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GROWTH AND MORPHOLOGY OF THE RHIZOME AND RHIZOID of *Pityrogramma calomelanos* (L.) LINK (PTERIDACEAE) AT VARYING COPPER SULFATE CONCENTRATIONS

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ABSTRACT

This study examined the growth and morphology of the rhizome and rhizoid of *Pityrogramma calomelanos* (L.) Link (Pteridaceae) at varying copper sulfate concentrations (0, 25, 50, 100, 200, and 400 ppm). *P. calomelanos* L. Link (Pteridaceae) was collected from an abandoned open-pit mine in Mogpog, Marinduque. Plant samples were transferred and incubated in a container with a SNAP solution for 2 days. Rhizomes and rhizoids from each individual plant were cut off and exposed to varying copper concentrations hydroponically. Growth of the rhizomes and rhizoids was measured every 15th day until the 75th day of experimentation. Gross morphology of the rhizome and rhizoid was observed, under the dissecting microscope on day 0 and day 75, of its exposure to the varying copper sulfate solution. Results showed an increase in the rhizome growth of *Pityrogramma calomelanos* (L.) Link (Pteridaceae) at copper sulfate concentrations of up to 50 ppm but inhibited at 100 ppm and higher copper sulfate concentrations. Significant differences on the rhizome and rhizoid lengths at varying copper sulfate concentrations were observed (F = 2.39, P < 0.05 and F = 3.19, P < 0.05, respectively) Rhizomes exposed to higher copper sulfate concentrations showed browned and cracked root cuticles. Damaged rhizomes and rhizoids were evident in plants exposed to 400 ppm of copper sulfate concentration.

Keywords: heavy metal, growth, morphology, fern.

1. INTRODUCTION

Mining in Marinduque Island is an important industry that provides benefits to those dependent on it. The mining industry continued to flourish in the island from 1969 to 1995 (Marges *et al*., 2011). In 1996, reports on the seepage of mine tailings and the mining accident in the island have been made. The mining accident brought about by the rupture in the drainage tunnel of the mine pit resulted to the release of huge amounts of copper mine wastes to both the surrounding land and waterways. The incident of the mining pollution in the nearby environment and waterways created an extensive impact to the lives of the individuals who were once dependent on the industry. Several years have passed since the time of the mining accident, but the situation in the island remained the same as copper concentrations continually persist in both the soil and water.

Environmental monitoring in the area has been done in the past and is continuously being done at present. The task remains to be an arduous and serious task especially that this helps in safeguarding the welfare of the community living in the area. There is a need to use measures that will help remove the heavy metals present in the environment. Concerted efforts are being undertaken to help the community rehabilitate the environment from the waste tailings and discharges that end up in the soil and water and contaminate the environment. Different technologies have been considered, but the problem lies on the exorbitant costs in using such technology. Currently, an increasing interest in rehabilitating and remediating the environment contaminated with mine wastes through the use of vegetation in a plant-based technology known as phytoremediation is being considered.

Phytoremediation is not a new topic in remediating and rehabilitating contaminated sites with mine wastes. Studies have shown that this technology is a feasible process that helps clean up the toxins from the waste sites as the plants absorb the toxins in their tissue (Mendez and Maier, 2008), prevent the leaching of toxins (Dong *et al.,* 2007), and promote the conversion of the tailing wastes to soil (Oh *et al.,* 2013). The numerous benefits that phytoremediation can provide are influenced by a number of factors that include the choice of plant that can grow in such an environment. There is a need to identify and use indigenous plants that grow in the area that can be used to perform the remediation of the contaminated site. To our knowledge, no study has explored on identifying and screening the indigenous plants in the area where the mining accident site occurred. Numerous plants are identified to be good candidates for phytoremediation, but there are a limited number of plants investigated especially among the indigenous plants thriving in the area. Hence, this study aims to identify an indigenous plant growing at the site and assess the growth and morphology of the rhizome and rhizoid of the plant at varying copper sulfate concentrations (0, 25, 50, 100, 200, and 400 ppm). Results of this study are vital as they provide baseline information on the indigenous plants' tolerance at varying copper concentrations and they will also provide important information in selecting the most useful plants for the remediation of the mine wastes contaminating this particular site.

