

RESEARCH PAPER

International Journal of Biosciences | IJB | ISSN: 2220-6655 (Print), 2222-5234 (Online) http://www.innspub.net Vol. 21, No. 6, p. 140-150, 2022

OPEN ACCESS

Bioaccumulation of Heavy Metals in Water Lilies (*Nymphaea* sp.) and Water Analysis in Lake Leonard and Lake PBCC, Mindanao Island, Philippines

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Key words: Bioaccumulation, Heavy metals, Water lilies (Nymphaea sp), Lake Leonard, Lake PBCC

http://dx.doi.org/10.12692/ijb/21.6.140-150

Article published on December 05, 2022

Abstract

Heavy metal refers to any metallic element that has a relatively high density, is hazardous or poisonous even at low concentrations, and is recognized as one of the environmental pollutants. The water lily is an herbaceous plant that grows in quiet, contained, stagnant water. Heavy metals are best accumulated by these hydrophytes. Absorption of heavy metals has detrimental effects on the morphology and architecture of plants, which have a direct impact on plant physiology. Moreover, heavy metals in the water sample from Lake Leonard and Lake PBCC shows that lead (Pb), nickel (Ni), Chromium (Cr) and Cadmium (Cd) has higher values than the permissible limits of the elements in the water. The roots and leaves of *Nymphaea sp*. from the two sites were below the detection limit of lead. On the other hand, it was detected that the stem of the plants in Lake Leonard was contaminated with nickel while below detected limit in Lake PBCC. Furthermore, the roots, stems and leaves of *Nymphaea sp*. from the study areas were contaminated with nickel and chromium since its concentration is beyond the permissible value. Samples of Nymphaea sp. and water samples from Lake Leonard were below the detection limit of cadmium, but greater than the permissible limit from Lake PBCC. On the other hand, the Cadmium concentration in the roots and leaves of Nymphaea sp. was higher than the limit.

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Introduction

Bioaccumulation is identified as the gradual build up over time of a chemical in a living organism. This occurs either because the chemical is taken up faster than it can be used or because the chemical cannot be broken down for use by the organism (that is, the chemical cannot be metabolized). Bioaccumulation is a big factor in environmental pollution moreover; chemical pollutants that are bioaccumulated can come from many sources like mining and agricultural activities.

Heavy metal can be referred to as any metallic element that has a relatively high density and is toxic or poisonous even at low concentration and are known to be among the contaminants of the environment. High concentrations of heavy metals in the plants and water clearly demonstrate pollution by heavy metals (Kabeer et al. 2014) furthermore; presence of higher concentrations of heavy metals in plants signifies biomagnification (Tiwari, et al. 2007) many industries release heavy metals such as Zn, Cd and Pb in water. Because of their toxicity, persistence, and non-degradability in the environment, heavy metals pollution in the aquatic systems is one of the largest threats to their environment that affects directly on flora, fauna and human health. High levels of Cd, Cu, Pb, and Fe can act as ecological toxins in aquatic and terrestrial ecosystems (Guilizzoni, 1991; Balsberg-Påhlsson, 1989). Aside from natural activities, almost all human activities also have potential contribution to produce heavy metals as side effects. Migration of these contaminants into non- contaminated areas as dust or leachates through the soil and spreading of heavy metals containing sewage sludge are a few examples of events towards contributing contamination of the ecosystems (Gaur and Adholeya, 2004) Furthermore, the concentrations of heavy metals in ponds or lake reflect the combined effects of weathering, floodplain, anthropogenic inputs, and water chemistry. Human activities especially which close to the water systems are the main heavy metals contributions in the ponds or lakes (Abdel-Baki et al., 2011). Heavy metals have the great tendency to accumulate in various aquatic

organisms including aquatic plants, which may enter into human body through consumption, thus can cause serious health problems. Heavy metals cannot be degraded (Linnik and Zabenko, 2000) but can change into the different redox forms. They can be accumulated in the food chain leading to chronic disease in humans. Cumulative effects of metals and chronic poisoning may occur as result of long-term exposure of low concentration metals (Mitra *et al.*, 2012).

