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The potential of acidophilic macroalgae as part of passive bioremediation technology for acid mine drainage in constructed wetlands

PH CHENG[1,3], **PJ OBERHOLSTER**[1,2], **A-M BOTHA**[3]
[1]CSIR Natural Resources and the Environment, PO Box 320, Stellenbosch, 7599, South Africa
[2]Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa
[3]Department of Genetics, University of Stellenbosch, Private Bag X1, Stellenbosch, 7601, South Africa
Email: pcheng@csir.co.za, poberholster@csir.co.za – www.csir.co.za

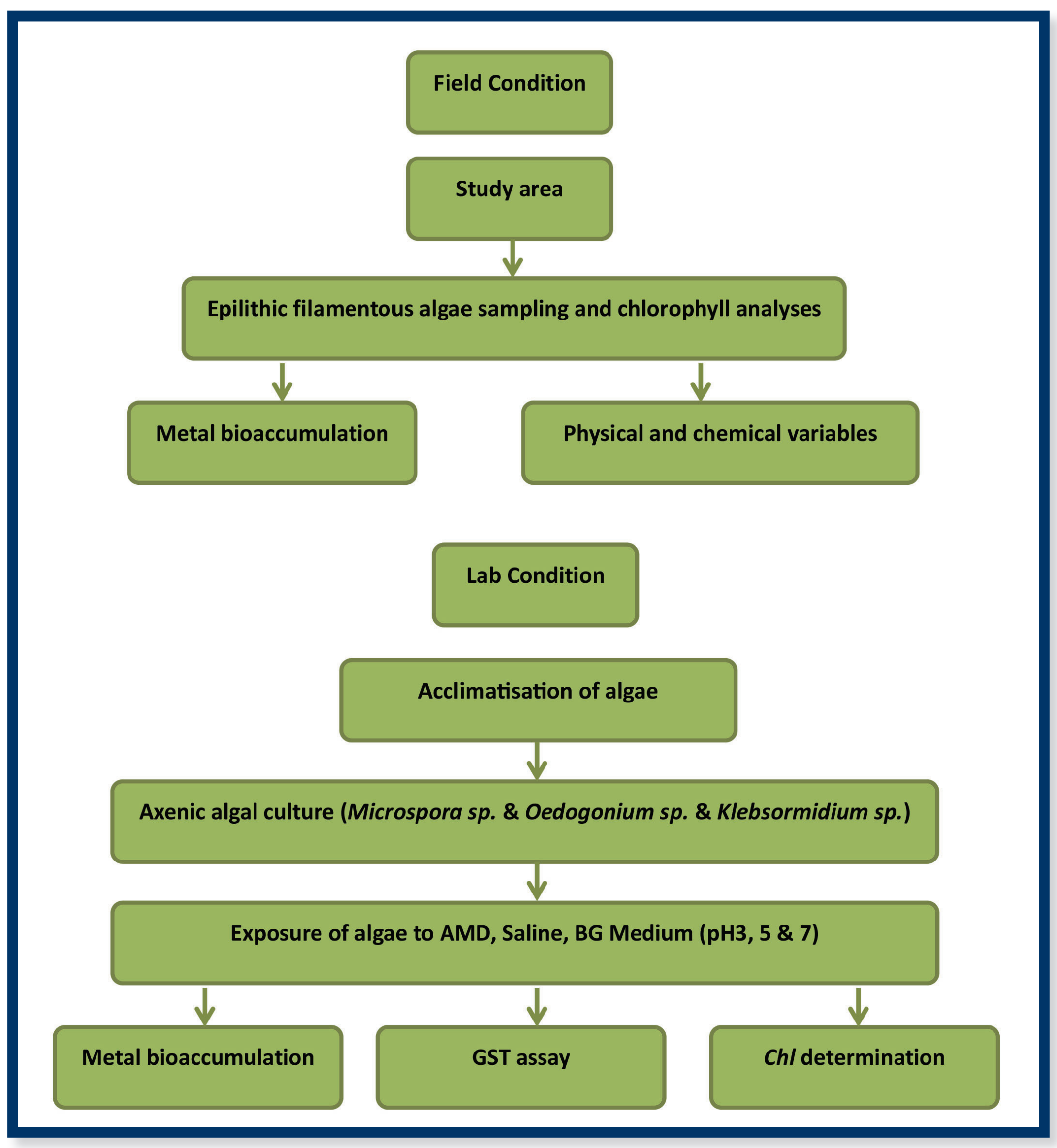
BACKGROUND

The properties of acid mine drainage (AMD) render water resources less habitable to various biota, hence waters that receive AMD are often characterised by very low biodiversity and the flora and fauna become dominated by highly tolerant organisms and acidophilic biota (Foster, 1982). Due to the adverse effects accompanying AMD, sensitive species are systematically reduced or eliminated. During the last three decades, passive treatment of AMD using technologies such as constructed wetlands has been developed as an alternative to conventional treatment in environments with medium to low flow regimes. Biological removal is perhaps the most important pathway for heavy metal removal in these wetlands (Sheoran and Sheoran, 2006). However, the metal storage capability of macrophytes in constructed wetlands can be lost in temperate areas during winter when wetland plants no longer take up metals and nutrients depending on the selected macrophyte species. However, during winter benthic green filamentous algae mats, as well as filamentous algae biofilm attached to submerged leaf surfaces of macrophytes, may play a major role in absorbing metals from the water column (Kalff, 2001).

