

# Effect of Copper Sulfate on *Armadillidium vulgare* Behavior

**Claim:** *Armadillidium vulgare* does not exhibit any significant kinesis or kinesis responses when exposed to relatively high concentrations of copper sulfate ( $\text{CuSO}_4$ ).

## Experimental Design:

Null Hypothesis: The distribution of *Armadillidium vulgare* between soil treated with a high concentration of copper sulfate ( $\text{CuSO}_4$ ) and soil treated with only distilled water does not differ significantly from the distribution observed in the control setup, where both chambers contain soil treated with only distilled water.

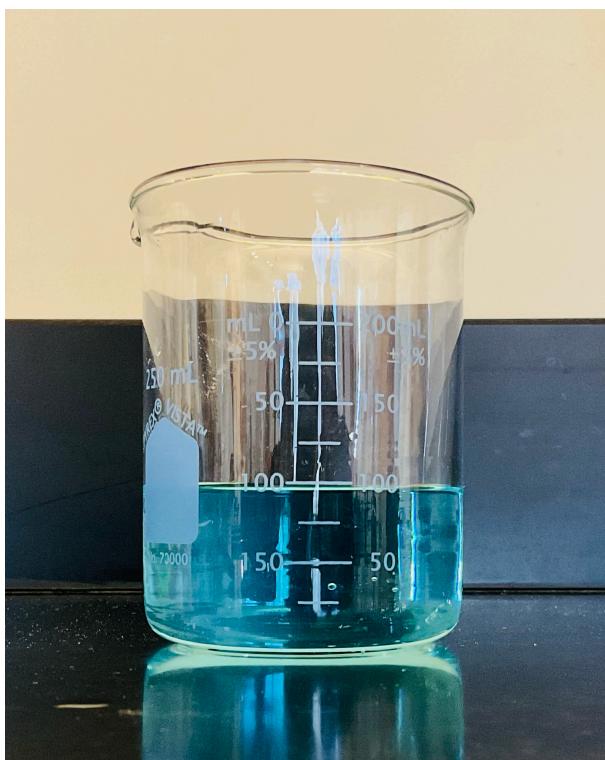
Alternative Hypothesis: The distribution of *Armadillidium vulgare* between soil treated with a high concentration of copper sulfate ( $\text{CuSO}_4$ ) and soil treated with only distilled water differs significantly from the distribution observed in the control setup, where both chambers contain soil treated with only distilled water.

## **Overview of the Experiment:**

The goal of the experiment was to determine the effect of an environment with high concentrations of heavy metals—generally toxic metallic elements with high densities and atomic mass such as lead (Pb), mercury (Hg), Arsenic (As), and Cadmium (Cd)—on kinesis or taxis response of *A. vulgare*. A type of heavy metal tested in the experiment was copper (Cu); specifically, the copper sulfate ( $\text{CuSO}_4$ ) solutions with varying concentration were applied to the soil since copper commonly exists as divalent ion ( $\text{Cu}^{2+}$ ) not only in aqueous environment and but also in soil environment. To test the effects of  $\text{Cu}^{2+}$  on taxis or kinesis responses of *A. vulgare*, two experimental groups with different concentrations of  $\text{CuSO}_4$  and one control group were tested.

## Experimental Groups:

The independent variable was the presence of  $\text{Cu}^{2+}$  in the soil. Different concentrations of copper sulfate—1.0 M and 0.10 M  $\text{CuSO}_4$ —were tested to assess whether a higher concentration would provoke a more pronounced kinesis or taxis response in *Armadillidium vulgare*; 0.10 M  $\text{CuSO}_4$  may not exhibit any statistically significant difference, but 1.0 M  $\text{CuSO}_4$  may potentially elicit stronger responses enough to show statistically significant results. Therefore, the two experimental groups were analyzed separately to evaluate the behavioral responses at each concentration independently.



**Figure 1:** 100 mL of 0.10 M  $\text{CuSO}_4$  used for the experiment

**For the first experimental group**, the Organic Materials Review Institute (OMRI) gardening soil was treated with 2.0 mL of 1.0 M  $\text{CuSO}_4$ . A volumetric pipet was used to accurately transfer and evenly distribute 2.0 mL of  $\text{CuSO}_4$  to the soil. Afterwards, the soil was gently mixed with spoons for uniform distribution of  $\text{Cu}^{2+}$ . Thus, 0.0020 mol  $\text{Cu}^{2+}$  is present in the soil:

$$\frac{1.0 \text{ mol Cu}^{2+}}{1 \text{ L}} \times \frac{1 \text{ L}}{1000 \text{ mL}} \times 2.0 \text{ mL} = 0.0020 \text{ mol Cu}^{2+}$$

Meanwhile, another batch of identical type of soil was treated with 2.0 mL of distilled water. Again, the volumetric pipet was used to accurately transfer and evenly distribute 2.0 mL of distilled water to the soil. Afterwards, the soil was gently mixed with spoons for uniform

distribution of distilled water. The purpose of preparing the soil treated with distilled water was to ensure an identical level of moisture for both chambers, which is an important variable determining the behaviors of pillbugs. Additionally, the Organic Materials Review Institute (OMRI) gardening soil was selected to ensure that the soil treated with distilled water does not contain any heavy metal ions; thus, it is possible to solely test the effects of  $\text{CuSO}_4$  by observing distribution of pill-bugs between chambers.

