Extended Essay Biology HL

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An assessment of the toxic effects of a landfill suspected as a source of pollution in Lomma Harbour, Sweden using the soil bioindicator species *Lepidium sativum* L. and *Lactuca sativa* L.

Date of submission 19 November 2012

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001286-034 May 2013

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Word count 4000

Abstract

The **aim of this investigation was to assess the toxicological effects of polluted soil being dispersed into unpolluted soil in a residential area in Lomma Harbour, Sweden, by using the species *Lepidium sativum* and *Lactuca sativa* as soil bioindicators.**

Soil samples were collected from the affected area in Lomma respectively an unaffected area in Löddeköpinge, which was to be used as a control when examining the soil from Lomma. The two species mentioned were used as soil bioindicators measuring germination of both species, seedling length of *L. sativum* and root length of *L. sativa* to test the soil from Lomma for toxicity affecting living organisms.

The results showed a significant difference ($p < 0.05$) between the two soils compared for germination, seedling length and root length. The results stated that *L. sativum* and *L. sativa* grown in soil from Lomma Harbour would have lower germination frequencies, 66% (± 9) compared to 89% (± 6) for *L. sativum* and 55% (± 15) compared to 84% (± 9) for *L. sativa*, grow shorter seedlings of *L. sativum*, 31 mm (± 4) compared to 35 mm (± 4) and shorter roots for *L. sativa*, 23 mm (± 6) compared to 41 mm (± 6), indicating that growth and germination of *L. sativum* and *L. sativa* is affected by the soil from Lomma compared to that from Löddeköpinge.

It can therefore be concluded that the soil in Lomma Harbour contains pollutants to such an extent that it has significant toxic biological effects. This result differs from the official examination which stated that the pollution levels were below the set threshold values and not hazardous. This investigation indicates that the soil from Lomma Harbour does have biological effects even if the pollution levels are below what by the County Administrative Board are set as accepted levels.

Word count: 300

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1 Introduction

The aim of this investigation was to **assess the toxicological effects of the soil pollution in area Lomma Harbour, Sweden and to find out if the pollution levels are significant enough to have harmful biological effects by examining the growth and germination of the bioindicator species *Lepidium sativum* and *Lactuca sativa*.**

1.1 Soil contamination in Lomma Harbour

During the work with the construction of a new residential area in Lomma in southern Sweden in 2004, called Lomma Harbour, there was a mix up of different soil masses[1,2]. Contaminated soil masses that should have been placed underneath buildings ended up mixed with clean soil and dispersed in gardens and public open areas of the new housing sites[3,4].

The mistake was not discovered until five years later when the building company's final report of the construction project was completed[5]. The case caught a lot of attention and was much debated in the media[6]. The building company was reported to the National Unit for Environmental- and Work Environment Cases by the County Administration of Scania and the case was taken to court where the company faced severe fines[7].

The municipality and Environmental Chief pressured for toxicological assessment of the soil in order to find out whether or not the contamination of the soil exceeded the threshold values set by the County Administrative Board[5,8]. Tests were considered necessary since polluted and clean soil were mixed and the degree of contamination was yet unknown.

The results of the analysis showed that the concentration of pollutants was below the threshold values set and that the soil was not hazardous[9,10]. However, the amount of contaminated soil spread out in the area was about nine hundred tons and such a quantity itself would not be completely harmless[6]. This leads on to this investigation which aims to examine whether the soil pollution has harmful biological effects.

The issue of the contaminated soil caused attention among the residents in the area who received information about the matter only after it had been brought up in media[6]. My own grandparents were among those affected and this motivated me to investigate the issue further. As the residents were discouraged from eating anything cultivated in the affected soil and to avoid inhaling dust from it, it seemed likely that the pollutants could have toxic effects[6]. Therefore this topic is highly significant to explore in order to determine eventual health risks. Since no research on humans would be possible I instead focused on investigating the effects on bioindicators to determine if the pollutant levels would be high enough to cause harmful biological effects.

1.2 Pollutants

The soil used for the new residential area in Lomma Harbour was found to be contaminated with various chemicals initially leaked from an asbestos factory active 1907-77[11]. Among the most prominent chemicals found were a range of substances referred to as polycyclic aromatic hydrocarbons, shortened PAHs[6].

PAHs are organic compounds naturally found in fossil fuels[12]. The different compounds vary in structure but are known to be both environmentally persistent and toxic[12]. They can also have carcinogenic effects in humans[14].

The contaminated soil masses were also reported to contain substantial amounts of copper[6]. Copper is well known for its toxic effects as a heavy metal, and although living organisms require certain amounts of copper for their regular activities, excessive amounts will have toxic effects[15,16].

1.3 Bioindicators

To examine the contaminated soil in this investigation, plant bioindicators were chosen to test for possible soil toxicity. Plant bioindicators are fast-growing plants, sensitive to changes in their environment[17]. By examining the state of plants grown in contact with the substances

studied and comparing those results with a control group, it can be deduced whether the substances tested have a detrimental effect or not[18].

