# Fate of Copper and Zinc in Cattle Manure

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***Abstract*****—**In view of optimizing the yield of cattle in terms of meat, milk and even fertility, famers are incorporating in the cattle's ratio excessive supplies of Copper and Zinc. These elements are not well assimilated by the cow and therefore, the majority of these elements will be present in the dung which will form the manure. Heavy metals are potentially toxic to humans and the environment. However, metal toxicity depends on chemical associations in soils. For this reason, determining the chemical form of a metal in soils is important to evaluate its mobility and bioavailability. Therefore, sequential extraction was used to fractionate copper and zinc. This research aimed at evaluating the influence of the application of enriched cattle manure on soils by applying 0.15 g of dried cattle manure on 20 g of an agricultural soil in the region of Zahle, Lebanon. Knowing that the mineralisation of the manure incorporated in the soil released an important amount of trace metals, a chemical approach was developed to follow the kinetic of carbon mineralization. The results showed the presence of the metals in the residual fraction to be dominant, followed by the fraction bound to organic matter in the copper's case and that bound to oxides in the zinc's case. Moreover, the addition of manure has a significant effect on the accumulation of heavy metals in the soil since a significant increase in the concentration of these elements in the soil was noted after the manure application.

***Keywords*****—**Copper, Fractionation, Manure, Mineralization, Zinc

## I. INTRODUCTION

DURING the last century, the agricultural sector underwent significant changes mainly seeking higher income. In the field of animal husbandry, modernization and intensification led to radical changes in the structure and techniques applied. One of the major changes concerned animal nutrition that is calculated nowadays according to the animals' growth stage and tailored to their needs [1]. Minerals present in the animal ration plays a significant role even if they are found in trace quantities [2].The essential role of trace metal elements (TME) is due to the fact that they are part of the composition of many enzymes, hormones and vitamins. However, these elements are not easily assimilated by animals [3]. Therefore, there is a need to administer them in excess in the diet of the animals in order to avoid any imbalance. These excesses are eliminated mainly in the faeces but also in smaller amounts in the urine or milk [4]. Cattle's faeces is the most used fertiliser in the world [2], hence, faeces containing significant amounts of the Cu and Zn are spread on soils and can have an important impact on the environment. Nowadays soil pollution by TME is a major problems of soil and water pollution [5].

The first purpose of the work presented here is to quantify the contributions of Cu and Zn and identify their forms in the diet of a dairy herd. The second purpose is to quantify the concentrations and define the forms of these metals in faeces and manure and finally to study the availability of these elements in the soil through a chemical fractionation.

## II. MATERIALS AND METHODS

### *A. Site Description and Sampling*

Dairy Khoury is one of the oldest dairy companies in Lebanon where it roots stretch back to the 1940's. The Farm, located in Bekaa Valley more specifically in Zahlah contains 2000 cattle heads spread over 85000 m$^2$ of land. Fig. 1 locates the region of Zahlah within the map of Lebanon.

Fig. 1 Geographical map of Lebanon

A total of 17 feed samples, 4 soil samples and 6 manure samples were taken from the farm.

Each cow receives approximately 30 kg of feed per day. Table I shows the complete diet of a cow per day.

TABLE I
QUANTITY OF FEED GIVEN BY THE FARM (KG/DAY)

| Feed | Quantity (kg/day) |
|---|---|
| Soja | 3.30 |
| Corn | 2.70 |
| Barley | 1.60 |
| Wheat Bran | 1.40 |
| MagnaPack | 0.20 |
| Dicalcium | 0.08 |
| Idafix | 0.01 |
| Vitamin | 0.20 |
| Sodium Carbonate | 0.08 |
| Salt | 0.07 |
| Ziocarb | 0.04 |
| Zinpro | 0.01 |
| Powder | 0.08 |
| Straw | 1.04 |
| Alfalfa | 6.66 |
| Cotton seed | 1.50 |
| Fermented corn | 12.5 |
| Total | 31.78 |

As seen in table I, cattle receive a wide variety of feed ranging from grains to mineral additives and vitamins. Soil samples were taken from an agricultural land located near the farm where application of manure from this farm was done over the past four years. The manure samples consisted of three fresh dung samples and three dry manure samples taken over a period of four days to ensure stability and representativeness in the composition.

*B. Soil Analyses*

Several experiments were made in order to evaluate the properties of the soil taken from Zahlah. In total, five analyses were performed: pH measurment (pH-water and pH-KCl); electrical conductivity of the soil, particle size analysis, determination of the total limestone and the soil water holding capacity.

