A comparison of the transition metal concentrations in the faeces, urine, and manure slurry from different livestock animals related to environmentally relevant microbial processes

Simon Svane and Henrik Karring
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Simon Svane¹ and Henrik Karring¹*

Abstract: The microbiological communities in livestock manure slurries produce gases of environmental concern such as ammonia, methane and nitrous oxide and require trace metals such as nickel, iron, and copper to synthesize active metalloenzymes that catalyse key biochemical reactions. Additionally, large quantities of trace metals are supplied to the soil when animal manure/manure slurry is used as a fertilizer, which has led to more strict legislation regarding metal contents in manure slurry. In this study, the concentrations of the environmentally relevant transition metals nickel (Ni), copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) in faeces and urine from pigs, cattle and horses were determined using graphite furnace and flame atomic absorption spectroscopy. We show that for all three animal species 97–100% of the metal contents in manure slurry originate from faeces. The analyses show that uncontaminated manure slurry from pigs has higher metal contents than the manure slurries from cattle and horses. Specifically, on a dry matter (dm) basis, pig manure slurry contains approximately 8 mg Ni/kg dm, 104 mg Cu/kg dm, 185 mg Zn/kg dm, 1134 mg Fe/kg dm, and 356 mg Mn/kg dm. Comparing the determined transition metal contents with published values for

ABOUT THE AUTHORS
The research group has expertise in the fields of microbiology, biochemistry, and analytical chemistry, and in characterization and study of the chemical and microbiological processes in livestock manure slurry. The group has investigated the degradation of urea by ureolytic bacteria and is working on different strategies to modulate specific biochemical and microbiological activities in manure slurry. The determination and comparison of transition metal concentrations in faeces, urine, and manure slurry from livestock animals is part of the overall characterization of livestock waste. Currently, the group’s research is focused on developing new technologies to mitigate ammonia and greenhouse gas emissions from manure slurry.

PUBLIC INTEREST STATEMENT
Intensive animal production influences the environment in a number of ways. The large amounts of manure slurry produced contain many different microorganisms which through metabolic processes produce and emit considerable quantities of ammonia and greenhouse gasses, which can affect both the environment and climate, and cause economic losses to the farmers. Common to these biogenic gasses is the metals needed by the microbial enzymes to produce the volatile substances. Generally, animal feed is rich in certain metals and, therefore, livestock manure slurries contain plenty of zinc, copper, nickel, iron and manganese. This means that large quantities of metals, especially copper and zinc, are supplied to the soil and affect the surrounding environment when animal manure slurry is used as a fertilizer. Therefore, it is important to measure and compare the metal contents in waste from different livestock animals to create responsible policies and regulations in agriculture.
manure/manure slurry reveals that especially Cu, Zn and Fe concentrations in manure slurry have decreased in recent years. Comparing our results with other observations suggest that the levels of Ni, Cu, Zn, Fe, and Mn in manure slurries do not limit the microbial processes involved in the production or assimilation of environmentally relevant biogenic gasses.

Subjects: Environment & Agriculture; Biochemistry; Microbiology
Keywords: agriculture; pig; cattle; horse; transition metals; metalloenzymes

1. Introduction
In 2016, the livestock production of pigs and cattle in the European Union (EU) reached 147 and 89 million heads, respectively (Forti, 2017). Consequently, the amount of faeces and urine from livestock that must be handled is significant. In 2011, in Denmark alone, the production of manure slurry from cattle and pigs was 31.7 million tonnes (Birkmose, Hjort-Gregersen, & Stefanek, 2013). This manure slurry is predominantly used as fertilizer in agriculture, but before the slurry is applied to fields and before the nutrients are assimilated by crops, both faecal and soil microorganisms will have metabolized a large amount of the substances present in the manure slurry. Several of the microbial processes in manure slurry start already in the gastrointestinal (GI) tracts of the animals, while others begin immediately after excretion in the livestock houses. Some of these metabolic processes continue in storage tanks and in the fertilized fields (Figure 1).

Microorganisms present in livestock faeces/manure slurry are responsible for producing different biogenic gases and most of the relevant metabolic reactions require transition metals such as nickel (Ni), copper (Cu), zinc (Zn), iron (Fe), or manganese (Mn). The metals are found in the active sites of the metalloenzymes where the metal ions participate in the catalysis of crucial biochemical reactions. Specifically, a subset of transition metal ion-dependent reactions catalysed by the gut microorganisms of livestock animals directly affect the atmosphere and environment through the formation or consumption of volatile substances, including ammonia (NH\textsubscript{3}), methane (CH\textsubscript{4}), nitrous oxide (N\textsubscript{2}O), carbon dioxide (CO\textsubscript{2}), and hydrogen (H\textsubscript{2}) (Figure 1) (Bertini, Gray, Stiefel, & Valentine, 2007; Glass & Orphan, 2012). For example, ureolytic bacteria produce gaseous NH\textsubscript{3} from the hydrolysis of urea in a nickel (Ni)-dependent enzymatic reaction (Callahan, Yuan, & Wolfenden, 2005; Dai & Karring, 2014; Mobley, Island, & Hausinger, 1995). In 2015, 72,759 tonnes of ammonia was emitted from livestock manure slurry in Denmark (EEA, 2017). Ni-dependent microbial processes also include the major methanogenic pathways, hydrogenotrophic and acetoclastic methane production, used by archaea to produce CH\textsubscript{4} during anaerobic respiration (Amara, Moesca, Volbeda, & Fontecilla-Camps, 2011; Ferry, 1992; Ragsdale, 2009; Thauer, 1998). Methane is a potent greenhouse gas (GHG), and its worldwide emission from livestock, 119.1 ± 18.2 teragrams (Tg) CH\textsubscript{4} in 2011, represents approximately 1/5 of the global CH\textsubscript{4} emission (Wolf, Asrar, & West, 2017). Finally, methanogenic archaea also produce nickel-iron [NiFe]
hydrogenase, which reversibly converts molecular H₂ into protons (H⁺). Other known hydrogenases typically depend only on Fe and are classified as [Fe] and [FeFe] hydrogenases (Bertini et al., 2007; Shima et al., 2008). Another microbial Fe enzyme related to livestock waste and environmental effects is nitric oxide (NO) reductase, which contains several heme prosthetic groups (Tavares, Pereira, Moura, & Moura, 2006) and catalyzes the reduction of NO into nitrous oxide (N₂O) during the denitrification process. The gas N₂O, one of the primary GHGs (alongside CO₂ and CH₄), can be further reduced to dinitrogen (N₂) by nitrous oxide reductase, which has a copper-sulphur [Cu₅S₇] cluster in its active site (Pomowski, Zumft, Kroneck, & Einsle, 2011). Other relevant Cu-dependent enzymes are nitrite reductase, which catalyses the reduction of NO₂⁻ to NO (another step in the denitrification process) (Tavares et al., 2006). In the context of using animal waste as fertilizer in agriculture, Mn is an essential trace element for crops such as manure slurry and soil predominantly due to heterotrophic respiration (Kuzyakov, 2006). However, all the generated gaseous CO₂ does not instantly evaporate, as CO₂ exists in equilibrium with H₂CO₃ in aqueous solutions. H₂CO₃ releases protons to form bicarbonate (HCO₃⁻), which can lead to acidification of the environment (Orr et al., 2005). The zinc (Zn)-dependent enzyme carbonic anhydrase catalyses the reversible interconversion of CO₂ and H₂O to HCO₃⁻ and H⁺, which is in equilibrium with H₂CO₃. Thus, the activity of carbonic anhydrase and the hydration of dissolved CO₂ help regulate pH and the rate of CO₂ emission.

