

Bioavailability of phosphorus of river sediments and its effect on growth of *Selenastrum capricornutum*

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April 28, 2020

Abstract

Increasing anthropogenic loading of phosphorus (P) threatens aquatic ecosystems. The bioavailability of P in sediments for algal growth depends on several physiochemical properties such as particle size distribution, mineralogy, pH, electrical conductivity (EC), and carbonate-content. This study was aimed on selecting the best chemical extraction method to characterize P-availability for the algae *Selenastrum capricornutum*. Principal component analysis (PCA) of the data identified two components that cover 79.3% of the total variation, and these components were dominated by particle size distribution, active calcium carbonate equivalence (ACCE), and EC. Many of the considered extractions were positively correlated with each other, with the exception being Bray-II. The sediments of some rivers had a higher Olsen-extractable P than 20 mg kg⁻¹, that is considered a threshold value above which the aquatic environment may become negatively affected. The average rank order of P extraction by single extractants was: Colwell > Mehlich III > NaOH 0.1 M > Olsen > Morgan > AB-DTPA > Bray II. The Colwell-extractable P concentrations of sediments varied from 1.44 to 88.0 mg kg⁻¹. This extractant significantly correlated with algal growth ($r^2=0.92$, $P<0.001$) and was the best single extraction method to characterize sediment P-bioavailability. Algae (*S. capricornutum*) growth was also well correlated with Mehlich III, NaOH 0.1 M, Olsen, and Morgan extractable-P concentrations. Algae growth was highly significantly correlated with EC.

Introduction:

Phosphorus is an essential macronutrient that is often limiting primary production in rivers and lakes. In terms of the Redfield ratio (106C:16N:1P), even a small addition of P under P-limited conditions could lead to a significant increase in the primary production of aquatic ecosystems. This may lead to eutrophication and hypoxia, a water dead zone, with significant loss of biodiversity and changes in the food web (Orihel et al., 2017; Dijkstra et al., 2018). As algae may bind nitrogen, an increase in P-availability may trigger algal outbreaks. This has been observed in many cases to occur due to excessive fertilizer and manure applications

For many aquatic systems, soil and particulate-P erosion together with runoff water are main sources of inputs of P to surface water (Brendel et al., 2019). Sediment pore water and desorption from its solid phase replenish P in overlying waters, and become available for consumption by aquatic organisms. It has long been recognized that soil and sediment bound P is only partly biologically available to plants and aquatic organisms as algae (Van Rotterdam et al., 2012). The bioavailable fraction of P can be evaluated by algal bioassays (Liu et al., 2016). Although algal assays are difficult to conduct and time-consuming, the bioavailable P estimated by assays is considered well to quantify the potential of sediment P to cause eutrophication (Young et al., 1985; Ekholm and Krogerus, 2003). Indeed, the quantification of bioavailable phosphorus for algal growth was shown to be essential to estimate eutrophication risks (Ellison and Brett,

2006; Okubo et al., 2012). However, it is attractive to predict bioavailable P using a chemical measure that is simple, routinely applicable, and cost-efficient. A single chemical extraction that allows to screen a large number of samples and monitor the health of aquatic ecosystems requires that the relationship between bioavailability of P from algal bioassay experiments and chemically extracted P is clear and unambiguous.

Several chemical extraction methods have been developed in the field of soil fertility to estimate bioavailable P in soil and sediments such as those developed by Morgan (1954), Mehlich (1983), Bray-Kurtz (1961), Colwell (1952) and Olsen et al. (1954). Chemical extractants are based on different mechanisms and abilities to extract P and may differ regarding the chemical used for extraction as well as e.g. sediment: water ratio, equilibration (shaking) time, and targeted P pools. For instance Bray-P may be useful for acid, but not for calcareous materials. Also Mehlich-P is based on extraction by acid, but performs better for high pH soils. Olsen-P extracts with NaHCO_3 and is more commonly used for pH-values exceeding slightly acid values, as it was developed for alkaline soils. Phosphorus extracted by 0.1 M NaOH is one of first methods developed to quantify algal available P (Young, 1982). Anderson and Magdoff (2005) reported that Olsen-P had the highest correlation with *Selenastrum capricornutum* growth in comparison with other extractants. Huettl et al. (1979) equilibrated sediment suspensions with hydroxy-Al saturated cation exchange resin and found that the P retained by resin correlated with *Selenastrum capricornutum* ($r^2 = 0.98$; $n = 5$). The well-known 0.01M CaCl_2 -extraction (Boekhold et al., 1993) provided excellent results for agricultural soils that are regularly limed. Sharpley (1993) used a selective extraction that was based on establishing contact between a soil suspension and Fe-oxide coated paper strips that were developed by Van Der Zee et al. (1987). The so-called Fe-paper extracted P content of runoff sediment was closely correlated ($r^2=0.96$) to the growth of P-starved *Selenastrum capricornutum* incubated for 29d with runoff as the sole source of P (Sharpley, 1993).

