Fachhochschule Köln
Cologne University of Applied Sciences

UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ
FACULTADES DE CIENCIAS QUÍMICAS, INGENIERÍA Y MEDICINA
PROGRAMAS MULTIDISCIPLINARIOS DE POSGRADO EN CIENCIAS AMBIENTALES
AND
COLOGNE UNIVERSITY OF APPLIED SCIENCES
INSTITUTE FOR TECHNOLOGY AND RESOURCES MANAGEMENT IN THE TROPICS AND SUBTROPICS

A COMPARISON OF THE EFFICIENCY OF SULFATE REDUCTION AND CADMIUM REMOTION USING CONSTRUCTED WETLANDS.

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SAN LUIS POTOSÍ, MÉXICO 23 de Agosto de 2011

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Ich erkläre mich mit einer späteren Veröffentlichung meiner Masterarbeit sowohl auszugsweise, als auch Gesamtwerk in der Institutsreihe oder zu Darstellungszwecken im Rahmen der Öffentlichkeitsarbeit des Institutes einverstanden.

Estoy de acuerdo con una publicación posterior de mi tesis de maestría en forma completa o parcial por las instituciones con la intención de exponerlos en el contexto del trabajo investigación de las mismas.

Unterschrift / *Firma*:

A mi familia:
Alejandro y Magdalena, Alejandro y Paty, Mariana y Juan Pablo

A mi abuela Bertha;
A la amada memoria de mis abuelos Bernardo y Manuel
y a la amada memoria de mi abuela Tello.

Todos en mi corazón por siempre.

To my Family:
Alejandro and Magdalena, Alejandro and Paty, Mariana and Juan Pablo

To my Grandmother Bertha;
To the loving memory of my Grandfathers Bernardo and Manuel
and to the loving memory of my Grandmother Tello

Everyone in my heart forever

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“The jade Columns have been erected,
in the middle of the lake the columns are rise
is the God who sustains the ground
and takes the Anahuac upon himself
over the celestial water.

There are beautiful flowers in your hands,
with green willows have tinged the city
to all that the waters surrounding,
and in the fullness of the day.

You have done a painting of the celestial water,
the land of Anahuac have tingeded
O ye Lords!
To you, Nezahualcoyotl
to you, Motecuhzoma
the Giver of Life hath invented,
hath wrought
our father, God
within the same water.”

Song of Nezahualcoyotl from Acolhuacan
(Excerpt)
Nezahualcoyotl

“Se han levantado columnas de jade,
de en medio del lago se yerguen las columnas,
es el Dios que sustenta la tierra
y lleva sobre sí al Anáhuac
sobre el agua celeste.

Flores preciosas hay en vuestras manos,
con verdes sauces habéis matizado la ciudad,
a todo aquello que las aguas rodean,
y en la plenitud del día.

Habéis hecho una pintura del agua celeste,
la tierra del Anáhuac habéis matizado,
¡oh vosotros señores!
A ti, Nezahualcóyotl,
a ti, Motecuhzoma,
el Dador de la Vida os ha inventado,
os ha forjado,
nuestro padre, el Dios,
en el interior mismo del agua.”

Canto de Nezahualcóyotl de Acolhuacan
(Fragmento)
Nezahualcoyotl

RESUMEN

La contaminación por Cadmio y sulfates ocurre como consecuencia de las actividades mineras y puede continuar por cientos o miles de años a pesar de haber cesado las actividades mineras. Los contaminantes mineros afectan el uso biológico, recreacional, industrial y municipal de grandes ríos y de afluentes a muchos kilómetros de distancia de la fuente contaminante. El drenaje ácido de minas y los metales pesados de minas abandonadas o jales mineros afectan las comunidades de invertebrados acuáticos, peces, mamíferos, vegetación riparia y abastecimientos de agua para uso domestico en todo el mundo.

Los humedales construidos son muy atractivos para el tratamiento debido a su relativo bajo costo de construcción y operación. La mayoría de la investigación relacionada con los humedales se basa en el tratamiento de aguas residuales de origen municipal para reducir las concentraciones de nitrógeno y fósforo, así como para disminuir la demanda biológica de oxígeno. Actualmente, es común encontrar muchos trabajos que refieren el uso de humedales construidos para la remediación de áreas contaminadas por desechos industriales, peligrosos y desechos mineros.

En los humedales construidos, los metales se concentran dentro de un área más manejable, la cual protege a los sistemas naturales adyacentes de sus efectos ecotoxicológicos. La eficiencia de los humedales construidos de forma experimental para la remoción de cadmio y la reducción de sulfato, así como el aminoramiento de la biodisponibilidad y el papel que tienen las plantas en el sistema, es el interés principal del presente documento.

Los humedales utilizados en la presente investigación, mostraron una alta eficiencia en el incremento del pH, partiendo de cerca de 3 y llegando a más de 8; la remoción de Cadmio fue muy elevada muy cercana al 100% y la reducción de sulfates mostró una eficiencia cercana al 76%. Siendo la principal fuente de acumulación de Cadmio el substrato pero las plantas mostraron una importante capacidad de absorción de Cadmio, y para el sulfato la principal fuente de acumulación fue el substrato, debido a las características de la mezcla utilizada (turba de *Sphagnum* y calcita)

PALABRAS CLAVES: humedales construidos, Typha, Cadmio, sulfato reducción.

ABSTRACT

The sulfate and metal contamination that occurs as a consequence of mining can continue for hundreds or thousands of years following the cessation of mining operations. Mine contaminants affect the biological, recreational, industrial, and municipal use of larger rivers many kilometers downstream from mining. The Acid Mine Drainage (AMD) and heavy metals from abandoned mines are affecting communities of aquatic invertebrates, fishes, mammals, riparian vegetation, and domestic water supplies, all over the world.

Constructed wetlands are attractive because they are relatively cheap to build and operate, and require little or no energy for operation. Most research in the use of wetlands for wastewater treatment has been directed towards municipal wastewaters to reduce concentrations of nitrogen and phosphorus and to lower biological oxygen demand. Nowadays, there are many works referring to the use of constructed wetlands for the remediation of polluted areas by industrial, hazardous and mining waste.

In constructed wetlands, metals are concentrated within a manageable area, which may protect adjacent, natural systems from ecotoxicological effects. The efficiency of experimental wetlands by removing cadmium and the reduction of the sulfates, to reduce their bioavailability and the role that plants have in the system, is the main concern of the present document.

The wetlands used in the present research, showed a high efficiency in the pH increase, from near 3 to more than 8; the Cadmium removal was very high almost 100% and the sulfate reduction showed an efficiency near 76%.

The main pool of Cadmium in the wetland was the substrate but the plants present a very important capacity of Cadmium absorption, and the pool for the sulfate was mainly the substrate, due to the characteristic of the present mixture, peat moss and limestone.

KEY WORDS: constructed wetlands, Typha, Cadmium, sulfate reduction.

ZUSAMMENFASSUNG

Das Sulfat und Metallen Kontamination, die als Folge des Bergbaus auftritt, kann für hunderte oder tausende von Jahren nach der Beendigung des Bergbaus weiter. Meine Verunreinigungen beeinträchtigen die biologische, Freizeit-, Industrie- und kommunalen Einsatz von größeren Flüssen vieleKilometer flussabwärts aus dem Bergbau. The Acid Mine Drainage und Schwermetalle aus stillgelegten Bergwerken beeinflussen Gemeinden von wirbellosen Wassertieren, Fische, Säugetiere, Ufervegetation undBrauchwasser versorgt, auf der ganzen Welt.

Pflanzenkläranlagen sind attraktiv, weil sie relativ billig zu bauen und zu betreiben sind, und benötigen wenig oder keine Energie für den Betrieb. Die meisten Untersuchungen in der Nutzung von Feuchtgebieten zur Abwasserbehandlung hat zur kommunalen Abwässern gerichtet, um Konzentrationen von Stickstoff und Phosphor zu reduzieren und die biologische Sauerstoffbedarf zu senken. Heutzutage ist es allgemein viele Werke mit Bezugder Einsatz von Pflanzenkläranlagen für die Sanierung von Flächen verschmutzt durch Industrie-, Ex-und Bergbau-Abfälle.

In Pflanzenkläranlagen werden Metalle in einem überschaubaren Gebiet, dasbenachbarte, natürliche Systeme aus ökotoxikologischen Auswirkungenschützen kann konzentriert. Die Effizienz der experimentellen Feuchtgebiete, indem Cadmium und die Reduktion der Sulfate, ihre dimishing der Bioverfügbarkeit und der rol, dass Pflanzen im System, ist das Hauptanliegendes vorliegenden Dokuments.

Die Feuchtgebiete in der vorliegenden Untersuchung verwendet werden, zeigten eine hohe Effizienz bei der pH-Wert zu erhöhen, aus der Nähe von 3 bis mehr als 8, die Cadmium Entfernung sehr hoch war fast 100% und die Sulfatreduktionzeigte einen Wirkungsgrad nahe 76%.

Der Haupt-Pool von Cadmium, in das Feuchtgebiet wurde das Substrat, sondern die Pflanzen stellen eine sehr wichtige Eigenschaft von Cadmium-Absorption, und das Sulfat seinem Pool war vor allem das Substrat aufgrund derCharakteristik der vorliegenden Mischung, Sphagnum Torf und Kalk.

STICHWORTE: Pflanzenkläranlagen, Typha, Cadmium, Sulfat-Reduktion.

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INTRODUCTION

The use of constructed wetlands as treatment systems has proved to be effective and low cost in the removal of heavy metals, organic and inorganic compounds harmful for environment, as well as, capable of pH increase in acid waters, as it was proved in other countries like Canada, Spain, USA, among others (EPA, 1988; López-Pamo, *et al.*, 2002).

The mineral recovery processes are partially effective, therefore, a percentage of the minerals that are wanted to extract remains in the tailings. The mineral recovery or extraction processes contain unwanted minerals inside the tailings (heavy metals, metalloids). As a result of mineral crushing, the big rocks are transformed in to small particles that finally constituted the tailings. Those particles from the tailings are made of fine to medium material (silt and Sandy material), are easily suspended and dispersed due the wind action in the form of dust that contain high concentrations of potentially toxic substances.

The tailings and waste piles are considered as potential places for the generation of Acid Mine Drainage (AMD), as it features a great permeability due to the small compaction facilitating the water percolation, as well as the air input. This conditions facilitates the sulfides oxidation process and the acidification of water. The acid waters have negative effects in rivers and lakes: destroying cultures and shores; producing, in most of the cases, fish and livestock mortalities; and affecting the human health.

In a broad sense, when a mine is functioning (coal, metal, open pits, etc) or abandoned, produce residues that can be altered in the environment (by oxidation) and generate acid waters with a great quantity of suspended solids and a high content of heavy metals, such as Fe, Cd, Mn, Zn, Cu, Pb and Mo, among others.

The wetland technology for passive treatment of acid water is being used successfully in some countries as an alternative to conventional treatment of Acid Mine Drainage that apply chemical additives as: CaO, $Ca(OH)_2$, NaOH, $CaCO_3$, Na_2CO_3, This technology is preferred due to its low investment its low cost and construction investment for operation and management, energy consumption, etc. Also, creates a small environment for flora and fauna in harmony with the landscape.