Water lily is herbaceous plant that grows in stagnant, calm and enclosed waters. This hydrophyte plant is floating on water surface with its big leaves support the floatation mechanism. Water lily has many colors such as pink, white and purple complete with sepals, stamen, carpels and petals (Skinner, 2006). It is an aquatic flowering plant that belongs to family Nymphaeaceae and the species that commonly found in tropical country is categorized under Nymphaea sp. (Slocum, 2005). Water lily has eight genera (e.g.Nuphar, Nymphaea, Victoria) and consist about 70 species. For example, for genus Nymphaea comprise of 35 species. It is a perennial aquatic rhizomatous stoloniferous herb. They are widely distributed in tropical and temperate regions, inhabiting stagnant fresh water, ponds, lakes and swamps (Bhattacharjee et al., 2001). Heavy metal accumulation can cause morphological changes on aquatic plants like Pistia straiotes which acquired gelatinous texture and dark color probably results from cell wall damage (Pimenta et.al., 2004). Moreover, heavy metal exposure can also promote lipid peroxidation via free radical generation, it causes breakdown of functional and structural integrity of biological membranes that would eventually effect to death (Upadhyay and Panda, 2009). Furthermore, in water lettuce it decreases the growth rate especially in high concentration additionally, according to Guimaraes et, al.2012, the decrease in the total chlorophyll and carotenoid content is one of the first symptoms of plant toxicity after exposure to various stress agents such as metals and metalloids. Heavy metals absorption has harmful effect in morphology and anatomy of plants, in turn,

directly influence the plant physiology (Han, et.al, 2004). Island of Mindanao has various lakes such as Lake Leonard and Lake PBCC where people living near the area that serves as their source of food without investigating its safety against toxic substances. Considering these facts, the researchers sought to explore the possible heavy metals contamination in Lake Leonard and Lake PBCC on the water and water lilies found in the area.

Materials and methods

Research locale

The study was conducted last November, 2017 at two lake sites one in Compostela Valley province and the other was in the Province of Agusan del Sur.

Lake leonard

Lake Leonard is a small freshwater crater lake in

Mount Leonard (also known as Leonard Kniassef), an andesitic-to-dacitic stratovolcano complex in the Leonard Mountain range which is located in one of the municipalities of Compostela Valley Province particularly, Brgy. New Leyte of Maco.

It has a coordinates of 7023'48"N 12603'36"E. Lake Leonard is actually the caldera of Leonard Kniassef Volcano, the only active volcano in Mindanao, the caldera was also known as crocodile lake, however several references mentions that the crocodiles were completely killed off by the mining activity of the area that somehow brought ruins to area in fact the edge of the lake is badly silted as a result of human activities on the upper slopes of the watershed, particularly road construction, shifting cultivation and logging. The disposal of mine tailings is also having direct adverse impact on the lake's resources.



Fig. 1. Sampling site of Lake Leonard.

Lake PBCC

Lake PBCC is found in Bayugan 3, Rosario, Agusan del Sur. According to the Philippine Statistics Authority, the municipality has a land area of 385.05 square kilometres (148.67 sq mi) constituting 3.85% of the 9,989.52-square-kilometre- (3,856.98 sq mi) total area of Agusan del Sur. This lake is a stretch of Agusan Marsh where in waters from Agusan River

will mix in the Lake PBCC during flooding or when heavy rains come. On its upper portion of the topography, there are small mining and large mining industries on its operation that probably wastes chemical will run –off to the lake. Lake PBCC is the good source of freshwater fishes consumed by the people in the locality.

Selection of the area

The area for the collection of Water Lilies (Nymphaea sp.) in the Lake Leonard and Lake PBCC.

Entry protocol

An Entry Protocol with the village officials at the Municipality of Rosario, Agusan del Sur and New Leyte, Maco, Compostela Valley province. The researchers observed research ethics particularly the entry protocols of indigenous people.

Preliminary survey of the sampling area

The researchers conducted reconnaissance survey to determine the possible habitats of the target species of Water Lilies (Nymphaea sp.) in the two sites.

Field works

The plants samples were collected from the different locations into polythene bags. All plants samples were labeled appropriately and Taken into Regional Soil Analysis, Department of Agriculture, Taguibo, Butuan

City, CARAGA Region.

Chemical analysis

Samples of plants were oven-dried at 100 degrees Celsius for 24 hours, then finely blended with an electric blender and sieved through a 2mm mesh sieve to facilitate digestion. 5ml of a mixture solution of 4:1 HNO3: HCIO4 was added to 1 g of plant components weighed with an analytical scale. It was cooked to dryness at a temperature of 105 degrees Celsius for one hour. Then, the flask was filled up to the mark with 1M HNO3 and left to cool. The solution was centrifuged for 30 minutes using a (HARRIER 15/80 model centrifuge) and transferred to sampling bottles for examination; all digested samples were tested using a Perl-Elmer Analyst AAS (Atomic Absorption Spectrophotometer).

