STONEWORTS (CHARA, NITELLA) AS A TOOL FOR SUSTAINABLE TREATMENT OF LOW STRENGTH WASTEWATERS

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Abstract: Lack of sustainability is evident in many existing wastewater treatment technologies. Wetlands and/or plant mediated remediation (phytoremediation) seemed to be a good answer in this regard. However greatest problem with this technology is the fate of plants that going to be rich in a particular contaminant, as upon senescence the contaminant it accumulated will get released to water. Thus as an answer to this shortcoming we have tested the applicability of stoneworts for the phytoremediation of low polluted wastewaters. Two independent experimentations were conducted to check the remediation of phosphorous (P) and heavy metals (chromium and cadmium). Results suggest stoneworts managed to store considerable portion of all these contaminants in redox insensitive forms. Thus we conclude stonewort mediated phytoremediation to be promising sustainable technique within the ranges and conditions investigated.

Keywords: *Heavy metals, Phosphorous, Phytoremediation, Stoneworts*

1. Introduction

The increasing shortage of water in the world along with rapid population increase and development gives reason for concern and the need for appropriate water management practices. Many present day systems are a “disposal-based linear system”, where the treated wastewater is disposed to waterbodies (e.g. river) or land (e.g. agricultural land). Even though the effluent meet the statutory requirement frequent disposal will ultimately make the ecosystem of the receiving body polluted in the long run due to processes like bioaccumulation. Thus the problem with the current treatment technologies is they lack sustainability. As such sustainability concepts like reuse, recycle etc., are not been practiced. It should be noted 99% of the wastewater is composed of water and the remaining contain vital nutrients like nitrogen, phosphorous (P), potassium and sulphur [1]. Disposal-based linear systems not only omit sustainable goals but also not cost effective.

Phytoremediation is the use of plants and their associated microbes for environmental cleanup [2]. This technology is an effective cleanup technology for variety of organic and inorganic pollutants. Inorganic pollutants that can be phytoremediated include plant macronutrients (nitrate and phosphate), plant trace elements [chromium (Cr), copper, iron, and manganese (Mn)) nonessential elements (cadmium (Cd), cobalt, fluorine, mercury). In case of aquatic plants, uptake of heavy metals to produce an internal concentration greater than in the external environment appeared widespread [3].

Phytoremediation generally takes place in a wetland system (either constructed or natural). In wetland treatment, natural forces (chemical, physical, and solar) act together to purify the wastewater [1]. Wetland treatment technology in developing countries offers a comparative advantage over conventional, mechanized treatment systems because the level of self-sufficiency, ecological balance, and economic viability is greater. The system allows for total resource recovery [4]. Thus in the first glance it can be concluded wetlands as a feasible option and green building rating systems such as LEED™ [5] explicitly recommend it as a potential technology. However greatest problem of phytoremediation would eventually be the fate of plants used for the purpose which become rich in that particular contaminant. Thus harvesting the plants at regular intervals is necessary if not upon senescence and decomposition, accumulated heavy metals or similar contaminants will get re-enter to the water column. Thus to make a wetland system and/or phytoremediation more sustainable, considerable attention need to be given to the above issue. Thus here we are going to check the applicability of the aquatic macrophyte stoneworts (also known as charophytes) for the phytoremediation of nutrients and heavy metals.

Charophytes the growth form of characean algae are an obvious form of aquatic vegetation in many quiescent water bodies: fresh to brackish and temporary to permanent with a worldwide distribution [6]. Many forms of charophytes are subject to calcification, which in the form of $CaCO_3$, takes place on stems, branchlets and on the surface of oogonia [7]. Calcification accompanies the photosynthetic utilization of bicarbonate [7].

2. Objectives and Methodology

The objective of this study is to assess the advantages of stoneworts, the growth form of characean algae in a wetland system. We specifically investigated the following:

1) To study the applicability of locally found stoneworts to accumulate P and selected metals and heavy metals
2) To discuss the suitability of stoneworts colonized shallow wetlands for ground water recharge

Experiment 1

Two sets of microcosms (5 L laboratory fish tanks) were maintained for a period of one year planted with: two stoneworts (*Chara* sp. and *Nitella* sp.). After one year plants were analyzed for total P and carbonate bound P as described by Siong and Asaeda [6] and, Gomes and Asaeda [7]. Plants were grown in a basic water sand combination: several times washed commercially available river sand and city tap water. The results will be compared with the results of two vascular angiosperm herbs (*Najas marina* and *Vallisneria gigantea*) as reported by Siong and Asaeda [6].