The main concern of the present work is to define the role of plants in the removal of Sulfates and Cadmium in constructed wetland.

OBJECTIVES

General Objective

- Determine the efficiency of the reduction of Sulfates and the removal of Cadmium using two experimental constructed wetlands.

Specific Objetives

- Compare the efficiency of the sulfate reduction between two constructed wetlands: one wetland with vegetation and another without vegetation.
- Estimate the efficiency in the total removal of Cadmium (Cd) and main process involved like sorption and/or complexation and compare these information between two constructed wetlands one with vegetation and another without vegetation.

BACKGROUND

1.- Sulfates, Cadmium and the Acid Mine Drainage (AMD) generation.

Sulfates (SO_4^{2-}) and Cadmium (Cd) can be found naturally in the environment. The sources of sulfur include geochemical weathering of minerals and wind-blown sea salt (Wetzel, 1983), but in the other hand, Cd is a naturally occurring heavy metal with no known nutritional requirement for biota (Kadlec, 1995) and is found naturally in small quantities in air, water and soil.

Large quantities of sulfur enter the atmosphere from natural and industrial sources, and return to terrestrial systems as an acid precipitation containing sulfates (sulfuric acid). The sulfates (SO_4^{2-}) are found in almost all natural water, and they are formed as a result of acid mine runoff from coal or other mineral extraction and from industrial sources. In human beings at concentrations higher than 250 mg/L, diarrhea occurs. In Mexico the safety levels of sulfates in drinking water have been set at 400 mg/L (DOF, 1995; NOM-127-SSA1-1994).

On the other hand, Cd can be released into the air when household or industrial waste, coal or oil are burned, during the waste incineration or mining activities (Laws, 1993; Plachy, 2000; cited by Kadlec and Knight, 1995), from semiconductors and solar cells, and from dyeing and printing textiles and pigments (Patnaik, 2007). The chronic toxicity of cadmium to humans and the environment has been well documented, the organs that accumulate this metal are liver and kidney, it is very slowly excreted, been its half-life between 20-30 years, also there is enough evidence of its carcinogenic effects in animals (Patnaik, 2007, Stoeppler, 1992). In Europe, the safety levels of Cd in drinking water have been set at 5 mg/L (Martins *et al.*, 2004), in Mexico it has been set at 0.005 mg/L (DOF, 1995; NOM-127-SSA1-1994).

The acid mine water is described as drainage of certain sulfide minerals in rocks exposed to oxidizing conditions (Skousen *et al.*, 1998) with a pH between 2 and 4 and high levels of dissolved metals (Table 1). In relation to the acid drainage, there are some reports on the mortality of fishes and shellfishes from rivers conditions, and in many cases, livestock mortality, destruction of crops and riverfronts, as well as human health condition. All this associated with yellow ocher-colored beds of rivers and lakes affected, and the increased turbidity of the water (Mills, 1995; Nordstron and Alpers, 1999; Morin and Hutt, 2001; López-Pamo *et al*., 2002).

Table 1.- Some important metal sulfides occurring in mining regions. The predominant acid producers are pyrite and marcasite (Taken from Skousen *et al.*, 1998).

	Name
FeS_2	pyrite
FeS_2	marcasite
Fe_XS_X	pyrrhotite
Cu_2S	chalcocite
CuS	covellite
$CuFeS_2$	chalcopyrite
MoS_2	molybdenite
NiS	millerite
PbS	galena
ZnS	*sphalerite*
FeAsS	arsenopyrite

The AMD, contain a large amount of suspended solids and high concentrations of sulfate (SO_4^{2-}) and dissolved metals (Fe, Al, Mn, Zn, Cu, Pb, Cd, Ca, Na, K, Mg, etc), the measured concentrations of the mine drainage range from 50 to 300 mg Fe/L, 20-30 mg Mn/L, 20-2000 mg SO_4^{2-}/L, 14-1700 mg Cd/L and pH of 3.0 to 5.5. These elements in these concentrations are harmful to biological activity, pollute water bodies and those waters may damage the structure and foundations of buildings. Due to the enormous cost of conventional sewage treatment, the long time it takes the generation of acidic waters, and the worsening of the situation in old mining areas, it was necessary to find a solution to this problem.

2.- The Acid Mine Drainage (AMD) Generation

Nordstrom y Alpers (1999) describe the oxidation process of pyrite as the main responsible for the formation of acidic waters, this oxidation is favored in mining areas due to the air that comes in contact with sulfides (through mining work access and the existing pores in the piles of tailings and waste) and by increasing the contact surface of the particles. These authors consider that the factors that most affect the acid generation are: the volume of pyrite, the concentration of it, the grain size and its distribution. The proton acidity is due to free protons (H^+) and the mineral acidity due to the dissolution of Fe, Al and Mn. These metals are considered acidic because by oxidation and hydrolysis can generate H^+ according to the following four reactions (Skousen *et al.*, 1998; Nordstron y Alpers, 1999; Mills, 1999; EPA, 1996 y 2000; Aduvire, *et al.*, 2009):

- $Fe^{2+} + ¼\ O_2 + {}^3/_2\ H_2O \leftrightarrow FeOOH + 2H^+$ (2.1)
- $Fe^{3+} + 2\ H_2O \leftrightarrow FeOOH + 3H^+$ (2.2)
- $Al^{3+} + 3\ H_2O \leftrightarrow Al\ (OH)3 + 3H^+$ (2.3)
- $Mn^{2+} + ¼\ O_2 + {}^3/_2\ H_2O \leftrightarrow MnOOH + 2H^+$ (2.4)

The tailings and waste piles (tailings) are considered as potential sites for the generation of acid drainage, these piles have a very low permeability, which facilitates the percolation of rainwater or runoff. Also, the air entering the mine workings and the tailings facilitates the oxidation of sulfides. The oxidation process of pyrite can be represented by the following typical reactions:

- $4FeS_2\ (s) + 4H_2O + 14O_2 \leftrightarrow 4\ FeSO_4 + 4H_2SO_4$ (2.5)
- $4FeSO_4 + O_2 + 2H_2SO_4 \leftrightarrow 2Fe_2(SO_4)_3 + 2H_2O$ (2.6)

- $4FeS_2(s) + 15O_2 + 2H_2O \leftrightarrow 2Fe_2(SO_4)_3 + 2H_2SO_4$ (2.7)

The reaction 2.5 shows the oxidation of pyrite, caused by the reaction with water or air, which ends with the iron released causing an increase in dissolved solids and increased acidity associated with a decreased pH with formation of ferrous sulfate, which is then oxidized to ferric sulfate (reaction 2.6). Wildeman and Laudon, (1989) proposed that the microorganisms can catalyze significantly the rate of oxidation of pyrite especially when mediate the oxidation of Fe(III) to Fe (II)". The ferric ion formed can then quickly oxidize the ore (reaction 2.8):

- $2FeS_2 + 2Fe_2(SO_4^{2-})_3 \leftrightarrow 6FeSO_4 + 4S°$ (2.8)

Or it can be hydrolyzed to produce ferric hydroxide and precipitated:

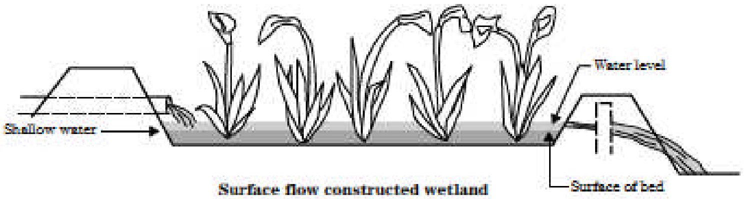
- $Fe^{3+} + 3H_2O \leftrightarrow Fe(OH)_3$ (s) + $Fe^{2+} + 3H^+$ (2.9)

The ferrous ion, Fe^{2+}, (reaction 2.8) can then be oxidized biologically to ferric ion, Fe^{3+}, and elemental sulfur to sulfuric acid. If the capacity for continuous neutralization to pH 3, the ferric ion precipitates as hydroxide by hydrolysis (reaction 2.9) and the pH value reached 3.5 the hydroxide stop forming and the activity of Fe^{3+} in solution is increased. In general, the geochemistry of acid mine drainage is a very complex phenomenon due to the production, motility, volume, concentration, grain size and distribution of pyrite on one side and the other to the various physical, chemical and biological processes which all contribute in the geochemistry of acid mine waters.

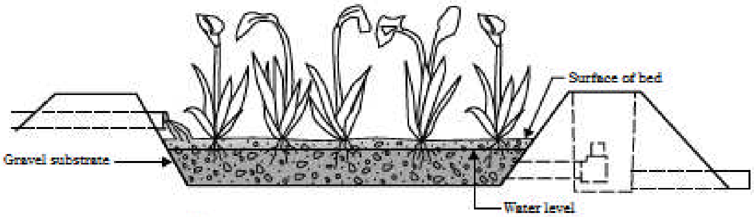
3.- Constructed Wetlands

The Constructed wetlands are defined as systems that simulate a transition zone between terrestrial and aquatic environment, but are specifically built for wastewater treatment under controlled conditions of location, size and treatment capacity (Gerba *et al.,* 1999).

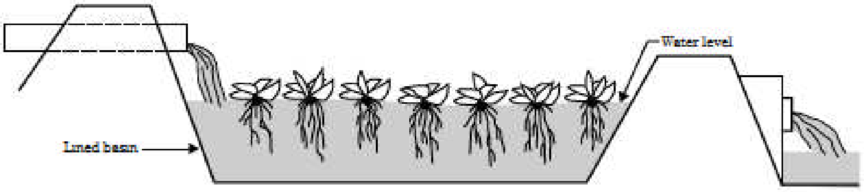
There are several types of constructed wetlands depending on the water flow: vertical flow or intermitent and horizontal or constant water flow. The last one could be: surface flow wetlands, floating aquatic plant and subsurface flow wetlands (Figure 1). Also the wetlands could be aerobic or anaerobic depending on the dissolved oxygen concentration found in the system. In this works, an anaerobic type wetland was tested.



Surface flow constructed wetland



Subsurface flow constructed wetland



Floating Aquatic Plant (FAP) system

Figure 1.- Types of constructed wetlands (USDA and NRCS, 2002).

The constructed anaerobic wetlands requires of anoxic conditions to properly be operated. There is a permeable substrate column (30-60 cm depth) composed of organic material (70-90 %), and a layer of limestone (Figure 2). The main principle is to eliminate dissolved oxygen and produce alkaline chemical processes or through the use of microorganisms. The emergent vegetation helps to stabilize the substrate in addition to providing additional organic matter (López-Pamo *et al.,* 2002) and create microenvironments for microorganisms that reduce or oxidize substances in the soil.

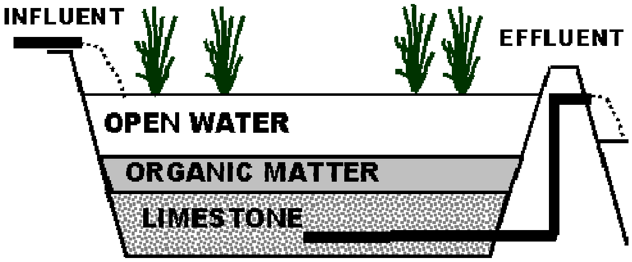


Figure 2.- Scheme of an Anoxic constructed wetlands.

