Observation of Varying Heavy Metal Concentrations on Triticum Aestivum Germination

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Introduction

Metals are found naturally in the environment by processes such are sedimentation and weathering of rocks in the environment and are vital in regulating crop yields, soil biomass and fertility (Sethy et al., 2013). However, concern grows when there is an input of these heavy metals when it is done so anthropogenically, which are man made causes including pesticide spray on crops, urban runoffs, fossil fuels, mining, etc. The purpose of the experiment is to understand the effects of Cadmium, Nickle, Copper, and Cobalt on the germination of the wheat seed *Triticum Aestivum* in order to understand metal toxicity at increasing concentrations. The seed will be germinated for 96 hours in varying concentrations and a zero control to understand what concentration of the metals inhibit the germination of the seeds. Selection for seeds that were not considered germinated were done so using a 2 mm cut off the radical or one that is less than half the size of the seed.

Germination Bioassays are useful to understand how something like the heavy-metal containing pesticides used to pray crops with can impact the health of plants and humans. Secondly, germination inhibition bioassays can help get a grasp at the gravity of damage these metal pollutants have for the future of our ecosystems. However, the degree of toxicity is variable in the dose, age, pH of environment, route of transmission, so the types of testing done cannot be transferrable to every type of plant or animal to study (Tchounwou et al., 2011).

Materials and Methods

- Please refer to Dr. C. Duxbury, Department of Biology, Spring 2021, Biology 354 Environmental Toxicology, Experiment 2: *Effects of Various Metals on Triticum Aestivum*., pp. 1-9, for a full list of the materials and methods conducted in the lab. No deviations were made from the original experiment (Duxbury, C., 2021).

Results

Table 1: Average number of seed germination after 96 hour germination time of Cadmium, Nickle, Cobalt, and Copper

Cadmium Concentration (mM)	Wheat Average # of seeds germinated	Nickle Concentration (mM)	Wheat Average # of seeds germinated	Cobalt Concentration (mM)	Wheat Average # of seeds germinated	Copper Concentration (mM)	Wheat Average # of seeds germinated
0	15	0	15	0	15	0	15
0.5	13	0.5	14.5	0.5	14	0.5	14
1	9.5	1	13	1	14.5	1	15
1.5	5	1.5	12	1.5	13.5	1.5	12.5
2	4	2	10.5	2	11.5	2	8.5
3	2	3	8.5	3	10	3	6
4	0	4	6	4	9	4	5.5
5	0	5	3	5	10.5	5	2.5
7	0	7	1.5	7	8.5	7	2
8	0	8	0.5	8	7	8	1

The average number of seeds germinated was done by counting the number of seeds with radicals protruding longer than 2 mm from the seed. 2 trials were run of the same experiment to add validity to results so average of 2 tests were taken then taken the average of those.

Table 2: Results of Varying Cadmium Concentration as A Percent Control of Germination and Inhibition

Concentration (mM)	Average # of seeds germinated	% Germination	% Inhibition
0	15	100	0.00
0.5	13	86.67	13.33
1	9.5	63.33	36.67
1.5	5	33.33	66.67
2	4	26.67	73.33
3	2	13.33	86.67
4	0	0.00	100.00
5	0	0.00	100.00
7	0	0.00	100.00
8	0	0.00	100.00

The average number of seeds germinated sample calculations are available in *Figure 3* in the appendix.

Table 3: Results of Varying Nickle Concentration as A Percent Control of Germination and Inhibition

Concentration (mM)	Average # of seeds germinated	% Germination	% Inhibition
0	15	100	0.00
0.5	14.5	96.67	3.33
1	13	86.67	13.33
1.5	12	80.00	20.00
2	10.5	70.00	30.00
3	8.5	56.67	43.33
4	6	40.00	60.00
5	3	20.00	80.00
7	1.5	10.00	90.00
8	0.5	3.33	96.67

Table 4: Results of Varying Cobalt Concentration as A Percent Control of Germination and Inhibition

Concentration (mM)	Average # of seeds germinated	% Germination	% Inhibition
0	15	100	0.00
0.5	14	93.33	6.67
1	14.5	96.67	3.33
1.5	13.5	90.00	10.00
2	11.5	76.67	23.33
3	10	66.67	33.33
4	9	60.00	40.00
5	10.5	70.00	30.00
7	8.5	56.67	43.33
8	7	46.67	53.33

Table 5: Results of Varying Copper Concentration as A Percent Control of Germination and Inhibition