*C. Feed and Manure Analyses*

In order to assess the quantities of Cu and Zn in the cattle's diet, cattle's manure and in the agricultural soils, a total digestion procedure was mandatory.

The samples were first dried and the dry matter content was determined. The drying process took place in a specialised oven at temperatures reaching 100 °C for the feed and soil samples and 60 °C for the manure samples over a period of 3 days. Then, approximately 0.5 g of each sample was weighed and underwent microwave digestion through a machine produced by ETHOS lab named "Milestone ETHOS lab station with easy WAVE". Due to the variety of samples taken from the farm, different digestion methods or programs were needed. Only two parameters varied: (1) the acids used; (2) the temperature and running time.

This method ensures the acid digestion of soil, manure and feed samples in a device through a closed vessel microwave heated under controlled temperature. It prepared the determination of total Cu and ZN by Atomic Absorption Spectroscopy through a machine called contrAA 600 [6]. AAS can measure up to the microgram per litre in a sample. These values were converted in mg/kg.

In order to use AAS to evaluate the Cu and Zn concentration in the samples, three distinct solutions were prepared:

- A stock solution of 1% nitric acid used for the dilution made by the machine in order to have a successful assay of the concentrations. The solutions digested by the microwave are diluted to its half (1/2) before being dosed in the ASS.
- A stock solution of Cu and Zn with known concentrations serving as a reference.
- A reference material (BCR 667) produced by the institute for reference materials and measurements possessing precise concentrations of Cu and Zn (60mg/kg of Cu and 175 mg/kg of Zn) was used to make sure the total acid digestion and AAS were measuring adequate concentrations.

*D. Carbon Mineralization*

The kinetics of mineralization of organic carbon in manure will be determined by incubation at a constant temperature of 22 °C for 35 days, according to the protocol presented by Parnaudeau et al. (2004) represented in Fig.2. Ten jars were used in this experiment (eight jars used as a repetition but each measured at different time, one jar used as a blank and one jar used as a control). Eight points of measurement were taken at different times: T+1,T+2, T+5, T+7, T+14, T+ 21, T+ 28, and T+ 35 days.

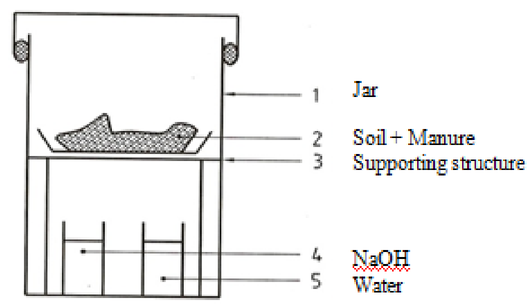


Fig. 2 Jar with soil and manure sample

The jar 1,2,3,6,7,8,9 and 10 contained 20 g of soil and 0.15 g of manure which represents an application of 30 T/ha which is the most used application of manure by the Lebanese farmers. The purpose of the NaOH in the jar is to catch the $CO_2$ released by the mineralisation of the manure. Water was added to the bottom of the jars in order to assure good humidity conditions. Jar number 5 was used as a blank and only contained NaOH. Jar 4 was used as control, it only contained soil and NaOH without manure. The purpose of jar 5 is to compare the carbon emissions between soil+manure samples and soil without manure samples.

*E. Chemical Fractionation*

Fate of heavy metals in soils is of great environmental concern. They represent major risks regarding contamination of natural waters after release by metal-bearing soil constituents and migration via the soil solution downward the water table [6]. Therefore, knowing the availability of the minerals in the soil is of importance and can be determined by chemical fractionation.

The chemical fractionation used is described in four stages shown in Table II: the acid fraction (soluble and exchangeable, bound to the carbonate fraction); the reducible fraction (bound to oxides); the oxidizable fraction (bound to the organic matter) and the residual fraction [7].

These four steps are characterised by adding acids of different strength in order to extract elements from fractions to which they are differently bound. All the solutions obtained from the chemical fractionation were analysed using the AAS to determine the quantity of Cu and Zn in each fraction.