To improve phosphorus management and deal effectively with adverse legacy effects of sedimentary phosphorus, we investigate bioavailability of P for the aquatic ecosystem. The scope of this research was to 1) study the bioavailability P in sediments according to several single chemical extractions and assessment of their relationship with P-inhibited algal growth, and 2) recognize influencing factors in P extraction in sustaining algal growth.

Material and Methods:

Study area

Urmia Lake is one of the 59 international parks of world heritage designated by the United Nations. It is located between west and east Azerbaijan in the uppermost northwest center of Iran. It has coordinates between 37 to 38 degrees and 15 minutes north latitude, and between 45 to 46 degrees east longitude. The Urmia National Park consists of three units: 1) a water unit, 2) a mountain and coastal plain unit, 3) rivers and wetlands. Urmia Lake basin has an area of 51876 km², which comprises 38% mountainous areas, 21% hills, 11.2% flats and terraces, 9% lakes, and the remainder (20.8%) includes plains and various lands utilized for different uses. From 19 rivers flowing into the lake, 14 are permanent and 5 others are seasonal rivers. The annual water discharge is estimated to be 5317 million m³. The desiccation of Urmia Lake and lowering of the river water level entering the lake, illustrates the need to give attention to the increasing pollution of rivers and wetlands around the lake. Water diversion and management of dryland regions for agricultural practices are having a serious impact on the water quality in the Urmia Lake basin.

Rivers are the main components of Urmia Lake ecosystems and connect the land to saltwater and form a land-freshwater-saltwater continuum. Although, they are often perceived as delivering dissolved, colloidal, and particulate P from the land to larger open waters, in fact also a series of biogeochemical processes along the continuum change the form and fate of P (Arfania et al., 2018). In the last decades, population growth, climate change, and intensification of agricultural practices such as land use change, overgrazing, and upland erosion have led to an increased loading of sediments to the downstream sections of rivers of the Urmia Lake basin. This resulted in dense algal blooms in downstream waters and wetlands around the western part of Urmia Lake basin which is an important habitat of aquatic organisms and birds.

Sediment Sampling

Twenty-five representative sediment samples from seven main western rivers of Urmia Lake basin were collected for this study (Figure 1). These included 9 sites in Nazloo Chai (S10 and S11), 3 sites in Shahar Chai (S13 to S15), 4 sites in Baranduz Chai (S12 to S15), 3 sites in Ghadar Chai (S16 to S18), 2 sites in Mahabad Chai (S19 and S20), 3 sites in Simineh Chai (S21 to S23) and 2 sites in Zola Chai (S24 and S25). Each river was sampled near the headwater and at the discharge point to the Urmia Lake Basin, and sometimes in-between. A total of two or three vertical sediment cores (0-10 cm) was collected from each sampling site and then mixed to have a composite sample. River sediments commonly dry out as water level recedes and this process can be expected to influence P speciation (Schönbrunner et al., 2012). For the Urmia Lake basin, water levels rise and overland flow occurs seasonally in the riverbed sediments, therefore most samples were collected from dry sites.

Characterization of the sediment

The sediments comprise a wide range in P bioavailability and P-retention capacity. In preparation for the algal assay, sediments were dried at 25°C and passed through a 2-mm sieve. Particle size distributions of the sediments were determined by hydrometer method and organic carbon content followed the dichromate oxidation method (Rowell, 1994). Soil pH and electrical conductivity (EC) were measured in a sediment-solution ratio of 1:5 with a pH and conductivity meter, respectively. Calcium carbonate equivalents (CCE) were determined by acid-neutralization method (Rowell, 1994) and the amount of active calcium carbonate equivalence (ACCE) by reaction with neutral ammonium oxalate (Loeppert and Suarez, 1996).