The higher removal of heavy metals is due to bond processes with the material that makes up the sediment in wetlands (Kadlec and Keoleian, 1986). Due to its positive charge, heavy metals are easily sorbed, complexed and bound to particles in suspension, which are subsequently deposited onto the substrate.

The precipitation of heavy metals in the form of insoluble salts such as carbonates, bicarbonates, sulfides and hydroxides, is another process that leads to long-term removal. These salts are formed by the reaction of heavy metals with other chemicals that are present in the water column and are insoluble and therefore precipitate salts fixed with the wetland substrate (Sheoran and Sheoran, 2006).

Peat its defined as a fibrous mass of organic matter, generally with a dark brown color, with a spongy consistency and is partially decomposed plant matter which is formed in wet areas under partially anaerobic conditions, where the rate of accumulation of plant matter is greater than the decomposition of that, hence, the decomposition is incomplete (Wase, *et al.*, 2003). Viraraghavan and Rao (1993), define it as an unconsolidated, hydrophilic, carbonaceous sediment formed by the accumulation of partially fragmented, decomposed and commonly heterogeneous plant remains which retain more than 75 % inherent moisture and less than 12 % mineral matter in saturated natural deposits. The physical and chemical properties of the peat depend on a variety of factors, such as the nature of the plants from which it has originated, the properties of the water in which the plants were growing (in particular the oxygen, the pH and the dissolved bases), and the moisture relations during and following its formation and accumulation (Stanek, 1977, cited by Wase, *et al.*, 2003).

In the present case, the peat moss is a complex material with lignin, cellulose and humic acids, that bears polar functional groups, such as alcohols, aldehydes, ketones, carboxylic acids, phenolic hydroxides and ethers that can be involved in chemical bonding. Due to this polar characteristic, the peat moss has a high adsorption potential for dissolved substances such as metals and polar organic molecules (Brown *et al.*, 1996).

Tan (2003) mentioned there are several studies had been made in the characterization of the major elements in humic matter of soils. An extract of that work is presented in the Table 2, where the soils formed with peat are compared with the ideal sample of humic acid on soil (reference) and the plant residue. The general composition of humic matter displays a close relation with that of the plant material from which it has been derived. The observed divergence from the plant residue is apparently the result of the humification process and other soil factors and the differences may be due to differences in origin or to the types of humic substances (Tan, 2003).

Table 2.- Summary of Elemental Composition of Humic Acids (HA) and Fulvic Acids (FA), extracted from Temperate Region Soils and Miscellaneous Environments (Modified from Tan, 2003).

Acid Type	**Soil/Environment**	**C**	**H**	**O**	**N**	**S**	**C/N**
		%					
Humic Acid	Histosols (Peat bogs)	58.7	5.0	32.9	3.4	n.d	17.3
Humic Acid	Reference	56.2	4.7	35.5	3.2	0.8	17.6
	Peat	55.5	5.4	33.1	3.0	n.d	18.5
	Plant Residue	49.6	6.3	41.6	2.5	n.d	19.8
Fulvic Acid	Spodsols	50.6	4.0	44.1	1.8	0.3	28.1
Humic Acid (artificial)	Merck Chemical Co.	59.8	4.8	33.6	1.8	n.d	33.2

3.1.- Sulfate Reduction Process

The plants generate microenvironments that assist in the reduction and oxidation processes, in those microenvironments certain bacteria species, such as those in the generas *Desulfovibrio* spp and *Delsulfotomaculuum* spp, employ sulfate in anaerobic respiration (Mitsch and Gosselink, 1986). According to Norton (1992), it is the most important metal removing mechanism in wetlands, that´s why it´s very important to provide enough organic matter inside the wetland. The presence of decaying organic matter in the wetland sediments and soils depletes oxygen and creates acid pore waters. Organic matter fuels sulfate reduction. For example:

- $SO_4^{2-} + 2CH_2O + 2H^+ \leftrightarrow H_2S + 2H_2O + CO_2$ (3.1)
- $SO_4^{2-} + CH_2O \leftrightarrow H_2S + HCO_3^-$ (3.2)

where CH_2O represents the organic substrate required by the microorganisms. The reaction 3.1 is favored at low pH, while the reaction 3.2 dominates at higher pH. When ion concentrations are low, or when sulfate and organic matter concentrations are high, significant hydrogen sulfide concentrations can occur. Several other metal sulfides are also very insoluble, including ZnS, CdS, and others (Kadlec and Knight, 1995).

The sulfates in the AMD are reduced to sulfides which form an insoluble precipitate that remains buried in the organic substrate of the substrate raising the pH as is shown in the reactions:

- $SO_4^{2-} + CH_2O \leftrightarrow H_2S + HCO_3^-$ (3.3)
- $SO_4^{2-} + H_2 \leftrightarrow H_2S + H_2O$ (3.4)

At the end of the reactions 3.3 and 3.4, Hydrogen sulfide is the common result. Part of this gas that is heavier than air, could be liberated to the atmosphere and another part could react with metal ions to form metal sulfides, which may be considered as the salts of hydrogen sulfide. Hydrogen sulfide is a reactive and toxic gas with problematic side effects, including a rotten egg odor, corrosion, and acute toxicity. The processing of sulfur in wetland ecosystems is represented by interconversions of several sulfur compounds in the different microregions of the ecosystem.

Oxidized forms, such as sulfite, sulfate, and thiosulfate, are found in the oxygenated portion of the FWS water column. Reduced forms, including sulfide, bisulfide, and elemental sulfur, are found in the soils and sediments under conditions of low redox potential. Ionic and molecular forms are prevalent. Hydrogen sulfide and methylated sulfur compounds are volatile, and may be lost from the wetland to the atmosphere. Sulfate is an essential nutrient because its reduced, sulfhydryl (-SH) form is used in the formation of amino acids. Because there are, usually, enough sulfates in surface waters to meet the sulfur requirement, sulfate rarely limits overall productivity in wetland systems (Figure 3).

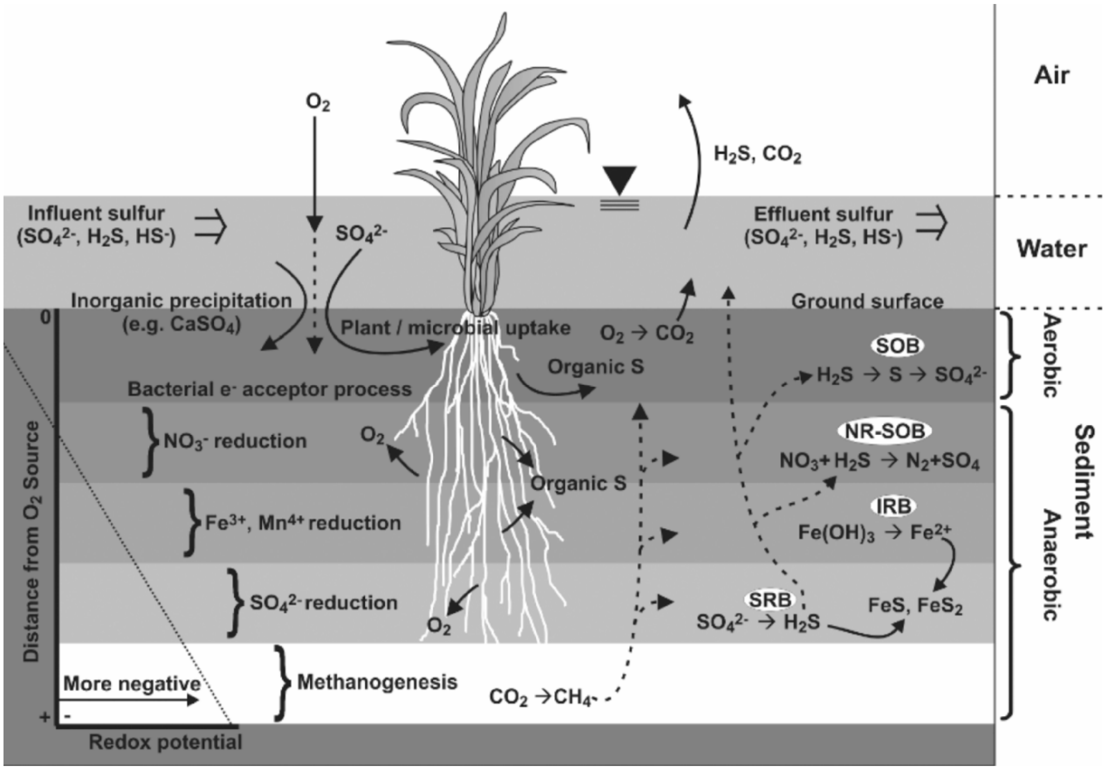


Figure 3.- Sulfur pathways, forms and their relation with Redox potential in constructed wetlands (From Sturman, *et al.*, 2008).

Treatment wetlands receive these atmospheric inputs as well as sulfur compounds that may be included in the chemicals in the water to be treated (Kadlec, 1995). Lamers (1998) document that sulfate has a negative effects on the growth rate of *Carex nigra*, *Juncus acutiflorus*, and *Gallium palustre*, at concentrations of 64 and 128 mg S/L. The presence of sulfide is coupled with anaerobic conditions in the root zone, but the effects of sulfide go beyond the anoxia (Koch *et al.*, 1990). Hydrogen sulfide apparently inhibits the activity of alcohol dehydrogenase enzyme, thereby limiting the ability of plants to avail themselves of alternative anoxic energy pathways (Kadlec and Knight, 1995).

3.2.- Cadmium Remotion Process

The average abundance of Cd in the earth’s crust is 0.16 ppm; in soils varied from 0.1 to 0.5 ppm; in streams it is 1 µg/L, and in groundwater´s concentrations are 1 - 10 µg/L (APHA, 1999). In surface waters, Cd typically occurs as Cd^{2+} and is most soluble at low pH in waters with low hardness, Cd is especially soluble in sulfide minerals that also contain Zn, Pb, or Cu, it is usually associated with Zn at a ratio of about 1 part Cd to 500 parts Zn in most rocks and soils. The solubility of Cd is controlled in natural waters by carbonate equilibrium. Guidelines for maximum Cd concentrations found in natural water are linked to the hardness or alkalinity of the water (i.e., the softer the water, the lower the permitted level of Cd). In solution, Cd is present as the free ion and complexed with some substances, and also as soluble compounds with carbonate, sulfate, chloride, hydroxides, and humates. The Cd complexes are highly adsorbable on organic particulates. It is nonessential element for plants and animals.

Cadmium storage in wetlands eventually creates sustained sediment concentrations that reflect the dilution of new cadmium deposits by the accretion of new wetland solids.

