Article

Geometric morphometrics of leaf blade shape in water hyacinth (*Eichhornia crassipes*: Pontederiaceae) population from Lake Mainit, Philippines

C.C. Cabuga, **Jr**[1, 2], **I.C.B. Delabahan**[1,2], **J.I.C. Dedel**[1,2], **M.A. Ayaton**[1,2], **L.A. Ombat**[2], **M.L.M. Budlayan**[3]
[1]Graduate School, Caraga State University-Main Campus, Ampayon, Butuan City 8600, Philippines
[2]Biology Department, College of Arts and Sciences, Caraga State University- Main Campus-Ampayon, Butuan City 8600 Philippines
[3]Physics Department, Mindanao State University-Iligan Institute of Technology, Andres Bonifacio Avenue, Iligan City 9200, Philippines
E-mail: cresenciocabugajr@gmail.com

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Abstract
This study was carried out in order to investigate the leaf blade shape variation in the populations of water hyacinth (*Eichhornia crassipes*) from Lake Mainit, Philippines using geometric morphometric analysis. A total of 120 leaf individuals were randomly collected in the four Barangays (Bunga, San Pablo, Dinarawan and San Roque) of which each area consists of 30 samples. To identify morphological variations in the leaf blade shape of water hyacinth, digital imaging was prepared and the acquired images were loaded into tpsDig2 program. Using thin-plate spline (TPS) series, landmark analysis was completed and subjected to symmetry and asymmetry in geometric data (SAGE) software. Results in Procrustes ANOVA showed high significant differences (P<0.0001) in the two factors analyzed: the sides and the individuals by sides which denoting high fluctuating asymmetry. This could be inferred that asymmetric variability might be associated to the outcomes of fluctuating asymmetry that have been derived from genetic and non-genetic influences. Moreover, differences of leaf blade shape have been observed from the collected leaf samples and among the study areas. Thus, using geometric morphometric analysis enables to identify morphological variations within and among species of the same taxa.

Keywords macrophytes; shape analysis; aquatic weeds; leaf morphology.

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1 Introduction

Eichhornia crassipes also known as water hyacinth is an aquatic weeds and widely inhabit lentic biota (Niaz and Razul, 1998). Its community is integral component in limnetic ecosystems since they influence biodiversity, produce a great biomass and a primary producer (Wetzel, 1993; Esteves, 1998). Also it's constitutes the recycling process to maintain a clear water system (Scheffer, 1998). Its population is identified to establish ecological amplitude (Esteves, 1998; Thomaz, 2002). The diverse characteristics of this plant associated to settle aquatic environments thus making them ecologically adaptive and tolerable for many environmental conditions (Esteves, 1998; Thomaz and Bini, 1998). Further, this plant species naturally with low movements and cannot escape from the various nutrient content, water current and some chemical and physical properties that affects their existence in aquatic environs (Pereira et al, 2012). Likewise, it has a limiting characteristic to occupy certain habitat and inversely respond to several ecological changes such as trophic condition (Barko et al., 1986; Van Geest, 2005). Nonetheless, the assemblages of this species either in a lake or a river gives efficient indication of the current ecological status from stress disorders and pressure modifying their habitat (Murphy, 2000).

