

# Importance of water quality on plant abundance and diversity in high-alpine meadows of the Yerba Loca Natural Sanctuary at the Andes of north-central Chile

Importancia de la calidad del agua sobre la abundancia y diversidad vegetal en vegas altoandinas del Santuario Natural Yerba Loca en los Andes de Chile centro-norte

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## ABSTRACT

Porphyry Cu-Mo deposits have influenced surface water quality in high-Andes of north-central Chile since the Miocene. Water anomalies may reduce species abundance and diversity in alpine meadows as acidic and metal-rich waters are highly toxic to plants. The study assessed the importance of surface water quality on plant abundance and diversity in high-alpine meadows at the Yerba Loca Natural Sanctuary (YLNS), central Chile (33°15' S, 70°18' W). Hydrochemical and plant prospecting were carried out on Piedra Carvajal, Chorrillos del Plomo and La Lata meadows the growing seasons of 2006 and 2007. Direct gradient analysis was performed through canonical correspondence analysis (CCA) to look for relationships among water chemistry and plant factors. High variability in water chemistry was found inside and among meadows, particularly for pH, sulphate, electric conductivity, hardness, and total dissolved Cu, Zn, Cd, Pb and Fe. Data on species abundance and water chemical factors suggests that pH and total dissolved Cu are very important factor determining changes in plant abundance and diversity in study meadows. For instance, *Festuca purpurascens*, *Colobanthus quitensis*, and *Arenaria rivularis* are abundant in habitats with Cu-rich waters while *Festuca magellanica*, *Patosia clandestina*, *Plantago barbata*, *Werneria pygmaea*, and *Erigeron andicola* are abundant in habitats with dilute waters.

**Key words:** porphyry deposits, phytotoxicity, plant diversity, acid mine drainage, copper toxicity.

## RESUMEN

Los megadepósitos de pórfidos de Cu-Mo han influido sobre la calidad de las aguas superficiales en las zonas altoandinas del centro-norte de Chile desde el Mioceno. Estas alteraciones en la calidad de las aguas podrían afectar negativamente a la vegetación presente en las vegas altoandinas, ya que las aguas ácidas y ricas en metales son altamente tóxicas para las plantas. En este estudio se evaluó el efecto de la calidad de las aguas en la abundancia y diversidad florística de las vegas altoandinas del Santuario de la Naturaleza Yerba Loca (SNYL), en Chile central (33° 15' S, 70° 18' O). Se realizaron prospecciones hidroquímicas y botánicas en las vegas de Piedra Carvajal, Chorrillos del Plomo y La Lata, durante las estaciones de crecimiento de 2006 y 2007. Se realizó un análisis directo de gradiente a través del Análisis de Correspondencia Canónica (CCA) para buscar relaciones entre los parámetros químicos del agua y los parámetros botánicos. Se encontró una alta variabilidad química del agua tanto dentro como entre las vegas, particularmente en cuanto a pH, sulfatos, conductividad eléctrica, dureza y contenidos totales disueltos de Cu, Zn, Cd, Pb y Fe. Los datos de abundancias específicas y los parámetros químicos del agua sugieren que el pH y el contenido de Cu total disueltos son factores importantes en determinar los cambios en la abundancia y la diversidad de las especies vegetales en las vegas estudiadas. Por ejemplo, *Festuca purpurascens*, *Colobanthus quitensis* y *Arenaria rivularis* son abundantes en ambientes de aguas con altos contenidos de Cu, mientras que *Festuca magellanica*, *Patosia clandestina*, *Plantago barbata*, *Werneria pygmaea* y *Erigeron andicola* son abundantes en lugares de aguas con bajos contenidos de Cu.

**Palabras clave:** depósitos porfíricos, fitotoxicidad, diversidad vegetal, drenaje ácido de mina, toxicidad por cobre.

## INTRODUCTION

Alpine meadows along streams are frequent in the Andes of north-central Chile (i.e., Ruthsatz 1993, Hoffmann et al. 1998, Arroyo et al. 2002, Cepeda et al. 2006, Squeo et al. 2006a). These landscape units are key components of the Andean ecosystems; they provide important habitat and resources for wildlife and trashumant livestock as they represent permanent water sources with high plant cover (70-100 %) and high floral and faunal diversity (Hoffmann et al. 1998, Cepeda & Morales 2006, Cepeda et al. 2006, Osorio et al. 2006, Squeo et al. 2006b, 2006c).

These azonal systems represent biogeographic islands scattered on the xeromorphic landscape of the alpine Mediterranean flora of the Andes of north-central Chile, as they markedly differ on their ecosystem structure and dynamic. Indeed, formation, persistence, size, and function of alpine meadows are more related to hydrologic processes than climatic conditions (Ruthsatz 1995, Carter 1996, Körner 2003, Osorio et al. 2006). According to Ruthsatz (1995), Alpine meadows located in north-central Chile most commonly are ground-water and surface-water discharge areas on slope breaks and depressions of mountain valleys derived from rain and snowmelt. Duration and seasonality of flooding and soil saturation, soil type, water chemistry, and drainage characteristics exert strong influence on the number, type, and distribution of plant species both among and inside alpine meadows (Chambers 1997, Körner 2000, Squeo et al. 2006b) as it also has commonly been described for lowland freshwater wetlands (Carter 1996). For example, differences in soil moisture and salinity have been normally described as abiotic factors that determine species distribution on alpine meadows, thus influencing the location of dry and moist alpine meadows (Chambers 1997, Körner 2003, Squeo et al. 2006b).

Plant abundance and distribution in freshwater wetlands has generally been related to water chemistry among other abiotic factors (Ehrenfeld & Schneider 1991, Carter 1996). Indeed, most floristic variation within north-west European wetland vegetation has been accounted for by water pH gradients (Wheeler & Proctor 2000). Studies on wetlands with secondary acidification of waters due to human activities in Great Britain have shown that acidified wetlands result in highly impoverished

vegetation with only some abundant plants such as the purple moor grass (*Molinia cerulea*) or the development of carpets of *Sphagnum* moss (Wheeler et al 2002). Metal content in surface waters has also been described as a factor determining plant distribution in wetlands. For example, *Juncus subnodulosus* dominates areas with high concentrations of iron in base-rich wetlands in Great Britain while *Epilobium hirsutum* is not present in those areas (Wheeler et al. 1985). Therefore, it has been suggested that plants can serve as indicators of wetland hydrochemistry (Ehrenfeld & Schneider 1991, Denninson et al. 1993)

In general, streams in high-elevation basins of world alpine areas are dilute because fast hydrologic flushing rates and minimal soil development limit interaction among high-flow discharge (spring snowmelt) and geologic materials that weather relatively slowly (Clow & Sueker 2000). However, relations among surface water chemistry and selected basin characteristics have been described in a number of studies. For example, several studies document that basins underlain predominantly by calcareous rocks tend to have surface water with higher alkalinity than basins with mostly noncalcareous rocks (Puckett & Bricker 1992, Rice & Bricker 1995). Brooks et al. (2001) document elevated concentrations of Zn and sulphate during snowmelt along some streams in Summit, Colorado, USA, due to historic mining and widely disseminated pyrite in the host rock of the catchments.

In the high-Andes of north-central Chile, a large porphyry copper-molybdenum (Cu-Mo) deposit (32-34° S; Skewes & Stern 1994a, 1994b) have influenced surface water quality in this alpine area since the late Miocene and early Pliocene (Henríquez 1974), such as in the Yerba Loca Natural Santuary (YLNS, 33° S, 70° W; Barceló 1984). Specifically, a large porphyry copper deposit (> 40 km<sup>2</sup>) with secondary formation of tourmaline and copper-zinc-molybdenum sulphides exist at high elevations of the Yerba Loca basin (Bassi 1982, Serrano et al. 1994, Stambuk et al. 1994<sup>1</sup>) which have long

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influenced surface water quality of streams in the area, particularly in terms of pH, sulphate content and mineral concentrations. For instance, the main stream along the YLNS is called Yerba Loca and it has acidic (4.1–5.3) and high sulphate ( $> 150 \text{ mg L}^{-1}$ ) and metal content waters (3.6–9.1  $\text{mg L}^{-1}$  of Cu and 0.2  $\text{mg L}^{-1}$  of Zn; R. Ginocchio unpublished data). These marked hydrochemical gradients in surface water may have an important role in structuring plant communities at meadows of the YLNS, as acidic and metal-rich waters are highly toxic to most plant species (Fernández & Henriques 1991, Adriano 2001, Ginocchio et al. 2002, Ginocchio & Baker 2004).

Even though differences on plant abundance and distribution have been described on alpine meadows of the Andes in north-central Chile, such as the occurrence of two main plant formations, grass-dominated meadows (i.e., *Deschampsia caespitose*, *Deyeuxia velutina*) and juncaceae cushion-like dominated meadows (i.e., *Patosia clandestina*, *Oxychloe andina*, *Juncus balticus*; Gajardo 1994, Squeo et al. 1999, 2006c, Luebert & Pliscoff 2006), scarce information exists on the importance of water chemistry on plant abundance and distribution, both inside and among alpine meadows. Therefore, the aim of this study was to assess the importance of water quality on plant abundance and distribution on high-alpine meadows at the YLNS as case study site.