The level of trace metals in manure slurry used as fertilizer is important since a level that is too low may limit plant growth, while a level that is too high can have detrimental effects. For example, Cu is essential for growth but is toxic if present in high concentrations (Gonzales, 1994; Kofoid & Kjellerup, 1984; Mengel & Kirkby, 1982; Wium-Andersen, 2012). Notably, Cu and Zn are added in large quantities to pig feed for medicinal purposes to prevent anaemia and diarrhoea and to help the animals gain weight (Burton & Turner, 2003; Poulsen, 1998; Smith, Tokach, Goddband, Nelssen, & Richbert, 1997). Since Cu and Zn are only retained by the animals to a small degree and are mostly excreted, it has been shown that soil fertilized with animal manure slurry can have a high content of these metals (Figure 1) (Gräber et al., 2005). In Denmark, the concentrations of Cu and Zn in soil fertilized with pig manure slurry increased in the period 1986–2014 by 21–28% and 2–5%, respectively (Bak, Jensen, & Larsen, 2015). The metal loading of agricultural soils fertilized with livestock manure is thus influenced by the metal content of the manure slurry and is known to affect the surrounding environment, especially streams and creeks (Figure 1) (Jensen & Bak, 2018). Consequently, this has led to stricter legislation on Cu and Zn contents of livestock feed and manure/manure slurry (Brandt, 2017; Gudbergersen, 2012). Therefore, we hypothesize that the levels of these transition metals and maybe others have decreased in livestock animal waste in recent years. In the present work, we have determined the concentrations of the enzymatically relevant first-row transition metals Ni, Cu, Zn, Fe, and Mn in faeces, urine, and manure slurry from three Danish livestock animal species with very different digestion systems, namely, pig (monogastric omnivore), cattle (true ruminant), and horse (monogastric herbivore). As the majority of the metal content of animal waste typically originates in the animal feed, we also analysed the pig and cattle compound feeds. Drinking water was analysed to establish the relative contribution of metals from this source. The metal contents of all samples were determined by graphite furnace or flame atomic absorption spectroscopy.

2. Materials and methods

2.1. Chemicals, reagents and equipment

All chemicals and standard solutions were purchased from Sigma-Aldrich and used as received unless otherwise stated. HNO₃ was purchased as puriss p.a.-grade. Graphite furnace atomic absorption spectroscopy (GFAAS) was carried out on an Agilent Technologies 240 AA system with a GTA 120 graphite tube atomizer and a PSD 120 automatic sampler (Agilent Technologies, Inc., CA, USA). Flame atomic absorption spectroscopy (FAAS) was carried out on a 55 AA Atomic
Absorption Spectrometer (Agilent Technologies, Inc., CA, USA). Coded single-element hollow cathode lamps (Ni, Cu, Zn, Fe, and Mn) were purchased from Agilent (Agilent Technologies Inc., CA, USA). Ultrapure water was obtained from an ELGA Purelab Chorus 1 system (Elga Veolia, UK). Sample digestion by electrothermal radiation was performed with a Multiwave GO microwave system (Anton Paar, Austria). The dry matter (dm) content was determined on an HR83 Halogen moisture analyser (Mettler Toledo, Glostrup, Denmark). Pipette tips were rinsed with 2 M HNO₃ and ultrapure water prior to use. All glassware was washed with 2 M HNO₃ and ultrapure water prior to use. Standards for GFAAS/FAAS were prepared by dilution of commercial certified standards (1000 mg/L, 4% HNO₃) with ultrapure water. The standards were freshly prepared on the day of analysis.

2.2. Collecting faeces and urine samples
Fresh faeces and urine samples were collected from fattening pigs, cattle, and horses. All samples were collected directly upon excretion from the animals to prevent any contamination from contact with the floor. Before measuring the metal and dry matter content, equal amounts of faeces or urine from several individual animals were pooled.

Pigs: The pigs (Landrace/Yorkshire and Duroc mix, 70–90 kg) were approximately 5–6 months of age, and they were kept in an intensive housing system with 2/3 slatted floor and 1/3 drained floor. The animals were given complete feed made from wheat, soy, sunflower seed, and sugar beet molasses and fortified with minerals and vitamins (Cu(SO₄) pentahydrate, 60 mg/kg; ZnO, 133 mg/kg; Fe(SO₄) monohydrate, 280 mg/kg and MnO, 68 mg/kg). The animals had free access to water. Faeces and urine samples from individual animals were collected, and each sample was packed separately in a clean plastic bag. Prior to the analyses, equal amounts of faeces or urine from 7–10 pigs were pooled and homogenized before being stored at −20°C for later use.

Cattle: The cattle consisted of three steers (1 year of age) and two dairy cows (4 and 10 years of age) of the Danish Red and Jersey breeds. The cattle were kept in a loose-housing system and were fed a mixture of 50% grain and 50% compound feed for dairy cattle that was fortified with minerals (Cu²⁺, 17.24 mg/kg; Zn²⁺, 51.71 mg/kg; Mn²⁺, 45.96 mg/kg) and supplemented with grass and hay. The cattle had free access to water. Faeces and urine samples from the individual animals were collected, and each sample was packed separately in a clean plastic bag. Subsequently, equal amounts of faeces or urine from the five specimens were pooled, homogenized, and stored at −20°C until use.