Experiment 2

Five sets of microcosms (1 L beakers) were maintained for a period of one year planted with *Nitella* sp. Three microcosms with plants; no heavy metals (control), 0.2 mg/L Cr^{6+} and 0.01 mg/L Cd. The other two without plants were given the same heavy metal treatments; 0.2 mg/L Cr^{6+} and 0.01 mg/L Cd. All units contained 40 mg/L calcium (Ca). After one year, plants and sediments were sampled. Plants were analyzed for the relevant heavy metal in alkaline and acidic regions of the main thalli. A sequential fractionation procedure to determine Cr or Cd speciation as exchangeable, carbonate bound, organic bound and residual was carried out according to Tessier et al. [8] for sediment. For other water analyses of both experiments were carried out according to APHA [9].

3. Phosphorous fractionation of plants

The total P and carbonate bound P fraction of plants of experiment 1 are shown in Table 1. The total P of *V. gigantea* and *N. marina* was significantly higher than *Chara* sp. and *Nitella* sp. (*ANOVA; $P<0.05$*). It should be noted even when compared on ash-free dry weight basis the total P of *V. gigantea* and *N. marina* observed to be significantly higher than the *Chara* sp. and *Nitella* sp. (data not shown). However the carbonate bound P content of *Chara* sp. and *Nitella* sp. observed to be significantly higher than *V. gigantea* and *N. marina* (*ANOVA; $P<0.05$*). The carbonate bound P fraction of *V. gigantea* and *N. marina* was less than 1 %. Thus the remaining 99% of the total P should contain water soluble P and/or organic P. Many water soluble and organic P compounds are bio-available. For example water soluble P contains inorganic P forms, which are immediately available for planktonic microorganism uptake. Unlike the two angiosperm species discussed the carbonate bound P fraction of stoneworts was notably high, 10% and 8% for *Chara* sp. and *Nitella* sp. respectively.

Table 1: *The total phosphorous (TP) and carbonate bound phosphorous (P) fraction of plants of experiment 1.*

Sample	TP (mg/g)	Carbonate bound P fraction (%)
Chara sp.	1.0 (0.05)	10.1 (3)
Nitella sp.	0.8 (0.01)	8.2 (2)
V. gigantea[1]	4.0 (0.12)	0.9 (0.01)
N. marina[1]	4.5 (0.06)	0.3 (0.00)

[1] source: Siong and Asaeda [6]

Rhizoid-bearing stoneworts are known to acquire P and also other nutrients primarily from the water column [45]. In contrast, vascular plants acquire P mainly from sediment via their roots and this result in high P content in plant biomass [7]. This is the main reason for the high P values observed in vascular plant tissues relative to charophytes [7]. Thus, vascular plants assimilation can result in reduction of nutrients in the water column. However, vascular submerged plants, upon senescence release the accumulated P again to the water column, making net P accumulation (in the long run) zero [9].

Decalcification, followed by co-precipitation of phosphate with $CaCO_3$ is an important process in the reduction of the bio-available P in the water column [10]. Calcium bound P in stoneworts has been discussed by Kufel and Kufel [11] referencing to sediment of lake that has been dominated by charophytes.

4. Hyperaccumulation of heavy metals by stoneworts

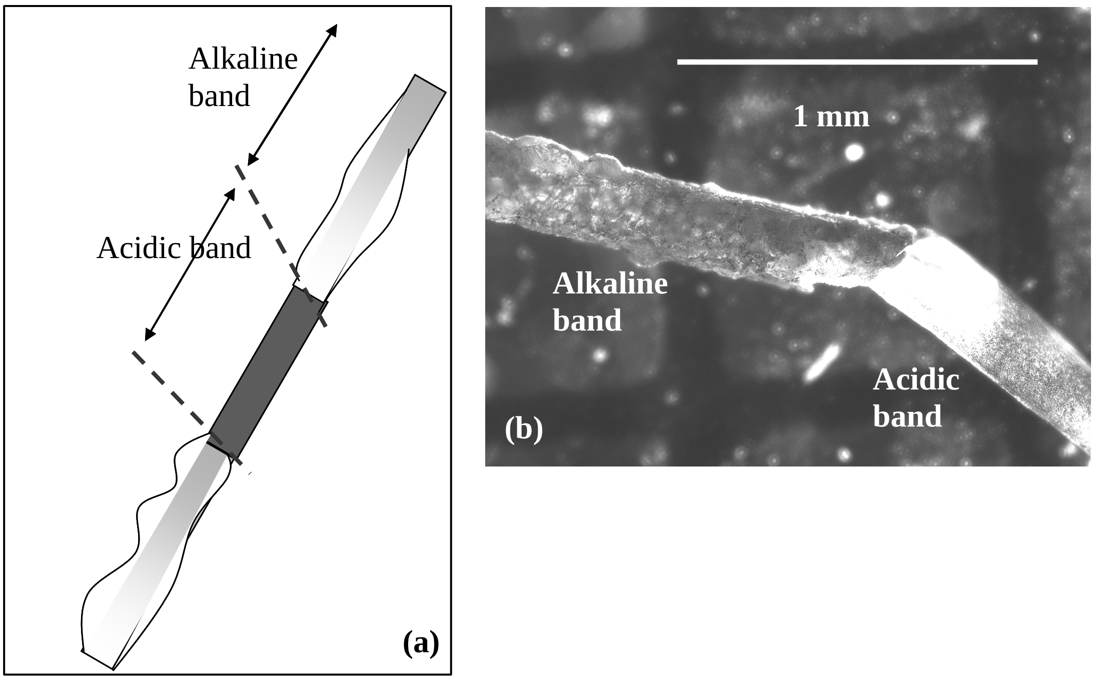

Figure 1: *(a) Conceptual layout and (b) microscopic view (Olympus, Japan) of acidic and alkaline bands, respectively.*

