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Evaluation of yeast strains as possible agents for trace enrichment of metal ions in aquatic environments

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Abstract

Sorption properties of six yeast strains were evaluated for trace enrichment of metal ions; Cd^{2+}, Cr^{3+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} from aqueous environments. Metal concentration was determined by flame atomic absorption spectrometry (FAAS). The results showed that trace enrichment of the metals under study with yeast, was dependent on the pH and available metal ions. Enrichment time of 30 min gave an optimum metal uptake. The presence of Na^{+}, K^{+}, and Ca^{2+} suppressed the uptake of Pb by less than 5%, but suppressed the uptake of Zn by between 15 and 25%. Mg^{2+}, Cu^{+}, Cu^{2+}, Cr^{3+} Cr^{6+}, Cd^{2+}, and Zn^{2+} suppressed the uptake of Pb by between 25 and 35%, and that of Zn by between 15 and 25%. For both Pb and Zn, Cd had the highest suppression of 35 and 30%, respectively for baker's yeast (*Saccharomyces cerevisiae*). Baker's yeast achieved enrichment factors (EF) of 23, 4, 100, and 1 for dam water, stream water, treated wastewater, and industrial effluent samples for Cu, Pb, Zn, and Cr, respectively. The recoveries of optimised Cd and Cr samples spiked with 2 $\mu g\,ml^{-1}$ of the metal could reach up to 90%, but never exceeded 66% for 10 $\mu g\,ml^{-1}$ samples. For Cu and Pb, the recoveries generally increased independent of concentration, however they were not as high as those for Zn, which exceeded 90% for all the samples spiked with 10 $\mu g\,ml^{-1}$ of the metal. *S. cerevisiae* PR 61/3 had the highest EF for Cr as compared to the other yeast strains. *S. cerevisiae* PRI 60/78 was the only yeast strain which was able to enrich Cd in all the samples. Baker's yeast had the highest EFs for Cu and Zn as compared to the other yeast strains without pH adjustment of the water samples. *Candida tropicalis* attained the highest EFs for Pb as compared to the other yeast strains. The results indicate that all the yeast strains used had a high affinity for Zn based on the EF values achieved. The results from these studies demonstrate that yeast is a viable trace metal enrichment agent that can be used freely suspended in solution to enrich metal ions at relatively low concentrations. This has ramifications on the traditional methods of sampling, sample collection, and transportation from remote sampling sites.

Keywords: Yeast strains; Trace metal enrichment; Aquatic environment

1. Introduction

Trace metal analysis of environmental samples normally requires sampling, pretreatment of samples at sampling site, storage of samples, sample clean up, analyte enrichment, and consequently detection. Most of the analytical methods that are currently in use for trace metal analysis employ conventional techniques of sample handling. In most cases conventional techniques fail to offer both sample clean up and enrichment [1]. Conventional techniques aimed at preconcentration of heavy metals usually include chemical precipitation [2], ion exchange [3], some adsorption process [4], membrane processes [5], crystallization, and electrochemical treatment [6,7]. It has been reported that such strategies can be ineffective and or expensive when very low concentrations of heavy metals in the range of 1–10 $\mu g\,ml^{-1}$ are present in natural waters [7–9].

Alternative, new sampling and sample clean-up techniques such as the use of microorganisms in trace metal analysis are finding their way in analytical chemistry [10]. Bacteria [11,12], fungi [13–16], and yeasts [11–18] are some

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of the microorganisms used. Both living and non-living microorganisms can be used in sampling of metal ions in solution. The ability of microorganisms to bind heavy metals in aqueous solution has long been of scientific interest [11–19]. Microorganisms have the potential to be employed for *in situ* sampling and sample clean up, giving a clean analyte that maybe subsequently determined [8]. Recently, microorganisms have been used for enrichment of trace metals [15,16] as well as for differentiation of metal species according to their toxicity [17,18]. In particular, yeasts are the most popular biomass investigated as a biosorbent for heavy metals in aquatic environments [19].

Much attention has been directed to laboratory studies on model systems containing yeast cells and heavy metal [20–23]. The efficiency of yeast cells on metal uptake can vary greatly depending on the physiological state and specific surface properties of cells, as well as pH and other physicochemical parameters of the metal containing solution [24–26]. Yeasts possess an acknowledged potential to accumulate a range of metal ions [27] and large amounts of this metal can remain associated with the yeast cell wall [28]. The cell wall is a multiamine, microfibrillar structure consisting of up to 90% polysaccharide, with mannan-β-glucan [29,30]. The cell wall components provide metal-binding groups including amino, hydroxyl, carboxyl groups, and sulphate as well as phosphate moieties.