Bioindicators were chosen as a method to examine the soil due to available resources and extent of the investigation. Cress, *Lepidium sativum* and lettuce, *Lactuca sativa* were used to test the soil from Lomma Harbour since both species have previously been successfully used as soil bioindicators[19,20].

The germination of both species in soil from the area in Lomma was investigated as well as seedling length of *L. sativum* and root length of *L. sativa*. Measuring the seedling length of *L. sativum* as a soil bioindicator has proved to be an effective method and the same goes for measuring the roots of *L. sativa* seedlings[19,20,21]. The measurements were then compared with plants grown in a control sample of uncontaminated soil collected from a field in Löddeköpinge to determine possible biological effects of the polluted soil from Lomma Harbour.

2 Research question

The research question of this essay is: **Are the pollution levels in the accidentally dispersed contaminated soil in a residential area in Lomma Harbour, significant enough to affect the growth and germination of *Lepidium sativum* and *Lactuca sativa*.** This question will be addressed by investigating the seed germination of *L. sativum* and *L. sativa* as well as the seedling length of *L. sativum* and root length of *L. sativa*

3 Hypothesis

The experimental hypothesis of this investigation is that the number of germinated seeds after five days in the soil from Lomma Harbour will be significantly less than the number in the control soil for both *Lepidium sativum* and *Lactuca sativa* because the contaminated soil will have higher levels of pollutants preventing normal germination rate of the species. Also *L. sativa* is expected to show a greater difference between the different soil samples as it has been reported to be more sensitive to its surroundings than *L. sativum*[21].

It is also expected that the *L. sativum* seedlings grown in the soil from Lomma Harbour will be significantly shorter in length after five days of growth than the ones grown in the control soil due to pollution present preventing normal growth.

The roots of the seedlings of *L. sativum* grown in the soil from Lomma Harbour are expected to be significantly shorter after five days of growth than on those in the control soil due to pollution preventing normal growth.

The null hypothesis for the previously stated experimental outcomes will be that no significant difference will exist between the samples grown in the soil from Lomma Harbour respectively in the control soil. That means no significant difference in germination between *L. sativum* in the different soils; the same for the germination of *L. sativa*; no difference in length of the seedlings of *L. sativum*; no difference in length of the roots of the *L. sativa* seedlings.

4 Investigation

4.1 Methods

4.1.1 Method for sample collection

1. Six plastic bags were labelled using masking tape and marker pen. Each label indicating soil sample and replicate number.

2. Five sites in the Lomma Harbour area were selected and another site in a field in Löddeköpinge with similar soil type, assumed to be unpolluted was selected as a control.

3. A garden spade was used to dig two decimetres down into the ground to reach the soil assumed to be polluted[6].

4. An 0.5 L measure was used to measure and collect 2.5 L of soil at the sampling sites.

5. The soil collected was put into 3 L plastic bags for transportation.

6. Five different 2.5 L samples of soil from the affected area in Lomma Harbour and one 2.5 L sample from Löddeköpinge were collected.

List of materials in *9.1.1 Materials for sample collection*.

4.1.2 Method for *Lepidium sativum*

1. Six plastic containers (5 × 9 × 11 cm) were labelled using masking tape and marker pen, each label indicating soil sample and replicate number.

2. A mixture of distilled water and 250 cm^3 of the first soil sample were prepared using beaker and spoon in order to achieve a similar soil consistency for all trials.

3. The soil mixture was placed in the plastic container indicating the corresponding sample number and evenly spread across the bottom of the container using the spoon.

4. Twenty cress seeds were spread out evenly on top of the soil using tweezers and carefully pushed down until embedded roughly five millimetres into the soil.

5. The plastic container was inserted into a plastic bag to prevent evaporation.

6. Step 2 to 5 was then repeated for each of the remaining soil samples including the control soil.

7. The containers were then placed under growth lights and left to grow at 20°C.

8. After five days the number of germinated seeds in each sample was noted.

9. The length of each germinated seedling was measured using a ruler.

10. Step 1 to 9 was repeated five times to give a total of five replicates of the experiment on each of the six samples.

List of materials in *9.1.2 Materials for Lepidium sativum experiment*.

4.1.3 Method for *Lactuca sativa*

1. Six plastic containers, identical to those used for the experiment with *L. sativum*, used for the first trials were labelled using masking tape and marker pen, each label indicating soil sample and replicate number.

2. A mixture of distilled water and 250 cm^3 of the first soil sample were prepared using a beaker and spoon in order to achieve a similar consistency for all trials.

3. One filter paper was cut to fit into the plastic container.

4. The soil mixture was put in the plastic container indicating the corresponding sample number and evenly spread across the bottom of the container using the spoon.

5. The filter paper from step 3 was put on top of the soil mixture and fitted tightly against the soil to become completely soaked by the wet soil.