**For the second experimental group,** the OMRI gardening soil was treated with 2.0 mL of 0.10 M  $\text{CuSO}_4$ . An identical procedure was performed to ensure accurate measurement and uniform distribution of  $\text{Cu}^{2+}$ . Thus, 0.00020 mol  $\text{Cu}^{2+}$  is present in the soil:

$$\frac{0.10 \text{ mol Cu}^{2+}}{1 \text{ L}} \times \frac{1 \text{ L}}{1000 \text{ mL}} \times 2.0 \text{ mL} = 0.00020 \text{ mol Cu}^{2+}$$

Similarly, the soil treated with 2.0 mL distilled water was prepared using an identical procedure used for the first experimental group.



**Figure 2:** Two-choice chamber with the soil treated with 2.0 mL of 0.10 M copper sulfate on the left (chamber #1) and the soil treated with 2.0 mL of distilled water on the right (chamber #2) before placing all pillbugs in the chambers

For both experimental groups, 15 pillbugs (all collected from the garden by myself) were placed in the center of the chambers. Temperature and light intensity were identical in both chambers and kept constant throughout the experiment. The pillbugs were allowed to roam freely for 5 minutes, after which the number of pillbugs in each chamber was recorded. Two trials were conducted in total. The dependent variable was the distribution of pillbugs between the two chambers.

### **Control Group:**

Since it is impossible to determine if kinesis responses of is due to the effects of presence of  $\text{CuSO}_4$  solely based on the results from the experimental groups, the control group with both chambers filled with 2.0 mL of distilled water was also tested. If the distribution of pillbugs from the control groups is significantly different from that from the experimental groups, then it is possible to conclude that kinesis responses of is due to the effects of presence of  $\text{CuSO}_4$ .

An identical procedure was performed for the control group. Afterwards, the number of pill-bugs located in each chamber were recorded.

To minimize carryover effects, the pillbugs used in the control group were tested first to ensure that exposure to copper did not influence their behavior during control trials. Additionally, the chambers were thoroughly cleaned between tests for both experimental and control groups to eliminate any residual chemical impurities from the chamber surfaces.

### **Evidence:**

**Table 1:** Raw data of observed counts of pillbugs in each chambers for experimental and control groups

| Experimental Group #1 |                        |          |          |         |
|-----------------------|------------------------|----------|----------|---------|
|                       |                        | Trial #1 | Trial #2 | Average |
| Chamber #1            | 1.0 M $\text{CuSO}_4$  | 9        | 5        | 7       |
| Chamber #2            | Distilled Water        | 6        | 10       | 8       |
| Experimental Group #2 |                        |          |          |         |
|                       |                        | Trial #1 | Trial #2 | Average |
| Chamber #1            | 0.10 M $\text{CuSO}_4$ | 7        | 8        | 7.5     |
| Chamber #2            | Distilled Water        | 8        | 7        | 7.5     |
| Control Group         |                        |          |          |         |
| Chamber #1            | Distilled Water        | 8        | 9        | 8.5     |
| Chamber #2            | Distilled Water        | 7        | 6        | 6.5     |

**Table 2:** Average observed counts for the 1.0 M CuSO<sub>4</sub> experimental group and average expected counts (observed counts from the control group) for chi-square test

|            | Observed | Expected | $\frac{(o - e)^2}{e}$ |
|------------|----------|----------|-----------------------|
| Chamber #1 | 7        | 8.5      | 0.2647                |
| Chamber #2 | 8        | 6.5      | 0.3462                |

$$\chi^2 = 0.2647 + 0.3462 = 0.6109$$

degree of freedom = 1

significance level = 0.05

critical value = 3.841

**Table 3:** Average observed counts for the 0.10 M CuSO<sub>4</sub> experimental group and average expected counts (observed counts from the control group) for chi-square test

|            | Observed | Expected | $\frac{(o - e)^2}{e}$ |
|------------|----------|----------|-----------------------|
| Chamber #1 | 7.5      | 8.5      | 0.1176                |
| Chamber #2 | 7.5      | 6.5      | 0.1538                |

$$\chi^2 = 0.1176 + 0.1538 = 0.2714$$

degree of freedom = 1

significance level = 0.05

critical value = 3.841

## **Reasoning:**

According to the results from the chi-square analysis, there is no statistically significant difference between the distribution of *A. vulgare* between soil treated with a high concentration of copper sulfate ( $\text{CuSO}_4$ ) and soil treated with only distilled water and the distribution observed in the control setup. The chi-square values for the 1.0 M and 0.10 M treatments were 0.6109 and 0.2714, respectively, both of which are well below the critical value of 3.841 at significance level of  $\alpha=0.05$  with one degree of freedom. Thus, the null hypothesis fails to be rejected. Thus, the presence of copper sulfate in the environment does not appear to induce any significant taxis or kinesis under the tested conditions.

