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Resistance of *Klebsormidium flaccidum* (Kützing) Silva, Mattox & Blackwell (Streptophyta) to heavy metals

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Abstract
The influence of copper ($1\text{-}10^{-10-2}$ M/L), nickel ($1\text{-}10^{-6-1}$ M/L) and manganese ($1\times10^{-4-1}$ M/L) chlorides on morphology of filaments and cells in *Klebsormidium flaccidum* (Kützing) Silva, Mattox & Blackwell (Streptophyta) were studied. Concentrations of $CuCl_2$ (1×10^{-5} M/L), $NiCl_2$ (1×10^{-3} M/L) and $MnCl_2$ (1×10^{-1} M/L) caused different morphological aberrations in algal cells, such as granulation, changing of cell form, damage of chloroplast, and complete destruction of the cell content. Heavy metals resulted in a decrease of *K. flaccidum* filament length. $CuCl_2$ caused increase of the cell length. *K. flaccidum* aplanospores formation occurred at a $MnCl_2$ concentration of 1×10^{-4} M/L.

Keywords: algae, *Klebsormidium flaccidum*, morphological characters, filaments, cells, resistance

Klebsormidium flaccidum (Kützing) Silva, Mattox & Blackwell (Streptophyta) is one of the most common soil algae and has been recorded from Europe, Asia and America (Ramanathan 1964; Lokhorst 1996). It is a very variable species with unusual powers of adaptation to different types of substrates and is comprised of several physiologically and hence biologically different forms. It is known that morphological characteristics of *Klebsormidium* taxa depend on environmental conditions (Wehr & Sheat 2003); however limits of their morphological variation under extreme ecological conditions are insufficiently studied.

The aim of this study was to evaluate *K. flaccidum* resistance to extreme levels of heavy metals pollution and assess the influence of these environmental stressors on morphological phenotype.

Material and methods

Algae isolated from soil in the territory of the Republic of Bashkortostan (Russia) were used in the experiments. The strains were cultivated on agar and liquid BBM (Andrejeva 1998). Light microscopy was carried out with a Biolam microscope with bright field optics. Algae were examined and photographed using Canon Power Shot A 620 digital camera.

The influence of copper ($1\text{-}10^{-10-2}$ M/L), nickel ($1\text{-}10^{-6-1}$ M/L) and manganese ($1\text{-}10^{-4-1}$ M/L) chlorides on morphological changes in protoplast structure and morphometrics of filaments and cells were studied. Fifty filaments and cells were examined in each experiment. The measurements were made on days 3, 6, 14 and 18 of the experiment. These metals were selected because they are considered as prevalent dangerous contaminants of the environment. Metal concentrations were selected based on literature data.

Statistical analysis of the results included calculations of arithmetic mean and its error, median, standard deviation, and coefficient of variation (Lakin 1990). Means were compared using Student's *t*-test at $\alpha = 0.05$ (Lakin 1990). Statistical analysis was performed using the Statistica for Windows program package.

Results and discussion

K. flaccidum is a filamentous alga with thin walled cylindrical cells, 5-8- (14) µm wide and 1-3 times longer than wide, cylindrical to barrel-shaped, thin walls, with a single parietal chloroplast, covering half the cell and containing a single pyrenoid (Ramanathan 1964; John et al. 2005) (Fig.1).

Typically, aberrations in *K. flaccidum* morphology caused by unfavourable conditions were evident from the 14th day of the experiment and did not change after that point.

Heavy metals caused decrease in *K.flaccidum* filament length (Fig.2). However, difference in the dynamics of this processes between different metals were noted.

Fig.1: *Klebsormidium flaccidum*, morphology of normal healthy filaments and cells.

