

# The Effect of Lime Stabilization on *E. coli* Destruction and Heavy Metal Bioavailability in Sewage Sludge for Agricultural Utilization

G. Petruzzelli, F. Pedron, M. Grifoni, A. Pera, I. Rosellini, B. Pezzarossa

**Abstract**—The addition of lime as  $\text{Ca}(\text{OH})_2$  to sewage sludge to destroy pathogens (*Escherichia coli*), was evaluated also in relation to heavy metal bioavailability.

The obtained results show that the use of calcium hydroxide at the dose of 3% effectively destroyed pathogens ensuring the stability at high pH values over long period and the duration of the sewage sludge stabilization. In general, lime addition decreased the total extractability of heavy metals indicating a reduced bioavailability of these elements. This is particularly important for a safe utilization in agricultural soils to reduce the possible transfer of heavy metals to the food chain.

**Keywords**—Biological sludge,  $\text{Ca}(\text{OH})_2$ , copper, pathogens, sanitation, zinc.

## I. INTRODUCTION

THE sludge is any solid, semisolid or liquid residual removed during the process of wastewater (domestic, industrial or mixed) treatment. The “water treatment line” of a wastewater treatment plant, is composed by three steps: a primary (mechanical, chemical/physical), a secondary (biological) and tertiary in which the nutrients (N, P and K) are removed, moreover the treatment plant is usually provided with a “treatment sludge line”. In this last line the originated sludge have “undergone biological, chemical or heat-term, the long-term storage or any other process significantly necessary to reduce its ability to ferment and health risks resulting from its use” (Directive 86/278/EEC, Art. 2b) [1]. The sewage sludge are the majority in volume among the by-products of this process and their amount depends on the type of treatment, the final achievable amount ranging between 0.25 to 12% by weight solids [2]. The increase of wastewater treatment plants, which led to an increase of sludge originated quantities, and an environmental legislation ever greater restrictive, require a more careful sewage sludge management by the national and international community. For example, the application of Directive 91/271/ECC, “Urban Waste Water Treatment” has planned an increase of 50% in the sludge production, between 1992 and 2005 [3], [4]. Therefore, it is necessary an optimization of the already existent destination strategies (composting, landfilling, and incineration) or the identification of additional innovative technologies for

treatment or recovery (production of energy). The use of sewage sludge, rich in mineral and organic nutrients, as fertilizer or organic amendments in agriculture is a good alternative to the problem of sewage sludge management. However, due to their origin sewage sludge may contain high concentration of organic and inorganic substances potentially harmful, i.e. pathogens, heavy metals and organic contaminants. The sewage sludge quality, depending on the characteristics of the production process, is strongly dependent on the pollution load of the original material. It is therefore necessary to eliminate toxic microorganisms and pathogens and to reduce the heavy metal content from sewage sludge before it is released back to the environment [5]. The European Union regulates the use of sewage sludge in agriculture to protect the soil, and the quality of surface and groundwater, and to prevent harmful effects on vegetation, animals and man [1]. However, being the topic rather complex, the regulatory framework is much wide and more legislative acts are often consulted in order to regulate or influence the production and use of sludge on land and to restrict the final destination of the waste. In particular, it is necessary to take account of: Directive 91/271/EEC concerning urban waste-water treatment; Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources; Directive 2000/60/EC establishing a framework for Community action in the field of water policy; Directive 2008/98/EC on waste and repealing certain Directives. One of the main and common purposes remains to encourage the reuse of sewage sludge in agriculture. In this frame, the Directive 86/278/EEC fixed limit values for concentrations of seven heavy metals (Cd, Cu, Ni, Pb, Zn, Hg, Cr) in soil (Annex IA), in sludge (Annex IB) and for the maximum annual quantities of heavy metals which may be introduced into the soil (Annex IC). The Directive establishes the biological characteristics of the sludge and the maximum quantity of sludge to apply to soil. Some Member States have introduced more stringent limit values for heavy metals and defined additional requirements for other contaminants. In Italy, the use of sewage sludge in agriculture is regulated by Legislative Decree 99/92 with identical limit values of heavy metal concentrations in soil (Annex IA) and in sludge (Annex IB), except for Cd and Cr, of the Directive 86/278/EEC. The Italian decree allows the use of sludge in agriculture only if all of the following conditions are present: sludges have been treated, stabilized and sanitized, are effective in supplying

