

PRODUCTION OF *Candida* BIOMASSES FOR HEAVY METAL REMOVAL FROM WASTEWATERS

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Abstract: Yeasts can accumulate heavy metals and grow in acidic media. In the present study, it was shown that *Candida* yeasts in an aqueous solution accumulate single Cu(II) and Ni(II) cations. The effect of heavy metal ions on the specific growth rate of biomasses and the uptake of metal ions during the growth phase was investigated in a batch system. Bioaccumulation efficiency decreased with increasing metal ion concentrations at constant sucrose concentrations. Both the specific growth rate and the biomass concentration were more inhibited in the bioaccumulation media containing Ni(II) ions singly as compared with the bioaccumulation media containing Cu(II) ions singly. The maximum specific growth rate and the saturation constant of yeasts were examined with a double-reciprocal form of Monod equation. Metal uptake performance decreased from 81.68% to 46.28% with increasing Ni(II) concentration from 25 mg/L to 250 mg/L for *Candida lipolytica*. *Candida* biomasses may be an alternative way of removal of heavy metals from wastewaters and may constitute a sample to produce new biomass. The study showed that *Candida* yeasts can be used as economical biomass due to their metal resistance and efficient production.

Özet: Mayalar, asidik ortamda büyüebilir ve ağır metalleri biriktirebilir. Bu çalışma, *Candida* türü mayaların sulu çözeltilerden tekli Cu(II) ve Ni(II) kationlarını biriktirdiğini göstermiştir. Ağır metal iyonlarının, biyokütlelerin spesifik büyüme hızı ve büyüme periyodu boyunca metal iyonlarını giderimi üzerindeki etkisi, bir kesikli sistemde araştırılmıştır. Sabit sakaroz derişiminde, metal iyonu derişimi arttıkça, biyobirikim verimi azalmıştır. Hem spesifik büyüme hızı hem de biyokütle konsantrasyonu, tek başına Cu(II) iyonları içeren biyoakümülyasyon ortamına kıyasla Ni(II) iyonları içeren biyoakümülyasyon ortamında daha fazla inhibe edilmiştir. Mayaların maksimum özgül büyüme hızı ve doyunluk sabiti, Monod denkleminin çift-karşılıklı formu ile incelenmiştir. *Candida lipolytica*'nın metal giderim performansı Ni(II) derişiminin 25 mg/L'den 250 mg/L'ye çıkmasıyla % 81,68'den % 46,28'e düşmüştür. *Candida* biyokütelleri, ağır metallerin atık sularından gideriminde alternatif bir yol olabilir ve yeni biyokütle üretimi için bir örnek oluşturabilir. Bu çalışma, *Candida* mayalarının, metal direnci ve verimli üretimleri nedeniyle ekonomik biyokütle olarak kullanılabilceğini göstermektedir.

Introduction

Clean water plays a vital role in living organisms. Industrial activities cause water contamination due to chemical, physical and biological components in water bodies. One of the important sources of water contamination is heavy metals. The presence of heavy metals in the environment may cause significant hazards to both animals and humans. Even in trace amounts, heavy metals play a vital role in human metabolic systems, and high concentrations of trace elements are toxic, and they cause physiological and neurological hazards (Tchounwou *et al.* 2012). Several methods are used for the treatment of wastewater effluents. These methods

include chemical precipitation, ion exchange, adsorption, membrane filtration, reverse osmosis, solvent extraction etc. (Wolowiec *et al.* 2019). Some adsorbents such as clay, zeolite, fly ash, agro wastes, and chitin have been reported as low-cost for the removal of contaminations from aqueous solutions. Biomass can be derived from both vegetables and animals, either living or dead, and is used as an adsorbent to efficiently remove heavy metals from wastewaters. Biomasses have some advantages such as high efficiency, minimal sludge formation, regeneration, and no additional supplementary of nutrients (Tripathi & Ranjan 2015). Yeasts which are used



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in the enzymatic industry and medicine can survive in a medium containing low or high concentrations of heavy metals (Cottet *et al.* 2020). One of the most important microbial source for biosorption of heavy metals is *Candida* species (Luna *et al.* 2016), which were shown to play an important role in the accumulation of metal ions (Honfi *et al.* 2016, Luk *et al.* 2017). Metal uptake capacity of *Candida* species under various experimental conditions depends on the metal type and the yeast species itself (Legorreta-Castañeda *et al.* 2020). Bioaccumulation, which contains some processes as complex formation, ion transfer, adsorption, and chelation is applied to eliminate toxic effects of heavy metals as a cheap, efficient, and green technology (Redha 2020, Fadel *et al.* 2017). The biosorption of metals is affected by several factors such as pH, temperature, concentration, type of biomass, contact time, and type of metal ions in solution. Bioaccumulation based on the accumulation of metals in living microorganisms is metabolism dependent (Açikel & Alp 2009). The bioaccumulation of heavy metals by living cells contains two stages. The first step is very fast due to surface adsorption carried out on the surface of the microorganism with physical adsorption and ion exchange. The second step is the intracellular metal uptake stage which occurs slower due to the metabolic activity of microorganisms (Podder & Majumder 2019). Bioaccumulation has been investigated in many studies for the removal of heavy metals from wastewaters. Cd(II) removal by *Candida tropicalis*, Cu(II) removal by *C. utilis*, Pb(II) removal by *C. albicans* can be given as examples (Gönen & Aksu 2008, Baysal *et al.* 2009, Rehman & Anjum 2011). In the bioaccumulation process, high concentrations of heavy metals may interact with microorganisms which would result in prolonged lag time and reduced growth rate. Therefore, microbial growth kinetics are affected by heavy metals. Nickel is a trace element necessary for microbial growth, but it may cause oxidative stress and disruption of the cell membrane when in higher concentrations, (Fashola *et al.* 2016). Cu(II) is one of the most stable metals and shows a high affinity for metalloproteins in cells (Waldron & Robinson 2009). Some microorganisms use Cu as a catalyzer for electron transfer reactions in cell metabolism. Microorganisms have different metal-binding proteins due to their nature (Dupont *et al.* 2011). Mathematical description of the growth kinetics can be explained by the Monod equation (Şengör *et al.* 2009), which is widely used to describe the empirical microbial growth of microorganisms as a simple model (1).

$$\mu = \frac{\mu_{max}S}{K_s + S} \quad (1)$$

Where μ_{max} is the maximum growth rate when there is enough substrate supplied to the cell and the value exceeds the limiting substrate concentration, $S > K_s$. The constant K_s is the saturation constant or half of the velocity constant and is equal to the concentration of the rate-limiting substrate when the specific growth rate is equal to one-half of the maximum specific growth rate (Monod 1949, Liu 2007). Microorganism cell consists of an outer

cover called a cell wall and contains a variety of functional sites such as amines, phosphates, sulfates, phenols, and hydroxyls with the ability for adsorption of metal ions (Javanbakht *et al.* 2013, Cottet *et al.* 2020). Metal ion adsorption by microorganisms is calculated by the mass balance equation (2).

$$q_e = \frac{(C_o - C_e)V}{m} \quad (2)$$

Where q_e is metal ion uptake per unit mass of biomass at equilibrium (mg/g biomass), C_e is the metal ion concentration in solution at equilibrium (mg/L), C_o is the initial metal ion concentration in solution (mg/L), V is volume of the initial metal ion's solution (L), and m is the mass of biomass (g) (Zha *et al.* 2020).