Horses: The horses were between 3 and 12 years of age and comprised six males and one mare of the breed Danish Warmblood. The grazing horses had free access to water and were also fed hay and complete horse feed that was supplemented with lucerne, beet, minerals and vitamins. Urine and faeces samples from individual horses were collected, and each sample was packed separately in a clean plastic bag. Equal amounts of faeces or urine from 3–4 horses were pooled, homogenized, and stored at −20°C.

2.3. Ethics statement
The faeces and urine samples were collected by using a homemade “‘bucket on a stick’“ and clean plastic bags. The animals were never touched and were never stimulated or forced to excrete faeces or urine. Because the animals experienced no “‘pain, suffering, anxiety or lasting harm’“, approval from the Danish Inspectorate for Animal Experiments was not necessary according to the relevant Danish legislation (Bekendtgørelse af lov om dyreforsøg, nr. 253 af 8. marts 2013—Executive order of law on animal experiments, no. 253 of 8. March 2013).

2.4. Dry matter determination
Dry matter determinations were carried out with the HR83 Halogen moisture analyser. All samples were dried by rapidly heating the samples to 130°C and then keeping the temperature constant until the mass change of the sample was less than 0.1 mg/90 sec. The drying times differed based
on the sample type and distribution of material but were generally approximately 4 min/g for feed, 4.3 min/g for urine, 9.5 min/g for pig/horse faeces, and 12.5 min/g for cow faeces.

2.5. Sample pretreatment and graphite furnace and flame atomic absorption spectroscopy analyses

Digestion and atomic absorption spectroscopy methods were adapted from elsewhere (Peters et al., 2003). Pooled faeces samples (201–369 mg) were mixed with 3 ml of 65% (v/v) HNO$_3$ and placed in HVT50 pressure vessels, followed by microwave digestion for 20 min at 180°C, yielding clear light blue solutions and a red/brown gas. The digestion followed a standard preprogrammed heating profile for organic sample digestion. The program consists of a ramp time of 20 min to reach 180°C, after which, the temperature is held constant for 10 min before cooling down. This program produced complete digestion of all sample types analysed here. The digested samples faded from the blue colour to colourless upon standing. Urine samples (30 ml) were acidified with 65% (v/v) HNO$_3$ to a final acid concentration of 9% (v/v) prior to analysis. For each sample, blanks were subjected to all pre-treatment steps. All samples, blanks, and commercial standards were analysed six times. For GFAAS, a few microliters of sample was injected into the graphite furnace by an auto sampler. The furnace was then heated according to a standard heating profile for each element, and the absorption of the element of interest was recorded. In cases when the element of interest was too concentrated, the sample was diluted and reanalysed. For FAAS, 1.5–2 ml of sample was manually injected into an air-acetylene flame, and the resulting absorption recorded. Every 6th sample analysed was a control containing a known concentration of the element being measured. For both techniques, the absorption was converted to concentration using a standard curve constructed from measurements of three dilutions of a commercial standard. The above methods were validated using standard solutions.

2.6. Statistics

Student’s t-test was used to determine whether the dry matter and metal concentrations of the pig and cattle feed and drinking waters, as well as the Zn concentrations in pig and cattle urine, were significantly different at a significance level of 0.05. One-way ANOVA followed by Tukey’s multiple comparisons test was used to determine whether the dry matter and metal concentrations in pig, cattle and horse faeces and urine were significantly different at a level of 0.05. All statistical analyses were carried out with GraphPad Prism 7.

3. Results

3.1. Transition metal contents of livestock animal feeds and drinking water

The Ni, Cu, Zn, Fe, and Mn contents of complete pig feed, unfortified cattle compound feed, pig drinking water, and cattle drinking water were measured by graphite furnace and flame atomic absorption spectroscopy (Table 1). Before being fed to the animals, the cattle feed was supplemented with a mineral and vitamin supplement containing CuSO$_4$ pentahydrate, 5.89 g/kg; ZnO, 5.60 g/kg; MnO, 5.17 g/kg; and other substances. The supplement was mixed into the feed on a 10.0 g/kg basis, enabling calculation of the estimated Cu, Zn and Mn concentrations in the final fortified cattle feed (Table 1). Ni and Fe are not added with the mineral supplement but are likely to be present to some degree as trace impurities. A true representative sample of horse feed could not be obtained and, therefore, horse feed and drinking water have not been included in the analyses.

The feeds contained almost the same amount of dry matter. The Ni contents of pig feed and drinking water were 1.51 ± 0.09 mg/kg dm and 3.84 ± 0.10 μg/L, respectively, which were higher than the contents for the corresponding cattle samples (Table 1). The pig feed also contained higher concentrations of Cu, Zn, Fe and Mn than the cattle feed. Interestingly, the cattle feed contained approximately 10 times less Fe than the pig feed, and apparently, no additional Fe was added with the mineral supplement. The cattle drinking water did have Fe and Cu concentrations approximately 20 and 250 times higher, respectively, than the concentrations found in the pig
drinking water (Table 1). The pig drinking water instead contained approximately 70 times more Mn than the cattle drinking water (Table 1). The large variations in the metal contents of drinking water likely reflect the different geographic origins of the water samples (pig drinking water from central Jutland and cattle drinking water from Funen).

3.2. Transition metal contents of faeces, urine, and manure slurry from livestock animals
The dry matter content was relatively similar for the pig and horse faeces at 25.35 ± 0.17% and 22.99 ± 0.50%, respectively, while the cattle faeces was wetter, containing only 15.44 ± 1.24% dry matter (Table 2). On the other hand, the cattle urine contained 7.95 ± 0.22% dry matter, which was more than twice the amount found in the pig or horse urine.

The contents of Ni, Cu, Zn, Fe, and Mn in the livestock faeces and urine samples were determined using the same analytical methods as described for the feed and drinking water (Table 2). The cattle urine was found to contain material that precipitated upon acidification of the samples with HNO$_3$. The precipitate was not identified but was not merely simple carbonates, which would dissolve at low pH. Consequently, the analyses of the different metals in cattle urine were carried out on both acidified and non-acidified samples to evaluate if any significant amount of the elements of interest were present in the precipitate. The concentration of Ni in the acidified cattle urine was 2.88 ± 0.64 μg/L compared to 5.01 ± 0.562 μg/L for the non-acidified sample, indicating that almost half the Ni precipitated during acidification (Table 2). The measured concentrations of Cu, Zn and Mn were the same for the acidified and non-acidified cattle urine samples. However, the Fe concentration determined when analysing the non-acidified sample was 45% lower than that for the acidified cattle urine, indicating that the Fe in cattle urine did not volatilize easily before being converted to nitrates. In summary, the acid-driven precipitate in samples of cattle urine did not appear to contain any of the metals of interest with the exception of Ni. The urine from pig and horse did not produce any visible precipitate after acidification.

