Form 2

Dissertation Abstract

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Dissertation title	**EFFECTS OF LOW OXYGEN ON SUBMERGED MACROPHYTES AND THE APPLICATION FOR MANAGEMENT (水生植物管理のための沈水植物の貧酸素化の影響の把握)**		

Abstract

※ The abstract should be in keeping with the structure of the dissertation (objective, statement of problem, investigation, conclusion) and should convey the substance of the dissertation.

Macrophytes, especially submerged macrophytes, are termed as 'ecosystem engineers' due to their roles in the oxygen production, nutrient cycling, control of the water quality, stabilization of the clear water state, restore damaged aquatic environments by concealing anti-algal allelochemicals, removing nutrients from the water and improving the water quality of aquatic areas. They frequently encounter a combination of biotic and abiotic stress factors during their life cycle. Among many other factors, soil anoxia, waterlogging, submergence and eutrophication are considered the major drivers for plant growth and species distribution in aquatic area. These factors can change the physical and chemical characteristics of the soil, which lead to higher respiration rates. In turn, these higher respiration rates result in reduced conditions or low dissolved oxygen (DO) in poorly mixed waters. Low oxygen in water can act as stressor alone for submerged macrophytes but it can be co-occur with other toxic substances, like hydrogen sulfide (H_2S). A preliminary laboratory experiment was conducted on soil to produce anoxic soil using glucose, as a reducing agent. The results confirmed that glucose can able to produce anoxic condition of soil along with the toxic substance H_2S.

H_2S plays a very important role in the biological, physical and chemical processes in aquatic ecosystems. Anoxic production of H_2S exerts strong toxicity in submerged plants and reduces growth by interfering with nutrient uptake, photosynthesis and metabolism. In addition, high H_2S can also responsible for the formation of reactive oxygen species (ROS), which can lead to protein degradation and peroxidation of the membrane lipid (lipid peroxidation, MDA). Plants that survive to transient submergence differ in the timing and duration of carbohydrate consumption and anaerobic metabolism. Under anoxic condition aerobic respiration is shift to an anaerobic fermentation which is less efficient, to provide energy for sustainable growth. Besides antioxidative enzymes, alcoholic fermentation is one of the most important metabolic adaptations which plants adopt for conserving energy to survive better under low O_2 environment. The present study hypothesized that, water column hypoxia and dissolved H_2S can affect plant species differentially and modify species composition in aquatic environments. Therefore, the general objective of the study is to explore the effect of water column oxygen and H_2S concentrations on the physiological, biochemical and anatomical variations of six submerged macrophytes. In total three laboratory studies were conducted to fulfil the general objectives of the present study.

The objective of first study was to select the threshold level of H_2S on submerged macrophytes. Four submerged macrophytes, *Egeria densa*, *Elodea nuttallii*, *Myriophyllum spicatum* and *Potamogeton crispus* were exposed to five treatments containing varying concentrations (0 to 1.0 mM, depending on species) of sodium hydrosulphide (NaHS) as the H_2S donor. NaHS can produce the desired levels of H_2S for the experiment. All the plants exposed to low concentrations of NaHS exhibited increased plant growth without showing oxidative stress. However, a decrease in growth rate, chlorophyll content, and an increase in hydrogen peroxide (H_2O_2) and malondialdehyde content (MDA) were observed after exposure to high sulfide concentrations, which indicated the presence of increased oxidative stress in the three plant species of interest. For *E. nuttallii* and *M. spicatum*, the activity of guaiacol peroxidase (POD) and ascorbate peroxidase (APX) levels decreased in the presence of 0.5 and 1.0 mM NaHS concentrations, suggesting that the antioxidative enzymes were not able to scavenge the reactive oxygen species responsible for oxidative stress, furthering plant senescence.