The present studies evaluate the possibility to employ freely suspended yeast in solution for the trace enrichment of metal ions in industrial wastewater, dam water, water from an electroplating plant, and processed wastewater from a sewage plant. The results indicate that freely suspended yeast is a good candidate for trace enrichment of metal ions in aquatic environments.

2. Experimental

2.1. Reagents

Stock solutions of 1000 $\mu g\,ml^{-1}$ Cd, Cr, Cu, Pb, and Zn were obtained from Saarchem (Muldersdrift, RSA). $CuCl_2$, $PbCl_2$, $CrCl_3{\cdot}6H_2O$, $ZnCl_2$, $Na_2CrO_4{\cdot}4H_2O$, KCl, NaCl, $CaCl_2$, $MgCl_2{\cdot}6H_2O$, NaOH, and HCl were obtained from Saarchem (Muldersdrift, RSA) while $CdCl_2{\cdot}2.5H_2O$ was from Acros Organics (New Jersey, USA). All 5 mM standard solutions were daily freshly prepared using ultra-pure water from a Millipore-Q system supplied by Millipore (Bedford, MA, USA).

2.2. Yeast biomass

Anchor Active Dry Yeast, by Anchor Yeast Inc. (Johannesburg, RSA) is a commercial preparation of baker's yeast, *S. cerevisiae* (production strain, approximately 90% cell viability) [31] was purchased from a grocery shop. Five other yeast strains were also used. These were *S. cerevisiae* PR 61/3, *S. cerevisiae* PRI 61/72, *S. cerevisiae* PRI 60/78, *Debaryomyces hansenii* strain 106, and *Candida tropicalis* strain 113. The five yeast strains are maintained and cultivated by the Department of Microbiology, University of Stellenbosch, (Stellenbosch, RSA).

2.3. Instrumentation

Two Varian models, SpectrAA-10 and SpectrAA-220 FS flame atomic absorption spectrometers from Varian (Sydney, Australia), were used in the determination of metals. Instrumental parameters for metal determination were those provided by the manufacturer. All pH measurements were carried out with a HANNA digital pH meter, HANNA instruments (Arvore-Vila do Conte, Portugal). The yeast suspensions were incubated in a Labcon (Maraisburg, RSA) model WBE-SPL 25 water bath with a shaking platform. A Heraeus SEPATECH (Osterode, Germany) model Labofuge 200 was used to centrifuge yeast suspensions at 5000 × *g*.

2.4. Sorption studies

Preliminary tests with all yeast strains to optimise pH for metal sorption were carried out in the pH range of 5.0–6.5. pH 2 was used for metal desorption. The pH of the metal solutions was adjusted using 0.01 M NaOH or 0.01 M HCl prior to incubation with yeast. The optimum pH was used for subsequent studies.

2.5. Adsorption and desorption of metal ions

10 ml of pH adjusted metal solution at 2 mM was added to 400 mg of yeast. The suspension was incubated at 25 °C for 30 min in a water bath with shaking at 150 rpm and subsequently centrifuged for 5 min. 5 ml of supernatant was separated from the yeast paste and diluted to 50 ml with ultrapure water. For desorption, the yeast paste was dislodged and homogenised with the remaining 5 ml of the supernatant to make a suspension. The pH of the mixture was lowered to pH 2 and the resulting suspension was incubated for 30 min, centrifuged and subsequently 5 ml of supernatant were separated from the yeast paste and diluted to 50 ml. The metal concentration in the supernatants was then determined by FAAS. For any subsequent experiments to optimise metal uptake, this sorption procedure was repeated and experiments were carried out in triplicates.

2.6. Effect of interfering ions on sorption of Pb and Zn

The effect of the alkali and alkali earth metal ions such as Na, K, Mg, and Ca termed here "interfering ions", on Pb and Zn uptake is important for efficient application of yeasts since these metal ions are ubiquitously present in environmental waters. Cd, Cr(III), Cr(VI), and Cu were also included as interfering ions, since it is apparent that yeasts can efficiently adsorb other heavy metals. The ratio of analyte to interfer-

ing ion was 1:1 by volume and concentration for portions of 400 mg of baker's yeast used.