2. METHODS

2.1. Plant collection, rhizome preparation, and labeling

A walk-through of the study site at Mogpog, Marinduque, Philippines, was done to identify the indigenous plants thriving in the area. Mogpog, Marinduque, is one of the municipalities in Marinduque Island. It is one of the areas that were affected when the mining accident occurred in Marinduque Island where one of the mining pits of the company operating in the area is situated. Plant samples within the area around the open-pit mine were collected, taxonomically identified, washed, and transferred in individual plant pots containing a SNAP solution and acclimatized for 2 days. After acclimatization, each individual plant was cut to isolate the rhizomes. All rhizoids were removed from the rhizome. A total of 90 individual rhizomes were used in the experimentation. Surface sterilization of the plant samples was conducted using a 10% (v/v) solution of a commercial bleach disinfectant. Samples were soaked in the bleach solution for 5 min.

2.2. Chemicals

Stock solutions of the heavy metals (1000 mg L^{-1}) were prepared. The metal salt used was $CuSO_4$ (Merck, Germany). The copper solutions used were prepared by diluting the metal salt to a SNAP solution for the hydroponics setup. Varying copper concentrations (25, 50, 100, 200, and 400 ppm) were prepared in a SNAP solution.

2.3. Hydroponics setup

The hydroponics setup was adopted from Kamal et al. (2004). The hydroponics setup is made of a steel framework with levels made with wood bases. Each level is affixed with timer-controlled light bulbs and air pumps. The entire setup was covered with aluminum foil to maximize the light emitted by the light bulbs to ensure that all plants receive enough light. The individual plant samples were placed in plastic tray containers, each containing a growth tray and oxygen pump. In the control setup, only the SNAP solution was placed in the container. In the treatments setup, varied copper concentrations (25, 50, 100, 200, and 400 ppm) were mixed with the SNAP solution and placed in each container. Each growth tray has 15 holes, and each hole contains one sample. There are 15 samples per treatment and control setup used in the experimentation. All samples were exposed to a 12-h dark and 12-h light cycle. All setups were continuously aerated and lighted equally in a controlled environment for 75 days.

2.4. Rhizome and rhizoid growth measurements and morphology

The rhizomes of the plants were exposed to the varying copper concentrations, and the rhizome growth was measured on the 15th, 30th, 45th, 60th, and 75th day using a Vernier caliper (mm). The rhizoids of the plant were measured on the 25th, 50th, and 75th day using a Vernier caliper (mm). Photo documentations on the morphology of the rhizomes and rhizoids of the plant exposed to varying copper concentrations were obtained using a Nikon dissecting microscope (Nikon, Japan).

2.5. Data analysis

The rhizome and rhizoid growth measurements were analyzed for significant differences across the control and treatment groups (at varying copper concentrations) using analysis of variance (ANOVA). A test with a $P < 0.05$ indicates that all statistical analyses are significant. All statistical analyses were performed using the GNU PSPP software.

3. RESULTS AND DISCUSSIONS

All the plants collected at Mogpog, Marinduque, were identified as *Pityrogramma calomelanos* (L.) Link (Pteridaceae). It is identified as silver fern and is indicated as an ideal phytoremediator as it is fast growing (Bondada *et al.,* 2004). The rhizome growth measurement of *P. calomelanos* (L.) Link (Pteridaceae) at varied copper sulfate concentrations is shown in Figure-1.

