UNIVERSITY OF LJUBLJANA
BIOTECHNICAL FACULTY
DEPARTMENT OF AGRONOMY

Ignacio PALAU SALVADOR

DYNAMICS OF ZINC AVAILABILITY FROM COMPOSTED AND NON-COMPOSTED BIOSOLIDS (SEWAGE SLUDGE)

GRADUATION THESIS

University studies

Ljubljana, 2008

UNVERSITY OF LJUBLJANA
BIOTECHNICAL FACULTY
DEPARTMENT OF AGRONOMY

Ignacio PALAU SALVADOR

DYNAMICS OF ZINC AVAILABILITY OF ZINC FROM COMPOSTED AND NON-COMPOSTED BIOSOLIDS (SEWAGE SLUDGE)

GRADUATION THESIS
University studies

DINAMIKA DOSTOPNOSTI CINKA IZ KOMPOSTIRANEGA IN NEKOMPOSTIRANEGA BLATA KOMUNALNE ČISTILNE NAPRAVE

DIPLOMSKO DELO
Univerzitetni študij

Ljubljana, 2008

Graduation thesis represents the finale of the university studies of agronomy. The thesis was done at the Centre of Soil and Environmental Sciences, at the Agronomy Department of Biotechnical Faculty at Ljubljana University. The practical work was done in the laboratories and in the experimental greenhouse of the named institution.

The Committee for graduation studies named doc. dr. Rok MIHELIČ as a supervisor for this thesis.

Committee for the evaluation and the defence of the thesis:

President:	prof. dr. Franc Batič Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo
Member:	prof. dr. Franc Lobnik Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo
Member:	doc. dr. Rok Mihelič Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo

Date of diploma thesis defence: 2nd October 2008

Undersigned I agree with publishing of my diploma thesis in full text on the internet page of the Digital Library of Biotechnical Faculty. I declare that the text in the electronic version is identical to the printed one.

Ignacio Palau Salvador

KEY WORDS DOCUMENTATION

ND	Dn
DC	UDC 631.879.4:628.473:631.41 (043.2)
CX	SEWAGE SLUDGE/COMPOST/ZINK AVAILABILITY /CHINESE CABBAGE/POT EXPERIMENT/HEAVY METALS
CC	AGRIS P30/P33/P35
AU	PALAU SALVADOR, Ignacio
AA	MIHELIČ, Rok (supervisor)
PP	SI-1000 Ljubljana, Jamnikarjeva 101
PY	2008
PB	University of Ljubljana, Biotechnical Faculty, Department of Agronomy
TI	AVAILABILITY OF ZINC FROM COMPOSTED AND NON-COMPOSTED BIOSOLIDS (SEWAGE SLUDGE)
DT	Graduation Thesis (University studies)
NO	XIII, 57, [18] p., 10 tab., 39 fig., 9 ann., 52 ref.
LA	en
AL	sl / en

AB The availability of zinc from composted and non-composted sewage sludge was studied during composting and in pot trial. This sludge was taken from the Domžale-Kamnik Wastewater Treatment Plan. It was known that this material contains a high concentration of zinc due to the proximity of some industries to the plant. During composting samples were taken and analysed in order to determine the total and available zinc content. After composting, a pot trial was established in a greenhouse consisted of four treatments, each with 5 replicates to asses Zn availability to plants. Two of the treatments were fertilized with composted and non-composted sludge and the other two were fertilized with NPK fertilizer in both cases and one of them in addition with $ZnCl_2$. The chosen plant for the pot trial was Chinese cabbage, a traditional vegetable crop in Slovenia, moderately sensitive to zinc. Zn availability decreased during composting process; however the total Zn concentration in compost increased in a small percentage. The plants with NPK grew faster than the plants with organic amendments. The absolute Zn availability shown by its content in the plants was lower with non-composted sewage sludge than with compost amendment; however the difference was not significant. The main conclusion is that the availability of zinc decreased during the composting process, but this decrease didn't mean lower concentration in the plants.

KLJUČNA DOKUMENTACIJSKA INFORMACIJA

ŠD	Dn
DK	UDK 631.879.4:628.473:631.41 (043.2)
KG	BLATO KOMUNALNE ČISTILNE NAPRAVE/KOMPOST/DOSTOPNOST CINKA/ORGANSKA SNOV/KITAJSKO ZELJE/LONČNI POSKUS/TEŽKE KOVINE
KK	AGRIS P30/P33/P35
AV	PALAU SALVADOR, Ignacio
SA	MIHELIČ, Rok (mentor)
KZ	SI-1000 Ljubljana, Jamnikarjeva 101
ZA	Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo
LI	2008
IN	DINAMIKA DOSTOPNOSTI CINKA IZ KOMPOSTIRANEGA IN NEKOMPOSTIRANEGA BLATA KOMUNALNE ČISTILNE NAPRAVE
TD	Diplomsko delo (univerzitetni študij)
OP	XIII, 57, [18] str., 10 pregl., 39 sl., 9 pril., 52 vir.
IJ	en
JI	sl / en

AI Proučevali smo dostopnost cinka (Zn) iz kompostiranega in ne-kompostiranega blata komunalne čistilne naprave Domžale-Kamnik. Predhodne analize so pokazale, da ima ta material veliko vsebnost Zn zaradi prispevka industrijskih vod na čistilno napravo. Med kompostiranjem smo analizirali skupno in dostopno obliko Zn. Po kompostiranju smo zasnovali lončni poskus v rastlinjaku, v štirih obravnavanjih, s petimi ponovitvami, z namenom ugotoviti rastlinam dostopno količino Zn iz kompostiranega in ne-kompostiranega blata. Pri dveh obravnavanjih smo uporabili kompostirano ali ne-kompostirano blato, pri drugih dveh pa obakrat NPK gnojilo in v enem še dodatno $ZnCl_2$. Izbrana rastlina za lončni poskus je bila zelje, tradicionalna vrtnina v Sloveniji, ki ima srednje potrebe po Zn. Med kompostiranjem se je dostopnost Zn zmanjšala, vsebnost skupnega Zn pa je rahlo narasla. Rastline so v obravnavanjih z dodanim NPK gnojilom hitreje rasle kot pri dodatku organskih gnojil. Absolutna količina Zn v rastlinah je bila nekoliko manjša pri dodatku ne-kompostiranega blata kot pri dodatku komposta. Razlika ni bila signifikantna. Glavna ugotovitev je, da se dostopnost Zn med kompostiranjem blata sicer zmanjša, vendar to ne vpliva na manjši sprejem Zn v rastline.

TABLE OF CONTENTS (INDEX)

Str.

Ključna dokumentacijska informacija III
Key words documentation IV
Table of contents V
Index of tables VII
Index of images VIII
Index of appendices X
Symbols and abbreviate words XII
Vocabulary XIII

1 PURPOSE OF THE EXPERIMENT AND HYPOTHESES 1
1.1 HYPOTHESES 1
2 INTRODUCTION 2
2.1 SEWAGE SLUDGE 2
2.1.1 Origin of sewage sludge. Wastewater Treatment Plants (WWTP) 2
2.1.2 The case of Domžale-Kamnik Wastewater Treatment Plant 5
2.1.3 Characteristics of sewage sludge 6
2.1.4 Heavy metals in sewage sludge 8
2.1.5 Composting process 8
2.1.6 Land application of sewage sludge 9
2.2 ZINC 10
2.2.1 Chemical properties 10
2.2.2 Zinc as essential element 10
2.2.3 Zinc deficiency, toxicity and tolerance 11
2.3 CHOSE VARIETY FOR THE POT TRIAL. CHINESE CABBAGE 13
2.3.1 Chinese Cabbage. **Cabbage. *Brassica rapa L. ssp. pekinensis.*** 13
3 MATERIAL AND METHODS 14
3.1 PREPARATION AND STUDY OF THE MIXTURE 14
3.2 PREPARATION OF THE COMPOSTING PROCESS 15
3.3 COMPOSTING PROCESS 16
3.4 PREPARATION OF THE PLANTING SUBSTRATE 16
3.5 PREPARATION OF THE POT TRIAL 17
3.5.1 Determination of the amount of composted and non-composted sewage sludge for the pot trial 18
3.5.2 Determination of the amount of fertilizers for the pot trial 19
3.5.3 Summary of the final amounts applied in the pot trial 20
3.5.4 Trasplant of the Chinese cabbage into the pots 20

3.6 PLANTS DEVELOPMENT 20
3.7 PLANTS HARVEST AND PROCESSING 22
3.8 PROCESSING OF SOIL AND SLUDGE SAMPLES 23
3.9 LABORATORY ANALYSES 24
3.9.1 Sewage sludge analyses 24
3.9.2 Soil analyses 30
3.9.3 Plants analyses 30
3.10 CALCULATION AND STATISTICAL METHODS 30
4 RESULTS AND DICUSSION 31
4.1 ANALYSES IN COMPOSTING PROCESS 31
4.1.1 PH, EC, total C, total N, NO_3-N and NH_4-N 34
4.1.2 Total Zn in sewage sludge in composting mixture during the composting process 32
4.1.3 CAT Zn in sewage sludge in compostung mixture during the composting process 36
4.1.4 Loss of dry weight 37
4.2 POT TRIAL AND SOIL ANALYSES 40
4.2.1 Total Zn analises at the beginning and at the end of the pot trial 40
4.2.2 Available Zn in soil at the beginning and at the end of the pot trial 42
4.3 PLANTS ANALYSES 44
4.3.1 Dry and wet weight, and percentage of dry matter after harvest 44
4.3.2 Total Zn in biomass after cabbage harvest 45
4.3.3 Greenness of leaves measured with hydro-N-tester 47
5 CONCLUSIONS 49
6 SUMMARY (POVZETEK) 51
6.1 SUMMARY 51
6.2 POVTEZEK 52
7 REFERENCES 54
ACKNOWLEDGEMENTS 58
APPENDICES 59

INDEX OF TABLES

Table 1: Water quality based on the BOD level (Ramalho,1996) 4

Table 2: Maximum allowed concentration of heavy metals in compost/anaerobic residue (Hege *et al*., 2003) 9

Table 3: Supply level of zinc (mg/kg soil) in arable soil (CAT method) (Hege *et al*., 2003) 11

Table 4: Fertilization advice (Hege *et al*., 2003) 11

Table 5: Amendments of fertilizers and sludge calculated for each experiment 20

Table 6: Chemical characteristics of the composting mixture before and after composting 31

Table 7: C/N ratio at the beginning and at the end of the composting process 33

Table 8: Statistic study of total zinc during composting process 34

Table 9: Correlation between availability of Zn and content in biomass 46

Table 10: Correlation between greenness and weight after harvest 48

INDEX OF FIGURES

Figure 1- Aerial picture of the Domžale-Kamnik WWTP 5

Figure 2- Chinese cabbage plant (var. Summer Bright) 13

Figure 3- 5 litres of mixture prepared for analysis. 14

Figure 4- Barrels with the swage sludge 14

Figure 5- The pot with mixture and other pot with water prepared for the analysis. 14

Figure 6- The pot with the mixture full of water losing it by gravity 14

Figure 7- The four pots with the mixture inside the chamber prepared for the composting process 15

Figure 8- The mixture, prepared and well mixed before the beginning of the process 15

Figure 9- Weighting the pots after taking the samples. 16

Figure 10- Taking the soil from a field at the Biotechnical Faculty 16

Figure 11- Mixture made with soil and quartz sand prepared for being screened 16

Figure 12- Preparation of the pot trial with pots, fertilizers and plants 17

Figure 13 The different amendments well mixed with the soil before planting the Chinese cabbage 17

Figure 15- Collection of the soil samples at the end of the pot trial process 20

Figure 16- Samples prepared to be kept in the freezer. 20

Figure 17- Hydro-N-tester measurement 21

Figure 18- Plants cut in four peaces drying in the chamber. 22

Figure 19- Plant weighed after being dried 22

Figure 20- Mashing a sample with a mortar and alcohol for avoiding contamination between samples 23

Figure 21- Classified samples prepared for being analysed 23

Figure 22- Reaction vessels used for the analysis of total zinc 24

Figure 23- Precision scale, used to weigh the samples, 24

Figure 24- Reaction vessels, reflux condensers and absorption vessels 25

Figure 25- Filtering the preparation 25

Figure 28- Total zinc during composting 34

Figure 29- CAT Zn during composting process 34

Figure 30- Weight changes during the composting process 37

Figure 31- Loss of dry weight in the composting process 38

Figure 32- Accumulative losses of dry weight indicating which was the amount lost in each measurement 38

Figure 33- Total zinc in soil at the beginning and at the end of the pot trial 40
Figure 34- Development of the CAT Zn in each experiment during the pot trial 42
Figure 35- Percentage of available zinc in relation to the total zinc content 43
Figure 36.- Dry/total biomass weight after harvest 44
Figure 37- Zinc content in biomass after harvest 45
Figure 38- Correlation between CAT zinc in soil and zinc content in biomass 46
Figure 39- Changes of greenness during the pot trial for each experiment 47

INDEX OF APPENDICES

APPENDIX A: Total and CAT Zn in mixture during composting process.

Appendix A.1: Total zinc

Appendix A.2: CAT zinc

Appendix A.3: Comparison between CAT and total zinc

APPENDIX B: Results of the calculations of the losses of dry weight during the composting process

Appendix B.1: Moisture of the mixture during the composting process

Appendix B.2: Losses of dry weight during the composting process

APPENDIX C: Calculation of the total amount of zinc in the soil of the four analyses during the pot trial

Appendix C.1: Amount of total zinc at the beginning of the pot trial

Appendix C.2: Amount of total zinc at the end of the pot trial

Appendix C.3: Comparison between values of total zinc at the beginning and at the end of the pot trial

Appendix C.4: Analyses of variance

APPENDIX D: Calculation of the available zinc content in soil during the pot trial

Appendix D.1: Calculation of the available Zn content in soil at the beginning of the pot trial

Appendix D.2: Calculation of the available Zn content in soil at the end of the pot trial

Appendix D.3: Comparison between available zinc content in percentage at the beginning and at the end of the pot trial

Appendix D.4: Comparison between available zinc content at the beginning and at the end of the pot trial

Appendix D.5: Analysis of variance

APPENDIX E: pH values of the soil at the beginning of the pot trial

APPENDIX F: Wet weight, dry weight and percentage of dry matter of the plants after harvest

APPENDIX G: Zinc content in biomass after harvest

Appendix G.1: Table with the zinc content values in biomass

Appendix G.2: Analysis of variance

APPENDIX H: Greenness of the leaves. Hydro-N-tester

APPENDIX I: Images of the plants in four different steps during the pot trial

SYMBOLS AND ABBREVIATE WORDS

CAT Zn	Soluble exchangeable fraction of total zinc
WWTP	Wastewater Treatment Plant
PE	Population Equivalent. Represents a production of waste water of 150 l/day
BOD	Biological Oxygen Demand
BOD5	Biological Oxygen Demand after 5 days
COD	Chemical
TSS	Total Suspended Solids
m.o.	microorganisms
^{o}C	Celsius degrees
O.M.	Organic Matter
d.m.	dry matter
NO_3-N	Nitrate-N
NH_4-N	Ammonium-N
mM	0.001 M or millimolar. It is 1 thousandth of a molar
ISO	International Organisation for Standardization
SIST	Slovenian Institute of Standardization

VOCABULARY

Biosolids	(Sewage sludge). A recyclable, primarily organic solid material produced by wastewater treatment processes
Smartrace	A fully chelated blend of micronutrients in most balanced form to attain optimum plant growth.
Chlorosis	It is a condition in which leaves produce insufficient chlorophyll. As chlorophyll is responsible for the green colour of leaves, chlorotic leaves are pale, yellow, or yellow-white
Perlite	It is an amorphus volcanic glass that has a relatively high water content, typically formed by the hydration of obsidian. It occurs naturally and has the unusual property of greatly expanding when heated sufficiently.
Lagooning	It is a natural treatment technique that consists in the accumulation of wastewater in ponds or basins, known as *biological or stabilization ponds*, where a series of biological, biochemical and physical processes take place.

1 PURPOSE OF THE EXPERIMENT AND HYPOTHESES

Zinc is a heavy metal but is also an essential plant nutrient, and therefore its application with biosolids can be beneficial. However its application can be also prejudicial, depending on the amount of Zinc which the plant uptakes. As soil organic matter has great capacity and strength of bonding with most trace metals, including zinc, application of composted or non-composted biosolids could enhance sorption of zinc to the soil colloids.

Leita *et al.* (2001) have found a diminishing ratio of soluble Zn/total Zn by the progressing of composting time. Zinc was probably bound to the humic fractions of compost, which increased 72% of dissolved organic carbon during the progression of composting.

It is however questionable whether composting technological process is really necessary for bonding of Zn to the humic fractions or perhaps identical process would occur within the fresh biosolids incorporated directly to the soil.

By the experiment the fate of Zn present at rather high quantity in biosolids after it is put as organic fertilizer to the soil directly or after composting will be examined. The dynamics of zinc availability during composting will be also examined.

During this diploma a greenhouse pot trial will also be done, with the aim of determining the uptake of zinc from different materials, as sewage sludge (composted or non-composted) and mineral fertilizers. The plant specie chosen for this experiment is the Chinese cabbage (*Brassica rapa var. pekinensis*). This is a common crop in Slovenia. This characteristic makes the election of Chinese cabbage a good chance for studying the possibility of use of this sludge as fertilizer in a future.

1.1 HYPOTHESES

- Soluble, exchangeable fraction of biosolids zinc decrease during organic matter degradation.
- Compost applied to the soil can prevent the release of zinc compared to the same amount of non-composted biosolids.
- Compost applied to the soil can also prevent its excessive uptake by crops compared to the same amount of non-composted biosolids.

2 INTRODUCTION

2.1 SEWAGE SLUDGE

First of all shall be explained that the terms biosolid and sewage sludge will be used in this document without any difference, considering them synonyms.

Sewage sludge is an end product of the wastewater treatment process. This material can be a wonderful source of nutrients for the soil. Using this material as a fertilizer can benefit the environment by turning wastes into valuable resources. These biosolids would otherwise have to be disposed of by landfilling, lagooning, incineration, or ocean dumping. On the other hand, heavy metals sometimes found in sewage sludge may present environmental problems. Several practices, similar to those used with other organic fertilizers, will maximize the benefits of using sewage sludge while minimizing the risks (Mitchel *et al.,* 1992).

In this first section of the introduction some aspects related with Sewage Sludge, since its formation until its use in agriculture will be presented.

2.1.1 Origin of Sewage Sludge; Wastewater Treatment Plants (WWTP)

The Wastewater Treatment Plants (WWTP) have as aim the transformation of urban and industrial water into clean water trying to avoid the direct wastes. There are two kinds of plants; the general plants which work with urban water or water which comes from small factories, and on the other hand private plants where the water of a big industry is treated. This is the main reason for which the sewage sludge of the WWTP has fewer amounts of heavy metals in the last times (Daims *et al.*, 2006).

There are several regulations related with the wastewater treatment, as the European regulation 271/91/CEE which means that most of the WWTP in Europe have had to adapt to more restrictive conditions on the discharge of water, mainly with reference to the concentration of nitrogen and phosphorous in effluent (Pijuan *et al*., 2006).

The transformation of urban and industrial water into clean water or water which is below the limits of contaminants is done using chemical, physical and biotechnical processes taking as references some normalized parameters like BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand) or TSS (Total Suspended Solids) (Daims *et al.*, 2006).

The treatment of wastewater in plant is divided into three main steps; the following explanation of these three steps is based on a publication of Ramalho (1996)

1. **Primary Treatment:** It bases on the physical removal of floatable and settleable solids. The three main actions in this treatment are:

    - Screening: to remove large objects, such as stones or sticks that could plug lines or block tank inlets.

    - Grit chamber: slows down the flow to allow grit to fall out.

