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Full Length Research Paper

Bioaccumulation of heavy metals by yeasts from Qua Iboe estuary mangrove sediment ecosystem, Nigeria

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The effect of heavy metals (Pb, Ni and Zn) on the growth rate, number of generation, generation time and bioaccumulation potential of *Saccharomyces*-ESY$_2$ and *Candida*-ESY$_{13}$ isolated from the epipellic sediment of Qua Iboe mangrove ecosystem was evaluated. The investigation was conducted over a period of 90 days in laboratory microcosms bearing epipellic sediment simulated with 1642.00 mgkg^{-1} of Zn, 208.00 mgkg^{-1} of Pb and 522.60 mgkg^{-1} of Ni. The growth attribute of yeasts were determined forth nightly, using the population dynamics of the inocula as an index of response to metal pollution and bioaccumulation capability. Results revealed that Ni and Zn increased the generation time of *Candida*-ESY$_{13}$ and *Saccharomyces*-ESY$_2$ respectively, resulting in a reduced number of generations and decrease in growth rate. Both yeasts exhibited a high Pb accumulating capability. However, *Candida*-ESY$_{13}$ with a bioconcentration factor (BCF) of 0.299 accumulated more Pb (41.87 mgg^{-1}) than *Saccharomyces*-ESY$_2$ with a BCF of 0.296. These findings have demonstrated that yeasts could tolerate and detoxify metals especially Pb in mangrove ecosystem and could be adapted for the detoxification of metals-impacted environments. However, the ability of these isolates to bioaccumulate this metal raises the question of biomagnifications in the food chain within a tropical mangrove ecosystem.

Key words: Heavy metals, bioaccumulation, growth rate, sediment.

INTRODUCTION

Metals persist in the environment and can become concentrated up the food chain (Eja et al., 2003). Lee et al. (2000) and Hargrave et al. (2000) have reported that metals may be bioconcentrated, bioaccumulated and biomagnified within food chains, causing higher trophic organisms to become contaminated with higher concentrations of chemical and metal contaminants than their prey. The risk for toxicity depends on the frequency, intensity and duration of contact with the metal contaminant along with exposure route (Calderon et al., 2003). Toxicity risk also depends on the inherent toxic potential of the metal itself. Thus, mercury (Hg), a non-essential metal, possesses more inherent toxic potential than copper (Cu), a metal essential for physiological function.

Heavy metals impact both the physiology and ecology of microorganisms (Sandrin and Maier, 2003) and are known to inhibit a broad range of microbial processes including methane metaboism (Capone et al., 1983), growth, nitrogen and sulphur conversions. Metals generate many of their deleterious effects through the formation of free radicals, resulting in DNA damage, lipid peroxidation and depletion of protein sulfhydryls (for example, glutathione) (Valko et al., 2005).

These reactive radicals comprise a wide range of chemical species including oxygen, carbon and sulfur radicals originating from the super oxide radical, hydrogen peroxide and lipid peroxides and also from chelates of amino acids, peptides and protein complexed with toxic metals (Naranmandura et al., 2006). Kachur et al. (1998) reported that toxic metal cations may substitute for physiologically essential cations within an enzyme molecule. For example, Cd^{2+} may substitute Zn^{2+}, rendering the enzyme non-functional. In response to toxic concentrations of heavy metals, many organisms including micro-organisms, can develop tolerance (Klerks and Weiss, 1987), resulting in the detoxification of such heavy metals. The development of heavy metal tolerance by micro-organisms presents the possibility of utilizing and optimizing microbially mediated reactions as a strategy

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for removing metal contaminants from the environment. Studies conducted in the last few decades have concentrated on hazards emanating from heavy metals contamination of the environment (Nriagu and Paujna, 1989). Studies have also been carried out on the tolerance and detoxification of heavy metals by bacterial species (Dean-Ross and Mills, 1989; Chikere and Okpokwasili, 2003; Faisal and Hasnain, 2006). The bacteria species tested for metal biosorption have revealed encouraging potential (Regine and Volesky, 2000).

On the other hand, much research have been conducted on the heavy metals biosorption potential of sea weeds and yeasts especially *Saccharomyces cerevisiae, Kluyveromyces fragilis, Candida tropicalis, Geotrichum capitatum, Rhodotorula minuta, Loddermyces elongisporus* and *Williopsis californica*. The results have shown that the yeasts are good biomass sources for metal biosorption (Rahatgaonkar and Mahore, 2008; Alluri et al., 2007; Sen and Ghosh Dastidar, 2006; Ting and Teo, 1994; Falih, 1998). However, the incessant cases of crude oil pollution in the Niger Delta of Nigeria and the rush for rapid industrial development coupled with lack of awareness about metal toxicity, suggest that there is an urgent need for research regarding the development of an economical and eco-friendly technology for the removal of pollutants in the region. In this study, we evaluated the effect of Pb, Zn and Ni on the growth rate, generation time, number of generations and detoxification potential of *Saccharomyces* and *Candida* prevalent in the epipellic sediment of the Qua Iboe Mangrove ecosystem.