The present study will investigate the population of *E. crassipes* through its leaf blade shape using geometric morphometric analysis from Lake Mainit, Phils. Morphometrics, deals to study shape and has been used to plants and its organs over the years. In plants, leaves are efficient structure that is readily available for examination as they are naturally growing in a year such as annual, deciduous and or perennial (Cope et al, 2012). Studying the leaf shape variations has been an utmost approach to discriminate characteristics between two taxa and known as an integral part in the field of plant taxonomy (Ellis et al., 2009; Goncalves and Lorenzi, 2011). As well as, leaf characters with intricate shape have been traditionally utilized for taxonomic basis in classification and identification (Cope et al., 2012). In differentiating shapes variation and co-variation, geometric morphometrics (GM) was employed to further identify shape differences. GM is known to be an efficient tool to characterize deviation between dorsal/ventral and bilateral symmetry of biotic elements (Cabuga et al., 2017). As such geometric morphometrics widely employed to distinguished shapes distinction (Zelditch et al., 2012). The shape composes of geometrical data sets out from filtered object undergone through rotational effects, location and scale (Dryden and Mardia 2016). Over the years, the development of phylogenetic reconstruction techniques applying morphological information is the furthest important advancement in comparative biology (Cope et al., 2012). Thus, this study aims to discriminate leaf blade shape variation in the populations of *E. crassipes* utilizing geometric morphometrics analysis.

2 Materials and Methods

2.1 Study area and samples collection

This study was conducted in Lake Mainit, Caraga Philippines (Fig. 1). Leaf samples were collected in the month of November-December, 2017. Utilize kitchen knife to cut the petiole of the plant. After which the collected leaves were placed in the ziplock and properly labelled of the area it was collected.

2.2 Fluctuating asymmetry of *E. crassipes*

2.2.1 Sample processing

The samples of *E. crassipes*were collected from the four Barangays (Bunga, Dinarawan, San Pablo, and San Roque located in Lake Mainit, Mindanao, Philippines. About 120 leaf individuals were randomly collected each consists of 30 samples per study area. Each of the leaves were placed into a Styrofoam to visibly see the samples point of origin for the land-marking process. Digital image of the dorsal and ventral side were exposed and photographed by Canon-DSLR camera (16 megapixels). To eliminate measurement error, the samples were tri-replicated and captured with the same position (Fig. 2).

