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Ph.D. in Agricultural Ecology
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Application of defecation lime in paddy field: effects on rice (*Oryza sativa* L.) plants and soil characteristics

Ph.D. Thesis

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Supervisor	**Academic Year**	*Coordinator*
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Ph. D. Thesis

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to my family

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Ph.D. in Agricultural Ecology - XXVI Cycle

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Chiodini, M. E., 2012. Application of defecation lime in paddy field: effects on rice (Oryza sativa L.) plants and soil characteristics. Ph.D. Thesis, University of Milano, 134 pp.

This work aimed to evaluate the effects of the defecation lime (DL) at the paddy rice field scale.

According to the prescriptions of the Italian D.Lgs (Legislative Decree) no. 75/2010 defecation lime is defined as a “product obtained by the chemical hydrolysis (or enzymatic attack) of biological materials by lime and/or sulphuric acid and subsequent calcium sulphate precipitation.” This product is classified as "lime and magnesia soil conditioner". They must contain at least 20% of calcium oxide (CaO) and 15% of sulphuric anhydride (SO_3) both related on a dry basis. According by law origin of the hydrolysed biological material must be reported on the label.

Raw biological material for the DL used in this experiment was sewage slugde deriving from municipal and industrial wastewater treatment plant.

DL effects were evaluated both during and at the end of growing season, on rice (*Oryza sativa* L.) plants and soil chemical characteristics. The analysis of the effects was conducted over two year – two growing season – in the same paddy field in order to know also the effects of repeated application of the product.

After spreading, incorporation of DL and soil tillage, rice, Volano variety, was immediately seeded by a row seeder. In order to evaluate the effects of depth of incorporation, for the first year DL was placed into soil by plowing instead for the second year by harrowing using a disc harrow.

For the rice plants the analysis were conducted over different variables i.e. number of emerged plants, development stage, aboveground biomass at the harvesting, yield, milling yield and trace metals concentration in grains while for the soil pH, TOC, CEC and trace metals concentration were evaluated.

Credits evaluation

Courses:

- Instrumental analysis.
- Image analysis.
- Scientific writing and communication.
- Geographical information system

Poster presentation at international/national congress:

- Confalonieri, R., Baldassar, A., Bertoglio, M., Bertone, G., Borsa, I., Bortone, L., Cairo, V., Cappelli, G., D'Incecco, P., Goldaniga, P., Iorio, V., Marziali, F., Mazza, M., Negrini, G., Pestarino, E., Piantanida, E., Pinnetti, M., Pirotta, M., Porta, R., Riva, M., Sessa, F., Uggeri, S., Urbinati, F., **Chiodini, M.**, Bregaglio, S., Acutis, M., 2010. Analisi e modellizzazione dell'effetto di agrotecniche sull'altezza della pianta: il progetto MIATA. Atti del Convegno Nazionale della Associazione Italiana di Agrometeorologia, Bari, 8-10 giugno 2010, 23-24.
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CONTENTS:

Chapter 1

INTRODUCTION

1.1 Defecation lime

Defecation lime, known also as defecation gypsum, is a material used as agricultural soil conditioner and obtained from liquid and solid biomass, such as the by-products of industrial processes i.e. wastes from paper making plants, sugar factories, and slaughterhouses (European Patent Office).

According to the prescriptions of the Italian D.Lgs (Legislative Decree) no. 75/2010 defecation lime is defined as a “product obtained by the chemical hydrolysis (or enzymatic attack) of biological materials by lime and/or sulphuric acid and subsequent calcium sulphate precipitation.” This product is classified as "lime and magnesia soil conditioner". They must contain at least 20% of calcium oxide (CaO) and 15% of sulphuric anhydride (SO_3) both related on a dry basis. According by law origin of the hydrolysed biological material must be reported on the label.

Chemical hydrolysis reactions leads to a break-up of the peptide bonds of the protein, with the result of the formation of fragments corresponding to peptides. If the reaction is driven on further, a break-up of all the peptide bonds and a release of amino-acids will ensue (European Patent Office).

1.2 Defecation lime producing process

European Patent Application - EP 2 135 854 A2 – define the “process for the production of defecation lime, named here defecation gypsum, for agricultural use”. This patent application refers to a process for the production of the agricultural soil conditioner registered as defecation gypsum, in various possible formulations, by alkaline hydrolysis with a calcium chemical selected form from calcium oxide and calcium hydroxide, of showable biological sludges and other proteic non dangerous, nontoxic and non noxious organic wastes to obtaining amino-acids and partly peptides. Hydrolysis reactions is stopped at a given time and pH value in order to avoid the degradation of the amino acids and is followed by the precipitation, through addition of sulphuric acid, of the calcium sulphate dihydrate intimately bound with particles of hydrolysed organic matter. During this process pathogenic organisms and microorganisms are killed. Trace metals concentration into defecation gypsum must be lower than stated by law or regulation limits (Bobbiesi, 2009).

The process essentially consists of two stages: a first stage with an alkaline hydrolysis of the raw sludge, and a second stage with a precipitation of calcium sulphate dihydrate, intimately linked to particles of organic matter, which actually constitutes the defecation gypsum.

- The first stage starts from a selected biological sludge or organic wastes. The hydrolysis reaction of the proteic material occurs over to 12 - 24 hours and the temperature is checked and maintained in a range from 40 to 60°C. The selected form of calcium is added in a sufficient quantity to ensure 20 - 22% concentration of CaO at the end of the process. Hydrolysis reaction started into the mixer but is completed on the storage pad, so is not necessary provide a mixers having extremely large size respect to the volumes of gypsum produced. Arranging the material on a pad also allows a better chance of monitoring the temperature and maintaining it within limits, if necessary, by simply turning the material over with a mechanical shovel. The first reaction that occurs is the following:

$$CaO + H_2O \rightarrow Ca(OH)_2 + \text{heat}$$

The relatively high pH value uphold for a period of 12 - 24 hours involves eliminating pathogenic organisms and microorganisms and preconditioning the product, to become an excellent nutritional basis for a useful microflora, which is also well suited for an enriching, if any, with a microflora specialized for agronomic useful activities.

- <u>In the second stage</u> occurs a fast kinetic reaction having an exothermic nature. The addition of the acid is done on the hydrolysed mass, obtained as above, by mixing it in a mixer. The temperature of the process is checked and maintained around 40 - 50°C in order to avoid the degradation of the organic matter. The sulphuric acid is used in diluted form, about 33%, to avoid an excessively rapid kinetic and allow the reaction to be more easily controlled. Also in this case, the reaction started in the mixer is completed on a pad where, if needed, the mass could be turned over with a mechanical shovel. The main reaction occurring is:

$$Ca(OH)_2 + H_2SO_4 \rightarrow CaSO_4{*}2H_2O + heat$$

In the second stage, concerning the water content, there is a slight addition due to the diluted sulphuric acid used. However this addition cannot compensate the water removed in the foregoing stage, so the final result of the process is a reduction of the initial content respect to the original raw materials. (Bobbiesi, 2009)

Figure 1.1 reported the diagram of the production process of the defecation lime, according the European Patent application defined above:

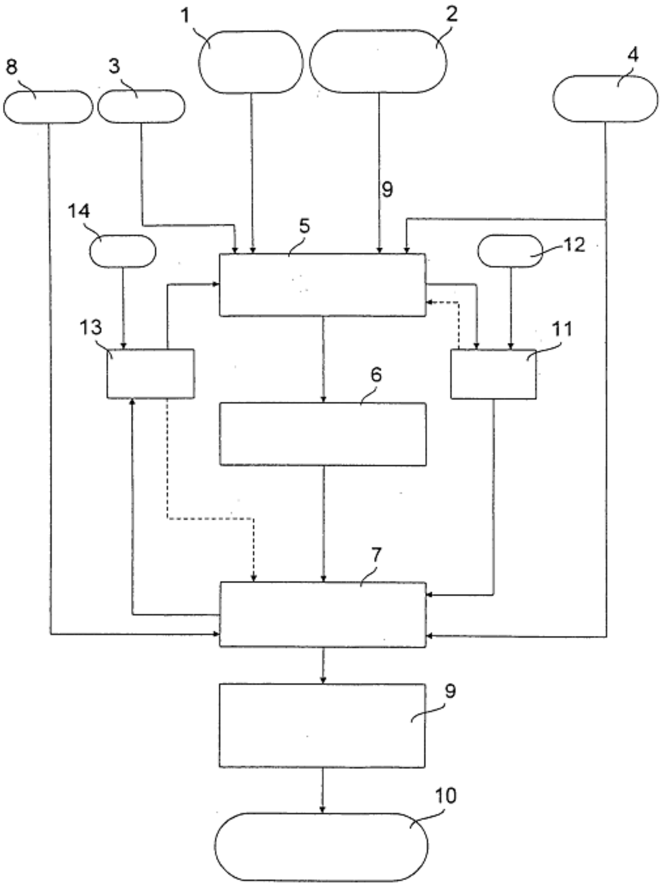

Figure 1.1: Step of the defecation lime production; components of chart are explained below

The sludges selected with other organic wastes (1), together with other special, non-organic wastes (2), a calcium chemical selected form between calcium oxide and calcium hydroxide (3) and optionally inert and/or reactive substances are, after appropriate weighing, conveyed to a first mixing stage (5) effected in a high efficiency mixer for the mixing of the supplied materials. The intimate mixing thus achieved highly favours the course of the reactions involved, all the way up to the formation of the desired defecation gypsum. The product obtained in said first mixing stage (5) is discharged to a heap where the hydrolysis (6), already initiated in the mixing stage (5), takes place. The product of this heap is, after the above mentioned retention period, conveyed to a second mixing stage (7) where an appropriately metered quantity of sulphuric acid (8), coming from a suitable storage tank, is added. The process may operate in a batch-type or in continuous manner: the mixer in which the second mixing (7) occurs may be the same wherein the first mixing (5) occurred. As an alternative, the second mixing (7) can of course be done in a second mixer having the same characteristics as the first one, and thus coming to operate in series to the same. Downstream of this second mixing stage (7), the product obtained is discharged onto a heap (9) where the precipitation of the calcium sulphate dihydrate, which already started during the mixing stage (7), occurs. The desired final product is then moved to the storehouse (10). The vapours released during the first mixing stage (5) are sucked and treated with an acid solution: when exhausted, this solution is conveyed to the second mixing stage (7) and replaced by the make-up solution (12). The washed vapours are returned to the first mixing stage (5), to a position in the mixer such as to favour the extraction of the vapours forming there. A suitable aspirator (not indicated in Figure 1.1) allows recycling the air. In a similar way, the vapours released during the second mixing stage (7) are sucked and conveyed to a washing stage (13) (basic scrubber) with a basic solution: when exhausted, said solution is conveyed to the first mixing stage (5) and replaced by the make-up solution (14). The washed vapours are returned to the second mixing stage (7) to a position, in the mixer, such as to favour the extraction of the vapours forming there. A suitable aspirator (not shown in Figure 1.1) allows recycling the air. If the two mixing stages (5) and (7) occur in the same mixer at different times, the aspirator is a single unit. If the two mixing stages are on the other hand occurring in two different mixers, the aspirators are two, one for each mixer. According to the plant capacity the mixers of the mixing stages (5) and (7) may also be more than two, also operating in parallel.

A comparable method to obtained defecation lime is included into the patent registered in the United States Patent and Trademark Office. This invention relates to methods for treating wastewater sludge cake with acid and calcium carbonate under acidic, low heat conditions, to produce a stable, soil-like or granular, finished product containing calcium carbonate, useful as a nitrogen fertilizer, synthetic soil component or soil conditioner for pH control (Burnham 1992).

Finally, is possible to find that defecation lime are also produced from sugar industry plant at the purification stage of raw juice by milk of lime and CO_2 (Baraldi et al., 2006; González-Fernándezet al., 2004; Paleckienė, et al., 2007).

1.3 Knowledge about defecation lime utilization in agricultural land

Little information was found about the effects following the defecation lime disposal.

Most of them are relate to the utilization of the defecation lime derived from sugar industry, named sugar factory lime (SFL).

González-Fernández et al. (2004) carried out a study to assay the SFL behaviour as an amendment material for an acid soil. Sugar factory refuse lime (SFRL) result from the purification-flocculation of colloid matter from the liquor extracted from sugar beet. In the process, slaked lime and carbon dioxide are used to purify the liquors. The composition varies in accordance with that of the limestone used in the manufacture of slaked lime. SFRL is the residue of these materials together with notable amounts of organic matter and micronutrients. In that work a finely pulverized dolomitic lime was used as a reference. Throughout six years and six crops it was observed how, in the plots amended with lime refuse, a slightly higher pH was maintained than that measured in the plots without lime, and with fewer fluctuations. At the end of their work they reported as SFRL had an effective correction effect, whose beneficial effects last for at least 9 years. They reported as the persistence of the effects of SFRL is greater than that shown by traditional liming materials such as dolomitic lime and its physical and chemical properties make it a valuable and efficient substitute for those other liming materials.

Data on sugar factory lime used for liming the soils are found also in other works: Moore et al., 2000; Lutin et al., 2002, Timmer, 2003; Wargo, 2002.

Paleckienė et al. (2007) also remember in their work that calcium is one of the main secondary nutrients necessary for healthy plant growth. Due to the presence of calcium, SFL is interesting as a calcium compound for fertilizer production or liming soils. Unfortunately, data concerning the use of SFL as calcium compound for production of compound fertilizers are lacking. Nitrogen, phosphorus, sodium, magnesium and other elements used for plant fertilization are an advantage of SFL chemical composition. Also into the European Patent Application EP 2 135 854 A2, is reported that a process for the preparation of defecation gypsum having high agronomic interest due to its high content of calcium and organic substances, such as amino acids and peptides, practically free from pathogenic substances and its different formulations from biological sludges and/or from other waste materials (European Patent Office).

