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EVALUATION OF *SPIROGYRA* SP. AS A BIOINDICATOR OF HEAVY METAL POLLUTION IN A TROPICAL AQUATIC ENVIRONMENT

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ABSTRACT

Due to the wide occurrence of environmental pollution, heavy metals need to be closely monitored in the aquatic environment because of its ecotoxicological effects on biota. In this regard, biomonitoring as a tool for the assessment of metal pollution in aquatic ecosystem is quite appealing. Therefore, this study was conducted to investigate algae as a potential bio indicator of heavy metal pollution in an aquatic environment in Fiji. *Spirogyra* sp. was sampled from a mining impacted water resource and analysed for heavy metals (manganese, copper, chromium, lead and zinc) using atomic absorption spectrometry. The order of metal accumulation in *Spirogyra* sp. was found as Mn > Cu > Cr > Pb > Zn. The results show that *Spirogyra* sp. is capable of accumulating heavy metals from polluted water and may be used a bioindicator species in environmental monitoring. Finally, the elevated heavy metal levels in the VGR aquatic environment could pose an ecological risk and appropriate remediation strategies are recommended to minimise adverse impacts to aquatic life.

KEY WORDS : Heavy metals, Pollution, *Spirogyra* sp., Bioindicator, Biomonitoring

INTRODUCTION

Environmental pollution of heavy metals has become a global issue due to increasing anthropogenic activities (Shafiuddin Ahmed *et al.,* 2021). Heavy metals are toxic to humans and produce multiple adverse health effects, even at low concentrations via their exposure in the environment and due to their ability to accumulate in human beings, organisms and in environmental media such as water, soil, sediment or plants (Obiri *et al.*, 2016). The human body uses metals like manganese (Mn), copper (Cu) and zinc (Zn) in small amounts, however, metals such as chromium (Cr) and lead (Pb) have negative impact on life in general even in small quantities. With regards to a significant economic and infrastructural growth in the past few decades, the small developing island states in the Pacific have been challenged by the heavy metal pollution and contamination of its environment (Chand and Prasad, 2013).

Multiple terrestrial sources of natural (erosion, leaching) and anthropogenic origin (e.g. mining and manufacturing waste) lead to the presence of high levels of toxic heavy metals in aquatic environments (Chand *et al.*, 2011). Mining is an important industry for Fiji's economy. Unfortunately, as shown in other parts of the world, this industry can be the chief source of metals entering into the environment. Mining can affect the environment through ore processing activities, such as discharged mine effluents and seepage from tailings (Mohapatra and Kirpalani, 2017), which can result in elevated concentration of toxic metals in the environment (Rashed, 2010). Previous studies have shown that mine tailings originating from gold mining activity in Fiji can be a significant source of heavy metals and there have been cases of mine tailings found to be interacting with the community and the environment (Ko *et al.* 2008).

While chemical analysis of water and sediment is the most direct approach to assess heavy metal pollution status in the environment, living organisms can be effectively utilised as bioindicators to screen the health of the natural ecosystem in the environment (Zhou *et al.*, 2008) (Parmar et al., 2016). Naturally occurring microorganisms such as algae has been used for detecting pollutants such as heavy metals due to their ability to take up and accumulate heavy metals from their surrounding environment (Wan Jusoh *et al*., 2020). For example, *Spirogyra* sp. is a benthic freshwater green algae widely distributed throughout the world (Lee and Chang, 2011) and already recognised as potential absorbent for heavy metals from mine or waste water (Bermanec *et al.*, 2018). In addition, due to its sensitivity to the presence of pollutants such as heavy metals in water, it has also been studied to some extent for the screening of heavy metals in aquatic ecosystems (Wan Jusoh *et al.*, 2020), particularly where point source of pollution exists (Rai et al., 2008). Therefore, the present work aims to determine the levels of Mn, Cu, Cr, Pb and Zn in *Spirogyra* sp. from a mining impacted water resource in Fiji and evaluate its potential as a bioindicator of heavy metal pollution in a tropical aquatic environment.

MATERIALS AND METHODS

Study Area

Situated in the northern part of Viti Levu, Fiji's main island, the Vatukoula gold mine has been in operation for the last eight decades. The mine has several tailings dam where all sludge waste material are deposited. Adjacent to the mine and tailings dams are the farmlands and residential areas which share the resources for subsistence use, sustenance and agriculture. The Toko tailings dam is used by the mine for discharging and storing mined sludge from the mines. Subsequently, the effluents from the dam are decanted and discharged into the Dakovono Creek, which then connects the main Nasivi River. The selected sampling stations were downstream of the dam wastewater discharge point into the Dakavono Creek as shown in Fig. 1.