## MATERIAL AND METHODS

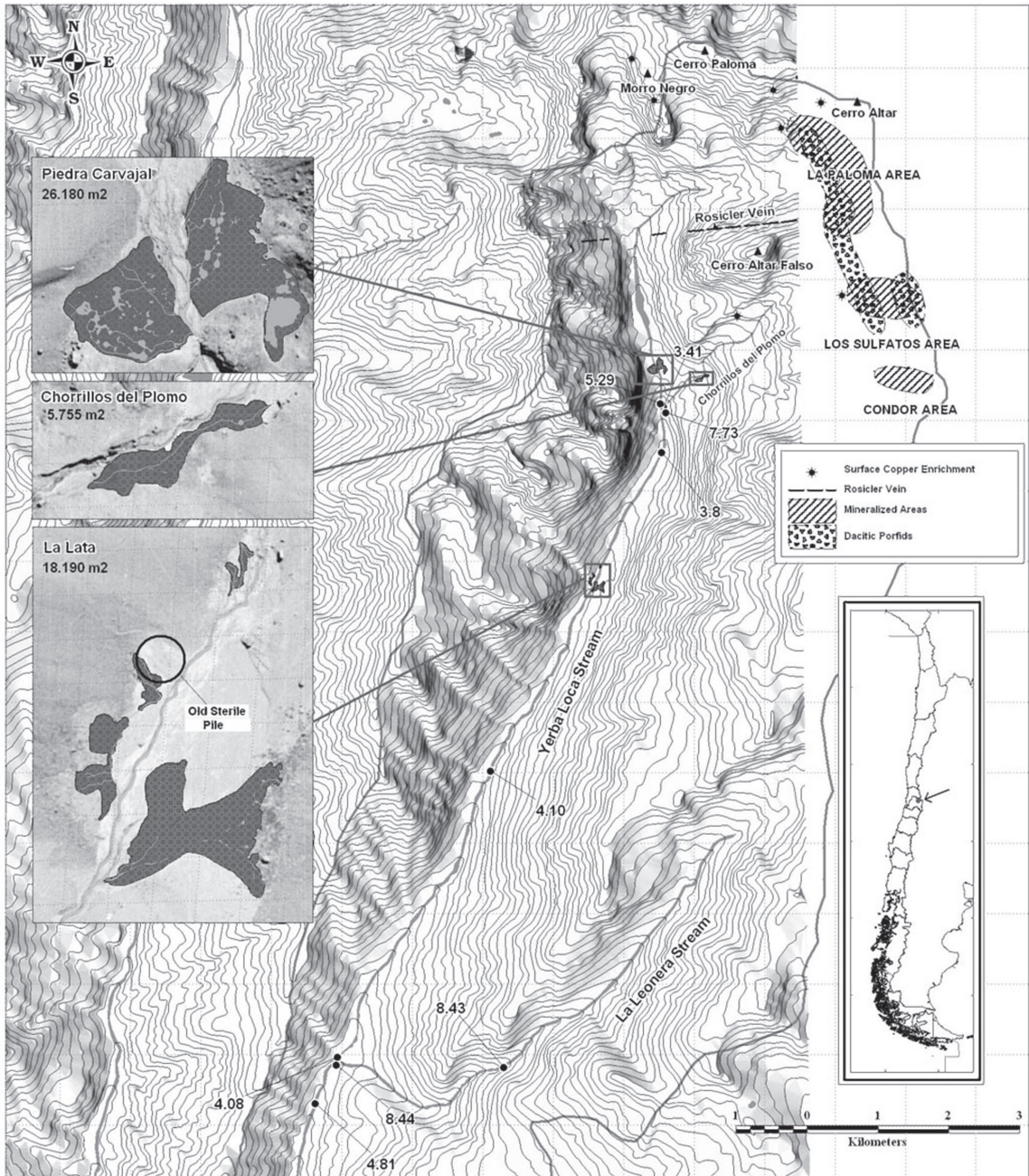
### *Study site*

The study was conducted in high-alpine areas of the Yerba Loca Natural Sanctuary (YLNS), the Andes of central Chile (33°15' S, 70°18' W). The area has Mediterranean influence, a 5- to 8-months snow-free growing season that typically extends from mid-October to mid-May (di Castri & Hajek 1976). Mean total annual precipitation at 2,500 m of altitude is about 445 mm, falling predominately as snow between May and September (Santibáñez & Uribe 1993). Mean monthly air temperature during the growing season ranges from 7 °C in April to 12 °C in February at 2,600 m of altitude (Cavieres & Arroyo 1999).

Three high-alpine meadows along the YLNS were selected for the study: Piedra Carvajal, Chorrillos del Plomo and La Lata (Fig. 1). They represent varied elevations (2,800 to 3,300 m), sizes (5,755 to 23,180  $\text{m}^2$ ) and downstream water sources from the Río Blanco-Los Bronces-Yerba Loca Cu-Mo deposit (Yerba Loca stream, upstream and downstream Chorrillos del Plomo confluence, and Chorrillos del Plomo stream, Fig. 1). Chorrillos del Plomo meadow is located in an open high-altitude terrace and is mainly drained by the main stream (Chorrillos del Plomo) while the other meadows are drained by the Yerba Loca stream but also by the water draining through the steep valley side slopes. An area of the north side of La Lata meadow is also influenced by the acid mine drainage (AMD) being generated by a nearby abandoned sterile pile of a historic small-scale silver exploitation (Fig. 1). Therefore, marked gradients in water chemistry are expected inside and among study meadows.

### *Vegetation sampling*

A number of sampling points per meadow (nine at Piedra Carvajal, 10 at Chorrillos del Plomo and 6 at La Lata) were systematically selected and georeferenced from aerial and satellite photographs in order to cover all meadow surfaces. Sampling points were located in the field with a GPS (Garmin, etrex Venture) and characterized in terms of plant richness and cover during the growing seasons of 2006 and 2007. A 2.5 x 2.5 m (5  $\text{m}^2$ ) quadrant was established in every sampling point and the identity of all plant species and the proportion of the quadrant area occupied by the different species were registered; plant cover was estimated with the Braun-Blanquet method as described by Kent & Coker (1994). Plant cover scales were +, less than 1 % cover; 1, 1-5 %; 2, 6-25 % cover; 3, 26-50 %; 4, 51-75 % cover; 5, 76-100 % cover. Additionally, a census of all the species present on study meadows was conducted in order to generate a floristic list of the species. Plant taxonomy followed *w*<sup>3</sup>TROPICOS, Missouri Botanical Garden (<http://mobot.mobot.org/W3T/Search/vast.html>) and Correa (1978). Plant species were classified according to origin and life form classification of Raunkiaer (1937).



*Fig. 1:* Location of Yerba Loca Natural Sanctuary (YLNS) in central Chile. Relative distribution of study high-alpine meadows, high-altitude mineral deposits and abandoned sterile pile from historic small-scale silver exploitation is indicated. Numbers in the map indicate pH values of surface water.

Ubicación del Santuario de la Naturaleza Yerba Loca (SNYL) en Chile central. Se indica la distribución relativa de las vegas altoandinas estudiadas, los depósitos minerales en altura y un botadero abandonado, producto de la explotación histórica de plata a pequeña escala en la zona. Los números en el mapa indican valores de pH de las aguas superficiales.

Aerial shoots of selected plant species were collected at 10 sampling sites with different water qualities in all three alpine meadows for metal determinations (copper, Cu, zinc, Zn, and iron, Fe). Samples were carried out to the laboratory, washed with ultrapure water ( $> 18 \text{ M}\Omega/\text{cm}$ ) to eliminate soil particles that were adsorbed on plant tissue surfaces (Steubing 1982). Shoots were then air dried at  $45^\circ\text{C}$  to a constant weight and Cu, Zn, and Fe contents were determined as described below.

#### *Water sampling*

Water samples were taken from all vegetation sampling point (where possible) with acid-washed polyethylene plastic vials (50 mL). An extra number of sites were also sampled (43 at Piedra Carvajal, 13 at Chorrillos del Plomo and 30 at la Lata) as all three study meadows may receive water from more than one source (i.e., ground-water from one or several slopes and surface water from streams). Method 1638 of the U.S. Environmental protection Agency (United States EPA: 1996; <http://www.epa.gov/cgi-bin/claritgw?op=display&document=clserv:OW:0569;rank=4&template=epa>) was used for the acid-washing of all polyethylene containers (24 h with 0.5 % Extran MA  $\text{O}_2$  neutral (Merk, Darmstadt, Germany), 24 h with 1 N  $\text{HNO}_3$ , 24 h with 1 N HCl, and four washings with ultrapure water ( $> 18 \text{ M}\Omega/\text{cm}$ )). The pH and EC determinations were done in the field up to 5 min after sample collection with a portable pH/EC tester (Hanna Instruments HI98130) which includes a combination pH electrode and a conductivity meter. Vials were closed and kept in double polyethylene plastic bags with hermetic seal according to method 1669, Sampling Ambient Water for Trace Metals at U.S. EPA Water Quality Criteria Levels (U.S. EPA 821-R-95-034, April 1995). Vials were carried out to the laboratory for general chemical determinations as described below.