6. Ten lettuce seeds were evenly spread out on top of the filter paper using tweezers.

7. The plastic container was inserted into a plastic bag to preserve the humidity.

8. Step 2 to 7 was repeated for each of the remaining soil samples including the control soil.

9. The entire setup was then placed under growth lights and left to grow at 20°C.

10. After five days the number of germinated seeds of each sample was collected.

11. The root length of each germinated seed was measured using a ruler.

12. Step 1 to 11 were repeated five times to give a total of five replicates of the experiment on each of the six samples.

List of materials in *9.1.3 Materials for Lactuca sativa experiment*.

4.2 Results

4.2.1 Seed germination of *Lepidium sativum* and *Lactuca sativa*

Tab. 1 Mean and standard deviation of number of germinated seeds as well as percentage of germinated seeds and its standard deviation of *L. sativum* and *L. sativa* in soil samples from Lomma Harbour versus control soil; results for *L. sativum* based on five soil samples with twenty seeds in each; for *L. sativa* based on five soil samples with ten seeds in each; p-values derived from unpaired t-tests comparing the results from the different soils with the level of significance set to p <0.05

		Mean number of germinated seeds	Standard deviation of number of germinated seeds	Mean number of germinated seeds/%	Standard deviation of number of germinated seeds/%	p
Lepidium sativum	Soil from Lomma Harbour	13	3	66	9	0.0016
	Control soil from Löddeköpinge	18	1	89	6	
Lactuca sativa	Soil from Lomma Harbour	6	2	55	15	0.0007
	Control soil from Löddeköpinge	8	1	84	9	

Raw data found in *9.2.1 Seed germination of Lepidium sativum* and *9.2.2 Seed germination of Lactuca sativa* and calculations shown in *9.3 Appendix C Calculations.*

The result of the germination of both *L. sativum* and *L. sativa* indicates that more seeds have germinated in the control soil than in the soil samples from Lomma Harbour. Comparing the two species can be done using the percentage germination which indicates that the amount of germinated seeds is greater for *L. sativum* than for *L. sativa*. The p-values from the unpaired t-tests for the number of germinated seeds for both *L. sativum* and *L. sativa* show that there is a significant difference between the soils tested (p <0.05).

The t-test was calculated to determine if any significant difference existed between the data from the bioindicators grown in the soil from Lomma Harbour and that from Löddeköpinge. This was a significant method of the investigation which would determine if any significant biological effect of the soil from Lomma actually exists.

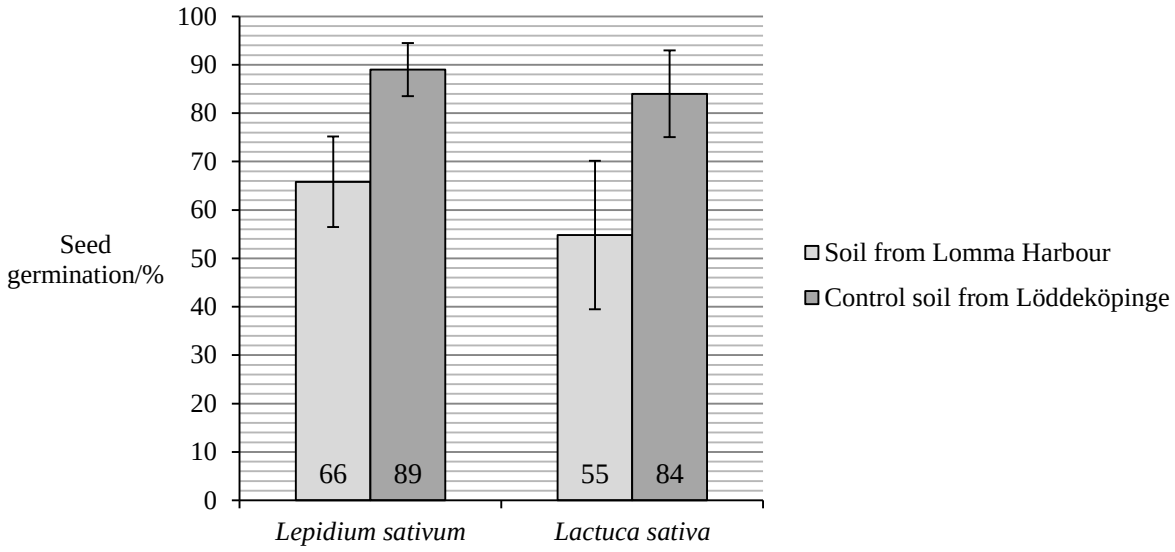

Fig. 1 The percentage of seeds germinated of *L. sativum* and *L. sativa*; results based on five different soil samples from Lomma Harbour and one sample for the control soil; five replicates performed for each sample; twenty seeds in each soil sample for *L. sativum* and ten for *L. sativa*; error bars indicate standard deviation.

The graph indicates that the difference between the control soil and the soil from Lomma Harbour is greater for *L. sativa* but also that the standard deviation for the germination of *L. sativa* is greater.

