Article

Metal removal from acid waters by an endemic microalga from the Atacama Desert for water recovery

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Abstract: The environmental problems generated by waste from the mining industry in the mineral extraction for business purposes are known worldwide. The aim of this work is to evaluate the microalga *Muriellopsis* sp. as a potential remover of metallic ions such as copper (Cu^{+2}), zinc (Zn^{+2}) and iron (Fe^{+2}), pollutants of AMD type waters. For this, the removal of these ions was verified in artificial acid waters with high concentrations of the ions under examination. As well as, the removal was evaluated in waters obtained from areas contaminated by mining waste. The results showed that *Muriellopsis* sp. removed metals in waters with high concentrations after 4 to 12 hours and showed tolerance to pH between 3 to 5. These results allow proposing this species as a potential bioremediator for areas contaminated by mining activity. In this work, some potential alternatives for application in damaged areas are proposed as a decontamination plan and future prevention.

Keywords: *Muriellopsis* sp., bioremediation, metallic ions, acid waters, removal

1. Introduction

The Atacama Desert located in Chile, is the aridest in the world due to its low rainfall and, scarce superficial and underground water resources, and also is one of the most important mining reserves of copper, gold, silver, molybdenum and lithium in the world [1]. These geographical conditions they have driven that the main economic development of our country to be based on mining production. Nevertheless, as a consequence of the mining waste, there is a production of acid mine drainages (AMD) which is a leachate that results from the oxidation of sulfides exposed to water, air, bacterial activity and heavy metal compounds, that are harmful to the environment and human health.

The AMD main characteristics are [2]: a) low pH values (between 2 and 5); b) high sulphate levels (several thousand mg/l), iron (between 50 and 1,000 mg/l), zinc (up to 200 mg/l), manganese (between 1 and 100 mg/l), aluminum, lead, copper, nickel, mercury, cadmium, chromium and other toxic elements such as arsenic, and c) high calcium and magnesium concentrations. The AMD formation begins when sulfide minerals present in coal or mine waste (such as pyrite) are exposed to air and

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water in mining operations [3]: Pyrite is chemically oxidized, creating a slightly acid environment suitable for the growth of the bacteria *Thiobacillus ferroxidans*. The resulting ferrous iron is regenerated to ferric by the action of *T. ferroxidans*. The ferric ion becomes available again to oxidize more pyrite and the cycle continues once it has started. The acid solution loaded with iron goes from a sulfide-rich environment to the encounter of rocks, soils and waters with a higher pH (>2.5); in this way, the ferric iron produced is hydrolyzed and generates greater acidity [4]. This ferric ion is responsible for dissolving many heavy metal sulfide minerals such as lead, copper, zinc, and cadmium.

In Chile, the discharge of these industrial waste is regulated by Supreme Decree 90: 2000 [5] which establishes the emission standard for pollutants associated with discharges of liquid waste in marine and continental waters superficial In order to comply with this regulation, have been used for water remediation methods such as chemical precipitation, ion exchange, adsorption, membrane purification [6], passive treatments, alkalinity production systems and in the last decades biosorption processes [7, 8, 9].

Biosorption processes use plants, including algae, which have the ability to bind metallic ions in negatively charged sites [10]. Several mechanisms have been proposed to explain metal tolerance in plants. These mechanisms can be divided into two broad categories: those that involves detoxification of metallic ions within the cell and those that prevent the metal from crossing the plasma membrane [11]. From these data, it has been proposed that the ability of metals to accumulate in microalgal cells by continuous exposures of the metal contaminant would lead to mechanisms of resistance through physiological adaptive processes [12]. In some microalgae there is a case of cross-resistance, which is when a species or population is resistant to more than one metal at the same time [13].

Microorganisms such as microalgae have demonstrated the ability to remove inorganic nutrients from wastewater such as nitrogen and phosphorus, which are assimilated for their growth [14]. Scientific support indicates the advantage of the use of microalgae in metal biosorption [15], its affinity to different metals has been recognized [16] and has been used in the remediation of metal ions [17]. For example, the use of marine algae and freshwater has been reported for the adsorption and elusion of gold, silver and cobalt [18, 19]. Based on these data, the aim of this work is to evaluate the viability of the microalgal biomass of *Muriellopsis* sp. to reduce the concentrations of metal ions (Cu^{+2}, Zn^{+2} and Fe^{+2}) from acid artificial water matrixes and with high metal concentrations, and from natural waters from acid drainages obtained from areas contaminated by local mining processes. This removal could be considered as a potential alternative to mitigate the contamination of areas with mining waste.