In this study, bioaccumulation, and growth properties of *Candida* biomasses for the uptake of Cu(II) and Ni(II) ions were investigated as a function of molasses sucrose and metal ion concentrations. Molasses sucrose was used as the main carbon source. The inhibition effects of metal ions on specific growth rates were examined. The results showed that *Candida* species can be used as a biosource for efficient removal of heavy metals from aqueous solutions at low costs.

Materials and Methods

Candida membranafiens (*C. membranafiens*-ATCC® 201377™), *C. utilis* (*C. utilis*-ATCC® 9950), *C. tropicalis* (*C. tropicalis*-ATCC® 13803™) and *C. lipolytica* (*C. lipolytica*-ATCC® 9733™) were obtained from the Biology Department of Ankara University. $(NH_4)_2SO_4$ and K_2PO_4 were purchased from Sigma-Aldrich Company. Molasses sucrose was supplied from a sugar factory in Ankara (Turkey). Molasses consisting of 47-48% sugar was used as the sole carbon source for the growth of the microorganisms. Total sugars constitute of the system included approximately 50% (w/w) of molasses, ash 11% (w/w), and total nitrogen compounds 7-8% (w/w). Non-sugar part of molasses contained minerals and trace elements such as K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Fe^{2+} , Cl^- , SO_4^- , PO_4^- , NO_3^- and metal oxides (as ferric 0.4-2.7%) (Açikel & Alp 2009).

Microorganism growth and bioaccumulation media

Yeasts were grown in aqueous media containing (1-10 g/L) molasses sucrose, (1 g/L) $(NH_4)_2SO_4$ and (1 g/L) K_2PO_4 at 25 °C (pH: 4.0). The growth and cultivation media were sterilized in an autoclave operating at 121°C at 0.99 bar for 15 minutes. Subcultures were grown for 4 days at a rotating speed of 150 rpm. 1 g/L Cu(II) and Ni(II) stock solutions were prepared by diluting Cu $(NO_3)_2 \cdot 3H_2O$ and Ni $(NO_3)_2 \cdot 6H_2O$ in distilled water. The pH of the working solutions was adjusted to desired value by adding 0.1 N NaOH and HNO_3 (pH: 4.0). The yeasts were adapted to the metal ions in culture medium by exposing them to single Cu(II) and Ni(II) ions during the growth phase to increase their metal resistance. The resistance to Cu(II) and Ni(II) ions was investigated as functions of initial metal ion and molasses sucrose

concentrations and the yeasts were adapted to higher metal ion concentrations after the first inoculation. Each yeast was adapted to each metal ion in its culture medium. Adapted yeasts were obtained from subcultures with different concentrations of metal ions in the range of 25-250 mg/L in varying concentrations from 1 g/L to 20 g/L for molasses. Yeast cultures which were resistant to 25 mg/L Cu(II) and Ni(II) ions at 10 g/L molasses sucrose concentration was used for further inoculation. 1 mL culture medium was used to inoculate the next culture medium containing 50 mg/L Cu(II) and Ni(II) ions at the same molasses sucrose concentration when growth culture reached to the exponential growth phase. Adaptation experiments by *Candida* species were carried out in 250 mL flask with 100 mL working volume.

Analytical procedure

5 mL samples were centrifuged at 3000xg for 5 min and the supernatant fluid was analyzed for metal ions. The precipitated cells were used for determination of the dry weight of the biomass and the biomass concentration. Yeast pellets were dried until constant mass at 60°C for 24 h. The amount of total metal ions was calculated from the calibration graph. Microorganism concentration was measured at 360 nm using a calibration curve relating the wet weight of the biomass to the dry weight of the biomass at 25°C. Residual metal concentrations were measured at 460 nm and 340 nm for Cu(II) and Ni(II), respectively by using Sodium diethyldithiocarbamate as the complexing agent (Sandell, 1950).

Abbreviations

- μ : Specific growth rate of yeast (h^{-1})
 μ_{max} : Maximum specific growth rate (h^{-1})
 C_{Cu} : Initial Cu(II) ion concentration (mg/L)
 C_{Ni} : Initial Ni(II) ion concentration (mg/L)
 $C_{\text{ac,Cu}}$: Bioaccumulated Cu(II) ion concentration at any time (mg/L)
 $C_{\text{ac,Ni}}$: Bioaccumulated Ni(II) ion concentration at any time (mg/L)
 K_s : Saturation constant (g/L)
 q_{Cu} : Specific Cu(II) uptake defined as bioaccumulated Cu(II) ion quantity per gram of dried yeast at the end of microbial growth (mg/g)
 q_{Ni} : Specific Ni(II) uptake defined as bioaccumulated Ni(II) ion quantity per gram of dried yeast at the end of microbial growth (mg/g)
 S_0 : Initial sucrose concentration (g/L)
 S : Sucrose concentration (g/L)
 T : Temperature ($^{\circ}\text{C}$)
 t : Reaction time (h)
 X : Dried yeast concentration in feed medium at any time (g/L)
 X_{max} : Maximum dried yeast concentration (g/L)

Results and Discussions

Microorganism growth and bioaccumulation properties were investigated as functions of initial metal

ion and molasses sucrose concentrations at pH: 4.0 and 25°C. The uptake yield (uptake %) was described as the ratio of bioaccumulated concentration of metal ion at the end of growth to the initial metal ion concentration. The results were expressed as the units of bioaccumulated metal ion concentration ($C_{\text{ac,m}}$: mg/L) and specific metal ion uptake determined as the amount of metal ion per unit of dry weight of cells (q_m : mg/g), dried cell concentrations at any time (X : g/L), specific growth rate of yeast (μ : h^{-1}). The specific growth rate of *Candida* yeasts was determined from the slope of $\ln X$ versus time plot at the exponential growth phase. The results indicated that biomass concentration was related to the metal concentrations in fermentation medium and the physiological properties of the yeasts. The ability of metal uptake by metal adapted yeasts were different due to the physiological properties of the yeasts. All experiments were conducted at 25°C. The effect of temperature on metal bioaccumulation depends on cellular metabolism. Uptake capacity of heavy metals by microorganisms decreased at low temperatures whereas high temperatures could damage cells and reduced uptake levels (Brady & Duncan 1994).