Sediment chemical and physical properties are shown in Table 1. The data was normally distributed according to the Kolmogorov-Smirnov test. The texture of sediments was often loamy sand and sandy loam. Sand (CV = 58.8%) and clay (CV = 54.3%) contents of sediments showed large variations in comparison with silt (CV = 39.3%). Electrical conductivity was low suggesting that the water was fresh (non-saline). Overall, sediment pH was alkaline (7.26-8.02), which might be related to higher calcium carbonate equivalents (CCE) in carbonate-rich sediments in the basin, and pH was quite similar in these samples. The average CCE and ACCE of sediments were 15.03 (2.50-23.75) and 2.08 (0.54-3.86), respectively.

Sediments were extracted using several single extraction methods that are summarized in Table 2. Olsen-P and Colwell-P extraction method have been designed for calcareous soils. Mehlich III-P and Bary II-P have been designed for acidic soil, and both contain HCl acid and ammonium fluoride for dissolution of Fe/Al-P minerals such as Strengite, (FePO₄·2H₂O) and Variscite, (AlPO₄·2H₂O). AB-DTPA extraction method is chelating agent for Fe, Al, Ca, and Mg and has been shown to be a good extraction method for acidic and calcareous soils. Morgan-P was designed for soil with a high association of Ca-P pools and usually dissolve soluble calcium phosphate (Ahmad and Jones, 1967). 0.1 M NaOH is for soil and sediment with high Fe/Al-P pools. Chemical analysis of P was done colorimetrically (Murphy and Riley, 1962).

Algal Bioassay

Selenastrum capricornutum, (a coccoid unicellular green algae) was cultivated in a nutrient stock solution containing macronutrients: NaNO₃ 25.5 mg L⁻¹, MgCl₂·6H₂O 12.2 mg L⁻¹, CaCl₂·2H₂O 4.41 mg L⁻¹, MgSO₄·7H₂O 14.7 mg L⁻¹, K₂HPO₄ 1.04 mg L⁻¹, and micronutrients: H₃BO₃ 185 µg L⁻¹, MnCl₂·4H₂O 416 µg L⁻¹, ZnCl₂ 3.27 µg L⁻¹, CoCl₂·6H₂O 1.43 µg L⁻¹, CuCl₂·2H₂O 0.012 µg L⁻¹, Na₂MoO₄·2H₂O 7.26 µg L⁻¹, FeCl₃·6H₂O 160 µg L⁻¹, Na₂SeO₄ 2.39 µg L⁻¹, at 25±1°C under continuous (250 µE m⁻² s⁻¹) "Cool-White" fluorescent light and 3 cm³/s aeration flow rate, and shake twice daily by hand until cells reached a stationary growth phase (10 d). To improve test performance Na₂EDTA·2H₂O 300 µg L⁻¹ was added. Culture medium was buffered with NaHCO₃ 15 mg L⁻¹. The culture medium containing microalgae at a stationary phase was centrifuged at 3000g, supernatant was discarded and pellets introduced to P-free nutrient growth medium. Severe phosphorus deficiency was estimated to occur when cells started to turn yellow (about 7 d), allowing cells to grow for an additional 3 d to ensure that the culture was starved (Figure. 2).

The test begins when the algae are added to the test flasks. Mix the inoculum well, and add 1 mL to the test solution in each flask. Flask positions in the incubator was randomly rotated each day to minimize possible spatial differences in illumination. Three replicates of each sediment were prepared by adding 100

mg of homogeneous sediment to 50 mL of P-free nutrient growth medium in separate 250-mL Erlenmeyer flasks, autoclaved at 121°C and 15 psi to avoid microbial contaminations. Flasks were inoculated with P-starved cell to give an initial flasks concentration 3×10^4 cell mL⁻¹, incubated at a constant temperature of $23 \pm 1^\circ$ C, light intensity of 110 μ E m⁻²s⁻¹ and stirred manually twice a day. Cell counting was done using a hemocytometer counting chamber for 14 d.