MATERIALS AND METHODS

Collection of sediment samples

The epipellic sediment was obtained from the mangrove ecosystem of the Q. Iboe estuary and the associated Stubbs creek (Figure 1). The estuary lies between latitude 4° 30' - 4° 45'N and longitude 7° 30' - 8° 00'E in the Niger Delta region of Nigeria. Three sampling sites designated locations 1, 2 and 3 were selected in fishing settlements at Mkpanak, Upenekang and the Stubbs creek respectively. A short core sampler was used to retrieve epipellic sediment with undisturbed sediment-water interfaces. Sediment samples obtained from the different locations were homogenized and the subsamples carefully transferred into clean glass containers and preserved in ice-cooled boxes. The samples were transported to the laboratory and analyzed within 12 h of collection.

Sediment characterization and background heavy metals analyses

Sediment physicochemical parameters were determined using standard procedures (Radojevic and Bashkin, 1999). Fast changing parameters, such as pH and salinity were measured in the field using a portable multi-probe quality meter (Model U7, Horiba Ltd). The soluble exchangeable cations (Ca^{2+}, Na^{+}, K^{+} and Mg^{2+}) were determined using a flame atomic absorption spectrophotometer after extraction with ammonium acetate (1 M = 1 mol dm^{-3}) at pH 7. The nutritive salts, CO_3^{2-}, SO_4^{2-}, Cl^{-}, NO_3^{-} and PO_4^{-} were determined by a titrimetric method (Radojevic and Bashkin, 1999), turbidimetric method (APHA, 1998), argentometric titration (Mohr's method) (APHA, 1985) and a colorimetric method (APHA, 1985) respectively.

Phosphorus as reactive orthophosphate was determined using the stannous chloride method (APHA, 1985) which is specially suited for determining low amounts of phosphate concentrations. Organic carbon content was determined by the rapid wet oxidation method based on the Walkey and Black procedure (Jakobsen, 1992; Page et al., 1982; AOAC, 1975). Total nitrogen was determined by classical Kjeldahl digestion followed by distillation. Total nitrogen in the distillates was determined by spectrophotometry. Particle size distribution (grain size analysis) was determined by the hydrometer method (AOAC, 1975; Juo, 1979).

For heavy metals analysis, the sediment samples were dried at 70 - 80°C for 48 h. These were then gently grounded with a rolling pin to disaggregate the samples but not to break down the grains themselves and sieved to collect less than 63 µ grain sizes. The sediment samples were digested as described by Ho et al. (2003) and Miroslav and Vladimir (1999). Precisely, 2.0 g of sediment was digested with a solution of concentrated HNO_3 (0.3 ml) and HCl (6.0 ml) to near dryness and allowed to cool before 20 ml of 5.0 M HNO_3 (1 M = 1 mol dm^{-3}) was added. The solutions were allowed to stand overnight and filtered. The filtrates were transferred into a 100 ml volumetric flask and made up to the mark with 0.5 M HNO_3 (Binning and Baird, 2001). The water sample was filtered through 0.45 µm filter paper. A reagent blank (without sample) was prepared using a mixture of HNO_3 and HCl, and the entire sequence of steps was followed as described for the sample preparation. The sample solution, filtered water sample and the blank were analyzed for the concentrations of extractable heavy metals (Cr, Pb, Ni, Zn, and Cu) using an inductively coupled plasma spectrophotometer (Optima 3000 – Perkin Elmer). The analysis was duplicated to verify the precision of the method of digestion. The instrumental detection limits (IDL) were, 0.02, 0.002, 0.01, 0.002, and 0.01 $mgkg^{-1}$ for Cr, Pb, Ni, Zn and Cu respectively. Duplicates and method blanks were employed to test for precision, accuracy and reagent purity used in the analytical procedures.

In order to reduce the detrimental effects of overlapping spectral interferences on element quantitation during metal analyses, an inter-element correction standard was prepared by using standardized solutions of metal ions prepared from their salts. A mixture of commercially available 100 mg/kg stock solutions (Analar grade) of Cr^{3+}, Pb^{2+}, Ni^{2+}, Zn^{2+} and Cu^{2+} were prepared as inter-element working standard solutions to verify that the overlapping lines do not cause the detection of elements at concentrations above the method's detection limits (MDLs) (Popek, 2003).

Simulation of sediment with metallic salts of Zn, Pb and Ni

Toxicity and response of yeasts to test metals were determined *ex situ* in laboratory microcosms containing 3 kg of epipellic sediment. For each metal, microcosms were prepared in replicates. The sediments were simulated with Pb, 208.00; Zn, 1642.00 and; Ni, 522.60 $mgkg^{-1}$.

