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Accumulation and toxicological effects of cadmium, copper and zinc on the growth and photosynthesis of the freshwater diatom *Planothidium lanceolatum (*Brébisson) Lange-Bertalot: A laboratory study

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Abstract
Studies on toxicity and tolerance of cadmium (Cd), copper (Cu) and Zinc (Zn) in the diatom *Planothidium lanceolatum (Brébisson) Lange-Bertalot* (*P. lanceolatum*) were conducted by short-term bioassays using cell density, mortality and photosynthesis apparatus parameters. Results showed that the growth of the diatom was acutely sensitive to high metal concentrations in the order: Cd>Zn>Cu. Also, the IC50 calculated for this diatom are 0.25; 0.35 and 0.62 mg.L^{-1} for Cd, Zn and Cu, respectively. The statistical analysis showed that there was a significant effect on Fv/Fm and on chlorophyll-a of *P. lanceolatum* at Cd, Zn and Cu concentrations of ≥0.1; ≥0.2 and >0.4 mg.L^{-1}, respectively, showing that photosynthesis is more affected then growth. Nevertheless, the diatom is tolerant to these metals in comparison with other works and most tolerant to Cu because the accumulation of this element was found to have the greatest affinity for the *P. lanceolatum* cells. Thus, this diatom widely available could be used as an economic biosorbent cheap material for the removal of these ions especially Cu from Tensift River waters. Further studies are needed to increase the biosorption capacities of biomass and develop appropriate biological technologies applicable in the wastewater treatment.

Keywords: Planothidium lanceolatum, toxicities and tolerances, metals, accumulation.

1. Introduction
Trace metals enter rivers from different sources including domestic and industrial wastewaters, agricultural runoff, and release from contaminated sediments or atmospheric deposition [1, 2]. The input of these pollutants into the aquatic ecosystems can mainly be diffuse (non-point-source pollution) or punctual (point-source pollution), depending on their source, and may have different environmental consequences. Some heavy metals are well-known as freshwater and marine pollutants, and much interest has been dedicated to elucidate their toxic effects on algae [3, 4].
Heavy metals such as Cd, Cu and Zn, in wastewaters are hazardous to the environment. These ions may cause toxic and harmful effects to living organisms in water and to the consumers of it [5]. For this reasons, the development of systems for their removing from polluted areas is necessary [6]. Recently, heavy metal

biosorption using biological material has emerged as a potential alternative instead of the existing conventional physicochemical methods [7-9]. Among the biological material, algae have proved to be advantageous because they present several advantages, i.e. economic regeneration, metal recovery potentiality, lower volume of chemical and / or biological sludge to be disposed off, high efficiency in dilute effluents and large surface area to volume ratio [10]. The bioconcentration degree by diverse phytoplankton has also been extensively studied [11]. Phytoplankton has the ability to detoxify excess metals by producing extracellular and intracellular binding compounds (e.g. phytochelatins) [12].
In Marrakech (Morocco), an important pollution is generated by the industry and mining which discharges metals into the Tensift River, mainly Cd, Cu and Zn [13]. Industrial wastewater and mining wastes flows directly into the environment without any treatment. In Morocco, freshwater microalgae especially diatoms are only used for ecological purpose or for diatom indices applications to evaluate river water quality. There are only few studies on metal toxicity and on diatom ability to remove metals from the environment [14].
The objective of this study was to investigate the ability of the diatom *P. lanceolatum* to tolerate Cd, Cu and Zn by the determination of a share of the toxicity over 72h under laboratory conditions, compare *P. lanceolatum* tolerance for the three heavy metals and their accumulation after the exposure time, and to evaluate, on the other hand, the relationships between toxicity and accumulation of Cd, Cu and Zn by this diatom because little information is available on the relationships between these two aspects (Accumulation, toxicity). The main interest of this microalga lies in its ability to adapt easily to environmental factors and it is dominant during all the year [15].

2. Material and methods

The places of procurement of chemicals and instruments used in the experimental study are the laboratory of Bioprocess Engineering at the Faculty of Sciences and Techniques and the Hydrobiology, Eco-toxicology and cleansing Laboratory at Semlalia Science Faculty in Marrakech.

2.1 Isolation and cultivation of P. lanceolatum:

The microalga used was the benthic *P. lanceolatum* isolated from the Tensift River. *P. lanceolatum* was grown in sterilized modified WC medium at pH 7.0 (Wright's Cryptophyta) [16]. Algal cultures were maintained in Erlenmeyer flasks of 2 liters-capacity in order to provide sufficient quantity of biomass for experiments. Cultures were incubated in a culture room illuminated at 72 µE m-2 s^{-1}, they were shacked under a light/dark cycle of 16/8 during 10 days at 25°C. Cultures were checked regularly microscopically. These cultures were deemed axenic.

2.2 Toxicity and Accumulation of Cd, Cu and Zn:

A batch method was used for growth-rate-inhibition bioassays and accumulation using 250 mL borosilicate glass Erlenmeyer flasks, coated with Coatasil silanising solution to prevent adsorption of heavy metals to the glass. Test flasks were soaked in 10% (v/v) nitric acid overnight and rinsed thoroughly with distilled water. A stocks solutions (100 $mg.L^{-1}$) of Cd, Cu and Zn were prepared from cadmium chloride ($CdCl_2$), copper sulfate ($CuSO_4$ $5H_2O$) and zinc chloride ($ZnCl_2$) using sterilized distilled water respectively, which was further diluted to different concentrations (0, 0.1, 0.2, 0.4, 0.8 and 1.6 $mg.L^{-1}$) of Cd, Cu and Zn using the WC medium lacking EDTA. It has been demonstrated that EDTA greatly decreases the toxicity of heavy metals by chelating them [17, 18]. The higher concentrations were used to better simulate concentrations rejected in the local environment. The sub-lethal of Cd, Cu and Zn on *P. lanceolatum* was determined using 72-h old diatom cultures. Sub-samples (5 ml) were taken from each flask at the beginning of each toxicity test and acidified, prior to determination of total dissolved Cd, Cu and Zn. Each flask was inoculated with 10^5 $cells.mL^{-1}$ of a prewashed *P. lanceolatum* suspension according to OECD [19]. Flasks microcosms were incubated under the same conditions as mentioned above.