Concentration (mM)	Average # of seeds germinated	% Germination	% Inhibition
0	15	100	0.00
0.5	14	93.33	6.67
1	15	100.00	0.00
1.5	12.5	83.33	16.67
2	8.5	56.67	43.33
3	6	40.00	60.00
4	5.5	36.67	63.33
5	2.5	16.67	83.33
7	2	13.33	86.67
8	1	6.67	93.33

Table 6: Data collected from Root and Shoot length of varying concentrations for Cadmium and Nickle

	Concentration (mM)	Mean Root Length (mm)	Standard Deviation	t-value	± 95% Cl	Mean Shoot Length (mm)	Standard Deviation	± 95% Cl
Cadmium	0	130.73	19.11	2.045	7.13	104.3	17.01	6.09
	0.5	36.6	9.57	2.052	3.59	39.28	13.75	5.32
	1	33.04	10.39	2.069	3.92	39.13	17.8	7.50
	1.5	18.73	6.18	2.145	2.42	15.87	5.11	2.81
	2	10.9	3.28	2.262	1.35	11.1	2.73	1.92
	3	6	1	4.303	0.79	7	2	3.67
	4	1.67	0.58	4.303	0.46	2.33	0.58	1.07
	5	1.5	0.5	12.706	1.16	1	0	0.00
	7	NA				NA		
	8	NA				NA		
Nickle	0	137.43	22.17	2.045	8.28	66.27	9.16	3.28
	0.5	112.63	13.22	2.045	4.94	64.33	10.18	3.64
	1	67.61	13.04	2.06	4.90	56.46	7.54	3.04
	1.5	31.42	11.81	2.069	4.46	44.88	9.08	3.83
	2	23.32	11.09	2.08	4.21	33.27	14.5	6.41
	3	10.82	5.16	2.12	2.00	22.65	7.52	3.85
	4	8.71	3.41	2.16	1.34	13.14	6.88	3.94
	5	6.69	2.29	2.179	0.91	12.38	3.23	1.94
	7	NA				NA		
	8	NA				NA		

Sample calculations for Mean root length, Standard deviation, and 95% C.I all found on *Figure 3* of the appendix. Data was calculated using different seeds germinated than ones used in above tables. The 95% CI values are what would be added or subtracted around the mean, as seen in *Figure 3* in the appendix if the mean root length for 2 mM of Cadmium is 10.9, we can say that 95% of our data will be in the bounds of 8.59 and 13.21 and the difference between the mean being 2.31.