In their study Paleckienė et al. (2007) analysed the usability for manufacturing granulated fertilizers containing calcium by using SFL. The results of investigations show, that waste from the beet sugar industry may be used in production of compound fertilizers as a calcium source. The results obtained are notable for environmental protection and plant fertilization.

1.4 Sewage sludge as defecation lime raw material

Among the biological product used as raw materials for defecation lime production, sewage sludge could be considered.

Sewage sludge is generated as a result of wastewater treatment processes. The progressive implementation in 2005 of the Directives 91/271/EEC and 98/15/EEC concerning urban wastewater treatment has increased the number of wastewater treatment plants operating in the EU and consequently the quantities of sewage sludge requiring disposal (Carmen Antolí et al., 2012)

The EU by the Directive 86/278/EEC seeks to encourage the use of sewage sludge in agriculture and regulate its use in such a way as to prevent harmful effects on soil, vegetation, animals and man. Safe disposal of the sewage sludge is one of the major environmental concerns (Ghanavati et al., 2012). Heather and Lloyd (2000), reported that sewage sludge, the solid portion which remains after wastewater treatment, is frequently disposed of in landfills.

Landfilling and land application of the sewage sludge are suggested to be the most economical sludge disposal methods (Metcalf and Eddy, 2003; Ghanavati et al, 2012).

The disposal of this products into agricultural land is widely interesting also because the presence of a large quantity of organic matter and plant nutrients. Tsadilas et al, (1995) reported that sewage sludge is found to be an effective organic fertilizer causing increments in the biomass of many crops. Heather and Lloyd (2000) reported that due to its high content of nutrients and organic matter, sludge has been applied worldwide in increasing amounts to agricultural lands for the past several decades. Even, according to different works (García et al., 2000; García-Gil et al., 2004; Fernández et al., 2009, Martinez et al., 2003) sewage sludge are defined as organic C-rich materials produced during wastewater treatment and represent a source of organic matter, nitrogen, phosphorus and other nutrients, which, if properly managed, can be used to improve organic fertility in intensively cropped degraded soils of Mediterranean climate zone.

About the effects of sewage sludge on soil biological characteristics Baneriee et al. (1997) reported that the analysis of the size and functional diversity of the soil microbial population indicate that sludge application resulted in a microbial population of reduced diversity but of equal or greater total biomass. The increased size of the soil microbial biomass was accompanied with unchanged or increased potential enzyme activity. Thus, although the sludge application affected the biology of the soil, there appeared to be little or no negative effects on the biochemical pathways related to nutrient cycling (N, P and S mineralization). According to Gibbs et al. (2006), the sludge cake applications generally increased soil microbial biomass C and soil respiration rates, whilst most probable numbers of clover *Rhizobium* were generally unchanged.

Prior to land application, sewage sludge needs to be stabilized (Figure 1.2). The stabilization procedure commonly reduces organic matter and water content, emission of unpleasant odors, and concentrations of pathogenic microorganisms (Straub et al., 1993). Common stabilization approaches include anaerobic (mesophilic or thermophilic) and aerobic digestion, lime stabilization, composting, and heat drying (Goldfarb et al. 1999).

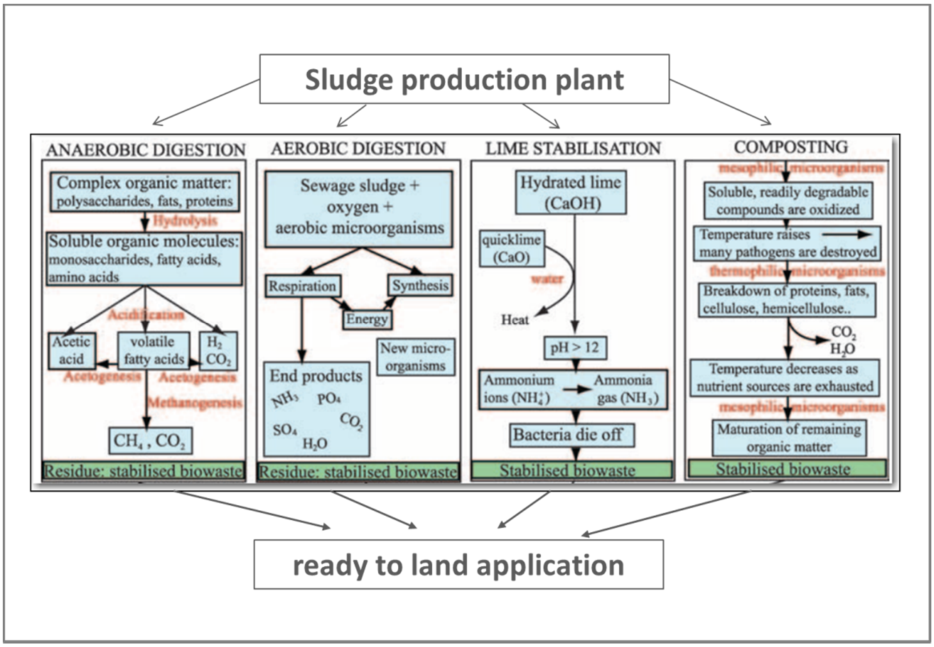

Figure 1.2: common stabilization approaches (Arthurson, 2008)

These procedures differ dramatically in their ability to reduce the pathogenic microbial content in sewage sludge (Gantzer et al., 2001). Traditionally, storage of sewage sludge was applied as the sole treatment, with the aim of sanitization in terms of destroying pathogenic microorganisms, a method proven not effective (Carrington, 2001, Gibbs et al., 1995) and therefore discontinued. The most frequently used stabilization methods for sewage sludge are biological anaerobic and aerobic digestion (Goldfarb et al. 1999). However, neither of these two procedures generates sludge that have a high quality, promoting a future shift to the use of alternative methods, such as alkaline stabilization and heat drying, to further reduce pathogen levels (Goldfarb et al. 1999), resulting in a quality higher that cited first. An additional promising option of producing hygienically safe material for arable recycling is to combine stabilization procedures, such as digestion, with pasteurization or liquid composting.

As well as the emission of unpleasant odors and concentrations of pathogenic microorganisms the presence of trace metals is another problem related to the sewage sludge disposal into agricultural land. Characteristics of sewage sludge depend upon the quality of sewage and type of treatment processes followed (Singh and Agrawal, 2008). Sewage sludge may contain high levels of toxic metals such as Pb, Cd, Ni, Cr, Hg, etc. due to the mixing of industrial wastewater with sewage (Mc Grath et al., 2000; Przewrocki et al, 2004; Dai et al, 2006; Singh and Agrawal, 2007, Stephen, 2009). Besides heavy metals, other harmful toxics such as pharmaceuticals, detergents, various salts, pesticides, toxic organics, flame retardants and hormone disruptorscan also be present in the sewage sludge (Antonious et al., 2003; Aparicio et al., 2007; Singh and Agrawal, 2008; Sánchez-Brunete et al., 2008).

The numerous trace metal contaminants in sewage sludge are either non-essential in plant metabolism such as Cd, Cr, Hg, Ni, Pb or essential in only trace quantities such as Cu, Fe, and Zn . These trace metals, which are difficult to remove, are the most significant restraint relative to land application of sludges and can often negate the benefits of land application (Heather and Lloyd, 2000).

There are social and legal concerns of uncontrolled use of sewage sludge for agriculture due to potential problems of elevated transfer of heavy metals to the food chain (Page et al., 1987), causing threat to human health (Wang et al., 2003). Heavy metal contamination in soil and groundwater may represent a long term threat for the environment and man (Ross, 1994; Baveye et al., 1999). Absorption, accumulation and tolerance to heavy metals may vary between different crops and at different levels of sewage sludge amendments (Bhogal et al., 2003; Garrido et al., 2005; Lavado, 2006; Singh and Agrawal, 2008). Crop plants may develop adaptive response to tolerate the heavy metal stress.

According to these problems is important to find a way to reduce the uptake of trace metals by plants.

Various methods have been developed for the remediation of heavy metal contaminated sites (e.g. Davis et al., 1999; Yoshizaki and Tomida, 2000). Therefore, the study of effective methods for heavy metal removal from sludge is very important to minimize the prospective health risk during application (Lasheen et al., 2000). The solubility or bioavailability of trace and toxic metals from sewage sludge is based on soil pH, soil cation exchange capacity, soil organic material, and soil water-holding capacity (Jamali et al, 2008). One approach to reduce the availability of heavy metals to plants is to decrease the concentrations of the available chemical species through increasing pH of compost (Petruzzelli, 1989; Ciavatta et al., 1993). Co-composting of solid waste with alkaline materials, such as bauxite residue, clay, and coal fly ash, has been carried out with the purpose of reducing the availability of heavy metals in compost and to supplement certain trace elements for plant growth (Qiao and Ho, 1997; Wong et al., 1997). Peles et al. (1998) found that Zn, Cu, Cd, and Pb contents in various species of crop are significantly lower in plants collected from the limed sludge compared to the unlimed sludge treated plots. An increase in Cu mobility caused by lime treatment of sewage sludge has been reported (Hsiau and Lo, 1998).

Jamali et al, (2008) reported that sludge amendment enhanced the dry weight yield of maize and the increase was more obvious for the soil with lime treatment. Liming the sewage sludge reduced the trace and toxic metal contents in the grain tissues, except Cu and Cd, which were below the permissible limits of these metals. This experiment demonstrates that liming was an important factor in facilitating the growth of maize in sludge-amended soil.

Also liming the soil reduces the bioavailability of metal for the plants uptake (Brallier S. et al., 1996; Fang and Wong, 1999; Krebs et al., 1998). Liming is a common practice in agriculture to maintain optimal soil pH, and has also been used to reduce the solubility of trace and toxic metals (Jamali et al, 2008). Speir.et al 2003 experiment results show that an intensive liming programme substantially raised soil pH values and resulted in much lower concentrations of metals in soil solution, in plant material and in groundwater.

The production of defecation lime from sewage sludge has acquired more importance after the D.G.R (regional decree) 9953/09 with which was limited the use of sewage sludge in agriculture.

Starting from 2011 for nitrate vulnerable zone (NVZ) and from 2013 for non nitrate vulnerable zone (nNVZ), the regional decree banned the industrial and municipal sewage sludge disposal; no restriction about sewage sludge originated from waste of food processing industries were defined. In order to remove this restrictions, the most important association, society and plants involved in sewage sludge management did an appeal to the Lombardy Regional Administrative Court (TAR of Lombardy).

At the end of legal dispute by the verdict n° 02822/2009 the restriction was repeal.

Despite is still possible to dispose this product, the defecation lime production starting from sewage sludge have a primarily importance. As reported above (chapter 1.2), the production of defecation lime is based, beside of sulphuric acid, to the addition to the raw material of lime. Based on the knowledge reported, the addition of lime is able to stabilize and hygienize the product and represent a valuable option to reduce the amount of plant available trace metals. Furthermore they could also be used to regulate the soil pH.

Unfortunately, no references are found on defecation lime made from sewage sludge and their effects following the agricultural land disposal.

Due to lack of knowledge about defecation lime application and positive effects presented above of the lime addition to sewage sludge, carrying out some study seem to be a valuable aspect.

1.5 Aim of the PhD thesis

According to the information reported in the previous chapters this PhD thesis was done to evaluate the effects of the defecation lime applied to agricultural land.

The experiment was carried out over two years in a paddy field located not far from the defecation lime production plant.

In this work were analysed both soil and plant variables at the start, at the end and during the growing seasons.

In order to collect the different samples of soil and plants a specific research works (Confalonieri et al 2007, Confalonieri et al. 2009) were previously done and the knowledge collected were then used.

Chapter 2

Analysis of sample size for variables related to plant, soil, and soil microbial respiration in a paddy rice field

Keywords. Visual jackknife, Resampling, Pre-sampling, Oryza sativa L., SISSI

Abstract

Pre-samplings for sample size determination are strongly recommended to assure the reliability of collected data. However, there is a certain dearth of references about sample size determination in field experiments. Seldom if ever, differences in sample size were identified under different management conditions, plant traits, varieties grown and crop age. In order to analyse any differences in sample size for some of the variables measurable in rice field experiments, the visual jackknife method was applied to pre-samples collected in a paddy rice field in Northern Italy, where a management typical for European rice was conducted. Sample sizes for 14 variables describing plant features (plant density, spikelet sterility, biomass, carbon and nitrogen concentration for the different plant organs and for the whole plant) and for 12 variables describing physical and chemical soil features (texture, pH, water holding capacity, soil organic matter, total carbon and nitrogen concentration, mineral nitrogen concentration) and soil microbial activity were estimated. The elementary units of observation were a 3-plant sample and an aggregate sample of four 125 cm 3 sub-samples respectively for plant- and soil-related variables. Sample sizes ranged between 15 and 27 for plant-related variables and between 5 and 6 for soil variables. Relating to plant features, remarkable differences in sample size were observed in carbon concentration values of different plant organs, probably due to maintenance respiration. Homogeneity among sample sizes for soil variables could be explained by the capability of aggregate samples in capturing a big part of the total variance. This study underlines

importance of carrying out pre-samplings aiming at sample size determination for different variables describing the cropping system.

2.1 Introduction

Preliminary samplings aiming at determining sample size should be carried out before performing measurements to avoid the collectionof data characterized by low reliability (Lapitanet al., 1979; Nath and Singh, 1989; Tsegaye and Hill, 1998; Ambrosio et al., 2004). However, sample size is often arbitrarily determined (Confalonieri et al., 2006), increasing the probability of Type II errors if the sample size is smaller than needed or expending critical resources or funds if the sample size is larger than necessary (James-Pirri et al., 2007).