#### *Chemical characterization of water and plant tissues*

Water samples were divided in two aliquots; one was acidified with 5 %  $\text{HNO}_3$  Suprapur (Merk) for elemental determinations. All samples and aliquots were kept at  $5^\circ\text{C}$  until analysis. Sulphate concentration was determined under

the United States EPA 300 method with an ionic chromatograph DIONEX 120X. Calcium (Ca) and magnesium (Mg) concentrations were determined with the United States EPA 251.1 and 242.1 methods, respectively, using an AAS (Perkin Elmer AAnalyst 300). Water hardness was estimated from Ca and Mg contents according to Clesceri et al. (1998). Total dissolved copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb) and iron (Fe) in water samples were determined using an inductively coupled plasma-mass spectrometer (ICP-MS; ELAN 6100 with autosampler AS90; Perkin-Elmer, Uberlinger, Germany) following U.S. EPA method SW-486 (U.S. EPA 1997). The standard calibration solution used was ICP-MS multielements, 99.99 % purity, high purity, and reference material ION-20 (National Water Research Institute, Burlington, Ontario, Canada). Sample, calibration standards, and reference material were in 0.2 %  $\text{HNO}_3$ .

Dried shoot tissues were finely crushed in a Retsch S100 agate ball mill (Newton, Pennsylvania, USA) and digested after a modified United States EPA protocol (United States EPA 1996). Every digestion batch included one blank sample, one standard reference material (SRM) sample (SRM 1573a tomato leaves; National Institute of Standards and Technology, Gaithersburg, Maryland, USA), one duplicated sample, and one quality-control sample for the quality-assurance and -control criteria. Metals were analyzed by ICP-MS (ELAN 6100 with autosampler AS90; Perkin-Elmer, Uberlinger, Germany).

#### *Copper tolerance testing*

Tolerance of *Festuca purpurascens* to copper was tested using standard dose-response metal testing in hydroponic culture as described by Schat & Ten Bookum (1992) and Harper (1996), as this species thrives in a broad range of water qualities in all study alpine meadows. Seeds of *F. purpurascens* were collected from individuals growing at different water quality sites at La Lata and Piedra Carvajal meadows as indicated in Table 1. Stock plants were grown in perlite supplemented with mineralized water and kept in a plant growth room until roots reached  $> 1 \text{ cm}$ -length. They were then transferred to 1-L polyethylene containers provided with a polystyrene floater with 6 perforations in which

the seedlings were plugged with a piece of polystyrene. The nutrient solution (1/5th strength Hoagland nutrient solution according to Harper 1996) was continuously aerated. Its composition was: 0.2 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 mM  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 0.5 mM  $\text{KNO}_3$ , 0.1 mM  $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ , 0.2  $\mu\text{M}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.2  $\mu\text{M}$   $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 2  $\mu\text{M}$   $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 10  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 0.1  $\mu\text{M}$   $\text{MoO}_3$ , and 10  $\mu\text{M}$  FeEDDHA. After 3 weeks of acclimatization to the new culture conditions, the tests were started. The length of the longest root was measured per seedling and the nutrient solution was replaced by a test solution of a similar composition that was spiked with 0, 0.125, 0.5, 1.0 and 2.0  $\text{mg L}^{-1}$  of Cu as  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ . Two to three replicated containers were used per treatment with a total of 12 or 18 seedlings per treatment, respectively. Test solutions were changed every two days to keep constant concentrations of nutrients and Cu. Containers were placed into a plant growth room under controlled temperature ( $23 \pm 2$  °C), photoperiod regime (day/night 12/12 h) and light intensity ( $103 \pm 14.3 \mu\text{m s}^{-1} \text{m}^{-2}$ ). After seven days of experimentation, the length of the longest root was measured per seedling and the root elongation per plant was estimated.

#### Data analysis

Univariate non-parametric procedures were used to analyze variation in water chemistry parameters among meadows and among

sampling sites (Zar 1984). The Kruskal-Wallis ANOVA by ranks test was used to compare water chemical parameters among meadows and to compare root elongation of *F. purpurascens* plants under the metal tolerance tests. Spearman correlations were used to evaluate relations among water chemistry factors in all sampling sites, to evaluate relations among water chemical parameters and plant richness of sampling sites, and to evaluate relations among metal content in shoots and metal contents in waters (Draper & Smith 1998). All analyses were performed with the Statistica 6.0 software.

A phytosociological classification of the flora identified at the 25 sampling sites was performed for exploratory analyses of plant associations, following the Braun-Blanquet system described by Kent & Coker (1994), as it was not always possible to take water samples from the sampling sites for a direct gradient analysis. A tabular comparison and sorting of sampling sites through identification of good differential species was performed after calculation of their degree of constancy (number of sampling points in which a given species occurs); differential species have intermediate ranges of constancy and tend to occur together in several sampling points but are absent or only sparingly present in the others, thus allowed formation of groups of floristic similarity.

Canonical correspondence analysis (CCA; Ter Braak 1986, Jongman et al. 1995) was used

TABLE 1

Water quality of high-alpine meadow sites at YLNS where seeds of *Festuca purpurascens* were collected for copper tolerance testing

Calidad del agua de las vegas altoandinas del SNYL donde se colectaron semillas de *Festuca purpurascens* para los ensayos de tolerancia a cobre

| Meadow-site                                 | Altitude<br>(m) | pH   | EC<br>( $\text{mS cm}^{-1}$ ) | Sulphate<br>( $\text{mg L}^{-1}$ ) | Hardness<br>( $\text{mg L CaCO}_3^{-1}$ ) | Total dissolved metal ( $\mu\text{g L}^{-1}$ ) |     |     |     |    |
|---|-----------------|------|-------------------------------|------------------------------------|---|--|-----|-----|-----|----|
|   |                 |      |                               |                                    |   | Cu   | Zn  | Cd  | Pb  | Fe |
| La Lata-Clear waters (V37)                  | 2,804           | 7.97 | 0.36                          | 90                                 | 208                                       | 34   | 20  | 0.1 | 0.1 | 12 |
| La Lata-Mine spoil (U5)                     | 2,823           | 5.13 | 0.62                          | 292                                | 401                                       | 5,959  | 328 | 0.8 | 2.6 | 19 |
| La Lata-Milky waters (L33)                  | 2,826           | 4.30 | 0.53                          | 211                                | 333                                       | 2,935  | 149 | BDL | 0.3 | 20 |
| Piedra Carvajal-Acidic/Cu-rich waters (K17) | 3,247           | 5.33 | 0.41                          | 249                                | 225                                       | 1,120  | 78  | 0.2 | 0.6 | 8  |

EC, electric conductivity; BDL, below detection limit  
EC, conductividad eléctrica; BDL, bajo límite de detección

to investigate relationships between water chemistry parameters and plant species abundance (direct gradient analysis) using data collected in a total number of 13 sampling sites (six at Piedra Carvajal, four at Chorrillos del Plomo and three at La Lata meadow). Species-specific absolute cover according to Braun-Blanquet classification (Kent & Coker (1994) was included in the analysis while water quality variables included were pH, sulphate, total dissolved Cu, and total dissolved Fe. Metal contents in waters were ln transformed. The ordination analysis was conducted using CANOCO software for windows version 4.5 (Biometrics – Plant Research International, Wageningen, The Netherlands; Ter Braak & Šmilauer 2002). Detrended correspondence analysis (DCA) was used for exploratory analyses of plant abundance – water quality relations with CANOCO software for either unimodal or linear models (Ter Braak 1995, Ter Braak & Šmilauer 2002). DCA gradient lengths were 5.2 standard deviations for the first axis, suggesting that the unimodal model was appropriate (Ter Braak & Šmilauer 2002). CCAs were subsequently used. CCAs were performed using default settings in CANOCO

and Monte Carlo permutation tests were performed to evaluate the significance of the plant abundance – water quality relationships (H0, no linear relationship between species and water quality matrices) using 499 permutations under full model, one based on the first canonical eigenvalue and one based on the sum of all canonical eigenvalues (Ter Braak 1986, Ter Braak & Šmilauer 2002). All selected water parameters were included in a forward-selection model to determine the contribution of each water quality parameter to the ordination model according to an unrestricted partial Monte Carlo permutation test (Lepš & Šmilauer 2003).

