

Studies of Water Arsenic and Boron Pollutants and Algae Phytoremediation in Three Springs, Iran

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Abstract Today drinking water quality, water pollutant and cleaning water are important topics in applied hydrology. Algae play an important role in controlling metal concentration in lakes and oceans. Their ability to absorption metals and taking up toxic elements from the environment has been recognized for many years. The study is examination of the possible extent of Arsenic and Boron algae phytoremediation in three springs from Sang-E-Noghreh area, Iran, by calculating bio-concentration and bio-accumulation factors in them. Water samples from the sites were separately collected for microscopy, ICP/MS analyzing and graphitic oven. Algae were identified and counted using available equipment and references. Data analyzed and bio-concentration (BCF) and bio-accumulation factors (BAF) were calculated for Boron and Arsenic. Results showed four algal divisions (Chlorophyta, Cyanophyta, Euglenophyta and Heterokontophyta) in all of sample sites waters with the exception of Gargab that lacked Euglenophyta. Boron and Arsenic BAF were higher than 10 in all three sites. It is believed that algal flora of three springs have absorbed and accumulated these elements from their environment into their bodies. Then these algae can be hyper-phytoremediator and their presence in water reduced water Arsenic and Boron pollutant.

Keywords Biological treatment, Algae, Phytoremediation, Arsenic, Boron, Iran

1. Introduction

Many environmental pollutants move through food chains and accumulate at sizeable levels in the tissues of animals and man[1,2]. A high environmental persistence and high lipophilicity chemicals should be thoroughly investigated for their potential toxicity through bioconcentration and bioaccumulation, both measured for long periods of exposure. Phytoremediation is one new approach that offers more ecological benefits and a cost efficient alternative. The plant used in the phytoremediation technique must have a considerable capacity of metal absorption, its accumulation and strength to decrease the treatment time[3]. The chemical compounds bioconcentration and bioaccumulation in aquatic and terrestrial organisms represent important criteria for ecotoxicological evaluation and hazard assessment[4,5]. Recent developments in environmental research have revealed that many living organisms can accumulate certain toxicants to body concentrations much higher than present in their environments[6].

Water pollution is one of the human problems in recent century. It is known as increasing some element

concentration having poisonous potential from the standards, can produce irreparable environmental effects[7]. A variety of both biotic and abiotic factors have been implicated in determining the boundaries of species distributions and water qualification. Biotic factors important to segregation include resources, predators and aquatic macrophytes. Abiotic factors such as dissolved oxygen, temperature and light can also influence the distribution of aquatic species[8,9]. So, studying biological and physico-chemical factors of water is necessary for finding suitable and economic ways for water quality improvement. Biological filtration of water is one of the standard and economical methods in comprising with other methods. Some living organisms such as algae are able to reduction or removing toxic elements by absorption from their environment. This method is called phytoremediation[10]. Phytoremediation is considered the remediation technique that generates the lowest negative environmental impact on the remediation sites and can be applied into newly developed urban areas because of its high public acceptance and its function as an aesthetical pleasure[11].

Algae are a group of living organisms that play an important role in controlling metal concentration in lakes and oceans[12,13] and the density and absorption of pollutants such as heavy metals in their environment[10]. The ability of algae to absorb metals has been recognized for many years. Algae possess the ability to take up toxic elements from the

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environment, resulting in higher concentrations than those in the surrounding water [11,14]. Bioaccumulation studies reveal the accumulation of the contaminant in the organism via uptake of food or water containing the contaminant [15].

Arsenic have been classified as group I carcinogens based on human epidemiological data [16]. Some aquatic macrophytic species (*Eichhornia crassipes*, *Lemna gibba*, *Lemna minor*, *Spirodela polyrhiza*, *Ipomoea aquatic*, *Azolla caroliniana*, *Azolla filiculoides*, *Azolla pinnata*, *Pistia stratiotes*, *Hydrilla verticillata* and *Lepidium sativum*) have been reported to accumulate high level of arsenic from contaminated water. Their arsenic uptake ability, mechanisms and their potential in phytoremediation technology have been studied and evaluated [9].