Generally, the faeces from cattle had the lowest metal concentrations, while in most cases, the pig faeces had the highest concentrations of the measured metals. Of note, the Ni content of the pig

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dry matter (%)</th>
<th>Ni (mg/kg dm)</th>
<th>Cu (mg/kg dm)</th>
<th>Zn (mg/kg dm)</th>
<th>Fe (mg/kg dm)</th>
<th>Mn (mg/kg dm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig feed</td>
<td>84.11 ± 0.14</td>
<td>1.51 ± 0.09</td>
<td>25.45 ± 0.65</td>
<td>149.1 ± 5.43</td>
<td>1728.6 ± 70.0</td>
<td>126.9 ± 0.05</td>
</tr>
<tr>
<td>Cattle feed</td>
<td>85.14 ± 0.13</td>
<td>0.48 ± 0.03</td>
<td>3.22 ± 0.13</td>
<td>48.72 ± 0.08</td>
<td>180.8 ± 5.50</td>
<td>39.99 ± 4.94</td>
</tr>
<tr>
<td>Fortified cattle feed*</td>
<td>85.14</td>
<td>0.48</td>
<td>20.46</td>
<td>100.4</td>
<td>180.8</td>
<td>85.95</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ni (μg/L)</th>
<th>Cu (μg/L)</th>
<th>Zn (μg/L)</th>
<th>Fe (μg/L)</th>
<th>Mn (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig drinking water</td>
<td>ND</td>
<td>3.84 ± 0.10</td>
<td>0.99 ± 0.07</td>
<td>&lt;10</td>
<td>3.41 ± 0.91</td>
</tr>
<tr>
<td>Cattle drinking water</td>
<td>ND</td>
<td>0.81 ± 0.03</td>
<td>247.8 ± 1.92</td>
<td>&lt;10</td>
<td>67.30 ± 3.71</td>
</tr>
</tbody>
</table>

*Estimated values based on the content of minerals in the cattle mineral supplement and a recommended dosage of 10.0 g/kg feed if the dry matter content is assumed to be the same as for the unfortified feed. The p-value obtained using Student’s t-test to compare the values for pigs and cattle is indicated below each pair of measurements. At a significance level of p < 0.05, the measured values for the respective pig and cattle samples are significantly different.
Table 2. Dry matter and metal (Ni, Cu, Zn, Fe, and Mn) contents of livestock animal faeces and urine. Metal contents are given on a dry matter basis (mg/kg) for faeces (mass fractions) and per volume (μg/L) for urine (mass concentrations). Values are reported as the mean±SD; dm, dry matter

<table>
<thead>
<tr>
<th>Sample</th>
<th>Animal</th>
<th>Dry matter (%)</th>
<th>Ni (mg/kg dm)</th>
<th>Cu (mg/kg dm)</th>
<th>Zn (mg/kg dm)</th>
<th>Fe (mg/kg dm)</th>
<th>Mn (mg/kg dm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pig</td>
<td>25.35 ± 0.17</td>
<td>10.38 ± 0.0002</td>
<td>131.7 ± 1.63</td>
<td>232.5 ± 2.80</td>
<td>1445.7 ± 46.2</td>
<td>453.7 ± 4.58</td>
</tr>
<tr>
<td>Faeces</td>
<td>Cattle</td>
<td>15.44 ± 1.24</td>
<td>1.03 ± 0.25</td>
<td>10.79 ± 0.10</td>
<td>101.0 ± 2.10</td>
<td>734.2 ± 20.4</td>
<td>196.6 ± 24.2</td>
</tr>
<tr>
<td></td>
<td>Horse</td>
<td>22.99 ± 0.50</td>
<td>2.04 ± 0.02</td>
<td>16.72 ± 2.02</td>
<td>85.69 ± 0.44</td>
<td>1204.4 ± 28.2</td>
<td>458.0 ± 0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p &lt; 0.02</td>
<td>p &lt; 0.003</td>
<td>p &lt; 0.007</td>
<td>p &lt; 0.002</td>
<td>p &lt; 0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(μg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pig</td>
<td>2.33 ± 0.18</td>
<td>26.15 ± 0.70</td>
<td>20.52 ± 0.36</td>
<td>251.6 ± 2.11</td>
<td>38.17 ± 4.40</td>
<td>6.68 ± 0.12</td>
</tr>
<tr>
<td>Urine</td>
<td>Cattle</td>
<td>7.95 ± 0.22</td>
<td>2.88 ± 0.64¹</td>
<td>28.57 ± 1.70</td>
<td>33.84 ± 0.001</td>
<td>58.87 ± 2.88</td>
<td>4.84 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>Horse</td>
<td>3.60 ± 0.18</td>
<td>1.77 ± 0.01*</td>
<td>64.96 ± 2.50</td>
<td>&lt;10</td>
<td>58.47 ± 1.68</td>
<td>7.89 ± 0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p &lt; 0.0005</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.003</td>
<td>p &lt; 0.0001²</td>
<td>p &lt; 0.0006</td>
</tr>
</tbody>
</table>

¹Urine without HNO₃ was found to contain 5.01 ± 0.562 μg Ni/L. Values marked by * in the same sub column are not significantly different between animal species at a significance level of p < 0.05 according to one-way ANOVA and Tukey’s test. Values not marked by superscript letters in the same sub column are significantly different. ²calculated with Student’s t-test (p < 0.05).
faeces was approximately 5 and 10 times higher than the Ni contents in faeces from horse and cattle, respectively. Furthermore, the Cu and Zn contents of the pig faeces were likewise approximately 10 and 2.5 times higher than the corresponding metal contents found in both the cattle and horse faeces. Finally, the measured amounts of Fe and Mn were equal in the pig and horse faeces, while the cattle faeces contained only approximately half the amounts of these metals (Table 2). For urine, the differences in the metal concentrations between the animal species were generally less pronounced with a few exceptions. Thus, there was little difference in the urine Fe and Mn contents between the three animal species, and the urine from pig and cattle contained approximately the same concentration of Cu. However, the horse urine contained more than twice as much Cu (64.96 ± 2.50 μg/L) as the pig and cattle urine contained. Furthermore, the pig urine had the highest Ni concentration (26.15 ± 0.70 μg/L), which was approximately 9 and 15 times higher than the Ni contents of the cattle and horse urine, respectively. The Zn concentration in urine covered a surprisingly large range with a value of 251.6 ± 2.11 μg/L for the pig urine but approximately 7 and more than 25 times less Zn in the cattle and horse urine, respectively (Table 2).

