# MANGROVE LEAF TISSUE SODIUM AND POTASSIUM ION CONCENTRATIONS AS SUBLETHAL INDICATORS OF OIL STRESS IN MANGROVE TREES

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**ABSTRACT:** *As part of an ongoing study of the long-term effects of the* Zoe Colocotroni *oil spill of March 17, 1973, in Bahia Sucia, Puerto Rico, the effects of the remaining oil on the red mangrove trees in the impact area were investigated. This study involved four sampling trips to the spill site and a reference area outside the spill zone between April 1979 and April 1981. The present study was based on the observation that stressed mangrove trees in the heavily oil-affected areas had a similar appearance to trees exhibiting stress due to hypersalinity in unoiled areas. The working hypothesis was that petroleum hydrocarbons induce stress in salt-excluding plants such as red mangroves by disrupting the ability of the roots to exclude ions from seawater. Measurements of sodium (the principal seawater cation) and potassium (a major physiological ion) were made on leaf samples taken from trees from oil-affected areas and reference areas. Sediment core samples were taken from the root zone of the trees sampled at each site and analyzed for hydrocarbons by gravimetric and gas chromatographic methods. The results show a relationship between sediment hydrocarbon concentration and the ratio of sodium to potassium for mangrove leaves sampled at each site. The results show that for trees exposed at the root zone to the least weathered oil, the values of this ratio for the leaves had the largest values, reflecting an oil-induced impairment of the salt (Na) exclusion mechanism. This approach to measuring the physiological health of mangrove trees at an oil spill site offers a potentially useful means of documenting oil stress and recovery from oil stress in salt-excluding halophytes. Because oil stress in mangroves appears to be a root membrane-directed effect, there may be a "window in time" between initial oil impact and plant damage, as oil penetrates the sediments, during which mitigation measures could be taken.*

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The present study is a continuation and extension of studies on the effects of the *Zoe Coloctroni* oil spill on Bahia Sucia, Puerto Rico, March 1973.[3,5] The work reported here deals with the effects of petroleum on red mangrove trees, *Rhizophora mangle,* an important feature of many tropical coastal ecosystems.

Mangrove environments are a major coastal feature of most tropical areas of the world and, in analogy to temperate coastal salt marshes, serve the following important functions:[4,8]

- *Shoreline protection.* Through the entrapment of waterborne detritus in the web of prop roots, *Rhizophora* species create new coastline and consolidate existing coastline. The coastal mangrove fringe also dissipates wave energy from tropical storms.
- *Creation of habitat.* The prop root web and associated organic-rich sediments of the *Rhizophora* forest provide a low-energy habitat for a diverse community of animals and plants.
- *Primary productivity.* Annual leaf drop by mangroves is a major input into the tropical coastal detritus cycle. It has been estimated that as much as two thirds of the world fish population depends on mangrove forests as a primary source of detritus energy.[1]

*Rhizophora* species are salt-tolerant and can normally exist in soil salinities as high as 60‰.[2] This salt tolerance results from the ability of the root membranes to exclude salts by developing a negative pressure in the plant conductive tissue due to leaf transpiration, a type of reverse osmosis process.[7]

The present study program was undertaken as a result of field observations by the authors:

- In the field, mangrove trees that are clearly stressed by oil exhibit behavior similar to that of mangroves stressed by hypersalinity.
- In real spill situations, heavily oiled mangrove trees have been observed to require periods of weeks or months to exhibit stress symptoms or death. This suggests that oil penetration to the roots is required for stress to occur, and rules out such mechanisms as interference with air-exchange tissues at the surfaces of aerial roots (Gilfillan and Page, unpublished results).

All of the present work was done with the red mangrove, *Rhizophora mangle,* a species constituting the major component of the seaward coastal fringe in the areas affected by the Zoe *Colocotroni* oil spill. The working hypothesis is that oil stress in salt-excluding halophytes, such as mangroves, results from interference by hydrocarbons with the root membrane-mediated salt exclusion process. Evidence for this includes studies by Scholander, who demonstrated that red mangroves exclude ions in seawater by a kind of reverse osmosis involving the root membranes.[6] It was shown that this process was not an active transport driven effect because electron transport uncoupling agents such as 2,4 dinitrophenol do not interfere with salt exclusion by *Rhizophora.* That membrane integrity is required is demonstrated by the fact that lipid solvents such as ether and chloroform interfere with salt exclusion. Teas has reported that chloride ion exclusion in the roots of mangrove seedlings is disrupted by exposure to diesel fuel and to toluene.[9] These observations support the hypothesis that oil stress in mangroves is an artificially induced hypersalinity syndrome in which the oil-exposed trees are less able to exclude salt from their root tissues. Therefore, the relative amounts of physiological ions in the tree's tissues should provide a relationship between extent of oil exposure and physiological well-being.

