MIC values to those observed in the present investigation. Furthermore, the seven yeast strains exhibited considerable MIC values (25–200 mM) for lead metal which were much superior than those observed in similar studies (Shakoori et al.2005; Chatterjee et al. 2011; Singh et al. 2013). The *Rhodotorula mucilaginosa* RO7 tolerance threshold remains nevertheless lower than that obtained in a different strain of the same species, which was endowed with an interesting capacity to survive until 500 mM of lead (Kan et al. 2018). It is important to notice that mercury was the most toxic metal for the studied yeast strains. According to Oyetibo et al. (2014), the autochthonous yeast strains of polluted estuary water must have molecular alterations to evolve the high mercury resistance. The high toxicity of mercury on yeast can inhibit the enzymatic activity of enzyme catabolic metabolism and lead consequently to cessation of respiratory activities and rapid depletion of ATP (Brunker 1976; Oyetibo et al. 2014).

Our findings demonstrate also the ability of two autochthonous yeast strains *R. mucilaginosa* RO7 and *Wickerhamomyces anomalus* WO2 to grow in the presence of lead. *R. mucilaginosa* is a yeast species known by its ability to remove lead (Jiang et al. 2015; Li et al. 2019). Furthermore, similar properties with high Pb2 + adsorption capacity were reported for *W. anomalus* from China (Li and He, 2020). Exposition to lead induced a remarkable change in the size and form of yeast cells. These results are in accordance with several earlier studies which demonstrated that the presence of lead in culture media can affect yeast cells and induce changes in their size and morphology (Bussche and Soares 2011; Sousa et al. 2014; Li et al. 2019). The cytotoxic effects of lead involve induction of apoptotic cell death in order to maintain the integrity of their plasma membrane, which was reported as a non-immediate target for lead toxicity (Van der Heggen et al. 2010; Bussche and Soares 2011). Lead can also be accumulated in the vacuoles, reducing the metal toxicity and cell swellings (Sousa et al. 2014). Other studies have proved that lead toxicity can even reach nucleic acids since it is able to reduce the ratio DNA/RNA and induce DNA damage (Yuan and Tang 1999; Chen and Wang 2007).

The present results also exhibited that the Pb uptake and the percentage of the yeast cell removal efficiency were highly significant and were affected by the initial lead concentration and the amount of biomass. In fact, at lead concentration of 31.25 ppm, the purification activity was more significant at low yeast biomass concentration than at high biomass concentration. These results are in accordance with those obtained by several authors (Ferraz and Teixeira 1999; Cho et al. 2004; Farhan and Khadom 2015) who reported that the purification activity of Pb decreases when the amount of yeast strains increases. This could be explained by the increased electrostatic interactions at

high biomass concentrations, inhibiting the metal biosorption, and the mass transfer limitations within yeast cells (Ferraz and Teixeira 1999; Cho et al. 2004). In contrast, the data from the investigations made with 125 ppm of lead concentration were contradictory with those obtained in lower lead concentration. Indeed, a direct relationship between the biomass concentration tested and the purification rate was observed at high Pb concentration. This finding may be attributed to the available active binding sites of the biomass for the available sorbate ions (Dahiya et al. 2008). It is noteworthy that the percentage of lead removal obtained in the present study is higher than those reported for other yeast strains such as *R. mucilaginosa* (32.5%) or *Saccharomyces cerevisiae* (52.94%, 79.9%) (Skountzou et al. 2003; Zhang et al. 2009; Li et al. 2019).

On the other hand, the removal efficiency of lead by the two strains reached its maximum levels at the highest lead concentration and under the lowest concentration of fresh biomass. These results confirm one more time the reverse relationship observed between biomass concentration and metal removal activity.

Conclusion

Based on the current research, we conclude that the seven yeast strains isolated from lake water polluted by lead and cadmium exhibited remarkable halotolerance and resistance to several heavy metals with MICs ranging between 10 to 300 mM for copper, zinc, lead, cadmium, chromium, and iron and between 0.1 to 0.5 mM for mercury. The two strains *W. anomalus* WO2 and *R. mucilaginosa* RO7 exhibited highly significant activities in lead removal, mostly at high lead concentration along with high biomass concentration. These results are very promising as a starting point for a potential application of these metal-resistant yeast strains in bioremediation of industrial effluent and the treatment of heavy metal-containing wastewater.

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Declarations

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

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