2.2.1 *Density and biomass algal:*

Algal cell density of each flask microcosm was measured daily using a hemocytometer Malassez cell and calculated as cell numbers ($cells.mL^{-1}$).

2.2.2 *Photosynthetic activity and photosynthetic pigment (Chlorophyll a):*
The maximal photosynthetic efficiency (Fv/Fm) and its kinetics were assessed in different concentration of Cd, Cu and Zn during ten days. Before recording the fluorescence, all samples were adapted to the dark for 2 min. Sufficient to allow complete re-oxidation of the photosystem (PS) II reaction centers. The fluorescence emission rates were evaluated using a handy Plant Efficiency Analyser fluorometer (Handy PEA fluorimeter; Hansatech Instruments Ltd, King's Lynn, UK) with a maximum light intensity of 3000 $\mu mol.m^{-2}.s^{-1}$.
Other parameters, based on the theory of energy flow in PSII and using the JIP test [20], were calculated. Such as ABS/CS (Absorbed energy flux per cross-section) expresses the total number of photons absorbed, TRo/CS (Trapped energy flux per CS) describes the maximal rate by which an excitation is trapped, ETo/CS (Electron transport flux per CS), DIo/CS (Dissipation energy per CS), RC/CSo and RC/CSm (Numbers of active reaction centres in the state of fully oxidized and reduced PSII reaction centre respectively) and PI (Performance Index).
After 72 hours of exposure, the spectophotometric analysis of chlorophyll a was based on the standard method of Rodier [21]. Subsamples (50 ml) were filtered through cellulose filter (0.45 µm). The chlorophyll extraction was carried out in 90% acetone. After centrifugation (4000 tr/min, 5 min), the supernatant was carried out by spectrophotometer at 630, 645, 663 and 750 nm.

2.2.3 *Metal accumulation:*
After 72 hours of exposure, the algal cells were filtered on a 0.45 µm Millipore filter, the resulting filtrate was acidified with ultrapure nitric acid and stored in the refrigerator before the analysis. The determination of Cd, Cu and Zn in the filtrate (free metal) was performed by a flame atomic absorption spectrophotometer (UNICAM 929). [Metal accumulated] = [total metal] (applied) - [free metal] [22]. The bioconcentration factor (BCF) was calculated as the ratio between the accumulation amount of metals (mg/g-dry wet) and their concentration in solution.

2.3 Statistical analysis
All the experiments were carried out in triplicate and the mean values with standard deviation are presented. The 72-h IC50, i.e. the inhibitory concentration to reduce the growth rate by 50%, was calculated, during exponential growth, using linear interpolation method for sub-lethal toxicity using statistical software (ICp Ver.2.0) [23]. Measured Cd, Cu and Zn concentrations were used in all calculations of toxicity endpoints. The data were tested for normality and homogeneity of variance, and tests for significance between treatments were determined using a one-way analysis of variance (ANOVA), and the Student-Newman-Keuls (S-N-K) ($P < 0.05$) test was used for detection of differences between groups. All analyses were carried out using the program SPSS 17.0 for Windows.

3. Results and discussion

3.1 Toxicity

3.1.1 Algal density:
The effect of increasing Cd, Cu and Zn concentrations on the cell density of *P. lanceolatum* is shown in Figure 1. The exposure of *P. lanceolatum* for 72h to different concentrations of Cd, Cu and Zn (0, 0.1, 0.2, 0.4, 0.8 and 1.6 $mg.L^{-1}$) showed an exponential negative relationship between cell density (R^2=0.941, R^2=0.969 and R^2=0.974 for Cd, Cu and Zn respectively) and the amount of metal supplied to the medium. The decrease in the cell density with increasing of metal concentration was most pronounced with Cd, followed by Zn and Cu. According to Toress et al. [24] growth is a good indicator of the toxic action of metals in microalgae and reflects the metabolism of the cell. During the 72 h of experiment duration, when metal added were increased, the growth of *P. lanceolatum* was inhibited. However *P. lanceolatum* were acutely sensitive to metals in the order: Cd>Zn>Cu, including therefore including *P. lanceolatum* in the group of the most tolerant organisms to copper. Indeed, other species are indicated tolerant to copper at less than 1.6 $mg.L^{-1}$ [25]. According to other works [25, 26] algal density was affected by these metals (Cd, Cu and Zn) but more tolerance was observed with Cu.