Due to differences in the dry matter content of and the ratio of faeces to urine of each of the animal species, the actual metal concentrations in manure slurry will significantly differ from the values measured in pure faeces and urine. The metal concentrations in manure slurry can be estimated based on the measured metal contents in faeces and urine and previously published feces:urine ratios for pigs, cattle and horses. The approximate weight:volume (w:v) ratios of feces:urine produced are 1:3 (w:v) for pigs (Canh et al., 1998), 3:2 (w:v) for cattle (ASABE, 2005), and 2:1 (w:v) for horses (Wartell et al., 2012). The metal concentrations in manure slurry were calculated as mg/kg dm in order to be able to compare our results with those published previously (Table 3).

The estimated concentrations of metals in the dried manure slurry (mass fractions) from the different animal species show that pig manure slurry has the highest concentrations of all the metals (Table 3). The concentrations of Ni, Cu, and Zn are rather similar between the cattle and horse manure slurry, while the Mn content of the cattle manure slurry is approximately half the Mn concentration in the pig and horse manure slurries (Table 3). The cattle manure slurry has the lowest Fe content (679.0 mg/kg dm), while the pig manure slurry contains 67% more Fe, equalling 1134.3 mg/kg dm. It is evident from the estimates that for all the metals, >97% of the total concentration in manure slurry originates from faeces (Table 3).

4. Discussion

4.1. Reduced Cu and Zn contents in feeds
In Denmark, there are threshold values of 25 mg Cu/kg dm and 150 mg Zn/kg dm in pig feeds meant for grower and finisher pigs according to the Danish Veterinary and Food Administration (Gudbergsen, 2012). The pig feed analysed in this study was found to contain 25.45 ± 0.65 mg Cu/kg dm and 149.1 ± 5.43 mg Zn/kg dm, thereby conforming to the threshold (Table 1). In an earlier English study in 1999, Nichololson et al. found significantly higher mean Cu concentrations of 159 and 128 mg/kg dm in grower and finisher pig feeds, respectively. The same study reported mean Zn concentrations of 356 and 308 mg/kg dm in grower and finisher pig feeds, respectively (Nicholson, Chambers, Williams, & Unwin, 1999). The lower Cu and Zn concentrations found in the present work are likely caused by relatively recent concerns that high amounts of metals, especially Cu and Zn, in animal manure slurry used as fertilizer may lead to increased antibiotic resistance of microorganisms, metal loading of fertilized soils and leaching to waterways, which can have negative environmental effects (Bak et al., 2015; Jensen & Bak, 2018; Sloth, 2015). A recent EU decision to completely phase out the use of medicinal ZnO in pig feed before 2022 is a direct consequence of these concerns (Brandt, 2017).

4.2. Pig manure/manure slurry as fertiliser
Previous studies have reported Cu concentrations in pig manure/manure slurries of 151–420 mg/kg dm for fattening pigs and a significantly higher concentration of approximately 784 mg/kg dm for
Table 3. Estimated dry matter and metal (Ni, Cu, Zn, Fe, and Mn) contents of livestock animal manure slurry. Dry matter and metal contents (mg/kg dm) in manure slurry from livestock animals (pig, cattle, and horse) are estimated based on the measured metal contents in faeces and urine and the previously found ratios of feces:urine (w:v) for each animal species. The percentage of the metal in manure slurry originating from faeces is listed in parentheses.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Animal</th>
<th>Feces:urine ratio (w:v)</th>
<th>Dry matter (%)</th>
<th>Ni (mg/kg dm) (97.11%)</th>
<th>Cu (mg/kg dm) (98.74%)</th>
<th>Zn (mg/kg dm) (99.86%)</th>
<th>Fe (mg/kg dm) (99.97%)</th>
<th>Mn (mg/kg dm) (99.98%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manure slurry</td>
<td>Pig</td>
<td>1:3</td>
<td>8.08</td>
<td>8.38</td>
<td>103.5</td>
<td>184.7</td>
<td>1134.3</td>
<td>355.9</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>3:2</td>
<td>12.53</td>
<td>0.97</td>
<td>10.15</td>
<td>93.60</td>
<td>679.0</td>
<td>181.7</td>
</tr>
<tr>
<td></td>
<td>Horse</td>
<td>2:1</td>
<td>16.52</td>
<td>1.54</td>
<td>12.84</td>
<td>64.34</td>
<td>903.9</td>
<td>343.7</td>
</tr>
</tbody>
</table>
weaners (Table 4). The Zn concentrations in manure/manure slurry ranged from 417 mg/kg dm to 1729 mg/kg dm for fattening pigs and to 3379 mg/kg dm for weaners (Table 4). In addition to the relatively large variations in Cu and Zn contents between different studies, the previously reported concentrations are significantly higher than those calculated in the present study for pig manure slurry, namely, 103.5 mg Cu/kg dm and 184.7 mg Zn/kg dm (Table 3). In recent years, the contents of Cu and Zn in pig feed have been regulated, and therefore, it is not surprising that the concentrations of these metals in manure slurry have decreased. The previously determined Fe concentration in pig manure/manure slurry ranged from 1559 to 6150 mg/kg dm, and Fe was typically the most abundant of the screened metals (Table 4). In the present study, pure pig faeces and urine showed Fe concentrations of 1445.7 ± 46.2 mg/kg dm and 38.17 ± 4.40 μg/L, respectively (Table 2), which correspond to approximately 1134.3 mg Fe/kg dm in manure slurry (Table 3). The previously reported content of Mn in pig manure/manure slurry is within the range 214–379 mg/kg dm (Table 4), which is in agreement with the 355.9 mg Mn/kg dm value calculated for pig manure slurry in our findings (Table 3). Finally, the previously reported Ni concentrations in pig manure/manure slurry ranging from 9 to 12.9 mg/kg dm (Table 4) are only slightly higher than the 8.38 mg Ni/kg dm calculated in our study (Table 3). Thus, with the exception of Mn, all the measured metal contents in manure slurry in our work were found to be lower than the concentrations observed in previous reports.