Although references about description of experiments where sample size was determined are not common, the effort invested in carrying out, describing and discussing results in the rare available examples demonstrates the importance of this practice.

According to the different situations, sample size determination is a process characterized by different degrees of complexity. Madhumita Das (2007) estimated sample size for saturated hydraulic conductivity of 129 topsoil samples (0.00–0.20 m depth) in India, founding values from 2 to 8 according to different levels of confidence and error percentage. Analyzing severity of Septoria leaf spot (caused by Septoria albopunctata) on blueberry plants, Ojiambo and Scherm (2006) identified 75 leaves (selected from 3 shoots per bush on 25 bushes) as the optimal sample size to determine disease severity as number of spots per leaf. A sample of 144 leaves (2 each sampled from 3 shoots per bush on 24 bushes) was required to detect disease severity as percent of necrotic leaf area. Araujo et al. (2004)identified 15% of total root mass of common bean plants as adequate to provide reliable root traits estimates. Lima e

Silva et al. (2005)calculated sample size for 4 sorghum traits: plant height, dry matter without panicle, panicle length, and panicle dry matter, finding sample sizes of, respectively, 14, 11, 14, and 24 plants per plot concerning the 4 variables were adequate. A single sample size of 25 plants was found by the same authors for all the variables using the experimental coefficient of variation alone instead of a formula for sample size derived by Thompson (1992). The estimation of different sample sizes for different traits was carried out also byStorck et al. (2007), who estimated sample size for the following maize traits: ear length, ear and cob diameter, ear weight, weight of grains per ear, cob weight and the weight of 100 grains, the number of grain rows per ear, the number of grain per ear and the length of grains. Results showed that the weight-related ear features needed 21 ears for a precise (5%) determination; 8 and 13 ears were needed respectively for size- and number-related features. Confalonieri et al. (2006), analysing paddy rice fields, estimated samples size values ranging from 15 to 33 plants under different management conditions (nitrogen fertilization, sowing techniques, sown variety) and development stages.

In these examples, different techniques to determine sample size were used and specific solutions were applied in the different conditions. Ojiambo and Scherm (2006)sampled plants at 3 hierarchical levels (leaf, shoot, bush) and related the sample size to the total time required for the determination (respectively 36 and 22 min in the two cases), thus taking into account the effort required in each case. Time required to determine a variable was taken in account in sample size determination also by Araujo et al. (2004). Storck et al. (2007)estimated sample size according to the formula $n_0 = t_{\alpha/2}^2 CV^2/D^2$, following the approach proposed by Martin et al. (2005), where CV is the percent coefficient of variation of the sampling error, D is the percent half-amplitude of the confidence interval for the average and this the critical value of the distribution. The same authors estimated sample size for different variables/parameters by clustering them into classes (weight-, size-, and number-related traits). Confalonieri et al. (2006) demonstrated how the sample size for rice aboveground biomass (AGB) determinations could vary according to management conditions using a resampling-based method, even when different managements affect plants growing in the same biophysical context.

The objective of this paper was to analyse the variability of different aspects of a paddy rice fields through sample size determinations for some of the plant and soil features of interest in agronomic field experiments. In Confalonieri et al. (2006), differences in variability and in sample size were analysed for aboveground biomass under different management conditions; in this study, the same objective was pursued in a standard rice field but concerning different variables related to soil, plant, and soil microbial activity.

2.2 Material and methods

2.2.1 Experimental data

Data were collected in a field located in the southern part of Milano (Northern Italy, 45.478N, 9.188E, 120 m a.s.l.) during 2006. The soil was loam, acid, with soil organic matter content next to 2.5%. Rice (Oryza sativaL., cv Libero, Indica type)was row seeded on April 12 and flooded at the third leaf stage (May 10; code 13 of the BBCH scale for rice; Lancashire et al., 1991). Rice received 140 kg N ha^{-1} split in 2 events: pre-sowing and top-dressed at the panicle initiation stage (June 29; code 34 of the BBCH scale for rice). 33.6 kg P_2O_5 ha^{-1} and 92.4 kg K^2O ha^{-1} were distributed in pre-sowing. Field management allowed prevention of water and nutrient stresses and kept the field weed and pest free.

Plant-related measured variables were aboveground biomass at physiological maturity (AGB; September 19; code 99 of the BBCH scale for rice), plant nitrogen concentration (PNC) and plant carbon concentration (PCC) at physiological maturity, spikelet sterility, and plant density. AGB, PNC and PCC were determined for leaves, stems and panicles separately. Measured soil variables were texture, mineral nitrogen concentration ($N\text{-}NO_3^-$ and $N\text{-}NH_4^+$), total carbon and nitrogen concentrations (SCC and SNC), soil organic matter (SOM), water holding capacity (WHC), pH (KCl), and pH (H2O) in the soil layer 0.0–0.2 m. Microbial activity in the soil (SMA) was estimated using a respirometric approach.

2.2.2 The visual jackknife

The visual jackknife method (Confalonieri, 2004; Confalonieri et al., 2006) was used in sample size determination. The standard jackknife (Tukey, 1958) is a resampling method based on the division of the original sample of N elements into groups of k elements, with k equal to 1 in case N is low. $N!/(N-k)!\,k!$ virtual samples (combinations without repetitions) of $N-k$ elements are generated by eliminating N!/(N – k)!k! times k different values from the original sample. In our case, the original sample is represented by the data coming from the pre-sampling. In the visual jackknife, different values of k are used. The process of generation of the $N!/(N-k)!\,k!$ virtual samples is repeated N – 1times with k assuming values from 1 to $N-2$, for a total of $\sum_{k=1}^{N-2} N!/(N-k)!\,k!$ different virtual samples. Mean and standard deviation are computed for all the generated samples and plotted on two charts, with the values of $N-k$ on the X-axis and the means (or standard deviations) on the Y-axis, in order to get a visual representation of how the means and the standard deviations of the generated samples vary with increasing $N-k$ values. Conceptually, the optimal sample size is considered equal to the $N-k$ value for which the variability among the means does not really decrease anymore with increasing sample size. The algorithm used for the determination of the optimal sample size consists of selecting $(N-k)'$ out of those $N-k$ higher than 2 and lower than $N-2$. Four weighted linear regressions are performed over the generated means as follows: the first and the second run, respectively, over the highest and lowest values of the $N-k \leq (N-k)'$; the third and the fourth run over the highest and lowest values of the $N-k > (N-k)'$. A global index

(SR^2) is computed by summing the coefficients of determination (R^2) of the four regressions. The reiteration of this procedure for all the possible $(N-k)'$ allows the identification of the optimum sample size, that is the $(N-k)'$ with the highest SR^2. The process stops when the next sample size does not produce SR^2 larger than 5% than the previous. A trimming process allows leaving extreme samples out of computation (for instance, the 5% most external means). This visual jackknife method overcomes the typical limitations of conventional methods (parametric statistics), requiring data-matching the statistical assumptions of normality and homoscedasticity.

The software SISSI 1.00 (Shortcut In Sample Size Identification; Confalonieri et al., 2007) was used to apply the visual jackknife. SISSI provides an easy access to the resampling-based computational procedures the visual jackknife is based on, and allows the user to easily customize the resampling settings. Numeric and visual outputs are displayed in the graphical user's interface, together with the sample size calculated with classical procedures based on Student's t. After the software has automatically applied the regression-based procedure to calculate the optimal sample size, the user is allowed to adjust manually the resampling estimated sample size. This is meant to further reduce sample size if the variability achieved (expressed as % coefficient of variation) is expected to be low enough to fall within what is considered by the researcher to be acceptable.

The SISSI's installation package is available free of charge for non-commercial purposes at http://www.robertoconfalonieri.it/ software_download.htm. The program is fully documented by the accompanying user's manual, which provides a detailed description of the scientific background and principles of usage.

2.2.3 Sample size determination

2.2.3.1 Plant-related variables

AGB, PNC, PCC and spikelet sterility were determined considering a randomly chosen 3-plant aggregate sample as basic unit of observation (Confalonieri et al., 2006), with N equal to 27 (see Section 2.2.2). AGB (kg ha^{-1}) was determined by drying the plant samples in oven at 1058C until constant weight to express them as dry matter. PNC (%) and PCC (%) were measured using an Elementary Analyser (model NA 1500, series 2, Carlo Erba, Italy), after milling the plant samples at 0.5 mm. Plant density (plants m^{-2}) was determined adopting a value of N equal to 20 and as basic unit of observation the value $L/n \cdot R$, where L is a segment of row measuring 100 cm, n is the number of emerged plants in L, and R is the number of rows in a 100 cm segment crossing the rows.

2.2.3.2 Soil variables

SMA (mg CO_2 g DM^{-1} 25 day^{-1}), texture, N-$NO_3^-$and N-$NH4^+$ (respectively kg N-$NO_3^-$$ha^{-1}$and kg N-$NH4^+$$ha^{-1}$), total carbon and nitrogen (%), SOM (%), WHC (%), pH (H_2O), and pH (KCl) (-) were determined assuming an aggregate sample (four 125 cm^3 sub-samples) as basic unit of observation, with N equal to 9. WHC was determined using the Stackman box method (Klute, 1986). SMA was measured as CO_2 release in a static system (ISO, 2002). Soil weights of 25 g (40% of the WHC) were incubated at 20°C in a closed vessel and the released CO_2 was adsorbed in a solution of sodium hydroxide (0.05 mol l^{-1}). The

CO_2absorbed was precipitated by adding $BaCl_2$. The unused NaOH was then titrated with HCl (0.1 mol l^{-1}). The respiration test was carried out for 25 days.

Table 2.1:

Features of the pre-samplings carried out for the different plant-related variables. Shapiro–Wilk normality test was carried out for all the variables; Bartlett homoscedasticity test was carried out among the groups of coherent variables. Levene homoscedasticity test was carried out in case of deviation from normality.

Variable		Number of pre-sampling units*	Units	Mean	Standard deviation	Normality†	Homoscedasticity‡
Biomass	Total (aboveground)	54	kg ha^{-1}	10250.36	2494.73		B-
	Leaves	54	kg ha^{-1}	858.94	269.61		
	Stems	54	kg ha^{-1}	6181.47	1509.92		
	Panicles	54	kg ha^{-1}	3209.95	799.20		
Nitrogen concentration	Total (aboveground)	54	%	0.72	0.09		B-
	Leaves	54	%	0.66	0.11		
	Stems	54	%	0.49	0.09		
	Panicles	54	%	1.16	0.14		
Carbon concentration	Total (aboveground)	54	%	42.02	0.37		B-, L+
	Leaves	54	%	40.67	0.50		
	Stems	54	%	40.99	0.51		
	Panicles	54	%	44.36	0.58	S ($P < 0.10$)	
Spikelet sterility		54	%	14.78	7.01		-
Plant density		20	plants m^{-2}	478.00	114.00	S ($P < 0.05$)	-

** Number of plants for variables describing plant features; number of determinations for plant density.*

† S-indicates not normal according to the Shapiro–Wilk test; blanks indicate normality.

‡ B and L—indicate not homoscedastic respectively according to the Bartlett and Levene test. The latter is used in case of deviation from normality; L+ indicates homoscedastic according to the Levene test; – indicates that homoscedasticity tests were not performed since the variable was not belonging to a group of coherent variables.

Table 2.2

Features of the pre-samplings carried out for the variables related to soil and soil microbial activity. Shapiro–Wilk normality test was carried out for all the variables; Bartlett homoscedasticity test was carried out among the groups of coherent variables. Levene homoscedasticity test was carried out in case of deviation from normality.

Variable		Number of pre-sampling units*	Units	Mean	Standard deviation	Normality†	Homoscedasticity‡
Soil microbial activity		9	mg CO_2 g DM $^{-1}$ 25 day^{-1}	22.49	4.23		-
N-NO_3 concentration		9	kg N-NO_3^- ha^{-1}	2.86	1.36		B-
N-NH_4 concentration		9	kg N-NH_4^- ha^{-1}	3.53	0.94		
Total carbon concentration		9	%	1.42	0.12		B-, L-
Total nitrogen concentration		9	%	0.13	0.01	S ($P < 0.10$)	
Soil organic matter		9	%	2.45	0.20		-
Water holding capacity		9	%	41.69	1.99		-
pH (H_2O)		9	-	5.69	0.12		B-
pH (KCl)		9	-	4.62	0.14		
Texture	Sand	9	%	39.46	5.97		B-
	Clay	9	%	17.18	0.97		
	Silt	9	%	43.36	5.48		

** Aggregated samples (four 125 cm3 sub-samples).*

† S—indicates not normal according to the Shapiro–Wilk test; blanks indicate normality.

‡ B and L—indicate not homoscedastic respectively according to the Bartlett and Levene test. The latter is used in case of deviation from normality; L+ indicates homoscedastic according to the Levene test; – indicates that homoscedasticity tests were not performed since the variable was not belonging to a group of coherent variables.

Texture was evaluated using the gravimetric method according to USDA. N-NO3- and N-NH4+ are measured with a continuous-flow analyzer (Flow Comp 1500, Carlo Erba, Italy). Total carbon and nitrogen concentration were determined using an Elementary Analyzer (model NA 1500, series 2, Carlo Erba, Italy).

