

RESPONSES OF BENTHIC MICROORGANISMS (THECAMOEBIANS) TO OIL SANDS PROCESS-AFFECTED MATERIALS; PROVIDING ENDPOINTS FOR GAUGING AQUATIC RECLAMATION SUCCESS

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ABSTRACT

Constructed wetlands and end-pit lakes will play an important role in reclamation options for fluid tailings (OSPW/M) at surface oil sands operations. Through time and with natural bioremediation viable aquatic habitats will develop, but currently few tools are available to determine the rates of remediation in produced ecosystems. A micropaleoecological environmental proxy (thecamoebians) has been demonstrated to provide a time-averaged indicator of ecosystem health. Thecamoebian communities in sediments from both impacted and non-impacted wetlands and lakes in the vicinity of oil sands operation have been compared. An index of response to stress has been compiled with the goal of using it as a predictor of the path of remediation that will produce sustainable ecosystems. This information also provides an endpoint for remediation efforts.

Thecamoebian assemblages in cores and surface samples from 63 natural lakes across the region were used to establish natural ecological ranges and remediation targets. These were compared to those present in wetland sediments impacted by oil sands materials (OSPW/M). The process-affected sites had lower thecamoebian diversity and were dominated by centropixid taxa, whereas more abundant and diverse assemblages dominated by difflugiid taxa characterized less-impacted sites. Moreover, assemblages responded quickly to changes in OSPW/M input and to various reclamation strategies, such as nutrient input. Preliminary results suggest that thecamoebians represent proxies for gauging ecosystem health, monitoring aquatic reclamation progression and developing target endpoints.

INTRODUCTION

The Alberta oil sands (AOS) are one of Canada's most economically important natural resources. Assessing and remediating potential detrimental environmental impacts to aquatic habitats resulting from sands developments in the Wood Buffalo region of northern Alberta requires innovative approaches that can follow ecological, temporal and spatial distribution of possible impacts.

During oil sands processing, large volumes of water are used, with most of it being recycled from tailings retention ponds. Over time, concentrations of dissolved constituents, mainly salts and dissolved organics, associated with oil sands operations become elevated in the OSPW/M. The waters released from tailings ("free" water) in surface zones or captured within the pore spaces of tailings (sands, fines) deposits have unique character and properties relative to natural non-OSPM impacted waters. In general, freshly produced OSPW will stress biota through elevated ionic content and presence of organic-acid constituents such as low-molecular-weight naphthenic acids, but the toxic character of the OSPW has been demonstrated to dissipate over time (Harris, 2007; Neville et al., 2011).

Ecological Indicators

Thecamoebians (testate amoebae, arcellacea) are protists (unicellular microorganisms) that comprise an important component within the microbial trophic level of the benthic community in lakes and wetlands (Patterson and Kumar, 2000; Beyens and Meisterfeld, 2001).

Species and strains are characterized by a simple sac-like decay-resistant organic test of pseudo-chitinous material that is variably agglutinated in different species (Patterson and Kumar, 2000; Scott et al., 2001). Thecamoebians are useful in

environmental research as they are characterized by rapid generation times, and sensitivity to environmental conditions at the sediment/water interface and epibenthic zone (Neville et al., 2010a). Their abundant fossilized remains preserve a record of contaminate responses and changing environmental conditions over time (McCarthy et al., 1995; Boudreau et al., 2005). Unlike most microfossil groups, thecamoebians are resistant to dissolution in lower pH environments (Swindles et al., 2007).

Variation in thecamoebian community assemblages have been successfully used in investigations of paleoclimate (Boudreau et al., 2005; McCarthy et al., 1995), sea-level change (Scott et al., 2001), and anthropogenic impact, including that of sulphide mining in acid-sensitive lakes in Ontario (Patterson et al., 1996; Reinhardt et al., 1998; Kumar and Patterson, 2000; Patterson and Kumar, 2000) and in Finland (Kauppila et al., 2006). These latter studies led us to investigate the sensitivity of thecamoebians to the by-products of oil sands production.

Wetland habitats, both constructed and opportunistic, will be important components of the reclaimed oil sands impacted landscapes, and the rate of progression from OSPM-stressed to more natural systems will be an important factor for gauging reclamation success. Simple and effective methods to monitor the early stages of remediation of these wetlands need to be developed to demonstrate a trajectory towards natural processes. This study investigates the use of benthic microbiota in assessing the effectiveness of the remediation process.