2.7. Enrichment of Cu, Cd, Cr, Pb, and Zn from environmental water samples using the different yeast strains

10 ml water samples taken from an industrial stream, a dam for drinking water, treated wastewater being discharged into a river from a sewerage treatment plant, and industrial effluent from a chromium electroplating factory were analysed for Cu, Cd, Cr, Pb, and Zn after enrichment with 400 mg of five strains of yeast. For all strains enrichment was carried out with and without pH adjustment of the samples.

2.8. Analysis of spiked environmental water samples after enrichment with baker's yeast

In order to evaluate the effect of metal concentration on enrichment by yeast, all water samples were spiked with 2 and 10 $\mu g\,ml^{-1}$ and analysed for the respective metals.

3. Results and discussion

Evaluation of the ability to enrich metal ions by yeast is expressed as a % metal or enrichment factor as in Eqs. (1) and (2), respectively.

$$\%\,\text{Metal uptake} = \left[\frac{C_{\text{des}} - C_{\text{ads}}}{C_{\text{std}}}\right] \times 100 \quad (1)$$

$$\text{EF} = \frac{C_{\text{yeast}}}{C_{\text{sample}}} \quad (2)$$

where C_{ads}, C_{des}, C_{std}, C_{yeast}, and C_{sample} are the concentrations of metal ions in the supernatants after adsorption, desorption, in the standard solution, concentration of the metal in the yeast, and sample, respectively.

3.1. Optimisation of the pH for metal ion uptake

The ability of microbial biomass to bind metals in solution has been shown to be dependent of pH. For example, change of less than 1 pH unit results in an increase in the amount of metal adsorbed from almost 0 to 100% [21,30,32]. pH of the solution influences both the solubility of metal ions and ionisation of the functional groups or ligands (i.e. carboxylate, phosphate, and amino groups) on the yeast cell wall [33]. These functional groups carry negative charges that allow the cells to have a high binding affinity for metal ions. Hence binding of metal ions occurs at the cell surface [34]. The effect of pH on metal ions in solution is often considerable and varies with the type of biomass and oxidation state of metal ion [35]. For instance Cr(VI) ions occur in aqueous solutions in the form of anions and hence this would result

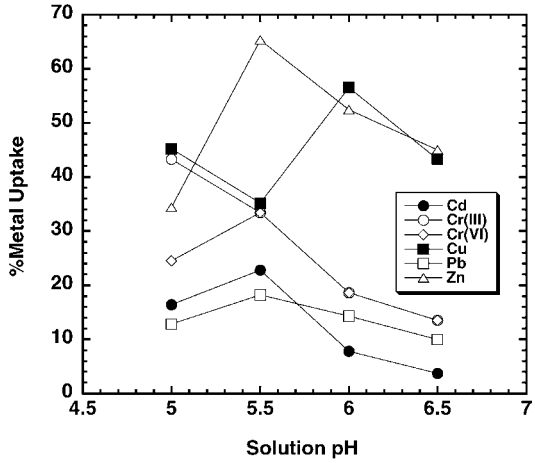

Fig. 1. Effect of pH on uptake of Cd^{2+}, Cr^{3+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} by *S. cerevisiae* PR 61/3. The yeast biomass used was 400 mg, for an incubation time of 30 min at 25 °C. Data are expressed as the mean and the S.D. < 2 for $n = 3$ samples.

in a different uptake at a different pH range as compared to the other metal ions. Leush et al. [36] reported that uptake of divalent ions by yeasts is reduced below pH 5.0. However, both monovalent and divalent ions are strongly bound by yeasts at pH ≥ 5.0 [35]. At low pH values (pH ≤ 2), the concentration of hydronium ions (H_3O^+) far exceeds that of metal ions and hence, these are bound to cell walls, leaving the metal ions unbound. In order to evaluate the effect of pH, studies were carried out using the five yeast strains for Cd, Cr(III), Cr(VI), Cu, Pb, and Zn, as shown by the results in Figs. 1 and 2. Metal uptake by the yeast strains showed a dependency on pH owing to competition between the metal ion and the hydronium ions for the negative active sites [37].