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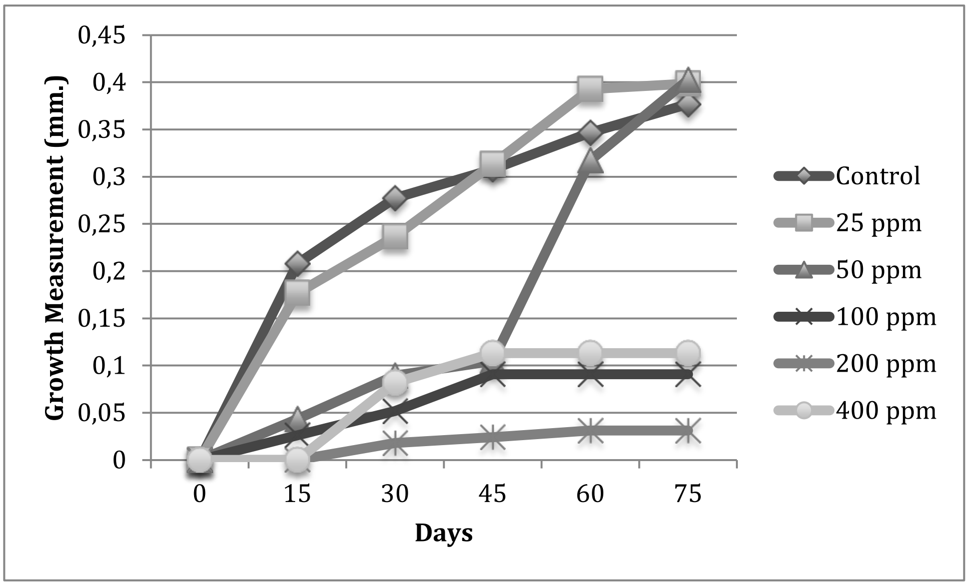

Figure-1. Rhizome growth of the *Pityrogramma calomelanos* (L.) Link (Pteridaceae) at varying copper concentrations.

The rhizomes in the control group showed an increase in the growth measurement from day 15 to day 75. In the treatment groups, the rhizomes exposed to 25–50 ppm of copper in a SNAP solution showed a marked increase in the growth measurement. This result is likely as copper is a micronutrient that plays an important role in the regulation of the different biochemical reactions in the promotion of plant growth (Gao *et al.*, 2008). Exposure to the rhizomes at 100-400 ppm showed a different result, where rhizome growth was slower and inhibited between day 45 and day 75. It is likely that the exposure of the rhizomes to higher copper concentrations may have damaged the rhizomes that include root cuticle disruption and root structure deformation (Sheldon and Menzies, 2005). A study (Gao *et al.*, 2008) presented that plants exposed to copper at high concentrations can be stressed and more free radicals are formed that can in turn damage the cell membranes of the plant cell, eventually leading to its death (Mittler, 2002). This deformation was evident among the rhizomes that were exposed to higher concentrations at day 75 of measurement.

The rhizoid growth of the *P. calomelanos* (L.) Link (Pteridaceae) rhizome exposed to the various copper concentrations was measured and is shown in Figure 2. The longest rhizoid growth was evident in the control group, whereas the shortest rhizoid growth was in the 400-ppm treatment. Results of the ANOVA show that significant differences on the rhizome and rhizoid growth were evident at varying copper concentrations ($F = 2.39$, $P < 0.05$ and $F = 3.19$, $P < 0.05$, respectively).