To further assess the applicability of thecamoebians as biomonitors of potential oil sands industrial impact, the project aims to determine reliable methods for discriminating between anthropogenic from natural sources and ecological impacts on natural areas surrounding oil sands operations. This could assist in evaluating whether OSPW/M emanating from oil sands operations is negatively impacting local aquatic habitats and whether reclaimed aquatic systems will perform as viable components in final lease-closure landscape. Thecamoebian populations from lakes sampled as part of this program were compared to populations found at oil sands sites where there was varying levels of stress from OSPW/M. A range in degree and timing of impacts provided various test sites that were analogous to what would be expected in aquatic

reclamation options including an indication of rates of progression to target endpoints.

METHODS

Test Pond Study Sites

Thirteen surface sediment samples were collected from the Constructed Wetland Test Facility (CWTF; located at Suncor Energy Inc.; Fig. 1; Table 1) in 2007; eight of those sites were re-sampled in 2008. Sediment samples for both years of study were collected using an Ekman grab sampler. The test site was comprised of four areas (Suncor CT Demonstration Study Site, Sustainable Lake South, Sustainable Lake North, and Crane Lake) that differed in construction and implementation, and each contained a series of wetlands (Fig. 1; Golder Associates Ltd., 2006). Between the sample collection of Set One (2007) and Set Two (2008), modifications were made to various CWTF sites causing increased or decreased OSPW inflow and subsequent changes in their chemistry.

Natural Study Sites

Surface sediments were collected from 8 lakes in August 2010 and 54 lakes in August 2011 (Fig. 2), sites were chosen to create a distal and proximal radius of natural lakes around the oil sands operation. The 2010 sample set was collected using a Glew gravity corer (Glew et al., 2001). The 2011 sample set was collected using an Ekman grab sampler.

Microfossil Analysis

Prior to thecamoebian analysis, 2 cc of sediment were passed through a 250 μm sieve to remove coarse organic debris and then a 37 μm sieve to remove fine organic and mineral detritus. The 37–250 μm aliquots were subdivided for quantitative analysis using a wet splitter. The wet aliquots were subsequently examined under an Olympus SZH10 dissecting binocular microscope (40 – 80X magnification) until a statistically significant number of specimens were quantified (Patterson and Fishbein, 1989). Identification of thecamoebians followed standard reference keys (e.g. Medioli and Scott, 1983; Kumar and Dalby, 1998). Scanning electron micrograph images of common species and strains were obtained using a Tescan Vega-II XMU VP scanning electron microscope at the Carleton University SEM facility (Fig. 3).

RESULTS

This study investigated 21 tailings-influenced lakes and wetlands and 53 natural lakes both up and

downstream of the Athabasca Oil Sands operation. The six samples in Set One from wetlands with relatively low OSPM impact (samples 8, 9, 16, 17, 20, and 21; average naphthenic acid (NA) concentration 8 mg/L, electrical conductivity 1400 $\mu\text{S}/\text{cm}$) contained a relatively abundant ($N=30/\text{cc}$) and diverse (mean $\text{SDI}=2.0$) thecamoebian fauna dominated by difflugiid species. The seven samples in Set One from the high OSPW character wetlands (samples 2, 5, 11, 13, 14, 15 and 18; average naphthenic acid concentration 47 mg/L, conductivity 2300 $\mu\text{S}/\text{cm}$) (Fig 4 & 5) yielded a generally less-diverse (mean $\text{SDI}=1.5$) and typically less-abundant fauna ($N=24.5/\text{cc}$). These sites were typically characterized by centropxyid thecamoebians, while difflugiids are rare in these samples. The eight sites resampled in June 2008 (Set Two) (Table 1) had average conductivity and naphthenic acid concentrations of 1656 $\mu\text{S}/\text{cm}$ and 24 mg/L, lower than the average values of 1850 $\mu\text{S}/\text{cm}$ and 27 mg/L, respectively, reported for the 2007 (Set One) samples. Typically centropxyid populations are ambiguous and can survive in a wide range of environments while difflugiid populations are sensitive to changes in environmental conditions, and populations will decrease as conditions deteriorate.