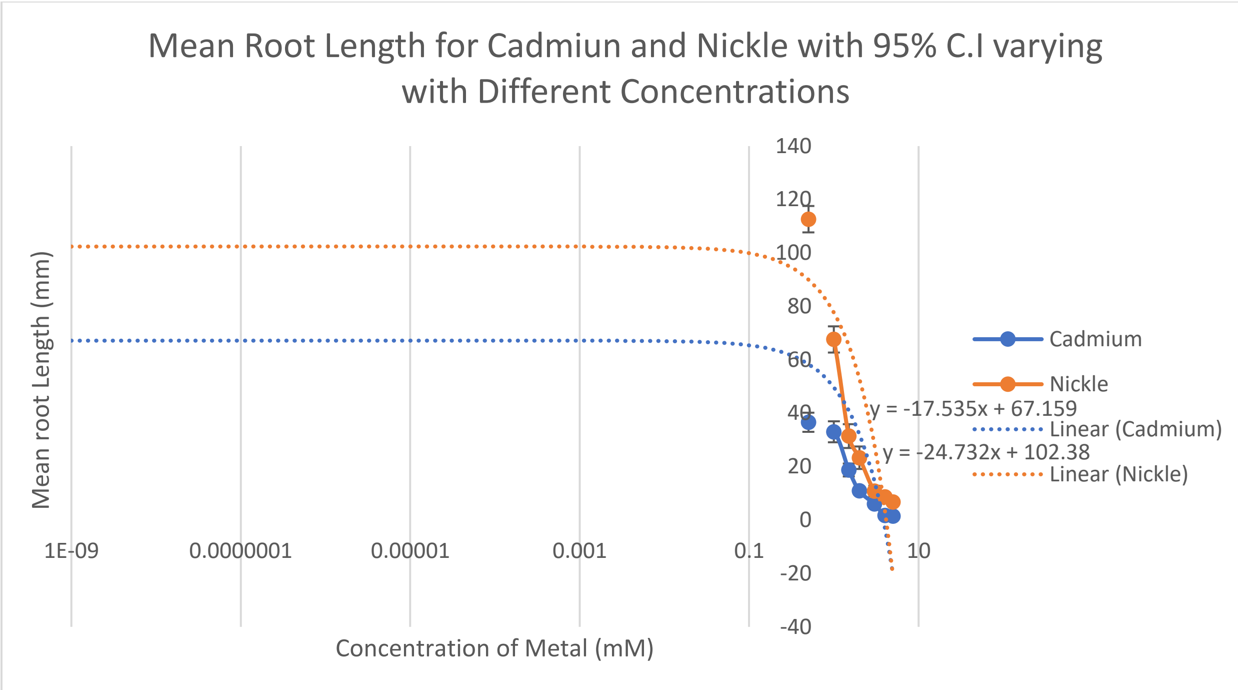

Figure 1: Mean Root Length for Cadmium and Nickle with 95% C.I varying with Different Concentrations

The control at 0.0 mM were not graphed as they cannot be displayed logarithmically on the x axis.

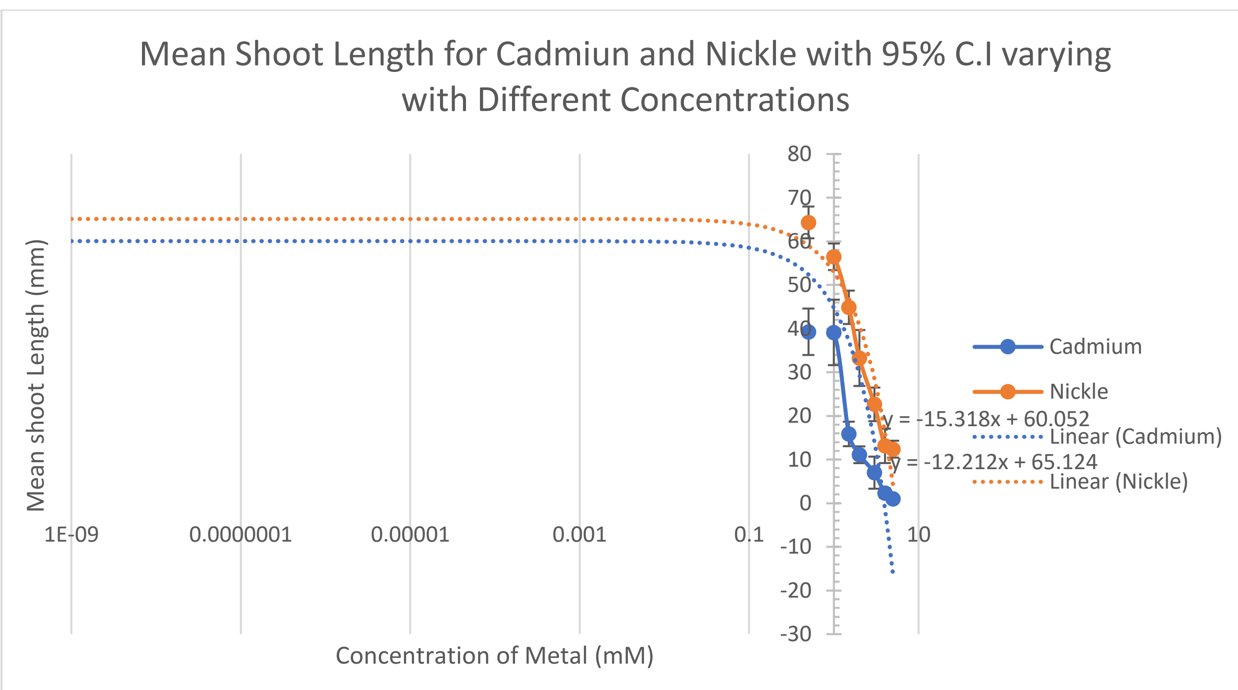

Figure 2: Mean Shoot Length for Cadmium and Nickle with 95% C.I varying with Different Concentrations

The control at 0.0 mM were not graphed as they cannot be displayed logarithmically on the x axis.

Sample Calculation as well as Probit graph for results can be found in Appendix.