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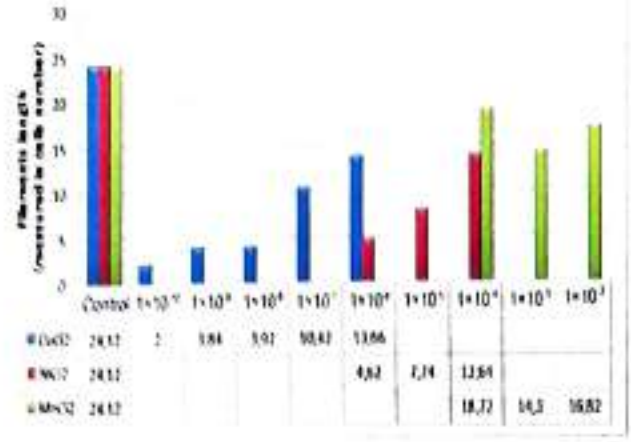

Fig.2: Influence of heavy metals on filament length of *Klebsormidium flaccidum*

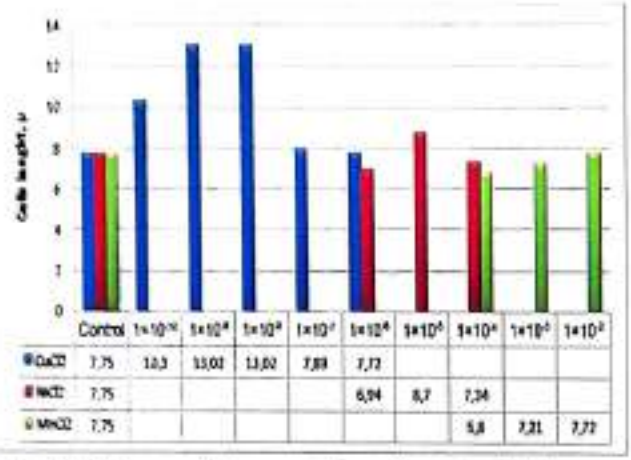

Fig.3: Influence of heavy metals on length of *Klebsormidium flaccidum* cells

With low concentrations of $CuCl_2$ and $NiCl_2$, length decreased considerably but then it increased with increasing concentration. With $MgCl_2$ cells were shortened at all concentrations. Heavy metals did not cause any change in cell width.

A gradual increase of length of filaments at $CuCl_2$ concentration from $1x10^{-10}$ to $1x10^{-8}$ and then their decrease was observed. These changes combined with increasing of cell length (Fig.3). It may be connected with disturbance of cell division. There was little change in cell length with $NiCl_2$ and $MnCl_2$.

Heavy metals also influenced morphological criteria of *K. flaccidum* (Table 1). All metals caused reduction of median and increase of variation coefficient for filaments length.

Heavy metal concentrations caused different morphological disturbances: influence of $CuCl_2$ and $MnCl_2$ were - damage of chloroplast, changing of cell form (Fig.4 and Fig.5) ; whereas that of $NiCl_2$ was formation of granulation. $MnCl_2$ induced aplanospore formation at a concentration of $1x10^{-4}$ M/L (Fig.6). We have established the influence of extreme factors on reproduction on *Xanthonema exile* (Xanthophyta) (Gaisina 2006). The influence of ecological factors on algae reproduction also has been discussed in the literature (Pecora & Rhodes 1973).

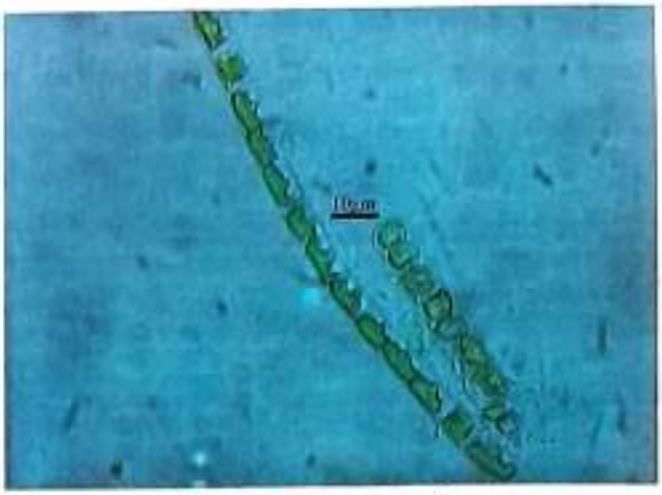

Fig.4: Influence of $CuCl_2$ on *K. flaccidum* at concentration $1x10^{-5}$– damage of chloroplast, changing of cell form