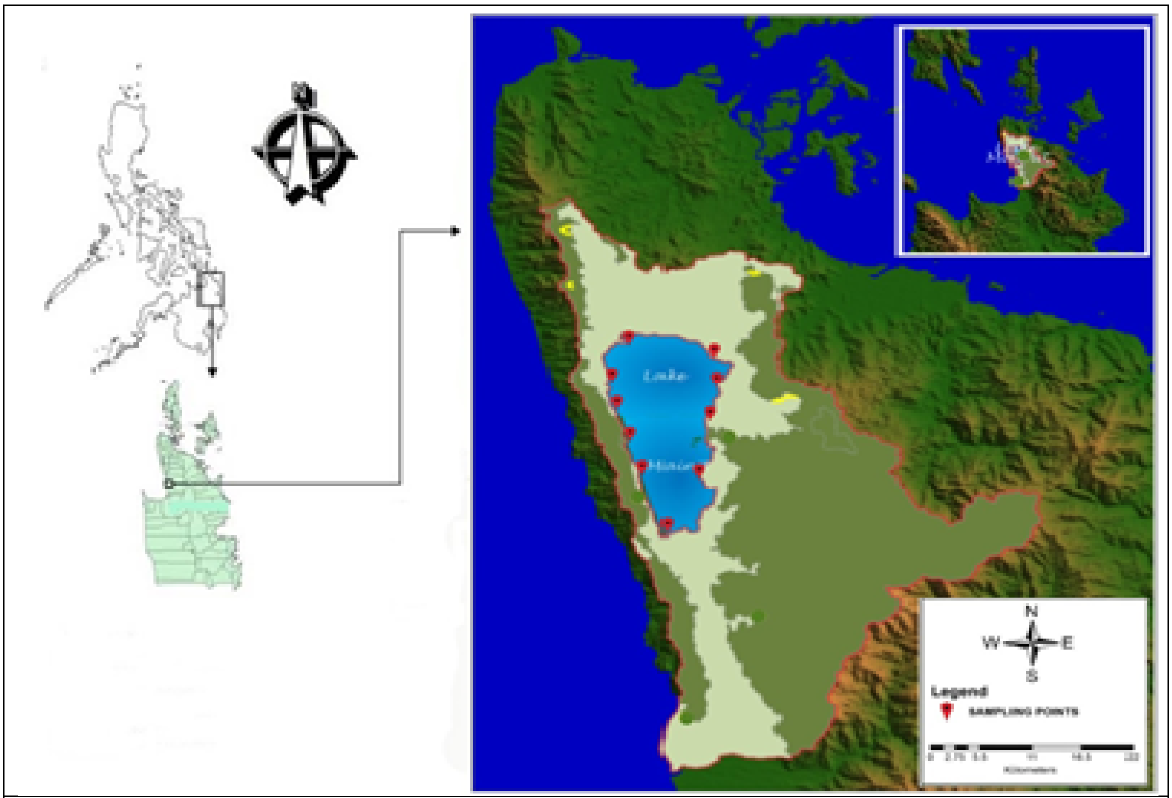

Fig. 1 Map of the study area, Lake Mainit, Caraga, Philippines.

2.2.2 Landmark selection and digitization

From the obtained images, it was transferred into tps files using tpsUtil. The samples were then digitized using the landmarking method of the tpsDig version 2 (Rohlf, 2004). A total of 16 anatomical landmarks points in dorsal and ventral side were used in this study (Table 1).

2.2.3 Shape analysis

The digitation for dorsal-ventral of the leaf samples undergone tri-replicated images through tpsDig2. The coordinate data was then subjected to (**SAGE**) Symmetry and Asymmetry in Geometric Data (version 1.04 Marquez, 2007) software and Procrustes ANOVA test was performed to determine the significant difference in the symmetry of the two factors considered –sides and individual by side. The significance level was confirmed at $P<0.0001$. The percentage (%) of FA were acquired and compared between the dorsal-ventral regions (Natividad et al., 2015).

2.2.4 Sediment collection

The samples of bottom sediments obtained from the same point where the plant samples collected. At each point, three sediment samples were taken from three established stations by using pre-cleaned 100 ml, wide-mouthed, disposable plastic containers and packed separately in pre-cleaned polyethylene bags. Each container has 500g of sediment samples from the study areas. Samples were placed in a ziplock polyethylene bags properly labeled with the area where it was collected and the number of replicates. The samples of bottom sediments were brought to the laboratory, air dried and then ground into fine powder using pestle and mortar. After which it was sieved manually with No. 80 mesh sieve.

