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Physa acuta snail as a biomonitor for the efficacy of bioremediation treatment of heavy metals (Fe III and Cd II) using the fungus (*Eupenicillium lapidosum*) in lined and unlined laboratory conditions

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ABSTRACT

The present study aimed to evaluate the effectiveness of *Physa acuta* snail as biomonitor for the efficacy of bioremediation treatment of heavy metals (Fe III and Cd II) using fungus *Eupenicillium lapidosum*. So, the effects of these heavy metals on the mortality percent of adult *P. acuta* snail, heavy metal concentrations in their soft tissues and on the antioxidant enzymes were evaluated under laboratory lined (sand cement) and unlined (mud) conditions for 2, 24 hrs and 7 days of exposure to the fresh mycelia of the fungus *E. lapidosum*, (50 ppm of Fe (III) & Cd (II)) and biosorption treatments of Fe and Cd by the fungus *E. lapidosum*. The data indicated that Cd was somewhat more toxic to the snails than Fe in cement lining than that of unlined conditions (mud). While, the lowest mortality percent was that of the biosorption treatments against tested heavy metals. The metal biosorption values by *E. lapidosum* against Cd were higher than that of Fe in cement lining than that of unlined ones. Concerning the results of iron and cadmium analysis in soft tissues of *P. acuta* snails demonstrated that the iron concentrations were higher than that of cadmium. While, during the biosorption processes the concentrations of accumulated metals were decreased by increasing the time of exposure. The data of the biochemical responses in tissues of exposed *P. acuta* snails showed alterations of some antioxidant parameters which was indicated by the significant increase in the lipid peroxidation (MDA) and decrease of endogenous antioxidant enzymes; Catalase (CAT) and glutathione (GSH) in lined conditions than that of unlined ones, while the effect decreased in the case of Fe and Cd biosorption. So, these results indicated that *P. acuta* snail and the fungus *E. lapidosum* are useful in the searches for biomarkers and biosorption, respectively.

INTRODUCTION

Metal pollution in aquatic ecosystems, especially river systems, is a major environmental concern. The ecological importance of metals emanates from their general toxicity and the fact that they are non-biodegradable and highly persistent and therefore, tend to accumulate in the environmrnt (Coetzee *et al*., 2002). Even minute

amounts of metals can cause subtle or chronic biological effects that may result in irreversible long-term changes in organisms (Bahobil *et al*., 2017).

According to the World Health Organization (WHO, 2004), the heavy metals, cadmium (Cd) and iron (Fe) are the most immediate concern. Cadmium increases lipid peroxidation, in addition it depletes antioxidants, glutathione and protein-bound sulfhydryl groups. It also promotes the production of inflammatory cytokines. Ingestion of any significant amount of cadmium causes immediate poisoning and damage to the liver and the kidneys. Compounds containing cadmium are also carcinogenic (Maret and Moulis, 2013). Iron plays an important role in biology, forming complexes with molecular oxygen in hemoglobin and myoglobin; these two compounds are common oxygen transport proteins in vertebrates. Iron is also the metal used at the active site of many important redox enzymes dealing with cellular respiration and oxidation and reduction in plants and animals (Boukhalfa and Crumbliss, 2002). Large amounts of ingested iron can cause excessive levels of iron in the blood. High blood levels of free ferrous iron react with peroxides to produce free radicals, which are highly reactive and can damage DNA, proteins, lipids, and other cellular components. Thus, iron toxicity occurs when there is free iron in the cell, which generally occurs when iron levels exceed the capacity of transferrin to bind the iron. Damage to the cells of the gastrointestinal tract can also prevent them from regulating iron absorption leading to further increases in blood levels. Iron typically damages cells in the heart, liver and elsewhere, which can cause significant adverse effects, including coma, metabolic acidosis, shock, liver failure, coagulopathy, adult respiratory distress syndrome, long-term organ damage, and even death (Cheney *et al*., 1995).

Heavy metal stress resulted in the production of $O_2^{\bullet-}$, H_2O_2 and $^{\bullet}OH$, which affect various cellular processes, mostly the functioning of membrane systems. Cells are normally protected against free oxyradicals by the operation of intricate antioxidant systems (Chandran *et al*., 2005). In molluscs, metal uptake may occur by facilitated diffusion, active transport, or endocytosis, and can be enhanced by metallothioneins (MT) synthesis or formation of mineralized granules. So, heavy metals have the ability to generate reactive radicals, resulting in cellular damage like depletion of enzyme activities, damage to lipid bilayer and DNA (Leonard *et al*., 2004). Basopo *et al*. (2014) mentioned that the activities of superoxide dismutase, catalase, glutathione S-transferase were enhanced as a result of the oxdative stress of copper on the freshwater snail *Helisoma duryi*.

Monitoring and prevention of heavy metal pollution is one of the hot topics in environmental researches. Heavy metal analysis in aquatic organisms can provide important information on the degree of environmental contamination and its impacts (Kowalczyk and Czepiel, 2013).Gastropods are one example of aquatic organisms that have demonstrated the ability as potential bioindicator and accumulate metals to high concentrations (Zhou *et al.*, 2008). Snails can be serve as biomonitors for pollution and bioremediation strategies (Lee, 2000; Lee *et al*., 2001*a* and *b*). Lee *et al*. (2002) stated that the snail survival, growth, and histopathological changes were monitored in the mystery snail, *Viviparus georgianus*, and the mimic ponds snail, *Pseudosuccinea columella*, to evaluate their suitability as biomonitors for the efficacy of bioremediation treatments of oil spill in a freshwater marshland.

The pond snail, *Physa acuta*, is a good model for testing acute and chronic toxicity of aquatic pollutants, as ease of field collection and laboratory maintenance, and efficacy in testing toxicity responses including mortality, reproduction and metabolism under both field and laboratory conditions (Woodard, 2006).