TABLE II
CHEMICAL FRACTION PROCEDURE

| Fraction | Extractant | Conditions |
|---|---|---|
| F1 : Soluble and Exchangeable | Magnesium Nitrate 0,01M | Steer for 24h |
| F2 : organic | Sodium Hydroxide 0,7M | Heat to 100 ˚C for 30 min with occasional steering |
| F3 : oxide | Oxalic acid 0,2M Ascorbic acid 0,1M Ammonium oxalate 0,2M | Heat to 100 ˚C for 30 min with occasional steering |
| F4 : residual | Distilled water | Digestion in Kjeldatherm Gerhardtat 160 ˚C |

Fraction 1: soluble and exchangeable. The metals recovered in this fraction can be divided into two families. The first one includes ion that are fixed by a non-specific link and that can be exchanged. The second family is the cations of metals precipitated and co-precipitated by the carbonate.

Fraction 2: bound to oxides. This fraction represents the amount of the metal mainly bound to iron, aluminium and manganese oxides. In order to make this extraction successful, pH was brought to levels close to 2 by addition of acids.

Fraction 3: bound to organic, Metals extracted in this fraction are considered related to the organic matter of the soil [8].

Fraction 4: residual, the remaining metal forms the residual fraction, mainly containing silicate minerals whose crystal structure may include trace metals (mainly substituted in clays). To determine the quantity specified in this fraction, a total digestion by strong acids is used [7].

## III. RESULTS AND DISCUSSION

### *A. Soil Analyses*

The main results of all the soil analyses are presented table III and table IV.

TABLE III
PH AND ELECTRICAL CONDUCTIVITY RESULTS OF STUDIED SOILS

| Measurment | Value |
|---|---|
| pH Water | 7.3 ±0.5 |
| pH KCl | 6.1 ± 0.0 |
| EC (dS/m) | 0.46± 0.00 |

According to the USDA (U.S. Department of Agriculture), a range between 0 and 2 dS/m means that the salt concentration in the soil is between 0 and 0.13 g/100 g, the osmotic potential is between 0 and 70 kPa [9]. As seen in table III, the pH-water of the soil is approximately neutral, whereas the pH-KCl is lower and considered acidic.

As presented in table IV, the soil tested is sandy since the sand fraction reached almost 90% whereas clay and silt fractions ranged between 1.7 and 5.5%.

The sandy soil is lightweight and holds little water and nutrients.

As a conclusion, this soil is not the best soil to grow crops due to the fact that it is not able to retain water and nutrient which means that we have to double the amounts of water and nutrients in order to supply the crop’s needs.

TABLE IV
PARTICLE SOIL ANALYSES RESULTS OF STUDIED SOIL

| Particles | Percentage (%) | *Standard Deviation* |
|---|---|---|
| Clay < 2 μm | 1.74 | ± 0.20 |
| Fine silt (2-20 μm) | 2.09 | ± 0.05 |
| Coarse silt (20-50 μm) | 5.25 | ± 0.25 |
| Fine sand (50-200 μm) | 51.53 | ± 0.15 |
| Coarse sand (200-2000 μm) | 39.29 | ± 0.15 |

### *B. Input and Output of Copper and Zinc*

After performing the quantification of Cu and Zn in each feed sample and in the manure sample, a calculation of the daily input and output per cow was performed.


Fig. 3 Daily input and output. d = day

As seen in Fig. 3 the daily intake of Cu and Zn is respectively 504 mg/d and 1596 mg/d, these values exceed by far the limits cited by Meschy (2007) (240 mg of Cu and 1200 mg of Zn). On the other hand, several studies showed that these elements are only absorbed in ruminants in a rate of 28.0% for copper and 26.5% for zinc [10]. According to these numbers, only 141.21 mg of copper and 422.83 mg of zinc will be assimilated per day by the cow. The quantity not assimilated will be eliminated in the faeces, urine and milk [10].

The releases of Cu and Zn in the manure are calculated using the following equation:

$$Total\ quantity\ released = Concentration\ of\ Cu\ and\ Zn\ in\ manure\ x\ Quantity\ of\ manure\ produced\ by\ day$$

The calculation shows that 348 mg of Cu are released every day in the cattle manure (faeces+ urine), while 1006 mg of Zn are released considering that each cow produces 20 kg of manure per day [3]. Therefore 69% and 63% respectively of Cu and Zn found in the output are eliminated in the faeces. The important side of these calculations remains the existence of copper and zinc in the faeces in a form yet undiscovered and these elements will find their way to the soil when the manure is spread, therefore causing considerable pollution to the soil and groundwater.

### *C. Carbon Mineralization*

The quantity of $CO_2$ emitted during 35 days of mineralisation was increased in the soil after addition of manure as compared to the control. In total, approximately 4000 mg of $CO_2$ were emitted by the control and 7500 mg after the addition of manure during 35 days. Significant differences were observed among C-$CO_2$ evolution rates during the incubation period, the most important emissions occurred during the first days after addition of the manure with a C-$CO_2$ emission of approximately 2500 mg (Fig. 4).