    - Sedimentation tank (settling tank or clarifier): settleable solids settle out and are pumped away, while oils float to the top and are skimmed off

2. **Secondary Treatment:** Secondary treatment typically utilizes biological treatment processes, in which micro organisms (m.o.) convert non-settleable solids to settleable solids. Sedimentation typically follows, allowing the settleable solids to settle out. Three options include:

    - Activated Sludge: The most common option uses m.o. in the treatment process to break down organic material with aeration and agitation, and then allows solids to settle out. Bacteria-containing "activated sludge" is continually circulated back to the aeration basin to increase the rate of organic decomposition.

    - Trickling Filters: These are beds of coarse media (often stones or plastic) 1-3. m. deep. Wastewater is sprayed into the air (aeration), and then allowed to trickle through the media. m.o., attached to and growing on the media, breaks down organic material in the wastewater. Trickling filters drain at the bottom; the wastewater is collected and then undergoes sedimentation.

    - Lagoons: These are slow, cheap, and relatively inefficient, but can be used for various types of wastewater. They rely on the interaction of sunlight, algae, m.o., and oxygen (sometimes aerated).

3. **Tertiary Treatment:** It bases on disinfection of the effluent choosing between some treatments like the addition of chloride or other compounds or using ultraviolet light. Tertiary treatment may include processes to remove nutrients such as nitrogen and phosphorus, and carbon adsorption to remove chemicals. These processes can be physical, biological, or chemical.

Continuing with the work in the Wastewater Purification Plants, the main parameters used for evaluating the quality of the water in any point of the process will be explained. The information shown in the following paragraphs is based on a publication Ramalho (1996):

1. **Biological Oxygen Demand (BOD):** It is a measure of the oxygen used by m.o. to decompose this waste. If there is a large quantity of organic waste in the water supply, there will also be a lot of bacteria present working to decompose this waste. In this case, the demand for oxygen will be high (due to all the bacteria) so the BOD level will be high. As the waste is consumed or dispersed through the water, BOD levels will begin to decline.

   Nitrates and phosphates in a body of water can contribute to high BOD levels. Nitrates and phosphates are plant nutrients and can cause plant life and algae to grow quickly. When plants grow quickly, they also die quickly. This contributes to the organic waste in the water, which is then decomposed by bacteria. This results in a high BOD level. The temperature of the water can also contribute to high BOD levels.

   When BOD levels are high, dissolved oxygen (DO) levels decrease because the oxygen that is available in the water is being consumed by the bacteria. Since less dissolved oxygen is available in the water, fish and other aquatic organisms may not survive.

Table.1- Water quality based on the BOD level (Ramalho, 1996)

BOD Level *(in ppm)*	**Water Quality**
1 – 2	**Very Good** There will not be much organic waste present in the water supply.
3 – 5	**Fair: Moderately Clean**
6 – 9	**Poor: Somewhat Polluted** Usually indicates organic matter is present and bacteria are decomposing this waste.
10 or greater	**Very Poor: Very Polluted** Contains organic waste.

2. **Chemical Oxygen Demand (COD):** It is used to express the amount of oxygen consumed during oxidation of a sample with hot acid dichromate solution under defined conditions: the test provides an estimate of the oxidisable matter present in the sample. The result is usually expressed as milligrams of oxygen consumed per litre of sample (mg l^{-1}) (Westwood, 2007).

3. **Total Suspended Solids (TSS):** These are solids in water that can be trapped by a filter. TSS can include a wide variety of material, such as silt, decaying plant and animal matter, industrial wastes, and sewage. High concentrations of suspended solids can cause many problems for stream health and aquatic life.

   To measure TSS, the water sample is filtered through a pre-weighed filter. The residue retained on the filter is dried in an oven at 103 to 105° C until the weight of the filter no longer changes. The increase in weight of the filter represents the total suspended solids.

   TSS can also be measured by analyzing for total solids and subtracting total dissolved solids.

2.1.2 The case of Domžale-Kamnik Waste Water Treatment Plant

This following information is based on an article written by N. Hvala *et al*. (2002).

The Domžale-Kamnik WWTP is a conventional two-stage activated sludge plant designed to remove organic matter from the wastewater. The capacity of the plant is 200,000 PE with an average daily inflow of approximately 20.000 m^3/day.

The plant influent consists of 45% municipal and 55% industrial wastewater. The BOD5 loading is about 10,000 kg/day (500 mg/l), while NH_4-N loading is about 700 kg/day (35 mg/l). The plant consists of a raw sewage pumping station, a screening structure, an aerated grit and grease chamber, primary settling tanks, highly loaded first biological stage (two parallel lines (aeration 2 × 1,000 m^3, settling tanks 2 × 1,200 m^3)), second biological stage (four parallel lines (aeration 4×1000 m^3, settling tanks 4 × 1,200 m^3)), four anaerobic digesters and sludge dewatering unit.

Figure 1- Aerial picture of the Domžale-Kamnik WWTP (CČN, 2007)

The existing plant satisfactorily eliminates carbon components; the reduction of COD is 92,7%, BOD5 96,2% and suspended solids 98,0%. As the plant is not planned for specific nitrogen elimination, it is unable to reduce NH_4-N by more than 50%.

The new stringent quality demands for effluent concentrations will require that the plant also eliminates nitrogen from the wastewater. According to the legislation, the following effluent concentrations must be met: TSS ≤ 35 mg/l, COD ≤ 100 mg/l, BOD5 ≤ 20 mg/l, NH4- N ≤ 10 mg/l (when the temperature of effluent from the aeration basin is equal to or above 12°C), total N ≤ 10 mg/l (only for plants on sensible areas, e.g. when effluent is discharged to the river with dams).

2.1.3 Characteristics of Sewage Sludge

The characteristics of biosolids play an important part of their use for land application. They can be broken down into three categories: physical, chemical and biological. (Eliot Epstein, 2002)

1) Physical Properties (Lindsay and Logant, 1998):

Physical properties affect the method of application, as well as the soil's physical and chemical properties. Several of these physical properties have an effect on the plant growth. They can affect the availability and accumulation of plant nutrients and trace elements. The solids content of biosolids affects the method of land application. There is a classification of sewage sludge based on the content of solids:

- Liquid or low-solids biosolids: these are normally injected into the soil to prevent vectors and provide better aesthetics. The addition of this kind of biosolids increases the moisture of the soil, and this could benefit plants growth. The Organic matter is diluted and consequently its benefit in improving soil structure will need a lot of time and several applications. The amount of trace elements and nutrients depends on the quantity and percent of solids of the biosolids.

- Dewatered or semisolid biosolids: are usually spread on the surface and subsequently plough into the soil. The concentration of solids adds organic matter to the soil improving its physical properties.

- High solid biosolids are usually compost or heat-dried products. Compost is an excellent source of organic matter and will improve the soil's physical properties, which include; Soil structure, soil-water relationships, water infiltration and permeability, soil erosion and runoff and soil temperature. Heat-dried products are used usually as fertilizers adding a little of organic matter since small amounts are applied without affecting the soil's physical properties.

The organic content of biosolids will vary, depending on the solids and extent of treatment. The organic matter can be as high as 70% depending on the wastewater treatment.

2) Chemical Properties (Zhang *et al*., 2002):

Chemical properties affect the plant growth as well as the soil's chemical and physical properties. The chemical properties of biosolids are affected by several factors:

a) Trace Elements, Heavy Metals, and Micronutrients

Biosolids contain trace elements, including heavy metals, primarily from industrial, commercial, and residential discharges into the wastewater system.

b) Organic Compounds

Organic compounds are found in biosolids as a result of industrial and commercial discharges, household discharges, pesticides from runoff and soil.

c) Acidity (pH)

The pH of most of biosolids (whether liquid, semisolid, or solid) is generally in the range of 7 to 8, unless lime is added during the wastewater treatment process. Lime, kiln dust and other alkaline products may be added to increase the pH and achieve the legal pathogen requirements. In some cases lime is added to reduce odours and avoid vectors during the transport to the composting facility.

d) Plant Nutrients

Plant nutrients are among the most important chemical characteristics of biosolids. Farmers value biosolids for the nitrogen and phosphorus content. The use of alkaline products in the Wastewater Purification Plants increases the Ca and Mg content.

3) Biological Properties (Zhang *et al*., 2002):

Biological properties affect the soil's microbial population and organic matter's decomposition in soil. Biological characteristics affect also human health and the environment.

a) Microbiological:

Pathogens are the most important biological property of biosolids for land application. The survival and potential movement of pathogens through soils to groundwater depend on several edaphic and climatic factors. The most important are soil moisture, pH, temperature, organic matter, soil texture, soil permeability, sunlight, and antagonistic microflora. The pathogens in the biosolids are bacteria, viruses and parasites.

b) Organic Matter:

Organic matter is an important constituent of biosolids. The use of biosolids for land application enhances the organic content of the soil. This is more important in sandy or clayey soils. In sandy soils the organic matter increases the water-holding capacity, the soil aggregation, and other soil physical properties. It reduces the soil bulk density. Also, organic matter increases the cation exchange capacity, a very important property for supplying plant nutrients.

2.1.4 Heavy metals in Sewage Sludge

Heavy metals are a group of elements found in the periodic table with a relatively high molecular weight (density > 5,0 mg/m^3) and when taken into the body can accumulate in specific body organs. The trace elements often referred to as heavy metals that have been regulated are: arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se)and Zinc (Zn). Chromium (Cr) was regulated in the first draft in 1993 but was deleted in 1995 (Duffus, 2002).

Biosolids contain heavy metals primarily from industrial, commercial and residential discharges into the wastewater system. As a result of the reduction of industrial discharges into the wastewater system and due to the existence of small cleaning plants in the industries, the quality of biosolids has improved. Although this is not an absolute truth as is possible to see in the case of this diploma. Changes in the materials used in domestic residences have also affected wastewater quality, decreasing its content of heavy metals (Dewil *et al*., 2006).

2.1.5 Composting process

Whether done on a small or large scale, composting is a method of solid waste management whereby the organic component of the solid waste stream is biologically decomposed under controlled conditions to a state in which it can be handled, stored, and/or applied to the land without adversely affecting human health or the environment (Golstein, 1977).

In this process, various m.o., including bacteria and fungi, break down organic matter into simpler substances. The effectiveness of the composting process is dependent upon the environmental conditions present within the composting system i.e. oxygen, temperature, moisture, material disturbance, organic matter and the size and activity of microbial populations (Gonzalez *et al*., 2005).

The essential elements required by the composting m.o. are carbon, nitrogen, oxygen and moisture. If any of these elements are lacking, or if they are not provided in the proper proportion, the m.o. will not flourish and will not provide adequate heat. A composting process that operates at optimum performance will convert organic matter into stable compost that is odour and pathogen free, and a poor breeding substrate for flies and other insects. In addition, it will significantly reduce the volume and weight of organic waste as the composting process converts much of the biodegradable component to gaseous carbon dioxide (Gonzalez *et al*., 2005).

The composting process is carried out by three classes of microbes (Gonzalez *et al.*, 2005):

• Psychrophiles - low temperature microbes
• Mesophiles -medium temperature microbes
• Thermophiles - high temperature microbes

2.1.6 Land application of Sewage sludge

The next paragraphs are based on information published by Epstein (2003).

Land application is defined as the spreading, spraying, injection, or incorporation of sewage sludge, including a material derived from sewage sludge (e.g., compost and pelletized sewage sludge), onto or below the surface of the land to take advantage of the soil enhancing qualities of the sewage sludge.

Sewage sludge quality is determined by these three parameters:

- The presence of pollutants (arsenic, cadmium, chromium, copper, lead, mercury, molybdenum, nickel, selenium, and zinc)

- The presence of pathogens (e.g., bacteria, viruses, parasites)

- The sewage sludge's attractiveness to vectors (e.g., rodents, flies, mosquitoes).

In the table below the quality of compost based on the content of heavy metal is shown.

Table 2- Maximum allowed concentration of heavy metals in compost/anaerobic residue (Hege *et al.*, 2003)

Heavy metal (mg kg^{-1} d.m.)	**Compost class 1* (EC)**	**Biosolids (U.S. EPA)**	**Biosolids (sewage sludge) (Slovenia)** Uradni list RS 84/2005	
			class 2 and sewage sludge for agriculture	Non agricultural use
Cd	0,7	85	2	5
Cr	100**	no limit***	150	500
Cu	100	4300	300	600
Hg	0,5	57	2	5
Ni	50	420	70	80
Pb	100	840	100	500
Zn	**200**	**7200**	**1200**	**2000**

* Such material can be utilised according criteria of good agricultural practice, without further restrictions; ** Even for Cr(III); *** Not even for Cr(VI)

2.2 ZINC

In this section the main element which will be studied in this diploma and will be analyzed like a chemical element, and also like essential plants nutrient.

2.2.1 Chemical properties

Zinc has atomic number 30 and atomic weight 65,38. It is relatively rare, being the 25th in order of abundance in the lithosphere; estimates vary from 0,005 to 0,02%. Zinc is usually found in nature as the sulfide; most other zinc minerals have probably been formed by oxidation of the sulfide. Zinc is widely distributed in the nature; it occurs in nearly all the igneous rocks, mainly as a replacement for iron. The principal commercial mineral is sphalerite, cubic ZnS, also called zinc blende or only blende (Gutierrez-Ríos, 1984).

Zinc is an essential trace element in plant and animal life. Zinc deficiency is recognized as a cause of various diseases in crops; in animals and man, deficiency may cause sexual immaturity and decreased fertility, especially in male. But these consequences due to the deficiencies and excess of Zinc in plants will be shown in the next point (Gutierrez-Ríos, 1984).

2.2.2 Zinc as an essential element

This section is based on studies done by Gutierrez-Ríos (1984).

Zinc is one of the essential micronutrients required for optimum crop growth. Plants take up zinc in its divalent form. At this time it still remains unclear whether this uptake is facilitated as diffusion through membranes specific for zinc ion or whether it is mediated by specific transporters. It has been concluded that both mechanisms operate, and about 90.5% of the total zinc required by plants moves towards the roots by diffusion. This lateral movement of zinc is highly dependent upon the soil moisture, and this may be the reason why, particularly in arid and semi-arid areas, zinc deficiency is more frequently seen.

The vast majority of zinc is present in the lattice structure of the soil and therefore, unavailable to meet the plant's nutritional requirements. Available soil zinc is dissolved in the soil solution in ionic or complex form and may be found on the exchange sites of clay minerals and organic matter. Zinc can also be found as adsorbed divalent cation, zinc hydroxide, or zinc chloride. The solubility of zinc is highly dependent upon soil pH, decreasing with high pH.

Zinc is transported in the xylem tissues from the roots to the shoots. However, high levels of zinc have been detected in the phloem tissues, which indicate that zinc moves through both transport tissues, and maybe remobilisation of zinc towards the grain during ripening. Substantial translocation of zinc takes place from the older leaves to the younger ones during grain development phase. Plants deficient in nitrogen do not show the retranslocation of zinc from the older leaves, indicating that the deficiency symptoms of zinc are more pronounced in the nitrogen deficient plants.

Table 3- Supply level of zinc (mg/kg soil) in arable soil (CAT method) (Hege *et al*., 2003)

Supply level	**mg Zn/kg soil**
A	< 1,1
C	1,1 - 3,0
E	> 3,0

Table 4.- Fertilization advice (Hege *et al*., 2003)

Soil supply level	**Fertilization to soil**	**Foliar fertilization**
	kg Zn/ha enough for 3 - 4 years	kg Zn/ha
A	7 - 10 [1)]	0,3
C	5 - 7	0,3
E	0	0

1) Lower values for light (sandy) soil, higher values for medium- to heavy soil

2.2.3 Zinc deficiency, toxicity and tolerance

- Zinc Deficiency (Brown *et al*., 1993)

Deficiency of zinc is widespread among crops grown in calcareous soils and highly weathered acid soils. The deficiencies in the calcareous soils are often associated with iron deficiency as well. Zinc deficiency symptoms in wheat appear between three to five weeks after emergence, and in rice about two to four weeks after transplanting. In severely deficient zinc soils, wheat and corn germination is poor and in these situations, seed treatment with Smartrace Zinc-Manganese or Smartrace Zinc can substantially improve seed germination and seedling vigour. Spray application of soluble zinc such as Smartrace Zinc during grain filling can improve the zinc level in seeds for better germination in such soils. The deficiency symptoms of zinc are;

1. Dusty brown spots of upper leaves of stunted plants
2. Uneven plant growth and patches of poorly established plants in the field
3. Decreased tillering, spike or spikelet sterility and interveinal chlorosis on l eaves
4. Dicots shows drastic decrease in leaf size, loss of lustre and shoots die off.
5. Premature leaf fall, chiefly in apples.

- Zinc Toxicity and Tolerance:

Excess Zn supply results in reduction of root growth and leaf expansion which is followed by chlorosis. Zinc toxicity may occur in areas particularly in the neighbourhood of Zn ore and spoil heaps.

Some plant species, however, are Zn tolerant and are able to grow in soils abnormally high in zinc. Antonovics *et al*. (1971), for example, quote Zn levels of between 600 to 7800 mg Zn/g dm of tolerant plant species growing on calamine soils. Generally concentrations in the order of 150 to 200 µg Zn/g dry matter of plant tissue are considered as toxic (Sauerbeck, 1982). Part of the zinc tolerance mechanism is dependent on the ability of the tolerant strains to bind Zn in the cell walls (Turner, 1969). In experiments with Zn Peterson (1969) observed that zinc is especially associated with the pectate fraction in tolerant ecotypes.

Some plants species and ecotypes are able to tolerate very high levels of Zn in the leaves and other upper plant parts. Wainwright and Woolhouse (1975) found almost equal levels of Zn in *Agrostis tenuis* plants growing in water culture high in Zn. The susceptible plants, however, had lost 50% of their chlorophyll, whereas the tolerant plants were not affected.

2.3 CHOSEN VARIETY FOR THE POT TRIAL - CHINESE CABBAGE

Cabbage is a very typical crop in Slovenia and this was one of the reasons for choosing this plant: This relation of Slovenia with this crop is presented by Žnidarčič *et al.* (2007) who show that Slovenia has a cultivated area of cabbage of around 450 ha, which represents around 20% of the fields in intensive vegetable production in this country and also explain that Slovenia has the favourable agroclimatic conditions for the production of cabbage varieties for fresh market and for processing.

2.3.1 Chinese Cabbage *Brassica rapa* L. ssp *pekinensis*

The following information related with Chinese cabbage has been written regarding to Maroto, 1995.

Popularly known as Chinese leaves, the hearted types of Chinese cabbage (known as Napa cabbage in the USA) form a barrel-shaped, rounded or tall cylindrical head of closely folded leaves, usually creamy to light green in colour, with crinkled texture, prominent white veining and white midribs broadening out at the base. The tall cylindrical types are generally later and slower-maturing.

“Loose or semi-headed” types are a distinct group that does not form hearts, used mainly as cut-and-come-again crops at the seedling and semi-mature stages. Varieties dubbed “fluffy tops” have beautiful butter-coloured centres of crĕpe-like leaves, ideal for salad use. All Chinese cabbages are crisp-textured with a delicate flavour.

They tolerate light frost in the open, but with cut-and-come-again treatment plants remain productive under cover in the winter months. In good conditions mature heads can be cut ten weeks after sowing. Chinese cabbage is a very thirsty crop; a single plant may need as much as 22l/5 gallons of water during its growing period.

Figure 2- Chinese cabbage plant (var. Summer Bright) (nickys-nursery, 2008)

The chosen variety for this thesis was the **Summer Bright**, due to its date for planting coincided with the realisation of the diploma.