The removal of heavy metals from the environment especially wastewater is now shifiting from the use of conventional adsorbents to the use of biosorbents in a process known as biosorption (Bahadir *et al*., 2007). Biosorbtion is a technique that can be used for the removal of pollutants from waters, especially those that are not easily biodegradable such as metals and dyes. Therefore, biosorption has been defined as the property of certain biomolecules (types of polymer or biomass) to bind and concentrate selected ions or molecules from aqueous solutions (Tszos, 2001). So, there has been continuing interest on the remediation of metals using microorganisms (fungi, algae, and bacteria) which could remove metals from their growth environment without making them available for their own metabolic processes (Joshi, 2018). Microorganisms can be important biosorbents for heavy metal remediation of contaminated soils and wastewaters (Pan *et al*., 2009). Due to unique chemical composition, microbial biomass sequesters metal ions by forming metal complexes from solution and obviates the necessity to maintain special growth-supporting conditions (Ahluwalia and Goyal, 2007). They have adventages over their chemical counterpart since they can remove ions at very low concentrations (on the order of 2-10 mg L^{-1}). Biosorbents are more specific and hence prevent the binding of alkaline earth material. Also, they have the potential of genetic modification and so can be tailored for increased specificity (Doble and Kumar, 2005). Biomolecules present in the biosorbent contain several chemical groups that act as ligands for the biosorption of the metal ions (Jerez, 2009).

Fungi are a diverse group of organisms belonging to the kingdom *Eumycota*. These microorganisms are known to detoxify metals by several mechanisms including ion exchange, chelation, adsorption, crystallization, valence transformation, extra and intracellular precipitation and active uptake (Gadd, 1993). The accumulation of metals from solutions by fungi can be divided into three categories: (1) biosorption of metal ions on the surface of fungi, (2) intracellular uptake of metal ions, and (3) chemical transformation of metal ions by fungi (Singh, 2006). Fungi offer a wide range of chemical groups that can attract and sequester the metals in biomass (Tomko *et al*., 2006). The fungal cell walls can be considered as a two phase system consisting of chitin framework embedded on an amorphous polysaccharide matrix (Yan and Viraraghavan, 2000).

So, this study aimed to evaluate the effectiveness of *Physa acuta* snail as biomonitor for the efficacy of bioremediation treatment of heavy metals (Fe III and Cd II) using fungus (*Eupenicillium lapidosum*) in lined and unlined laboratory conditions.

MATERIALS AND METHODS

Experiment animal: *Physa acuta* snails (synonymous with *P. integra* and *P. heterostropha*) (Dillon *et al*., 2002) were collected from lined and unlined water bodies in Behaira (Nubaria) and Giza Governorates, then carried to the laboratory to be maintained under laboratory conditions (25 ± 2ºC) according to the method described by WHO (1965). The 2nd generation of these snails were used as a laboratory ones for the current experiment (El-Sayed, 2006).

Fungal biomass: (The fungus *Eupenicillium lapidosum*) which isolated in a previous study (Abdel-Motlb, 2016) from the water samples of the investigated lined and unlined water bodies in Behaira (Nubaria) and Giza Governorates.

Kingdom : Fungi

Phylum : *Ascmycota*

Subphylum : *Pezizomycotina*
Class : *Eurotiomycetes.*
Order : *Eurotiales*
Family : *Trichocomaceae*
Genus : *Eupenicillium*
Species : *lapidosum*

Experimental design: Metal salt solutions of Fe (III) and Cd (II) ($FeCl_3$ or $CdCl_2$) of 50 ppm were prepared. The pH of metal solution was adjusted at 7.0 using 1N HCl or 1N NaOH. Subsequent to metal solution preparation, 5 gm of fungal biomass was suspended in 1000 ml of metal solution in a white plastic container (25 x 13 x 6.5 cm) provided with mud substratum or sand cement (the most common material used in lining of the surveyed lined and unlined water canals in the investigated governorates) with contact time of 2, 24 hrs and 7 days at 28 ± 2°C (Kumar *et al*., 2011). The pH and temperature were recorded daily.

Sets of 360 mature snails with (8-10 mm) shell height were divided into 12 groups; six groups in cement lining material and the other six groups in unlined ones (mud), each group of 30 snails was exposed in 3 replicates, each of 10 snails /L. The 1st two groups were kept as non-treated in lined and unlined aquaria (control). The 2nd groups were treated with *E. lapidosum* in lined and unlined aquaria whereas the 3rd ones in lined and unlined were treated with 50 ppm of Fe and the 4th groups also, exposed to Cd. The 5th groups were exposed to both the fungus *E. lapidosum* + Fe and the last groups 6th were exposed to the fungus + Cd. After 2, 24 hrs and 7 days exposure periods, the number of dead snails in each a aquarium was recorded and the mortality percentage was calculated. From each specified group, concentration of the tested heavy metals was measured in the solution of the experiments and in the tissues of snails. Also, the tissue biochemical parameters (Catalase (CAT), glutathione (GSH) and The lipid peroxidation, represented by malondialdehyde (MDA) concentration) were determined.

Estimation of heavy metals in tested samples: Were performed according to the method of A.O.A.C.(1995) using Atomic Absorbtion Spectrophotometer (Solar M 600531 v1 .27) at Central Agricultural Pesticides Laboratory, Agricultural Research Center ARC, Dokki, Giza, Egypt. The current, wavelength and slit band width of each element were adjusted automatically by the instrument software.

Standards: Metals stock standards of Cd and Fe were obtained from Merck, Darmstadt, Germany (Merck's ampoules; 1000 mg).

Analysis of heavy metals in water: For the analysis of total heavy metals, water samples (200 ml) were digested with 5 ml of di-acid mixture (HNO3: HCIO4: 9:4 ratio) on a hot plate and filtered by Whatman No. 42 filter paper and made up the volume to 50 ml by double distilled (ddH_2o).

Digestion of snail tissues: The snail's soft tissues were separated from their shells carefully, 0.01 gm of the soft tissues of each snail species, dried in an oven at 50°C, weighted, and then digested in 1ml of conc. HNO_3 at 70°C for 2h. The digested samples were then diluted with 5 ml ultra pure deionized water for analyzing heavy metals (Federici *et al*., 2007). The Bioconcentration factor (BCF), which is the ratio of the chemical concentration in an organism or biota to the concentration in water (Gobas and Morrison, 2000), was calculated as follow:

$$BCF = \frac{Concentration\ biota}{Concentration\ water}$$

Metal uptake and percent biosorption efficiency: The equilibrium sorption capacity of fungal biomass at the corresponding equilibrium conditions was

determined using a mass balance equation expressed as in equation (1) (Akar *et al*., 2009):

$$q = \frac{V(C_i - C_f)}{W} \qquad (1)$$

where,

V= the volume of metal solution (L);

C_i = initial metal concentration (mg L^{-1});

C_f =Final/ residual concentration (mg L^{-1});

W = amount of biomass (mg L^{-1});

The percent biosorption of metal ion was calculated as follows (Sari and Tuzen, 2009):

$$\text{Biosorption (\%)} = \left[\frac{C_i - C_f}{C_i}\right] x\ 100 \qquad (2)$$

Effect of metal biosorption on certain biochemical parameters in tissue of *Physa acuta*:

For preparation of tissue homogenates, the snail's shell was gently crushed between two glass slides and the snail's soft tissues were separated under a dissecting microscope in cold distilled water. Snail's soft tissues were homogenized in phosphate puffer at a ratio 1:10 W/V, centrifuged (3000 rpm) for 10 min and the supernatant was used. Some biochemical activity (MDA, CAT and GSH) determined in this study were analyzed spectrophotomerically by using reagent kits Purchased from Biodiagnostic Company, Dokki, Giza, Egypt.