2. Materials and Methods

2.1. Obtaining microalgal strains

The microalga *Muriellopsis* sp. was obtained from the strain collections of the Unidad de Microbiología Aplicada (UMA) at the Universidad de Antofagasta. It was cultivated in environment F/2 [20] modified and incubated at 20 ± 1°C. It was cultivated in a 25-liter Photobioreactor at 20°C with a continuous photoperiod of 70 μE m-2s^{-1} continuous exposure (24h light) for 30 days.

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2.2. Metal removal by *Muriellopsis* sp. from artificial acid drainage (AAD)

The AAD consisted in the simulation of water with similar characteristics to acid drainage obtained from mining waste. For this, 2 acid matrixes were established at pH 5 and 3, standardized with HCl 0.1 N in 100 ml Erlenmeyer flasks with 50 ml of 35% sterilized Marine Saline Solution (7 mg L^{-1} $MgSO_4.7H_2O$; 0.8 mg L^{-1} KCl; 24 mg L^{-1} NaCl). Then, Cu^{2+}, Zn^{2+} and Fe^{2+} ions (Trizol of 1000 mg/l, Merck) [21] were inoculated to the solutions with the aim of obtaining concentrations of 20, 50 and 100 mg/l. As control 35% SSM at pH 5 and 3 without metallic ions were used. Once the solutions were prepared, a $1.1x10^7$ cél/ml concentration of *Muriellopsis* sp. was added. The treatments and controls were incubated at room temperature with constant shaking to keep the sample homogenized in Shaking (JSSI-100T). The microalgal count was recorded at 4, 8 and 12 hours through the Neubauer chamber with an OLYMPUS BX microscope. The pH was measured through pH-meter (PHS-W-LIDA) and the metal removal was recorded with the Cu^{+2}, Zn^{+2} and Fe^{+2} kits (Spectroqant®, Merck) using a spectrophotometer (Pharo 300, Merck).

2.3. Removal of Fe^{2+} ion by *Muriellopsis* sp. from natural acid drainage (NAD)

The NAD sample from mining waste was obtained 45 km northeast from Antofagasta, an area affected by mining activity (coordinates U.T.M 7,406,500 - 7,409,000 N and 389,000 - 494,500 E). At the laboratory, the sample was recorded, Fe^{2+} concentration with Spectroqant® Kit through a Pharo 300 spectrophotometer, pH (pHmeter PHS-W-LIDA) and salinity (ATAGO-ATC-S/MILL-E). The sample was kept at room temperature.

Based on the natural parameters of metal concentration, pH, and salinity of NAD sample from the contaminated area, 3 artificial waters were prepared as controls. For this, 100 ml Erlenmeyer flasks were inoculated with 30 ml of SSM (35%) sterilized and acidified to pH 4 with HCl 0.1 N. In order to obtain concentrations of 50, 100, 800 mg/l, Fe^{+2} (Trisol 1000 mg/l, Merck) was added. Likewise, a negative control was prepared with 35% SSM and pH 4 without inoculating metallic ions. In parallel, 100 ml flasks were used with 30 ml of NAD as a treatment. Then, a $1.0x10^7$ cél/ml concentration of *Muriellopsis* sp. was added to treatments and controls. Controls and treatments were incubated at room temperature with constant shaking to keep the sample homogenized (Shaking JSR JSSI-100C/JSSI-100T). The microalgal count was recorded at 6 and 12 hours through the Neubauer chamber with an OLYMPUS BX microscope. The pH was measured by pH-meter (PHS-W-LIDA) and the metal removal was recorded with the Iron kit (Spectroqant®, Merck) through a spectrophotometer (Pharo 300, Merck).

Data Analysis

Tests of each treatment and control were carried out in triplicate. The relation in the microalga *Muriellopsis* sp., of the variables of density, metal removal, and pH variations were evaluated through analysis of variance (ANOVA) and differences of means by multiple comparisons Tukey's, previous verification of normality and homocedasticity of data. The analysis was performed using the GraphPad PRISM 5.0 statistical software (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Density and metal removal by *Muriellopsis* sp. in AAD