Sampling

In each of the selected sites, algae samples were collected using hand and plastic gloves, placed in clean polyethylene zip-lock bags and transported to the laboratory in ice. At all locations, the depth of water was <0.5 m. Taxa identification was carried out with the assistance of a plant botanist and the algae samples were identified as *Spirogyra* sp. Physicochemical parameters such as temperature, pH, ORP, conductivity, turbidity, dissolved oxygen, total dissolved solids, salinity and depth at each sampling station were also taken using a calibrated handheld multi-parameter digital meter (Model 85, YSI Inc., USA).

Sample Preparation

In order to separate *Spirogyra* sp. samples from sediment and organic debris, the samples were washed with distilled water and then separated using acid-washed plastic knives. The samples were dried in an oven for 48-72 h in 80 °C until reaching the constant weight and ashed in a furnace in 450 °C for 48 h. Ashed samples were digested using concentrated nitric acid (HNO_3) on a hot plate and diluted by 1% HNO_3. The sample was then filtered into a beaker, transferred to a volumetric flask and diluted to 50 ml with distilled water. The procedural blank and certified reference material were subjected to the same digestion procedure.

Fig. 1. Map showing the location of the study area and sampling sites in Fiji.

Analytical Method

All sample containers, reagent bottles and glassware were acid washed with 0.1 N nitric acid before rinsing with copious amounts of ultrapure water and drying in air before use. All solutions were prepared in ultrapure water (Millipore system). 1 g/L mono-elemental standards of Mn, Cu, Cr, Pb and Zn (C.P.A. Ltd) were used for calibration, from which working and calibration standards of each metal were prepared daily before use. Metal concentrations of the digests were determined using an AAnalyst 400 flame atomic absorption spectrometer (Perkin Elmer, USA) with an air/acetylene flame.

Quality Control

The accuracy and precision of the analytical procedure were verified with use of a certified reference material BCR-482 lichen (IRMM, Belgium), spiked samples and replicate analysis of samples. The relative difference between the measured and certified concentrations of Mn, Cu, Cr, Pb and Zn were -3.2, -6.3, -5.5, -6.6 and -4.7, respectively. The mean recoveries of Mn, Cu, Cr, Pb and Zn from spiked samples were 97.6, 94.3, 91.8, 102.3, and 99.5%, respectively. The relative standard deviation for replicate analyses was within 5% (n = 3). The limit of detection (LOD) was calculated as the metal concentration that corresponded to three times the standard deviation of seven independent measurements of the procedural blank.

RESULTS AND DISCUSSION

The physicochemical characteristics of the sampling stations are outlined in Table 1. The study region had a temperature range of 26.01-28.71 °C in the study period. The slightly alkaline pH (mean = 7.87) was within the WHO drinking water guideline range of pH 6.5-8.5 (WHO, 2008). High pH values could be related to the discharge of highly alkaline tailings dam effluent (Ko *et al.*, 2008). The sampling stations showed high turbidity and exceeded the WHO Guideline value of 5 NTU (Obiri *et al.*, 2016). TDS for most stations were above WHO guideline value of 1 g/l as well. The tailings dam discharge could be the contributing factor for these observations since mining activities have been shown to contribute to high turbidity and TDS of surrounding water resources (Acheampong *et al.*, 2013). DO, EC and salinity had mean values of 8.58

Table 1. Physicochemical characteristics of the sampling stations.

Sampling Station	Latitude Longitude	Temperature (°C)	pH	Oxidation-Reduction Potential (mV)	Conductivity (mS/cm)	Turbidity (NTU)	Dissolved Oxygen (mg/L)	Total Dissolved Solids (g/L)	Salinity (ppt)
SS10	17°28'55.32"S 177°52'4.23"E	28.71	7.69	226	0.64	9.91	10.02	0.45	0.2
SS11	17°28'55.46"S 177°51'48.65"E	26.91	7.58	200	2.38	12.63	8.98	1.86	1.2
SS12	17°28'50.17"S 177°51'39.74"E	27.41	8.00	181	2.46	16.36	8.16	1.58	1.3
SS13	17°28'46.80"S 177°51'27.37"E	27.48	7.90	177	2.50	16.67	7.32	1.60	1.3
SS14	17°28'42.40"S 177°51'11.18"E	27.64	8.44	198	2.32	49.42	9.88	1.48	1.2
SS15	17°28'43.00"S 177°50'52.00"E	26.01	7.59	193	0.95	6.43	7.10	0.61	0.5
Average ± SD		27.36 ±0.89	7.87 ± 0.33	196±17	1.88±0.84	18.57±15.61	8.58±1.26	1.26±0.58	1.0±0.5