The concentration of MDA (n mol/g. tissue) (the end product of lipid peroxidation) was estimated colorimetrically at 534 nm according to the method of Satoh (1978) using lipid peroxide kit (Cat. No. MD 2529).

Catalase (CAT) (U/g) activity measured at 240 nm by the method of Aebi (1984) using catalase assay kit (Cat. No. CA 2517).

The concentration of GSH (n mol/g. tissue) was measured at 405 nm using colorimetric GSH kit (Cat. No. GR 2511) according to Beutler *et al.* (1963).

Statistical Analysis:

The comparison between means and standard deviations of different groups were tested for significance using two independent samples *t*-test. The differences were considered significant at $p<0.05$.

RESULTS

P. acuta snails were used as biominitor for assessing the efficiency of Fe (III) and Cd (II) biosorption by the fungus *E. lapidosum*. So, the results in Table (1) and Figure (1) claimed that Cd exposure was somewhat more toxic to the snails than Fe exposure in cement lining than that of unlined conditions (mud) and it was also observed that increasing the time of exposure caused a marked increasing in the mortality percent. While, the lowest mortality percent was that of the biosorption treatments against tested heavy metals.

Table 1: Mortality percent of *Physa acuta* snails during Fe (III) and Cd (II) biosorption by the fungus *E. lapidosum* at different periods post exposure.

Time of exposure	Mortality % of *Physa acuta* snails during bioremediation of Fe (III) and Cd (II) by The fungus *Eupenicillium lapidosum* in lined and unlined laboratory conditions											
	Lined (cement)						Unlined (mud)					
	Fe (III)		Cd (II)		Fungus /Snail	Control Snail/ cement	Fe (III)		Cd (II)		Fungus /Snail	Control Snail/ mud
	Fe/snail	Fe/ fungus/ snails	Cd/ snail	Cd/ fungus/ snails			Fe/ snail	Fe/ fungus/ snails	Cd/snail	Cd/ fungus/ snails		
2 hrs	16.67	6.67	23.33	6.67	0	0	13.33	3.33	20	6.67	0	0
24 hrs	43.33	13.33	50	16.67	10	0	36.67	10	40	13.33	6.67	0
7 days	73.67	30	80	36.67	30	0	70	26.67	76.67	33.33	23.33	0

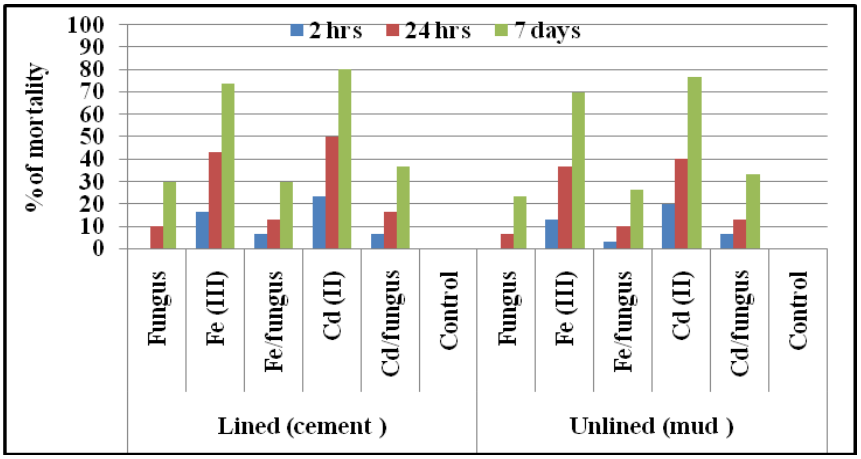

Fig. 1: Mortality percent of *P. acuta* snails during Fe (III) and Cd (II) biosorption by the fungus *E. lapidosum* at different periods post exposure.

The highest metal biosorption values by *E. lapidosum* were that against Cd for 2, 24 hrs and 7 days of exposure in cement lining (41.07, 48.66 and 49.41 mg g^{-1}, respectively) with biosorption efficiencies values of (81.24, 94.74 and 99.72 %) without any significant differences ($P > 0.05$) if compared to that of Cd in unlined conditions (mud) (37.10, 45.84 and 49.14 mg g^{-1}) with biosorption efficiencies of (74.20, 92.22 and 98.16%, respectively) (Table 2 and Figure 2). Fe biosorption value after 2 hours of exposure in cement lining was significantly decreased ($P < 0.01$), being 19.33 mg g^{-1} in comparison with that of mud condition.

Table 2: Metal biosorption (mg g^{-1}) and biosorption efficiency (%) of the fungus *E. lapidosum* against Fe (III) and Cd (II) in lined and unlined conditions at different periods post exposure.

Time of exposure	Lined (cement)				Unlined (mud)			
	Fe (III)		Cd (II)		Fe (III)		Cd (II)	
	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%
2hrs	19.33 ± 2.46**	39.38	41.07 ± 6.11	81.24	34.26 ± 2.02	68.52	37.10 ± 2.98	74.20
24 hrs	33.67 ± 5.94*	67.40	48.66 ± 4.14	94.74	43.55 ± 1.08	86.8	45.84 ± 1.71	92.22
7 days	48.08 ± 3.98	96.16	49.41 ± 3.43	99.72	46.19 ± 1.99	92.20	49.14 ± 3.49	98.16

Data are expressed as mean ± SD * : Significant difference at *($p < 0.05$)* ** : Highly Significant *($p < 0.01$)*.