2.2.3.3 Data pre-processing

Shapiro–Wilk (Shapiro and Wilk, 1965) and D’Agostino– Pearson (D’Agostino, 1970, 1986; D’Agostino et al., 1990) statistical tests were applied to test the assumption of the normality of the distributions of the data from the original N-element samples. Homoscedasticity for coherent variables (e.g., carbon concentration in the different plant organs) was verified with the Bartlett’s test (Bartlett, 1937)and, in case of departures from normality, with the Levene test (Levene, 1960) which is less sensitive than Bartlett’s to normality despite Bartlett’s better performance (Snedecor and Cochran, 1967).

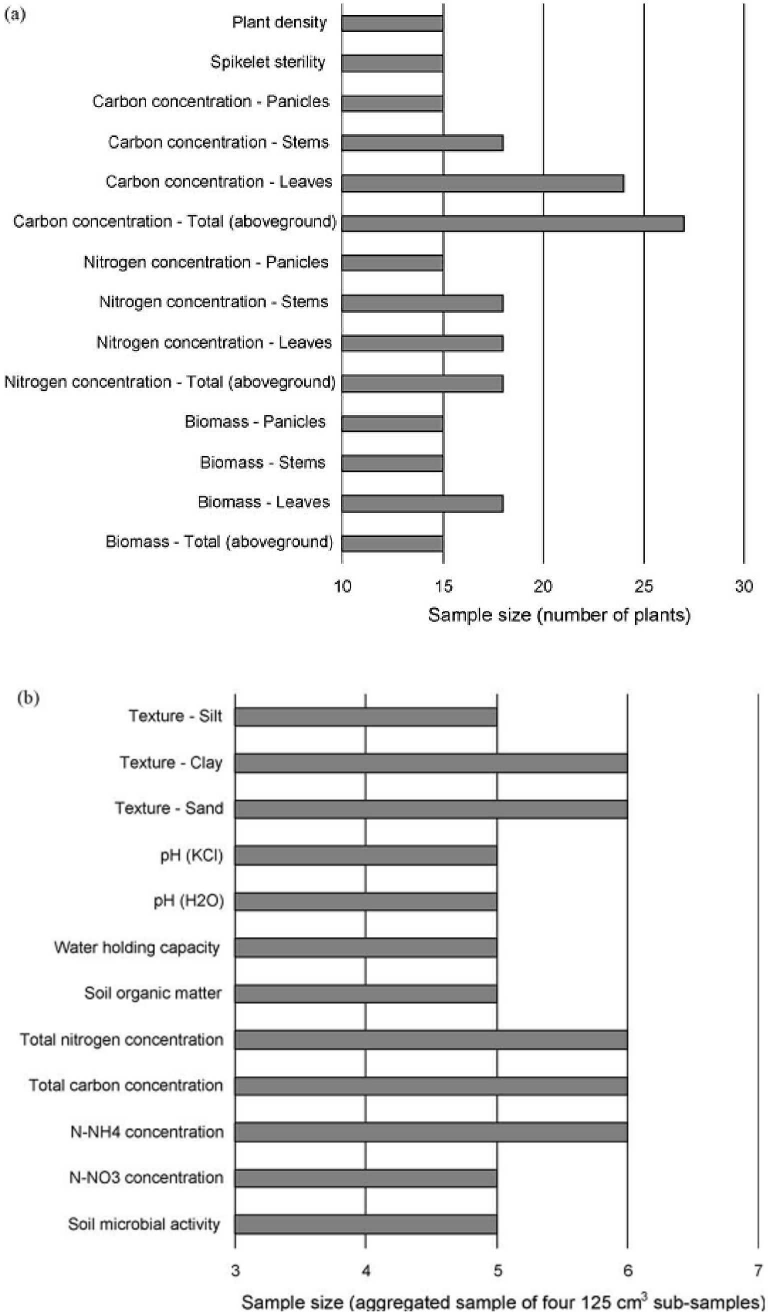

Fig. 2.1 Sample sizes obtained for the different variables. (a) Plant-related variables; (b) Soil-related variables.

2.3 Results and discussion

2.3.1 Preliminary analysis

Panicles carbon concentration, plant density and SNC showed deviation from normality, whereas homoscedasticity was not verified for practically all the variables with a coherent meaning (e.g., total, leaves, stems, panicle biomass) (Tables 2.1 and 2.2). Variances of total plant, leaves, stems and panicles carbon concentrations were considered homogeneous according to the Levene test.

2.3.2 Plant-related variables

Higher sample sizes tended to be associated with carbon concentration variables; whereas, lower sample sizes were associated with plant density, spikelet sterility, and biomass variables. Among biomass-related variables, leaves presented the highest variability, probably due to senescence phenomena and loss of the oldest leaves in the last part of the crop cycle and during sampling procedures (Fig. 1a). This effect disappears when considering the variability of total biomass because of the low relative weight of leaves compared to the other plant organs (see Table 1a). Lower sample size values for panicles nitrogen concentration with respect to the other plant organs is probably due to translocation during the grain filling and ripening phases. Nitrogen translocation processes are characterized by a single sink (grains) and by multiple sources, with leaf blades playing a major role, followed by stems and leaf sheaths (Mae and Ohira, 1981).Differences in nitrogen translocation efficiencies from different plant organs could be modulated according to nitrogen availability and uptake rates (driven by microscale phenomena) before anthesis, when most of the nitrogen uptake in rice plants occurs (Ntanos and Koutroubas, 2002), and to conditions experienced during the grain filling. According to this hypothesis, the variability in the sink nitrogen concentration at maturity would be lower than that of the sources.

The highest differences were observed in the sample sizes for the carbon concentration in the different plant organs. A possible explanation is related to their maintenance respiration rates. According to Van Diepen et al. (1988), relative maintenance respiration rates (kg CH_2O $kg^{-1}day^{-1}$) in leaves are about 30% higher than in stems and almost 7 times that of grains. For all these plant organs, respiration rate is strongly dependent on temperature. According to the morphology of the canopy and to the non-homogeneity of the plant density, leaves belonging to different plants can be exposed to different micrometeorological conditions (Uchijima, 1976). Even small differences in temperature exposure among plants can have an impact in modulating the high leaves' maintenance respiration rates, affecting the final variability in leaves carbon concentration. This effect can be even clearer when the field is not perfectly levelled, when water pools persist during drying events. Even a few centimetres of water can affect the vertical thermal profile (Confalonieri et al., 2005), generating variability between the plants growing in pools and those growing where the field is already dried.

2.3.3 Soil variables

The variability in sample size for soil-related variables is lower than the one discussed for plant variables (Fig. 1b). Sample size is 5 in 60% of the cases and 6 in the other ones. The definition of a sampling unit consisting in an aggregate sample of four sub-samples allowed surely capturing a significant amount of the total variance in the aggregate sample. The resulting low variability among aggregated soil samples is able to explain the low and homogeneous sample sizes obtained for soil variables. Moreover thepresence of floodwater formost of the crop cycle’s days represents a kind of buffer for the physical and biochemical environment, thus reducing the spatial variability (e.g., the elements transformation rates).

2.4 Conclusions

Following a study where rice aboveground biomass sample size variability was discussed under different management conditions and different development stages (Confalonieri et al., 2006), we analysed here the sample size variability for different variables describing plant, soil and microbial activity under a standard rice management for European conditions.

In many cases, the statistical assumptions (normality and homoscedasticity) required by classic procedures in sample size determination based on t-distribution were not met. Moreover, the t-distribution methods require as input the maximum acceptable error (difference between sample and population means), which in many cases cannot be easily identified, since it varies according (i) to biophysical factors which could change from an experimental field (or situation) to another and (ii) to the resources for carrying out the experimentation (Confalonieri, 2004). Consequently, a resampling-based method was used for sample size determination. In general, sample size values of plant features were higher than those estimated for soil-related variables. Among plant variables, whose sample size ranges between 15 and 27 plants, sample size for carbon concentration in the different plant organs presented the highest variability. For soil, sample sizes are similar for variables describing biochemical and physical aspects and microbial activity.

This work confirmed the need of carrying out pre-samplings aiming at sample size determination to guarantee the representativeness of the measurements. In a previous study, Confalonieri et al. (2006)underlined the importance of sample size determination for aboveground biomass under different management conditions, sown varieties, and development stages. Here, the importance of determining specific sample sizes also for the different variables describing a rice-based cropping system has been demonstrated. Besides these theoretical considerations, this paper could be used as support for identifying suitable sample sizes for the variables analysed in case of lack of resources for extensive pre-sampling investigations.

Chapter 3

Application of defecation lime in paddy field: effects on soil chemical properties

Keywords. Defecation lime, rice (Oryza sativa L.), soil, pH, trace metals.

Abstract

Defecation lime is the product obtained by the hydrolysis of biological materials by lime and/or sulphuric acid and subsequent calcium sulphate precipitation”. This product is defined by the Italian L.D 75/2010 “as “calcium and magnesium corrective products”. Among biological material also sewage sludge can be used being an alternative option to give a value and to dispose them in a most economical and useful way.

Despite there are some knowledge and work about the effects of defecation lime application into agricultural land, no reference are found about defecation lime made from sewage sludge.

The aim of this paper is to evaluate the effect of defecation lime on paddy soil chemical characteristics over two years of experiments.

Results of their application suggest that, according to the acquired knowledge, there is no contraindication for their disposal in paddy field. It seem that after two year they are able to determine a little variation of the soil pH. At the end of second year results of Anova test ($\alpha = 0.1$) showed that plots treated only with defecation lime had a upper pH value compared with other treatments. Over two years, started from 5.80, they reached a value of 6.11. Instead at the end of each year not significant differences ($P(F) > 0.05$) was recorded for soil total trace metals concentration.

3.1 Introduction

According to Legislative Decree 75/2010 “defecation lime is the product obtained by the hydrolysis of biological materials by lime and/or sulphuric acid and subsequent calcium sulphate precipitation”. This product is defined as “calcium and magnesium corrective products”. They must contain at least 20% of calcium oxide (CaO) and 15% of sulphuric anhydride (SO_3). The origin of the hydrolyzed biological material must be reported on the label.

Several biological materials could be used to produce defecation lime. Masharipova (2006), reported the production by sugar industry waste. Among biological material also sewage sludge can be used being an alternative option to give a value and to dispose them in a most economical and useful way.

The EU by the Directive 86/278/EEC seeks to encourage the application of sewage sludge in agriculture and regulate its use in such a way as to prevent harmful effects on soil, vegetation, animals and man. Safe disposal of sewage sludge is one of the major environmental concerns (Ghanavati et al., 2012). Sewage sludge, being organic waste, is a good source of plant nutrients such as N, P, K, Ca, Mg, Fe, etc. as well as other organic constituents (Martinez et al., 2003). As recommended, sewage sludge should be applied with caution in accordance with the plant nutrient needs without impairing the quality of the soil, surface and ground water. Sewage sludge intended for arable land use needs to be previously evaluated for quality regarding content of heavy metals, persistent organic pollutants and pathogenic microorganism (Arthurson, 2008). Prior to land application stabilization is needed. The stabilization procedure commonly reduces organic matter and water content, emission of unpleasant odors, and concentrations of pathogenic microorganisms (Straub et al, 1993). Common stabilization approaches include anaerobic (mesophilic or thermophilic) and aerobic digestion, lime stabilization, composting, and heat drying (Goldfarb et al, 1999). The most frequently used stabilization methods for sewage sludge are biological anaerobic and aerobic digestion (Goldfarb et al, 1999). A stabilization process similar to liming one is used in the defecation lime producing process. Attention on defecation lime increased in Italy in the last years as a consequence of DGR 9953/09. According to this regional decree there was a limitation for the industrial and municipal sewage sludge arable crop land application. It banned their use into NVZ (nitrate vulnerable zone) starting from 2011 while starting from 2013 into nNVZ (non-nitrate vulnerable zone). No restriction about sewage sludge originated from waste of food processing industries were defined. This restriction was repealed in 17 May 2011 as a consequence of Lombardy court sentence.

Furthermore it should be remembered that from years a few number of livestock are bred in paddy field area. In northern Italy (Lombardy, Piedmont), according to sixth agricultural census return, there are 27952 head of cattle and 854543 hectares of cereal cultivated area. Paddy field area is 227608 hectares (ISTAT, 2011). As a result there is little quantity of organic matter available to maintain paddy soil fertility. In this way the chance of defecation lime application is important to preserve this valuable production factor. Despite there are few papers about the effects of defecation lime originated from sugar industry waste no reference are found about defecation lime made from sewage sludge. In the other side there are much work about sewage sludge and their effect into different arable crop land.

The aim of this paper is to evaluate the effect of defecation lime on paddy soil chemical characteristics over two years of experiments.

3.2 Materials and Method

3.2.1 Experimental field and design

A two years experiment was done in order to evaluate the effects of defecation lime on soil chemical characteristics. The experiment was performed in a paddy field having 1.27 hectares area and located in Cava Manara (Pavia, Lombardy, 45.151 N, 9.084 E, 80 m a.s.l.), a municipality sited in an area where rice is widely cultivated.

Four blocks and four plots for each block were done.

Four level were tested:

0. CONTROL: never defecation lime nor fertilizer are applied,
1. USUAL MANAGEMENT: management according to the usual agricultural technique,
2. DEFECATION LIME + CHEMICAL FERTILIZER: pre-sowing defecation lime and top-dressing chemical fertilizer application,
3. DEFECATION LIME: pre-sowing defecation lime application.

“Zero” level represented the background of the field; it is the natural evolution of the soil without any fertilizer input. Level “one” was the typical managing situation. Level “two” was done in order to verify the ability of the defecation lime to provide to a part – about half – of plant N nutrition, while left amount was supplied by chemical fertilizer by two different applications: tillering and panicle initiation development stage. Level “three” wanted to test the corrective ability of the product and, at the same time, its capacity to ensure the N needed for rice growth. Paddy field management of the experiment is reported in table 3.1.