3. MATERIALS AND METHODS

The practical part of this diploma was made from March 2008 to July 2008 at the Biotechnical Faculty of the University of Ljubljana. The different steps will be presented in the following paragraphs

3.1 PREPARATION AND STUDY OF THE MIXTURE

At the beginning sewage sludge, taken from the Domžale-Kamnik Municipal WWTP, was mixed with bark and perlite due to the physical properties of both materials.

The fractions, in volume, which formed the mixture, were: sludge (75%), pine bark (15%), and perlite (15%). The volumes were: sludge (3,5 l), pine bark (0,75 l), perlite (0,75 l).

Figure 3- 5 litres of mixture prepared for analysis

Figure 4- Barrels with the Swage Sludge

After the preparation of the 5 l of mixture, whose final weight was 2.907,0 g, the first test was done: watering the mixture in a 5 l pot and waiting until all the water which could get lost by gravity was lost. After this the sample was weighed again to measure the water capacity of the material (= "container capacity").

Figure 5- The pot with mixture and other pot with water prepared for the analysis

Figure 6- The pot with the mixture full of water losing it by gravity

Meanwhile two samples of 100 g were taken, one from the mixture and other from the sludge. These samples were used later for several analyses which will be explained in this thesis.

3.2 PREPARATION OF THE COMPOSTING PROCESS

After the determination of the water content of the mixture the second step began. This consisted of preparing the mixture for the composting process. It had the same proportions like the first mixture, that is:

- Sludge = 70% (in volume) = 2924 g
- Bark = 15% (in volume) = 212 g
- Perlite = 15% (in volume) = 82 g

The total amount of mixture was 3218 g. Four pots were prepared with about 800 g of this material. The mixture was heated in the thermostatically controlled chamber with a temperature of 30°C. The four pots were placed inside the chamber and their top was covered by paper tissues and paper bags to avoid excessive drying of the surface. This simple simulation of the composting process lasted 37 days.

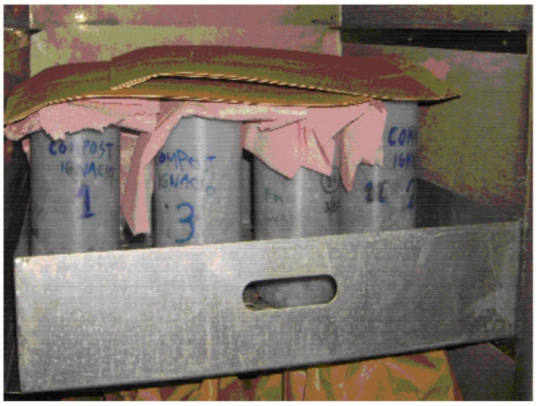


Figure 7- The four pots with the mixture inside the chamber prepared for the composting process

Figure 8- The mixture prepared and well mixed before the beginning of the process

3.3 COMPOSTING PROCESS

The prepared mixture was composted at 30 ºC for 37 days in four replicates. On the days 1, 3, 6, 12, 18, 24, 30 and 37 two average samples of 50 g were composed: around 25 g from each pot. The pots were also weighed in these same days. All the samples which were taken were immediately frozen.

Figure 9- Weighting the pots after taking the samples.

It is important to indicate that on the day 12 after taking the samples and weighting the pots, 200 ml of water were added to each pot to adjust compost to the optimal moisture content.

3.4 PREPARATION OF THE PLANTING SUBSTRATE

About 80 kg of earth were taken from a field of the Biotechnical Faculty for the preparation of the substrate in which the plants were going to be planted. It was known that on these fields no fertilizers had been used in the lasts 15 years. This was important to avoid the presence of trace elements and plant nutrients in a high amount in the soil. The vegetable parts (plant debris) found in the used soil were manually eliminated from the substrate.

Figure 10- Taking the earth from a field at the Biotechnical Faculty

Figure 11- Mixture made with soil and quartz sand prepared for being screened.

Collected soil was mixed with quartz sand; about 10% of the total amount of soil (80-90 kg) which equates 10 kg of sand. Finally the mixture was screened through 1 cm mesh and thus prepared for the pot trial.

3.5 PREPARATION OF THE POT TRIAL

This analysis consisted of the preparation of four different treatments:

1. Soil without organic amendments (fertilized with mineral NPK; the same P level as the given by non-composted sludge; N and K application according to normal fertilization advice).

2. Soil without organic amendments (fertilized with mineral NPK and mineral zinc equivalent to total amount of Zn applied with organic amendments).

3. Soil with amendment of non-composted sludge.

4. Soil with amendment of composted sludge (amount equivalent to non-composted sludge in terms of total Zn quantity).

For each treatment five pots with a plant of Chinese cabbage per pot were used. Each pot had 5 l of the soil already prepared. To prepare the treatments completely, the required amount of fertilizers and sludge needed to be calculated. In the next paragraphs these calculations will be explained.

Figure 12- Preparation of the pot trial with pots, fertilizers and plants

Figure 13 The different amendments well mixed with the soil before planting the Chinese cabbage

3.5.1 Determination of the amount of composted and non-composted sludge for the pot trial

In the case of sewage sludge (composted and non-composted) the amount of 3 Mg of sludge (D.M.)/hectare was used as a normal application. Based on this value the final amount of sludge which was going to be applied was calculated as follows:

- Calculation of the moisture of the sewage sludge. This value was 37,9%.

- The second step was to calculate the amount of dry matter of sludge which should be used for a pot of 5 l. In this case 20 cm depth was taken as reference for the typical application of organic matter in a field due to the use of superficial farm implements. Being in this case the total volume of a hectare 2000000 l.

$$3 \text{ Mg DM/ha} = (3*10^6 \text{g}/2*10^6 \text{l}) = 1{,}5 \text{ g/l} * 5 \text{ l} = 7{,}5 \text{ g of sludge (DM)/ pot} \quad \ldots(1)$$

- The next step was to calculate the amount of sludge which was going to be used based on the fraction of dry matter of this sludge.

$$\begin{array}{c} 7{,}5 \text{ g (DM)} \rightarrow 37{,}9\% \\ \text{X g} \rightarrow 100\% \end{array} \quad \ldots(2)$$

X = 19,8 g of no-composted sludge per pot

For the calculation of the amount of composted sewage sludge the process was exactly the same, taking also the value of 3 Mg of dry matter per hectare. Only changed when was necessary to use the percentage of dry matter of the composted sewage sludge. In this case the moisture was 41,2%. And the final amount which was going to be applied in the soil of the pots was:

$$\begin{array}{c} 7{,}5 \text{ g (DM)} \rightarrow 41{,}2\% \\ \text{X g} \rightarrow 100\% \end{array} \quad \ldots(3)$$

X = 18,2 g of composted sludge per pot

3.5.2 Determination of the amount of fertilizers in the pot trial

The amount of fertilizers was calculated on the base of the mineral concentration of the sewage sludge, with the aim to make equal the amendment of the studied nutrients.

- Amount of NPK: The determination of this fertilizer was based on the amount of phosphorus applied by the non-composted sludge. The analysis of this sludge showed a concentration of phosphorus of 15,15 kg/Mg. This means that for 3 Mg it was 45,45 kg of P which corresponds to 104,1 kg of P_2O_5. The chosen fertilizer was a formulated fertilizer NPK 15-15-15, obtaining then to the amount of fertilizer which should be used; 694/ha.

  The only necessary calculation to get the final amount of fertilizer NPK (15-15-15) was to transform this amount into the volume of a pot, which is 5 litres. The chosen depth was 20 cm or 2000000 litres volume per ha.
  .

$$694 \text{ kg NPK} \rightarrow 2*10^6 \text{ litres}$$
$$X \text{ kg NPK} \rightarrow 5 \text{ litres}$$

X = 1,735 g fertilizer NPK (15-15-15) per pot

- Amount of zinc: In this case first the amount of Zn was calculated and afterwards the amount of the chemical compound -ZnCl2- which was going to be used. The applied amount of zinc was the same than the applied amount with the non-composted sludge. The value of 3 Mg of sludge per hectare was taking as base, but it was necessary to know the concentration of zinc in this sludge. 3 kg of zinc per Mg of sludge should be the reference for this experiment, based on previous analyses of this sludge some years ago. Having both values and taking the same application volume than in the rest of the determinations (2000000 litres) the calculation of the applied amount of zinc of non-composted sludge was:

1 Mg Sludge = 3 kg Zn
3 Mg Sludge = 9 kg Zn

$$9 \text{ kg Zn} \rightarrow 2000000 \text{ litres}$$
$$X \text{ kg Zn} \rightarrow 5 \text{ litres}$$

X = 22,5 mg Zn per pot

This amount was transformed into values of $ZnCl_2$ which meant **46,9 mg of $ZnCl_2$ per pot**. This was a difficult work to apply this very small amount into the soil due to the product's consistence as a powder. The $ZnCl_2$ was diluted by deionized water to 0,2345 g of $ZnCl_2$/litre, applying 2 litres into each pot, in which were the 0,469 g of $ZnCl_2$.

3.5.3 Summary of the final amounts applied in the pot trial

Table 5- Amendments of fertilizers and sludge calculated for each treatment

Treatment	Amendment (in each of the five pots)
1-Soil with amendment of NPK fertilizer	**1,735 g** of fertilizer NPK (15-15-15)
2-Soil with amendment of NPK and Zn fertilizers	**46,9 mg** of $ZnCl_2$ and **1,735 g** of fertilizer NPK (15-15-15)
3-Soil with amendment of non-composted sewage sludge	**19,8 g** of non-composted sewage sludge
4-Soil with amendment of composted sewage sludge	**18,2 g** of composted sewage sludge

3.5.4 Transplanting of the Chinese cabbage into the pots

When the soil was prepared and the different additives added to the pots the transplanting of the Chinese cabbages was done. The plants had only two cotyledons and two or three leaves. The transplanting was manual and the pots were watered after the transplanting, for making easier the development of the roots.

3.6 PLANTS DEVELOPMENT

After the transplanting the plants were placed inside the greenhouse at the Biotechnical Faculty of Ljubljana, and were watered as often as it was necessary depending on the development state and the ambient temperature. The plant growing process took 72 days, since the 25th of April until the 8th of July of 2008. During this time was possible to observe differences between lines, which will be discussed in the conclusions.

Soil sampling and plant greenness measurements during the pot experiment

Soil samples of each pot were taken at the beginning and the end of the growing process, being these soil samples immediately frozen.

Figure 15- Collection of the soil samples at the end of the pot trial process

Figure 16- Samples prepared to be put in the freezer

The level of greenness was measured at the beginning and at the end of the growing process getting a measure for each treatment. The measurement of greenness was taken by an apparatus called hydro-N-tester. This apparatus works by measuring the chlorophyll content of the leaf which is related to the nitrogen status of the plant. Thirty random measurements across the treatment give an average value which is used to indicate how much nitrogen the crop requires. The N-tester measurements are strongly influenced by crop variety and growth stage. For this reason the N-tester measurements has only relative meanings – they should always be compared only among the measurements taken from the same variety, and at same light radiation conditions.

Figure 17- Hydro-N-tester measurement

3.7 PLANT HARVEST AND PROCESSING

After the growing process the aerial part of the plants were harvested. Due to the weight of the roots was, in all the cases, less than 5% of the total plant weight.

The plants were weighed immediately after the harvest. They were cut into segments and transferred to the laboratory, air dried (65ºC) and ground with achat mill (in order to avoid zinc contamination) to pass a 1 mm sieve. The samples were stored at room conditions until analyses. Total dry matter was determined by drying the samples at 10 ºC until constant weight

Figure 18- Plants cut in four parts dry in the chamber

Figure 19- Plant weighed after drying

3.8 PROCESSING OF SOIL AND SLUDGE SAMPLES

Samples of sludge compost and soil taken were frozen, for keeping their properties unalterable. When the pot trial was going to finish the preparation of these samples for being analyzed in the laboratory was done. This consisted of drying them at 30 degrees C during 24 hours, mashing and keeping them in plastic boxes. All the samples were coded and the necessary analyses for each sample were documented in the Soil Information System of the laboratory of the Centre of Soil and Environmental Science at the Biotechnical Faculty. The mashing was done manually using a pestle and mortar, and using alcohol for avoiding contaminations between samples.

Figure 20- Mashing a sample with a pestle and mortar and alcohol for avoiding contamination between samples

Figure 21- Classification and documentation of samples.. Samples prepared for being analysed

3.9 LABORATORY ANALYSES

The samples were prepared (dried and mashed) and kept and documented in the lab. But not all the amount of the sludge samples taken during the composting process were dried, part of these samples (around 5 g) were weighed and put into the oven at 105 ^{o}C for 24 hours, and then were placed in the desiccators for 30 minutes. After this time the samples without any water were weighed again, and with both measurements were possible to calculate the losses of dry matter during the composting process. These calculations will be presented in the section 5; Results.

3.9.1 Sewage Sludge Analyses

- Measurement of total Zn in sewage sludge and in compost mixture (sludge + structural agent)

  The measurements were done at the start, at the end of composting and at six times in between (day 1, 3, 6, 12, 18, 24, 30, 37). This analysis was done using the samples which were mashed, classified and kept in the lab as was explained in the point 4.7.

  The first step of this analysis was to make the extraction of zinc from the samples. This extraction was done following the protocol SIST ISO 11466 (1996):

  Scope: This International Standard specifies a method for the extraction, with *aqua regia*, of trace elements from soils and similar materials, prepared according to ISO 11464, and containing less than about 20% (m/m) organic carbon according to ISO 10694. Materials containing more than about 20% (m/m) organic carbon will require treatment with additional nitric acid. The resulting solution is suitable for the determination of trace elements using appropriate atomic spectrometric techniques. With high solute concentrations in extract solutions, spectral interferences and background enhancement should be expected.

Figure 22- Reaction vessels used for the analysis of total zinc

Figure 23- Precision scale, used to weigh the samples

Summary of the procedure: Weigh approximately 3g, to the nearest 0,001 g, of the sample into the 250 ml reaction vessel. Moisten with about 0,5 ml to 1,0 ml of water and add, while mixing, 21 ml of hydrochloric acid followed by 7 ml of nitric acid, drop by drop if necessary, to reduce foaming. Add 15 ml of nitric acid to the absorption vessel. Connect the absorption vessel and condenser to the reaction vessel and allow standing for 16 hours at room temperature to allow for slow oxidation of the organic matter in the soil.

Raise the temperature of the reaction mixture slowly until reflux conditions are reached and maintain for 2 hours, ensuring that the condensation zone is lower than 1/3 of the height of the condenser, then allow to cool. Add the contents of the absorption vessel to the reaction vessel, via the condenser, rinsing both the absorption vessel and condenser with a further 10 ml of nitric acid.

Allow the reaction vessel to stand so that most of any insoluble residue settles out of suspension. Decant the relatively sediment-free supernatant carefully onto a filter paper, then wash the insoluble residue onto the filter paper with a minimum of nitric acid. Collect this filtrate with the first.

Apparatus: Grinding Mill, Test sieve, Dessicator, Reaction Vessel, Reflux Condenser, Absorption Vessel, non-return type, Roughened glass beads, Temperature-controlled heating apparatus, Funnel and Volumetric flask.

When the extraction was finished, the measurement of total zinc was done. This analysis was done following the protocol SIST ISO 11047 (1999), Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc by electrothermal atomic absorption spectrometry.

Figure 24- Reaction vessels, reflux condensers and absorption vessels.

Figure 25- Filtering the preparation

- Measurement of CAT Zn in Sewage Sludge in compost mixture (sludge + structural agent):

  These measurements were also done at the beginning and at the end of the composting and five times in between (day 1, 3, 6, 12, 18, 24, 30, 37). This analysis was done with the same samples used for the total Zn test.

  This analysis was done following the protocol presented by Hoffman (1991), Determination of main and microelements in soils in Calciumchlorid/DTPA-Ectract:

  Principle: Extraction of the main and micronutrients N, P, K, Mg, Fe, Cu, Mn, Zn, Na and B in relation 1/ 10 (m/v) with a weak buffered Calciumchlorid/DTPA-solution (CAT) and determine the nutrients in the filtrate.

  Summary of the procedure: 5 g are taken from each sample. Then, the solution must be prepared taking 14,7 g of $CaCl_2$ and 0,88 g of DTPA (Diethylenetriamine-pentaacetic acid) and mixing both with hot water at 80 ^{o}C until having 1 l of preparation. When the solution is ready 100 ml of it were diluted in 900 ml of distillate water. Then 50 ml of this last solution must be mixed with the 5 grams of sludge sample. All the mixtures are shaken for one hour using a horizontal shaking machine. The measurement should be done latest one day after the filtration or the filtrates should be conserved in the cooling chamber.

- pH measurement:

  This measurement was done following the protocol SIST ISO 10390 (1996):

  Scope: This International Standard specifies an instrumental method for the routine determination of pH using a glass electrode in a 1:5 (v/v) suspension of soil in water (pH-H_2O), in a solution of 1 mol/l potassium chloride (pH-KCl) or in a solution of 0,01 mol/l calcium chloride (pH-$CaCl_2$).

  This International Standard is applicable to all types of air-dried soil samples.

  Summary of the procedure:

  - Preparation of the suspension: Take a representative test portion of at least 5 ml from the laboratory sample using the spoon. Place the test portion in the sample bottle and add five times its volume of water potassium chloride solution or calcium chloride solution. Shake or mix the suspension vigorously, for 5 minutes, using the mechanical shaker or mixer, and wait at least 2 hours, but not longer than 24 hours.

  - Calibration of the pH-meter: Calibrate the pH-meter as prescribed in the manufacturer's manual.

- Measurement of the pH: Adjust the pH-meter as indicated in the manufacturer's manual. Measure the temperature of the buffer solution and the soil suspension thoroughly just before measurement of the pH. Measure the pH in the settling suspension. Read the pH after stabilization is reached. Note the recorded values to two decimal places.

- Determination of Total Nitrogen:

  This analysis was done using titanium dioxide as catalyst (ISO 11261)

  Scope: Organic nitrogen in the sludge is digested and oxidised to ammonium. Nitrate-N, and nitrite-N are reduced to ammonium-N. Ammonium-N already present in the sludge as well as ammonium-N formed by the reactions is determined by the procedure total soil N.

  Summary of the procedure: a portion of air dried sludge sample of about 0,2 g-1 g is placed into the digestion flask. Salicylic/sulphuric acid is added and thoroughly mixed with soil. The mixture stays several hours, and then sodium thiosulphate is added. The mixture is heated cautiously until the frothing cease. A catalyst mixture is added to the cool flasks. The heating is repeated until the digest clears. The mixture is boiled for several hours until the sulphuric acid condenses about 1/3 of the way up to the neck of the flask. After the digestion the content of flask is transferred into the distillation apparatus. Boric acid and than sodium hydroxide are added slowly. Indicator (bromcresol green, methyl red, ethanol) is added to the obtained distillate, which is then titrated with 0,01 M sulphuric acid to a violet end point.

- Determination of soluble nitrogen forms (NO_3-N, NH_4-N, N_{total}-N, N_{org}-N) (SIST ISO 14255; Houba et al, 1999):

  Sample (sludge) extraction is made by shaking (2 hours) the portion of sludge sample in 0,01M $CaCl_2$ (1:10 soil : solution - w/v). After the extraction, the solution is centrifuged (10 min.; 1800 g). Clear supernatant is used for the analyses. Extracted samples are stored in a freezer (at 4ºC) up to one week. If analyses are not performed within a week, then the extracts are stored deep frozen (at least -18ºC). Prior to the analyses, frozen samples are thawed at room temperature over night.

  Determination of soluble nitrogen forms are proceeded by flow injection analyses (FIAS Perkin-Elmer 3000; Müller et al., 1992).