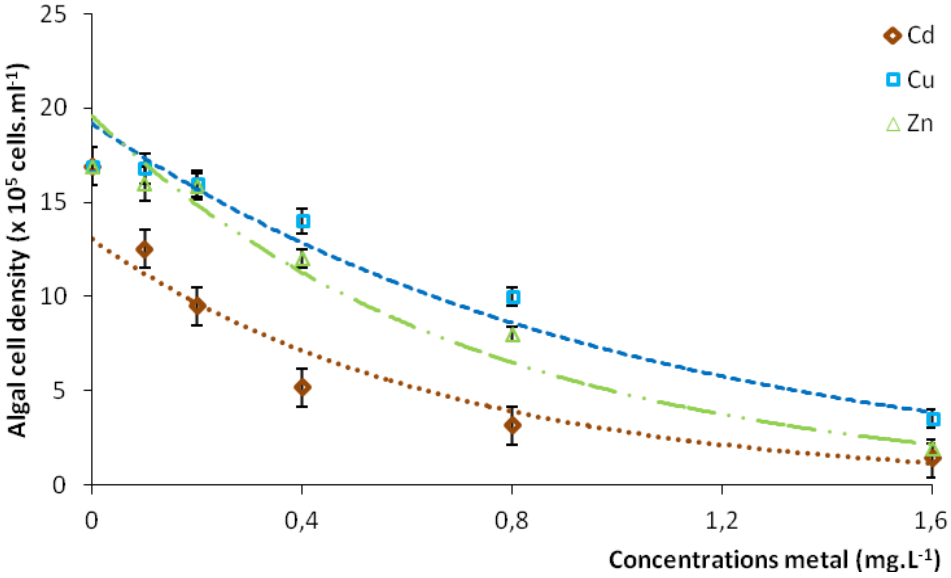

Figure 1: Effect of cadmium. copper and zinc concentrations on diatom *P. lanceolatum* growth after 72h exposure on WC medium. (mean ±S.E.)(n=3).

In addition, the IC50 values calculated are 0.25, 0.35 and 0.62 for Cd, Zn and Cu, respectively (Table 1). The comparison of the IC50 values of heavy metals in *P. lanceolatum* is interesting for discussion. The results indicated that the alga was most sensitive to Cd (0.25 $mg.L^{-1}$), followed by Zn (0.35 $mg.L^{-1}$) and Cu (0.62 $mg.L^{-1}$). Although Cu and Zn are essential metals for living organisms, these metals can be toxic and can cause algal cell death at elevated concentrations. Comparing these IC50 values with those just mentioned in table 1, our results show, generally, higher values for Cu, Cd and Zn, but no study of toxicity was made for *P. lanceolatum* for such a comparison, Taken together, there are both similarity and difference in the toxicity data from the present and previous studies. However, this is unavoidable since it is generally problematic to compare IC and EC values between different studies, in which toxicity tests have been carried out with various laboratory apparatus, toxicity criteria, algal species and experimental conditions (e.g., temperature, culture medium, salinity, exposure time, CO_2 availability, light source and light intensity).

3.1.2 Photosynthetic activity and Photosynthetic pigment (Chlorophyll a):

At the physiological level, the measurement of photosynthetic efficiency (Fv/Fm) and the JIP test are useful and effective parameters to assess the photosynthetic status of alga under heavy metals stress conditions. The above described physiological measurements provide a first insight into the changes of the photosynthetic apparatus upon the action of Cd, Cu and Zn. A physicochemical approach, based on fluorescence induction measurements, according to Strasser and Strasser [27], was chosen to get more information about the changes in structure and functioning of the photosynthetic apparatus of *P. lanceolatum* on metal addition. The Effect of Cd, Cu and Zn at different concentrations on maximum quantum efficiency (Fv/Fm) of photosystem II of diatom *P. lanceolatum* after 72h is presented in Figure 2. Data were analyzed by ANOVA and Student-Newman-Keuls (S-N-K) test ($P \leq 0.05$). As the Cd, Cu and Zn concentrations increased in the medium, Fv/Fm decreased. The statistical analysis showed that there was a significant effect on Fv/Fm of *P. lanceolatum* at Cd, Zn and Cu concentrations of ≥ 0.1 , ≥ 0.2 and ≥ 0.4 $mg.L^{-1}$, respectively.

Figure 2 indicating that the three metals had an inhibitory effect in the following order Cd>Zn>Cu on the photochemical activity of *P. lanceolatum*. In our study, metal toxicity was quantified as the inhibition of the maximum photosystem II quantum yield. The Fv/Fm can reveal the mechanisms involved in metal toxicity [28]. Cd was thought to inhibit PSI and PSII activity [29, 30] ultimately resulting in a significant reduction of Fv/Fm which indicated direct or indirect photosynthetic activity impairments [28, 31]. However, Zhou *et al.* [32] proposed that the inhibitory site of Cd in *Microcystis aeruginosa* was not located at the PSII or PSI level, but was probably situated on the ferredoxin/NADP+-oxidoreductase enzyme at the terminus of full electron

transport chain. Miao *et al*. [28] suggested that the Fv/Fm and the cell-specific growth rate had comparable sensitivities in quantifying the toxic effects of Cd, Cu and Zn in marine phytoplankton. In fact, it is well known that Cd^{2+} disorganizes chloroplast causing the damage of photosynthetic pigments [33]. As a consequence of this, the photosynthetic activity could severely be affected, causing the growth inhibition or complete death of the cells. Biosynthesis of phycocyanin and carotenoid could also be affected by the heavy metal ions [34]. However, for zinc, growth inhibition may not be related to the intracellular metal concentration, but to extracellular zinc [35]. In fact, the possible mode of toxic action of zinc is related to the cell membrane, where it may disrupt the uptake of calcium which is necessary for the Ca-ATPase activity in cell division [36]. For copper, Stauber and Florence [37] found that it toxicity resulted from a reaction in the cytoplasm between copper and glutathione (reduced form,GSH), leading to oxidation of glutathione (oxidised form, GSSG), disturbance of the GSH:GSSG ratio, and suppression of mitosis. Copper concentrations that were inhibitory to cell division had no effect on other cell functions such as photosynthesis, respiration, ATP production, electron transport, and membrane ultrastructure to explain this resistance of *P. lanceolatum* for copper, cells continued to photosynthesise but were unable to divide, leading to an increase in cell size, similar to that reported for *Chlorella* sp. [38]. The reduction of Cu toxic effects with lower concentrations (<0.4 $mg.L^{-1}$) can be accounted for either as a result of algal requirement of this element in metabolic processes or explained by production of some organic compound which decreases metal toxicity. Albergoni *et al*. [39] and Rijstenbil *et al*. [40] suggested that some of the algae capable to produce metal binding compounds therefrom get the ability to bind and sequester copper ions in the cytoplasm and reduce toxicity. The corresponding compounds are thiols, glycoproteins and carbohydrates. Torres et al. [41] demonstrated that algae *Cylindrothica fusiformis* produces carbohydrate as a defense mechanism against copper toxicity in stationary phase when cells are exposed to 0.5 $mg.L^{-1}$. At longer exposure times, copper may affect subcellular organelles such as the chloroplast and mitochondria. Wong *et al*. [38] reported structural alterations to thylakoid membranes of *Chlorella* sp. and inhibition of photosynthesis.
There were no significant differences on Fv/Fm between control cultures and cultures with 0.1 and 0.2 $mg.L^{-1}$ concentrations (P>0.05) for Cu and cultures with 0.1 $mg.L^{-1}$ concentrations (P>0.05) for Zn (Figure 2), confirming that its effect on the photosynthetic apparatus is not a stress response.