Appropriate weights of the following metallic salts, Pb $(NO_3)_2$, $ZnSO_4.7H_2O$ and $NiSO_4.6H_2O$ were separately dissolved in 1000 ml of sterile deionized water. The solutions were used to contaminate the sediment, thoroughly mixed and incubated at room temperature (28 ± 2°C) for 90 days. Sterile distilled water obtained from the investigated ecosystem was used to moisten the sediments periodically.

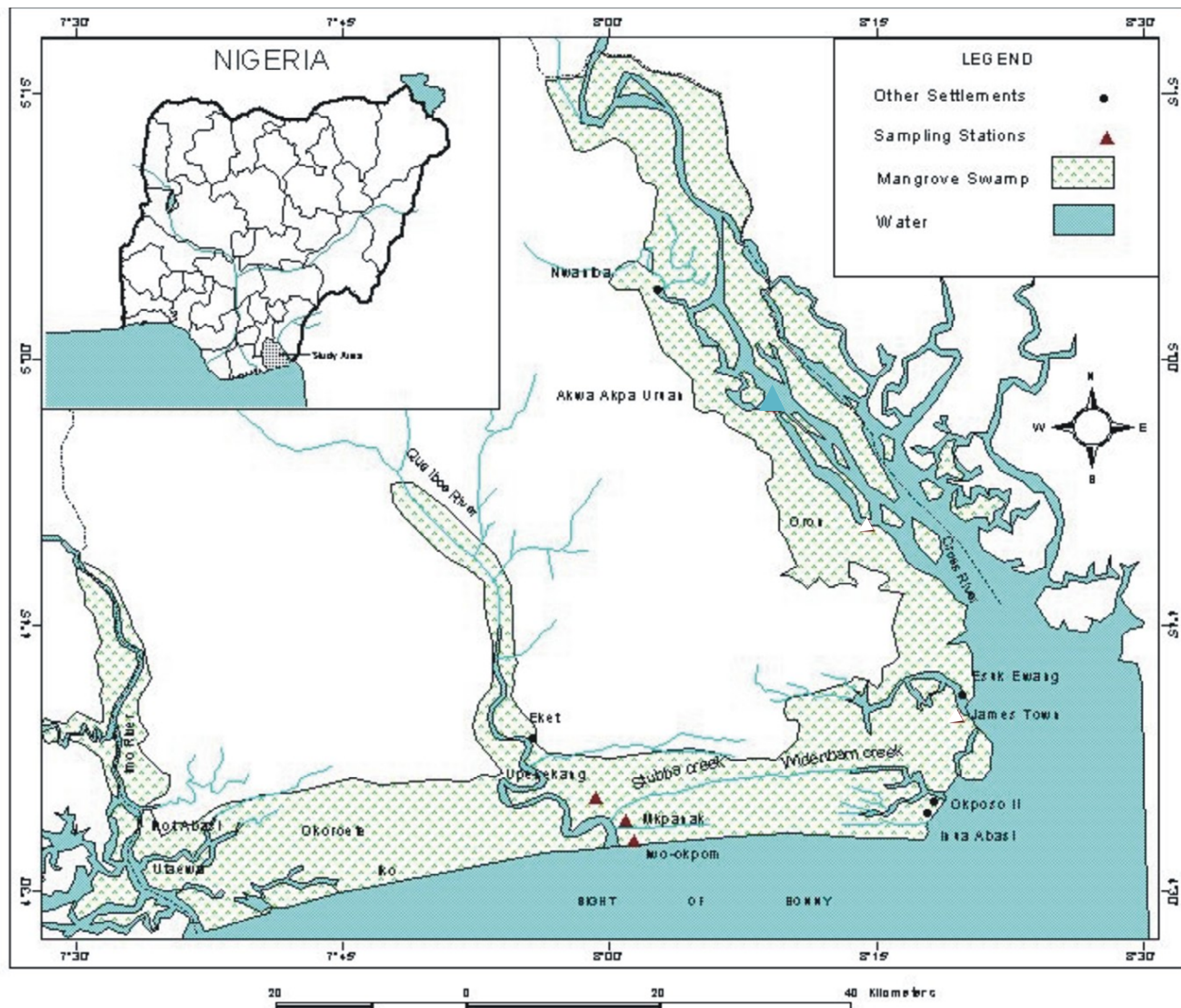

Figure 1. Location of study (the mangrove ecosystem of the Qua Iboe estuary and the associated Stubbs creek).