Effect of initial pH on microbial growth

Effect of initial pH on specific growth rate and maximum microorganism concentrations of *Candida* yeasts was examined in the pH range of 2.0-5.0 at 10 g/L molasses sucrose concentration. Maximum specific growth rate and microorganism concentrations were obtained at pH: 4.0. Initial pH was a major factor in the quantity of metal ion bioaccumulation. All *Candida* species showed growth at pH: 2.0-5.0. The highest value of specific growth rate and microorganism concentration was found as 0.308 h^{-1} and 3.111 g/L respectively using *C. lipolytica* in metal free media (Table 1). Bioaccumulation experiment was conducted at pH: 4.0 which showed maximum growth.

Effect of initial sucrose concentration on microbial growth

The effect of initial sucrose concentration on growth rates of *Candida* yeasts in metal-free media was investigated in the sucrose concentration range of 1.0-20.0 g/L, at pH: 4.0 and 25°C. The relationship of specific growth rate to substrate concentration was explained with saturation kinetics. It was observed that the specific growth rate and biomass concentration increased with increasing initial sucrose concentration up to 20.0 g/L. Microorganism concentration increased from 1.52 g/L to 3.48 g/L with an increase in the initial sucrose concentration from 1.0 to 20.0 g/L for *C. lipolytica* in metal-free media (Fig. 1). Lower growth performance among the yeast cells was seen using *C. utilis*. We have found that molasses was a suitable carbon source for fermentation medium of *Candida* species. It was also reported in previous studies that molasses could be used as feasible and economical for microbial growth (Aksu & Dönmez 2000, Açikel & Alp 2009, Evirgen & Sağ Açikel 2014).

Effect of initial metal ion concentrations on growth of *Candida species*

The effect of initial Cu(II) and Ni(II) ion concentrations on microbial growth of *Candida* species were examined at different Cu(II) and Ni(II) ion concentrations. The metal ion concentrations in the fermentation medium varied in the range of 25-250 mg/L for Cu(II) and Ni(II). The range of molasses sucrose concentrations of prepared fermentation media varied between 1 and 20 g/L. Both metal ions inhibited specific growth rates and biomass concentrations for all yeasts. We found that the inhibition effect of Ni(II) ions on

specific growth rate and microorganism concentration was higher than Cu(II) ions for all yeasts. *Candida lipolytica* showed the highest specific growth rate and microorganism concentration among the yeasts in metal media. Maximum specific growth rate significantly decreased from 0.302 h⁻¹ to 0.278 h⁻¹ with an increase in the initial Ni(II) concentration from 100 to 200 mg/L for *C. utilis*. Inhibition kinetics was determined using the double reciprocal plot of the Monod equation. When initial Cu(II) and Ni(II) ion concentrations were increased in the range of 25-250 mg/L, the maximum specific growth rate (h⁻¹) of the yeasts decreased whereas saturation constants (K_s) increased (Table 2).

Table 1. Effect of initial pH on the maximum specific growth rate, maximum dried microorganism concentration in metal-free medium (So: 10 g/L; T:25°C).

pH	<i>C. membranaefaciens</i>		<i>C. utilis</i>		<i>C. tropicalis</i>		<i>C. lipolytica</i>	
	μ_{max} (h ⁻¹)	X_{max} (g/L)	μ_{max} (h ⁻¹)	X_{max} (g/L)	μ_{max} (h ⁻¹)	X_{max} (g/L)	μ_{max} (h ⁻¹)	X_{max} (g/L)
2	0.198	2.001	0.177	1.985	0.206	2.112	0.211	2.223
3	0.241	2.552	0.222	2.443	0.253	2.601	0.261	2.751
4	0.253	2.854	0.238	2.658	0.289	2.999	0.308	3.111
5	0.251	2.851	0.235	2.651	0.279	2.995	0.306	3.005

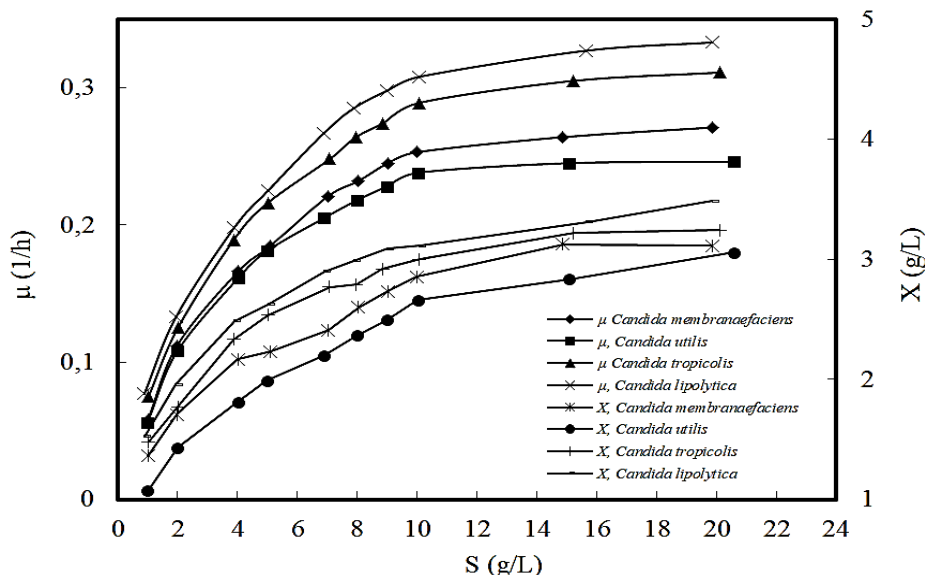


Fig. 1. Effect of initial sucrose concentration on specific growth rate and microorganism concentration for *Candida lipolytica*, *Candida utilis*, *Candida tropicalis* and *Candida membranaefaciens* (pH: 4.0; SR: 150 rpm; T: 25°C).

Table 2. Comparison of the maximum specific growth rates and the saturation constants in the presence of increasing concentrations of single Cu(II) and Ni(II) ions (So: 1-20 g/L; pH: 4, T: 25°C; SR: 150 rpm).