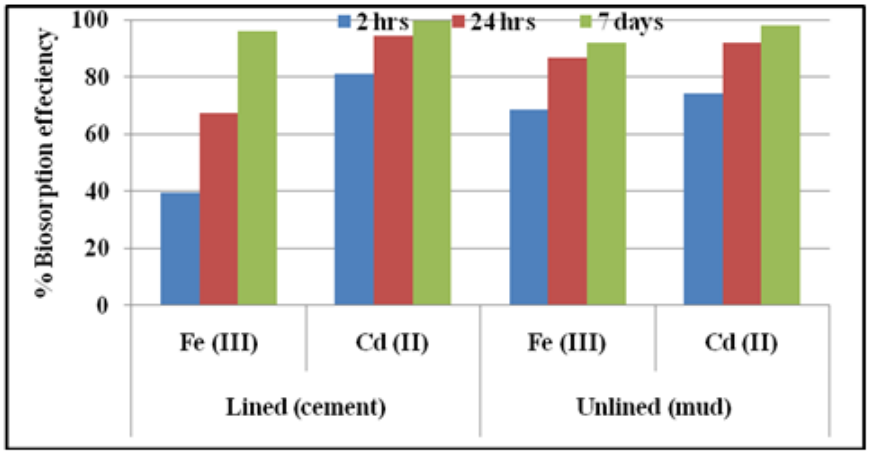

Fig 2: Biosorption efficiency (%) of the fungus *E. lapidosum* against Fe (III) and Cd (II) in lined and unlined conditions at different periods post exposure.

Also, 24 hrs of exposure was significantly decreased (P<0.05), being 33.67 mg g^{-1}, while at 7 days of exposure (48.08 mg g^{-1}), non significantly increased (P>0.05), if compared with unlined ones and the biosorption efficiencies values were (39.38, 67.40 and 96.16%). In mud substratum the biosorption values were (34.26, 43.55 and 46.19 mgg^{-1}) with biosorption efficiencies values of (68.52, 86.8 and 92.20%). So, the biosorption efficiency of the fungus *E. lapidosum* against Cd was higher than that of Fe.

The results of iron and cadmium analysis in soft tissues of *P. acuta* snails at 2, 24 hrs and 7 days of exposure demonstrated considerable variability in tissue metal concentrations. Generally, The iron concentrations were high than the concentrations of cadmium. Fe and Cd concentrations were highly significantly increased (P<0.001) in lined conditions if compared with that of unlined ones and the concentrations of accumulated heavy-metals were increased by increasing the time of exposure (Table 3, Figures 3A and B).

Table 3: Heavy-metal concentrations in soft tissues of *P. acuta* snails and bioconcentration factor (BCF) during Fe (III) and Cd (II) biosorption by the fungus *E. lapidosum* at different periods post exposure in lined and unlined conditions.

Time of exposure	Lined (cement)								Unlined (mud)							
	Fe (III)				Cd (II)				Fe (III)				Cd (II)			
	Fe /snail	BCF	Fe /fungus/snails	BCF	Cd /snail	BCF	Cd /fungus/snails	BCF	Fe /snail	BCF	Fe /fungus/snails	BCF	Cd /snail	BCF	Cd /fungus/snails	BCF
2 hrs	316.22*** ±5.58	18.35	98.97 ±5.58	28.56	200.30*** ±5.03	15.57	85.03** ±4.57	8.42	153.06 ±2.96	5.03	96.62 ±6.22	13.02	127.95 ± 6.53	4.55	71.62 ± 1.85	11.07
24 hrs	497.64*** ±7.04	27.36	36.87 ±3.97	16.22	255.94** ±5.16	18.87	25.29 ±6.05	1.13	385.78 ±7.18	21.69	31.35 ±9.55	5.17	230.75 ± 5.47	18.49	23.95 ± 5.60	1.98
7 days	1803.07*** ±7.39	46.06	11.45 ±2.12	1.05	601.17*** ±5.56	40.45	9.33 ±2.03	0.05	1190.55 ±9.05	42.22	10.27 ±4.02	2.38	411.01 ± 3.95	38.66	7.22 ± 6.02	0.003

Data are expressed as mean ± SD Significant difference at *($p < 0.05$)*, ** : Highly Significant *($p < 0.01$)*, *** : More Highly Significant *($p < 0.001$)*.

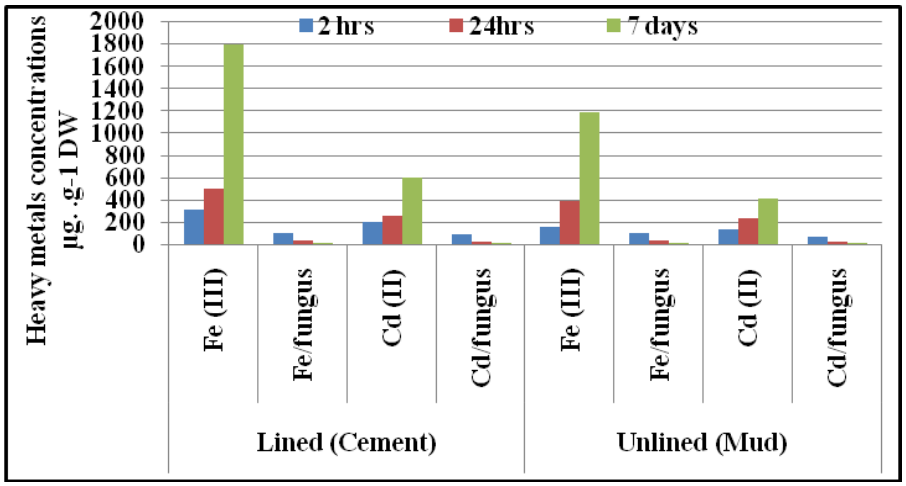

Fig. 3A: Heavy-metal concentrations in soft tissues of *P. acuta* snails during Fe (III) and Cd (II) biosorption in lined and unlined conditions.