4.3. Cattle manure/manure slurry as fertiliser

Cattle manure/manure slurry generally has Cu concentrations in the range of 20–139 mg/kg dm, which are quite low compared to the concentrations reported for pig manure/manure slurry (Table 4). Our analyses revealed a very low Cu content of only 10.15 mg/kg dm in cattle manure slurry (Table 3). Interestingly, our analyses showed nearly the same level of Cu in the pig compound feed (25.45 ± 0.65 mg Cu/kg dm) as that calculated for fortified cattle feed (20.46 mg Cu/kg dm) (Table 1). The cattle were, however, also fed grain and some grass and hay, whereas the pigs were fed only dry complete feed. Zn concentrations between 79 and 280 mg/kg dm in cattle manure/manure slurry have previously been reported (Table 4), which are consistent with our findings of 93.60 mg Zn/kg dm in cattle manure slurry (Table 3). The lower levels of Zn in faeces and manure slurry from cattle compared to those found for pigs are in accordance with the higher concentrations of Zn in pig feed than in cattle feed, which is mainly due to the ZnO present in the pig feed fortifier (Table 1). Only two previous studies tested cattle manure for the presence of Fe and Mn (Table 4). In those studies, the Fe concentrations were found to be 1970 and 3527 mg/kg dm, which is approximately 3–5 times higher than the Fe content found in cattle faeces/manure slurry in the present study (Tables 2 and 3). The estimated Mn concentration of 181.7 mg/kg dm in cattle manure slurry (Table 3) is, on the other hand, quite similar to the 111 and 180 mg Mn/kg dm values reported earlier for cattle manure (Table 4). The large difference between the Fe contents in cattle faeces/manure slurry found in our and previous studies is most likely caused by differences in feed additives and the different types of samples analysed. Additionally, while we have analysed uncontaminated cattle faeces, the previous studies most likely analysed stored manure slurry from storage tanks. When analysing stored manure/manure slurry, substances such as metal fixtures, metal pipes, and stable disinfectant products based on iron sulphate (e.g. Stalosan F from Vilofoss) will all contribute Fe to the manure slurry. Finally, the Ni concentration in cattle manure slurry was calculated to be 0.97 mg/kg dm (Table 3), which is significantly lower than the previously reported values of 6–9 mg Ni/kg dm (Table 4). The relatively large difference between the Ni contents in cattle manure determined in the present study and those in previous work may be related to different types of feed among the tested animals or the fact that we used uncontaminated cattle faeces for our analyses.

4.4. Horse faeces as fertiliser

While a number of studies have investigated the content of various metals in the manure/manure slurry of pigs and cattle, very few have examined the faeces and urine from animal species such as horse, which are less important in industry. Thus, to the best of our knowledge, the Cu, Zn, Fe, and Mn contents in horse manure have been reported in only one study (Moreno-Caselles, Moral, Perez-Murcia, Perez-Espinosa, & Rufete, 2002). The Cu concentration in horse manure reported by Moreno-Caselles et al. (2002) was 22 mg/kg dm (Table 4), which is almost twice as much as the
Table 4. Dry matter and metal (Ni, Cu, Zn, Fe, and Mn) contents of pig, cattle and horse manure and slurry reported from 1999–2017. The results from the oldest studies are listed first. The sample terms are the same as those reported in the studies. Mean±SD values and ranges (numbers in parentheses) are included when reported in the studies. ND, not determined; NR, not reported.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dry matter</th>
<th>Ni</th>
<th>Cu</th>
<th>Zn</th>
<th>Fe</th>
<th>Mn</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig slurry</td>
<td>4.4 (0.5–21.6)</td>
<td>10.4 (&lt;0.1–49.8)</td>
<td>351 (&lt;1.0–807)</td>
<td>575 (&lt;5–2500)</td>
<td>ND</td>
<td>ND</td>
<td>(Nicholson et al., 1999)</td>
</tr>
<tr>
<td>Cattle slurry (beef)</td>
<td>12.0 (2.2–21.0)</td>
<td>6.4 (1.9–20.4)</td>
<td>33.2 (17.5–48.7)</td>
<td>133 (68–235)</td>
<td>ND</td>
<td>ND</td>
<td>(Nicholson et al., 1999)</td>
</tr>
<tr>
<td>Cattle manure (dairy)</td>
<td>NR</td>
<td>8 ± 9.5 (2–35)</td>
<td>139± 242 (18–1100)</td>
<td>191 ± 115 (87–488)</td>
<td>ND</td>
<td>ND</td>
<td>(McBride &amp; Spiers, 2001)</td>
</tr>
<tr>
<td>Horse manure</td>
<td>NR</td>
<td>ND</td>
<td>22 (20–29)</td>
<td>167 (129–205)</td>
<td>729 (284–1173)</td>
<td>110 (108–111)</td>
<td>(Moreno-Caselles et al., 2002)</td>
</tr>
<tr>
<td>Cattle manure</td>
<td>NR</td>
<td>ND</td>
<td>20 (9–30)</td>
<td>79 (61–114)</td>
<td>3527 (2541–8054)</td>
<td>111 (91–142)</td>
<td>(Moreno-Caselles et al., 2002)</td>
</tr>
<tr>
<td>Pig manure</td>
<td>NR</td>
<td>ND</td>
<td>170 (138–226)</td>
<td>427 (307–563)</td>
<td>1559 (1464–1597)</td>
<td>328 (201–435)</td>
<td>(Moreno-Caselles et al., 2002)</td>
</tr>
<tr>
<td>Pig manure compost</td>
<td>26.1</td>
<td>ND</td>
<td>174</td>
<td>417</td>
<td>6150</td>
<td>379</td>
<td>(Liu, Imai, Ukita, Sekine, &amp; Higuchi, 2003)</td>
</tr>
<tr>
<td>Cattle liquid manure</td>
<td>5.53 ± 2.71</td>
<td>6.31 ± 7.15</td>
<td>64.24 ± 58.42</td>
<td>231.76 ± 63.07</td>
<td>ND</td>
<td>ND</td>
<td>(Schwaert &amp; Grant, 2003)</td>
</tr>
<tr>
<td>Pig liquid manure</td>
<td>1.77 ± 1.34</td>
<td>10.22 ± 2.69</td>
<td>263.33 ± 108.63</td>
<td>1016.67 ± 1085.81</td>
<td>ND</td>
<td>ND</td>
<td>(Schwaert &amp; Grant, 2003)</td>
</tr>
<tr>
<td>Pig slurry (#1, fattening)</td>
<td>5.4</td>
<td>9</td>
<td>640</td>
<td>770</td>
<td>ND</td>
<td>ND</td>
<td>(Moller, Jensen, Tobiasen, &amp; Hansen, 2007)</td>
</tr>
<tr>
<td>Pig slurry (#2, fattening)</td>
<td>1.7</td>
<td>ND</td>
<td>494</td>
<td>911</td>
<td>ND</td>
<td>ND</td>
<td>(Moller et al., 2007)</td>
</tr>
<tr>
<td>Cattle slurry (dairy)</td>
<td>6.4</td>
<td>9</td>
<td>100</td>
<td>280</td>
<td>ND</td>
<td>ND</td>
<td>(Moller et al., 2007)</td>
</tr>
<tr>
<td>Cattle manure</td>
<td>NR</td>
<td>6.3</td>
<td>51 (8–117)</td>
<td>164 (49–405)</td>
<td>1970</td>
<td>180 (40–312)</td>
<td>(Sager, 2007)</td>
</tr>
<tr>
<td>Cattle manureb</td>
<td>NR</td>
<td>ND</td>
<td>31.04 (19.48–66.58)</td>
<td>126.33 (65.27–319.27)</td>
<td>ND</td>
<td>ND</td>
<td>(Zhang, Li, Yang, &amp; Li, 2012)</td>
</tr>
<tr>
<td>Pig manure</td>
<td>NR</td>
<td>ND</td>
<td>420.39 (77.62–890.023)</td>
<td>475.99 (156.47–860.32)</td>
<td>ND</td>
<td>ND</td>
<td>(Zhang et al., 2012)</td>
</tr>
<tr>
<td>Pig manure</td>
<td>NR</td>
<td>12.9 (3.1–97.3)</td>
<td>388.5 (22.4–3387.6)</td>
<td>1199.2 (93.0–8239.0)</td>
<td>ND</td>
<td>ND</td>
<td>(Holzel et al., 2012)</td>
</tr>
<tr>
<td>Sample</td>
<td>Dry matter (%)</td>
<td>Ni (mg/kg dm)</td>
<td>Cu (mg/kg dm)</td>
<td>Zn (mg/kg dm)</td>
<td>Fe (mg/kg dm)</td>
<td>Mn (mg/kg dm)</td>
<td>References</td>
</tr>
<tr>
<td>-------------------------</td>
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<td>---------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>Pig (weaners) slurry</td>
<td>2.48 ± 1.87</td>
<td>ND</td>
<td>784.83 ± 262.74</td>
<td>3379 ± 1998.93</td>
<td>ND</td>
<td>ND</td>
<td>(Birkmose &amp; Tybirk, 2013)</td>
</tr>
<tr>
<td>Pig (fattening) slurry</td>
<td>4.78 ± 2.78</td>
<td>ND</td>
<td>297.0 ± 187.30</td>
<td>1729.6 ± 1070.41</td>
<td>ND</td>
<td>ND</td>
<td>(Birkmose &amp; Tybirk, 2013)</td>
</tr>
<tr>
<td>Pig manure</td>
<td>17.8 ± 0.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2754 ± 272</td>
<td>214 ± 17</td>
<td>(Gondek &amp; Mierzwa-Hersztek, 2016)</td>
</tr>
<tr>
<td>Pig manure</td>
<td>NR</td>
<td>9.27</td>
<td>151.11</td>
<td>538.29</td>
<td>ND</td>
<td>ND</td>
<td>(Wang et al., 2017)</td>
</tr>
</tbody>
</table>