Discussion

The goal of the lab was successful in observing the effects different concentration of varying metals have on the germination of seed growth, the overall trend for each metal displayed that higher concentrations of metals produce toxic effects. The metals in order of most to least toxic are Cadmium, Nickle, Copper, and Cobalt with their respective EC50 values at 96 hours were 1.4 mM, 2.33 mM, 2.5 mM, and 7 mM. Cadmium's virulent toxicity in plants works by triggering oxidative species in the environment which prevent the plant's ability to uptake important minerals and nutrients to maintain metabolic processes and photosynthetic machinery, resulting in cell death of the plant tissue (Qadir et al., 2014). This mode of action is shared amongst many metals when exposed at a high enough concentration, however, referring to *Table 2,* cadmium achieved 100% germination inhibition after only 4 mM of exposure. Nickle, Copper, and Cobalt are considered essential to plant growth and are taken up from the soil by their concentration gradients. According to the probit paper, these 3 essential metals show visibly less inhibition at smaller traces like 0.5 mM than Cadmium, being 6.66% for the maximal of the 3 and nearly double at 13.33% for Cadmium.

Triticum Aestivum is a monocot seed which germinates and produces a single leaf while dicot seeds germinate containing 2 leaf seeds which germinate slower and require a higher moister content inside the seed (Scarpella et al., 2004). A study done at the University of Constantinus by Pirselova and Colleagues examined these differences by exposing monocotyledonous maize and dicotyledonous soybeans to cadmium. They found that the soybean was more resistant to the heavy metal than the monocot seed as it has 13.9% greater production of b-1,3-glucanases, a subgroup of a pathogen response defence protein (Piršelová et al., 2011). This provides insight in the difficulties of seed bioassays as different seed types will have varying levels of tolerance to toxicants and how they uptake it from their environment. But does highlight the relevance of the direct impact humans have on the microecology that is vital for plants to sustain life through anthropogenic activities.

Some areas of variability that occurred within this lab is the process used to gather data for *Tables 2-5,* the cut off value used to screen for seeds considered successfully germinated is 2 mm. This method may yield by inability to accurately measure the exact length of the radical on the screen so eyeball estimates will not yield exact results across multiple rounds of testing. In person measurements with a measuring device would provide better means to approach this problem and provide greater validity within the lab.

Question 1. If you were conducting this assay in the field, explain in general how modifying the following factors may play a role in the toxic response of metals on seeds/plants.

- Soil conditions
    - All soils are not the same soil, there are vast differences in the microbiota and concentrations of organic and inorganic particulates, so conducting the experiment with different soil could produce positive or negative effects on growth of seeds/plants in its response to a metal toxicant. Areas near farmlands where pesticides are used, or other anthropogenic activities induces a greater number of these metal toxicants which could perpetuate the toxic response in plant giving inaccurate results (Quan, M., & Liang, J. 2017).
- pH
    - The affect pH has on the toxic response on plants/seeds relates to the ability for heavy metals to concentrate and absorb in the plant, as pH decreases, the presence of the toxic metal increases. This is a result of metal ion increasing in solubility as the presence of free H+ molecules in solution increases (Riba et al., 2004).

Question 2. At low concentrations of some of the metals, you may find that the plants showed improved performance relative to controls. Discuss possible explanations for this response.

- As seen in Table 4, Cobalt has a %inhibition at 0.5 mM of 6.66 and at 1.0 mM it had a percent inhibition of 3.33%. This indicates that a plant actually thrives in an environment with a greater amount of cobalt up to a level where it becomes toxic, compared to an environment with lesser cobalt. This is because Cobalt is an essential metal for plant growth as it is used in cobalamin, an important component of enzymatic function for the plant

(Witte et al., 2004).

Question 3. Not all the metals used in this experiment are harmful. Using a few specific examples from the metals used in this experiment discuss the role of "essential" metals. Give an example of a "non-essential" metal used and indicate how these differ from essential.

- Similarly to the above example, copper is an essential metal used in this experiment that is necessary for plant growth and development in small traces but can be fatal at higher levels. A non-essential metal would be Cadmium, at 0.5 mM it has a % inhibition of 13.33% and at 4 Mm a full 100% inhibition, Cadmium is found in significantly smaller traces in the environment than Cobalt (Piršelová, B et al., 2011).