Table 3.1
Paddy field management

		Application timing			
Year	Level	Before tillage	Tillering	Panicle initiation	Total N applied (kg ha^{-1})
First year	0	-	-	-	0
	1	org. fert.	chem. fert.	chem. fert.	160
	2	def. lime	chem. fert.	chem. fert.	184
	3	def. lime	-	-	195
Second year	0	-	-	-	0
	1	org. fert.	chem. fert.	chem. fert.	160
	2	def. lime	chem. fert.	chem. fert.	162
	3	def. lime	-	-	317

org. fert. (organic fertilizer): was represented to the hoof and horn fertilizer (12-2-0; 40% of TOC); chem. fert. (chemical fertilizer: at the tillering was applied ENTEC 24:0:29 while at the panicle initiation a 20:20:20 fertilizer was applied); def. lime (defecation lime)

The amounts of defecation lime applied are reported below (table 3.2).

Table 3.2
Amounts of defecation lime applied in the experiment

Year	Treatment	Amount (t ha^{-1})
First year	0	-
	1	-
	2	2.4
	3	7.0
Second year	0	-
	1	-
	2	3.3
	3	13.9

Amounts as reported on dry matter

Tillage operations done during the two years are reported in table 3.3.

Table 3.3
Tillage operation

First year	**Second year**
1. defecation lime speading	1. defecation lime speading
2. plowing	**2. disc harrowing**
3. harrowing by rotary harrow	3. harrowing by rotary harrow
4. sowing by row seeder	4. sowing by row seeder
5. herbicide treatments	5. herbicide treatments
6. tillering fertilization (where needed)	6. tillering fertilization (where needed)
7. fungicide treatment	7. fungicide treatment
8. harvesting	8. harvesting

Plowing was conducted at 25 cm while disc harrowing at 15 cm depth.

As shown in table above except for the main tillage operation needed to bury the defecation lime into the soil, the same works were done over two years. The different main tillage techniques were chosen in order to verify the effect of defecation lime on the plant germination depending on the burying depth.

3.2.2 Defecation lime used in the experiment

Defecation lime used was produced in the Alan s.r.l. specific plant located in Bascapè (Pavia, Lombardy).

The production process is based on the patent presented at the chapter 1.2 and specifically is reported below (figure 3.1):

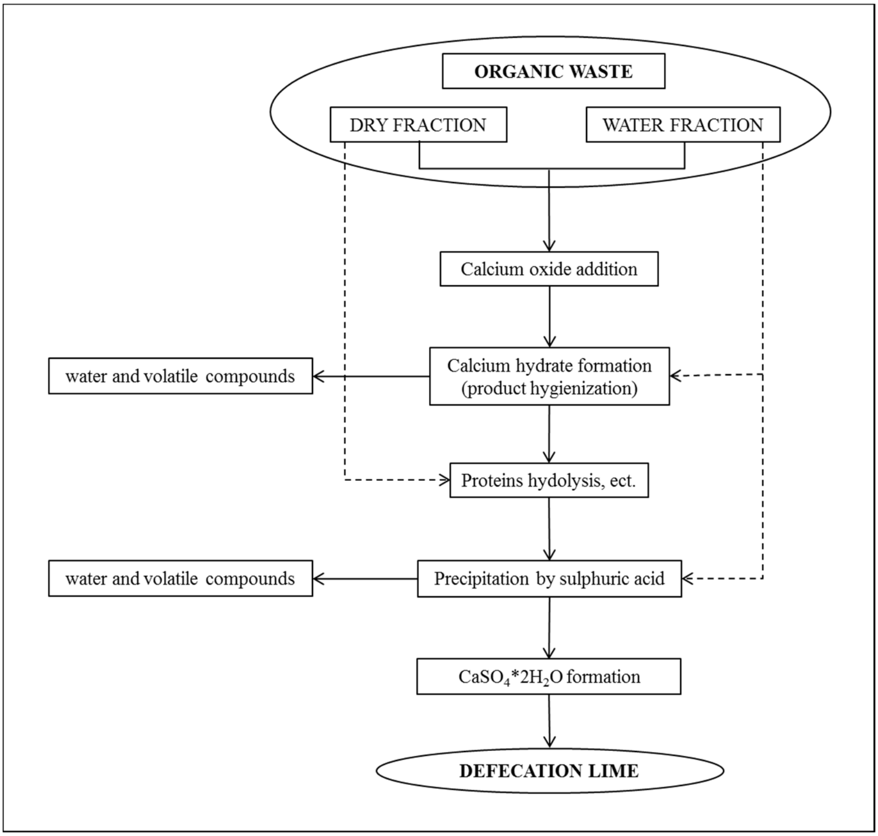

Figure 3.1: process for defecation lime production adopted by Alan s.r.l

Raw materials used for the defecation lime production derived from industrial and municipal wastewater plants from Lombardy region.

Defecation lime was applied on paddy soil using Franzosi FVR 110 muck spreader while chemical fertilizer was applied by a centrifugal fertilizer spreader.

Chemical characteristic of the defecation lime are reported below (table 3.4)

Table 3.4
Chemical characteristics of the defecation lime applied

Parameter	First year	Second year	Limit (d.lgs 75/2010)
CaO (%)	26.50	24.70	> 20 %
SO_3 (%)	18.00	20.10	> 15 %
TOC (g kg-1)	29.00	39.21	
Ntot (%)	2.80	2.30	
Ni (mg kg^{-1})	22.00	58.98	100
Cd (mg kg^{-1})	0.50	0.46	1.5
Zn (mg kg^{-1})	390.00	213.25	500
Hg (mg kg^{-1})	0.72	0.07	1.5
Cu (mg kg^{-1})	130.00	70.43	230
Pb (mg kg^{-1})	29.70	56.98	100
Cr (mg kg^{-1})	53.50	55.81	n.d.
As (mg kg^{-1})	0.40	3.52	n.d.

“Limit” represent the maximum concentration allowed into defecation lime by law (D.lgs 75/2010)

3.2.3 Soil sampling and characterization

Soil was sampled both before the experiment and at the end of the two rice-growing seasons.

In the first case samples were required to know the initial situation while in the second case to evaluate the effect of the different treatments. Each sample was taken from the surface of soil - cleaned from straw of cultivation - to 25 cm deep.

In the first case fifteen samples were collected randomly into the experimental area while, in the second case one sample – merging different subsamples taken randomly – for each plot was collected.

The samples were air-dried, sieved to 2 mm mesh size and stored for further chemical analysis.

In order to define the soil characteristics the variables reported below were analysed:

- pH (H_2O)
- CEC (cmol$^{(+)}$ kg^{-1})
- TOC (g kg^{-1})
- Cr (mg kg^{-1})
- Cu (mg kg^{-1})
- Ni (mg kg^{-1})
- Pb (mg kg^{-1})
- Zn (mg kg^{-1})
- Cd (mg kg^{-1})
- Hg (mg kg^{-1})
- As (mg kg^{-1})

Soil total concentration of trace metals reported were determined while just for five of them – Copper (Cu), Nickel (Ni), Lead (Pb), Zinc (Zn) and Cadmium (Cd) – also the plants bioavailable amount were analysed.

Moreover the texture of the soil was established.

Soil common chemical parameters were defined according to standard soil science procedures (Faithfull, 2002).

Total trace metals concentrations were defined as follows: a representative sample of up to 0.5 g is digested in 10 mL of concentrated nitric acid using microwave heating with a suitable laboratory microwave unit. The sample and acid are placed in a fluorocarbon (PFA) microwave vessel. The vessel is capped and heated in the microwave unit. After cooling, the vessel contents are diluted to volume of 50 mL, filtered and then analyzed. Elements contents were determined by inductively coupled plasma mass spectrometry (ICP-MS, Varian. Fort Collins, USA). A certified standard reference material (GBW 07405, soil) from the National Centre for Standard Materials (Beijing, China) was used in the digestion and analysis. Average recovery was 92 ± 4% for all the metals determined. To ensure the accuracy and precision in the analyses, reagent blanks were run with samples.

In order to define bioavailable amount of trace metal specific extraction method (ISO/DIS 14870, ISO 14876, ISO 11047) was applied. After the extraction, the analysis was performed with inductively coupled plasma mass spectrometry (ICP-MS, Varian. Fort Collins, USA) as the same way reported for the total trace metals concentrations.

Soil physical and chemical characteristics before the experiment are defined in table 3.5.

3.2.4 Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) with F test used to compare means (SPSS statistical software, SPSS Chicago IL). In order to separate statistically different means, Ryan-Einot-Gabriel-Welsch F post hoc test were performed. P value was set at $\alpha = 0.05$. According to Acutis et al (2012) data where checked for homogeneity of variances and normality, and experimentwise test are used for multiple comparison of means

3.3 Results

3.3.1 First year

As presented above result of chemical analysis done over soil samples collected at the start of experiment are reported in table 3.5:

Table 3.5
Soil chemical characteristics before the experiment

Parameter	Value	Limit (D.Lgs. 99/92)
pH $_{H2O}$	5.8	
Texture (USDA)	loamy sand	
TOC (g kg^{-1})	10.17 ± 1.5	
CEC (c mol$^{(+)}$ kg^{-1})	5.78 ± 0.15	
Cr (mg kg^{-1})	20.20 ± 0.50	n.d.
Cu (mg kg^{-1})	8.90 ± 1.20	100
Ni (mg kg^{-1})	23.90 ± 1.7	75
Pb (mg kg^{-1})	16.80 ± 0.05	100
Zn (mg kg^{-1})	33.90 ± 0.80	300
Cd (mg kg^{-1})	0.10 ± 0.002	1.5
Hg (mg kg^{-1})	0.07 ± 0.02	1
As (mg kg^{-1})	2.65 ± 0.004	n.d.

Trace metals is reported as total concentration; concentration limit are related at the maximum amount of heavy metals in agricultural soil for the application of sewage sludge

Because no restriction are defined for the soil heavy metal amount related to the soil calcium and magnesium corrective products application, in table above, soil heavy metals concentration limits for the application of sewage sludge (L.D. 99/92) was used.

Accoding to United States Department of Agriculture soil had a moderate acid reaction and a lomy sand texture (Soil Survey Manual, 1993).

Statistical analysis performed on the data of main soil characteristics (table 3.6) did not show any difference between treatments.

Table 3.6
First year results about chemical characteristics of soil samples

Treatment	pH (H_2O)	TOC ($g\ kg^{-1}$)	CEC ($c\ mol\ kg^{-1}$)
0	5.95	7.90	7.61
1	5.90	8.78	7.51
2	5.70	7.55	7.90
3	5.80	8.53	8.16
Sig.	0.401	0.360	0.540

Values in table are the mean for each treatment; P(F) in all casas was >0.05

Concerning the total trace metals concentration, no statistical differences were shown.

Table 3.7
First year results about total trace metals in soil

Treatment	Cr (mg kg-1)	Cu (mg kg-1)	Ni (mg kg-1)	Pb (mg kg-1)	Zn (mg kg-1)	Cd (mg kg-1)	Hg (mg kg-1)	As (mg kg-1)
0	17.95	7.25	14.60	15.35	32.30	0.22	0.06	2.44
1	17.75	7.25	12.08	16.85	31.95	0.20	0.07	2.49
2	17.90	6.95	11.80	16.20	31.75	0.26	0.07	2.80
3	19.56	6.97	13.85	17.30	33.80	0.19	0.07	2.48
Sig.	0.274	0.935	0.256	0.531	0.664	0.948	0.262	0.465

P(F) in all casas was >0.05

Also concerning the plants bioavailable trace metals, no difference were found.

Table 3.8
First year results about plants bioavailable trace metals in soil

Treatment	Ni (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
0	0.111	0.012	0.367	0.058	0.002
1	0.192	0.012	0.395	0.047	0.003
2	0.115	0.013	0.402	0.049	0.003
3	0.124	0.013	0.420	0.048	0.003
Sig.	0.581	0.455	0.511	0.687	0.819

P(F) in all casas was >0.05

3.3.2 Second year

Also for the second year, no difference over all soil characteristics and trace metals concentrations were show.

Table 3.9

First year results about chemical characteristics of soil samples

Treatment	pH (H_2O)	TOC (g kg-1)	CEC (c mol kg-1)
0	5.63	6.53	5.97
1	5.65	8.56	6.58
2	5.78	7.34	5.99
3	6.11	9.65	6.89
Sig.	0.053	0.053	0.529

Values in table are the mean for each treatment; P(F) in all casas was >0.05

Table 3.10

Second year results about total trace metals in soil

Treatment	Cr (mg kg-1)	Cu (mg kg-1)	Ni (mg kg-1)	Pb (mg kg-1)	Zn (mg kg-1)	Cd (mg kg-1)	Hg (mg kg-1)	As (mg kg-1)
0	23.82	14.03	16.94	16.93	60.22	0.15	0.13	5.56
1	26.84	13.78	19.19	17.27	54.83	0.12	0.08	5.86
2	26.66	13.05	19.24	17.75	64.40	0.14	0.08	5.22
3	26.66	13.93	18.81	19.38	64.79	0.15	0.06	5.87
Sig.	0.574	0.076	0.568	0.635	0.477	0.626	0.269	0.569

P(F) in all cases was >0.05

Even bioavailable plant metals concentration did not show statistical difference.

Table 3.11

Second year results about plants bioavailable trace metals in soil

Treatment	Ni (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
0	0.126	0.015	1.103	0.641	3.121
1	0.135	0.018	1.212	0.796	3.630
2	0.132	0.017	1.286	0.685	2.093
3	0.130	0.019	1.231	0.765	2.264
Sig.	0.887	0.366	0.462	0.474	0.601

P(F) in all casas was >0.05

3.4 Discussion

As reported above no statistical difference were evaluated for the main soil characteristics even if for some variables – treatment three year 2009 CEC; treatment three year 2010 all variable – a upper values were observed.