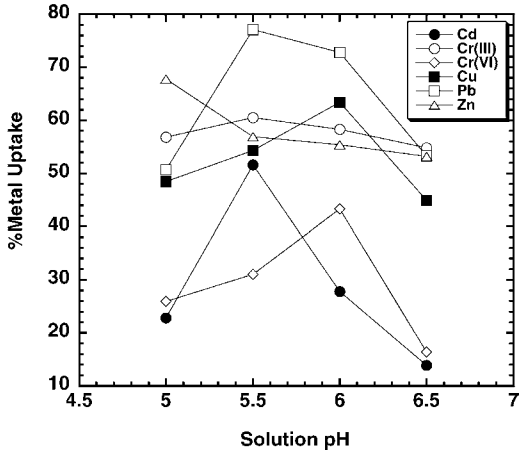

Fig. 2. Effect of pH on uptake of Cd^{2+}, Cr^{3+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} by *S. cerevisiae* PRI 60/78. The yeast biomass used was 400 mg, for an incubation time of 30 min at 25 °C. Data are expressed as the mean and the S.D. < 1 for $n = 3$ samples.

3.2. Effect of pH for metal ion uptake by S. cerevisiae PR 61/3

As shown in Fig. 1 the optimum pH for uptake of Cd, Cr(VI), Pb, and Zn by this yeast strain is pH 5.5. The uptake of Cr(III) decreased with an increase in pH. The optimum pH for uptake of Cu was observed at pH 6.0. From the metals studied uptake of Zn and Pb by *S. cerevisiae* PR 61/3 were highest and lowest, respectively. For the metals studied more of the metal uptake occurred between pH 5.0 and 5.5 with exception to Cu.

3.3. Effect of pH for metal ion uptake by S. cerevisiae PRI 60/78

From Fig. 2, the uptake of Cd^{2+}, Cr^{3+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} by *S. cerevisiae* PRI 60/78 was in the pH range 5.0–6.5. The optimum pH for uptake of Cd, Cr(III), and Pb was observed at pH 5.5 and that of Cr(VI) and Cu was at pH 6.0. The uptake of Zn by this yeast strain decreased with increase in pH. *S. cerevisiae* PRI 60/78 was the most efficient yeast strain in metal uptake. An increase in metal uptake occurred in the pH range 5.0–6.0. For all the metals studied as the pH was increased beyond the optimum pH values, the metal uptake decreased. Uptake of Pb and Cr(VI) by *S. cerevisiae* PRI 60/78 were highest and lowest, respectively. *S. cerevisiae* PRI 60/78 had the highest uptake of Pb compared to the other yeast strains.

An increase in metal uptake in all the yeast strains studied occurred in the pH range 5.0–6.0. A decrease in metal uptake in all the yeast strains was observed in the pH range 6.0–6.5. The decrease in metal uptake at higher pH values may be attributed to the competition between the functional groups of the cell walls and the ligands in solution. This effect leads to fewer metal ions being available for the functional groups with increase in pH and also at higher pH values the functional groups uptake fewer metal ions [18]. The lower uptake was also probably due to the formation of anionic hydroxide complexes at higher pH values [38] by bonding of metal ions to hydroxyl groups as in Eq. (3) [39]:

$$M^{n+} mOH^{-} \leftarrow M(OH)m^{(m-n)-} \quad (3)$$

The decrease in the uptake of Cd, Cr(III), Pb (from Fig. 1) and Cd, Pb, and Zn (from Fig. 2) could mean that these metals have a tendency to form stable hydroxides at higher pH values. At lower pH values the uptake of these metals was slightly high. However, lower pH values do not guarantee high metal uptake as the positive charge on the yeast cells [35], may inhibit the approaching positively charged metal ions. The positive charge of yeast cells is due to the protonation of acid and weakly basic coordinating functional groups such as amino and carboxylate groups on the cell wall surface at lower pH values [18]. All the yeast strains exhibited a poor uptake in Cr(VI) ions. This is because Cr(VI) ions are anionic and could be electrostatically bonded to unprotonated carboxyl oxygen and sulphate, therefore the interaction of these ions with microbial cells is primarily electrostastic in nature [40]. Hence its uptake is not influenced by pH.