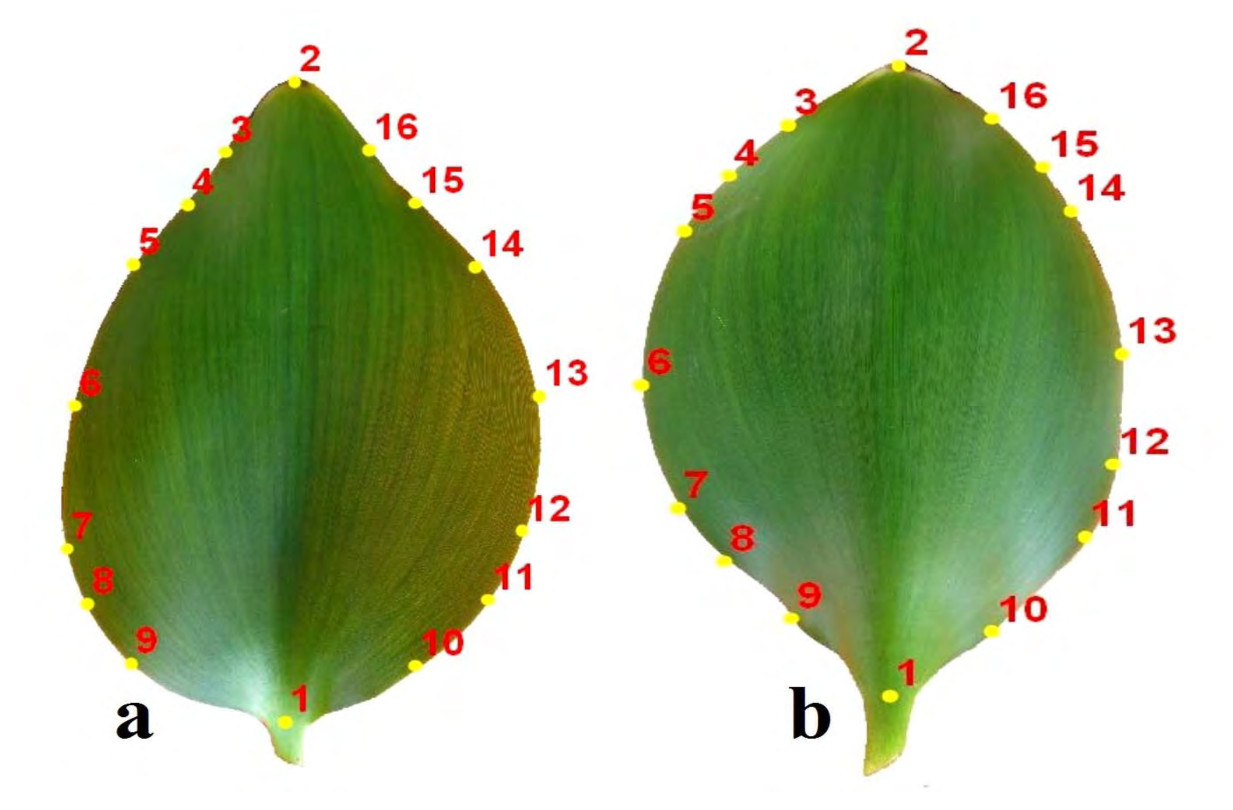

Fig. 2 Landmarks description of *E. crassipes* (a) Ventral (b) Dorsal.

2.2.5 Determination of levels of Nitrogen, Phosphorous and Potassium (NPK), Cadmium, Chromium, Lead & Nickel in sediments

About 100g of dried sediments was weighed using an electronic weighing scale and placed in a polyethylene bags and properly labeled. The prepared sediment samples were further submitted for the analysis for the percent (%) of Nitrogen, Phosphorous and Potassium (NPK). The analysis was done by the Department of Agriculture- (Regional Soils Laboratory located at Brgy. Taguibo, Butuan City, Agusan del Norte, Phils. The analysis was administered using the Agilent Technologies®MY14300001 Atomic Emission Spectrophotometer.

2.2.6 Physico-chemical water parameters

The Physico-chemical water parameters that comprise temperature, total dissolve solids (TDS), conductivity, pH, Dissolve Oxygen (DO) and salinity, was evaluated with the same date mentioned above. In the study area three sites (Stations 1, 2 & 3) was established along with three replicates and randomly selected for the measurements. This was done using the multi-parameter water quality meter (EUTECH PCD). Data were presented as mean ± standard error mean (SEM) and were calculated using Paleontological Statistics and Software (PAST).

Table 1 Description of the landmark points adapted from Vieira et al. (2014).