Appendix

Table 6 calculations

Slope of a line for copper

$y_1 = .8 \quad x_1 = .1$
$y_2 = .5 \quad x_2 = .825$ } obtained from probit line of best fit

$y = mx + b$

$y = \left(\frac{y_2 - y_1}{x_2 - x_1}\right) + b$

$y = (.5 - .8 / .825 - .1) + .375$

$y = 9.9x + .375$

Standard deviation / mean of 2 mM root length in Cadmium

mean = Sum / n of (10, 12, 15, 16, 8, 6, 7, 11, 13, 11) / 10 = 10.9

$$S.D = \sqrt{\frac{\Sigma(h - \bar{h})^2}{(N-1)}} = \sqrt{\frac{96.9 - 9.69}{10 - 1}} = 3.28$$

95% C.I of Cadmium (2 mM)

$= \bar{h} \pm t * [S.D / \sqrt{N}]$

$= 10.9 \pm 2.228 * [3.28 / \sqrt{10}]$

$= 8.59$ and 13.21

Tables 2-5 calculations

% Germination of control (0.5 mM sample of Cobalt)

$= 14/15_{(control)} * 100\% = 93.33\%$

% inhibition

$100 - 93.33 = 6.67\%$

Figure 3: Sample calculations for Tables 2-5 and 6 in results

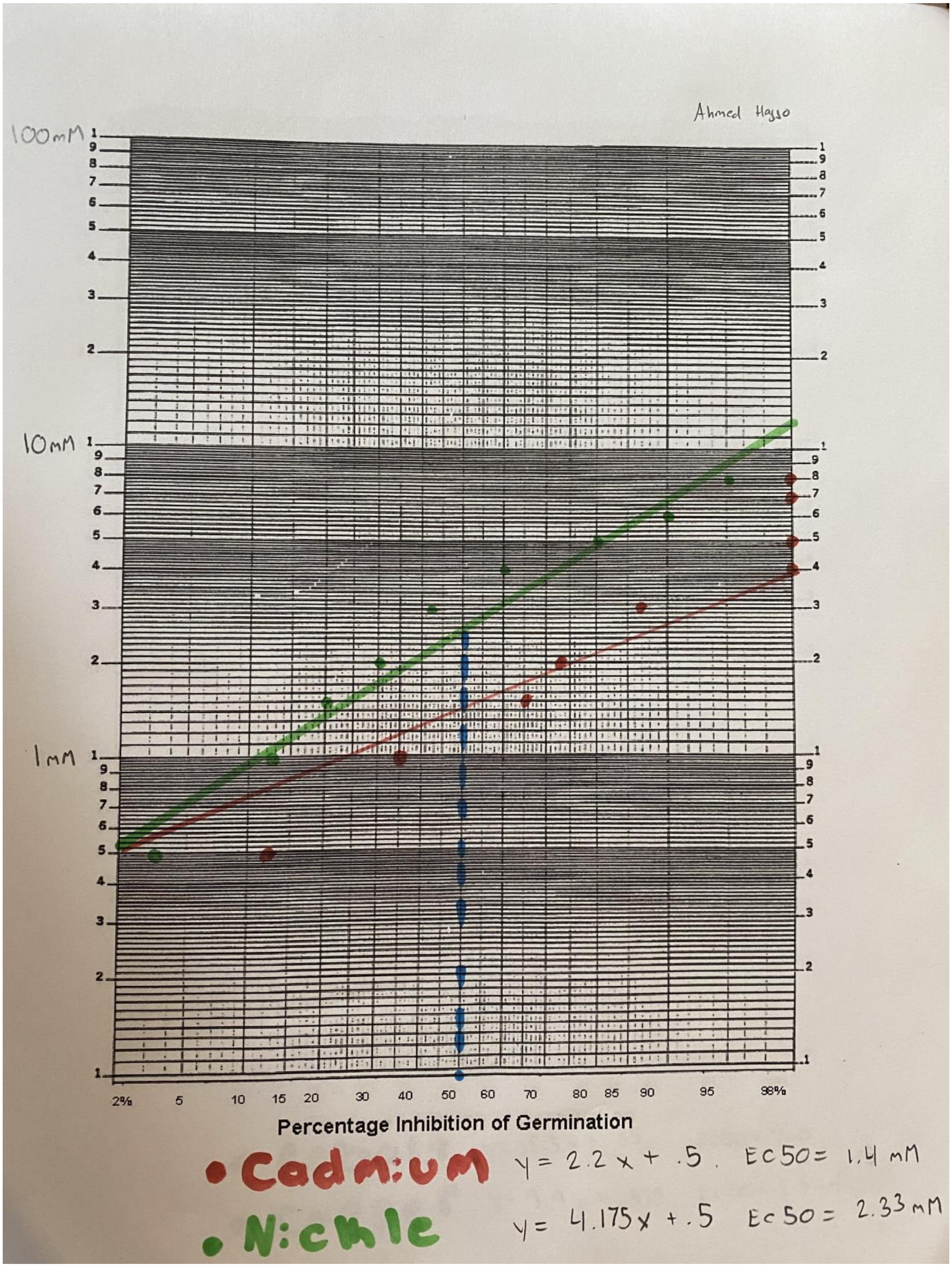

Figure 4: Probit graph of percent inhibition of germination for Cadmium and nickle