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nutrients and do not contain toxic, harmful, persistent substances. In Italy, although the sludge production has increased of about 30% from 1992 to 2006, the percentage of reuse for agriculture has remained the same (26% of total product) [6].

The microbial community in sludge includes a large number of pathogens, mostly parasites (bacteria, viruses, fungi and yeast), which can vary in quality and quantity on the basis of the sewage source. The main sources of enteric pathogens (*Escherichia coli*, *Clostridium* spp, *Salmonella* spp, *Mycobacterium* spp.) are the human and domestic animals excreta [7]. Due to the potential risk for human populations, much attention is directed to the presence of the bacterium *E. coli* resulting by improper disposal of agricultural waste through the contamination of fruits and vegetables [8]. Usually, the concentrations of microbiological parameters in the sewage sludge are higher than the established regulatory limits (especially with regard to fecal coliforms) [9]. Therefore, the correct use of sewage sludge in agriculture must consider the risks associated with the possible spread of these pathogens and must provide the best treatment for their reduction. Stabilization has as main objectives: the reduction of fermentability, of odour emissions (through chemical and thermal treatment), and of bacteria and suspended solids (through biological process, aerobic or anaerobic). In the chemical stabilization the inhibition of the biological reactions is performed by the increase of the pH of the substrate, up to values around 12 [9]. In fact, the microorganisms' survival is a function of several factors (temperature, humidity, pH, etc...) according to the characteristics of individual species. The rise in pH above 12 has the effect of destroying most of the indigenous microorganisms present in the sludge, reducing the hazard by 2 or more log<sub>10</sub> [7], and of inhibiting the growth of pathogens [10], [11]. Often, the lime is used in the stabilization processes as an effective mean to reduce environmental risks deriving from the pathogens [12]. The stabilization treatment with lime is carried out by mixing the sludge with lime and water resulting in the formation of an alkaline environment. At the end of the process, pH values above 10 are achieved for a certain period [4], thus ensuring the pathogenic microorganisms elimination [11]. However, a pH reduction may occur due to atmospheric CO<sub>2</sub>, leading to a subsequent reactivation of pathogens [9].

The aim of this paper was to evaluate the effect of lime, as calcium hydroxide, during the stabilization process of sewage sludge on *E. coli* enumeration and on heavy metal (Cu, Ni, Pb and Zn) speciation.

## II. MATERIALS AND METHODS

### A. Experimental Setup

The raw primary sludge was collected in urban wastewater treatment plants of different cities in Tuscany. The experiments were carried out by mixing 150 g of sludge with various doses of lime as calcium hydroxide. A volume of 150 mL of water was added to facilitate the homogenisation of the mixtures that was performed by a mechanical mixer for 30

minutes. Four different sample mixtures were prepared:

- Sewage sludge without addition of Ca(OH)<sub>2</sub>, Control sample (F1)
- Sewage sludge with addition of Ca(OH)<sub>2</sub> at 1% percentage of dry matter (F2)
- Sewage sludge with addition of Ca(OH)<sub>2</sub> at 2% percentage of dry matter (F3)
- Sewage sludge with addition of Ca(OH)<sub>2</sub> at 3% percentage of dry matter (F4)
- Sewage sludge with addition of Ca(OH)<sub>2</sub> at 5% percentage of dry matter (F5)

All the reported data are the mean of three replicates. Experiments lasted 35 days and during this period the pH of the samples was determined using a glass electrode.