Coordinates	Locations
1	Petiole-leaf blade midrib junction
2	Apex of leaf blade midrib
3	Intersection of left-hand leaf margin and the projection of the 1st secondary vein on the left from the leaf apex
4	Intersection of left-hand leaf margin and the projection of the 2nd secondary vein on the left from the leaf apex
5	Intersection of left-hand leaf margin and the projection of the 3rd secondary vein on the left from the leaf apex
6	Intersection of left-hand leaf margin and the projection of the central secondary vein on the left, if there is an odd number of veins on that side. If the number of veins is even, then the landmark is the point along the leaf margin intermediate between the projections of the two central secondary veins onto the leaf margin
7	Intersection of left-hand leaf margin and the projection of the 3rd secondary vein on the left from the leaf base
8	Intersection of left-hand leaf margin and the projection of the 2nd secondary vein on the left from the leaf base
9	Intersection of left-hand leaf margin and the projection of the 1st secondary vein on the left from the leaf base
10	Intersection of right-hand leaf margin and the projection of the 1st secondary vein on the right from the leaf base
11	Intersection of right-hand leaf margin and the projection of the 2nd secondary vein on the right from the leaf base
12	Intersection of right-hand leaf margin and the projection of the 3rd secondary vein on the right from the leaf base.
13	Intersection of right-hand leaf margin and the projection of the central secondary vein on the right, if there is an odd number of veins on that side. If the number of veins is even, then the landmark is the point along the leaf margin intermediate between the projections of the two central secondary veins onto the leaf margin
14	Intersection of right-hand leaf margin and the projection of the 3rd secondary vein on the right from the leaf apex
15	Intersection of right-hand leaf margin and the projection of the 2nd secondary vein on the right from the leaf apex
16	Intersection of right-hand leaf margin and the projection of the 1st secondary vein on the right from the leaf apex

3 Results and Discussion

Procrustes ANOVA was used to identify the significant values of variation of asymmetry and measurement errors among the leaf blade shape of water hyacinth (*E. crassipes*) (Klingenberg, 2003) (Table 2). There were 2 factors analyzed: Sides and Individual x sides. Among the populations examined, Barangay San Roque has the highest FA level (67.0637%), followed by Barangay San Pablo (63.0821%), Barangay Bunga (55.2820%) and Barangay Dinarawan (53.2719%). It was observed that highly significant difference ($P<0.0001$) occurs in the individual leaves resulting fluctuating asymmetry when one of the leaf samples compared into another. Its sides also showed highly significant variance signifying fluctuating asymmetry in its dorsal and ventral region of the samples. Thus, the relation among the individuals and sides also exhibited highly significant difference which suggested fluctuating asymmetry influenced amongst the interaction of individuals and sides.

Furthermore, variation in leaf morphology among species at different sites is an indication that some species adapt better in some particular habitats using a different type of leaf form (Kusi, 2013). The variability in leaves has also been attributed to both genetic and environmental influences (Hovenden and Vander Schoor, 2004) and showed that leaf traits such as the length, width, and area are controlled by both environmental and genetic factors. However, the ecological causation has an overriding influence on these traits. While several studies concluded that leaf shape differences i.e. small and thick leaves are adaptive to dry habitats to conserve water while large and thin leaves are adaptive to wet habitats (Rowland et al., 2001). The patterns of variation in leaf morphology affect organisms whose feeding and reproduction depend on leaves (Suomela and Ayres 1994). The leaf in particular has characteristic development and structure which varies across plant species (Simpson, 2010). On the other hand, it has long been known that the climate in which a plant grows has an effect on the shape of its leaves. In addition, temperature has a direct result of the leaf shape and also associated to a wider variety of environments (Royer et al., 2005).

Table 2 Procrustes ANOVA on leaf blade shape of *E. crassipes* from Lake Mainit, Philippines.

Effect	SS	DF	MS	F	P-VALUE
			Barangay Bunga		
Sides	0.651	24	0.0023	55.2820	0.0001**
Individual x Sides	0.0534	1275	0	5.2316	0.0001**
Measurement Error	0.0443	500	0	--	--
			Barangay San Roque		
Sides	0.0574	30	0.0030	67.0637	0.0001**
Individual x Sides	0.0648	1279	0	4.5654	0.0001**
Measurement Error	0.0411	600	0	--	--
			Barangay Dinarawan		
Sides	0.545	27	0.0021	53.2719	0.0001**
Individual x Sides	0.0346	1285	0	5.3261	0.0001**
Measurement Error	0.0444	600	0	--	--
			Barangay San Pablo		
Sides	0.0698	40	0.0029	63.0821	0.0001**
Individual x Sides	0.0324	1281	0	5.3410	0.0001**
Measurement Error	0.0311	500	0	--	--