The dotted blue line indicated where the EC50 value was taken for the respective metals

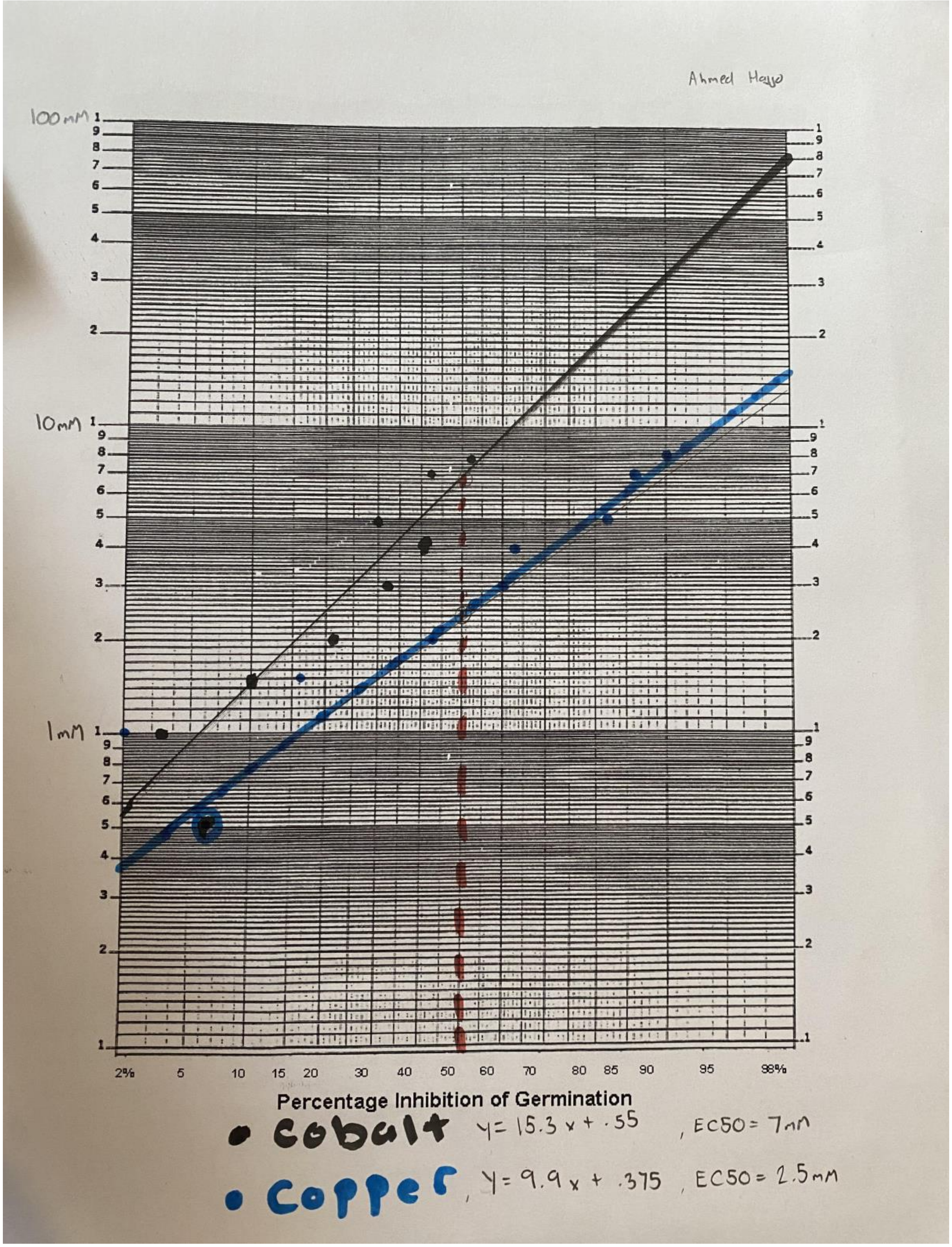

Figure 5: Probit graph of percent inhibition of germination for Cobalt and Copper

The circle around the black data point at 0.5 mM indicated that copper has the same value as cobalt. The dotted red line indicated where the EC50 value was taken for the respective metals

Work Cited

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