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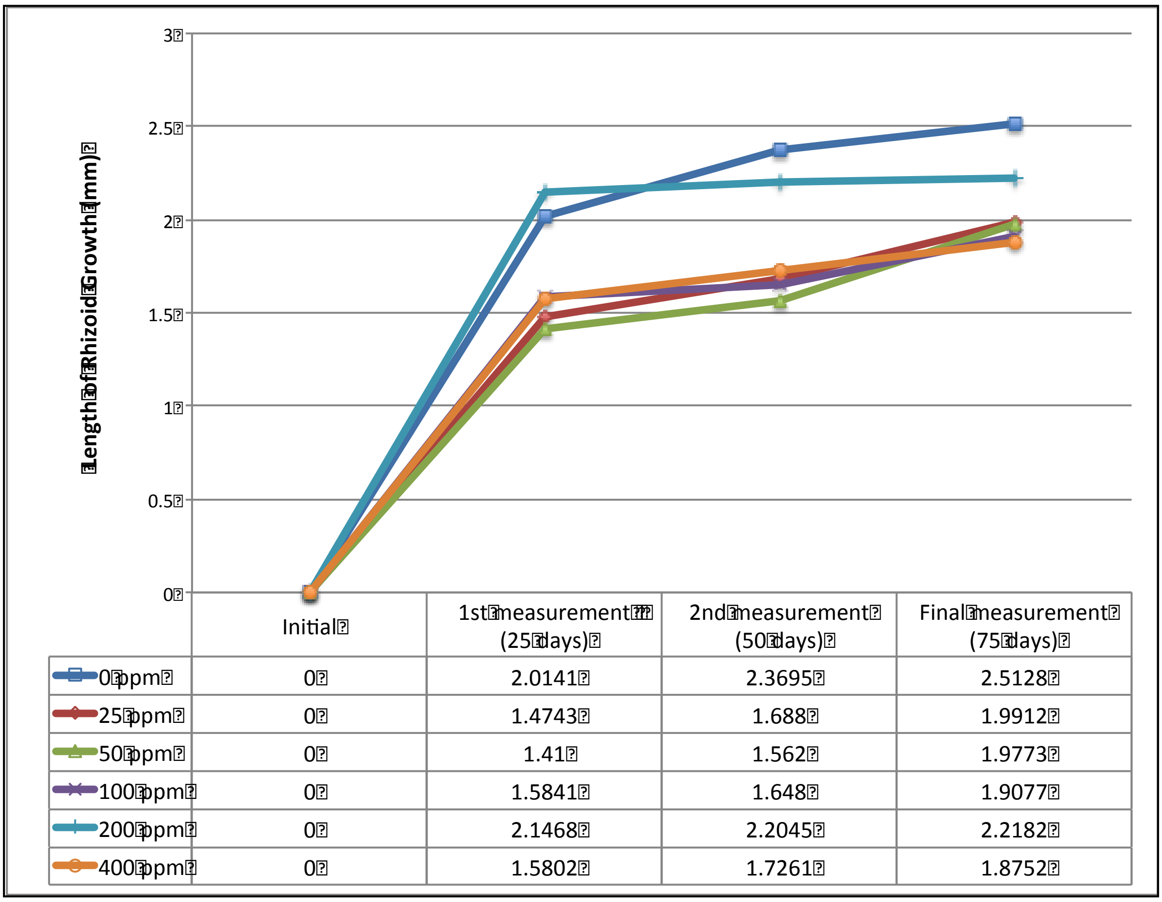

	Initial	1st measurement (25 days)	2nd measurement (50 days)	Final measurement (75 days)
0 ppm	0	2.0141	2.3695	2.5128
25 ppm	0	1.4743	1.688	1.9912
50 ppm	0	1.41	1.562	1.9773
100 ppm	0	1.5841	1.648	1.9077
200 ppm	0	2.1468	2.2045	2.2182
400 ppm	0	1.5802	1.7261	1.8752

Figure-2. Rhizoid growth of *Pityrogramma calomelanos* (L.) Link (Pteridaceae) at varying copper concentrations.

The morphology of *P. calomelanos* (L.) Link (Pteridaceae) rhizome and rhizoids at varying copper concentrations was viewed using a Nikon dissecting microscope and is shown in Figure-3. The rhizomes and rhizoids of the *P. calomelanos* (L.) Link (Pteridaceae) at varying copper concentrations showed undamaged rhizomes and rhizoids from the control group (A) up to the exposure treatments of 25–100 ppm (B–D). However, at 200-400 ppm, the *P. calomelanos* (L.) Link (Pteridaceae) showed damages in the root cuticle and root structure deformations. Cracks on the root meristem were evident in the exposure treatment of 400-ppm copper concentration. The damaged root system of *P. calomelanos* (L.) Link (Pteridaceae) may be responsible for the inhibition of the growth and development of the rhizomes (Allaoui-Sosse *et al*., 2004).