At the end of the treatment after 12 hr., the lowest density of the microalga *Muriellopsis* sp., was observed in treatments of 100 mg/l in pH5 and pH3. Considering as initial inoculum 1.0x10^{7} cél/ml, final values were: Cu^{2+} 7.9x10^{6} cél/ml in pH 5 and 3, in Zn^{2+} 8.8x10^{6} cél/ml in pH5 and 8.5x10^{6} cél/ml in pH3 and in Fe^{2+} 8.9x10^{6} cél/ml in pH5 and 7.9x10^{6} cél/ml in pH3. In addition to observing a tolerance of the microalga to survive acid pH, an increase of pH in the medium was recorded at the end of the experiment. For example, the pHs maximums observed in treatments were: in Cu^{2+} pH 7.0 (pH5) and 6.3 (pH3); in Zn^{2+} pH 7.1 (pH5) and 6.7 (pH3), and in Fe^{2+} pH 7.0 (pH5) and 6.3 (pH3). Unlike the other ions, in Fe^{2+} a decrease in pH was registered in the 100 mg/l concentration at the end of the experiment as 2.4 (pH5) and 1.7 (pH3). In controls (without metals) in pH 5 and 3 a maximum pH of 7.6 was registered. Finally, the microalgal survival (%) fluctuated among the different concentrations of metals between 72-99% in pH5 and 65-95% in pH3 (Figure 1, 2 and 3).

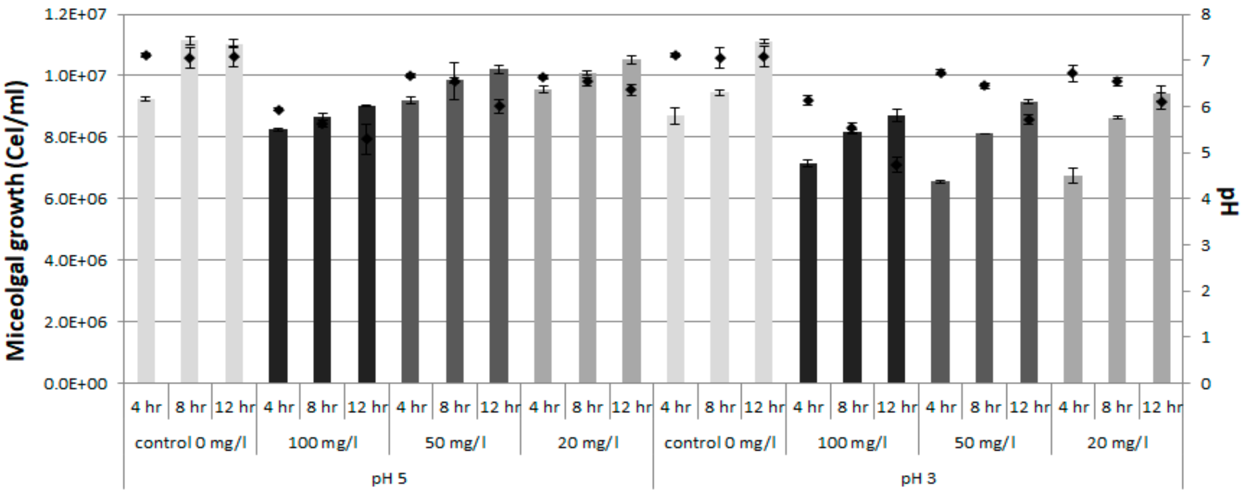

Figure 1. Growth of the microalga *Muriellopsis* sp. in AAD, cultivated at different pHs and copper concentrations.