Metal Ion		<i>C. membranefeciens</i>		<i>C. utilis</i>		<i>C. tropicalis</i>		<i>C. lipolytica</i>	
C_{iCu} (mg/L)	C_{iNi} (mg/L)	μ_{max} (h ⁻¹)	K_s (g/L)	μ_{max} (h ⁻¹)	K_s (g/L)	μ_{max} (h ⁻¹)	K_s (g/L)	μ_{max} (h ⁻¹)	K_s (g/L)
25.0	0.0	0.354	5.399	0.341	5.805	0.374	4.555	0.422	5.080
100.0	0.0	0.337	5.354	0.341	6.472	0.363	4.677	0.388	4.568
200.0	0.0	0.336	6.586	0.299	6.033	0.338	4.887	0.378	5.378
250.0	0.0	0.310	6.771	0.285	6.519	0.313	4.984	0.335	4.920
0.0	25.0	0.346	5.725	0.319	5.600	0.352	4.243	0.345	4.473
0.0	100.0	0.333	5.718	0.302	5.408	0.356	5.013	0.395	5.268
0.0	200.0	0.309	7.004	0.278	6.374	0.309	5.111	0.328	4.918
0.0	250.0	0.267	6.265	0.264	7.196	0.284	5.269	0.317	5.796

Candida utilis was sensitive to high concentrations of Cu(II) with an extension in lag phase duration, correlated with a decrease in yeast production. The increase of Cu(II) and Ni(II) concentrations led to a drastic decrease in microbial growth for *C. utilis*. *Candida lipolytica* was highly resistant to Cu(II) when compared with three other yeasts. The increase in Cu(II) concentration also caused a decrease in biomass production. For instance, microorganism concentrations were found as 2.854 g/L, 2.658 g/L, 2.999 g/L and 3.111 g/L for *C. membranaefaciens*, *C. utilis*, *C. tropicalis* and *C. lipolytica*, respectively, in the metal-free media (Table 1). Biomass concentrations decreased as 2.666 g/L, 2.456 g/L, 2.831 g/L and 2.968 g/L for *C. membranaefaciens*, *C. utilis*, *C. tropicalis* and *C. lipolytica*, respectively, at 100 mg/L Cu(II) concentration (Table 3). The decrease in biomass concentration was found higher for fermentation medium containing 100 mg/L Ni(II). When initial Ni(II) concentration was 100.0 mg/L, microorganism concentrations were found as 2.574 g/L, 2.371 g/L, 2.735 g/L and 2.868 g/L for *C. membranaefaciens*, *C. utilis*, *C. tropicalis* and *C. lipolytica*, respectively (Table 3).

Bioaccumulation experiments

The combined effect of heavy metals and molasses sucrose on the bioaccumulation properties of adapted *Candida* yeasts was investigated. Initial metal ion concentration in the fermentation medium varied in the range 25-250 mg/L for Cu(II) and Ni(II) at changing sucrose concentrations in the range of 1-20 g/L. We found that bioaccumulated Cu(II) and Ni(II) ions and microbial growth increased with increasing initial molasses sucrose concentrations for all *Candida* yeasts. The uptake performance of Cu(II) ions was higher than Ni(II), and *C. lipolytica* showed the maximum removal efficiency in a medium containing both single metal ions. An increase in sucrose concentration significantly increased the growth and metal uptake capacity of the microorganisms. The amount of Cu(II) and Ni(II) ions uptake per gram dry microorganism increased with increasing the initial metal concentrations up to 250 mg/L. We found that microbial growth was supported by increasing initial sucrose concentrations in metal- stressed fermentation media. In other words, bioaccumulation of Cu(II) and Ni(II) ions by *Candida* species was metabolism dependent. Specific Cu(II) uptake was determined as 31.77 mg/g, 29.71 mg/g, 33.04 mg/g, and 34.39 mg/g for *C. membranaefaciens*, *C. utilis*, *C. tropicalis*, *C. lipolytica*, respectively, at fermentation media containing 10 g/L constant sucrose

and 200 mg Cu(II) mg/L (Fig. 4). At the same conditions, the specific Ni(II) uptake significantly decreased for all yeasts in medium containing Ni(II) ions when compared to Cu(II) ions. For example, the specific growth rates were obtained in a medium containing 200 mg Ni(II)/L and 10 g/L sucrose were determined as 25.96 mg/g, 24.76 mg/g, 26.13 mg/g, and 28.80 mg/g for *C. membranaefaciens*, *C. utilis*, *C. tropicalis*, *C. lipolytica*, respectively (Fig. 5). Although removal efficiency increased with increasing initial sucrose concentration for all yeasts, it decreased with increasing initial metal ion concentrations. Uptake efficiency significantly decreased from 88.80% to 56.44% with an increase in the initial Cu(II) concentration from 25 to 200 mg/L for *C. lipolytica* at 20 g/L constant sucrose concentration (Table 4). This decrease may result from metal-binding sites on yeast surfaces. It was reported that binding sites were occupied first rapidly, then decreased with increasing metal concentrations (Honfi *et al.* 2016).

The bioaccumulated Cu(II) concentrations were higher than bioaccumulated Ni(II) concentrations in growth media containing 25 mg/L single Cu(II) and Ni(II) ions (Figs 2, 3).

The intolerance of living cells to high metal concentrations limits bioaccumulation process, but living cells have the potential genetic recombination to improve the metal-adapted strain (Malik 2004). Heavy metal bioaccumulation capacity of *Candida* species was strongly pH-dependent due to changing solution chemistry and active functional groups on the biomass. Due to the Ni(II) inhibition effect, metal uptake performance decreased from 81.68% to 46.28% with increasing Ni(II) concentration from 25 to 250 mg/L for *C. lipolytica*. According to Gönen and Aksu (2008), *C. utilis* accumulated toxic heavy metals. The uptake efficiency of Cu(II) by *C. utilis* was observed as 27.0% in a growth medium containing 11.2 g/L sucrose and 101.3 mg/L Cu(II) ions (Gönen & Aksu 2008). Pawan and Devi (2018) investigated bioaccumulation of Ni(II), Zn(II), Cr(VI) by *Aspergillus awamori*, *A. flavus* and *A. niger* and found that *A. niger* was a highly tolerant strain. Maximum dry weight of *A. flavus* was found as 12.31 g/L for Ni(II) in medium containing 100 mg/L initial broth concentration (Pawan & Devi 2018). Rehman and Anjum (2010) reported that *Candida tropicalis* was a metal-resistant yeast. They reported that *C. tropicalis* bioaccumulated 64% copper from industrial wastewater after 4 days (Rehman & Anjum 2010).