## RESULTS

### *Water chemistry*

Water chemistry factors of study high-alpine meadows are shown in Table 2. Significant differences were found among meadows for mean pH, electric conductivity (EC), sulphate, hardness, and total dissolved Cu and Zn values. Specifically, water at Chorrillos del Plomo

TABLE 2

General water chemistry characteristics of the three high-alpine meadows at the YLNS, central Chile

Características químicas generales de las aguas de las tres vegas altoandinas del SNYL, en Chile central

| Meadow               |      | Altitude (m) | pH    | EC (mS cm <sup>-1</sup> ) | Sulphate (mg L <sup>-1</sup> ) | Hardness (mg L CaCO <sub>3</sub> <sup>-1</sup> ) | Total dissolved Metal (µg L <sup>-1</sup> ) |       |      |      |       |
|----------------------|------|--------------|-------|---------------------------|--------------------------------|--|---|-------|------|------|-------|
|                      |      |              |       |                           |                                |  | Cu  | Zn    | Cd   | Pb   | Fe    |
| Piedra Carvajal      | Mean | 3,256        | 7.60  | 0.38                      | 160.3                          | 168.6  | 475.5                                       | 29.4  | 0.15 | 0.25 | 55.8  |
|                      | SD   |              | 1.50  | 0.23                      | 125.1                          | 132.2  | 690.2                                       | 29.9  | 0.14 | 0.27 | 130.5 |
|                      | Max  |              | 10.81 | 0.89                      | 396.7                          | 475.0  | 1970.0                                      | 78.0  | 0.50 | 0.80 | 403.0 |
|                      | Min  |              | 4.72  | 0.08                      | 31.0                           | 40.0   | 1.0   | 3.0   | 0.07 | 0.03 | 3.0   |
|                      | N    |              | 52    | 52                        | 9                              | 9  | 9   | 9     | 9    | 9    | 9     |
| Chorrillos del Plomo | Mean | 3,265        | 5.60  | 0.70                      | 327.5                          | 499.4  | 40.7  | 114.7 | 0.32 | 0.24 | 38.3  |
|                      | SD   |              | 0.69  | 0.10                      | 27.5                           | 30.4   | 23.6  | 13.3  | 0.10 | 0.03 | 7.2   |
|                      | Max  |              | 6.50  | 1.00                      | 358.0                          | 523.0  | 67.0  | 126.0 | 0.40 | 0.30 | 45.0  |
|                      | Min  |              | 3.96  | 0.59                      | 320.0                          | 465.0  | 22.0  | 100.0 | 0.20 | 0.20 | 31.0  |
|                      | N    |              | 23    | 23                        | 3                              | 3  | 3   | 3     | 3    | 3    | 3     |
| La Lata              | Mean | 2,810        | 6.43  | 0.42                      | 82.9                           | 81.1   | 2579.3                                      | 138.6 | 0.32 | 1.02 | 18.9  |
|                      | SD   |              | 1.42  | 0.15                      | 82.9                           | 81.1   | 2579.3                                      | 138.6 | 0.32 | 1.02 | 18.9  |
|                      | Max  |              | 8.70  | 0.69                      | 300.6                          | 401.0  | 6006.0                                      | 337.0 | 0.80 | 2.60 | 73.0  |
|                      | Min  |              | 4.30  | 0.21                      | 90.1                           | 176.0  | 10.0  | 7.0   | 0.07 | 0.10 | 10.0  |
|                      | N    |              | 36    | 36                        | 9                              | 9  | 9   | 9     | 9    | 9    | 9     |
| Kruskal Wallis       | df   |              | 2     | 2                         | 2                              | 2  | 2   | 2     | 2    | 2    | 2     |
| ANOVA by ranks       | H    |              | 27.04 | 36.35                     | 6.29                           | 11.04  | 7.32  | 8.75  | 5.02 | 3.90 | 5.41  |
|                      | P    |              | <0.01 | <0.01                     | <0.05                          | <0.01  | <0.05                                       | <0.05 | 0.08 | 0.14 | 0.07  |

meadow is more acidic and has higher EC, hardness and sulphate values than the other ones. Water of La Lata meadow is slightly acidic and have high levels of total dissolved Cu and Zn while water of Piedra Carvajal meadow is neutral and has intermediate EC and total dissolved Cu levels. In general, each study meadows show high variability in water chemistry (Table 2), particularly in terms of pH (two to six orders of magnitude), EC (one order of magnitude), sulphate (one order of magnitude), hardness (one order of magnitude), total dissolved Cu (one to three orders of magnitude) and total dissolved Zn (one order of magnitude).

There are some significant relations among water chemistry factors as shown in Table 3. Indeed, EC of water is significantly and positively correlated to sulphate, hardness, and total dissolved Zn and Cd while sulphate is significantly and positively correlated to hardness and total dissolved Zn and Cd (Table 3); total dissolved Cu is significantly and positively correlated to total dissolved Zn, Cd, and Pb and concentration of all these metals is significantly and negatively correlated to pH (Table 3). In other words, concentration of Cu, Zn, Cd, and Pb increases with increasing water acidity while salinity (measured as EC) increases with sulphate contents and hardness of water.

### Flora and vegetation

Total richness of study high-alpine meadows at YLNS reached 32 vascular plant species belonging to 19 families and 26 genres (Table 4). A number of two non-vascular species (bryophytes) were also identified but they were not taxonomically classified to the species level. Species richness varies among meadows from 14 to 18, being higher in the smaller one (18 species at Chorrillos del Plomo, Table 5). Chorrillos del Plomo meadow has the highest number of unique species (not shared with the other two meadows) and a total number of only three species is shared by all three study meadows (Table 5). Native plant species dominates the flora of study high-alpine meadows (85 %) while endemic and exotic species are less common (3 and 6 %, respectively). In terms of the plant life forms, hemicryptophytes are the most common (88 %) while therophytes (6 %) and nanophanerophytes (6 %) are less represented. Total plant cover of study meadows is high (53 to 88 %, Table 5) in comparison with zonal vegetation found in high-alpine areas of central Chile.

Results of the classification analysis indicate that vascular plant species of study meadows at YLNS can be grouped into three plant associations or groups. Group I is located among 3,147 and 3,265 m of altitude and is represented

TABLE 3

Spearman correlation coefficients ( $r_s$ ) among water chemistry parameters of sampling sites at the three high-alpine meadows of the YLNS, central Chile;  $n = 21$ , bold numbers are correlations statistically significant at  $P < 0.05$

Coefficientes de correlación de Spearman ( $r_s$ ) entre parámetros químicos del agua de los sitios de muestreo en las tres vegas altoandinas del SNYL, en Chile central;  $n = 21$ , los números destacados en negro corresponden a correlaciones significativas  $P < 0,05$

|          | pH    | EC    | Sulphate | Hardness | Cu    | Zn    | Cd    | Pb    | Fe   | Richness |
|----------|-------|-------|----------|----------|-------|-------|-------|-------|------|----------|
| pH       | 1.0   |       |          |          |       |       |       |       |      |          |
| EC       | -0.38 | 1.0   |          |          |       |       |       |       |      |          |
| Sulphate | -0.42 | 0.94  | 1.0      |          |       |       |       |       |      |          |
| Hardness | -0.22 | 0.86  | 0.83     | 1.0      |       |       |       |       |      |          |
| Cu       | -0.82 | 0.27  | 0.28     | 0.18     | 1.0   |       |       |       |      |          |
| Zn       | -0.74 | 0.62  | 0.56     | 0.51     | 0.83  | 1.0   |       |       |      |          |
| Cd       | -0.72 | 0.50  | 0.52     | 0.44     | 0.80  | 0.91  | 1.0   |       |      |          |
| Pb       | -0.67 | 0.30  | 0.33     | 0.32     | 0.85  | 0.80  | 0.89  | 1.0   |      |          |
| Fe       | 0.03  | 0.39  | 0.23     | 0.39     | -0.15 | 0.17  | -0.06 | -0.14 | 1.0  |          |
| Richness | 0.68  | -0.05 | -0.09    | 0.01     | -0.59 | -0.40 | -0.34 | -0.44 | 0.22 | 1.0      |



TABLE 4

Vascular flora of high-alpine meadows at YLNS in the Andes of central Chile. L, La Lata; C, Chorrillos del Plomo; P, Piedra Carvajal meadow