### B. Counts for *Escherichia coli* Colonies

The colony counts of *Escherichia coli* (5) were performed immediately after the homogenisation and after 1 day, 7 days, 15 days and 35 days on *E. coli*/Coliform Chromogenic Medium (Oxoid). Each sample was serially diluted (1:10) and plated in duplicate onto agarised medium. The agar plates were incubated in thermostat at 37°C for 24 h. Plates of those dilutions yielding 20-200 colonies were enumerated. All purple colonies were counted and recorded as typical *E. coli*.

### C. Microcosm Experiment

Microcosm test was carried out using *Frumentum durum* grown in an agricultural soil characterized by a basic pH (8.0). Microcosm pots were prepared according to previous studies [13] using 200 g of soil and 15 seeds of wheat. The plants were allowed to grow for 35 days. Wheat seedlings were collected and analysed for heavy metal contents. In the microcosms the soil was treated with two different doses of F4 sewage sludge, low (L) corresponding to 22.5 tons /ha and high (H) corresponding to 40.5 tons /ha.

### D. Heavy Metals Analysis

At the end of the experiment, the samples were dried at 110°C and ground to pass a 0-2 mm sieve to determine the total content and the speciation of copper, zinc, nickel and lead. Total content was determined using an acid attack with a mixture of 5 ml HNO<sub>3</sub> and 2 ml HClO<sub>4</sub> added to 1 g of dry material and an overnight digestion. The samples were mineralised using a microwave system "ETHOS 900" (MILESTONE S.r.l., Bergamo, Italy) with a pulsed-mode emission in Teflon vials.

The speciation of the four heavy metals was determined by a sequential extraction procedure using H<sub>2</sub>O, KNO<sub>3</sub> 1 M and DTPA 0.01 M [14]. In the sequential extraction, the sludge/liquid ratio was 1/5, using 4 g of sludge in 20 mL of extracting solution. Each extraction lasted 3 hours. At the end of this period the samples were centrifuged at 15000 rpm for 15 minutes and then filtrated.

Flame Atomic Absorption Spectrometry (Varian AA 240FS) was used to determine concentrations of heavy metals in samples obtained both from acidic digestion and from sequential extraction. Vegetal samples were analysed following the described previous acid attack after one night

digestion. Quality control of the data was performed using the certified reference material, CRM ERM – CC141.

### E. Statistics

All statistical analysis was performed using STATISTICA version 6.0 (Statsoft, Inc., USA).  $\text{Ca}(\text{OH})_2$  additions effects were analyzed using one-way analysis of variance (ANOVA). Differences between means were compared and a post-hoc analysis of variance was performed using the Tukey honestly significant difference test ( $P < 0.05$ ).

## III. RESULTS AND DISCUSSION

The addition of increasing amounts of calcium hydroxide to sludge samples induced an increase in pH from 6.5 to about 10.0 (F2) and 12.0 (F3, F4 and F5). However, after 7 days, the pH of F2 and F3 samples began to decrease and after 35 days the pH reached values of 8.0 and 9.0, respectively (Fig. 1).

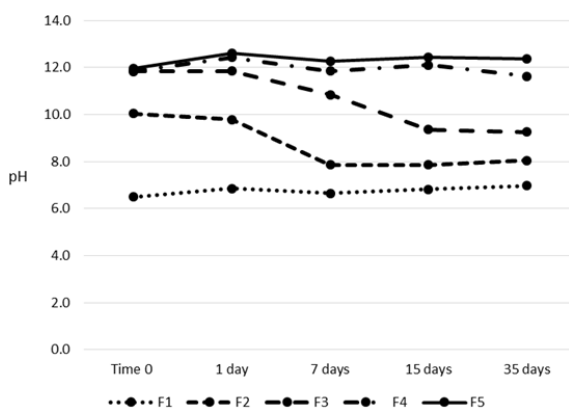


Fig. 1 pH changes with time in sewage sludge samples treated with  $\text{Ca}(\text{OH})_2$