  Ammonium-N determination: sample (soil extract) is mixed with the solution of sodium hydroxide (NaOH) in a short reaction coil (200 μL 400 μL: depends upon the concentration of ammonium-N). Ammonium presented in the extract is converted to ammonia gas which penetrates a gas-diffusion membrane (teflon) in a special on-line diffusion cell. Ammonia is fused with a parallel flow of the acceptor (acid indicator: bromcresol violet, bromothymol blue, cresol red and potassium hydroxide, and alkali reagent: EDTA Na-salt + boric acid; pH = 13,5). A colour is developed (red) and its intensity is measured by spectrophotometer (Perkin-Elmer Lambda 2) at 590 nm (Müller *et al.*, 1992).

  Nitrate-N (+ nitrite-N) determination: soil extract is reduced in a Cd-Cu column to a nitrite-N, which forms a diazo- compound in an acid solution with sulphanilamid. In a combination with N-(1-naphtil) ethylendiamin dihydroxide a red/violet colour is formed. The colour intensity is measured at 540 nm (Müller *et al*., 1992; Möller, 1988; Tecator: Application Note 62/83; Tecator GmbH, 6054 Rodgau 6).

- Determination of total carbon:

  This analysis was done following the protocol ISO/DIS 10694 (1994):

  Scope: This International Standard specifies a method for the determination of the total carbon content in soil after dry combustion. This International Standard is applicable to all types of air-dry soil samples.

  Summary of the procedure:

  - Calibration of the apparatus: Calibrate the apparatus as described in the relevant manual. For the purpose of calibration or establishing a calibration graph.

  - Determination of the total carbon: The amount of test portion taken into analysis depends on the expected total carbon content and on the apparatus used. Weigh out the air-dried sample in a crucible. Carry out the analyses in accordance with the manufacturer's manual of the apparatus.

- Electrical Conductivity:

This analysis was done following the protocol SIST ISO 11265 (1994):

Scope: This International Standard specifies an instrumental method for the routine determination of the specific electrical conductivity in an aqueous extract of soil. The determination is carried out to obtain an indication of the content of water-soluble electrolytes in a soil.

This International Standard is applicable to all types of air-dried soil samples.

Summary of the procedure:

- Extraction: Weigh 20 g of the laboratory sample and transfer to asking bottle. Add 100 ml of water at a temperature of 20^{o}C ± 1^{o}C. Close the bottle and place it in a horizontal position in the shaking machine. Shake for 30 minutes. Filter directly through a filter paper. Carry out a blank determination in the same way. The value of the blank shall not exceed 1 mS/m. If the value of the blank exceeds this, repeat the extraction.

- Checking the cell constant: Measure the conductivity of the potassium chloride solutions according to the instruction manual of the instrument. Calculate for each potassium chloride a cell constant. Use the average of the calculated values as the cell constant of the instrument. The calculated cell constant shall not differ by more than 5% from the value given by the manufacturer. Adjust the cell constant on the conductivity meter.

- Measurement of the electrical conductivity of the filtrates: Measure the electrical conductivity of the filtrates according to the instructions provided by the manufacturer of the conductivity meter. Carry out the measurements with the temperature corrected to 25^{o}C. Note the results to one decimal place, expressed in millisiemens per metre.

3.9.2 Soil Analyses

Some Analyses were also done using the soil samples taken at the beginning and at the end of the pot trial.

- Total Zn and CAT Zn at the beginning and at the end of the pot trial:

  This analyses were done identically like in the case of sewage sludge following the protocol SIST ISO 11466 (1996) in the case of the total zinc and the protocol presented by Hoffman (1991) in the case of the CAT zinc. The only difference was the amount of sample necessary in the total Zn analysis, in which 5 grams of each sample were added instead of 3 grams in the case of the sludge.

- pH measurement at the beginning of the pot trial:

  In this case the followed protocol was the SIST ISO 10390 (1996) as well as I the case of the pH measurement done with sewage sludge.

3.9.3 Plants Analyses

After the plants were harvested and dried, they were transported into the laboratory where they were mashed and classified. Afterwards the zinc content was determined. This analysis was done following the protocol SIST ISO 11466 (1996) which has been already explained for the sewage sludge.

3.10 CALCULATIONS AND STATISTICAL METHODS

Two software programmes were used for the realisation of this diploma: Microsoft Excel and Statgraphics.

Microsoft Excel was used for preparation of all the tables and graphics shown in the diploma. In the case of the graphics, some of them were created using averages of the different values obtained during the pot trial, in these graphics the standard deviation of these group of values is also shown with the aim of determining the statistical significance of the differences.

The statistical programme Statgraphics was used for the analyses of variance (ANOVA) presented in the appendices. In this case the statistical significance of the data is studied, confirming id the values are statistically reliable

4 RESULTS AND DISCUSSION

In this section the results of the analyses, done during this diploma, as well as the discussions developed from these results will be presented. First, the results will be divided into three groups according to the origin of the analysed material; composting process, pot trial and harvest.

4.1 ANALYSES IN COMPOSTING PROCESS

4.1.1 pH, Electrical Conductivity, Total C, Total N, NO_3^- and NH_4^+

Some characteristics of the mixture used for composting at the beginning and at the end of the process will be studied in the following lines.

Table 6- Chemical characteristics of the composting mixture before and after composting

	pH	EC (mS/cm)	Ctot (%)	Ntot (%)	NO_3-N (mg/l)	NH_4-N (mg/l)
Compost Day 1	**7**	**1,15**	**28**	**2,3**	**11**	**5,6**
Compost Day 37	**6,9**	**1,71**	**25,2**	**2,2**	**42,5**	**23,5**

a) pH

PH is a measurement of the concentration of hydrogen ions in a solution expressed as a negative logarithm.

The pH of most biosolids (whether liquid, solid or semisolid) is generally in the range of 7 to 8, unless lime is added during the wastewater treatment process (Epstein, 2003)

The pH value at the beginning of the process was 7, this equals a neutral level; and it is quite good for the decomposition of organic matter because as is written by Taiwo and Oso (2004) compost decomposes fastest with a pH of around 6.5 (slightly acidic).

The pH level at the end of the process was 6,9. This is a good level for a compost to be added to plants because the availability of nutrients is good with neutral levels of pH as is explained by Jugsujinda and Patrick (1977).

b) Electrical Conductivity

Electrical conductivity is a measurement of soluble salt content. High salt levels can be harmful to germinating seeds and plants when the compost is a component of the growing medium. The desired ranges may not apply when compost is used as an amendment because of the diluting effect of mixing the compost with soil (Hanlon *et al*., 2002).

In the studied case the level of EC at the end of the composting process amounts **1,71 mS/cm**, which is a low level even for sensitive plants

c) Total C

Total carbon (C) describes a direct measurement of all organic and inorganic carbon in the compost sample. In the case of the sewage sludge the rate of total carbon was 28%. The final material obtained at the end of the composting process had a content of total C of 25,2%.

d) Total N

Nitrogen is an essential nutrient for plants. When compost is applied to land, organic nitrogen undergoes numerous transformations. These transformations are extremely important as they affect plant growth, microbial activity, and reactions through the soil. These transformations are affected by several conditions in the soil and the compost: moisture of soil, rate of mineralization, soil porosity, temperature, etc. Total nitrogen (N) includes all forms of nitrogen: organic N, ammonium N (NH_4-N), and nitrate N (NO_3-N). Total N will normally range from less than 1 % to around 5 % (dry weight basis) in most sewage sludge and from 0.5 to 2.5% (dry weight basis) in finished composts.

In the case which is being studied in this diploma, the content of nitrogen is 2,2% at the end of the composting process, this means a normal level of nitrogen, inside the range presented by Epstein (2003) in his study of 38 sewage sludge in USA and which was between 0,5% and 7,6%.

e) C/N Ratio

Using the values of total N and total C the C:N ratio is presented in the table below, as well as the change of this ratio after the composting process.

Table 7- C/N Ratio at the beginning and at the end of the composting process

	Ctot (%)	Ntot (%)	C/N Ratio
Compost Day 1	28	2,3	**12,17**
Compost Day 37	25,2	2,2	**11,45**

As can be observed in the table the relation of C:N is smaller than the normally recommended for a good quality compost mixture at the start of composting process, being this value usually between 20 and 30. Although this doesn't mean that the composting process is not possible, this is only the best value for having a good composting without major N losses via volatilization. On other hand the value of this ratio after the composting decreased for 0,72 which means a decrease of 5,91%. And this value is normal for sewage sludge as is indicated by Wortmann C. (2006) showing in his work that the normal ratio C:N for this material is between 5 and 16.

f) NH_4-N

This is one of the forms resulted from the mineralization organic N, the formation NH_4-N is the first step of this mineralization and is called ammonification. Plants are able to absorb this N-form

g) NO_3-N

NO_3-N is the mineralized form of organic N which is formed after NH_4-N and NO_2-N by a process termed nitrification. Depending on the conditions of the soil the transformation of NH_4-N into NO_3-N can have higher or lower speed.

Considering NH_4-N and NO_3-N values it can be considered the amount of total nitrogen on a normal level and these two forms in a low amount, the source of nitrogen from organic form into mineral forms will be available for a long time. Although the amount of mineral nitrogen or in other words available nitrogen, is very low. This can mean a problem for the plants which will be not fertilized with nitrogen fertilizers.

h) Relation between NO_3-N and NH_4-N

This relation between NO_3-N and NH_4-N is one of the indicators of maturity and quality of composts. Iglesias-Jimenez et al. (2008) explain how a higher concentration of NO_3-N in comparison to the concentration of NH_4-N is a good indicator of the mature state of the compost.

In the case of the composting process developed in this diploma the relation NO_3-N/NH_4-N was since the beginning higher than the unity.

4.1.2 Total Zn in sewage sludge in compost mixture during composting process

This analysis consisted of measuring the content of zinc in all the samples taken during the composting process. Average of total zinc content was done for each sampling date. In the Fig. 28 it is possible to see the tendency of the zinc content in the composting mixture during the 37 days of process. A tendency line is presented to see better the zinc content changes.

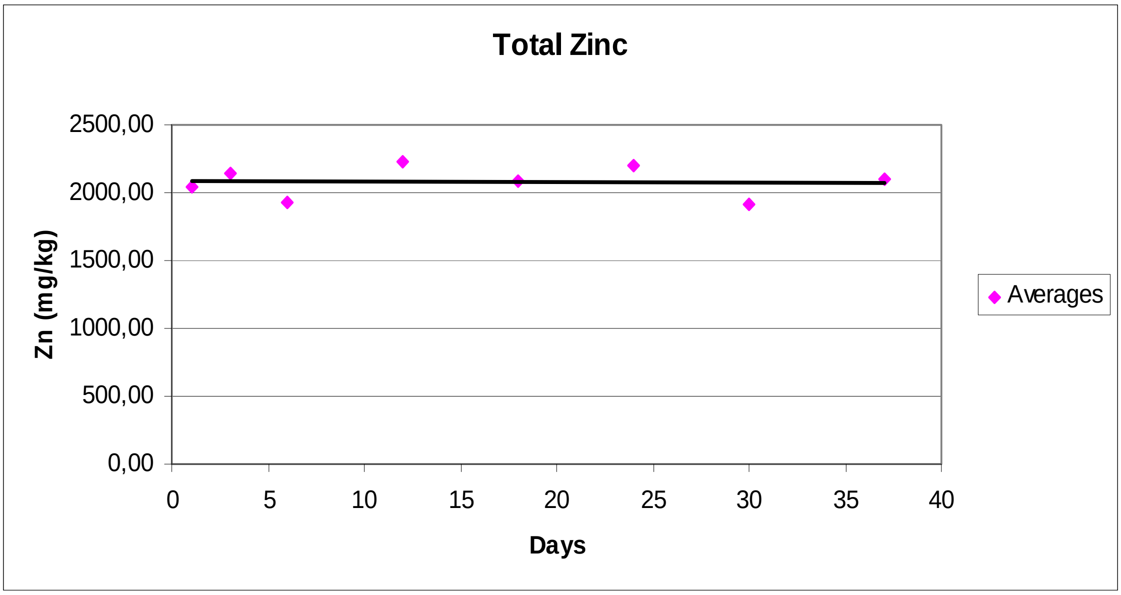


Figure 28- Total zinc during composting

In the table below a statistic study of the values is presented.

Table 8- Statistic study of Total zinc during composting process

Standard deviation	113 mg Zn/kg compost
Total average	2080 mg Zn/kg compost
Standard deviation (percentage)	5,5 %

Based on this first analysis it is possible to discuss the idea presented in the point 3.5.2, where the calculation of the amount of fertilizers is shown. In the case of the amendment of Zn fertilizer the amount was calculated with the means of the concentration of Zn in the non-composted sewage sludge as reference. The decision was to take 3000 mg Zn/kg dry matter (dm) as the concentration of zinc in the non-composted sludge, based on analyses of sludge from the same WWTP some years ago. Now it is possible to see that this prediction was incorrect, because as is presented in the table Appendix A the final concentration of zinc after 37 days of process was 2097 mg zinc/kg dm.

The total amount of Zn of this compost is very high, according to results of other works related to the composting of sewage sludge. This is the case of Topcuoglu and Onal (2007) who analyzed non-composted sewage sludge of different WWTP from the region of Antalya (Turkey) and had average results of 1220 mg Zn/kg dm instead of 2097 mg Zn/kg dm which is the result obtained in this diploma. Also Gondek and Filipek-Mazur (2006) obtain a result of total zinc content of 419 mg Zn/kg d.m for a composted sewage sludge taken from a WWTP in Poznan (Poland).. Also the maximum accepted levels of total zinc for sewage sludge, shown in table 2, confirm the assumption that the total amount of Zn of this compost is too high. It would be only accepted by the USA law, not in Slovenija, there even not for non agricultural uses.

On the other hand, reminding to the tendency of the zinc concentration during the composting process in the figure 28 it is clear that the concentration of zinc in the composting mixture didn’t change significantly during the approximately 5 weeks of process. The final amount of zinc increased for 55,3 mg Zn/kg, which means in percentage a 2,7 % of the initial amount. This result can be explained by the work of Barker and Bryson (2002) in which the authors explain that metallic contaminants are not lessened quantitatively during composting and because of the loss of carbon during composting, the concentrations of metallic contaminants in solid wastes may be increased. However, as it will be presented in the next section, the bioavailability of metallic contaminants may be lessened by composting.

The results obtained in this analysis can be compared with the results gotten by Jakubus (2003), who calculated the total content of zinc at the beginning and at the end of the composting process in sewage sludge. In this case the time used for the composting was 6 months instead the 37 days used in the case of this diploma. The results were: 1024 mg Zn/kg at the beginning and 1104 mg Zn/kg at the end of the process. This means an increase of 80 mg Zn/kg or 7,81 % talking in percentage. In this work the increase of the total Zinc was due to the high presence of the next fractions in the sewage sludge: bonds with amorphous Fe oxides, residual and mobile fractions, in which the increase of total zinc content happened. Knowing that the time of composting was longer in this experiment, the results can be considered similar.

Standard deviation from the average value of total Zn during the composting process was rather low (5,5%; Tab. 8). This was necessary because the variance of the values in the graphic is very high,. This is normal when the tendency is completely constant (horizontal).

4.1.3 CAT Zn in sewage sludge in compost mixture during composting process

CAT Zn results represent the amount of available zinc for the plants. In this case analyses were done using the samples taken during the composting process as well as in the case of Total Zn. Averages for each day were done and with them the tendency presented in the graphic below was calculated.

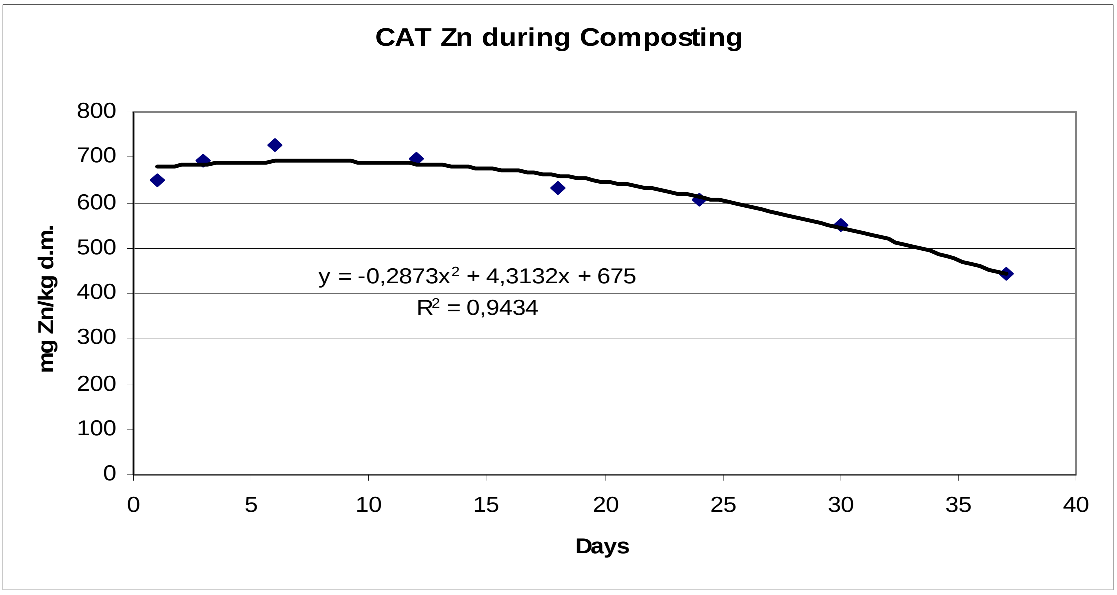


Figure 29- CAT Zn during composting process

Observing the results of the analysis and its graphical representation it is possible to develop some ideas related with the amount of CAT Zn at the beginning and at the end of the composting process as well as during it:

- First of all, the values of zinc concentration are extremely high, as it was already known due to the characteristics of the effluent of the WWTP from where was taken the sewage sludge. This affirmation can be confirmed checking some articles published related with this topic. For instance Topcuoglu and Onal (2007) remind to values around 70 mg available Zn/kg d.m. of composted greenwaste, really far from the value obtained from the studied sludge.

- On the other hand it is easy to observe in the figure 29 presented in this analysis, that there is a decrease tendency in the amount of the available zinc. This is an important result because in this case the first **hypothesis** presented in the section 1 (hypotheses) is thus being **confirmed**. This reduction in the amount of soluble, available fraction of zinc is explained by the degradation of organic matter during the composting as is shown by Krebs et al. (1998) who meant the relation between the decrease of availability of heavy metals during composting and the formation of the humic fraction as a direct relation. Also Amlinger (2002) explains that the availability of all metals decreases after the first week of composting, and this also happened as he studied in the case of Cd and Zn. His conclusion was that the humic fraction of the dissolved organic carbon (DOC) and the insoluble organic complexes play an essential role for the removal of metals from soluble phase of soil.

4.1.4 Loss of Dry Weight

In these paragraphs the losses of dry weight measured during the composting process will be studied. The loss of weight in a composting process means production of metabolic H_2O and CO_2. This production of CO_2 is due to the mineralization of O.M. and it's subsequent transformation into humus, this process is called humification (García-Gomez, 2005).

In the figure 30 all the weight changes are shown. As can be seen in the case of the day 12, a big change happened, this is due to the amendment of 200 ml of water to each of the four pots in which the composting was done. In this graphic the changes in the dry fraction of the total weight are also shown.