An inverse relationship ($r^2=0.60$) between the relative abundance of difflugiid thecamoebians and conductivity was also noted (Neville et al., 2010). In addition, conductivity shows a strong relationship ($r^2=0.71$) with the relative abundance of centropxyids (Fig. 6).

Thecamoebian assemblages from the two largest test sites, Sustainable Lake North and Sustainable Lake South, differed markedly from each other, even though the chemical characteristics of the overlying water differed only slightly. These sites were created at the same time, as a layer of fluid fine tails (FFT) was capped by a layer of about 2.5m of fresh OSPW from an active tailings basin. After filling, they were isolated from fresh OSPW recharge. Also, these ponds were hydrologically isolated from surface water recharge, with water balances controlled by precipitation/evaporation from the basin itself and the release of OSPW from the FFT zone (Fig. 5). High concentrations of NAs and high conductivity values at both sites remained fairly constant between Set One and Set Two, but Sustainable Lake South had the more diverse and difflugiid-rich assemblage both years. In addition, a much greater change in the thecamoebian assemblage was observed at Sustainable Lake North between 2007 and 2008, with an increase in

the relative abundance of difflugiid taxa from 3.1% to 23.5% of the total assemblage, and a change in SDI from 0.83 to 1.76.

Cluster analysis allows for grouping of sites that contain similar populations, while Principal Component Analysis (PCA) indicates the relationship between sites and measured environmental variables. Both cluster (Fig. 7) and PCA (Fig. 8) identified a gradual change in the thecamoebian communities from tailings influenced sites to those found at natural lakes (non OSPW/M). The cluster analysis produces four main faunal groupings. The majority of tailings sites cluster in Groups 3 & 4 with the exception of site 14 and 14-2, which cluster in Group 2 with the natural lakes. The PCA shows two main groupings; one containing the tailings influenced sites clustering on the left of the diagram, and the other containing the natural lakes clustering on the right.

DISCUSSION

Samples from the constructed wetland test facility (CWTF) at Suncor Energy Inc. demonstrated the sensitivity of thecamoebians to the by-products of mining and processing oil sands. The relative abundance of centropxyid thecamoebians showed a strong correlation with NA concentrations ($r^2=0.74$) and electrical conductivity ($r^2=0.71$) in the combined 2007 and 2008 sample sets, whereas an inverse correlation exists with difflugiid thecamoebians (Fig. 6). The resampling of eight sites in 2008 further confirmed the sensitivity of thecamoebians to by-products of oil sands mining and extraction, with the additional data slightly raising the r^2 values. The regression line created by plotting the relative abundance of difflugiid or centropxyid thecamoebians against NA concentration or conductivity (Fig. 6) may be used to extrapolate how quickly the health of an aquatic ecosystem undergoing remediation is changing. Future work will establish baselines to estimate endpoints.

The constructed wetlands showing high impacts from OSPW/M contained lower diversity thecamoebian assemblages that were dominated by centropxyid-type (Fig. 4 & 5). Less impacted sites contained more diverse assemblages dominated by difflugiid-type thecamoebians (Fig. 4 & 5). Thecamoebian assemblages responded quickly to a deliberate reduction in the rate of OSPW/M input, with an increase in species diversity and the relative abundance of difflugiid thecamoebians. At sites with little change in water

quality thecamoebian assemblages were comparable to the previous year (Fig. 4 & 5).

Test sites with low OSPW character (i.e., low concentrations of salt [conductivity] and dissolved organics [mainly naphthenic acids]) contained assemblages similar to those found in natural lakes in the Boreal Forest region of Alberta (Neville et al, 2010b). These sites cluster with Group 3 (Fig. 7) indicating that thecamoebian populations are similar to those found in natural non-OSPW/M impacted lakes surrounding the oil sands developments. Less impacted aquatic test sites from the active Leases result from low inputs of OSPW or natural bioremediation occurring over time. When results from these locations are plotted, they tended to cluster within the natural lakes (Group 3, Fig. 7), even though their water chemistry was different than that seen in natural lakes. Thecamoebian populations found in tailings influenced lakes are strongly influenced by ion content (Fig. 8), while populations in natural non-OSPW/M lakes appeared to be more influenced by temperature. In examination of the water quality properties and thecamoebian communities, it appears that the ecology of the less impacted tailings influenced lakes are developing towards and are becoming similar to the natural lakes found in northern Alberta. This encouraging evolution of a viable microbial trophic level in oil sands reclamation sites provides confidence that given sufficient time these systems should become viable aquatic habitats capable of supporting higher trophic level organisms.