Although algae have been reported to be quite efficient in heavy metal removal from acid mine water in wetlands, very little is known about different benthic mat algal species and AMD remediation technologies (Das et al., 2009). Therefore, the presence and tolerance of diverse benthic algal species to AMD provides the option to utilise them in AMD remediation as part of passive bioremediation technology in constructed wetlands.

The purpose of the study was to investigate the bioaccumulation of metals and trace metal loads in algae exposed to AMD generated from gold and coal mining activities under different environmental conditions. The data generated from this study will be used to inform passive treatment technologies.

MATERIALS AND METHODS



Field Condition

Study area

Epilithic filamentous algae sampling and chlorophyll analyses

Metal bioaccumulation

Physical and chemical variables

Lab Condition

Acclimatisation of algae

Axenic algal culture (*Microspora sp.* & *Oedogonium sp.* & *Klebsormidium sp.*)

Exposure of algae to AMD, Saline, BG Medium (pH3, 5 & 7)

Metal bioaccumulation

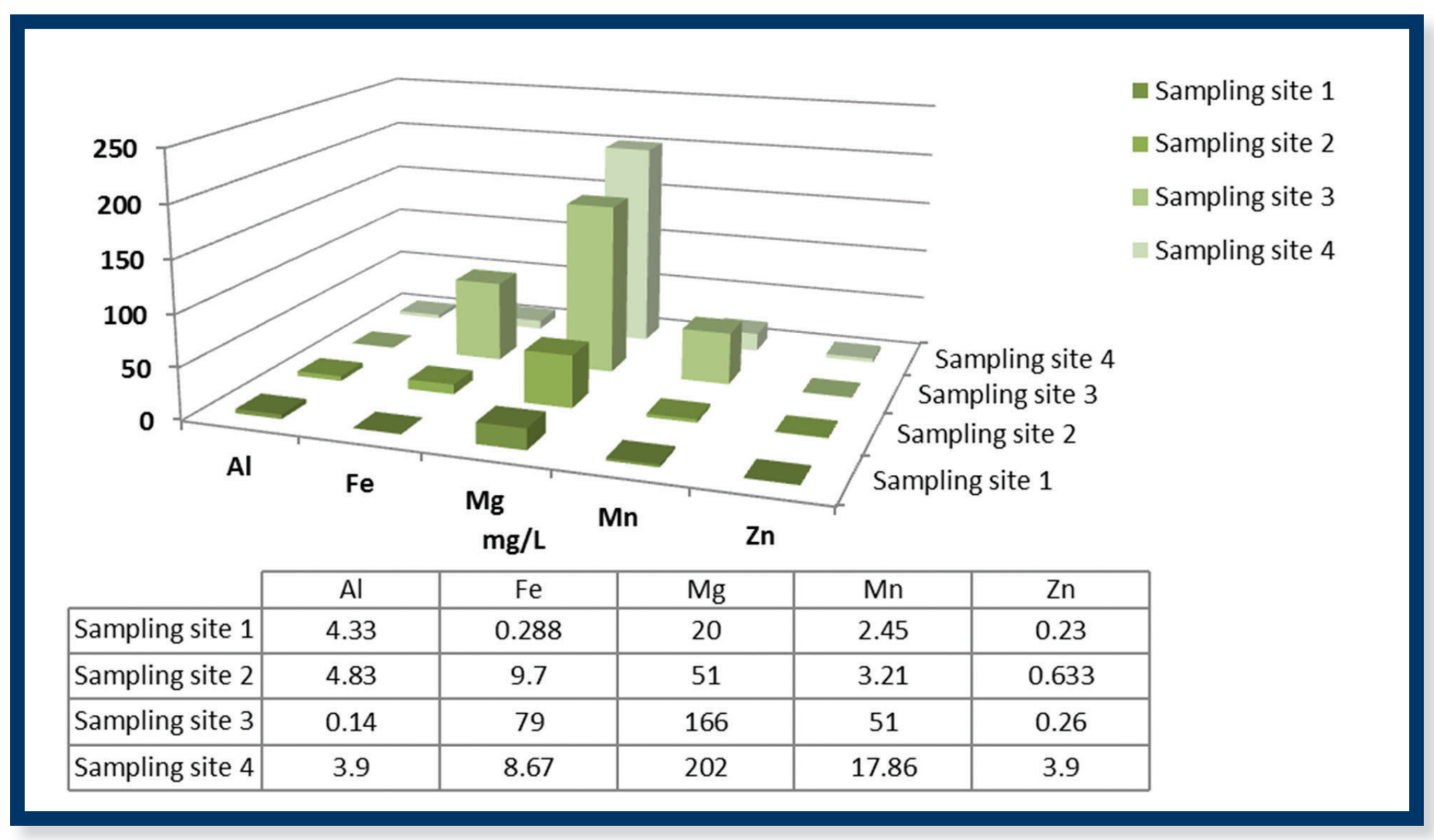
GST assay

Chl determination

RESULTS

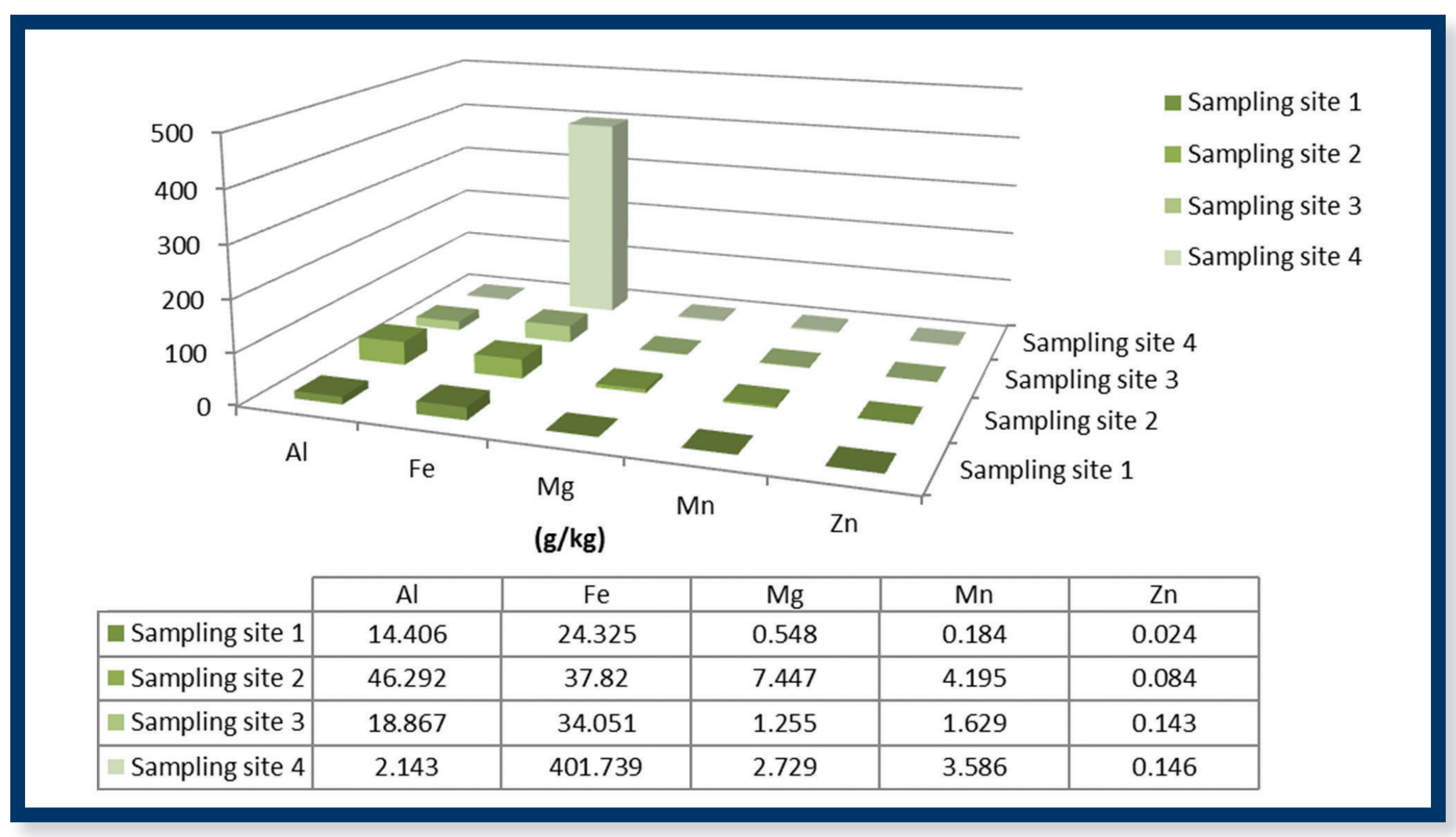
Table 1: Summary of descriptive selected physical and chemical characteristics of the different sampling sites

