The Effects of Various Concentrations of Lead Solutions on Behavior and Regeneration in Planaria

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Introduction:

Have you ever been to an old mining town? If you have ever been to Joplin Missouri, you probably know it used to be known as the lead and zinc mining capital of the world (1). Mining and smelting of lead and zinc in southwest Missouri, southeastern Kansas and northeastern Oklahoma (known as the Tri-State District) began around 1850 and ended in the 1970’s. According to the Environmental Protection Agency (EPA), the mining operations in the Tri-State District produced 150 million tons of waste. Of this, there are still 9 million tons that remain (9). According to the EPA, Missouri is ranked number one in the nation for the most lead released to land and the 6th highest state for amount of lead released into the water (10).

The surface and underground mining in Joplin and the Tri-State area left the landscape with many open mine pits and mine shafts. Locally called chat piles, the small hills of gravel and dirt removed from the pits are called tailing piles and are often contaminated with heavy metals, including lead (1). Because of the mining activities, the ground water, surface water and soil in the area contain unhealthy amounts of lead and other heavy metals. In the Oronogo-Duenweg Mining area alone, there are more than 2,500 homes whose yards are contaminated with lead (9). The town of Picher, Oklahoma, the location of the Tar Creek Superfund site, is so contaminated that the EPA has recommended a government buyout of residents and is relocating families and closing the town.
So why is lead contamination so dangerous to our health? Lead is toxic to living organisms. Lead is a heavy metal that was once used in household plumbing, paint and as an additive to gasoline (4). People can be exposed to lead through lead dust. Dust containing lead could enter the body through breathing or by putting hands or objects that have the dust on them in their mouths. Children are often exposed to lead by eating paint chips or contaminated soil (3). The symptoms in children are often more severe than in adults. Symptoms include brain damage and mental retardation. Lead poisoning can also cause behavioral and learning problems. Other symptoms are slowed growth, hearing problems and headaches (2). Adult lead poisoning symptoms include high blood pressure, problems with digestion, memory loss, lack of concentration, muscle and joint pain (2). During pregnancy, lead can be passed to the unborn baby through the mother’s blood. This can lead to a baby that is born with brain damage or mental disabilities (3).

Planaria are free living flatworms that have the remarkable ability to regenerate any missing body part. If a planaria were cut into three pieces, each piece would form an entirely new worm. Planaria are very simple organisms that range in size from 2 mm to 5 cm long (7). Planaria show cephalization or the development of a head. The head has a very simple brain and two eyespots that can detect light or dark. The head also has two feelers called auricles that act as chemical receptors. The nervous system of the worm is a nerve net that extends to all parts of the body. If a worm is touched on its tail or other region the entire body responds to the touch (7). All living things have the capability to regenerate. Some organisms, like humans, can only regenerate tissues. For example, if a person gets a cut, that cut will eventually heal by replacing the damaged skin tissue with new skin. Other organisms, such as lizards, crayfish and planaria can replace an entire
missing body part. Within the body of the planaria certain cells called neoblasts can be found. These neoblasts make up 30% of the cells within an adult planaria. Neoblasts are embryonic stem cells that are called totipotent cells (8). Totipotent cells are cells that have the ability to become any other cell type; that is why they are said to have total potential (6). Because planaria possess these totipotent cells, they are often used in studies to evaluate the effect of various substances on stem cells. Planaria are also very sensitive to their environment and will display different body posture or behavior than when in a healthy environment. For these reasons, planaria were chosen for my experiment.

Purpose:

The purpose of this study was to determine if certain concentrations of lead affect the behavior and regeneration of planaria. To determine this, I observed their swimming behavior and body posture. I also observed the rate of regeneration and any abnormalities in their eyespots or head region.

Hypotheses:

I believe that the planaria in all concentrations of lead solutions will show eyespot abnormalities. I also believe that they will be able to detect the lead in the solutions and will show abnormal swimming behavior and body posture.