Test isolates

Using the spread plate technique (Harley and Prescot, 1996) and Sabouraud dextrose agar (SDA) as the culture medium, the yeasts were isolated on the 90th day from epipellic sediment contained in microcosms simulated with test metals. The prevalent isolates from sediments simulated with high levels of Pb, Zn and Ni were purified and identified according to the schemes of Barnett and Pankhurst (1974) as *Candida*-ESY_{13} and *Saccharomyces*-ESY_2.

Determination of the tolerance and detoxification potential of yeasts

The tolerance and detoxification potential of the isolates (*Candida*-ESY_{13} and *Saccharomyces*-ESY_2) to heavy metals were determined determined in 200 ml of sterile malt extract broth (MEB) contaminated with metal solutions of Pb (140 mgl^{-1}), Zn (328 mgl^{-1}) and Ni (241 mgl^{-1}) contained in 250 ml Erlenmeyer flasks. The media were seeded with a 24 h old broth culture containing 5.0 x 10^2 $cfuml^{-1}$ of *Saccharomyces*-ESY_2 and 6.0 x 10^2 $cfuml^{-1}$ of *Candida*-ESY_{13}. Thereafter, the inoculated flasks were incubated at room temperature (28 ± 2°C) for 21 days (504 h). A control experiment with flasks containing inoculated MEB without metal pollutants was also prepared. The cultures were incubated on a mechanical shaker (SGM-300, Gallenkamp, England) at 60 rpm. The culture pH optical density and total viable count (TVC) were determined every 72 h for a period of 21 days.

The pH was determined using a pH meter (EIL 7020; Kent Industrial Measurement Ltd.). The attenuance was measured at 550 nm wavelength (Malakul et al., 1998) with a spectrophotometer while the TVC was determined by the pour plate method (Harrigan and

Table 1. Mean S.D. and coefficient of variation of some physicochemical attributes and background heavy metals load of epipellic sediment of Qua Iboe Mangrove Ecosystem

Parameters	Location			Mean	S.D.	C.V.%
	1	2	3			
pH	6.90	6.31	6.71	6.64	0.30	4.52
Total organic carbon (%)	10.18	9.71	10.11	10.00	0.25	2.5
Total organic nitrogen (%)	0.26	0.22	0.23	0.24	0.017	-
Available phosphorus ($mgkg^{-1}$)	5.30	4.93	4.88	5.04	0.14	2.76
Exchangeable bases						
Ca ($mgkg^{-1}$)	6.56	5.47	6.14	6.06	0.30	4.95
Mg ($mgkg^{-1}$)	3.23	2.71	3.11	3.02	0.27	8.94
Na ($mgkg^{-1}$)	9.58	7.71	8.21	8.50	0.97	11.41
K ($mgkg^{-1}$)	0.30	0.31	0.29	0.30	0.0001	0.01
Nutritive salts ($mgkg^{-1}$)						
CO_3^{2-}	98.10	94.21	96.41	96.24	1.95	2.03
Cl^-	106.55	100.31	99.31	102.06	3.92	3.84
SO_4^{2-}	70.61	68.40	69.21	69.41	1.12	1.61
NO_3^-	26.13	23.41	27.12	25.55	1.95	7.63
Salinity%	5.41	4.61	5.01	5.01	0.22	4.39
Particle size distribution (%)						
Sand	44.70	43.81	44.63	44.38	0.49	1.10
Silt	34.60	34.20	33.71	34.17	0.45	1.32
Clay	20.70	21.99	21.66	21.45	0.67	3.12
Heavy metals ($mgkg^{-1}$)						
Zn	101.5	103.75	102.65	102.48	1.23	1.20
Cu	43.30	44.75	43.55	43.53	1.00	2.29
Ni	20.70	21.20	21.10	21.00	0.05	0.24
Pb	44.80	45.75	43.20	44.58	1.11	2.49
Cr	0.013	0.015	0.014	0.014	0.000006	-

McCance, 1990).

Enumeration was done after 5 days of incubation. Population dynamics of the inocula was used as an index of growth response and detoxification of metals. Thus, using the TVC data, the number of generations (n), generation time (gt) and growth rate (Gr) of the isolates were estimated as described by Pelczer et al. (1982).

Determination of the heavy metals bioaccumulation potential

At the end of the toxicity testing, cells were harvested by centrifugation at 10,000 rpm for 15 min and washed with sterile deionized water 3 times. The process yielded about 1.00 g of fresh cell pellets which were subjected to drying in an oven at 60°C for 48 h. Dried cell pellets obtained were digested and the amount of metal accumulated by each isolate was determined using a flame atomic absorption spectrophotometer at 540 nm wavelength. This procedure has been previously adopted by Faisal and Hasnain (2006).

RESULTS AND DISCUSSION

The test organisms were isolated from microcosms containing the halomorphic and highly psammitic epipellic sediments (Table 1) which were exposed to varying concentrations of the heavy metals for 90 days. The incidence and tolerance of the diverse species of yeasts found in mangrove sediment to elevated levels of metals are presented in Table 2. Among them, *Candida*-ESY_{13} and *Saccharomyces*-ESY_2 were the most prevalent and tolerant isolates and were selected for the metal accumulation study.