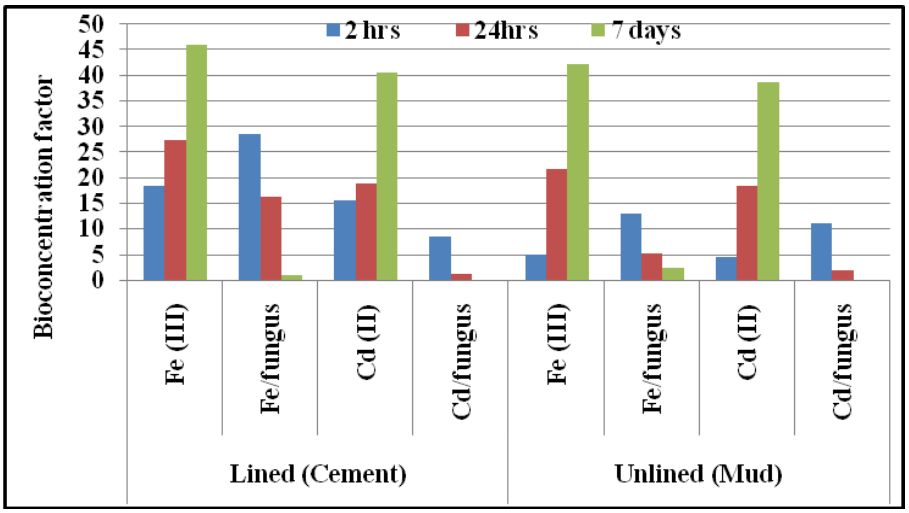

Fig. 3B: The bioconcentration factors (BCF) of heavy metals accumulated in soft tissues of *P. acuta* snails during Fe (III) and Cd (II) biosorption in lined and unlined conditions.

On the other hand, during the biosorption processes the concentrations of accumulated metals were decreased by increasing the time of exposure as a result of the action of *E. lapidosum* against Fe and Cd. The snail groups tested in the biosorption processes against Fe and Cd in lined conditions at 2, 24 hrs and 7 days showed non-significant increased ($P>0.05$) than that of unlined ones, except the concentration at 2 hrs of exposure in lined conditions during Cd biosorption (Cd/fungus/snails) was significantly increased ($P<0.01$) if compared to that of unlined. The highest concentration of accumulated metals in soft tissues of *P. acuta* snails was that of Fe at 7 days of exposure, being 1803.07µg.g^{-1}DW with bioconcentration factor (BCF) (46.06) and the lowest concentration (7.22 µg.g^{-1}DW) was that of snails exposed to the biosorption process against Cd (Cd/fungus/snails) with BCF (0.003).

The biochemical responses in tissues of *P. acuta* snails obtained after 2, 24 hrs and 7 days exposure to Fe, Cd, the fungus *E. lapidosum* and the bioremediation process against the tested metals in lined and unlined conditions are presented in (Tables 4 & 5) and (Figures 4A & B). The data revealed alterations of some

antioxidant activities as indicated by the increase in lipid peroxidation and decrease of endogenous antioxidant enzymes (CAT and GSH) in lined conditions than that of unlined ones and the more time of exposure the more effect on the biochemical parameters. On the other hand, as a result of biosorption process the concentrations of heavy metals decreased and the effect decreased, especially in the case of cadmium.

Table 4: Effect of iron (Fe III) biosorption by *E. lapidosum* on some antioxidant activities in tissues of *P. acuta* snails at different periods post exposure in lined and unlined conditions.

Enzymes / Fe		Lined (cement)			Control	Unlined (mud)			Control
		Fungus/ Snail	Fe/ snail	Fe/ fungus/ snails	Snail/ cement lining	Fungus / Snail	Fe/ snail	Fe/ fungus/ snails	Snail/ Mud
Catalase (CAT) (U/gm)	2 hours	7.11 ± 0.09	5.99* ± 1.08	7.81 ± 0.31	8.72 ± 1.01	7.31 ± 1.32	6.13 ± 1.29	7.58 ± 2.55	8.70 ± 1.80
	24 hours	6. 94 ± 1.14	5.20*** ± 0.11	7.96 ± 1.26	8.70 ± 0.41	7. 45 ± 0.95	5.70* ± 1.06	7.65 ± 0.64	8.67 ± 1.48
	7 days	6.33*** ± 0.22	4.76*** ± 0.56	8.10 ± 0.80	8.69 ± 0.29	6.93 ± 0.80	5.01** ± 0.45	7.88 ± 0.96	8.69 ± 0.79
Lipid peroxidation (Malondialdehyde) (MDA) (nmol/gm)	2 hours	445.10** ± 20.11	612.24*** ± 36.48	448.12*** ± 11.88	370.15 ± 9.23	416.30*** ± 5.82	548.35*** ± 1.67	459.39*** ± 7.44	370.11 ± 4.73
	24 hours	508.56*** ± 13.56	650.44*** ± 7.33	411.86* ± 8.33	371.55 ± 15.47	428.56* 19.22	616.33*** ± 10.44	439.23** 17.99	371.56 10.55
	7 days	591.01*** ± 18.67	701.22*** ± 13.40	393.44* ± 7.29	371.38 ± 11.42	516.01*** ± 24.48	664.99*** ± 24.48	416.33* ± 10.75	371.33 ± 14.68
Glutathione (GSH) (nmol/gm)	2 hours	89.62** ± 5.22	57.24*** ± 2.71	118.78* ± 7.46	155.14 ± 13.98	119.42* ± 20.59	76.89*** ± 4.13	112.58*** ± 5.68	157.14 ± 3.39
	24 hours	77.31*** ± 1.89	50.17*** ± 5.62	130.76 ± 9.89	158.01 ± 15.32	103.24*** ± 8.44	66.96*** ± 2.60	123.14** ± 6.55	153.01 ± 2.06
	7 days	70.45*** ± 2.97	35.24*** ± 4.17	142.56** ± 0.95	158.20 3.21	59.15*** 2.75	44.55*** ± 0.79	132.77** ± 1.65	155.20 ± 5.59

Data are expressed as mean ± SD Significant difference at *(p< 0.05)*, **: Highly Significant *(p< 0.01*), ***: More Highly Significant *(p< 0.001).*

Table 5: Effect of cadmium (Cd II) biosorption by *E. lapidosum* on some antioxidant activities in tissues of *P. acuta* snails at different periods post exposure in lined and unlined conditions.

