Effects of different concentrations of a heavy metal chloride on the growth of cress seedlings

Introduction
Heavy metals are everywhere, be it lead in car exhausts and industrial emissions, cadmium in paints, mercury in amalgams or zinc in batteries. These compounds invariably dissolve in rain, enter soil and are taken up by plant roots. If the metal is not present in sufficient quantities to kill the plant outright, it accumulates and is passed on to anything that eats it. Thus heavy metal accumulates down a food chain, causing the food we eat to be very contaminated.

However I am more interested in those times when the metal is present in sufficient quantities to either kill the plant or inhibit growth.

Contamination can affect plants in many ways:

It can disrupt the normal plant/water relationship.

It can indirectly affect plant metabolism, for example by disrupting nutrient availability.

It may be directly toxic to plant cells. Heavy metals are systemic killers not contact killers. That is to say that the metal is not directly toxic but indirectly disrupts normal plant functioning.

Heavy metals have a very high affinity for sulphur. Thus when they enter plants, they are immediately attracted to disulphide bridges between molecules of the amino acid cysteine (in proteins). The heavy metal ion reacts with the sulphur molecules removing them from the bond.

This changes the shape of the protein, usually denaturing it. This is the basis of heavy metal inhibition of enzymes.

The first proteins to be affected by heavy metal uptake are the chelates. These are transport proteins responsible for transport of micronutrients (e.g. iron). Heavy metals bind irreversibly with the sulphur in the chelate molecule, thus inhibiting iron transport. General discolourisation of the plant leaves then occurs due to iron deficiency. It is this deficiency that ultimately kills the plant.

Toxicity of the heavy metal ion is therefore determined by its ability to bind with the chelate protein (and thus by its affinity to sulphur). Even though some heavy metals are essential micronutrients (e.g. Zn^{2+} and Cu^{2+}), all heavy metals are toxic in very low concentrations, though each plant species has a different tolerance.

Growth is inhibited for different reasons, principally because mitosis is inhibited in the meristem (the growing shoot).

My investigation involves determining the relative toxicity of one heavy metal chloride (lead) on cress. I expect growth to be inhibited at low concentrations, with death of the plant occurring at slightly higher ones. I will measure the height of the plants after 5 days growth.

AIMS

The aim is to investigate the effect of varying concentrations of a heavy metal ion on the growth of cress seedlings. Variables are the concentrations of the heavy metal, all other variables will be controlled where possible.

HYPOTHESES
$PbCl_2$ will inhibit growth of cress at low concentrations, higher concentrations of the salt will have greater effect on cress growth.

METHOD-PILOT
Short experiments were conducted for several reasons:

To determine whether the heavy metal compound actually had a negative effect on cress growth.
To determine appropriate conditions.

To establish how long it takes for cress seedlings to have grown sufficiently to observe differences in growth.

Pilot involved planting cress on cotton wool (soaked in concentrations ranging from 0.2M to 0.002M of the heavy metal salt). This pilot proved that the heavy metal salt did have an adverse effect on growth. At 0.2M almost all seeds failed to germinate; lower concentrations would be needed. It was also noticed at this stage that at low concentrations shoot growth was relatively unaffected, but root growth was greatly decreased. However, roots became very tangled with cotton wool and were hard to remove. Because of this I decided not to measure dry mass and to grow the seeds on filter paper.

EXPERIMENTAL PROCEDURES

In order to control the variables, 40 seeds were planted in each Petri dish. All dishes received the same amount of light at constant temperature in a Dewpoint propagator. The propagator will also keep a constant humidity and prevent evaporation of the various solutions. If the solutions of lead chloride had suffered evaporation, the concentration of the solutions would alter. This would make it difficult to 'top up' the solutions. The seedlings were all measured after 5 days. Distilled water alone acted as a control.

The only variable was therefore the concentration of metal salt.
5 x distilled water (control)
5 x 0.00025M $PbCl_2$, 5 x 0.001M $PbCl_2$, 5 x 0.01M $PbCl_2$, 5 x 0.02M $PbCl_2$, 5 x 0.1M $PbCl_2$ and 5 x 0.2M $PbCl_2$

APPARATUS

Dewpoint propagator

35 Petri dishes

Lead Chloride

Filter paper circles

Acetate circles (marked with grid)

1500 cress seeds

Distilled water

Measuring cylinders, 1x500 cm^3 , 1x100 cm^3

Chinagraph pencil

Syringes – 50 ml, 20 ml , 10 ml and 5 ml

Spatula

Plastic gloves

Eyeglasses

SAFETY PRECAUTIONS

Plastic gloves and eyeglasses were worn when handling toxic lead chloride to avoid unnecessary contact. If any solid lead chloride is spilt, this must be removed into a bucket and the area well rinsed with water.

Any solutions should be clearly labelled as TOXIC.

All excess chemicals will be disposed of following the school safety policy.