Different metal ions have different pH optima, possibly due to the different solution chemistry of metal ions [40]. The optimum pH for heavy metal uptake is also organism dependent because of different adsorptive sites for different species of microorganisms [40]. Hence each metal binds to the different sites on the cell surface because of the associated affinities of the ligands for the metals on the cells. The different optimum pH values for metal uptake by the yeast strains supports this hypothesis.

3.4. Effect of interfering cations on uptake of Pb and Zn by baker's yeast

The alkali and alkali earth metal ions such as Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+} have been known to interact with yeasts through weak electrostatic bonding [41] and play an important role in many biological functions, such as in metal transport channels or pores in the membrane of yeast cells [42].

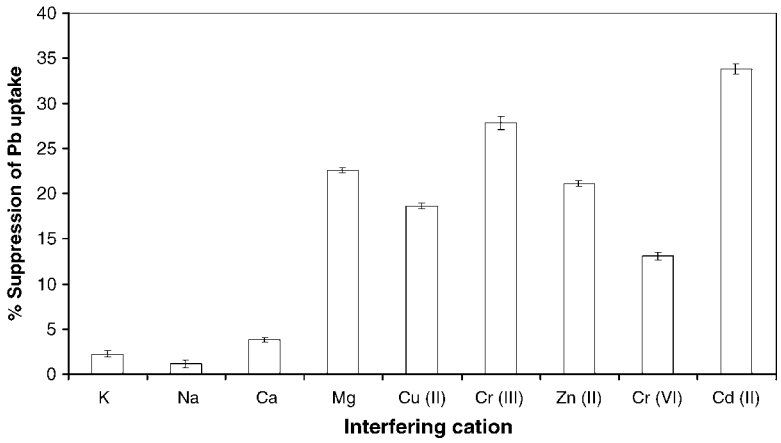

Fig. 3. Effect of interfering ions on the uptake of Pb by *S. cerevisiae*. The yeast biomass used was 400 mg, for an incubation time of 30 min at 25 °C. The data are expressed as the mean ± standard deviation for $n = 3$ samples.

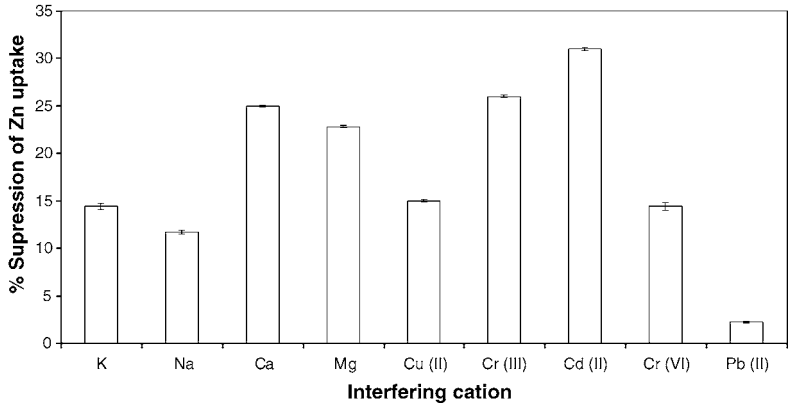

Fig. 4. Effect of interfering ions on the uptake of Zn by *S. cerevisiae*. The yeast biomass used was 400 mg, for an incubation time of 30 min at 25 °C. The data are expressed as the mean ± standard deviation for $n = 3$ samples.

They may also be used in cationic exchange with metals such as Co^{2+}, Cd^{2+}, Cu^{2+}, and Zn^{2+} resulting in their uptake [41]. This cationic exchange can occur either in the cell wall, cell membrane or cytoplasm. Thus, the knowledge of the effect of these metals ions, is important for efficient application of the yeast biomass.

Fig. 3 shows that K, Na, and Ca had virtually no effect on Pb uptake by *S. cerevisiae*. In contrast the presence of Mg, Cd, Cr(III), Cr(VI), Cu, and Zn resulted in a decrease in Pb uptake. The presence of Mg resulted in a decrease in Pb uptake. Its effect in decreasing Pb uptake was much greater than that of Ca. Mg is known to protect yeasts against several heavy metal cations, including Pb [43–45]. This protection is generally considered to result from competitive inhibition of metal uptake. The presence of Cd caused the highest decrease in Pb uptake by about 35%, followed by 28% from Cr(III).