Statistical Analysis

All P concentrations data generated in this study were analyzed with Minitab16. A simple Pearson correlation was developed for each parameter (EC, pH, CCE, OM, ACCE, Clay, Sand, Silt, Vfs, and D50) by using the regression procedure in Minitab 16 to estimate the influence of these parameters in P concentration. Phosphorus concentrations in different pools are dependent on geochemical variables and the parameters studied were chosen as independent variables.

Results and Discussion:

Multivariate analysis of sediment properties

To recognize the physico-chemical properties and sediment that controls the variability of the data, the results of a principal component analysis (PCA) of sediment characteristic variables are shown in Table 3. The first and second components together represent 79.3% of the variation. First PC is mainly determined by ACCE, Clay, and silt, and is negatively dependent on Vfs, D50 and Sand. This implies that correlated variables control variation in the data set. The second component showed strong negative correlations with pH and CCE, negatively correlated with OM, and EC. There were large differences between observations from various samples (Figure 3). This may be due to sampling from different rivers representing a wide diversity of properties such as texture, pH, EC, and ACCE. Overall, the PCA analysis revealed that variation of sediments properties is dominated by particle size distribution, and to a slightly smaller extent by EC, ACCE and OM. Particle size distribution varied, which was partly due to differences of geomorphological features of rivers, slope of terrain, and geological structure of region.

Relationships between various sediment P extractants

Table 4 shows the descriptive statistics of the P extractions. The results indicated that different extraction methods showed large differences in P extractability for the same sediment samples. The average ranking order of extracted-P by single extractants was: Colwell > Mehlich III > NaOH 0.1 M > Olsen > Morgan > AB-DTPA > Bray II. Some sediments had a higher Olsen-extractable P than the threshold value of 20 mg kg⁻¹, which value indicates possible release that may adversely affect the aquatic environments (McDowell et al., 2001; Sims et al., 2002).

In order to assess, the correlation of extractants on the concentrations of different P extraction, additional analyses were made for each extractant and the key results are shown in Table 5. Although there were large variations between the P-extractants, they were significantly correlated with each other with r² values ranging from 0.68 to 0.95. The strongest correlation is observed between sediment Mehlich III-P and Olsen-P. Considering that the pH of most sediments in this study was less than 7.6, potentially less neutralization of the Mehlich III-P extracting solution resulted from sediment CaCO₃ (Kuo, 1996; Wang et al., 2015). In Mehlich III, the extraction with nitric acid, acetic acid, and fluoride, lead to the release of P from sediment by dissolution and the formation of complexes with iron and aluminum and calcium. As fluoride and calcium form precipitates of calcium fluoride, this leads to dissolution of calcium phosphates (Kamprath and Watson, 1980). The weakest relationship was found between sediment Olsen- P and Bray-II-P, likely due to small extraction by the Bray-II P test for sediments with pH >6.8 or sediments with a high degree of base saturation (Sims, 2000). However, the lack of correlation between Mehlich III and Bray II was not expected, because there is a common belief that these two methods extract similar P pools from soils and sediments by cation hydrolysis (Kleinman et al., 2001; Ebeling et al., 2008; Wang et al., 2016). We attribute the poor correlation between these two methods was to the high pH of some sediment samples (pH >7.3) (Sotomayor-Ramírez et al., 2004) and a range of sediment P levels studied with Mehlich III-P <50 mg kg⁻¹. There was a significant

positive correlation between all P extractions except with Bray II and Total-P contents. Zhou et al. (2001) reported a good correlation between extracted Total-P with Olsen-P and 0.1 M NaOH. However, regression analysis showed that correlation between NaOH 0.1 M and Total-P in sediments was smaller than for other extractants.