Boron contamination is a serious environmental problem affecting both ecosystems and human activities [17]. Exposure to large amounts of boron over short periods of time can affect the stomach, intestines, liver, kidney, and brain and can eventually lead to death [18]. In order determining the environmental fate of released chemicals from different pollutant sources, to determine their bioconcentration in aquatic species is essential. Bio-concentration factor (BCF) of a chemical compound is defined as the ratio between the concentration of that chemical in an organism (Corg) and the concentration of the chemical compounds in the aqueous environment (Cm) [2,19-26]. The calculation of BCFs can be based on the wet weight (BCFw) or on the lipid content (BCFl), of the aquatic organism or its tissue. BCFs are usually determined for various species of fish, but other aquatic organisms can be used, such as algae or mussels. Aquatic organisms can accumulate chemical compounds both directly from the environment (via skin or respiratory surface) and indirectly (by collecting and concentrating a chemical compound from food). This process is called bioaccumulation, and is measured with the bioaccumulation factor (BAF) which is defined as the ratio of the concentration of a chemical accumulated inside an organism (Corg) to the concentration in the surrounding environment (Cm) [1,2,4,16]. The bioaccumulation level depends on the nature of the chemical compound, species, duration of exposure, concentration in water and its accumulation level in food [27]. BAF was categorized as: <1 excluder, 1-10 accumulators, >10 hyper accumulators [28,29]. Shamsuddoha et al. (2006) in their studies on growing algae in rice fields showed that algae can hyper accumulate as from water [14]. Also a rice variety receiving arsenic-contaminated irrigation water ranging from 0 to 2.0mg as/L, and grown with and without algae, showed that the presence of algae in the growth medium reduces as accumulation in rice. The pot experiment showed that algae could reduce accumulation of arsenic in rice plants by as much as 71%. Algal growth was also found to depress arsenic accumulation in soil [15].

The present study is an attempt to examine the possible extent of phytoremediation Arsenic and Boron by algae in three spring waters from Sang-E-Noghreh area (Khorasan-e-Razavi), Iran by calculating bio-concentration and

bio-accumulation factors in algae.

2. Materials and Methods

2.1. Study Site

Experiments were carried out in Sang-E-Noghreh Village in 115 Km to South-East of Mashhad-Khorasan Razavi, Iran and 40 km to South of Fariman. The area is located on long. 35° 26' to 35° 30' N and lat. 59° 50' to 59° 52' E with 1077 hectare surface area, 1600-2830 m altitude from sea surface. For studies of algal flora and Arsenic and Boron algae phytoremediation with calculating bio-concentration and bio-accumulation factors in Sang-E-Noghreh, three springs (Arrehe kamar=A1, Zireh Ghaleh=A2 and Gargaab=A3) were selected (Figure 1).

2.2. Sampling and Analyses

In March, 2010 water samples from the sites were collected for ICP/MS analyzing after measuring some water physical factors such as pH and temperature using HDPE vials. Each water sample was filtered using 0.45 μ micro filter and then poured in the labeled vial. Then samples pH was regulated by adding nitric acid and stored at 4°C until sending to the Canadian Acme Laboratory. Also for algal flora studies three groups of algal samples were collected.

First group of algal samples were collected for studying density without any fixation, Second group was fixed for identification using fixative (0.1 M Sodium Cacodylate and %6 Glutaraldehyde) and third group were collected in sterile vial for element concentration measurement. All of samples were put into the aluminum cover and kept in 4°C. Then, samples were transferred to the laboratory. Algae of first and second group samples were identified using light microscopy and available references [30-34]. Counting of samples was undertaken based on Lobban et al (1998) [35] method. Third group samples were dried using 80°C oven for 24 hours. After sample digestion and filtration, boron and arsenic concentration was measured using Shimaduz-AA-670 graphitic oven.

2.3. Data Analysis

Data were analyzed using the EXCEL₂₀₀₇ software. Figure 2 show four identified algal division concentrations in three springs of Sang-E- Noghreh area in winter. Also bio-concentration (BCF) and bio-accumulation factors (BAF) were calculated for Boron and Arsenic elements in three examined spring waters. Results have been shown in Table 1.

3. Results

Results of algal flora studies showed existing four algal divisions (Chlorophyta, Cyanophyta, Euglenophyta and Heterokontophyta) in all of sample sites waters with the exception of A₃ site that lacked Euglenophyta. Table 1 and Figure 2 show the divisions concentrations in three springs (Arrehe kamar=A₁, Zireh Ghaleh=A₂ and Gargaab=A₃) of Sang-E- Noghreh area in winter. The most algal genera

variation was observed in A₃. A₁ had the highest level of family and order numbers (Table 1). A₁ site showed the highest Chlorophyta and Cyanophyta density (mm³), while, Heterokontophyta had the highest concentration in A₃ site (Figure 1). Also Arsenic and Boron concentrations in water and algae samples from these springs (BFCs) and their bio-accumulation factors (BAFs) have been shown in Table 1. As the Table shows algal Arsenic concentration (ppb) of A₁ site is higher than two others, while, A₂ had the most algae Boron concentration (ppb). A₁ site showed the highest level of Arsenic and Boron bio-accumulation factors (BAFs) (Table 1).