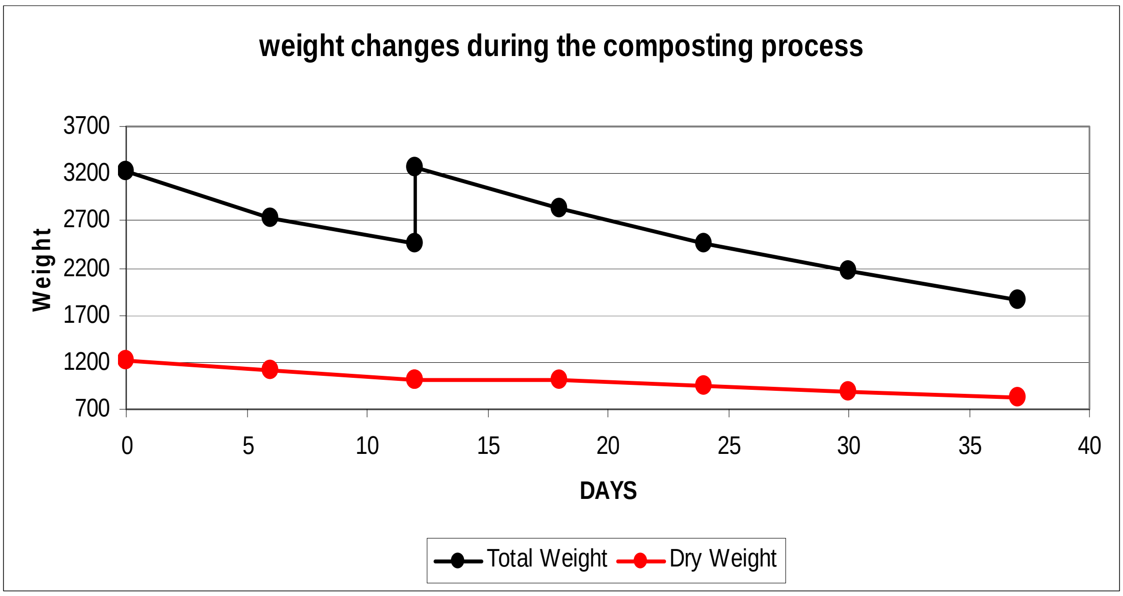


Figure 30- Weight changes during the composting process

The figure 31 shows the loss of dry weight during the composting process. The graphic 34 represents the same measurement but in an accumulative way.

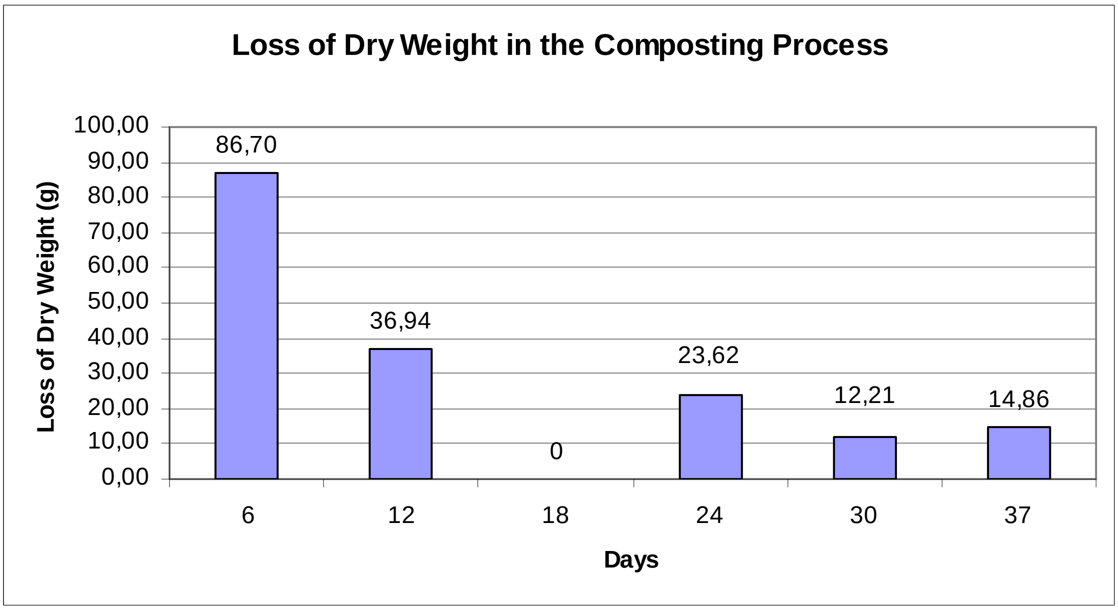


Figure 31- Loss of Dry Weight in the composting process

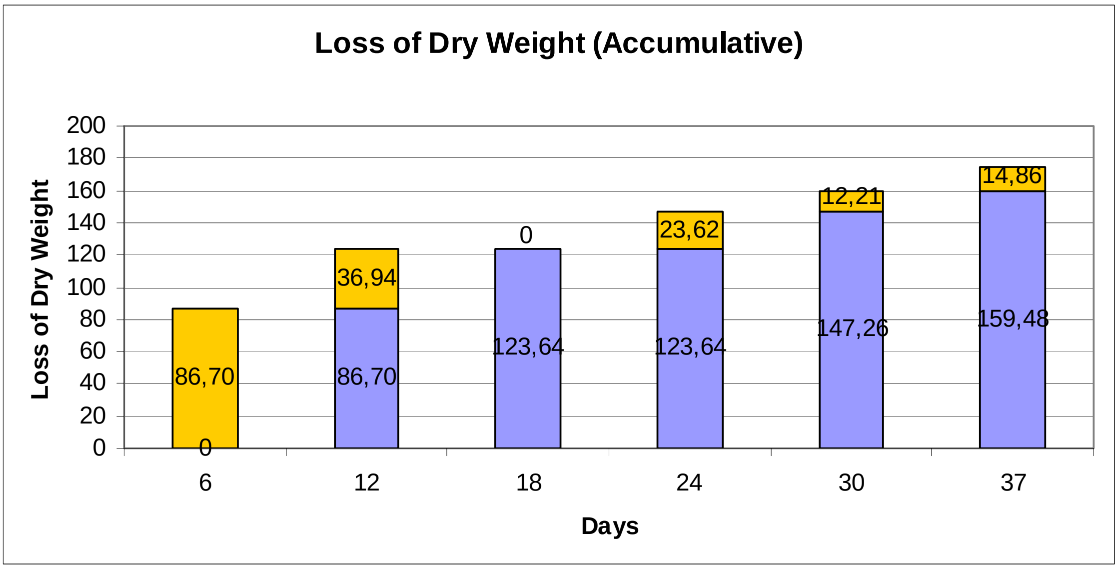


Figure 32- Accumulative losses of Dry Weight (g); yellow bars indicate which was the amount lost in each measurement

In this section in which the loss of dry weight during the composting process is being studied, some aspects can be discussed. For example the necessity of adding water to mixture the day 12. This amendment of water was necessary due to the dryness of the air in the chamber in which the pots were located for the realisation of this experiment; although some issues were put on the pots with the aim of protect the mixture of this dry air, as can be seen in the figure 7, but this was not enough as it is possible to observe in the appendix D.1 where the moisture of the composting matter is shown. This amendment of water had as consequence the inexistence of losses of dry matter during the period between the day 12 and the day 18, all the apparent lost weight during these days was H_2O.

Regarding to the losses of dry matter, as can be seen in the figures of this section the amount of lost dry matter is becoming smaller with the time, being the lost dry matter during the first week the 49,7 % of the total. The loss of dry matter (175 g) during the composting process meant a 14,4 % of the total amount of dry matter at the beginning of the experiment.

- Relation between loss of dry weight and changes in the availability of zinc in the composting mixture:

Once the losses of dry weight and the CAT Zn values during the composting process have been studied, it is possible to discuss the relation between both factors. Comparing the decrease of availability and the losses of dry weight a direct relation between them can be seen. This is due to the loss of dry weight means formation of humic fraction and this fraction acts decreasing the availability of heavy metals, as is the case of Zn. This transformation has been explained in the section 4.1.3.

4.2 POT TRIAL AND SOIL ANALYSES

4.2.1 Total zinc analyses of soil at the beginning and at the end of the pot trial

The graphic below represents the concentration of total zinc in the soils used for the pot trial at the beginning and at the end of this time.

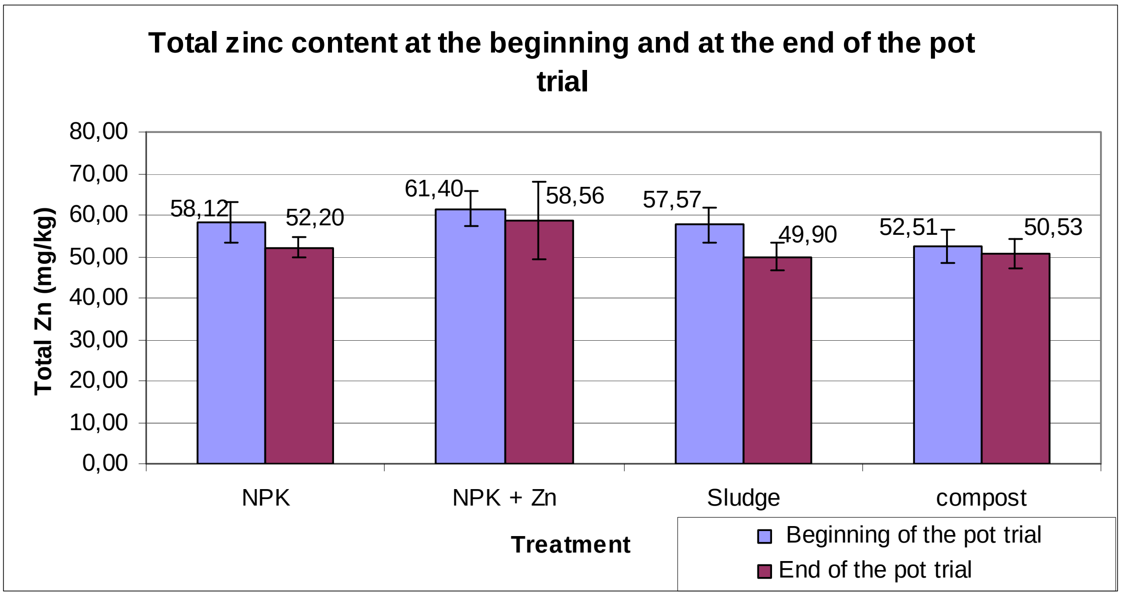


Figure 33- Total zinc in soil at the beginning and at the end of the pot trial

The first observation which is necessary to do looking at this graphic is the high concentration of zinc in the case of the soil without any amount of zinc fertilizer or sewage sludge. Looking at this value the theoretically assumed low level of zinc in this soil is confirmed as false. It is also strange that the value of total zinc for this soil is higher than in the soils with amendment of organic fertilizers. This can have same explanations, like some mistake in the take of the sample or the analyses; although the differences with the values of the soils with organic amendment are not statistically significant. For this it is possible to consider them in the same level.

Comparing the other three experiments, the soil with $ZnCl_2$ treatment has the highest concentration of total zinc; this is explained by higher amendment of zinc comparing with the other treatments. The lowest concentration of total zinc is found in the case of composted sewage sludge, being the soil treated with non-composted sludge between the both values.

The values of total zinc content are in a normal range, as can be confirmed comparing them with values shown in similar works. For instance, the results presented by Gondek K. and Filipek-Mazur B. (2006) show a value of 73,5 mg Zn/kg, a bit higher than the concentrations presented in this diploma. This value was obtained from analyses done in soils with amendment of composted green waste. Other case on which it is possible to base the affirmation of normal level of total zinc in the soils used for the pot trial is the information presented by Brinton (2000) who estimates the typical values for soils between 14 and 125 mg Zn/kg.

The changes in the concentration of total zinc from the beginning to the end of the pot trial are different depending on the treatment done in each treatment. As can be seen in the figure 33, the decrease of total zinc for the non-composted sewage sludge has been bigger than for the other treatments, being the smallest decrease the observed for the composted sewage sludge, very close to what happened in the soils with amendment of mineral zinc fertilizer. As can be seen in the mentioned graphic, at the beginning of the pot trial the lowest total zinc level was for the compost soil, but at the end this was the case with the soil treated with non-composted sewage sludge. This higher decrease in the case of the fresh sewage sludge could be due to the higher transformation of organic matter into the humic fraction during the pot trial

As can be seen in Appendix C.4 an analysis of variance was done with the aim of determining the quality of the results. Based on this analysis it is possible to say that the statistic difference between values can be accepted as significant.

4.2.2 Available zinc in soil at the beginning and the end of the pot trial

The aim of this analysis was to know the amount of available zinc in each of the four treatments taking samples and making the analyses of the 20 pots which formed the pot trial. This analysis was done in samples taken at the beginning and at the end of the experiment, trying to study the changes in the fraction of available zinc.

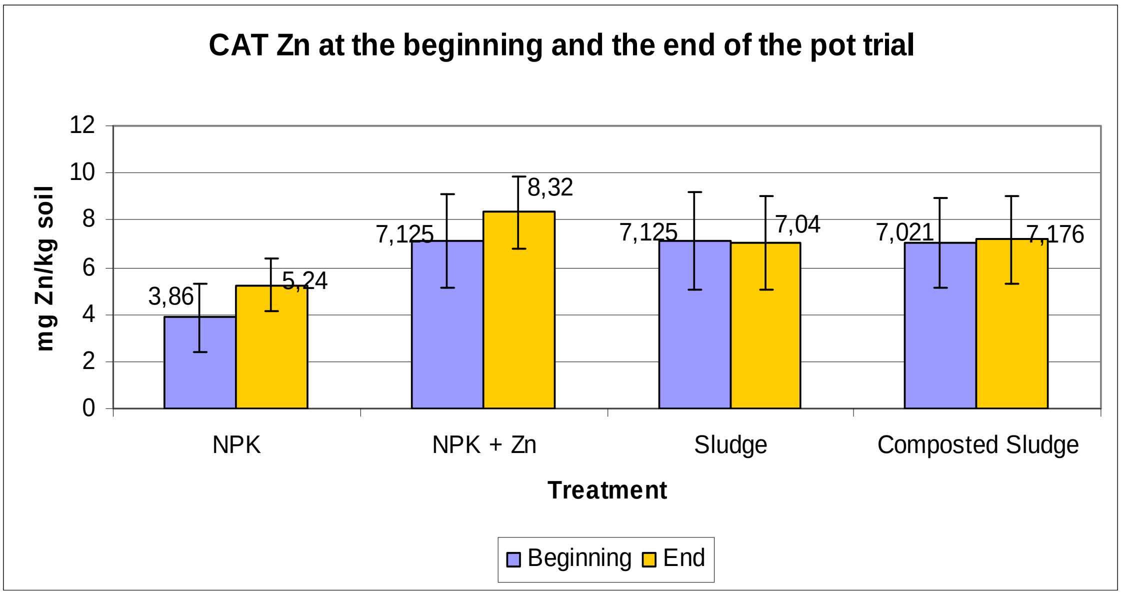


Figure 34- Development of the CAT Zn amount in each experiment during the pot trial

First of all, as can be seen in the graphic above, the amount of available zinc at the beginning of the pot trial is very similar between the three treatments which amendment of zinc. The lowest value corresponds to the soil with amendment of NPK fertilizer, due to no amount of zinc was added to this soil. However the amount of available zinc should be lower in this case, but as has been discussed in the section before, the soil which was taken at the Biotechnical Faculty of Ljubljana and which was considered free of zinc, had a relatively high concentration of this heavy metal; anyway the amount of available zinc was smaller than in the other three treatments.

The different amounts of available zinc in these four treatments have high values, as can be proofed looking at similar analyses in other studies, one of these cases is the work done by Hanč et al. (2002) in which three different soils where fertilized with 3 different sewage sludge. In this case the analyses of available zinc in the soils gave values much smaller than in the case of this diploma, these where; 0,028, 0,095 and 0,322 (mg Zn/kg).

On the other hand, observing the figure 34 which shows the changes of the availability of zinc after the 72 days of pot trial, it is possible to distinguish the treatments into two groups, one formed by the experiments with amendment of composted and non-composted sludge and the other formed by the amendment of chemical fertilizers. In the first group the tendency is constant, that is the amount of available zinc didn't change significantly during the pot trial. But in the case of the experiments with inorganic fertilizers the tendency was to grow up, in very different levels due to the initial concentration but similar in percentage. These differences of behaviours of availability of zinc have several explanations. For instance, Hanč A. *et al.* (2002) write about the relation between availability of zinc and humic fraction in the soil, as well as the changes in the soil pH. It is positive for the prevention of zinc availability a high content of humic compounds and a middle-acid pH.

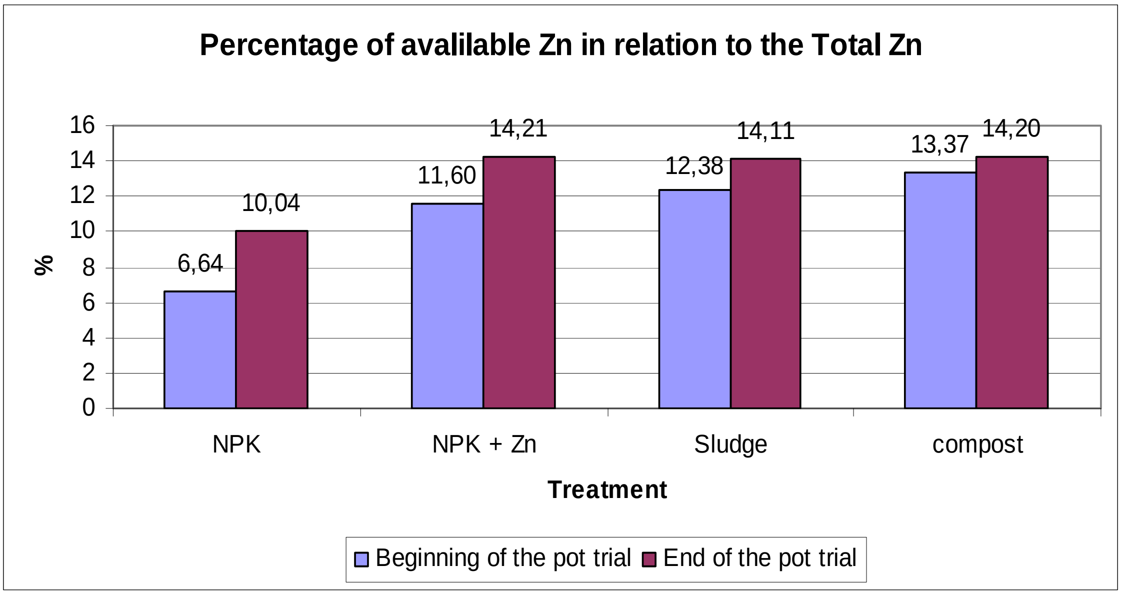


Figure 35- Percentage of available Zinc respect to the total at the beginning and at the end of the pot trial

The figure 35 shows the changes of available fraction of zinc in relation to the total zinc at the beginning and at the end of the pot trial. As can be seen in the graphic above the lowest increase of available zinc in percentage happened in the case of the soil treated with composted sewage sludge. This result gives the opposite idea made using as reference the figure 34, where the highest decrease of available zinc in absolute figures happened in the case of the soil treated with non-composted sewage sludge. This difference between both graphics is because of the different decrease of total zinc in both treatments. In the figure 33 it is possible to observe how the decrease of total zinc is much bigger in the soil with non-composted sludge than in the soil with composted sludge, this difference makes that the changes in percentage give different results comparing with the absolute values.

An analysis of variance was done with the aim of determining the quality of the results of CAT zinc as well as in the case of Total zinc. Based on this analysis it is possible to say that the statistic difference between values can be considered as significant.

4.3 PLANT ANALYSES

4.3.1 Wet Weight, Dry Weight and Dry matter percentage after harvest

After cabbage was harvested, the plants were measured without their roots, as was explained in the section 3.6. After 24 hours drying the plants were measured for second time. With these two measures was possible to obtain the value of dry percentage.

In the following graphics the three values obtained from the cited measurements are shown comparing them between the four treatments which were studied in the pot trial.