The results of Zn uptake in the presence of interfering ions are shown in Fig. 4. Na, K, Mg, and Ca caused an appreciable decrease in Zn uptake by *S. cerevisiae* compared to Pb uptake (see Fig. 3). The presence of Cr(VI) and Cu also resulted in a decrease in Zn uptake, but the effect was less than that of Cd and Cr(III). Zn uptake by *S. cerevisiae* was not greatly affected by the presence of Pb. It seems *S. cerevisiae* has a higher affinity for Zn over Pb. However, in the presence of interfering ions, the yeast cells could still uptake about 65% of the analytes (see Figs. 3 and 4) present in the mixture. Cd had the highest interference in both the uptake of Pb and Zn by *S. cerevisiae*. It suppressed the uptake of Pb by about 35% (see Fig. 3) and for Zn by about 33% (see Fig. 4). Cd strongly binds to functional groups on yeast cell walls [41]. Hence this results in formation of a Cd-complex with a high formation constant, which facilitates the displacement of other metals [41].

The interactive effects of a mixture of metals on a microorganism can be extremely complex and three types of responses may be produced by an organism: (i) the effect of the mixture is greater than that of each of the individual effects of the constituents in the mixture (synergism); (ii) the effect of the mixture is less than that of each of the individual effects of the constituents in the mixture (antagonism); (iii) the effect of the mixture is equivalent to each of the individual effects of the constituents in the mixture (non-interaction) [46].

In mixtures of two or more metal species in solution, the synergistic or antagonistic interactions occurring between metal ions may affect individual metal uptake by the microorganism.

Synergistic effects were recently demonstrated for dual metal solutions [23]. Brady et al. [23] reported that more than one metal ion could be accumulated simultaneously by *S. cerevisiae* and in greater quantities than when a single metal was present in solution. Ting and Teo [20] have demonstrated non-interaction of co-ions in dual metal ions systems in respect of their uptake by *S. cerevisiae*. However, long-term incubation (24 h) resulted in an inhibitory effect of Cd on Zn uptake by yeast cells [20]. The same phenomenon was observed in studies carried out, but rather over short incubation time (30 min). The most logical reason for the antagonistic action is claimed to be competition for adsorption sites on the yeast cells and or the screening effect by the second metal ion [40].

3.5. Analysis of metal ions from environmental water samples after enrichment with yeast

As shown in Table 1, enrichment factors (EF) ranged from 1 to 98 when the samples were analysed without pH adjustment (EF 1) with baker's yeast. For the detected metals, the lowest and highest EF were achieved for Cr and Zn, respectively. Cd was not detected in all samples, with or without pH adjustment. Adjusting the pH significantly enhanced the metal uptake as shown by the increased EF for all metals (EF 2). The dam water and treated wastewater samples seemed to benefit most from pH adjustment.

The EFs for *S. cerevisiae* PR 61/3, *S. cerevisiae* PRI 60/78, *S. cerevisiae* PRI 61/72, *C. tropicalis*, and *D. hansenii* ranged from 1 to 71, 1 to 60, 1 to 80, 1 to 31, and 1 to 44, respectively (data not shown). *S. cerevisiae* PR 61/3 gave the highest EF

Table 1
Enrichment factors for Cr^{3+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} in water samples after enrichment by *S. cerevisiae*[a]

Sample Type and pH	Cu			Pb			Zn			Cr		
	Direct Analysis ($\mu g\,ml^{-1}$)	EF 1	EF 2 (pH 5.4)	Direct Analysis ($\mu g\,ml^{-1}$)	EF 1	EF 2 (pH 5.2)	Direct Analysis ($\mu g\,ml^{-1}$)	EF 1	EF 2 (pH 5.8)	Direct Analysis ($\mu g\,ml^{-1}$)	EF 1	EF 2 (pH 5.2)
Stream Water (pH 7.26)	0.006 (0.002)	6	8	0.051 (0.005)	1	4	0.029 (0.001)	54	75	0.090 (0.008)	1	2
Dam Water (pH 7.89)	0.002 (0.001)	10	23	0.056 (0.004)	1	4	0.026 (0.000)	80	84	0.030 (0.006)	1	2
Treated Wastewater (pH 9.36)	0.004 (0.001)	7	11	0.060 (0.007)	1	2	0.021 (0.002)	98	100	0.027 (0.000)	1	2
Metal Effluent (pH 5.60)	0.076 (0.03)	3	3	0.194 (0.006)	1	2	0.434 (0.050)	3	6	2.008 (0.060)	1	1

[a] Data for enrichment factors after enrichment by *S. cerevisiae* with and without pH adjustment of the water samples. The yeast biomass used was 400 mg, for an incubation time of 1 h at 25 °C. Data are expressed as the mean and the numbers in parentheses represent the standard deviation for $n = 3$ samples.