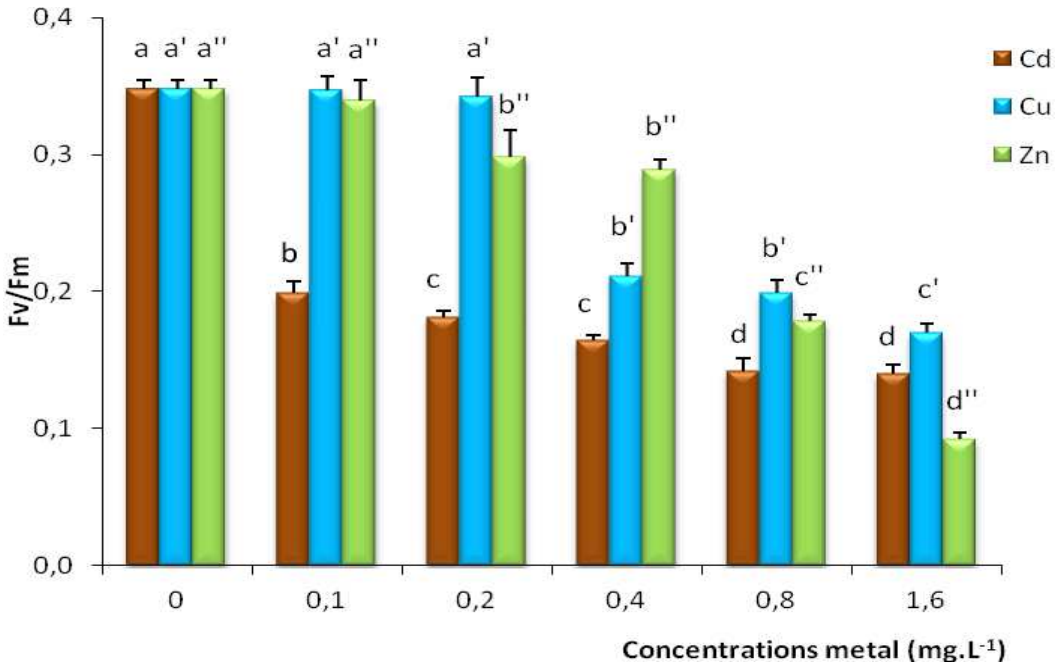

Figure 2: Effect of cadmium. copper and zinc at different concentrations on maximum quantum efficiency (Fv/Fm) of photosystem II of diatom *P. lanceolatum* after 72h exposure. Different lower-case letters next to the bars indicate significant differences ($p < 0.05$) among treatments (Student-Newman-Keuls (S-N-K) from ANOVA one way test) (mean ±S.E.)(n=3).

Table 1: Toxicity of Cd. Cu and Zn for different microalgae. according to different authors.

Microalgae species	Endpoint	Metal toxicity (mg.L^{-1})			Exposure time (h)	References
		Cd	Cu	Zn		
Planothidium lanceolatum	IC50	0.25	0.62	0.35	72h	This study
Micractinium pusillum	IC50	0.28	-	0.34	72h	[22]
Isochrysis galbana	EC50	0.91	1.4	0.6	120h	[27]
Chaetoceros sp.	EC50	-	0.088	-	72h	[50]
Phaeodactylum tricornutum	IC50	-	0.008	-	72h	[51]
Thalassiosira pseudonana	IC50	0.0078	-	-	96h	[52]
Chlorella vulgaris	EC50	-	-	0.153	72h	[53]
Nitschia palea	IC50	0.0276	-	-	120h	[54]

Regarding changes in pigment composition (chlorophyll a), in autotrophic cultures of *P. lanceolatum*, grown under different concentrations of Cd, Cu and Zn (Figure 3), the same results were observed with respect to chlorophyll-a concentrations as another indicator for algal growth, which had offered evidence that Cu, Zn and Cd may affect the chlorophyll synthesis or photosynthetic activity at concentrations of ≥0.1, ≥0.2 and ≥0.4 mg.L^{-1} for Cd, Zn and Cu respectively (Figure 3), like the Fv/Fm, except for Cu metal were a significant effect on the diatom was at concentrations more than 0.4 mg.L^{-1}. Thus a good linear relationship was observed between the chlorophyll a concentration and maximum quantum efficiency (Fv/Fm) of photosystem II. There were no significant differences on chlorophyll a between control cultures and cultures with <0.4 mg.L^{-1} (P>0.05) for Cu and culture with 0.1 mg.L^{-1} concentrations (P>0.05) for Zn.