The second study was conducted to investigate the effect of hypoxia on four submerged macrophytes *P. aguillanus*, *P. crispus*, *E. densa* and *M. spicatum*. The four plants were hydroponically treated with 5% Hoagland solution after deoxygenated the water with anaerobic gas and placed in a 2.5 L AnaeroJar. Anaerobic indicators were used to check the low oxygen level ($<0.1\%$) inside the jar. All the plants exposed to hypoxia increased the plant growth and Indole acitic acid (IAA) content. However, the increase in H_2O_2 and antioxidative enzymes, under the exposure of hypoxic conditions, provided the indication of enhance oxidative stress in four subjected plants, which can able to scavenge the oxidative stress. The growth of adventitious roots and the formation of air spaces confirmed by electron microscopic images in four study plants were also observed, which recognized as the adaptive responses to hypoxic conditions. The results of the present study can provide the information that the four submerged macrophytes can survive under the hypoxic condition; *P. anguillanus* and *P. crispus* can outcompete the other two macrophytes in the laboratory condition under hypoxia. One important finding, chloroplast movement was observed by Transmission Electron Microscope (TEM) photographs for *P. crispus* leaves. Where, chloroplast and mitochondria changed their position from epidermis cell wall to opposite cell wall and mitochondria shifted to the epidermis area. Hence, the correct positioning or movement of chloroplasts and mitochondria are essential for cellular homeostasis of plants from external stress, suggested that low oxygen exerts stress in *P. crispus* compared to *P. anguillanus*. Other possible adaptation mechanisms under low

oxygen stress are the amino acid metabolism in plant. To observe the amino acid metabolism and metabolic changes, under hypoxia and anoxia, *P. anguillanus* was further treated with the exposure to hypoxic and anoxic conditions for 21 days. High growth rates and an increased indole acetic acid (IAA) content in *P. anguillanus* were observed under hypoxic conditions (4.0-fold to control) compared to anoxic conditions (1.5-fold to control). The activation of glycolysis and fermentation processes was further established by the enhancement of alcohol dehydrogenase activity and pyruvate content in the study plants while exposed to low oxygen concentrations. The capillary electrophoresis–mass spectrometry (CE-MS) analysis of the metabolome identified metabolite accumulations (e.g., lactate, alanine, glutamate, glutamine, aspartate, asparagine, valine, malate, citrate, isocitrate, proline and γ-amino butyric acid) in response to the anoxia. Consequently, we concluded that *P. anguillanus* could survive in low oxygen concentrations by the alterations of certain physiological and metabolic functions.

The third study was conducted to investigate the combined effect of hypoxia and H_2S on submerged macrophytes. A hydroponic experiment was performed to investigate the stress responses and biochemical adaptations of four submerged macrophytes, *Potamogeton crispus*, *Myriophyllum spicatum*, *Egeria densa*, and *Potamogeton oxyphyllus*, to the combined exposure of hypoxia and hydrogen sulfide (H_2S, provided by NaHS). The investigated plants were subjected to a control, hypoxia, 0.1 mM NaHS+hypoxia and 0.5 mM NaHS+hypoxia conditions. All experimental plants grew optimally under hypoxic and control conditions in comparison to that grown in the presence of H_2S. For *P. crispus* and *M. spicatum*, significant decreases of total chlorophyll and increases in oxidative stress (H_2O_2 and MDA) were observed with exposure to both sulfide concentrations. However, the decrease in CAT and APX from exposure to 0.5 mM NaHS suggests that the function of the protective enzymes reached their limit under these conditions. In contrast, for *E. densa* and *P. oxyphyllus*, the higher activities of the three antioxidative enzymes and their anaerobic respiration abilities (ADH activity) resulted in higher tolerance and susceptibility under high sulfide concentrations.

In plants, different sulfide tolerance mechanisms were discussed, which included mechanisms of avoiding sulfide exposure, oxidizing sulfide, or excluding sulfide from the body and metabolic adaptations (cytochrome c oxidase and ADH activity). The present study suggests that the increase in antioxidative enzymes could be another possible mechanism for aquatic plants to become sulfide tolerant in high sulfide environments. Based on the present study the sequence of hypoxia and H_2S tolerance in the submerged macrophytes are *P. oxyphyllus*> *P. anguillanus*> *E. densa* > *P. crispus*> *M. spicatum* > *E. nuttallii*.