Flora vascular de las vegas altoandinas del SNYL en los Andes de Chile central. L, vega de La Lata; C, vega de Chorrillos del Plomo; P, vega de Piedra Carvajal

| Scientific name                           | Family           | Life form          | Origen  | Meadow |
|---|------------------|--------------------|---------|--------|
| <i>Acaena magellanica</i>                 | Rosaceae         | Hemicriptophyte    | Native  | C      |
| <i>Arenaria rivularis</i>                 | Caryophyllaceae  | Hemicriptophyte    | Native  | L      |
| <i>Calandrinia affinis</i>                | Portulacaceae    | Hemicriptophyte    | Native  | L      |
| <i>Calceolaria biflora</i>                | Scrophulariaceae | Hemicriptophyte    | Native  | L      |
| <i>Carex atropicta</i>                    | Cyperaceae       | Hemicriptophyte    | Native  | C,P    |
| <i>Carex macloviana</i>                   | Cyperaceae       | Hemicriptophyte    | Native  | L,C,P  |
| <i>Cerastium arvense</i>                  | Caryophyllaceae  | Hemicriptophyte    | Exotic  | L,C,P  |
| <i>Colobanthus quitensis</i>              | Caryophyllaceae  | Hemicriptophyte    | Native  | L,C    |
| <i>Deschampsia venustula</i>              | Poaceae          | Hemicriptophyte    | Native  | L,P    |
| <i>Calamagrostis chrysostachya</i>        | Poaceae          | Hemicriptophyte    | Native  | C      |
| <i>Calamagrostis velutina</i>             | Poaceae          | Hemicriptophyte    | Native  | P      |
| <i>Draba gilliesii</i>                    | Brassicaceae     | Hemicriptophyte    | Native  | C      |
| <i>Eleocharis albibracteata</i>           | Juncaceae        | Hemicriptophyte    | Native  | C,P    |
| <i>Empetrum rubrum</i>                    | Empetraceae      | Nanophanerophyte   | Native  | C      |
| <i>Erigeron andicola</i>                  | Asteraceae       | Hemicriptophyte    | Native  | L,C,P  |
| <i>Erigeron</i> sp.                       | Asteraceae       | Hemicriptophyte    | Native  | C      |
| <i>Festuca magellanica</i>                | Poaceae          | Hemicriptophyte    | Native  | C,P    |
| <i>Festuca purpurascens</i>               | Poaceae          | Hemicriptophyte    | Native  | L,C,P  |
| <i>Gaultheria caespitosa</i> <sup>1</sup> | Ericaceae        | Nanophanerophyte   | Native  | C,P    |
| <i>Gentiana prostrata</i>                 | Gentianaceae     | Therophyte         | Native  | C,P    |
| <i>Hypochaeris thrincoides</i>            | Asteraceae       | Hemicriptophyte    | Endemic | L,C,P  |
| <i>Hypsela reniformis</i>                 | Campanulaceae    | Hemicriptophyte    | Native  | L      |
| <i>Juncus stipulatus</i>                  | Juncaceae        | Hemicriptophyte    | Native  | L      |
| <i>Patosia clandestina</i>                | Juncaceae        | Hemicriptophyte    | Native  | C,P    |
| <i>Phleum alpinum</i>                     | Poaceae          | Hemicriptophyte    | Exotic  | P      |
| <i>Plantago barbata</i>                   | Plantaginaceae   | Hemicriptophyte    | Native  | L,C,P  |
| <i>Poacea</i> sp.                         | Poaceae          | Hemicriptophyte    |         | P      |
| <i>Trifolium polymorphum</i>              | Fabaceae         | Hemicriptophyte    | Native  | L      |
| <i>Werneria pygmaea</i>                   | Asteraceae       | Hemicriptophyte    | Native  | L,C,P  |
| <i>Lemna</i> sp. <sup>1</sup>             | Lemnaceae        | Hydrofita flotante |         | L      |
| <i>Mimulus glabratus</i>                  | Scrophulariaceae | Therophyte         | Native  | L      |
| <i>Stuckenia filiformis</i> <sup>1</sup>  | Potamogetonaceae | Hemicriptophyte    | Native  | P      |

<sup>1</sup> New records for the area and for central Chile. Nuevos registros para el área y para Chile central

TABLE 5

General characteristic of the flora present in the high-alpine meadows at YLNS, central Chile

Características generales de la flora presente en las vegas altoandinas del SNYL, en Chile central

|                           | Piedra Carvajal | Chorrillos del Plomo | La Lata |
|---------------------------|-----------------|----------------------|---------|
| Surface (m <sup>2</sup> ) | 26,180          | 5,755                | 18,190  |
| Total cover (%)           | 87.8            | 75.6                 | 52.4    |
| Richness                  | 14              | 18                   | 16      |
| Unique species            | 3               | 6                    | 8       |
| Shared species            | 3               |                      |         |

in all three meadows. Group II is only represented at Chorrillos del Plomo meadow from 3,242 to 3,270 m of altitude while group III is found in all three meadows from 2,799 to 3,293 m of altitude. Dominant (cover > 26 %), secondary (cover < 25 %) and rare species (cover < 5 %) characteristics of these plant formations are listed in Table 6. Besides these groups, some aquatic plant species dominates small lagoons present in Piedra Carvajal and La Lata meadows, such as *Juncus stipulatus*, *Lemna* sp. and *Stuckenia filiformis* (Pers.) Börner (ex *Potamogeton strictus* Phil.); micro algae are also quite common in these lagoons.

Group I corresponds to a well defined high-diversity plant formation (26 species) that is dominated by *Patosia clandestina*, *Erigeron andicola*, *Festuca magellanica*, *Plantago barbata*, *Carex macloviana* and *Werneria pygmaea*, with a diverse group of secondary species. This plant formation is present in all study meadows in areas where water quality is close to dilute waters described for streams in the Andes of central Chile (i.e., neutral, low hardness and EC, and low in minerals). However, it shows some differences among meadows; it is very common at Piedra Carvajal where it grows as continuous grassland, reaching up to 75 % of total cover but it is restricted to very small high-slope patches at Chorrillos del Plomo meadow. In this last meadow, secondary species change and two new species occur, *Gaultheria caespitosa* and *Empetrum rubrum*. Both species have not been previously described in high-alpine meadows of central Chile. This plant formation reaches up to 60 % total cover in La Lata meadow but as patches with lower plant height than in Piedra Carvajal meadow (4-cm height versus 8-cm height, respectively). This may be the result of higher cattle impact at La Lata than in Piedra Carvajal meadow during the spring-summer periods.

Group II is a plant formation with 14 different plant species (half the species number of group I); it is dominated by *Calamagrostis chrysostachya* (ex *Deyeuxia chrysostachya*), *Carex atropicta* and *C. macloviana* (Table 6) with three main secondary species (*Werneria pygmaea*, *Hypochaeris thrincioides*, and *Cerastium arvense*) and eight less represented species. This plant formation is only represented at Chorrillos del Plomo meadow where it dominates. It has high cover values (46 to 100

%) and reaches up to 17 cm height. According to the soil humidity level, *C. atropicta* (dominant in low water content areas) is replaced by *C. macloviana* (dominant in humid areas) as a secondary species. This plant formation seems to be associated with slightly acidic to acidic waters, having both high hardness (i.e., Ca levels) and levels of sulphate but low mineral contents (i.e., total dissolved Cu and Fe).

TABLE 6

Plant formations (groups) according to the phytosociological classification analysis of the high-alpine meadows at YLNS, Andes of central Chile. Numbers indicate plant covers; 1, 1-20 %; 2, 21-40 %; 3, 41-60 %; 4, 61-80 %; 5, 81-100 %

Formaciones vegetales (grupos) de acuerdo al análisis de clasificación fitosociológica de las vegas altoandinas del SNYL, en Chile central. Los números indican la cobertura vegetal; 1, 1-20 %; 2, 21-40 %; 3, 41-60 %; 4, 61-80 %; 5, 81-100 %

| Species                            | Group |    |     |
|------------------------------------|-------|----|-----|
|                                    | I     | II | III |
| <i>Acaena magellanica</i>          | 1     | .  | .   |
| <i>Calamagrostis velutina</i>      | 1     | .  | .   |
| <i>Gentiana prostrata</i>          | 1     | .  | .   |
| <i>Poacea</i> sp.                  | 1     | .  | .   |
| <i>Trifolium polymorphum</i>       | 1     | .  | .   |
| <i>Calandrinia affinis</i>         | 1     | .  | .   |
| <i>Calceolaria bifolia</i>         | 1     | .  | .   |
| <i>Phleum alpinum</i>              | 1     | .  | .   |
| <i>Hypsela reniformis</i>          | 1     | .  | .   |
| <i>Empetrum rubrum</i>             | 1     | .  | .   |
| <i>Gaultheria caespitosa</i>       | 2     | .  | .   |
| <i>Patosia clandestina</i>         | 3     | .  | .   |
| <i>Plantago barbata</i>            | 4     | .  | .   |
| <i>Erigeron andicola</i>           | 4     | .  | .   |
| <i>Werneria pygmaea</i>            | 5     | 2  | .   |
| <i>Carex atropicta</i>             | 1     | 3  | .   |
| <i>Festuca magellanica</i>         | 3     | 1  | .   |
| <i>Hypochaeris thrincioides</i>    | 2     | 2  | .   |
| <i>Eleocharis albibracteata</i>    | 1     | 1  | .   |
| <i>Sphagnum</i> sp.                | 1     | 1  | .   |
| Bryophyta                          | 3     | 2  | 2   |
| <i>Cerastium arvense</i>           | 1     | 2  | 2   |
| <i>Carex macloviana</i>            | 3     | 3  | 4   |
| <i>Festuca purpurascens</i>        | 2     | 1  | 5   |
| <i>Colobanthus quitensis</i>       | .     | 1  | 1   |
| <i>Calamagrostis chrysostachya</i> | .     | 5  | .   |
| <i>Draba gilliesii</i>             | .     | 1  | .   |
| <i>Erigeron</i> sp.                | .     | 1  | .   |
| <i>Juncus stipulatus</i>           | 1     | .  | 1   |
| <i>Deschampsia venustula</i>       | 1     | .  | 1   |
| <i>Arenaria rivularis</i>          | .     | .  | 1   |
| Total number of species            | 26    | 14 | 8   |
| Number of sampling points          | 11    | 6  | 8   |

Group III corresponds to a very low diversity plant formation (eight species) that reaches up to 30-40 cm height (Table 6). Its total cover varies from 32 to 99 %, but it dominates the areas where very acidic blue or milky coloured waters flows in all study meadows. Dominant species are *Festuca purpurascens* and *Carex macloviana* with only two important secondary species (*Cerastium arvense* and a bryophyte).