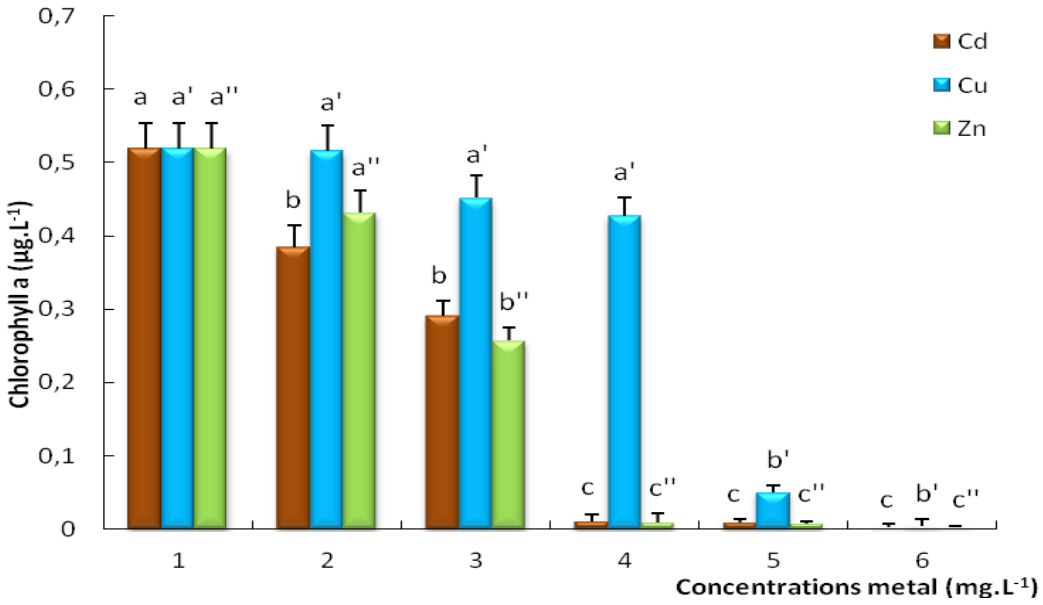

Figure 3: Effect of cadmium. copper and zinc at different concentrations on diatom *P. lanceolatum* Chlorophyll a after 72h exposure. Different lower-case letters next to the bars indicate significant differences ($p < 0.05$) among treatments (Student-Newman-Keuls (S-N-K) from ANOVA one way test) (mean ±S.E.)(n=3).

The effect of Cd, Cu and Zn on kinetic of maximum quantum efficiency (Fv/Fm) of photosystem II in *P. lanceolatum* was also determined. The three heavy metals treatments with 0.2 mg.L^{-1} concentrations presented clear evidence of disturbing photosystem II (Figure 4). Since fluorescence responses (Fv/Fm) were much lower than controls for Cd treatments, due to the rapid decreases in Fv/Fm, which produced measurements lower than 0.18 relative units. This decrease was rather slow in the presence of Zn, while 0.2 mg.L^{-1} reduced

Fv/Fm to 0.26 but then increased again after 2 days of incubation. However, during these 10 days of exposure, Cu reflected a less significant toxic impact on photosynthesis, with the Fv/Fm of 0.33 compared to 0.36 in control. This situation is reversed quickly to be stabilized, where proteins and compounds photosynthetic are resynthesized [42], this mechanism is often found in resurrection plants [43], therefore, *P. lanceolatum* have an adaptation mechanism of photosynthetic alteration produced by heavy metals (Cd, Cu and Zn) stress conditions.

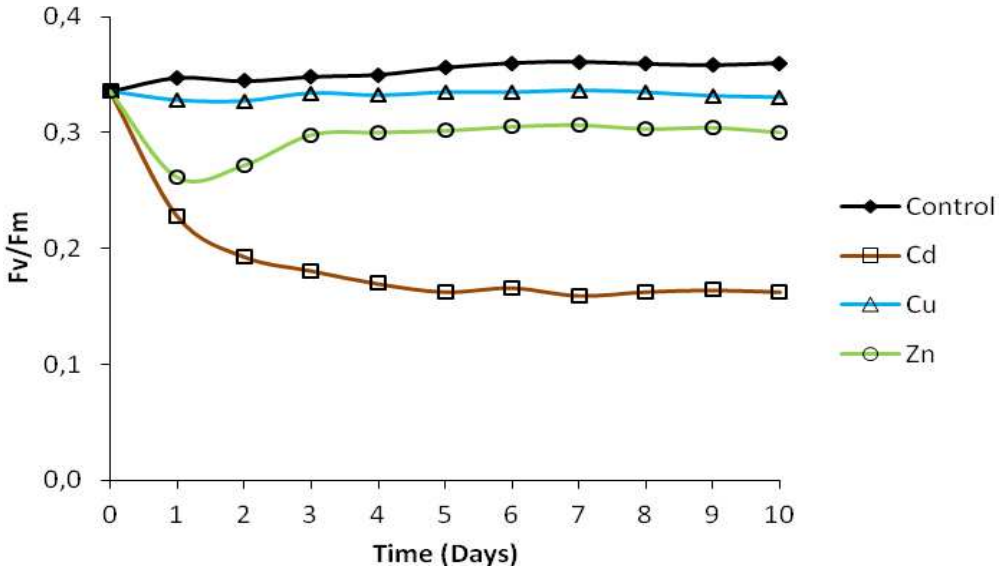

Figure 4: Kinetic of maximum quantum efficiency (Fv/Fm) of photosystem II in *P. lanceolatum* treated with 0.2 mg.L^{-1} of Cd. Cu and Zn (mean ±S.E.)(n=3).