The pH values were almost constant in untreated sludge samples (F1) during the whole experiment. In samples F2 and F3, where the amounts of the added  $\text{Ca}(\text{OH})_2$  were respectively 1% and 2% with respect to the used sludge amount, pH initially increased, but starting from the 7th day pH decreased from 10.0 to 7.9 in F2 sample and from about 11.8 to 10.8 in F3 sample. The increasing of the percentage of  $\text{Ca}(\text{OH})_2$  added to sludge up to 3% (F4) and 5% (F5) produced a raise in pH to 12.0 that remained unchanged with time.

The influence of pH on the concentration of *E. coli* colonies during the 35 days of the experiment is showed in Fig. 2.

In sample F1, without added calcium hydroxide, *E. coli* colonies remained fairly constant with time varying in a short range around an average value of  $5.8\text{E}+06$  CFU/g of dry sludge.

The addition of calcium hydroxide to the sludge caused a decrease in *E. coli* concentration. At 2% of alkaline agent addition (F3), a total destruction of this microorganism since the first day of the experiment was observed.

Our results show that the percentage of 2% (F3) is the minimum amount of calcium hydroxide necessary to completely destroy the pathogens in one day. However, due to inefficient buffering capacity, at 2% of  $\text{Ca}(\text{OH})_2$  added,

several parts of the sludge remained unstable, thus allowing a certain microbial activity, the production of  $\text{CO}_2$  and a consequent pH decrease. In the case, there may be the risk that it is not ensured the maintenance of a highly alkaline environment necessary to avoid the survival of pathogenic microorganisms. Thus, the minimum amount of  $\text{Ca}(\text{OH})_2$  necessary to maintain stable pH values of the sludge in order to prevent odour problems and to destroy pathogens was the 3% addition (F4). After this treatment, the pH remained unchanged at a value around 12 during the whole period of the test.

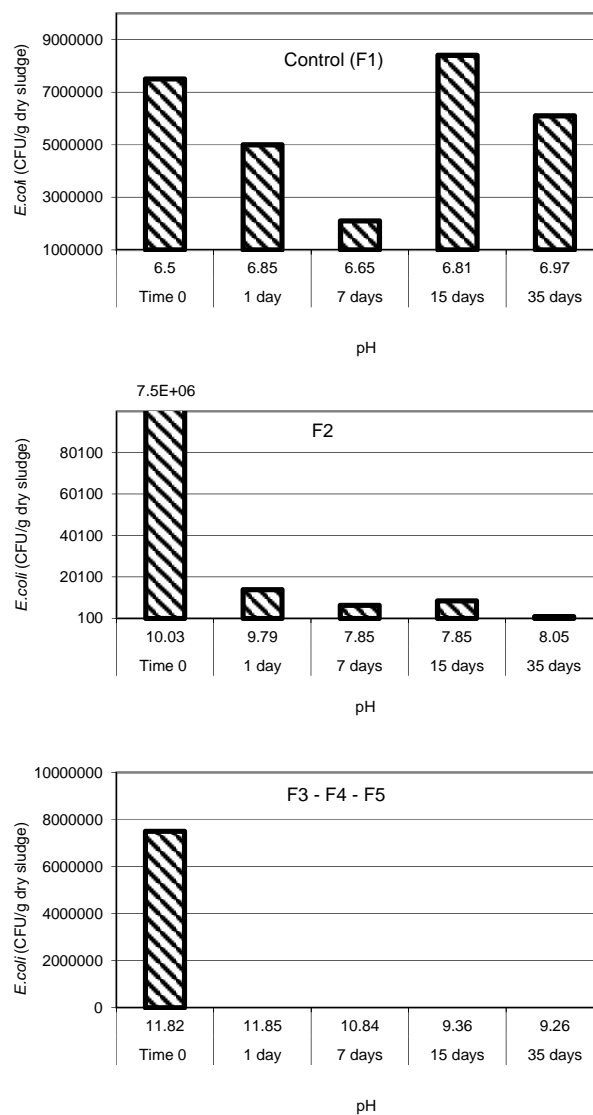


Fig. 2 Relationship between *E. coli* colonies and the amount of  $\text{Ca}(\text{OH})_2$  from sample F1 (control) to F5