4.2.2 Seedling length of *Lepidium sativum*

Tab. 2 Mean and standard deviation of seedling length in *L. sativum* as well as mean and standard deviation of root length in *L. sativa* grown in soil from Lomma Harbour from Lomma Harbour versus control soil; results for *L. sativum* based on five soil samples with twenty seeds in each; for *L. sativa* based on five soil samples with ten seeds in each; p-values derived from unpaired t-tests comparing the results from the different soils with the level of significance set to p <0.05.

		Mean length/mm	Standard deviation /mm	p
Lepidium sativum seedling length	Soil from Lomma Harbour	31	4	0.0112
	Control soil from Löddeköpinge	35	4	
Lactuca sativa root length	Soil from Lomma Harbour	23	6	<0.0001
	Control soil from Löddeköpinge	41	6	

Raw data found in *9.2.3 Seedling length of Lepidium sativum* and *9.2.4 Root length of Lactuca sativa* and calculations shown in *9.3 Appendix C Calculations*.

The means shown in the table indicates that the seedlings of *L. sativum* grow longer in the control soil compared to the soil from Lomma Harbour. The p-value from the unpaired t-test of the seedling length of *L. sativum* shows that the difference between the two groups is significant ($p < 0.05$).

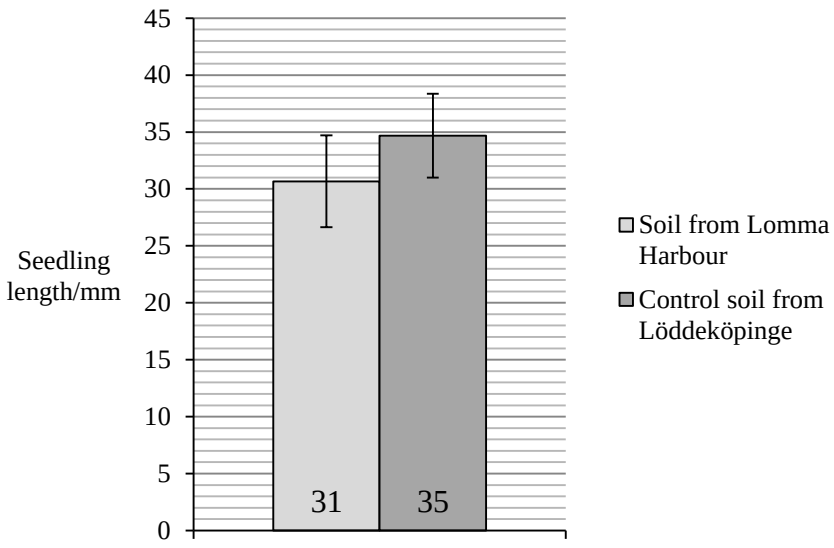

Fig. 2 Mean seedling length of *L. sativum*; results based on five soil samples from Lomma Harbour with twenty seeds in each and one control soil with twenty seeds; error bars indicate standard deviation.

The graph shows that there is a difference between the seedling lengths of *L. sativum* grown in the control soil and in the soil from Lomma Harbour where the control soil gives longer seedlings.

4.2.3 Root length of *Lactuca sativa*

The mean root length of *L. sativa* (Tab. 2) indicates that the roots of *L. sativa* grow longer in the control soil than in the soil from Lomma Harbour. The p-value shows that the difference between the two groups is significant ($p < 0.05$).

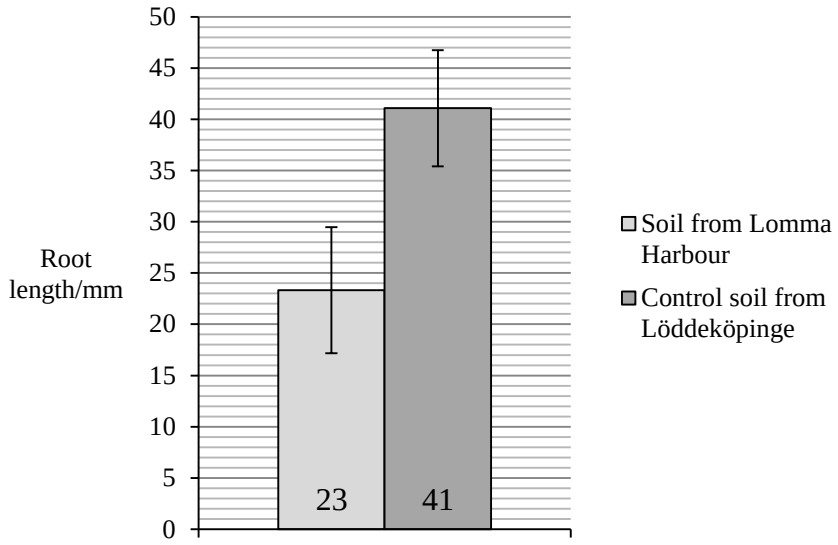

Fig. 3 Mean root length of *L. sativa*; results based on five soil samples from Lomma Harbour with ten seeds in each and one control soil with ten seeds; error bars indicating standard deviation.