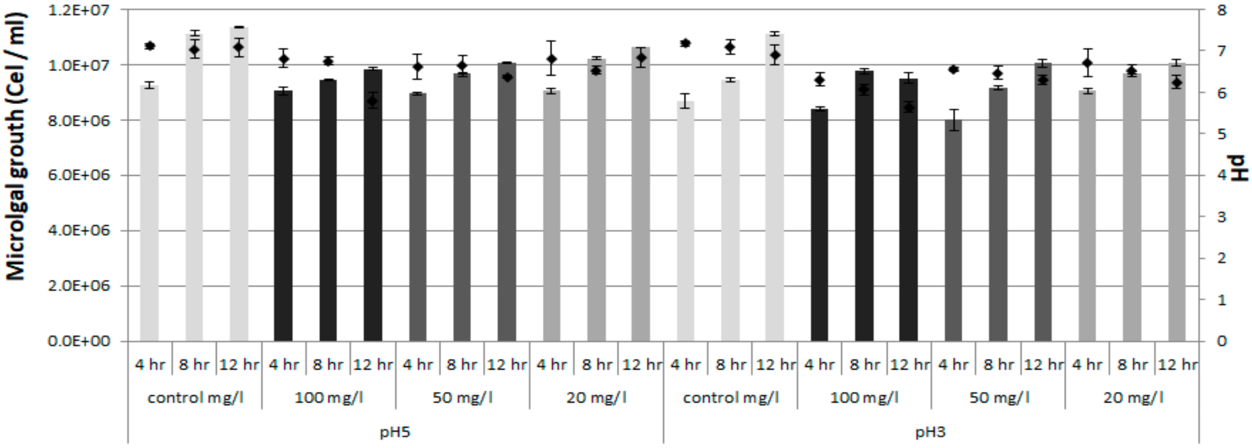

Figure 2. Growth of the microalga *Muriellopsis* sp. in AAD, cultivated at different pHs and zinc concentrations.

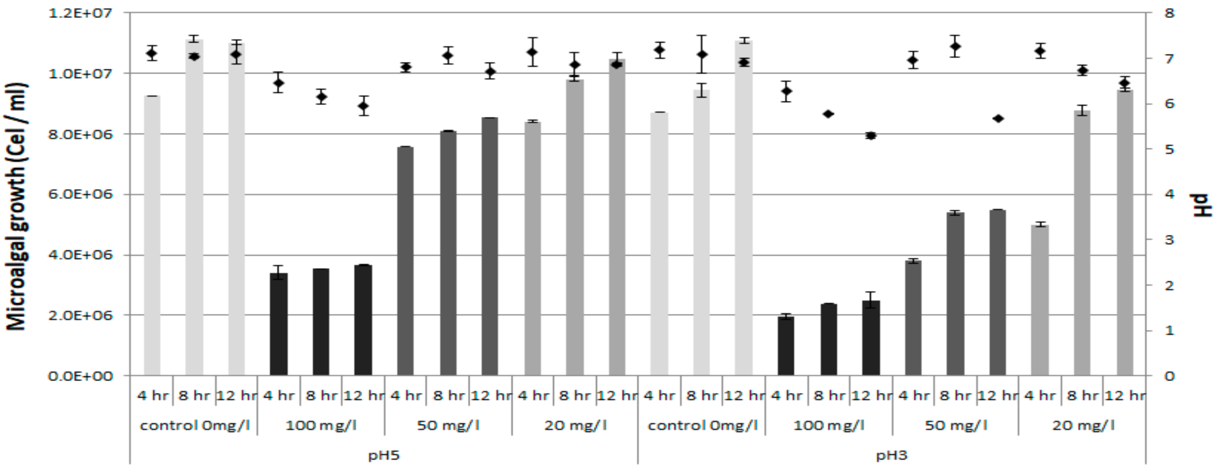

Figure 3. Growth of the microalga *Muriellopsis* sp. in AAD, cultivated at different pHs and iron concentrations.

The results showed that from 4 to 12 hours of treatment, an increase in metal removal was observed (Cu^{2+}, Zn^{2+} and Fe^{2+}). At 12 hours of treatment, it was observed that the best removal in all metals was obtained in 20 mg/l, in a range from minimum to maximum of 63 to 99.6% in pH3 and 84 to 99.9% in pH5. Followed by 50 mg/l with 37.6 to 85.5% in pH3 and between 71.5 to 99.7% in pH5. Finally, in 100 mg/l with 18.6 to 80.9% in pH3 and 41 to 93.6% in pH5 (Table 1). The highest percentage of removal was obtained in Fe^{2+} in pH 5 and 3, this result encouraged that in samples of natural acid drainage we focused only on measuring Fe^{+2} removal at different concentrations.

Table 1. Copper, zinc and iron removal from AAD by the microalga *Muriellopsis* sp., cultivated in SSM (35%), different pHs and metal concentrations.