#### Plant species and water quality relationships

There are few significant correlations among water chemistry factors and plant species richness (Table 3). The pH of water was significantly and positively correlated to plant

species richness of sampling sites while both total dissolved Cu and Pb were significantly and negatively correlated to plant species richness. Acidification and metal-enrichment of waters result in a marked reduction on the species number. Indeed, acidic and Cu-rich waters were dominated by few species, such as *Festuca purpurascens* and *Colobanthus quitensis*.

Investigations on relationships among metal contents in waters and metal content in aerial plant tissues from field collected plants show significant and positive correlations among both Cu and Zn contents in shoots and total dissolved levels of these metals in water ( $r_s = 0.86$  and  $P < 0.01$  for Cu;  $r_s = 0.62$  and  $P < 0.05$  for Zn) (Table 7). It is interesting to note the

TABLE 7

#### Metal content in shoots of plant species growing in sites with different water qualities of high-alpine meadows at YLNS

Contenido de metales en los vástagos de especies vegetales creciendo en sitios con distintas calidades de agua en las vegas altoandinas del SNYL

| Species                            | Meadow               | Site | Metal in shoot          |                         |                         | Water chemistry                                |     |    | pH   |
|------------------------------------|----------------------|------|-------------------------|-------------------------|-------------------------|--|-----|----|------|
|                                    |                      |      | Cu                      | Zn                      | Fe                      | Total dissolved metal ( $\mu\text{g L}^{-1}$ ) |     |    |      |
|                                    |                      |      | ( $\text{mg kg}^{-1}$ ) | ( $\text{mg kg}^{-1}$ ) | ( $\text{mg kg}^{-1}$ ) | Cu   | Zn  | Fe |      |
| <i>Festuca purpurascens</i>        | Piedra Carvaja       | 1    | 1026                    | 43                      |                         |  |     |    |      |
|                                    |                      | 2    | 348                     | 57                      |                         |  |     |    |      |
|                                    |                      | 3    | 286                     | 34                      |                         |  |     |    |      |
|                                    |                      | 4    | 282                     | 70                      | 144                     | 1120   | 78  | 8  | 5.33 |
|                                    |                      | 5    | 131                     | 27                      |                         |  |     |    |      |
|                                    |                      | 6    | 62                      | 48                      |                         |  |     |    |      |
| <i>Festuca purpurascens</i>        | La Lata              | 1    | 324                     | 72                      | 610                     | 6143   | 337 | 22 | 4.96 |
|                                    |                      | 2    | 299                     | 122                     | 132                     | 5959   | 328 | 19 | 5.13 |
|                                    |                      | 3    | 145                     | 47                      | 1103                    | 34   | 20  | 12 | 7.97 |
|                                    |                      | 4    | 78                      | 28                      | 257                     | 2410   | 97  | 10 | 5.76 |
|                                    |                      | 5    | 45                      | 34                      | 422                     | 754  | 76  | 73 | 4.66 |
| <i>Festuca purpurascens</i>        | Chorrillos del Plomo | 1    | 14                      | 188                     | 111                     | 33   | 118 | 38 | 5.65 |
|                                    |                      | 2    | 17                      | 54                      | 137                     | 33   | 118 | 38 | 5.65 |
| <i>Calamagrostis chrysostachya</i> | Chorrillos del Plomo | 1    | 20                      | 54                      | 191                     |  |     |    |      |
|                                    |                      | 2    | 17                      | 54                      | 137                     | 33   | 118 | 38 | 5.65 |
| <i>Carex macloviana</i>            | La Lata              | 1    | 487                     | 88                      | 312                     | 2410   | 97  | 10 | 5.76 |
|                                    |                      | 1    | 181                     | 58                      | 553                     | 446  | 34  | 16 | 6.20 |
| <i>Gaultheria caespitosa</i>       | Chorrillos del Plomo | 1    | 31                      | 54                      | 439                     |  |     |    | 5.82 |
|                                    |                      | 1    | 18                      | 88                      | 226                     |  |     |    |      |
| <i>Juncus stipulatus</i>           | La Lata              | 1    | 69                      | 70                      | 1,146                   | 10   | 7   | 31 | 8.27 |
| <i>Empetrum rubrum</i>             | Chorrillos del Plomo | 1    | 38                      | 52                      | 390                     |  |     |    | 5.70 |
| <i>Deschampsia venustula</i>       | Piedra Carvajal      | 1    | 32                      | 42                      | 236                     | 11   | 18  | 34 | 6.67 |
| <i>Werneria pygmaea</i>            | Piedra Carvajal      | 1    | 13                      | 43                      | 97                      | 5  | 3   | 14 | 9.56 |
| Briophyte                          | La Lata              | 1    | 2,731                   | 375                     | 3,054                   | 5,959  | 328 | 19 | 5.13 |
|                                    |                      | 2    | 6,034                   | 259                     | 2,270                   | 6,006  | 328 | 19 | 5.08 |

very high total Cu contents (2,731 to 6,034 mg kg<sup>-1</sup> dry weight, dw) detected on aerial tissues of a moss species (Briophyte) that thrives on Cu-rich (approximately 6,000 µg L<sup>-1</sup>) and acidic (approximately 5.0) waters at La Lata meadow (Table 7). Furthermore, *Festuca purpurascens* plants are able to thrive along a broad water quality gradient in terms of pH (4.7 to 7.9) and total dissolved Cu (34 to 6,143 µg L<sup>-1</sup>; Table 7), thus determining a broad range of Cu content in shoots (45 to 1,026 mg kg<sup>-1</sup> dw). Laboratory results of Cu tolerance testing of *F. purpurascens* seedlings grown from seeds collected in four sites with different water qualities indicate that all tested individuals of this species are tolerant to Cu,

irrespective of the water quality of the site of provenance of the seeds (i.e., low or high in total dissolved Cu; Tables 1 and 8), as root elongation of seedling of all sampling sites is not significantly inhibited by increasing Cu concentrations of testing solutions (Table 8).

CCA among plant species abundance and selected water quality variables (pH, sulphate, total dissolved Cu, and total dissolved Fe) indicates that the first axis (eigenvalue,  $\lambda = 0.62$ ) is the most important for explaining overall variation of species abundance-water quality relation (56.4 %, Table 9). This axis is strongly related to total dissolved Cu (inter-set correlation = 0.877, Table 10). However, the global Monte Carlo permutation test shows that

TABLE 8

Copper tolerance testing of *Festuca purpurascens* plants belonging to sites with different water qualities at the high-alpine meadows of the YLNS. The mean and standard deviation are given

Ensayos de tolerancia a cobre en plantas de *Festuca purpurascens* colectadas en sitios con distintas calidades de agua en las vegas altoandinas del SNYL

| Cu added (nominal)            | Root elongation (cm)          |                           |                              |  |
|-------------------------------|-------------------------------|---------------------------|------------------------------|--|
|                               | La Lata<br>Clear waters (V37) | La Lata<br>Mine spoil(U5) | La Lata<br>Milky waters(L33) | Piedra Carvajal<br>Acidic/Cu-Rich<br>waters(K17) |
| 0.0 mg L <sup>-1</sup>        | 1.1 ± 0.80                    | 1.4 ± 0.31                | 1.8 ± 0.66                   | 2.2 ± 0.49                                       |
| 0.125 mg L <sup>-1</sup>      | 1.4 ± 0.74                    | 1.2 ± 0.22                | 1.6 ± 0.54                   | 2.0 ± 0.61                                       |
| 0.25 mg L <sup>-1</sup>       | 1.1 ± 0.59                    | 1.7 ± 0.78                | 2.0 ± 0.69                   | 2.4 ± 0.56                                       |
| 0.5 mg L <sup>-1</sup>        | 1.9 ± 0.55                    | 1.4 ± 0.33                | 2.0 ± 0.54                   | 2.2 ± 0.48                                       |
| 1.0 mg L <sup>-1</sup>        | 0.8 ± 0.71                    | 1.7 ± 1.02                | 2.2 ± 0.65                   | 2.1 ± 1.01                                       |
| Kruskal-Wallis ANOVA by ranks |                               |                           |                              |  |
| H                             | 6.2                           | 10.9                      | 8.3                          | 2.1  |
| df                            | 4                             | 4                         | 4                            | 4  |
| N                             | 27                            | 89                        | 89                           | 58   |
| P                             | 0.183                         | 0.065                     | 0.08                         | 0.716  |

TABLE 9

Summary of CCA (first four axes) of the species abundance - water quality variables

Resumen del CCA (primeros cuatro ejes) para las variables de abundancia específicas y calidad del agua

| Axes                                   | 1     | 2     | 3     | 4     |
|--|-------|-------|-------|-------|
| Eigenvalue ( $\lambda$ )               | 0.627 | 0.310 | 0.145 | 0.029 |
| Species-water quality correlations     | 0.921 | 0.891 | 0.647 | 0.453 |
| Cumulative percentage of variance (%): |       |       |       |       |
| (a) species                            | 19.3  | 28.8  | 33.3  | 34.2  |
| (b) species-water quality relation     | 56.4  | 84.3  | 97.4  | 100.0 |

both the first canonical axis (F-ratio = 1.91, P = 0.24) and all canonical axes (F-ratio = 1.21, P = 0.36) are not significant. This could have been the result of the small number of sampling sites were both water chemistry and plants factors were available for the analysis (n = 13). Therefore, no significant relationship among plant species abundance and all four water chemistry variables considered in the analysis was found. The forward-selection procedure to determine the contribution of each water quality parameter to the ordination model indicates that total dissolved Cu is the water quality variable that has a significant contribution (F-ratio = 2.4, P = 0.002) to variation of species abundance, according to an unrestricted partial Monte Carlo permutation test (Table 11). Therefore, a second CCA was performed among plant species abundance and total dissolved Cu in water.