Cadmium is removed by particulate settling and trapping, chemical precipitation and coprecipitation, partitioning to sediments, and lesser extent by the plant uptake. Cadmium forms very insoluble compounds with sulfide and carbonate (see reactions 3.5 and 3.6 respectively), among others:

- $S^{2-} + Cd^{2+} \leftrightarrow CdS$ (3.5)
- $CO_3^{2-} + Cd^{2+} \leftrightarrow CdCO_3$ (3.6)

The precipitation of the carbonate can be microbiologically mediated (reaction 3.6), for example by *Alcaligenes denitrificans* (Remacle *et al.,* 1992). The removal of cadmium can also take place through co-precipitation with iron, manganese, and aluminum oxyhydroxides. Cadmium may be incorporated into the lattice of iron oxides, Cd-hematite, and Cd-goethite being formed. With increasing concentrations of Cd in a system, a percentage of Cd may replace Fe in the iron oxides.

4.- Process that take place in the plant (Phytoremediation)

Phytoremediation strategies refer to the predominant mechanisms made by plants, but also, in some cases, indicate the role of the microbial communities during the remediation process. Thus, it becomes evident that phytoremediation is a complex process that involves the participation of the microbial community associated with its root system. The processes involved in phytoremediation are:

a) **Phytodegradation**: Through enzymatic reactions between plants and microorganisms in the rhizosphere, the pollutants are partially or completely degraded or processed; TNT, DNT, RDX, antrazina, solvents clorinated, DDT, pesticides, phenols and nitriles, etc.

b) **Phytostimulation**: The exudates from the roots of plants stimulate the growth of microorganisms, this facilitate the establishment of microbial communities in the surrounding soil (fungi, bacteria, protozoa) whose activities cause metabolic mineralization of pollutants. Pollutants that can be removed by means of this process are Cd, Co, Cr, Ni, Hg, Pb, Se, Zn, radioactive isotopes, phenols.

c) **Phytovolatilization:** Some plants are capable to volatilize certain contaminants in soil, sediment or water; Hg, Se and chlorinated solvents.

d) **Phytostabilization:** plants that develop a dense root system, using mechanisms of sequestration, lignification or humidification to stabilize contaminants in mine waste Mine waste lagoons, phenols and chlorinated compounds.

e) **Phytoacumulation or Phytoextraction:** some plants have the ability to accumulate pollutants in their roots, stems or foliage, which can be easily harvested. Certain plants, are called hyperaccumulators, this means that they can absorb unusually large amounts of metals in comparison to other plants and the ambient metals concentration. In other words, they can contain toxic element levels about 100 times more than non accumulator plants growing in the same soil, with some species and metal combinations exceeding conventional plant levels by a factor of more than 1000 (Cornish, *et al.*, 1995). Contaminants as Cd, Co, Cr, Ni, Hg, Pb, Se, Zn can be removed by plants through this process.

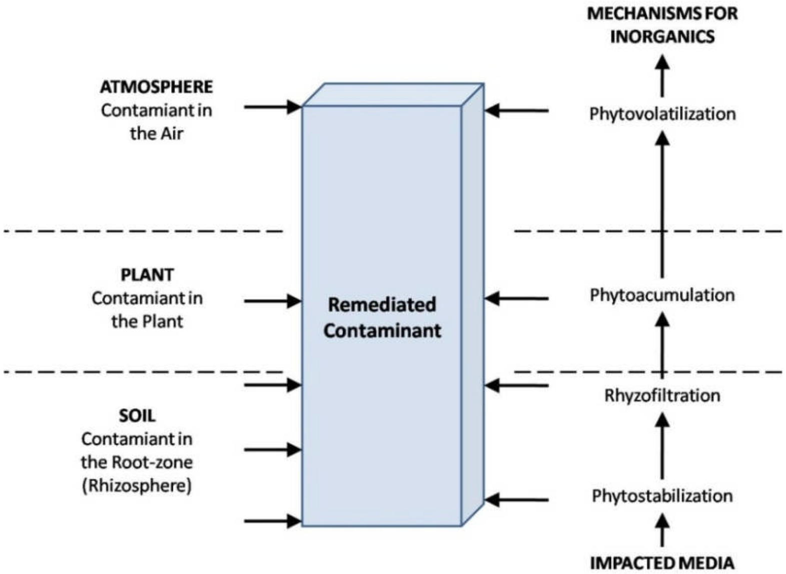


Figure 4.- Potential contaminant fates during phytoremediation in the soil-plant-atmosphere continuum (modified from Suthersan, 2002).

5.- Biology of the Typhacea

The Typhaceae (Cattail family) are represented worldwide by the genus *Typha*, comprising about 18 species of herbaceous plants which provide fibrous leaves used for matting and construction. The fruit is a dehiscent nutlet (Ellis, *et al.*, 1985). This perennial freshwater aquatic herb can grow three or more meters in height (Motivans and Apfelbaum, 1987), with linear, thick, and spongy leaves from subterranean stems (Heywood 1978). The plants have unisexual wind pollinated flowers that are condensed into thickened terminal spadix structures (close cylindrical spikes) which consist of two portions; the pistillate, or female, portion is below, and the staminate, or male, portion is above (Figure 5). North American cattails have male flowers located close to the terminus of the spadix. Achenes are produced from female florets and have an elongated embryo and a stalk covered with fine, unmated hairs that aid in wind dispersal.

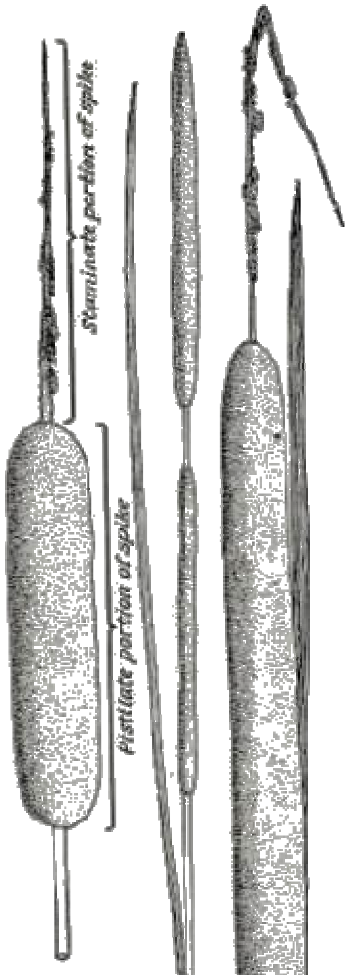


Figure 5.- Male and Female portions (Modified from Fasset, 1940).

Cattail produces a dense rhizome mat and the clustered leaves cause litter. Dense Cattail growth and litter may reduce the opportunity for other plants to establish or survive (Weson and Waring 1969). Mineralized substrates necessary for many plants to germinate are buried by this litter. Beneath the litter, substrates are cool and moist, optimal conditions for survival of a seed bank (Van der Valk and Davis 1976) but not for seed germination. Vegetative reproduction by rhizomes creates characteristic cattail colonies. Survival of rooted aquatic plant rhizomes does not occur for more than several growing seasons without the gas exchange supported by live and dead stems reaching above the water's surface (Whigham and Simpson 1978). Minimal carbohydrate reserves occur immediately prior to flowering (Linde *et al.* 1976, Gustafson 1976). Dispersal can occur by seeds, rhizomes, corms, and by the movement of parts of colonies torn by wind, water, ice, or animals. Cattail control or reduction may be desirable where noticeable increases threaten natural plant diversity and habitat heterogeneity.

According to Apfelbaum (1985), Motivans and Apfelbaum (1987) and Selbo and Snow (2004), three species of cattail and several hybrids occur in North America, this includes broad-leaved (*T. latifolia*), narrow-leaved (*T. angustifolia*), and tall cattails (*T. domingensis*) (Smith 1962, Hotchkiss and Dozier 1949), also there are hybrids of intermediate appearance that have been reported, and are often referred to as the species *Typha x glauca* (Motivans and Apfelbaum, 1987).

Cattails are considered to have a cosmopolitan distribution and wide ecological amplitude. They may appear in almost any wet place like wetlands, sedge meadows, along slow moving streams, river banks, and lake shores and are often the first invaders in a newly excavated poll. The underground stems spread extensively, so that a stand of Cattail an acre in extent may actually consist of but a few plants.

Cattails can grow on a wide gradient of substrate types. Wet pure sand, peat, clay and loamy soils have been documented under cattail stands.

Typha spp plants are mined by caterpillars of the moths *Arzama opbliqua* and *Nonagria oblonga* (Klots 1966). Aphids and *Colandra pertinaux* (the snout beetle) also feed on *Typha* spp leaves and stems. The stems may have many species of pupa living within them (Klots 1966). The cattail rhizomes provide food to mammals such as the muskrat (*Ondatra* spp). The grazing of muskrats may greatly influence cattail communities.

According to many authors, Cattails have a high capacity to take heavy metals into their structure (Mc Naughton et al., 1979; Taylor and Crowder, 1984; Pip and Stepaniuk, 1992; Ye *et al.*, 1997; Suthersan, 2002; Sasmaz *et al.*, 2008). *Typha* spp plants tolerate high concentrations of heavy metals in his tissue without serious physiological damage as was presented by Dunbain and Bowmer (1992).

METHOD

In this research, the methodology was structured in four main parts: a) the collection of plants (cattail, *Typha* spp) and its acclimatization; b) the design and construction of the wetland and the preparation of the experimental solution; c) the measurement of pH, Cd and sulfate quantification in percolates from the wetland, and, d) the analysis and interpretation of the results to evaluate the wetland efficiency and the statistical analysis.

1.- Collection of Plants (**Typha** ***spp) and climatization.***

1.1.- Collection of Plants

Plants were collected and analyzed from different sites. The plants from the Tangamanga I park were selected. This is a recreational park located in the city of San Luis Potosi (San Luis Potosi, Mexico). The plants at this place grow naturally in the irrigation and drainage channels. In this site, the plants did not contain significant concentrations of metals or metalloids. The selected plants had a height between 30 and 50 cm, and were collected trying to preserve most of rhizomes. The collection was realized trying to avoid the damage of aerial and roots areas (Carranza-Álvarez, 2005; Alonso-Castro, 2005). Figure 6 shows the collection site at the Tangamanga I park.

Figure 6.- Collect area in the Tangamanga I Park, irrigation and drainage channels where *Typha* spp is growing.

After the collection, the plants were planted in a commercial soil substrate (Pro-Mix™), which is a mixture of Canadian *Sphagnum* spp peat moss, perlite, wetting agent and limestone, other type of nutrients were not added (Figure 7). This is aimed to keep plants alive by simulating the conditions of their natural habitat (Carranza-Álvarez, 2005; Alonso-Castro, 2005). Once the plants adapted to the conditions mentioned, they shall be conditioned to granular substrates chosen as carrier for the constructed wetland (Gravel with pea gravel, 50% each). Running water is added and the liquid and the liquid level was maintained below of the support material for the plants to simulate the same conditions of a subsurface wetland, no extra nutrients were added.

Figure 7.- Acclimatized plants in the soil substrate.

1.2.- Canadian *Sphagnum* spp peat moss as a organic matter source

Pro-Mix® is Canadian *Sphagnum* spp peat moss (CSPM) partially decomposed *Sphagnum* moss (75 to 85 %), mixed with limestone, agrolite (perlite) and moisturizing agents (Premier Tech).