*Median value was 64 mg/kg dm. ° Farm size 100–300 animals. ° Farm size 200–800 animals.
12.84 mg Cu/kg dm value estimated in the present study (Table 3). Likewise, the authors report average Zn concentrations of 167 mg/kg dm in horse manure, which is approximately 2.5 times the amount estimated in the present study (Table 3). In contrast, Moreno-Caselles et al. (2002) report relatively low concentrations of Fe and Mn (Table 4) compared to our results (Table 3). The most likely reason for the observed differences in metal concentrations in horse manure is the composition of the locally used feed and the amount of grazing performed by the horses. To the best of our knowledge, no previous studies have reported the concentration of Ni in horse faeces (2.04 ± 0.02 mg/kg dm) or urine (1.77 ± 0.01 μg/L); we found that these values were comparable to the amounts found in cattle faeces and urine, respectively (Table 2). Among the samples from the three animal species, the horse and cattle manure slurries contain lower amounts of Ni, Cu, Zn, Fe and Mn (on a dry mass basis) than pig manure slurry. In particular, the Ni, Cu and Zn contents are higher in pig manure slurry than in manure slurry from cattle and horse, while the Fe and Mn contents are more similar across the species (Table 3).

4.5. “Wet” concentrations of metals

It is common to report nutrient concentrations from livestock waste as mass fractions on a dry matter basis to enable comparison between studies. However, when comparing different animal species with different faeces:urine ratios of excretion and different dry matter contents of manure slurry, it is advantageous to also consider the “wet” concentrations (molar concentrations) of nutrients, as these are the true concentrations experienced by excreted gut microbes (Table 5). Cattle and horses excrete faeces and urine in ratios (w:v) of approximately 3:2 and 2:1, respectively, whereas the ratio is 1:3 for pigs. Combined with the different dry matter contents of the manure slurries, this information about the ratios means that while pig manure slurry has significantly higher metal contents than cattle and horse manure slurries on a dry mass basis (Table 3), the differences between the metal contents are smaller when considering the true wet concentrations (Table 5). Interestingly, the Fe molar concentration (µmol/L) in manure slurry is almost the same for pig and cattle, while horse manure slurry contains almost twice as much Fe (2673.91 µM) (Table 5). This finding is in contrast to the mass fraction contents calculated on a dry matter basis, where pig manure slurry contains 1134.3 mg Fe/kg dm and cattle manure slurry only contains 679 mg Fe/kg dm (Table 3). On the same note, wet horse manure slurry has a significantly higher Mn concentration (1033.36 µM) than pig or cattle manure slurries (Table 5), underscoring the importance of not considering the contents of chemical elements, vitamins, etc. on a dry matter basis only.