Initial Concentrations		20 mg/l				50 mg/l				100 mg/l			
Time		0 hr	4 hr	8 hr	12 hr	0 hr	4 hr	8 hr	12 hr	0 hr	4 hr	8 hr	12 hr
pH_3	Cu^{+2}	0% (0 mg/l)	50,9% (10,2mg/l)	68,5% (13,7mg/l)	92,7% (18,6mg/l)	0% (0 mg/l)	30,2% (15,1mg/l)	40,3% (20,2mg/l)	56,2% (28,1mg/l)	0% (0 mg/l)	37,3% (37,3mg/l)	46% (46mg/l)	80,6% (80,6mg/l)
	Zn^{+2}	0% (0 mg/l)	34,7% (6,9mg/l)	41,8% (8,4mg/l)	62,2% (12,4mg/l)	0% (0 mg/l)	16% (8mg/l)	32,8% (16,4mg/l)	37,6% (18,8mg/l)	0% (0 mg/l)	12,3% (12,3mg/l)	16,2% (16mg/l)	17,8% (17,8mg/l)
	Fe^{+2}	0% (0 mg/l)	68,6% (13,7mg/l)	70,4% (14mg/l)	99,6% (19,9mg/l)	0% (0 mg/l)	59,5% (29,7mg/l)	60,7% (30,3mg/l)	85,2% (42,6mg/l)	0% (0 mg/l)	8,4% (8,4mg/l)	17,6% (17,6mg/l)	47,2% (47,2mg/l)
pH_5	Cu^{+2}	0% (0 mg/l)	78,8% (15,8mg/l)	80,6% (16,1m/l)	89,7% (17,9mg/l)	0% (0 mg/l)	66,4% (33,2mg/l)	68,8% (33,2mg/l)	71% (35,5mg/l)	0% (0 mg/l)	46,8% (46,8mg/l)	57,5% (57,5mg/l)	79% (79mg/l)
	Zn^{+2}	0% (0 mg/l)	59,2% (11,8mg/l)	82,6% (16,52mg/l)	83,7% (16.73mg/l)	0% (0 mg/l)	33,6% (16,8mg/l)	70% (35mg/l)	74,4 (37,2mg/l)	0% (0 mg/l)	30,3% (30,3mg/l)	38,1% (38,1mg/l)	40,4% (40,4mg/l)
	Fe^{+2}	0% (0 mg/l)	76,6% (15,3mg/l)	88,7% (17,7mg/l)	99,9% (19.98mg/l)	0% (0 mg/l)	90,3% (45,2mg/l)	90,7% (45,4mg/l)	99,7% (49,8mg/l)	0% (0 mg/l)	62% (62mg/l)	92,7% (92,7mg/l)	93,5% (93,5mg/l)

The ANOVA statistical analysis performed to compare effects between the measured variables (pH variation, density, and metal removal) indicated statistically significant differences between the variables in Cu^{2+} ($F=662.4$, $p<0.0001$), Zn^{2+} ($F=1235$, $p<0.0001$) and Fe^{2+} ($F=666$, $p<0.0001$). The analysis of means differences by Tukey's multiple comparisons revealed that in metals, the microalga density was influenced by the pH of the culture medium, observing significant differences

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($p<0.001$). In addition, the pH variation recorded at the end of the experiment was not significant ($p<0.001$) between pH5 and pH3 in all treatments with metals. As well as, no significant differences were observed in the microalgae density obtained at the end of the experiment at both pH in all treatments with metals. Regarding removal, the statistical analysis indicated that it is related to the microalgae density in the samples at pH5 and pH3 when significant differences were observed between these variables ($p<0.001$). However, metal removal from the microalgae is not related to the pH of the culture medium, as there are no significant differences between these variables.

3.3. Density and metal removal by *Muriellopsis* sp. in NAD

The collected NAD from a contaminated area presented an orange color, with pH 4, salinity of 35‰ and a concentration of Fe^{+2} of 80 mg/l ± 0.1 mg/l. At the end of the treatment after 12 hours and considering as initial inoculum 1.0×10^7 cél/ml, the lowest density of the microalga *Muriellopsis* sp. in AAD was observed in 800 mg/l with 2.7×10^6 cél/ml and in NAD 8.7×10^6 cél/ml was registered. Considering pH4 as initial, it was observed that the microalga presented a tendency to increase the pH of the medium, registering 8.7 in control, pH 4.7 in AAD 50 mg/l, and pH 4.6 in NAD 80 mg/l. However, there was a tendency to lower the pH in AAD of 100 mg/l (pH 3.1) and 800 mg/l (pH 1). Finally, the microalgal survival percentage fluctuated between 28% (AAD 800 mg/l) and 127% (control) (Figure 4). The results of the removal showed that from 6 hours of sampling, Fe^{2+} removal by the microalga was recorded in controls and treatment. The greatest removal was 71.6 mg/l (71.6%) in AAD 100 mg/l, followed by NAD 80 mg/l with 64.5 mg/l (80.6%). Similar trend was recorded at the end of the treatment (12 hours) with 91.3 mg/l (91.3%) in AAD 100 mg/l and 74 mg/l (92.5%) in NAD 80 mg/l. Although in treatment of 800 mg/l, the Fe^{2+} concentration was exaggerated, the recorded removal was 63.3 mg/l (7.9%), the result was important since the high resistance of the microalga and the effective removal could be verified (Table 2).