2.- Design and Construction of the Wetlands

2.1.-Elaboration of Experimental solution

A synthetic solution was prepared, containing known concentrations of sulfate and Cd. Once the Cattail plants were planted in the wetland, both wetlands were exposed to a solution with 676 mg/L of sulfates and 1 mg/L of cadmium, during the first week. From the second to the fifth week the concentrations of sulfate and Cadmium were increased and maintained at 1,000 mg/L and 20 mg/L, respectively.

The sulfate solution was made with Sodium sulfate ($Na_2SO_4 \cdot 10H_2O$) (Reyes Máquez, 2008) and the Cd solution was made with cadmium nitrate ($Cd(NO_3)_2 \cdot 2H_2O$) (Carranza-Álvarez *et al.,* 2008). Also, 10 mL of nitric acid 70% (HNO_3) were added in each tank to reach a pH around 3 and to be sure that Cd is in the dissolved form. The substances were all added in each tank of 50 L before to feed the wetlands. The level of the solution was maintained at the mark of 41.5 L. The pH was measured by duplicate with a pH meter Orion™ model 520At.

2.2.- Design and Construction of the wetlands

Two constructed wetlands were made: one without plants (from now on it will be denominated as Wetland 1) and another one with *Typha* spp (from now on it will be denominated as Wetland 2). The wetlands were of 53 cm length, 39 cm height and 30 cm depth. The Substrate was made with a mixture of pea gravel (1/2 in, maximum size), gravel (3/4 in, maximum size) and Pro-Mix® (60% organic matter and 40% of gravel mixture).

In other hand, each wetland had 2 tanks, one with the inflow solution (affluent) and the other one with the outflow solution (effluent). Both tanks were marked at every 5 L, in order to obtain a visual reference of solution volume (Figure 8). Previously that the soil mix and solution were added to the vessels to construct the wetland, all the materials (tanks, boxes, furnaces, hoses) were treated with a solution of nitric acid 3%. The inflow was controlled 2 times a day with screwing the faucet reaching the required inflow or the hydraulic residence time.

Figure 8.- The two constructed wetlands, in the back appears the wetland 1 (without plants) and in the front the Wetland 2 (with plants), also showing the inflow (affluent) tanks and the outflow (effluent) tanks.

2.3.- Calculations

The calculations that will be described below were used to design the wetland and to measure the residence time of the experimental solutions.

A) Porosity

The importance of porosity is due the soil pores that are filled with air and water. The air and water moves trough through the open spaces in the soil, so the amount of water and oxygen for the plant growth and the water movement rate in the soil are related with the pore size and the air amount. It can be calculated in the next equation (Aguilera, 1989):

$$\%\,\varepsilon = \left(1 - \frac{\rho_b}{\rho_p}\right) X\ 100$$

Where: $\%\varepsilon$ is the Porosity percentage; ρ_b is the Bulk density and is defined as the proportion of the weight of a soil relative to its volume. It is expressed as a unit of weight per volume, and is commonly measured in units of grams per cubic centimeters (g/cc); ρ_p is the Particle density or real density is defined as the weight of a known volume compared with the same water weight.

B) Actual Wetland Detention Time

The Actual Wetland Detention Time (τ) is defined as the wetland water volume involved in flow dividedby the volumetric water flow, and is given with the next equation (Kadlec and Wallace, 2009):

$$\tau = \frac{\mathrm{A_w h}\varepsilon}{Q}$$

Where: τ is the Actual Wetland Detention Time (time units); A_w is the wetland area (m^2); h is the wetland depth (m); ε is the Wetland Porosity (dimentionless); Q is the Flow (m^3/time units).

Note: *EPA (1999b and 1993) define the same equation as the hydraulic residence time (HRT) of a treatment wetland, which is the average time that water remains in the wetland, expressed as mean volume divided by mean outflow rate, and is given with the next equation:*

$$\tau = \frac{V\varepsilon}{Q}$$

*Where: τ is the hydraulic residence time (time units, t); V is the Wetland volume (cubic units, L^3; Volume is calculated from the following equation: A_w*h; where: A_w is the wetland area, and h is the depth); ε=Wetland Porosity (dimentionless); Q is the Flow rate (cubic units per time units L^3/t).*

C) Vegetation density

The vegetation or stem density of wetland plants is important because the resistance to water flow is determined in part by stem density (Kadlec and Wallace, 2009), thus, according to EPA (1999) and Koottatep *et al.* (1999), the Cattails (*Typha* spp) has a relatively large basal diameter, and occurs at about 40-50 stems per square meter in treatment wetlands, denser vegetation can contribute significantly to internal nutrient loading as the plants decompose (Sartoris *et al.*, 2000), a thick detrital mats will contribute to short-circuiting of flow (Groeneveld and French, 1995, cited by Thullen *et al.*, 2005), both mature and standing dead plants shade algal and autotrophic microbial communities, thus, reducing the nutrient-retention capacities (even if small and of short duration) of those communities (Hansson and Granéli, 1984; Moore et al., 1994; Grimshaw et al., 1997; Richardson, 1999; Wetzel, 2000a; Présing et al., 2001, cited by Thullen *et al.,* 2005) and, finally will not represent a significative difference, the number of stems increase, for the contamiant removal (Legacé, *et al.*, 2000).

3.- Analytical Chemistry

Sulfates and Cd concentrations in the affluent and effluent solutions were determined at Autonomous University of San Luis Potosí (UASLP) in the Trace Elements Laboratory of the Faculty of Chemistry, Water treatment Laboratory from Engineering Faculty and the Microwave acid digestion of soil with high content in organic matter was performed in the Environmental Restoration Laboratory from the Metallurgical Institute.

For Cadmium concentrations higher than 2 mg/L were determined by duplicate using a Varian™ Flame Atomic Absorption Spectrophotometer with Direct Air-Acetylene (model SpectrAA 220 FS with 228.8 nm of wavelength). For concentrations lower than 0.01 mg/L were measured by duplicate, using a Varian™ Graphite Furnace Atomic Absorption Spectrophotometer (model SpectrAA 220Z with 228.2 nm of wavelength) was used to measure; the samples were measured by duplicates.

For the sulfates were determined by duplicate using the standard turbidimetric method (APHA, AWWA, WEF, 1999, EPA, 2007), was used using duplicates.

A) Cadmium quantification.

A.1.- Direct Air-Acetylene Flame Method (AAS-F)

According to APHA (1999) and EPA (2007), the method followed was 3111B or EPA 7000B, all the reagents and standards were prepared with deionized water trace metals free. Aninternal calibration standard was prepared with a concentration of 1.5 mg/L of Cdand 5 standards of cadmium and a blank (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L). were prepared in a nitric acid solution 0.02N (Anachemia Environmental Grade). A calibration curve was obtained and the samples Cd concentrations were determined at 228.8±0.02 nm; the accuracy was ±0.05 mg/L.

Also, this method was used for the quantification of Cd associated to the soil organic matter in previous microwave assisted acid digestion samples (Section A.3; Ortega-Morales, 2011).

A.2.- Graphite Furnace Atomic Absorption (GFAAS)

According to EPA (2007), the method followed was EPA 7010, all the solutions including the blank for the calibration curve, the standards, the dilution of the samples were prepared with deionized water trace metals free. For the analytical quality control, we used the Standard Reference Material NIST 1643e that contain 6.57µg/L of Cadmium. The calibration curve was done with 5 standards (2, 4, 8, 16 and 20 µg/L) prepared in a nitric acid solution 0.02N (Anachemia Environmental Grade); absorbance was determined at a wavelength of 228.2±0.02 nm. All the measures were done by duplicate; the accuracy was ±0.05 µg/L.

A.3.- Microwave assisted acid digestion of soil with high content of organic matter (Ortega-Morales, 2011)

The soil samples were taken from each wetland using a PVC tube of 40 cm long and 1 cm of diameter (previously washed with nitric acid), using it as a corer. The soil cores of each wetland were taken at 10 cm distance from each other, in the direction of the length trying to cover all the wetland, after this, the three cores were mixed in order to obtain a homogenate sample from the wetland. The homogenate soil was sieved, using a sieve with openings of 2 mm, to obtain the fine particles fraction; then the sieved soil were put into plastic bags and weighted in an analytical balance (Ohaus™ model Adventurer) to obtain the wet weight. The soil samples were dried at 45°C during 72 h (stove VWR™ model 1324) and the dry weight was determined.

Duplicates of 0.25 g of dried soil were weighted (analytical balance Sartorius™ model BL605) from each wetland, and one Standard Reference Material NIST 27210. Each soil samples and reference material were mixed with 15 mL of concentrated nitric acid; the acid mixtures were placed in a Teflon™ microwave vessel and digested using a microwave heating unit CEM™ model Mars 5. The digestion program applied to the samples is shown in the Table 3.

Table 3.- Specified Variables for the Microwave assisted digestion with CEM™ Mars 5.

Stage	**1**	**2**	**3**	**4**	**5**
Power (%)	25	100	100	100	100
Pressure (PSI)	20	40	85	150	200
Time (min)	30	30	30	30	30
Time at Pressure	10	10	10	10	10

After cooling, the vessel´s content were filtered with Whatman™ 5 filter papers (2.5 µm), then diluted to a final volume of 25 mL in a volumetric flask. Cadmium concentrations were determined by AAS-F (Varian SpectrAA220FS) using the appropriate calibration curve and sample dilutions. All the measures were made by duplicates.

B) Sulfate quantification by Turbidimetric method.

The method followed was the EPA 375.4 method (EPA, 2007) for high range, using a Spectrophotometer HACH™ model DR-4000, program 5008, with 880 nm of wavelength. Previously, all the samples were filtered using 2 filters types: Whatman™ filter paper (No. 5, 2.5 µm) and Cellulose Whatman™ filter (0.45 µm). All the reagents, buffer solution and the standard were prepared with deionized water.

The buffer solution was made dissolving 30g of magnesium chloride ($MgCl_2 \cdot 6H_2O$), 5 g of sodium acetate ($CH_3COONa \cdot 3H_2O$), 1 g of potassium nitrate (KNO_3) and 20 mL of Acetic acid (CH_3COOH), all in 500mL deionized water and make up to 1 L.

In each tube 5 mL of sample, 1 mL of buffer solution and 0.5 g of barium chloride ($BaCl_2$). After the adding of the reagent, the tube was stirred for 1 min. The tube was placed inside the spectrometer in the cell holder and wait for 30 seconds wait for 30 seconds to develop the reaction. After that the sulfate concentration of each sample was read at 880nm. It is needed to say that the samples were diluted 1:5. The spectrophotometer is internally calibrated to give automatically the sulfates concentration (mg/L). Every 4 samples the blank was read in order to verify the calibration; the accuracy of this method is ±2.25mg/L.