mg/l, 1.26 ms/cm and 0.48 ppt, respectively. The high values of the EC could be possibly due to the presence of electrolytes in the tailings dam wastewater (Acheampong *et al.*, 2013) being discharged into the Dakavono Creek. The ORP revealed a comparatively high variation with a mean value of 196 ± 17 mV.

The mean concentrations of metals in the algae samples at the different sampling stations is given in Table 2. It was evident that *Spirogyra* sp. was able to accumulate high levels of Mn (107.5 - 360.4 mk/kg) in a polluted environment, which is consistent with similar studies carried out elsewhere. Kaonga *et al.* (2008) studied the ability of *Spirogyra aequinoctialis* to accumulate Mn from water and concluded it can be used as a biological indicator for long term heavy metal water pollution monitoring. Similarly, *Spirogyra subsalsa* recorded maximum bioaccumulation of Mn among other algae in another study by Aladdin and Aziz (2020.

The next highest concentrations in *Spirogyra* sp. were of Cu (54.6 - 260.2 mg/kg), Cr (27.0 - 116.1 mg/kg) and Pb (13.7 - 96.0 mg/kg). Heavy metals such as Cu, Pb and Zn are associated with many of the gold deposits (Hamidian *et al.*, 2016) and therefore, end up in mine tailings. Minerals comprising Cr like chromite is common in rock and soil particles while atmospheric deposition and mining sludge are the other sources. The levels of Cr in *Spirogyra* sp. were also found to be in accordance with literature values for *Spirogyra* sp. from the Shoor River (Iran), an area influenced by local agriculture and Mn mining activities (Hamidian *et al.*, 2016). They found high Cr levels in the 12 stations at the river (102.31 ± 58.45 mg/kg), which has agriculture and mining in the vicinity in addition to dumping of wastes. (Kaonga *et al.*, 2008) have also shown that *Spirogyra aequinoctialis* was able to accumulate Pb from polluted water. The concentrations of Zn in the *Spirogyra* sp. samples were much lower than for all the other metals. This indicates their lower potential availability of Zn in this aquatic ecosystem or regulated uptake of these metals by *Spirogyra* sp. The accumulation abilities of the algae *Spirogyra* sp. for different metals have been reported by other authors as well (Rajfur and K^{3}os, 2015). For example, Bermanec *et al.* (2018) found that Cd, Co, Sr, and Zn preferentially accumulated in *Spirogyra* sp. taken from the acid mine drainage impacted Majdanska River, suggesting that this algae species have great ability to accumulate metals from the aquatic environment.

The accumulation order of heavy metals in *Spirogyra* sp. samples was found as Mn > Cu > Cr > Pb > Zn, determined from the average levels of these metals from all sampling sites (Fig. 2). Significant difference (ANOVA, á = 0.05) between the levels of metal accumulation at difference sampling stations as well as between the metals was observed. Differences observed in metal concentrations in the *Spirogyra* sp. at different sampling stations might be due to differences in environmental conditions or nature of the *Spirogyra* (Çelekli and Bozkurt, 2021). It has been reported that the absorption of metals highly depends on the algal taxon, age of *Spirogyra* sp., seasonal variation, the metal, the age of the material, and other

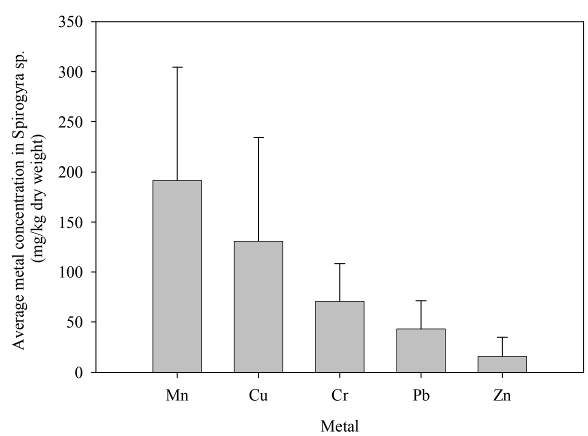

Fig. 2. Mean concentrations of metals in *Spirogyra* sp. (mg/kg dry weight). The error bars represent the standard deviations.