In the broth screen flasks during toxicity studies, the yeasts grew well, producing reasonable turbidity and TVC as an indication of tolerance and detoxification (Table 3). *Candida*-ESY_{13} grew remarkably better in MEB simulated with Ni and Pb while *Saccharomyces*-ESY_2 performed better in MEB simulated with Zn and Pb. The growth of *Candida*-ESY_{13} exposed to Ni contamination is depicted in Figure 2. The exponential growth phase was observed at 216 h with a Gr of 0.042 h^{-1} resulting from n = 9.00 and gt of 23 h. For *Candida*-ESY_{13} exposed to Pb, the exponential growth phase was noticed at 288 h. The Gr recorded was 0.073 h^{-1} with n = 21.00 and gt of 14.00 h (Figure 3). The growth rate of *Saccharomyces*-ESY_2 cultured in Zn-contaminated broth was 0.045 h^{-1} at 360 h with 16.00 numbers of generations recorded within 22 h (Figure 4). For *Saccharomyces*-ESY_2 exposed to Pb contamination (Figure 5), the exponential growth phase

Table 2. Incidence and heavy metals tolerant levels of yeasts isolated from the Qua Iboe Estuary mangrove sediment.

Microbial Isolates' I. D.	Control	Zn1 (821.00) $mgkg^{-1}$	Zn2 (1231.00) $mgkg^{-1}$	Zn3 (1642.00) $mgkg^{-1}$	Pb1 (90.00) $mgkg_{-1}$	Pb2 (144.00) $mgkg^{-1}$	Pb3 (208.00) $mgkg^{-1}$	Cu1 (471.00) $mgkg^{-1}$	Cu2 (668.00) $mgkg^{-1}$	Cu3 (864.00) $mgkg^{-1}$	Cr1 (38.00) $mgkg^{-1}$	Cr2 (46.00) $mgkg^{-1}$	Cr3 (58.00) $mgkg^{-1}$	Ni1 (253.60) $mgkg^{-1}$	Ni2 (388.00) $mgkg^{-1}$	Ni3 522.60 $mgkg^{-1}$
Candida sp. ESY13	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Cryptococcus sp. ESY4	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-
Geotrichum sp. ESY22	+	+	+	-	+	-	-	-	-	-	+	-	-	+	-	-
Saccharomyces sp. ESY2	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Sporobolomyces sp. ESY13	+	+	-	-	-	-	-	+	+	-	+	-	-	+	-	-
Rhodotorula sp. ESY16	+	+	+	-	+	-	-	-	-	-	+	-	-	+	+	-

Table 3. Amount of Pb, Zn and Ni accumulated by test isolates.

Isolate	Concentration of Metal Accumulated Mg/g (dry weight of cells)		
	Pb	Zn	Ni
Saccharomyces-EYS_2	41.44	4.28	NRT
Candida-ESY_{13}	41.87	NRT	0.83

NRT = No remarkable turbidity (isolates with this attribute were not selected for the respective metal accumulation potential analysis).

was attained at 144 h. The growth rate recorded was 0.080 h^{-1} with 11.60 numbers of generation and 12.50 h generation time. In contrast, the control culture of the test microorganisms revealed a generally higher growth rate and number of generations with a lower generation time.

For instance, the growth rate recorded for *Saccharomyces*-ESY_2 at the exponential phase (144 h) was 0.112 h^{-1} with 16.20 and 8.90 h number of generations and generation time respectively.

The delay in cell replication of metal impacted yeast cultures may be ascribed to metal toxicity. The reduction in growth of yeasts could be attributed to the leng-thening acclimation period (defined as time taken to observe an increase in viable counts of the organism) as a consequence of the metal stress. This observation is supported by the report of McEldowney (1994) who reported that toxic metals decreased the physiological activities of micro-organisms. Variation in the level of res-ponse to metal toxicity between yeast species is in agreement with the observation of Roane and Pepper (1997) in that toxicity of metal depends on on the nature of the organism.