Table 3. Comparison of decrease in maximum dried microorganism concentrations obtained in the metal-free fermentation media with Cu(II) and Ni(II) ions present as the single metal at 100 and 250 mg/L (So: 10 g/L; pH: 4, T: 25°C; SR: 150 rpm).

Metal Ion		<i>C. membranaefaciens</i>		<i>C. utilis</i>		<i>C. tropicalis</i>		<i>C. lipolytica</i>	
C _{iCu} (mg/L)	C _{iNi} (mg/L)	X _{max} (g/L)	Decrease %	X _{max} (g/L)	Decrease %	X _{max} (g/L)	Decrease %	X _{max} (g/L)	Decrease %
100.0	0.0	2.666	2.4	2.456	7.6	2.831	5.6	2.968	4.6
250.0	0.0	2.335	14.5	2.148	19.2	2.483	17.2	2.607	16.2
0.0	100.0	2.574	5.8	2.371	10.8	2.735	8.8	2.868	7.8
0.0	250.0	2.175	20.4	1.199	54.9	2.315	22.8	2.432	21.8

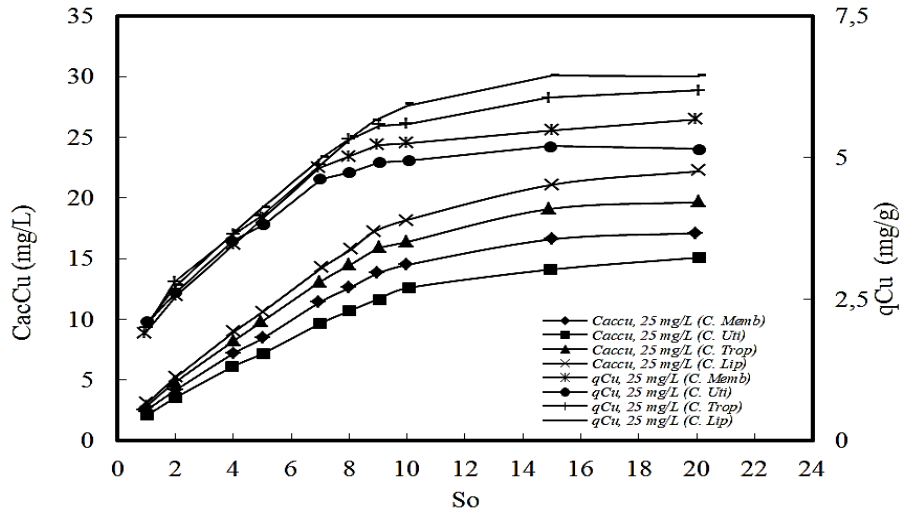


Fig. 2. Effect of initial sucrose concentration on the bioaccumulated Cu(II) ion quantity per unit weight of dried biomass and specific Cu(II) uptake in the presence of 25 mg/L Cu(II) (pH: 4.0; SR: 150 rpm; T: 25°C).

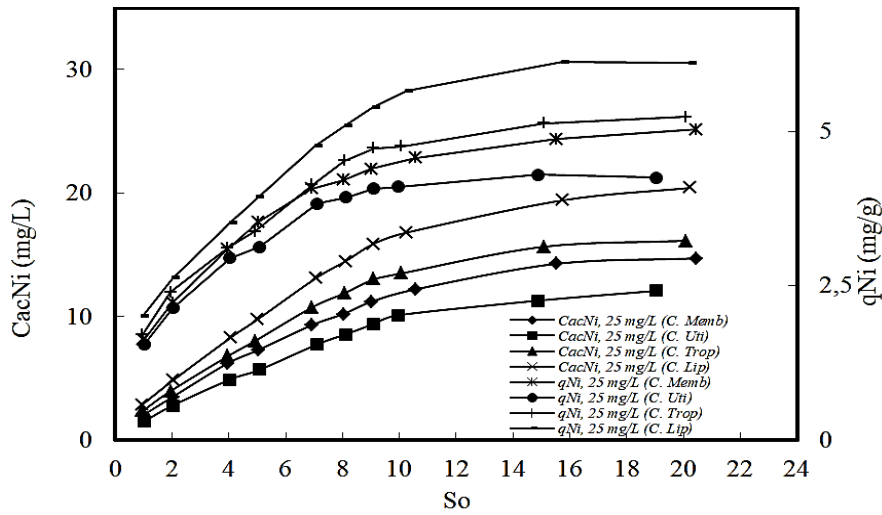


Fig. 3. Effect of initial sucrose concentration on the bioaccumulated Ni(II) ion quantity per unit weight of dried biomass and specific Ni(II) uptake in the presence of 25 mg/L Ni(II) (pH: 4.0; SR: 150 rpm; T: 25°C).

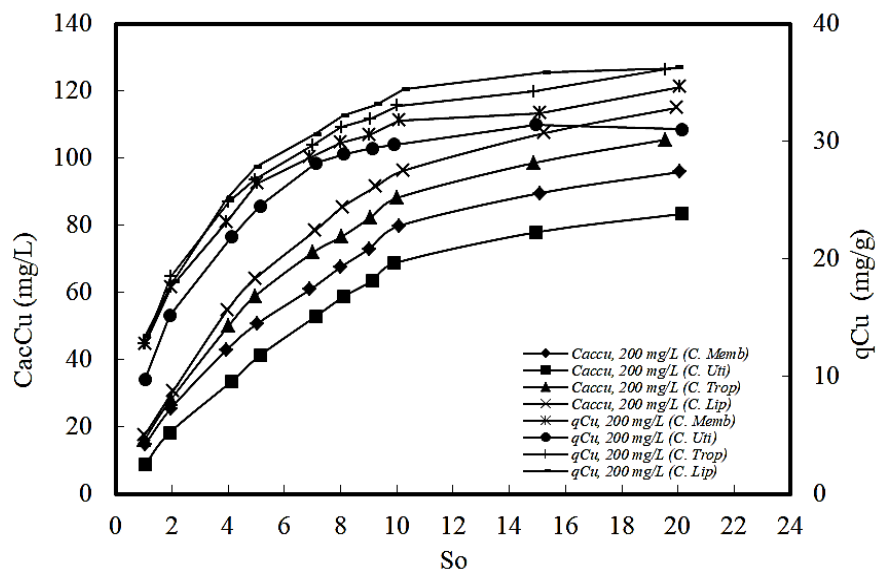


Fig. 4. Effect of initial sucrose concentration on the bioaccumulated Cu(II) ion quantity per unit weight of dried biomass and specific Cu(II) uptake in the presence of 200 mg/L Cu(II) (pH: 4.0; SR: 150 rpm; T: 25°C).