Fig.5: Influence of $NiCl_2$ on *K. flaccidum* at concentration1M/L - granulation

Fig.6: Influence of $MnCl_2$ on *K. flaccidum* at concentration $1x10^{-4}$ - aplanospore formation

The results of our investigations are summarized in Table 2. Our study confirmed previous data on *Klebsormidium* resistance to extreme levels of ecological factors. Some of *Klebsormidium* species live in acidic environments under the heavy metals pollution (Wehr and Sheat 2003). Our previous investigations have shown, that for *X. exile* (Xanthophyta) heavy metals toxicity was the same (Cu > Ni > Mn), but

Table 1: Heavy metals influence on morphological criteria of *Klebsormidium flaccidum*

Concentrations, M/l	X_{min}	X_{max}	σ	Me	cv, %	t_{fact}
$CuCl_2$						
Filaments length (measured in cells number)						
Control variant	15,00	51,00	9,09	21,00	37,69	-
1×10^{-4}	3,00	41,00	7,76	12,50	56,81	6,36
1×10^{-5}	3,00	23,00	5,31	9,00	50,96	9,86
1×10^{-6}	2,00	6,00	1,76	4,00	44,90	15,82
1×10^{-7}	2,00	6,00	1,61	4,00	41,93	15,75
1×10^{-10}	2,00	2,00	0,00	2,00	-	-
Cells length, μ						
Control variant	6,80	8,50	0,85	8,50	10,97	-
1×10^{-6}	3,40	10,20	2,07	8,50	26,81	0,11*
1×10^{-7}	3,40	11,90	1,97	8,50	24,97	0,37*
1×10^{-8}	11,90	13,60	0,81	13,60	6,22	33,89
1×10^{-9}	11,90	13,60	0,81	13,60	6,22	33,89
1×10^{-10}	8,50	11,90	1,30	10,20	12,62	12,29
$NiCl_2$						
Filaments length (measured in cells number)						
Control variant	15,00	51,00	9,09	21,00	37,69	-
1×10^{-4}	2,00	34,00	10,06	11,00	73,75	6,09
1×10^{-5}	2,00	26,00	6,77	5,00	87,47	10,42
1×10^{-6}	2,00	15,00	3,35	4,00	72,51	13,73
Cells length, μ						
Control variant	6,80	8,50	0,85	8,50	10,97	-
1×10^{-4}	6,80	8,50	0,80	6,80	10,89	2,58
1×10^{-5}	6,80	10,20	1,71	8,50	19,66	4,88
1×10^{-6}	5,10	8,50	1,23	6,80	17,72	3,34
$MnCl_2$						
Filaments length (measured in cells number)						
Control variant	15,00	51,00	9,09	21,00	37,69	-
1×10^{-2}	2,00	32,00	9,71	16,00	57,73	4,10
1×10^{-3}	2,00	29,00	8,45	13,50	59,09	5,22
1×10^{-4}	2,00	51,00	12,48	22,00	66,67	2,36
Cells length, μ						
Control variant	6,80	8,50	0,85	8,50	10,97	-
1×10^{-2}	5,10	8,50	1,25	8,50	16,19	0,15*
1×10^{-3}	3,40	11,90	2,19	6,80	30,37	1,70*
1×10^{-4}	3,40	10,20	1,50	6,80	22,06	3,99

X_{min}, minimum value of character; X_{max}, maximum value of character; Me, median; σ, standard deviation; *CV*, variation coefficient; asterisks indicate values that lack statistical significance according to Student's *t*-test at $P = 0.05$

Table 2: *Klebsormidium flaccidum* resistance to heavy metals

Metal	Minimal concentration, caused strong morphological disturbance (impossibility of cells measurement)
Cu	1×10^{-5}
Ni	1×10^{-3}
Mn	1×10^{-1}

K.flaccidum was more resistant for copper pollution (Gaisina and Khaibullina 2007).

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