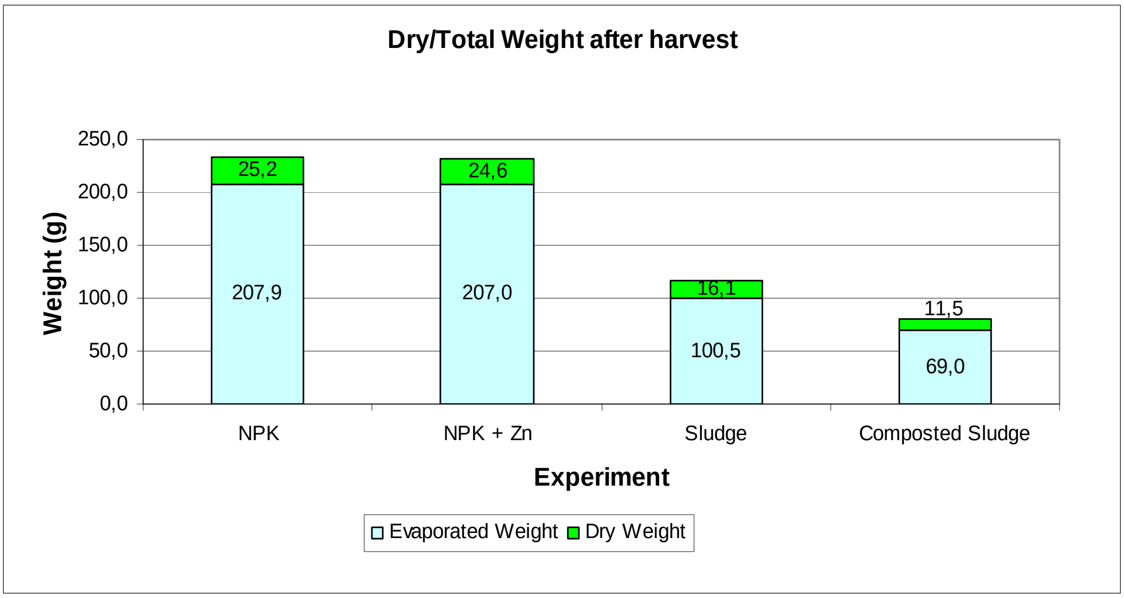


Figure 36- Comparison between total and dry weight after harvest

Based on the graphic above the difference level of yield of the four treatments can be analyzed. As can be seen the NPK treatment produced the heaviest plants. However the weight of the plants of the second treatment (NPK + Zn) is very close to the weight measured for the NPK treatment. The treatments with amendments of non-composted and composted sewage sludge produced significantly lower yields. In the case of the non-composted sludge the yield is significantly higher than the development of the plants of the composted sludge.

This higher development in the two first treatments is due to the amendment of the NPK fertilizer which makes these important elements available for the growth by the plants.

Regarding to Raigón *et al.* (2005) the time necessary to grow Chinese cabbage (var. Summer Bright) is around 75-80 days and its final weight around 1,5 kg. Although the growing period of the studied plants consisted of 72 days, it is possible to observed their low development, most of all in the cases of the plants treated with organic amendments, or in other words without amendment of NPK fertilizer.

4.3.2 Total Zn in biomass after cabbage harvest

Zinc content in biomass at the end of the pot trial was measured using the plants after being harvested and dried in the oven. This results are shown in the graphic below.

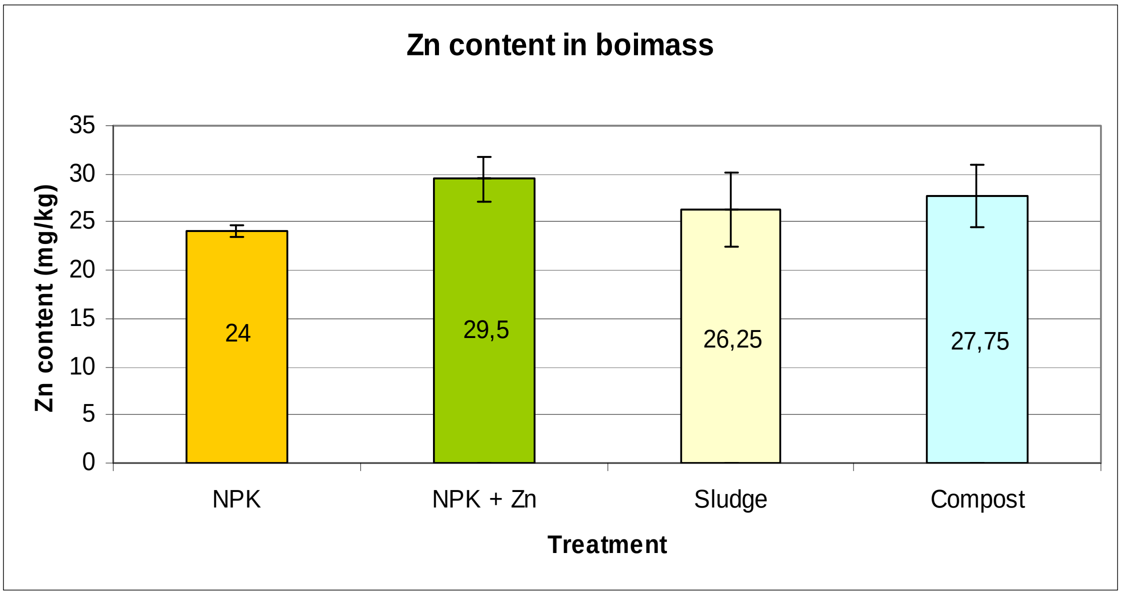


Figure 37- Zn content in biomass after harvest

This analysis is the last one referred to the zinc bioavailability. In the figure 37 can be seen the different zinc contents in cabbage plants, expressed in mg of zinc per kg of dry matter. The cabbage plants which were planted in soil without any amendment of zinc (NPK treatment) have the lowest content. On the other hand the plants which grew with amendment of $ZnCl_2$ have the highest content. These two values were in a normal range for vegetative plant parts, as it is shown by Jara-Peña *et al.*, 2002 regarding to normal ratios between 10-100 mg Zn/kg. In the case of the two treatments with amendment of sewage sludge the plants which grew in soil with non-composted sludge have lower zinc content than the plants which grew with composted sewage sludge, but the difference is small and not statistically significant.

Based on this analysis the third hypothesis can not be confirmed or denied, because the values obtained with this analysis have not significant differences. Although the uptake of zinc was higher by the plants which grew with compost than the plants which grew with amendment of fresh sewage sludge, their differences are not big enough to have a clear conclusion.

There is also an interesting correlation between available zinc in soil and zinc content in biomass as it can be seen in the table 9. This means that there is a relation between availability of zinc in the soil and uptake of this element by the plants. This relation can be also observed in the figure 38 seeing how with more available zinc in the soil the zinc content in the plants is also bigger.

Table 9- Correlation between Availability of Zn and content in biomass

Treatment	Available Zn (mg Zn/kg)	Zn content in Biomass (mg Zn/kg)
NPK	**5,2**	**24**
NPK + Zn	**8,3**	**29,5**
Sludge	**7,0**	**26,2**
Compost	**7,2**	**27,7**

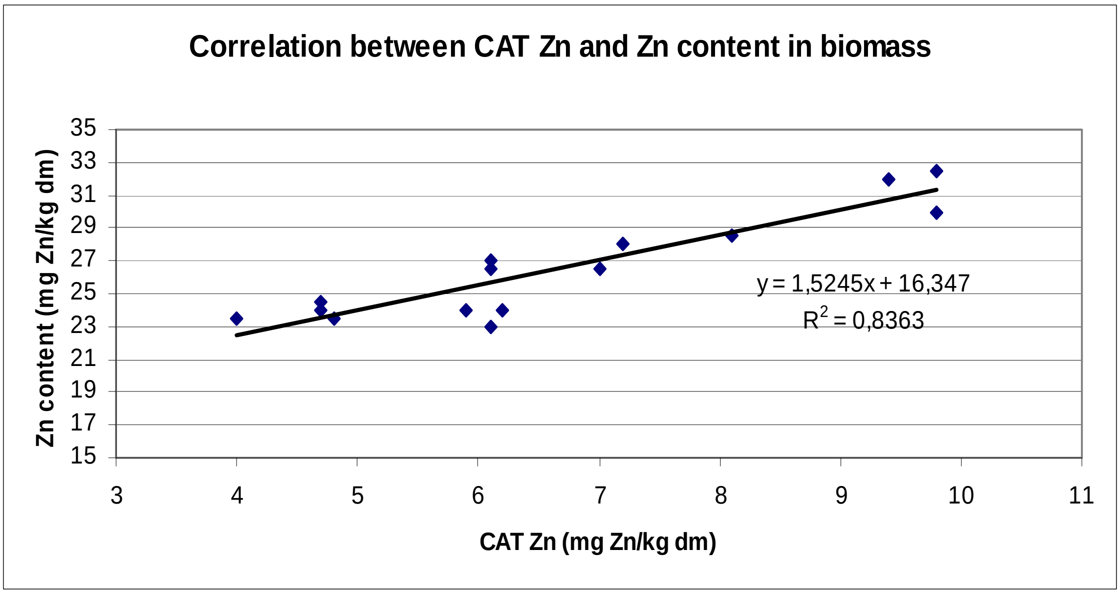


Figure 89- Correlation between CAT zinc in soil and zinc content in biomass

4.3.3 Leaves' greenness. Hydro N-tester measurement

Last analysis presented in this diploma is the measurement of the greenness of the leaves of the Chinese cabbages used in the pot trial. These measurements were done two times as it is possible to observe in the graphic below; at the beginning and at the end of the pot trial

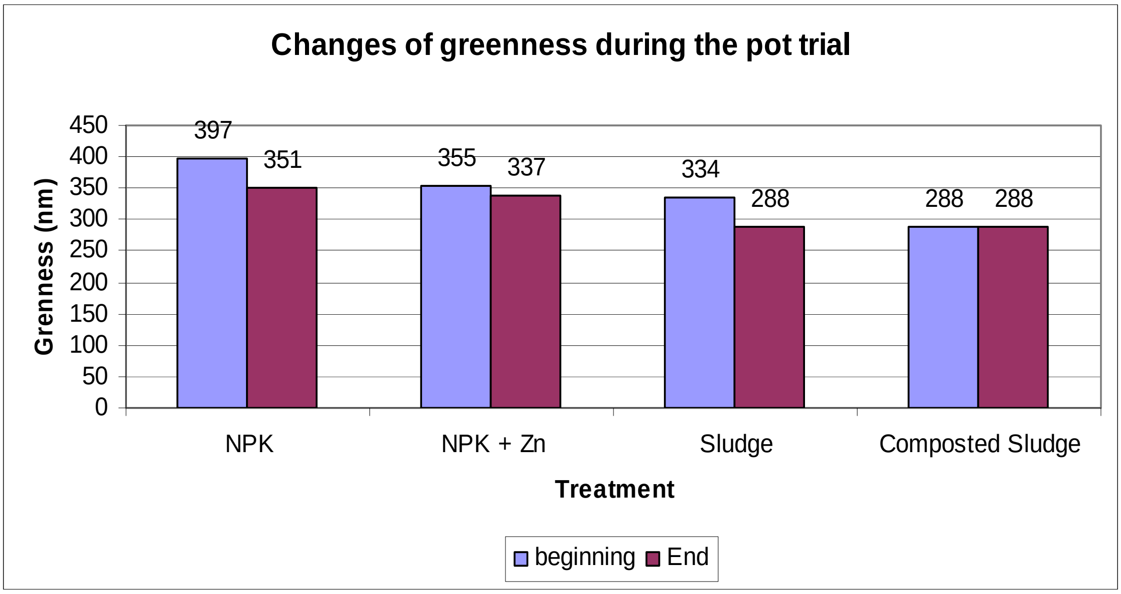


Figure 39- Changes of greenness during the pot trial for each experiment

As can be seen in the graphic above there was a change in the greenness of the leaves during the plants growth. The greenness level of the leaves has a direct relation with the nitrogen content of these leaves (Koteva, 2001). And based on this relation some discussions can be done.

Only in the case of the plants fertilized with composted sludge there was no change, being also true that their level of nitrogen was the lowest of the four treatments. The leaves of the treatment with amendment of NPK fertilizer had the highest level of nitrogen followed by the treatment with this same fertilizer plus Zn fertilizer. It is interesting to see how the only treatment without change of greenness was the one with compost amendment, even when composting decreases the availability of nitrogen. This has as explanation that the lower growth of these plants in comparison to the plants of the other treatments, maintaining their greenness in a same level.

There is also a direct relation between content of nitrogen and weight of the plants, as can be observed in the table 10. The highest value for the greenness coincides with the heaviest plants, as well as happens in the rest of the cases. Although it is also possible to see how the relation is not equal in all the cases. This confirms a relation between leaves greenness and development of the plants. In this relation the R^2 obtained is 0,8, which means that the in the 80% of the case the relation is confirmed.

Table 10- Correlation between greenness and weight after harvest

Treatment	Greenness (nm)	Weight
NPK	**397**	**233**
NPK + Zn	**355**	**231**
Sludge	**334**	**116**
Compost	**288**	**81**

5 CONCLUSIONS

- After 37 days of composting process no reduction of total zinc could be observed. Moreover the amount even increased in a small percentage (2,7%) from 2041 mg Zn/kg dm to 2097 mg Zn/ kg dm These coincide with the results of some authors who explain the different possible behaviours of heavy metals concentration during the composting process. For instance Barker and Bryson (2002) explain in their work the possibility of increase of heavy metals during composting. Jakubus (2003) obtained similar results in his work and could determine an increase of total zinc of 7,81% after 6 months of composting.

- There was a clear reduction in the amount of available zinc after the 37 days of composting. This decrease was from 647,5 mg available Zn/ kg dm to 445 mg available Zn/ kg dm, which means 202,5 mg available Zn/ kg dm or 31,3% in percentage. These results confirm the idea shown in the first hypothesis of this diploma, due to the reduced availability of zinc by 31,3% in the composted sewage sludge in comparison to the non-composted sludge. This reduction of available zinc during composting has been studied by several authors, for instance Krebs et al. (1998) and Amlinger (2002) who explain how the formation of the humic fraction from the organic matter, contained in the composted material, has a direct relation with the decrease of available zinc within the composting process.

- The decrease of the soluble, exchangeable fraction of zinc during organic matter degradation is confirmed by the measurements of losses of dry weight including the losses of organic matter during the composting process of the studied sewage sludge.

- The prevention of the release of available zinc in the pot trial allows two interpretations depending on which results are considered; absolute value of available zinc or available zinc content in relation to the total. In the first case the prevention is higher in soil with fresh sludge, but in the second one soil with compost presents a higher prevention. Anyway the obtained results don't present differences statistically significant.

- The contents of total zinc in the harvested cabbages were also determined. In this case it is possible to see significant differences between the mineral treatments, but not between the two organic. Although it can be seen how the zinc level is a bit higher in the plants treated with compost this difference can not be used to confirm which treatment had a bigger zinc uptake because the differences are not statistically significant.

- The EC and pH analyses indicate good quality values for the compost. In the case of Total C and Total N these values were used to get the rate C/N which is in this case a bit low, nevertheless normal for sewage sludge. The ratio NO_3-N/NH_4-N showed a good quality of composting process.

- pH analysis for soil was also done, obtaining as results values of pH between 6,7 and 7. This values are optimal for the application of biosolids (Jugsujinda and Patrick, 1997) and due to their similarity no differences of behaviour of zinc can be related with this factor

- According to the results of greenness analysis and dry matter measurement it can be affirmed that the plants with amendment of mineral fertilizers were more developed than the plants treated with composted and non-composted sewage sludge. The picture presented in the Appendix I show this as well. Comparing both treatments amendment of composted and amendment of non-composted sewage sludge the pictures show how the plants treated with non-composted sludge had better development, although not very significant.

- After all the conclusions have been presented can be seen how the quality of the compost in the pot trial was not better than the non-composted sewage sludge referred to the availability of zinc.

6 SUMMARY (POVZETEK)

6.1 SUMMARY

The aim of this diploma was to determine the availability of zinc from sewage sludge taken from the Domžale-Kamnik Waste Water Treatment Plant. It was known that this material contains a high concentration of zinc due to the proximity of some industries. The first step of the diploma consisted of composting this sewage sludge and making a comparison between characteristics of composted and non-composted sewage sludge, including the amount of total and available zinc, and determination of losses of dry weight during the composting process. Afterwards a pot trial was done. This was formed by four treatments, each with 5 replicates. Two of the treatments consisted of fertilizing with composted and non-composted sludge and the other two ones were both fertilized with fertilizer NPK and one of them with addition of $ZnCl_2$. The chosen plant for the pot trial was Chinese cabbage, due to it is a common vegetable crop in Slovenia. The duration of the pot trial was eleven weeks. After this time the plants were harvested and their content of zinc measured. The soil in which the plants grew was analysed at the beginning and at the end of the pot trial. Apart from the determination of zinc contents other analyses were also done, like the greenness of the plants' leaves at the beginning and the end of the pot trial with hydro N-tester and of the plants' weight after the harvest. Based on the results of all the analyses the availability of zinc in the four treatments was determined.

During the composting process the amount of total zinc maintained nearly constant with an increase of 2,7% at the end of the 37 days of composting process. By contrast the amount of available zinc decreased significantly during the process. This decrease of 31,3% has been explained during the diploma as the action of the O.M. – when degrades it becomes part of the humic fraction which bound Zn into less available form (Jakubus, 2003).

Some characteristics of the sewage sludge before and after composting were also determined (pH, EC, total N, total C, NO_3-N and NH_4-N). Upon the measured values the quality of the composting can defined as good.

After the compost was prepared the pot trial started. The zinc content of the soils of the 20 pots was determined at the beginning and at the end of the pot trial. Total zinc content decreased in all the treatments. Comparing the decrease between the soils with composted and non-composted sewage sludge, the latter one had bigger decrease. In the case of available zinc the content increased in the soils treated with mineral fertilizers and decreased in the soils with amendment of composted and non-composted sewage sludge. The decrease was bigger in the case of the non-composted sludge. According to this result it can be deduced that the composted sludge doesn't prevent the release of zinc compared to the same amount of non-composted sewage sludge, as it was assumed by the second hypotheses.

The last analysis related with the zinc bioavailability was the determination of zinc content in biomass after the plants were harvested. The results of this analysis show a bit lower content of zinc in treatments with non-composted sewage sludge than with composted sewage sludge, but the differences are very small, and not statistically significant.

As a final conclusion it can be stated that although the zinc availability decreased during the composting process, zinc release during the pot trial was more or less equal for both composted and non-composted sludge treatments.

6.2 POVZETEK

Namen te diplome je izmeriti dostopnost cinka za rastline iz blata komunalne čistine naprave Domžale – Kamnik. Znano je bilo, da to blato vsebuje precej cinka zaradi prispevka odpadnih vod industrije na čistilno napravo.
Najprej smo blato kontrolirano kompostirali in nato primerjali karakteristike kompostiranega in ne-kompostiranega blata, vključno glede količin skupnega in dostopnega cinka. Izmerili smo tudi izgube suhe snovi med kompostiranjem.
Nato smo zasnovali lončni poskus s štirimi obravnavanji, v petih ponovitvah. Pri dveh obravnavanjih smo v tla dodali bodisi kompost, bodisi ne-kompostirano blato. V tretjem obravnavanju smo tla pognojili z mineralnim NPK gnojilom z odmerkom P primerljivo z obravnavanjem, kjer smo dodali ne-kompostirano blato. V četrtem obravnavanju smo tla pognojili z NPK gnojilom (enako kot v tretjem obravnavanju) ter dodali še Zn v obliki $ZnCl_2$.
Izbrana rastlina za lončni poskus je bila kitajsko zelje, pogosta zelenjavna rastlina v Sloveniji. Lončni poskus je trajal 11 tednov. Zatem smo rastline poželi in izmerili skupno vsebnost Zn v nadzemnem delu rastlin. Tla, v katerih smo gojili kitajsko zelje, smo vzorčili na začetku in ob koncu poskusa. Poleg meritev Zn smo opravili še meritve drugih elementov oz. parametrov, kot npr. intenziteto zelene barve listov zelja s Hydro N-testerjem in maso rastlin ob žetvi. Na podlagi rezultatov vseh analiz smo določili dostopnost cinka v vseh štirih obravnavanjih.