Table 2 illustrates the levels of Cr and Ca observed in alkaline and acidic areas of plants of the experiment 2. Alkaline areas contained 0.75 mg/g Cr, whereas 0.61 mg/g Cr in acidic areas. Thus alkaline areas contained about 55% of the total Cr of plants. It should be noted the alkaline areas had an ash content of > 90 % from its dry weight, compared to < 1 % in acidic areas. The ash content is comparable to the Ca levels of the respective regions and they can be used as alternatives when only one is available [12]. Thus after correcting for ash alkaline regions will give significantly high (*ANOVA*, $P < 0.05$) Cr content relative to the acidic regions. Similar results were obtained for Cd treated units (data not shown).

Table 2: *Chromium (Cr) and calcium (Ca) levels measured in alkaline and acidic areas of experiment 2. Parentheses give standard deviation for mean.*

Fraction	Alkaline band		Acidic band	
	Cr	Ca	Cr	Ca
Fraction (%)	55.1	99.8	44.9	> 0.2
Concentration (mg/g)	0.75 (0.09)	74.8 (1.1)	0.61 (0.13)	0.1 (0.0)

Sequential fractionation of sediment for heavy metals

Table 3 shows sequential fractionation of sediments of Cr treated units (experiment 2). The carbonate bound Cr observed to be the highest fraction. Next highest fractionation was organic bound. When compared with the results of microcosoms without plants but treated with heavy metals it was evident that these two fractions were indeed from plant detritus. Similar results were obtained for Cd treated units (data not shown).

Table 3: *Sequential fractionation of sediments carried out for the chromium treated units of the experiment 2. Parentheses give standard deviation for mean.*

Fraction	%	mg/g
Exchangeable	19.3	0.013(0.001)
Carbonate bound	35.4	0.025(0.009)
Organic bound	34.5	0.024(0.010)
Residual	10.8	0.007(0.000)

A thick marl bottom sediment layer frequently found beneath charophytes meadows is an evident that charophytes calcite can function as long term storage of Ca. Subsequent analysis conducted for these sediments found it contain not only Ca but also other elements in high levels. Alkaline areas to have extremely high levels of Ca are a well documented observation and similar results were obtained with this experiment. Apart from Ca, Mg is also known to get precipitated [7]. Alkaline areas to have precipitations of strontium (Sr) and Mn was reported by McConnaughey [13].

High Cr content in alkaline areas and carbonate bound fraction suggest Cr can get accumulated in processes associated with calcite. Two processes are possible: absorption/adsorption and coprecipitation. As significant percentage of Cr are in redox insensitive forms makes frequent harvesting unnecessary.

5. Advantages of using stoneworts in a wetland designed to treat low polluted wastewaters

The intention of sustainable design is to "eliminate negative environmental impact completely through skillful, sensitive design". Often domestic wastewaters after the secondary treatment could be regarded as low polluted. In many cases the effluent might have already met the standards stipulated by the authorities. However the effluent still contains pollutants at trace levels. Thus such an effluent might not be good for sustainable activities like groundwater recharge or irrigation. Freshwater wetlands can, in some circumstances, renovate added secondarily treated wastewater, thus providing an alternative to land or water disposal or expensive physical-chemical treatment processes [14]. Successful remediation of wastewater in wetlands is advantages in many ways. Wetlands can be used for groundwater recharge. This makes the wetland a closed loop treatment. Researchers have discovered ground water recharge of up to 20% of wetland volume per season [15]. However this needs extreme caution considering the possible contamination of groundwater if the recharge is meant by wetlands used for wastewater treatment. The plants we are proposing such as stoneworts are advantages in many ways. It not only accumulates nutrients like P, but also in redox insensitive forms. After the plant senescence most of the accumulated P bound with calcite will get precipitated in the wetland bottom. Thus in the long run resource recovery will be possible. It should be noted that P is an especially important nutrient to recycle, as the P in chemical fertilizer comes from limited fossil sources. Ability to accumulate heavy metals when present in trace levels will make this plant ideal for the treatment of industrial wastewaters. It should be noted treatment of industrial and domestic wastewaters should be done in separate wetlands. It is recommended to carryout pilot scale experimentation in this regard.

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