	Location (lat, long)	Substrate tape	AMD source	Surface water composition	Vegetated or unvegetated at sampling location	Stream cross-sectional area (m^2)
Site 1: Klip stream	S 25° 37.290′	Mud/sediment	Decanting surface flows from coal mines	Mixture of AMD (coal mining) and untreated sewage effluent	vegetated	5–7
	E 29° 12.752′					
Site 2: Brug stream	S 25° 51.424′	Mud/sediment	Seepage from decanting coal mines forming a stream	AMD effluent (coal mining)	vegetated	2–3
	E 29° 08.139′					
Site 3: Black reef incline	S 26° 11527′	Mud/sediment	Decanting water from gold mines	Location	vegetated	5–15
	E 27° 72314′					
Site 4	S 26° 11110′	Mud/sediment	Bore hole effluent from decanting gold mines forming a stream	AMD effluent (gold mining)	vegetated	2–4
	E 27° 72273′					



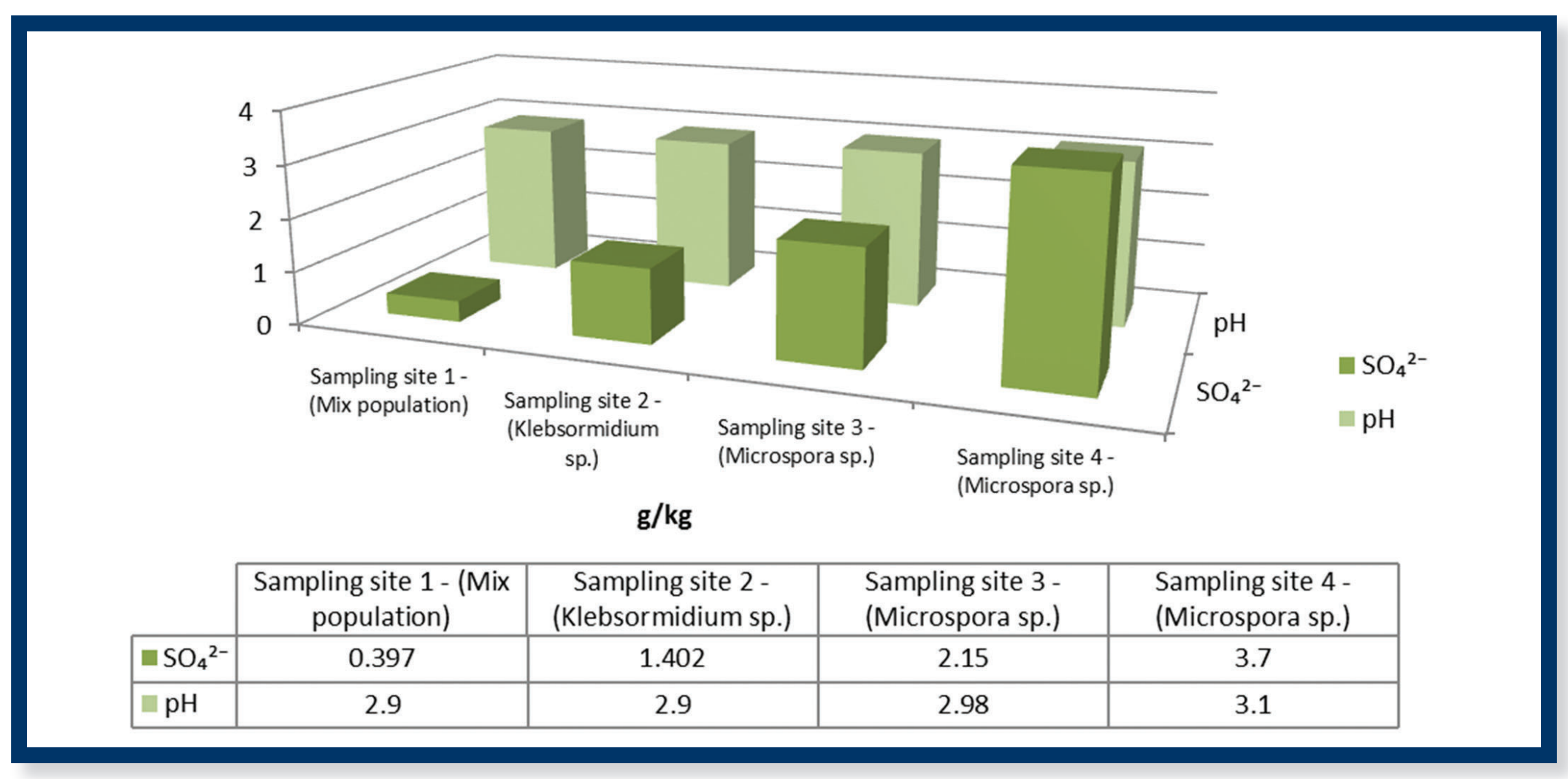
	Al	Fe	Mg	Mn	Zn
Sampling site 1	4.33	0.288	20	2.45	0.23
Sampling site 2	4.83	9.7	51	3.21	0.633
Sampling site 3	0.14	79	166	51	0.26
Sampling site 4	3.9	8.67	202	17.86	3.9