JIP-test parameters (Table 2) showed that Cd, Cu and Zn treatment decreased the absorbed energy flux per cross-section (ABS/CS), trapped energy flux per CS (TRo/CS), electron transport flux per CS (ETo/CS), dissipation energy per CS (DIo/CS), RC/CSo and RC/CSm. Finally, these changes resulted in a decrease in the performance index (PI). In addition to the results mentioned above, the JIP analysis, was done to assess the effect of Cd, Cu and Zn on electron transport at the acceptor site of PS II, which show that the three heavy metals induce changes in energy fluxes in the photosynthetic apparatus of *P. lanceolatum*, there is reduction of all parameters JIP for three metals, but this reduction is more accentuated for Cd than Zn and Cu . The total light energy flux absorbed (ABS) significantly decreased with the increase in the concentration; however, this energy was slightly dissipated (DIo). These changes were accompanied by an incomplete supply of energy to the reaction centres (RC) of the PS as the trapping energy (TRo) decreased as did the number of RC. This reduction was reflected in a low electron transport flux (ETo) involved in CO_2 fixation. These changes in the photosynthetic apparatus have been observed in different organisms affected by diverse heavy metals [44-46].

3.2 *Metal accumulation:*

In addition, the physiology itself may have an overall effect on the way in which the metal is accumulated in the cell. It is demonstrated that there are two phases in metal adsorption by microalgae: a first phase, not dependent on cellular metabolism, where metal binds to the cellular surface, and a second, slower phase, dependent on metabolism, where metal is accumulated in the interior of the cell [18]. Table 3 shows the total amount of three metal element biosorbed by this microalga as function of different metals concentrations in the medium after 3 days of exposure, and the resulting concentration factor (ratio of metal concentration/g dry weight of cells to concentration per ml of metal test solution). BCF of the Cu was found to have the greatest affinity for the cells (1918.86), while Cd had intermediate, and Zn the lowest. To explain this lowest affinity for Zn (BCF=549.03), Macfie and Welbourn [47] found that metals in increasing order of affinity for the cell wall in *Chlamydomonas reinhardtii* were Ni, Co, Cd and Cu, wich matche the order of total metal content of the cell with Ni being accumulated in the least amounts.

Table 2: Summary of selected JIP-test parameters of *P. lanceolatum* after 72h exposure to various metal concentrations of Cd. Cu and Zn.

Metals	C ($mg.L^{-1}$)	P.I.	ABS/CS	TRo/CS	ETo/CS	DIo/CS	RC/CSo	RC/CSm
Cd	0	0.18±0.001	25.16±0.310	8.35±0.140	5.08±0.002	16.81±0.540	2.64±0.025	5.13±0.170
	0.1	0.12±0.001	15.01±0.120	2.52±0.080	2.66±0.090	12.48±0.160	2.01±0.001	2.02±0.110
	0.2	0.07±0.001	14.01±0.100	1.80±0.007	2.47±0.100	12.19±0.002	1.54±0.071	1.72±0.130
	0.4	0.04±0.007	13.15±0.090	1.55±0.001	1.56±0.030	10.45±0.050	0.75±0.002	1.02±0.056
	0.8	0.03±0.001	12.83±0.110	1.26±0.005	1.38±0.006	09.87±0.070	0.64±0.001	0.93±0.002
	1.6	0.01±0.003	12.50±0.080	1.08±0.003	1.11±0.001	09.82±0.170	0.57±0.001	0.85±0.009
Cu	0	0.18±0.001	25.17±0.310	8.35±0.140	5.08±0.002	16.81±0.540	2.64±0.025	5.13±0.170
	0.1	0.16±0.001	20.17±0.330	6.97±0.110	4.84±0.120	14.19±0.230	1.90±0.140	3.23±0.150
	0.2	0.14±0.001	18.83±0.210	4.91±0.100	3.66±0.076	13.92±0.170	1.74±0.010	2.41±0.007
	0.4	0.11±0.001	17.67±0.170	3.68±0.060	2.79±0.012	13.88±0.007	1.21±0.002	2.09±0.013
	0.8	0.06±0.001	17.02±0.090	2.80±0.007	2.71±0.021	13.49±0.032	1.05±0.006	1.28±0.001
	1.6	0.05±0.001	16.83±0.013	2.05±0.090	2.66±0.004	12.98±0.001	0.91±0.001	1.06±0.003
Zn	0	0.18±0.001	25.17±0.310	8.35±0.140	5.08±0.002	16.81±0.540	2.64±0.025	5.13±0.170
	0.1	0.18±0.001	24.56±0.430	7.19±0.100	5.02±0.091	17.31±0.420	2.82±0.020	4.49±0.310
	0.2	0.16±0.001	21.83±0.260	6.91±0.002	3.79±0.054	15.92±0.089	2.34±0.001	3.57±0.008
	0.4	0.16±0.001	21.58±0.050	6.11±0.016	4.62±0.130	15.38±0.005	1.85±0.034	3.44±0.014
	0.8	0.14±0.001	20.83±0.120	4.76±0.002	2.84±0.008	14.07±0.120	1.59±0.002	2.96±0.001
	1.6	0.02±0.001	15.53±0.007	1.26±0.001	1.86±0.001	13.23±0.190	0.01±0.001	1.05±0.005

Values represent mean of six measurements.

The recently developed biotic ligand model, which suggested that metal toxicity to the organisms resulted from metal bioaccumulation in the algal cells, has been used to predict metal toxicity in organisms [48], which explains high BCF for Cd (1223.24) with large toxicity for *P. lanceolatum*, since the variations of the inhibition percentage of the toxicity parameters (Cell density, Fv/Fm and chlorophyll) were much more dependent on the intra-Cd concentration.

Table 3: Cadmium. Copper and Zinc biosorbed by *P. lanceolatum* and Bioconcentration factor after 72h exposure for different concentrations (mean ±S.E.)(n=3).