The interest in the agricultural utilization of sewage sludge from wastewater treatment derives from its content in valuable fertilizing components such as N, P, K and organic matter. However, sewage sludge contains high levels of heavy metals that is necessary to evaluate prior to sludge application to land to avoid their accumulation in soil. The use of sewage sludge

in agriculture is allowed on the basis of heavy metals content. Threshold values are established by European legislation [1] and Italian national legislation [15]. The sludge utilized in this study was characterized by the following mean values of total concentration: Cu 369 mg kg<sup>-1</sup>, Zn 773 mg kg<sup>-1</sup>, Ni 42.4 mg kg<sup>-1</sup> and Pb 55.8 mg kg<sup>-1</sup>. All the heavy metal concentrations were within the allowed limits and tended to decrease from sample F1 to sample F5 due to the diluting effect of the added lime that did not contain heavy metals (data not shown).

Beyond the total metal concentrations, mobility and bioavailability are the key parameters that determine the transfer of these elements into the food chain. Mobility and bioavailability of heavy metals depend on their chemical forms in sludge, that greatly influence the fate, and transport of the microelements in soils where sludge is utilized. Single or sequential extractions are used to operationally identify the different chemical forms of heavy metals associated with particular linkages to the components of the sewage sludge. In the present experiment we used a 3 step sequential extraction procedure with H<sub>2</sub>O, KNO<sub>3</sub> and DTPA (diethylenetriamine pentaacetic acid). This procedure [14], [16] has been successfully applied to different matrices, including natural and contaminated soil, sewage sludge and compost:

- First step (H<sub>2</sub>O): heavy metals extractable in water are those present in the sludge in soluble forms either as single and soluble complexed ions
- Second step (1M KNO<sub>3</sub>): this extractant solubilizes metals

in exchangeable forms, linked with ionic bonds to organic and inorganic components of sludge. The amounts released are related to the ionic composition of the extractant, which influences the heavy metals adsorption-desorption processes.

- Third step (0.01M DTPA): this organic acid is able to extract the metals bound to organic matter. These metals may be complexed or adsorbed by the organic substances in sewage sludge. In addition, DTPA may partially solubilize Fe and Mn oxides, to which heavy metals are strongly linked. DTPA extraction provides a chemical information on the metals that are potentially available for plant uptake [17].

The scheme was applied to the original sewage sludge (F1) and to the four types (F2-F5) of sludge derived from Ca(OH)<sub>2</sub> addition with the aim to determine the influence of the stabilization method on the mobility of the heavy metals contained in the sewage sludge. The results obtained for the four investigated metals are reported in Fig. 3.

In general, the distribution of each metal in the three fractions of the sequential extraction procedure (SEP) was influenced by the Ca(OH)<sub>2</sub> treatment used. Ni was extracted in higher amounts during the first extraction step, and similar results were reported also for different kinds of sewage sludge [18]. The extractability of Ni was similar in all the sludge samples, suggesting that it was not influenced by the addition of Ca(OH)<sub>2</sub>.