4.- Statistical Analysis

4.1.- Coefficient of variation

Represent the ratio of the standard deviation ($\mathcal{S}$) of a set of numbers to their mean ($\bar{X}$) expressed as percent. It relates standard deviation (or precision) of a set of data to the size of the numbers:

$$CV = 100\left(\frac{\mathcal{S}}{\bar{X}}\right)$$

4.2.- Bias error

Represent the ratio of the error calculated as the difference between an observed (estimated) value and the true value.

$$\Delta e = 100\left(\frac{X\ obs - \tau}{\tau}\right)$$

Where: X obs is the Observed value (estimated); τ is the True value

4.3.- Recovery percentage

Represent the fraction, in percentage, of the total quantity of a substance recoverable following a specific chemical procedure:

$$R\ \% = 100\left(\frac{X\ obs}{\tau}\right)$$

4.4.-Wetland efficiency

Nowadays, different methods are being using to express the performance of wetlands in the retention of contaminants. A comparative analysis of performance assessment method to treat coal mine drainage was made by Tarutis *et al.* (1999). As shown in that work, traditionally it has been used the removed concentration of the pollutant calculated as the difference between the concentrations in the affluent (input) and the effluent (output) to calculate the removal efficiency. This measure has been called in for specialized treatment efficiency:

$$Wef. = \left(\frac{Cin - Cout}{Cin}\right)100$$

Where: *Wef* is the Wetland efficiency; *Cin* is the Concentration of analyte in the inflow (affluent); *Cout* is the Concentration of analyte in the outflow (effluent).

4.5.- Analysis of Variances (ANOVA)

The statistical analysis was performed using data analysis software STATISTICA version 8 (StatSoft, 2007), and the test was an Univariate Tests of Significance for each variable, in order to see if there are significant differences between the two wetlands. Those analyses were done to evaluate the effectiveness of the experimental conditions proposed in the present research in the removal of pollutants by the wetlands.

RESULTS

As it can be seen in the table 5 and from the dimensions presented in the section 4, each wetland had an area of 2067 cm^2. According to EPA (1999) the number of plants per square meter of Cattails planted in Wetland 2 was calculated at 9.

Applying the equation 1, percentage of Porosity (% ε) was of 42% and so, the porosity is 0.42. This value was applied in the calculation of Actual Wetland Detention Time (eq. 2) for the present constructed wetlands obtaining 4 L/day (more precise 3.65 L/day) or 5 mL/min (more precise 4.63 mL/min).

Table 4.- Design parameters of the Wetlands and the variables initial concentrations.

	Area (m^2)	**Plant density**	**τ (L/day)**	**Q (L/day)**	**Evap (L/day m^2)**		**pH Initial (Average)**	**Cd^{2+} Initial (mg/L)**	**SO_4^{2-} Initial (mg/L)**
W1	0.2067	0	3.65	6.67	3.48		2.88	41	4676
W2	0.2067	9	3.65	6.67	6.96		2.87	41	4676

HRT- Actual Wetland Detention Time; Q - inflow; Evap - Evapotranspiration

1.- pH increase

The initial pHs were around 3 (pH of the Wetland 1 was 2.88, pH of the Wetland 2 was 2.87, see Table 4), at the end of the treatment the average pH were 8.39 and 8.32 in Wetland 1 and 2 respectively.

2.- Efficiency of the wetlands in the removal of Cd from the solution

As previously mentioned, the total Cadmium concentration was 20±0.01 mg/L in each tank (per week) and after the 40 days of treatment and after 40 days of treatment the concentration of Cd in the solution decreased until 10.66 µg/L for Wetland 1 and 3.85 µg/L for Wetland 2. The recovery of the reference material concentration was 97.03±2.97% (see Table 5).

According to equation 7 (Tarutis *et al.*, 1999), the wetland efficiency obtained was 99.99% for the Wetland 1 and 100% for the Wetland 2 (Table 6).

3.- Cadmium concentration in the substrate.

The Cadmium average concentration associated to the substrate using the microwave assisted acid digestion of soil with high organic matter (Ortega-Morales, 2011) were 8043.02 mg/Kg in the wetland 1 and 6850.07 mg/Kg in the wetland 2. The recovery of the certified material was 112.84±12.84%. Table 5 shows the results obtained for each one of the duplicates analyzed.

4.- Efficiency of the wetlands in the removal of sulfate from the solution

The total concentration of sulfates added to the wetland was 4676±2.5 mg/L, the average concentration of sulfate found in the outflow was 1195 mg/L for wetland 1 and 1135 mg/L for wetland 2. It is important indicate that there was a sulfate background value measured in both wetlands was 575 mg/L of sulfates, therefore, the real average value for wetland 1 was 620 mg/L and 560 mg/L for wetland 2.

Table 5.- Final pH, Cadmium and Sulfates concentrations in the Wetlands (by duplicates).

Wetland	**Cd Solution (µg/L)**	**Cd substrate (mg/Kg)**	**Sulfates (mg/L)**	**pH**
W1	12.68	8094.17	660	8.41
W1	8.64	7991.88	580	8.37
W2	3.94	6863.44	565	8.31
W2	3.76	6836.71	555	8.33
RM – certified conc	6.57	21.80	1000	
RM – determined conc	6.37	24.60	965	

Note: *shows the values obtained for each duplicate of Wetland 1 (W1) or for Wetland 2 (W2). RM refers to the reference material used for the analytical quality control.*

Taking into account the total amount of sulfate added and the final concentration found in the outflow (Table 6), the efficiency estimated for the wetland 1 was 74.4 % and for the wetland 2 was 75.7%.

Table 6.- Efficiencies (%) in the removal of Cadmium in solution, sulfate reduction.

	Wetland 1	**Wetland 2**
Cd solution	99.99	100.00
Sulfates	74.44	75.73

5.- Cadmium distribution in the wetland components.

The pool of Cd in the wetland 1 was, as expected, was the soil substrate due to 100% of the Cd was associated to this part of the wetland. In the wetland 2 most important pool of Cadmium was the soil substrate which had 85% of the total Cd concentration and, the 15% was found in the plant (Figure 9).

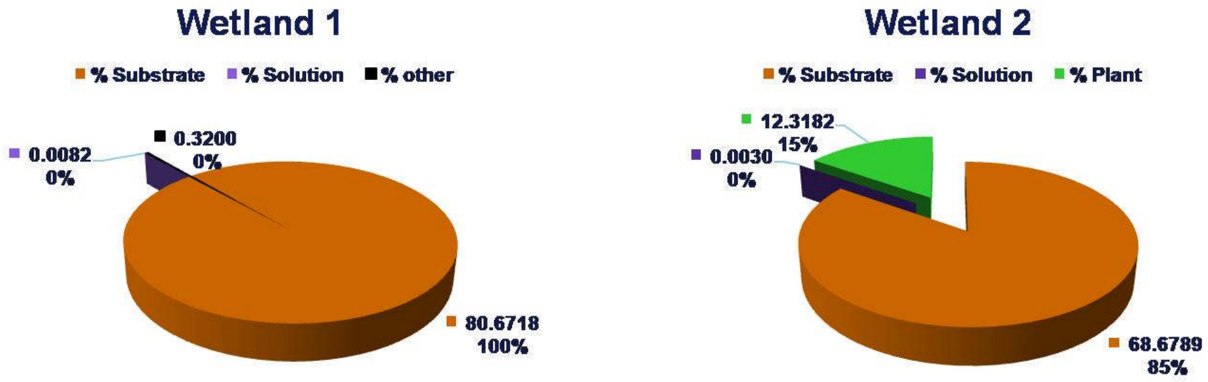


Figure 9.- Percentage of Cadmium distribution in the wetland components

DISCUSSION

In the present research, the pH increased in both wetlands, from 3 to 8 (Tables 5) that may be caused by an acid dissolution of Calcium Carbonate from the limestone (pKa=9.0, $K_{sp}=4.8e^{-9}$). This pH increasing helps to avoid, in nature and in mine sites, the metals and metalloids dissolution caused by the oxidation of pyrite (acid mine drainage). Table 7 shows an example of pH increasing through the dissolution of limestone using acetic acid.

Table 7.-The pH increasing (with pKa=4.76) and decreasing total acid concentration ([A]) by the dissolution of limestone.

[A] (mol/L)	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-10}
Initial pH	2.38	2.88	3.39	3.91	4.47	5.15	6.02	6.79	7.00
Final pH	6.75	7.25	7.75	8.14	8.25	8.26	8.26	8.26	8.27
Dissolved $CaCO_3$ (g per liter of acid)	49.5	4.99	0.513	0.0848	0.0504	0.0474	0.0471	0.0470	0.0470

The Cadmium removal occurs by different aspects: 1) by the soil substrate in the wetland and 2) by the plant, being the soil matrix the most important part of the system because removes the highest amount of Cadmium.

Removal of Cd by the soil substrate of the wetlands. Cadmium concentrations are limited by the dissolution of carbonates and its concentrations in alkaline or neutral soils as Santillán-Medrano and Jurinak (1975) mentioned. However, at pH higher than 6, Cd is adsorbed by the soil or precipitated, and the solution concentrations of Cadmium are greatly decreased (McLean and Bledsoe, 1992). Some authors (Kalmykovaa *et al.*, 2008; Tan, 2003; Crist *et al.*, 1999; Benedetti *et al.*, 1995),– have proposed that *Sphagnum* peat moss is a good biosorbent for the metal removal from wastewater and mine drainage. The sorption of these metals onto peat may be explained on the basis of the chemical constituents of peat, since peat chemical constituents are humic and fulvic acids with a high presence of anionic groups (Ulmanu *et al.*, 2008; Allen, 1987). Their results indicated that the interaction of Cadmium with the humic acids fraction in *Sphagnum* peat moss increase with an increasing pH and H^+ ions are released from carboxylic and phenolic groups. Also, with the increasing pH there are an increasing of negatively charged macromolecules repelling among them and making a greater number of active sites available for adsorption of other metal ions. Cadmium adsorption to phenolic groups is favored at high pH and low Cd concentrations. Benedetti *et al.* (1995) showed the contribution of carboxylic and phenolic sites to the binding of Cd and other metals on peat humic acid at pH 6 and 8. They observed that Cd was mainly bound to carboxylic groups at high concentrations and to phenolic groups at low concentrations (Figure 9). Balan *et al.* (2008) mentioned that sorptive removal of Cd increased with an increasing contact time. The influence of pH on the adsorption process has been reported from other studies (Petroni *et al.*, 2001; Viraraghavan and Rao, 1993; D’Avila *et al.* 1992; Gusset *et al*, 1986), where the best removal efficiencies happened within the range of pH 3.5 to 8.5; below pH 3.0 peat capacity metals adsorption decrease and above pH 6.5, most of the metal ion species precipitate and at pH 9.0 peat degrades due to the solubilization of humic substances (Coulliard, 1994). Also beyond pH 9 the metals would be precipitated as metal hydroxides (Viraraghavan and Rao, 1993).

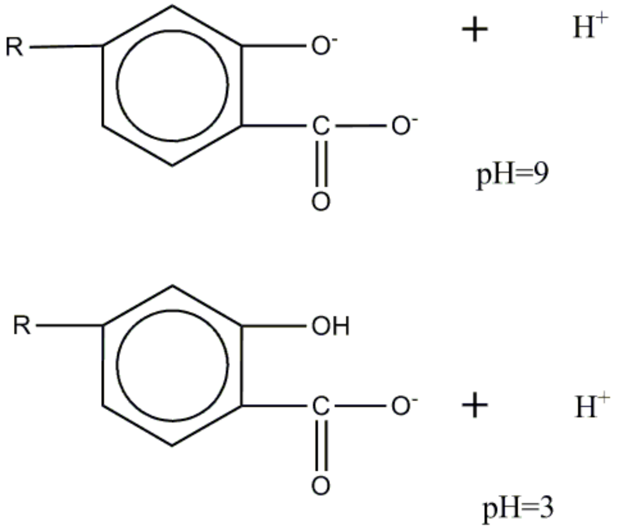


Figure 10.- Variable charges in a humic molecule by the dissociation of protons from phenolic group at pH 9 and from carboxylic group at pH 3 (Modified from Tan, 2003).