Algal growth and sediment test phosphorus

A linear relationship between algal growth and 7 of the sediment P tests is shown in Figure 4, which shows that algal growth was different in the growth medium incubated with different sediments. Moreover, this figure reveals that there is a significant relationship between the P tests and *Selenastrum capricornutum* growth (Figure 4). Of the seven sediment P test measurements, Colwell-P correlated best with algal growth ($r^2=0.92, P < 0.001$). Part of the explanation for the high correlation between Colwell-P and Olsen-P with algal growth may be that the pH of algal growth solution, buffered at pH 7.5 with NaHCO_3 , was much closer chemically to the Olsen and Colwell extracts of 0.5 M NaHCO_3 at pH 8.5 than the other extractants. The solid : solution ratio (1:50) and the long extraction time of Colwell-P are more similar to the *S. capricornutum* growth conditions (1:100), which is the plausible reason that it outperformed the Olsen-P (1:20) extraction.

Similarly, 0.1 M NaOH had a significant relationship with algal growth. Fe/Al-P represents phosphorus bound to Fe and Al oxides and is exchangeable with OH^- and other inorganic phosphorus compounds, which are soluble in bases (Wu et al., 2011). Dorich et al. (1984) also found that 0.1 M NaOH-extractable phosphorus was significantly correlated with 2-day and 14-day available phosphorus for an alga (*S. capricornutum*). The high correlation between 0.1 M NaOH and algal growth indicated the sensitivity of P bioavailability to redox conditions in river system.

Morgan-P (NaAcetate pH = 4.8) was expected to extract P from Ca-P pools bound to the authigenic carbonate fluorapatite (CFAP) + biogenic apatite (Ruttenberg, 1992). The correlation between Morgan-P and algal growth was therefore significant. Actually, in calcareous soils, it is the high activity of Ca^{2+} cations, rather than that of carbonate anions, that affects P mobility, due to the precipitation of Ca-phosphates with low solubility (Jalali and Jalali., 2107). Torbert et al. (2002) studied relationship of soil test P and runoff in calcareous and non-calcareous soils. They indicated that in calcareous soils losses of P in runoff was reduced due to the reaction of Ca with soluble P to form insoluble Ca-P minerals. William et al. (1980) reported that uptake of P by the cells alga (*Scenedesmus quadricauda*) varied from 8 to 50% of total P and from 38 to 83% of nonapatite inorganic P when measured directly. Xiao-Fei et al. (2015) reported that part of Ca-P can be uptaken by *Microcystis aeruginosa*.

All sediment P measurements were consistently high in sediments with high P concentrations, however, in some sediments, the response was very high during the two-week growth period and even continued for a long period of time. One possible explanation is the presence of internal conversion of P from recalcitrant P pool to bioavailable P is high in this sediment (S20). The Colwell extractant separated sediments well against the algal population. It means that sediments with high bioavailable P had high algal growth. Some sediment P can be easily desorbed and released, particularly when the concentration of phosphorus in the water column is depleted. It is expected that Colwell- extracted P would separate from potentially available P to immediately available to algae. So, 14 days cultural time was well correlated with Colwell P. The proportion of Colwell-P to Total-P in the sediments of rivers was the most consistent of all the extractions used (Table 4).

Algal growth and sediment properties

There was a highly significant correlation between EC and *Selenastrum capricornutum* populations ($r^2 = 0.87$) (Figure 5). This implies that the sediments with high salinity supply enough P for the growth of the algae. The sediment of the present investigation had an EC of only 0.23 to 1.22 dS m^{-1} and our data suggest that an increasing EC might lead to larger growth. This larger growth could be related with greater P availability, but it might also be due to other factors that affect algal growth. Several earlier studies also showed an increase in P bioavailability with increasing salinity (Jordan et al., 2008; Hartzell et al., 2010; Li et al., 2015); however, this salinity is in the range that does not hinder organism survival (EC = 0.46

± 0.28). From the negative relationship between algae growth and both CCE and pH could be deduced that these sediment properties restricted the degree of eutrophication (Figure 5). Therefore, *Selenastrum capricornutum* is not calcium-loving and occurs in calcareous streams with deposits of travertine and tufa. There is also a positive relationship between OM and the algae growth, what might indicate the availability of organic P for the growth of algae.