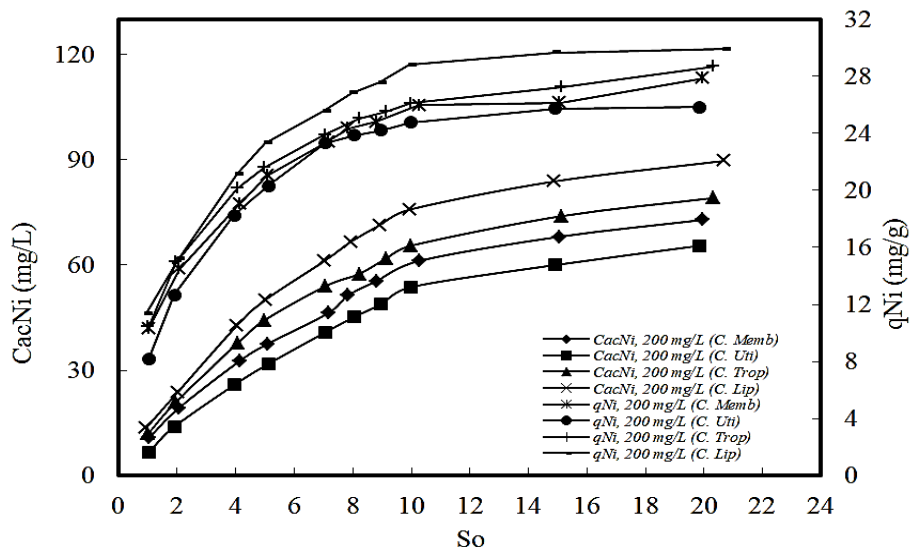


Fig. 5. Effect of initial sucrose concentration on the bioaccumulated Ni(II) ion quantity per unit weight of dried biomass and specific Ni(II) uptake in the presence of 200 mg/L Ni(II) (pH: 4.0; SR: 150 rpm; T: 25°C).

Table 4. Comparison of the bioaccumulated Cu(II) and Ni(II), the Cu(II) and Ni(II) metal ion bioaccumulation efficiency in the fermentation media containing single Cu(II) and Ni(II) ions (pH: 4.0; T: 25°C; SR: 150 rpm).

Yeast		<i>C. membranaefaciens</i>		<i>C. utilis</i>		<i>C. tropicalis</i>		<i>C. lipolytica</i>	
S_i (g/L)	$C_{i,Cu}$ (mg/L)	$C_{ac,Cu}$ (mg/L)	Uptake (%)	$C_{ac,Cu}$ (mg/L)	Uptake (%)	$C_{ac,Cu}$ (mg/L)	Uptake (%)	$C_{ac,Cu}$ (mg/L)	Uptake (%)
1		2.50	10.00	2.13	8.52	2.88	11.52	3.13	12.52
10	25	14.50	58.00	12.60	50.40	16.40	65.60	18.20	72.80
20		17.10	68.40	15.1	60.4	19.67	78.68	22.20	88.80
1		6.88	6.88	5.50	5.50	7.56	7.56	8.70	8.70
10	100	46.80	46.80	38.40	38.40	51.48	51.48	59.20	59.20
20		58.47	58.47	46.77	46.77	64.31	64.31	73.96	73.96
1		14.60	7.30	8.65	23.38	16.06	8.03	17.51	8.75
10	200	79.80	39.90	68.70	20.67	88.20	44.10	96.30	48.15
20		95.88	47.94	83.37	34.35	105.46	52.73	114.96	57.48
1		11.60	4.64	8.50	3.40	15.20	6.08	16.42	6.56
10	250	94.81	37.92	79.01	31.60	104.29	41.71	112.60	45.04
20		115.05	46.02	95.88	38.35	126.56	50.62	141.10	56.44
S_o (g/L)	$C_{i,Ni}$ (mg/L)	$C_{ac,Ni}$ (mg/L)	Uptake (%)	$C_{ac,Ni}$ (mg/L)	Uptake (%)	$C_{ac,Ni}$ (mg/L)	Uptake (%)	$C_{ac,Ni}$ (mg/L)	Uptake (%)
1		2.04	8.16	1.51	6.04	2.36	9.44	2.88	11.52
10	25	12.20	48.80	10.10	40.40	13.50	54.00	16.8	67.20
20		14.71	58.84	12.08	48.32	16.13	64.52	20.42	81.68
1		5.84	5.84	4.47	4.47	6.50	6.50	7.39	7.39
10	100	39.70	39.70	31.20	31.20	44.30	44.30	50.70	50.70
20		49.70	49.70	38.80	38.80	55.31	55.31	62.86	62.86
1		10.80	5.40	6.66	3.33	12.05	6.02	13.65	6.82
10	200	61.20	30.60	53.70	26.85	65.50	32.75	75.80	37.90
20		72.87	36.43	65.50	32.75	79.10	39.55	89.67	44.83
1		8.82	3.52	6.63	2.65	10.50	4.20	13.46	5.38
10	250	72.20	28.88	62.10	24.84	79.26	31.70	92.33	36.93
20		87.44	34.97	78.70	31.48	96.18	38.47	115.70	46.28

The physiological and biochemical properties of microorganisms can be changed by the presence of heavy metals. Heavy metals such as copper (Cu(I) and Cu(II)) carry soluble electrons and can catalyze Fenton and Haber-Weiss reactions. Cytoplasmic molecules in microorganisms can cause serious damage to DNA, lipids and other proteins (Giner-Lamia *et al.* 2014). Heavy metal can cause ion imbalance by binding to the cell surface and

entering through ion channels or transmembrane carriers (Chen *et al.* 2014). High vitamin and mineral contents of molasses sucrose stimulate microorganisms growth (Razack *et al.* 2013). The structure of metal-binding agents (homopolysaccharides, single saccharides, and acid components) in microorganisms and their distributions in the cell wall determine the metal accumulation capacity (Raspor & Zupan 2006). Heavy

metal cations can interact with some cations on the cell wall. The cell wall and heavy metal interactions may inhibit the function of physiological cations in the cell structure. As a result of this inhibition, oxidative stress occurs in the cell. For example, Fe, Zn, and Ca affect the uptake and toxicity of heavy metals in microorganisms. Ca and Fe ions reduce the uptake of Cd (Volland *et al.* 2014). Metal uptake is mainly related to the concentrations of metals in solution (Modak & Natarajan 1995). Also, intercellular electrostatic interactions in cells play an important role in metal uptake capacity. At a certain equilibrium, biomass adsorbs more metal ions at lower cell concentrations (Gourdon *et al.* 1990). The inhibition effect of heavy metals on microorganism growth depends on the total metal ion concentration, the chemical structure of the metal, and redox potential of metal. Environmental factors such as temperature, pH, organic acids, and humic acids can alter the conversion, transport, valence of heavy metals, and the resistance of microorganisms to heavy metals.