Results and discussion

The maximum allowable lead concentration in water is 0.05 mg/l (WHO, 1996). In addition, the concentration of lead in water samples from Lake Leonard and Lake PBCC is 11 mg/l and 12 mg/l, respectively, as shown in Table 2.

Consequently, the results of the analysis demonstrated that the concentration of the element in the water of Lake Leonard and Lake PBCC exceeds the maximum concentration of lead in water.

Table 1. Concentration of Lead (Pb) (mg/kg) in the different parts of Water Lilies (*Nymphaea sp.*) from Lake Leonard and Lake PBCC.

Study Areas	Roots mg/kg	Leaves mg/kg	Stems mg/kg	Permissible Limit (WHO, 1996)
Lake PBCC	BDL	BDL	54 mg/kg	2.0 mg/kg
Lake Leonard	BDL	BDL	BDL	2.0 mg/kg

BDL - Below Detection Limit.

As suggested by the World Health Organization, the lead limit for water lilies (*Nymphaea spp.*) is 2.0 mg/kg (1996). Nonetheless, table 1 reveals that the lead content in the roots and leaves of Nymphaea sp. is below the detection limit for Lake Leonard and Lake PBCC. On its stem, 54 mg/kg were found in Lake Leonard, which is below the detection limit in Lake PBCC. This suggests that the roots and leaves of Nymphaea species are toxic. Lead levels at both sites were below the detection threshold.

On the other hand, it was determined that the stems of the plants in Lake Leonard were polluted with nickel since their content exceeded the allowable amount of 2.0 mg/kg, although the concentration in Lake PBCC was below the limit discovered. Table 2. Concentration of Lead (Pb) (mg/l) in water samples from Lake Leonard and Lake PBCC.

Study Areas	Water Samples mg/l	Permissible Limit (WHO, 1996)
Lake PBCC	11 mg/l	0.05 mg/l
Lake Leonard	12 mg/l	0.05 mg/l

Table 3. Concentration of Nickel (Ni) (mg/kg) in the different parts of Water Lilies (*Nymphaea sp.*) from Lake Leonard and Lake PBCC.

Study Areas	Roots	Leaves	Stems	Permissible Limit
	mg/kg	mg/kg	mg/kg	(WHO, 1996)
Lake PBCC	330 mg/kg	185 mg/kg	929mg/kg	10 mg/kg
Lake Leonard	19 mg/kg	105 mg/kg	31 mg/kg	10 mg/kg

Nickel (Ni) is regarded as a crucial trace element for human and animal health (Hassan et. al, 2012). In terms of its toxicity, nickel is comparable to other substances. The primary attribute of nickel is that it is not degraded in the body, but its chemical structure can be altered. According to World Health of Organization (1996), the maximum acceptable content of Nickel is 0.2 mg/l. In addition, the concentration of Nickel in water samples from Lake Leonard and Lake PBCC is 2 mg/l, as shown in Table 4. In addition, the reported concentration of the element in Lake Leonard and Lake PBCC exceeds the maximum Nickel concentration in water.

As suggested by the World Health Organization, the maximum allowable level of Nickel for plants is 10 mg/kg (1996). Nevertheless, table 3 demonstrated that Nymphaea sp.

Table 4. Concentration of Nickel (Ni) (mg/l) in water samples from Lake Leonard and Lake PBCC.

Study Areas	Water Samples mg/l	Permissible Limit (WHO, 1996)
Lake PBCC	2 mg/l	0.2 mg/l
Lake Leonard	2 mg/l	0.2 mg/l

Table 5. Concentration of Chromium (Cr) (mg/kg) in the different parts of Water Lilies (*Nymphaea sp.*) from Lake Leonard and Lake PBCC.

Study Areas	Roots mg/kg	Leaves mg/kg	Stems mg/kg	Permissible Limit (WHO, 1996)
Lake PBCC	362 mg/kg	211 mg/kg	1102mg/kg	1.30 mg/kg
Lake Leonard	24 mg/kg	67 mg/kg	18mg/kg	1.30 mg/kg

The Nickel concentrations in Lake Leonard are 19 mg/kg and Lake PBCC is 330 mg/kg. On its stem, Lake Leonard contained 31 mg/kg and Lake PBCC,

929 mg/kg. In addition, the nickel concentration of the leaves in Lake Leonard is 105 mg/kg and in Lake PBCC it is 185 mg/kg.