The graph shows a notable difference between the root lengths in the control soil and that of the soil from Lomma Harbour where the root length is greater in the control soil. The standard deviations are similar for both sets.

4.3 Associated qualitative observations

The plants of both species varied in their appearance; the majority grew properly and had a light green colour; however, some seedlings of both species turned out dry and dead and some of the seedlings of *Lepidium sativum* had a dark green colour and slimy consistency. The abnormal seedlings were most frequent in the soil from Lomma Harbour.

5 Conclusion

The **aim of this investigation was to assess the toxicological effects of polluted soil being accidentally dispersed in a residential area in Lomma Harbour, Sweden to find out if the pollution levels are significant enough to have harmful biological effects by examining the growth and germination of the bioindicator species *Lepidium sativum* and *Lactuca sativa*.**

The results of this investigation show that the soil from Lomma Harbour contains pollutants to such an extent that seed germination and plant growth are affected (Fig. 1, Fig. 2, Fig. 3).

The mean percentage germinated seeds of *L. sativum* was 89 % (± 6) for the control soil and only 66 % (± 9) for the samples from Lomma Harbour. For *L. sativa* the percentage of germinated seeds in the control soil was 84 % (± 9) while the germination of the seeds grown in the soil from Lomma Harbour was only 55 % (± 15). The p-values received from the t-tests, 0.0016 for *L. sativum* and 0.0007 for *L. sativa*, comparing the percentage germination between control soil and soil from Lomma Harbour were lower than the set level of significance of 0.05 for both species indicating that there is a significant difference between the soils. The comparison of germination (Fig. 1) shows that *L. sativa* generally has a lower germination frequency based on the control soil samples of both species.

Conclusively it can be said that the germination of *L. sativum* and *L. sativa* is affected by soil from Lomma Harbour in such way that the amount of germinated seeds decreases when grown in it compared to unpolluted soil. According to the results of the t-tests performed, the experimental hypothesis is to be accepted for the seed germination.

The mean seedling length of *L. sativum* shows a difference in length between seeds grown in soil from Lomma Harbour and in the control soil. The mean length of seedlings grown in the control soil was 35 mm (± 4) while the mean length of those grown in the soil from Lomma Harbour was 31 mm (± 4). However, the difference is not great. Nevertheless there is a difference showing that seeds of *L. sativum* grown in soil from Lomma Harbour give rise to shorter seedlings than those grown in the control soil.

The result of the t-test where $p = 0.0112$ shows that a significant difference exists ($p < 0.05$) despite the contradicting appearance of the graph (Fig. 2). Based on the results of the t-test the experimental hypothesis is to be accepted and it can be said that the effect of the soil from Lomma Harbour on seedling length of *L. sativum* is that they grow shorter compared to those of seeds grown in the control soil.

The mean root length of *L. sativa* show a significant difference ($p < 0.05$) between the seedlings grown in soil from Lomma Harbour and the control soil since $p < 0.0001$. The graph (Fig. 3) shows the mean root length of *L. sativa* shorter in the soil from Lomma harbour than in the control soil since the mean length of the roots in the control soil is 41 mm (± 6) and 23 mm (± 6) in the soil from Lomma Harbour. The results clearly indicate that seeds grown in the polluted soil give shorter roots than those grown in unaffected soil. This result suggests that the experimental hypothesis is to be accepted and the conclusion can be said to be that the effect of the soil from Lomma Harbour on root length of *L. sativa* is that they grow shorter than when grown in unpolluted soil.

The research question can after the analysis of the results be answered: The growth of *L. sativum* and *L. sativa* show a significant difference when grown in the soil from Lomma Harbour compared to the growth of the same species in uncontaminated soil. The difference consists of less germinated seeds, shorter seedlings and shorter roots of those grown in the contaminated soil. Generally, the soil from the new construction area in Lomma Harbour affects the species *L. sativum* and *L. sativa* in a negative way by inhibiting the growth in all factors investigated suggesting that the pollution levels in Lomma Harbour are significant enough to have biological effects.

The results of this investigation does not correspond to the one executed by officials giving rise to further questions on the validity of either study. The official study is likely to be more extensive than this one and is probably more accurate. This investigation has still used valid methods and should not be dismissed too easily, the weakness is probably that by using bioindicators the contamination cannot be determined to be above or below set threshold values.

The investigation shows that it could be that living organisms are affected by the presence of the contaminated soil although the pollution levels are below the set threshold values and therefore not considered harmful. This would give implications such as lessened production from cultivation and maybe even medical repercussions and recommendations previously given to the inhabitants of the area and described in *1.1 Soil contamination in Lomma Harbour* should still be considered.

6 Evaluation

6.1 Materials

The materials used in the experiments for the investigation could be evaluated as faulty equipment and other components could affect the entire outcome of the investigation.

The soil used in the investigation plays an important part as it is what is being examined and investigated. The soil taken from Lomma Harbour did not have the desirable consistency causing difficulties in the experiments by containing varying amounts of gravel and having an inconsistent consistency it created a varied basis for the experiments.