Initial metal concentration ($mg.L^{-1}$)	Metal Biosorbed ($mg.g^{-1}$ DW)			Bioconcentration factor		
	Cd	Cu	Zn	Cd	Cu	Zn
0.1	2.21±0.2	1.65±0.4	1.55±0.5	221	165	51.67
0.2	5.82±0.4	3.65±0.2	3.52±0.3	291	365	70.4
0.4	22.63±1.2	8.56±0.5	19.2±0.5	1131.5	856	120
0.8	71.98±1.5	53.8±1.2	53.58±1.1	1199.67	1054.87	357.2
1.6	275.51±3.1	134.32±1.9	118.66±1.7	1223.24	1918.86	549.03

DW : Dry Weight

The higher Cu accumulated in the exposed *P. lanceolatum*, could be due to induction of heavy metal peptides sequestration (phytochelatin) and detoxifying metals in vegetal cells. The induction of phytochelatin in phytoplanktonic algae has been widely demonstrated both in the laboratory cultures and in field studies. Naciri *et al*. [49] found correlation of the concentrations of Cu with the metal-binding polypeptides phytochelatin in *Tetraselmis suecica*. Thus, the highest level of metal biosorbed are 275.51±3.1, 134.32±1.9 and 118.66±1.7 $mg.g^{-1}$ DW occurred in cultures, respectively, with highest Cd, Cu, and Zn concentrate of 1.6 $mg.L^{-1}$. In comparison with other_algal species, this diatom accumulates largely more. Yap et al. [27] found that *Isochrysis galbana* is able to accumulate only 0.02, 0.11, and 0.30 $mg.g^{-1}$ at the initial concentrations of 1 mg $Cd.L^{-1}$, 0.5 mg $Cu.L^{-1}$, 0.5 mg $Zn.L^{-1}$, respectively. There is considerable potential for using *P. lanceolatum* to

treat wastewaters discharged into the Tensift River taking into account Cd, Cu and Zn concentrations in Tensift River wastewaters of 1.66, 8.85 and 15.9 $\mu g.L^{-1}$, respectively [22]. In addition this microalga is present during all the seasons.

Conclusion

This study indicates that the diatom *P. lanceolatum* which is widely available can be used as biosorbent material for removal of heavy metals ions, especially Cu and Zn from Tensift River waters. In comparison with other studied algae, *P. lanceolatum* is more resistant to Cu, Cd and Zn and can accumulate high amounts of these elements. Therefore, when the concentration of these three metals is higher, *P. lanceolatum* cells are able to accumulate more metal, until they reach a toxic level. Nevertheless, the diatom is most tolerant to Cu. And the accumulation of this element was found to have the greatest affinity for the cells. Changes in the photosynthetic apparatus have been also observed; they decrease with the increase of metal concentrations and are more affected then growth. Further studies are needed to increase the biosorption capacities of biomass and develop appropriate biological technologies in the treatment of wastewaters because the natural field conditions might contain other metals and pollutants and the biochemical and physiochemical interactions among these collective pollutants could be complicated process. Later on, practical applications of such techniques at larger scales would be useful for bioremediation of heavy metal polluted wastewaters since there is a lack of industrial wastewater treatment. Therefore, *P. lanceolatum* - and possibly other microalgae - may have the potential to be used as an ecofriendly and economic biosorbent cheap material for the removal of toxic metals in polluted waters.

References

1. Nriagu, J.O., Pacyna, J.M. *Nature* 333 (1988) 134.
2. Allen, H.E., Garrison, A.W., Luther III, G.W. Metals in Surface Waters. Ann Arbour Press, Chelsea, MI (1998).
3. Reed, R.H., Gadd, G.M. Metal tolerance in eukaryotic and prokaryotic algae. In: Shaw, A.J. (Ed.), Heavy Metal Tolerance in Plants: Evolutionary Aspects. CRC Press, Boca Raton, FL, USA, (1990) 105.
4. De Filippis, L.F., Pallaghy, C.K. Heavy metals: Sources and biological effects. In: Rai, L.C., Gaur, J.P. (Eds.), Phycological Perspectives of Water Pollution. E. Schweizerbart Verlag, Stuttgart (1992).
5. Aksu, Z., Kutsal, T. *Environ. Technol.* 11 (1990) 979.
6. Torres, E., Cid, A., Herrero, C., Abalde, J. *Bioresource Technol.* 63 (1998) 213.
7. Aksu, Z. Biosorption of heavy metals by microalgae in batch and continuous systems. In: Wong YS, and Tam NFY (eds) Algae for waste water treatment. Springer Germany 99 (1998) 37.
8. Pavasant, P., Apiratikul, R., Sungkhum, V., Suthiparinyanont, P., Wattanachira, S., Marhaba, T.F. *Bioresour. Technol.* 97 (18) (2006) 2321.
9. Gupta, V.K., Shrivastava, A.K., Jain, N. *Water Res.* 35 (2001) 4079.
10. Gupta, V.K, Rastogi, A. *Colloid. Surface. B.* 64 (2008) 170.
11. Fisher, N.S., Reinfelder, J.R. The trophic transfer of metals in marine systems. In Metal Speciation and Bioavailability in Aquatic Systems, Tessier, A., Turner, D.R., Eds., John Wiley, New York, (1995) 363.
12. Moffett, J.W., Brand, L.E. *Limnol. Oceanogr.* 41 (1996) 388.
13. El Adnani, M., Rodriguez-maroto, J.M., Sbai, M.L., Loukili idrissi, L., Nejmeddine. A. *Environ. Technol.* 28 (2007) 969.
14. *Zerhouni, R. A. PhD Thesis, University Sidi Mohamed Ben Abdellah, Fès, Morocco (2003).*
15. Sbihi, K. *Mémoire DESA, University Cadi Ayyad*, Marrakech, Morocco (2006).
16. Guillard, R.R.L., Lorenzen, C.L. *phycologia.* 8 (1972) 10.
17. Sunda, W.G., Guillard, R.L. *J. Mar. Res.* 34 (1976) 511.
18. Moreno-Garrido, I., Lubián, L.M., Soares, A.M.V.M. *Ecotoxicol. Environ. Saf.* 47 (2000) 112.
19. OECD guideline 201. *Freshwater alga and cyanobacteria, growth inhibition test.* Organisation for Economic Co-operation and Development, Paris, 2006a.
20. Strasser, R.J., Srivastava, A., Tsimilli-Michael, M. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In Yunus, M., Pathre, U., Mohanty, P. [Eds.] Probing Photosynthesis: Mechanism, Regulation and Adaptation. Taylor and Francis, New York (2000) 445.