Table 6. Concentration of Chromium	n (Cr) (mg/l) in water san	mples from Lake Leonard and Lake PBC
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Study Areas	Water Samples mg/l	Permissible Limit (WHO, 1996)
Lake PBCC	1 mg/l	0.1 mg/l
Lake Leonard	1 mg/l	0.1 mg/l

This suggests that the roots, stems, and leaves of Nymphaea sp. The study regions were contaminated with nickel since its content exceeds the allowable limit of 10 mg/kg. Chromium (Cr) is the most abundant element in the earth's mantle that are widely used in industry as alloying, tanning of animal

hides, plating, inhibition of water corrosion, textile dyes, ceramic glazes, refractory bricks and pressuretreated lumber (Avudainyagam *et al.*, 2003). It is also considered as a human carcinogen (Clar, 1993) and it also toxic to many plants (Shanker A, *et al* 2005), aquatic animals (Velma *et al*, 2005) and microorganisms (Petrilli and De Flora, 1977). On the other hand, it is considered a micronutrient in humans, being necessary for sugar and lipid metabolism and is generally not harmful.

Table 7. Concentration of Cadmium (Cd) (mg/kg) in the different parts of Water Lilies (*Nymphaea sp.*) from Lake Leonard and Lake PBCC.

Study Areas	Roots mg/kg	Leaves mg/kg	Stems mg/kg	Permissible Limit (WHO, 1996)
Lake PBCC	BDL	BDL	21mg/kg	0.02 mg/kg
Lake Leonard	BDL	BDL	BDL	0.02 mg/kg

BDL – Below Detection Limit.

Table 8. Concentration of Chromium (Cr) (mg/l) in water samples from Lake Leonard and Lake PBCC.

Lake PBCC BDL 0.01 mg/l Lake Leonard BDL 0.01 mg/l	Study Areas	Water Samples mg/l	Permissible Limit (WHO, 1996)
Lake Leonard BDL 0.01 mg/l	Lake PBCC	BDL	0.01 mg/l
	Lake Leonard	BDL	0.01 mg/l

BDL – Below Detection Limit.

The permissible limit in water is 0.1 mg/l (WHO, 1996). Moreover, the level of concentration of chromium in water samples as shown in table 6 from Lake Leonard has 1 mg/l and Lake PBCC has 1 mg/l.

Hence, the results of the analysis revealed that the concentration of the element in the water from the Lake Leonard and Lake Lake PBCC is greater than the maximum concentration of chromium in the water.



Fig. 2. Sampling site of Lake PBCC.

On the other hand, the permissible limit of chromium for plants is 1.30 mg/kg as recommended by World Health Organization (1996). Table 5 revealed that the roots of Nymphaea sp. concentration of chromium in Lake Leonard are 24mg/kg and 362 mg/kg in Lake PBCC. On its stem, 18 mg/kg was detected in Lake Leonard and 1102 mg/kg in Lake PBCC. Moreover, leaves in Lake Leonard chromium concentration is 67 mg/kg and in Lake PBCC is 211 mg/kg.



Fig. 3. Summary of the lake's Nymphae sp. Heavy Metal Accumulation.

The graph evidently shows the high level of Chromium and Nickel accumulation on the stem followed by roots and leaves of *Nymphae sp*.

This implies that the roots, stems and leaves of Nymphaea sp. from the two sites were contaminated with chromium since its concentrations are beyond the permissible value which is 1.30 mg/kg. Cadmium (Cd) is the most dangerous metal that characterized by high stability and toxicity is non- degradable and this property allow it to stay in circulation when it is released to environment (Denise *et al*, 1989).

This element causes cancers (Banderdki *et al*, 2005) and oxidative stress when it binds with essential respiratory enzymes (Nies DH, 2003).





Graph shows that among other heavy metal tested Lead (Pb) has the highest level of content in water.

If we compare the two graphs of *Nymphae sp.* and water analysis of Lake PBCC Chromium and Nickel was high in Plants however lead is below detection level in plants, in contrast water has low Chromium and Nickel content but evidently high in Lead. This data tells us that *Nymphae sp.* has slow or not able to accumulate lead at all.

According to World Health of Organization (1996), the maximum permissible concentration of Cadmium is 0.2 mg/l in water. Moreover, Table 8 shown the level of concentration of Cadmium in water samples from Lake Leonard and Lake PBCC are both below detection limit. Furthermore, the recorded concentration of the element from the Lake Leonard and Lake PBCC is greater than the maximum concentration of Cadmium in the water.