Table 2. Metals concentrations in *Spirogyra* sp. (average ± SD; mg/kg dry weight) at the different sampling stations.

Sampling Station	Mn	Cu	Cr	Pb	Zn
SS10	360.4 ± 17.2	251.5 ± 13.5	27.0 ± 2.5	13.7 ± 2.4	18.9 ± 2.4
SS11	118.0 ± 4.9	260.2 ± 23.1	96.3 ± 8.5	38.7 ± 3.2	42.2 ± 1.2
SS12	250.9 ± 24.1	113.7 ± 5.2	66.8 ± 7.2	96.0 ± 5.3	22.4 ± 1.7
SS13	248.0 ± 17.3	96.7 ± 2.3	116.1 ± 15.3	45.3 ± 2.4	22.3 ± 2.2
SS14	319.0 ± 14.8	137.3 ± 9.2	82.2 ± 5.6	40.9 ± 4.6	39.0 ± 1.4
SS15	107.5 ± 4.2	54.6 ± 4.5	57.0 ± 3.2	37.5 ± 2.1	12.0 ± 0.8

Table 3. Comparison of heavy metal levels in *Spirogyra* sp. in the present study with that of similar studies done elsewhere.

Country	Water system	Metal levels (mg/kg, dry weight)	Reference
Fiji	Dakavono Creek and Nasivi River	Cr 70.9; Cu 130.7; Mn 191.3; Pb 43.2; Zn 16.1	This study
Iran	River Shoor	Cr 102.31 ; Cu 19.64 ; Mn 97.65 ; Pb 10.77 ; Zn 274.68	(Hamidian *et al.*, 2016)
Malawi	Chirimba, Mudi, Nasolo, Michiru, Mangunda, Limbe and Naperi streams	Mn 0.432 -16.132 ; Pb 0.042 - 0.972	(Kaonga *et al.*, 2008)
Poland	Turawa, Nysa and Otmuchow reservoirs	Cu 1.24 ; Mn 22.60 ; Pb 4.38 ; Zn 9.12	(Rajfur and K³os, 2015)
Poland	Turawa Lake	Mn 12330 ; Cu 47.5 ; Pb 684 ; Zn 1411	(Rajfur *et al.*, 2011)
Pakistan	River Swat	Cu 0.04-0.08 ; Pb 0.06-0.1 ; Zn 0.53-1.56	(Alam *et al.*, 2020)
India	Lake Nainital	Mn 176 ; Cu 47; Cr 4 ; Pb 95 ; Zn 34	(Ali *et al.*, 1999)
India	Water bodies of Lucknow, Unnao and Kanpur	Mn 18.22 ; Cu 11.02 ; Cr 0.61 ; Zn 31.42	(Rai *et al.*, 2008)
India	Drinking water facilities, hand pumps and covered wells	Mn 729; Cu 235 ; Cr 450; Pb 315	(Rai *et al.*, 1996)
Turkey	Tigris River	Mn 43-187.8 ; Cu 1.15-7.13 ; Zn 1.40-6.85	(Karadede-Akin and Ünlü, 2007)

environmental factors (Hamidian *et al.*, 2016). The wide variations of metal concentrations observed could be due to the physicochemical characteristic of the sampling sites discussed earlier.

A comparison of heavy metal levels in *Spirogyra* sp. in the present study with that of similar studies done elsewhere is summarised in Table 3, which shows that there can be significant variation in metal levels accumulated by *Spirogyra* sp. depending on the environmental factors.

CONCLUSION

Spirogyra sp. collected from an aquatic environment was successfully used for the indication of Mn, Cr, Cu, Pb and Zn pollution. This study showed that *Spirogyra* sp. has the ability of accumulating Mn, Cr, Cu, Pb and Zn in a polluted aquatic environment and therefore, it can be used as a biological indicator for long term heavy metal water pollution monitoring in tropical environments. Furthermore, differences in environmental conditions showed variations in metal level accumulations in *Spirogyra* sp. The results further confirms that the aquatic environment in VGR are impacted by mining activities and contributing to elevated heavy metal levels in the immediate water resources. This study recommends safer environmental strategies and proper monitoring of the water resources in the VGR in order to minimise ecological risks and adverse impacts on the aquatic life. Further studies could explore the potential of *Spirogyra* sp. for phytoremediation and the removal of toxic elements from the aquatic ecosystem.

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Conflict of interest

The authors declare that there is no conflict of interest.

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