Phosphorus tests and sediment properties

Pearson's correlations were performed to investigate the relationship between P extraction efficiency and sediment physico-chemical properties (Table. 6). The P extractants were negatively correlated with pH and the correlation was strongest with 0.1 M NaOH. Wang et al. (2012) who found that pH was important soil parameter to all the equations relating P concentration in leachates with Olsen-P, Mehlich III-P, and Bray II- P concentrations. Obviously, the correlation factor was higher for Colwell than other P extractants for EC ($r = 0.93$, $P < 0.001$). The study by Arfania et al. (2018) revealed that sediment chemical properties such as electrical conductivity, organic matter, pH and calcium carbonate, had effects on the distribution and bioavailability of P pools. Calcium carbonate equivalence (CCE) was negatively correlated with Colwell and NaOH 0.1 M. Olsen, Colwell and 0.1 M NaOH extractant were positively correlated with organic matter content Jalali and Jalali. (2017) reported that pH, and EC made important contributions to most P release from leaching column, while OM and silt content was less contributed on P availability. A positive correlation of Olsen and Mehlich III with the silt fraction indicated the influence of sediment particle size on P bioavailability (Table 6).

Conclusion:

Due to population growth and the associated agricultural developments and upland soil erosion, western rivers discharging in Urmia Lake release massive quantities of P-loaded water and sediment. This is the main reason for eutrophication of wetlands at this lake. In this investigation, a selection of extraction methods was considered in their capability to extract sediment-bound P. The extracted quantities appeared to vary significantly between these methods, even though most methods showed a good correlation with each other. The extracted P was also compared with algae growth in a bio-assay, for the algae *Selenastrum capricornutum*. Correlations with algal growth were modest, except for the Colwell-P that correlated very well. For sediments as discharged into Urmia Lake, where pH and EC appears to have an effect on algae growth, the Colwell-P extraction therefore looks as the best option.

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Table 1. Physiochemical properties of sediments

Table 6. Correlation between P extractant and physical and chemical properties of sediments	Table 6. Correlation between
	Clay
AB-DTPA	0.18
Olsen	0.25
Colwell	0.18
Mehlich III	0.18
Morgan	0.31
0.1 M NaOH	-0.05

Table 2- Used phosphorus extraction methods	Table 2- Used phosphorus extraction methods	Table 2- Used phosphorus
Reference	Reference	Sediment: Water
Olsen et al (1954)	Olsen et al (1954)	1:20
Colwell (1963)	Colwell (1963)	1:50
Mehlich (1984)	Mehlich (1984)	1:10
Morgan (1941)	Morgan (1941)	1:10
Bray and Kurtz (1954)	Bray and Kurtz (1954)	1:10
Soltanpour and Schwab (1977)	Soltanpour and Schwab (1977)	1:20
Sharpley (1991)	Sharpley (1991)	1:100

Table 3. Results of the principal component analysis with the loadings of sediment properties	Table 3. Results of the prin
Variable	PC1
Clay	0.98
Silt	0.72
Sand	-0.89
vfs	-0.96
D50	-0.71
OM	0.11

Table 3. Results of the principal component analysis with the loadings of sediment properties	Table 3. Results of the prin
CCE	0.14
ACCE	0.78
pH	0.02
EC	0.12
Eigenvalue	5.16
% Variance	51.60
Cumulative % variance	51.60

Table 5. Correlation between extracted P by different sediment extraction methods (n=25)	Table 5. Correlation between
AB-DTPA	AB-DTPA
Olsen	1
Colwell	0.72**
Mehlich III	0.74**
Bray II	0.74**
Morgan	-0.04
NaOH 0.1 M	0.75**
Total	0.36
	0.75**

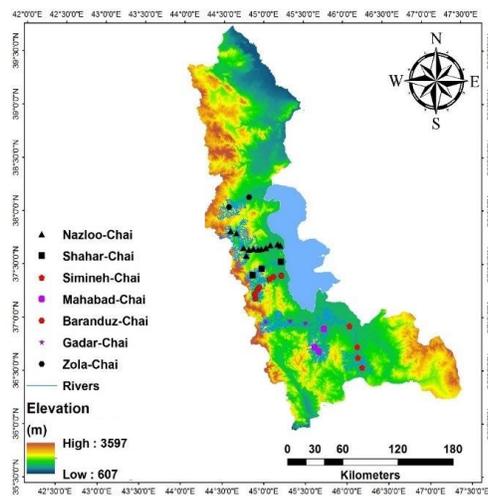


Figure 1. Study area showing location of sampling sites in western rivers in the Urmia Lake basin

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