After the initially high mineralization rate, a gradual decrease was observed in the following samples; the second day, $CO_2$ emissions dropped nearly 40% and reached 1600 mg, and while from day 7 till day 35 (end of the experience) the C-$CO_2$ emissions were almost constant ranging from 600 to 642 mg/l.

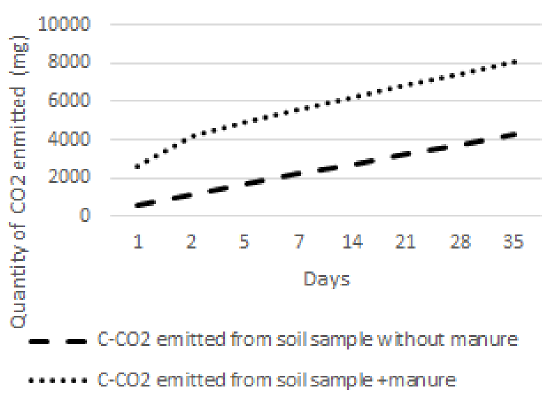


Fig. 4 Comparison between $CO_2$ emissions in control and in soil + manure sample

If we compare the evolution of carbon mineralisation in the soil with manure and the soil without manure (Fig.4), we observe that the control samples reached only 50% of the mineralisation of the sample+ manure. The reason behind that is the lack of manure in the soil, therefore the organic matter that should be mineralised in the soil is low and has already been partly mineralized. However, the control mineralized an excessive quantity of carbon as compared to the average annual mineralization of soils given in the literature [11]–[12], this can be explained by the favourable conditions of temperature and humidity created in the experiment.

### *D. AAS Results after Carbon Mineralization*

After the carbon mineralisation was successfully done, the soil samples from all the jars were digested to measure the accurate total concentration of Cu and Zn in them using the AAS. The purpose of this measure is to assess the increase of Cu and Zn content in the soil after the manure addition.

The fig. 5 shows that the Cu and Zn concentrations after adding manure have drastically increased than the older concentration of these elements found in the soil before adding the manure. First of all a 25% increase was noted for the Cu concentration after adding manure from the farm, while the concentration of Zn increased approximately 15% after adding the same amount of manure.

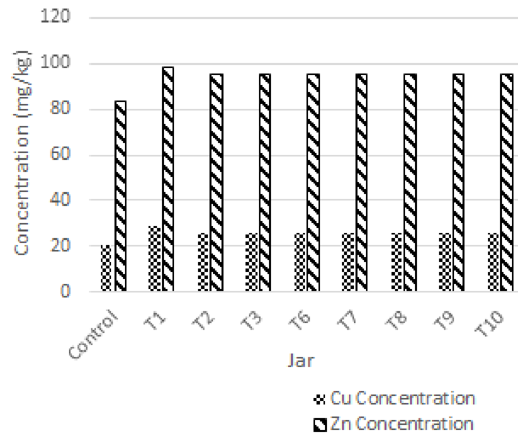


Fig. 5 Cu and Zn concentration after carbon mineralization

### *E. Chemical Fractionation*

The sequential extraction used in this study is useful to assess the potential mobility and bioavailability of heavy metals in the soils. The four chemical fractions are operationally defined by an extraction sequence that follows the order of decreasing solubility. Assuming that bioavailability is related to solubility, then metal bioavailability decreases in the order: soluble and exchangeable >bound to OM >bound to oxide > residual.

The accuracy of the sequential chemical extraction procedure was validated by comparing the sum of the 4 fractions obtained by extraction with the results of total digestion of samples. The chemical extraction showed recovery rates of respectively 96 % and 98% for Cu and Zn.