On the other hand, Cd could be also affected by the reduction of S. In the anaerobic conditions of the wetlands, the sulfates will be reduced to sulfide producing a very insoluble complex of Cd sulfide (CdS(s)), as it can be observed on figure 10 obtained by modeling with the software Medusa™. Kadlec and Knight (1995) mentioned that at high pH the compounds formed by the reduction of sulfates and organic matter produces hydrogen sulfide (H_2S) and bicarbonate (HCO_3^-), the redox conditions did not reach the reductive conditions to produce hydrogen sulfide.

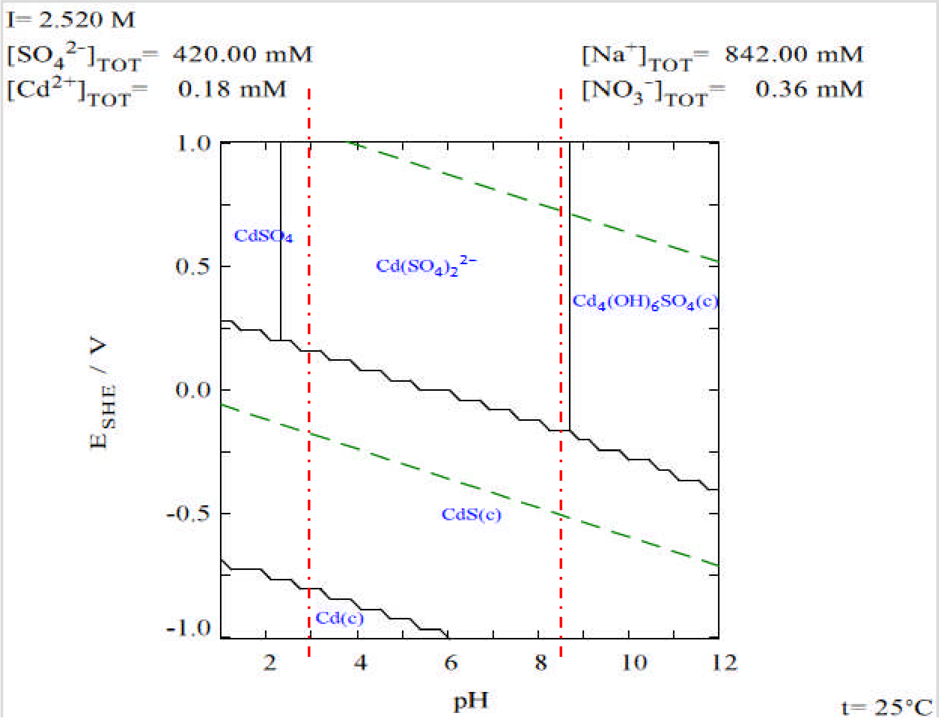


Figure 11.- Diagram Eh vs pH showing the dominant species of Cd at the experimental conditions of total dissolved sulfate concentration and ionic strength used in the wetlands. the punted lines represent the pH range, from 2.8 (inflow) to 8.4 (outflow). The temperature was considered at 25°C. The modeling was done using the software MEDUSA.

Removal of Cd by the plants. The Cd sorption by *Typha* spp, despite it is not as significant as the metal concentration removed by the soil, represents a very important metal removal process because the plants removed more than 15% of Cd from the solution. Alonso-Castro *et al.* (2009), reported 89% of Cd removal by *Typha* spp from solutions of 5 mg/L. According to Jindal and Samorkhom (2005), the plant is able to tolerate up to 3200 ppm and, in his research, the roots accounted more than 50% of the total uptake by the plant. This agrees with the review conducted by Suthersan (2002) where *Typha* spp plants typically had higher values of Cd in roots and rhizomes than in shoots (Table 8). Plant shoots contained less than 4 µg/g dry weight when they were exposed to solutions non contaminated with mine residues, but concentrations can become elevated in wetland plant tissues in response to exposure to very high concentrations of Cd as Carranza-Álvarez *et al.* (2007) mentioned for *T. latifolia* and *S. americanus* growing in wastewater where the plants accumulated 25 mg Cd/Kg roots. Taylor and Crowder (1984) said that it is not likely that there is an evolutionary selection for heavy metal tolerance, but rather it is inherent in the species. Alonso-Castro *et al.* (2009) reported a significant decrease in leaf elongation of the plants exposed to solutions with 7.5 mg/L of Cd and symptoms of toxicity such as fragility, chlorosis in leafs when the *T. latifolia* plants were treated with a solution containing Cd and Pb. In the wetland 2 none of those signs appeared in the plants, even the *Typha* spp plants in that wetland develop new shoots. This new shoots development can occur due to an increase in tolerance of the oxidative stress or an increase in the amount of Phytochelatins or class III metallothioneins, which are natural compounds engaged in the detoxification and homeostasis of heavy metals through metal thiolate formation, both process could occur due to the *Typha* spp plants acclimatization to the Cd solution concentration or the period of time where the *Typha* spp plants shows those signs of toxicity was not reached, whatever the reason was, the Typha spp plants in the wetland 2 tolerated Cd concentrations almost 11 times (10.8 times) higher than those reported by Alonso-Castro *et al.* (2009).

Table 8.- Examples of Cadmium content of Plant tissues and Sediments in treatment Wetlands (Kadlec and Knight, 1995).

Plant Part	***Typha* spp**	**Water (μg/l)**	**Solid (μg/g)**	**References (cited by Kadlec and Knight, 1995)**
Shoots	*T. latifolia*	Sewage	0.21	Obarska-Pempkowiak *et al.*, 2005
	T. latifolia	6.5	3.40	Mungur *et al.*, 1995
	T. latifolia	< 1	1.36	Eckhart *et al.*, 1999
	T. latifolia	Sewage	0.47	Maddison *et al.*, 2005
	T. latifolia	22.0	0.56	Lan *et al.*, 1990
Roots	*T. latifolia*	< 1	0.59	Eckhart *et al.*, 1999
	T. latifolia	Sewage	0.33	Obarska-Pempkowiak *et al.*, 2005
	T. latifolia	6.5	4.00	Mungur *et al.*, 1995
	T. latifolia	22.00	4.03	Lan *et al.*, 1990
	T. latifolia	Sewage	0.39	Maddison *et al.*, 2005
Rhizomes	*T. latifolia*	< 1	0.09	Eckhart *et al.*, 1999

The sulfate removal was not as effective as the Cd removal because theoretically the sulfates concentration should be lesser than 300 mg/L, the concentration feed to the wetlands. However, the sulfate concentration in the wetland solution before the sulfate addition was 575mg/L. I have considered this as the sulfate background concentration that probably comes from the peat moss. Many researchers had been reporting a sulfur accumulation on peat moss in in the order of 0.1 – 0.9 g/m^2/y (Moore *et al.*, 2004), 700–1400 mg/g (Botrell and Novák, 1997), 0.3–1.5 g/m^2/y (Novák *et al*., 1994). Novák and Wieder (1992) assumed that most of that sulfur is in the form of organic sulfur and most of the organic sulfur in all of these peat deposits was sulfur bonded to carbon, rather than sulfur bonded to ester. Depth profiles and the distribution of sulfur among inorganic and organic fractions in *Sphagnum* derived peat deposits appear to be controlled largely by depth dependent patterns in reduction/oxidation and in immobilization/mineralization (Novák and Wieder, 1992). Moore *et al.,* (2004) studied Eastern Canadian bogs and concluded that the plant canopy and upper peat control the subsurface dynamics of sulfur and nitrogen through their filter function, converting mineral nitrogen and sulfur into organic forms. Therefore, the content of S bonded to the *Sphagnum* peat will depend on the time, place and depth from where the peat is collected. Based on that, the sulfate removal was calculated subtracting the sulfate background concentration to those determined in the wetland effluent solution (outflow).

On the other hand, the efficiencies obtained were between 74 % and 76 %. This is a lower efficiency that that reported by Wiessner *et al.,* (2010). They found a 90 % sulfate removal from the inflow up to the middle of a planted wetland and observed a weak but continuous remobilization. Wiessner *et al.,* (2010) suggested a simultaneous reoxidation of immobilized sulfur from pools caused by the diffusion of oxygen through the unsaturated top-gravel layer also, the plants might have enough potential to enable reoxidation of the immobilized and dissolved reduced sulfur to sulfate, as can be supposed from the figure 11.

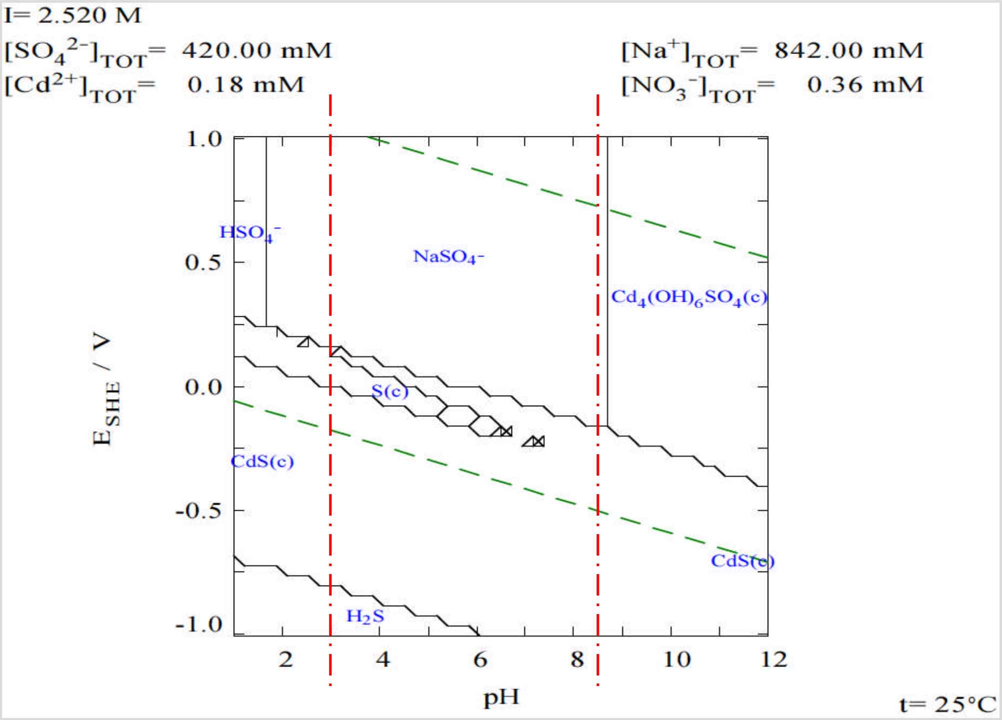


Figure 12.- Diagram Eh vs pH showing the dominant species of S at the experimental conditions of total dissolved sulfate concentration and ionic strength used in the wetlands. the punted lines represent the pH range, from 2.8 (inflow) to 8.4 (outflow). The temperature was considered at 25°C. The modeling was done using the software MEDUSA.