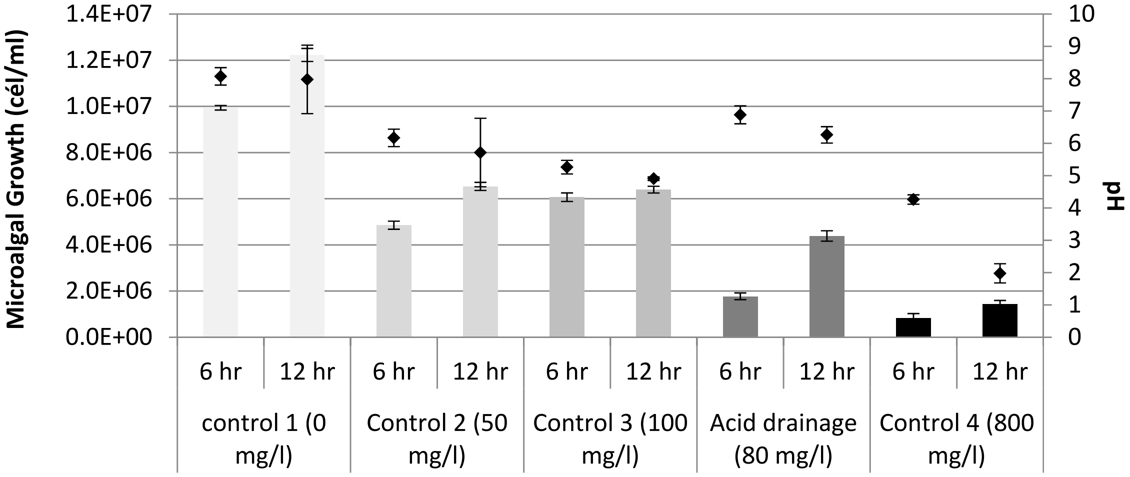

Figure 4. Growth of the microalga *Muriellopsis* sp. in NAD (80 mg/l of iron, pH4 and salinity 35‰)

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Table 2. Iron removal from AAD by the microalga *Muriellopsis* sp.

	Initial Concentrations of Fe^{2+}			
Hours	**50 mg/l**	**100 mg/l**	**Acid Drainage (80 mg/l)**	**800 mg/l**
6 h	59,9% (29,9 mg/l)	71,6% (71,6 mg/l)	80,6% (64,5 mg/l)	0,42% (0,33 mg/l)
12 h	95,6% (47,8 mg/l)	91,6% (91,6 mg/l)	92,8% (74,2 mg/l)	7,5% (6 mg/l)

The ANOVA statistical analysis of the variables (pH variation, density, and metal removal) indicated significant differences between the evaluated variables (F=82 p<0.0001). The analysis of means differences by Tukey's multiple comparisons revealed that the microalgae density was influenced by the pH from the culture medium when significant differences were observed (p<0.001). Regarding the removal, the analysis indicates that the microalgae density is related to metal removal from the samples when significant differences were observed (p<0.001). However, metal removal of the microalga is not related to the pH of the culture medium as no significant differences were observed.

4. Discussion

Acid mine drainages contain dissolved metals, being iron one of the main compounds of AMD [22], an important aspect to be considered is the impact caused by these discharges, since it strongly affects biodiversity (flora and fauna), both in the soil and in the water, since the acidity condition of the AMD alters the natural cycle of the affected ecosystems [7–23]. Considering the toxicity and duration of these drainages, it is essential to prevent its formation or apply the most appropriate treatment for its mitigation and control, which must comply with the maximum acceptable limits [24], which in the case of Chile is regulated by the Decree 90/2000 [25].

The aim of simulating AAD with NAD parameters was due to the fact that we previously needed to verify if the microalga *Muriellopsis* sp. had the capacity to tolerate acid pH and remove metals from samples with high concentrations, without affecting its viability. After this analysis, this behavior was compared in NAD samples naturally contaminated by mining processes.