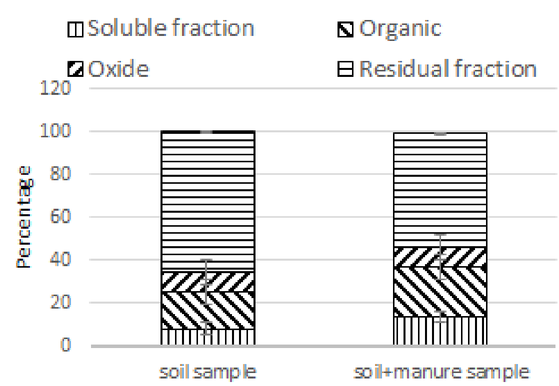


Fig. 6 Comparison between Cu availability through the fractions in soil sample and soil + manure sample

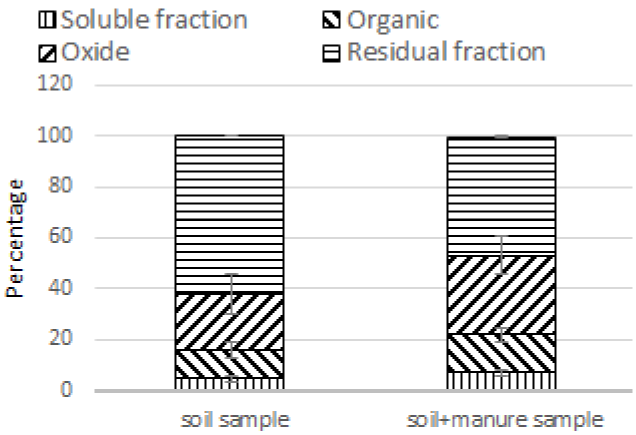


Fig. 7 Comparison between Zn availability through the fractions in soil sample and soil + manure sample

Zinc:

Zinc was mostly concentrated in the residual fraction, although it was also present in other fractions (fig. 7). The greater percentage of Zn in the residual fraction probably reflects the greater tendency for Zn to become unavailable with time after reaching the soil. Among the non-residual fractions, Zn was mostly present in the oxide fraction with a percentage close to the residual fraction (31%), this may be partially due to the high stability constants of Zn oxides [13].

Copper:

Like Zn, most of the Cu in the soil was present in the residual fraction (53%). Among the non-residual fraction, Cu existed mostly in the organic fraction with a percentage reaching 23%. The major association of Cu with the organic fraction in our soil may be due to highly constant organic-Cu complexes [14]. This relation between Cu and the organic fraction indicate the effectiveness of organic matter as a scavenger of Cu in soil, this is attributed to Cu having high affinity for humic substances which are a fraction of natural organic matter chemically active in the complexion of such metal [15].

In addition, in fig. 6 and 7, a significant difference in the percentage of the residual fraction is noted between the soil samples without manure application and the soil samples amended with manure. The samples that contain soil only showed a high percentage 65% and 62% of Cu and Zn respectively in the residual fraction, this high percentage shows an aging process for Cu and Zn. Therefore, time plays a big role in the mobility of TME since when more time passes, Cu and Zn tend to be converted from non-residual fractions to residual fractions. The positive side of this aging process is that the majority of Cu and Zn in the soil cannot be leached to the underground water.

As a conclusion, Zn has a higher mobility value than Cu since 54% of the Zn in the soil is in the non-residual fractions while Cu only presented 46% of its content in the non-residual fraction, this is due to non- residual fractions accumulating reasonably high amount of Zn in the soil.

## IV. Conclusions and Perspectives

This study has been conducted in order to assess the quantities and forms of Cu and Zn in the diet of cattle, and to quantify the rates and determine the forms of these metals in the faeces, manure and finally in the ground after burial. Dairy Khoury, the industry chosen for all the sampling, is giving a wide variety of feed and mineral supplements to the cows with more than 16 constituents in the ratio. The input of Cu and Zn is respectively 564 mg/day and 1596 mg/day. The carbon mineralisation showed that after the addition of manure, the soil experienced more mineralisation since a higher quantity of $CO_2$ was emitted. Therefore, the manure brought a lot of organic matter and trace metal elements to the soil, this increase in the concentration of TME was clear in the AAS results showing an increase of 25% and 15% for the Copper and Zinc respectively in the soil samples. The sequential chemical fractionation showed that almost 50% of Copper and Zinc belonged to the residual fraction and were not mobile. When it comes to the non-residual fractions, Copper was found in the organically bound fraction with a percentage reaching 23% while Zinc was found in the bound to oxide fraction with a percentage reaching 31%.

As perspectives, future studies should focus on the long-term effects of the application of manure rich in Cu and Zn. In fact, any change in soil conditions could alter the compartmentalization of metals and release large accumulated amounts. To finalize the chemical aspect, a study on the vertical mobility would be interesting to consider whether these metals pass into the deeper horizons and if they are therefore likely to pollute groundwater. Finally, it would be interesting to study the concentration of Copper and Zinc in the milk of the cows to assess if the milk poses any threat to the human health due to excessive quantities of trace metals in it.


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