Fig. 5. Summary of the lake's Nymphae sp. Heavy Metal Accumulation.



Fig. 6. Summary on the Heavy Metal content of Lake's waterLake Leonard's *Nymphae sp.* and water analysis for heavy metal graphs shows that Chromium and Nickel accumulated higher in Leaves compared to roots and stems, moreover water analysis shows high contend in lead the same with Lake PBCC lead is high in water but in below detection level in plants, this provides us a consistent data that *Nymphae sp.* has slow or not able to bioaccumulate lead.

In connection to this, 0.02 mg/kg as recommended by World Health Organization (1996) as the permissible limit of Cadmium for plants. However, table 7 revealed that the roots and leaves of Nymphaea sp. concentration of Cadmium in Lake Leonard and Lake Lake PBCC were below the

detection limit. On the other hand, on its stem, below detected limit was detected in Lake Leonard and 21 mg/kg in Lake PBCC. This implies that the roots, stems, and leaves of N. nucifera. and water samples from Lake Leonard were below the detection limit of cadmium. Moreover, the Cadmium concentration in the stem of Nymphaea sp. from Lake PBCC is greater than the permissible limit that is 0.02 mg/g.

On the other hand, the cadmium concentration in the roots and leaves of Nymphaea sp. and water samples from Lake PBCC were below detection limit.



Fig. 7. Water Lily (Nymphae *sp.*) Profile Scientific Classification Kingdom: Plantae Clade: Angiosperms Order: Nymphaeales Family: Nymphaeaceae Genus: Nymphaea

Nymphaea is a genus of hardy and tender aquatic plants in the family Nymphaeaceae. The genus has a cosmopolitan distribution. Many species are cultivated as ornamental plants, and many cultivars have been bred. Water lilies are aquatic rhizomatous perennial herbs, sometimes with stolons, as well. The leaves grow from the rhizome on long petioles. Most of them float on the surface of the water. The blades have smooth or spine-toothed edges, and they can be rounded or pointed. The flowers rise out of the water or float on the surface, opening during the day or at night.

Source: Wikipidia

https://en.wikipedia.org/wiki/Nymphaea

Conclusion

Heavy metals are chemical elements with a high metallic atomic number and constitute a significant class of environmental pollutants. In addition, the levels of the heavy metals lead (Pb), nickel (Ni), chromium (Cr), and cadmium (Cd) in the water samples from Lake Leonard and Lake PBCC exceeded the allowable limits for the elements in the water. The

roots and leaves of Nymphaea sp. lead levels at both locations were below the detection threshold. On the other hand, nickel was identified in the stems of plants in Lake Leonard, whereas levels were below the detection limit in Lake PBCC.

Moreover, the roots, stems, and leaves of Nymphaea species are toxic. The research regions were polluted with nickel and chromium because their concentration exceeded the allowable limit of 10 mg/kg. Last but not least, the roots, stems, and leaves of Nymphaea spp. cadmium levels in Lake Leonard water tests were below the detection limit. In addition, the Cadmium content in the stem of Nymphaea sp. the discharge from Lake PBCC exceeds the legal limit. In contrast, the quantity of cadmium in the roots and leaves of Nymphaea sp. Lake PBCC water samples were below the detection limit.

Recommendations

Researchers may conduct an intensive investigation of assessing the level of heavy metals in the water and Nymphaea sp. in Lake Leonard and Lake PBCC such as Mercury, Copper, Iron, Manganese, Cobalt and Zinc that is not part of the scope of the study.

The Local Government Unit where the two sites belong can consider conducting a program that would aware the residents near the area of study on the effects of the heavy metals in the human system.

The Legislative department of the sites can consider crafting an ordinance that would solve or may lessen the amount of the heavy metals in the bodies of water. 4. Further researches may be conducted to determine which of the plants in the Lakes considered as Best accumulator of the heavy metals.

Longitudinal studies can be conducted in the two lakes to monitor presence of heavy metals not just in water lilies but in other macrophytes, macro invertebrates and in the sediments as well.

Acknowledgment

The authors would like to express their sincere gratitude to the Municipalities of Rosario, Agusan Del Sur and New Leyte, Maco, Compostela Valley province that made this research undertaking possible. Also to the various people who are very instrumental to us. Your significant efforts are appreciated and gratefully acknowledged.

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