Accumulation of toxic metal is not by the fast constitutive uptake system, rather, it is by inducible uptake systems. Therefore some amount of time is needed for the inducible enzyme system to be expressed and this is species-dependent (Prescot et al., 1999). The effects of the metals were most apparent on the generation time of the test organisms. Higher generation time is indica-

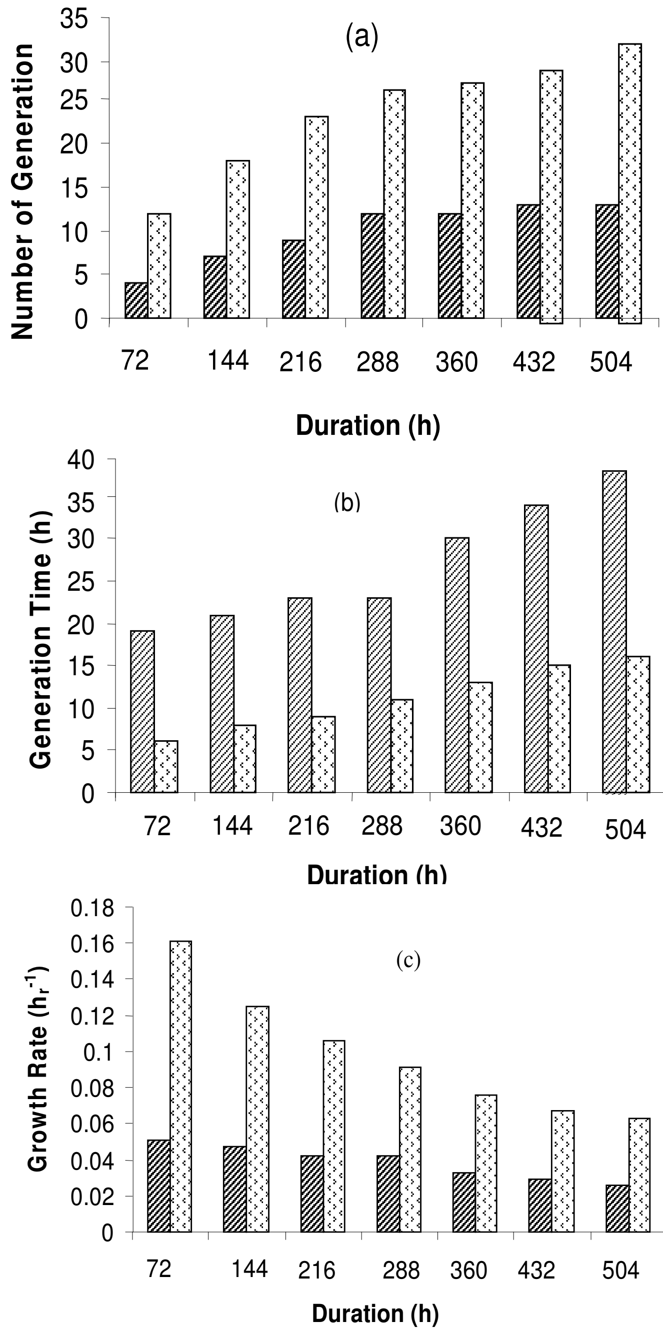

Figure 2. Influence of nickel on the number of generations (a), generation time (b) and growth rates (c) of *Candida* – ESY_{13}.