Figure 1: Metal and trace metal concentrations (mg/L) in water column from the selected sampling sites



	Al	Fe	Mg	Mn	Zn
🟩 Sampling site 1	14.406	24.325	0.548	0.184	0.024
🟩 Sampling site 2	46.292	37.82	7.447	4.195	0.084
🟩 Sampling site 3	18.867	34.051	1.255	1.629	0.143
🟩 Sampling site 4	2.143	401.739	2.729	3.586	0.146

Figure 2: Metal and trace metal concentrations (mg/kg d.wt) in algae samples from the selected sampling sites (n=4)



	Sampling site 1 - (Mix population)	Sampling site 2 - (Klebsormidium sp.)	Sampling site 3 - (Microspora sp.)	Sampling site 4 - (Microspora sp.)
🟩 SO_4^{2-}	0.397	1.402	2.15	3.7
🟩 pH	2.9	2.9	2.98	3.1

Figure 3: Concentration of sulphate and pH from the selected sampling sites where dominant algae species were found.

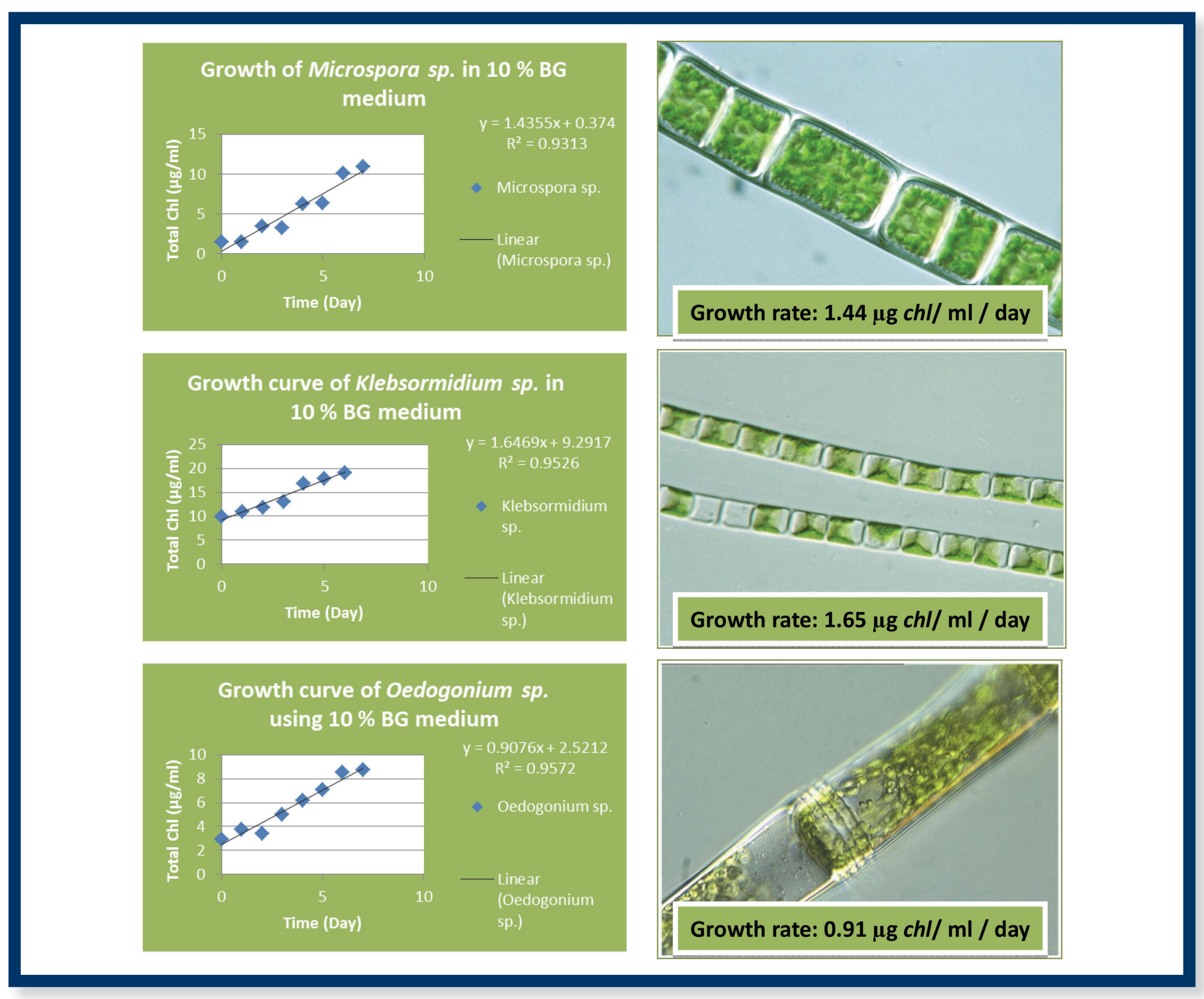


Figure 4: Growth curve of macroalgae expressed relative to total chlorophyll (monitored over a period of 7 days in laboratory conditions)