Concerning pH results and known that defecation lime is defined as calcium and magnesium corrective product, hypothesis is that the specific amounts of defecation lime dispose for the two year were not enough to determine a significant variation of the soil pH. This assumption is in agreement as required according to the tables of lime requirements for soil correction. Through the analysis of that table and the required elaborations, in order to rise pH from 5.8, started value of paddy soil, to 6.11, last and upper value for the treatment three, would have been necessary 4.49 t ha^{-1} of CaO while by defecation lime, in the second year, only 2.74 t ha^{-1} were applied.

Results presented above were obtained by processing the data as define in the chapter above: P value set to 0.05 and R-E-G-W F test that is a true experimentwise test.

Moreover if the analysis was conducted by setting P value to 0.1, the same test are able to define homogeneous subset: 3^{a}, $2^{a,b}$, 1^{b}, 0^{b} (table 3.12), so the mean separation indicates at least a tendency in the data.

Comparing this new observation with those presented just above, we could hypothesize that defecation lime was more efficient that CaO only for the soil reaction correction.

In order to be sure of this hypothesize specifically experiment to test defecation lime versus CaO effects in paddy field should be done.

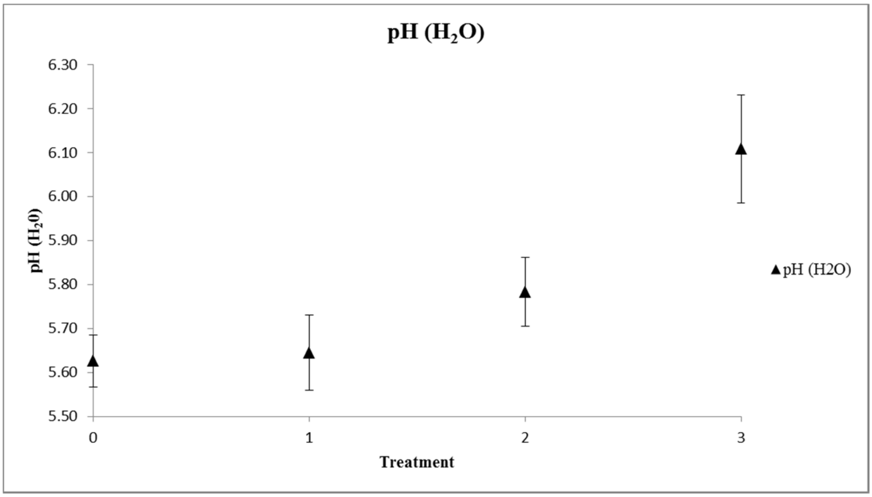

Chart 3.1 Soil pH values at the end of second year for each treatments

Table 3.12

Results of the Anova post hoc for the pH values at the end of second year. P value set to 0.1

	Treatment	Homogeneous subset	
Ryan-Einot-Gabriel-Welsch F	0	b	
	1	b	
	2	a,b	a,b
	3		a
Sig.		0.390	0.106

The same effect was observed if the analysis, setting P value to 0.1, is conducted for soil total organic carbon (TOC, g kg^{-1}; chart 3.2, table 3.13).

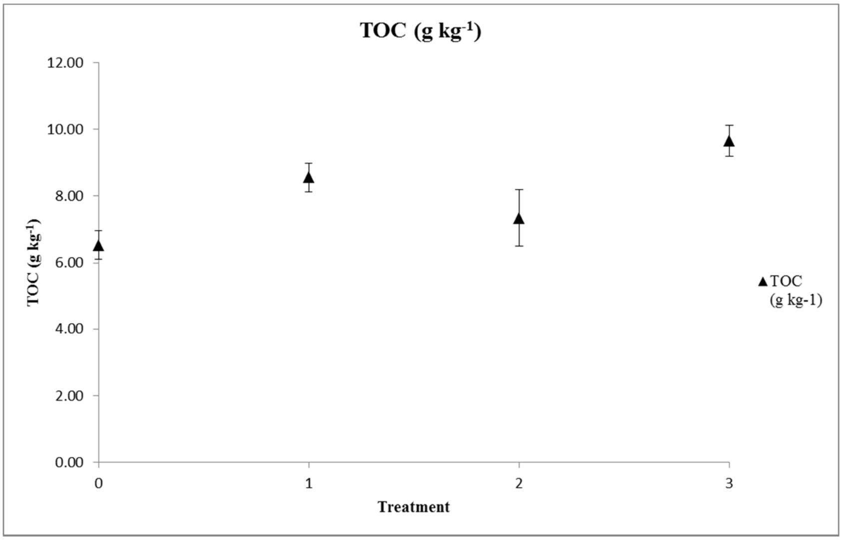

Chart 3.2 Soil TOC values at the end of second year for each treatments

Table 3.13
Results of the Anova post hoc for the TOC values at the end of second year. P value set to 0.1

	Treatment	Homogeneous subset	
Ryan-Einot-Gabriel-Welsch F	0	b	
	2	b	
	1	a,b	a,b
	3		a
Sig.		0.112	0.345

Regarding the value shown for treatment three the hypothesis is that the effect is due to the organic matter added by defecation lime application (TOC applied was 0.54 t ha^{-1}) while, for the treatment one is probably due to the organic fertilizer – hoof and horn – applied (TOC applied was 0.06 t ha^{-1}).

For the first year, concerning total trace metals concentration in soil no difference between treatments were show. This lack of significant effects could be due to (i) low trace metals concentration introduced by defecation lime (table 3.13), (ii) leaching phenomena occurred by field flooding and weekly repeated flooding-dry rounds, (iii) amount of plants bioavailable trace metals uptaken from soil.

About the variations observed from first to second year both in total and in bioavailable trace elements was possible to note that also Garrison et al. (1983) for elements as Ni, Cu, Zn, Cd, and Pb, observed changes of similar magnitude over the years.

Table 3.14

First year trace metals amounts added with the defecation lime and percentage of increasing respect initial soil concentration

	Treatment	D.L. dry weight (t ha^{-1})	Ni (mg kg^{-1})	Cd (mg kg^{-1})	Zn (mg kg^{-1})	Hg (mg kg^{-1})	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Cr (mg kg^{-1})	As (mg kg^{-1})
	2	2.40	0.0151	0.0003	0.2675	0.0005	0.0892	0.0204	0.0367	0.0003
	3	6.98	0.0439	0.0010	0.7782	0.0014	0.2594	0.0593	0.1068	0.0008
% respect initial concentration	2		0.06%	0.34%	0.79%	0.71%	1.00%	0.12%	0.18%	0.01%
	3		0.18%	1.00%	2.30%	2.05%	2.91%	0.35%	0.53%	0.03%

First two lines reported the amount of trace metals applied by defecation lime.

Initial soil trace metals concentration are reported in table 3.4

Even for plants bioavailable trace metals no difference were identified. These effects could be determined from many variables. Violante et al (2010) reported that bioavailability and mobility of trace metals in soil are determined by different variables as pH, sorbent nature, presence and concentration of organic and inorganic ligands, root exudates and nutrients, biotic and abiotic red-ox reaction.

Furthermore Sauve et al. (2000) confirming this knowledge, by evaluating that the amount of bioavailable trace metals in soil are not directly linked to the total trace metal concentration.

The same effects we hypothesize be at the basis of the absence of difference shown in the second year: table 3.15 reported the part of trace metals added through defecation lime application and their weight on the total soil concentration before the product disposal. As shown the percentage respect to the total amount seems to be very little (table 3.15) so as justify the results observe.

Table 3.15

Second year trace metals amounts added with the defecation lime and percentage of increasing respect initial soil concentration

	Treatment	D.L. weight (t ha-1)	Ni (mg kg-1)	Cd (mg kg-1)	Zn (mg kg-1)	Hg (mg kg-1)	Cu (mg kg-1)	Pb (mg kg-1)	Cr (mg kg-1)	As (mg kg-1)
	2	3.33	0.06	0.00	0.20	0.00	0.07	0.05	0.05	0.00
	3	13.89	0.23	0.00	0.85	0.00	0.28	0.23	0.22	0.01
% respect initial concentration	2		0.29%	0.33%	0.43%	0.11%	0.59%	0.30%	0.19%	0.06%
	3		1.21%	1.35%	1.73%	0.61%	2.40%	1.21%	0.79%	0.26%

First two lines reported the amount of trace metals applied by defecation lime.

3.5 Conclusions

Analysed results suggest that are not contraindication for the application of defecation lime in paddy fields.

Also if other evaluation should be needed, these products seem could be potentially used to control soil pH. According to González-Fernández (2004) it would be interesting to evaluate the soil pH following some – eight to ten – years of the defecation lime application. In fact they reported that defecation lime are able to have a beneficial correction effects from year – at least nine – respect others commonly used correction products.

No restriction could be reported respect the variation in heavy metals concentration occurred over the experiment.

Analysis of the soil chemical characteristics have to be done before the defecation lime spreading and, as suggested by the good management practices, these analysis should be carried out every five years in order to evaluated the evolution of the soil.

Chapter 4

Application of defecation lime in paddy field: effects on rice (Oryza sativa L.) plants

Keywords. Defecation lime, rice (Oryza sativa L.), plants growing variables, trace metals in rice grain.

Abstract

The effects of application of a defecation lime - defined as a “product obtained by the chemical hydrolysis (or enzymatic attack) of biological materials by lime and/or sulphuric acid and subsequent calcium sulphate precipitation (Italian D.Lgs 75/2010”) - derived from sewage sludge on rice plants development, yield and trace elements content in the grain were assessed in a 2-years experiment. At the end of two year no difference over crop development related variables was observed. Concerning concentration of trace metals in rice grains some difference were showed on cadmium (Cd) for the first year: plots treated just with defecation lime showed the lower value. For the same element at the end of experiment upper concentration (on average 0.36 mg kg^{-1}) than admitted by law (0.2 mg kg^{-1}) was highlighted in rice grains. According to the result observed, seem that defection lime could be considered an useful way to furnish nitrogen to rice plant, without risk of cumulating heavy metals

4.1 Introduction

According to the prescriptions of the Italian D.Lgs (Legislative Decree) no. 75/2010 defecation lime is defined as a “product obtained by the chemical hydrolysis (or enzymatic attack) of biological materials by lime and/or sulphuric acid and subsequent calcium sulphate precipitation.” This product is classified as "lime and magnesia soil conditioner". They must contain at least 20% of calcium oxide (CaO) and 15% of sulphuric anhydride (SO_3) both related on a dry basis. According by law origin of the hydrolysed biological material must be reported on the label.

There is little knowledge about defecation lime. Masharipova, 2006, reported different methods to produce defecation lime and also different ways of utilization. Defecation lime analysed in the work of Masharipova derived from waste of sugar industries but other biological material i.e. sewage sludge as base product could be used. As largely know sewage sludge is generated as a result of wastewater treatment processes. The quantity of sludge produced has been, for several years, increasing greatly in Europe because of the implementation of Council Directive 91/271/EEC of 21 May 1991 concerning urban waste water treatment. The Sewage Sludge Directive 86/278/EEC seeks to encourage the use of sewage sludge in agriculture and to regulate its use in such a way as to prevent harmful effects on soil, vegetation, animals and man. Safe disposal of sewage sludge is one of the major environmental concerns. Sludge is rich in nutrients such as nitrogen and phosphorous and contains valuable organic matter that is useful when soils are depleted or subject to erosion.

Sewage sludge is found to be an effective organic fertilizer causing increments in the biomass of many crops (Tsadilas et al., 1995). However, its long-term use can cause heavy metal accumulation in the soil (Dai et al., 2006).

Wheat (Triticum aestivumL. var. holly) grown in sewage sludge (2 and 10 kg m^{-2}) amended Hartsells sandy loam and Decatur silty clay loam soils of USA showed significantly higher concentrations of Zn, Cd and Ni in wheat grains at increasing sewage sludge amendment rates in both the soils (Tadesse et al., 1991), but absorption, accumulation and tolerance to heavy metals may vary among different crops and at different levels of sewage sludge amendments (SSA) (Bhogal et al., 2003; Garrido et al., 2005; Lavado, 2006; Singh and Agrawal, 2008).

Crop plants may develop adaptive response to tolerate the heavy metal stress. Positive effects on yield components, such as shoot height and surface area of leaves, of dwarf bean (Theodorates et al., 2000) and growth and productivity of flax (Tsakou et al., 2002) have been reported at different rates of sewage sludge amendments in the soil. Also according to Singh and Agrawal (2010) shoot length, number of leaves, leaf area and total biomass of rice plants increased significantly when grown under various sewage sludge amendment rates; yield of rice increased by 60%, 111%, 125%, 134% and 137% at 3, 4.5, 6, 9 and 12 kg m^{-2} sewage sludge amendment rates, respectively, as compared to those grown in un-amended soil.

Prior to land application, sewage sludge needs to be stabilized and evaluated for quality regarding content of heavy metals, persistent organic pollutants and pathogenic microorganism (Arthurson, 2008).

The stabilization procedure commonly reduces organic matter and water content, emission of unpleasant odors, and concentrations of pathogenic microorganisms (Straub et al, 1993).

Common stabilization approaches include anaerobic and aerobic digestion, lime stabilization, composting, and heat drying (Goldfarb et al, 1999). A stabilization process, similar to the liming one, which can be applied to sewage sludge as raw material is used for defecation lime producing process. After this process defecation lime can be disposed on agricultural crop land.