Regarding metal removal in the AAD and NAD tests, based on Decree 90:2000 which establishes that the maximum discharge limit is 4.8 mg/l in Cu^{+2}, 1 mg/l in Zn^{+2} and Fe^{+2}. In our tests, it was observed that *Muriellopsis* sp., at 12 hours of treatment, managed to remove high concentrations of these metals. These results are preliminarily interesting to be used as potential bioremediators, since microalga *Muriellopsis* sp. removes metals from liquid samples in a short time, survives in high metal concentrations, and acid pH. Tolerance tests have been carried out in other microalgae at high metal concentrations, whose results have demonstrated that they are below the tolerated concentrations by *Muriellopsis* sp. in our work. For example, with respect to copper, studies by Cordero (2005) [26] demonstrated that the microalga *Tetraselmis chuii* in LC50 tests tolerated a maximum of 6.4 +/- 3.2 mg/l of copper. In toxicity tests with Zinc, it was found that the tolerance of *Selenastrum capricornut* and *Nannochloropsis oculata* microalgae were around 0.76 and 3.22 mg/l respectively at 24 hours [27]. In iron, Estupiñan (2015) [28] observed that in acid drainage samples (36.9 mg/l) from a coal mine the *Chlorella Vulgaris* and *Scenedesmus Quadricauda* microalgae managed to absorb 86.75% and 92.77%. The potential of bioremediation of heavy metals, of the microalgae have

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been studied extensively, establishing that they are very efficient in this task. Below is a review of studies on the capacity of metal uptake of microalgae (Table 3).

Table 3. Heavy metal absorption capacity from different microalgal species.

Metal	Microalgal species	Maximum Absorption (mg g-1)	pH Optimization	Initial metal concentration (mg.L-1)	Biomass Concentration (g L-1)	Temperature (°C)	Time (Hours)	References
Al (II)	*Scenedesmus sp.*	0.75	7.68-8.61	0.88			336	[29]
As (II)	*Thalassiosira sp. & Tetraselmis sp.*	0.111	7	0.13			0.3	[30]
Cd (II)	*Chlorella vulgaris*	85.3	4	200	0.75	20	2	[31]
	Chlorella sp.	11.9	7.8-8	20			1.2	[32]
	Chlorella sp.	36.4	7.8-8	100			1.2	[32]
	Chlorella sp.	59.86	7.8-8	200			1.2	[32]
	C. vulgaris	86.6	4	150	1	25		[33]
	C. vulgaris	200-250		300	1	30	0.2	[34]
	Chamydomonas reinhardtii	42.6	6			25	1	[58]
	C. Reinhardtii	145	7	989.21		23		[35]
	Scenedesmus obliquus	50	6	50	0.6	30		[36]
	S. obliquus	12.56	7	25	5	20	168	[37]
	S. obliquus	25.33	7	50	5	20	168	[37]
	S. obliquus	50.48	7	100	5	20	168	[37]
Cr (III)	*Chlorella miniata*	41.12	4.5	100		25	24	[38]
	C. sorokiniana	58.8	4		1	25		[39]
	Scenedesmus sp.	2.85	7.68-8.61	3.23			336	[29]
Cr (VI)	*Chlorella vulgaris*	140	1.5	250	1	25		[40]
	Chlamydomona reinhardtii	18.2	2		0.6	25	2	[41]
	C. reinhardtii	18.2	2		0.6	25	2	[41]
	C. reinhardtii	18.2	2		0.6	25	2	[41]
	Dunaliella Sp. 1	58.3	2	100	1	25	72	[42]
	Dunaliella Sp. 2	45.5	2	100	1	25	72	[42]
	Scenedesmus inclassatulus	4.4	8.9			25	24	[43]
	Scenedesmus obliquus	79.1		85.6			40	[44]
Cu (II)	*Scenedesmus quadricauda*	75.6	5			22	120	[45]
Cu(III)	*Chlorella vulgaris*	89.19	3.5		0.005	25	0.5	[46]
	C. vulgaris	14.48	3.5		0.1	25	0.5	[46]
	C. vulgaris	420.67	3.5	31.77		25	3	[47]
	C. vulgaris	714.892	3.5	31.77		25	3	[47]
Hg (II)	*Chlamydomonas reinhardtii*	72.2	6			25	1	[48]
	Chlorella sp.	0.0058	6.2	0.007		28.5	288	[49]
	Pleurococcus sp.	0.0059	6.2	0.007		28.5	288	[49]
	Scenedesmus sp.	0.00455	6.2	0.007		28.5	288	[49]
Ni (II)	*Chlorella miniata*	1.367	7.4				24	[50]
	C. sorokiniana	48.08	5	200	1	25	0.33	[51]