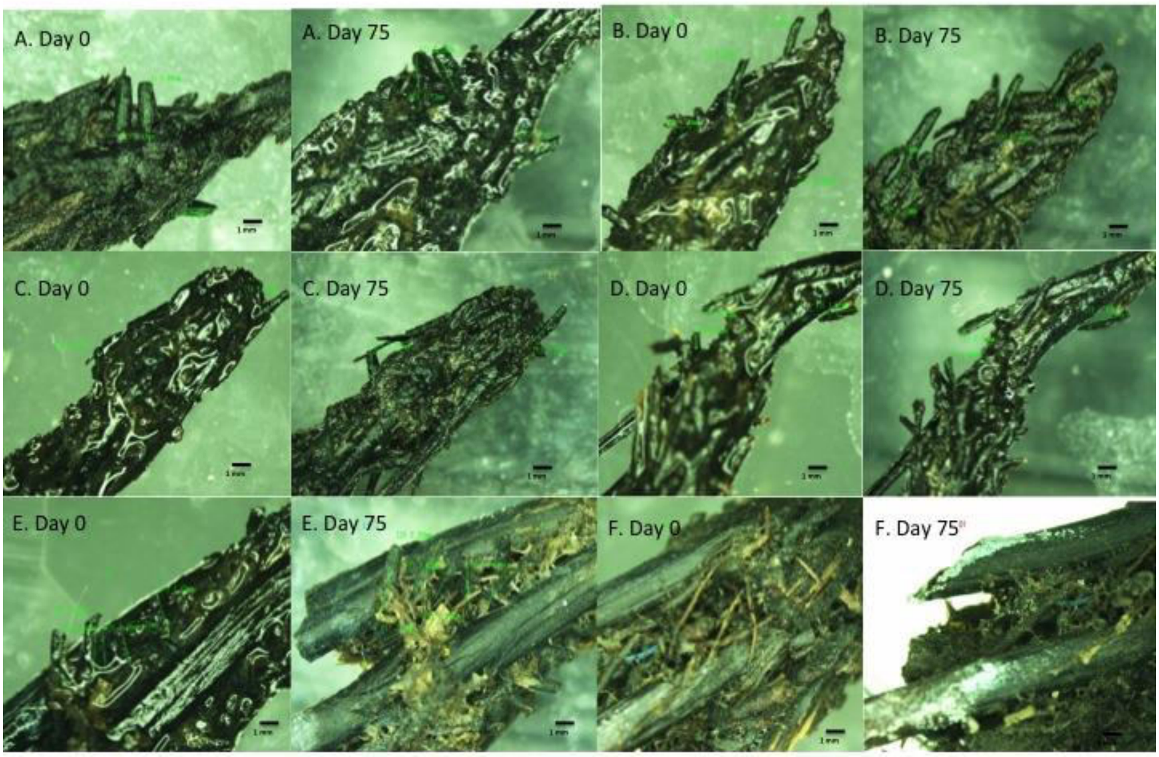

Figure-3. Rhizome and rhizoid morphology at various copper concentrations: (A) control group, 400×; (B) exposure at 25-ppm copper concentration, 400×; (C) exposure at 50-ppm copper concentration, 400×; (D) exposure at 100-ppm copper concentration, 400×; (E) exposure at 200-ppm copper concentration, 400×; (F) exposure at 400-ppm copper concentration, 400×.

4. CONCLUSIONS

Results of this study have shown that the rhizome and rhizoid growth were affected as rhizomes and rhizoids are exposed to higher copper concentrations. The inhibition of the rhizome growth at higher copper concentrations is indicated by damages on the rhizome of *P. calomelanos* (L.) Link (Pteridaceae).

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