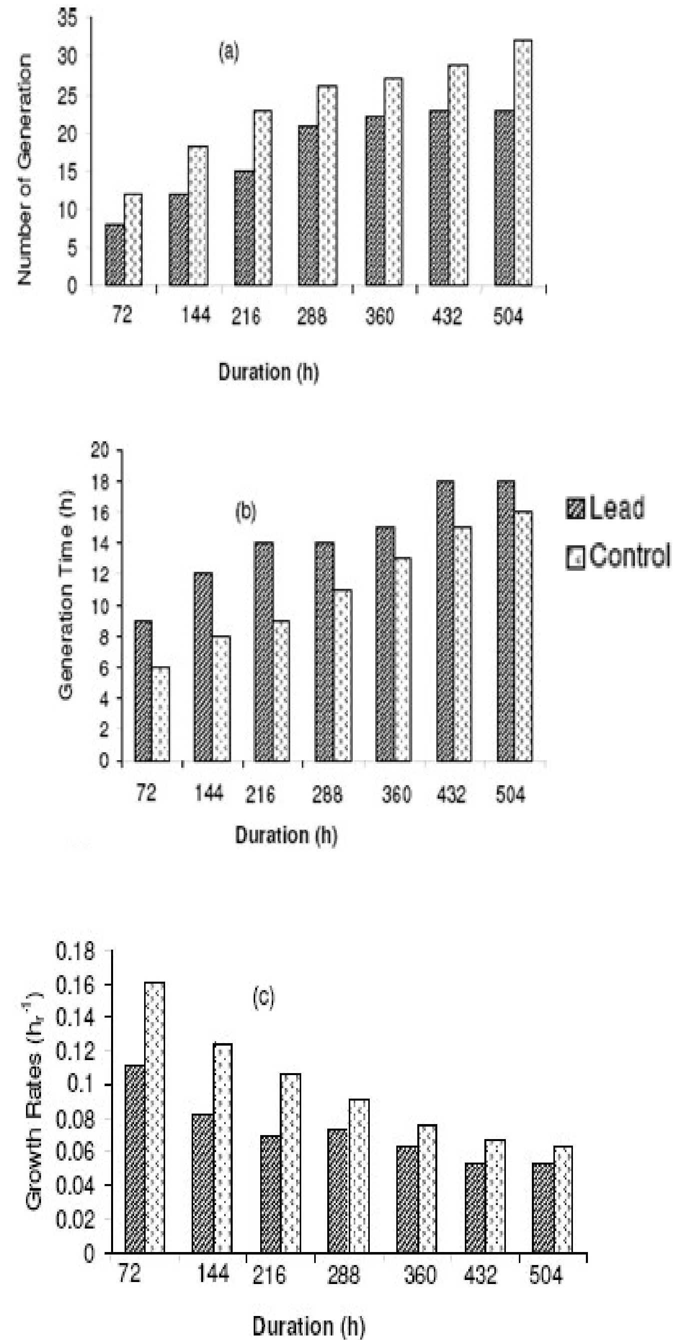

Figure 3. Influence of lead on the number of generations (a), generation time (b) and growth rates (c) of *Candida* - ESY_{13}.

tive of the amount of time needed for the inducible enzymes such as permeases and complexing protein molecules to be synthesized. This may adversely affect the number of generation. The increase in generation time of organisms as a result of metal toxicity also resulted in a reduction in the number of generations, thereby affecting the microbial population which is essential in detoxification, decontamination and other density dependent processes. The result has revealed that Ni and Zn increased the generation time of *Candida* and *Saccharomyces* species respectively, resulting in their decreased growth rate and reduced number of generations. This amounts to a decrease in the Ni and Zn detoxification ability of the organisms. The bioaccumulating capability of the isolates (Table 3) indicated that the yeast *Candida* – ESY_{13} accumulated more Pb than *Saccharomyces* – ESY_2. It specifically accumulated 41.87 mg/g (dry weight of cells) of Pb. However, *Saccha-*

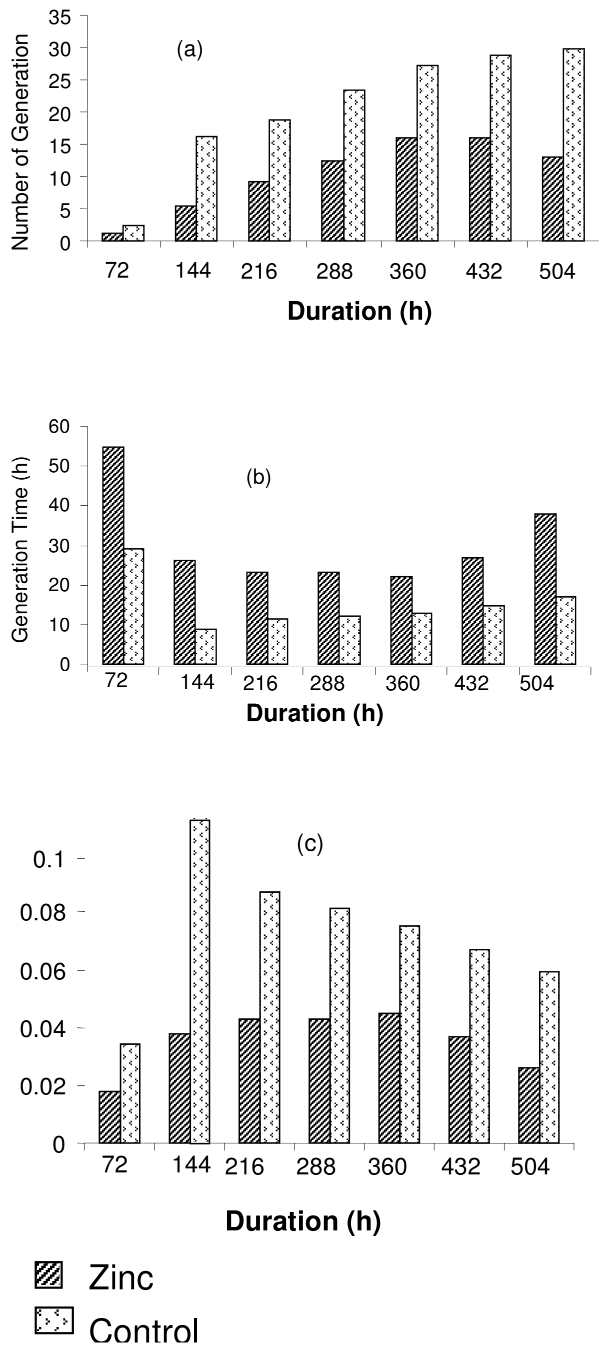

Figure 4. Influence of zinc on the number of generations (a), generation time (b) and growth rates (c) of *Saccharomyces* – ESY_2.