Med procesom kompostiranja je vsebnost skupnega cinka ostala ves čas skoraj konstantna oz. se je ob koncu 37 dnevnega kompostiranja povečala le za 2,7%. Nasprotno pa se je v času kompostiranja vsebnost dostopnega Zn značilno zmanjšala. To zmanjšanje za 31,3% je v diplomi razloženo kot učinek organske snovi – med njeno razgradnjo se tvorijo huminske snovi, ki vežejo Zn v manj dostopno obliko (Jakubus, 2003).

Nekatere izmerjene lastnosti blata pred kompostiranjem in po njem (pH, EC, skupni N, skupni C, NO_3-N in NH_4-N) kažejo, da je bilo kompostiranje dobro vodeno in da je pridobljeni kompost kakovosten.

Po kompostiranju smo pričeli z lončnim poskusom. Vsebnost Zn v tleh v 20 loncih smo določili na začetku in ob koncu poskusa. Skupna vsebnost Zn se je glede na začetno vsebnost ob koncu zmanjšala pri vseh obravnavanjih. Zmanjšanje pri obravnavanju s kompostom je bilo manjše kot pri obravnavanju z ne-kompostiranim blatom. Vsebnost dostopnega cinka se je povečala v variantah z dodanim mineralnim NPK gnojilom ter zmanjšala v variantah s kompostom ali ne-kompostiranim blatom. Zmanjšanje je bilo večje v primeru dodatka ne-kompostiranega blata. Na podlagi tega rezultata lahko sklepamo, da kompostirano blato ne prepreči sproščanja Zn v primerjavi z enako količino dodanega ne-kompostiranega blata, kot smo domnevali v drugi hipotezi.

Meritev cinka v biomasi rastlin po žetvi je bila zadnja analiza pri določevanju dostopnosti cinka. Rezultati so pokazali nekoliko nižjo vsebnost cinka pri obravnavanju z ne-kompostiranim blatom v primerjavi z obravnavanjem s kompostiranim blatom, vendar razlike niso bile signifikantne.

Kot sklepno ugotovitev lahko povzamemo, da čeprav se je dostopnost cinka med procesom kompostiranja zmanjšala, je bilo sproščanje cinka v lončnem poskusu približno enako iz kompostiranega kot iz ne-kompostiranega blata.

7 REFERENCES

Antonovics J., Bradshaw A.D., Turner R.G. 1971. Heavy metals tolerance in plants. Advetn. Ecological Research, 7: 1-85 p

Barker A., Bryson G. 2002. Bioremediation of heavy metals and organic toxicants by composting. The scientific world Journal, 2: 407-420 p

Brinton W.F. 2000. Compost quality: Standards and guidelines. Woods end research laboratory, Inc. 45 p

Brown P.B., Cakmak I., Zhang Q. 1993. Form and function of zinc in plants. In: Zinc in soils and plants. A.D. Robson (ed). Dordrecht, Kluwer Academic Publishers: 93-106 p

CČN Domžale Kamnik. 2007**.** http://www.ccn-domzale.si (5.5.2008)

Daims H., Taylor M.W., Wagner M. 2006. Wastewater treatment: a model system for microbial ecology. Trends into Biotechnology, 24: 483-489 p

Dewil R., Baeyens J., Neyens E. 2006. Reducing the heavy metal content of sewage sludge by advanced sludge treatment methods. Environmental Engineering Science, 23: 994-999 p

Duffus J.H. 2002. Heavy metals, a meaningless term. Chemistry International, 23: 793-807 p

Epstein E. 2003. Characteristics of sewage sludge and biosolids. In: Land application of sewage sludge and biosolids. New York, Lewis Publishers: 13-26 p

García-Gómez A. 2005. Organic matter fractions involved in degradation and humification processes during composting. Compost science & utilization, 13. 127-135 p

Goldstein J. 1977. Composting improves the final product. In: Sensible Sludge. A new look at a wasted natural resource. Emmaus, Rodale Press Inc: 22-43 p

Gondek K., Filipek-Mazur B. 2006. Selected soil properties of some microelements from soil with compost supplement. Electronic Journal of Polish Agricultural Universities, 39: 15-21 p

González P., Millares R., Lobo M. 2005. Composición del compost de lodos de depuradora utilizado en ensayos. Uso de lodos de depuradora compostados como enmienda orgánica en suelos de olivar. Madrid, Ministerio de Educación y Ciencia: 21-43 p

Gutiérrez-Ríos E. 1984. Metales pesados. Química Inorgánica. Barcelona, Editorial Reverté: 135-158 p

Hanč A., Tlustoš P., Száková J., Balik J. Pavlikova D. 2002. The zinc mobility in three different soils amended by sewage sludge incubated with limestone and lime, and Zn uptake by oats. Rostlinná Výrova, 48: 518–524 p

Hanlon E.A., McNeal B.L., Kidder G. 2002. Soil and container media electrical conductivity interpretations. Florida, Institute of Food and Agonomical Science: 10 p

Hege U., Peretzki F., Diepolder M., Capriel P. 2003. Leitfaden für die Düngung von Acker- und Grünland, 7. überarbeitete Auflage 2003. Herausgeber: Bayerische Landesanstalt für Landwirtschaft, Institut für Agrarökologie, Ökologischen Landbau und Bodenschutz: 85 p

Hoffman G. 1991. VDLUFA-Verlag. Darmstadt.1 p

Houba V.J.G., Temminghoff E.J.M., Gaikhorst G.A., Vark W. 2000. Soil analysis procedures using 0.01 M Calcium Chloride as extraction reagent. Communications in Soil Science and Plant Analysis 31: 1299–1396 p

Hvala N., Vrečko D., Burica O., Stražar M., Levstek M. 2002. Simulation study supporting wastewater treatment plant upgrading. Water science and technology 46: 325-332 p

Iglesias-Jimenez E., Barral-Silva M.T., Marhuenda-Egea F.C. 2008. Relación NO^3-N/NH^4-N. In: Indicadores de madurez y estabilidad del compost. Madrid, Mundiprensa (ed): 243-283 p

ISO/DIS 10694. 1994. Soil quality- Determination of organic and total carbon after dry combustion: 7 p

Jakubus M. 2003. Changes in zinc combinations in sewage sludge composted with and without an addition of lime. Electronic Journal of Polish Agricultural Universities, 6: 20-25 p

Jara-Peña E., Villegas A., Sánchez P. 2002. Contenido de P, N y K y rendimiento de frambuesa roja (*Rubís idaeus* L). Revista Peruana de Biología, 9: 84-93 p

Jugsujinda A., Patrick W.H. 1977. Growth and nutrient uptake by rice in a flooded soil under controlled aerobic-anaerobic and pH conditions. Agronomic Journal, 69: 705-710 p

Krebs R., Gupta S.K., Furrer G., Schulin R. 1998. Solubility and plant uptake of metals with and without liming of sludge-amended soils.. Environmental Quality Journal, 27**:** 18–23 p

Koteva V. 2001. Hydro-N-tester diagnostic of the wheat and barley nitrogen fertilizing. Soil Science Agrochemistry and Ecology, 36: 53-58 p

Lindsay B.J., Logant T.J. 1998. Field response of soil physical properties to sewage sludge. Journal of environmental quality, 27. 534-542 p

Leita L., Fornasier F., Mondini C., Cantone P. 2001. Organic matter evolution and availability of metals during composting of MSW. Applying compost - benefits and needs. Seminar Proceedings, Brussels, 22 – 23 November 2001. Amlinger F. *et al.* (ed). Copyright: Federal Ministry of Agriculture, Forestry, Environment and Water Management, Austria, and European Communities, 2003. ISBN 3-902 338-26-1. 282 p

Maroto J.V. 1995. Crucíferas. In: Horticultura herbácea especial. Madrid. Mundiprensa. (ed): 135-195 p

Mitchell C. 1992. Using sewage sludge as fertilizer. Environmental Education Series, 97: 15-16 p

Muller C.J**.,** Ruitenbeek J.M., Jong L.J. 1992. Conductance and supercurrent discontinuities in atomic-scale metallic constrictions of variable width. Physical Review Letters, 69: 140-143 p

Nickys Nursery Ltd. 2008. http://www.nickys-nursery.co.uk (5.5.2008)

Peterson PJ. 1969. The distribution of zinc-65 in *Agrostis tenuis* Sibth. and *A. stolonifera* L. tissues. Journal Expert Botanical, 20**:** 863-875 p

Pijuan M., Guisasola A., Baeza J.A., Carrera J., Casas C., Lafuente J. 2006. Net P-removal deterioration in enriched PAO sludge subjected to permanent aerobic conditions. Journal Biotechnol, 123: 117-126 p

Raigón M.D., Domínguez-Gento A., Vidal E., Carot-Sierra J.M. 2005 Estudio de la conservación de la col china (*Brassica pekinensis*) cultivada ecológicamente. Colección cursos y conferencias, 4: 7-9 p

Ramalho R.S. 1996. Tratamientos realizados en EDAR. Tratamiento de Aguas Residuales. Barcelona, Reverté (ed): 175-187 p

Sauerbeck D.R. 1982. Influence of crop rotation, manorial treatment and soil tillage on the organic mater content of German soils. In: Soil degradation. Proceedings of the EEC similar held in Wageningen, Netherlands. D. Boels, D.B. Davies and A.E. Johnson (eds): 163-179 p

SIST ISO 11466. 1996. Soil quality- Extraction of trace elements soluble in aqua regia. 1996. 6 p

SIST ISO 11047. 1999. Soil quality-Determination of cadmium, chromium, cobalt copper, lead, manganese, nickel and zinc- Flame and electrothermal atomic absorption spectrometric methods: 5 p

SIST ISO 14255. 1999. Soil quality- Determination of soluble nitrogen fractions: 5 p

SIST ISO 11261. 1995. Soil quality- Determination of total nitrogen: 4 p

SIST ISO 10390. 1996. Soil quality- Determination of pH: 4p

SIST ISO 11265. 1994. Soil quality- Determination of the specific electrical conductivity: 3 p

Taiwo L.B., Oso B.A. 2004. Influence of composting techniques on microbial succession, temperature and pH in a composting municipal solid waste. African Journal of Biotechnology, 3: 239-243 p

Topcuoglu B., Onal M. 2007. Heavy metal accumulation in eggplant (solanum melongea) grown in MSW compost applied to soil. Antalaya, Akdeniz University Vocational High School of Technical Sciences publication: 15 p

Turner R.G. 1969. Heavy metal tolerance in plants. In: Ecological Aspects of the Mineral Nutrition of Plants. Oxford, I.H. Rorison (ed): 399-410 p

Wainwright S.J., Woolhouse H.W. 1975. Physiological mechanisms of heavy metal tolerance in plants. In: The ecology of resource degradations and renewal. New York, M.J. Chadwick and G.T. Goodman (ed): 231–257 p

Westwood D. 2007. Methods for the Examination of Waters and Associated Materials. Leicester, Environment Agency (National Laboratory Service): 8 p

Wortmann C. 2006. Composting Manure and Other Organic Residues. NebGuide, 1315. 25-30 p

Zhang X, Zhou L, Shen Q, Zong L, Zhan X, Wu Q, Liao X, Wong WC. 2002. Changes of chemical and biological properties during composting sewage sludge by using forced aerated static pile model. Ying Yong Sheng Tai Xue Bao,13: 467-470 p

Žnidarčič D., Kacjan-Maršic N., Ozvald J., Požrl T., Trdan S. 2007. Yield and quality of early cabbage in response to within-row plant spacing. Acta agriculturae Slovenica, 89: 15-23 p

ACKNOWLEDGEMENTS

I would like to express my gratitude to a lot of people of the Biotehniška Fakulteta. First of all to Rok Mihelič, supervisor of this diploma, who accepted my as pupil and helped me a lot in every moment, and gave me always his support. I would like to say thank you also to Frank Batič, who was my Erasmus coordinator in the last academic year and who helped me to contact professor Mihelic.

I would like to say also thank you very much to all the people of the laboratory of the Centre of soil and environmental science in which I did all the analyses shown in this thesis, and very specially to two very nice and intelligent women: Andreja Hodnik and Zalka Ilc, with whom I have had very nice work atmosphere, feeling as another member more of the laboratory. I would like to say some thing very special to Zalka because she spent a lot of time explaining me every thing but we have a big language barrier. I only can say "Hvala Lepa Zalka". And in general to all the teachers and laboratory technicians with whom I have had the pleasure of shearing my time during my stance in this very nice country.

APPENDIX A

Total and CAT zinc of sewage sludge in compost mixture during composting process

Appendix A.1- Total Zn results

num. Sample	average (mg/l)	weight (g)	Zn (mg/kg)	Zn (mg/kg)
DAY 1	1,677	2,0076	2088,63	
DAY 1	1,608	2,0143	1995,11	**2041,87**
DAY 3	1,719	2,0144	2133,70	
DAY 3	1,723	2,0104	2142,30	**2138,00**
DAY 6	1,614	2,0319	1985,21	
DAY 6	1,516	2,0155	1880,12	**1932,66**
DAY 12	1,823	2,0572	2215,09	
DAY 12	1,818	2,0299	2238,41	**2226,75**
DAY18	1,846	2,02	2284,03	
DAY 18	1,517	2,0024	1893,98	**2089,01**
DAY24	1,795	2,0618	2176,80	
DAY24	1,820	2,048	2221,37	**2199,09**
DAY 30	1,528	2,0112	1899,36	
DAY 30	1,576	2,0426	1928,91	**1914,14**
DAY 37	1,718	2,054	2091,45	
DAY 37	1,746	2,076	2102,90	**2097,17**

Appendix A.2- CAT Zn results

Num.Sample	Average (mg/l)	Weight (g)	Zn CAT (mg/Kg)	Average (mg Zn/Kg)
DAY1 (S1-2)	62,5	5	625	
DAY1 (S1-2)	67	5	670	**647,5**
DAY3 (S-1)	70	5	700	
DAY3 (S-2)	68,5	5	685	**692,5**
DAY6 (S-1)	73,5	5	735	
DAY6 (S-2)	72	5	720	**727,5**
DAY12 (S-1)	70	5	700	
DAY12 (S-2)	69,5	5	695	**697,5**
DAY18 (S-1)	62,5	5	625	
DAY18 (S-2)	64	5	640	**632,5**
DAY24 (S-1)	61,5	5	615	
DAY24 (S-2)	60	5	600	**607,5**
DAY30 (S-1)	55	5	550	
DAY30 (S-2)	55	5	550	**550**
DAY37 (S-1)	55	5	550	
DAY37 (S-2)	34	5	340	**445**

Table 5.3.- CAT Zn during composting process

Appendix A.3- Comparison between CAT zinc and total zinc

num. Sample	TOTAL Zn (mg/kg)	CAT Zn (mg/kg)	CAT Zn/TOTAL Zn (%)
DAY 1	2041,87	647,50	**31,71**
DAY 3	2138,00	692,50	**32,39**
DAY 6	1932,66	727,50	**37,64**
DAY 12	2226,75	697,50	**31,32**
DAY18	2089,01	632,50	**30,28**
DAY24	2199,09	607,50	**27,63**
DAY 30	1914,14	550,00	**28,73**
DAY 37	2097,17	445,00	**21,22**

Table 5.4.- Relation between CAT and TOTAL Zn during composting

APPENDIX B

Results obtained for the determination of the loss of dry weight during the composting process

Appendix B.1- Calculation of the composting mixture moisture during the composting process

Sample	Weight (g) (9/06/2008)	Dry weight (g) (10/06/2008)	Lost Weight (g)	Dry weight (%)	Moisture Water Weight (%)	Moisture after watering
DAY 1	13,92	5,24	8,68	37,6	**62,4**	
DAY 3	13,61	5,52	8,09	40,6	**59,4**	
DAY 6	15,38	6,29	9,09	40,9	**59,1**	
DAY 12	16,69	7,06	9,63	42,3	**57,7**	**68,4**
DAY 18	17,22	6,08	11,14	35,3	**64,7**	
DAY 24	14,48	5,55	8,93	38,3	**61,7**	
DAY 30	16,83	6,92	9,91	41,1	**58,9**	
DAY 37	15,49	6,88	8,61	44,4	**55,6**	

Appendix B.2- Calculation of the losses of Dry Weight during the composting process using the values obtained in the table shown in the Appendix D.1

DAY 0 (11/03/2008)			DAY 6 (18/03/2008)			Loss Weight	Loss H2O	**Loss Dry Weight**
Final Weight	Humidity	Weight H2O	Start Weight	Humidity	Weight H2O			
3218	62,36	2006,63	2750,00	59,10	1625,33	468,00	381,30	**86,70**
DAY 6 (18/03/2008)			DAY 12 (25/03/2008)			Loss Weight	Loss H2O	**Loss Dry Weight**
Final Weight	Humidity	Weight H2O	Start Weight	Humidity	Weight H2O			
2642,50	59,10	1561,79	2467,50	57,70	1423,73	175,00	138,06	**36,94**
200 ml of water were added into the mixture								
DAY 12 (25/03/2008)			DAY 18 (31/03/2008)			Loss Weight	Loss H2O	**Loss Dry Weight**
Final Weight	Humidity	Weight H2O	Start Weight	Humidity	Weight H2O			
3163,00	68,40	2163,49	2837,50	64,69	1835,64	325,50	327,85	**-2,35**
DAY 18 (31/03/2008)			DAY 24 (7/04/2008)			Loss Weight	Loss H2O	**Loss Dry Weight**
Final Weight	Humidity	Weight H2O	Start Weight	Humidity	Weight H2O			
2732,50	64,69	1767,71	2455,50	61,67	1514,34	277,00	253,38	**23,62**
DAY 24 (7/04/2008)			DAY 30 (14/04/2008)			Loss Weight	Loss H2O	**Loss Dry Weight**
Final Weight	Humidity	Weight H2O	Start Weight	Humidity	Weight H2O			
2349,00	61,67	1448,66	2160,00	58,88	1271,87	189,00	176,79	**12,21**
DAY 30 (14/04/2008)			DAY 37 (21/04/2008)			Loss Weight	Loss H2O	**Loss Dry Weight**
Final Weight	Humidity	Weight H2O	Start Weight	Humidity	Weight H2O			
2054,00	58,88	1209,46	1868,00	55,58	1038,31	186,00	171,14	14,86

APPENDIX C

Calculation of the Total amount of zinc in the soil of the four analyses during the pot trial

Appendix C.1- Amount of Total zinc at the beginning of the pot trial

num.Sample	average (mg/l)	weight (g)	Zn tot (mg/Kg)	Average (mg Zn/ kg)
1-1 (NPK)	1,9	3,028	62,75	
1-2(NPK)	1,8	3,0542	58,94	
1-4(NPK)	1,6	3,0591	52,30	
1-5(NPK)	1,79	3,0591	58,51	**58,12**
2-1 (NPK+Zn)	1,75	3,0678	57,04	
2-2(NPK+Zn)	1,91	3,0103	63,45	
2-3(NPK+Zn)	1,81	3,072	58,92	
2-4(NPK+Zn)	2	3,0225	66,17	**61,40**
3-1(Sludge)	1,5	3,0449	49,26	
3-2(Sludge)	1,78	3,0166	59,01	
3-3(Sludge)	1,7	3,0294	56,11	
3-4(Sludge)	1,65	3,0241	54,56	**54,82**
4-1(Compost)	1,59	3,0325	52,43	
4-2(Compost)	1,75	3,0162	58,02	
4-3(Compost)	1,47	3,0223	48,64	
4-5(Compost)	1,55	3,0427	50,94	**52,51**