Conclusion

In the present study, microbial growth and bioaccumulation properties of *Candida* biomasses were investigated as a function of molasses sucrose and metal ion concentrations in a batch reactor. Optimum pH was found 4.0 for each yeast and all experiment conducted at this pH value. Biomass concentrations and specific growth rates increased with increasing initial sucrose concentration in metal and metal-free media. Both Cu(II) and Ni(II) inhibited specific growth rates and the contribution of Cu(II) inhibition was lower than Ni(II). Thus, the maximum specific growth rates of yeasts decreased with increasing initial metal ion concentrations. The saturation constants were determined in both metal and metal-free fermentation medium. Saturation constants

and the amounts of Cu(II) and Ni(II) ions uptake per gram dry biomass increased with the increase of the initial concentration of heavy metals ions. The removal efficiency of Cu(II) ions was higher than Ni(II), and *Candida lipolytica* showed maximum uptake efficiency in fermentation medium containing Cu(II) and Ni(II) ions. Results showed that bioaccumulation of Cu(II) and Ni(II) ions by *Candida* biomass was metabolism dependent. *Candida* biomasses showed different bioaccumulative capacities for the same metal ions. The yeast surface properties and cell wall components may change affinity and cell-metal interaction. In addition, environmental conditions can affect the accumulation capacity of yeasts. Although Cu(II) and Ni(II) have similar chemical properties, genetic differences of yeasts and the chemical nature of metals caused differences in bioaccumulation performances. This study showed that *Candida* biomasses are useful for removal Cu(II) and Ni(II) ions from aqueous solutions. The comparison of bioaccumulation properties of *Candida* yeasts may help to an effective selection of *Candida* strains for water treatments.

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References

- Açıkel, U. & Alp, T. 2009. A study on the inhibition kinetics of bioaccumulation of Cu(II) and Ni(II) ions using *Rhizopus delemar*. *Journal of Hazardous Materials*, 168(2-3): 1449-1458. <https://doi.org/10.1016/j.jhazmat.2009.03.040>
- Aksu, Z. & Dönmez, G. 2000. The use of molasses in copper (II) containing wastewaters: effects on growth and copper (II) bioaccumulation properties of *Kluyveromyces marxianus*. *Process Biochemistry*, 36(5): 451-458. [https://doi.org/10.1016/S0032-9592\(00\)00234-X](https://doi.org/10.1016/S0032-9592(00)00234-X)
- Baysal, Z., Çınar, E., Bulut, Y., Alkan, H. & Dogru, M. 2009. Equilibrium and thermodynamic studies on biosorption of Pb(II) onto *Candida albicans* biomass. *Journal of Hazardous Materials*, 161(1): 62-67. <https://doi.org/10.1016/j.jhazmat.2008.02.122>
- Brady, D. & Duncan, J.R. 1994. Bioaccumulation of metal cations by *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology*, 41: 149-154. <https://doi.org/10.1007/BF00166098>
- Chen, S., Yin, H., Ye, J., Peng, H., Liu, Z., Dand, Z. & Chang, J. 2014. Influence of co-existed benzo[a]pyrene and copper on the cellular characteristics of *Stenotrophomonas maltophilia* during biodegradation and transformation. *Bioresource Technology*, 158: 181-187. <https://doi.org/10.1016/j.biortech.2014.02.020>
- Cottet, C., Ramirez-Tapias, Y.A., Delgado, J.F., de la Osa, O., Salvay, A.G. & Peltzer, M.A. 2020. Biobased Materials from Microbial Biomass and Its Derivatives. *Materials*, 13(6): 1263. <https://doi.org/10.3390/ma13061263>
- Dupont, C.L., Grass, G. & Rensing, C. 2011. Copper toxicity and the origin of bacterial resistance-New insights and applications. *Metallomics*, (3): 1109-1118. <https://doi.org/10.1039/c1mt00107h>
- Evirgen, O.A. & Sag Acikel, Y. 2014. Simultaneous copper bioaccumulation, growth and lipase production of *Rhizopus delemar* in molasses medium: optimisation of environmental conditions using RSM. *Chemistry and Ecology*, 30(1): 39-51. <https://doi.org/10.1080/02757540.2013.827670>
- Fadel M., Hassanein, N.M., Elshafei, M.M., Mostafa, A.H., Ahmed, M.A. & Khater, H.M. 2017. Biosorption of manganese from groundwater by biomass of

Saccharomyces cerevisiae. *HBRC Journal*, 13(1): 106-113. <https://doi.org/10.1016/j.hbrcej.2014.12.006>