The soil used could vary between the different trials as well as within the same trials for the different specimens. That could subsequently have led to a variation in the final results and outcomes; for example this issue could be behind the larger standard deviation of the germination of the seeds of *Lactuca sativa* in the soil from Lomma Harbour (Tab. 1). By having such a great impact on the final outcome of the investigation, this issue is a very significant weakness of the investigation. To reduce this effect as much as possible the method has been design to decrease the variation within the same soil samples: Each soil sample has been thoroughly mixed with distilled water to create an even consistency, the same for all samples and as much gravel as possibly has been removed. The same procedure was used for the control soil.

Considering the soil of the control group its similarity to the soil from Lomma Harbour was based on qualitative observations only hence unseen differences between the two types of soil could have affected the evaluation of the biological effects of the soil from Lomma Harbour. If the soil from Löddeköpinge naturally gives different conditions for *L. sativum* and *L. sativa* independent of the conditions of the soil from Lomma Harbour it could create distinct uncertainty of this investigation making it a very significant weakness. This weakness could be prevented in further investigation by examining the soil used as control more closely for suitability for the task.

The water used plays a role in the results of the experiments as well due to its importance to plants and is therefore an important issue to consider. While stirring the soil with the water, the consistency and water content between the different trials and samples could vary as no fixed water amount could be used due to the soil initially having different consistencies and human errors could occur when estimating the consistency between different containers. Therefore the amount of water constitutes a significant variation. The water resources for the different specimens did vary as some seedlings were found dry and dead when being measured. This could then lead to a remaining variation in the results. As a significant weakness, the effects of this was attempted to be avoided by carefully comparing the soil of the different containers to achieve as equal foundations for all trials and repeats.

The equipment used in the experiments affect the results as well. The filter paper used for the experiment with *L. sativa* was kept constant using the same sort by the same manufacturer and type. However, the filter paper still constituted an obstacle to perfect results as the shape cut and the area of it actually being in contact with the soil might vary and could by that affect the water uptake hence have an effect on the growth of the plants. This weakness could have be reduced by having a template shape after which to cut all papers after to assure a consistent area of all and the soil was shaped as even as possible to allow as much of the filter paper as possible to come in contact with the damp soil.

6.2 Methods

When it comes to the methods used in the experiments for the investigation, there are aspects worthy of evaluation: The design of the method could be improved to give a more extensive and perhaps more accurate result.

The way used to collect the soil in Lomma Harbour was restricted and could not be done randomly due to private property, paving and similar obstacles. This constitutes a significant weakness of the investigation as it limits the range of the soil collected, hence the variable under examination. This could be redressed by performing a more extensive investigation examining all areas affected and would most likely require more official research.

A consistent depth for sampling was used in this investigation but the depth where the affected soil could actually be found might have varied without one knowing about it when sampling. This could then restrict the actual amount of affected soil collected. To prevent this in future investigations some way of analysing the soil to determine if it actually contains pollutants should be done before the method with bioindicators is performed.

The number of seeds used in each trial could be increased to give more trials and specimens to measure thus making the final processed data more reliable and avoid the risk of random errors or ensuring that would be to simply increase the number of repeats of each trial.

The light conditions under which the plants were kept to grow was kept constant. Maybe a more normal light cycle resembling the one of the plants natural habitat would make the results more specific to what would be the ordinary growing of the two species. It might also create a more realistic environment for the plants adjusting the experiment to the natural conditions of Lomma Harbour.

The results of this investigation are limited by the methods and material used as well as the extent of the investigation itself. To further explore the topic, more extensive investigations could be done using other methods. The effects on animals, hence likely effect on humans, could be investigated by for example examining the rabbit population in the area known to consume plants grown in gardens of the housing area and comparing this result with rabbits elsewhere. Also chemical investigations could be performed to determine what pollutants actually are present; for example the ISAL (Infrared Soil Analysis Laboratory) method could be used[22].

7 Acknowledgements

I would like to thank my granddad Hans Lunga greatly, who with his personal interest and dedication has encouraged and helped me with this essay by sharing his knowledge about and insight in the issue of the soil pollution in Lomma Harbour.

I would also like to express my deepest appreciation for Annette Carlström, my respected and highly competent biology teacher and supervisor of this essay, whose encouragement and great devotion in my work still amazes me.

For the proofreading of this essay a special thanks to my grandparents Jan Erik Övermo and Monika Diehl Övermo for helping me completing this essay

To all my dear friends and my family for their continuous support and encouragement a great thank you.