In the comparison of the Sustainable Lake North (site 14) and Sustainable Lake South (site 15) sites from Suncor's constructed wetland facility (Fig. 5), an illustration of the potential power and applicability of using thecamoebian assemblages as a biomonitoring tool for assessing reclamation management options has been presented. These adjacent small lakes of similar dimension were constructed at the same time and underwent the same seasonal changes. As they evolved with time, the surface waters showed similar characteristics of OSPW. In both 2007 and 2008 studies, thecamoebian assemblages in Site 15 (15 and 15-2) were more diverse and difflugiid-rich than would have been expected for a lake with such high OSPW/M influence. Sites 14 and 15 have been managed differently since their construction in 1992, with intensive nutrient-loading practiced at Sustainable Lake South but not at Sustainable Lake North. The properties of the sediment in each site also suggest that

Sustainable Lake South showed higher organic content from detrital build-up on the underlying OSPM, which resulted from its higher productivity. Our analysis suggests that nutrient loading may speed up the remediation process by boosting productivity and, consequently, detrital deposition rates, even if the site remains highly impacted by OSPM. Cluster analysis (Fig. 7) places the thecamoebian assemblage found in Sustainable Lake North within Group 2, which is mainly composed of natural lakes surrounding the operation. This suggests that the benthic ecology of this lake is becoming similar to that found in natural Boreal lakes and, in terms of ecology, the remediation endpoint is on the trajectory toward being met.

CONCLUSIONS

Thecamoebians are sensitive proxies of environmental quality, as demonstrated at the Suncor Energy Inc. Constructed Wetlands Test Facility (CWTF) near Fort McMurray, Alberta. They provide a relatively easy and inexpensive method for assessing remediation practices and efficacy in oil sands aquatic-reclamation systems.

When thecamoebian populations in a reclaimed water body are compared to those in natural environments of the region, a goal for aquatic reclamation habitats can be defined. In OSPW/M impacted environments, the changes in numbers and composition of the thecamoebian community can be applied as an indication of ecosystem health. Over time the trajectory to natural remediation endpoints may be established.

The thecamoebian community structure at CWTF sites responded to changes in water chemistry produced by a deliberate reduction of OSPW flux. The high degree of similarity between the 2007 and 2008 sample sets where there was no marked change in water quality suggests that the use of thecamoebians as a remediation metric is both sensitive and reproducible. Chemically, the OSPW/M impacted reclamation systems are likely to be quite different from the natural lakes but the thecamoebian populations in these lakes and wetlands have still begun to establish and progress toward natural ecosystems. Currently, in some of the less OSPW/M impacted reclamation test ponds and wetlands, thecamoebian populations already resemble natural benthic Boreal communities.

ACKNOWLEDGEMENTS

We thank Suncor Energy Limited for allowing access to the Suncor wetlands and resources, and Syncrude Canada Limited for supporting the tailings study. The investigation of natural sites surrounding the oil sands operation was supported by the Coal and Oil Resources (CORES) research program, through the Geological Survey of Canada (GSC) and Natural Resources Canada (NRCan).

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Table 1: Identification of samples collected at each site from the Suncor constructed wetlands test facility (CWTF) in 2007 (Set One) and 2008 (Set Two).

Year	Dyke 4 Seepage	Dyke 4 Reservoir	Control Reservoir	V-notch Weir	Gooseneck	Weir B	Sustainable Lake North
2007	2	5	8	9	11	13	14
2008	2-2	5-2	8-2	9-2			14-2
Year	Sustainable Lake South	Sodic Wetland	Muskeg Wetland	Jan's Pond	Pond A	Crane Lake	
2007	15	16	17	18	20	21	
2008	15-2			18-2		21-2	



Figure 1: Satellite photographs of the Suncor Constructed Wetlands Test Facility (CWTF) in the Athabasca Oil Sands (Neville et al., 2011).