METHOD

1. A 0.2M solution was prepared; the appropriate amount (see appendix for calculations) of metal chloride was added to l000 cm^3 of water. This was diluted to give further concentrations.

2. An acetate circle was prepared for each Petri dish. This was ruled with a grid of 4 x 5 squares,(draw)

3. Filter paper was then placed over the top of this and 4 cms^3 of the 0.2M solution was added. This will make the grid visible.

4. Then 2 cress seeds were placed in each square.

5. The Petri dish was labelled using a chinagraph pencil- indicating concentration and date planted.

6. Stages 2-5 were repeated for another 4 Petri dishes, each containing 0.2M PbCl2.

7. Stages 2-6 were then repeated for each of the other PbC_{l2} solutions.

8. The dishes are then placed in a Dewpoint propagator for 5 days. This propagator controls temperature, light and humidity.

9. After 5 days growth, a random number table was used to remove a maximum of 10 germinated seedlings from 5 squares in each Petri dish. This gave a random sample, while avoiding having to measure all 40 seedlings per dish. To ensure uniformity, growth was measured as the total length of the plant.

RESULTS SUMMARY.

Whilst all the seedling lengths were only measured to the nearest whole millimetre, all of the means are given to one decimal place.

Control of water

CONC. (M)	Mean Length (mm)	Size of sample
0.00000	29.7	39

PbC_{l2}

CONC. (M)	Mean Length (mm)	Size of sample
0.00025	29.0	39
0.00100	27.9	40
0.01000	10.2	39
0.02000	7.2	40
0.10000	5.6	39
0.20000	4.1	38

OBSERVATIONS

The seedlings displayed a leaf necrosis, they were all dark blue/green.

This was probably a result of inhibition of root proteins (chelates) which transport metal ions (e.g. iron). Discolourisation was therefore caused by iron deficiency in leaves.

It was also observed that at low concentrations, shoot height was relatively unaffected, but root growth was greatly inhibited. This implies that inhibition of root proteins occurs before mitosis in the meristem is inhibited.

CONCLUSION

Results from the investigation show that the lead chloride has a negative effect on cress growth (as measured by height). The general trend is of decreasing growth with increasing concentration of PbCl2. The greatest effect of lead chloride is up to 0.02M. Any further increases have less effect.

Therefore even small concentrations of lead could inhibit enzyme action or mitosis, reducing cell division and therefore growth. This is supported by the *t* tests carried out where all but the 0.00025M concentration show a significant effect (see appendix). The *t* values also support the greatest significance up to 0.02M as the *t* value increases less for higher concentrations.

The effect of the lead in such small concentrations has implications for mining waste tips which may leave the soil with low lead concentrations, but high enough to inhibit enzyme action and mitosis, therefore slowing or preventing recolonisation.

Further experiments using other plants e.g. mosses and lichens would be needed to see if these groups were also affected.

SOURCES OF ERROR

It is possible that the lead chlorides were not properly mixed. This would lead to pockets of high and low heavy metal concentration, respectively encouraging and inhibiting growth. This would lead to poor results.

Also, not all dishes may have received the same volume of solutions.

MODIFICATIONS

Lower concentrations should have been used to determine the point at which lead affects germination (down to 0.0001M).

If the cress had been allowed to grow for longer, more difference in growth might have been recorded. It might have been preferable to have used nitrates, which are more water-soluble than chlorides.

Dry mass measurements could have been taken.

(graphs to show effects of lead chloride concentration on growth(height) of cress seedlings) (Height(mm) on left, concentration on bottom(M))

APPENDIX

Make up of solutions:

PbCl2: RMM = 278

\0.2M $\Rightarrow$ 55.6g PbCl2/1000 cm^3 distilled water.

From the 0.2M solution prepared serial dilutions were carried out to give 0.1M, 0.02M, 0.01M, 0.001M and 0.00025M solutions.

Lead Chloride concentration (M)	0.2M lead chloride (cm^3)	Distilled water (cm^3)
0.20000	1000.00	0.00
0.10000	500.00	500.00
0.02000	100.00	900.00
0.01000	50.00	950.00
0.00100	5.00	995.00
0.00025	1.25	998.75
0.00000	0.00	1000.00

STATISTICAL ANALYSIS

***t* test**

The *t* test is a test of significance. It is used to tell whether two results are statistically significant.

In general when using more than 30 measurements per set of data, the value of should be compared with the critical value at ∞ degrees of freedom. This critical value is 1.96 for p = 0.05, and if the calculated value of *t* is greater than (or equal to) this critical value then the null hypothesis can be rejected.

In all the following cases, the null hypothesis is that there is no difference between the data from the two samples being compared.

t values calculated for control v various heavy metal concentrations

	Lead chloride concentration (M)					
	0.00025	0.00100	0.01000	0.02000	0.10000	0.20000
t value	1.61*	3.72	38.26	45.45	51.78	59.79

The results marked with a *, are not significant, all other results are significant.