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9 Appendices

9.1 Appendix A Materials

9.1.1 Materials for sample collection

garden spade
1 L measure ± 50 cm^3
6 3 L plastic bags
marker pen and masking tape

9.1.2 Materials for the *Lepidium sativum* experiment

6 × 5 500 cm^3 FixPack® plastic containers with the dimensions 5 × 9 × 11 cm
marker pen and masking tape
5 separate ≈ 5 × 250 cm^3 soil samples collected from Lomma Harbor
1 ≈ 5 × 250 cm^3 control soil sample collected from a field in Löddeköpinge
distilled water
spoon
20 × 6 × 5 seeds of *Lepidium sativum*
tweezers
6 × 5 1 L plastic bags
growth lamps OSRAML 18w/77 Floura
15 cm ruler ± 0.1 cm

9.1.3 Materials for the *Lactuca sativa* experiment

500 cm^3 beaker 50 cm^3

6 × 5 500 cm^3 FixPack® plastic containers with the dimensions 5 × 9 × 11 cm

marker pen and masking tape

5 separate 5 × 250 cm^3 soil samples collected from Lomma Harbor

1 5 × 250 cm^3 control soil sample collected from a field in Löddeköpinge

distilled water

spoon

10 × 6 × 5 seeds of *Lactuca sativa*

tweezers

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scissors

6 × 5 1 L plastic bags

growth lamps OSRAML 18w/77 Floura

15 cm ruler ± 0.1 cm

9.2 Appendix B Raw data

9.2.1 Seed germination of *Lepidium sativum*

Tab.1 Number of germinated seeds of *L. sativum* out of twenty seeds in the different soil samples where number one to five are samples of the soil from Lomma Harbour and the control sample consists of soil from Löddeköpinge; five repeats performed for each samples times the number of seeds

Soil sample	Number of germinated seeds				
	1	2	3	4	5
1	18	18	12	12	16
2	16	14	16	13	17
3	13	11	9	13	13
4	13	13	14	14	14
5	9	9	8	10	14
Control soil	16	18	19	18	18

Calculations performed shown in *9.3 Appendix C Calculations.*

9.2.2 Seed germination of *Lactuca sativa*

Tab.2 Number of germinated seeds of *L. sativa* out of ten seeds in the different soil samples where number one to five are samples of the soil from Lomma Harbour and the control sample consists of soil from Löddeköpinge; five repeats performed for each samples times the number of seeds

Soil sample	Number of germinated seeds				
	1	2	3	4	5
1	5	4	6	7	3
2	6	6	4	5	8
3	7	5	6	2	5
4	5	6	3	5	6
5	8	5	7	4	9
Control soil	7	9	9	9	8

Calculations performed shown in *9.3 Appendix C Calculations.*

9.2.3 Seedling length of *Lepidium sativum*

Tab. 3 Length of seedlings of *L. sativum* seeds in the different soil samples where number one to five are samples of the soil from Lomma Harbour and the control soil consists of soil from Löddeköpinge; the length measured in millimetres with an uncertainty of one millimetre; five repeats performed for each samples times the number of seeds

Soil sample		Seedling length/mm ± 1 mm				
		1	2	3	4	5
1	1	46	36	41	41	35
	2	37	35	50	29	21
	3	37	33	45	46	40
	4	47	37	40	34	44
	5	48	50	41	48	52
	6	40	32	44	38	41
	7	36	49	47	27	38
	8	35	31	14	40	46
	9	42	46	13	15	13
	10	40	39	20	19	25
	11	17	31	45	22	23
	12	44	37	39	18	37
	13	48	31			24
	14	8	18			36
	15	9	7			32
	16	25	53			31
	17	9	18			
	18	11	13			
	19					
	20					

Table continued on the next page.

2	1	45	43	32	40	52
	2	38	59	47	32	49
	3	40	41	27	41	27
	4	37	39	40	43	50
	5	47	30	42	51	44
	6	40	52	43	36	47
	7	36	36	34	29	49
	8	40	41	37	37	31
	9	35	3	41	41	26
	10	50	46	35	11	41
	11	27	35	51	43	46
	12	4	44	25	31	12
	13	24	21	50	8	41
	14	42	9	26		11
	15	34		23		29
	16	2		42		41
	17					4
	18					
	19					
	20					
3	1	35	45	36	34	30
	2	24	32	49	32	19
	3	36	19	27	15	43
	4	30	36	32	26	34
	5	32	52	22	39	22
	6	39	49	20	37	29
	7	36	27	11	31	24
	8	34	6	5	42	41

Table continued on the next page.

	9	24	7	18	38	51
	10	35	49		35	48
	11	15	4		9	29
	12	17			31	21
	13	28			6	8
	14					
	15					
	16					
	17					
	18					
	19					
	20					
4	1	42	41	34	44	37
	2	31	38	47	38	26
	3	29	43	50	17	30
	4	38	42	34	18	25
	5	40	51	27	31	40
	6	30	17	40	28	25
	7	21	16	43	34	31
	8	13	14	50	36	12
	9	39	11	41	29	21
	10	34	39	22	26	25
	11	24	39	29	30	16
	12	7	33	35	25	17
	13	11	8	36	9	9
	14			14	14	22
	15				13	
	16				19	

Table continued on the next page.