Enzymes / Cd		Lined (cement)			Control	Unlined (mud)			Control
		Fungus/ Snail	Cd/ snail	Cd/ fungus/ snails	Snail/ cement lining	Fungus / Snail	Cd/ snail	Cd/ fungus/ snails	Snail/ Mud
Catalase (CAT) (U/gm)	2 hours	7.11 ± 0.09	5.78* ± 0.47	8.02 ± 0.96	8.72 ± 1.01	7.31 ± 1.32	6.01 ±1.20	7.82 ± 0.55	8.70 ± 1.80
	24 hours	6. 94 ± 1.14	4.70*** ± 0.10	8.13 ± 1.24	8.70 ± 0.41	7. 45 ± 0.95	5.04* ± 0.56	7.98 ± 0.87	8.67 ± 1.48
	7 days	6.33*** ± 0.22	3.99*** ± 0.26	8.28 ± 1.49	8.69 ± 0.29	6.93 ± 0.80	4.41** ± 0.62	8.03 ± 1.52	8.69 ± 0.79
Lipid peroxidation (Malondialdehyde) (MDA) (nmol/gm)	2 hours	445.10** ± 20.11	628.33*** ± 17.66	403.14* ± 14.27	370.15 ± 9.23	416.30*** ± 5.82	609.23*** ± 25.22	429.02*** ± 3.56	370.11 ± 4.73
	24 hours	508.56*** ± 18.56	750.18*** ± 22.45	399.06 ± 10.88	371.55 ± 15.47	428.56* 19.22	728.57*** ± 23.18	415.94* ± 14.78	371.56 10.55
	7 days	591.01*** ± 12.67	989. 77*** ± 29.15	387.10 ± 3.75	371.38 ± 11.42	516.01*** ± 8.13	838.84*** ± 35.27	407.26** ± 10.22	371.33 ± 14.68
Glutathione (GSH) (nmol/gm)	2 hours	89.62** ± 5.22	54.07*** ± 2.78	139.10 ± 12.42	155.14 ± 13.98	119.42* ± 20.59	68.42*** ± 8.77	129.13*** ± 0.93	157.14 ± 3.39
	24 hours	77.31*** ± 1.89	44.11*** ± 2.07	142.55 ± 8.20	158.01 ± 15.32	103.24*** ± 8.44	55.37*** ± 1.85	135.22*** ± 1.46	153.01 ± 2.06
	7 days	70.45*** ± 2.97	31.16*** ± 1.88	149.35* ± 2.89	158.20 ± 3.21	59.15*** ± 2.75	40.64*** ± 2.03	141.09* ± 6.03	155.20 ± 5.59

Data are expressed as mean ± SD Significant difference at *(p< 0.05)*, **: Highly Significant *(p< 0.01*), ***: More Highly Significant *(p< 0.001).*

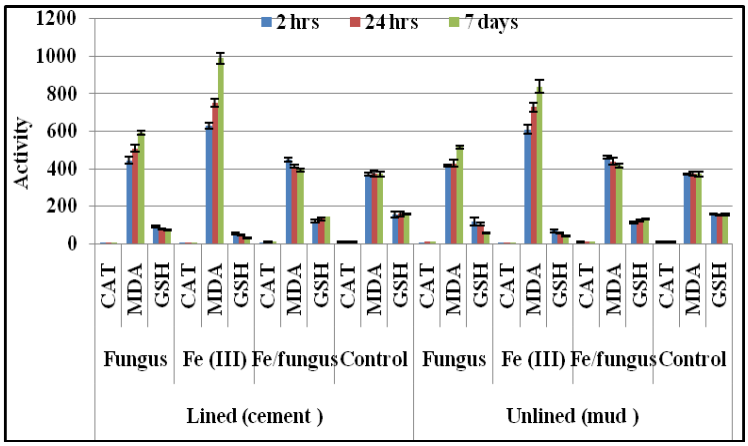

Fig. 4A: Effect of iron (Fe III) biosorption by *E. lapidosum* on some antioxidant activities (CAT(U/gm), MDA (nmol/gm) and GSH (nmol/gm) in tissues of *P. acuta* snails at different periods post exposure in lined and unlined conditions.

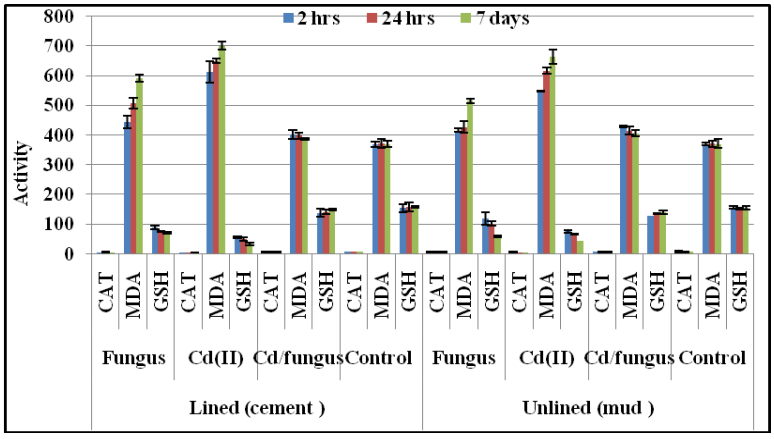

Fig. 4B: Effect of cadmium (Cd II) biosorption by *E. lapidosum* on some antioxidant activities (CAT(U/gm), MDA (nmol/gm) and GSH (nmol/gm)) in tissues of *P. acuta* snails at different periods post exposure in lined and unlined conditions.

Catalase (CAT) activity was significantly decreased in exposed snails compared to their corresponding control in lined and unlined conditions. At 7 days post exposure highly significant decreases ($P< 0.001$) were observed at fungus, Fe and Cd treartments, being 6.33, 4.76 and 3.99 compared to 8.69 U/gm of their corresponding control in lined conditions (cement), while Fe/fungus have no significance ($P>0.05$). Also, in unlined conditions, the fungus and Fe/fungus treatments non-significantly reduced catalase activity ($P> 0.05$) than that of control group, while at 24 hrs and 7 days of Fe and Cd exposure significantly decreased, being 5.70, 5.01 and 5.04, 4.41, respectively compared to 8.69 U/gm of their control. The results in Tables (4&5) and Figures (4A&4B) also disclosed that glutathione (GSH) concentrations were also significantly decreased in exposed snails in comparison with their concentrations in their control. As their concentrations decreased with increasing exposure time to 7 days, being 70.45, 35.24, 31.16, 142.56 and 149.35 nmol/gm, respectively if compared to 158.20 nmol/gm of their control in cement lining. While in mud condition were 59.15, 44.55, 40.64, 132.77 and 141.09, nmol/gm compared to 155.20 nmol/gm of untreated control ones.