In general, unlike more volatile or noxious chemical stimuli, copper does not necessarily trigger immediate sensory detection that leads to instantaneous negative chemotaxis—where an organism rapidly moves away from a repellent source at varying speeds depending on the chemical concentration or the organism's sensitivity. Instead, copper typically causes intoxication through gradual cellular damage, which can reduce locomotion or result in apparent positive chemotaxis, where organisms remain longer in contaminated environments—not due to attraction, but due to impaired movement. A similar experiment was performed on *Caenorhabditis elegans*, where three nematodes were placed equidistantly from a point source of copper sulfate, and their movement was recorded over four minutes. The nematodes moved an average speed of  $0.24 \pm 0.11$  mm/s in water,  $0.15 \pm 0.05$  mm/s in 5.308 mmol/L  $\text{CuSO}_4$ ,  $0.10 \pm 0.01$  mm/s in 10.616 mmol/L  $\text{CuSO}_4$ , and  $0.06 \pm 0.00$  mm/s in 15.924 mmol/L  $\text{CuSO}_4$ , which shows that locomotion was significantly hindered by copper sulfate intoxication (Lai, 2014). Such behavior—where apparent positive chemotaxis arises from toxicity-induced immobility—has been observed in other organisms as well. However, no similarly nuanced chemotactic responses were observed in *A. vulgare* during this experiment.

The lack of chemotaxis is explained by how *A. vulgare* physiologically interacts with copper. *A. vulgare* possesses hepatopancreatic cells capable of sequestering heavy metals, including copper, potentially mitigating immediate toxic effects. The hepatopancreas comprises small (S) cells, primarily responsible for accumulating metals, and big (B) cells, containing large stores of glycogen and lipids, serving as the main energy reserve for isopods. Despite representing about 5% of the dry weight of the animal, the hepatopancreas is capable of containing more than 75% of zinc, 95% of cadmium, 80% of lead, and 85% of total copper (Panza, 2024). The ability of *A. vulgare* to regulate internal copper levels reduces the need for immediate behavioral changes in response to external copper concentrations.

Such unique physiological structure of *A. vulgare* explains why it did not display any significant taxis or kinesis responses to copper sulfate under the tested conditions.

## **Rebuttal**

The confidence level of the data is relatively low, primarily due to the limitations of the chi-square test with small sample sizes. The chi-square test assumes a sufficiently large sample size—typically with expected frequencies of at least 5 in each category and a total sample size greater than 30—to ensure reliable statistical interpretation. The limited number of pillbugs used in this experiment may be insufficient to detect subtle behavioral changes, increasing the risk of a Type II error.

Although *A. vulgare* is capable of tolerating elevated levels of copper due to the presence of hepatopancreatic cells that sequester and store heavy metals like copper (Panza, 2024), the 1.0 M copper sulfate solution used in the experiment may still have been lethal or highly toxic. This acute toxicity could suppress normal movement, thereby masking any clear taxis or kinesis responses. In other words, more significant behavioral effects may have been observable with a larger sample size, additional trials, or an extended observation period that allowed for the development of intoxication effects.

Another potential confounding variable is the soil itself. Although the same type of soil—Organic Materials Review Institute (OMRI) gardening soil—was used across all trials, the inherent heterogeneity of the soil may have introduced variability. Differences in particle size, organic content, or micro-distribution of the copper solution across samples may have influenced pillbug behavior. To control for this, future experiments might consider eliminating soil altogether and applying copper sulfate and distilled water directly to flat, inert surfaces such as filter paper or agar plates. This would help isolate the chemical stimulus as the sole independent variable and minimize inconsistencies caused by substrate composition.

Additionally, it is possible that copper sulfate may cause a form of kinesis not easily detected through simple distribution counts. For example, intoxication may reduce movement speed or alter turn frequency, which would not be captured in a binary chamber-count measurement. A more detailed video analysis tracking pillbug movement paths or velocity might reveal subtler responses to copper exposure that cannot be inferred from final positions alone.

**Works Cited:**

Lai, J., Mao, E., & Mao, L. (2013). Effect of different concentrations of copper sulphate on the speed of *Caenorhabditis elegans*. *The Expedition*, 3. <https://ojs.library.ubc.ca/index.php/expedition/article/view/184808>

Panza, G., Montanari, M., Lopez, D., Burattini, S., Ciacci, C., Fumelli, P. P., Pasini, G., Fusi, V., Giorgi, L., Grandoni, F., Papa, S., Santolini, R., & Canonico, B. (2024). Flow cytometric analysis of hepatopancreatic cells from *Armadillidium vulgare* highlights terrestrial isopods as efficient environmental bioindicators in ex vivo settings. *Environmental science and pollution research international*, 31(6), 9745–9763. <https://doi.org/10.1007/s11356-023-31375-x>