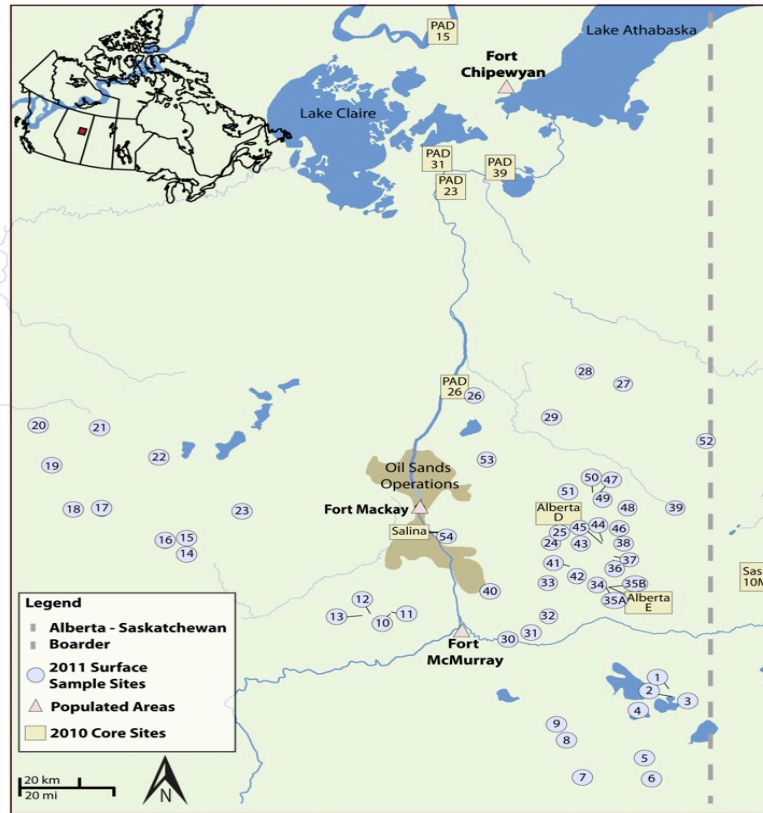


Figure 2: Map depicting the location of the natural lakes sampled surrounding the Athabasca Oil Sands operation.

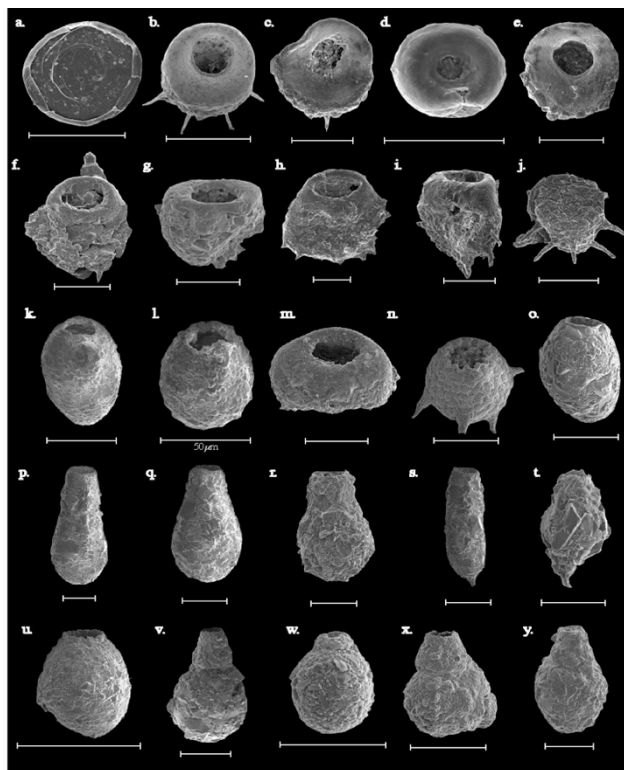


Figure 3: Examples of common thecamoebians found in Alberta.