Baraldi et al. (2006) reported the positive effects following the application of defecation lime on sugar beet production. In our knowledge no references are available about the effects of defecation lime in paddy soil and on rice plants. Furthermore no studies about defecation lime produced starting from sewage sludge were identified.

The aim of this work is to evaluate the effects of defecation lime on rice plants in order to define if they could be used to support plants production.

4.2 Material and methods

4.2.1 Paddy field and experimental design

A two years experiment was done in order to evaluate the effects of defecation lime on soil chemical characteristics. The experiment was performed in a paddy field having 1.27 hectares area and located in Cava Manara (Pavia, Lombardy, 45.151 N, 9.084 E, 80 m a.s.l.), a municipality sited in an area where rice is widely cultivated.

Four blocks and four plots for each block were done.

Four level were tested:

0. CONTROL: never defecation lime nor fertilizer are applied,
1. USUAL MANAGEMENT: management according to the usual agricultural technique,
2. DEFECATION LIME + CHEMICAL FERTILIZER: pre-sowing defecation lime and top-dressing chemical fertilizer application,
3. DEFECATION LIME: pre-sowing defecation lime application.

"Zero" level represented the background of the field; it is the natural evolution of the soil without any fertilizer input. Level "one" was the typical managing situation. Level "two" was done in order to verify the ability of the defecation lime to provide to a part – about half – of plant N nutrition, while left amount was supplied by chemical fertilizer by two different applications: tillering and panicle initiation development stage. Level "three" wanted to test the corrective ability of the product and, at the same time, its capacity to ensure the N needed for rice growth. Paddy field management of the experiment is reported in table 4.1.

Table 4.1

Paddy field management

		Application timing			
Year	Level	Before tillage	Tillering	Panicle initiation	Total N applied (kg ha^{-1})
First year	0	-	-	-	0
	1	org. fert.	chem. fert.	chem. fert.	160
	2	def. lime	chem. fert.	chem. fert.	184
	3	def. lime	-	-	195
Second year	0	-	-	-	0
	1	org. fert.	chem. fert.	chem. fert.	160
	2	def. lime	chem. fert.	chem. fert.	162
	3	def. lime	-	-	317

org. fert. (organic fertilizer): was represented to the hoof and horn fertilizer (12-2-0; 40% of TOC); chem. fert. (chemical fertilizer: at the tillering was applied ENTEC 24:0:29 while at the panicle initiation a 20:20:20 fertilizer was applied); def. lime (defecation lime)

The amounts of defecation lime applied are reported below (tale 4.2).

Table 4.2
Amounts of defecation lime applied in the experiment

Year	Treatment	Amount (t ha^{-1})
First year	0	-
	1	-
	2	2.4
	3	7.0
Second year	0	-
	1	-
	2	3.3
	3	13.9

Defecation lime amounts are reported as dry matter

Tillage operations done during the two years are reported in table 4.3.

Table 4.3
Tillage operation

First year	**Second year**
1. defecation lime speading	1. defecation lime speading
2. plowing	**2. disc harrowing**
3. harrowing by rotary harrow	3. harrowing by rotary harrow
4. sowing by row seeder	4. sowing by row seeder
5. herbicide treatments	5. herbicide treatments
6. tillering fertilization (where needed)	6. tillering fertilization (where needed)
7. fungicide treatment	7. fungicide treatment
8. harvesting	8. harvesting

As shown in table above except for the main tillage operation needed to bury the defecation lime into the soil, the same tillages were done over two years. The different main tillage techniques (bold type in table 4.3) were chosen in order to verify the effect of defecation lime on the plant germination depending on the burying depth.

Concerning the water management paddy field was flooded the first time when rice plants were at three unfolded leaves (code 13 BBCH).

Then paddy field was subjected to flooded and unflooded stages that occurred every week.

4.2.2 Rice variety

The rice variety sown into paddy field was Volano, a Japonica medium cycle variety. Volano was in 2012 cultivated on 19550.45 hectares equal to 8.32% of total Italian paddy field area (ENR, 2012). According to the results of SIQURISO (2009) project, Volano was named as high cadmium (Cd) exclusion capacity variety.

Rice was row seeded by a specific cereal seeder in May both for the first and second year. The sowing was done, after the soil tillage, on unflooded soil. Rice plants were then flooded at three unfolded leaves according to the traditional management technique.

4.2.3 Defecation lime used in the experiment

Defecation lime used was produced in the Alan s.r.l. specific plant located in Bascapè (Pavia, Lombardy).

The production process is based on the patent presented at the chapter 1.2 and specifically is reported in figure 4.1.

Raw materials used for the defecation lime production derived from industrial and municipal wastewater plants from Lombardy region.

Defecation lime was applied on paddy soil using Franzosi FVR 110 muck spreader while chemical fertilizers were applied by a centrifugal fertilizer spreader.

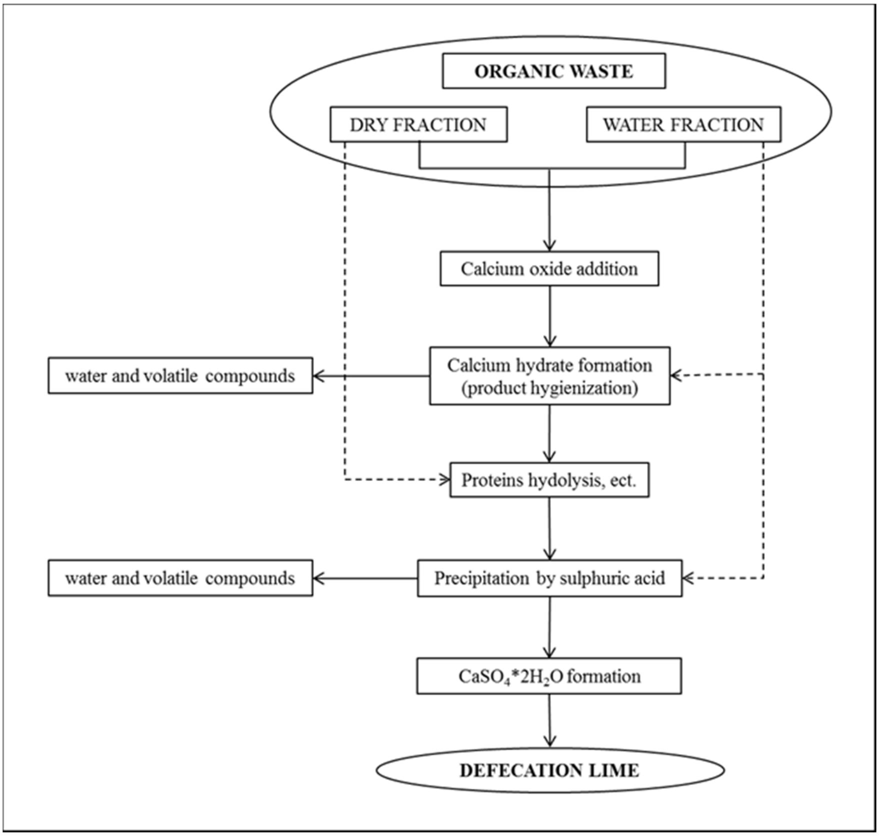

Figure 4.1: process for defecation lime production adopted by Alan s.r.l

Chemical characteristic of the defecation lime are reported below (table 4.4)

Table 4.4
Chemical characteristics of the defecation lime applied

Parameter	First year	Second year	Limit (d.lgs 75/2010)
CaO (%)	26.50	24.70	> 20 %
SO_3 (%)	18.00	20.10	> 15 %
TOC (g kg-1)	29.00	39.21	
Ntot (%)	2.80	2.30	
Ni (mg kg^{-1})	22.00	58.98	100
Cd (mg kg^{-1})	0.50	0.46	1.5
Zn (mg kg^{-1})	390.00	213.25	500
Hg (mg kg^{-1})	0.72	0.07	1.5
Cu (mg kg^{-1})	130.00	70.43	230
Pb (mg kg^{-1})	29.70	56.98	100
Cr (mg kg^{-1})	53.50	55.81	n.d.
As (mg kg^{-1})	0.40	3.52	n.d.

Elements amounts are reported on dry matter. Limit represent the maximum concentration allowed into defecation lime from law (D.lgs 75/2010)

4.2.4 Variables under analysis

Different variables were analysed in order to evaluate the effect of defecation lime (table 4.5)

Table 4.5
Variable analysed during the experiment

Type of variable	Name of variable	Measure unit
Growing and production	Number of plants at three unfolded leaves	n° of plants m^{-2}
	Plant development stage	BBCH
	Number of steam	n° of stem m^{-2}
	Aboveground biomass (AGB)	t ha^{-1}
	Rice yield	t ha^{-1}
	Rice milling yield	%
Trace metals concentration in rice grains	Chromium (Cr)	(mg kg^{-1})
	Copper (Cu)	(mg kg^{-1})
	Nichel (Ni)	(mg kg^{-1})
	Lead (Pb)	(mg kg^{-1})
	Zinc (Zn)	(mg kg^{-1})
	Cadmium (Cd)	(mg kg^{-1})
	Mercury (Hg)	(mg kg^{-1})
	Arsenic (As)	(mg kg^{-1})

The number of plants at three unfolded leaves was determined counting them into one linear meter along the row. Measure was repeatedly four times and randomly for each plot. This sample size was determined by SISSI software (Confalonieri et al., 2007).

Development stage of rice was evaluate by a BBCH rice development code stage according to Lancashire et al., 1991. Time step among two observations was around fifteen days.

Number of stem was determined counting the stem before rice harvest in 0.25 m^2 area. As above, measure was repeatedly four times according to the result of SISSI software (Confalonieri et al., 2007). The four measures was take randomly into each plot. The number of stem was later reported in one square meter.

After drying at 105° C till constant weight, rice aboveground biomass (t ha^{-1}) was determined. The aboveground material was collected by cutting rice plants at the root collar into one square meter. Samples were collected randomly. Sampling was repeatedly four times for each plot.

Yield (t ha^{-1}) of plants was determined by weighing paddy grains after drying at 105° C till constant weight. Paddy grain were collected reaping the ears of plants harvested to the aboveground biomass determination.

Rice milling yield (%) was determined by processing paddy grains with a specific husking machines. In order to do this operation about two hundred grams of paddy grain were sent to Italian National Rice institute (ENR).

Finally trace metals concentration in grains was establish as follow: an amount of rice grains, taken randomly from the total quantity collected for the yield determination, was milled to 0.2 mm size, then a representative sample of up to 0.3 g is digested in 10 mL of concentrated nitric acid using microwave heating with a suitable laboratory microwave unit. The sample and acid are placed in a fluorocarbon (PFA) microwave vessel. The vessel is capped and heated in the microwave unit. After cooling, the vessel contents are diluted to volume of 50 mL, filtered and then analyzed. Elements contents were determined by inductively coupled plasma mass spectrometry (ICP-MS, Varian. Fort Collins, USA).

A certified standard reference material (GBW 07405, soil) from the National Centre for Standard Materials (Beijing, China) was used in the digestion and analysis. Average recovery was 92 ± 4% for all the metals determined. To ensure the accuracy and precision in the analyses, reagent blanks were run with samples.

4.2.5 Statistical analysis

Statistical analysis were performed using analysis of variance (ANOVA) with F test used to compare means (SPSS statistical software, SPSS Chicago IL). In order to separate statistically different means, Ryan-Einot-Gabriel-Welsch F post hoc test were performed. P value was set at $\alpha = 0.05$. According to Acutis et al. (2012) data where checked for homogeneity of variances and normality, and experimentwise test are used for multiple comparison of means. Due to the differences in the amount of defecation lime used, the two years was analyzed separately.

4.3 Results

4.3.1 First year

The results of plants related variable observed for the first year are reported below

Table 4.6
Some plants related variable for the first years

Treatment	n° plant m^{-2}	n° stem m^{-2}
0	397	561
1	366	562
2	403	570
3	423	629
Sig.	0.381	0.197

Values in table are the mean for each treatment; P(F) in all casas was >0.05

Table 4.7
Some plants related variable for the first years

Treatment	AGB (t ha^{-1})	yield (t ha^{-1})	milling yield (%)
0	14.95	7.21	77.99
1	16.08	7.73	76.22
2	16.39	8.07	74.83
3	15.85	7.37	79.17
Sig.	0.633	0.490	0.152

Values in table are the mean for each treatment;AGB and yield values are reported on a drye matter; P(F) in all casas was >0.05

By the analysis of reported tables is possible to show that no differences were observed.

Considering the data collected for the development stage, all treatments presented the same code at the same evaluation time step.