Table 2
Determination of recovered metal ions in spiked water samples with and without pH adjustment of the samples after enrichment with baker's yeast[a]

Metal spiked	Spiked concentration ($\mu g\,ml^{-1}$)	% Recovery			
		Stream water	Dam water	Wastewater	Metal effluent
Cd	0	ND[b]	ND[c]	ND[d]	ND[e]
		ND[f]	ND[f]	ND[f]	ND[f]
	2	61.3 ± 0.9[b]	67 ± 1[c]	18 ± 3[d]	55 ± 3[e]
		88 ± 1[f]	78 ± 2[f]	47 ± 1[f]	74.0 ± 0.8[f]
	10	51.1 ± 0.9[b]	48.1 ± 0.8[c]	51 ± 1[d]	36 ± 3[e]
		57.2 ± 0.2[f]	57 ± 2[f]	57 ± 1[f]	51 ± 1[f]
Cu	0	50 ± 1[b]	23 ± 1[c]	28 ± 1[d]	49 ± 2[e]
		64 ± 1[g]	58.1 ± 0.8[g]	35.9 ± 0.6[g]	51.7 ± 0.4[g]
	2	73 ± 3[b]	62.5 ± 0.6[c]	42.50 ± 0.07[d]	60 ± 3[e]
		82 ± 2[g]	81.9 ± 0.9[g]	71.7 ± 0.7[g]	88.8 ± 0.9[g]
	10	41 ± 1[b]	39 ± 1[c]	53 ± 1[d]	66.2 ± 0.7[e]
		85.9 ± 0.5[g]	47 ± 1[g]	78 ± 2[g]	70 ± 2[g]
Cr	0	39.4 ± 0.9[b]	32 ± 2[c]	56 ± 2[d]	69 ± 1[e]
		68 ± 1[h]	49 ± 2[h]	63 ± 2[h]	78.5 ± 0.5[h]
	2	52 ± 1[b]	51.7 ± 0.4[c]	15 ± 3[d]	87 ± 1[e]
		66.3 ± 0.6[h]	77 ± 2[h]	42 ± 1[h]	91 ± 2[h]
	10	33 ± 1[b]	48.1 ± 0.8[c]	38.2 ± 0.6[d]	49 ± 2[e]
		45.7 ± 0.6[h]	57 ± 2[h]	50 ± 2[h]	66 ± 1[h]
Pb	0	ND[b]	ND[c]	24 ± 2[d]	29 ± 1[e]
		ND[h]	ND[h]	43 ± 1[h]	52 ± 1[h]
	2	41 ± 2[b]	35 ± 1[c]	43.0 ± 0.8[d]	35.8 ± 0.4[e]
		66.3 ± 0.7[h]	77 ± 2[h]	52 ± 1[h]	72.5 ± 0.8[h]
	10	34 ± 1[b]	37 ± 1[c]	37 ± 2[d]	50.2 ± 0.7[e]
		71 ± 2[h]	74 ± 2[h]	79 ± 1[h]	79 ± 1[h]
Zn	0	61.3 ± 0.3[b]	45 ± 2[c]	29.3 ± 0.3[d]	37 ± 2[e]
		80 ± 2[i]	75.6 ± 0.5[i]	68 ± 2[i]	53 ± 1[i]
	2	78 ± 2[b]	75 ± 2[c]	66 ± 2[d]	80 ± 2[e]
		84 ± 2[i]	79 ± 3[i]	77.10 ± 0.07[i]	97 ± 3[i]
	10	47.7 ± 0.7[b]	49.4 ± 0.8[c]	51.0 ± 0.6[d]	64.3 ± 0.1[e]
		94.4 ± 0.1[i]	95 ± 2[i]	103 ± 2[i]	93.50 ± 0.08[i]