4. Discussion and Conclusions

Based on our results it is believed that absences (in A₃) or low density presences (in A₂ and A₃) of Euglenophyta in all three studies sample sites in winter depended on low water temperature (Figure 1). As Shayestehfar *et al* (2010) showed temperature regulates the density and diversity of zooplankton in freshwater. Their data showed that maximum zooplankton density occurred in 26.5°C to 28.1°C air and water temperature [36]. Also Khodaparast (1998) studies showed the most frequency of Euglenophyta was observed in increasing water temperature and entering organic compounds into the water [37]. Chlorophyta density was nearly equal in all three sample sites. But it was more in A₁ comparing to A₂ and A₃ and Cyanophyta density was much more in A₁ too. Our data comparison with WHO (World Health Organization) standard showed water Arsenic concentration is higher than drinking water standard limit in A₃ site, but A₁ and A₂ are in standard limit. Boron in A₂ and A₃ water samples was higher than drinking water standard limit, while A₁ was in standard limit [38] (Table 1). Also bio-accumulation factors (BAFs) for these elements were higher than 10. Therefore Chlorophyta and Cyanophyta are hyper-absorbent and hyper-accumulator for Arsenic and Boron [28]. Comparison of Arsenic and Boron BAFs in three studied sites showed the highest level in A₁ for both elements (Table 1). It is believed that Chlorophyta and Cyanophyta have absorbed and accumulated these elements from

their environment into their bodies. Then these algae can be hyper-phytoremediator and their presence in water reduced water Arsenic and Boron pollutant. In A₃, by the reason Heterokontophyta high density and low density of Cyanophyta, its Arsenic and Boron water is more than two other sites. Based on these results it can be concluded that existing pectocellulose compounds in Chlorophyta and silica in Heterokontophyta cell walls have prevented of Arsenic and Boron high level absorption from their environment. Peptidoglycan cell wall presence in Cyanophyta cell wall and absence of silica and pectocellulose may be caused more absorption of these two elements from water in comparison with Chlorophyta and Heterokontophyta. Studies of physico-chemical effects of water especially heavy metals concentrations on algae density in three springs of Sang-E-Noghreh area, Iran in winter showed that positive correlations between Arsenic concentration and Heterokontophyta density [10]. Phytoremediation of waters by aquatic organisms such as algae has been recently explored for the removal of organic pollutants possessing endocrine disrupting capacity (39). Also Sjahrul (2012) reported using marine phytoplanktons (*Tetraselmis chuii* and *Chaetoceros calcitrans*) as phytoremediators (40). Sekabirak *et al* (2010) studies showed that algae have the potential to accumulate Pb, Cd, Cu, and Zn. Algae can thus be used in bio-monitoring of heavy metal pollution in urban stream water since it can be used in quantification of pollutants. Concentrations of heavy metals in algal biomass reflect metal load in the stream water. Algae can therefore be used in the phytoextraction process of heavy metals in aquatic urban stream water and wastewater effluents (41). Imamul Huq *et al* (2007) showed that *Navicula* (a Heterokontophyta genus) was able to Arsenic bio-absorption from its environment [15]. According to our results and above cases pure cultivation of each algae genus from Chlorophyta, Cyanophyta and Heterokontophyta in different pollutant media is separately suggested. Then, it can be exactly help us for a clear conclusion about accumulated or remediated algae taxa.