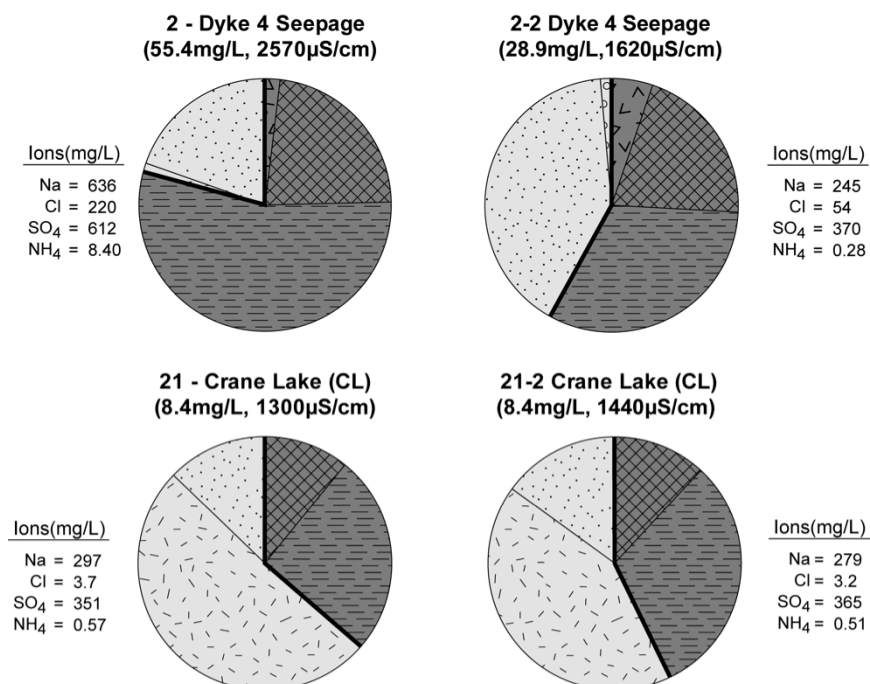


Figure 4: Pie diagrams showing the change in assemblages at sites 2 and 21 between 2007 (Set One) and 2008 (Set Two). There is virtually no change in water chemistry at site 21 and nearly identical thecamoebian assemblages are observed both years.

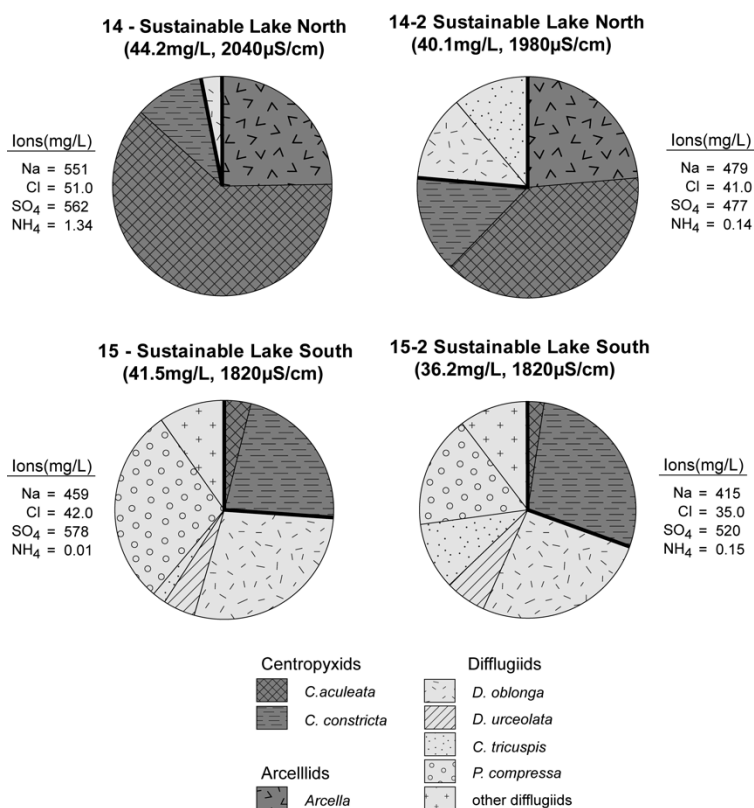


Figure 5: Diagrams showing the change in assemblages at sites 14 and 15 between 2007 (Set One) and 2008 (Set Two). Pies show assemblage differences between adjacent sites despite similar water quality.