It is needed to say that the pH of the wetland probably did not interfere. Birnbaum and Wireman (1984) reported that the optimum growth of the sulfate reductive bacteria occurred at pHs lower than 8.5; at pHs higher that 9.2 no growth was observed by the authors. In the both wetlands studied in this work, the pH was below 8.5. Dvorak *et al.* (1992) mentioned that a low pH (below 6) the sulfate reduction activity is inhibited and the solubility of metal sulfides is increased.

According to the statistical analysis, Univariate test of significance of ANOVA between the two wetlands, for each variable the *p*-level presented significant differences ($p \leq 0.05$) in two of three variables analyzed (Table 8): Cd concentration in the substrate (soil) and sulfate concentrations in solution. the concentration of Cadmium in solution was not significant, also the F test, thru F value < 1, which corroborates the variance estimates between the two wetlands for each variable, shows a significantly F value greater than 1, in Cd in substrate and Sulfate concentration in the solution, hence the *Typha* spp plants create different microbiological conditions for the reduction of sulfates and conditions the removal of Cd from the solution and helping to precipitate it in the soil.

Table 9.- Results of the Univariate ANOVA analysis at a significance level of 95%. The significant values are represented in bold. F test value is greater than 1.

	***p* level**	**F value**
Cd in Solution	0.0614	8.5335
Cd in Substrate	**0.0002**	**439.0131**
Sulfate in Solution	**0.0001**	**609.8102**

CONCLUSIONS

- The wetland design was highly effective for the Cd removal and less effective in the sulfate removal. Also, the wetland buffered the low pH of the solution promoting the removal of the metal.
- The soil substrate, in both wetlands, is highly efficient in the sorption of Cd.
- The Cd removal was dominated by the substrate and was probably promoted by the pH increasing, the content of carboxylic and phenolic groups in the humic acids of the peat moss and the reduction of sulfate to sulfides. The acidity of the solution was buffered by carbonate dissolution. The sulfate reduction could be produced by the anaerobic conditions of the wetlands and so, Cd could be precipitated as CdS.
- There are significant differences between the two wetlands, being more efficient in sulfate reduction and Cadmium removal by the planted wetland than the unplanted wetland.
- This type of wetland can be used for the treatment of acid drainage under these experimental conditions.

RECOMMENDATIONS

- Taking in to account that the redox potential is very important to know in which oxidizing states are the substances under the experimental conditions, this parameter needs to be measured and used to determine the microbiological activity in the substrate. To avoid the fast pH increase by the acid dissolution of limestone, it will be necessary to put the limestone in a layer at the bottom of the wetland and reduce the amount of it, perhaps in 5 to 10%, hoping to reach a pH slightly above the 7 and not above 8, for a better removal of metals and better conditions of sulfate reduction.
- The same type of wetland should be tested with the interaction of more metals and metalloids, to evaluate its efficiency in the removal of several contaminants as those reported in mine wastes.
- To measure more variables like pH, dissolved oxygen, nutrients, etc, it is necessary to put some faucet sampler every 10 cm.
- In the near future, it will be necessary to test this technology for the application to the treatment of acid waters and test its efficiency in the removal of contaminants.

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APPENDIXES

APPENDIX 1.- Premier Tech´s Peat Bogs, Production Plants and Depot (Taken from Premier Tech, 2008).

legend

	Shipping Points	8
	Depots	5
	Production Plants	10
	Peat Bogs	21
◇	Int'l Head Office	1
	Research Center	1
☆	Sales Offices	2

Shipping Regions

Western	Central	Eastern
☐ CANADA	☐ CANADA	☐ CANADA
☐ USA	☐ USA	☐ USA
	☐ MEXICO	

ALL AROUND THE WORLD, PREMIER HORTICULTURE's customers rely on the **QUALITY** and consistency of our growing media. The superior quality of PRO-MIX products helps growers **OPTIMIZE** their commercial production while minimizing the labor and management costs involved. At the retail level, the results can be observed in the homes and gardens that blossom from PREMIER's **PERFORMANCE.**

APPENDIX 2.- Flame Atomic Absorption Spectrophotometer with Direct Air-Acetylene Report for the Cadmium solution in the outflow.

```
Peso nominal                       1.0000
Volumen nominal                    1.0000
Método:                            Cd (Zeeman)
Elemento - Matriz:                 Cd -
Tipo de instrumento:               Zeeman
Unidades de Conc.:                 µg/L
Modo del instrumento:              Absorbancia
Modo de muestreo:                  Normal automático
Modo de calibración:               Concentración
Modo de medida:                    Área de pico
Réplicas Patrones:                 2
Réplicas muestras:                 2

Longitud de onda:                  228.8 nm
Anchura de rendija:                0.5 nm
Ganancia:                          71 %
Corriente de lámpara:              4.0 mA
Posición de la lámpara:            1
Corrección de fondo:               C. Fondo activado

PATRÓN 1:                          2.000 µg/L
PATRÓN 2:                          4.000 µg/L
PATRÓN 3:                          8.000 µg/L
PATRÓN 4:                          16.000 µg/L
PATRÓN 5:                          20.000 µg/L

Altura del cabezal:                0.0 mm
Volumen total:                     15 uL
Volumen de muestra:                10 uL
Factor de reducción del Vol.:      2
Conc. de partida:                  20.000 µg/L

CdCERO CAL       0.000       µg/L   -0.0035    -0.0020    -0.0050
CdPATRÓN 1       2.000       µg/L    0.1479     0.1445     0.1512
CdPATRÓN 2       4.000       µg/L    0.2666     0.2633     0.2700
CdPATRÓN 3       8.000       µg/L    0.4920     0.4914     0.4925
CdPATRÓN 4       16.000  A   µg/L    0.7659     0.7584     0.7734
CdPATRÓN 5       20.000  A   µg/L    0.8965     0.8787     0.9143
Ajuste de curva             = Racional Nuevo
Conc.característica         = 0.059 µg/L
r                           = 1.0000
Concentración calculada     = -0.046  2.069   3.913   8.104   15.414  20.528
```

Residuales = 0.046 -0.069 0.087 -0.104 0.586 -0.528

Cdh1	10.660	µg/L	0.6025	0.8846
Cdblanco	0.122	µg/L	0.0091	0.0081
Cdstandard	6.373	µg/L	0.4060	0.4055
Cdh2	3.850	µg/L	0.2628	0.2512

APPENDIX 3.- Direct Air-Acetylene Flame Method Report for theCadmium associated to the soil in the wetland.

Peso nominal 1.0000
Volumen nominal 1.0000
Método: Cd (Llama)
Elemento - Matriz: Cd -
Tipo de instrumento: Llama
Unidades de Conc.: mg/L
Modo del instrumento: Absorbancia
Modo de muestreo: Manual
Modo de calibración: Concentración
Modo de medida: Área de pico
Réplicas Patrones: 3
Réplicas muestras: 2

Longitud de onda: 228.8 nm
Anchura de rendija: 0.5 nm
Ganancia: 39 %
Corriente de lámpara: 10.0 mA
Posición de la lámpara: 3
Corrección de fondo: C. Fondo desactivado

PATRÓN 1: 0.500 mg/L
PATRÓN 2: 1.000 mg/L
PATRÓN 3: 1.500 mg/L
PATRÓN 4: 2.000 mg/L
PATRÓN 5: 2.500 mg/L

Tiempo de medida: 5.0 s
Retraso previo a la lectura: 5 s
Tipo de llama: Aire/Acetileno
Flujo de Aire: 13.50 L/min
Flujo de acetileno: 2.00 L/min
Altura del quemador: 0.0 mm

CdCERO CAL	0.000	mg/L	0.0020	0.0010	0.0025	0.0025
CdPATRÓN 1	0.500	mg/L	0.7511	0.7504	0.7497	0.7533
CdPATRÓN 2	1.000	mg/L	1.4468	1.4450	1.4467	1.4487
CdPATRÓN 3	1.500	mg/L	1.9975	1.9920	2.0005	2.0000
CdPATRÓN 4	2.000	mg/L	2.5804	2.5669	2.5888	2.5854
CdPATRÓN 5	2.500	mg/L	3.0314	3.0301	3.0291	3.0350

Ajuste de curva = Racional Nuevo
Conc.característica = 0.003 mg/L

r = 0.9998
Concentración calculada = 0.001 0.506 1.012 1.460 2.010 2.514
Residuales = -0.001 -0.006 -0.012 0.040 -0.010 -0.014

Cdblanco	0.005	mg/L	0.0082	0.0080
Cdstandard	1.502	mg/L	2.0457	2.0459
Cd1	1.160	mg/L	1.6368	1.6300
Cd2	**0.246**	**mg/L**	**0.3693**	**0.3661**
Cd3	1.375	mg/L	1.8981	1.8963
Cd4	1.356	mg/L	1.8760	1.8739
Cd5	1.165	mg/L	1.6404	1.6400

Note: Cd1 and Cd5 are for Wetland 2, Cd3 and Cd4 are for Wetland 1, Cd2 is for Reference Material NIST 27210.

APPENDIX 4.- Sulfate concentration values determined by HACH using the Turbidimetric Method, without subtracting the original value of the sulfates in the wetland.

Dilution Factor (DF) 1:5, except tap water.		
	w/o dilution factor mg/L	**Applying DF mg/L**
H_2O tap	**3**	**3**
H_2O wetland w/o sulfates	**115**	**575**
H_2O initial (1g/L)	**193**	**965**
W1.1	**247**	**1235**
W1.2	**231**	**1155**
W2.1	**228**	**1140**
W2.2	**226**	**1130**

APPENDIX 5.- Redox potential range in soil and sediment, showing the microbial metabolism process and electron acceptor

Table 10.- Redox potential range in soil and sediment, showing the microbial metabolism process and electron acceptor (Taken from Delaune and Reddy, 2005).

<table>
<tr><td colspan="11">Anaerobic</td><td colspan="4">Aerobic</td><td>Sediment condition</td></tr>
<tr><td colspan="3">Highly reduced</td><td colspan="5">Reduced</td><td colspan="3">Moderately reduced</td><td colspan="4">Oxidized</td><td>Redox condition</td></tr>
<tr><td colspan="2">CO_2</td><td colspan="3">SO_4^{2-}</td><td colspan="3">Fe^{3+}</td><td colspan="3">Mn^{4+}
NO_3^-</td><td colspan="4">O_2</td><td>Electron Acceptor</td></tr>
<tr><td colspan="16"></td></tr>
<tr><td colspan="4">Anaerobic</td><td colspan="7">Facultative</td><td colspan="4">Aerobic</td><td>Microbial Metabolism</td></tr>
<tr><td>-300</td><td colspan="3">-200</td><td colspan="2">-100</td><td>0</td><td colspan="2">+100</td><td>+200</td><td>+300</td><td>+400</td><td>+500</td><td>+600</td><td>+700</td><td></td></tr>
</table>