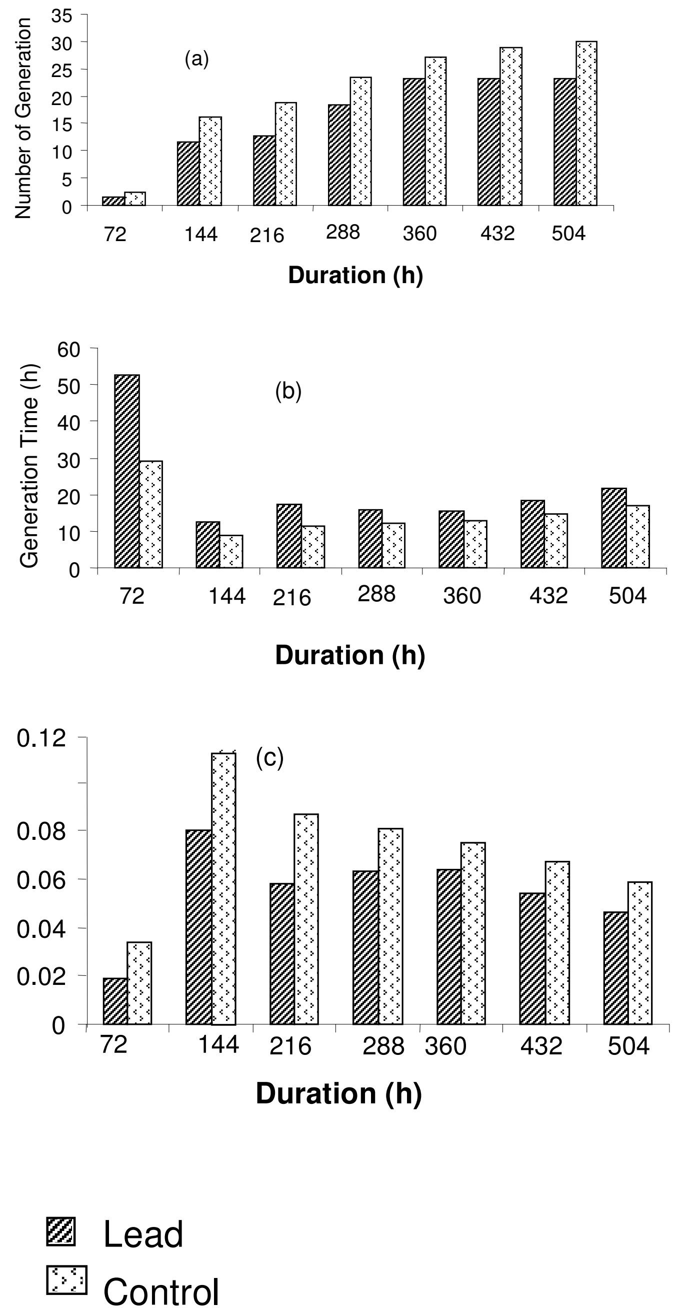

Figure 5. Influence of lead on the number of generations (a), generation time (b) and growth rates (c) of *Saccharomyces* – ESY_2.

romyces-ESY_2 accumulated 4.23 mg/g (dry weight of cells) of the Zn while *Candida*-ESY_{13} accumulated 0.83 mg of Ni/g dry weight of cells. It is obvious that *Candida*-ESY_{13} and *Saccharomyces*-ESY_2 have high Pb accumulating capability. The slight differences in the concentrations of metals accumulated in the cells might account for the variation in the detoxification levels recorded for the diverse species. On the other hand, the concentration of Ni and Zn bioaccumulated by yeasts were low with Ni bioconcentrated in fractions.

Conclusion

Metals accumulation by micro-organisms may lead to

their removal from the environment or a reduction in the concentration of bioavailable metal. Specifically, *S. cerevisiae* can remove toxic metals, recover precious metals and clean radio-nuclides from aqueous solution to various extents (Alluri et al., 2007; Wang and Cheng, 2006). *S. cerevisiae* is a product of many single cell and alcohol fermentations and like the fodder yeast (*C. utilis*) it can be procured in large quantities at low cost (Wang and Cheng, 2006; Muter et al., 2002). *S. cerevisiae* also has the ability to differentiate between different metals such as selenium, antimony and mercury based on their toxicity (Wang and Cheng, 2006).

Therefore, the mangrove yeasts tested may have strong potential for heavy metals biosorption. However, the accumulation of a highly toxic element like Pb is worrisome. The ability of these micro-organisms to bioaccumulate Pb raises the question of biomagnification in mangrove food chains since they play a functional role, occupying diverse niches in various ecosystems. This not withstanding, our findings have demonstrated how well yeasts could tolerate and detoxify metals, especially Pb in mangrove ecosystem and could be adapted for the remediation of metals-impacted environments.

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