In the present study, two ions were measured. Sodium ion, the principal seawater cation, would be elevated in the tissues of trees unable to exclude salt efficiently from their roots. Potassium ion, a major physiological cation, would serve as a reference. In a healthy tree, the ratio of sodium to potassium would be smaller than in a tree unable to exclude salt effectively. Because salt exclusion in *Rhizophora* is driven by a negative osmotic pressure generated by water transpiration from leaf tissues, leaf samples were taken from the field sites for Na/K analysis from individual trees. Sediment samples were taken for hydrocarbon analysis from the sediments associated with the roots of each tree sampled.

## Methods

Samples of leaves were collected in the field, wrapped in aluminum foil, placed in plastic bags, returned to the laboratory on ice, and then frozen until analyzed. Sediment samples were taken using a 7.5 by 50 cm coring device. The core at the root zone was transferred to a solvent-washed glass container and sealed with a foil-lined closure. The sediment samples were returned to the labortory on ice and frozen until analyzed.

For each tree sampled, a subsample of 5 mature leaves was taken from the frozen field sample. The leaf surfaces were rinsed with distilled water and the leaves were dried in an oven to constant weight. The dried tissues were digested in $HNO_3$ and analyzed for Na and K by atomic absorption spectrophotometry. Each value reported here represents the mean of replicate determinations made on three subsamples from each field sample. Petroleum and biogenic hydrocarbons were determined in the sediment samples by methods described elsewhere.[3,5]

## Results

The sampling site locations given in Table 1 are described in detail elsewhere.[3,5] The data in Table 1 give Na/K ratios for the leaves analyzed and the values for the sediment hydrocarbon concentrations in the sediments associated with the trees sampled.

## Discussion

The data in Table 1 show a rough correlation between hydrocarbon concentration and Na/K ratio in the leaf tissues. This relationship would be expected if impairment of the salt exclusion process in the roots resulted in the enrichment of the plant tissues by Na due to seawater ion intrusion. While many of the sediments were still heavily oiled during the sampling period 6–8 years after the spill, the material was very extensively biodegraded.[3,5] Furthermore, the oil was distributed through the reduced, poorly consolidated organic sediments as globules of varying size. This explains the variability of the overall gravimetric hydrocarbon data in Table 1 for such locations as Hermit I as a function of time.

Because the extent of weathering of the oil at a given site appeared to have a greater effect on the trees than the absolute quantity of oil, the hydrocarbon data was corrected to partially take weathering into account. This was done by taking the ratio of (a) oil and grease (total lipid extractables) to (b) total hydrocarbon concentration (aliphatics plus aromatics) : (O&G) : (total HC) in Table 1. The justification for this is that, for unoiled locations, most of the total extractable lipids are not hydrocarbons, but fats, waxes and other fat-soluble material.[5] For freshly oiled locations, a larger fraction of the total lipid soluble material will be hydrocarbons, and this fraction will decrease as the oil breaks down in the sediments by physical and biological processess. Therefore, the ratio will be large for an unoiled site and small for an oiled site. This ratio is called the "weathering ratio," WR, and correlates well with Na/K. The data in Table 1 show that the oiled Bahia Sucia sites had lower weathering ratios than the unoiled Guanica reference sites.

The Na/K data show higher values for the oiled sites than for the reference sites. In the present study, only limited measurements of the interstitial salinities of the sediment samples were made in 1980. It is clear that the Na/K values also reflect differences in the salinity regimes of the plants at each sampling site. For example, the Guanica reference site GIE was an interior site with a higher soil salinity than the seaward fringe GI80 and GII80 sites, thus accounting for a higher Na/K value, even though all three reference samples were oil-free with high WR values. The North mangrove sites, with restricted seawater circulation, had higher soil salinities (40–48 ‰) than the Hermit I site, which had free seawater circulation and a lower soil salinity (ca. 35 ‰).

The Na/K data were interpreted by means of a simple exponential dose-response relationship, with weathering ratios for hydrocarbons

**Table 1. Analytical results for mangrove leaf Na/K analyses and hydrocarbon analyses of associated sediments**

| | | Hydrocarbon contents of sediments (ppm, dry weight) | | | | Na/K ratio |
|---|---|---|---|---|---|---|
| Site | Date | Oil and grease | Aliphatic | Aromatic | Weathering ratio[1] | of leaves[2] |
| Bahia Sucia spill zone | | | | | | |
| Hermit I (oiled) | 4/79 | 64,700 | 12,000 | 13,300 | 2.6 | 8.5 |
| | 9/79 | 13,700 | 2,140 | 2,270 | 3.1 | 7.4 |
| | 10/80 | 187,200 | 72,400 | 36,400 | 1.7 | 6.0 |
| | 4/81 | 122,300 | 28,700 | 34,500 | 1.9 | 3.3 |
| North Mangrove | | | | | | |
| NM5 (oiled) | 10/80 | 144,860 | 28,200 | 14,600 | 3.4 | 4.7 |
| | 4/81 | 368,000 | 91,800 | 98,000 | 3.9 | 1.9 |
| NM4 (oiled) | 10/80 | 343,000 | 94,700 | 79,200 | 2.0 | 4.9 |
| | 4/81 | 526,000 | 135,000 | 145,000 | 1.9 | 3.2 |
| NM3 (recovering interior) | 10/80 | 33,200 | 3,840 | 4,300 | 4.1 | 3.0 |
| NM1 (recovering fringe) | 10/80 | 136,000 | 25,500 | 23,400 | 2.8 | 2.9 |
| Guanica reference site | | | | | | |
| GIE | 9/79 | 26,400 | 201 | 1,240 | 18.0 | 4.0 |
| GI80 | 10/80 | 5,430 | 254 | 172 | 12.8 | 1.8 |
| GII80 | 10/80 | 24,500 | 1,030 | 254 | 17.7 | 1.3 |