Materials and Methods:

Materials Needed:

1. Planaria (Dugesia japonica) – 40 worms
2. Petri Dishes – 12
3. Colored Tape
4. 500 mL Flasks – 3
5. Lead solutions
6. Bottled water – Nestle Pure Life
7. Stereoscope
Methods:

**Set up of Blind Study and Solution Preparation:**

I chose to do a blind study for this experiment. In this blind study, the identity of the solutions the planaria were placed in was not known to me. There were five flasks: 1. 0.15 ppm lead solution (10x EPA) 2. 0.75 ppm lead solution (50x EPA) 3. 1.5 ppm lead solution (100x EPA) 4. 15 ppm lead solution (1000x EPA) 5. bottled water. My designated supervisor made the solutions to the correct concentration and placed them in colored flasks and kept the identity of the solutions in the flask unknown to me. Each solution was made by dissolving the lead salt (lead nitrate) in bottled water. Since planaria are very sensitive to their environment, they can only live in a certain brand of bottled water, Nestle Pure Life, which was used throughout the experiment. Four concentrations of lead solution were used. The first concentration was 0.15 parts per million. This concentration is 10 times stronger than what the EPA recommends for drinking water safety. The second concentration was 0.75 parts per million or 50 times stronger than the EPA recommended value. The third was 1.5 parts per million or 100 times the EPA value and the fourth was 15 parts per million which is 1000 times the EPA value for safe drinking water (10).

**Cutting Procedures:**

1. Several Petri dishes were filled with water and frozen.
2. The surface of the ice was covered with plastic wrap.
3. Filter paper was placed on top of plastic wrap and bottled water was used to wet the surface of the filter paper. The frozen plate was used to immobilize the planaria for cutting.

4. A pipet was used to transfer a planaria worm from the stock container to the frozen Petri dish.

5. The dish was placed under the stereoscope and each worm was checked to make sure there were no abnormalities before cutting them.

6. A scalpel was used to cut the worm into three equal segments (see diagram).

7. After each worm was cut, they were checked for neatness of the cut and discarded if incorrectly cut.

8. Each segment was placed into a labeled, color-coded Petri dish. Dishes were labeled as Heads, Middles or Tails.

9. The scalpel was cleaned with alcohol every 2\textsuperscript{nd} or 3\textsuperscript{rd} worm to remove any slime build up.
**Experimental Groups:**

Ten worms per group were used. Each worm was cut into thirds, making a total of thirty fragments per experimental group. There were five groups or colors used in the experiment:

<table>
<thead>
<tr>
<th></th>
<th>Red</th>
<th>Turquoise</th>
<th>Green</th>
<th>Pink</th>
<th>Orange</th>
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<tr>
<td>Heads</td>
<td>Heads</td>
<td>Heads</td>
<td>Heads</td>
<td>Heads</td>
<td>Heads</td>
</tr>
<tr>
<td>Middles</td>
<td>Middles</td>
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<td>Middles</td>
<td>Middles</td>
<td>Middles</td>
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<tr>
<td>Tails</td>
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<td>Tails</td>
<td>Tails</td>
<td>Tails</td>
<td>Tails</td>
</tr>
</tbody>
</table>

**Observations:**

1. Literature research and photographs of regeneration were used as a guideline to let me know how regeneration should look for each day. *Dugesia japonica*, the species of planaria used in this experiment, are known to take 7 to 10 days to completely regenerate missing parts (7).