Results of the new CCA indicate that the canonical eigenvalue of the first canonical axis is high ( $\lambda = 0.58$ ), explaining 17.9 % of the

overall variation of species abundance-water quality relation; the Monte Carlo permutation test shows that all canonical axis are significant (F-ratio = 2.39, P = 0.002). Total dissolved Cu concentration in water is strongly related to the first canonical axis (inter-set correlation = 0.896; Table 12), thus explaining most of the variation of plant species abundance. Plot of the first two axes from CCA ordination is shown in Fig. 2; the first axis (constrained) represents the variation of species abundance that is explained by total dissolved Cu concentration in water while the second axis (unconstrained) represents residual variation that is not explained by total dissolved Cu. Relative location among plant species (solid triangles) and sampling sites (solid circles) in the CCA ordination diagram indicates the sites where plant species show highest relative cover (Fig. 2). Position of the species in the diagram is in agreement with sampling sites. The arrow in the diagram represents the gradient of total dissolved Cu and its relative location indicates

TABLE 10

Canonical coefficients and the inter-set correlations (correlation coefficients) of water chemistry variables with the first two axes of the CCA

Coefficientes de correlación canonical y correlaciones inter-ser (coeficientes de correlación) de las variables de la química del agua con los primeros dos ejes del ACC

| Axis-variable | Canonical coefficients |         | Correlation coefficients |         |
|---------------|------------------------|---------|--------------------------|---------|
|               | 1                      | 2       | 1                        | 2       |
| pH            | 0.1795                 | -0.8125 | -0.5579                  | -0.4773 |
| Sulphate      | -0.1382                | 0.8701  | 0.2392                   | 0.7484  |
| ln Cu         | 1.1425                 | -0.8048 | 0.8772                   | 0.1758  |
| ln Fe         | -0.2139                | -0.1734 | -0.2569                  | 0.0551  |

TABLE 11

Marginal ( $\lambda_1$ ) and conditional effects ( $\lambda_A$ ) obtained from forward selection procedure. Significance was confirmed by unrestricted partial Monte Carlo permutation test

Efectos marginales y condicionales de los procedimientos de selección creciente. La significancia fue confirmada con el test no restringido y parcial de permutación de Monte Carlo

| Water chemistry variables | $\lambda_1$ | $\lambda_A$ | P-value | F-value |
|---------------------------|-------------|-------------|---------|---------|
| ln Cu                     | 0.58        | 0.58        | 0.002   | 2.40    |
| pH                        | 0.37        | 0.32        | 0.230   | 1.33    |
| Sulphate                  | 0.33        | 0.20        | 0.602   | 0.83    |
| ln Fe                     | 0.08        | 0.07        | 0.940   | 0.28    |

the relationship with plant abundance and sampling points. The length of the arrow indicates the importance of the water chemistry factor and the perpendicular projection of solid triangles on the arrow indicates the species distribution in the total dissolved Cu gradient. In the first axis of the CCA diagram the species follow a total dissolved Cu gradient that increases from left to right of the diagram (i.e. low to high total dissolved Cu concentration in waters). For instance, *Festuca purpurascens*, *Colobanthus quitensis*, and *Arenaria rivularia* are more abundant in habitats with high concentrations of total dissolved Cu while *Erigeron andicola*, *Festuca magellanica*, *Patosia clandestina*, *Werneria pygmaea*, and *Plantago barbata* are more abundant in habitats with low concentrations of total dissolved Cu (Fig. 2).

#### DISCUSSION

Marked surface water chemistry gradients have been created in the mountain valley of the YLNS from different origins; natural from oxidative processes of high-altitude Cu-Mo deposits (acid rock drainage, ARD), and anthropogenic from acid mine drainage (AMD) resulting from an abandoned pile of mine wastes left by historic silver exploitations located nearby La Lata meadow. Snow melting during spring/summer months followed by water percolation through permeable mineral deposits and dumped mine wastes result in oxidation and hydrolysis processes that leads to surface water acidification, increased salinity, and mineral enrichment. Indeed, it is well documented that mineral deposits and massive mine wastes rich in metal sulphurs (i.e., pyrite,  $\text{FeS}_2$ ) generate ARD or AMD when exposed to

air and water (Ritcey 1989, Gray 1996, Gray 1998, Kontopoulos 1998, Benner et al. 1999, Peppas et al. 2000, Dinelli et al. 2001, Evangelou 2001) as pyrite weathers relatively rapid (Clow & Sueker 2000).

In most cases, alpine meadows at YLNS receive water from more than one source (i.e. ground-water from one or several slopes and surface water from streams), so environmental heterogeneity in water quality is high, both among and inside meadows. For example, waters among meadows varied in terms of pH (mean values) in the following order of increasing acidity, Piedra Carvajal (7.6) < La Lata (6.4) < Chorrillos del Plomo (5.6), but high pH variability exists inside each meadow: two units in Chorrillos del Plomo and 4-5 units in the other two meadows. On the other hand, surface waters followed the following order in terms of increasing total dissolved Cu levels (mean values), Chorrillos del Plomo ( $41 \mu\text{g L}^{-1}$ ) < Piedra Carvajal ( $476 \mu\text{g L}^{-1}$ ) < La Lata ( $2,579 \mu\text{g L}^{-1}$ ). Again, high variability exists for this parameter inside each meadow: three times at Chorrillos del Plomo and from 600 to almost 2,000 times in the other two meadows.

Increased levels of metals, changes in pH from acidic to alkaline, marked changes in hardness, electric conductivity and sulphate levels seems to be common on streams at high elevation as shown by preliminary studies performed by this group and scarce published information (Cepeda & Morales 2006). Furthermore, information available in literature indicates that ARD is a natural phenomenon that has occurred in the longitudinal belt of porphyry Cu-Mo deposits along the north-central Andes (32-34° S, 70° W) called Río Blanco-Los Bronces-El Teniente (Arias & Cruzat 1974, Henríquez 1974, Barceló 1984), a

TABLE 12

Canonical coefficients and the inter-set correlations (correlation coefficients) of water chemistry variables with the first two axes of the CCA

Coeficientes de correlación canonical y correlaciones inter-ser (coeficientes de correlación) de las variables de la química del agua con los primeros dos ejes del ACC

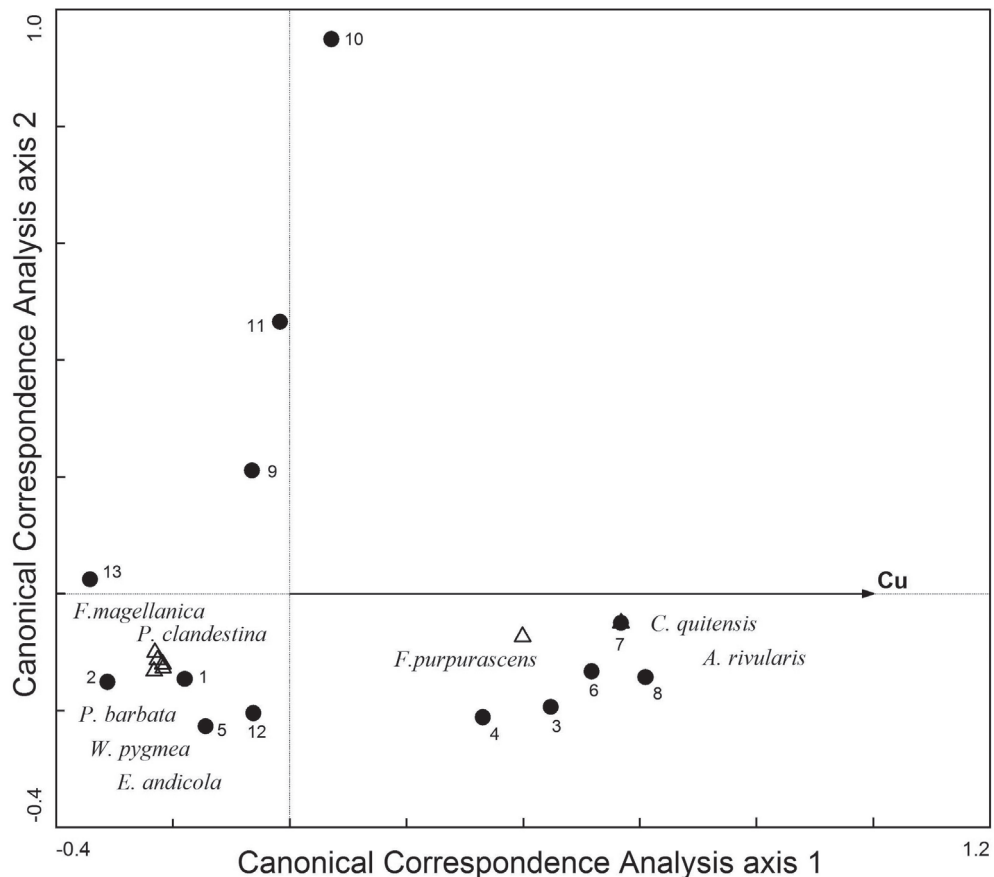
| Axis-variable | Canonical coefficients |   | Correlation coefficients |   |
|---------------|------------------------|---|--------------------------|---|
|               | 1                      | 2 | 1                        | 2 |
| ln Cu         | 1                      | 0 | 0.8958                   | 0 |