Appendix C.2- Amount of Total zinc at the end of the pot trial

num.Sample	average (mg/l)	weight (g)	Zn tot (mg/Kg)	Average (mg Zn/ kg)
1-1(NPK)	1,65	3,017	54,69	
1-2(NPK)	1,5	3,0084	49,86	
1-3(NPK)	1,53	3,0072	50,88	
1-4(NPK)	1,7	3,075	55,28	
1-5(NPK)	1,55	3,0818	50,30	**52,20**
2-1(NPK+Zn)	1,57	3,0377	51,68	
2-2(NPK+Zn)	1,9	3,0635	62,02	
2-3(NPK+Zn)	1,53	3,0207	50,65	
2-4(NPK+Zn)	2,25	3,0712	73,26	
2-5(NPK+Zn)	1,66	3,0074	55,20	**58,56**
3-1(Sludge)	1,59	3,0109	52,81	
3-2(Sludge)	1,4	3,0162	46,42	
3-3(Sludge)	1,64	3,0279	54,16	
3-4(Sludge)	1,44	3,0129	47,79	
3-5(Sludge)	1,48	3,0639	48,30	**49,90**
4-1(Compost)	1,5	3,0305	49,50	
4-2(Compost)	1,47	3,0717	47,86	
4-3(Compost)	1,45	3,0844	47,01	
4-4(Compost)	1,6	3,0444	52,56	
4-5(Compost)	1,68	3,0146	55,73	**50,53**

Appendix C.3- Comparison between values of total zinc at the beginning and at the end of the pot trial

Treatment	Total Zn Beginning (May)	Total Zn End (July)
NPK	**58,12**	**52,20**
NPK + Zn	**61,40**	**58,56**
non-composted Sewage Sludge	**54,82**	**49,90**
Composted Sewage Sludge	**52,51**	**50,53**

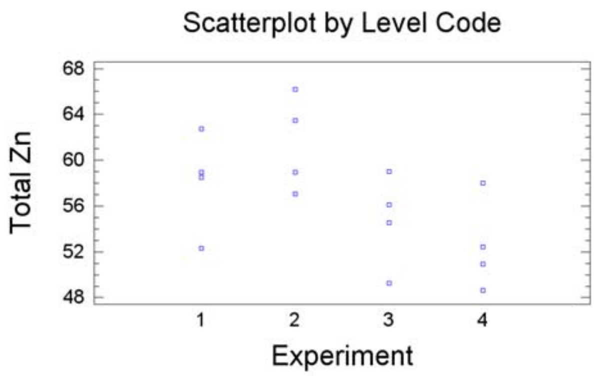
Appendix C.4- Analyses of variance for the total zinc values at the beginning and at the end of the pot trial

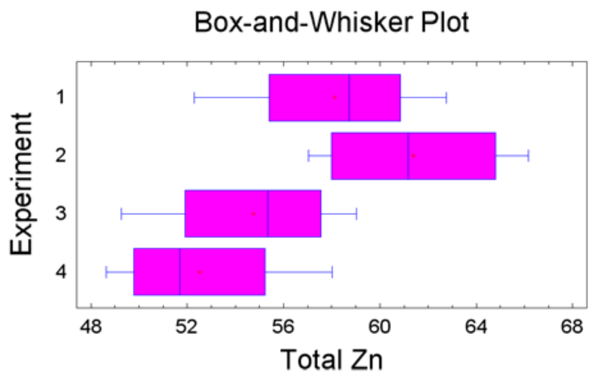
a) Total zinc at the beginning of the pot trial

ANOVA Table for Total Zn by Treatment

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	182,046	3	60,6821	3,53	0,0485
Within groups	206,23	12	17,1858		
Total (Corr.)	388,276	15			

The ANOVA table decomposes the variance of Total Zn into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 3,53, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0,05, there is a statistically significant difference between the mean Total Zn from one level of Experiment to another at the 95,0% confidence level. To determine which means are significantly different from which others, select Multiple Range Tests from the list of Tabular Options.



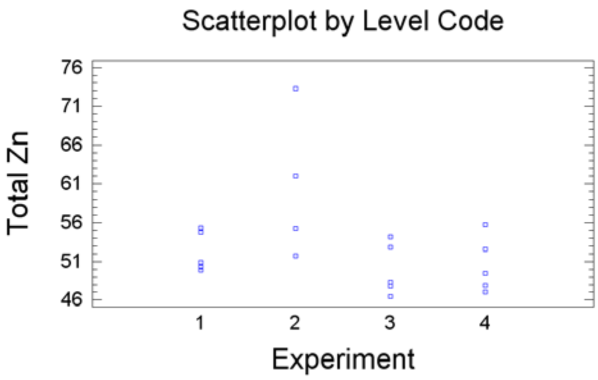


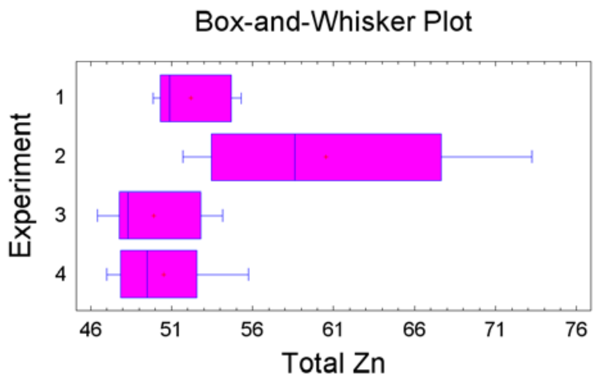
b) Total zinc at the end of the pot trial

ANOVA Table for Total Zn by Treatment

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	309,069	3	103,023	3,91	0,0301
Within groups	394,998	15	26,3332		
Total (Corr.)	704,067	18			

The ANOVA table decomposes the variance of Total Zn into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 3,91, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0,05, there is a statistically significant difference between the mean Total Zn from one level of Experiment to another at the 95,0% confidence level.





APPENDIX D

Calculation of the available zinc content in the soil of the four experiments at the beginning and the end of the pot trial

Appendix D.1- Available zinc at the beginning of the pot trial

num.Sample	average (mg/l)	weight (g)	Zn CAT (mg/Kg)	Average (mg Zn/kg)
1-1(NPK)	0,59	5	5,9	
1-2(NPK)	0,28	5	2,8	
1-3(NPK)	0,29	5	2,9	
1-4(NPK)	0,49	5	4,9	
1-5(NPK)	0,28	5	2,8	**3,86**
2-1(NPK+Zn)	0,93	5	9,3	
2-2(NPK+Zn)	0,83	5	8,3	
2-3(NPK+Zn)	0,56	5	5,6	
2-4(NPK+Zn)	0,53	5	5,3	**7,125**
3-1(Sludge)	0,54	5	5,4	
3-2(Sludge)	0,55	5	5,5	
3-3(Sludge)	0,8	5	8	
3-4(Sludge)	0,96	5	9,6	**7,125**
1-4(Compost)	0,98	5	9,8	
4-2(Compost)	0,73	5	7,3	
4-3(Compost)	0,63	5	6,3	
4-5(Compost)	1,02	5	10,2	**7,021**

Appendix D.2- Available zinc at the end of the pot trial

num.Sample	average (mg/l)	weight (g)	Zn CAT (mg/Kg)	Average (mg Zn/kg)
1-1(NPK)	0,47	5	4,7	
1-2(NPK)	0,59	5	5,9	
1-3(NPK)	0,4	5	4	
1-4(NPK)	0,68	5	6,8	
1-5(NPK)	0,48	5	4,8	**5,240**
2-1(NPK+Zn)	0,61	5	6,1	
2-2(NPK+Zn)	0,81	5	8,1	
2-3(NPK+Zn)	0,98	5	9,8	
2-4(NPK+Zn)	0,98	5	9,8	
2-5(NPK+Zn)	0,78	5	7,8	**8,320**
3-1(Sludge)	0,61	5	6,1	
3-2(Sludge)	0,62	5	6,2	
3-3(Sludge)	1,06	5	10,6	
3-4(Sludge)	0,62	5	6,2	
3-5(Sludge)	0,61	5	6,1	**7,040**
1-4(Compost)	0,7	5	7	
4-2(Compost)	0,47	5	4,7	
4-3(Compost)	0,94	5	9,4	
4-4(Compost)	0,53	5	5,3	
4-5(Compost)	0,72	5	7,2	**7,176**

Appendix D.3- Comparison between available zinc at the beginning and at the end of the pot trial

Treatment	MAY	JULY
	Average (mg Zn/kg)	Average (mg Zn/kg)
NPK	**3,86**	**4,85**
NPK + Zn	**7,125**	**8,875**
Non-composted Sludge	**7,125**	**6,15**
composted Sludge	**7,021**	**6,854**

Appendix D.4- Comparison between available zinc at the beginning and at the end of the pot trial in percentage respect to the total zinc amount

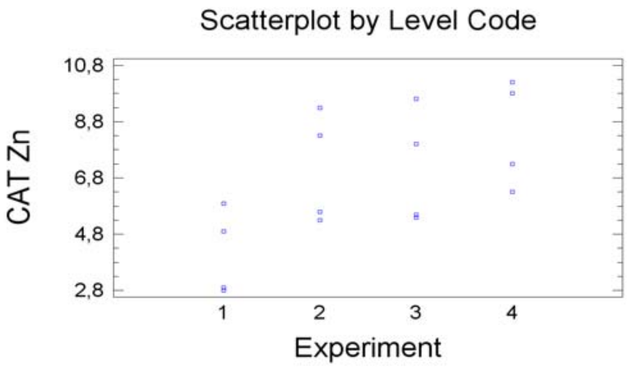
Treatment	MAY	JULY	Increase (%)
	Percentage CAT Zn/Total Zn (%)	Percentage CAT Zn/Total Zn (%)	
NPK	**6,64**	**10,04**	**3,40**
NPK + Zn	**11,61**	**14,21**	**2,60**
non-composted Sludge	**12,38**	**14,11**	**1,73**
Composted Sludge	**13,37**	**14,20**	**0,83**

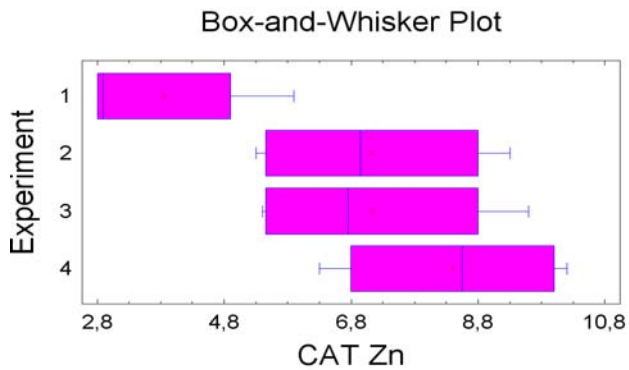
Appendix D.5- Analyses of variance for the CAT Zn values at the beginning and at the end of the pot trial

a) CAT Zn at the beginning of the pot trial

Source	Sum of Squares	Df	Mean Square	F-Ratio	**P-Value**
Between groups	52,3918	3	17,4639	5,22	**0,0139**
Within groups	43,507	13	3,34669		
Total (Corr.)	95,8988	16			

The ANOVA table decomposes the variance of available zinc into two components: between-group component and within-group component. The F-ratio, which in this case equals 5,22, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0,05, there is a statistically significant difference between the mean available zinc from one level of Experiment to another at the 95,0% confidence level.

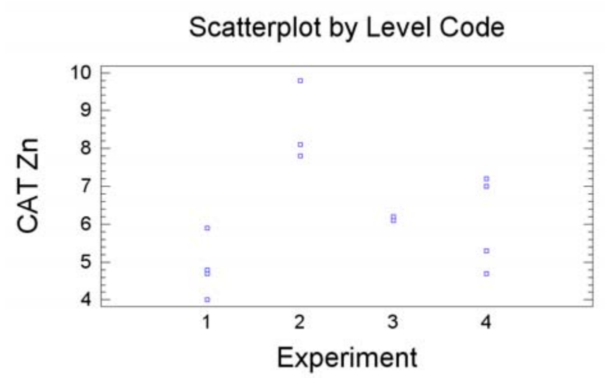


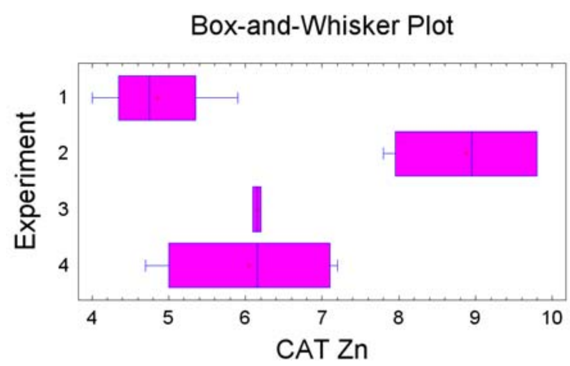


b) CAT Zn at the end of the pot trial

Source	Sum of Squares	Df	Mean Square	F-Ratio	**P-Value**
Between groups	34,7469	3	11,5823	13,99	**0,0003**
Within groups	9,9375	12	3,34669		
Total (Corr.)	44,6844	15			

The ANOVA table decomposes the variance of Available zinc into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 13,99, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0,05, there is a statistically significant difference between the mean Available zinc from one level of Experiment to another at the 95,0% confidence level.





APPENDIX E

pH values of the soils used in the pot trial

Sample	pH value	pH Average
1.1(NPK)	6,77	
1.2(NPK)	6,72	
1.3(NPK)	6,77	
1.4(NPK)	6,77	**6,76**
2.1(NPK+Zn)	6,69	
2.2(NPK+Zn)	6,77	
2.3(NPK+Zn)	7,02	
2.5(NPK+Zn)	6,9	**6,85**
3.1(Sludge)	6,9	
3.2(Sludge)	7,04	
3.3(Sludge)	6,64	
3.4(Sludge)	6,67	**6,81**
4.1(Compost)	6,77	
4.3(Compost)	6,77	
4.4(Compost)	7,03	
4.5(Compost)	6,8	**6,84**

APPENDIX F

Wet Weight, Dry Weight and percentage of dry matter of the harvested plants after the pot trial

pot number	wet weight (g)	dry weight (g)	Dry percentage (%)	Wet Weight Average (g)	Dry Weight Average (g)	Dry Percentage Average (%)	weight lost drying (g)
1.1(NPK)	208,0	23,0	11,06				
1.2(NPK)	224,0	23,0	10,27				
1.3(NPK)	249,5	24,5	9,82				
1.4(NPK)	258,0	29,5	11,43				
1.5(NPK)	226,0	26,0	11,50	**233,1**	**25,2**	**10,8**	**207,9**
2.1(NPK+Zn)	203,0	22,0	10,84				
2.2(NPK+Zn)	241,0	26,5	11,00				
2.3(NPK+Zn)	257,0	25,0	9,73				
2.4(NPK+Zn)	225,5	25,0	11,09	**231,6**	**24,6**	**10,7**	**207,0**
3.1(Sludge)	112,5	16,0	14,22				
3.2(Sludge)	103,0	15,0	14,56				
3.3(Sludge)	127,5	17,0	13,33				
3.4(Sludge)	124,0	16,5	13,31				
3.5(Sludge)	116,0	16,0	13,79	**116,6**	**16,1**	**13,8**	**100,5**
4.1(Compost)	67,5	11,5	17,04				
4.2(Compost)	77,0	10,5	13,64				
4.3(Compost)	83,5	11,5	13,77				
4.4(Compost)	94,0	12,5	13,30	**80,5**	**11,5**	**14,4**	**69,0**

APPENDIX G

Values of content of zinc are shown with the average of these values for each treatment. As well as an Anova study

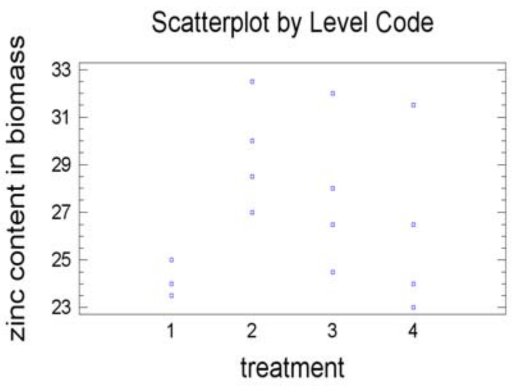
Appendix G.1- Table with the values of Zinc content in biomass

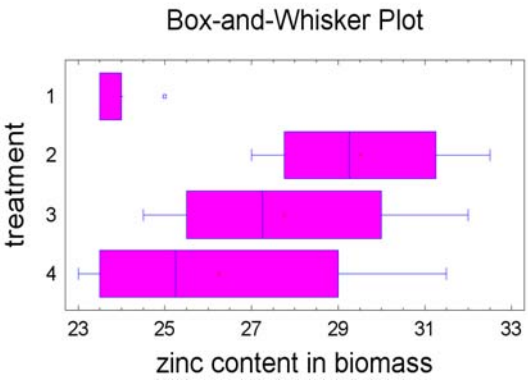
Treatment	Zn content (mg Zn/kg dry biomass)	Average (mg Zn/kg dry biomass)
1.1(NPK)	23,5	
1.2(NPK)	25	
1.3(NPK)	24	
1.4(NPK)	24	
1.5(NPK)	23,5	**24**
2.1(NPK+Zn)	28,5	
2.2(NPK+Zn)	32,5	
2.3(NPK+Zn)	27	
2.5(NPK+Zn)	30	**29,5**
3.1(Sludge)	26,5	
3.2(Sludge)	31,5	
3.4(Sludge)	24	
3.5(Sludge)	23	**26,25**
4.1(Compost)	24,5	
4.2(Compost)	26,5	
4.3(Compost)	28	
4.5(Compost)	32	**27,75**

Appendix G.2- Analysis of Variance of the zinc content in biomass after harvest

Source	Sum of Squares	Df	Mean Square	F-Ratio	**P-Value**
Between groups	73,0294	3	24,3431	3,46	**0,0482**
Within groups	91,5	13	7,03846		
Total (Corr.)	164,529	16			

The ANOVA table decomposes the variance of zinc content in biomass into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 3,45859, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0,05, there is a statistically significant difference between the mean zinc content in biomass from one level of treatment to another at the 95,0% confidence level.





Appendix G.3- Correlation between CAT zinc at the end of the pot trial and zinc content in biomass

Treatment	Available Zn (mg Zn/kg)	Zn content in Biomass (mg Zn/kg)
1-1(NPK)	4	23,5
1-1(NPK)	4,7	24
1-1(NPK)	5,9	24
1-1(NPK)	4,8	23,5
2-1(NPK + Zn)	8,1	28,5
2-1(NPK + Zn)	9,8	32,5
2-1(NPK + Zn)	6,1	27
2-1(NPK + Zn)	9,8	30
3-1(Sludge)	6,1	26,5
3-1(Sludge)	6,2	31,5
3-1(Sludge)	6,2	24
3-1(Sludge)	6,1	23
4-1(Compost)	4,7	24,5
4-1(Compost)	7	26,5
4-1(Compost)	7,2	28
4-1(Compost)	9,4	32

APPENDIX H

Leaves' greenness during the pot trial. These values were measured using Hydro-N-tester

Experiment	beginning (13/05/08)	The End (8/07/08)
	16th day (nm)	72nd day (nm)
NPK	397	351
NPK + Zn	355	337
Non-composted Sludge	334	288
Composted Sludge	288	288

APPENDIX I

Images of the plants in four different dates (May, 5th, June, 10th, June, 25th, July, 5th) during the pot trial. Each column represents a treatment.