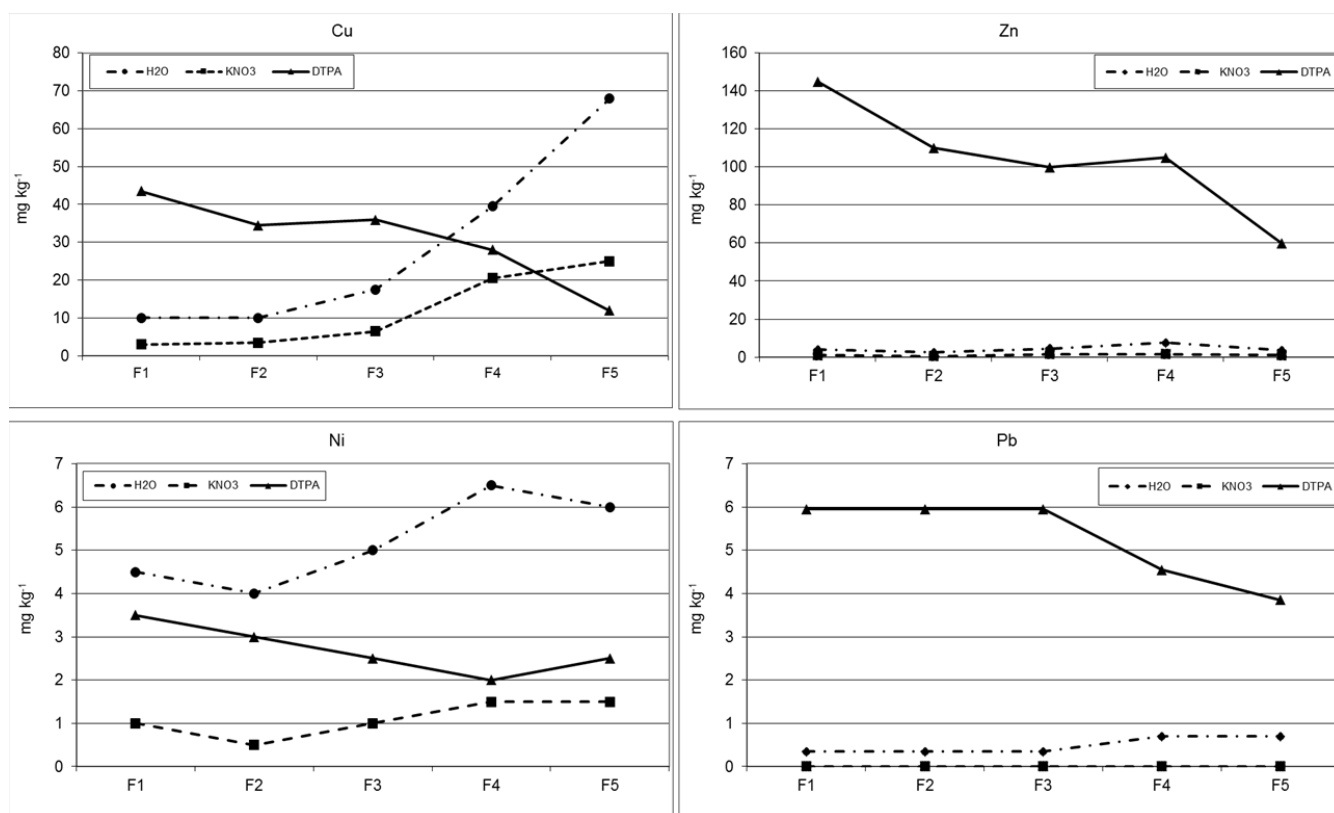


Fig. 3 Heavy metal sequential extraction in different sludge samples: concentrations are expressed as mg kg<sup>-1</sup> dry matter

The total extractability, defined as the sum of the concentrations (C) of a metal in the three steps of the SEP:

$$\sum C(\text{H}_2\text{O}) + C(\text{KNO}_3) + C(\text{DTPA})$$

where about 21% of the total Ni content in the sewage sludge is accounted for, in accordance with previous research findings [18], [19].

In the original sewage sludge, the greatest extraction percentage of Cu, Zn and Pb was obtained with DTPA extraction. The results indicate that the metals were mainly associated with the organic matter (OM); this was to be expected given the affinity of OM for these elements with the formation of stable complexes [20].