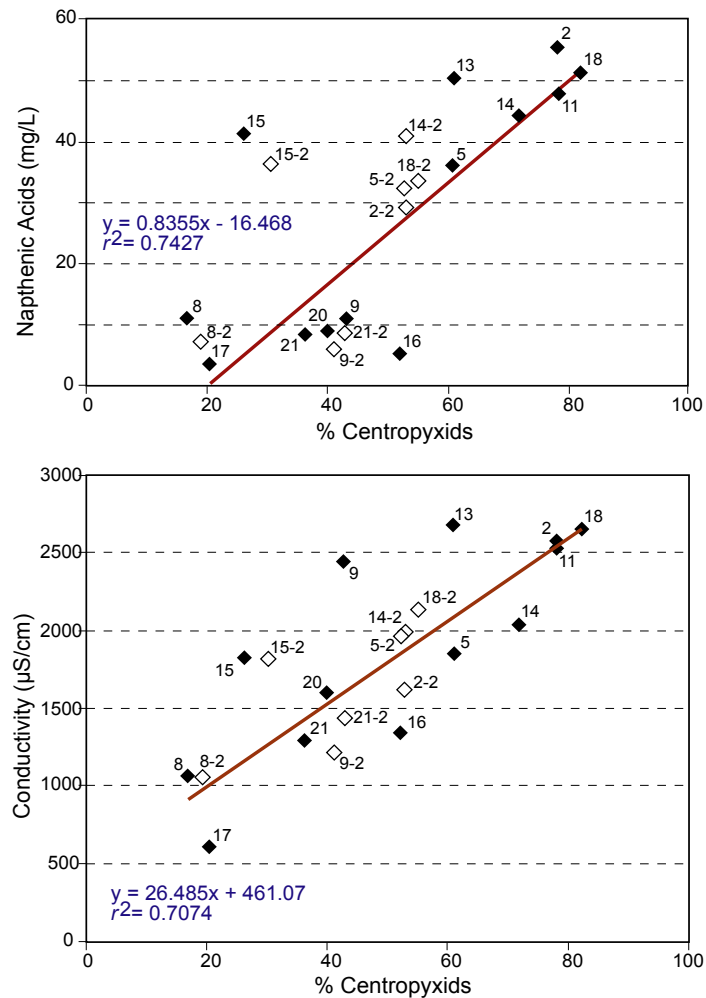


Figure 6: Graphs showing the strong correlations between the relative abundance of centropyxid thecamoebians and two of the major by-products of oil sands extraction—naphthenic acid concentrations ($r^2=0.74$) and electrical conductivity ($r^2=0.71$). The 2008 values (hollow diamonds) have shifted down the line of best fit compared to the 2007 values (solid diamonds) reflecting improvement in water quality. The enhanced water and consequent decline in the relative abundance of centropyxid thecamoebians is most apparent at sites 2 (Dyke 4 Seepage) and 18 (Jan's Pond), where they reflect the deliberately reduced inflow of oil sands process-affected water (OSPW) between 2007 and 2008.