*Note: ** (P<0.0001) highly significant,* ***F****= fluctuating asymmetry*

The present study identifies leaf shape variations among the population of *E. crassipes* as observed in the percentage of FA. This could be inferred that asymmetric variability might be associated to the outcomes of fluctuating asymmetry that have been derived from non-genetic influences (Viscosi, 2015). As much as, FA stands as an observable mechanism to understand dissimilarities along the two axes (dorsal-ventral or left-right side). The relative amount of FA could be perceived along the left/right part of leaves has been associated to noise created from random errors during development, influenced by environmental stresses (Miller, 1995;

Kozlov et al., 1996; Hodar, 2002). Moreover, FA is the variance in subtle differences between the left and the right sides in bilaterally symmetrical organisms or parts of them, and it provides a measure of how well an individual can buffer its development against internal genetic and external environmental stress during ontogeny (Van Valen, 1962; Palmer, 1996). FA serves as an indicator of environmental stress should distinguish between genetic and environmental effects (Sinclair and Hoffmann, 2003). Nonetheless, environmental perturbation and nutrient availability may also influence developmental stability (Lappalainen et al., 2000; Black-Samuelsson and Andersson, 2003). Deficiencies in nutrients such as nitrogen, phosphorous, and potassium (NPK) lead to slower growth, alterations in shoot and root biomass, and changes in leaf coloration and development (Bottrill et al., 1970; Sultan and Bazzaz, 1993; Yeh et al., 2000). Nevertheless, any change of nutritional components, whether increase or decrease, may result in decreased developmental stability (Lappalainen et al., 2000). In addition, geographical location might also influence leaf shapes differences since organisms required to adapt with the environments they inhabit. Altitudinal gradients caused decrease in soil depth, nutrient retention and water, and significantly an increase in rainfall intensity, variation in dry season and solar effect (Korner, 2007). Study shows light conditions and trophic state of the area might change the establishment and development of aquatic macrophytes (Mitchell, 1974; Wetzel, 1990; Esteves, 1998; Scheffer, 1998; Thomaz and Bini, 1998; Thomaz, 2002; Nurminen, 2003; Kocic et al., 2008; Penning et al., 2008a). In general, the results of the Procrustes ANOVA showed significant leaf shape differences between species of *E. crassipes*.

Table 3 Mean values of NPK & Heavy Metals from the collected sediments in Lake Mainit, Philippines.

Elements	Standard Values	Station 1 Mean ± SEM	Station 2 Mean ± SEM	Station 3 Mean ± SEM	Mean ± SEM
Nitrogen (%)	1.22%	0.1 ± 0.0058	0.17 ± 0.0058	0.06 ± 0115	0.11 ± 0.0321
Phosphorous (%)	1.06%	0.02 ± 0.0058	0.06 ± 0.0058	0.04 ± 0.0058	0.04 ± 0.0115
Potassium (%)	2.04%	0.0533 ± 0.0033	0.0767 ± 0.0033	0.19 ± 0.0058	0.1067 ± 0.0422
Chromium (ppm)	≤25^	354.333 ± 8.3732	130.333 ± 14.1461	2256.33 ± 6.6416	913.665 ± 674.439
Nickel (ppm)	≤75*	168.333 ± 9.5277	89 ± 6.3509	1626.67 ± 18.1873	628.001 ± 499.859
Cadmium (ppm)	≤85*	29.3333 ± 3.1798	48 ± 2.3094	52.6667 ± 4.3333	43.3333 ± 7.1285
Lead (ppm)	≤420*	BDL	BDL	BDL	BDL

***Note**: 0.1ppm detection limit of heavy metals, ***FAO**- Food Agriculture Organization, **^USEPA**-United States Environmental Protection Agency*