Results of the trace metals concentration into rice grain are following reported:

Table 4.8
Trace metals concentration in rice grains for the first year of experiment reported on dry matter

Treatment	Cu (mg kg-1)	Cr (mg kg-1)	Ni (mg kg-1)	Pb (mg kg-1)	Zn (mg kg-1)	Cd (mg kg-1)	Hg (mg kg-1)	As (mg kg-1)
0	3.29	19.59	9.40	0.17	27.80	0.13	0.06	0.21
1	3.54	20.03	9.49	0.17	25.97	0.13	0.06	0.23
2	3.30	14.72	6.72	0.19	26.85	0.06	0.05	0.24
3	4.05	19.24	9.04	0.18	32.59	0.05	0.04	0.26
Sig.	0.451	0.447	0.309	0.975	0.082	0.016*	0.274	0.973
S.E.	0.511	3.538	1.578	0.062	2.376	0.026	0.011	0.109

Values in table are the mean for each treatment; P(F) was >0.05 for each trace metals with the exception of Cadmium; S.E. = standard error

About cadmium result and analysing them by Ryan-Einot-Gabriel-Welsch F test, the homogeneous subset identified were: 3^{a}, $2^{a,b}$, $1^{b,c}$, 0^{c}

Table 4.9

Correlation results for the first year from Cd concentration in grains and analyzed soil variables

		SOIL VARIABLES										
		pH (H_2O)	TOC	CEC	Cr	Cu	Ni	Pb	Zn	Cd	Hg	As
grains_Cd	Pearson	0.554	0.050	-0.158	-0.558	0.542	-0.057	-0.564	-0.528	-0.404	-0.419	-0.469
(mg kg^{-1})	Sig. (2-tails)	0.154	0.907	0.708	0.150	0.165	0.893	0.146	0.178	0.320	0.302	0.241

Based on n = 8; TOC (g kg-1); CEC (c mol+ kg-1); Trace metals (mg kg-1) reported on dry matter; P(F) was >0.05

Table 4.10

Correlation results for the first year from Cd concentration in grains and bioavailable heavy metal from soil

		BIOAVAILABLE HEAVY METALS				
		Ni	Cd	Cu	Zn	Pb
grains_Cd	Pearson	-.710*	-0.349	0.477	0.359	-0.286
(mg kg^{-1})	Sig. (2-tails)	0.049	0.396	0.232	0.383	0.492

Based on n = 8; Heavy metals (mg kg-1) and Cd in grains are reported on dry matter; P(F) was >0.05 except for Ni

Below, is reported the Ni bioavailable vs. Cd in grains correlation chart:

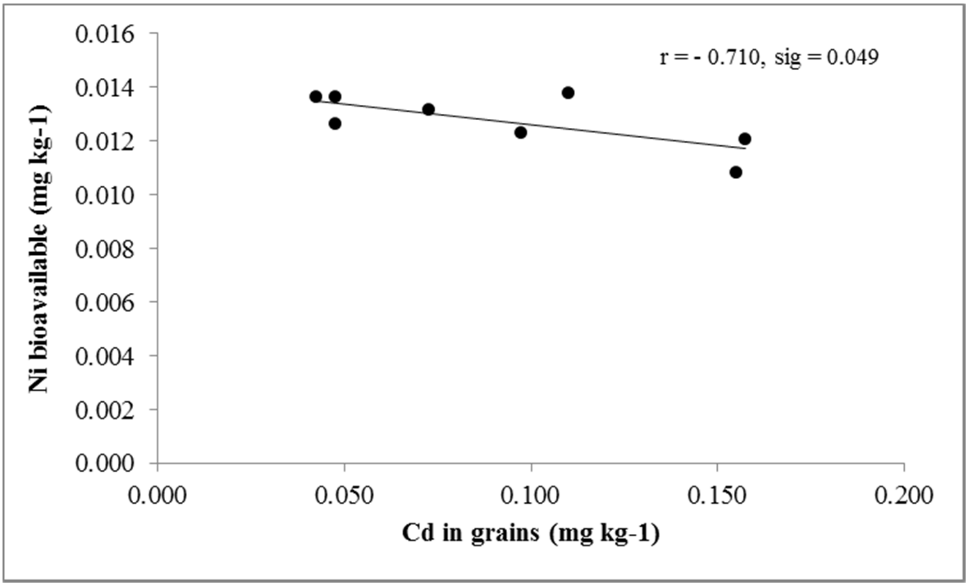

Chart 4.1: correlation between bioavailable Ni from soil and grains Cd concentration for the first year

4.3.2 Second year

In the second year plant relate variables showed the following results:

Table 4.11

Some plants related variable for the second years

Treatment	n° plant m^{-2}	n° stem m^{-2}
0	426	537
1	384	599
2	417	559
3	392	563
Sig.	0.907	0.643

Values in table are the mean for each treatment; P(F) in all casas was >0.05

Table 4.12

Some plants related variable for the second years

Treatment	AGB (t ha-1)	yield (t ha-1)	milling yield (%)
0	18.20	6.45	87.26
1	18.31	6.45	89.41
2	18.20	6.31	89.57
3	18.00	6.03	88.73
Sig.	0.954	0.657	0.729

Values in table are the mean for each treatment;AGB and yield values are reported on a drye matter; P(F) in all casas was >0.05

As shown in tables above, is possible to note that no difference were observed over the considered variables.

About development stage, all treatments presented the same code at the same evaluation time step.

Results of the trace metals concentration in rice grain are reported below:

Table 4.13

Trace metals concentration in rice grains for the second year of experiment reported on dry matter

Treatment	Cu (mg kg-1)	Cr (mg kg-1)	Ni (mg kg-1)	Pb (mg kg-1)	Zn (mg kg-1)	Cd (mg kg-1)	Hg (mg kg-1)	As (mg kg-1)
0	4.44	39.59	18.94	0.18	33.51	0.42	0.03	1.47
1	4.15	34.26	16.38	0.10	32.12	0.30	0.05	1.23
2	4.85	36.25	17.49	0.20	38.98	0.39	0.03	1.22
3	4.78	40.75	18.69	0.12	36.96	0.34	0.02	0.91
Sig.	0.600	0.625	0.759	0.083	0.449	0.317	0.406	0.529
S.E.	0.567	5.411	0.445	0.038	4.512	0.062	0.016	0.364

Values in table are the mean for each treatment; P(F) was >0.05 for each trace metals. S.E. = standard error

Table 4.14

Correlation results for the second year from Cd concentration in grains and analyzed soil variables

		SOIL VARIABLES										
		pH (H_2O)	TOC	CEC	Cr	Cu	Ni	Pb	Zn	Cd	Hg	As
grains_Cd	Pearson	0.039	0.024	0.021	0.098	-0.577	0.049	0.009	0.417	-0.035	-0.182	-0.374
mg kg^{-1}	Sig. (2-tails)	0.926	0.955	0.960	0.817	0.135	0.908	0.984	0.305	0.935	0.665	0.361

TOC (g kg-1); CEC (c mol+ kg-1); Trace metals (mg kg-1) reported on dry matter; P(F) was >0.05

Table 4.15

Correlation results for the second year from Cd concentration in grains and bioavailable heavy metal from soil

		BIOAVAILABLE HEAVY METALS				
		Ni	Cd	Cu	Zn	Pb
grains_Cd	Pearson	0.618	-0.503	-0.250	0.062	0.188
mg kg^{-1}	Sig. (2-tails)	0.102	0.204	0.550	0.884	0.656

Heavy metals (mg kg-1) and Cd in grains are reported on dry matter; P(F) was >0.05

4.4 Discussions

Observing and analysing plants related variables results, we can hypothesize that over two years of experiment paddy field was quite stable: no statistical difference was shown among treatments, especially between control versus other treatments, where different input, both in terms of quantity and quality, were applied.

Differently from the result of Sabey and Hart (1975) work's, where the application of sewage sludge near the crop seeding determined a reduction in plants emergence, no negative effects were showed following the application of defecation lime, deriving from sewage sludge, at the same day of rice seeding (tables 4.6 and 4.12: n° plants m^2).

Results of trace metals concentration in rice grains showed that, for the first year, for cadmium (Cd), statistical difference was observed (tables 4.8 and 4.9). This effect do not seem to be directly related to the bioavailable amount of cadmium in soil because, as reported in the table 3.8, no difference were observed.

However is also important to note that soil bioavailable concentrations are referred to the samples collected at the end of the year but not reflect the dynamism of the process occurred during the growing season.

The hypothesys is that bioavailability of cadmium was different and was changed during the growing season due to the flooded and unflooded stages that occurred every week.

Some author reported that, when paddy field is flooded and the soil is in a reducing condition, any Cd in the soil combines with sulphur (S) to form CdS, which has a low solubility in water. However, when the field is drained and the soil is in an oxidative condition, CdS is converted into $CdSO_4$, which is soluble in water. This means that the solubility of Cd changes depending on the redox potential of the soil. (Arao et al., 2009; Iimura,, 1978; Ito, 1976) This effect could have been enhanced to the soil – acid in this experiment – pH which promote the heavy metals mobility and bioavailability (Tlustoš et al., 2006; King, 1988; Heckman et al., 1987; Kuo and McNeal, 1984; Harter, 1983; Dijkshoorn et al., 1981; Street et al., 1978; Chaney et al., 1977)

Finally, for the first year, and according to the Commission regulation (EC) n° 1881/2006 and its modification, where is reported the maximum level allowed for some contaminant elements in foodstuffs, we observed that for cadmium the concentration determined were less than the maximum – 0.2 mg kg^{-1} –admitted.

At the end of second year, for each analysed variables, no statistical difference were observed. Cadmium concentrations were over to the law maximum concentration admitted. On the contrary and over two years lead (Pb) – a Cd competitor in plants uptake (Tingqiang et al., 2012) – concentrations in grains were under the maximum limit established by law equal to 0.2 mg kg^{-1} as cadmium.

Analysing the tables 4.8 and 4.14, is possible to show that for the first year Cd concentration in grain were lower than Pb while, for the second year, opposite situation was observed.

This situation was not reflected for the soil total concentration where Pb was more present than Cd overall years. We hypothesize that the competition effects are not just between Cd to Pb but also from the other elements and also soil variable as pH, sorption site, redox potential plays an important role in metal uptake. No correlation from Cd in grains to other trace metals in soil – total and bioavailable – pH, CEC and TOC, for the second years were observed while fir the first year Cd was slightly and inversely correlated with bioavailable Ni (table 4.11; Sig. 0.049; chart 4.1). Bing L. et al. (2012) observed a strong correlation ($p < 0.0001$) between brown rice concentration of Cd and micronutrients elements as Ni ($p < 0.0001$) but, according to their result was observed a direct correlation ($r = 0.41$). No results were collected about redox potential evolution during the years so additional studies should be done in order to define its effects on trace metals behaviour. Further study could be also helpful to observe if the correlation show from bioavailable Ni and Cd in grains will change over the year.

4.5 Conclusions

Defecation lime application, according to the management applied and result collected, did not show negative effects over all plants related variables.

Due to the behaviour of the system analysed, where no difference were observed from treatments, especially from the control to the others, we suggest that, to evaluate its evolutions, further years of studies should be done.

Concerning trace metals concentration and especially for Cd, attention must be carrying out to its concentration in grains because at the end of second years its values were upper than the maximum level imposed by law.

Also to this results and due to the high variation from Cd concentration from first year to second year and the correlation between bioavailable Ni and Cd in grains, further analysis are required in order to well establish which are the process involved.

Chapter 5

Conclusions

Reusing of bio-processing waste and biological by-products has risen of importance over last years. By anthropogenic activity in fact high quantity of those products are generated daily.

If managed and controlled properly they could be used for agricultural purposes. Depending of the process by which they were produced and by which they were stabilized they could be used as fertilizer, soil conditioner, corrective or growing media.

Stabilization is required in order to reduce organic matter and water content, emission of unpleasant odors, and concentrations of pathogenic microorganisms (Straub et al., 1993).

Among biological processing waste obtained from industrial and municipal wastewater plans, sewage sludge have great importance.

Sewage sludge, after proper stabilization are largely disposed in agricultural soil as fertilizer thanks to their value in terms of organic matter, nitrogen, phosphorus and other nutrients contents.

Different process can be used to stabilize these products such as alkaline stabilization, composting, aerobic and anaerobic digestion. A process similar to the alkaline stabilization is the one used for defecation lime producing process. In this process biological products is processed with calcium oxide (CaO) and then with sulphuric acid (H_2SO_4).

Specifically and according to Legislative Decree 75/2010, defecation lime is “the product obtained by the hydrolysis of biological materials by lime and/or sulphuric acid and subsequent calcium sulphate precipitation”. Defined by law as “calcium and magnesium corrective products", they must contain at least 20% of calcium oxide (CaO) and 15% of sulphuric anhydride (SO_3).

Unfortunately no references about the application of defecation lime, from sewage sludge, were found.

The aim of this PhD thesis was to evaluate the effects of defecation lime in agricultural land, following their disposal, on soil chemical characteristics and plants.

In order to evaluate their effects a two years experiments were done.

Field area was selected not far from defecation lime producing plants, specifically a paddy field located in Cava Manara (Pavia, Lombardy) was used.

At the end of experiment and by the result collected over the soil chemical characteristics, no restriction or contraindication can be established related to the application of defecation lime in paddy fields. Also if other evaluation should be needed, these products seem could be potentially used to control soil pH. This conclusion is not ultimate because the two years period of the study could be not sufficient for asses the evolution of this soil characteristics: in fact according to the good management practices, soil analysis should be carried out every five years in order to evaluated the evolution of the soil and in any case before the defecation lime spreading.

Concerning plants related variables, no statistical difference were observed so, also in this case, no contraindication can be established. It is important that there is not depressive effects on plan emergence, as highlighted for sewage sludge.

Regarding trace metals concentration in grains and especially for Cd, attention must be carrying out due to the values, upper than the limit fixed by law, observed at the end of second years.

According to these results, we suggest that further years of studies would be required to well establish what are the process involve in the observed phenomena and to know the evolution of the system.

It would be interesting to consider all the input that could be affect trace metals concentration as: fertilizer applied, irrigation water, fungicide by which seeds are coated, and eventually all the plant protection products used.

Furthermore is needed to evaluate the rexod potential of the soil and its variation induced by soil flooding. Eventually the behaviour of different rice variety could be assessed.

In conclusion we reported that to establish the effects of defecation lime over different crops and different soils, thanks to the partnership of the defecation lime production plant and a farm located in a different area (Broni, Pavia, 45.087 N, 9.266 E, 70 m a.s.l.), compared to paddy field, a new study is doing on winter wheat since 2012.

The first result about the effects on winter wheat about the last growing season are still under analysis so are not reported here.

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