Following the addition of increasing quantities of lime, Zn and Pb showed a decrease in the mobile fractions. The total extractable amounts diminished from 18% to 7% for Zn and from 13% to 8% for Pb. This is due to the sharp decrease in the DTPA extractable fraction as a consequence of the increase of sewage sludge pH following  $\text{Ca}(\text{OH})_2$  additions. The increase of  $\text{Ca}(\text{OH})_2$  addition induced a decrease of DTPA extractable amounts of Cu, but for this metal the lime addition promoted an higher water and  $\text{KNO}_3$  extractability. This behavior can be ascribed to the effect of lime addition on the Cu-organic matter complexes. Dissolved organic matter (DOM) concentration in sludge and soils has been showed to be positively correlated with pH increase [21]. Increased alkalinity modified the pH dependent charge surfaces of solid phase of sludge. Negative charged surfaces, at alkaline pH, repel negatively charged molecules of organic matter increasing as a consequence their concentration in the solution phase of sewage sludge. The behavior of Cu depends on the association of the metal with DOM. Cu has a strong affinity for organic matter and forms complexes of high stability thus increasing the concentrations of soluble organically complexed forms of Cu [21]. Moreover when organic matter is attacked by  $\text{Ca}(\text{OH})_2$ , also free Cu ionic forms are liberated and remained in solution due to the competition with the high amounts of  $\text{Ca}^{2+}$  ions for the negative charged surfaces of sewage sludge. As a consequence the total extractability of Cu raised from 14% in the original sludge to 24% in F5 sludge.

Bioavailability results from SEP were confirmed by data from microcosm experiments reported in Table I. In the microcosms the soil was treated with two different doses of F4 sewage sludge, low (L) and high (H). The results of heavy metal concentrations in plants were compared with the data obtained adding to the same soil the untreated sludge at (L) doses.

Sewage sludge addition did not influence the biomass production (data not shown). F4 addition to soil decreased Zn uptake by wheat seedlings in comparison with OS addition. This effect was not statistically significant for Cu, while Ni and Pb concentrations in plants were always below the detection limit.

TABLE I  
HEAVY METAL CONCENTRATIONS IN WHEAT SEEDLINGS FROM MICROCOSM EXPERIMENT

Sample	Cu	Zn	Ni	Pb
CT	7.39±0.50	46.5±0.83	<LQ	<LQ
Soil + F4 (L)	8.68±0.62	43.9±0.62	<LQ	<LQ
Soil + F4 (H)	8.79±0.70	50.8±0.70	<LQ	<LQ
Soil + OS (L)	9.53±0.71	57.8±0.82	<LQ	<LQ

Data Are Expressed as  $\text{mg kg}^{-1}$ .

CT: Original soil; L: low dose ; H: high dose; OS: original sludge; LQ (detection limit) Ni: 8  $\mu\text{g/L}$ ; LQ Pb: 5  $\mu\text{g/L}$ .

#### IV. CONCLUSION

In conclusion, lime can be added to the sludge at different stages of the treatment process before thickening, before or after dewatering. Added as  $\text{Ca}(\text{OH})_2$  is able to destroy pathogens, and to reduce the biological oxygen demand, reducing in the meantime the offensive odors. Our results show that the use of calcium hydroxide, as stabilization procedure, is of high interest in particular for small sewage treatment plants for economic reasons due to the cost of lime.  $\text{Ca}(\text{OH})_2$  addition at the dose of 3% has effectively destroyed the pathogens of the sludge, decreasing in the meantime most of heavy metals in bioavailable forms. Even if positive results were obtained with lower doses, the use of  $\text{Ca}(\text{OH})_2$  at 3-5% ensure the stability of high pH values over long period and the duration of the sewage sludge stabilization. In general, lime addition decreased the total extractability of heavy metals indicating a reduced bioavailability of these elements. This is particularly important for a safe utilization in agricultural soils in order to reduce the transfer of heavy metals to the food chain.

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