The lipid peroxidation, represented by Malondialdehyde (MDA) concentration, measured as thiobarbituric acid reactive substances, was significantly increased in exposed snails, as their concentrations in tissues of *P. acuta* at 2hrs post exposure were 445.10, 612.24, 628.33, 448.12 and 403.14 nmol/gm of the fungus, Fe, Cd and Fe & Cd/fungus treatments compared to 370.15 nmol/gm of unexposed snails, and reached 591.01, 701.22, 989.77, 393.44 and 387.10 compared to 371.38 nmol/gm of their control at 7 days post exposure in cement lining, respectively. On the other hand in unlined condition (mud), the lipid peroxidation concentration were 416.30, 54.35, 609.23, 459.39, 429.02 nmol/gm, compared to 370.11 nm/gm for control ones at 2 hrs of snail exposure, and increasing time of exposure to 7 days reached their concentration to 516.01, 664.99, 838.84, 416.33, 407.33 nmol/gm, respectively if compared to untreated snails (371.26 nm/gm) Tables (4 & 5) and Figures (4A & 4B). From these results also, it was clear that Cd treatment was more toxic than that of Fe.

DISCUSSION

In the present study *P. acuta* snails were used as biominitor for assessing the efficiency of biosorption treatments of the fungus *E. lapidosum* against Fe (III) and Cd (II). Snails can be serve as biomonitors for pollution and bioremediation strategies (Lee, 2000; Lee *et al*., 2001*a* and *b*). Lee *et al*. (2002) stated that the snail survival, growth, and histopathological changes were monitored in the mystery snail, *Viviparus georgianus*, and the mimic ponds snail, *Pseudosuccinea columella*, to evaluate their suitability as biomonitors for the efficacy of bioremediation treatments of oil spill in a freshwater marshland.

The obtained results claimed that the mortality percent of the adult *P. acuta* snails exposed for 2, 24 hrs and 7 days to the fresh mycelia of the fungus *E. lapidosum*, (50 ppm of Fe (III) & Cd (II)), the biosorption process of the fungus against Fe and Cd in lined conditions (cement lining) were higher than unlined conditions (mud). These results are in accordance with El-Said *et al*. (2009) who reported that the two types of soils (alluvial and sandy) had a significant reducing effect on the molluscicidal activity of the plants *Agave attenuata* and *Agave filifera* against *B. alexandrina* snails. They attributed these findings to the adsorption or absorption of some plant compounds on the soil particles, suggested that the lining of water bodies may be effective in the success of molluscicidal operations. Also, the results indicated that Cd exposure is somewhat more toxic to the snails than Fe exposure and the lowest mortality percents was that of the biosorption treatments against heavy metals. It was also observed that increasing the time of exposure caused a marked increasing in the mortality percent. It can be speculated that Fe and Cd induced mortality of *P. acuta* snails and this attributed to the damage caused by these chemicals to different body organs and functions within the snails.

Moreover, the effects of Cd and Fe on the mortality of *P. acuta* observed during the present study generally were similar to those reported by Ravera (1991); Abd-Allah *et al.*(2003); Hill (2005) and Wadaan (2007) for *Physa gyrina*, *B. alexandrina*, *Biomphalaria glabrata* and *P. acuta*, Respectively.

The present results recorded that the metal biosorption values by *E. lapidosum* against Cd for 2, 24 hrs and 7 days of exposure in cement lining were higher than that of Fe. Similar results obtained by Baik *et al*. (2002) who studied the biosorption of heavy metals by whole mycelia and selected components of *Aspergillus niger*, *Rhizopus oryzae* and *Mucor rouxii*. Also, Shivakumar *et al*. (2014) stated that,

Aspergillus niger and *Aspergillus flavus* showed almost similar metals uptake ability. *Aspergillus niger* showed high accumulation of Fe (75%) followed by Zn(49%)> Cu(45%)> Cr(41%) >Ni(25%). *Aspergillus flavus* has accumulated high percentage of Pb(82%) followed by Zn (40%)>Cu(34%)> Ni(20%). These results also were similar to those reported previously for several fungal species exposed to levels of heavy metals at different periods of exposure, e.g. *Penicillium purpurogenum* (Say *et al*., 2004), *Rhizopus arrhizus* (Preetha and Viruthagiri, 2005), *Pencillium chrosogenum* (Pazouki *et al*., 2007) *Pencillium simplicissimum* (Fan *et al*., 2008), *Pencillium janthinellum* (Kumar *et al*., 2008), *Aspergillus niger* (Amini *et al*., 2009), *Aspergillus fumigatus* (Bhainsa and D'Souza, 2009) and *Alternaria alternata* (Bahobil *et al*., 2017) .

The effect of the tested fungus, *E. lapidosum*, on the snail's life in the presnt work could be attributed to secondary metabolites of that fungus. This genus has been recognized as a rich source of biosctive secondary metabolites (Fill *et al*., 2007). Recent examples include the anticancer berkelic acid from *Penicillium* sp., polyketides with HIV integrate inhibitory activity from *P. chrysogenum* and insecticidal paraherquamides H and I from *Penicillium cluniae* (Singh, 2003 and Stierle *et al*., 2006). *P. janthinellum* produced polyketides, basically hydroxyan-thraquinones, ergosterol and poliols (Marinhoa *et al*., 2005). The active metabolites of such species were identified as methyl gallte (phenolic compound). Lahlou (2004) investigated the activity of 14 phenolic compounds against *B. truncatus* where the most effective one was gallic acid compound with *para*-methoxy group. Recently, Gohar *et al*. (2013) isolated methyl gallate from the molluscicidal plant *Callistemon viminalis*.

Concerning the results of iron and cadmium analysis in soft tissues of *P. acuta* snails at 2, 24 hrs and 7 days of exposure demonstrated considerable variability in tissue metal concentrations. Generally, the iron concentrations were higher than the concentrations of cadmium. Fe and Cd concentrations were highly significantly increased in lined conditions if compared with that of unlined ones and the concentrations of accumulated heavy-metals were increased by increasing the time of exposure. On the other hand during the biosorption processes the concentrations of accumulated metals were decreased by increasing the time of exposure as a result of the action of the fungus *E. lapidosum* against Fe and Cd. These results were similar to those reported previously for several gastropod species exposed to levels of heavy metals at different periods of exposure, e.g. *Biomphalaria glabrata* (Abd-Allah *et al*., 2003), *Onchidium struma* (Lia *et al*., 2009), *Lymnaea luteola L.* (Das and Khangarot, 2011), *Melanoides tuberculata* (Shuhaimi *et al*., 2012), *Cepaea nemoralis* (Kowalczyk and Stryjecki, 2013), *Biomphalaria alexandrina* (Mostafa *et al*., 2014) and *Bellamya bengalensis* (Mahajan, 2015).