2. Observations made were: 1. behavior/body posture and movement 2. overall rate of regeneration 3. eye regeneration/abnormalities. 4. survival rate. These four categories were chosen after discussing them with my designated supervisor who had experimented with planaria previously.
**Scoring Guides:**

**Behavior/Body Posture and Movement:**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
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<tbody>
<tr>
<td>0</td>
<td>no movement</td>
</tr>
<tr>
<td>1</td>
<td>normal/gliding</td>
</tr>
<tr>
<td>2</td>
<td>contracting/head weaving</td>
</tr>
<tr>
<td>3</td>
<td>wriggling/spastic movement</td>
</tr>
<tr>
<td>4</td>
<td>dead</td>
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</tbody>
</table>

**Overall Rate of Regeneration:**

<table>
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<tbody>
<tr>
<td>0</td>
<td>behind schedule</td>
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<tr>
<td>1</td>
<td>on schedule</td>
</tr>
<tr>
<td>2</td>
<td>ahead of schedule</td>
</tr>
<tr>
<td>3</td>
<td>dead</td>
</tr>
</tbody>
</table>

**Eye Regeneration:**

<table>
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</thead>
<tbody>
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<td>none</td>
</tr>
<tr>
<td>1</td>
<td>black dots</td>
</tr>
<tr>
<td>2</td>
<td>circles beneath spots forming</td>
</tr>
<tr>
<td>3</td>
<td>crescent pigment</td>
</tr>
<tr>
<td>4</td>
<td>round pigment/ no pigment</td>
</tr>
<tr>
<td>5</td>
<td>extra eye</td>
</tr>
<tr>
<td>6</td>
<td>missing eye</td>
</tr>
<tr>
<td>7</td>
<td>other description</td>
</tr>
<tr>
<td>8</td>
<td>dead</td>
</tr>
</tbody>
</table>

3. Scoring guides were used to simplify the observations. Detailed sketches were also made daily. Sketches were made of the tail sections that were regenerating
heads. The head section is where most abnormalities in regeneration will be seen. Also, any other notations were made for anything unusual or important.

4. Worms were placed in a dark location and left undisturbed while regenerating.

5. Observations were made daily for ten days.

**Results:**

**Average Percent of All Fragments of Planaria Regenerating on Schedule When Exposed to Various Concentrations of Lead Solutions**

![Average Percent of All Fragments of Planaria Regenerating on Schedule When Exposed to Various Concentrations of Lead Solutions](image)

Figure 1

Figure one graph is showing the average number of planaria that were on schedule when exposed to certain concentrations of lead solutions. To determine if they were on schedule I looked on a diagram showing what the planaria looked like day one through seven. In the Blind Control 90.3% were regenerating on schedule. The Blind control fragments that were alive were always on schedule, but the fragments that died on Day 1 and Day 3 put the group behind schedule slightly. The 10x EPA lead group showed that 42% showed that they were on schedule. In the 50x EPA lead group showed
that only 26% had been on schedule. The 100x EPA lead group only had 21.3% planaria on schedule. The 1000x EPA lead group also only had a very low number of 5.0% that where on schedule. A statistical analysis test was run to determine if these results were significant. The ANOVA shows that there was significant differences among all of the groups except the between 50x and the 100x.

**Figure 2**

Figure two graph is showing the percent of planaria that showed abnormal behavior while regenerating. Some abnormalities observed were contracting or scrunched into a ball, head weaving, wriggling spastically, or dead. Because the different fragments showed varying results, I decided to display data for all fragments. In the Blind Control group only a small percentage, ranging from 4% to 15% showed abnormal behaviors. In the 10x group, the percentage of worms exhibiting abnormal behavior ranged from 53% to 68% for all fragments, which was much higher than the control group. The 50x group
showed an even higher percentage of abnormal behavior, ranging from 66 to 80%. The 100x EPA lead group had 100 percent of the heads, 79% of the middles, and 89% percent for tails exhibiting abnormal behavior during the 10-day observation period. The 1000x EPA group had 95% percent for heads, 99% for middles, and 100% for tails. The reason in this group there was so many planaria that had abnormal behavior is because they all died either on Day 1 or Day 2. While alive, all fragments showed contracted body posture. The ANOVA statistical test showed that there were significant differences among all groups in their behavior except between the 100x and the 1000x.