10. Fashola, M.O., Ngole-Jeme, V.M. & Babalola, O.O. 2016. Heavy metal pollution from gold mines: Environmental effects and bacterial strategies for resistance. *International Journal of Environmental Research and Public Health*, (13): 1047. <https://doi.org/10.3390/ijerph13111047>
11. Giner-Lamia, J., L'opez-Maury, L., Florencio, F.J. & Janssen, P.J. 2014. Global transcriptional profiles of the copper responses in the cyanobacterium *synechocystis* sp. PCC 6803. *PLOS ONE*, 9 (9): e108912. <https://doi.org/10.1371/journal.pone.0108912>
12. Gönen, F. & Aksu, Z. 2008. Use of response surface methodology (RSM) in the evaluation of growth and copper(II) bioaccumulation properties of *Candida utilis* in molasses medium. *Journal of Hazardous Materials*, 154(1-3): 731-738. <https://doi.org/10.1016/j.jhazmat.2007.10.086>
13. Gourdon, R., Bhende, S., Rus, E. & Sofer, S.S. 1990. Comparison of cadmium biosorption by Gram-positive and Gram-negative bacteria from activated sludge. *Biotechnology Letters*, 12: 839-842. <https://doi.org/10.1007/BF01022606>
14. Honfi, K., Táros, K., König-Péter, A., Kilár, F. & Perneszi, T. 2016. Copper(II) and Phenol Adsorption by Cell Surface Treated *Candida tropicalis* Cells in Aqueous Suspension. *Water, Air & Soil Pollution*, 227: 1-14. <https://doi.org/10.1007/s11270-016-2751-0>
15. Javanbakht, V., Alavi, S.A. & Zilouei, H. 2013. Mechanisms of heavy metal removal using microorganisms as biosorbent. *Water Science and Technology*, 69(9): 1775-1787. <https://doi.org/10.2166/wst.2013.718>
16. Legorreta-Castañeda, A.J., Lucho-Constantino, C.A., Beltrán-Hernández, R.I., Coronel-Olivares, C. & Vázquez-Rodríguez, G.A. 2020. Biosorption of Water Pollutants by Fungal Pellets. *Water*, 12(4): 1155-1193. <https://doi.org/10.3390/w12041155>
17. Liu, Y. 2007. Overview of some theoretical approaches for derivation of the Monod equation. *Applied Microbiology and Biotechnology*, 73: 1241-1250. <https://doi.org/10.1007/s00253-006-0717-7>
18. Luk, C.H.J., Yip, J., Yuen, C.W.M., Pang, S.W., Lam, K.H. & Kan, C.W. 2017. Biosorption Performance of Encapsulated *Candida krusei* for the removal of Copper(II). *Scientific Reports*, 7: 1-9. <https://doi.org/10.1038/s41598-017-02350-7>
19. Luna, J.M., Rufino, R.D. & Sarubbo, L.A. 2016. Biosurfactant from *Candida sphaerica* UCP0995 exhibiting heavy metal remediation properties. *Process Safety and Environmental Protection*, 102: 558-566. <https://doi.org/10.1016/j.psep.2016.05.010>
20. Malik, A. 2004. Metal bioremediation through growing cells. *Environment International*, 30(2): 261-78. <https://doi.org/10.1016/j.envint.2003.08.001>
21. Modak, J.M. & Natarajan, K.A. 1995. Biosorption of metals using nonliving biomass-A review. *Mining, Metallurgy & Exploration*, 12: 189-196. <https://doi.org/10.1007/BF03403102>
22. Monod, J. 1949. The growth of bacterial cultures. *Annual Reviews in Microbiology*, 3(1): 371-394. <https://mcb.berkeley.edu/labs/garcia/sites/mcb.berkeley.edu/labs/garcia/files/Teaching/2017-MCB137/Monod1949.pdf> (Date accessed: 22.11.2020)
23. Pawan, K.R. & Devi, R. 2018. Heavy metal tolerance and adaptability assessment of indigenous filamentous fungi isolated from industrial wastewater and sludge samples. *Beni-Suef University Journal of Basic and Applied Sciences*, 7(4): 688-694. <https://doi.org/10.1016/j.bjbas.2018.08.001>
24. Podder, M.S. & Majumder, C.B. 2019. Bacteria immobilization on neem leaves/MnFe₂O₄ composite surface for removal of As(III) and As(V) from wastewater. *Arabian Journal of Chemistry*, 12: 3263-3288. <https://doi.org/10.1016/j.arabjc.2015.08.025>
25. Raspor P. & Zupan J. 2006. Yeasts in Extreme Environments. pp. 371-372. In: Péter G. & Rosa C.A. (eds). *Biodiversity and Ecophysiology of Yeasts*. Springer-Verlag, Berlin, 580 pp. https://doi.org/10.1007/3-540-30985-3_15
26. Razack, S.A., Velayutham, V. & Thangavelu, V. 2013. Medium optimization for the production of exopolysaccharide by *Bacillus subtilis* using synthetic sources and agro wastes. *Turkish Journal of Biology*, 37: 280-288. <https://doi.org/10.3906/biy-1206-50>
27. Redha, A.A. 2020. Removal of heavy metals from aqueous media by biosorption. *Arab Journal of Basic and Applied Sciences*, 27(1): 183-193. <https://doi.org/10.1080/25765299.2020.1756177>
28. Rehman, A. & Anjum, M.S. 2011. Multiple metal tolerance and biosorption of cadmium by *Candida tropicalis* isolated from industrial effluents: glutathione as detoxifying agent. *Environmental Monitoring and Assessment*, 174: 585-595. <https://doi.org/10.1007/s10661-010-1480-x>
29. Rehman, A. & Anjum, M.S. 2010. Cadmium Uptake by Yeast, *Candida tropicalis*, Isolated from Industrial Effluents and Its Potential Use in Wastewater Clean-Up Operations. *Water, Air and Soil Pollution*, 205: 149-159. <https://doi.org/10.1007/s11270-009-0062-4>
30. Şengör, S.S., Barua, S., Gikas, P., Ginn, T.R., Peyton, B., Sanı, R.K., & Spycher, N.F. 2009. Influence Of Heavy Metals On Microbial Growth Kinetics Including Lag Time: Mathematical Modeling And Experimental Verification. *Environmental Toxicology and Chemistry*, 28: 2020-2029. <https://doi.org/10.1897/08-273.1>
31. Sandell, E.B. 1950. Colorimetric Determination of Traces of Metals Volume III. pp. 304-475. In: Clarke, B.L. & Kolthoff, I.M. (eds). *Chemical Analysis A Series of Monographs on Analytical Chemistry And Its Applications*. Interscience Publishers INC, London, 688 pp.
32. Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. & Sutton, D. J. 2012. Heavy metal toxicity and the environment. *Experientia Supplementum*, 101: 133-164. https://doi.org/10.1007/978-3-7643-8340-4_6
33. Tripathi, A. & Ranjan, M.R. 2015. Heavy Metal Removal from Wastewater Using Low Cost Adsorbents. *Journal of Bioremediation & Biodegradation*, 6: 315-320. <http://dx.doi.org/10.4172/2155-6199.1000315>
34. Volland, S., Bayer, E., Baumgartner, V., Andosch, A., Lütz, C., Sima, E. & Lütz-Meindl, U. 2014. Rescue of

- heavy metal effects on cell physiology of the algal model system *Micrasterias* by divalent ions. *Journal of Plant Physiology*, 171(2): 154-163.
<https://doi.org/10.1016/j.jplph.2013.10.002>
35. Waldron, K.J. & Robinson, N.J. 2009. How do bacterial cells ensure that metalloproteins get the correct metal? *Nature Reviews Microbiology*, 7: 25-35.
<https://doi.org/10.1038/nrmicro2057>
36. Wołowiec, M., Komorowska-Kaufman, M., Pruss, A., Rzepa, G. & Bajda, T. 2019. Removal of Heavy Metals and Metalloids from Water Using Drinking Water Treatment Residuals as Adsorbents: A Review. *Minerals*, 9(8): 487-504. <https://doi.org/10.3390/min9080487>
37. Zha, F., Wang, H., Xu, L., Yang, C., Kang, B., Chu, C., Deng, Y. & Tan, X. 2020. Initial feasibility study in adsorption capacity and mechanism of soda residue on lead (II)-contaminated soil in solidification/stabilization technology. *Environmental Earth Sciences*, 79: 1-12.
<https://doi.org/10.1007/s12665-020-08990-9>