The present investigation suggested that, Fe, Cd and the fungus *E. lapidosum* induced alterations of some antioxidant activity indicated by the significant increase in the lipid peroxidation, represented by MDA concentrations and decrease of endogenous antioxidant enzymes (Catalase (CAT) and glutathione (GSH)) in tissues of *P. acuta* in lined conditions than that of unlined ones which reflect the dysfunction of exposed snails compared to control unexposed ones and the more time of exposure the more effect on the biochemical parameters.

On the other hand the effect of antioxidant activity decreased with increasing exposure time in the case of bioremediation treatment against Fe and Cd using the fungus *E. lapidosum* as a result of the action of the biosorption process and decreasing the metals concentrations, especially in the case of cadmium. From these

results also, it was clear that Cd treatment is more toxic than that of Fe. The results are in accordance with general statements that these deteriorations in the biochemical parameters of exposed snails is due to metal-induced oxidative stress which is responsible for physiological changes and disorders in the body function (Chen *et al*., 2006 and Espín *et al*., 2014).

The data obtained here demonstrated that, Fe and Cd increasing lipid peroxidation is attributed to the observed deficiency in CAT and GSH. These antioxidants are essential for prevention against lipid peroxidation in all aerobic organisms. They indirectly protect cells against the adverse effects of xenobiotics, carcinogens and toxic radicals (Griffith, 1999). Lipid peroxidation has been suggested to play a role in metal toxicity, with numerous studies undertaken using malondialdehyde (MDA) as biomarker of oxidative stress (Steven and Nerishi, 1992; Khalil, 2015 and Ogunka-Nnoka *et al*., 2018).

Studies on Cd toxicity showed that it inhibits the activity of majority of enzymes involved in anti-oxidations (Casalino *et al*., 2002) inducing an increased production of free radicals, lipid peroxidation, and destruction of cell membranes (Ognjanovic *et al*., 2003). Cd is also reported to inhibit the activities of many enzymes by binding to their sulfhydryl groups or by inhibiting the protein synthesis (Waisberg *et al*., 2003).Similar to present findings, Siwela *et al*. (2010); Bakry *et al*., (2013) and Basopo *et al*., (2014) were observed on *Biomphalaria alexandrina, Lymnaea natalensis* and *Helisoma duryi*, respectively.

Finally, it can be concluded that *P. acuta* snail and the fungus *E. lapidosum* were useful in the searches for biomarkers and biosorption, respectively.

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ARABIC SUMMARY

كفاءة قوقع *Physa acuta* كمؤشر حيوى لتقييم كفاءة عملية المعالجه الحيويه لعنصري الحديد والكادميوم بإستخدام فطر *Eupenicillium lapidosum* فى الظروف المعمليه المبطنه وغير المبطنه.

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كان الهدف من هذه الدراسه هو تقييم كفاءة قوقع *Physa acuta* كمؤشر حيوى لتقييم كفاءة عملية المعالجه الحيويه لعنصري الحديد والكادميوم بإستخدام فطر *Eupenicillium lapidosum*. لذلك تم إختبار تأثير هذه المواد الملوثة على نسبة الوفيات لهذا القوقع وتركيز عنصري الحديد والكادميوم فى أجسامها وأنشطة الأنزيمات المضادة للأكسدة تحت الظروف المعملية المبطنة (أسمنت و رمل) والظروف الغير مبطنة (الطين). وقد أوضحت النتائج أيضا أن نسب وفيات قواقع *Physa acuta* بعد التعريض لمدة ساعتين ، ٢٤ ساعة و٧ أيام لتركيز ٥٠ جزء فى المليون من محلول عنصرى الحديد والكادميوم ، ميسيليا فطر*Eupenicillium lapidosum* ، و أخيرا التعريض لعملية المعالجة الحيوية للعنصرين السابقين كانت أعلى فى الظروف المبطنة بالاسمنت عنها فى الظروف غير المبطنة. وأيضا خلصت النتائج إلى ان عنصر الكادميوم أكثر سمية للقواقع عن عنصر الحديد ، بينما اقل نسب وفيات للقواقع كانت خلال عملية المعالجة الحيوية. وقد أوضحت النتائج أن كفاءة إمتصاص فطر *Eupenicillium lapidosum* للحديد والكادميوم انها كانت أعلى لعنصر الكادميوم عنها لعنصر الحديد فى الظروف المبطنة بالأسمنت عنها فى الظروف غير المبطنة (الطين). أما بالنسبة لنتائج تحليل عنصري الحديد والكادميوم فى أنسجة قواقع *Physa acuta* أوضحت أن تركيزات عنصر الحديد كانت أعلى من تركيزات عنصر الكادميوم فى النسيج، وعلى العكس خلال عملية المعالجة الحيوية فإن تركيزات العناصر تقل بزيادة وقت التعريض. أما بالنسبة للقياسات الكيميائية الحيوية لقواقع*Physa acuta* المعرضة لعنصرى الحديد والكادميوم، فطر*Eupenicillium lapidosum* و عملية المعالجة الحيوية للعنصرين السابقين فى الظروف المعملية المبطنة وغير المبطنة فقد اوضحت النتائج وجود تغيرات فى أنشطة الأنزيمات المضادة للأكسدة وهذا واضح فى زيادة تأكسد الدهون وإنخفاض فى الإنزيمات المضادة للأكسدة الذاتية (إنزيم الكاتلاز (CAT) والجلوتاثيون (GSH) فى الظروف المبطنة (الاسمنت) عنها فى الظروف غير المبطنة (الطين) ويزيد التأثير بزيادة وقت التعريض، وعلى العكس فإن التأثير يقل على القواقع الموجودة خلال عملية المعالجة الحيوية لعنصرى الحديد والكادميوم نتيجة لفعل عملية الإمتصاص الحيوى للعناصر بواسطة الفطر وبالتالى تقل تركيزات العناصر وخاصة مع عنصر الكادميوم.ولذا فإن هذه الدراسة تؤكد أن قواقع *Physa acuta* وفطر *Eupenicillium lapidosum* يمكن إستخدامهما فى الدراسات الخاصة بالمؤشرات الحيوية أو الإمتصاص الحيوى على التوالى.