1. The Na and K concentration ratios represent average values of triplicate determinations for samples of 5 leaves each.
2. The weathering ratio, (oil and grease)/(aliphatics plus aromatics), reflects the degree to which hydrocarbons in the sediments resemble naturally occurring (biogenic) hydrocarbons (high ratios) or petroleum hydrocarbons (low ratios).

extracted from the sediment substrate as shown in Figure 1. Exponential regression of the data in Table 1 yields the following empirical equation:

$$\mathrm{Na/K} = 5.2e^{-.08\mathrm{WR}} \qquad r^2 = 0.50\ (P < .01)\ n = 12$$

For the purposes of the exponential regression, the GIE reference site data was omitted and the regression line in Figure 1 reflects this. A relationship including soil salinities at each site would probably produce a better regression. This overall result supports the observation by Gilfillan that unweathered oil exerts a greater stress on mangroves that weathered oil.[3]

These results demonstrate that the Na/K ratio of leaf tissue is a readily measured and potentially useful sublethal indicator of oil stress for *Rhizophora* and possibly for other salt excluding halophytes as well. The state of the oil in the sediments associated with the trees is very important in terms of its toxic effects; the ranking of sites within the framework of the tissue Na/K ratios generally follows the extent of oil weathering at each site. The reference stations exhibited high weathering ratio values (a large fraction of nonhydrocarbon lipids) and low Na/K values. Sites with the least weathered oil had the highest Na/K ratios.

For the Hermit I site, the Na/K ratio decreases with time, from 8.5 to 3.3 over a two year period, thus indicating progressive recovery of the oil stress and progressive weathering and lowering of toxicity of the oil in the sediments associated with the trees. The sites in the North Mangrove area (Table 1) behaved in a similar fashion as far as the Na/K ratio was concerned. The hydrocarbon distribution in the sediments in the interior North Mangrove sites (NM5 and NM4) was far less homogeneous than in the Hermit I sediments. Therefore, the hydrocarbon data for the NM5 and NM4 sites may not reflect oil stress as accurately as the Na/K data, which integrates the total hydrocarbon exposure of the entire root system of the tree. Moreover, the interior trees in North Mangrove did not receive free seawater circulation as did the Hermit I trees. This means that elevated soil salinity may contribute to the existing hydrocarbon-induced stress in these plants.

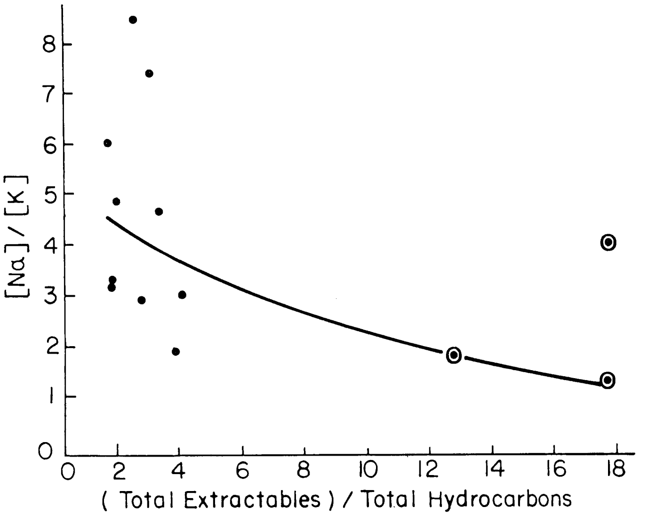

Figure 1. Plot of Na/K versus weathering ratio (see Table 1). The regression line is for the exponential regression of the data in the table, excluding the hypersaline reference site point (weathering ratio = 18, Na/K = 4.0). The points corresponding to unoiled reference sites are circled in the figure.

## Conclusions

Because the value of Na/K can be determined quite readily, this approach offers a potentially useful means of documenting oil stress and recovery from oil stress in salt-excluding halophytes. These data also suggest that, because oil stress is a root membrane-directed effect in which spilled oil must penetrate the sediments to the root zone, there is a period of time of weeks or months between initial oil impact and plant damage, during which mitigation measures could be undertaken to lessen the overall long term impact on the mangrove environment.

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