21. J. Rodier, *L'analyse de l'eau*, 7ème édition Dunod, Paris, 1984.
22. Toumi, A., Belkoura, M., Benabdallah, S., El Alami, M., Loukili Idrissi, L., Nejmeddine, A. *Environ. Technol*. 28 (2007) 19.
23. EPA, A linear interpolation method for sublethal toxicity: The Inhibition Concentration (ICp) Approach (Version 2.0). U.S. Environmental Protection Agency. Environmental Research Laboratory, Duluth, MN 55804, USA (1993).
24. Toress, E., Cid, A., Fidalgo, P., Herrero, C., Abalde, *J. Aquat Toxicol* 39 (1997) 231.
25. Yan, H., Pan, G. *Chemosphere* 49 (2002) 471.
26. Yap, C.K., Ismail, A., Omar, H., Tan, S.G. *Environ. Int.* 29 (2004) 1097.
27. Strasser, B., Strasser, R. Measuring fast fluorescence transients to address environmental questions: the JIP-test, in: P. Mathis (Ed.), Photosynthesis: From Light to Biosphere, vol. V, Kluwer Academic Press, Dordrecht (1995) 977.
28. Miao, A.J., Wang, W.X., Juneau, P. *Environ. Toxicol*. Chem. 24 (2005) 2603.
29. Atal, N., Saradhi, P., Mohanty, P. *Plant Cell Physiol*. 32 (1991) 943.
30. Neelam, A., Rai, L.C. *J. Microbiol. Biotechnol*. 13 (2003) 544.
31. Krupa, Z., Oquist, G., Huner, N.P.A. *Physiol. Plant*. 88 (1993) 626.
32. Zhou, W., Juneau, P., Qiu, B. *Chemosphere* 65 (2006) 1738.
33. Leborans, G.F., Novillo, A. *Water Res*. 30 (1996) 57.
34. Atri, N., Rai, L.C. *J. Microbiol. Biotech*. 13 (2003) 544.
35. Wilde, K.L., Stauber, J.L., Markich, S.J., Franklin, N. M., Brown, P. L. *Arch. Environ. Contam. Toxicol.* 51 (2006) 174.
36. Stauber, J.L., Florence, T.M. *Mar. Biol.* 105 (1990) 519.
37. Stauber, J.L., Florence, T.M. *Mar. Biol.* 94 (1987) 511.
38. Wong, S.L., Nakamoto, L., Wainwright, J.F. *J Appl. Phycol.* 6 (1994) 405.
39. Albegoni, V., Piccini, E., Coppellotti, O. *Comparative Biochemistry and Physiology* 67 (1980) 121.
40. Rijstenbil, J.W., Dehairs, F., Ehrlich, R., Wijnholds, J.A. *Aquatic Toxicology* 42 (1998a) 187.
41. Torres, M., Goldberg, J., Jensen, T.E. *Microbios* 96 (1998) 141.
42. Hader, D., Lebert, M., Sinha, R.P., Barbieri, E.S, Helbling, E.W. *Photochemical and Photobiological Sciences* 1 (2002) 809.
43. Farrant, J.M., Vander willigen, C., Loffell, D.A., Bartsch, S., Whittaker, A. *Plant, Cell and Environment* 26 (2003) 1275.
44. Zhang, D., Pan, X., Mu, G., Wang, J. *J Appl Phycol* 22 (2010) 479.
45. Pan, X., Chen, X., Zhang, D., Wang, J., Deng, C., Mu, G., Zhu, H. *J. Phycol.* 45 (2009) 386.
46. Pinto, E., Sigaud-Kutner, T.C.S., Leitao, M.A.S., Okamoto, O.K., Morse, D., Colepicolo, P. *J. Phycol.* 39 (2003) 1008.
47. Macfie, S.M., Welbourn, P.M. *Arch Environ Contam Toxicol* 39 (2000) 413.
48. De Schamphelaere, K.A.C., Stauber, J.L., Wilde, K.L., Markich, S.J., Brown, P.L., Franklin, N.M., Creighton,N.M., Janssen, C.R. Environ. Sci. Technol. 39 (2005) 2067.
49. Nassiri, Y., Ginsburger-Vogel, T., Mansot, J.L. *Biol Cell* 84 (1995) 221.
50. Debelius, B, Forja, J.M., Del Valls, A., Lubian, L.M. *Ecotoxicol. Environ. Saf.* 72 (2009) 1503.
51. Levy, J.L., Angel, B.M., Stauber, J.L., Poon, W.L., S.L. *Aquat. Toxicol.* 89 (2008) 82.
52. Wang, M.J. Wang, W.X. *Aquat. Toxicol.* 95 (2009) 89.
53. Muyssen, B.T.A. Janssen, C.R. *Chemosphere*. 45 (2001) 507.
54. Branco, D., Lima, A., Almeida, S.F.P., Figueira, E. *Aquatic Toxicology* 99 (2010) 109.