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	C. vulgaris	0.641	7.4				24	[50]
	C. vulgaris	15.4	5	100	2.5	25	2	[52]
	C. vulgaris	23.47	5.5		0.005	25	0.5	[46]
	C. vulgaris	15.6	5	100	2.5	25	2	[52]
	C. vulgaris	20.23	5.5		0.1	25	0.5	[46]
	C. vulgaris	58.4	4.5	150	1	25		[33]
	C. vulgaris	59.29	4.5	5			1	[46]
	C. vulgaris	264.7	5.5	29.34	0.1	25	3	[47]
	C. vulgaris	437.84	5.5	29.34		25	3	[47]
	Scenedesmus quadricauda	30.4	5			22	120	[45]
Pb (II)	*Chlamydomonas reinhardtii*	96.3	5			25	1	[48]
	Chlorella vulgaris	200-250		300	1	30	0.2	[54]
	Spirullina sp.	41	4	50	0.1		0.3	[55]
	Spirullina sp.	45	8	50	0.1		0.3	[55]
	Spirullina sp.	5	2	100	0.1		0.3	[55]
	Scenedesmus obliquus	296.16	6.5	300	0.1	25	96	[56]
	Thalassiosira sp. & Tetraselmis sp.	0.049	7	0.06			0.3	[57]
	Tetraselmis suecica	3.56	8.3-9.9	5		21.1-22.5	168	[58]
	Tetraselmis suecica	1.944	8.3-9.9	10		21.1-22.6	168	[58]
U (VI)	*Chlorella vulgaris*	14.3	4.4	23.8	0.76		0.08	[59]
	C. vulgaris	26.6	4.4	23.8	0.76		96	[59]
	C. vulgaris	27	4.4	23.8	0.76		96	[59]
Zn (II)	*Scenedesmus obliquus (ACO1598)*	75	6-7	429.6	0.02	25	24	[60]
	S. obliqus (L)	75	6-7	836.5	0.02	25	24	[60]
	S. obliqus (L)	50	6-7	209.6	0.02	25	1.5	[60]
	Scenedesmus quadricauda	55.2	5			22	120	[53]
	Spirullina sp.	37.5	4	50	0.1		30	[55]
	Spirullina sp.	44.5	8	50	0.1		30	[55]
	Spirullina sp.	35	2	100	0.1		30	[55]

Regarding pH in the test with AAD, it was observed that in most cases it tended to rise in the culture medium. This can be explained because due to photosynthesis, the microalgae produce bicarbonate (HCO_3^-) and carbonates (CO_3^{2-}) [61] which could be basifying the culture medium. With the exception of the iron controls (NAD test) in treatments of 100 and 800 mg/l, the pH decreased coinciding with the decrease in the microalgal density that was probably affected by the high copper concentrations and as a consequence prevented that these regulate pH. This fall could also have occurred because the ion's standard solution is dissolved in sulfuric acid, which provides the solution with Fe^{2+}, SO_4^{2-}, H^+ that upon exposure to water and oxygen generates oxidation, producing an increase in acidity [62]. In the NAD treatment (80 mg / l) the tendency to raise the pH ranged from 4.0 to 4.5. Likewise, since it is a natural sample, it is probable that other dissolved solids or its components interfere in the development of the microalga to regulate pH, although the absorption of the microalga was not affected.

5. Conclusions

Our results allow us to conclude that the microalga *Muriellopsis* sp. can survive 12 hours exposed to acid pH (between 3 and 5), to high concentrations of metallic ions up to 100 mg/l in Cu^{2+}, Zn^{2+} and 800 mg/l in Fe^{2+}. This is the first work that reports the tolerance of the microalga *Muriellopsis*

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sp. to parameters similar of acid drainages in mining. Based on these results, we propose the microalga *Muriellopsis* sp. as a potential bioremediator of waters contaminated by mining processes. As a biotechnological application, reactors could be used which allow the entry of contaminated water that will be inoculated with microalgae for a period of 12 hours, then the treated liquid it will separate by precipitation (In our tests we have been able to observe qualitatively that the microalga without agitation has the capacity to precipitate in a short time). Another treatment alternative is the use of raceway pools with contaminated waters which could be inoculated with the microalgae *Muriellopsis* sp. as a treatment. Parallel to field work, it is necessary to make a more specific study that identifies the feasibility of applying this treatment system at an industrial scale.

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