phenomenon that can be magnified by current and future mine operations (Dold & Fontboté 2001). Therefore, broad environmental gradients on hydrochemistry at high alpine areas of the Andes in north-central Chile may be a more common phenomenon than previously considered. However, this phenomenon has not been systematically evaluated yet.

#### *Relation among water quality and high-Alpine meadow vegetation*

Our data suggests that environmental gradients in surface water, such as pH and total dissolved

Cu, are important factors determining plant distribution, diversity and abundance in high-alpine meadows at YLNS. Large (mean values) and small (variability values) scale variations in water quality parameters may explain changes in plant community structure among and inside alpine meadows. On one hand, we have found quite even plant diversities (14 to 18) among study meadows, which are in the range of six to 19 species described for alpine meadows in the Andes of South America (Squeo et al. 1994, Teillier 2005). However, this is an unexpected result from the viewpoint of the species-area relationship (Begon et al. 1990) as study meadows have marked



**Fig. 2:** Canonical ordination diagram illustrating the distribution of water chemistry factors (arrows,  $n = 1$ ), sampling sites (solid circles,  $n = 13$ ) and plant species (solid triangles,  $n = 8$ ) in ordination space. Water chemistry factor correspond to total dissolved Cu. Only the species well related to the first ordination axis are included (17 % of minimal adjust).

Diagrama de ordenación canónico que ilustra la distribución de los factores químicos del agua (flechas,  $n = 1$ ), las unidades de muestreo (círculos sólidos,  $n = 13$ ) y las especies vegetales (triángulos sólidos;  $n = 8$ ) en el espacio de ordenación. El factor químico del agua corresponde al Cu total disuelto. Se incluyen sólo las especies bien relacionadas con el primer eje de ordenación (ajuste mínimo al 17 %).

differences in size (5,755 to 26,180 m<sup>2</sup>). A possible explanation for this finding may be the occurrence of high environmental variability at small spatial scales, particularly in the small meadow (Chorrillos del Plomo) as it has previously suggested (Stewart et al. 2000, Wilson 2000). Indeed, Chorrillos del Plomo meadow showed the highest diversity in terms of both species diversity and plant associations. Besides the high variability in surface water quality found in this study, Chorrillos del Plomo meadow could have more marked gradients in soil moisture and salinity due to its higher slope and complex microtopography. Therefore, a number of environmental factors may be involved in structuring high-alpine meadow communities, surface water quality being another one not well considered until now.

On the other hand, Chorrillos del Plomo meadow has, in general, more acidic waters with higher contents of sulphate but low contents of metals than La Lata and Piedra Carvajal meadows. The last two meadows have neutral to slightly acid waters, low in sulphate but high in metal contents. This general difference (mean values) may explain changes in dominant plant formations found among study meadows. Chorrillos del Plomo meadow has a unique plant formation (group II according to the phytosociological classification), dominated by *Calamagrostis chrysostachya* (ex *Deyeuxia chrysostachya*), *Carex atropicta* and *C. macloviana* with few secondary species. The other two plant formations (group I and III), are less represented in areas with different water qualities. La Lata and Piedra Carvajal meadows share similar water qualities, with well represented areas with either dilute waters or AMD/ARD impacted waters. On these areas, two different plant formations dominate. Plant formation defined as group I, with high diversity (26 species) and dominated by *Patosia clandestina*, is common in dilute waters (low contents of sulphate and metals), while plant formation defined as group III, with very low diversity (eight species) and dominated by *Festuca purpurascens*, is common in areas where very acidic and high metal content waters flows. Squeo et al. (1999) has described two main plant formations for the high-alpine meadows of the Andes in north-

central Chile; grass-dominated meadows (i.e., *Deschampsia caespitose*, *Deyeuxia velutina*), such as group II and III in this study, and juncaceae cushion-like dominated meadows (i.e., *Patosia clandestina*, *Oxychloe andina*), such as group I in this study. This second vegetation type has been described by other authors as *Patosia clandestina*-*Juncus balticus* formation and it is considered as the representative vegetation of alpine meadows in north-central Chile (Gajardo 1994, Luebert & Plissock 2006). It will be interesting to further evaluate if this two general vegetation types of the high-alpine meadows may be considered as indicative of specific water qualities.

Spatial variation in pH and metal levels (i.e., total dissolved Cu) in surface waters would select for tolerant species in those particular areas having acidic and metal-rich waters, as these factors can limit plant establishment and growth. For example, copper and zinc are essential micronutrients for all organisms, but it is well known that they are toxic at high concentrations (Baker & Walker 1989, Fernández & Henriques 1991, Adriano 2001). Levels of total dissolved Cu and Zn found in this study were in most cases well above the acute quality criteria for aquatic life recommended by United States EPA (calculated value of 16.5 µg L<sup>-1</sup> for Cu in pristine waters of north-central Chile according to Villavicencio et al. (2005); 120 µg L<sup>-1</sup> for Zn according to United States EPA 1999), and they should be therefore toxic for aquatic organisms and plants. However, organisms differ widely in their tolerance to metals with some organisms being able to store certain metals with no adverse physiological response (Peters et al. 2007). Indeed, some species thriving on highly Cu-rich waters in alpine meadows at YLNS, such as *Festuca purpurascens* and mosses, did not show metal toxicity symptoms and were able to reproduce on these field conditions even though they reached very high Cu contents (2,731 to 6,034 mg kg<sup>-1</sup> dw) on aerial tissues. As a reference, the commonly observed range of Cu in aerial tissues (dw) of crops and fruit trees varies from 4 to 40 mg kg<sup>-1</sup> (Adriano 2001) and from 2.7 to 21 mg kg<sup>-1</sup> in aquatic plants (Harrison 1998).

Our results showed that plant richness was significantly reduced by increasing acidity and Cu content in surface waters and only some



species were able to growth and reproduce in this stressful condition, such as *Festuca purpurascens*, *Colobanthus quitensis*, and *Arenaria rivularia*. This species were less represented in other areas and they can be defined as indicator plants for water anomalies (i.e., metal-rich waters). On the other hand, species like *Carex macloviana*, *Patosia clandestina*, and *Erigeron andicola*, more abundant in habitats with dilute waters, can be used as indicators of dilute waters. However, further standard dose-response laboratory tests (Ginocchio et al. 2002, Ginocchio & Baker 2004) are needed to determine the degree of tolerance of these species to pH and metals. Reduction in grass diversity, density and productivity has been described for other meadows impacted by historic mine exploitations (Stoughton & Marcus 2000), but development of tolerance to metals in plants has been demonstrated in several studies around the world (Bradshaw 1984, Bradshaw & Hardwick 1989, Kruckberg & Wu 1992, Macnair & Baker 1994, Brooks 1998). Plant adaptation to increased metal levels can occur rapidly, sometimes within few years of disturbance (Bradshaw 1984, Baker 1987, Tyler et al. 1989), therefore, under a long history of interaction, porphyry Cu-Mo deposits and historic mine spoils affecting surface water quality at the YLNS may have resulted in directional selection for metal and acidic water tolerant plant species. Indeed, copper tolerance testing of *F. purpurascens* seedlings under laboratory conditions showed the constitutive tolerance of this species to elevated Cu concentrations. Further research to objectively evaluate degree of tolerance of high-alpine meadow species and populations to metal-rich and acidic waters are, however, required.

This pioneer study suggests the importance of surface water chemistry factors, besides other hydrological factors such as soil humidity and salinity, in determining plant abundance and diversity in high-alpine meadows at the YLNS, the Andes of north-central Chile. However, a need for increasing the sampling effort is needed to be able to get more conclusive results and further studies are needed to verify this phenomenon in other alpine areas of north-central Chile, in order to get more conclusive and general results.

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