	17					
	18					
	19					
	20					
5	1	31	39	45	37	42
	2	39	40	5	26	37
	3	34	15	43	10	32
	4	23	37	42	38	29
	5	32	38	8	28	37
	6	14	30	23	23	31
	7	13	31	24	33	29
	8	19	6	30	37	37
	9	4	11		42	31
	10				20	29
	11					8
	12					31
	13					28
	14					4
	15					31
	16					38
	17					
	18					
	19					
	20					
Control soil	1	33	44	46	36	18
	2	32	35	37	50	35
	3	28	39	39	44	36
	4	27	32	64	39	25

Table continued on the next page.

	5	32	51	42	38	41
	6	33	32	36	41	34
	7	34	46	36	30	29
	8	38	23	39	46	20
	9	40	16	57	38	40
	10	24	37	41	43	39
	11	34	32	32	29	33
	12	25	29	19	31	38
	13	15	44	21	37	41
	14	28	18	26	53	35
	15	40	43	55	39	30
	16	16	37	18	22	37
	17		53	4	44	14
	18		47	52	28	23
	19			41		
	20					

Calculations performed shown in *9.3 Appendix C Calculations.*

9.2.4 Root length of *Lactuca sativa*

Tab. 4 Length of roots of *L. sativa* seeds in the different soil samples where number one to five are samples of the soil from Lomma Harbour and the control soil consists of soil from Löddeköpinge; the length measured in millimetres with an uncertainty of one millimetre; five repeats performed for each samples times the number of seeds

Soil sample		Root length/mm ± 1 mm				
		1	2	3	4	5
1	1	32	33	29	11	23
	2	22	21	36	15	33
	3	25	34	37	24	10
	4	36	4	34	13	
	5	20		3	12	
	6			4	25	
	7				29	
	8					
	9					
	10					
2	1	37	38	29	10	29
	2	14	42	17	33	22
	3	27	7	9	14	23
	4	39	13	4	18	21
	5	36	28		3	19
	6	4	26			15
	7					6
	8					31
	9					

Table continued on the next page.

	10					
3	1	22	42	27	49	35
	2	52	35	30	3	39
	3	27	50	35		31
	4	39	8	26		21
	5	23	9	5		8
	6	3		4		
	7	6				
	8					
	9					
	10					
4	1	4	29	9	24	28
	2	42	26	53	2	22
	3	43	24	47	19	23
	4	3	30		18	28
	5	7	29		5	5
	6		7			
	7					
	8					
	9					
	10					
5	1	52	13	11	21	20
	2	46	23	34	7	41
	3	63	8	26	26	29
	4	54	4	34	23	14
	5	43	12	30		17
	6	16		27		44

Table continued on the next page.

	7	11		43		20
	8	12				36
	9					12
	10					
Control soil	1	52	46	59	4	23
	2	63	37	49	64	49
	3	54	58	54	48	16
	4	44	61	29	53	48
	5	67	43	15	58	35
	6	51	50	37	43	31
	7	6	51	27	62	43
	8		8	66	63	21
	9		11	14	6	
	10					

Calculations performed shown in *9.3 Appendix C Calculations.*

9.3 Appendix C Calculations

The mean value

$$\bar{x} = \frac{\sum x_i}{n}$$

$\bar{x}$ = the mean value

$\sum$ = the sum

x_i = each individual value of the data set

n = the total number of values in the data set

An example of the calculation of the mean; the mean of *L. sativum* seed germination in the soil from Lomma Harbour in the first repeat:

$$\bar{x} = \frac{\sum x_i}{n} \leftrightarrow \frac{69}{5} = 13.8$$

All calculations of the mean in the investigation were done using Microsoft Excel.

The standard deviation

$$s_x = \sqrt{\sum \frac{(x_i - \bar{x})^2}{n-1}}$$

s_x = the standard deviation

$\sum$ = the sum

x_i = each individual value of the data set

$\bar{x}$ = the mean value

n = the total number of values in the data set

All calculations of the standard deviation in the investigation were done using Microsoft Excel.

The percentage

$$P = \frac{F}{n} \times 100$$

$P =$ the percentage

$F =$ the frequency, the part wanted in percentage, in the investigation the mean values are used for the calculations of the percentage

$n =$ the total number of values in the data set

An example of the calculation of the percentage; the percentage of germinated *L. sativum* seeds in the control soil:

$$P = \frac{F}{n} \times 100 \leftrightarrow \frac{13.2}{20} \times 100 = 65.8\%$$

This example shows a more precise answer than the values shown can give due to simplification. The values used in the real calculations are more precise.

All calculations of the percentage were done in *Microsoft Excel*.

Unpaired t-test

$$t = \frac{(\bar{x}_A - \bar{x}_B)\sqrt{n}}{\sqrt{{s_A}^2 + {s_B}^2}}$$

$t =$ the t-value

$\bar{x}_A =$ the mean of the first set

$\bar{x}_B =$ the mean of the second set

$n =$ the total number of values

$s_A =$ the standard deviation of the first set

$s_B =$ the standard deviation of the second set

All calculations of the unpaired t-test were done using an internet-based calculator[23].