ND: not detected.
[a] Metal recoveries after enrichment by *S. cerevisiae* in spiked water samples. Data are expressed as the mean and the standard deviation of three determinations.
[b] pH 7.26.
[c] pH 7.89.
[d] pH 9.36.
[e] pH 5.60.
[f] pH 6.00 (adjusted).
[g] pH 5.40 (adjusted).
[h] pH 5.20 (adjusted).
[i] pH 5.80 (adjusted).

for Cr as compared to the other yeast strains (data not shown). *S. cerevisiae* PRI 60/78 was the only yeast strain which was able to enrich Cd in all the samples (data not shown). Baker's yeast had the highest EFs for Cu and Zn as compared to the other yeast strains without pH adjustment of the water samples. *C. tropicalis* had the highest EFs for Pb as compared to the other yeast strains (data not shown). Most yeast strains had poor EFs for Cd and Pb. The results indicate that all the yeast strains used had the highest EF values for Zn, more especially from treated wastewater. These findings are very much welcome considering that the pH of treated wastewater was 9.36. Hence yeasts are able to enrich Zn from water even if the pH is alkaline whereas it has been reported that Zn is said to be available in acidic pH [37].

It seems the yeast strains studied have a high affinity for Zn and low affinity for Cd and Pb. Although Zn is an essential nutrient as a trace element for animals, plants, and microorganisms, it is toxic when present at millimolar concentration, and exerts its toxicity at much higher concentrations than cadmium, copper, lead, and mercury [47]. Baker's yeast gave the highest EF for Zn in all the samples as compared to the other yeast strains.

The different EF ranges given by the yeast strains may have been attributed to the initial concentrations of the metal species present in the water samples. The cell wall composition of the yeast strains might have a key role in affecting enrichment even though the cell wall structure of the yeast strains was not investigated. It has been reported that the cell wall structure determines binding of metal ions to the active sites [48]. Other factors include the specific surface properties of the organism and the physicochemical parameters of the solution such as temperature, pH, and biomass concentration [49,50].

3.6. Analysis of metal ions from spiked environmental water samples after enrichment with baker's yeast

Our finding to date, indicate the baker's yeast can concentrate metal ions in low concentration in environmental water samples. Hence to verify this capability the water samples were spiked in order to determine how much of the metal can be enriched. The recovery values were evaluated using the following equation:

$$\%\,\text{Recovery} = \frac{\text{Recovered value}}{\text{Spiked value}} \times 100 \quad (4)$$

where the recovered value is obtained by subtracting the analytical value of an original sample from the enriched value of the spiked sample this is after desorption of the metal from the yeast phase. The spiked value in the sample is the known amount of analyte added to the sample.

The results given in Table 2 show good recoveries of metals in samples with their pH adjusted to their optimum sorption pH. This occurred in both samples spiked and non-spiked. Recoveries of metals in samples spiked with $2\,\mu g\,ml^{-1}$ of the metal were relatively high as compared to samples spiked with $10\,\mu g\,ml^{-1}$ of the metal with exception to Zn. This was observed in samples spiked with $10\,\mu g\,ml^{-1}$ of Zn. The recoveries increased almost two-fold.

4. Conclusion

Metals have different solubility and pH optima, possibly due to the different solution chemistry of metal ions. The pH optimum for heavy-metal uptake is also organism dependent because of different adsorptive sites of different species of microorganisms. Analysis for Cd, Cr, Cu, Pb, and Zn in different types of water samples showed that enrichment factors of 100 could be achieved for metals such as Zn without pH adjustment of the water samples. Adjusting the pH of the water samples significantly enhanced metal uptake by yeast strains. From the yeast strains used in the enrichment studies, it is evident that all the yeast strains had a high uptake of Zn as compared to the other metals, without pH adjustment of the water samples. Adjusting the pH of the water samples to an optimum sorption pH for the studied metals did enhance the recovery of the metals from both samples spiked and not spiked. Recoveries of metals in samples spiked with $2\,\mu g\,ml^{-1}$ of the metal were relatively high as compared to samples spiked with $10\,\mu g\,ml^{-1}$ of the metal with exception to Zn. This indicates that yeasts are suitable to enrich metal ions in solution at relatively low concentrations. Such an observation is encouraging since many conventional methods often fail in detecting the low concentrations of metals in aquatic systems. The present study has demonstrated that freely suspended yeast cells can retain their ability to accumulate a broad range of metals to varying degrees under a wide range of external conditions which makes them more suitable for trace metal analysis.

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