The levels of Nitrogen, Phosphorous, Potassium and Heavy Metals: Chromium, Nickel, Cadmium and Lead from the collected sediments in Lake Mainit, Phils. was presented in Table 3. It was observed that Potassium has the highest mean value (0.1067 ± 0.0422) followed by Nitrogen (0.11 ± 0.0321) and Phosphorous (0.04 ± 0.0115) however these values were very low when compared to the standards. Nitrogen, phosphorous and potassium (NPK) are important soil elements that control the fertility and crop production

(Singh and Mishra, 2012). The low amount of nitrogen in the soils indicating a low amount of carbon deposits. While organic matter also influences nutrient availability to the soil (Bullock and Burton, 1996). The natural variability of soil results from complex interactions between geology, topography, climate as well as soil use (Jenny, 1980; Quine and Zahng, 2002). Phosphorus is the second most important macronutrient available in the biological systems, which constitutes more than 1% of the dry organic weight.

On the other hand, the levels of heavy metals were also analyzed and found out that Chromium has the highest mean value (913.665 ± 674.439) and followed by Nickel (628.001 ± 499.859), Cadmium (43.3333 ± 7.1285), while lead showed below detection limit (BDL). The values of these heavy metals were higher and exceeded the safe limits set by international agencies in soil. Sediments are considered to be an essential carrier as well as a sink of heavy metals in the hydrological cycle (Celo et al., 1999).

Table 4 Mean values of physico-chemical parameters from four Barangays in Lake Mainit, Philippines.

Water Parameters	Standard DAO 90-34 & Water Watch Australia National Technical Manual (2002)	Barangay Bunga Mean ± SEM	Barangay San Roque Mean ± SEM	Barangay Dinarawan Mean ± SEM	Barangay San Pablo Mean ± SEM
Conductivity	100-2000 uS/cm	168.789 ± 1.1279	168.533 ± 0.5393	159.911 ± 78.0185	174.944 ± 2.0345
DO	>5mg/L	6.3556 ± 0.1026	8.03 ± 0.2478	6.3622 ± 0.1520	6.6544 ± 0.2507
pH	6.5-8.5	7.7356 ± 0.0449	8.0578 ± 0.0773	7.6344 ± 0.0435	7.7133 ± 0.1061
Salinity	<0.5ppt	0.08 ± 0	0.08 ± 0	0.08 ± 0	0.08 ± 0
Temperature	3^0C rise[a]	28.65 ± 0.1239	29.5778 ± 0.1222	29.1778 ± 0.3696	28.4667 ± 0.1748
TDS	<1000mg/L	80.1778 ± 0.5243	79.9667 ± 0.2539	171.778 ± 2.3713	82.9667 ± 1.0018

*Note: **DAO** – DENR Administrative Order*

It is also counted that sediments works as a habitat where most of the aquatic organism directly acquire its source of nutrients (Suciu et al., 2008). Further, heavy metals are potential human health toxicant when higher concentrations in soils. Meanwhile, the physico-chemical components of the Lake were also determine (Table 4). From the obtained results the values were in the suggested limits set by the international agencies. Water variables play as an important determinant in assessing water quality for many aquatic environments. Further, its physical properties that include, temperature and rate of suspended solids, chemical parameters like hardness, alkalinity, pH and metals are important for aquatic components growth and production (Viadero, 2005).

4 Conclusion

This study was carried out to determine the leaf blade shape variations in water hyacinth (*E. crassipes* population from Lake Mainit, Phils. The result shows high significant difference (P<0.0001) in the two factors analyzed sides and individual by sides. This could be inferred that asymmetric variability might be associated to the outcomes of fluctuating asymmetry that have been derived from non-genetic influences. Likewise, asymmetrical influences of the leaf blade shape associated with ecological perturbations, extreme weather condition, and nutrient availability, geographical settings and genetic influences. Further, the present study observed variations among species of the same population. Thus, the importance of geometric morphometrics in identifying morphological variances has been continually applied in studying biological shapes.

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