Figure 3 graph is showing regeneration abnormalities of planaria when exposed to certain concentrations of lead solutions. There were many abnormalities observed during the 10-day regeneration period. These abnormalities included fragments that regenerated a head region that was missing an eye, some fragments had one normal eye and one undeveloped eye, other
worms regenerated eyes but several days later than expected and many showed abnormal
growths or spots where the pigment was missing. The Blind Control showed only one
abnormality which was that 13.7% of the worm fragments developed eyes at a delayed rate,
however when their eyes did develop, they were normal. In the 10x it did not show abnormal
growth, but it did show all other abnormalities. In this group 8% of the worms were missing
eyes. Also 47% showed one normal eye and one undeveloped eye. And 94% of the worms had
delayed eye regeneration. The 50x planaria group also did not show abnormal growth, but, like
the 10x group, it showed all other regeneration abnormalities. In this group 55% of the planaria
showed missing eyes, 64% showed one normal eye and one developed eye. And 100% of the
worms had delayed eye regeneration. The 100x lead group showed every abnormality.
Fourteen percent of the worm fragments that developed heads had eyes that were missing when
they regenerated. Other worm fragments in the 100x group (29%) developed one normal eye
and one abnormal eye. Sixty two percent of the 100x group showed delayed eye regeneration.
By Day 7 all the fragments in the 100x group had spots that developed where the pigment was
beginning to disappear. The 1000x lead group had such a large percentage of death by Day 2
that none of the fragments had the chance to regenerate. During the two days of regeneration,
the worm fragments that were living all showed depigmentation and at the site of the cut, the
worm seemed to be “eaten away” by the solution.
Figure 4 Graph shows a comparison of the percent of worm fragments that survived during the ten days of regeneration. The Blind Control had an 89.5% survival rate compared to the 10x group that showed that 76% survived and the 50x group showed that only 57% survived. The 100x had 35.7% that survived and the 1000x with only 8.7% that survived. The chi-square analysis showed that these differences were significant.

**Conclusions**

The ANOVA statistical test showed that there were significant differences among all groups (except the 50x and 100x) in their behavior. What this means is that the lead solution and how concentrated the solution was had significant effects on their behavior. For the regeneration rate, the ANOVA showed that there was significant difference between all groups (except the 100x and 1000x). This means that the higher the concentration of lead, the longer it takes the worms to fully regenerate. The lead must have been slowing down the stem cell activity. For survival rate, the worms in the most
concentrated lead solution (1000x) died after only one or two days. This means that the lead was toxic at this concentration. In the 100x group, the head fragments all died on Day 1, but the middles and tails survived longer. What this means is that at 100 times EPA concentration, the lead is toxic or nearly toxic to the planaria. The ones that survived showed a lot of abnormalities. The ANOVA test showed that survival rate is dependent on the concentration of the lead solution because there were significant differences between the control and all lead groups. There was a definite steady decrease in the number of fragments that survived. And what is more interesting is in the 10x and 50x groups, because the concentration was not high enough to kill the worms, I was able to see more abnormalities in these two groups. Even in the group where the lead concentration was only 10 times the EPA value, eye regeneration abnormalities were very common. This could be related to the effect the lead has on the nervous system, since the eyes are part of this system.
Further Research

Since other heavy metals besides lead are also contaminating our environment. I would like to see if zinc and cadmium, two other heavy metals mined in this area, could affect the regeneration and behavior of the planaria.

It would be interesting to see if the abnormalities that developed in the planaria would be passed on to future generations. To test this, I would take the planaria that had been exposed to lead that developed abnormalities (like missing an eye). I would cut them using the same cutting procedures as before only I would place them back into clean spring water to regenerate. If the abnormality showed up in the newly regenerated worm, then I would know that it had somehow altered the cells so that they would never be normal again.

I also think it would be very interesting to see what concentrations of lead are in the local water sources to see if it could affect any other living organisms since Joplin and surrounding areas were once mining towns.

Acknowledgements

I would like to thank my designated supervisor, Shannon Sample, for all her help and wisdom.
Bibliography