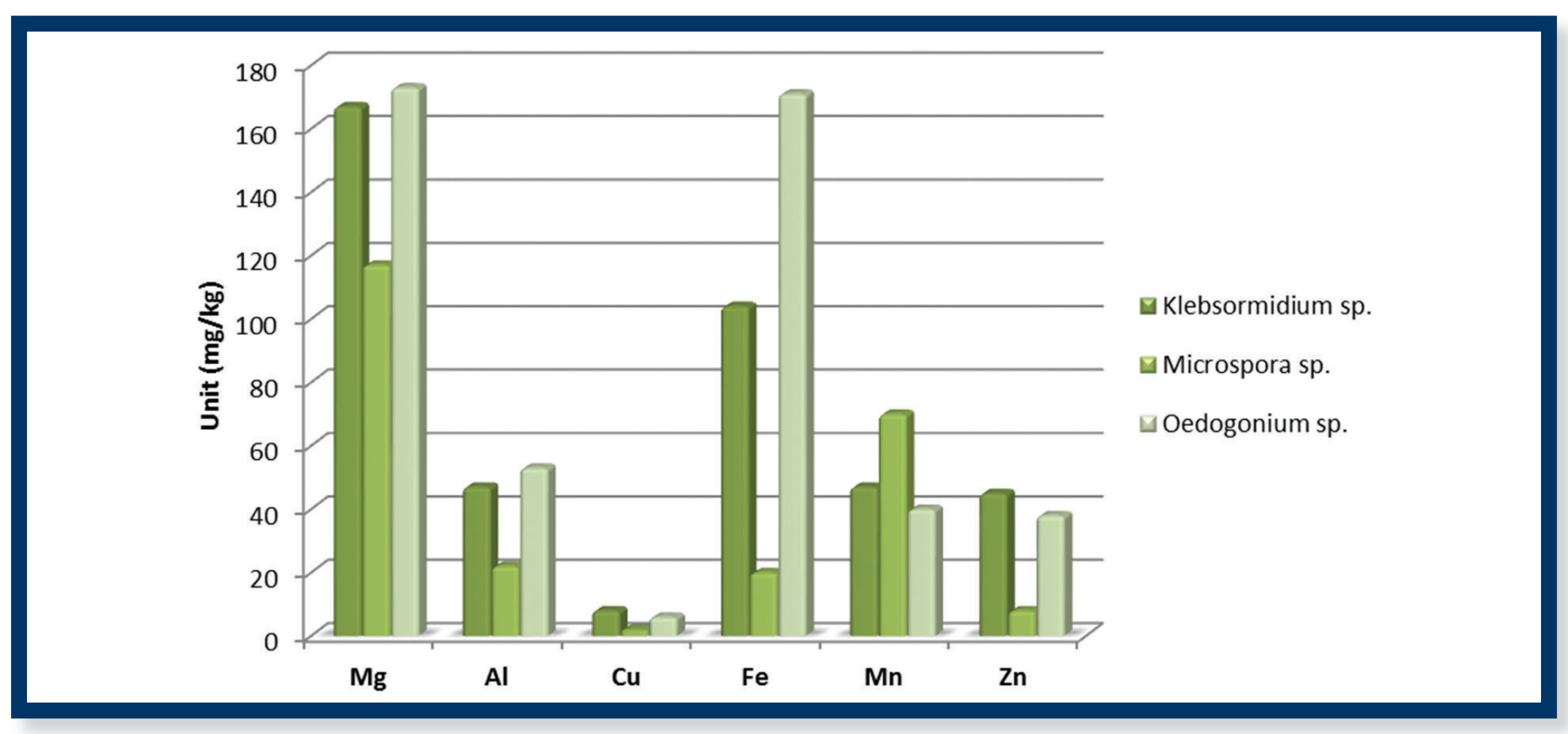


Figure 5: Metal and trace metal concentrations (mg/kg) in algae samples at day 7

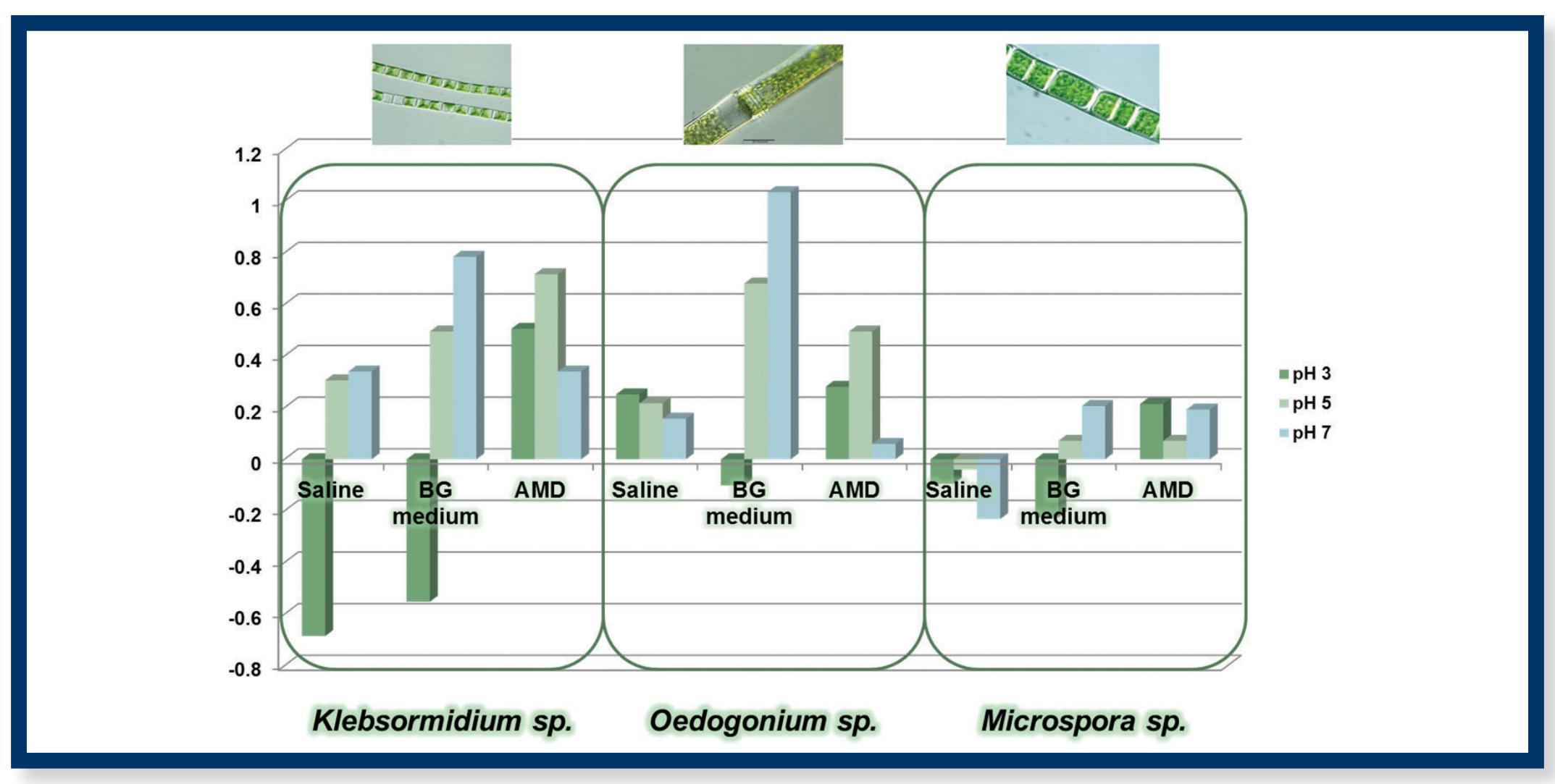


Figure 6: Total chorophyll production rate of algae samples exposed to various mediums and monitored over a period of 7 days

This study shows that certain species of benthic filamentous algae can play an important role as part of passive treatment technology by absorbing metals under different environmental conditions.

Figure 7: Mats of filamentous green algae *Microspora sp.* at sampling site 3

Key findings to date

1. There are three major interacting stressors from AMD on aquatic biota namely 1) acidity, 2) heavy metals and 3) metal oxide deposition.
2. Nutrients also play a major role to stimulate algal growth under AMD conditions.
3. Low algae biomass at sites 3 and 4 was likely due to two factors, namely 1) metal oxide deposition onto algal cells which may inhibit photosynthesis, and 2) substrate being covered by mineral crystals.
4. A significant decrease in chlorophyll content of all algae species when exposed to low pH medium. From this study it is evident that metal concentrations may be more influential than pH in determining algal distribution in streams receiving acid mine drainage.
5. Very high concentrations of Al and Fe are bioaccumulated by the algae *Klebsormidium klebsii*.
6. Algae absorb sulphates as well as different types of metals at different pHs.