4.6. Ni, Cu, Zn, Fe and Mn concentrations required for microbial growth and metabolism

The high number of diverse microbial metalloenzymes makes it clear that the availability of relevant transition metals is important depending on which microorganisms thrive in a given environment and which metabolic pathways the microorganisms use. Some studies have investigated whether nickel-restricted diet/feed would reduce ammonia production as the gut microbiota would be unable to synthesize enough active urease. One study showed good eradication of the ureolytic bacterium H. pylori when patients were placed on nickel-free diets (Campanale et al., 2014), while Spears and co-workers studied the influence of nickel restriction on various parameters, including urease activity and ammonia concentration, in neonatal pigs (Spears, Jones, Samsell, & Armstrong, 1984). However, in the latter case, no reduction in ammonia synthesis or

<table>
<thead>
<tr>
<th>Sample</th>
<th>Animal</th>
<th>Ni (µmol/L)</th>
<th>Cu (µmol/L)</th>
<th>Zn (µmol/L)</th>
<th>Fe (µmol/L)</th>
<th>Mn (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manure slurry</td>
<td>Pig</td>
<td>11.54</td>
<td>142.43</td>
<td>228.22</td>
<td>1641.13</td>
<td>523.44</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>2.07</td>
<td>21.67</td>
<td>179.36</td>
<td>1523.55</td>
<td>414.46</td>
</tr>
<tr>
<td></td>
<td>Horse</td>
<td>4.34</td>
<td>36.12</td>
<td>162.55</td>
<td>2673.91</td>
<td>1033.36</td>
</tr>
</tbody>
</table>
urease activity was detected, suggesting that the Ni requirement of the ureolytic bacteria was lower than the baseline Ni content (120–160 µg Ni/kg dm) of the feed used in the study. In the present study, the Ni content of pig faeces was found to be 10.38 ± 0.0002 mg/kg dm, which was approximately five times higher than that found in the faeces from horse and 10 times higher than that in the cattle faeces (Table 2). Interestingly, an earlier study showed that the rate of urea hydrolysis was in fact 2-fold higher in pig faeces than in cattle faeces, which may indicate that Ni concentration has some influence on urease activity (Dai & Karring, 2014). To the best of our knowledge, the urease activity in horse manure has not been investigated. Additionally, some studies have suggested that the production of CH₄ is approximately 1.5–2 times higher in pig manure/manure slurry than in cattle manure/manure slurry (Matulaitis, Juskiene, & Juska, 2015; Petersen, Olsen, Elsgaard, Triolo, & Sommer, 2016). Notably, methanogenesis is also a Ni-dependent metabolic process. Under controlled microbial culture conditions, the concentration of Ni required to orchestrate Ni-dependent metabolic processes such as urea hydrolysis by urease and methane anabolism is, however, very low. For example, the maximal growth of Hydrogenomonas bacteria required 0.3 µM Ni, while cultures of mixed methanogens were reported to be growth limited only below 0.44 µM Ni. Finally, H. pylori has been successfully grown in media containing 0.2–1.0 µM Ni (Hutner, 1972; Kida et al., 2001; Wolfram, Haas, & Bauerfeind, 2006). It has been shown that the minimal inhibitory concentration (MIC) of Ni²⁺ and Cu²⁺ towards E. coli is 1.0 mM (Nies, 1999). Together, these findings suggest that the faeces and manure slurry from all three animal species have Ni concentrations that exceed the amount required by ureolytic and methanogenic microorganisms but that do not reach toxic levels, indicating that Ni does not limit the microbial processes in these ecosystems (Tables 2, 3 and 5). Furthermore, it has been estimated that approximately 10⁵ atoms of Fe and Zn and 10⁶ atoms of Cu and Mn are required per E. coli cell volume, corresponding to approximately 100 µM and 10 µM concentrations, respectively, in the cytoplasm. However, microorganisms are adept at concentrating metals; for example, E. coli grown in minimal media was shown to have an intracellular Zn concentration that was 2000 times higher than the ambient concentration (200 µM Zn in the cytoplasm compared to only 0.1 µM Zn in the growth medium), suggesting that very low metal concentrations can be tolerated (Outten & Ó’Halloran, 2001). According to those observations, the manure slurries from pig, cattle and horse therefore contain more than enough Ni, Cu, Zn, Fe and Mn to fulfil the requirements for microbial metabolism and growth (Table 5).

4.7. Future perspectives

Agriculture is a significant source of the greenhouse gases CH₄, CO₂ and N₂O and other volatile compounds (e.g. NH₃) affecting the environment and climate. With the increasing concern about the impact of intensified livestock production on the environment, a detailed understanding of the composition of animal wastes and the conditions of growth for the microbial communities in these ecosystems is essential to mitigate the emission of gaseous compounds from animal manure slurry. Microbial metalloenzymes using transition metals as cofactors are essential in metabolic processes with major importance to the production, assimilation, and conversion of biogenic gaseous compounds such as NH₃, CH₄, CO₂, and N₂O. In this study, we determined the concentrations of the transitions metals Ni, Cu, Zn, Fe, and Mn in the feed, drinking waters, faeces and urine of three livestock animal species with very different digestion systems, namely, pig (monogastric omnivore), cattle (true ruminant), and horse (nonruminant/monogastric herbivore). Comparing our results with those previously reported show that in almost all cases, our values are the lowest, likely because of the increased focus on livestock diet and the environmental impact of soil metal loading from fertilizer use. Furthermore, our results suggest that microbiological metabolic processes depending on the transition metals Ni, Cu, Zn, Fe, and Mn are not limited by restricted access to the metals in the faeces, urine or manure slurry from any of the animal species analysed in this work. Finally, due to the presence of high amounts of all the analysed metals, particularly Cu and Zn, in pig manure slurry compared to those in cattle manure slurry, it seems that cattle manure/manure slurry may be a better choice as fertilizer in cases where metal overloading of soil is a concern. Nickel-restricted diet strategies have been investigated previously as a possible way to reduce ammonia emissions from livestock but with little effect, probably due to the minute
amounts of Ni required by ureolytic microorganisms. For NH\textsubscript{3} emissions, other strategies such as manure slurry acidification or the use of urease inhibitors could hold great promise (Sigurdarson, Svane, & Karring, 2018). A better understanding of the chemical composition of feed and manure slurry may lead to the combination of diet-related and waste treatment strategies. Thus, knowing the concentrations of these essential micronutrients in livestock faeces and manure slurry also provides the basis to optimize and possibly modulate transition metal-dependent microbial processes in the future to reduce the emissions of polluting gaseous compounds from livestock animal production and/or to enhance the fertilizer value of manure slurry.

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Competing Interests
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Declaration of interest statement
None of the authors has any affiliations with or involvement in any companies, trade associations, unions, or other groups with a direct financial interest in the subject matter discussed in the manuscript and/or their immediate family.

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