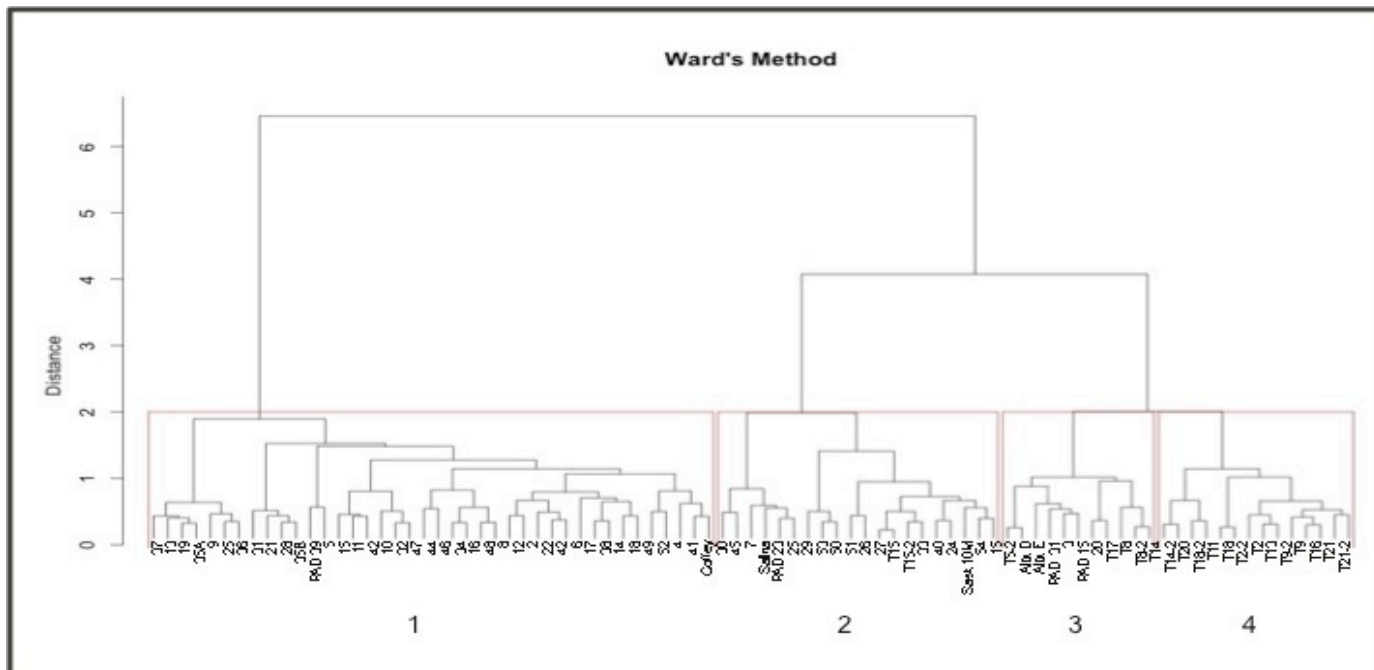


Figure 7: Cluster analysis using Wards method showing 4 main assemblage groupings. Samples collected from tailings impacted sites are prefixed with T and mainly a cluster in groups 3 & 4.

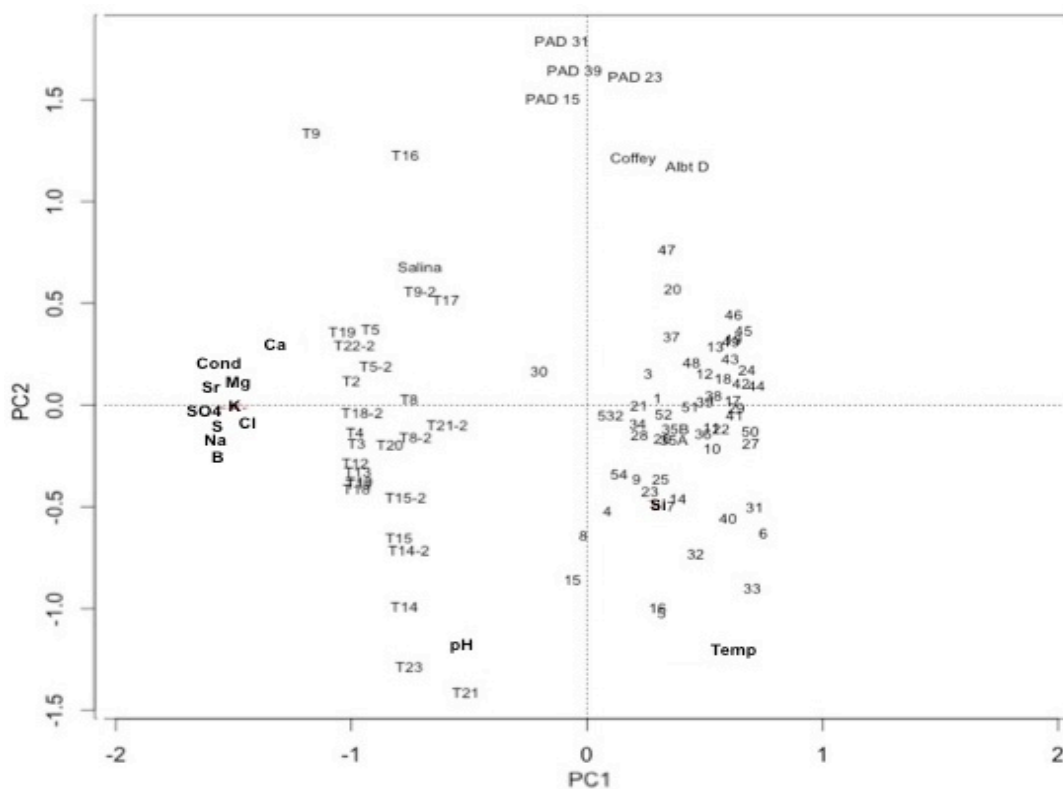


Figure 8: Ordination diagram of sites and environmental parameters created using Principal Component Analysis (PCA). Samples collected from tailings impacted sites are prefixed with T and cluster on the left half of the diagram while natural sites cluster on the right and are primarily influenced by Temperature and Si.