Table 1. Results of water samples algal studies and their analysis using microscopy, ICP/MS and graphite oven methods from three springs in Sang-E-Noghreh area, Iran (Arreh kamar=A₁, Zireh Ghaleh=A₂ and Gargaab=A₃) and their Arsenic and Boron bio-concentration (BCF) and bio-accumulation factors (BAF) in winter

Factors	Sample sites (Springs)		
	ArrehKamar (A1)	Zireh Ghaleh (A2)	Gargaab (A3)
Algae Arsenic concentration (ABCFA) ppb	75086	18386	16198
Water Arsenic concentration (WBCFA) ppb	6.5	2.3	22.70
BAF of Arsenic (ppb)	11551.69	7993.91	713.57
Algae Boron concentration (ABCFB) ppb	165210	267370	191200
Water Boron concentration (WBCFB) ppb	211	1194	1260
BAF of Boron (ppb)	782.99	223.93	151.75
Chlorophyta density in mm ³ (in winter)	381	300	307
Cyanophyta density in mm ³ (in Winter)	5347	1753	1824
Euglenophyta density in mm ³ (in Winter)	19	6	0
Heterokontophyta density in mm ³ (in Winter)	106	33	427
Algal orders numbers	9	5	5
Algal families number	13	6	9
Algal genera numbers	18	7	20

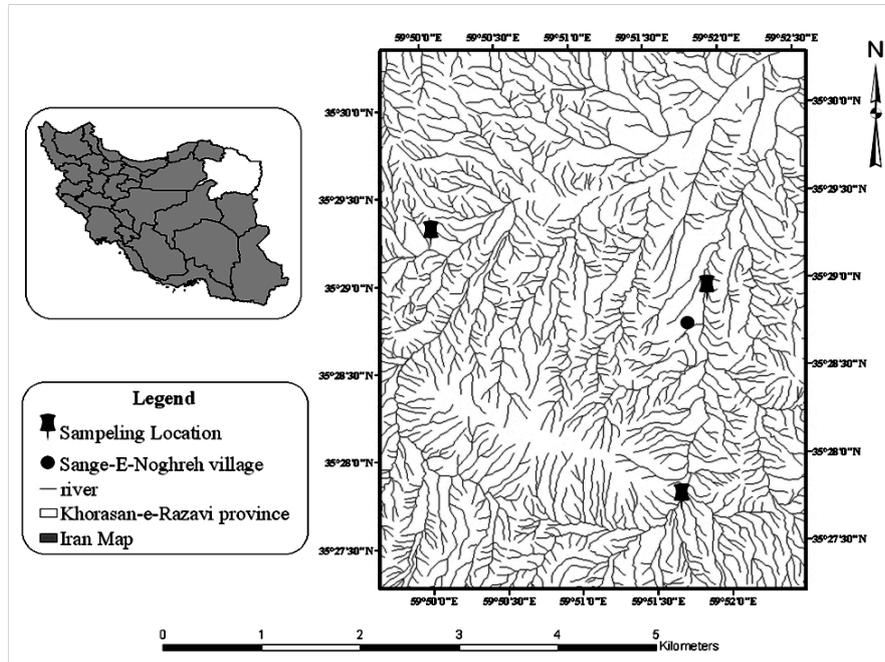


Figure 1. Water and algae sampling sites location in Sang-E- Noghreh area, Iran

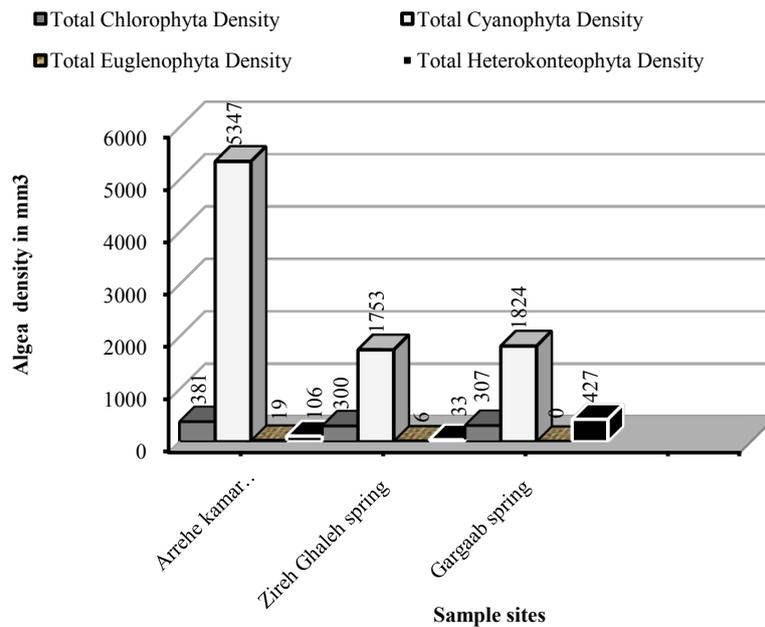


Figure 2. Four identified algal division concentrations in three springs of Sang-E- Noghreh area, Iran in winter

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