Ongoing research

1. Currently, oxidative stress responses to metal levels impacted by metal contamination are monitored using algae.
2. Oxidative damage and antioxidant responses involved in sulphate assimilation metabolism are investigated in the three acidophilic algae species used in this experiment. The relationship with metal accumulation and pH, as well as with environmental chemical data (metals and nutrients), is currently investigated.
3. An evaluation of the suitability of a combined approach which looks at external levels of exposure and bioaccumulation, as well as biomarkers such as oxidative stress responses, to monitor and assess metal pollution in aquatic environments exposed to AMD.

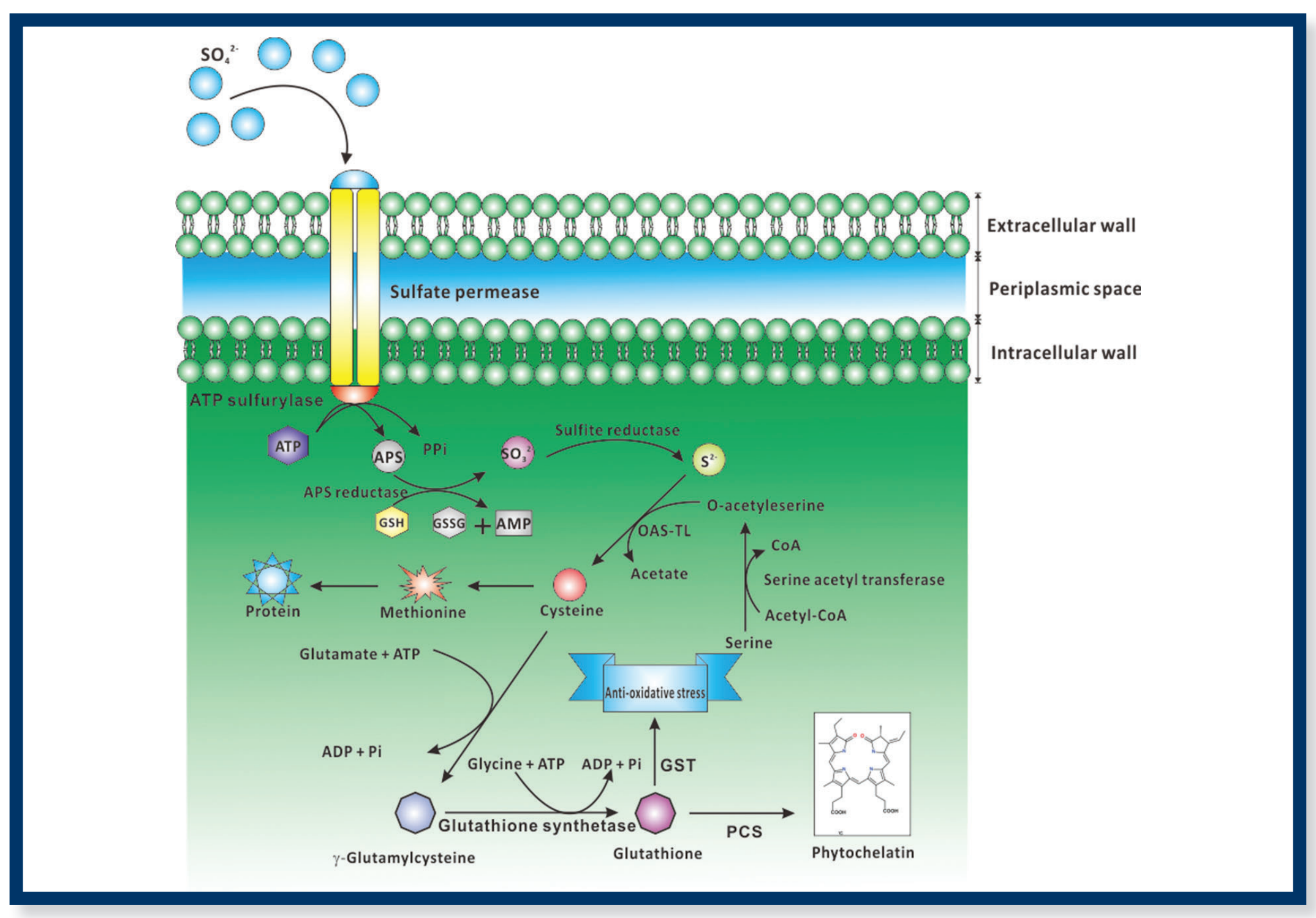


Figure 8: Putative sulfate reduction and assimilation pathway in algae (APS, adenosine 5′-phosphosulfate; Fdred, Fdox, reduced and oxidized ferredoxin; RSH, RSSR, reduced and oxidized glutathione; SQDG, sulfoquinovosyl diacylglycerol)

CONCLUSION

It was evident that certain species of benthic filamentous algae can play an important role as part of passive treatment technology by absorbing metals under different environmental conditions.

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